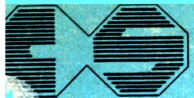

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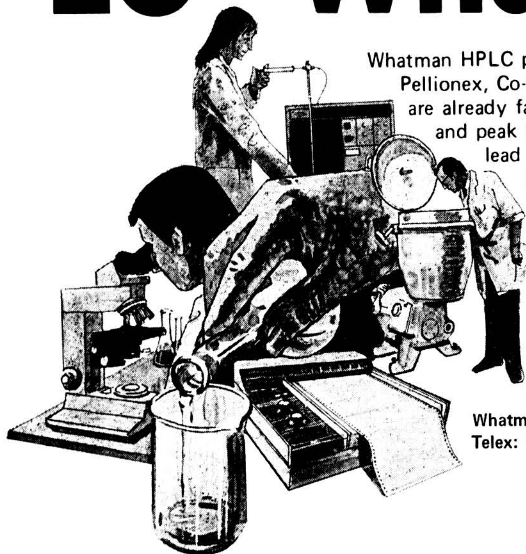

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Summaries of Papers in this Issue

Transformation and Decomposition of β-Molybdosilicic acid

Information about the reaction in which β-molybdosilicic acid is transformed into the α-acid is required in the context of both general chemical studies of the molybdosilicic acids and analytical studies. Previously, only Massart seems to have studied the reaction kinetics in any detail. Although our study confirmed Massart's observation that the rate of transformation of β-acid is first order with respect to β-acid concentration, it supported an alternative and simpler explanation for the changes in the apparent rate constant that accompany changes in the hydrogen ion concentration in the reaction mixture. The mechanism consists of a unimolecular transformation reaction with an acid - base pre-equilibrium involving the transformable β-acid species: when the pH is lowered, the shift in the acid - base equilibrium reduces the proportion of the total β-acid concentration present as the transformable species and thereby decreases the apparent rate constant. The apparent rate constant was found to be zero order with respect to molyddate concentration (0.005–0.050 M molybdate-molybdenum). The activation energy of the elementary reaction was found to be 15.7 ± 1.2 kcal mol^{-1}. The study has shown that the α-acid decomposes at pH values less than approximately 0.75 and greater than 3.5 (in 1.0 M sodium chloride solution). It is suggested that the β-acid decomposes more rapidly than the α-acid at any given pH less than 0.75. Methods of non-linear parameter estimation have been used extensively in fitting theoretical models to observed data.

Keywords: *Molybdosilicic acids*; *silicate*; *water*

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Analyst, 1977, **102**, 73–85.

Rapid Versatile Method for Determining Mercury at Sub-nanogram Levels by Cold-vapour Atomic-absorption Spectroscopy

This paper describes a rapid, dual-channel method of cold-vapour atomic-absorption spectroscopy for determining mercury which produces reproducible results to within ±2% of the average peak height for a number of 50-ng injections giving 50% of full-scale deflection (f.s.d.). The method has a detection limit of 0.25 ng (2:1 signal to noise ratio) and the blank values are low, *e.g.*, 10 ml of a mixture of 5% nitric acid and 0.01% potassium dichromate at a nitrogen flow-rate of 500 ml min^{-1} yields a blank of 1% f.s.d. (recorder: 1.0 mV, × 6 scale expansion).

Keywords: *Mercury determination*; *sub-nanogram level*; *atomic-absorption spectrophotometry*; *cold-vapour technique*

W. R. SIMPSON and G. NICKLESS

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Analyst, 1977, **102**, 86–94.

Determination of Ruthenium in Biological Material by Atomic-absorption Spectrophotometry Using Electrothermal Atomisation

A sensitive and rapid method was developed for the determination of ruthenium in faeces and other products of digestion by ruminants. The sample was ashed at 350 °C with a mixture of potassium nitrate and potassium hydroxide, dissolved in dilute nitric acid, and analysed by atomic-absorption spectrophotometry using a carbon rod atomiser. The method is particularly free from interferences and is suitable for the determination of ruthenium at concentrations between 5 and 50 μg per gram of dry matter.

Keywords : Ruthenium determination; biological material; atomic-absorption spectrophotometry

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Analyst, 1977, **102**, 95–98.

Nitrate Determination by Reduction to Ammonia and Gas-phase Ultraviolet Absorption Spectrometry

Nitrate can be rapidly reduced to ammonia by the action of titanium(III) sulphate, even at room temperature. Subsequent displacement of the ammonia by a current of air enables the gas-phase molecular absorbance of the ammonia to be measured and thus provides a sensitive and selective method for the determination of nitrate. Ions which affect the reduction of nitrate by titanium(III) salts interfere with the method and must be removed if present in large amounts.

Keywords: Nitrate determination; ammonia; ultraviolet absorption spectrometry

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Analyst, 1977, **102**, 99–103.

Comparison Between an Ultraviolet Spectrophotometric Procedure and the 2,4-Xylenol Method for the Determination of Nitrate in Groundwaters of Low Salinity

An ultraviolet spectrophotometric procedure for the determination of nitrate in groundwaters of low salinity has been investigated and compared with the recommended 2,4-xylenol method. The ultraviolet procedure is not subject to interference from the major cations present in typical groundwaters and interference from high levels of nitrite is overcome by the addition of sulphamic acid. Good agreement is demonstrated between nitrate values obtained by both methods on a number of groundwater samples.

The ultraviolet procedure offers a rapid and sensitive means of determining nitrate in such waters, which are generally low in content of organic matter, particularly where the volume of sample is limited, *e.g.*, studies on pore water samples.

Keywords: Groundwater; nitrate determination; ultraviolet spectrophotometry; 2,4-xylenol

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Analyst, 1977, **102**, 104–109.

Spectrophotometric Determination of Niobium and Germanium in Superconducting "Nb_3Ge" Sputtered Films

The determination of niobium and germanium in single-phase and crystalline radiofrequency sputtered "Nb_3Ge" thin films by rapid colorimetric methods is described. The niobium to germanium molar ratios obtained are compared with those obtained by electron-microprobe analysis.

Keywords: *Niobium determination*; *germanium determination*; *thin films*; *spectrophotometry*

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Analyst, 1977, **102**, 110–113.

Determination of Iron with Bathophenanthroline Following an Improved Procedure for Reduction of Iron(III) Ions

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) forms a red complex with iron(II) but not with iron(III) ions and is used in the determination of total iron when present at low molarities in aqueous solutions. The reduction of iron(III) ions is a critical step in such determinations. A modified procedure is described which brings about a 5-fold increase in the amount of iron(III) ions reduced compared with the original procedure, involving replacement of hydroxylammonium chloride with L-ascorbic acid, addition of concentrated hydrochloric acid and heating the solution in a steam-cabinet.

Keywords: *Iron determination*; *bathophenanthroline*; *reduction*; *L-ascorbic acid*

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Analyst, 1977, **102**, 114–119.

Improved Method for the Determination of Ethylenediaminetetraacetic Acid in Aqueous Environmental Samples by Gas - Liquid Chromatography

An improved method for the gas - liquid chromatographic determination of ethylenediaminetetraacetic acid in aqueous environmental samples is described. The separation of the major peaks is increased by preparing the ethyl derivatives of the sample components, 1,6-hexanediaminetetraacetic acid being used as internal standard. The lower limit of detection of the method is approximately 15 $\mu g\,l^{-1}$ with 25-ml samples. This limit can be improved, if necessary, by using a larger sample volume.

Keywords: *Ethylenediaminetetraacetic acid determination*; *sewage*; *water*; *gas - liquid chromatography*

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Analyst, 1977, **102**, 120–123.

The Analyst

Transformation and Decomposition of β-Molybdosilicic Acid

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Information about the reaction in which β-molybdosilicic acid is transformed into the α-acid is required in the context of both general chemical studies of the molybdosilicic acids and analytical studies. Previously, only Massart seems to have studied the reaction kinetics in any detail. Although our study confirmed Massart's observation that the rate of transformation of β-acid is first order with respect to β-acid concentration, it supported an alternative and simpler explanation for the changes in the apparent rate constant that accompany changes in the hydrogen ion concentration in the reaction mixture. The mechanism consists of a unimolecular transformation reaction with an acid - base pre-equilibrium involving the transformable β-acid species: when the pH is lowered, the shift in the acid - base equilibrium reduces the proportion of the total β-acid concentration present as the transformable species and thereby decreases the apparent rate constant. The apparent rate constant was found to be zero order with respect to molybdate concentration (0.005–0.050 M molybdate-molybdenum). The activation energy of the elementary reaction was found to be 15.7 ± 1.2 kcal mol^{-1}. The study has shown that the α-acid decomposes at pH values less than approximately 0.75 and greater than 3.5 (in 1.0 M sodium chloride solution). It is suggested that the β-acid decomposes more rapidly than the α-acid at any given pH less than 0.75. Methods of non-linear parameter estimation have been used extensively in fitting theoretical models to observed data.

Keywords: *Molybdosilicic acids*; *silicate*; *water*

Relatively small amounts of silicate present in a variety of materials, *e.g.*, natural waters,[1] metals,[2] rocks[3] and biological materials,[4] are mostly determined colorimetrically after converting the silicate into molybdosilicic acid. In such determinations, the reaction by which the β-molybdosilicic acid is transformed into α-molybdosilicic acid is important for two major reasons. Firstly, in those procedures which rely upon formation of β-acid, any uncontrolled transformation reaction can lead to error because of loss of the β-acid. Secondly, in the alternative procedures in which the α-acid is formed, the transformation reaction can be used to ensure that any β-acid inadvertently formed is converted into the α-acid. In a similar way, the transformation can be either a nuisance or an assistance in studies of the general chemistry of the molybdosilicic acids.[5–7]

Despite the importance of the transformation reaction in the context of the chemistry of the molybdosilicic acids, little seems to be known about it. Indeed, Massart[8] seems to have been the only worker to have studied the kinetics of the reaction in any detail, although Strickland,[5] who discovered the existence of the two acids and also the transformation reaction, carried out exploratory kinetic experiments.

We became interested in the transformation reaction because ignorance of its behaviour, under various conditions not dealt with previously, hindered interpretation of the results of our studies of the general chemistry of the molybdosilicic acids.[1,7,9] Although in many instances we avoided such problems by using an *ad hoc* approach, we anticipated that we

would require more rigorous control of the reaction in our studies of the kinetics of the formation of the molybdosilicic acids.

During this work, we discovered that α-molybdosilicic acid decomposes in saline solutions at pH values greater than approximately 3.5 (depending on ionic strength) or less than 1.0. The β-acid is believed to follow the same behaviour but to decompose more rapidly. Although little attention is paid here to these decomposition reactions, they are undoubtedly of importance to the analyst. Indeed, we believe that their existence probably accounts for the unexplained behaviour of the molybdosilicic acids when molybdosilicic acid solutions were treated with oxalic acid in order to suppress phosphate-phosphorus interference.[1]

Experimental

Apparatus and Reagents

Absorbance measurements were made on a Unicam SP 1800 spectrophotometer equipped with a constant-temperature housing for the cell. An EIL, Model 23A, direct-reading pH meter was used, which was standardised at pH 4.0 by means of Soloid buffer tablets (Burroughs Wellcome & Co.) and at pH 1.0 by using a mixture of 25.0 ml of 0.20 M potassium chloride solution and 67.0 ml of 0.20 M hydrochloric acid.[10] Readings of pH relating to those solutions to which sodium chloride had not been added could be used directly to obtain the hydrogen-ion concentration. However, when a background concentration of chloride (as sodium chloride) was maintained, the observed pH had to be corrected for salt effect on the electrodes before it was converted into a hydrogen-ion concentration (in 1.0 M sodium chloride the observed pH values were 0.08 too low). Other reagents were prepared in the manner described previously.[9] Ammonia solution was prepared by distilling and re-dissolving ammonia from a mixture of ammonium chloride and sodium hydroxide. The "distilled" ammonia solution was stored in a polythene container; storage in glass vessels resulted in uptake of silicate, which interfered in the determination of the apparent rate constant of transformation.

Method

In principle, a concentrated solution of β-acid was used to inoculate each reaction mixture in which the transformation was studied. This concentrated solution of β-acid was prepared at room temperature, where the rate of transformation is slow relative to the rate of formation. The β-acid was formed according to conditions described previously.[9] A stock molybdate solution was prepared by mixing 500 ml of 0.25 M molybdate-molybdenum solution, 985 ml of distilled water and 15.0 ml of 10.0 N sulphuric acid. The concentrated solutions of β-acid were mixed 10 min prior to inoculation from 15.0 ml of the stock molybdate solution and 10.0 ml of 100.0 mg l^{-1} silicate-silicon solution. Thus, the β-acid (40.0 mg l^{-1} of silicon) was prepared repeatedly in 0.050 M molybdate-molybdenum and at a pH of 1.8. To inoculate the reaction mixture, 5.00 ml of the β-acid solution were added to 45.0 ml of a solution of ammonia that had been heated to 53.3 ± 0.1 °C in a water-bath. After inoculation, a portion of the reaction mixture was transferred quickly into a spectrophotometer cell kept in the cell carriage at 50.0 ± 0.1 °C. The remainder of the reaction mixture was used for pH determination.

In these studies of the transformation reaction, and also in our unpublished studies of the kinetics of the formation of the molybdosilicic acids, it has been necessary to fit experimental data to mathematical models. In order to do this fitting, we used a non-linear least-squares method. In principle, each fitting is accomplished by using an iterative scheme in which the parameters of the model (*e.g.*, a and b in the exponential model $y = ae^{bx}$) are changed so that the deviation of the fitted line from the experimental data is minimised. The deviation is measured as the sum, over all data points, of the squares of the differences between the observed and fitted values. Expressed generally, the model is

$$Y_i = \mathrm{f}\,(X_i, \boldsymbol{\theta}) + \epsilon_i$$

where ϵ_i is the ith error of n observations of the form (X_i, Y_i) for $i = 1, \ldots, n$, $\boldsymbol{\theta}$ is the vector of p parameters to be estimated and f is a function non-linear in $\boldsymbol{\theta}$. Assuming that the errors are normally distributed and independent, the error sum of squares is defined as

$$S(\boldsymbol{\theta}) = \sum_{i=1}^{n} [Y_i - f(X_i, \boldsymbol{\theta})]^2$$

Given an initial estimate $\hat{\boldsymbol{\theta}}^0$, $\hat{\boldsymbol{\theta}}$ is the estimate of $\boldsymbol{\theta}$ that minimises $S(\boldsymbol{\theta})$. $\hat{\boldsymbol{\theta}}$ is found by the Newton iterative method[11]:

$$\boldsymbol{\theta}^{j+1} = \boldsymbol{\theta}^j - [H^{-1}\,\boldsymbol{v}]_{\boldsymbol{\theta}=\boldsymbol{\theta}^j}$$

$j = 0, 1, \ldots,$ where H is the Hessian matrix:

$$H_{lk} = \frac{\partial^2 S}{\partial\theta_l \partial\theta_k}$$

$l = 1, \ldots, p$, $k = 1, \ldots, p$ and $\boldsymbol{v}$ is the gradient vector:

$$v_l = \frac{\partial S}{\partial\theta_l}$$

$l = 1, \ldots, p$. When testing the validity of arguments based on results obtained using the non-linear least-squares method, it is not possible to use the correlation coefficient and F-test that would be used in a linear case. Here, therefore, use has been made of the coefficient of determination[12]:

$$r^2 = 1 - \frac{S(\hat{\boldsymbol{\theta}})}{\Sigma(Y_i - \bar{Y})^2}$$

where $\bar{Y} = \Sigma Y_i/n$, to describe how well each set of experimental data fit a given model. To compare the fitting of two alternative models we have used the likelihood ratio test.

The confidence limits for the estimated parameters ($\hat{\boldsymbol{\theta}}$) are obtained from the co-variance matrix:

$$V_{\hat{\boldsymbol{\theta}}} \approx \hat{\sigma}^2 H^{-1}$$

where $\hat{\sigma}^2 = S(\hat{\boldsymbol{\theta}})/(n - p)$ and H^{-1} is evaluated at $\boldsymbol{\theta} = \hat{\boldsymbol{\theta}}$. The diagonal elements of $V_{\hat{\boldsymbol{\theta}}}$ give the variance ($\sigma_{\hat{\boldsymbol{\theta}}}{}^2$) of the parameters $\hat{\boldsymbol{\theta}}$, and hence the 95% confidence limits are approximately

$$\hat{\boldsymbol{\theta}} \pm t_{(n-p)} \cdot \sigma_{\hat{\boldsymbol{\theta}}}$$

Where it has been useful to compare data with predicted values from a model (Fig. 1) involving estimated parameters ($\hat{\boldsymbol{\theta}}$), confidence limits of the predicted values ($\hat{Y}$) have been calculated. So,

$$\hat{Y}_i = f\,(X_i, \hat{\boldsymbol{\theta}})$$

and the variance of $\hat{Y}$ for a given x is

$$V_{\hat{Y}} = \hat{\sigma}^2 + \boldsymbol{w} V_{\hat{\boldsymbol{\theta}}} \boldsymbol{w}^T = \sigma_{\hat{Y}}{}^2$$

where $\boldsymbol{w} = \left[\frac{\partial f}{\partial \boldsymbol{\theta}}\right]_{\boldsymbol{\theta} = \hat{\boldsymbol{\theta}}}$ and T denotes the transpose.

Hence the 95% confidence limits for $\hat{Y}$ are approximately

$$\hat{Y} \pm t_{(n-p)} \hat{\sigma}_{\hat{Y}}$$

Results and Discussion

The objective of this study was to find how the rate of transformation varies with the concentrations of molybdosilicic acid, molybdate and hydrogen ions, and with temperature. A spectrophotometric technique, in which both α- and β-molybdosilicic acids contribute to the measured absorbance at 390 nm, was used. With this technique, changes in the concentrations of β-acid and α-acid are not readily separable. Thus, the absorbance (A_t) of any molybdosilicic acid solution at time t, after correcting for blank absorbances, is

$$A_t = \alpha^* \alpha_t + \beta^* \beta_t \quad \ldots \quad (1)$$

where α^* and β^* are the specific absorbances of the acids and α_t and β_t the concentrations of the two acids at time t.

Effect of Changing the β-Acid Concentration

Our results confirm Massart's original observation[8] that the rate of transformation of the β-acid is first order with respect to β-acid concentration. During transformation, the absorbance of each solution (A_t) was found to decrease exponentially with time, thus

$$A_t = \theta_1 + \theta_2 \exp(-\theta_3 t) \quad \ldots \quad (2)$$

where θ_1, θ_2 and θ_3 are parameters of the exponential model. Typical examples of the results are given in Table I. The coefficient of determination for each fitting of the data to the

TABLE I

CHANGE IN ABSORBANCE AT 390 nm ACCOMPANYING TRANSFORMATION OF THE β-ACID AT 30 °C

A: pH 2.99, chart scale 0.00–1.00, absorbance equivalence 0.0–0.2 A.
B: pH 2.99, chart scale 0.00–1.00, absorbance equivalence 0.0–1.0 A.

A			B		
Time/min	Observed chart reading	Value calculated from exponential model	Time/min	Observed chart reading	Value calculated from exponential model
1.0	0.930	0.931	1.0	0.948	0.956
2.0	0.887	0.890	2.0	0.912	0.914
3.0	0.852	0.852	3.0	0.878	0.875
4.0	0.818	0.816	4.0	0.842	0.839
5.0	0.786	0.783	5.0	0.807	0.805
6.0	0.756	0.753	6.0	0.777	0.774
7.0	0.727	0.725	7.0	0.749	0.746
8.0	0.699	0.699	8.0	0.722	0.719
9.0	0.676	0.675	9.0	0.695	0.694
10.0	0.651	0.652	10.0	0.671	0.672
11.0	0.629	0.632	11.0	0.650	0.651
12.0	0.611	0.612	12.0	0.629	0.631
13.0	0.592	0.595	13.0	0.611	0.613
14.0	0.578	0.578	14.0	0.594	0.596
15.0	0.562	0.563	16.0	0.562	0.566
16.0	0.548	0.549	18.0	0.538	0.541
17.0	0.538	0.536	20.0	0.516	0.519
18.0	0.525	0.524	22.0	0.498	0.500
20.0	0.503	0.502	24.0	0.482	0.484
22.0	0.482	0.484	26.0	0.471	0.470
24.0	0.470	0.468	28.0	0.459	0.458
∞	<0.392	0.372	30.0	0.450	0.448
—	—	—	32.0	0.441	0.439
—	—	—	36.0	0.425	0.425
—	—	—	∞	<0.390	0.386

exponential model exceeded 0.999 8 in every instance; a perfect fit would have given a value of 1.000 0. Further, we were unable to detect any difference between the rate constant, θ_3, obtained by using different initial concentrations of β-acid but otherwise identical conditions. Triplicate determinations of apparent rate constants at 30.0 °C, pH 3.0 ± 0.1,

molybdosilicic acid concentrations of 4.00 and 0.80 mg l^{-1} of silicon and no added sodium chloride gave values of 0.819, 0.767 and 0.847, and 0.766, 0.801 and 0.811, respectively; the mean apparent rate constant for the two concentrations did not differ significantly at the 5% level. This behaviour is consistent with the rate of transformation of the β-acid being first order with respect to the β-acid concentration provided that there is only transformation, because, in this instance

$$\beta_t = \beta_0 \, e^{-kt} \qquad (3)$$

and

$$\beta_t + \alpha_t = Z \qquad (4)$$

where Z is the total concentration of molybdosilicic acid and k the apparent rate constant. As all of the molybdosilicic acid is initially in the β-form (*i.e.*, $\alpha_t = 0$), then

$$\beta_t = \beta_0 = Z$$

Therefore, from equations (3) and (4)

$$\alpha_t = Z(1 - e^{-kt}) \qquad (5)$$

From equations (1), (3) and (5), therefore

$$A_t = \alpha^* Z + (\beta^* Z - \alpha^* Z) \, e^{-kt}$$
$$= A^\infty + \Delta A e^{-kt}$$

where A^∞ and ΔA are the final absorbance and total change in absorbance, respectively, and, on comparing coefficients with equation (2), $\theta_1 = A^\infty$, $\theta_2 = \Delta A$ and $\theta_3 = -k$.

Initially, a difficulty was experienced in our study of the effect of changing the initial β-acid concentration on the apparent rate constant. The decrease in absorbance with time, resulting from transformation of the β-acid, was found to have been offset by a concomitant increase in absorbance caused by the formation of additional α-acid. The α-acid was formed from small amounts of silicate present in the ammonia solution that was used to increase the pH of the molybdosilicic acid solution to its final value (prior to the inoculation of the reaction mixture with the solution of β-acid, the pH of the reaction mixture was too high for formation of α-molybdosilicic acid). Such an increase in absorbance can cause errors in two ways. Firstly, it can decrease the magnitude of the apparent rate constant, and secondly, it can distort the graphs of absorbance against time so that they are not exponential. We observed the second of these effects in the graphs relating to the solutions that contained the low initial concentration of β-acid (0.80 mg l^{-1} of silicon). At these concentrations, an expanded absorbance range was utilised to give the required resolution and the effect was manifested as an induction period; the graph of absorbance *versus* time was flat for the first few minutes. At the higher concentration of β-acid the effect was not discernible. The difficulty was eliminated by using an ammonia solution prepared from gaseous ammonia as described above.

Salt Effects

Addition of sodium chloride to the reaction mixtures accelerated the transformation reaction markedly (Table II). As will be shown later, the major effect of the salt is to alter the constant for an acid - base equilibrium that precedes transformation. In view of the marked salt effect, our studies were usually conducted with a background of 1.0 M sodium chloride solution in order to suppress other minor changes in ionic strength. However, some experiments were conducted without added sodium chloride in order to obtain information about the transformation reaction under conditions approaching those encountered in the determination of silicate in natural fresh waters.[1]

TABLE II

EFFECT OF ADDING SODIUM CHLORIDE ON THE APPARENT RATE CONSTANT

Temperature, 30.0 °C; 4.0 mg l^{-1} of silicon as β-acid; 0.005 M molybdate-molybdenum.

Sodium chloride added/mol l^{-1}	Apparent rate constant/min^{-1}	Hydrogen-ion concentration/N
0.0	4.4×10^{-3}	2.69×10^{-3}
0.3	1.95×10^{-2}	3.16×10^{-3}
0.5	2.55×10^{-2}	3.16×10^{-3}
0.7	3.45×10^{-2}	3.23×10^{-3}
1.0	5.20×10^{-2}	3.16×10^{-3}
2.0	8.10×10^{-2}	3.09×10^{-3}
3.0	9.80×10^{-2}	2.75×10^{-3}
4.0	1.05×10^{-1}	3.31×10^{-3}

Effect of Changing the Molybdate Concentration

The rate of transformation at 17 °C was found to be zero order with respect to the molybdate concentration. At pH 3.48 $\pm$ 0.05 the apparent rate constant was 0.032 $\pm$ 0.002 min^{-1} at molybdate-molybdenum concentrations of 0.005, 0.010, 0.020, 0.040 and 0.050 M.

Effect of Changing the Hydrogen-ion Concentration

Our study of the effect of hydrogen-ion concentration on the kinetics of transformation has been complicated by the decomposition of the molybdosilicic acids. It is convenient, therefore, to discuss decomposition first.

Decomposition

Decomposition of either α- or β-molybdosilicic acid was shown to have occurred in an experiment in which a set of reaction mixtures (1.0 M in sodium chloride), each containing the same amount of β-acid but at different pH, were incubated in a water-bath for 6 h at 50.0 °C. A second set of reagent blanks (0.0 mg l^{-1} of added silicon) were treated in the same way. After cooling, the absorbance (390 nm) (due to the α-acid formed by transformation) was constant between pH 0.75 and 3.50 (Table III). Outside these limits the absorbance was

TABLE III

RESIDUAL ABSORBANCE OF SOLUTIONS OF THE β-ACID (CONTAINING 1.0 g-ion l^{-1} OF CHLORIDE) AFTER INCUBATION FOR 6.0 h AT 50.0 °C

pH	Absorbance (390 nm, 2-cm cell)	pH	Absorbance (390 nm, 2-cm cell)
0.40	0.421	1.45	0.494
0.49	0.437	1.60	0.491
0.55	0.452	1.83	0.490
0.61	0.468	3.00	0.503
0.65	0.477	3.20	0.499
0.75	0.493	3.35	0.500
0.81	0.494	3.50	0.500
0.98	0.497	3.60	0.470
1.05	0.489	3.70	0.444
1.10	0.489	3.81	0.406
1.15	0.494	3.90	0.343
1.22	0.494	4.02	0.240
1.31	0.493		

lower, indicating decomposition. Similar studies, in which solutions of either the α-acid, or the β-acid, formed from the same amount of silicon, were incubated at 50.0 °C and pH less than 1.0 for up to 21.5 h, strongly suggest that both of the molybdosilicic acids decompose at low pH. The α-acid was prepared in the same way as the β-acid, except that during its preparation the pH of the solution was 4.0 instead of 1.8.[9] After incubation for 21.5 h only the α-acid remained; the spectra of the molybdenum blues, formed from each yellow molybdosilicic acid solution after treatment with excess of acid and tin(II) chloride, were characteristic of the α-acid.[7] At pH 1.03, the yellow solutions of the α-acid and the transformed β-acid yielded similar absorbances at 390 nm (Table IV), showing that no significant decompo-

sition (less than 2%) had occurred at that pH, as was to be expected from the previous experiment. However, at lower pH the absorbances at 390 nm were lower in both sets of solutions, indicating decomposition. Moreover, at pH less than 1.0 the absorbance at 390 nm of any solution of β-acid was lower than that of the corresponding solution of α-acid. Relative to the series of solutions of α-acid, therefore, enhanced loss of molybdosilicic acid occurred in the series of solutions of β-acid. The most likely explanation for these observations is that the β-acid itself also decomposes.

TABLE IV

COMPARISON OF THE EXTENT OF THE DECOMPOSITION OF MOLYBDOSILICIC ACID (APPROXIMATELY 4.0 mg l^{-1} OF SILICON) AT pHs OF 1.0 AND BELOW (0.005 M MOLYBDATE-MOLYBDENUM, 1.0 g-ion l^{-1} IN CHLORIDE)

The results are absorbances (390 nm, 2-cm cell), corrected for blank absorbance

		pH					
	Time/h	1.42	1.03	0.70	0.45	0.30	0.10
Sample originally β-acid	0	0.848	0.858	0.860	0.864	0.878	0.866
	1.2	0.560	0.606	0.601	0.574	0.531	0.472
	2.75	0.547	0.551	0.532	0.498	0.451	0.388
	21.5	0.548	0.549	0.539	0.484	0.424	0.335
Sample originally α-acid	0	—	0.557	0.552	0.553	0.558	0.562
	1.2	—	0.565	0.554	0.555	0.554	0.554
	2.75	—	0.561	0.551	0.547	0.545	0.541
	21.5	—	0.561	0.553	0.542	0.522	0.467
Blank absorbance, identical for both α- and β-acid solutions	0	0.010	0.011	0.011	0.007	0.009	0.009
	2.75	0.009	0.011	0.010	0.007	0.007	0.009
	21.5	0.009	0.012	0.011	0.006	0.006	0.009

Without major modification, our spectrophotometric technique cannot tolerate reactions that decompose the molybdosilicic acids, as it is assumed in equation (4) that there are no side-reactions. If this technique is applied when this assumption is invalid, the measured rate constant will be too large as the absorbance of the reaction mixture will decrease at an enhanced rate. Evidence of this effect can be seen in our earliest results, which were obtained prior to our discovering that decomposition accompanied transformation at the extreme pH values in the range 0.0–5.0. An example of one set, relating to 1.0 M sodium chloride

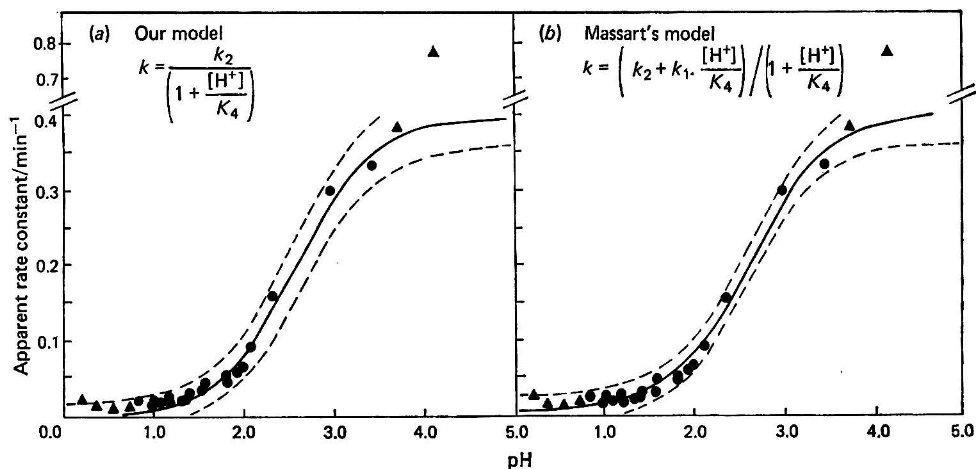

Fig. 1. Variation of the apparent rate constant with pH for results relating to 50.0 °C and 1.0 M sodium chloride solution. Additional points (▲) to those from Table VI (●) have been added to show the total pH range studied. The increase in ordinate values observed below pH 0.75 and above pH 3.5 probably result from the decomposition of the molybdosilicic acids. The solid lines in (*a*) and (*b*) show the results of fitting the data to the simple and Massart models, respectively. The broken lines give the confidence limits at the 95% level.

solution at 50.0 °C, is given in Fig. 1. At the extreme pH settings, the apparent rate constant is higher than extrapolation of the graph between pH 1.0 and 3.7 would suggest. This anomaly also appears at low pH in Massart's data.[8]

As it was difficult to modify our spectrophotometric technique to overcome the problem of decomposition, our further studies have been restricted to the pH regime where only transformation occurs.

Transformation

The rate of transformation of the β-acid increases with decrease in the hydrogen-ion concentration (Table V). Initially, it seemed that two simple mechanisms could have led to this behaviour. Firstly, a base-catalysed transformation, or secondly, a transformation reaction preceded by an acid - base equilibrium involving the reactive β-acid species. However, as plots of the logarithm of the apparent rate constants against pH did not yield the classical graphs[13] associated with acid - base catalysis, the former mechanism was not considered further. For the second mechanism to be feasible, it seemed that only one (or perhaps two) of the four protons of the β-acid would be involved. This followed from Tsigdinos

TABLE V

APPARENT RATE CONSTANTS FOR THE TRANSFORMATION OF β-MOLYBDOSILICIC ACID UNDER VARIOUS CONDITIONS

30.0 °C; 1.0 M sodium chloride		50.0 °C; 1.0 M sodium chloride		50.0 °C; 0.0 M sodium chloride	
$[H^+]$	$k \times 10^2$/min^{-1}	$[H^+]$	$k \times 10^2$/min^{-1}	$[H^+]$	$k \times 10^2$/min^{-1}
3.54×10^{-4}	10.6	3.63×10^{-4}	33.9	4.17×10^{-5}	35.3
4.90×10^{-4}	9.08	1.07×10^{-3}	29.9	6.17×10^{-5}	31.1
6.61×10^{-4}	8.68	4.57×10^{-3}	16.1	1.02×10^{-4}	27.9
9.55×10^{-4}	8.47	8.31×10^{-3}	9.07	1.07×10^{-4}	26.1
1.66×10^{-3}	7.54	1.07×10^{-2}	6.69	3.16×10^{-4}	17.6
2.04×10^{-3}	6.84	1.18×10^{-2}	6.09	6.06×10^{-4}	14.8
2.51×10^{-3}	6.02	1.59×10^{-2}	5.45	9.33×10^{-4}	9.79
3.39×10^{-3}	5.28	1.59×10^{-2}	4.74	2.46×10^{-3}	5.90
5.62×10^{-3}	3.64	2.63×10^{-2}	4.47	3.98×10^{-3}	3.75
7.94×10^{-3}	2.55	2.82×10^{-2}	3.40	5.62×10^{-3}	2.33
1.00×10^{-2}	2.05	3.98×10^{-2}	2.82	7.94×10^{-3}	1.96
1.23×10^{-2}	1.55	4.17×10^{-2}	2.27	1.12×10^{-2}	1.18
1.69×10^{-2}	1.31	4.37×10^{-2}	2.06	2.51×10^{-2}	0.81
1.99×10^{-2}	1.08	6.46×10^{-2}	2.28	5.01×10^{-2}	0.77
3.09×10^{-2}	0.89	6.46×10^{-2}	1.96		
		8.51×10^{-2}	1.96		
		9.77×10^{-2}	2.30		
		1.05×10^{-1}	1.59		
		1.41×10^{-1}	2.27		

and Hallada's observations[14] that, in water, the β-acid is a strong tetrabasic acid with its titration graph displaying only a single point of inflection at the addition of four equivalents of base. It seemed that our postulated mechanism could be compatible with Tsigdinos and Hallada's strong acid,[14] provided that the pK value relating to the last proton ($\beta H^{3-} \rightarrow \beta^{4-} + H^+$) was sufficiently low to make successive dissociations indiscernible on the titration graph. As our results suggested that the mechanism would demand a pK value between 2.0 and 4.0 (which is the range where the rate of transformation changed markedly with pH), it seemed likely that this condition would be satisfied. Subsequently, it was found that Massart[8] had already modelled the kinetics of transformation with an acid - base pre-equilibrium. However, whereas in our model only one β-acid species was transformed, Massart's model allowed two β-acid species to be transformed. When generalised, the mechanisms involve the following:

(*a*) an acid - base equilibrium:

$$\beta H^{3-} \overset{K_4}{\rightleftharpoons} \beta^{4-} + H^+ \quad \ldots \quad (6)$$

where $K_4 = [\beta^{4-}]\,[H^+]/[\beta H^{3-}]$ or $pH = pK_4 + \log\,([\beta^{4-}]/[\beta H^{3-}])$;

(b) a continuity equation:

$$\beta^{4-} + \beta H^{3-} = \beta_{Total}$$

if only two β-acid species are present in significant amounts. This gives

$$\beta^{4-} = \frac{[\beta_{Total}]}{\left(1 + \frac{[H^+]}{K_4}\right)}$$

(c) elementary transformations:

$$\beta H^{3-} \xrightarrow{k_1} \alpha \quad \ldots \quad (7)$$

$$\beta^{4-} \xrightarrow{k_2} \alpha \quad \ldots \quad (8)$$

Considering our model, *i.e.*, $k_1 = 0$, the transformation velocity is given by

$$k_2\,[\beta^{4-}] = \frac{k_2[\beta_{Total}]}{\left(1 + \frac{[H^+]}{K_4}\right)}$$

Therefore, the rate of reaction is first order with respect to the total concentration of the β-acid and the apparent rate constant, k, is

$$k = \frac{k_2}{\left(1 + \frac{[H^+]}{K_4}\right)} \quad \ldots \quad (9)$$

Considering the Massart model,[8] *i.e.*, $k_1 \neq 0$, the apparent rate constant, k, is given by

$$k = \frac{\left(k_2 + k_1 \cdot \frac{[H^+]}{K_4}\right)}{\left(1 + \frac{[H^+]}{K_4}\right)} \quad \ldots \quad (10)$$

At any pH, the amount of β-acid transformed via the fully ionised species is a given multiple (η) of that transformed via the protonated species. That is,

$$k_2\,[\beta^{4-}] = \eta k_1\,[\beta H^{3-}] \quad \ldots \quad (11)$$

Solving for $[\beta^{4-}]/[\beta H^{3-}]$ and substituting in equation (6), we obtain

$$pH = pK_4 + \log\left(\eta \cdot \frac{k_1}{k_2}\right) \quad \ldots \quad (12)$$

Our problem, therefore, was to determine which of the two models gave the more satisfactory fit to the observed data. This was accomplished using the likelihood ratio (R) test, defined as

$$R = \frac{\text{likelihood of } k_1 = 0}{\text{likelihood of } k_1 \neq 0}$$

where $-n \ln R$ has a χ^2 distribution with 1 degree of freedom.

TABLE VI

FIT OF THE EXPERIMENTAL DATA TO THE MODELS

				$k = \frac{k_2}{\left(1 + \frac{[H^+]}{K_4}\right)}$			$k = \frac{\left(k_2 + k_1 \cdot \frac{[H^+]}{K_4}\right)}{\left(1 + \frac{[H^+]}{K_4}\right)}$				Comparison of the models	
Temperature/°C	Sodium chloride concentration/M	No. of points	pH range	k_2/min^{-1}	pK_4	Coefficient of determination (r^2)	k_1/min^{-1}	k_2/min^{-1}	pK_4	Coefficient of determination (r^2)	Likelihood ratio (R)	$-n \ln R$ (χ^2 distribution with 1 degree of freedom)
30.0	1.0	15	1.51–3.45	0.115 ± 0.008	2.59 ± 0.16	0.988 1	−0.006 ± 0.005	0.112 ± 0.005	2.50 ± 0.25	0.990 8	0.768 4	3.95
50.0	1.0	19	0.85–3.45	0.397 ± 0.025	2.57 ± 0.15	0.989 6	0.004 ± 0.006	0.402 ± 0.024	2.60 ± 0.22	0.990 4	0.920 5	1.57
50.0	0.0	14	1.30–4.38	0.372 ± 0.026	3.48 ± 0.23	0.991 3	0.009 ± 0.011	0.380 ± 0.026	3.54 ± 0.29	0.992 9	0.818 0	2.81
31.0	2.0											
Massart's data (*a*)		8	1.15–3.40	0.148 ± 0.016	2.47 ± 0.38	0.979 0	0.001 ± 0.016	0.148 ± 0.018	2.47 ± 0.54	0.979 0	1.000	0.00
Massart's data (*b*)		10	−0.25–3.40	0.148 ± 0.016	2.47 ± 0.33	0.984 0	0.003 ± 0.007	0.150 ± 0.016	2.50 ± 0.40	0.985 3	0.919 7	0.84

The results of fitting the observed data to equations (9) and (10) by means of the non-linear least squares method are given in Table VI. The experimental data fitted both models very well because in every instance the coefficient of determination (Table VI) was greater than 0.979; a value of 1.000 indicates an exact fit. Massart's data (for 31.0 °C and 2.0 M sodium chloride concentration)[8] were also treated in the same way. However, as Massart was unaware that the β-acid decomposes at pH values less than 1.0 it is probable that his apparent rate constants for pH 0.23 and −0.25 include a decomposition component. Therefore, in one instance, (*a*), in order to obtain results free of the decomposition effect only data relating to pH values above 1.0 were included. In another instance, (*b*), in order to obtain results that can be compared directly with Massart's, we re-evaluated Massart's data on the Massart model [equation (10)]. Whereas Massart's values for k_1, k_2 and pK_4 were 0.004, 0.143 and 2.5, respectively, our values were 0.003, 0.150 and 2.50, respectively, showing good agreement.

The results in Table VI can be used to yield a clearer picture of the operation of the Massart model. Thus, substitution of the relevant values of pK_4, k_1 and k_2 into equation (12) shows that in 2.0, 1.0 and 0.0 M sodium chloride solution, the pH values at which the velocities of transformation via the fully ionised species are ten times that via the protonated species ($\eta = 10$) are 1.8, 1.6 and 2.9, respectively. Therefore, in sodium chloride solution transformation via the protonated species would be significant only at pH values less than approximately 1.8 (Fig. 1); in similar solutions, but without sodium chloride, the reaction would be significant at pH values approximately 1 unit higher. The absence of a significant amount of transformation via the protonated species at even higher pHs, *e.g.*, 3.5, demands that the values of the rate constant k_2 for the Massart model should be similar to those for the simpler model; this result has been demonstrated (Table VI, Fig. 1).

The likelihood ratio test shows that in all instances except that at 30.0 °C and 1.0 M sodium chloride concentration there is no significant improvement in the fit using the Massart model [equation (10)] instead of the simpler model [equation (9)] at the 5% significance level (for significance, $-n\ln R \geqslant 3.84$ at the 5% level with 1 degree of freedom). Moreover, although in the anomalous instance an improved fit was given by the Massart model [equation (10)], this fit relied upon a negative value of k_1. As such a value is not feasible, the best possible fit is once again given by the simpler model [equation (9)], as $k_1 = 0$ is the nearest feasible value. It should also be noted that the result for the case in which sodium chloride was not added was obtained by using data that must be slightly biased in favour of the Massart model. That is, the apparent rate constants relating to the lower pH settings will be too high because of an increase in ionic strength derived from additional acid.

The discussion, so far, shows that existing information about the transformation reaction (including that obtained by Massart[8]) strongly supports the simpler model as being correct. Nevertheless, we admit that in all instances studied except that by Massart, the constraint of being unable to include points relating to solutions in which decomposition is known to occur (pH values less than 0.75 in 1.0 M sodium chloride solution), might have biased the argument in favour of this conclusion. We feel, therefore, that the ultimate test of the credibility of the simpler model can be provided only by further study of the transformation reaction over a wide pH range, and including pH values at which we have found decomposition to occur. We have not extended our studies in this way because our exploratory work suggests that such an extension would present formidable problems. Even the first step, that of confirming our suspicion that the rates of the decomposition reactions are first order with respect to concentration of molybdosilicic acid, is difficult. The magnitude of the apparent rate constants of each of the contributing reactions changes rapidly with decrease in pH in this low pH range, and we have found it difficult to control the hydrogen-ion concentration to the required degree. Moreover, at the low pH, the rates of some of these reactions will be low even at elevated temperatures (50 °C); with our equipment, this effect would demand excessive amounts of time. Notwithstanding such practical problems, it seems unlikely that a composite apparent first-order rate constant for both decomposition and transformation of the β-acid will be split easily to give individual apparent rate constants. Although simpler reaction systems that involve two competitive first-order reactions have been studied successfully before,[13] a knowledge of the concentrations of the products at the end of the reaction has been necessary in order to achieve a mathematical solution. In these studies, however, such information is not readily available. We also feel that the spectro-

photometric method used here has only limited value in any extended study as it does not readily discriminate between changes in α-acid and β-acid concentrations.

Activation energy for transformation

Determination of the apparent rate constant for the transformation of the β-acid at various known temperatures (Table VII) but constant pH has allowed the activation energy of the reaction to be calculated. A plot of the natural logarithm of the apparent rate constant against the reciprocal of the absolute temperature (Arrhenius plot[13]) fitted a straight line well (correlation coefficient 0.997 5). The gradient and its standard error were −788 1 and 279 K^{-1}, respectively, giving an activation energy of 15.7 kcal mol^{-1} with a standard error of 0.6 kcal mol^{-1}. It was possible to use the apparent rate constant (k) instead of the true rate constant (k_2) in the Arrhenius plot because the magnitude of the dissociation constant (K_4) was not markedly dependent on temperature (Table VI). It was reasonable to assume, therefore, that the apparent rate constant was a multiple (ϕ) of the true rate constant, *i.e.*,

TABLE VII

VARIATION WITH TEMPERATURE OF THE APPARENT RATE CONSTANT FOR THE TRANSFORMATION OF THE β-ACID

Temperature/°C (± 0.05 °C)	pH	k/min^{-1}
50.10	3.42	0.466
	3.45	0.500
45.10	3.50	0.372
	3.50	0.358
40.10	3.52	0.244
	3.50	0.249
34.82	3.50	0.172
	3.52	0.172
23.02	3.51	0.056 2
	3.50	0.056 0
17.00	3.51	0.030 3
	3.55	0.032 1

$$k = \frac{1}{\left(1 + \frac{[H^+]}{K_4}\right)} k_2 = \phi k_2$$

provided that $[H^+]$ and K_4 remain constant. From values of pK_4 (Table VI) it follows that at a constant pH of 3.5 the magnitude of ϕ changes only from 0.895 at 50.0 °C to 0.890 at 30.0 °C.

Some Analytical Implications

In addition to satisfying our requirement for precise values of the rate of transformation over a wide range of conditions, this study shows that, as a result of the effect of ionic strength on both the transformation of β-acid and the decomposition of α-acid, the conditions for analysis of saline waters will differ from those used for fresh waters. We suggest that the effect of ionic strength on both transformation of the β-acid and decomposition of the α-acid (in weakly acidic conditions) are analogous. Thus, we suggest that in both instances at constant pH, the reaction rate increases with increase in ionic strength (earlier experiments[9] at low ionic strengths were not designed in such a way that would emphasise the presence of the decomposition reaction at pH>5.0; for the present, therefore, the occurrence of the decomposition reaction at low ionic strengths and pHs close to 5.0 must remain conjectural). Therefore, as the ionic strength is increased the transformation reaction tends to increase the proportion of α-acid in any β-acid or α- + β-acid mixture, that is, it tends to shorten the pH range where β-acid is formed. Similarly in the case of the decomposition reaction an increased ionic strength tends to shorten the pH range where the α-acid is formed.

The fact that, in 1.0 M sodium chloride solution, decomposition of the α-acid occurs at pHs greater than 3.5 emphasises the latter point. Thus, while the use of a pH of 4.0 in fresh-water silicate-silicon analysis proved acceptable,[1] its use in the determination of silicate-silicon in 1.0 M sodium chloride solution would lead to reduced and possibly non-stoicheiometric yields of the α-acid. Unfortunately, at this stage the magnitude of the shortening of the pH ranges appropriate for α- and β-acids cannot be predicted more precisely because the rates of formation of the two acids will also be affected by the ionic strength. Our continuing studies of the formation kinetics are directed partly towards solving this problem.

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Rapid Versatile Method for Determining Mercury at Sub-nanogram Levels by Cold-vapour Atomic-absorption Spectroscopy

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This paper describes a rapid, dual-channel method of cold-vapour atomic-absorption spectroscopy for determining mercury which produces reproducible results to within ±2% of the average peak height for a number of 50-ng injections giving 50% of full-scale deflection (f.s.d.). The method has a detection limit of 0.25 ng (2:1 signal to noise ratio) and the blank values are low, *e.g.*, 10 ml of a mixture of 5% nitric acid and 0.01% potassium dichromate at a nitrogen flow-rate of 500 ml min^{-1} yields a blank of 1% f.s.d. (recorder: 10 mV, ×6 scale expansion).

Keywords: *Mercury determination*; *sub-nanogram level*; *atomic-absorption spectrophotometry*; *cold-vapour technique*

The very low concentrations of mercury that are found in protein solutions collected in fractions from gel-permeation columns are normally determined by the use of labelled mercury (mercury-203), a process requiring highly specialised laboratory facilities (for example, see reference 1). To avoid the need for such facilities the apparatus described here was developed; with its use up to sixty samples per hour can be analysed. The method has been applied to the analysis of soils, natural waters, sediments and biological samples, some examples of which are given below.

A somewhat similar principle has been used by Hawley and Ingle,[2] but the method described here utilises a conventional, single-beam spectrometer and up to double the number of analyses can be completed in the same time.

Experimental and Results

Development and Description of Apparatus

The apparatus is shown schematically in Fig. 1. The salient features are as follows. A medium porosity frit is used in the bubblers, which allows for better aeration of the sample compared with the traditional Drechsel head.[3] Fine sinters tend to become blocked with tin(II) chloride or protein precipitates, while coarse sinters cause the sample to carry over into the drying tube. The bubblers are enlarged at their tops. By increasing the diameter a reduction of foaming and sample carry-over is obtained in comparison with straight-sided bubblers. A bubbler of about 50-ml volume has been found to be the most efficient. Bubblers of 100- and 250-ml total volume have also been tried, but the smaller bubbler gives faster response times and better detection limits for smaller samples at lower flow-rates. The bubblers are dual channel, which allows for a maximum sample size of 20 ml of, for example, river water, giving a detection limit as low as 12.5 p.p.t. (0.25 ng per 20 ml of sample). In addition, a tube containing gold wire can be introduced parallel to the drying tube containing magnesium perchlorate in order to check for molecular absorption, a system used by Jones and Nickless.[3]

Reagents and Instrumentation

All reagents were of analytical-reagent quality, and all glass-ware was soaked for 24 h in 20% nitric acid followed by a mixture of 0.01% potassium dichromate and 5% nitric acid, made up with doubly distilled water, the latter mixture being left in the glass-ware until it was required for use.

Tin(II) chloride.

Magnesium perchlorate, 14–22 *mesh.*

Potassium dichromate.

Nitric acid, fuming.

Sulphuric acid.

Mercury(II) chloride.

Tin(II) chloride was prepared as a 20% solution in 1 M sulphuric acid. Standards containing 0.01, 0.1 and 1.0 p.p.m. of mercury(II) in a mixture of 5% nitric acid and 0.01% potassium dichromate solution[4] were obtained by dilution of a 1 000 p.p.m. stock solution.

The determinations were carried out on a Varian Techtron Model AA5 atomic-absorption spectrometer, both with and without the use of the Varian Techtron BC-6 background corrector unit, using an Oxford Instrument 3000 Series potentiometric recorder.

The principle used was that described by Poluektov *et al.*[5] and later developed by Hatch and Ott,[6] whereby mercury(II) is reduced to mercury(0) with a solution of tin(II) ions.

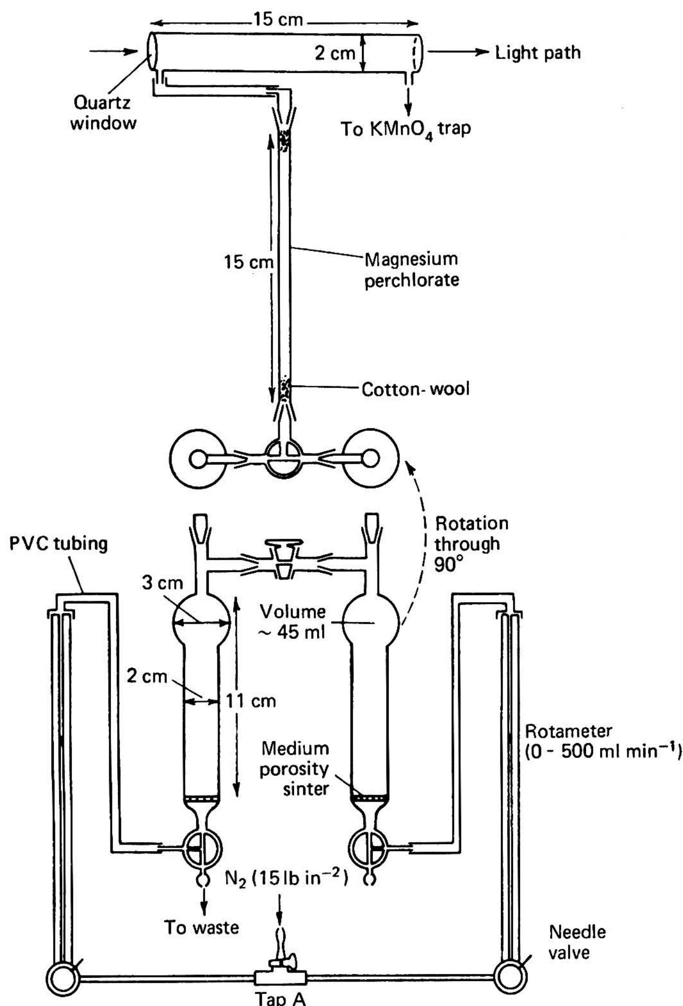

Fig. 1. Dual-bubbler system for determination of mercury by cold-vapour atomic-absorption spectroscopy. Scale: ¼ full size. All taps are Teflon Interflow, three-way, 3.4 mm diameter and all joints are B10.

Operation

The sequence of events for the determination is shown in Fig. 2, starting from operation 1 in the upper left-hand corner and progressing sequentially through to operation 8 in the lower right-hand corner. In operation 1 tin(II) chloride is injected into the bubblers, the stoppers

replaced and residual mercury volatilised from the solution (operation 2). The tap adjacent to the drying tube is switched to allow the passage of nitrogen through the left or right channel (operation 3), the sample injected and the stopper replaced (operation 4). Operations 5 and 6 describe a similar procedure for the alternative channel, while 7 and 8 depict the evacuation of spent solution from the bubblers. This complete sequence takes less than 2 min if tin(II) chloride solution that has been previously de-gassed with nitrogen is used.

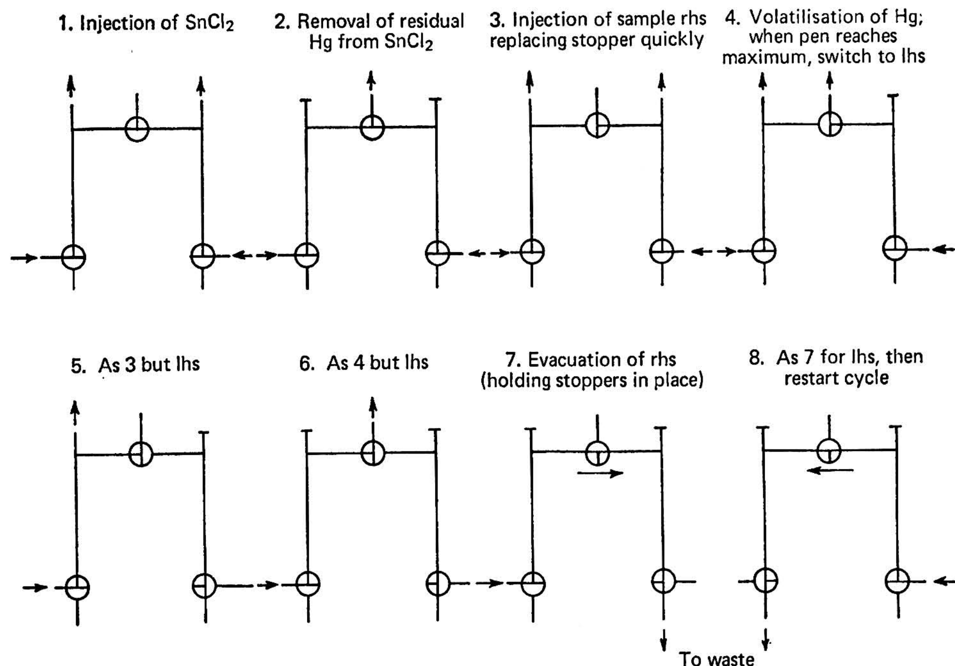

Fig. 2. Diagrammatic representation of the position of the three-way taps and the nitrogen flow during a determination when using the bubblers individually. T represents a stopper in place, ↑ the direction of nitrogen flow and ⊖ a three-way tap.

For small sample volumes (less than 1 ml) the nitrogen flow can be left on throughout a series of analyses; for larger volumes and for dual injections it is necessary to turn off the nitrogen supply at tap A (Fig. 1) to prevent sample loss.

Optimisation of Conditions

Optimisation of the conditions was carried out by using 50-μl injections of a 1 p.p.m. standard solution, *i.e.*, 50 ng of mercury. The best operating conditions consist of a compromise of reagent and sample volumes, bubbler volume (see above) and flow-rate to give an appropriate total time for volatilisation (0.5–1 min) and peak height. Fig. 3 shows the effect on peak heights obtained by varying the flow-rate and reagent volume . Both bubblers gave identical results.

It can be seen that at a flow-rate of 500 ml min^{-1}, the peak height remained approximately constant for volumes of tin(II) chloride solution of 6–12 ml. This phenomenon enabled a series of sample injections to be carried out on a starting volume of 6 ml of tin(II) chloride solution without replacing the solution. A total drop in sensitivity of less than 5% was observed on the further addition of approximately 3 ml of sample as, for example, 6 $\times$ 0.5 ml of 0.1 p.p.m. standard or 8 $\times$ 0.4 ml of 0.1 p.p.m. standard. There were no cross-contamination problems as the nitrogen flow was maintained during and between injections and the tin(II) chloride remained effective because it was in great excess.

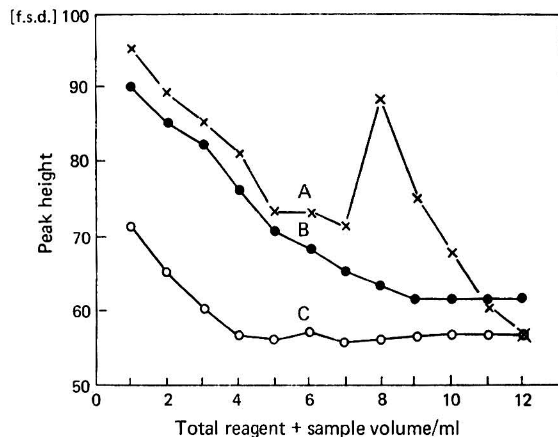

Fig. 3. Aeration characteristics: peak height *versus* reagent + sample volume for various flow-rates. Operating conditions: lamp current, 3.5 mA; wavelength, 253.6 nm; slit width, 125 μm; scale expansion, ×7; damping, C; recorder, 10 mV; chart speed, 1 cm s^{-1}; and amount of mercury, 50 ng (as 50-μl injections of 1 p.p.m. standard). Flow-rates: A, 300; B, 400; and C, 500 ml min^{-1}.

For injections of less than 0.25 ml, 1 ml of tin(II) chloride solution gave a large and reproducible response at a flow-rate of 400 ml min^{-1} of nitrogen but at 300 ml min^{-1} of nitrogen the peak height obtained was erratic, the time for total volatilisation varying from 0.75 to 2.5 min. This is a very long time for an open-ended system and may be caused by poor aeration and mixing of the solutions or turbulence in the dead space of the bubbler. For these reasons, flow-rates of 400 and 500 ml min^{-1} only are used for analysis, and some recommended operating conditions are shown in Table I.

TABLE I

SUGGESTED OPERATING CONDITIONS

For volumes of 6–10 ml of initial solution, sample volume effects are negligible.

Concentration of mercury*	Volume of sample/ml	Volume of tin(II) chloride solution/ml	Recorder reading/mV	Scale expansion	Flow-rate/ ml min^{-1}
10 p.p.t.–1 p.p.b.	20	2	10	×12	500
1–100 p.p.b.	1–5	7–3	10	×6	500
	Combined volume 6–10 ml				
	1	1	10	×6	400
10–100 p.p.b.	0.1–1	1	10	×6	400
0.1–0.5 p.p.m.	0.1–1	1	50	×1	400

* p.p.b. = parts per 10^9; p.p.t. = parts per 10^{12}.

Calibration and Blanks

The calibration graphs are linear from 0 to 400 ng of mercury. Table II gives the operating conditions and slopes of calibration graphs at various flow-rates and volumes of tin(II) chloride.

TABLE II

GRADIENTS OF CALIBRATION GRAPHS FOR VARIOUS OPERATING CONDITIONS

	9 ml of $SnCl_2$ at 500 ml min^{-1}				1 ml of $SnCl_2$ at 400 ml min^{-1}		
Amount of mercury	Slope, % of f.s.d. per ng	Recorder reading/mV	Scale expansion	Amount of mercury	Slope, % of f.s.d. per ng	Recorder reading/mV	Scale expansion
1–10 ng injections of 0.01 p.p.m. solution	3.0	10	×11	1–10 ng injections of 0.1 p.p.m. solution	4.5	10	×11
10–100 ng injections of 0.1 p.p.m. solution	0.64	10	×6	10–100 ng injections of 1.0 p.p.m. solution	1.0	10	×6

The calibrations were carried out on an average of three injections for each of 1, 2, 4, 6, 8, 10 and 10, 20, 40, 60, 80 and 100 ng of mercury, the range being ±2% of the mean of the peak height for each reading.

The blank for 10 ml of 5% nitric acid and 0.01% potassium dichromate with diluted doubly distilled water was 1% f.s.d. at a nitrogen flow-rate of 500 ml min^{-1}, a 10-mV recorder voltage and a scale expansion of ×6.

Some Applications of the Method

Standard sediments

The standard sediments were those circulated by the Environmental Protection Agency (EPA)[7] in a "round-robin" survey.

Samples of 0.05–2 g were weighed into boiling-tubes, which were then placed in an ice-bath. Fuming nitric acid (5 or 10 ml) was added to each sample and the digestion was allowed to proceed overnight, followed by warming to 45 °C for 3 h. The resulting suspensions were diluted with 0.01% potassium dichromate solution, filtered through Whatman 541 ashless filter-paper and the filter-paper collected in a calibrated flask, the residue being rinsed with dichromate solution until the volume reached the calibration mark.

Aliquots of these solutions were injected into 6 ml of tin(II) chloride solution. The results are shown in Table III and, as can be seen, compare very favourably with the results obtained from the EPA survey.

Urine

Urine samples were obtained from a patient who had undergone retrograde root filling treatment at the Bristol Dental Hospital.

Samples were taken morning and afternoon following treatment for 1 week and one other sample was taken 2 weeks after treatment. Samples were stored in silanised screw-top bottles; 20-ml aliquots of urine were pipetted into boiling-tubes, which were left to stand in an ice-bath. A total of 10 ml of fuming nitric acid was added to each tube in 0.5-ml aliquots in order to avoid excessive heating and foaming. The digestion was carried out at 45 °C for 3 h, after which time the solutions were decanted into 100-ml calibrated flasks and made up to the mark with 0.02% potassium dichromate solution. Care should be exercised at this stage because the solutions may foam violently.

On injection of a portion of the solution (1–2 ml) into the bubbler, foaming, resulting in sample carry-over, sometimes occurred but could be rectified by the addition of 1 ml of 1% tributyl citrate in ethanol as an anti-foaming agent. Because of these varying foaming characteristics it is necessary to inject a standard on top of each sample, a method similar to that described below for fish samples.

Immediately after the dental treatment the amount of mercury in the urine rose to 53 $\mu g\ l^{-1}$, but was rapidly dissipated over 3 d to a background concentration of 7 $\mu g\ l^{-1}$.

Water

Silanised glass reagent bottles (500 ml) were used to transport samples from the River Avon. The bottles had been washed previously with 25% nitric acid and one bottle con-

TABLE III

DETERMINATION OF MERCURY IN STANDARD SEDIMENTS

All volumes given are the average of three injections.

Sediment number (72C564-)	Amount of sample/g	Volume of acid/ml	Volume of— dilution/ml	Volume of— injection/ml	Amount of mercury in injection/μg	Total mercury/ μg g^{-1}	EPA results, "round-robin" survey[7]: Analytical method	EPA results: Number of laboratories	EPA results: Values obtained/μg g^{-1}: Mean	EPA results: Values obtained/μg g^{-1}: Standard deviation
3	0.129 84	5	100	1.0	1.155	119.4	Various	135	109.55	21.5
3	0.054 33	10	100	2.0	0.124	114.1	—	—	—	—
3	0.147 81	10	100	0.8	0.142 5	120.5	Aqua-regia digestion	52	110.5	29.27
4	0.136 18	5	100	2.0	0.124	45.5	Various	138	43.91	9.13
				0.2	0.012 75	46.8	—	—	—	—
4	0.150 12	10	100	0.2	0.013 75	45.8	Aqua-regia digestion	51	44.9	10.16

taining 0.01% potassium dichromate solution had also been allowed to stand until required; the samples were introduced into the bottles through Whatman 541 ashless filter-paper.

On to 1 ml of tin(II) chloride solution, in each bubbler, 10 ml of river water, *i.e.*, a 20-ml total sample volume, was injected immediately on returning to the laboratory. The flow of nitrogen was switched off for this operation, the apparatus having previously been flushed to eliminate any memory effects.

Aliquots of 50 ml were also taken from each bottle and 5 ml of fuming nitric acid added; 20-ml aliquots of this solution were then analysed to determine their mercury content. Doubly distilled water treated in exactly the same way gave a blank of 0.5 ng. The results are shown in Table IV.

TABLE IV

MERCURY IN RIVER WATER

Post-sampling treatment of water	Pre-sampling treatment of bottles			
	Acid wash		Dichromate wash	
	ng per 20 ml	$\mu g\ l^{-1}$	ng per 20 ml	$\mu g\ l^{-1}$
Untreated	0.15	0.007 5	0.25	0.012 5
Acid	0.65	—	0.85	—
Acid — blank (0.5 ng)	0.15	0.007 5	0.35	0.017 5

Feldman[4] has suggested that chromium binds with the active sites on the glass and helps to prevent absorption of mercury on to the glass, and this suggestion appears to be correct as zero blanks are obtained with dichromate solution alone.

Fish

Sammut,[8] in this laboratory, has used the method described here for the analysis of mackerel caught off Malta. The effect of varying acid volume, time and temperature of digestion was examined. Fish are difficult to analyse to determine mercury because of protein precipitation, the formation of fat emulsions and subsequent foaming problems., Because of these effects, 1 ml of 1% tributyl citrate was added to the 1 ml of tin(II) chloride solution in the bubbler and standards were injected after the addition of the sample. The results obtained are presented in Table V.

Judging by the range of results, it appears that the digestion method and the interferences mentioned above have very little effect. The range is 283–295 ng g^{-1} with a mean of 291.4 ng g^{-1}.

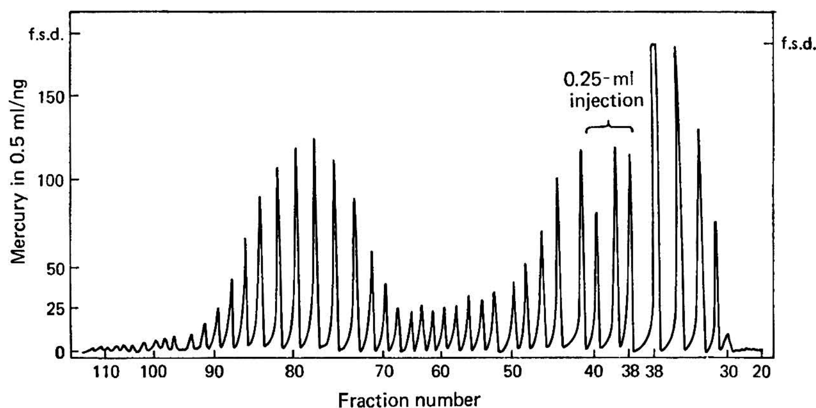

Fig. 4. Mercury content of protein solutions collected in 5-ml fractions from a Sephadex G-75 gel permeation column. Total analysis time, 40 min; chart speed, 1 cm min^{-1}; recorder voltage, 10 mV.

TABLE V

DETERMINATION OF MERCURY IN MACKEREL

Blank peak height = 1.5 mm for 20% fuming nitric acid solution.

Wet mass/g	Volume of fuming nitric acid/ml	Digestion time	Tempera- ture/°C	Dilution volume/ ml	Injection volume/ ml	Peak height/ mm	Calibration			Mercury found/ ng g^{-1}
							Mass of mercury/ng	Peak height/ mm	Peak height for 10 ng/mm	
2.83	5.0	Overnight	50 (2 h)	25	0.5	75	20	90	45	294
2.988	5.0	2 h	85	25	1.0	120	30	129	43	283
2.755	5.0	2 h	85	25	1.0	152	30	140	47	293
2.39	5.0 + 0.5 ml of 10% dichromate solution	Overnight	Room temperature	25	1.0	107	30	115	38.3	292
2.43	10.0	2 h (using iced cold finger on tube)	85	50	1.0	67	20	94	47	293
2.55	5.0 + 0.5 ml of 10% dichromate solution	2 h	85	25	1.0	119	20	123	61.5	290
2.48	10.0	2 h	85	50	1.0	38	20	52	26	295

Protein solutions

Protein-bound mercury is normally determined by using radioactive tracers and gel-permeation chromatography. Fig. 4 shows the spectra of the amount of mercury in 0.5-ml aliquots of 5-ml fractions collected from a Sephadex G-75 column. The separation was carried out on mercury-binding proteins obtained from a micro-organism. Alternate tubes were taken for analysis, the contents of 49 tubes being analysed in 40 min. Injections of standards and changes of tin(II) chloride solution took a further 12 min but these results have been omitted in the interests of simplification.

Six millilitres of tin(II) chloride solution were used at a nitrogen flow-rate of 500 ml min^{-1} and five reagent changes were made in all.

Acknowledgement is made to M. Sammut for the use of his results and to Dr. Stack of the Bristol Dental Hospital.

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Determination of Ruthenium in Biological Material by Atomic-absorption Spectrophotometry Using Electrothermal Atomisation

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A sensitive and rapid method was developed for the determination of ruthenium in faeces and other products of digestion by ruminants. The sample was ashed at 350 °C with a mixture of potassium nitrate and potassium hydroxide, dissolved in dilute nitric acid and analysed by atomic-absorption spectrophotometry using a carbon rod atomiser. The method is particularly free from interferences and is suitable for the determination of ruthenium at concentrations between 5 and 50 μg per gram of dry matter.

Keywords: *Ruthenium determination; biological material; atomic-absorption spectrophotometry*

Ruthenium, as the trisphenanthroline complex, has been used as a solid-phase marker in studies of digestion by ruminants, analyses having been carried out by either radiochemical[1] or X-ray fluorescence techniques.[2] Recent experiments at this laboratory required the use of relatively low levels of inert ruthenium, and it was therefore necessary to develop a sensitive analytical method suitable for faeces and solid samples from the digestive tract.

Atomic-absorption spectrophotometry, usually an attractive method for the determination of trace amounts of metals, is not particularly sensitive for ruthenium; Montford and Cribbs,[3] in their study using the air - acetylene flame, used ruthenium concentrations ranging from 20 to 140 μg ml^{-1}. Work in this laboratory confirmed that 10 μg ml^{-1} was the lowest analytically useful concentration in aqueous solution. Solvent extraction or concentration by distillation[4] was considered to be too time consuming for routine work.

Preliminary work with the carbon rod atomiser indicated that this method was particularly sensitive for ruthenium and that it warranted detailed investigation of its possible application. This view was confirmed recently by Everett.[5]

No information is available in the literature on the preparation of organic samples for analysis for ruthenium; however, work by Marshall and Rickard[6] and by Biswas and Mukerji[7] on inorganic material showed that ruthenium could be recovered after fusion with mixtures of potassium nitrate and hydroxide at 350 °C, suggesting that a similar procedure would be successful with organic material.

Experimental

Apparatus

A Varian, Model AA6, atomic-absorption spectrophotometer with a Varian, Model 63, carbon rod atomiser and a BC-6 background corrector was used for all ruthenium determinations. A Linear Instruments chart recorder was used to monitor the absorbance signals. Operating parameters for the system are shown in Table I.

Standards

A stock solution of tris(1,10-phenanthroline)ruthenium(II) chloride was prepared by the method described by MacRae and Evans.[2] This solution contained approximately 2 000 μg ml^{-1} of ruthenium.

Tris(acetylacetonato)ruthenium(III) was used as a reference standard as previously described by Braca *et al.*[8] and was prepared by a method similar to that of Veening *et al.*[9] for related complexes. Five millilitres of acetylacetone were refluxed with 0.2 g of ruthenium(III)

TABLE I

OPERATING PARAMETERS FOR THE ATOMIC-ABSORPTION SPECTROPHOTOMETER

Parameter	Value
Wavelength	349.9 nm
Lamp current	10 mA
Spectral band width	0.2 nm
Read-out mode	Absorbance
Drying time	20 s, 3.2 units
Ashing time	10 s, 5.5 units
Atomisation time	2.5 s, 7.5 units
Shielding gas	Nitrogen, flow-rate 3 l min^{-1}
Sample volume	5 μl

chloride in 30 ml of 10% hydrochloric acid for 1 h. The mixture was neutralised with 10% sodium carbonate solution and, after cooling, the deep red crystals were filtered off, washed with water and recrystallised from 20% aqueous ethanol.

A solution containing 100 μg ml^{-1} of ruthenium was prepared by dissolving 0.039 4 g of this complex in 100 ml of 50% ethanol containing 8.4% of uranyl nitrate.[3] This reference solution was used to standardise the stock solution of the phenanthroline complex that had been suitably diluted, also with 50% ethanol containing 8.4% of uranyl nitrate. The atomic-absorption spectrophotometer was used with an air - acetylene flame to compare these concentrated solutions.

Sub-standards (for carbon rod analyses) in the range 0–1 μg ml^{-1} of ruthenium were prepared by dilution of the stock solution of the phenanthroline complex with 1 M nitric acid. The acetylacetone complex was not suitable for preparation of these standards because of its volatility.

Sample Preparation

A representative portion of the sample was dried at 80 °C and ground to pass a 1-mm sieve. A sub-sample was ground to a fine powder using a Tema mill and 0.500 g of this material weighed into a 25-ml glass beaker. Then, 0.06 g of potassium nitrate and 0.02 g of potassium hydroxide dissolved in 2 ml of water were added and the contents of the beaker dried at 100 °C prior to ashing at 350 °C for 16 h.

The residue was suspended in 6 ml of 4 M nitric acid, heated on a water-bath and diluted with water to 25 ml without filtration. A 5-μl aliquot was taken for analysis.

Results and Discussion

Instrument Sensitivity

Fig. 1 shows a typical calibration graph for 5-μl aliquots of standard solutions. The carbon

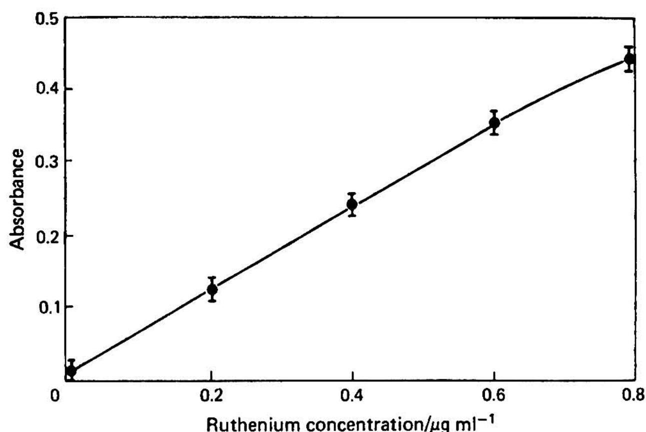

Fig. 1. Calibration graph for the carbon rod atomiser, using 5-μl aliquots. Mean absorbance values are shown (●) from the chart read-out, together with the standard errors.

tube was replaced after approximately 100 samples or when the sensitivity and precision had deteriorated significantly.

Standards prepared from the phenanthroline complex were stable indefinitely and gave the same calibration graphs as those prepared by taking aliquots of the complex through the ashing procedure, during which it was converted into potassium ruthenate (K_2RuO_4), which is not stable in solution.[6]

Ashing Recoveries

The removal of organic material from such samples presents unusual problems because of the potential volatility of ruthenium under oxidising conditions. Simple dry ashing at 500 °C gave very low and erratic recoveries while digestion with a perchloric - nitric - sulphuric acid mixture resulted in complete loss of ruthenium as ruthenium(VIII) oxide.

Ashing of the sample at 350 °C removed most of the organic material and the residue was a finely divided carbonaceous material. Higher ashing temperatures resulted in losses of ruthenium.

It was necessary to re-suspend the undissolved residue before an aliquot was taken for analysis as it contained a significant amount of ruthenium. Unshaken samples gave readings that were approximately 30% low; however, samples that were shaken immediately before analysis gave good recoveries and precision (see Table II). The recovery was unaltered for samples kept for periods of up to 1 week after preparation.

Samples having a known concentration of ruthenium were prepared by shaking a sample of ruthenium-free faeces for 16 h with a known amount of the phenanthroline complex in solution. After centrifugation, the supernatant liquid was analysed for non-adsorbed ruthenium and the concentration in the dried and weighed pellet of faeces calculated by difference. Over 96% of the total ruthenium was bound to the solid, in agreement with the findings of Tan *et al.*[1] These samples were analysed by the proposed method and the replicated recoveries are shown in Table II (samples 1 and 2).

Further recovery tests were performed by adding known amounts of the complex directly to the ashing beaker, and the recoveries are also shown in Table II (samples 3–5). No naturally occurring ruthenium was detected in any sample.

Varying the amount of potassium nitrate and hydroxide added had no effect on the recovery of ruthenium except that large amounts caused spattering of the sample. The mixture of potassium nitrate and hydroxide was added in a volume of 2 ml of water to allow thorough mixing with the sample during the drying and ashing stages.

TABLE II

RECOVERY OF KNOWN AMOUNTS OF RUTHENIUM FROM SOLID SAMPLES

Sample No.	Sample	Ruthenium added/ $\mu g\ g^{-1}$	Ruthenium found*/ $\mu g\ g^{-1}$
1(a)	Faeces: 6 samples ashed	22.6	21.8 ± 0.7
1(b)	Faeces: 6 aliquots analysed	22.6	21.8 ± 0.5
2(a)	Faeces: 6 samples ashed	12.5	12.0 ± 0.2
2(b)	Faeces: 6 aliquots analysed	12.5	12.0 ± 0.3
3	Spear grass	0	—
		10.0	9.8 ± 0.3
		20.0	20.0 ± 0.7
4	Abomasal solids	0	—
		10.0	10.1 ± 0.2
		20.0	19.2 ± 0.6
5	Faeces (lucerne diet)	0	—
		10.0	9.6 ± 0.2
		20.0	19.0 ± 0.5

* Mean ± standard deviation.

Interferences

As no naturally occurring ruthenium was detected in a range of faeces, the procedure for interference testing was considerably simplified. No non-atomic absorption was detected for any sample. The satisfactory recoveries for spiked faeces from different diets indicate a general freedom from interferences; however, a range of potential interferents was tested

and the results are shown in Table III. Chromium EDTA was included as it was used in the present animal experiments as a liquid-phase marker. It is interesting to note that uranyl nitrate was the only interfering substance of those tested; in flame atomic-absorption spectrophotometry, uranium is used as a releasing agent for ruthenium and enhances the sensitivity.

TABLE III

EFFECT OF POTENTIAL INTERFERENTS ON THE DETERMINATION OF 1 $\mu g\ ml^{-1}$ OF RUTHENIUM

Element	Chemical form	Concentration/ $\mu g\ ml^{-1}$	Ruthenium found*/ $\mu g\ ml^{-1}$
S	$(NH_4)_2SO_4$	100	1.02
		200	1.03
		400	1.00
P	KH_2PO_4	100	1.02
		200	1.01
		400	1.00
Ca	$Ca(NO_3)_2$	100	1.00
		200	1.01
		400	0.99
Na	NaCl	100	1.01
		200	1.02
		400	1.02
Si	Na_2SiF_6	100	0.99
		200	0.97
		400	1.02
Al	$Al_2(SO_4)_3$	100	0.98
		200	0.96
		400	0.97
Zn, Fe, Cu, Mn ..	Chlorides	10 of each	1.01
		20 of each	1.00
		40 of each	1.00
		100 of each	0.98
U	$UO_2(NO_3)_2$	10	0.71
		100	0.08
Cr	NaCr(EDTA)	1	1.03
		10	1.00
		100	0.91

* Mean of three readings.

Precision

Readings were reproducible to $\pm 0.01\ \mu g\ ml^{-1}$ (see Fig. 1), corresponding to $\pm 0.5\ \mu g\ g^{-1}$ in the samples. The standard deviations shown in Table II include some additional error due to sample preparation.

The lowest level of ruthenium that could be determined accurately was 5 μg per gram of dry matter, although this figure could be considerably improved by lowering the dilution ratio or taking multiple aliquots for analysis.

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Nitrate Determination by Reduction to Ammonia and Gas-phase Ultraviolet Absorption Spectrometry

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Nitrate can be rapidly reduced to ammonia by the action of titanium(III) sulphate, even at room temperature. Subsequent displacement of the ammonia by a current of air enables the gas-phase molecular absorbance of the ammonia to be measured and thus provides a sensitive and selective method for the determination of nitrate. Ions which affect the reduction of nitrate by titanium(III) salts interfere with the method and must be removed if present in large amounts.

Keywords: *Nitrate determination*; *ammonia*; *ultraviolet absorption spectrometry*

The author has recently shown[1] that ammonium-nitrogen in solution can be determined by displacement of ammonia from alkaline sample solutions with a current of air and measurement of the absorbance at 201 nm of the ammonia thus displaced. Because nitrate can be readily reduced to ammonia, it was decided to investigate the feasibility of applying a similar procedure to the determination of nitrate in solution. In the author's original method for the determination of ammonium-nitrogen, a steam-heated, 500-mm absorption cell was used. In the present investigation it was decided to employ a shorter absorption cell, which would fit into the space provided for the atomiser in most conventional atomic-absorption spectrometers.

Experimental

Apparatus

A Shandon Southern A3600 atomic-absorption spectrometer was used in conjunction with an Auto-graph flat-bed recorder. The absorption cell used was the standard silica absorption tube from a Shandon Southern A3490 arsenic - selenium detection unit. However, any silica, glass or plastic tube of approximately 8 mm internal diameter and 180 mm in length with a central side-arm for introduction of the air containing the ammonia will suffice. It is unnecessary to heat a tube of this length provided that the temperature of the sample solution is less than approximately 300 K, as no condensation problems are experienced under these conditions. The absorption cell was connected to the reaction cell by narrow-bore nylon tubing.

Reagents

Stock 1 000 μg ml^{-1} *nitrate-nitrogen solution.* Dissolve 7.221 g of analytical-reagent grade potassium nitrate in water and dilute to 1 l.

Solutions containing diverse ions. A range of 1 000 μg ml^{-1} solutions of cations and anions was prepared, from analytical-reagent grade salts whenever possible, by dissolution in water containing a few drops of dilute hydrochloric acid, if necessary, to prevent precipitation.

Titanium(III) sulphate solution. BDH Chemicals Ltd. (Poole, Dorset) technical grade 15% titanium(III) sulphate solution in 23% sulphuric acid was used as supplied or (where specified) after fresh, 10-fold dilution.

Results and Discussion

Choice of Reducing Agent

Although several reducing agents can be used to reduce nitrate to ammonia, it was decided to investigate the use of titanium(III) sulphate. This reagent can be used in solution form, which renders it more suitable than solid reagents for use (ultimately) in automated systems. Moreover, it is available in solution at a high concentration, so that it is possible to add rapidly the required aliquot of reducing agent in manual systems. The reagent is inexpensive and the evolution of hydrogen is relatively slow.

Amount of Titanium(III) Required

In order to assess the amount of titanium(III) required, triplicate 5-ml aliquots of 50 μg ml^{-1} solutions of nitrogen, both as ammonium sulphate and as potassium nitrate, were treated with 20-ml aliquots of 40% sodium hydroxide solution and various volumes of 10-fold diluted titanium(III) solution. In each instance the mixture was shaken in a tightly stoppered plastic centrifuge tube[1] for 1 min, and the absorbance at 201 nm of the ammonia displaced by an air-flow of 660 ml min^{-1} was measured. The results are shown in Fig. 1, and indicate 98% recovery of nitrate-nitrogen under the conditions described above. A similar recovery

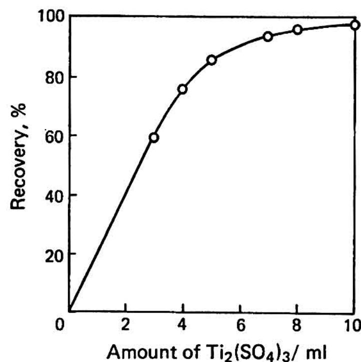

Fig. 1. Effect of volume of 10-fold diluted titanium(III) sulphate solution upon recovery of nitrate-nitrogen.

was obtained by using 1- and 2-ml aliquots of undiluted titanium(III) solution, although the signal obtained was greater because of the smaller dilution factor. Moreover, the latter, smaller, aliquots can be added more rapidly, thus minimising the possibility of ammonia being lost during mixing.

Effect of Temperature

In the author's original method for the determination of ammonium-nitrogen, it was shown that the sample temperature had a substantial effect upon the absorbance obtained; similar dependence was observed for nitrate determination. Using the conditions outlined above, but with 2 ml of undiluted titanium(III) solution, recoveries of 93, 98 and 99% were obtained by thermostatically controlling the temperature of the solution in 30-min steps at 273, 303 and 328 K, respectively. It was found that raising the temperature did not improve the percentage reduction of nitrate when the amount of undiluted titanium(III) solution used was less than 1 ml. Moreover, at 328 K the precision of the analysis was conspicuously poorer, which was attributed partly to condensation problems at this elevated temperature and partly to a build-up of hydrogen pressure in the stoppered centrifuge tubes over the 30-min constant temperature period.

Effect of Time

Duplicate samples containing 50 μg ml^{-1} of nitrogen as ammonium or nitrate were treated with 20 ml of 40% sodium hydroxide solution and 1 ml of undiluted titanium(III) solution, and the mixtures shaken for 1 min in stoppered tubes. The absorbances of the solutions were measured both immediately and, for separate pairs of duplicates, after thermostatically controlling the solutions at 303 K for 2, 6, 10, 20, 30, 45, 60, 90, 120 and 240 min and the mean percentage recoveries calculated. A graph of percentage recovery against time showed the reaction to proceed to 98% completion virtually immediately; subsequent periods of standing merely caused poorer precision owing to the build up of hydrogen pressure mentioned earlier. It was therefore decided to take all subsequent measurements immediately after shaking.

Effect of Order of Reagent Addition

The effect of the order of reagent addition depended both upon the amount of reagent added and upon the temperature of the sample solution. For additions of up to approximately 0.2, 0.3 and 0.4 ml of undiluted titanium(III) solution at temperatures of 273, 303 and 328 K, respectively, the percentage recovery of nitrogen was actually enhanced when the titanium(III) was added before the 40% sodium hydroxide solution. However, at higher concentrations of titanium(III) at these same temperatures, the percentage recovery of nitrogen was reduced drastically when the titanium(III) was added first. Thus, at 303 K, 2 ml of titanium(III) added first gave a recovery of only 32%; this reverse was attributed to the formation of an unstable intermediate. The higher acid concentration at higher titanium levels accelerated the decomposition of the intermediate and the characteristic smell of nitrogen dioxide was observed. It was found that the percentage recovery obtained depended critically upon the speed with which the operator managed to add the 40% sodium hydroxide solution following the addition of the titanium. However, in routine use the alkali should be added first and no problems are then encountered.

Calibration Graphs

A typical calibration experiment recorder trace is shown in Fig. 2, for the range 0–5 $\mu g\ ml^{-1}$ of nitrate-nitrogen at an 8-fold scale expansion. Ten-millilitre aliquots of sample solution were used, with 20-ml portions of 40% sodium hydroxide solution, 1-ml portions of titanium(III) solution and an air flow-rate of 660 ml min^{-1}. A linear calibration graph was obtained over the range 0–5 $\mu g\ ml^{-1}$ but appreciable curvature was obtained over the range 0–50 $\mu g\ ml^{-1}$, as is shown in Fig. 3. The linear working range can be extended upwards by employing one of the less sensitive ammonia absorption peaks.[1] The sensitivity of the technique can be made poorer by using a wider monochromator slit width (a spectral band width of less than 0.2 nm is required for optimum sensitivity) or by decreasing the amount of sample used. The latter alternative also extends the linear working range upwards.

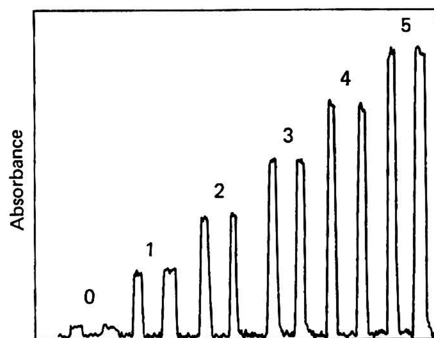

Fig. 2. Typical recorder trace showing results for 0–5 $\mu g\ ml^{-1}$ of nitrate-nitrogen. Total time of run, 21 min. Numbers represent $\mu g\ ml^{-1}$ of nitrate-nitrogen.

Interferences

A range of solutions was prepared in duplicate containing 10 $\mu g\ ml^{-1}$ of nitrate-nitrogen and 500 $\mu g\ ml^{-1}$ of possible interferent. Ten-millilitre aliquots of each solution were mixed with 20-ml portions of 40% sodium hydroxide solution, and 1-ml portions of undiluted titanium(III) solution were added. The ammonia was determined as described above. Under these conditions aluminium, calcium, cadmium, chromium(III), caesium, gallium, indium, potassium, lanthanum, lithium, magnesium, manganese, sodium, nickel, rubidium, strontium, zirconium, chloride, phosphate, sulphate and silicate ions caused less than 3% interference. Cobalt, copper, iron(III) and zinc ions caused a depression in signal of 55, 60, 50 and 7%, respectively. The same concentrations of these elements did not interfere in

the determination of the same concentration of nitrogen as ammonium-nitrogen under these conditions. This contrast indicates that the mechanism of the interference must either involve oxidation of part of the titanium(III) or the formation of an unstable intermediate complex with these ions, which decomposes with loss of nitrogen in some form other than ammonia.

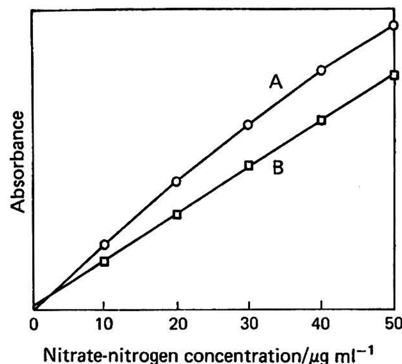

Fig. 3. Calibration graphs for nitrate-nitrogen. A (○), 0–50 μg ml^{-1}; no scale expansion; absorbance scale 0–1. B (□), 0–50 μg per 10 ml; 8-fold scale expansion (*i.e.*, 0–5 μg ml^{-1} of nitrogen); absorbance scale 0–0.125.

The degree of interference could be reduced considerably by doubling the amount of titanium(III) used. However, these interfering cations can be removed rapidly from solution by total ion exchange. In order to examine this procedure 20-ml aliquots of samples containing interfering ions and nitrate-nitrogen were shaken for 2 min with 2-g portions of washed Amberlite IR-120(H) cation-exchange resin; interference-free standard nitrate-nitrogen solutions were treated in the same way. When 10-ml aliquots of the supernatants were treated with sodium hydroxide and titanium(III) solutions in the normal way, it was found that the depression caused by these four elements had been eradicated completely.

Nitrate and Ammonium-nitrogen Mixtures

In the absence of interfering ions, the proposed method can be used to determine the sum of ammonium- and nitrate-nitrogen. As ammonium-nitrogen can be determined separately by using the same method [in the absence of titanium(III)[1]], the amount of nitrate-nitrogen can be determined by difference. In the presence of excess amounts of cobalt, copper, iron(III) or zinc ions, the ion-exchange pre-separation method must be employed. As ammonium is 95% absorbed by the resin under the conditions outlined, nitrate-nitrogen can be measured directly after the ion-exchange treatment; a correction should be applied for residual ammonium-nitrogen unless the nitrate-nitrogen to ammonium-nitrogen ratio is greater than 10:1.

Conclusions

Gas-phase molecular absorption spectrometry constitutes the basis of a sensitive and selective method for the determination of nitrate. Interferences are few, and readily overcome. As in the determination of ammonium-nitrogen,[2] sensitivity can be further improved by using a solid alkaline reagent, such as sodium or potassium hydroxide or carbonate, but only at the expense of poorer precision with the present system. With some minor modifications the proposed method should be ideally suited to the fully automated determination of nitrogen in solution, although the simplicity of the instrumentation requirements when an atomic-absorption spectrometer is available also makes the proposed method particularly suitable for use in laboratories in which nitrate is not determined on a routine basis, and the capital outlay for, and maintenance of, an AutoAnalyzer system cannot readily be justified.

Moreover, the problems in colorimetric methods associated with the analysis of coloured or turbid solutions should be eradicated; this is particularly true with extracts from certain types of soils.[3]

The author is indebted to Mrs. E. Haw for assistance with much of the experimental work, and to the Science Research Council for financial support.

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Comparison Between an Ultraviolet Spectrophotometric Procedure and the 2,4-Xylenol Method for the Determination of Nitrate in Groundwaters of Low Salinity

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An ultraviolet spectrophotometric procedure for the determination of nitrate in groundwaters of low salinity has been investigated and compared with the recommended 2,4-xylenol method. The ultraviolet procedure is not subject to interference from the major cations present in typical groundwaters and interference from high levels of nitrite is overcome by the addition of sulphamic acid. Good agreement is demonstrated between nitrate values obtained by both methods on a number of groundwater samples.

The ultraviolet procedure offers a rapid and sensitive means of determining nitrate in such waters, which are generally low in content of organic matter, particularly where the volume of sample is limited, *e.g.*, studies on pore water samples.

Keywords: *Groundwater; nitrate determination; ultraviolet spectrophotometry; 2,4-xylenol*

Groundwaters provide almost 40% of the domestic water supply in England and Wales, and the concentration of nitrate present has an important bearing on potability. The current WHO standard for European drinking waters[1] recommends that levels of nitrate should not exceed 11.3 mg l^{-1} of nitrate-nitrogen (50 mg l^{-1} of nitrate). High levels of nitrate can give rise to harmful physiological effects, such as methaemoglobinaemia, particularly in infants,[2] and at present there is concern over evidence that suggests that nitrate concentrations in some major aquifers are increasing to an undesirably high level.[3,4] Analytical methods for nitrate therefore need to be accurate so that threshold values can be determined with confidence and precise so that small trends in concentration can be detected quickly.

Numerous colorimetric methods for the determination of nitrate in natural waters have been reported. Reagents proposed for direct nitration include phenoldisulphonic acid,[5] 2,4-xylenol[6] and 2,6-xylenol,[7,8] while diazotisation following reduction to nitrite has also been used.[9–12] These procedures suffer from the general disadvantage of being lengthy, as well as, in some instances, being subject to specific interferences, *e.g.*, from chloride. Ion-selective electrode methods have been used recently for the determination of nitrate[13,14] and offer certain advantages in terms of speed, but experience in this laboratory and elsewhere has shown that interferences, especially from chloride and hydrogen carbonate, can lead to significant and unacceptable errors.[15,16]

An ultraviolet spectrophotometric method for determining nitrate in alkaline earth metal carbonates has been described[17] and subsequently proposed as the basis for procedures for the measurement of nitrate in waters,[18–22] effluents[18] and soil extracts.[23] The typical major inorganic constituents of groundwaters have been reported to have little effect on ultraviolet absorption at the wavelengths used (210–220 nm) and it has been suggested that correction for interference from organic matter can be made by measuring the absorbance also at 275 nm, where nitrate does not absorb strongly.[18,19] In an early study, good agreement was demonstrated between nitrate values obtained by the phenoldisulphonic acid method and from ultraviolet absorption measurements for certain well and river waters.[18] Preliminary experiments in this laboratory suggested that ultraviolet spectrophotometry offered a sensitive, precise and extremely rapid procedure for determining nitrate in potable groundwaters and the work described here was carried out to investigate the technique in more detail and, in particular, to compare it with the recommended 2,4-xylenol method.[24]

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Experimental

Apparatus

Absorbance measurements were made with a Unicam SP500, Series 2, ultraviolet - visible light spectrophotometer and absorption spectra were plotted using a double-beam Unicam SP1800 ultraviolet - visible light scanning spectrophotometer coupled to a chart recorder.

Reagents

Standard nitrtae solution. A stock nitrate solution containing 100 mg l^{-1} of nitrate-nitrogen was prepared by dissolving 0.721 8 g of potassium nitrate (dried at 105 °C) in water and diluting the solution to 1 l.

Standard nitrite solution. A stock nitrite solution containing 100 mg l^{-1} of nitrite-nitrogen was prepared by dissolving 0.492 6 g of sodium nitrite in water and diluting the solution to 1 l.

Sodium hydroxide solution. A stock sodium hydroxide solution was prepared by dissolving 200 g of sodium hydroxide in water and diluting the solution to 1 l. A working solution was prepared as required by diluting 40 ml of the stock solution to 200 ml with water.

Sulphuric acid, 85% V/V. This acid was prepared by the careful addition of 850 ml of Aristar sulphuric acid, sp. gr. 1.84, to 150 ml of water.

2,4-Xylenol solution in acetic acid. A 1% solution of 2,4-xylenol was prepared by dissolving 1 g of the reagent in 100 ml of glacial acetic acid.

Sulphamic acid solution, 1%. A 1% solution of sulphamic acid was prepared by dissolving 10 g of sulphamic acid in 1 l of water.

Mercury(II) sulphate solution, 2%. A 2% solution of mercury(II) sulphate was prepared by dissolving 2 g of mercury(II) sulphate in a mixture of 80 ml of water and 20 ml of sulphuric acid, sp. gr. 1.84.

Toluene. AnalaR toluene was used for extraction.

Iron solution. BDH iron(III) chloride standard solution for atomic-absorption spectroscopy.

$$1 \text{ ml} \equiv 1.00 \text{ mg of Fe.}$$

Procedure

Filter the samples through 0.45-μm membrane filters on collection and acidify by addition of 2 ml of 50% *V/V* Aristar sulphuric acid per litre.

Ultraviolet spectrophotometric procedure

Dilute the samples as necessary to bring their concentrations of nitrate into the range 0–1.0 mg l^{-1} of nitrate-nitrogen and add sufficient 1% sulphamic acid solution to give a final sulphamic acid concentration of 0.1% *m/V* if the concentration of nitrite in the final solution is likely to exceed 20 μg l^{-1} of nitrite-nitrogen. Measure absorbances at 206 nm in 1.0-cm cells.

2,4-Xylenol method

To 10 ml of sample, or an aliquot containing not more than 30 μg of nitrate-nitrogen, in a 100-ml conical flask add 2 ml of 2% mercury(II) sulphate and 0.5 ml of 1% sulphamic acid solutions; allow the flask to stand for 5 min then add 1 ml of 1% 2,4-xylenol solution and mix. Slowly add 24.5 ml of 85% *V/V* sulphuric acid, while cooling the flask in running water. Place the flask in a water-bath at 30 °C for 30 min, then transfer the contents to a 125-ml separating funnel and rinse the flask with two successive 25-ml volumes of water. Add 10 ml of toluene, shake the funnel gently for 3 min, allow the phases to separate and run the aqueous layer to waste. Rinse the funnel and contents with 10 ml of water, allow to separate and run the water to waste. Add 10 ml of working-strength sodium hydroxide solution to the toluene extract, shake for 1 min, allow the phases to separate, run off the sodium hydroxide layer into a 1.0-cm cell and measure the absorbance at 445 nm. Run a blank and a set of standards in the range 1–20 mg l^{-1} of nitrate-nitrogen.

Results and Discussion

Standard solutions containing 0.2, 0.5 and 1.0 mg l^{-1} of nitrate-nitrogen were scanned between 190 and 280 nm. The resultant spectra (Fig. 1) showed a strong peak at 206 nm and the absorbances at this wavelength varied linearly with concentration of nitrate. Identical

spectra were obtained for nitrate in solutions containing 100 mg l^{-1} of sodium chloride. In order to establish whether any gross interference from a typical groundwater matrix occurred, the absorption spectra of a membrane-filtered (0.45 μm) sample of groundwater from the Bunter Sandstone and that of a standard solution were plotted. It can be seen from Fig. 2 that no significant alteration in the over-all shape of the nitrate spectrum, or shift in the position of the peak, occurred.

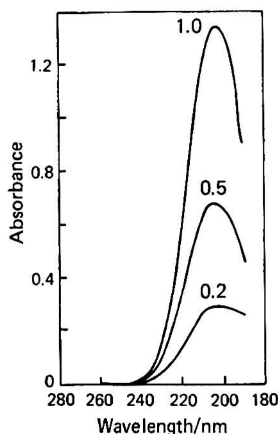

Fig. 1. Absorption spectra of standard solutions containing 0.2–1.0 mg l^{-1} of nitrate-nitrogen.

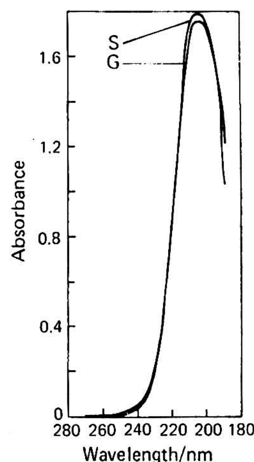

Fig. 2. Absorption spectra of standard nitrate solution (3.33 mg l^{-1} of nitrate-nitrogen), S, and groundwater sample from the Bunter Sandstone, G.

Nitrite, which absorbs in a similar part of the ultraviolet region, is a potential source of interference. A solution containing 1 mg l^{-1} of nitrite-nitrogen was scanned between 190 and 260 nm and the spectrum is shown in Fig. 3, together with that of a 1 mg l^{-1} solution of nitrate-nitrogen for comparison. The nitrite absorption maximum is at about 211 nm

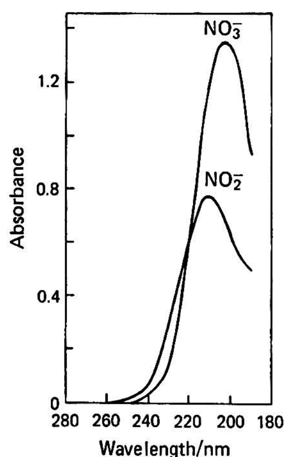

Fig. 3. Absorption spectra of nitrate and nitrite standard solutions containing 1 mg l^{-1} of nitrogen.

and it absorbs less strongly than nitrate at a similar concentration. Solutions containing 0.5 mg l^{-1} of nitrate-nitrogen and up to 100 $\mu g\ l^{-1}$ of nitrite-nitrogen were prepared and the absorbances obtained at 206 nm are given in Table I. It can be seen that interference from nitrite is significant only at concentrations above 20 $\mu g\ l^{-1}$. All groundwaters so far analysed have contained less than 17 $\mu g\ l^{-1}$ of nitrite so that it has not proved necessary to make a correction for the absorbance due to nitrite in these waters. Dilution of the sample, which is usually required in the ultraviolet procedure, will further decrease any potential nitrite interference. Interference by higher concentrations of nitrite can be removed by the addition of 0.1% *m/V* of sulphamic acid, as shown in Table I.

TABLE I

EFFECT OF SULPHAMIC ACID ON NITRITE INTERFERENCE IN A SOLUTION CONTAINING 0.5 mg l^{-1} OF NITRATE-NITROGEN

Nitrite-nitrogen present/$\mu g\ l^{-1}$	Absorbance at 206 nm in 1-cm cell	
	Sulphamic acid absent	0.1% *m/V* of sulphamic acid present
0	0.329	0.332
5	0.330	0.331
10	0.331	0.332
20	0.336	0.333
50	0.349	0.331
100	0.367	0.334
200	0.406	0.333

Absorption by iron in the ultraviolet region has been reported[17] and, although iron levels in potable groundwaters are typically low, the effect of iron over a range of concentrations was investigated. A 10-ml volume of standard iron solution (1 mg ml^{-1}) was evaporated to dryness and heated to fuming twice with perchloric acid to destroy trace amounts of nitrate. The residue was dissolved in a few drops of Aristar hydrochloric acid and diluted to give a 200-mg l^{-1} solution, from which nitrate solutions containing also 0–1 000 $\mu g\ l^{-1}$ of iron were prepared. In most oxidising groundwaters at pH 7, the amount of iron(II) in solution is unlikely to exceed 100 $\mu g\ l^{-1}$ and at this level interference is insignificant (Table II), being equivalent to approximately 0.015 mg l^{-1} of nitrate-nitrogen. In waters containing initially higher concentrations of iron(II), normal handling procedures should ensure aeration of the samples which, combined with omission of the acidification step, will lower iron(II) levels considerably, with a consequent reduction in interference.

TABLE II

EFFECT OF IRON ON THE ABSORBANCE OF NITRATE SOLUTIONS CONTAINING 0.5 mg l^{-1} OF NITRATE-NITROGEN

Iron present/$\mu g\ l^{-1}$	0	8	20	40	80	200	400	1 000
Absorbance at 206 nm in 1-cm cell	0.331	0.332	0.333	0.335	0.339	0.350	0.370	0.429

Absorbances from a typical calibration graph are given in Table III and demonstrate the sensitivity of the ultraviolet spectrophotometric procedure.

TABLE III

ABSORBANCES OF NITRATE STANDARDS

Nitrate-nitrogen/mg l^{-1}	0.2	0.4	0.6	0.8	1.0
Absorbance at 206 nm in 1-cm cell ..	0.133	0.265	0.400	0.531	0.661

As an over-all check on accuracy, nitrate was determined on several groundwater samples by both the ultraviolet procedure and the 2,4-xylenol method. Typical results are given in Table IV and further comparative results are summarised in Fig. 4. Good agreement between the ultraviolet and 2,4-xylenol methods was obtained for all the groundwater samples analysed. Three samples of River Thames water were analysed by both procedures with equally good

agreement, although it is recognised that the ultraviolet method may not be satisfactory for all types of river water.

TABLE IV

NITRATE CONCENTRATIONS DETERMINED BY ULTRAVIOLET AND 2,4-XYLENOL METHODS

Absorbances measured in 1-cm cells.

Type of water	Nitrate-nitrogen/mg l^{-1} Ultraviolet method	Nitrate-nitrogen/mg l^{-1} 2,4-Xylenol method
Bunter Sandstone	0.04	0.04*
Bunter Sandstone	8.3	8.2
Bunter Sandstone	0.005*	0.04*
Bunter Sandstone	0.54	0.55
Bunter Sandstone	4.5	4.6
Lincolnshire Limestone	7.9	7.9
Lincolnshire Limestone	5.8	5.5
River Thames, Chiswick	11.0	10.9

* 4-cm cell.

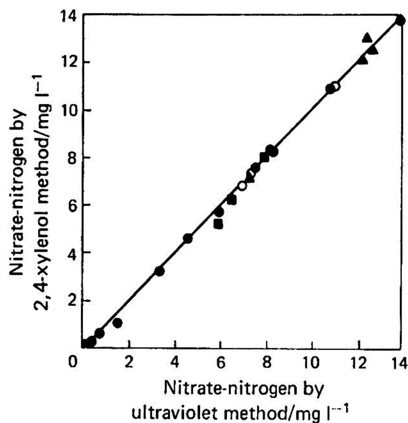

Fig. 4. Correlation between values for nitrate in groundwaters measured by the 2,4-xylenol and ultraviolet methods. ○, River Thames; ●, Bunter Sandstone; ■, Lincolnshire Limestone; and ▲, Middle Chalk.

The 2,4-xylenol method is slow and laborious; separation of the aqueous and organic phases presents problems because of the formation of stable emulsions. There is a significant reagent blank and the sensitivity is of the order of 0.04 absorbance unit per mg l^{-1} of nitrate-nitrogen. By comparison, the ultraviolet procedure is rapid and sensitive. Up to 30 samples per hour can be measured, depending on whether or not dilution is required. No reagents other than the stabilising acid and, if high nitrite levels are suspected, sulphamic acid are required and the sensitivity is over an order of magnitude greater than that of the 2,4-xylenol method ($A = 0.66$ per mg l^{-1} of nitrate-nitrogen). Levels of iron(II) typical of oxidising groundwaters do not cause significant interference. Interference by dissolved organic matter on the determination of nitrate by ultraviolet absorption is well known and the technique should not be employed for groundwaters that contain significant amounts of organic material. A procedure for correcting for such interference, which involves a second absorbance measurement at 275 nm, has been described.[19]

In this laboratory, the ultraviolet procedure has been used routinely for analysis of groundwaters for nitrate and its inherent sensitivity, often necessitating dilution of samples by a

factor of 10–20, has proved particularly useful in the analyses of interstitial waters extracted by centrifugation[25] from the chalk and other UK aquifer material, when as little as 1 ml of sample has been available.

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Spectrophotometric Determination of Niobium and Germanium in Superconducting "Nb_3Ge" Sputtered Films

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The determination of niobium and germanium in single-phase and crystalline radiofrequency sputtered "Nb_3Ge" thin films by rapid colorimetric methods is described. The niobium to germanium molar ratios obtained are compared with those obtained by electron-microprobe analysis.

Keywords: *Niobium determination*; *germanium determination*; *thin films*; *spectrophotometry*

The determination of the total film compositions for a series of "Nb_3Ge"† thin films was necessary in order to assist in the interpretation of the annealing results[3] obtained on these thin films deposited on polished, polycrystalline α-alumina substrates. Assuming a compositionally homogeneous "Nb_3Ge" thin film[1] with a density of 8.7 g cm^{-3} the total mass of the deposited film is calculated to range from approximately 220 to 350 μg.

The determination of microgram amounts of germanium and niobium in "Nb_3Ge" films on alumina substrates by "wet" chemistry is limited to the application of colorimetric methods. Sensitive colorimetric procedures with few interferences are available for the determinations, provided close attention to detail is maintained. The extraction of germanium with carbon tetrachloride from 9 M hydrochloric acid furnishes a clean separation from niobium[4] following the dissolution of the film with hydrofluoric, nitric and sulphuric acids.

A number of reagents have been proposed for the colorimetric determination of germanium[5] but 2,6,7-trihydroxy-9-phenylxanthen-3-one (usually referred to as phenylfluorone), proposed by Cluley,[6] is one of the most sensitive. Several elements, including niobium, interfere with the determination of germanium with this reagent; however, they are separated by the extraction of germanium with carbon tetrachloride in hydrochloric acid solution. Only arsenic is extracted together with germanium but it does not react with the reagent.

Germanium(IV) forms a slightly soluble orange - red complex with phenylfluorone in which the molar ratio is 1:2. In 0.5–1.5 M hydrochloric acid the colour that develops has an adsorption maximum at 504 nm, and a molar absorptivity of 38 500 l mol^{-1} cm^{-1}. A suspension that forms is stabilised by the addition of a protective gelatin solution. However, at hydrochloric acid concentrations below 0.3 M the reagent precipitates. The Beer - Lambert law is obeyed at the concentrations encountered in the analysis of the films in this study.

Niobium forms a 1:1 orange - red complex with 4-(2-pyridylazo)resorcinol (PAR) in an acetate - tartrate medium at pH 5.8. The absorption maximum is at 550 nm and the molar absorptivity is 38 700 l mol^{-1} cm^{-1}, permitting the determination of a niobium concentration as low as 0.1 μg ml^{-1}. The reaction of PAR with niobium in the presence of EDTA is highly selective. Cobalt, copper, uranium, vanadium and silver interfere with the determination of niobium, while interference from tantalum is overcome by the addition of a large amount of tartrate. The presence of fluoride has a slight effect on the colour development but is removed by heating with sulphuric acid. The method used for this study is essentially that of Belcher *et al.*[7]

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† The convention of putting quotation marks around the formula Nb_3Ge is used to indicate that the stoicheiometry of this A15 compound exists over a range of composition.[1,2]

Preparation of Thin Films

The "Nb_3Ge" thin films were deposited on high-purity, polished, polycrystalline α-alumina substrates (2.5 × 12.5 × 0.6 mm) by radiofrequency getter sputtering. They formed part of a detailed study on the preparation and characterisation of such phases reported elsewhere.[3] Typical sputtering conditions were: radiofrequency power density, 5.2 W cm^{-2}; argon sputtering gas pressure, 80 × 10^{-2} torr; substrate heating table temperature, approximately 930 °C; and deposition time, 4 h. The sputtering target consisted of a 5 cm diameter, 99.99% pure niobium disc with an approximately 3-cm^2 island of electronic-grade germanium cemented off-centre to it. The substrates were positioned on a molybdenum heating table approximately 2.9 cm below the target. The substrate - target configuration was arranged such that a composition gradient extending from a germanium-rich region to a niobium-rich region resulted during the deposition. Film thicknesses ranged from approximately 0.7 to 1.3 µm.

Analytical Procedure

Apparatus

A Bausch & Lomb Spectronic 70–6 spectrophotometer was used.

Reagents

Carbon tetrachloride.

Gelatin solution, 1% m/V.

Tartaric acid solution, 10% m/V. Dissolve 100 g of tartaric acid in 700 ml of water. Filter and dilute to 1 l with water.

Ammonium acetate buffer solution. Dissolve 80 g of ammonium acetate and 6.5 ml of glacial acetic acid in 700 ml of water. Filter and dilute to 1 l with water.

Ethylenediaminetetraacetic acid, disodium salt solution, 0.02 M. Dissolve 7.445 g of EDTA disodium salt in 1 l of water.

2,6,7-*Trihydroxy*-9-*phenylxanthen*-3-*one* (*phenylfluorone*) *solution,* 0.05% *m/V.* Dissolve 50 mg in 100 ml of 96% ethanol containing 0.5 ml of 6 M hydrochloric acid with gentle heating.

4-(2-*Pyridylazo*)*resorcinol* (*PAR*) *solution,* 10^{-3} M. Dissolve 0.295 g of the disodium salt in 700 ml of water, filter and dilute to 1 l with water.

Standard germanium stock solution, 100 µg ml^{-1}. Dissolve 0.036 g of Specpure germanium(IV) oxide in 20 ml of water containing 0.5 g of sodium hydroxide in a small beaker. When dissolution is complete, transfer into a 250-ml calibrated flask and dilute to volume with water.

1 ml ≡ 100 µg of Ge.

Standard germanium working solution, 5 µg ml^{-1}. Transfer 50.0 ml of the standard germanium stock solution into a 1-l calibrated flask and dilute to volume with water.

1 ml ≡ 5 µg of Ge.

Standard niobium stock solution, 100 µg ml^{-1} *in* 10% *tartaric acid.* Fuse 71.6 mg of ignited Specpure niobium(V) oxide and 2 g of potassium pyrosulphate in a platinum crucible. Cool and dissolve the melt in 10% tartaric acid solution. Transfer the solution into a 500-ml calibrated flask with 10% tartaric acid solution and dilute to volume with 10% tartaric acid.

1 ml ≡ 100 µg of Nb in 10% tartaric acid solution.

Standard niobium working solution, 10 µg ml^{-1} *in* 1% *tartaric acid.* Transfer 25.0 ml of the standard niobium stock solution into a beaker and dilute to 150 ml. Adjust the pH of the solution to 5.8 by adding 2 M sodium hydroxide solution (about 20 ml is required). Cool the solution and transfer into a 250-ml calibrated flask. Dilute to volume with water.

1 ml ≡ 10 µg of Nb in 1% tartaric acid solution.

Preparation of Niobium Calibration Graph

Transfer 0-, 1.0-, 2.0-, 4.0-, 6.0-, 8.0- and 10.0-ml aliquots of the standard niobium working solution (10 µg ml^{-1}) into a series of beakers. In the same order add 10.0, 9.0, 8.0, 6.0, 4.0, 2.0 and 0 ml of 1% tartaric acid solution to the beakers. Dilute each solution to 40 ml with water and adjust the pH to 5.8 by adding 2 M sodium hydroxide solution. Cool the solutions and transfer them into 100-ml calibrated flasks. Add 10 ml of 0.02 M EDTA solution, 10 ml of

10^{-3} M PAR solution and 20 ml of ammonium acetate buffer. Mix and dilute to volume with water and allow to stand for 1 h. Measure the absorbances of the solutions against the blank solution in a spectrophotometer at 550 nm using 20-mm cells. Plot a graph of the absorbance against the concentration of niobium.

Preparation of Germanium Calibration Graph

Transfer 0-, 1.0-, 2.0-, 3.0-, 4.0- and 5.0-ml aliquots of the standard germanium working solution (5 μg ml^{-1}) into a series of 25-ml calibrated flasks. Add to each 5 ml of dilute hydrochloric acid (2 + 3) and enough water to obtain a volume of 20 ml. Add 1 ml of 1% gelatin solution followed by 1.5 ml of phenylfluorone reagent, dilute to volume and mix. Measure the absorbances of the solutions after 1 h against the blank solution in a spectrophotometer at 504 nm using 10-mm cells. Plot a graph of the absorbance against the concentration of germanium.

Preparation of Sample Solution and Separation of Niobium and Germanium

To the specimen in a platinum (or PTFE) dish add 1 ml of dilute sulphuric acid (1 + 1), 1 ml of dilute nitric acid (1 + 1) and 5 ml of hydrofluoric acid. Place the dish on a hot-plate and evaporate until copious fumes of sulphur trioxide are evolved. Cool, add 5 ml of water and heat until fumes of sulphur trioxide are again evolved. Avoid prolonged fuming with sulphuric acid. Cool, add 5 ml of water and transfer into a 250-ml separating funnel with the aid of 5 ml of concentrated hydrochloric acid. Add an additional 10 ml of hydrochloric acid to give an acid concentration of 9 M. Mix thoroughly and cool to below 25 °C. After 10 ml of carbon tetrachloride, stopper and shake the funnel vigorously for 2 min. After the layers have separated, withdraw the organic layer into a second separating funnel. Repeat the extraction with two successive 10-ml portions of carbon tetrachloride. Reserve the aqueous portion for the determination of niobium.

To the combined organic extracts add exactly 5 ml of water containing 1–2 drops of 0.01 M sodium hydroxide solution and shake for 2 min. Allow the layers to separate and discard the organic phase. Transfer the aqueous phase containing the germanium into a 50-ml calibrated flask and dilute to volume with water.

Determination of Germanium

Transfer a 5.0-ml aliquot of the aqueous solution into a 25-ml calibrated flask and add, in order, 5 ml of dilute hydrochloric acid (2 + 3), 10 ml of water, 1 ml of 1% gelatin and 1.5 ml of phenylfluorone solutions. Dilute to volume with water, mix and allow to stand for 1 h. Measure the absorbance against a blank solution at 504 nm using a 10-mm cell. Determine the concentration of germanium from the calibration graph.

Determination of Niobium

Evaporate the aqueous phase reserved from the germanium extraction to light fumes of sulphur trioxide in a platinum dish. Cool the solution, add 25 ml of 10% tartaric acid solution, warm the solution and transfer into a beaker containing 25 ml of 10% tartaric acid solution. Adjust the pH to 5.8 with 2 M sodium hydroxide solution, cool, transfer into a 250-ml calibrated flask and dilute to volume with water.

Transfer a 50.0-ml aliquot of this solution into a 100-ml calibrated flask. Add 10 ml of 0.02 M EDTA solution, 10 ml of 10^{-3} M PAR solution, 20 ml of ammonium acetate buffer, dilute to volume with water and mix. Allow to stand for 1 h and measure the absorbance against a blank solution in 20-mm cells at 550 nm. Determine the concentration of niobium from the calibration graph.

Results and Discussion

No problems were encountered when using the proposed techniques provided that close attention was paid to the details. The results obtained for amounts of germanium and niobium in a series of thin films are listed in Table I. The niobium to germanium ratios are compared with ratios obtained on the same samples by electron-microprobe analysis. The electron-microprobe ratios represent the average composition resulting from both random length and width scans across the surface of the film. The depth of the region analysed by

the electron microprobe was calculated to be 0.27 μm. The results show that the total niobium to germanium ratio in the film is always lower than the ratios obtained by electron-microprobe analysis. This result is consistent with analysis by ion-scattering spectroscopy that showed that these same "Nb_3Ge" radiofrequency sputtered films had a relatively constant niobium to germanium ratio through their bulk but contained a thin (approximately 13% of the film thickness) germanium-rich film - substrate interface region.[8]

TABLE I

MICROGRAM AMOUNTS OF NIOBIUM AND GERMANIUM IN RADIOFREQUENCY SPUTTERED "Nb_3Ge" THIN FILMS AND NIOBIUM TO GERMANIUM RATIOS

Film	Niobium/μg	Germanium/μg	Niobium to germanium ratio: Proposed procedure	Niobium to germanium ratio: Electron-microprobe analysis
02 137 513	195	46.0	3.31 ± 0.08	3.61 ± 0.04
02 137 515	205	45.1	3.55 ± 0.08	4.29 ± 0.06
02 137 516	182	36.4	3.91 ± 0.07	4.70 ± 0.10
02 137 517	171	32.7	4.09 ± 0.08	4.93 ± 0.07
02 137 518	150	26.7	4.39 ± 0.08	5.54 ± 0.08
02 137 519	126	21.4	4.60 ± 0.08	5.77 ± 0.10

The electron-microprobe results showed that, depending upon the substrate position relative to the sputtering target configuration, the films varied from being relatively homogeneous to inhomogeneous (on a millimetre scale). The homogeneity of the sample decreases from film 02137513 to 02137519. The results are also supported by the phase relationships resulting from annealing studies, which suggested that all of the total niobium to germanium ratios in the film were lower than the crystal chemistry niobium to germanium ratio (*i.e.*, 4.9) that is assumed to characterise the stable germanium-rich "Nb_3Ge" equilibrium homogeneity range boundary phase.[3]

We have shown that it is possible to determine microgram amounts of niobium and germanium in "Nb_3Ge" radiofrequency sputtered thin films by the proposed procedures. The analysis of the total film is an important parameter to be considered in the compositional characterisation of these sputtered films and the understanding of their thermodynamic behaviour.

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Determination of Iron with Bathophenanthroline Following an Improved Procedure for Reduction of Iron(III) Ions*

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Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) forms a red complex with iron(II) but not with iron(III) ions and is used in the determination of total iron when present at low molarities in aqueous solutions. The reduction of iron(III) ions is a critical step in such determinations. A modified procedure is described which brings about a 5-fold increase in the amount of iron(III) ions reduced compared with the original procedure, involving replacement of hydroxylammonium chloride with L-ascorbic acid, addition of concentrated hydrochloric acid and heating the solution in a steam-cabinet.

Keywords: *Iron determination*; *bathophenanthroline*; *reduction*; *L-ascorbic acid*

Bacteriological growth media containing iron concentrations of less than 10^{-6} M are required for the study of bacterial iron transport systems.[1] Fortunately there are a number of extraction procedures available to reduce the iron content of media to this level.[1] A subsequent problem is the accurate determination of the iron content in the prepared medium.

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) is a highly specific compound suitable for the determination of extremely low concentrations of iron in aqueous solutions.[2] Used as the chromophore in the ferroin reaction [formation of a red complex of iron(II) ions with bathophenanthroline], iron concentrations as low as 10 parts in 10^9 can be detected.[3] Although 100-ml samples are required for detection at these low levels, the coloured complex is extracted into a small volume of 3-methylbutan-1-ol.[2,3] The coloured complex is very stable and contaminating iron in the reagents used in the assay can be removed by following essentially the same procedure as that used for the analysis of the samples.[2,3]

Bathophenanthroline forms the red complex with iron(II) ions but not with iron(III) ions. Most metal cations do not interfere with the detection of iron(II) ions.[2] A light yellow complex is formed with cobalt but is not extracted by 3-methylbutan-1-ol.[2,3] Copper forms a yellow complex but it is colourless under the acidic conditions of the assay.[2,3] However, Penner and Inman[4] have observed interference by cadmium, copper and zinc; furthermore, cobalt, copper, manganese and nickel appear to form complexes with bathophenanthroline and thus interfere to some extent by reducing the amount of bathophenanthroline available for the formation of the ferroin complex.[5]

The use of bathophenanthroline as a sensitive chromophore for the detection of iron was described by Smith *et al.*[3] Numerous modifications of this procedure have been described. Hydroxylamine, hydrazine, dithionate, ascorbic acid and tin(II) chloride have been used to reduce iron(III) ions. Amyl acetate, amyl alcohol, chloroform, hexan-1-ol and nitrobenzene have been used to extract the ferroin complex.[6] Marczenko[6] has recently suggested that samples be boiled after the addition of 0.1 g of hydroxylamine and 2.0 ml of 50% sodium acetate solution. Penner and Inman[4] have used a combination of hydroxylamine and ascorbic acid for the reduction of iron(III) ions in high-purity molybdenum, niobium, tantalum and tungsten metals.

In this laboratory, attempts to construct standard graphs with iron(III) chloride by using the original procedure of Smith *et al.*[3] were unsuccessful; however, reproducible and reliable standard graphs were obtained with iron(II) sulphate. Thus the reduction of iron(III) to iron(II) ions appears to be the chief problem in the original procedure. The modified procedure presented in this paper achieves a much greater reduction of iron(III) ions and gives reliable standard graphs with both iron(III) chloride and iron(II) sulphate.

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Experimental

Apparatus

Absorbance measurements were made on a Perkin-Elmer double-beam spectrophotometer (Coleman 124).

Erlenmeyer flasks (125 ml) were used to process the samples before their transfer into separating funnels (250 ml). All glassware was immersed overnight in a 50% *V*/*V* solution of concentrated hydrochloric acid and then rinsed thoroughly with de-ionised distilled water.

Reagents

Bathophenanthroline solution, 0.001 M. Dissolve 0.334 g of bathophenanthroline (Sigma) in 50 ml of glass-distilled ethanol (95%) and dilute to 100 ml with double-distilled de-ionised water.[2,3]

L-*Ascorbic acid solution*, 15% *m*/*V*. Dissolve 15.0 g of L-ascorbic acid in 100 ml of double-distilled de-ionised water. Add 0.1 ml of concentrated hydrochloric acid and place the solution in a 250-ml separating funnel. Add 4.0 ml of 0.001 M bathophenanthroline solution, shake thoroughly and allow to stand. After 30 min, add 10.0 ml of 3-methylbutan-1-ol, repeat the shaking and allow 8 h for the complete separation of the alcoholic and aqueous phases. Draw off the lower layer into a prepared container and discard the alcoholic phase containing any iron present as impurity.

Sodium acetate solution, 10% *m*/*V*. Dissolve 10.0 g of sodium acetate in 100 ml of double-distilled de-ionised water. Transfer the solution into a 250-ml separating funnel, add 0.1 ml of concentrated hydrochloric acid and 4.0 ml of 15% *m*/*V* L-ascorbic acid solution and shake the mixture vigorously. Add 4.0 ml of 0.001 M bathophenanthroline solution, repeat the shaking and allow the mixture to stand. After 30 min, add 10.0 ml of 3-methylbutan-1-ol, shake thoroughly and allow 8 h for the complete separation of the phases; discard the alcoholic layer.

3-*Methylbutan*-1-*ol*. Reagent-grade 3-methylbutan-1-ol (Aldrich Chemical Co.) can be used with no prior extraction process.

Ethanol, 95% *V*/*V*. Distil 95% ethanol in an all-glass apparatus to remove impurities.

Standard solutions of iron. Stock solutions containing 1.0 μg ml^{-1} of iron(II) and 1.0 μg ml^{-1} of iron(III) ions in double-distilled de-ionised water were prepared from iron(II) sulphate and iron(III) chloride, respectively [4.978 g of $FeSO_4.7H_2O \equiv 1.0$ g of iron(II) and 4.84 g of $FeCl_3.6H_2O \equiv 1.0$ g of iron(III)]. Standard solutions ranging in concentration from 0.5 to 8.0 μg of iron per 100 ml were prepared by dilution of 0.5–8.0-ml volumes of the iron stock solutions to a final volume of 100 ml.

Procedure

Add 4.0 ml of the L-ascorbic acid reagent and 0.1 ml of concentrated hydrochloric acid to 100 ml of the sample. Heat in a steam-cabinet for 1 h, cool the sample to room temperature and transfer it into a 250-ml separating funnel. Add 5.0 ml of the sodium acetate reagent and mix thoroughly, then add 4.0 ml of the bathophenanthroline reagent, shake vigorously and allow the mixture to stand for 30 min. Add 10.0 ml of 3-methylbutan-1-ol and shake vigorously. Allow the mixture to stand for 8 h for complete separation of the alcoholic and aqueous phases. Draw off and discard the lower phase. Read the absorbance of the alcohol phase at 533 nm.

The above modified procedure differs from the original procedure, described by Smith *et al.*,[3] in the following respects: firstly, 2.0 ml of 10% *m*/*V* hydroxylammonium chloride were replaced with 4.0 ml of 15% *m*/*V* L-ascorbic acid solution and 0.1 ml of concentrated hydrochloric acid and the solution was heated for 1 h in a steam-cabinet; secondly, 5.0 ml instead of 4.0 ml of 10% *m*/*V* sodium acetate solution were added to adjust the pH; thirdly, 30 min were allowed for the development of the iron(II) - bathophenanthroline complex (the original procedure began extraction of the complex immediately after addition of the bathophenanthroline); fourthly, the two phases were allowed to separate for 8 h instead of 5 min; and fifthly, only one extraction with 3-methylbutan-1-ol was performed (the original procedure utilised two extractions with a final dilution of the extract with ethanol to a total volume of 50 ml).

A blank was prepared by using a 50% *V*/*V* ethanol solution in place of the 0.001 M batho-

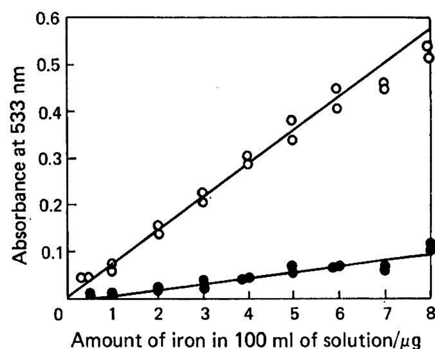

Fig. 1. Standard graphs for iron(II) sulphate (○) and iron(III) chloride (●) prepared by the original method.[3]

TABLE I

STANDARD GRAPHS GENERATED BY THE ORIGINAL METHOD OF SMITH *et al.*[3] WITH IRON(II) SULPHATE AND IRON(III) CHLORIDE

Amount of iron in 100 ml/μg	Absorbance at 533 nm: Iron added as iron(II) sulphate			Iron added as iron(III) chloride		
	Experimental*	Adjusted	Calculated	Experimental*	Adjusted	Calculated
0	0.020	—	—	0.022	—	—
0	0.022	—		0.025	—	
0.5	0.066	0.045	0.040	0.029	0.006	0.001
0.5	0.066	0.045		0.032	0.008	
1	0.086	0.065	0.075	0.032	0.008	0.007
1	0.097	0.076		0.027	0.004	
2	0.161	0.140	0.146	0.046	0.023	0.019
2	0.172	0.151		0.045	0.022	
3	0.229	0.208	0.217	0.046	0.023	0.031
3	0.244	0.223		0.056	0.033	
4	0.305	0.284	0.288	0.066	0.043	0.043
4	0.314	0.293		0.066	0.043	
5	0.367	0.346	0.359	0.076	0.053	0.055
5	0.395	0.374		0.086	0.063	
6	0.432	0.411	0.430	0.092	0.069	0.067
6	0.469	0.448		0.092	0.069	
7	0.472	0.451†	0.501	0.094	0.071	0.079
7	0.478	0.457†		0.097	0.074	
8	0.553	0.532†	0.572	0.131	0.108	0.091
8	0.534	0.513†		0.125	0.102	

* Experimental absorbance values of samples minus the averaged absorbance value of the samples of zero iron concentration.

† Absorbance values not used in the linear regression analyses. Statistical analyses yielded the following results: (1) for iron(II) sulphate $y = 0.071x + 0.004$, correlation coefficient = 0.997 and standard deviation = ± 0.009; (2) for iron(III) chloride $y = 0.012x - 0.005$, correlation coefficient = 0.981 and standard deviation = ± 0.006.

phenanthroline reagent. In the preparation of standard graphs, absorbance values of samples of zero iron concentration (no added iron) were subtracted from absorbance values of the samples in order to correct for any residual contaminating iron in the double-distilled de-ionised water and in the assay reagents.

Results

Original Procedure

The standard graphs for iron(II) and iron(III) ions obtained by a slightly modified form of the original assay procedure are shown in Fig. 1 and the experimental results set out in Table I. These modifications comprised allowing 8 h for the separation of phases, omitting the second extraction with 3-methylbutan-1-ol and omitting the dilution of the extract with ethanol to a final volume of 50 ml. When the original procedure was followed exactly, absorbance values of samples containing 0–1.0 μg of added iron were not significantly different (results not reported). The results indicate that hydroxylammonium chloride fails to reduce iron(III) ions effectively. A comparison of the slopes of the standard graphs for iron(III) and iron(II) ions {[iron(III) slope/iron(II) slope] × 100%} indicates that approximately 16% of the iron(III) ions are reduced to iron(II) ions by hydroxylammonium chloride.

Modified Procedure

The standard graphs for iron(II) and iron(III) ions obtained by our modified procedure are shown in Fig. 2 and the experimental results set out in Table II. The results indicate that approximately 82% of the iron(III) ions are reduced, a 5-fold increase over the reduction obtained with hydroxylammonium chloride.

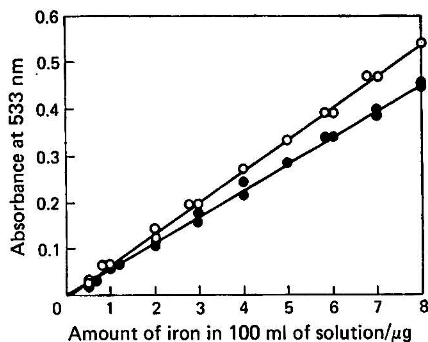

Fig. 2. Standard graphs for iron(II) sulphate (○) and iron(III) chloride (●) prepared by the modified method.

Determination of the remaining iron content in the 15% *m/V* L-ascorbic acid reagent indicates that 0.049 μg of iron is derived from 4.0 ml of this reagent. The 10% *m/V* sodium acetate reagent does not add a significant amount of iron to the samples. The iron content of the concentrated hydrochloric acid was not determined because of the small volume added to the samples. Analyses of 3-methylbutan-1-ol and 0.001 M bathophenanthroline solution were not performed because of difficulties encountered in extraction and solubility. The pH values of samples at various steps in both procedures are shown in Table III. In the original procedure, the pH values of the samples were slightly higher than those in our modified procedure.

The use of glassware that has not been treated in hydrochloric acid is a significant source of contaminating iron. The iron content of samples of double-distilled de-ionised water processed in untreated glassware was 3.67 μg per 100 ml. Double-distilled de-ionised water from the same source, processed in treated glassware, had an iron concentration of 0.387 μg per 100 ml.

TABLE II

STANDARD GRAPHS GENERATED BY THE MODIFIED METHOD WITH IRON(II) SULPHATE AND IRON(III) CHLORIDE

Amount of iron in 100 ml/ µg	Absorbance at 533 nm: Iron added as iron(II) sulphate			Iron added as iron(III) chloride		
	Experimental*	Adjusted	Calculated	Experimental*	Adjusted	Calculated
0	0.027	—	—	0.027	—	—
0	0.027	—		0.027	—	
0.5	0.051	0.024	0.028	0.056	0.029	0.029
0.5	0.057	0.030		0.046	0.019	
1	0.092	0.065	0.062	0.092	0.065	0.057
1	0.092	0.065		0.086	0.059	
2	0.149	0.122	0.130	0.147	0.120	0.113
2	0.164	0.137		0.137	0.110	
3	0.222	0.195	0.193	0.192	0.165	0.169
3	0.222	0.195		0.208	0.181	
4	0.299	0.272	0.266	0.246	0.219	0.225
4	—	—		0.260	0.233	
5	0.357	0.330	0.334	0.310	0.283	0.281
5	—	—		—	—	
6	0.423	0.396	0.402	0.367	0.340	0.337
6	0.423	0.396		0.367	0.340	
7	0.498	0.471	0.470	0.417	0.390	0.393
7	0.498	0.471		0.426	0.399	
8	0.569	0.542	0.538	0.485	0.458	0.449
8	—	—		0.475	0.448	

* Experimental absorbance values of samples minus the averaged absorbance values of the samples of zero iron concentration. These values were used in the linear regression analyses. Statistical analyses yielded the following results: (1) for iron(II) sulphate $y = 0.068x - 0.006$, correlation coefficient = 0.999 and standard deviation = ± 0.004; (2) for iron(III) chloride $y = 0.056x + 0.001$, correlation coefficient = 0.999 and standard deviation = ± 0.007.

Discussion

The higher pH values of samples processed by the original procedure do not account entirely for the poor reduction of iron(III) ions, as elimination of the steam heating in the modified procedure also results in poor reduction. Concentrated hydrochloric acid, 15% *m/V* L-ascorbic acid solution and steam heating for 1 h are all required for the improved reduction of iron(III) ions; elimination of any one of these three conditions results in incomplete reduction. Penner and Inman[4] have observed that extraction of the ferroin complex is less complete at pH 4.0 than is extraction at pH 5.5. We obtained the opposite result; when concentrated hydrochloric acid was omitted (resulting in a final pH of 5.1), reduction of iron(III) ions was poor. As Penner and Inman[4] lowered the pH of the sample to approximately 1.0 for reduction of iron(III) ions, with subsequent elevation to pH 5.5 for formation and extraction of the ferroin complex, this apparent discrepancy occurs because the original procedure of Smith *et al.*[3] does not lower the pH of the sample below 3.7 for the reduction of iron(III) ions.

The results indicate that the original procedure fails to reduce iron(III) ions effectively (only 16% reduction), whereas our modified procedure achieves an 82% reduction. A second method for determining the percentage reduction of iron(III) ions, using the average of the absorbance of the iron(III) chloride samples divided by the absorbance of the iron(II) sulphate samples at each concentration, shows a reduction of iron(III) ions of 87% by our modified method and 15% by the original procedure.

The modified procedure has been used extensively in this laboratory and always yields reproducible values in various media, as well as reliable standard graphs. For the standard graph generated with iron(III) chloride by the modified procedure, the following equation can be used to determine iron concentrations (x) in water or media samples:

$$x = \left(\frac{y - 0.001}{0.056} \times \frac{1}{0.82}\right) - 0.049$$

where y is the observed absorbance. This equation adjusts for the 82% conversion of iron(III) ions and the 0.049 μg of iron added from the ascorbic acid reagent. The equation generated by an iron(III) ion standard graph should be used for the determination of iron in aqueous solutions, as most of the iron present will be in the iron(III) state. By adjusting for the percentage reduction of iron(III) ions and the addition of iron from reagents, an accurate determination of iron can be achieved.

Normally washed glassware contains significant amounts of contaminating iron, which must be removed by treatment with hydrochloric acid (or another acid) in order to eliminate erroneously high values.

Attempts to analyse complex media containing peptone, yeast extract and similar preparations containing peptide fragments by this procedure will yield apparent iron concentrations lower than those actually present. This conclusion has been drawn from numerous comparisons of growth and ionophore production by various bacterial strains in media both with and without peptide fragments (unpublished results). It appears likely that these peptide fragments chelate iron and make it unavailable for both extraction and reduction to iron(II) ions.

TABLE III

VALUES OF pH IN SAMPLES AFTER EACH STEP OF THE ORIGINAL AND MODIFIED PROCEDURES

Additions	Original procedure	Modified procedure
100 ml of water	6.9	6.9
4.0 ml of 15% *m/V* L-ascorbic acid solution . .	—	2.5
2.0 ml of 10% *m/V* hydroxylammonium chloride solution	3.7	—
0.1 ml of concentrated hydrochloric acid	—	1.9
After steam heating for 1 h	—	2.3
4.0 ml of 10% *m/V* sodium acetate solution	5.0	—
5.0 ml of 10% *m/V* sodium acetate solution	—	3.8
4.0 ml of 0.001 M bathophenanthroline solution . .	5.1	3.8

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Improved Method for the Determination of Ethylenediaminetetraacetic Acid in Aqueous Environmental Samples by Gas - Liquid Chromatography

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An improved method for the gas - liquid chromatographic determination of ethylenediaminetetraacetic acid in aqueous environmental samples is described. The separation of the major peaks is increased by preparing the ethyl derivatives of the sample components, 1,6-hexanediaminetetraacetic acid being used as internal standard. The lower limit of detection of the method is approximately 15 $\mu g\ l^{-1}$ with 25-ml samples. This limit can be improved, if necessary, by using a larger sample volume.

Keywords: *Ethylenediaminetetraacetic acid determination; sewage; water; gas - liquid chromatography*

A method was required for the determination of ethylenediaminetetraacetic acid (EDTA) in sewage, sewage effluent and river water because of the effect it might have in mobilising trace metals, *i.e.*, in reducing their tendency to be removed from solution by adsorption and precipitation reactions and possibly causing their desorption from contaminated sediments. EDTA salts are present in low concentrations in detergent preparations and some food products and are not readily biodegradable.

Use of colorimetric and complexometric methods was unsuccessful and a method based on the work of Rudling[1] and Chau and Fox,[2] in which the methyl ester was determined by gas - liquid chromatography using 1,2-cyclohexanediaminetetraacetic acid (CDTA) as internal standard, presented problems as the retention times of the methyl esters of EDTA and those of certain C-18 fatty acids (stearic and oleic), which were also usually present in the samples being investigated, were very similar and the peaks were not resolved under the conditions employed. It was considered unlikely that any stationary phase would completely resolve EDTA from the range of C-18 fatty acids that might be found in samples of sewage and sewage effluent. It was also found that humic acid in the sample, most of which failed to react with the esterifying agent, produced a greater than theoretical recovery of EDTA, apparently because of interaction between CDTA and the non-reactive fraction of humic acid.

In the method proposed here the ethyl derivatives of the sample components were prepared so that the major peaks would be well separated. The ethyl esters of fatty acids up to and including the C-18 fatty acids eluted well before the EDTA derivative and did not interfere. Fatty acids of greater chain length are invariably present at insignificantly low concentrations in environmental samples. It was also found that the linear analogue of CDTA, 1,6-hexanediaminetetraacetic acid (HDTA), when used as internal standard, did not interact with the humic acid present in the sample. A series of small peaks occurred in the region of the peak for HDTA but did not interfere; these peaks may have originated from detergent components.

Chau and Fox[2] have described a method of lowering the detection limit in the gas - liquid chromatographic determination of nitrilotriacetic acid (NTA) by incorporating an anion-exchange pre-concentration step. A similar pre-concentration step was investigated for EDTA, using a strongly basic anion-exchange resin, but was found to be unsuitable for samples of sewage, sewage effluent and most river waters because humic acids blocked the column. The step could be used for samples that were almost entirely free of humic acid, *e.g.*, drinking water, but recovery at low concentrations of EDTA (1–20 $\mu g\ l^{-1}$) was found to be low and variable, necessitating the addition of radioactively labelled EDTA as tracer so that the recovery achieved by each pre-concentration could be determined independently.

Experimental

Reagents

EDTA, disodium salt. Laboratory-reagent grade.

HDTA. Laboratory-reagent grade. Obtained from Kodak Ltd. In addition this reagent was prepared by condensing 1,6-hexanediamine with chloroacetic acid and, following the method of Schwarzenbach and Ackermann,[3] was purified via the ester.

Esterifying solution. Add slowly, with stirring and cooling, 2.5 ml of sulphuric acid (Aristar grade) to approximately 40 ml of absolute ethanol. Add 25 μl of acetic acid (Aristar grade) and make up to 50 ml with ethanol.

Phosphate buffer solution. Dissolve 14.1 g of anhydrous disodium hydrogen orthophosphate (AnalaR grade) in approximately 75 ml of distilled water, add hydrochloric acid (AnalaR grade) until pH 7.0 is reached and make up to 100 ml with distilled water.

Chloroform containing 2% of ethanol. Laboratory-reagent grade. Re-distil before use.

All other reagents should, if possible, be of AnalaR grade.

Apparatus and Conditions

All glassware with which the sample came into contact was rinsed with concentrated nitric acid followed by distilled water in order to reduce potential interferences to a minimum.

Gas - liquid chromatography

Instrument. A Pye 104 chromatograph with dual flame-ionisation detectors. Use of a reference column to compensate for column bleed at high temperature is optional.

Columns. One or two 1.5-m glass columns of 4 mm i.d., containing 3% OV-1 on 80–100-mesh Chromosorb W(HP).

Gas flow-rates. Nitrogen (carrier) 65 ml min^{-1}; hydrogen 60 ml min^{-1}; and air 730 ml min^{-1}.

Temperature programme. The temperature of the column oven was maintained at 150 °C for 4 min after injection, then increased at a rate of 10 °C min^{-1} to 285 °C. This temperature was maintained for 2 min. The injection-port temperature was approximately 200 °C at the time of injection.

Ionisation amplifier. An attenuation factor of between 500 and 5 000, depending on the concentrations of EDTA and HDTA, was used.

Recorder. A Servoscribe RE 541.20 chart recorder was used in the linear mode at a chart speed of 10 mm min^{-1}.

Syringe. A Hamilton 10-μl syringe with an 11.5-cm needle was used to inject the sample directly on to the column packing.

Measurement of peak area. The areas of the peaks were calculated from their heights and their widths at half-height.

Procedure

Filter the sample through a 0.45-μm Millipore filter with a Whatman GF/A pre-filter and extract the filtrate once with two thirds of its volume of chloroform. Acidify, with a few drops of 90% formic acid, a 25–200-ml aliquot of the aqueous layer (containing 10^{-8}–10^{-7} mol of EDTA) and add the internal standard (10^{-8}–10^{-7} mol of HDTA). After evaporation on a water-bath to approximately 2 ml, and before precipitation of solids has occurred, rinse the concentrate into a medium-wall Pyrex test-tube (150 × 10 mm). Continue evaporation in the tube at 100 °C under a stream of nitrogen and finally in an oven at 110 °C. Draw out the middle of the tube in a gas - oxygen flame, add between 1.0 and 1.5 ml of freshly prepared esterifying solution, seal the tube and place it with the bottom 2–3 cm in boiling water for 5 h. After cooling, open the tube and rinse the contents, using 1 ml of chloroform, into a centrifuge tube containing 3.0–4.5 ml of phosphate buffer solution. Shake the tube and contents for 1 min. Separate the layers by centrifuging, transfer the chloroform layer into a small sample tube and evaporate the solvent at 50 °C under a stream of nitrogen. Dissolve the residue in 50 μl of acetone and inject approximately 7 μl of the solution on to the gas - liquid chromatographic column.

Results and Discussion

When EDTA and HDTA, over a range of relative concentrations, were added directly

to the esterification tubes the ratio of the area of the peak due to EDTA to that due to HDTA was 0.73, with a coefficient of variation of 4%. Known concentrations of EDTA were also added to various aqueous samples and the recoveries determined (Table I). The tap-water sample (Sample 2) originated from a chalk aquifer. Samples 6 and 7 consisted of settled sewage from a purely domestic source and Sample 4 was of effluent from a biological filter that was treating sewage from the same source. Sample 5 was the final effluent from a sewage works treating a mixture of domestic and industrial sewage by the activated-sludge process.

TABLE I

RECOVERY OF EDTA FROM AQUEOUS SAMPLES TO WHICH KNOWN CONCENTRATIONS OF EDTA HAD BEEN ADDED

Each of the results given in this table is from a single determination.

The apparent original EDTA concentration in samples 1 and 2 has been assumed, from a knowledge of the origin of the samples, to be zero. Where the concentration found exceeds the concentration added, this is probably due to experimental error.

From the n estimates (n = 3, 4 or 5) of the apparent original EDTA concentration in each of samples 3 to 7, the weighted average was calculated by giving the first figure, in the order given in the table, a weighting of n, the second of $n-1$, and so on. This procedure, which was designed to compensate for the increase in experimental error in the difference between the "added" and "found" concentrations of EDTA as both increased, gave, for samples 3 to 7, values of 769, 178, 219, 80 and 202 μg l^{-1}, respectively. These values were used to calculate the recoveries given in the last column.

Sample number	Sample type	Concentration of EDTA/μg l^{-1}		Apparent original concentration of EDTA/μg l^{-1}	Recovery, %
		Added	Found		
1	Distilled water	46	40	0	87
		92	86	0	94
		221	202	0	91
		368	370	0	101
2	Tap water	46	54	0	117
		92	83	0	90
		221	194	0	88
		368	325	0	88
3	River water	0	860	860	112
		368	1180	810	112
		736	1350	610	79
		1105	1710	600	85
4	Sewage effluent	0	187	187	104
		96	263	167	89
		184	360	176	99
		293	473	180	100
5	Sewage effluent	0	251	251	115
		294	554	260	114
		589	777	188	96
		883	1007	124	91
		1178	1361	183	97
6	Settled sewage	0	109	109	136
		96	166	70	91
		184	226	42	79
		293	356	63	95
7	Settled sewage	0	176	176	88
		184	402	218	109
		293	539	246	115

All except Samples 1 and 2 contained EDTA on collection, and the recovery was calculated from the weighted average of the apparent original concentration of EDTA. The mean recovery was 98.6% with a standard deviation of 13.1%. Approximately half of the experimental error was thought to arise from the measurement of the chromatographic peak areas. Except in these recovery determinations, two aliquots of each sample, usually of different volumes, were analysed in order to reduce the error in the final result.

The sensitivity obtained was approximately 5×10^{-8} mol l^{-1} (15 μg l^{-1}) with 25-ml samples. Increasing the sample volume up to a probable convenient maximum of 200 ml gave a corresponding increase in sensitivity.

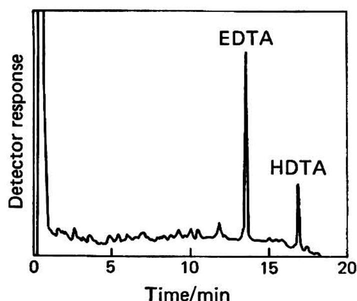

Fig. 1. Typical chromatogram showing EDTA in 25 ml of primary (settled) sewage 24 h after collection. [EDTA] = 4.5×10^{-8} mol per 25 ml (520 μg l^{-1}); [HDTA] = 1.26×10^{-8} mol per 25 ml (internal standard).

A typical chromatogram is shown in Fig. 1. Peaks due to compounds other than EDTA and the internal standard were usually relatively small. Primary sewage that was processed immediately after collection, however, showed prominent peaks closely following the solvent peak and in positions corresponding to long-chain fatty acids (Table II).

TABLE II

RELATIVE RETENTION TIMES OF THE ETHYL ESTERS OF EDTA AND OTHER COMPOUNDS

Chelating agents			Long-chain fatty acids			
HDTA	EDTA	NTA	C-18	C-16	C-14	C-12
1.00*	0.80*	0.32	0.73, 0.71, 0.69	0.58	0.47	0.35

* Kováts retention indices: HDTA, 2 737.86; EDTA, 2 320.42.

The method is sensitive to NTA, but none has been detected so far in environmental samples. A series of small peaks was obtained in the region of the peak for EDTA in positions corresponding to the alkanes C-24 to C-30. These peaks may have derived from alkyl sulphonate detergents; for example, under the esterification conditions used, a C-13 alkyl sulphonate gave a large peak in the position corresponding to the alkane C-26. The detergents appeared to originate at least partly from laboratory glassware, despite rinsing with concentrated nitric acid before use.

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Rapid Enzymatic Determination of Lactose in Food Products Using Amperometric Measurement of the Rate of Depletion of Oxygen

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An enzymatic method for the rapid and inexpensive measurement of free lactose in food products is described, based on the amperometric measurement of the rate of consumption of oxygen when lactose is hydrolysed by β-galactosidase, followed by oxidation of the product glucose in the presence of glucose oxidase and molecular oxygen. A Beckman glucose analyser with a Clark oxygen electrode is used for signal processing. Only minor pre-treatment of the sample, with no reaction incubation, is required. The measurement normally takes less than 5 min for samples that do not require extraction. The results are precise and accurate. Results for the analysis of milk and powdered milk samples are compared with those obtained by a phenol - sulphuric acid method.

Keywords: *Enzymatic analysis; electrochemistry; lactose determination; food*

The disaccharide lactose is present uniquely in milk; no other foodstuffs contain lactose, except those to which milk or lactose from milk may have been added. There has been considerable interest in the development of methods for the determination of lactose, as the lactose content in foodstuffs is indicative of the amount of skimmed milk powder that has been added.

The classical redox titration methods for the determination of lactose, such as the iodimetric method developed by Hinton and Macara[1] over 50 years ago and the Lane and Eynon titration,[2] are not only cumbersome but also give erratic results owing to under- and over-oxidation in the presence of certain ions in foodstuffs.

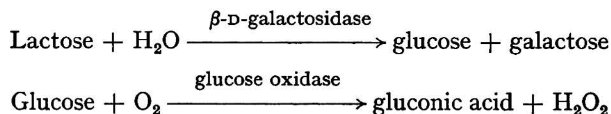
Colorimetric methods for the determination of lactose, involving the use of various chromogenic compounds, have been available for some time.[3] The most widely used procedure of this category is perhaps the phenol - sulphuric acid method.[3–5] While it is simple and precise, it has disadvantages such as non-specific colour absorption, non-specific reaction and the use of highly corrosive reagents.

Recently, chromatographic methods for the determination of lactose have gained attention. Gas chromatography has been applied to the measurement of lactose in physiological fluids after oral administration of disaccharides,[6] and high-performance liquid chromatography has been employed for the routine determination of lactose in milk.[7] The former method requires sample pre-treatment and has a long operation time, while the latter is not suitable for low concentrations of sugar.

The most specific method for the determination of lactose is the enzymatic method. Two enzyme-coupled procedures have been utilised, in both of which the specific determination of lactose is started by hydrolysing the substrate with β-galactosidase. In one method, the hydrolysis product, galactose, is monitored spectrophotometrically by coupling with galactose dehydrogenase and nicotinamide adenine dinucleotide (NAD).[8–10] In the other method, another product, D-glucose, is measured by coupling with two enzymes, hexokinase and glucose-6-phosphate dehydrogenase, in the presence of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP).[11–16] Although both of these methods have been proved to be specific and accurate, disadvantages such as high reagent cost and generally long operation procedures are major drawbacks in applying them in routine analysis.

Infrared spectrophotometry has recently been used for the routine analysis of milk.[17,18] Fat, protein and lactose contents in fresh and preserved milk samples, for example, are determined by a commercial infrared milk analyser (IRMA) (Sir Howard Grubb Parsons and Co. Ltd.).

This paper describes an electrochemical method for measuring lactose concentrations in foodstuffs. It is based on the amperometric measurement of the rate of depletion of oxygen in the following stepwise enzymatic reactions:



The rate of consumption of oxygen is proportional to the amount of glucose produced from the hydrolysis of lactose, and is directly related to the lactose concentration. The hydrogen peroxide generated by the enzymatic reaction is catalytically reduced by iodide and an alcohol, and therefore does not disproportionate to give oxygen.

The principle of the measurement is similar to the polarographic oxygen method described by Kadish *et al.*[19] for the determination of glucose, which has been adapted as the basis for the Beckman glucose analyser; the reader should refer to this paper for further details. The procedure is rapid and simple, only minor pre-treatment of samples is required and no incubation is necessary.

Experimental

Apparatus

A glucose analyser (Beckman Instruments Inc.) with a Clark-type oxygen electrode was used to measure the amperometric current and electronically take its derivative. Both the direct-current output and the derivative output were recorded on a two-pen strip-chart recorder (Omni Scribe, Houston Instruments Corp.). A Beckman DU-2 spectrophotometer was used for the colorimetric measurements of lactose by the phenol - sulphuric acid procedure.[3–5] A Finn pipette (Kemistien, Helsinki, Finland) with a volume range of 5–50 μl was used for delivering small volumes of samples.

Reagents

Imidazole buffer solution, pH 6.5. Dissolve 6.8 g of imidazole in 2 l of distilled and de-ionised water and adjust the pH of the solution to 6.5 with concentrated hydrochloric acid.

Buffer - iodide - molybdate stock solution. Dissolve 30 g of potassium iodide, 0.5 g of ammonium molybdate and 3.0 g of magnesium chloride in 1 l of the buffer solution and check and adjust the pH to 6.5.

Glucose oxidase solution. Dissolve 3.5 g of glucose oxidase (β-D-glucose:oxygen oxido-reductase, E.C. 1.1.3.4) powder containing about 50 000 U (Sigma Type II; Catalogue No. G6125) in 400 ml of the above stock solution. Add 15 ml of 96% ethanol and a few drops of octanol (to prevent foaming). Shake the mixture vigorously in order to dissolve the enzyme and then dilute it to 500 ml with the above stock solution.

β-Galactosidase solution. Dissolve 50 mg (containing about 5 000 U) of partially purified β-galactosidase (β-D-galactoside galactohydrolase, E.C. 3.2.1.23) powder in 2.5 ml of the buffer solution. The enzyme used was obtained from Worthington Biochemical Corp. (Catalogue No. 4099); it is available in purified form from different sources but these preparations are more expensive and were considered unnecessary in the present work (see below).

Stock standard solution, 7.5 g *per* 100 ml. Dissolve 15.00 g of reagent-grade lactose monohydrate (Matheson, Coleman and Bell Manufacturing Chemists) in 200 ml of distilled and de-ionised water. Each working day, dilute this stock solution in order to obtain working standard solutions containing the desired lactose monohydrate concentrations.

Preparation of Sample

Pasteurised and homogenised whole milk was purchased and diluted with different proportions of water, but no other pre-treatment was applied. Skimmed milk powder, canned soup, baby foods, cottage cheese and ice cream were obtained from local supermarkets.

The cheese sample is ground, extracted and filtered according to the method used by Barnett and Tawab.[4] The aqueous extracts of other solid or semi-solid samples are prepared by a method similar to that used by Bahl.[13–15] Sufficient amounts of accurately weighed

sample are macerated in a blender at high speed with 100 ml of water heated to 40–50 °C. The mixture is filtered through filter-paper and an appropriate volume of the filtrate is diluted to 100 ml with water so as to adjust the lactose concentration of the final solution to within the range 50–500 mg per 100 ml.

Procedure

Deliver 1.0 ml of glucose oxidase solution (containing about 100 U of glucose oxidase) to the sample cell of the analyser. Pipette 10–50 μl of standard solution or sample into the cell and allow the oxygen concentration of the solution inside the cell to equilibrate with atmospheric oxygen. There is an initial rapid change in oxygen concentration in the sample cell, which is indicated by a rapid change on the digital display panel of the analyser (with the selector switch in the "Check" position). This sudden change in oxygen concentration is due to the addition of a sample that has an oxygen concentration different from that of the reagent solution already in the cell. Also, some carbohydrates in the sample, in the presence of glucose oxidase, can react with oxygen. It takes about 0.5–5 min to complete oxidation and reach equilibrium, depending on the amount of carbohydrates catalysable by glucose oxidase.

Finally, the desired reaction of lactose is triggered by pipetting 15 μl of β-galactosidase reagent (containing 30 U) into the cell and the rate of depletion of oxygen is recorded. The derivative peak, which represents the maximum rate of current change, is obtained in about 30 s, and is directly related to the lactose concentration. After the peak has been reached, it is not necessary to continue recording.

Interference Studies

The interferences were determined by using aqueous solutions containing different compositions of oligosaccharides except lactose.

Results and Discussion

Effects of Instrument Variables

The oxygen membrane electrode normally degrades with time, and its response (peak heights) decreases gradually. Therefore, it is necessary to re-charge the electrode after a certain period of time (usually once a week), according to the manufacturer's instructions. The signal also varies from one electrode to another, depending on the surface areas of the cathode (rhodium or platinum) and the anode (silver).

In order to obtain the best analytical results, it is desirable to prepare a new calibration graph each working day. However, because of the linearity of the calibration graph (see below), only a few points are normally required on each graph.

The Beckman glucose analyser has a thermostatically controlled reagent and cell compartment, which controls the operating temperature to 33 $\pm$ 0.1 °C. Therefore, experiments were carried out at this temperature with the precisions reported below. No effort was made to study the effect of temperature on the reaction rate by changing the thermostat setting.

Each of the linear and derivative amplifiers of the glucose analyser has a gain-control potentiometer ("Air Adjust" and "Glucose Sensitivity" controls, respectively). By working at full scale of both gain-control potentiometers, the signals can be increased significantly.[20] The analyser has two modes of operation; by operation in the U-mode (higher gain designed originally for cholesterol tests), the signal is enhanced about four-fold over that in the G-mode (for glucose tests).

Effect of pH

The effect of pH on the activity of β-galactosidase has been studied by Wallenfels and co-workers.[10,21] By using lactose as substrate, a flat pH optimum is estimated between 6.5 and 7.0. Glucose oxidase shows a wide, usable range of pH between 4 and 7, with maximum activity at about pH 5.5.[22]

The effect of pH on the coupled reaction rate of both enzymes was studied for two buffers, imidazole and 2-amino-2-methylpropan-1-ol (AMP), both at 0.05 M concentration. The optimum pH is about 6.5 (Fig. 1).

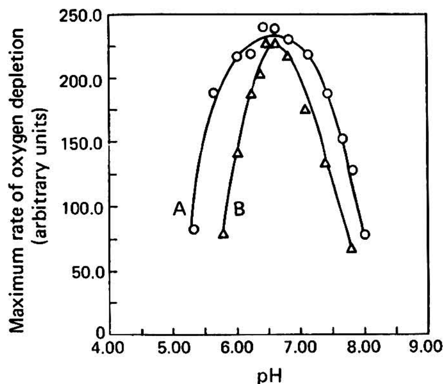


Fig. 1. Effect of buffer and pH on rate of consumption of oxygen. A, Imidazole, 0.05 M; B, 2-amino-2-methylpropan-1-ol, 0.05 M.

Effect of Enzyme Activities

Figs. 2 and 3 show the effects of enzyme concentrations on the maximum rate of the coupled reaction. The rate of consumption of oxygen seems to become independent of glucose oxidase activity above 50 U ml^{-1} at a lactose concentration of 400 mg per 100 ml. It is preferable to use a larger amount (100 U ml^{-1}) in order to ensure that the reaction rate is not limited by the glucose oxidase activity.

The rate of the coupled reaction tends to level off slightly at higher β-galactosidase activities. We chose to use 30 U for each test, to give a compromise between the linearity of the signal - substrate concentration relationship in the desired concentration range and the cost of enzyme.

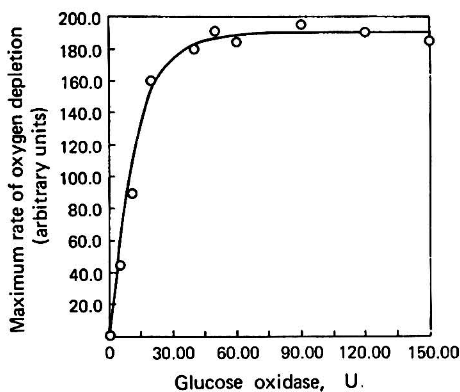


Fig. 2. Effect of glucose oxidase activity on the rate of consumption of oxygen in imidazole buffer of pH 6.5. To 1 ml of buffer, containing different activities of glucose oxidase, in the measurement cell 10 μl of a 400 mg per 100 ml lactose monohydrate standard solution and 30 U of β-galactosidase were added.

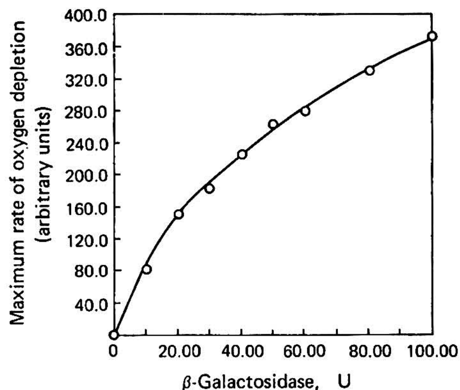


Fig. 3. Effect of β-galactosidase activity on the rate of consumption of oxygen in imidazole buffer of pH 6.5. To 1 ml of buffer, containing 10 μl of a 400 mg per 100 ml lactose monohydrate standard solution and 100 U of glucose oxidase, 10 μl of different activities of β-galactosidase were added to trigger the reaction.

Effect of Alcohol Concentrations

Shifrin and Hunn[23] reported that in the presence of low concentrations (about 5%) of methanol, ethanol and propan-1-ol, the catalytic activity of β-galactosidase from *E. coli* K_{12} is increased when *o*-nitrophenyl β-D-galactopyranoside is used as the substrate. In addition,

ethanol at a level of 5–10% is routinely added to Beckman glucose oxidase reagent[19,24,25] in order to remove the hydrogen peroxide that is produced from glucose solution in the presence of catalase, which exists as an impurity in most enzymes.

We have examined the effect of alcohols on the over-all reaction rate. Fig. 4 shows the graph of maximum reaction rate against alcohol concentrations. It indicates that only a slight increase (about 10%) in the coupled reaction rate is observed at low concentrations (about 3%) of methanol and ethanol in the presence of 15 mmol l^{-1} of Mg^{2+}. An increase in the concentration of methanol and ethanol or addition of propan-1-ol in the coupled reaction caused the reaction rate to decrease.

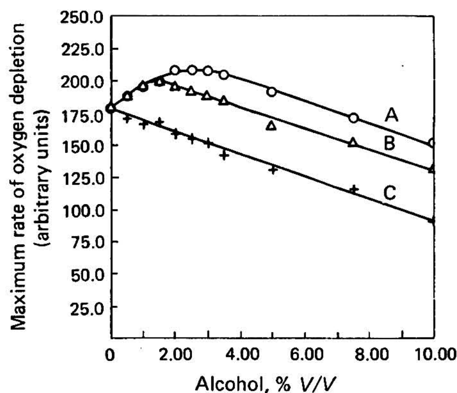


Fig. 4. Effect of alcohols on the rate of oxygen consumption. A, Methanol; B, ethanol; and C, propan-1-ol.

Calibration Graph

Fig. 5 shows recorded signals (rate of depletion of oxygen *versus* time) for aqueous lactose standards. The peak values give the maximum rate of consumption of oxygen and are directly proportional to the lactose concentration. The sample size was 10 μl and signals were recorded at two different recorder sensitivities, 10 and 1 V full scale. The numbers on the peaks represent the concentration of lactose monohydrate. The results can also be read directly from the digital display panel after injecting β-galactosidase (with the selector switch in the "Operate" position). The calibration graph (Fig. 6) obtained by plotting peak signals against lactose concentration is linear from 0 to 850 mg per 100 ml (0–83 μg ml^{-1}

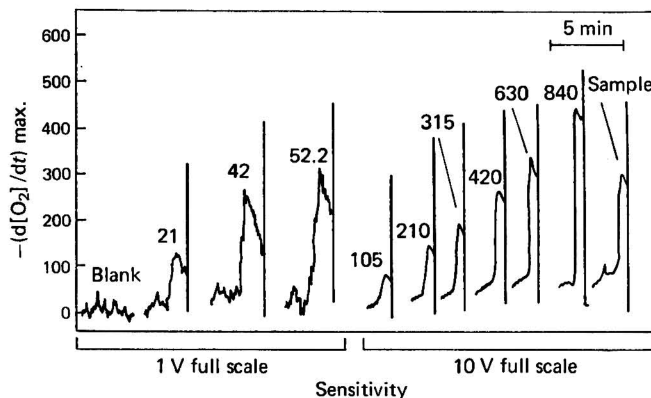


Fig. 5. Signals recorded for aqueous 10-μl lactose monohydrate standards. The numbers on the peaks represent the concentration of lactose monohydrate in milligrams per 100 ml; the peak heights are measured from the base line to the top.

in the measurement cell when 10 μl of sample and 10 μl of β-galactosidase were added to glucose oxidase - buffer reagent).

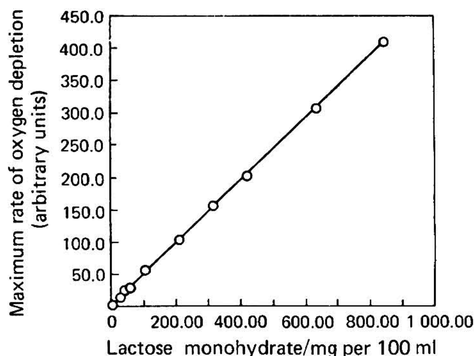


Fig. 6. Calibration graph for aqueous lactose monohydrate standards (10-μl samples).

Precision

For 15 determinations on a milk sample, a coefficient of variation of ±2.95% was obtained.

Recovery

Table I shows the efficiency of recovery of the proposed method. The lactose standard solution (containing the desired amount of lactose monohydrate) was added to milk and skimmed milk powder and the lactose concentration in the resulting mixture was determined. The recovery was within ±2.8% (standard deviation) of 100%, with an average error of about −1.6% in the range 150–250 mg of lactose monohydrate per 100 ml.

TABLE I

RECOVERY OF LACTOSE ADDED TO MILK AND SKIMMED MILK POWDER

Values corrected for lactose already present in samples.

Sample	Lactose added/mg	Lactose recovered/mg	Relative error, %
Milk*	50.0	48.8	−2.4
	75.0	76.2	1.6
	100	103	3.0
	125	121	−3.2
	150	143	−4.7
		Average:	−1.1
Skimmed milk powder† ..	50.0	47.9	−4.2
	75.0	73.0	−2.7
	100	102	2.0
	125	123	−1.6
	150	145	−3.3
		Average:	−2.0

* 2 g of milk sample added to a solution containing the desired amount of lactose and then diluted to 100 ml.

† 0.2 g of skimmed milk powder mixed with lactose and then diluted to 100 ml.

Interferences

Two enzymes are involved in the procedure described and, in order to reduce the cost of the analysis, it is desirable not to use highly purified enzyme preparations. Glucose oxidase samples from various sources normally contain different amounts of impurities.[24] Table II lists the non-specific reactions between three commercial glucose oxidase reagents and some common carbohydrates present in foods.[26] The glucose equivalents were measured in the

reagent solution prepared under our conditions and, therefore, the values are different from those reported by Fischl *et al.*[24] In our procedure, because we add the sample to glucose oxidase reagent prior to the addition of β-galactosidase in order to hydrolyse lactose, the presence of impurities in glucose oxidase does not affect the results. Carbohydrates that react with oxygen in the presence of impurities are removed from the sample. However, the actual time required to allow the oxygen concentration in the sample cell to attain equilibrium with atmospheric oxygen is dependent upon the amount of carbohydrates present in the sample. Hence, in some samples, especially sweetened food products that contain high concentrations of sucrose, it is desirable to remove impurities from glucose oxidase. As suggested by previous investigators,[24,27] disaccharidase impurities can be inactivated in glucose oxidase reagents by incubation at 55 °C for 2 h. Thus, by using the impurity-free enzyme, the time required to remove interferences and to equilibrate the oxygen concentration can be eliminated.

TABLE II

NON-SPECIFIC REACTIONS OF GLUCOSE OXIDASE PREPARATIONS

Substance (100 mg per 100 ml)	Glucose (lactose*) equivalents/mg per 100 ml		
	Sigma Type II	Beckman Grade I	Beckman Grade III
Maltose	3.5 (210)	13 (780)	0.0 (0)
Sucrose	1.0 (60)	2.6 (156)	1.4 (84)
Cellulose	0.0 (0)	0.0 (0)	0.0 (0)
Dextrin	1.5 (90)	4.2 (252)	0.0 (0)
Starch	2.1 (126)	1.3 (78)	0.0 (0)

* Lactose equivalents, given in parentheses, are calculated on the basis that 1 mg per 100 ml of glucose gives a signal (peak height) equivalent to about 60 mg per 100 ml of lactose monohydrate under the reagent conditions used, with 30 U of β-galactosidase used for lactose hydrolysis.

It should be noted that in the normal mode of operation of the instrument for the determination of glucose, none of the substances listed in Table II interferes, even at high concentrations, because the signal response is much slower (as well as smaller) than for glucose, *i.e.*, the glucose peak is reached before the instrument responds to the other substances. It is for this reason also that any interference from other substances in determinations of lactose is likely to be smaller than is indicated from the individual peaks given in Table II, because the lactose signals are also more rapid.

The specificity of β-galactosidase used in our method was studied by employing the impurity-free glucose oxidase and the partially purified β-galactosidase and tested with the carbohydrates listed in Table II. No detectable reaction was observed for any carbohydrate up to concentrations of 2 g l^{-1}.

Accuracy

The accuracy of the proposed method was established by analysing pasteurised milk and skimmed milk powder by this method and comparing the results with those obtained by the phenol - sulphuric acid method[3–5] (Table III). The results obtained by the two methods did not differ significantly.

TABLE III

LACTOSE CONTENTS OF MILK AND SKIMMED MILK POWDER

Sample	Lactose, % *m/m*	
	Present method	Phenol - sulphuric acid method
Milk—		
Brand I	4.63	4.73
Brand II	4.81	4.90
Brand III	4.72	4.54
Skimmed milk powder—		
Brand I	50.2	51.3
Brand II	51.1	52.0
Brand III	48.3	49.5

Application

By applying the above procedure, the lactose contents of a number of different food products were determined. Table IV shows the lactose contents of some foodstuffs obtained from local supermarkets.

TABLE IV

LACTOSE CONTENTS IN FOODSTUFFS

Sample	Lactose, % *m/m*		
	Brand 1	Brand 2	Brand 3
Canned soup	0.80	0.68	0.63
Gravy mix	4.05	11.3	4.50
Baby food	0.80	0.95	1.05
Cottage cheese	4.12	4.38	4.41
Ice cream	3.65	3.47	3.51

As suggested by Bahl,[13–15] the amount of skimmed milk powder added to food products can be determined from the lactose content with a relative error within 10%, on the basis that average skimmed milk powder contains about 51% of lactose. Although the effect of non-enzymic browning reactions during food processing and storage on the degradation of free lactose needs to be studied further, our method nevertheless provides a convenient means of calculating the amount of skimmed milk powder added to food products.

Conclusions

The method described here utilises a specific enzymatic procedure for determining free lactose in foodstuffs. The method is reliable in accuracy and precision, is suitable for small samples and is rapid and simple. The whole determination takes less than 5 min and is free from interferences from other species present in food products. An important factor in this method is that the reagents employed are much less expensive than those used in other enzymatic methods, and it is therefore suitable for use in routine food analysis.

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SHORT PAPERS

Non-stoicheiometric Copper Sulphide Membrane Electrode

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Keywords: *Copper sulphide membrane electrode*

The dependence of the potential of electrodes made from a metal sulphide on their stoicheiometry and on the activity of sulphide ions and the ions of the same metal in the solution undergoing measurement was described by Sato[1] by means of a general equation derived thermodynamically:

$$E_{M_iX_j} = E^0_{M_iX_j} + \frac{RT}{2ijF}\ln\frac{[M^{j+}]^i_{aq}\,[X]^j_{M_iX_j}}{[X^{i-}]^j_{aq}\,[M]^i_{M_iX_j}} \quad .. \quad .. \quad .. \quad (1)$$

where the standard potential of the electrode can be defined as

$$E^0_{M_iX_j} = \tfrac{1}{2}\,(E^0_{M,M^{j+}} + E^0_{X^{i-},X}) \quad .. \quad .. \quad .. \quad .. \quad (2)$$

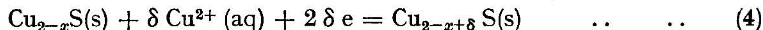
In the above equations i and j denote the stoicheiometric coefficients, M a metal, X a non-metallic element and subscripts aq and M_iX_j (or s) indicate whether particular activities refer to the component in the aqueous or the solid phase of the non-stoicheiometric compound. In practice, equation (1) is not applied. A slightly modified approach was applied in the paper by Koebel.[2]

In an earlier paper Sato[3] presented a means of calculating the potential of the chalcocite electrode when immersed in solutions containing copper ions in an amount exceeding that of the sulphide ions and compared the results obtained with experimental data, achieving good agreement. He described the potential of the chalcocite electrode by use of Nernst's equation:

$$E_{eq} = 0.530 + RT\,(2F)^{-1}\ln\,[Cu^{2+}] \quad .. \quad .. \quad .. \quad (3)$$

where the experimental value of the constant was 0.505. The copper - sulphur system and its standard potentials have since been investigated by Mathieu and Rickert.[4]

In this paper we propose a model membrane electrode, made from a non-stoicheiometric copper sulphide ($Cu_{2-x}S$), as presented below. During the reduction of the infinitesimal amount (δ mol) of Cu^{2+} ions, the non-stoicheiometric phase composition changes slightly in agreement with the reaction



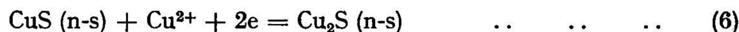
$$Cu_{2-x}S(s) + \delta\,Cu^{2+}\,(aq) + 2\,\delta\,e = Cu_{2-x+\delta}\,S(s) \quad .. \quad .. \quad (4)$$

Contrary to Sato,[1] who assumed that as components of the compound M_iX_j the elements M and X would be of zero charge (as in an amalgam), the authors of the present paper assume[5] the following presentation of the phase of $Cu_{2-x}S$:

$$[Cu_{2-x}S] = [(1 - x)\,Cu_2S\,(n\text{-}s) \times x\,CuS\,(n\text{-}s)] \quad .. \quad .. \quad (5)$$

where square brackets denote the single-phase mineral unit and symbols Cu_2S (n-s) and CuS (n-s) the components of the non-stoicheiometric solid phase. The essential difference between equation (5) and Sato's approach[1] is that in reality both univalent and bivalent copper occurs in the $Cu_{2-x}S$ phase, taken into consideration in equation (5), whereas in Sato's approach all of the metal atoms are considered to be equivalent to one another.

Following from the above, the reaction (4), taking place on the electrode, will be described by the equation



$$CuS\ (n\text{-}s) + Cu^{2+} + 2e = Cu_2S\ (n\text{-}s) \qquad (6)$$

If pure crystals of CuS (covellite) and Cu_2S (chalcocite) are taken as the standard state, then the value 0.530 V is obtained for the standard potential in reaction (7), it having been calculated earlier by Sato.[3]

For the copper sulphide electrode Nernst's equation thus takes the form

$$E_{eq}^{(ms)} = E^{o(ms)} + RT\ (2F)^{-1} \ln\ [CuS\ (n\text{-}s)]\ [Cu_2S\ (n\text{-}s)]^{-1} + RT\ (2F)^{-1} \ln\ [Cu^{2+}] \qquad (7)$$

or in standard conditions

$$E_{eq}^{(ms)} = E_{n\text{-}s}^{o\ (ms)} + RT\ (2F)^{-1} \ln\ [Cu^{2+}] \qquad (8)$$

where

$$E_{n\text{-}s}^{o\ (ms)} = 0.530 + 0.029\ 55 \times \log\ [CuS\ (n\text{-}s)]\ [Cu_2S\ (n\text{-}s)]^{-1} \qquad (9)$$

and the superscript (ms) indicates the metastable equilibrium state. Considering the influence of Cu^+ ions on the copper sulphide electrode potential we obtain

$$E_{eq}^{(ms)} = E_{n\text{-}s}^{o\ (ms)} + RT\ (2F)^{-1} \ln \{[Cu^{2+}] + [Cu^+]^2 K_{n\text{-}s}\}$$

where the coefficient of selectivity $K_{n\text{-}s}$ is defined by the following equation:

$$K_{n\text{-}s} = \frac{K_{So,\ CuS}}{K_{So,\ Cu_2S}} \times \frac{[CuS_{(n\text{-}s)}]}{[Cu_2S_{(n\text{-}s)}]} = K^o \frac{[CuS_{(n\text{-}s)}]}{[Cu_2S_{(n\text{-}s)}]} = K^o \exp \left[\frac{530 - E_{n\text{-}s}^{o\ (ms)}}{12.83}\right]$$

K_S being the solubility product and the subscript zero in K_{S0} indicating that the solid substance dissolves with the formation of non-complexed ions. (In this last equation the experimental value $E_{n\text{-}s}^{o\ (ms)}$ should be expressed in millivolts.)

The value of the standard coefficient of selectivity K^o was calculated on the basis of generally accepted thermodynamic data (ΔG_f^o for chalcocite is 20.6 kcal mol^{-1}, for covellite -11.70 kcal mol^{-1}, for Cu^{2+} ions 15.53 kcal mol^{-1} and for Cu^+ ions 12.00 kcal mol^{-1}) and was taken to be $10^{12.74}$.

Experimental

Potentiometric measurements were carried out by using a vessel of 200-ml capacity, with a cover, in which the electrode under examination and the electrolytic junction with the saturated calomel electrode were fixed. Copper(II) sulphide embedded in epoxy resin was sealed into a glass tube. Electrical contact with the mineral was achieved by means of a spring clamping bolt with a gold-plated tip. Solutions were prepared using triply distilled water and the analytical-grade reagents [ammonium chloride, copper(II) chloride and copper(II) nitrate] were recrystallised. The copper sulphide electrodes were synthesised from spectroscopically pure metallic copper and elemental sulphur in silica ampoules under a 10^{-5} torr vacuum at 1 250 °C. The samples investigated had a net composition of $Cu_{1.98}S$ and $Cu_{1.86}S$. Before measurement the solutions were deoxygenated by passing purified argon saturated with water vapour through them. The potential of the copper sulphide electrodes was measured by using a digital voltmeter with an accuracy of 0.05%. The results were recorded on a printer controlled by pulses from a generator of frequency 1–10^{-2} Hz.

Results

A number of potentiometric measurements were carried out by using the non-stoicheiometric copper sulphide membrane electrode in solutions of composition $x\ Cu(NO_3)_2 + HNO_3$ at a constant ionic strength of 1 mol dm^{-3}. The characteristic potential of the electrode examined, $E_{n\text{-}s}^{o\ (ms)}$, was established by extrapolation of the experimental relationship E/log $[Cu^{2+}]$ to the copper activity representing 1 mol dm^{-3}. The ionic activity coefficient

of Cu^{2+} ions was assumed to be 0.96, obtained from the nomogram described by Zanker.[6] On the basis of the definition of $E^{o\,(ms)}_{n-s}$ discussed, the relationship of the activities of the electrode components was calculated.

For the electrode with the composition $Cu_{1.98}S$ it was found that

$$E^{o\,(ms)}_{n-s} = 502 \text{ mV } (\textit{versus} \text{ SHE}) \text{ and } \frac{[\text{CuS (n-s)}]}{[\text{Cu}_2\text{S (n-s)}]} = 0.1$$

and for the electrode of composition $Cu_{1.86}S$

$$E^{o\,(ms)}_{n-s} = 519 \text{ mV } (\textit{versus} \text{ SHE}) \text{ and } \frac{[\text{CuS (n-s)}]}{[\text{Cu}_2\text{S (n-s)}]} = 0.4$$

During further investigations potentiometric measurements were also carried out in solutions of a composition NH_4Cl + Cu(I) at an ionic strength of 1 mol dm^{-3}. The over-all copper(I) ion concentration varied in the range 5×10^{-6}–10^{-2} mol dm^{-3}. In these solutions the complex $CuCl_3^{2-}$ was predominant. Its dissociation constant, β_3, is 1.45×10^{-6}, according to references 7 and 8. The activity of the non-complexed Cu^+ ion in the solutions investigated was established by independent measurement of the potential of a metallic copper electrode (copper of spectroscopic purity, obtained from Johnson Matthey Chemicals Ltd.). The standard potential of the copper electrode in solutions of Cu^+ ions was assumed to be 520 mV (*versus* SHE). The dependence of the potential of the copper sulphide membrane electrode on Cu^+ ion activity is indicated by the following equations:

$$E = 0.849 + 0.054 \log [\text{Cu}^+] = (E^{o\,(ms)}_{n-s} + \frac{0.054}{2} \log K_{n-s}) + 0.054 \log [\text{Cu}^+]$$

for the $Cu_{1.98}S$ electrode and

$$E = 0.874 + 0.056 \log [\text{Cu}^+] = (E^{o\,(ms)}_{n-s} + \frac{0.056}{2} \log K_{n-s}) + 0.056 \log [\text{Cu}^+]$$

for the $Cu_{1.86}S$ electrode.

In Table I the values of the selectivity coefficient, calculated from these relationships, are presented. They are in good agreement with theoretical values.

TABLE I

COMPARISON OF CALCULATED AND THEORETICAL SELECTIVITY COEFFICIENTS

Electrode	[CuS (n-s)]/[Cu_2S (n-s)]	K_{n-s} theoretical	K_{n-s} experimental
1	0.1	$10^{11.74}$	$10^{11.8}$*
2	0.4	$10^{12.34}$	$10^{12.3}$

* The same value was obtained by Hulanicki *et al.*[9]

The authors are indebted to Professor A. Pomianowski for his kind interest in this work.

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Determination of Carbon in Iron and Steel by an Air-combustion Method

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Keywords: Carbon determination; air combustion; iron; steel

For many years the determination of the carbon content of steels, pig and cast irons and other ferrous alloys has been carried out by combustion of a sample, usually in the form of drillings or turnings, and collection and measurement of the carbon dioxide produced. In the British Standard method this measurement is made gravimetrically, after absorption of the carbon dioxide in soda-asbestos.[1] The Ströhlein gas-volumetric method is also used. More recently, to meet the demand for rapid methods enabling adjustments to be made to the molten metal, other techniques, such as thermal conductivity, infrared absorption, coulometric titration and electrical conductivity have been employed.[2]

All of these methods involve the use of pure oxygen as the supporting gas for combustion, which is effected at temperatures ranging from 1 000 to 1 250 °C, depending on the type of sample. Indeed, it would appear that the direct-combustion gravimetric method was only able to supplant the tedious separation and wet-oxidation methods used in the 19th century[3] when plentiful supplies of oxygen and electric furnaces capable of maintaining the necessary high temperature became available. As far as can be ascertained, no attempt was made to burn the sample directly in a stream of air rather than pure oxygen, although in some early versions of the method air was used to sweep out the combustion train after the sample had first been ignited in a stream of oxygen. In the analogous combustion method for determination of sulphur air has been used,[4] but a much higher temperature is employed (1 400 °C) and the purpose of using air is to avoid the formation of iron(III) oxide, which can react with some of the sulphur-containing gases and reduce the yield of titratable sulphur.

The aim of the work described in this paper was to find whether and to what extent air can be used instead of oxygen for the combustion of steels and cast irons in the determination of carbon. Owing to limited resources it has been possible, at this stage, to carry out tests only on relatively non-refractory materials.

Experimental

The procedure used was essentially that described by Westwood and Mayer[5] but, instead of the oxygen supply, air was drawn through the train at a flow-rate of about 250 $cm^3 min^{-1}$ by means of a water pump connected to the exit end of the Midvale bottle containing the soda-asbestos absorbent. Before entering the combustion tube the air was dried by drawing it through a Midvale bottle containing silica gel; it was purified from carbon dioxide in a second Midvale bottle containing soda-asbestos, and finally drawn through a 250-cm^3 Drechsel bottle containing about 100 cm^3 of liquid paraffin (to indicate the air flow-rate). A 1-g amount of pure lead foil was added to each sample to act as a flux. Preliminary tests indicated that a total time of 15 min was sufficient to ensure complete combustion of the sample and for all of the carbon dioxide to be absorbed in the Midvale bottle.

Results

Tests were carried out on standard samples of steel, white cast irons, grey cast irons and an alloyed austenitic cast iron with a range of carbon contents from 0.10 to 4.0%. The compositions of these samples are shown in Table I. The results obtained for the carbon contents are given in Table II together with the standard deviations found at the 1, 2, 3 and 4% carbon levels. All of these results were obtained using a combustion-tube temperature of 1 000–1 050 °C.

Discussion

The results obtained show that it is possible to carry out satisfactory determinations of

TABLE I

CERTIFIED COMPOSITION OF SAMPLES

Sample	Description	Element, %										
		C	Si	S	P	Mn	Ni	Cr	Mo	Cu	V	Mg
BCS 163/1	Carbon steel	1.21	0.28	0.026	0.026	0.32	—	—	—	—	—	—
BCS 170/3	Grey cast iron	2.13	2.47	0.080	0.71	0.76	—	—	—	0.60	—	—
BCS 173/1	Austenitic cast iron	2.42	2.73	0.020	0.029	0.82	13.2	4.06	—	5.05	—	—
BCS 221/1	Carbon steel	0.60	0.25	0.031	0.025	0.66	0.15	0.08	—	—	—	—
BCS 407	Low-alloy steel	0.50	0.69	0.012	0.033	0.13	0.61	3.00	0.82	0.43	0.23	—
GIRI 2257	Cast iron	3.60	3.03	0.073	0.45	0.57	—	—	—	—	0.10	—
BCIRA D1/1	White cast iron	4.01	1.89	—	—	0.03	1.01	1.0*	—	—	—	0.106
BCIRA D1/2	White cast iron	2.87	2.76	—	—	0.72	0.84	1.0*	—	—	—	0.058
Steel 3	Low-alloy steel	0.10	0.20	0.022	0.012	0.45	0.21	2.10	0.98	0.13	—	—

* Approximate figure (not standardised).

carbon content on samples of carbon and low-alloy steels, grey cast irons, white cast irons and even a highly alloyed austenitic cast iron using air instead of oxygen as the supporting gas in the direct combustion method. The time required for a determination is not appreciably longer than that required when oxygen is used. Combustion appears to take place very smoothly and the residues after combustion are found to be sintered and not fused as occurs when oxygen is used. Consequently, there is negligible contamination of the interior of the combustion tube by slag, so that the useful life of the tube can be considerably extended. This saving, together with the fact that oxygen cylinders are not required, gives the method an economic advantage.

TABLE II

CARBON CONTENT (%) DETERMINED BY PROPOSED METHOD

	Sample								
	BCS 163/1	BCS 170/3	BCIRA D1/1	BCIRA D1/2	BCS 221/1	BCS 173/1	BCS 407	GIRI 2257	Steel 3
	1.18	2.11	3.99	2.90	0.60	2.42	0.51	3.58	0.10
	1.17	2.12	3.98	2.93	0.56	2.41	0.52	3.62	—
	1.20	2.11	4.05	2.88	—	2.43	0.51	—	—
	1.16	2.13	3.97	2.86	—	2.39	—	—	—
	1.19	2.14	4.03	2.88	—	—	—	—	—
	1.22	2.15	4.04	2.85	—	—	—	—	—
	1.20	2.11	4.03	2.86	—	—	—	—	—
	1.19	2.11	3.97	2.86	—	—	—	—	—
	1.19	2.10	4.00	2.84	—	—	—	—	—
	1.21	2.11	3.99	2.83	—	—	—	—	—
Mean	1.19	2.12	4.00_5	2.86_5	0.58	2.41	0.51	—	—
Standard deviation ..	0.019	0.016	0.030	0.029	—	—	—	—	—

In addition to the main series of tests reported in Table II, a small number of other tests were carried out. Samples BCS 170/3 and BCS 173/1 were tested without the addition of flux to the samples before ignition. Under these conditions and using a furnace temperature of 1 000 °C results of 2.13 and 2.41% of carbon, respectively, were obtained after a combustion time of 20 min. It was also found that a result of 2.10% of carbon could be obtained for BCS 170/3 using a furnace temperature of 850 °C, when 1 g of lead foil was added as flux. In this experiment a combustion time of 30 min was necessary.

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Spectrophotometric Determination of Beryllium in Air by a Sensitised Chrome Azurol S Reaction

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Keywords: *Beryllium determination*; *air*; *Chrome Azurol S*; *spectrophotometry*

As beryllium is highly toxic, it is essential to monitor air for the presence of trace amounts of this metal in areas where it is produced or fabricated. Although many spectrophotometric reagents for beryllium are known,[1] their sensitivities do not approach that provided by the fluorimetric method using morin.[2] We report here a spectrophotometric method of comparable sensitivity wherein addition of cetylpyridinium bromide considerably enhances the colour intensity of the beryllium - Chrome Azurol S complex. As usual, EDTA serves as a suitable masking agent for eliminating interferences from commonly associated ions. The results obtained by this method on air samples from the beryllium laboratory at the Bhabha Atomic Research Centre are compared with those obtained with the morin method. Similar sensitisation of the beryllium - Chrome Azurol S system by polyoxyethylene dodecylamine and poly(vinyl alcohol) has been reported.[3,4]

Experimental

Reagents

Chrome Azurol S (*Mordant Blue* 29). The sodium salt (Fluka) was purified by double recrystallisation as the free acid.[5]

Beryllium solutions. Solutions were prepared from the sulphate, $BeSO_4.4H_2O$ (Merck), and standardised gravimetrically.

Cetylpyridinium bromide solution, 1%. The solution was prepared from material as supplied by BDH Chemicals.

EDTA, disodium salt, solution, 1%.

Hexamine solution, 5%.

Gum arabic solution, 1%.

Sulphuric acid, 0.1 N.

Apparatus

Spectrophotometric measurements were made on a Beckman DU spectrophotometer and fluorescence measurements on a BARC, Model P1, fluorimeter.

Samples

Samples were collected by pumping air through a Whatman 41 filter-paper for 30 min at a pumping rate of 17.6 ft^3 min^{-1}. The paper was leached with 15–20 ml of 0.1 N sulphuric acid and the leachings made up to 50 ml with water. Suitable aliquots were taken for analysis.

Procedure

To a 5.0-ml aliquot of the sample, add 1 ml of 1% Chrome Azurol S solution, 10 ml of 1% gum arabic solution and 2 ml of 1% EDTA (disodium salt) solution. Adjust the pH to about 2.0 and add 1 ml of 1% cetylpyridinium bromide dropwise with constant stirring and 3.0 ml of 5% hexamine solution. Adjust the final pH to 5.0 and make the volume up to 50 ml. Measure the absorbance at 605 nm after 1 h against the corresponding reagent blank. Obtain a calibration graph by using a set of standards containing from 0 to 3.0 μg of beryllium under the same conditions.

As suggested by Sandell,[6] EDTA was used as a masking agent in place of sodium pyrophosphate during the fluorimetric determination.[2]

Results and Discussion

The results for two air samples (with standard additions) are given in Table I.

The Be^{2+} - Chrome Azurol S system has been studied by many workers.[7,8] Using an acetate buffer and a large concentration of EDTA, Pakalns[9] found ϵ at 570 nm to be 4.06×10^3 l mol^{-1} cm^{-1}. As both of these compounds were found to have a bleaching effect on the coloured complex, we employed a much lower concentration of EDTA and replaced acetate with hexamine as the buffer, obtaining an absorptivity at 570 nm of 2.35×10^4 l mol^{-1} cm^{-1}.

TABLE I

DETERMINATION OF BERYLLIUM IN AIR SAMPLES

Air sample	Amount of beryllium added/μg	Aliquot taken/ml	Beryllium found/μg	
			Proposed method	Fluorimetry
I	—	5.0	0.12	0.11, 0.13
	0.4		0.52	—
	1.0		1.10	—
	0.1		0.22	0.21
II	—	1.0	0.06	0.06
	0.08		0.14	—
	0.16		0.21	—
	0.05		0.11	0.10
	0.10		0.16	0.15

Bailey *et al.*[10] have suggested the sensitisation of binary metal ion complexes with organic dyestuffs by the addition of long-chain cationic surfactants. The addition of cetylpyridinium bromide to the Be^{2+} - Chrome Azurol S complex shifts $\lambda_{max.}$ to 605 nm and increases ϵ to 9.6×10^4 l mol^{-1} cm^{-1}. Beer's law is obeyed for Be^{2+} concentrations in the range 0–3.0 μg per 50 ml. The masking action of EDTA successfully prevents the interference of a 100-fold excess of ions such as Al^{3+}, Ca^{2+}, Ba^{2+}, Cu^{2+}, Mg^{2+} and Fe^{3+}. The detection limit for the commonly used fluorimetric method with morin is 5.0 ng ml^{-1}, which, under the most favourable conditions, can be decreased to 1 ng ml^{-1}.[6] The method proposed here achieves this limit (1.0 ng ml^{-1} for an absorbance of 0.010 against a blank) and is free from a number of constraints to which the former is subject.[11]

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Improved Method for the Synthesis of 3-Propyl-5-hydroxy-5-D-*arabino*-tetrahydroxybutyl-3-thiazolidine-2-thione (PHTTT) and its Homologues, Specific Spectrophotometric Reagents for Copper

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Keywords: *3-Propyl-5-hydroxy-5-*D-arabino*-tetrahydroxybutyl-3-thiazolidine-2-thione reagent; spectrophotometry; copper*

3-Propyl-5-hydroxy-5-D-*arabino*-tetrahydroxybutyl-3-thiazolidine-2-thione (PHTTT) has been reported by Stiff[1] to be a specific spectrophotometric reagent for the determination of copper in water, but we have found that the synthetic method suggested by Stiff gave low yields of this reagent. However, on modifying the method slightly, we were able to obtain the product in a higher yield, and its purity was also higher than that which could be obtained using Stiff's method. In this paper we describe the modified method, which has also been found to be satisfactory for the synthesis of the 3-ethyl homologue (EHTTT) and the 3-butyl homologue (BHTTT), both of which can be used as spectrophotometric reagents for copper.

Experimental

Synthesis of the Reagents

Add 9 g of glucose to a mixture of 6 ml of methanol and 4.5 ml of propylamine (ethylamine and butylamine for the 3-ethyl and 3-butyl homologues, respectively). Stir the mixture until a gel is formed. Add 30 ml of hot ethanol (50–60 °C) to the gel and stir until the gel dissolves and then re-forms. Allow the gel to stand overnight at room temperature, then add a mixture of 20 ml of ethanol and 7 ml of carbon disulphide and heat the mixture below 50 °C for 1 h. Allow it to stand overnight at room temperature for complete crystallisation. Recrystallise the product once from ethanol - water (7 + 3). The yield is 48%.

Elemental analyses of the products gave the following results: (1) C 40.8, H 6.6 and N 4.7%; PHTTT requires C 40.4, H 6.4 and N 4.7%. (2) C 37.9, H 6.4 and N 4.8%; EHTTT requires C 38.2, H 6.1 and N 4.9%. (3) C 42.9, H 6.8 and N 4.6%; BHTTT requires C 42.5, H 6.8 and N 4.5%.

Determination of Copper

Place an appropriate amount of a 100 p.p.m. standard solution of copper in a 100-ml calibrated flask, followed by 3 ml of a 0.15% *m*/*V* solution of PHTTT (or EHTTT or BHTTT) in ethanol - water (15 + 85), and make up to the mark with de-ionised water. Allow the solution to stand for 10 min, then measure its absorbance with a spectrophotometer. Prepare a calibration graph from solutions containing 0–1 000 $\mu g\ l^{-1}$ of copper and with pH values within the range 6–8.

Results and Discussion

In the procedure suggested by Stiff,[1] heat is applied to the mixture of glucose and alkylamine and the dark-coloured viscous mass obtained is difficult to handle. The most crucial modification we have suggested is to avoid heating at this stage, as good yields of *N*-alkylglycosylamines can be obtained by reaction at room temperature.[2] With this modification, white or very nearly white PHTTT or its homologues can be obtained and it is necessary to recrystallise it only once, and the yield is increased from 33 to 48%.

We have also found that 3 ml of the 0.15% reagent solution is necessary in order to complex all of the copper when its concentration is close to 1 000 $\mu g\ l^{-1}$.

The spectral properties of the copper complexes are listed in Table I. It can be seen that the absorption maxima for the various copper complexes are virtually identical and the molar absorptivity increases slightly with increase in the chain length of the alkylamines; however, the Sandell sensitivities are not significantly different.

TABLE I

SPECTRAL PROPERTIES OF THE COMPLEXES FORMED BETWEEN COPPER AND PHTTT OR ITS HOMOLOGUES

Reagent	Absorption maximum/nm	Molar absorptivity/$l\ mol^{-1}\ cm^{-1}$	Sandell sensitivity/$\mu g\ cm^{-2}$
MHATT*	430–434	1.35×10^4	—
EHTTT	433	1.39×10^4	0.004 6
PHTTT	433	1.45×10^4	0.004 4
BHTTT	433	1.48×10^4	0.004 3

* The 3-methyl homologue; data from reference 3.

The reagent EHTTT has the advantage over MHATT, the methyl homologue, that it can be prepared more easily, and it has a slight advantage over PHTTT and BHTTT in being more soluble in water, so that aqueous solutions can easily be prepared.

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Communications

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Novel Sampling System for the Direct Analysis of Powders by Atomic-absorption Spectrometry

Keywords: *Powders; minerals; sampling; atomic-absorption spectrometry*

The analysis of soils, rocks, minerals, cement and similar materials by atomic-absorption spectrometry has received widespread attention. These materials, however, usually require time-consuming and often complex dissolution procedures prior to their analysis and the possibility of using a simple, direct method of analysis for this type of material would therefore be particularly welcome.

Willis[1] has recently studied the factors that govern the analysis of powders by flame atomic-absorption spectrometry using a slurry procedure. After grinding solid samples, then dispersing and suspending them in an aqueous medium, by using constant agitation, it was possible to nebulise the samples directly and to determine the concentrations of several elements. Unfortunately, there are two limitations to this technique: in order to achieve a satisfactory atomisation efficiency[1] it is necessary to grind the solid samples to a particle size of less than 10 µm and secondly constant mixing of the suspended material, using either ultrasonic agitation or a magnetic stirrer, is required during the analysis.

In the work outlined here, these two problems have been largely overcome. Firstly, by using electrothermal atomisation (Perkin-Elmer HGA 74 and Model 360 atomic-absorption spectrometer) rather than flame atomisation, it is possible to tolerate larger particle sizes for the samples. This advantage is borne out by work carried out on the direct introduction of powdered solid samples to electrothermal atomisers.[2–5] The limiting factor often becomes dependent more on the ability to achieve homogeneous sampling from the suspension. Secondly, by using a thixotropic thickening agent it is possible to achieve a suspension which is stable for several days but which can still be repeatedly and reproducibly sampled.

The thixotropic thickening agent used in this work was a commercially available material, Viscalex HV30 (Allied Colloids Ltd., Bradford), which is an acrylic copolymer containing carboxyl groups, supplied in the form of an acidic, low-viscosity emulsion. Dilution and neutralisation of this emulsion produces a highly viscous gel over the pH range 6–10. The production of this viscous gel together with the incorporation of the powdered sample unfortunately introduces a large number of air bubbles, which makes reproducible sampling of the suspension extremely difficult. The bubbles can be removed, however, by ultrasonic agitation of the gel, for about 60 s, so that the trapped bubbles rise to the surface as a froth. An alternative and more convenient procedure is to add a commercial de-foaming agent (*e.g.*, NOPCO NPZ, supplied by Diamond Shamrock Chemical Co., Morristown, N.J., USA) to the gel during preparation, which considerably reduces the amount of air trapped in the gel.

Fig. 1 illustrates the variation in signal response for chromium in a rock sample (0.1 g) suspended in various concentrations of Viscalex HV30 (0.5, 1.0 and 2.0% *V/V* in a volume of 100 ml). The suspensions were analysed by atomic-absorption spectrometry using electrothermal atomisation, over a period of several days, without agitation of any type. The results show that the 2% *V/V* concentration was stable for the period of testing; a 5% *V/V* concentration, while also providing a very stable suspension, could not be sampled satisfactorily owing to its high viscosity.

Checks on the reproducibility of sampling from a 2% *V/V* Viscalex HV30 gel produced the following results. The average mass of 20 consecutive samples taken with a 50-µl micropipette was 0.0466 g with a relative standard deviation of 0.01 (the specific gravity of the gel at 20 °C was 0.999).

There is a reduction in sample size, therefore, of approximately 7% compared with aqueous samples.

Recommended Procedure

Grind the sample to pass a 325-mesh ($\leqslant$44-μm particle size). Place up to 1 g of sample in a 200-ml beaker together with 1 ml of a 1% *m*/*V* solution of sodium hexametaphosphate (a wetting agent) and mix the two together with a glass rod. Add 2 ml of Viscalex HV30, dilute to approximately 50 ml with water, add 0.1 ml of NOPCO NPZ and then a few drops of ammonia solution to neutralise the mixture. Stir well with the glass rod for 1–2 min to disperse the sample as the gel forms. Either dilute the solution to approximately 100 ml in the beaker or transfer it into a 100-ml calibrated flask prior to dilution with water. Finally, stir or shake the sample to effect efficient mixing. The resulting suspension is stable for several days.

Results and Discussion

The only problems with sampling are that it is essential to wipe the outside of the plastic tip of the micropipette to remove excess of sample, and the best results are obtained by employing a ramp pyrolysis stage to avoid sputtering of the sample.

Calibration graphs produced using aqueous standards and standards prepared in a 2% *V*/*V* Viscalex HV30 gel are linear (over normal calibration ranges) and were almost identical in slope when using electrothermal atomisation.

Possible applications of this technique are numerous and wide ranging. These applications are illustrated by the determination of copper in rock samples. The rocks were prepared as described above using 0.1-g samples and analysed by atomic-absorption spectrometry using electrothermal atomisation.

Fig. 2 shows a comparison between values obtained for copper in five rock samples using (i) direct analysis of the solid samples, by atomic-absorption spectrometry using electrothermal atomisation, after suspension in 2% *V*/*V* Viscalex HV30 as described above, and (ii) analysis of the rock samples

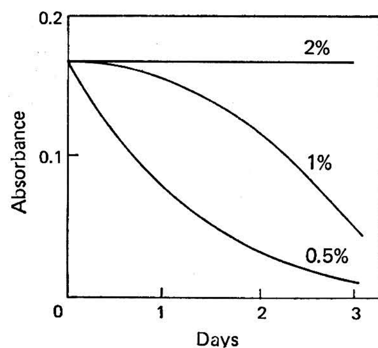

Fig. 1. Variation in the absorbance reading for chromium, in a rock sample suspended in various concentrations of Viscalex HV30, as a function of time.

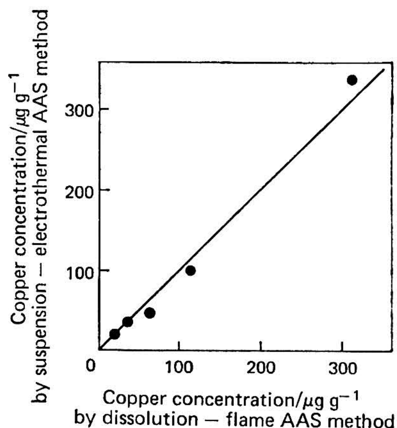

Fig. 2. Comparison of the concentrations of copper determined in five rock samples by dissolution - flame atomic-absorption and suspension - electrothermal atomic-absorption procedures.

by flame atomic-absorption spectrometry after dissolution of the samples. The results are particularly good when one bears in mind that the direct solid analysis values were obtained by comparison with aqueous standards prepared in a 2% *V*/*V* Viscalex HV30 gel and secondly that the total analysis time required after grinding was only 45 min.

Finally, preliminary results show that these gels can also be used with flame atomisation provided

that a micro-sampling cup system is used for nebulisation. It is probable that a less viscous 1% *V/V* Viscalex HV30 gel would be preferable. At this concentration the reduction in sensitivity compared with aqueous standards is of the order of 30%. Using this instrumental set-up, one of the problems described by Willis[1] is overcome, *i.e.*, the need for constant agitation of the sample. The major requirement that the sample particle size must be below 10 μm is still essential, however, in order to obtain a high atomisation efficiency.

In summary, the use of a thixotropic thickening agent enables the advantages of the slurry procedure to be exploited to the full. Lengthy sample dissolution procedures, which are required for normal methods of analysis, are avoided. Multiple determinations can be achieved from a single sample weighing compared with the multiple sample weighings that are required for direct solid analysis.

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Simple Technique for the Pyrolysis - Mass Spectrometry of Polymeric Materials

Keywords: *Pyrolysis - mass spectrometry*; *polymers*

Pyrolysis - mass spectrometry has been extensively studied by Meuzelaar *et al.*[1] and has been shown to have considerable potential for the characterisation and discrimination of natural and synthetic polymers. Their equipment was specially built for the technique and combined a Curie-point pyrolyser and quadrupole mass spectrometer operated in an optimum geometric arrangement. Few laboratories can afford to duplicate this equipment and the technique is likely to find greater use only if commercially available mass spectrometers can be conveniently modified. This paper describes the linking of a Curie-point pyrolyser to a magnetic-sector mass spectrometer to provide a method of generating pyrolysis mass spectra. The pyrolysate is passed through an empty glass chromatographic column and jet separator before entering the mass spectrometer. Forty or more sequential mass spectral scans are integrated by computer processing to give a composite mass pyrogram. The technique has been used to study a variety of polymeric materials of forensic interest.

Experimental

A Pye Curie-point pyrolyser, a Varian 2700 gas chromatograph and a V.G. Micromass 12F mass spectrometer were used. The pyrolyser was mounted on the front of the gas chromatograph and was purged with helium at a flow-rate of 5 ml min^{-1}. Samples were pyrolysed at 610 °C (heating time 15 s) and the pyrolysate was swept into an empty 45 cm × 6.35 mm o.d. × 2 mm i.d. glass column maintained at 200 °C in the gas chromatograph. A make-up flow of helium (10 ml min^{-1}) was introduced at the end of the column by using a Swagelok stainless-steel $\frac{1}{4}$–$\frac{1}{16}$ in reducing union with a length of $\frac{1}{16}$ in o.d. stainless-steel tubing brazed into it. The mixed outlet flow was passed through a length of glass-lined stainless-steel microbore tubing to the jet separator of the mass spectrometer.

The mass spectrometer was operated under standard electron impact conditions: electron energy 70 eV, emission current 100 μA, accelerating voltage 4 kV and source temperature 240 °C. The mass range (25–200) was scanned at 3 s per decade with a magnet re-set time of 3 s. About 40–50

scans of the pyrolysate were made with acquisition, storage and processing of spectra carried out with a V.G. 2040 data system, supplemented with an X - Y plotter. The standard software and specially written Fortran IV programs were used to produce composite X - Y plots (mass pyrograms) of the integrated spectra after background subtraction. The intensities of the ions 28, 32, 40 and 44 (*i.e.*, ions associated with air in the system) were set to zero before normalisation and only those peaks with intensities above 0.1% of the base peak were processed.

The system was used to study a wide range of polymeric materials using amounts of sample down to 5 μg.

Results and Discussion

To obtain maximum reproducibility in pyrolysis - mass spectrometry it is essential to integrate a large number of spectra from a given pyrolysate, and a fast-scanning quadrupole mass spectrometer is inherently better suited to this task than the relatively slow-scanning instrument we have used. Nevertheless, by introducing the pyrolysate into the instrument over a period of several minutes it has been found possible to generate reproducible mass pyrograms. The empty glass column spreads out the pyrolysate vapour into a band, the width of which is dependent on the column dimensions and the gas flow-rate through it. In practice, the jet separator requires a helium flow-rate of 15 ml min^{-1} for optimum operation—hence the use of the make-up flow.

The materials studied have included alkyd and acrylic paint flakes, synthetic and natural fibres, bitumens, adhesives, putties and greases. The sensitivity is sufficiently high to allow samples of 5 μg or less to give adequate electron-impact spectra, but in the chemical ionisation mode larger samples are necessary. Mass pyrograms are usually characteristic of the sample type and frequently allow discrimination between samples of similar composition provided that the analysis is reproducible. In a few instances where this is not so, the irreproducibility appears to be associated with the partial segregation of the pyrolysate during passage through the glass column.

The advantages of pyrolysis - mass spectrometry over pyrolysis - gas chromatography for generating information about polymeric materials are its speed, sensitivity, ease of producing data that can be computer processed and the elimination of the variables associated with gas chromatography. A major disadvantage of pyrolysis - mass spectrometry is that a complex mixture is produced by a combination of pyrolysis and electron-impact fragmentation, which makes a mass pyrogram more difficult to interpret than the chromatograms produced in pyrolysis - gas chromatography, in which only a pyrolytic breakdown stage is involved.

Conclusions

The procedure described enables workers using magnetic-sector mass spectrometers with data-processing equipment to generate mass pyrograms of polymeric materials. The instrumental modifications are simple and do not involve permanent changes to the mass spectrometer.

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Book Reviews

ANALYTICAL ATOMIC SPECTROSCOPY. By WILLIAM G. SCHRENK. *Modern Analytical Chemistry.* Pp. xvii + 375. New York and London: Plenum Press. 1975. Price $39.

This book is one of the first two in a series under the title "Modern Analytical Chemistry" to be expected from this publisher under the general editorship of Professor Hercules of the University of Georgia.

"Analytical Atomic Spectroscopy" is set out to provide a reasonably comprehensive account of the basic principles, instrumentation and applications of the analytical techniques of atomic spectroscopy that involve the outer valency electrons, *viz.*, arc and spark emission spectrography, flame atomic-emission spectrometry and atomic-absorption and atomic-fluorescence spectroscopy. The text is based on the author's experience as a teacher of graduate students and is intended to fill the gap that is seen by him to occur between those books that can be classed as laboratory manuals or methods books and monographic treatises on specialised techniques.

To this end the author has concentrated on the fundamentals of theory and instrumentation and has quite rightly in this context left out reference to detailed methods of analysis for elements.

The opening chapter sets the historic scene from the time of the early observations of Greek philosopher-scientists such as Aristophanes through the aptly called "dark ages" to the Newtonian era, the classic work of Kirchhoff and Bunsen and up to the present time. This does not occupy much space (9 pages), but it forms a pleasant and well balanced introduction to the next chapter, which gives as good a basic account of the essential theories of the origins of atomic spectra as I have seen for some considerable time. The treatment of such topics as energy level diagrams, broadening effects, spectroscopic term symbols, transition probabilities, etc., is particularly well done. This is followed by two chapters on (1) Filters, Prisms, Gratings and Lines, and (2) Composite Spectrometers, which again are, in my opinion, topics that are not too relevantly covered in many student texts on analytical atomic spectrometry. The next four chapters give a well balanced and comprehensive treatment of arc/spark emission spectrometry and the book continues with individual chapters on flame emission, atomic-absorption and atomic-fluorescence spectrometry and ends with appendices on basic definitions, two lists of spectral lines with gf and intensity values arranged according to wavelength and element, specimen spectral charts, an absorbance/percentage transmittance table, Seidel Function tables, etc. There are comprehensive subject and author indexes.

This is an unusually good book and is highly recommended. T. S. WEST

TRACE ANALYSIS. SPECTROSCOPIC METHODS FOR ELEMENTS. Edited by J. D. WINEFORDNER. *Chemical Analysis Series, Volume* 46. Pp. xii + 484. New York, London, Sydney and Toronto: John Wiley & Sons. 1976. Price £18; $29.45.

This addition to the well known series edited originally by Professors Kolthoff and Elving and now with Professor J. D. Winefordner as co-editor adds considerably to the reputation of the series in a very special way.

This text provides background rather than fundamental information on a selection of techniques for elemental analysis such as atomic-absorption and atomic-fluorescence spectrometry, molecular absorption and fluorescence spectrometry, nuclear techniques such as neutron-activation and isotope-dilution analysis, X-ray absorption and fluorescence spectrometry and spark-source mass spectrometry. This book is not meant to be a reference work on any one technique, and this is obvious at first sight, but rather to provide a useful critical means of comparing these techniques with each other and several others not reviewed in its pages. The text is provided liberally with tabular and graphical presentations of data, which help in the process of selecting procedures and sample preparation. There are also preliminary chapters on topics such as pre-concentration, loss of sample, contamination, sources of error and the characteristics of optical instruments. The appendices on quantitation (largely based on the concepts of Professor H. Kaiser) and the relative merits of sequential, multi-channel and multiplex systems are an unusual and most valuable feature, which is perhaps characteristic of the Editor's outstanding knowledge and experience on these topics.

The text avoids the trap of describing current commercial instruments, but does survey their present-day performances, capabilities and prices in order to assess the relative merits of the techniques. This may tend to become obsolescent rather quickly, but it is a very minor fault. The

chief fault, if such it be, is, in this reviewer's opinion, that the book tends to fall between two stools. It is, I feel, sufficiently detailed, and indeed the dust-jacket says it is, although the Editor's preface suggests otherwise, for the unwary student or technician to be led to think that the text must be a definitive one on the main topics. None of the authors would, I am sure, think that this is so. Nevertheless, the volume of detail in these chapters (6–10) is overwhelming and I feel that shorter and more rudimentary chapters on the techniques and more detail in Chapters 1–5 and 11 would have suited the purpose of the text somewhat better. I also feel that the working techniques of spark and arc emission spectrography are dismissed too lightly in comparison with their widespread use in industry at present.

However, this is an admirable book, which, in my opinion, presents more than any other an excellent over-all picture of these techniques in relation to each other. The synoptic view presented by the six authors is very good, owing, no doubt, to the teacher - pupil relationship between the Editor and his co-authors. As a reference source book for comparison of spectroscopic techniques of elemental analysis, it is uniquely valuable and should be most useful to all concerned with such matters.

T. S. West

The Determination of Sulphur-containing Groups. Volume 2. Analytical Methods for Thiol Groups. By M. R. F. Ashworth. *The Analysis of Organic Materials. Number 2.* Pp. xii + 288. London, New York and San Francisco: Academic Press. 1976. Price £9.80; $21.50.

The thiol function exhibits the properties of an acid, an alcohol and a reducing agent, and a choice of chemical methods is available for its determination. The properties of the function can be influenced by the remainder of the molecule; this fact widens considerably the range of analytical methods to be considered, and in the present work Professor Ashworth has decided to limit the methods mainly to those applicable to compounds in which the thiol group is carried primarily by a carbon atom joined either to hydrogen or to another carbon atom. Exceptions are made in order to include the important mercaptobenzthiazole and mercaptopurines but the xanthates and dithiocarbamates are omitted.

Much of the book (217 pages) is devoted to chemical methods of analysis, although many of these methods have instrumental finishes. The methods are reviewed according to the chemical reactions involved. For example, 55 pages are concerned with oxidation reactions and 73 pages with mercaptide formation. This systematic approach should be helpful to the analyst who needs to determine a thiol for which no method has been reported.

Polarography, physical methods (spectrophotometry, mass spectrometry, phosphorimetry and the use of radioactive reagents), chromatography and enzymic methods are rather briefly discussed (36 pages), but a considerable amount of information on chromatographic procedures is provided in tabular form. The recently developed procedure for the separation of thiols by covalent chromatography on immobilised disulphides has escaped mention; however, the equilibrium reactions that form the basis of the method rate a full chapter in the book.

This work should form a useful addition to the library of the organic analytical chemist. The references, about 1 500, are comprehensive and cover literature published up to mid-1974.

E. J. Greenhow

Wilson & Wilson's Comprehensive Analytical Chemistry. Volume VI. Analytical Infrared Spectroscopy. Edited by G. Svehla. Pp. xiv + 555. Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company. 1976. Price Dfl190; $76.50.

This expensive book maintains in every way the traditions now well-established for the Wilson and Wilson Series. Trivial misprints are virtually non-existent, although some well-known authors' names appear as Goley (p. 93), Flawler (Fowler) p. 479, and Harrich (p. 494); both author and editor are to be congratulated on producing a good, clear text. A total of 1 749 references is cited, and the subject is treated from the viewpoint of the practising analytical chemist rather than that of the theoretical molecular spectroscopist. Theory is treated in some 73 pages, then follow chapters that deal with: Apparatus (77 pp.); Experimental Techniques (48 pp.); Qualitative Analysis (176 pp.); Quantitative Analysis (47 pp.); Possible Practical Applications (39 pp.); and a brief but useful guide to the relevant literature.

There are two main difficulties. First of all, infrared spectroscopy is now too big a subject to be covered adequately, even in broad, general terms, in 500 pages. The coverage given here appears to be rather uneven, but priorities and preferences can vary widely from person to person; for example, the value of the inclusion of about 50 pages of performance details and design characteristics of commercial spectrometers, with diagrams showing their optical layout, could be questioned. Secondly, so many quite outstanding books of about the same length, depth of treatment and coverage have appeared previously that it is doubtful if any author could succeed in producing a refreshingly "new" book. Unfortunately this one has, in too many places, an appearance that can only be described as *déjà vu*; for example, many of the figures are reproduced from the well known text of Jones and Sandorfy while the tables of characteristic bond and group frequencies come either from the same source or from the text by A. D. Cross.

In short, the established infrared spectroscopist will get nothing new out of this book; the subscriber to this series will get another quality production of undoubted worth if he has not had anything on infrared on his shelf before; and the student, with it all to learn, will get a sound introduction to the subject (if he has a greater source of unearned income than usual, or can find a library with a copy to lend) but no better an introduction than has been available in the well known standard texts for quite a few years. D. M. W. Anderson

Modern Optical Methods of Analysis. By Eugene D. Olsen. Pp. x + 629. New York: McGraw-Hill Book Company. 1975. Price £11.75.

The author of this book should be congratulated on producing what, in my view, must represent the most satisfactory text-book currently available for undergraduate courses on spectroscopy. All quantitative and qualitative or structural techniques are covered, and while no specialist will be entirely satisfied with the treatment of his own subject, the balance and perspective of this book are exactly what today's graduates need. With the increasing price of books, the adoption of a single text to cover many branches of spectroscopy would be a great attraction to students and this single volume might satisfy the requirements of undergraduate spectroscopy courses at many UK universities. It could also be considered to be a suitable starting point for many of our MSc courses in analytical chemistry. From the academic viewpoint, the only important technique omitted is mass spectrometry. The book covers ultraviolet - visible and infrared spectrophotometry; atomic-emission, -absorption and -fluorescence spectrometry; X-ray methods, particularly fluorescence; fluorimetry, phosphorimetry, turbidimetry and nephelometry; refractometry, interferometry, spectropolarimetry and circular-dichroism spectrometry; Raman, microwave, nuclear magnetic resonance and electron spin resonance spectroscopy; and gamma-ray and Mössbauer spectroscopy.

The perspective of the book is akin to that developed by Meites and Thomas several years ago in their "Advanced Analytical Chemistry" and should be acceptable to most analytical chemists. The style and presentation are also similar to this earlier text, only the coverage has been altered by restriction to spectroscopic techniques and extension to cover many more of these. The range and limitations of each technique for quantitative and qualitative analysis are lucidly explained, accepted nomenclature is used, many worked examples are given and the design and operational requirements of modern instruments are carefully described. Sufficient theory is given to satisfy most courses, but where this is not the case, supplementary reading could be used. However, this is the type of book that the student should have for himself to pick up and read in idle moments and to refer back to at times of examination stress. J. M. Ottaway

Practical Pharmaceutical Chemistry. Third Edition. By A. H. Beckett and J. B. Stenlake. Part 1. General Pharmaceutical Chemistry. Revised by J. B. Stenlake with contributions by W. D. Williams. Pp. x + 370. London: The Athlone Press. 1975. Price £10.

Practical Pharmaceutical Chemistry. Third Edition. By A. H. Beckett and J .B. Stenlake. Part 2. Physical Methods of Analysis Pp. xii + 552. London: The Athlone Press. 1976. Price £15.

In 1968, the original text-book was radically restructured to meet the dramatic advances in pharmaceutical (especially instrumental) analysis as well as the greatly increased number of new

drugs and tighter monograph requirements. At the same time, the authors sought to meet the differing needs of elementary and advanced students by splitting the book into two different parts: one for general methodology and principles of quality control and a second part as a vehicle for specialist techniques described by individual experts. In the ensuing 9 years, the major changes have been political and social rather than analytical. In Britain, implementation of the Medicines Act has imposed what hitherto was implicit, stringent quality control in the manufacture of medicines and more explicit examination for impurities, stability and availability of dosage form. The BP in 1970, and latterly the BPC and BVetC, have been "nationalised," while the European Pharmacopoeia now runs to three volumes, which have prior status in signatory countries. In Western Europe recognition of inspection has advanced and within the EEC harmonisation moves slowly but inexorably. The Third Edition of Beckett and Stenlake has to reflect these key developments.

In Part 1, the opening chapter on chemical purity and control recognises pharmacopoeial and industrial sophistication; infrared spectrometry properly has much more prominence but surprisingly little is made of bioavailability (four short paragraphs in Chapter 1) considering the importance currently attached to *in vitro* comparative testing, not to mention the preoccupation of one senior author. There are, however, specific examples of official disintegration and dissolution tests in the final formulation chapter. There are minor improvements in chapters on the theoretical basis and techniques of quantitative analysis and the more specific presentations on acid - base, non-aqueous, redox, argentimetric and complexometric titrations, as well as ion exchange, gel filtration, solvent extraction and (still useful) gravimetry. The text has been meticulously updated, particularly in accord with the change between the 1968 and 1973 editions of the BP. Whether another edition will be needed in 1978, to take account of substances, but not necessarily the corresponding methods, imported from the BPC, is an interesting speculation.

Two new chapters merit special note. That on the analysis of formulations includes ointments and creams, suspensions, lozenges, suppositories and aerosol preparations, as well as the traditional tablets, capsules and injections. Convenient monographic sections supplement the terse requirements of the corresponding BP appendices or BPC formulary. Then of particular interest is an agreeably written and authoritative chapter on product registration. This chapter presents factual summaries of current (1975) British licensing requirements, distils guidance on good manufacturing practice and discusses the rationale of various particulars required in Product Licence applications. This is a cogent and comprehensive guide and is warmly commended to the would-be registration scientist, whether ultimately recruited to industry or to government service; the chapter could usefully be reprinted under a separate (soft) cover to supplement guidance already available through the licensing authority. However, it is a matter for debate whether the bulk of this chapter need (or should) form part of an introductory student text-book. The guide lines are liable to periodical review and future students in Britain would need revisions, whereas for those in other countries the minutiae of British practice are merely illustrative of one system.

Part 2 is organised in 14 chapters with guest authors presenting particular techniques. Two chapters provide an impressive account of general methods for determining the physical properties of substances and the solid state of drugs, neatly balancing the advanced student's need to understand the underlying physics, with exercises pertinent to pharmaceutical analysis. A minor quibble is that although SI viscosity units are defined and related to the CGS system, no explicit mention is made of the threatened demise of the poise and stokes. Useful additions include succinct references to Cotton effects and spectropolarimetry, and descriptions of differential thermal analysis and differential scanning calorimetric examination of polymorphic forms. Concepts and methods of determining particle size are briefly introduced, with a reference to the SAC/AMC review for further information; the table of BP/BPC drugs currently subject to particle size requirements is useful and a good description of the Coulter counter is supported with well chosen experiments.

In the ever expanding field of chromatography there are adequate expositions and practical details for column, paper, thin-layer and gas - liquid techniques, backed with realistic pharmaceutical exercises, a rather short table of common spray reagents, and a list of BP monographs that incorporate paper or gas - liquid chromatographic tests, but not thin-layer chromatography, which is very widely used. Minor criticisms include too limited reference to (semi-)quantitative thin-layer chromatography and the use of those very convenient aluminium- or polyester-backed sheets; exercises largely taken from publications of the 1960s; and a similarly dated account of columns and detectors available for gas - liquid chromatography. Curious, too, is the use of the symbol x, rather than t_R, for characteristic retention time. To this new edition has been added

a very readable account of the mode of operation of high-performance liquid chromatography, with brief notes on pumps, ultraviolet and refractive-index detectors, columns and packing materials. Appended are some student exercises and a list of pharmaceutical applications culled from manufacturer's literature.

Three further chapters are concerned with electrochemical analysis: measurement of e.m.f.; specific electrodes for hydrogen and other ions; potentiometric, conductimetric and high-frequency titrations; and theoretical and organic polarography, supplemented with amperometric titrations and a new section on coulometry.

Seven chapters, about 40% of the volume, are devoted to spectrometric analysis. The basic physics is followed by pharmaceutical applications of molecular absorption (quantitative and diagnostic) with an extensive tabulation of drugs ordered by ultraviolet maxima. There is very brief treatment of atomic emission and absorption and of spectrofluorimetry. A longer chapter introduces the diagnostic use of infrared spectrometry; the standard Colthup chart is reproduced and illustrative examples are given. The chapter on nuclear magnetic resonance spectrometry has a particularly clear exposition of proton spectroscopy and a useful 4-page summary of chemical shifts that is clearer than some other published examples. This chapter has been considerably updated and includes double resonance, shift reagents and brief mention of CAT and FT techniques and the use of carbon-13 spectra. The chapter on mass spectrometry is, appropriately, shorter but nevertheless manages succinctly to deal with low and high resolution, gas chromatograph - mass spectrometer interfacing, selected ion monitoring and chemical and field ionisation; the applications cited are of general organic, rather than strictly pharmaceutical, interest.

The final chapter, on radiochemical techniques, occupies a disproportionate amount (20%) of the volume and seems the least relevant. Counting equipment and its general use are extensively surveyed and cogent hygiene and safety warnings are given but there are far more experiments than are likely in a pharmaceutical course; the final one, however, is topical: an introduction to radioimmunoassays.

Both volumes are well written and illustrated and, in a pretty thorough reading, very few misprints were encountered. Subject to the minor criticisms specified, this new edition can be warmly commended.

G. F. Phillips

THE PHYSICAL BASIS FOR HETEROGENEOUS CATALYSIS. Edited by EDMUND DRANGLIS and ROBERT I. JAFFEE. *Proceedings of the Ninth Battelle Colloquium in the Materials Sciences, Gstaad, Switzerland, September 2–6, 1974.* Pp. xxvi + 596. New York and London: Plenum Press. 1975. Price $54.

The twenty-six papers presented at this colloquium are given in full in this volume, together with the discussion of each paper and the concluding general discussion of the conference. The majority of the papers will be of little interest to analytical chemists but several include interesting applications of new analytical techniques for the study of metal surfaces and of materials adsorbed thereon. Those interested in surface studies using electron diffraction, electron microscopy or photoelectron spectroscopy should find something to interest them.

J. M. Ottaway

ORGANIC FUNCTIONAL GROUP ANALYSIS BY GAS CHROMATOGRAPHY. By T. S. MA and ATHANASIOS S. LADAS. *The Analysis of Organic Materials, Number* 10. Pp. x + 173. London, New York and San Francisco: Academic Press. 1976. Price $16.75; £6.80.

This book deals with the application of existing instruments and known separation techniques to the quantitative gas-chromatographic analysis of organic compounds by means of the formation of suitable derivatives.

While not aimed primarily at a user new to the technique of gas chromatography, the principles of the technique and the operating parameters are discussed and presented in such a way that a beginner would have no difficulty in making good use of the contents of the book.

As well as a good, clear introduction to the subject, there are six other divisions of the contents dealing with coupling of functional group reactions with gas chromatography and oxygen, nitrogen, sulphur, unsaturated and miscellaneous functions.

The chapters dealing with the analysis of various groups are taken mainly from published literature, much of it by T. S. Ma and his former students. Each is presented clearly, giving good discussion of techniques and examples, and with detailed descriptions of operating conditions and any special equipment required. Efforts have been made to convert the functions into derivatives

that can be separated readily by gas chromatography from similar groups or products of side-reactions, and care has been taken to select the most suitable columns for the work in question.

The layout and presentation of the contents are first class and for each chapter there is an extensive list of literature references. This is a handbook which should be of considerable value to students and lecturers in educational establishments and to analysts in industry and in research laboratories.

D. SIMPSON

QUANTITATIVE ANALYSIS BY GAS CHROMATOGRAPHY. By JOSEF NOVÁK. *Chromatographic Science Series, Volume 5.* Pp. x + 218. New York and Basle: Marcel Dekker, Inc. 1975. Price SWFr.58.

In the preface to this book, the author makes the observation that quantitative gas chromatography is normally considered mainly from the practical aspect, and the book under review is an attempt to redress this situation and present a detailed theoretical consideration of the topic. In this respect the book is well presented, and contains a great deal of detailed theoretical consideration and mathematical treatment. Unfortunately, there are virtually no points in the book where theoretical predictions and experimental observations meet for comparison and the reader is thus left with little idea of the validity of the theory when he embarks upon practical quantitative chromatography. For example, there is a chapter on detectors in which various gas-chromatographic detectors (both commonly used and apparently randomly chosen uncommon detectors) are neatly classified according to whether the response is mass or concentration sensitive and whether they are destructive or non-destructive. These are not, of course, new concepts. The gas-density balance, an almost ideal example for predicting quantitative data, is dismissed merely with the comment that it should display certain properties. No evidence is presented to support this statement and no clear distinction is made between the Martin and Nerheim designs. Similarly it is stated that the katharometer, the response of which is notoriously difficult to predict, obeys a particular equation. No consideration is given to the extensive experimental evidence to confirm or refute these predictions. Detectors, which are fundamental to any discussion on quantitation, are thus briefly treated at this point in the book. There is a separate chapter on the prediction of responses of detectors, but even here there is little attempt to compare theory with experimental observation. There are in this chapter references to several papers in which detector responses have been measured, but these are neither complete, nor are they discussed, and the reader is left to consult the original literature for himself. The flame-ionisation detector, surely demanding a lengthy discussion in view of its extensive use in quantitative analysis, is not given such treatment, although an observation relating to changes in response with stationary phase bleed is very useful. There is a mention of Scott's detector early in the book with no literature reference or indication of the type of detector under discussion, and it is not until the following chapter that it is identified as a flame thermocouple detector, a device never in common use and not available commercially. In the section on electron-capture detectors the reader is not given any warning of the difficulties of predicting response, or information on the alternative modes of operation.

The various methods of calibrating detectors are described in detail, but the reader's attention is not drawn forcefully enough to the practical difficulties associated with some of these techniques. There is a section on quantitation and capillary chromatography that is very interesting, but again little attention is paid to the practical difficulties. The section on liquid - liquid extraction is comprehensive but covers 13 pages before the use of multiple extractions to overcome difficulties associated with unfavourable partition coefficients is discussed. There is a useful section on the special problems associated with headspace analysis.

The chapter on peak areas is helpful, and the reviewer fully supports the comment that "the operator's experience, along with the quality of the chromatogram, determines the precision of the result."

There are very few misprints and the book is well set out, although the use of a "typewriter" type-face is not very attractive. References 32 and 74 are identical, and quoted as a pre-print of a symposium; the proceedings of this symposium have been available for about 14 years.

In general, the book is a well devised theoretical exercise embracing all steps in quantitative gas chromatography. It will not, however, enable the reader to gain an insight into the practical applications or limitations of the topic. The reviewer therefore concludes that the book has been given an incorrect title, which is unfortunate both for the author and reader.

T. A. GOUGH

A LABORATORY MANUAL OF QUALITATIVE ORGANIC ANALYSIS. By H. T. OPENSHAW. Pp. xii + 92. London, New York and Melbourne: Cambridge University Press. 1976. Price £2.75.

Older chemists will remember and recognise the text of this little manual, which has now appeared in paperback some 20 years after the last (third) edition was published.

In the intervening years, considerable changes have occurred in the teaching of practical organic chemistry and less and less time can be devoted to the practice of qualitative analysis, be it organic or inorganic. Regrettable though this situation is, one must face facts and ask why this book has been allowed to re-appear without any significant change since 1955. The analytical chemist now has on hand such informative books as Feigl's "Spot Tests in Organic Analysis" and the modern organic chemist is far more attuned to the utilisation of spectroscopic techniques to provide a qualitative picture of his unknown material.

Based strictly on classical lines, this manual directs the reader to chemical detection of the characteristic groupings after simple preliminary tests and qualitative elemental analysis (Lassaigne's test). Once the functional group is established, then suitable derivatives can be prepared to establish the identity of the original substance. Countless generations of students can testify to the effectiveness of such procedures, which not only taught them an appreciable amount of chemistry but also enabled them to acquire reasonable manipulative skill. Alas, too often one hears today of students unable to perform a simple recrystallisation with any real competence.

But time does not stand still, and although Dr. Openshaw's book has served the chemical community well, there will be too few young people interested in purchasing this little book, even at the lower paperback price, simply because the subject is no longer a formal part of their teaching syllabus. To those more discriminating individuals, this book is still good chemical value, provided its present-day limitations are accepted.

W. I. STEPHEN

PRACTICAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. Edited by C. F. SIMPSON. Pp. x + 315. London and Rheine: Heyden & Son. 1976. Price $18.50; £9.30; DM 59.50.

During the last 4 years, at least six books have appeared that deal with various aspects of high-performance liquid chromatography (HPLC). Hence any new books on this topic are likely to include much material that has been adequately covered previously. This is so with this book, but its emphasis on the practical aspects of HPLC does result in the inclusion of much material that is both novel and invaluable to the less experienced chromatographer.

The book is based on the lectures and practical sessions at the 1975 Chemical Society School on HPLC. The introductory third is devoted to the now well documented theoretical background to the technique. This part is followed by a section dealing with the various methods of separation, each chapter written by an expert in the respective method. These chapters are informative but the references quoted are rarely later than 1974, which means that some of the recent innovations in ion-pair and bonded-phase chromatography are not dealt with adequately. One chapter in this section deals with bioaffinity chromatography, which is a useful addition to a general treatise. The final section of the book concentrates on the practice of HPLC and includes a selection of detailed experiments. These experiments embrace a variety of problems that practising chromatographers have encountered and, no doubt, spent many hours solving in their own laboratories. They provide an excellent introduction to the field and should have value for those designing courses in HPLC.

The book is attractively presented and reasonably priced. I would recommend that all newcomers to the field of HPLC should read it.

BRIAN B. WHEALS

HIGH PRESSURE LIQUID CHROMATOGRAPHY IN CLINICAL CHEMISTRY. Edited by P. F. DIXON, C. H. GRAY, C. K. LIM and M. S. STOLL. *Proceedings of a Symposium held at Kings College Hospital Medical School, December* 15–16, 1975. Pp. xxvi + 224. London, New York and San Francisco: Academic Press. 1976. Price £4.80; $12.25.

This book is a collection of symposium papers with one general introductory paper by Professor J. H. Knox and two brief papers on techniques (moving-wire detector and trace analysis). Most of the book is devoted to papers on applications of HPLC to individual clinical chemical and bio-

chemical problems, with papers on amino-acids, lipids, oligosaccharides, steroids, porphyrins, bile pigments, nucleotides, biologically active amines and various classes of drugs. While generally useful and interesting, the quality of the papers is very variable, some being merely abstracts (surely not warranting inclusion) and others detailed, well written papers on the practical aspects of separation and quantitation, comparison with established methods where appropriate and examples of clinical applications.

The editors are over-optimistic and, in my opinion, misleading about the future of HPLC (surely high-*performance*, not pressure, to judge from some of the papers given) and its application in routine clinical chemistry. Comparing the technique with gas chromatography (GC), they state that GC "promised to be one of the most powerful tools for clinical investigation" but that "with the exception of a few specialised fields, the gas chromatograph is of secondary importance in most clinical laboratories." The first statement *has* certainly proved to be true especially when combined as GC - MS, perhaps more so outside the UK, and the latter fact is surely the fault of the (routine) clinical chemists themselves, with their general unwillingness to devote much time or dedicated personnel to more specialised techniques and analytical methods. This fact is borne out by the absence of the well established HPLC system, amino-acid analysis, from many routine clinical chemistry departments. For good results and trouble-free working, liquid chromatography requires considerably more experience and skill than does GC, and much better instrumentation will be required before the former competes adequately with the latter in many applications, particularly when coupled on-line with secondary identification systems such as mass spectrometers. Professor Knox gives a more balanced view of where progress has been made (in column technology), where it is still required (detectors, pumps and injection systems) and of some of the inherent problems of liquid chromatography in general, some of which are presently being "rediscovered." Professor Knox ends his paper on the optimistic note that virtually all compounds of interest will "yield to HPLC." However, is this really necessary or required? The real value of HPLC is in the study of those compounds which are not amenable to study by techniques such as GC because of their intractable involatility, and those which can be studied directly in biological fluids without the need for prior extraction or derivative preparation or for subsequent analysis of collected fractions. HPLC and GC, with other chromatographic techniques, are complementary and neither will be superior for all applications. It is unfortunately this message that the book and the editors in particular have failed to present, falling into their own trap of over-enthusiasm for the "new" technique. I feel that HPLC in routine or service clinical chemistry (as opposed to research clinical biochemistry) will, in the 10 years suggested by the editors, be very much in the position that GC finds itself today.

Overall, the production of the book is good, as are the illustrations, and the editors and publishers are to be congratulated on producing the book so quickly. The index is sub-divided but still useful. Some of the legends to the figures are unfortunately on pages separated from the figure to which they refer, and there are too many references to work in the press or in preparation, making it difficult to evaluate some papers. There is little introductory text and the book is most suitable for chemists and biochemists who are already familar with the technique. Although the low price brings the book within the scope of the individual, the work will probably be more suitable for inclusion on the library shelves of institutions and departments where there is already a working interest in HPLC.

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THE CHEMICAL SOCIETY

Rapid Enzymatic Determination of Lactose in Food Products Using Amperometric Measurement of the Rate of Depletion of Oxygen

An enzymatic method for the rapid and inexpensive measurement of free lactose in food products is described, based on the amperometric measurement of the rate of consumption of oxygen when lactose is hydrolysed by β-galactosidase, followed by oxidation of the product glucose in the presence of glucose oxidase and molecular oxygen. A Beckman glucose analyser with a Clark oxygen electrode is used for signal processing. Only minor pre-treatment of the sample, with no reaction incubation, is required. The measurement normally takes less than 5 min for samples that do not require extraction. The results are precise and accurate. Results for the analysis of milk and powdered milk samples are compared with those obtained by a phenol - sulphuric acid method.

Keywords: *Enzymatic analysis*; *electrochemistry*; *lactose determination*; *food*

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Department of Chemistry, University of Washington, Seattle, Wash. 98195, USA.

Analyst, 1977, **102**, 124–131.

Non-stoicheiometric Copper Sulphide Membrane Electrode

Short Paper

Keywords: *Copper sulphide membrane electrode*

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Analyst, 1977, **102**, 132–134.

Determination of Carbon in Iron and Steel by an Air-combustion Method

Short Paper

Keywords: *Carbon determination*; *air combustion*; *iron*; *steel*

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Analyst, 1977, **102**, 135–137.

Spectrophotometric Determination of Beryllium in Air by a Sensitised Chrome Azurol S Reaction

Short Paper

Keywords: *Beryllium determination*; *air*; *Chrome Azurol S*; *spectrophotometry*

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Analytical Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay-85, India.

Analyst, 1977, **102**, 137–139.

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All completed application forms must reach the Director of Personnel, Electricity Headquarters, 24/25 Marina—Lagos Nigeria not later than Friday, 11th March, 1977.

Improved Method for the Synthesis of 3-Propyl-5-hydroxy-5-D-*arabino*-tetrahydroxybutyl-3-thiazolidine-2-thione (PHTTT) and its Homologues, Specific Spectrophotometric Reagents for Copper

Short Paper

Keywords: *3-Propyl-5-hydroxy-5-*D-arabino-*tetrahydroxybutyl-3-thiazolidine-2-thione reagent*; *spectrophotometry*; *copper*

Y. M. CHOW, K. S. MAK and O. W. LAU

Department of Chemistry, Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

Analyst, 1977, **102**, 139–140.

Novel Sampling System for the Direct Analysis of Powders by Atomic-absorption Spectrometry

Communication

Keywords: *Powders*; *minerals*; *sampling*; *atomic-absorption spectrometry*

C. W. FULLER and I. THOMPSON

Tioxide International Limited, Stockton-on-Tees, Cleveland.

Analyst, 1977, **102**, 141–143.

Simple Technique for the Pyrolysis - Mass Spectrometry of Polymeric Materials

Communication

Keywords: *Pyrolysis - mass spectrometry*; *polymers*

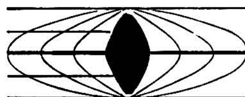
J. C. HUGHES, B. B. WHEALS and M. J. WHITEHOUSE

Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London, SE1 7LP.

Analyst, 1977, **102**, 143–144.

Annual Reports on Analytical Atomic Spectroscopy

VOLUME 5, 1975



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