The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly International Publication
dealing with all branches of
Analytical Chemistry

Published by the
SOCIETY FOR ANALYTICAL CHEMISTRY

Volume 97
No. 1157, Pages 585-680 **August, 1972**

ANALAO

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

OFFICES OF THE SOCIETY: 9/10 SAVILE ROW, LONDON, W1X 1AF

Telephone 01-734 9864

Advertisement Agent: J. Arthur Cook, 9 Lloyd Square, London, WC1X 9BA.
Telephone 01-837 6315

Published by The Society for Analytical Chemistry
Distributed by The Chemical Society, Publications Sales Office,
Blackhorse Road, Letchworth, Herts., SG6 1HN

Volume 97, No. 1157

August, 1972

Summaries of Papers in this Issue

The Influence of Diffusion on the Determination of Particle-size Distribution by Gravitational and Centrifugal Sedimentation

In previous papers expressions for the influence of diffusion on gravitational sedimentation were calculated for products with characteristics $[C(k)]^n = ak$ for $n = \frac{1}{2}$, 1 and 2, and $a = 5$, and the results were, to a certain extent, generalised.

In the present paper the expressions for the arbitrary distributions are extended to cover all constant fields, and they may also be applied with sufficient accuracy to centrifugal sedimentation when the average acceleration in the sedimentation vessel is the same as the acceleration in the constant field.

Further, the expressions for heterodisperse products of arbitrary distributions are extended to cover very fine products and products for which the difference between the density of the particles and that of the suspending medium approaches zero.

Finally, the influence of diffusion by the examination of monodisperse products is calculated, and agreement is shown with the author's earlier papers dealing with these problems.

SØREN BERG

Nybrovej 401 (Sophienholm), DK-2800 Lyngby, Denmark.

Analyst, 1972, **97**, 585–600.

The Determination of Trace (Parts per 10^9) Amounts of Gold in Plants by Non-destructive Activation Analysis

A non-destructive activation analysis method for the routine determination of trace amounts (parts per 10^9) of gold in the ashes of various plant materials, grass and maize has been developed. The homogeneity of the gold distribution in the samples is of particular importance and several different methods of sample preparation have been studied. The lower limit for the determination of gold is below 1 part per 10^9, calculated for dried plant material. The method was developed for a geochemical research programme and permits the determination of submicrogram amounts of gold in plants to be carried out more simply than do techniques that involve chemical separation.

P. SCHILLER, G. B. COOK, A. KITZINGER and E. WÖLFL

I.A.E.A. Laboratory, A-2444 Seibersdorf (near Vienna), Austria.

Analyst, 1972, **97**, 601–604.

Determination of Thorium in Bovine Bone by Neutron-activation Analysis

Thorium has been determined in bovine bone by neutron-activation analysis. A radiochemical procedure is described in which protactinium-233 resulting from the reaction

$$^{232}\mathrm{Th}(\mathrm{n},\gamma)^{233}\mathrm{Th} \xrightarrow{\beta \text{ decay}} {}^{233}\mathrm{Pa}$$

is extracted with trioctylamine in xylene and subsequently back-extracted with distilled water, following collection of the thorium in the bone by Korkisch's anion-exchange method and irradiation. The protactinium-233 activity is measured by gamma-ray spectrometry.

The precision of the method is better than ± 15 per cent. for samples with a thorium content exceeding 0·0037 p.p.m.

S. OHNO and T. ICHIKAWA

National Institute of Radiological Sciences, 9–1, 4-chome, Anagawa, Chiba-shi, Japan.

Analyst, 1972, **97**, 605–608.

THE ANALYST

NOTICE TO SUBSCRIBERS

(other than Members of the Society)

Subscriptions for *The Analyst*, *Analytical Abstracts* and *Proceedings* should be sent to:

The Chemical Society, Publications Sales Office,
Blackhorse Road, Letchworth, Herts.

Rates for 1972

(a)	*The Analyst*, *Analytical Abstracts*, and *Proceedings*, with indexes	£33·50
(b)	*The Analyst*, *Analytical Abstracts* printed on one side of the paper (without index), and *Proceedings*	£34·50
(c)	*The Analyst*, *Analytical Abstracts* printed on one side of the paper (with index), and *Proceedings*	£40·50
	The Analyst *and* Analytical Abstracts *without* Proceedings—	
(d)	*The Analyst* and *Analytical Abstracts*, with indexes	£31·00
(e)	*The Analyst*, and *Analytical Abstracts* printed on one side of the paper (without index)	£32·00
(f)	*The Analyst*, and *Analytical Abstracts* printed on one side of the paper (with index)	£38·00

(Subscriptions are NOT accepted for *The Analyst* and/or for *Proceedings* alone)

Identification of Species of Heat-sterilised Canned Fish by Polyacrylamide-disc Electrophoresis

The heat-denatured proteins of canned fish obtained by treating the fish with cyanogen bromide yield polypeptides that are soluble in 6 M urea solution. These fragments of proteins, which have been cleaved selectively at the methionyl peptide bond, can be separated by electrophoresis in 6 M urea solution. The separation patterns for canned herring, tuna, plaice, salmon, lemon sole, sprat, haddock and mackerel are presented. The method extends the electrophoretic method of species identification to include canned fish.

I. M. MACKIE and T. TAYLOR

Ministry of Agriculture, Fisheries and Food, Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, AB9 8DG.

Analyst, 1972, **97**, 609-611.

The Determination of Sulphur in Lubricating Oil Fractions and in Fuel Oils—A Coulometric Method

The original combustion - microcoulometric method in which the Dohrmann microcoulometer is used for the determination of trace amounts of sulphur in organic liquids has been modified. The inlet to the microcoulometer has been re-designed to permit complete combustion of oils to take place in the inlet. The modified inlet and flow systems have been used for the determination of sulphur in oil fractions obtained from preparative layer chromatography and in fuel oils.

JEAN P. DIXON

Shell Research Ltd., Thornton Research Centre, P.O. Box No. 1, Chester, CH1 3SH.

Analyst, 1972, **97**, 612-619.

The Effect of Infrared Radiation on the Potentiometric Behaviour of Gold-wire Electrodes

Gold-wire and infrared-irradiated gold-wire electrodes have been used in potentiometric titrations in conjunction with the saturated calomel electrode or molybdenum-wire reference electrode. The step heights obtained in pH titrations by using the gold electrode are less than those obtained with other pH electrodes. In iron(II) - potassium dichromate titrations, the performance of the gold electrode is comparable with that of the platinum electrode, whereas in iron(II) - potassium permanganate titrations, a double step is obtained with reductant and oxidant solutions of 10^{-5} and 10^{-4} N, respectively.

When the infrared-irradiated electrode is used in pH titrations, the curve height increases by 200 mV. A similar increase is also obtained for iron(II) - potassium dichromate titrations, but not for iron(II) - potassium permanganate titrations. However, the asymmetry in the permanganate titration vanishes, giving rise to a single curve. The reasons for this behaviour are discussed.

M. R. DHANESHWAR and R. G. DHANESHWAR

Analytical Division, Bhabha Atomic Research Centre, Modular Laboratories, Trombay, Bombay-85, A.S., India.

Analyst, 1972, **97**, 620-625.

The Determination of Ammonia in Condensed Steam and Boiler Feed-water with a Potentiometric Ammonia Probe

An electrochemical probe, based on the diffusion of gas across a polymer membrane, has been investigated for possible use in determining ammonia in condensed steam and boiler feed-water. A simple method that gives results of adequate precision and accuracy has been developed; the standard deviations at concentrations of 1·0, 0·5 and 0·1 μg ml^{-1} were 0·05, 0·01 and 0·005 μg ml^{-1}, respectively. Results obtained by the method described and by the ammonium-sensitive glass electrode method differed by a maximum of 6 per cent. and an average of only 0·3 per cent. Of the substances to be expected in feed-water, only cyclohexylamine and octadecylamine had a significant effect. For routine purposes this method is preferred to the more precise, but more time consuming, indophenol blue method. The principal advantage of this probe over the ammonium-sensitive glass electrode is that no correction is required in the presence of alkali metal ions.

D. MIDGLEY and K. TORRANCE

Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey.

Analyst, 1972, **97**, 626–633.

Potentiometric Determination of *p*-Urazine with an Ion-selective Electrode

The behaviour of *p*-urazine in 1 M sodium hydroxide solution when silver nitrate solution is added has been interpreted on the basis of potentiometric and spectroscopic measurements and elemental analysis. A potentiometric method has been developed for the quantitative determination of *p*-urazine.

V. P. IZVEKOV, M. KUCSERA-PÁPAY, K. TÓTH and E. PUNGOR

Institute for General and Analytical Chemistry, Technical University, Budapest, Hungary.

Analyst, 1972, **97**, 634–638.

The Assay of Potassium 6-[L-(+)-α-Phenoxypropionamido]-penicillanate and 6-[D-(+)-α-Phenoxypropionamido]penicillanate in Phenethicillin Potassium by Circular Dichroism Spectrometry

Circular dichroism spectra of potassium 6-[L-(+)-α-phenoxypropionamido]penicillanate and 6-[D-(+)-α-phenoxypropionamido]penicillanate have been recorded, and form the basis of an assay procedure with which to determine the proportions of these two isomers in Phenethicillin Potassium. Eight commercial samples of Phenethicillin Potassium have been assayed by this method and the results are compared with those obtained by microbiological assay.

J. B. STENLAKE, G. C. WOOD, H. C. MITAL and SHEENA STEWART

Department of Pharmaceutical Chemistry, University of Strathclyde, George Street, Glasgow, G1 1XW.

Analyst, 1972, **97**, 639–643.

Determination of Riboflavine in Injections Containing Liver Extract

Interference by the pigments of liver extract in the spectrophotometric determination of riboflavine by the method of the British Pharmacopoeia 1968 is obviated by chromatography on a column of activated acidic alumina with the mixture chloroform - ethanol (98 per cent.) - glacial acetic acid (50 + 50 + 0·3 v/v); riboflavine passes through the column while liver pigments are retained. The eluate is concentrated by evaporation, buffered at pH 4, then measured spectrophotometrically at wavelength 444 nm.

SAAD A. ISMAIEL

Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El Mataria St., Post El Zeitoun, Cairo, Egypt.

Analyst, 1972, **97**, 644–646.

THE ANALYST

The Influence of Diffusion on the Determination of Particle-size Distribution by Gravitational and Centrifugal Sedimentation

BY SØREN BERG

(*Nybrovej* 401 (*Sophienholm*), *DK*-2800 *Lyngby*, *Denmark*)

In previous papers expressions for the influence of diffusion on gravitational sedimentation were calculated for products with characteristics $[C(k)]^n = ak$ for $n = \frac{1}{2}$, 1 and 2, and a = 5, and the results were, to a certain extent, generalised.

In the present paper the expressions for the arbitrary distributions are extended to cover all constant fields, and they may also be applied with sufficient accuracy to centrifugal sedimentation when the average acceleration in the sedimentation vessel is the same as the acceleration in the constant field.

Further, the expressions for heterodisperse products of arbitrary distributions are extended to cover very fine products and products for which the difference between the density of the particles and that of the suspending medium approaches zero.

Finally, the influence of diffusion by the examination of monodisperse products is calculated, and agreement is shown with the author's earlier papers dealing with these problems.

IN earlier papers[1–3] I calculated the influence of diffusion by gravitational sedimentation on suspensions of products of different arbitrary distributions. In this paper these calculations are summarised as a basis for analogous calculations concerning centrifugal sedimentation, for which the results of these previously performed, difficult and time-consuming calculations and numerical integrations are used.

The equilibrium condition for sedimentation of monodisperse suspensions by gravity (see Symbols used, p. 599) is—

$$6\pi r\eta v = \frac{4}{3}\pi r^3 (\rho_k - \rho_f)g - \frac{RT\delta \ln c_{h,t}}{N\delta h} \quad .. \quad .. \quad .. \quad (1)$$

Solving equation (1) for v gives

$$v = \frac{2r^2 (\rho_k - \rho_f)g}{9\eta} - \left(\frac{RT}{6\pi r\eta N c_{h,t}} \times \frac{\delta c_{h,t}}{\delta h}\right)$$

Through a cross-section of area of 1 cm² at a depth h will pass per unit of time (s)

$$vc_{h,t} = \frac{2r^2 (\rho_k - \rho_f)gc_{h,t}}{9\eta} - \frac{RT\delta c_{h,t}}{6\pi r\eta N\delta h}$$

grams of particulate matter.

Through a cross-section at the depth $h + \Delta h$ there will pass

$$vc_{h,t} + \Delta(vc_{h,t})$$

grams of particulate matter.

Therefore, the concentration in the layer bounded by the horizontal sections increases by

$$-\frac{\delta(vc_{h,t})}{\delta h}$$

per unit of time as Δh approaches zero. Hence—

$$\frac{\delta c_{h,t}}{\delta t} = -\frac{\delta(vc_{h,t})}{\delta h}$$

and therefore

$$\frac{\delta c_{h,t}}{\delta t} = \left(\frac{RT}{6\pi r\eta N} \times \frac{\delta^2 c_{h,t}}{\delta h^2}\right) - \left[\frac{2r^2 (\rho_k - \rho_f)g}{9\eta} \times \frac{\delta c_{h,t}}{\delta h}\right]$$

where

$$\frac{RT}{6\pi r\eta N} = \frac{\left(\frac{4\pi}{3}\right)^{\frac{1}{3}} RT}{6\pi k\eta N} = \frac{m}{k} = D$$

m being a constant and D the diffusion coefficient.

If

$$\frac{2r^2(\rho_k - \rho_t)g}{9\eta} = \frac{2k^2(\rho_k - \rho_t)g}{9\eta\left(\frac{16\pi^2}{9}\right)^{\frac{1}{3}}} = i(\rho_k - \rho_t)k^2 = jk^2 = iuk^2 = B$$

where i, j, u and B are constants, then

$$\frac{\delta c_{h,t}}{\delta t} = D\frac{\delta^2 c_{h,t}}{\delta h^2} - B\frac{\delta c_{h,t}}{\delta h} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

and

$$vc_{h,t} = B\,c_{h,t} - D\frac{\delta c_{h,t}}{\delta h}$$

are obtained.

In a layer bounded by the upper surface and a plane at depth h, the increase in concentration per unit of time is determined solely, if h is sufficiently small, by the amount of material passing the plane at the depth h. Therefore

$$\lim_{h\to 0}\frac{\delta c_{h,t}}{\delta t} = \lim_{h\to 0}\left(\frac{-vc_{h,t}}{h}\right)$$

and it follows that

$$\lim_{h\to 0}\left(\frac{h\delta c_{h,t}}{\delta t}\right) = \lim_{h\to 0}(-vc_{h,t})$$

from which

$$vc_{0,t} = 0$$

Hence

$$D\frac{\delta c_{h,t}}{\delta h} - Bc_{h,t} = 0 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

when $h = 0$.

From analogous considerations it can be seen that

$$D\frac{\delta c_{h,t}}{\delta h} - Bc_{h,t} = 0 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

for $h = l$, where l is the depth of the vessel.

The problem, which has been solved by Mason and Weaver,[4] was to find a solution of equation (2) that could satisfy the conditions (3) and (4).

By introducing

$$\frac{D}{Bl} = \frac{m}{il(\rho_k - \rho_t)k^3} = \frac{m}{jlk^3} = \alpha$$

$$\frac{h}{l} = y$$

$$\frac{Bt}{l} = \frac{it(\rho_k - \rho_t)k^2}{l} = \frac{jtk^2}{l} = t'$$

and assuming that one starts with a homogeneous suspension of concentration $c_{.,0}$, Mason and Weaver's general solution was

$$\frac{c_{h,t}}{c_{.,0}} = F(\alpha, y, t')$$

$$= \frac{e^{\frac{y}{\alpha}}}{\alpha\,(e^{\frac{1}{\alpha}}-1)} + 16\alpha^2\pi e^{\frac{2y-t'}{4\alpha}} \sum_{m=1}^{\infty} \frac{e^{-\alpha m^2\pi^2 t'}\, m\left[1-(-1)^m e^{-\frac{1}{2\alpha}}\right](\sin m\pi y + 2\pi m\alpha \cos m\pi y)}{(1+4\pi^2 m^2 \alpha^2)^2} \quad (5)$$

When the sedimentation vessel can be considered to extend downwards without limit, the equation reduces to

$$\frac{c_{h,t}}{c_{.,0}} = G(k,h,t) = -\frac{B\sqrt{t}}{\sqrt{D\pi}} e^{-\frac{(Bt-h)^2}{4Dt}} + \tfrac{1}{2}\left[1-\Phi\left(\frac{Bt-h}{\sqrt{4Dt}}\right)\right] + \tfrac{1}{2}e^{\frac{Bh}{D}}\left[1+\frac{B}{D}(Bt+h)\right]\left[1-\Phi\left(\frac{Bt+h}{\sqrt{4Dt}}\right)\right] \quad .. \quad .. \quad (6)$$

where $\Phi\left(\frac{Bt-h}{\sqrt{4Dt}}\right)$ and $\Phi\left(\frac{Bt+h}{\sqrt{4Dt}}\right)$ signify probability integrals whose arguments are $\frac{Bt-h}{\sqrt{4Dt}}$ and $\frac{Bt+h}{\sqrt{4Dt}}$, respectively.

Equation (6) remains true provided that the concentration in the lower part of the vessel remains constant and equal to the original concentration.

Graphs of the function $G(k,h,t)$ were calculated for products of density 2·5 g cm^{-3} suspended in water at 20 °C for such values of t that $k_{h,t} = b\sqrt{\frac{h}{tu}}$ (for $h = 2$ cm) has values as follows: 0·15, 0·10, 0·07, 0·05, 0·035 and 0·025 μm. These results imply that particles of the given size at their respective sedimentation times would have fallen 2 cm if no diffusion had occurred.

In Table I the calculated values of $G(k,h,t)$ are given and, for comparison, values of the function $F(\alpha,y,t')$ calculated on the assumption that $y = 0{\cdot}3$, *i.e.*, that the depth l of the vessel is about 7 cm, for $k_{h,t} = 0{\cdot}035$ μm and 0·025 μm. In this way an indication can be obtained of how long a sedimentation time can be allowed if the concentration is to be calculated from the function $G(k,h,t)$, as 7 cm is a convenient depth for the sedimentation vessel. Graphs with calculated values of $G(k,h,t)$ as ordinate and k as abscissa are shown in Fig. 1. In such graphs k can be used as a measure for h because $k = b\sqrt{\frac{h}{tu}}$. Fig. 1 thus shows the distribution of concentration as functions of h for the particle sizes and times indicated in Table I.

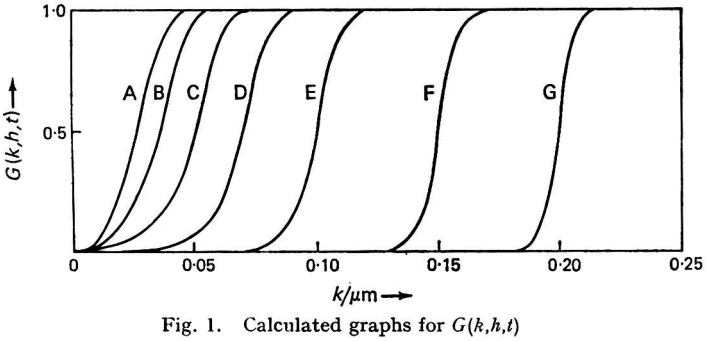


Fig. 1. Calculated graphs for $G(k,h,t)$

Curve	$k_{h,t}$/μm	t/minutes
A	0·025	433 000
B	0·035	221 000
C	0·05	108 000
D	0·07	55 200
E	0·10	27 000
F	0·15	12 000
G	0·20	6760

Suspensions of particles of unequal size are next considered. The assumption that one can calculate the characteristic of a material from the concentrations $c_{h,t}$, determined by the pipette or the diver method, requires the equation

$$C(k_{h,t}) = C\left(b\sqrt{\frac{h}{tu}}\right) \approx \frac{c_{h,t}}{c_{.,0}}$$

to hold with sufficient accuracy that a random error of about 2 per cent. is not exceeded. If this condition is fulfilled, then

$$\frac{c_{h,t}}{c_{.,0}} = \int_0^{k_{max.}} F(\alpha,y,t')V(k)\mathrm{d}k \qquad (7)$$

where $V(k)$ is the distribution $\frac{\mathrm{d}C(k)}{\mathrm{d}k}$. If $C\left(b\sqrt{\frac{h}{tu}}\right) - \frac{c_{h,t}}{c_{.,0}}$ is symbolised by $\Delta C(k_{h,t})$, it follows that

$$\Delta C(k_{h,t}) = C\left(b\sqrt{\frac{h}{tu}}\right) - \int_0^{k_{max.}} F(\alpha,y,t')V(k)\mathrm{d}k \qquad (8)$$

where $\Delta C(k_{h,t})$ must not exceed 2 per cent. of $c_{.,0}$.

The value $\Delta C(k_{h,t})$ can be calculated if the characteristic and therefore the distribution curve $V(k)$ are known. Assuming arbitrarily that the characteristic of the material satisfies the following equation, where n and a are constants,

$$[C(k)]^n = \mathrm{a}k$$

we obtain

$$n[C(k)]^{n-1}\mathrm{d}C(k) = \mathrm{a}\mathrm{d}k$$

and

$$V(k) = \frac{\mathrm{d}C(k)}{\mathrm{d}k} = \frac{1}{n}\mathrm{a}^{\frac{1}{n}}k^{\frac{1-n}{n}}$$

In this instance, therefore,

$$\Delta C(k_{h,t}) = \mathrm{a}^{\frac{1}{n}}\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}} - \int_0^{k_{max.}} F(\alpha,y,t')\frac{1}{n}\mathrm{a}^{\frac{1}{n}}k^{\frac{1-n}{n}}\,\mathrm{d}k$$

$$= \frac{\mathrm{a}^{\frac{1}{n}}}{n}\left[n\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}} - \int_0^{k_{max.}} F(\alpha,y,t')k^{\frac{1-n}{n}}\mathrm{d}k\right] \qquad (9)$$

When the depth of the vessel is so great that

$$F(\alpha,y,t') \approx G(k,h,t)$$

we obtain

$$\Delta C(k_{h,t}) \approx \frac{\mathrm{a}^{\frac{1}{n}}}{n}\left[n\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}} - \int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\mathrm{d}k\right] \qquad (10)$$

By using the calculated curves for $G(k,h,t)$, the quantity

$$\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,\mathrm{d}k$$

is determined by numerical integration for $n = 0{\cdot}5$, $n = 1{\cdot}0$ and $n = 2{\cdot}0$, *i.e.*, for those instances where the characteristic satisfies the equations $[C(k)]^{\frac{1}{2}} = ak$, $C(k) = ak$ and $[C(k)]^2 = ak$, respectively, with the same experimental conditions as in Table I and Fig. 1.

In Table II (*a*) the sedimentation time in minutes is recorded, together with the values of the functions

$$k_{h,t} = b\sqrt{\frac{h}{tu}},\quad n\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}},\quad \int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk \quad \text{and} \quad \frac{n\Delta C(k_{h,t})}{a^{\frac{1}{n}}}$$

for $n = 0{\cdot}5$, as well as

$$a^{\frac{1}{n}}\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}},\quad \frac{a^{\frac{1}{n}}}{n}\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk \quad \text{and} \quad \Delta C(k_{h,t})$$

in per cent. for $n = 0{\cdot}5$ and $a = 5$. In Tables II (*b*) and (*c*) corresponding values are given for $n = 1$ and $n = 2$, respectively.

The value of $\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk$ was calculated by using Simpson's formula; the uncertainty apparently does not exceed 2 to 3 figures in the last decimal place. Consequently, the uncertainty of $\Delta C(k_{h,t})$ can be evaluated as being 1 to 2 figures in the last decimal place in Tables II (*a*) and (*b*), and 2 to 3 figures in Table II (*c*).

It can be seen from the tables that the calculated systematic errors caused by diffusion will not exceed 1·8 (1·5 *plus* 0·3) per cent. of $c_{.,0}$ for any of the instances given in references 1 (p. 62) and 2 (p. 206).

In Fig. 2 the characteristics corresponding to the special instances when $C(k)$ satisfies the equations $[C(k)]^{\frac{1}{2}} = 5k$, $C(k) = 5k$ and $[C(k)]^2 = 5k$ are plotted.

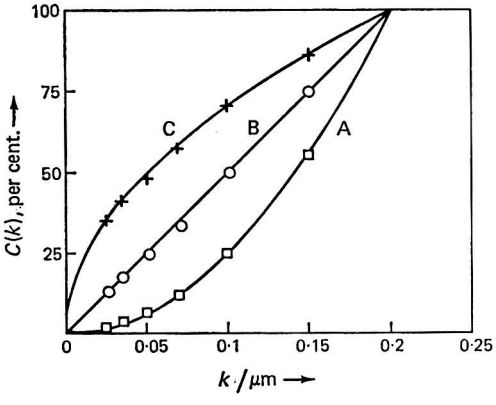


Fig. 2. Graphs of characteristics for particular values of $C(k)$. Curve A, $[C(k)]^{\frac{1}{2}} = 5k$; curve B, $C(k) = 5k$; and curve C, $[C(k)]^2 = 5k$. The marked points in the figure correspond to calculated values of the function

$$\frac{5^{\frac{1}{n}}}{n}\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk$$

□, $n = \frac{1}{2}$; O, $n = 1$; ×, $n = 2$. The small deviations of these points from the corresponding lines are due to the systematic error caused by diffusion

TABLE I

CALCULATED VALUES OF $G(k,h,t)$ AND $F(\alpha,y,t')$ FOR $\rho_k - \rho_f = 1{\cdot}5$ g cm^{-3}, $\eta = 0{\cdot}0102$ POISE, TEMPERATURE = 20 °C AND SUCH VALUES OF t THAT $k_{h,t}$ for $h = 2$ cm ASSUMES THE INDICATED VALUES OF $k_{h,t}$

	$G(k,h,t)$						$F(\alpha,y,t')$	
k/μm	t = 12 000 minutes $k_{h,t}$ = 0·15 μm	t = 27 000 minutes $k_{h,t}$ = 0·10 μm	t = 55 200 minutes $k_{h,t}$ = 0·07 μm	t = 108 000 minutes $k_{h,t}$ = 0·05 μm	t = 221 000 minutes $k_{h,t}$ = 0·035 μm	t = 433 000 minutes $k_{h,t}$ = 0·025 μm	t = 221 000 minutes $k_{h,t}$=0·035μm	t = 433 000 minutes $k_{h,t}$=0·025μm
0·17	1·000	—	—	—	—	—	—	—
0·16	0·945	—	—	—	—	—	—	—
0·15	0·491	—	—	—	—	—	—	—
0·14	0·083	—	—	—	—	—	—	—
0·13	0·005	—	—	—	—	—	—	—
0·12	—	0·9983	—	—	—	—	—	—
0·11	—	0·9096	—	—	—	—	—	—
0·10	—	0·4990	1·0000	—	—	—	—	—
0·09	—	0·1351	0·9959	—	—	—	—	—
0·08	—	0·0242	0·8800	1·0000	—	—	—	—
0·07	—	0·0043	0·4979	0·9933	—	—	—	—
0·06	—	—	0·1831	0·8521	—	—	—	—
0·055	—	—	—	—	0·9911	1·0000	—	—
0·050	—	—	0·0604	0·489	0·9462	0·9995	—	—
0·045	—	—	—	—	0·830	0·991	—	—
0·040	—	—	0·024	0·2150	0·655	0·941	0·655	0·941
0·035	—	—	—	—	0·4728	0·8127	0·473	0·812
0·030	—	—	0·0130	0·0932	0·3207	0·6241	0·320	0·619
0·025	—	—	—	—	0·2087	0·4305	0·207	0·417
0·020	—	—	—	0·0427	0·1296	0·2685	0·126	0·244
0·015	—	—	—	—	0·0732	0·1467	0·068	0·119
0·010	—	—	—	0·0141	0·0332	0·0623	0·029	0·041
0·005	—	—	—	—	0·0080	0·0136	—	—

TABLE II

VALUES FOR VARIOUS FUNCTIONS WITH SEDIMENTATION TIMES

Sedimentation time (t)/ minutes	$k_{h,t} = b\sqrt{\frac{h}{tu}}$ for h = 2 cm	$n\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}}$	$\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk$	$\frac{n\Delta C(k_{h,t})}{a^{\frac{1}{n}}}$	$a^{\frac{1}{n}}\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}}$, per cent. (a = 5)	$\frac{a^{\frac{1}{n}}}{n}\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk$, per cent. (a=5)	$\Delta C(k_{h,t})$, per cent. (a = 5)
(*a*) $n = \frac{1}{2}$, $[C(k)]^{\frac{1}{2}} = ak$							
12 000	0·1500	0·011 25	0·0113	0·0000	56·3	56·5	0·0
27 000	0·1000	0·005 00	0·004 96	0·000 04	25·0	24·8	0·2
55 200	0·0700	0·002 45	0·002 41	0·000 04	12·3	12·1	0·2
108 000	0·0500	0·001 25	0·001 24	0·000 01	6·25	6·15	0·1
221 000	0·0350	0·000 613	0·000 648	−0·000 035	3·06	3·24	−0·18
433 000	0·0250	0·000 313	0·000 385	−0·000 072	1·56	1·92	−0·36
(*b*) $n = 1$, $C(k) = ak$							
12 000	0·1500	0·1500	0·150	0·000	75·0	75·0	0·0
27 000	0·1000	0·1000	0·0992	0·0008	50·0	49·6	0·4
55 200	0·0700	0·0700	0·0685	0·0015	35·0	34·2	0·8
108 000	0·0500	0·0500	0·0481	0·0019	25·0	24·0	1·0
221 000	0·0350	0·0350	0·0342	0·0008	17·5	17·1	0·4
433 000	0·0250	0·0250	0·0260	−0·0010	12·5	13·0	−0·5
(*c*) $n = 2$, $[C(k)]^2 = ak$							
12 000	0·1500	0·775	0·774	0·001	86·6	86·5	0·0
27 000	0·1000	0·632	0·629	0·003	70·7	70·4	0·3
55 200	0·0700	0·529	0·522	0·007	59·2	58·4	0·8
108 000	0·0500	0·447	0·434	0·013	50·0	48·5	1·5
221 000	0·0350	0·374	0·364	0·010	41·8	40·7	1·1
433 000	0·0250	0·316	0·317	−0·001	35·4	35·5	0·1

Points are also plotted in Fig. 2 that correspond to the calculated values of

$$\frac{5^{\frac{1}{n}}}{n}\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk$$

to show the systematic error.

Table I and Fig. 1 give results calculated for $u = \rho_k - \rho_f = 1{\cdot}5$ g cm^{-3} and for such values of t that $k_{h,t}$ for $h = 2$ cm assumes the indicated values, but for other values of h and u values of sedimentation time and particle size can be stipulated such that the graphs will give the concentration under the new conditions.

This fact has already been proved in my earlier papers[1,2] by consideration of the following—

$$F(\alpha,y,t') = F\left(\frac{D}{Bl},\frac{h}{l},\frac{Bt}{l}\right) \quad .. \quad .. \quad .. \quad .. \quad (11)$$

and in the case $F'y(\alpha,y,t')$ is approximately zero and $F(\alpha,y,t')$ at the same time is nearly unity when $y_1 \leqslant y \leqslant y_2$ and $l = l_1$. Hence the values of $F(\alpha,y,t')$ for $y < y_1$ must be independent of l, provided that $l > l_1$. It follows, therefore, that for $y < y_1$ and $l > l_1$,

$$F\left(\frac{D}{Bl},\frac{h}{l},\frac{Bt}{l}\right) \approx F\left(\frac{D}{Bl_1},\frac{h}{l_1},\frac{Bt}{l_1}\right) \quad .. \quad .. \quad .. \quad .. \quad (12)$$

Therefore,

$$F(\alpha,y,t') \approx F(\alpha_1,y_1,t'_1)$$

when the following condition is satisfied—

$$\frac{\alpha}{\alpha_1} = \frac{y}{y_1} = \frac{t'}{t'_1}$$

This is identical with

$$\frac{D}{B}\times\frac{B_1}{D_1} = \frac{h}{h_1} = \frac{Bt}{B_1t_1} \quad .. \quad .. \quad .. \quad .. \quad (13)$$

As $B = iuk^2$ and $D = \frac{m}{k}$, and $B_1 = iu_1k_1^2$ and $D_1 = \frac{m}{k_1}$, it follows that

$$\frac{k_1^3u_1}{k^3u} = \frac{h}{h_1} = \frac{uk^2t}{u_1k_1^2t_1}$$

$$k_1 = k\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\times\left(\frac{u}{u_1}\right)^{\frac{1}{3}} \quad .. \quad .. \quad .. \quad .. \quad (14)$$

$$t_1 = t\left(\frac{h_1}{h}\right)^{\frac{5}{3}}\times\left(\frac{u}{u_1}\right)^{\frac{1}{3}} \quad .. \quad .. \quad .. \quad .. \quad (15)$$

For $l = \infty$, $F(\alpha,y,t') = G(k,h,t)$, and therefore

$$G(k,h,t) = G(k_1,h_1,t_1) \quad .. \quad .. \quad .. \quad .. \quad (16)$$

The correctness of this equation can also be shown by combining $B_1 = iu_1k_1^2$, $D_1 = \frac{m}{k_1}$, as well as equations (14) and (15), with equation (6). We obtain—

$$\frac{B_1\sqrt{t_1}}{\sqrt{D_1}} = \frac{iu_1k_1^{\frac{5}{2}}t_1^{\frac{1}{2}}}{m^{\frac{1}{2}}} = \frac{B\sqrt{t}}{\sqrt{D}}$$

$$B_1t_1 = iu_1k_1^2\,t_1 = \frac{Bt}{h}\times h_1$$

$$B_1t_1 \pm h_1 = Bt\frac{h_1}{h} \pm h_1 = (Bt \pm h)\frac{h_1}{h}$$

$$D_1 t_1 = \frac{\mathrm{m}}{k_1} \times t_1 = Dt\left(\frac{h_1}{h}\right)^2$$

$$\frac{\mathrm{B}_1 t_1 \pm h_1}{\sqrt{D_1 t_1}} = \frac{\mathrm{B}t \pm h}{\sqrt{Dt}}$$

$$\frac{\mathrm{B}_1 h_1}{D_1} = \frac{\mathrm{i u}_1 k_1^2 h_1}{\mathrm{m}} = \frac{\mathrm{B}h}{D}$$

$$\frac{\mathrm{B}_1}{D_1}(\mathrm{B}_1 t_1 + h_1) = \frac{\mathrm{i u}_1 k_1^3}{\mathrm{m}}(\mathrm{B}t + h)\frac{h_1}{h} = \frac{\mathrm{B}}{D}(\mathrm{B}t + h)$$

The numerical integrations were carried out on the assumption that $h = 2$ cm and $\rho_k - \rho_f = 1{\cdot}5$ g cm^{-3}. If the depth at which the measurements are made is h_1, the difference between the densities of the particles and the medium is u_1, and $[C(k)]^n = \mathrm{a}k$, it follows from equations (14), (15), (16) and (10), that

$$\Delta C(k_{\mathrm{h}_1,\mathrm{t}_1}) = \mathrm{a}^{\frac{1}{n}}\left[\left(b\sqrt{\frac{h_1}{\mathrm{u}_1}}\right)^{\frac{1}{n}} - \frac{1}{n}\int_0^{k_{\max.}} G(k_1,h_1,t_1)k_1^{\frac{1-n}{n}}\,\mathrm{d}k_1\right]$$

In order to express

$$\Delta C(k_{\mathrm{h}_1,\mathrm{t}_1}) \text{ by } \int_0^{k_{\max.}} G(k,h,t)k^{\frac{1-n}{n}}\,\mathrm{d}k$$

which is already known for certain values of n from the numerical integrations, we insert the values of k_1, t_1 and $G(k_1,h_1,t_1)$ and thus obtain:

$$\Delta C(k_{\mathrm{h}_1,\mathrm{t}_1}) = \mathrm{a}^{\frac{1}{n}}\left\{\left[b\sqrt{\frac{h_1}{t\left(\frac{h_1}{h}\right)^{\frac{5}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}\mathrm{u}_1}}\right]^{\frac{1}{n}} - \frac{1}{n}\int_0^{k_{\max.}} G(k,h,t)k^{\frac{1-n}{n}}\left[\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}\right]^{\frac{1-n}{n}} \times \mathrm{d}\left[\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}k\right]\right\}$$

$$= \mathrm{a}^{\frac{1}{n}}\left\{\left[\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}b\sqrt{\frac{h}{t\mathrm{u}}}\right]^{\frac{1}{n}} - \left[\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\frac{1}{n}\int_0^{k_{\max.}} G(k,h,t)k^{\frac{1-n}{n}}\,\mathrm{d}k\right\}$$

$$= \left[\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\frac{\mathrm{a}^{\frac{1}{n}}}{n}\left[n\left(b\sqrt{\frac{h}{t\mathrm{u}}}\right)^{\frac{1}{n}} - \int_0^{k_{\max.}} G(k,h,t)k^{\frac{1-n}{n}}\,\mathrm{d}k\right] \quad .. \quad .. \quad (17)$$

Therefore,

$$\Delta C(k_{\mathrm{h}_1,\mathrm{t}_1}) = \left[\left(\frac{h}{h_1}\right)^{\frac{1}{3}}\left(\frac{\mathrm{u}}{\mathrm{u}_1}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}} \times \Delta C(k_{\mathrm{h,t}}) \quad .. \quad .. \quad .. \quad (18)$$

For $h = 2$ cm and $\mathrm{u} = 1{\cdot}5$ g cm^{-3} we have

$$\Delta C(k_{\mathrm{h}_1,\mathrm{t}_1}) = \left[\left(\frac{2}{h_1}\right)^{\frac{1}{3}}\left(\frac{1{\cdot}5}{\mathrm{u}_1}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\Delta C(k_{\mathrm{h,t}}) \quad .. \quad .. \quad .. \quad (19)$$

and if the concentration is measured at depths h and h_1 in the same suspensions at times t and t_1, respectively,

$$\Delta C(k_{\mathrm{h}_1,\mathrm{t}_1}) = \left(\frac{h}{h_1}\right)^{\frac{1}{3n}}\Delta C(k_{\mathrm{h,t}}) \quad .. \quad .. \quad .. \quad .. \quad (20)$$

Therefore, for $h = 2$ cm,

$$\Delta C(k_{h_1,t_1}) = \left(\frac{2}{h_1}\right)^{\frac{1}{3n}} \Delta C(k_{h,t}) \quad .. \quad .. \quad .. \quad .. \quad (21)$$

These considerations have dealt exclusively with sedimentation in a constant field where the acceleration was that due to gravity ($g = 981$ cm s^{-2}). They will now be extended to include constant fields in general, including those with a higher acceleration qg. Later, the results obtained will be applied to centrifugal fields, in which the acceleration is proportional to the distance from the axis of revolution.

In situations when Stokes' law and the expression for the influence of diffusion are valid the equilibrium condition for a constant field with acceleration qg becomes

$$6\pi r\eta v = \frac{4}{3}\pi r^3 (\rho_k - \rho_f)qg - \frac{RT\delta lnc_{h,t}}{N\delta h}$$

Then the parameters in $F(\alpha,y,t')$ must be replaced by α_2, y_2 and t'_2, and we have

$$F(\alpha,y,t') = F(\alpha_2,y_2,t'_2)$$

when

$$\frac{\alpha}{\alpha_2} = \frac{y}{y_2} = \frac{t'}{t'_2}$$

These conditions are identical with

$$\frac{D}{B} \times \frac{B_2}{D_2} = \frac{h}{h_2} = \frac{Bt}{B_2 t_2}$$

because

$$B = iuk^2 \text{ and } D = \frac{m}{k}$$

and

$$B_2 = iu_2 q k_2^2 \text{ and } D_2 = \frac{m}{k_2}$$

Therefore,

$$\frac{qk_2^3 u_2}{k^3 u} = \frac{h}{h_2} = \frac{uk^2 t}{qu_2 k^2 t_2}$$

$$k_2 = \frac{k}{q^{\frac{1}{3}}} \times \left(\frac{h}{h_2}\right)^{\frac{1}{3}} \times \left(\frac{u}{u_2}\right)^{\frac{1}{3}} \quad .. \quad .. \quad .. \quad .. \quad (22)$$

$$t_2 = \frac{t}{q^{\frac{1}{3}}} \times \left(\frac{h_2}{h}\right)^{\frac{5}{3}} \times \left(\frac{u}{u_2}\right)^{\frac{1}{3}} \quad .. \quad .. \quad .. \quad .. \quad (23)$$

When $h_2 = h$ and $u_2 = u$, then

$$k_2 = \frac{k}{q^{\frac{1}{3}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (24)$$

and

$$t_2 = \frac{t}{q^{\frac{1}{3}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (25)$$

For $l = \infty$, $F(\alpha,y,t') = G(k,h,t)$ and therefore $G(k,h,t) = G(k_2,h_2,t_2)$ .. .. (26)

The validity of the above expressions can also be shown by combining $B_2 = iu_2 q k_2^2$ and $D_2 = \frac{m}{k_2}$, as well as equations (22) and (23), with equation (6).

According to considerations analogous to those leading to equation (17), remembering that $[C(k)]^n = ak$, and that b must be replaced by $b_2 = \frac{b}{q^{\frac{1}{2}}}$ as the acceleration is qg, we

obtain, from equations (22), (23), (26) and (10),

$$\Delta C(k_{h_1,t_1}) = a^{\frac{1}{n}}\left[\left(b_2\sqrt{\frac{h_2}{t_2u_2}}\right)^{\frac{1}{n}} - \frac{1}{n}\int_0^{k_{max.}} G(k_2,h_2,t_2)k_2^{\frac{1-n}{n}}\,dk_2\right] \quad .. \quad .. \quad (27)$$

In order to express $\Delta C(k_{h_2,t_2})$ in terms of the known integral, we insert the values of b_2, k_2, t_2 and $G(k_2,h_2,t_2) = G(k,h,t)$ into equation (27) and obtain

$$\Delta C(k_{h_2,t_2}) = a^{\frac{1}{n}}\left\{\left[\frac{b}{q^{\frac{1}{3}}}\sqrt{\frac{h_2}{\frac{t}{q^{\frac{1}{3}}}\left(\frac{h_2}{h}\right)^{\frac{5}{3}}\times\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\times u_2}}\right]^{\frac{1}{n}}\right.$$

$$\left. - \frac{1}{n}\int_0^{k_{max.}} G(k,h,t)\left[\frac{k}{q^{\frac{1}{3}}}\left(\frac{h}{h_2}\right)^{\frac{1}{3}}\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\right]^{\frac{1-n}{n}} d\left[\frac{k}{q^{\frac{1}{3}}}\left(\frac{h}{h_2}\right)^{\frac{1}{3}}\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\right]\right\}$$

$$= a^{\frac{1}{n}}\left\{\left(\frac{b}{q^{\frac{1}{3}}}\sqrt{\frac{q^{\frac{1}{3}}h^{\frac{5}{3}}}{th_2^{\frac{2}{3}}u^{\frac{1}{3}}u_2^{\frac{2}{3}}}}\right)^{\frac{1}{n}} - \frac{1}{n}\left[\frac{1}{q^{\frac{1}{3}}}\left(\frac{h}{h_2}\right)^{\frac{1}{3}}\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\times\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk\right\}$$

$$= \frac{a^{\frac{1}{n}}}{q^{\frac{1}{3n}}}\left\{\left(b\sqrt{\frac{h}{tu}}\sqrt{\frac{h^{\frac{2}{3}}}{h_2^{\frac{2}{3}}}\times\frac{u^{\frac{2}{3}}}{u_2^{\frac{2}{3}}}}\right)^{\frac{1}{n}} - \frac{1}{n}\left[\left(\frac{h}{h_2}\right)^{\frac{1}{3}}\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\int_0^{k_{max.}} G(k,h,t)k^{\frac{1-n}{n}}\,dk\right\}$$

$$= \frac{1}{q^{\frac{1}{3n}}}\left[\left(\frac{h}{h_2}\right)^{\frac{1}{3}}\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\times\frac{a^{\frac{1}{n}}}{n}\left[n\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}} - \int_0^{k_{max}} G(k,h,t)k^{\frac{1-n}{n}}\,dk\right] \quad .. \quad (28)$$

Therefore

$$\Delta C(k_{h_2,t_2}) = \frac{1}{q^{\frac{1}{3n}}}\left[\left(\frac{h}{h_2}\right)^{\frac{1}{3}}\left(\frac{u}{u_2}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\Delta C(k_{h,t}) \quad .. \quad .. \quad (29)$$

and for $h = 2$ cm and $u = 1{\cdot}5$ g cm^{-3} we have

$$\Delta C(k_{h_2,t_2}) = \frac{1}{q^{\frac{1}{3n}}}\left[\left(\frac{2}{h_2}\right)^{\frac{1}{3}}\left(\frac{1{\cdot}5}{u_2}\right)^{\frac{1}{3}}\right]^{\frac{1}{n}}\Delta C(k_{h,t}) \quad .. \quad .. \quad (30)$$

and for $h_2 = h$ and $u_2 = u$,

$$\Delta C(k_{h_2,t_2}) = \frac{\Delta C(k_{h,t})}{q^{\frac{1}{3n}}} \quad .. \quad .. \quad .. \quad .. \quad (31)$$

Equations (29), (30) and (31) are valid in constant fields with acceleration qg. In centrifugal fields the acceleration is $\omega^2 x$, and is therefore not constant. With the application of beaker centrifuges, conoidal or sector-shaped sedimentation vessels are to be preferred.[1,2] In a typical example the concentration might be determined at a depth of 2 cm, while the distance from the surface of the suspension to the axis of revolution and to the bottom of the vessel could be 12 and 5 cm, respectively.

Great relative accuracy is not necessary to calculate $\Delta C(k_{h_2,t_2})$. When the dimensions indicated are maintained (approximately), equations (29), (30) and (31) will be valid for calculating $\Delta C(k_{h_2,t_2})$ with sufficient relative accuracy provided that the mean acceleration within the sedimentation vessel is qg. Then

$$\omega^2 \times \frac{(12 + 14)}{2} = qg$$

where $\omega = \frac{\pi\nu}{30}$ and $g = 981$ cm s^{-2}. From this we obtain

$$q = \frac{13\pi^2\nu^2}{900 \times 981} = 0{\cdot}000145\ \nu^2 \quad .. \quad .. \quad .. \quad (32)$$

where ν is the number of revolutions per minute (r.p.m.). For further reliability one could ensure that ν is so large that the calculated value of $\Delta C(k_{h_s,t_s})$ is less than, for instance, 1 per cent. of $c_{.,0}$.

Influence of Diffusion on Heterodisperse Products of Arbitrary Distributions

From Fig. 2, Tables II (*a*), (*b*) and (*c*) and equations (18) and (21), it will be seen that the influence of diffusion on determination by gravitational sedimentation can in general be neglected. Thus, the determination of heterodisperse products with particle sizes down to 0·035 μm and of density 2·5 g cm^{-3} can be performed satisfactorily by gravitational sedimentation by using the diver or pipette method with a minimum of apparatus and manual effort.[1–3,5] However, the diver method requires long sedimentation periods, even when using spherical divers with a diameter of about 7 mm, although measurements can be taken in a depth as small as 10 mm.

The influence of diffusion is noticeable for products with a very small particle size so that centrifugal sedimentation must be applied in order to shorten the sedimentation periods. The procedure necessitates the carrying out of a determination by the diver or pipette method[1–3,5] in a beaker centrifuge, arranged as a thermostat and provided with conoidal sedimentation vessels.

Because of the short sedimentation periods, and in accordance with equations (29), (30) and (31), combined with equation (32), the influence of diffusion is much less noticeable with this method than with gravitational sedimentation, and can be neglected except when the product is extremely fine or the difference between the density of the particles and that of the suspension approaches zero. In such instances the following procedure should be followed. The determination is carried out by centrifugal sedimentation as usual, but as soon as a sufficient number of points on the graph of the characteristic have been determined, the graph is constructed and produced to cut the line at which the value of function $C(k)$ is equal to 100 per cent., by which the maximum particle size $k_{\mathrm{a\ max.}}$ is determined. The characteristic graph found is compared with the three graphs of characteristics given in Fig. 2 and the one is chosen that, by altering the scale of delineation of particle size, could be made to cover the newly drawn graph most accurately. The characteristic found will, by the calculation of $\Delta C(k_{h_s,t_s})$, be presupposed to fit the equation

$$[C(k)]^n = \mathrm{a}k$$

for a value of n that is identical with the value of n in the characteristic graph chosen from Fig. 2, and for the value of a determined by

$$[C(k_{\mathrm{a\ max.}})]^n = \mathrm{a}k_{\mathrm{a\ max.}} = 1$$

$$\mathrm{a} = \frac{1}{k_{\mathrm{a\ max.}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (33)$$

The numerical integrations given in Table II (*a*), (*b*) and (*c*) are performed for products with the characteristic $[C(k)]^n = 5k$.

Thus

$$\mathrm{a} = 5 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (34)$$

and

$$\frac{1}{k_{5\ \mathrm{max.}}} = \mathrm{a} = 5 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (35)$$

Combining equations (34) and (35) with equation (10), we obtain

$$\frac{\Delta C(k_{h,t})}{\frac{\left(\frac{1}{k_{5\ \mathrm{max.}}}\right)^{\frac{1}{n}}}{n}} = n\left(b\sqrt{\frac{h}{tu}}\right)^{\frac{1}{n}} - \int_0^{k_{\mathrm{max.}}} G(k,h,t)k^{\frac{1-n}{n}}\,\mathrm{d}k \quad .. \quad .. \quad .. \quad (36)$$

Combining equations (33) and (36) with equation (28), we obtain

$$\Delta C(k_{h_2,t_2}) = \left[\left(\frac{h}{h_2}\right)^{\frac{1}{3}} \left(\frac{u}{u_2}\right)^{\frac{1}{3}} \left(\frac{\frac{1}{k_{a\ max.}}}{\frac{1}{k_{5\ max.}} \times q^{\frac{1}{3}}} \right) \right]^{\frac{1}{n}} \Delta C(k_{h,t}) \quad .. \quad .. \quad (37)$$

where $\Delta C(k_{h,t})$ is given as a percentage of $c_{.,0}$ in Table II (*a*), (*b*) and (*c*) for a = 5 and $n = \frac{1}{2}$, 1 and 2; k_2 is given by equation (22), t_2 by equation (23) and q by equation (32).

Because, as previously stated, the uncertainties in the values of $\Delta C(k_{h,t})$ can be evaluated at 1 to 2 units in the last decimal place in Table II (*a*) and (*b*), and at 2 to 3 units in Table II(*c*), the calculated systematic errors due to diffusion will not exceed:

For Table II (*a*) ($n = \frac{1}{2}$)	.. ..	0·2 + 0·2 = 0·4 per cent. of $c_{.,0}$
For Table II (*b*) ($n = 1$)	.. ..	1·0 + 0·2 = 1·2 per cent. of $c_{.,0}$
For Table II (*c*) ($n = 2$)	.. ..	1·5 + 0·3 = 1·8 per cent. of $c_{.,0}$

When the particles and the suspending medium are of almost the same density, $\frac{u}{u_2}$ may be large.

When $h_2 = h$, and $u_2 = u$

$$\Delta C(k_{h_2,t_2}) = \left(\frac{\frac{1}{k_{a\ max.}}}{\frac{1}{k_{5\ max.}} \times q^{\frac{1}{3}}} \right)^{\frac{1}{n}} \Delta C(k_{h,t}) \quad .. \quad .. \quad .. \quad (38)$$

When $k_{a\ max.} = k_{5\ max.}$, we again obtain equation (31) and, by inserting $q = 1$ into equation (37), the latter can be used for calculating gravitational sedimentation.

If the values of $\Delta C(k_{h_2,t_2})$, calculated by means of equation (37) or (38), exceed, for instance, 0·5 per cent. of $c_{.,0}$, a new centrifugal experiment with either a higher speed, or a shorter sedimentation period, or both, must be performed.

A systematic error in $\Delta C(k_{h_2,t_2})$ can, of course, be introduced by the simplifying assumption concerning the particle-size distribution of the product examined.

NOTE—

When $C(k)$ approaches the 100 per cent. line slowly, it is better to use the particle size $k_{(50\ per\ cent.)}$, corresponding to $C(k) = \frac{1}{2}$, as the parameter. Further, by determining $C(k_{(80\ per\ cent.)})$ and $C(k_{(25\ per\ cent.)})$, and solving the equations $0{\cdot}8^n = a k_{(80\ per\ cent.)}$ and $0{\cdot}25^n = a k_{(25\ per\ cent.)}$ for n and a, analogous, but more complicated expressions could be deduced.

On the other hand, as previously stated, great relative accuracy is not necessary in calculating $\Delta C(k_{h_2,t_2})$, but the calculated values of $\Delta C(k_{h_2,t_2})$ should not exceed, for example, 0·5 per cent. of $c_{.,0}$. In instances when the centrifuge has other dimensions, or when greater accuracy is required, the number can be reduced to 0·25 per cent. of $c_{.,0}$ or less.

It must be emphasised that it is an important condition of obtaining correct results by this method that the material of the sample to be examined is divided into single particles before the analysis and that coagulation does not occur during the sedimentation. These factors can usually be ensured by adequate pre-treatment, and by application of an appropriate peptising agent.[1–3,5] The above calculations, as well as those following for monodisperse products, cover all particle sizes for which equation (1) is valid.

INFLUENCE OF DIFFUSION ON THE EXAMINATION OF MONODISPERSE PRODUCTS

For a monodisperse product the characteristic (the cumulative particle-size distribution) is represented by a vertical line. The influence of diffusion can be examined by the following considerations.

It is apparent that the $G(k,h,t)$ graphs in Fig. 1 are similar to the characteristics that would be found by examining the respective suspensions in the ordinary way by the pipette or diver method and by using the sedimentation times given in Table I. Further, it can be seen that the family of graphs for $F(\alpha,y,t')$, which is approximately equal to $G(k,h,t)$, tend towards the vertical to the abscissa $k_{h,t}$, and this tendency increases with increasing values of $k_{h,t}$. For $k_{h,t} = 0{\cdot}2\ \mu m$ the deviation between the graph and the vertical, which is the real characteristic of the product, can be neglected.

For the $G(k,h,t)$ graph in question, $t = 6760$ minutes, $k_{h,t} = 0{\cdot}2$ μm and the acceleration is 981 cm s^{-2}. It is in accordance with experience that the influence of diffusion on gravitational sedimentation begins to be significant for particle sizes smaller than 0·2 μm. If the acceleration is qg and equations (22) and (23) are satisfied, then $F(\alpha,y,t')$ and $G(k,h,t)$ will be unchanged.

Further, when $h_2 = h = 2$ cm and $u_2 = u = 1{\cdot}5$ g cm^{-3} equations (22) and (23) will be reduced to equations (24) and (25).

Introducing $k = 0{\cdot}2$ μm and $t = 6760$ minutes, we have

$$k_2 = \frac{0{\cdot}2}{q^{\frac{1}{3}}} \qquad (39)$$

and

$$t_2 = \frac{6760}{q^{\frac{1}{3}}} \qquad (40)$$

Arbitrarily making t_2 equal to 150 minutes,

$$q = \left(\frac{6760}{150}\right)^3 = 45^3$$

$$k_2 = \frac{0{\cdot}2}{45} = 0{\cdot}0045\ \mu\text{m}$$

This value of 0·005 μm for k_2 was obtained in earlier work[3,5] by considerations based on the curve $U(k,h,t)$,[1,2] which is valid for coarser particles than $G(k,h,t)$ and $F(\alpha,y,t')$.

According to equation (32)

$$\nu = \left(\frac{45^3}{0{\cdot}000\,145}\right)^{\frac{1}{2}} = 25\,000 \text{ r.p.m.}$$

which will be valid for centrifugal sedimentation with a beaker centrifuge of the dimensions indicated.

If, instead, we arbitrarily put $t_2 = 450$ minutes, we obtain

$$q = \left(\frac{6760}{450}\right)^3 = 15^3$$

$$k_2 = \frac{0{\cdot}2}{1{\cdot}5} = 0{\cdot}0133\ \mu\text{m}$$

and, according to equation (32)

$$\nu = \left(\frac{15^3}{0{\cdot}000\,145}\right)^{\frac{1}{2}} = 4800 \text{ r.p.m.}$$

Again, if we arbitrarily put $t_2 = 1500$ minutes, we obtain

$$q = \left(\frac{6760}{1500}\right)^3 = 4{\cdot}5^3$$

$$k_2 = \frac{0{\cdot}2}{4{\cdot}5} = 0{\cdot}045\ \mu\text{m}$$

and, according to equation (32)

$$\nu = \left(\frac{4{\cdot}5^3}{0{\cdot}000\,145}\right) = 790 \text{ r.p.m.}$$

It will be seen that the critical particle size will decrease with increasing speed of revolution; it can be calculated by reference to the special equations relating to centrifugal sedimentation.

I thank the Foundation for Applied Chemistry of the Technical University of Denmark for financial support, and Mr. A. Hald, Professor in Statistics at the University of Copenhagen, for having carried out the numerical calculations and integrations for my earlier papers.

SYMBOLS USED

k	Particle size (cube root of particle volume)
r	$\frac{k}{\left(\frac{4\pi}{3}\right)^{\frac{1}{3}}}$
t	Time elapsed after homogenising the suspension
h	Distance from the surface of the suspension
η	Viscosity of the medium at 20 °C
v	Velocity of particle, $\frac{h}{t}$
ρ_k	Density of the particle
ρ_f	Density of the fluid
g	Acceleration due to gravity
R	Gas constant
T	Absolute temperature
N	Avogadro's constant
$c_{h,t}$	Concentration at the depth h after the sedimentation period t
D	Diffusion coefficient $= \frac{RT}{6\pi r\eta N} = \frac{m}{k}$
m	kD
B	$\frac{2r^2}{9\eta}(\rho_k - \rho_f)g = i(\rho_k - \rho_f)k^2 = jk^2 = iuk^2$
i	$\frac{2g}{9\eta\left(\frac{16\pi^2}{9}\right)^{\frac{1}{3}}}$
j	$i(\rho_k - \rho_f)$
u	$\rho_k - \rho_f$
l	Depth of the vessel
α	$\frac{D}{Bl} = \frac{m}{jlk^3}$
ν	$\frac{h}{l}$
t'	$\frac{Bt}{l} = \frac{jtk^2}{l}$
$c_{.,0}$	Original uniform concentration of the suspension
$\Phi(p)$	$= \frac{2}{\sqrt{\pi}}\int_0^p e^{-p^2}dp$ is the probability integral with the argument p
$F(\alpha,\nu,t')$	Defined by equation (5)
$G(k,h,t)$	Defined by equation (6)
$U(k,h,t)$	$= \frac{1}{2}\left(1 - \Phi\frac{Bt-h}{\sqrt{4Dt}}\right)$
$k_{h,t}$	The size of a particle that in time t could have fallen a distance h if no diffusion had occurred
b	$\left(\frac{4\pi}{3}\right)^{\frac{1}{3}}\left(\frac{9\eta}{2g}\right)^{\frac{1}{2}}$
$C(k)$	Fraction of material that is of smaller particle size than the particle size k
$V(k)$	$V(k) = \frac{dC(k)}{dk}$

$$\Delta C(k_{h,t}) = C\left(b\sqrt{\frac{h}{ut}}\right) - \frac{c_{h,t}}{c_{.,t}} = C\left(b\sqrt{\frac{h}{ut}}\right) - \int_0^{k_{max.}} F(\alpha,y,t')\,V(k)\,dk$$

n	Constant in the equation $[C(k)]^n = ak$
a	Constant in the equation $[C(k)]^n = ak$
q	$\frac{\text{acceleration}}{g}$
ω	Angular velocity
ν	Revolutions per minute
x	Distance from the axis of rotation

References

1. Berg, S., *Ingvidensk. Skr.*, 1940, **2**, 1.
2. ——, *Kolloidbeihefte*, 1941, **53**, 149.
3. ——, A.S.T.M. Special Technical Publication No. 234, American Society for Testing Materials, Philadelphia, Pa., 1958, p. 143.
4. Mason, M., and Weaver, W., *Phys. Rev.*, 1924, **23**, 412.
5. Berg, S., *Acta Polytech. Scand., Ser. A*, 1965, **43**, 1.

Received *April 28th*, 1971
Accepted *February 1st*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 601–604

The Determination of Trace (Parts per 10^9) Amounts of Gold in Plants by Non-destructive Activation Analysis*

BY P. SCHILLER, G. B. COOK, A. KITZINGER† AND E. WÖLFL
(*I.A.E.A. Laboratory, A-2444 Seibersdorf (near Vienna), Austria*)

A non-destructive activation analysis method for the routine determination of trace amounts (parts per 10^9) of gold in the ashes of various plant materials, grass and maize has been developed. The homogeneity of the gold distribution in the samples is of particular importance and several different methods of sample preparation have been studied. The lower limit for the determination of gold is below 1 part per 10^9, calculated for dried plant material. The method was developed for a geochemical research programme and permits the determination of submicrogram amounts of gold in plants to be carried out more simply than do techniques that involve chemical separation.

SEVERAL investigations have already been made into the determination of trace amounts of gold in plant materials. Warren and Delavault[1] used the dry-ashing method for the determination of gold in twigs and needles and Morris and Gupte[2] determined this element in kale by activation analysis. Beardsley, Briscoe, Růžička and Williams[3] reported on the substoicheiometric determination of trace amounts of gold in kale by neutron-activation analysis. This last method involves the wet ashing of irradiated samples followed by solvent extraction of gold diethyldithiocarbamate from a sulphuric acid or hydrochloric acid medium following the addition of a substoicheiometric amount of copper diethyldithiocarbamate in chloroform. All these methods involve chemical treatment of the samples.

A non-destructive method based on activation analysis is presented in this paper. The method was developed for an I.A.E.A. coordinated research programme that involved the use of activation analysis for geochemical and geobotanical prospecting. Geobotanical prospecting is based on a knowledge of the relationship between the concentration of the investigated metal in ores from the metal-bearing area and that in plants from the same area.

EXPERIMENTAL

APPARATUS AND REAGENTS—

A 20-cm^3 lithium-drifted germanium gamma-ray detector inside a Compton anti-coincidence shield was used. This shield is a large tank containing about 1 m^3 of liquid scintillator solution.[4] The tank is scanned from above by eight photomultipliers. The use of several vertical and horizontal tunnels allows the germanium detector and the sample whose activity is to be measured to be positioned inside the tank.

The liquid scintillator shield described above reduces the Compton continuum by a factor of between 2 and 8 (depending on the channel number) for single gamma-rays that are not emitted as part of a cascade decay scheme. The Compton anti-coincidence shield therefore enhances the selectivity and sensitivity of the gold determination. Spectra from emitters of cascade gamma-rays show an even larger reduction of the Compton continuum, typically by a factor of 25 to 40.

Gamma-ray spectra of up to 1024 channels were recorded with a pulse height analyser and then transferred directly on to computer cards by means of an IBM 523 gang summary punch. A computer program written in FORTRAN IV for an IBM 360/30 computer and a special subroutine, written in basic machine language, to interpret the cards generated by the IBM 523 punch, were used. The data processing methods described by Parr[5] were applied.

* Presented at the Third SAC Conference, Durham, July 12th to 16th, 1971.

† Present address: Institute for Experimental Mineralogy and Geochemistry ČSAV, Prague, Czechoslovakia.

All reagents were of analytical-reagent grade. Metallic gold was used as a standard, being dissolved in aqua regia and the solution diluted with 6 M hydrochloric acid. The gold concentration of the stock solution was 1 g l^{-1}.

PROCEDURE—

The plant samples were dried at 105 °C for 24 hours, milled in a planetary ball mill for 20 minutes (leaves) or 30 minutes (needles) and then homogenised for 30 minutes in a laboratory mixer. Possible contamination resulting from this procedure was tested for by using proved blank materials. No gold was detected. That the procedures used for cleaning the apparatus between samples were adequate was shown by results obtained with materials from blank areas milled after samples with high gold contents had been analysed, when only 2 p.p.b. (parts per 10^9) or less were found.

Ashing was carried out in two steps, first for 1 hour at 350 °C and then for 4 hours at 650 °C. The losses that occurred during dry ashing were examined by using the plant reference material provided by Bowen[6]; the results were in good agreement with those obtained by other workers (see Table I).

The samples (1 g of ash or 10 g of dried material), placed in polythene tubes, were irradiated together with gold standards in a TRIGA reactor for 8 hours at a flux of $1{\cdot}75 \times 10^{12}$ neutrons cm^{-2} s^{-1}. After a decay period of about 6 days, the samples were counted for 10 minutes each and the complete gamma-ray spectrum up to 2 MeV was recorded. The calculation of the results was based on the gold-198 photopeak at 412 keV. Four replicate analyses were carried out on each sample.

RESULTS AND DISCUSSION

In this paper, some experiments on plant samples from a gold-bearing area in lowlands near the River Danube in South Slovakia, Czechoslovakia (Fig. 1), are reported. The plants examined were grass and maize, the only vegetation grown in this area.

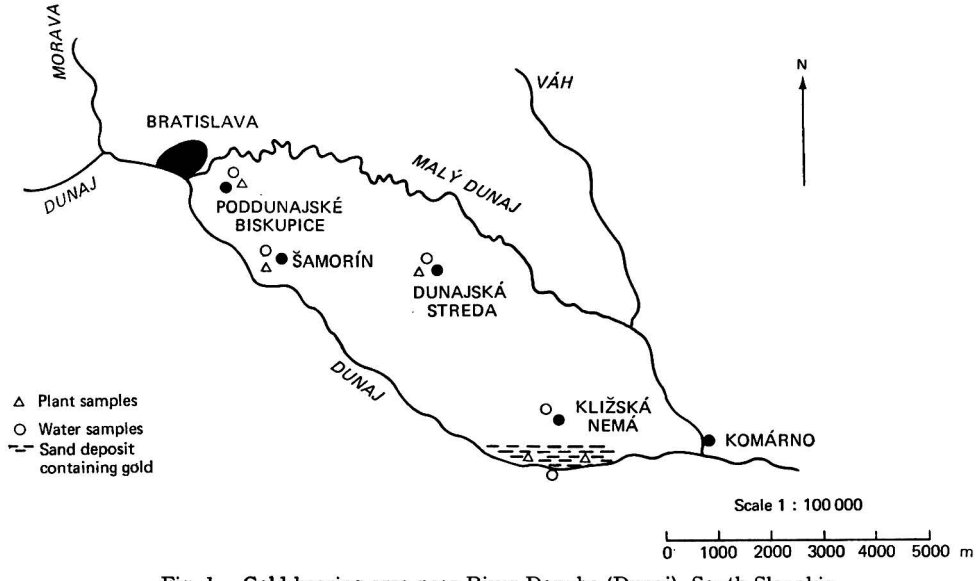


Fig. 1. Gold-bearing area near River Danube (Dunaj), South Slovakia

Because the method is non-destructive, it is particularly important that the gold distribution in the samples analysed should be homogeneous. For this reason several different methods of sample preparation were studied. The simplest method seemed to be the direct ashing of the sample before irradiation. However, with this procedure, the differences between replicates were too great. The relative standard deviation (S_r) was 34 per cent.

for grass from the south-eastern zone (zone A) and 65 per cent. for grass from the south-western zone (zone B). For maize, the relative standard deviation was 29 per cent. for zone A and 30 per cent. for zone B. A substantial improvement was obtained when, in addition, the ash was milled to a fine powder and homogenised. The relative standard deviation decreased with grass samples to 14 per cent. for zone A and 21 per cent. for zone B, and with maize to 12 per cent. for zone A and 7 per cent. for zone B.

As Table I shows, the mean concentration of gold in a biological reference material (*Brassica oleracea*, provided by Bowen[6]) after ashing and milling to homogenise the samples, was found to be 2·1 p.p.b., which is in excellent agreement with the results of Morris and Gupte[2] and Beardsley, Briscoe, Růžička and Williams.[3] Therefore, a 1-g sample of ash is sufficient for the non-destructive determination of gold even for an extremely low concentration, such as was present in the kale samples (Fig. 2).

TABLE I

RESULTS (ON DRIED MATERIAL) OBTAINED FOR GOLD IN KALE (*Brassica oleracea*)

	Gold, p.p.b.	Mean value, p.p.b.	S_r, per cent.
Morris and Gupte[2]	2·1, 2·0, 2·4, 2·4	2·2	9·3
Beardsley *et al.*[3]	1·1, 2·5, 2·7	2·1	41·5
Bowen[7]	(two measurements)	2·9	—
This work	2·1, 2·3, 1·9, 2·1	2·1	7·7

S_r = relative standard deviation as percentage of the mean.

It would clearly be an advantage if ashing, which prolongs sample preparation by several hours, could be avoided. However, as the ash content of the plants investigated varied between 7 and 17 per cent., about ten times more material would have to be irradiated and measured, and the use of relatively large samples reduces the detection efficiency because of geometrical and self-absorption factors. Moreover, the different packing densities of the various dried plant materials would introduce different geometries during the measurement of the gamma-ray spectrum. Although these differences could be corrected for, a series of factors would be necessary to cover the various sample sizes. Efficiency is therefore greater and reproducibility better when ashed samples are used, despite the extra preparation time.

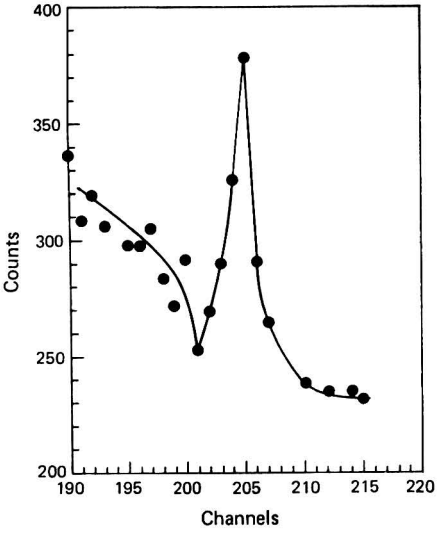


Fig. 2. Gold peak in kale (gold content 2·1 p.p.b). One channel is equivalent to 2 keV

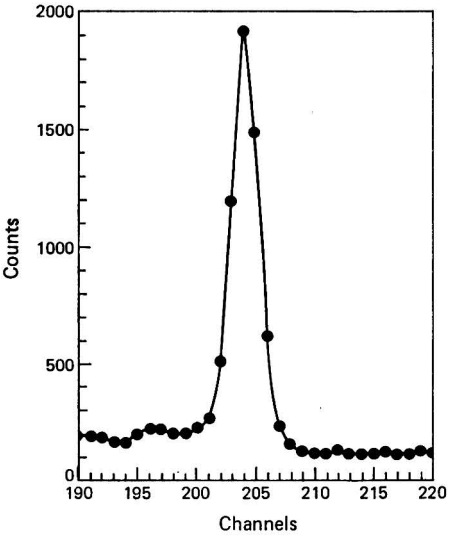


Fig. 3. Gold peak in maize from gold-bearing area (gold content 54 p.p.b). One channel is equivalent to 2 keV

In the evaluation of the gamma-ray spectra from the plants under investigation, no interfering peaks were observed near the gold-198 peak. For example, Fig. 3 shows the gold peak for maize collected from the gold-bearing area in South Slovakia and Table II shows the gold concentrations determined in maize and grass from that area. The amount of gold determined in plants collected at a distance of 10 km or more from the area of the gold deposit was only about 2 p.p.b.

TABLE II

RESULTS (ON DRIED MATERIAL) OBTAINED FOR GOLD IN MAIZE AND GRASS FROM A GOLD-BEARING AREA OF SOUTH SLOVAKIA

	Gold, p.p.b., from			
	Maize		Grass	
	Zone A	Zone B	Zone A	Zone B
	65	51	56	37
	57	51	70	33
	50	56	66	52
	58	47	80	39
	61	74	49	52
	81	50	46	54
	60	49	51	39
	81	53	69	43
Mean value	64	54	61	43
S_r, per cent.	17	16	20	18

S_r = relative standard deviation as percentage of the mean.

The method reported here enables a selective quantitative submicro-determination of gold to be performed in plants, and gives good precision. The non-destructive nature of the method, which avoids the current need for chemical treatment of the samples, represents a considerable simplification of the determination in comparison with other methods in current use. This is very important in geobotanical studies, in which the number of samples to be analysed is large.

We thank Dr. R. M. Parr for providing assistance with and advice on the computer program.

REFERENCES

1. Warren, H. V., and Delavault, R., *Bull. Geol. Soc. Amer.*, 1950, **61**, 123.
2. Morris, D. F. C., and Gupte, J. C., private communication, *quoted by* Beardsley, D. A., Briscoe, G. B., Růžička, J., and Williams, M., *Talanta*, 1965, **12**, 829.
3. Beardsley, D. A., Briscoe, G. B., Růžička, J., and Williams, M., *Talanta*, 1965, **12**, 829.
4. I.A.E.A. Laboratory Activities, Seventh Report, Technical Reports Series No. 103, I.A.E.A., Vienna, 1970, p. 46.
5. Parr, R. M., Proceedings of the Symposium on "Radioaktive Isotope in Klinik und Forschung," Bad Gastein, Austria, 1970, Urban and Schwarzenberg, Munich, Berlin and Vienna, 1970, p. 97.
6. Bowen, H. J., *in* Shallis, P. W., *Editor*, "Proceedings of the SAC Conference 1965, Nottingham," W. Heffer & Sons Ltd., Cambridge, 1965, p. 25.
7. ——, *Adv. Activation Analysis*, 1969, **1**, 101.

Received *October 7th*, 1971
Accepted *February 21st*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 605–608

Determination of Thorium in Bovine Bone by Neutron-activation Analysis

BY S. OHNO AND T. ICHIKAWA*

(*National Institute of Radiological Sciences, 9–1, 4-chome, Anagawa, Chiba-shi, Japan*)

Thorium has been determined in bovine bone by neutron-activation analysis. A radiochemical procedure is described in which protactinium-233 resulting from the reaction

$$^{232}Th(n,\gamma)^{233}Th \xrightarrow{\beta\text{-decay}} {}^{233}Pa$$

is extracted with trioctylamine in xylene and subsequently back-extracted with distilled water, following collection of the thorium in the bone by Korkisch's anion-exchange method and irradiation. The protactinium-233 activity is measured by gamma-ray spectrometry.

The precision of the method is better than ± 15 per cent. for samples with a thorium content exceeding 0·0037 p.p.m.

THORIUM is likely to be an important element for nuclear fuel material in the future and many investigators have determined thorium in environmental materials[1–6] from toxicological and geological standpoints. For example, Twitty and Boback[7] determined thorium in biological materials by neutron-activation analysis, while Alian and Sanad[8] determined this element in aluminium by neutron-activation analysis and a combined method involving standard addition and solvent extraction of protactinium-233.

On the other hand, other workers[9] investigated the separation and extraction of thorium and protactinium-233. Khosla[10] investigated the separation of thorium with *N*-benzylaniline in chloroform in a sulphuric acid medium, whereas Norton and Stoenner[11] separated thorium by an anion-exchange method.

In this paper, a method is described for the determination of trace amounts of thorium in bone tissue by an anion-exchange method and solvent extraction of the radioisotope protactinium-233 produced in its pure state by the reaction

$$^{232}Th(n,\gamma)^{233}Th \xrightarrow{\beta\text{-decay}} {}^{233}Pa$$

EXPERIMENTAL

APPARATUS—

A Hitachi RAH 403 100-channel gamma-ray spectrometer with a $1\frac{3}{4} \times 2$-inch well-type sodium iodide (thallium) crystal was used.

REAGENTS—

All reagents were of recognised analytical grade.

Thorium standard solution—Prepare an aqueous solution containing 10 μg ml^{-1} of thorium.

Trioctylamine - xylene solution—Dissolve 25 g of trioctylamine in 475 ml of xylene. Store the solution in an amber glass bottle.

Methanol - nitric acid solution—Prepare a stock solution by mixing 90 ml of methanol and 10 ml of 5 M nitric acid.

Anion-exchange resin—Wash Dowex 1-X8 resin (100 to 200 mesh) with nitric acid (about 2 N). Pack the resin into an ion-exchange tube to give a column 5 cm in length and 1 cm in diameter, and then wash the column with 100 ml of a 90 per cent. solution of methanol in 5 M nitric acid.

PREPARATION OF SAMPLE FOR IRRADIATION—

Weigh accurately 10 g of ashed bovine bone sample into a beaker and dissolve it in a few millilitres of about 2 N nitric acid. Evaporate the solution to dryness on a hot-plate and then dissolve the residue in 200 ml of the methanol - nitric acid solution. After filtering,

* Present address: Japan Atomic Energy Research Institute, Oarai Establishment, Ibaraki Pref., Japan.

if necessary, pass the sample solution through the prepared anion-exchange resin column at a flow-rate of 50 to 60 ml h^{-1}. After washing the column with 100 ml of methanol - nitric acid solution, elute the adsorbed thorium on the anion-exchange resin with 50 ml of 1 N nitric acid. Evaporate the eluted solution almost to dryness and then transfer it to a 10-ml calibrated flask, wash the beaker with a small amount of distilled water and dilute to the mark. Transfer an aliquot of this solution equivalent to 1 g of ashed bone sample with a pipette on to a filter-paper (Toyo Roshi No. 5C) previously washed with 2 N nitric acid solution and dry the paper and sample under an infrared lamp. Place the paper in a polyethylene bag and heat-seal the bag.

IRRADIATION—

After packing four samples and two standard specimens together in a pneumatic tube, the irradiation was carried out in a thermal neutron flux of 7×10^{13} neutrons cm^{-2} s^{-1} in the JRR-2 reactor of the Atomic Energy Research Institute of Japan for 20 minutes.

RADIOCHEMICAL PROCEDURE FOR INDUCED PROTACTINIUM-233—

Open the irradiated sample into a beaker and decompose completely the organic matter by treating the filter-paper containing the irradiated sample with concentrated nitric acid and 30 per cent. hydrogen peroxide solution, finally evaporating off the nitric acid. Add a few millilitres of 12 N hydrochloric acid to the contents of the beaker and evaporate off the hydrochloric acid. Next add a few millilitres of 12 N hydrochloric acid and 1 ml of 10 per cent. ascorbic acid solution and warm the beaker on a hot-plate for several minutes. Transfer the solution into a separating funnel with a few millilitres of distilled water. Then, after adding 10 ml of 5 per cent. trioctylamine - xylene solution, extract the protactinium-233 by shaking the mixture for 1 minute and allowing it to stand for 5 minutes. After removing the aqueous layer, wash the organic phase with 10 ml of 12 N hydrochloric acid and then back-extract any protactinium-233 with 10 ml of distilled water. Place the aqueous phase and washings in a polyethylene counting vial for gamma-ray spectrometry.

ACTIVITY MEASUREMENT—

A Hitachi-303 100-channel pulse-height analyser was used for the quantitative measurement of the photopeaks, principally that of protactinium (0·32 MeV). The photopeak area was evaluated according to Covell's method[12] and protactinium-233 was identified from half-life values reported by Covell as determined by observing decay and from the gamma-ray energy associated with this radioactive decay.

RESULTS AND DISCUSSION

The thorium content of bovine bone was determined by neutron-activation analysis and the results are given in Table I. For the purpose of avoiding unnecessary radiation exposure

TABLE I

RESULTS (CALCULATED ON THE ASHED SAMPLE) OBTAINED FOR THORIUM IN BOVINE BONE (FEMUR)

Sample number	Age/years	Activity of protactinium-233/ counts s^{-1} ± standard deviation	Concentration, p.p.m. ± standard deviation
1	2	0·9464 ± 0·0374	0·0202 ± 0·0008
2	7	0·1223 ± 0·0200	0·0026 ± 0·0004
3	0·8	0·1714 ± 0·0215	0·0037 ± 0·0005
4	5	0·5171 ± 0·0281	0·0111 ± 0·0006
5	3	3·1175 ± 0·0444	0·0667 ± 0·0011
6	4	1·3315 ± 0·0420	0·0285 ± 0·0009
Standard (10 μg) ..		461·6200 ± 2·1051 473·6533 ± 2·1269	
Reagent blank		Not detectable	
Literature values—			
Refs. 4 and 5			0·004
Ref. 9			0·004 to 0·006
Ref. 2			0·023

Counting time: 5000 s for the sample and 300 s for the standard specimen.

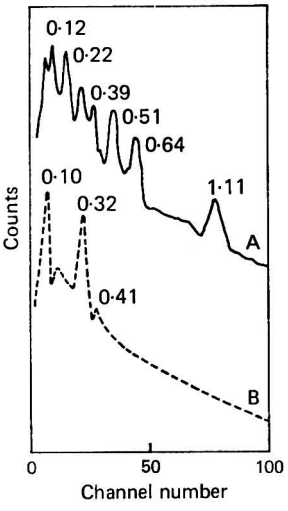


Fig. 1. Gamma-ray spectra of A, irradiated sample, and B, protactinium-233 from the irradiated sample after solvent extraction. The values on the peaks are measured in MeV

to various radionuclides induced by radioactivation of target material, thorium in bone was concentrated by Korkisch's anion-exchange method.[6] Although this method of concentration of thorium is a conventional technique, it is clear from Fig. 1 that the other element present, *i.e.*, chromium in the form of chromate, is simultaneously adsorbed on the anion-exchange resin. Moreover, the main uncertainty in the separation and determination of thorium when using neutron-activation analysis as described above arises prior to dissolution of the sample with nitric acid and its concentration with anion-exchange resin. Also, as reported by Alian and Sanad,[8] who determined thorium by solvent extraction and radioactivation techniques with tridecylamine as the solvent for the extraction of protactinium-233, the other induced radionuclide, *i.e.*, iron-59, is co-extracted with protactinium-233. However, the solvent extraction described here appeared to be satisfactory for the separation of protactinium-233 and the proposed method was used for this purpose. Moreover, a reagent blank test was carried out (Table I); it appears that the thorium content of the reagent is negligible. Also in Table I, the results obtained by this method are compared with those given in the available literature, which were obtained partly by neutron-activation analysis and partly by a spectrophotometric technique.

As shown in Table II, the solvent extraction recovery was in the range 96 to 98 per cent. The limit of detection was calculated, from the determination on a standard specimen, to be about 0·003 p.p.m. for thorium, and the precision of the method is better than ± 15 per cent. for a sample with a thorium content in excess of 0·0037 p.p.m.

TABLE II

EFFECT OF ACIDITY ON EXTRACTION OF PROTACTINIUM-233 WITH 5 PER CENT. TRIOCTYLAMINE IN XYLENE

Hydrochloric acid/N	Extraction, per cent.	Hydrochloric acid/N	Extraction, per cent.
1	1·67	7	97·84
2	2·91	8	98·00
3	3·00	9	96·63
4	14·68	10	97·12
5	55·96	11	97·96
6	93·77	12	96·80

We thank Dr. F. Yamazaki, Director of the Japan Atomic Energy Research Institute, and Dr. M. Suzuki, Head of the Fourth Laboratory of the Environmental Hygiene Division of the National Institute of Radiological Sciences, for encouragement during this investigation.

References

1. Morgan, J. W., and Lovering, J. E., *Analytica Chim. Acta*, 1963, **28**, 405.
2. Picer, M., and Storohal, P., *Ibid.*, 1968, **40**, 131.
3. Awwal, M. A., and Carswell, D. J., *J. Inorg. Nucl. Chem.*, 1968, **30**, 1057.
4. Edgington, D. N., *Int. J. Appl. Radiat. Isotopes*, 1967, **18**, 11.
5. ——, *Rep. Congr. Atom. Energy Commn U.S.*, ANL-7060, 1964, 77.
6. Korkisch, J., and Tera, F., *Analyt. Chem.*, 1961, **33**, 1264.
7. Twitty, B. L., and Boback, M. W., *Analytica Chim. Acta*, 1970, **49**, 19.
8. Alian, A., and Sanad, W., *Ibid.*, 1967, **38**, 327.
9. Petrow, H. G., and Strehlow, C. D., *Analyt. Chem.*, 1967, **39**, 265.
10. Khosla, M. M. L., *Analytica Chim. Acta*, 1971, **54**, 315.
11. Norton, E. F., and Stoenner, R. W., *Ibid.*, 1971, **55**, 1.
12. Covell, D. F., *Analyt. Chem.*, 1959, **31**, 1785.

Received *January 12th*, 1972
Accepted *April 4th*, 1972

Identification of Species of Heat-sterilised Canned Fish by Polyacrylamide-disc Electrophoresis

BY I. M. MACKIE AND T. TAYLOR

(*Ministry of Agriculture, Fisheries and Food, Torry Research Station, P.O. Box* 31, 135 *Abbey Road, Aberdeen, AB*9 8*DG*)

The heat-denatured proteins of canned fish obtained by treating the fish with cyanogen bromide yield polypeptides that are soluble in 6 M urea solution. These fragments of proteins, which have been cleaved selectively at the methionyl peptide bond, can be separated by electrophoresis in 6 M urea solution. The separation patterns for canned herring, tuna, plaice, salmon, lemon sole, sprat, haddock and mackerel are presented. The method extends the electrophoretic method of species identification to include canned fish.

ELECTROPHORESIS of the water-soluble proteins of the muscle of fish is now a widely used method for identifying species.[1–3] It has been shown to be a particularly sensitive method for differentiating between species that are difficult or impossible to identify on morphological criteria alone.[4,5] It is also of value in identifying the species of portions, such as fillets, of commercially important fish when the usual identifying features have been removed during processing. With the introduction of the Labelling of Food Regulations[6] in 1973 it is likely that there will be an even greater need for objective methods of identifying both raw and cooked fish.

Heating denatures and precipitates proteins of muscle and hence the identification of species from the electropherograms of the water-soluble proteins is possible only for raw and partially cooked fish. The electrophoretic method for the identification of species was extended to cooked fish by Mackie,[7] who showed that fragments of the heat-precipitated proteins could be extracted in high concentrations of urea and that species-specific patterns were obtained on separating these proteins by electrophoresis. This method, however, is limited to fish that have been cooked under normal conditions and cannot be used for canned products as the proteins are rendered inextractable in solutions of urea, presumably because more severe denaturation of the proteins takes place at the high temperatures that are required in the canning process. Similar findings have been obtained in the identification of meat species after heating.[8]

This paper describes an extension of the electrophoretic method of identifying species to include heat-sterilised canned fish. It is based on the specific cleavage of the methionyl peptide bonds of the heat-sterilised proteins with cyanogen bromide[9] to release protein fragments that can be extracted with 6 M urea solution.

METHODS

CANNED PRODUCTS—

Fish of the species herring (*Clupea harengus* L.), plaice (*Pleuronectes platessa* L.), lemon sole (*Microstomus kitt*), *sprat* (*Sprattus sprattus*) and haddock (*Melanogrammus aeglefinus*) were canned in the Torry Research Station. Commercial cans of sockeye salmon, mackerel and tuna were also examined. The fish or fish portions were removed from the cans, separated as completely as possible from the oil or sauce and allowed to drain.

REACTION WITH CYANOGEN BROMIDE—

A portion of the canned fish (5·0 g), drained free from liquor, was added to 100 ml of 70 per cent. v/v formic acid solution containing 2·5 g of cyanogen bromide in a stoppered flask. The suspension was shaken for a few minutes to break up the flesh and was then allowed to stand for 24 hours at room temperature. The formic acid and the cyanogen bromide were removed on a rotatory evaporator at 40 °C, and the residue was suspended in distilled water, dialysed against running water for 24 hours and re-evaporated to dryness.

UREA EXTRACT—

The dry residue was dissolved as completely as possible in 15 ml of 10 M urea solution by allowing the suspension to stand overnight at room temperature. After neutralisation, if required, with a few drops of 1 N sodium hydroxide solution, the solid was removed by centrifuging the suspension at 80 000 *g* for 20 minutes. The clear solution was then removed and retained for electrophoresis. For extracts of oily fish, it was necessary to remove the solution by using a pipette so as to obtain the solution free from the oil.

ELECTROPHORESIS—

The electrophoretic procedure used was that described by Mackie[3,10] involving the use of polyacrylamide gels containing 6 M urea solution. A 20-μl volume of the urea extract was usually applied and electrophoresis was allowed to proceed for 40 minutes at room temperature. The gels were stained with Amido black and de-stained either by solvent extraction or by an electrolytic method, and transferred finally to tubes containing the de-staining solvent.

RESULTS AND DISCUSSION

A comparison of the electropherograms of the eight species (Fig. 1) shows that the separation patterns can be distinguished from one another. A duplicate examination of all of these species showed that the patterns are also reproducible for each species.

Certain zones are common to all the patterns but there is a sufficient number of other zones that have different mobilities to allow a clear differentiation of the individual patterns to be made. Previous preliminary work on five samples each of canned cod and canned salmon showed that such patterns are constant for each species and as such they are of potential value in the identification of species. These patterns do not show the wide differences in the relative intensity of staining that are found for aqueous extracts of the myogens of raw muscle or urea extracts of cooked muscle. They are characterised by many well separated zones that have similar intensities of staining.

Extracts prepared from canned fish that had been treated with formic acid in the absence of cyanogen bromide did not give any zones when they were subjected to electrophoresis. A similar result was obtained when canned muscle was extracted directly with 6 M urea solution.[3,10] In the latter work, one or two zones, although insufficient for individual species to be identified, could occasionally be discerned against an intense background staining. In the present experiment (Fig. 1), a certain amount of background staining occurred but it did not interfere with the interpretation of the separation patterns.

Although cyanogen bromide has not previously been applied to rendering residues from heat-precipitated proteins soluble, it has been used extensively in studies on protein structure.[9] In this reaction, which is carried out under mildly acidic conditions at room temperature in either 70 per cent. formic acid or 0·1 N hydrochloric acid, the methionyl peptide bond is cleaved selectively with the release of two peptides, of which one has a terminal carboxyl group and the other a terminal amino group.

It has been found that the use of formic acid gives a higher yield of the polypeptides than when hydrochloric acid is used and also the formic acid can readily be removed by rotary evaporation at low pressure rather than by freeze-drying, which is required for the removal of hydrochloric acid. A 200-fold excess of cyanogen bromide relative to methionine in fish muscle was used in this experiment to obtain the maximum conversion of the methionine residues.[9] It has been assumed for the purposes of this calculation that all the methionine residues of raw muscle, approximately 2·0 g per 100 g of protein,[11] are available for this reaction. It must be concluded from the results of this experiment that a proportion of the fragments released by cyanogen bromide are of a size that is suitable for separation by electrophoresis, and as species-specific separation patterns have also been obtained it is clear that genetically determined differences in either the primary amino-acid sequence or in the composition of these fragments still exist at this level of protein structure. This method clearly has potential value in the identification of canned fish products or fish products that have been subjected to high temperatures or extensive heating at normal cooking temperatures.

Further work could, however, be necessary to determine to what extent various pre-canning treatments, such as frozen storage, will have on the separation pattern. It has

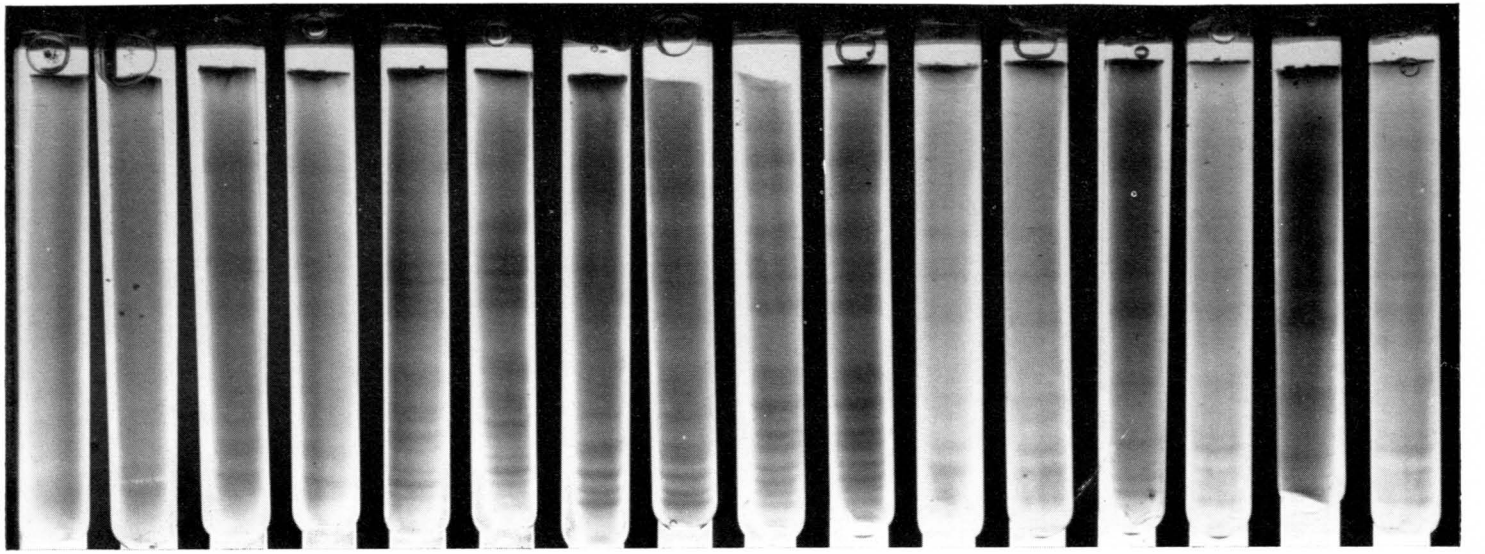


Fig. 1. Electrophoretic separation patterns of protein fragments, produced by treatment with cyanogen bromide, from canned fish (in pairs): (*a*), herring; (*b*), tuna; (*c*), plaice; (*d*), salmon; (*e*), lemon sole; (*f*), sprat; (*g*), haddock; and (*h*), mackerel

[To face page 610

not previously been possible to identify heat-sterilised canned products by an objective method, although recent work by McLay and Parsons[12] has shown that thin-layer chromatograms of the 2,4-dinitrophenylhydrazones of the carbonyls present in such products are also species-specific.

Conclusion

Cyanogen bromide can be used to render soluble protein fragments from canned fish products. Species-specific separation patterns of these protein fragments are obtained by electrophoresis in 6 M urea solution. The method extends the electrophoretic method for the identification of species to include fish products that have been processed at high temperatures.

The work described in this paper was carried out as part of the programme of the Torry Research Station.

References

1. *J. Ass. Off. Analyt. Chem.*, 1967, **50**, 205.
2. Cowie, W. P., *J. Sci. Fd Agric.*, 1968, **19**, 226.
3. Mackie, I. M., *J. Ass. Publ. Analysts*, 1969, **7**, 83.
4. Tsuyuki, H., Roberts, E., and Vanstone, W. E., *J. Fish. Res. Bd Can.*, 1965, **22**, 203.
5. Jones, B. W., and Mackie, I. M., *Comp. Biochem. Physiol.*, 1970, **32**, 267.
6. "The Labelling of Food Regulations," S.I. 1970 No. 400, H.M. Stationery Office, London.
7. Mackie, I. M., *Analyst*, 1968, **93**, 458.
8. Mattley, M., Parsons, A. L., and Lawrie, R. A., *J. Fd Technol.*, 1970, **5**, 41.
9. Gross, E., *Meth. Enzym.*, 1966, **9**, 238.
10. Mackie, I. M., *J. Ass. Publ. Analysts*, 1972, **10**, 18.
11. Connell, J. J., and Howgate, P. F., *J. Sci. Fd Agric.*, 1959, **4**, 241.
12. McLay, R., and Parsons, E., *Analyst*, 1972, **97**, 376.

Received *February 21st*, 1972
Accepted *April 4th*, 1972

The Determination of Sulphur in Lubricating Oil Fractions and in Fuel Oils—A Coulometric Method

By JEAN P. DIXON

(Shell Research Ltd., Thornton Research Centre, P.O. Box No. 1, Chester, CH1 3SH)

The original combustion - microcoulometric method in which the Dohrmann microcoulometer is used for the determination of trace amounts of sulphur in organic liquids has been modified. The inlet to the microcoulometer has been re-designed to permit complete combustion of oils to take place in the inlet. The modified inlet and flow systems have been used for the determination of sulphur in oil fractions obtained from preparative layer chromatography and in fuel oils.

Analytical procedures that involve coulometric determinations have the advantages of very high sensitivity and speed and their application to determinations of trace amounts of substances is therefore of great importance. The microcoulometric detection system manufactured by the Dohrmann Instrument Company, according to the design of Coulson and Cavanagh,[1,2] was first used as a detector for sulphur compounds in gas chromatography. In their work, the quantitative performance of this system was also assessed, and, subsequently, its use for total elemental analysis was described in the literature.[3–5]

The sample is vaporised in a stream of helium, which is subsequently mixed with oxygen and passed through a combustion tube to the titration cell. Sulphur dioxide formed as a combustion product reacts in the cell with coulometrically generated titrant (I_3^-)—

$$SO_2 + I_3^- + H_2O \longrightarrow SO_3 + 3I^- + 2H^+$$

The cell is operated at a pre-set constant titrant-ion concentration, which is maintained during the titration by the reaction

$$3I^- \longrightarrow I_3^- + 2e^-$$

The current thus produced passes through a series of resistors and the potential drop *versus* time relationship is recorded. This relationship is equivalent to that of current *versus* time, and by Faraday's law the number of coulombs of electricity used in maintaining a constant titrant-ion concentration is proportional to the amount of reacting species (sulphur dioxide). This number of coulombs of electricity is determined by the integrated area beneath the current *versus* time graph.

The titration is controlled by two pairs of electrodes situated within the cell and connected to the coulometer. One pair of electrodes monitors the concentration of the titrant (which is normally set by means of the "Bias Set" control on the coulometer); the other pair generates titrant, as required, so as to maintain a constant titrant-ion concentration.

Unfortunately, complete recovery is usually not obtained when the oxidative method of operation is used (*i.e.*, when sulphur is measured only as sulphur dioxide in the titration cell) and it is customary to use a conversion factor (0·6 to 0·95) in these methods. This incomplete recovery arises because some of the sulphur is converted into sulphur trioxide and is not measured in the cell. The operating conditions used have varied considerably, particularly with respect to the temperature and flow-rates required to obtain optimum conversion of sulphur into sulphur dioxide. Our experience has indicated that, of the variables that can affect the conversion factor, the actual preparation of the cell, notably the iodine reference electrode, may be the most critical (see below). A selection of operating conditions known to have been used is given in Table I.

In this work, a Dohrmann, Model C-250A, microcoulometer equipped with an S-250 pyrolysis furnace, a T-300-P iodine cell and a Servoscribe recorder with a disc integrator, Model RE 512, were used. The equipment was set up according to the instructions in the Dohrmann Manual, with the exceptions that a combustion tube of larger bore (27 mm o.d.) and potassium iodide - sulphamic acid electrolyte solution were used.

TABLE I

OPERATING CONDITIONS OF DOHRMANN MICROCOULOMETER FOR SULPHUR (OXIDATIVE) DETERMINATION

Conditions	Combustion tube: Small bore (Dohrmann) + auxiliary vaporiser	Larger bore	Larger bore	Small bore (Dohrmann)	Small bore (Dohrmann)
Furnace temperatures—					
Inlet/°C	250 to 300	400	400 to 500	500	650
Centre/°C	700 to 725	1000	900	700	800
Outlet/°C	700 to 725	900	800	700	800
Flow-rates—					
Oxygen/ml min^{-1} ..	130	100	90 to 125	200	60
Inert gas/ml min^{-1} ..	30	100	30 to 70	50	140
Cell—					
Electrolyte	KI - CH_3CO_2H	KI - NH_2SO_3H	KI - CH_3CO_2H - NaN_3	KI - CH_3CO_2H - NaN_3	KI - CH_3CO_2H - NaN_3
Bias/mV	150	130 to 150	120 to 140	155 to 160	160
Gain		Low		Low	Low
Recovery, per cent. ..	80 to 85	80 to 95	80	95	60 to 70

The results for typical gasolines, various gasoline blending components and two distillate fuels are given in Table II. A calibration blend, containing dibenzyl disulphide in heptane, was run frequently during this work, and the recovery was consistently close to 75 per cent.

The Dohrmann microcoulometer, with an oxidation tube of increased capacity, has given satisfactory results for the determination of total sulphur for a wide range of sulphur contents in volatile petroleum products. Samples with sulphur contents in excess of about 100 p.p.m. must be diluted with a suitable sulphur-free solvent. We used heptane and also xylene. Xylene is a more satisfactory solvent than heptane for distillate fuel oils because some fuel oils do not dissolve completely in heptane and settle out on standing. Fuel oils in general, however, apart from those included in Table II, did not give satisfactory results; in most instances results below 50 per cent. of expected values for sulphur content were obtained.

TABLE II

SULPHUR CONTENT OF GASOLINES, GASOLINE BLENDING COMPONENTS AND LIGHT DISTILLATE FUELS

Sample	By X-ray fluorescence method	By Dohrmann method
Alkylate	19 p.p.m.	23 p.p.m.
Light straight-run distillate	52 p.p.m.	62 p.p.m.
Steam-cracked gasoline	74 p.p.m.	64 p.p.m.
Thermal reformate	140 p.p.m.	137 p.p.m.
Light catalytically cracked gasoline (diluted 1 + 9 with diluent*)	380 p.p.m.	375 p.p.m.
Light catalytically cracked gasoline (diluted 1 + 49 with diluent)	490 p.p.m.	505 p.p.m.
Full-range gasoline (diluted 1 + 99 with diluent)	0·13 per cent. w/w†	0·12 per cent. w/w
Distillate fuel (diluted 1 + 199 with diluent)	0·22 per cent. w/w‡	0·22 per cent. w/w
Distillate fuel (diluted 1 + 499 with diluent)	0·50 per cent. w/w‡	0·49 per cent. w/w

* See text.
† Wickbold result.
‡ American Petroleum Institute correlation samples.

The problems inherent in the determination of sulphur in fuel oils are currently being extensively examined. Permitted limits of sulphur in fuel oils are being drastically reduced in some countries; concomitant attention to the accuracy and repeatability of methods for the determination of sulphur is therefore important. In a recent American Petroleum Institute (Committee for Air and Water Conservation) report,[6] the correlation programme carried out showed that the microcoulometric procedure had better precision and greater speed than some of the other methods, but that its usefulness was at present restricted to

the lighter distillate fuel oils. Consequently, we decided to investigate possible modifications of the microcoulometric procedure in an attempt to make it suitable for use with heavier fuel oils.

Development of a modified procedure for less volatile products

The paramount consideration in attempting to extend the coulometric procedure to the determination of sulphur in less volatile products would appear to be to improve the volatilisation or optimum oxidation conditions, or both.

Quartz baffle tube system—

We attempted to obtain improved performance, *i.e.*, optimum oxidation conditions to deal with heavier products, by including a quartz baffle tube between the inlet heater and the combustion tube. In this tube, which is widely used in microanalysis for the complete oxidation of organic microsamples for the determination of carbon, hydrogen, sulphur and the halogens, sample vapour and oxygen are mixed by a series of concentric quartz baffles within the quartz core of the tube. With this modification, the recovery of sulphur dioxide, as measured in the cell, was very low (less than 40 per cent.), but by using an appropriate factor (0·37), we obtained good results for some of the heavier American Petroleum Institute fuel-oil samples.

Killer and Underhill[3] have reported that the quartz-chip packing of the original Dohrmann combustion tube catalysed the oxidation of sulphur dioxide to sulphur trioxide. This reaction is an example of the apparent adverse effect of quartz on a favourable yield of sulphur dioxide. The baffle tube introduced a vastly increased surface area of quartz that may have contributed to the poor conversion to sulphur dioxide, but was accompanied by complete decomposition of the fuel oil, as correct results were obtained by the use of the appropriate factor. A modified approach, described below, was therefore examined.

Modified arrangement for sample oxidation—

The development of the Dohrmann system for the determination of total sulphur follows from the use of the coulometric cell as a detector in gas chromatography, in which the injection of the sample and the transport of its gaseous products in a carrier gas are standard procedures. However, such procedures are not necessarily standard in total elemental analysis, and therefore an oxidising injection system was investigated.

Simple alterations to the inlet end of the quartz tube were carried out and are illustrated in Fig. 1. Essentially, the gas flows are reversed—oxygen flows through the injection part of the apparatus and helium enters later as the carrier gas. The injection part becomes a small furnace, heated to 800 °C, into which the sample is injected with a platinum needle as the oxidising atmosphere rapidly corrodes a steel needle.

The new quartz insert fits directly into the Dohrmann tube. Power supply for the heater winding is taken from the same source in the Dohrmann furnace module as that used for the standard inlet heater block. The thermocouple built into the ceramic insulation is connected to an external meter, with a suitable temperature range.

Injection and flow-rates—

A standard blend containing 178 p.p.m. of dibenzyl disulphide in xylene was used to test the system. Flow requirements were unknown and arbitrary flow-rates of 120 ml min^{-1} of oxygen and 40 ml min^{-1} of helium were used.

The first injections of 5-μl samples indicated a recovery of 98 per cent. However, difficulty was encountered in maintaining a suitable steady injection rate of about 0·2 $\mu l\ s^{-1}$ with the Hamilton 701SN syringe, and sometimes multi-peaks (as opposed to Gaussian peaks) and low recoveries were obtained. A Hamilton Automatic Dispenser, with 0·2-μl increments, was then used, and the effect of using different flow-rates was examined. Gaussian peaks and optimum recovery, 99 to 101 per cent., were obtained with a lower oxygen flow-rate (50 ml min^{-1}) and a higher flow-rate of purge gas (120 ml min^{-1}).

Blending base for oils—

A heavy fuel oil containing 1·85 per cent. w/w of sulphur, blended with xylene to give a sulphur concentration of about 150 p.p.m., again gave low but consistent results (0·80,

0·83 and 0·81 per cent. of sulphur). The probable reason for this was that the mixture contained components of such different boiling-points that distillation was taking place in the injection needle; the solvent was flashing off and leaving some residual sample. Examination of the tip of the platinum needle revealed the presence of partially decomposed carbonaceous material. We therefore blended the fuel with a sulphur-free medicinal white oil, and obtained results of 2·01, 1·98 and 2·04 per cent. of sulphur. The blending oil should have a viscosity such that it can be drawn into the syringe without causing excessive resistance to flow. If necessary, a small amount (5 to 10 per cent.) of petroleum spirit of boiling range 80 to 100 °C can be added to the blending base to produce a suitable mixture.

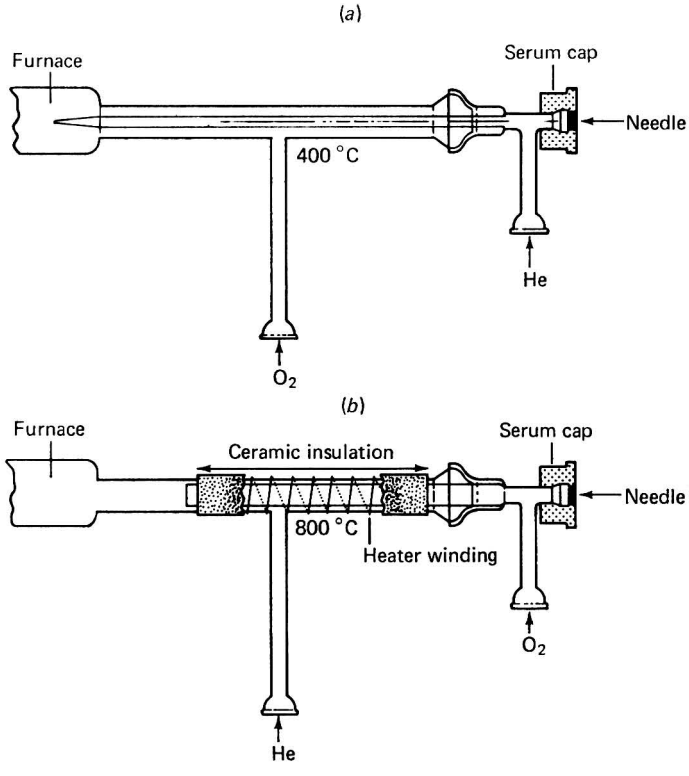


Fig. 1. Modifications to inlet end of quartz tube. (*a*) Original system: (*i*) sample pyrolysed in helium, (*ii*) meets oxygen in the furnace and (*iii*) needle inserted to centre of inlet heater. (*b*) Modified system: (*i*) sample burnt in oxygen, (*ii*) helium transports oxidised products and (*iii*) needle inserted only to inlet of hot zone

Experimental

Operating conditions for the Dohrmann microcoulometer are given in Table III.

Preparation of apparatus—

Adjust the temperature of the inlet heater to 800 °C, the centre furnace to 900 °C and the outlet furnace to 800 °C. Adjust the oxygen flow-rate to 50 ml min^{-1} and the helium flow-rate to 120 ml min^{-1}.

The initial preparation of the cell is described in the Dohrmann Manual. On a daily basis, rinse the cell and side-arms with distilled water, followed by the freshly prepared diluted electrolyte (10 ml of 0·5 per cent. w/v potassium iodide, 10 ml of 2·0 per cent. w/v sulphamic acid and 80 ml of distilled water). Most of the 100-ml volume of electrolyte should be used so that both side-arms are thoroughly flushed with the fresh electrolyte.

With the coulometer set to "Low Gain" and the measuring resistance at 100 Ω, turn the coulometer function switch to "Generator Read" and adjust the bias potential until the generator potential is zero. Turn the coulometer function switch to "Operate," set the recorder range at 20 mV full scale and adjust the recorder to zero (Note 1).

NOTE 1—

The recorder should be previously adjusted to +5 per cent. of full scale as the zero, so that the extent of over-titration can be recorded.

Decrease the bias potential in small steps (10 mV) and examine the recorder trace. When the over-titration becomes small, inject a 5-μl standard and examine the trace. It is likely that no further reduction in bias potential will be necessary. (Too much reduction in bias potential leads to broad tailing peaks.)

TABLE III

OPERATING CONDITIONS FOR THE MODIFIED PROCEDURE

Furnace temperatures—	
Inlet (oxidation)/°C	800
Centre/°C	900
Outlet/°C	800
Flow rates—	
Oxygen/ml min^{-1}	50
Helium/ml min^{-1}	120
Cell—	
Electrolyte	KI - NH_2SO_3H
Bias/mV	120 to 140
Gain	Low

PROCEDURE—

Flush the syringe several times with the standard sulphur solution and eliminate air bubbles. Adjust the volume of the syringe barrel to 5 μl, wipe the needle with a tissue, and insert the needle through the serum cap until it is level with the inlet end of the hot zone. Inject the solution in increments of about 0·2 μl s^{-1} with the automatic dispenser. Withdraw the needle and observe the trace of the titration on the recorder chart. Insert the needle in the solution again and draw up sufficient liquid to permit the gas space to be measured. This space is equivalent to the volume of solution that has evaporated from the needle during insertion near the hot zone, and should be added to the 5-μl volume of solution injected from the syringe barrel.

Mark the total sample volume on the chart and also the coulometer resistance and the recorder range. Repeat the injection of standard solution twice more, or until repeatable results are obtained.

Weigh the sample (Note 2) by difference from a suitable glass rod or spatula into a 25-ml flask, using the values given below as a guide—

Sulphur, per cent. w/w	<0·5	0·5 to 2·0	>2·0
Sample weight/mg	500 to 250	250 to 100	100 to 50

Weigh into the flask 5 to 10 g of blending oil and mix the contents of the flask thoroughly. Warm the flask, if necessary, to facilitate mixing.

Inject 5-μl samples at the inlet end of the hot zone. Measure the gas space (Note 3) caused by volatilisation from the needle, as described above. Observe the trace and mark the chart.

NOTE 2—

Sample sizes can be reduced by factors of 10 and 100, if necessary, by adjusting the instrument settings.

NOTE 3—

In order to measure the gas space when white oil is used as the diluent, extra care is required so that the syringe plunger is withdrawn without air bubbles entering the syringe.

CALCULATION—

Instrument constant, K—The equation

$$\text{Sulphur, p.p.m. w/w} = \frac{\text{Peak area (cm}^2\text{)} \times \text{Recorder sensitivity (mV cm}^{-1}\text{)} \times 16 \times 10^6}{\text{Chart speed (cm s}^{-1}\text{)} \times F \times \text{Resistance } (\Omega) \times W \times 96\,500}$$

where W mg is the weight of sample and F is a factor defined below, can be simplified by the use of a constant, K, which incorporates all those terms that remain constant during operation.

For example,

$$\text{K} = \frac{\text{Recorder sensitivity (mV cm}^{-1}\text{)} \times 16 \times 10^6}{\text{Chart speed (cm s}^{-1}\text{)} \times 96\,500}$$
$$= 15{\cdot}54$$

if (*i*) recorder range is 10 mV full scale; (*ii*) measuring resistance is 100 Ω; and (*iii*) recorder span is 16 cm (Servoscribe, Model RE512).

Other values of K can be read from Table IV, or calculated from it.

TABLE IV

INSTRUMENT CONSTANTS, K

Resistance/Ω	Recorder range/mV	K
999	5	0·777
999	10	1·554
999	20	3·108
999	50	7·77
100	5	7·77
100	10	15·54
100	20	31 08
100	50	77·7

Factor, F—

$$F = \frac{\text{Area} \times \text{K}}{\text{Sulphur content in standard (p.p.m.)} \times W \text{ (mg)}}$$

Sulphur content—

$$\text{Sulphur content} = \frac{\text{Area} \times \text{K}}{F \times W \text{ (mg)}} \text{ p.p.m.}$$
$$= \frac{\text{Area} \times \text{K}}{F \times W \text{ (mg)} \times 10^4} \text{ per cent. w/w}$$

RESULTS

The modified procedure was used for the determination of sulphur in oil fractions obtained from preparative layer chromatographic separations and in fuel oils. The fractions from preparative layer chromatography (2 to 20 mg according to availability) were diluted with white oil (5 to 10 g) and shaken thoroughly. Results for coulometric determinations on 5-μl samples of the blends are given in Table V.

TABLE V

SULPHUR CONTENTS OF AROMATICS AND PREPARATIVE LAYER CHROMATOGRAPHIC FRACTIONS FROM BASE OILS

		Sulphur content referred to total aromatics, per cent. w/w				
Original oil	Sulphur content in aromatics (by coulometry), per cent.	F1 (resins and other polar compounds)	F2 (polyaromatics with some diaromatics)	F3 (monoaromatics with some diaromatics)	Total (F1 + F2 + F3)	Recovery, per cent. w/w
A	0·90	0·02	0·44	0·44	0·90	100
B	0·74	0·36	0·05	0·30	0·71	96
C	1·20	0·06	0·59	0·49	1·14	95
D	1·16	0·08	0·69	0·44	1·21	104
E	0·96	0·05	0·49	0·33	0·87	91
F	1·09	0·06	0·65	0·47	1·18	108
G	1·15	0·16	0·52	0·39	1·07	93
H	0·97	0·05	0·51	0·36	0·92	95

Fuel oils (30 to 200 mg according to sulphur content) were diluted with about 10 g of diluent. Results for 5-μl samples are given in Table VI.

The repeatability of the modified procedure, which is of importance in view of the current interest in improved methods for determining the sulphur content of fuel oils, was assessed by analysing several separate blends of the same two fuel oils. The results are given in Table VII.

Table VI

Sulphur content of fuel oils

Sample	Sulphur contents determined by— quartz-tube (IP 63/55) method, per cent. w/w	Dohrmann method, per cent. w/w
Automotive gas oil	0·25	0·274, 0·275, 0·277, 0·270, 0·275
	0·60	0·597, 0·600, 0·597
Marine diesel oil	1·05	1·08, 1·10, 1·10, 1·06, 1·09
Medium fuel oil	1·85	2·05, 2·01, 2·06, 2·08, 2·05
Heavy fuel oil	2·20	2·23, 2·23, 2·27, 2·22, 2·23
Marine fuel oil	3·05	3·10, 3·14, 3·27, 3·16, 3·21
Very heavy fuel oil	3·5	3·51, 3·51, 3·49, 3·55, 3·49
Long residue	4·05	4·07, 4·04, 4·11, 3·98, 4·00, 4·00

Statistical analysis of the results given in Table VI showed that the repeatability is considerably better than those of the bomb, quartz-tube and flask combustion methods in the range 0·2 to 3 per cent. of sulphur; above 3 per cent. of sulphur the repeatability of the bomb method is greater, but those of the quartz-tube and oxygen-flask combustion methods remain lower than that of the modified coulometric method.

Table VII

Sulphur content of diluted heavy fuel oils

Material and number of diluted sample	Sulphur content, per cent. w/w*	Average, per cent. w/w
Heavy fuel oil containing 2·20 *per cent. w/w of sulphur*†—		
I	2·23, 2·23, 2·27, 2·22, 2·23	2·24
II	2·15, 2·22, 2·19, 2·14, 2·16	2·17
III	2·12, 2·18, 2·19, 2·12, 2·13	2·15
IV	2·23, 2·23, 2·21, 2·23, 2·22	2·22
	Over-all average	2·20
Heavy fuel oil containing 0·94 *to* 0·98 *per cent. w/w of sulphur*‡—		
I	0·97, 1·00, 0·97, 0·99, 0·98, 0·98	0·98
II	0·98, 0·96, 0·96, 1·00, 0·96	0·97
III	1·00, 0·98, 0·98, 0·99, 0·97, 0·97	0·98
IV	1·02, 1·02, 1·00, 1·01, 1·02	1·01
V	0·99, 0·99, 1·00, 0·99, 0·95, 0·98	0·98
	Over-all average	0·98

* Results for separate 5-μl injections of each sample.
† Determined by quartz-tube method.
‡ American Petroleum Institute correlation sample.

Discussion

The "oxidising" injection, together with optimum flow-rates and a suitable dilution medium, provide adequately for the determination of sulphur in oils that have previously proved to be difficult or even impossible to analyse by the coulometric procedure. Reference has previously been made to our experience with the operating conversion factor. We have shown that during the development of the modified procedure, the optimum conversion factor reached 100 per cent. This level was maintained during all the work on fuel oils, which had been started with a freshly prepared cell. All the results for oil fractions were obtained after the cell had been cleaned out and prepared afresh. Complete (100 per cent.) conversion of the sulphur into sulphur dioxide was not reached again, either with the present cell or with another one that was also used. Conversion sometimes reached 90 per cent. but was generally about 85 per cent., which is better than in the original procedure. In

conclusion, therefore, it seems that one particular preparation, notably of the iodine reference electrode, was solely responsible for a prolonged period of operation at 100 per cent. efficiency.

While it would be of interest and value to be able to repeat these conditions again, and thus eliminate the need for the use of a conversion factor, failure to achieve these conditions does not impair the use of the modified procedure for the analysis of oils.

References

1. Coulson, D. M., and Cavanagh, L. A., *Analyt. Chem.*, 1960, **32**, 1245.
2. ——, ——, "Microcoulometric Detection in Gas Chromatography," Pittsburgh Conference on Analytical and Applied Spectroscopy, March, 1961.
3. Killer, F. C. A., and Underhill, K. E., *Analyst*, 1970, **95**, 505.
4. Marsh, W. W., jun., *Analyt. Lett.*, 1970, **3**, 341.
5. "A.S.T.M. Tentative Method of Test for Trace Quantities of Total Sulphur in Volatile Organic Liquids (Microcoulometric Method)," American Society for Testing Materials, Philadelphia, 1969.
6. Crawford, A. R., and Dyroff, G. V., "An Evaluation of Methods for Determining Sulphur in Fuel Oils," American Petroleum Institute, Washington, D.C., 1969.

Received *March 9th*, 1972
Accepted *April 7th*, 1972

The Effect of Infrared Radiation on the Potentiometric Behaviour of Gold-wire Electrodes

By M. R. DHANESHWAR and R. G. DHANESHWAR

(*Analytical Division, Bhabha Atomic Research Centre, Modular Laboratories, Trombay, Bombay-85, A.S., India*)

Gold-wire and infrared-irradiated gold-wire electrodes have been used in potentiometric titrations in conjunction with the saturated calomel electrode or molybdenum-wire reference electrode. The step heights obtained in pH titrations by using the gold electrode are less than those obtained with other pH electrodes. In iron(II) - potassium dichromate titrations, the performance of the gold electrode is comparable with that of the platinum electrode, whereas in iron(II) - potassium permanganate titrations, a double step is obtained with reductant and oxidant solutions of 10^{-5} and 10^{-4} N, respectively.

When the infrared-irradiated electrode is used in pH titrations, the curve height increases by 200 mV. A similar increase is also obtained for iron(II) - potassium dichromate titrations, but not for iron(II) - potassium permanganate titrations. However, the asymmetry in the permanganate titration vanishes, giving rise to a single curve. The reasons for this behaviour are discussed.

It is well known that light affects the performance of electrodes, especially photosensitive electrodes such as those of silver and silver halides.[1–4] Recently, the study of these electrodes has been extended to include the glass electrode, which is not truly photosensitive.[5] The gold electrode, which shows photosensitivity, was chosen for this study because irradiation of the electrodes by ultraviolet or infrared rays may induce some changes in the surface conditions which, in turn, might cause differences in their behaviour.

Moreover, unlike the platinum electrode, the gold electrode is not widely used in potentiometric work, probably because of its sluggishness. In the present work, unirradiated and irradiated gold indicator electrodes were used in pH and redox titrations at various concentrations. The reference electrodes used were the saturated calomel electrode (S.C.E.) and the molybdenum-wire electrode, the latter having been established[6] as a very useful secondary reference electrode. The effect of irradiation on both the gold and molybdenum electrodes could thus be studied. Throughout this paper gold* and molybdenum* denote infrared-irradiated electrodes.

Experimental

Apparatus—

The potentiometric work was carried out on a Vibron, Model 33B-2, direct-reading potentiometer (Electronic Instruments Ltd.).

Infrared lamp—A Philips 250-W lamp was used.

Electrodes—

A gold-wire electrode of length either 10 or 5 mm and diameter 1 mm was prepared by sealing the wire in a soft-glass tube of length 3 inches and diameter 5 mm. Platinum-wire (length 14 mm and diameter 1 mm) and molybdenum-wire (length 12 mm and diameter 1 mm) electrodes were prepared by sealing the respective wires in Pyrex glass tubing. A Beckmann dip-type calomel electrode was used as a reference electrode.

Cell—

A 50-ml beaker fitted with a rubber bung that had five holes served as the potentiometric cell. Gold, platinum, molybdenum and standard calomel electrodes were inserted through the four holes and into the fifth hole the tip of the burette was inserted. A magnetic stirrer was used to stir the solution.

CHEMICAL SOLUTIONS—

Stock 0·1 N solutions of sulphuric acid, sodium hydroxide, potassium permanganate, potassium dichromate and ammonium iron(II) sulphate were prepared from their respective analytical-reagent grade reagents and standardised according to well established procedures. Subsequent dilutions were made from these stock solutions.

PROCEDURE—

The electrode to be irradiated was placed directly beneath and at a distance of 17 cm from the infrared lamp, generally for a period of 2¼ hours. The lamp was then switched off and the electrode was allowed to cool for 1½ hours. Together with the reference electrode, it was then dipped into the solution to be titrated and the titration was performed in the usual way. The end-points obtained with the gold indicator electrode, whether unirradiated or irradiated, in both pH and redox titrations were checked with visual end-points whenever possible and were found to agree to within 0·05 ml.

For the gold - S.C.E. system, pre-treatment of the gold electrode with dilute nitric acid (1 + 1) for 1 minute, and for the gold - molybdenum system, cathodisation at —1·5 V in 1 + 4 sulphuric acid for 2 minutes, were found to give reproducible results with maximum step heights. The latter were measured by using the tangent method, as in polarography.

RESULTS

pH TITRATIONS—

The titrations could be performed only down to 0·01 N concentration with the gold - S.C.E. system. The step height for the gold electrode is much less than those for the molybdenum[7–9] and platinum electrodes,[10] as can be seen in Table I. However, the response of the gold electrode is better than that of the silver electrode as the latter gives no response in pH titrations.[6]

TABLE I

GOLD-WIRE AND IRRADIATED GOLD-WIRE ELECTRODES IN pH TITRATIONS
Apparatus: Vibron electrometer, Model 33B-2

Solution No.	Indicator electrode	Reference electrode	Titrant, H_2SO_4, concentration/N	Titrand, NaOH, concentration/N	Step height/mV	Remarks
1	Au	S.C.E.	0·1	0·1	186	S-type curve
1A	Au	S.C.E.	0·1	0·1	60	S-type curve
2	Mo	S.C.E.	0·1	0·1	344	S-type curve
3	Pt	S.C.E.	0·1	0·1	380	S-type curve
4	Au*	S.C.E.	0·1	0·1	306	S-type curve
4A	Au*	S.C.E.	0·1	0·1	268	S-type curve
4B	Au*	S.C.E.	0·1	0·1	280	Irradiation for ¼ hour
5	Mo*	S.C.E.	0·1	0·1	350	S-type curve
6	Au	Mo	0·1	0·1	125	Peak
7	Au*	Mo	0·1	0·1	203	Peak
8	Au*	Mo	0·1	0·1	50	Peak irradiation for ½ time
9	Au	Mo*	0·1	0·1	175	S-shape curve
10	Au*	Mo*	0·1	0·1	75	Peak
11	Au	S.C.E.	0·01	0·01	110	S-shape curve
12	Au*	S.C.E.	0·01	0·01	238	S-shape curve
13	Au	S.C.E.	0·001	0·001	Nil	—
14	Au*	S.C.E.	0·001	0·001	30	S-shape curve

* Denotes irradiated electrode.
Time of irradiation 2¼ hours, unless otherwise stated.
For 1A, 4A, 11 and 12 the pre-treatment consisted of cleaning with emery paper.

When the gold electrode, after being cleaned with No. 0 emery paper, is irradiated for 2¼ hours, the step height for titration at 0·1 N concentration increases by 208 mV (solutions Nos. 1A and 4A), while the increase is only 120 mV for a gold electrode that has been cleaned with 1 + 1 nitric acid (solutions Nos. 1 and 4). The difference is reduced to 128 mV for 0·01 N

solutions (*cf.* Nos. 11 and 12). At 0·001 N concentration, a suitable titration curve is obtained only for the irradiated electrode. It is of interest to note that when the molybdenum - S.C.E. system is used, with the irradiated molybdenum wire, only a marginal increase of 6 mV is obtained and then only with 0·1 N solutions.

When the time of irradiation is increased for gold* - S.C.E. from ¼ hour to about 2¼ hours, the step height increased from 280 to 306 mV (Nos. 4 and 4B), with no further increase with the time of irradiation.

Four combinations, gold - molybdenum, gold* - molybdenum, gold - molybdenum* and gold* - molybdenum* are possible when the bimetallic gold - molybdenum system is used. When only the gold electrode is irradiated, the step height increases by 78 mV, but is smaller by 103 mV compared with the gold* - S.C.E. system. Reduction of the irradiation time by half, from 2 to 1 hour, results in the significant reduction of 153 mV for the gold* - molybdenum system. The characteristics of the curve obtained with the different possible combinations in a bimetallic system such as gold - molybdenum, in which the electrodes are subjected to irradiation, are summarised in Table I. Generally, irradiation of the molybdenum electrode leads to decreased step heights.

REDOX TITRATIONS WITH THE UNIRRADIATED GOLD - MOLYBDENUM SYSTEM—

With the gold - molybdenum system in potassium permanganate - iron(II) titrations, both electrodes were previously cleaned with No. 0 emery paper.

At higher concentrations, the step height for gold - molybdenum is smaller than that for the platinum - molybdenum system, but as the concentration decreases, the step height for the former system becomes greater than for the latter, as shown in Table II. At an iron(II) concentration of 10^{-4} N, the curve does not rise smoothly near the equivalence point, but the ascending and descending portions appear to have two different slopes because of the sluggishness of gold when changing over to MnO_4^-/Mn^{2+}. With the decrease in iron(II) concentration to 10^{-5} N, two distinct curves are obtained, whereas for the platinum - molybdenum system only one curve is obtained.

TABLE II

GOLD INDICATOR ELECTRODE IN REDOX TITRATIONS

Apparatus: Vibron electrometer, Model 33 B-2; electrode lengths: gold 5, molybdenum 12 and platinum 14 mm; diameter of each electrode 1 mm

Solution No.	Indicator electrode	Reference electrode	Oxidant (titrant) concentration/N	Reductant (titrand), Fe^{2+}, concentration/N	Step height/mV	Remarks
1	Au	Mo	10^{-1} $KMnO_4$	10^{-1}	497	S-type curve
2	Pt	Mo	10^{-1} $KMnO_4$	10^{-1}	628	S-type curve
3	Pt	S.C.E.	10^{-1} $KMnO_4$	10^{-1}	660	S-type curve
4	Au	Mo	10^{-2} $KMnO_4$	10^{-2}	456	S-type curve
5	Au	Mo	10^{-3} $KMnO_4$	10^{-3}	352	Double wave discernible
6	Au	Mo	10^{-3} $KMnO_4$	10^{-4}	380	Double wave discernible
7	Au	Mo	10^{-4} $KMnO_4$	10^{-5}	363	Double wave (Height of the first wave, 210 mV)
8	Au	Mo	10^{-5} $KMnO_4$	10^{-6}	16	Single wave
9	Pt	Mo	10^{-3} $KMnO_4$	10^{-4}	335	Single wave
10	Pt	Mo	10^{-4} $KMnO_4$	10^{-5}	185	Single wave
11	Pt	Mo	10^{-5} $KMnO_4$	10^{-6}	Nil	—
12	Au	Mo	10^{-1} $K_2Cr_2O_7$	10^{-1}	206	S-type curve
13	Pt	Mo	10^{-1} $K_2Cr_2O_7$	10^{-1}	245	S-type curve
14	Pt	S.C.E.	10^{-1} $K_2Cr_2O_7$	10^{-1}	220	S-type curve
15	Au	Mo	10^{-2} $K_2Cr_2O_7$	10^{-2}	143	S-type curve

The indication of the equivalence point at an iron(II) concentration of 10^{-6} N is obtained for the gold - molybdenum system but not for the platinum - molybdenum system. At this concentration also, two curves are obtained for the former system, although the second curve is very poorly formed. The equivalence point is obtained from the first wave, as can be verified from the results of the titration of 10^{-4} N potassium permanganate against 10^{-5} N iron(II) with the platinum - molybdenum system, in which only a single curve is obtained.

Gold - molybdenum system in titrations of iron(II) with potassium dichromate—For titrations at 0·1 N concentration, the step heights for the gold - molybdenum and platinum-molybdenum systems are reduced by 300 and 375 mV, respectively, compared with the permanganate titration (Table II). The titrations were performed only down to 0·01 N concentration, below which the curve becomes deformed.

REDOX TITRATIONS WITH THE GOLD* ELECTRODE—

Gold - molybdenum system in titrations of iron(II) with potassium permanganate—Compared with the gold electrode, the step heights obtained with the gold* electrode are smaller by 7 to 10 mV down to an iron(II) concentration of 10^{-4} N, the most significant change occurring in 10^{-5} N solution. Instead of a double curve, only a single curve is obtained for the gold* electrode (Fig. 1). The height of this wave is 250 mV compared with the height of 210 mV of the first wave obtained for the unirradiated electrode. However, no end-point could be detected at 10^{-6} N concentration (Table III).

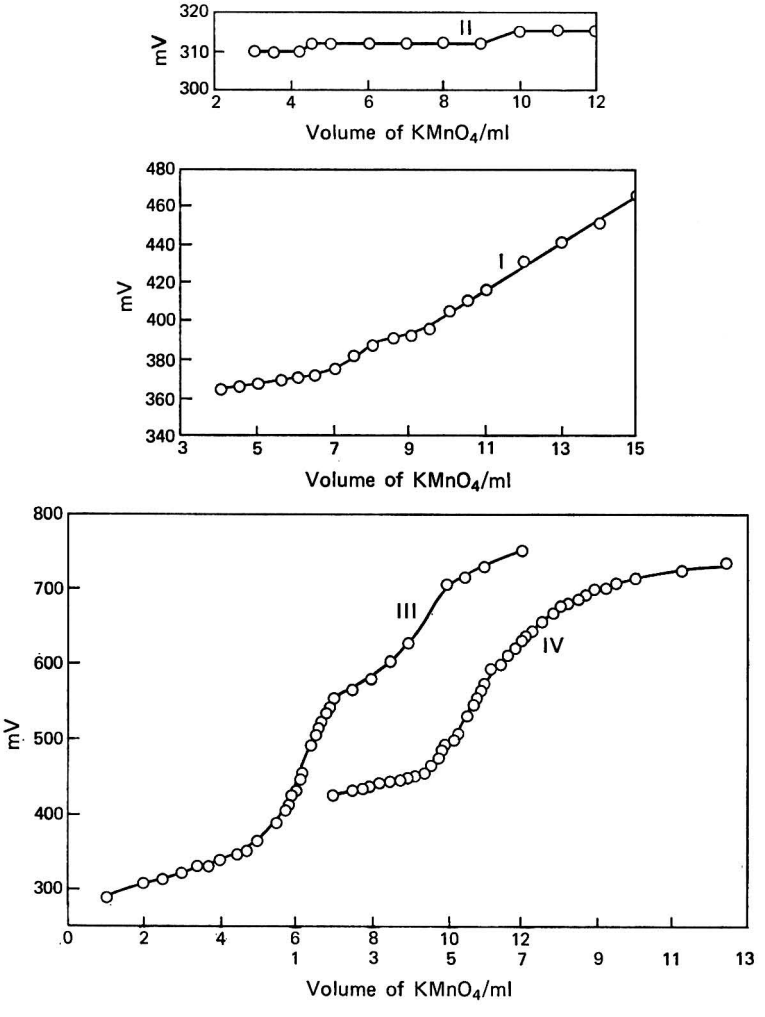


Fig. 1. Gold - molybdenum electrode in redox titrations of $KMnO_4$ *versus* Fe^{2+}: I, gold unirradiated, 10^{-5} N $KMnO_4$, 10^{-6} N Fe^{2+}; II, gold irradiated, 10^{-5} N $KMnO_4$, 10^{-6} N Fe^{2+}; III, gold unirradiated, 10^{-4} N $KMnO_4$, 10^{-5} N Fe^{2+}; and IV, gold irradiated, 10^{-4} N $KMnO_4$, 10^{-5} N Fe^{2+}

For dichromate titrations, gold irradiation alone is of no avail, as the curve obtained is deformed. When, however, both the gold and molybdenum electrodes are irradiated at 0·1 N concentration, a curve height of 345 mV is obtained compared with 206 mV with the unirradiated electrode (Table III). For permanganate titrations also a very marked increase of almost 100 mV in the curve height is obtained at 0·1 N concentration when both gold and molybdenum-wire electrodes are irradiated.

TABLE III

IRRADIATED GOLD ELECTRODE IN REDOX TITRATIONS

Apparatus: Vibron electrometer, Model 33B-2; reference electrode: molybdenum; electrode lengths: gold 10 and molybdenum 12 mm; electrode diameter 1 mm for both electrodes

Solution No.	Oxidant (titrant) concentration/N	Reductant (titrand) concentration/N	Step height/mV	Remarks
	$KMnO_4$	Fe^{2+}		
1	10^{-1}	10^{-1}	490	S-type curve
2	10^{-2}	10^{-2}	428	S-type curve
3	10^{-3}	10^{-3}	345	S-type curve
4	10^{-3}	10^{-4}	370	S-type curve
5	10^{-4}	10^{-5}	250	Single S-type curve
6	10^{-5}	10^{-6}	Nil	—
7	10^{-1}	10^{-1}	584	Both Au and Mo electrodes were irradiated
	$K_2Cr_2O_7$	Fe^{2+}		
8	10^{-1}	10^{-1}	345	S-type curve, both Au and Mo electrodes were irradiated

PLATINUM - MOLYBDENUM SYSTEM—

The irradiated platinum electrode shows peculiar properties. For titration at 0·1 N concentration, the wave height is reduced by 58 mV compared with the unirradiated system, whereas at an iron(II) concentration of 10^{-5} N, the height increases by 53 mV with the irradiated electrode. However, no curve is obtained at 10^{-6} N concentration.

DISCUSSION

pH TITRATIONS—

Any metal electrode functions as a pH electrode because of the formation of a sparingly soluble oxide or hydroxide layer on its surface. The performance of the electrode is always better if an oxide of lower valency is present, as was confirmed earlier with the molybdenum electrode.[9]

However, gold is too resistant to oxidation and oxides of lower valency have a strong tendency to disproportionate.[11] Therefore, a gold - gold(II) oxide electrode could not be prepared and the oxide film formed on the electrode surface is reported to be gold(III) oxide.[11] Because of the presence of this layer of oxide of higher valency, it can be appreciated that in pH titrations the performance of the gold electrode is not as satisfactory as that of the molybdenum electrode, on the surface of which oxide of lower valency is present. Another reason is that, unlike platinum, gold is a non-occlusive element and does not occlude oxygen to any significant extent.

Under the influence of irradiation, some interconversion of gold(III) oxide into its only other stable oxide of lower valency, gold(II) oxide, must occur, as the performance of this electrode for pH titrations greatly improves. Moreover, the effect of time of irradiation suggests that this conversion reaches a steady state and is stable over a long period of time. Another observation that supports the above considerations is the behaviour of the irradiated molybdenum electrode. As, initially, the oxide layer on the surface of the molybdenum is in its lowest valency state, it cannot be reduced to a still lower valency state after irradiation, and hence the irradiated molybdenum electrode shows no change from that obtained with the unirradiated electrode.

It may be stressed here that the changes observed with the irradiated electrodes are not caused by the heating effect. The heated electrode suffers a loss in efficiency, thus reducing the step height resulting from the formation of oxides of higher valency on the electrode surface. Moreover, the results are not reproducible with the heated electrode and, therefore, have not been tabulated.

REDOX TITRATIONS—

The noble metals act only as electron exchangers in the redox systems and hence the nature of the oxide or hydroxide layer is not as important in these titrations as in pH titrations. Consequently, irradiation of the gold electrode has only marginal effects as regards curve heights.

The effect of irradiation is observed in another way in these titrations. The titration reaction is asymmetrical, because, as stated,[12] for every MnO_4^- ion, five Fe^{2+} ions are required, resulting in asymmetry of the titration curve around the equivalence point. Before this point is reached the potential-determining couple is Fe^{2+}/Fe^{3+} and afterwards it is MnO_4^-/Mn^{2+}. Around the equivalence point, the gold electrode becomes sluggish in changing over from one couple to another. This effect is manifested in the formation of a double curve at an iron(II) concentration of 10^{-5} N. With prolonged irradiation of the electrode, the lattices and grain structure of the electrode material undergo some change, which facilitates the electrode response, thus enabling it to change over from one potential-determining couple to another much more rapidly so that the delay is not manifested in the appearance of two curves.

Another noteworthy feature is the increased susceptibility to mutual polarisation when the electrodes of the bimetallic system are irradiated, with the consequent steep increases in curve heights, even though the molybdenum electrode is a constant-potential electrode and its irradiation alone does not give satisfactory results. This aspect merits further detailed study.

We thank Dr. M. Sankar Das, Head of the Analytical Division, for his keen interest in this work.

REFERENCES

1. Dhaneshwar, R. G., Ph.D. Thesis, University of Exeter, 1962.
2. Moody, G. J., Oke, R. B., and Thomas, J. D. R., *Analyst*, 1969, **94**, 803.
3. Van Peski, A. C. H., de Voogs, D. A., and Engel, D. J. C., *Nature, Lond.*, 1969, **223**, 177.
4. Hill, M. P. L., Moody, G. J., Oke, R. B., and Thomas, J. D. R., *Lab. Pract.*, 1970, **19**, 593.
5. Milward, A. F., *Analyst*, 1969, **94**, 154.
6. Dhaneshwar, M. R., Ph.D. Thesis, University of Poona, 1967.
7. Athavale, V. T., Dhaneshwar, M. R., and Dhaneshwar, R. G., *Indian J. Chem.*, 1969, **7**, 602.
8. ——, ——, ——, *Ibid.*, 1969, **7**, 1221.
9. Athavale, V. T., Apte, V. P., Dhaneshwar, M. R., and Dhaneshwar, R. G., *Ibid.*, 1968, **6**, 656.
10. Apte, V. P., and Dhaneshwar, R. G., *Talanta*, 1966, **13**, 1595.
11. Ives, D. J. G., and Janz, G. J., "Reference Electrodes," Academic Press, New York, 1961. pp. 333 and 481.
12. Lingane, J. J., "Electroanalytical Chemistry," Second Edition, Interscience Publishers, New York, 1958, p. 139.

Received *January 10th*, 1972
Accepted *March 14th*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 626–633

The Determination of Ammonia in Condensed Steam and Boiler Feed-water with a Potentiometric Ammonia Probe

BY D. MIDGLEY AND K. TORRANCE

(*Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey*)

An electrochemical probe, based on the diffusion of gas across a polymer membrane, has been investigated for possible use in determining ammonia in condensed steam and boiler feed-water. A simple method that gives results of adequate precision and accuracy has been developed; the standard deviations at concentrations of 1·0, 0·5 and 0·1 μg ml^{-1} were 0·05, 0·01 and 0·005 μg ml^{-1}, respectively. Results obtained by the method described and by the ammonium-sensitive glass electrode method differed by a maximum of 6 per cent. and an average of only 0·3 per cent. Of the substances to be expected in feed-water, only cyclohexylamine and octadecylamine had a significant effect. For routine purposes this method is preferred to the more precise, but more time consuming, indophenol blue method. The principal advantage of this probe over the ammonium-sensitive glass electrode is that no correction is required in the presence of alkali metal ions.

AMMONIA is present in the steam - water circuit of most power stations utilising high pressure steam either from direct addition or from decomposition of hydrazine. Concentrations normally in the range from 0·1 to 1·0 μg ml^{-1} are required to be measured with a precision of 10 per cent. Absorptiometric analysis by use of the indophenol blue method,[1] although of high precision, is time consuming. A recently developed method involving the use of an ammonium-sensitive glass electrode[2] is simple and rapid, if less precise, and has been shown to be suitable for most power station applications, despite interferences that occur in solutions containing alkali metal ions.

Electrodes, based on the Severinghaus principle[3] for determining carbon dioxide, have been available commercially for some time, and have been reviewed by Severinghaus[4] and Smith and Hahn.[5] A version is now available for determining ammonia (Electronic Instruments Ltd.). Alkali metal ions do not interfere with this ammonia sensor, which is similar in precision and convenience to the ammonium-sensitive glass electrode. The term probe, as distinct from electrode, is used hereafter, as the probe is itself an electrochemical cell and no external reference electrode is necessary.

BASIS OF THE METHOD—

The sensing surface of a flat-ended, glass pH electrode is pressed tightly against a hydrophobic polymer membrane that is acting as a seal for the end of a tube containing ammonium chloride solution. Only a thin film of solution remains between the membrane and the glass electrode and this is virtually sealed off from the bulk of the solution, in which is immersed a silver - silver chloride electrode. The membrane permits the diffusion of free ammonia, but not of ions, from the sample to the electrolyte film. Little, if any, transport of water takes place and it is neglected in the following considerations. At equilibrium the partial pressures of ammonia on either side of the membrane are equal, and so changes in the ammonia concentration in the sample are reflected by activity changes in the film, and thus indirectly by pH changes arising from the ammonium - ammonia equilibrium:

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

The relationship between the e.m.f. of the probe and the ammonia concentration in the film can be established by considering the cell

$$\underset{\text{(bulk)}}{Ag|AgCl|m\text{M } NH_4Cl|}\underset{\text{(film)}}{m\text{M } NH_4Cl,\ v\text{ M } NH_3|\text{glass electrode}}$$

where mM is the molar concentration of the ammonium chloride solution and v is the number of moles of ammonia that have diffused through the membrane per litre of solution in the

film. The potential (E) generated by this cell can be described by

$$E = E^{\circ}_{\text{glass}} + k \log a_{H^+} - E^{\circ}_{\text{Ag . AgCl}} + k \log a_{Cl^-} \quad .. \quad .. \quad (1)$$

where $k = 2{\cdot}3 \frac{RT}{F}$. Collecting the constant terms, we obtain

$$E = E' + k \log a_{H^+} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

From its dissociation equilibrium, the dissociation constant, K, of the ammonium ion is

$$K = \frac{a_{H^+} a_{NH_3}}{a_{NH_4^+}} = 10^{-9{\cdot}25} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

and we can substitute for a_{H^+} in equation (2)—

$$E = E' + k \log \left(K \frac{a_{NH_4^+}}{a_{NH_3}} \right) \quad .. \quad .. \quad .. \quad .. \quad (4)$$

As the system is at constant ionic strength, the activity coefficients can be assumed to be constant. Collecting the constant terms in equation (4) we have

$$E = E'' + k \log [NH_4^+] - k \log [NH_3] \quad .. \quad .. \quad .. \quad (5)$$

The dissociation constant, K, is so small that changes in the ammonium-ion concentration in the film due to changes in the ammonia equilibrium across the membrane also become very small and for practical purposes the ammonium-ion concentration can be considered to be constant. For example, when using standard solution A (1000 μg ml^{-1} ammonia solution) as the internal filling solution there is less than 1 per cent. variation in the ammonium-ion concentration for an ammonia concentration in the sample ranging from 10^{-3} to 3×10^4 μg ml^{-1}. Within this range, the temperature being constant, we can write equation (5) as

$$E = \text{Constant} - k \log [NH_3]$$

The probe therefore has a Nernstian response to the ammonia concentration in the film and hence to the ammonia concentration outside the membrane.

Experimental

Apparatus—

The probe potentials were measured with an Electronic Instruments Ltd. (E.I.L.) Vibron, Model 33B-2, electrometer used in conjunction with a calibrated back-off device, such that readings were taken from the 30 mV scale. The potentials were recorded on a Sunvic 10S chart recorder.

The ammonia probes were the E.I.L. Laboratory Model 8002-2 and Industrial Model 8002-6, which in all the tests were used in a dip-type mode, *i.e.*, the probe was placed in the test solution, which was contained in a beaker, stirring being effected by a magnetic stirrer.

Between the analyses of the various batches the probe should be stored with its tip immersed in a solution consisting of ten volumes of a standard ammonia solution and one volume of 1 M sodium hydroxide solution; the lowest standard solution used for the analyses is convenient. For periods between use of greater than 2 weeks the probe should be stored with the tip immersed in standard solution A; in this event a short re-activation period will be necessary, similar to that found useful following initial assembly (see below).

Reagents—

Water—Water, low in ammonia, was prepared by passing distilled water (from a Manesty still) through a column of strongly acidic cation-exchange resin in the hydrogen form. This "cation-water" was used unless otherwise stated.

A few experiments were carried out with water prepared by passing distilled water through a mixed-bed de-ioniser ("mixed-bed water") and also with water that was distilled twice before being distilled from alkaline permanganate solution in an all-glass still ("permanganate-water").

Sodium hydroxide solutions—Sodium hydroxide solutions were made up from ampoules of B.D.H. concentrated volumetric reagent.

Standard ammonia solution A—Dissolve 3·141 g (±0·001 g) of ammonium chloride (AnalaR, dried at 100 °C) in water, make up to the mark with water in a 1-litre calibrated flask, and mix. Store the solution in a glass-stoppered glass bottle. This solution was found to be stable for at least 20 weeks.

1 ml of solution ≡ 1000 μg of ammonia.

Standard solution B—Introduce by pipette 50 ml of standard solution A into a 500-ml calibrated flask, make up to the mark with water, and mix. Store in a glass-stoppered glass bottle. This solution was found to be stable for at least 4 weeks.

1 ml of solution ≡ 100 μg of ammonia.

Standard solution C—Introduce by pipette 10 ml of standard solution B into a 1-litre calibrated flask, make up to the mark with water, and mix. Prepare this solution daily as required.

1 ml of solution ≡ 1 μg of ammonia.

All the other chemicals were of AnalaR grade, except morpholine, cyclohexylamine, hydrazine and octadecylamine, which were of analytical-reagent grade.

Sample collection—

Ammonia may be lost from samples at temperatures above 50 °C. Samples should be collected at below 40 °C, by using a cooling coil if necessary. The sample bottle should be filled completely and immediately stoppered.

Because the concentration of ammonia in the sample tends to decrease with time, the sample should be analysed without undue delay, and in any event within 8 hours.

Analytical procedure—

Solutions are prepared for analysis by adding one volume of 1 M sodium hydroxide solution to ten volumes of sample or standard ammonium chloride solution. A 25-ml beaker containing 20 ml of sample and 2 ml of base, together with a magnetic follower, is almost filled when the probe is inserted and the exposed surface of the solution is small, thus reducing losses of ammonia during measurement. Losses are also reduced by not adding the base until the probe is about to be immersed. Ideally, the measurements should be made at a fixed temperature.

Remove the probe from the solution in which it is stored, rinse it with distilled water and immerse it in a beaker of distilled water stirred by means of a magnetic stirrer. Prepare a test solution for analysis, remove the probe from the distilled water, dry it with a soft tissue, and immerse it in the test solution. Stir, and when the probe gives a steady reading, usually within 5 minutes, note the millivolt reading. Then return the probe to the beaker of distilled water.

In a batch of analyses a test solution prepared from standard solution C (1 μg ml^{-1} ammonia solution) is analysed first, followed by the unknown sample solutions. The frequency of re-standardisation depends on the stability of the equipment and the precision required. Let the millivolt readings for a solution prepared from standard C and an unknown sample be V_S and V_x, respectively. The concentration, C, of ammonia in the sample, can be calculated from

$$C = \text{antilog}\left(\frac{V_S - V_x}{k}\right) \mu\text{g ml}^{-1}$$

where k is the slope of the probe calibration graph obtained by the procedure described below.

Preparation of the calibration graph—

By dilution of standard solution B prepare sufficient volumes of 5·0, 2·0, 0·5, 0·2 and 0·1 μg ml^{-1} ammonia solutions to be able to carry out duplicate determinations. These solutions, together with standard solution C, are analysed according to the above analytical procedure. The determinations should be repeated at least once on another day, and then again, as required, until the calibration graph is defined with the required precision.

Calculate the average differences in millivolt readings between standard solution C and each of these five solutions. The differences are plotted on the y-axis against the logarithm of the ammonia concentration on the x-axis. Standard solution C should be included as the point (log 1, 0).

Although the calibration graph should be linear, in practice it may be slightly curved

below 0·5 μg ml^{-1} because of the presence of ammonia (or other interfering substances) in the water. The slope, k, should be obtained from the linear portion of the graph at concentrations well above the curved section. The slope of the calibration graph increases by approximately 1 mV per decade change in concentration for an increase in temperature of 1 °C. The temperature of the samples and standard should not differ by more than 1 °C and should be within 5 °C of the temperature at which the calibration graph was prepared, if corrections for temperature are to be avoided.

ASSEMBLY AND RESPONSE OF THE PROBE—

Successful operation of the probe depends on the pressure of the glass electrode against the membrane. If insufficient pressure is applied, ammonia is not trapped in a film at the glass surface but diffuses into the bulk of the filling solution. In this event the response time is long (a matter of hours) and the sensitivity low. Too much pressure results in a noisy signal and may even damage the membrane. The probe may have a sluggish response after assembly, and conditioning in a solution comprising ten volumes of 0·1 μg ml^{-1} ammonia solution and one volume of 1 M sodium hydroxide solution for 30 minutes before use is recommended.

SOURCES OF ERROR—

Variation of sensitivity—The probe has been shown to have an essentially Nernstian response but deviations can arise because of faulty glass electrodes, damaged membranes or imperfect assembly of the probe.

Effect of temperature—Changes in temperature affect the sensitivity of the probe, but for most practical purposes this effect is negligible. More important are short-term fluctuations in room temperature (such as may be caused by air-conditioning units) because these affect a number of equilibria in the electrode system. The standard solution should be at room temperature and the samples within 1 °C of this temperature.

Bias—Bias will be produced by any ammonia present in the water used to prepare the standard solutions. The magnitude of the bias can be calculated as follows. From the results used to prepare the calibration graph calculate the concentrations of the nominally 0·1 and 0·2 μg ml^{-1} standard solutions as described in the analytical procedure above. The concentration of ammonia in the water is obtained by subtracting the nominal from the observed concentrations. The mean concentration, δ μg ml^{-1}, of ammonia in the water can then be calculated.

If the value of δ is considered to be significant, the concentration of a sample calculated as in the analytical procedure should be multiplied by a factor of $(1 + \delta)$.

Checking precision and bias—It is recommended that a second standard ammonia solution (with a concentration of 0·1 μg ml^{-1}) should be analysed with each batch of sample and standard determinations. The results obtained are then plotted on a control chart. The ordinate of the chart should be the difference between the millivolt readings obtained with the 1 and 0·1 μg ml^{-1} standards, the standard deviation of which is known from precision tests.

RESULTS

SENSITIVITY OF THE PROBE—

The probes gave a Nernstian response (58·5 mV per decade change in concentration) from 100 to 0·1 μg ml^{-1}, but only when permanganate-water was used. The calibration graph became curved at concentrations of the ammonia solution below 1 μg ml^{-1} with standards made from various batches of cation-water and mixed-bed water, resulting in millivolt differences in the ranges 58 to 54 mV and 54 to 47 mV, respectively, between nominal concentrations of 1·0 and 0·1 μg ml^{-1}. Curvature arises either because the probe does not behave in a Nernstian fashion at low absolute levels of free ammonia, or because of the presence of ammonia or other interfering species in the water used for preparing the standards. The former explanation is unlikely to be correct because pairs of test solutions (with ammonia concentrations of 1·0 and 0·1 μg ml^{-1}) showed no significant differences in Δ mV, whether adjusted to pH 8·7, when only 25 per cent. of the total ammonia is free, or pH 12·8, when the ammonia is 100 per cent. free (see Table I). Assuming that the curvature resulted from residual ammonia in the water, it was calculated that permanganate, cation and mixed-bed waters contained 0·01, 0·01 to 0·03 and 0·03 to 0·09 μg ml^{-1} of ammonia, respectively.

RESPONSE TIME OF THE PROBE—

The probe takes from 2 to 5 minutes after immersion in a solution to reach its equilibrium potential, provided that the solution has a higher free ammonia concentration than the previous one; in the recommended procedure this is always the case as the probe is rinsed in a beaker of de-ionised water between measurements.

INTERNAL FILLING SOLUTION—

In addition to the filling solutions supplied by the manufacturers, ammonium chloride solutions corresponding to 1000, 100 and 10 μg ml^{-1} ammonia solutions were tried. There was no significant difference in sensitivity or response time. We used the 1000 μg ml^{-1} solution for almost all the work reported here because it was convenient to use the stock standard solution as the internal filling solution.

EFFECT OF DIFFERENT pH REGULATORS—

The pH of the test solution governs the free ammonia concentration presented to the probe by samples of a given total ammonia concentration. The effect of different pH regulators was tested and the results are summarised in Table I. Below pH 8, interference from carbon dioxide can become significant.

It can be seen that the pH of the test solution does not affect the sensitivity of the probe, *i.e.*, Δ mV values, although the millivolt reading at a particular total ammonia concentration does depend on the pH of the test solutions. The use of sodium hydroxide solution was preferred because borax solutions gave longer response times. The procedure adopted was to add 1 ml of 1 M sodium hydroxide solution per 10 ml of sample, as this gave the least dilution of the sample and the shortest response time.

The effect of a small change in the concentration of the sodium hydroxide solution was also tested. Alternate measurements were made on 1 μg ml^{-1} ammonia samples, with 1·0M and 0·9 M sodium hydroxide solutions used to control the pH. The millivolt differences between successive samples ($E^{1\cdot0} - E^{0\cdot9}$) were +1·3, −0·2, 0·0, −0·5, −0·3 and 0·0. As the within-batch standard deviation with 1 μg ml^{-1} ammonia solutions was found to be 1·16 mV (from the precision tests) the 10 per cent. variation in concentration of sodium hydroxide solution produced no significant error in the measured concentration of ammonia.

TABLE I

EFFECT OF DIFFERENT pH REGULATORS

Base	Ratio of volume of sample to volume of base	pH	Millivolt readings with total ammonia concentrations: 1 μg ml^{-1}	0·1 μg ml^{-1}	Δ mV
1 M Sodium hydroxide ..	10:1	12·8 (100)*	−104·5	−57·5	47†
0·1 M Sodium hydroxide ..	10:5	12·5 (100)	−100·0	−54·0	46†
0·1 M Sodium hydroxide ..	10:1	11·9 (100)	−98·0	−51·0	47†
0·03 M Borax	10:1	10·3 (90)	−84·0	−37·0	47†
1 M Sodium hydroxide ..	10:1	12·8 (100)	−9·8	+42·2	52
Borax - hydrochloric acid ..	10:1	8·7 (25)	+30·1	+81·8	52

* The figures in brackets denote the calculated percentage of free ammonia at the corresponding pH.

† Standards prepared with mixed-bed water. Standard solution B was used as the internal filling solution.

STABILITY OF SOLUTIONS—

The stability of the test solutions after adjustment of the pH was examined as follows. A series of 25-ml beakers containing 1 ml of 1 M sodium hydroxide solution and 10 ml of 0·1 μg ml^{-1} ammonia solution were allowed to stand for 0, 10, 20 and 30 minutes before the probe was immersed and readings taken. The results are shown in Table II, together with the result for a solution which had stood for 45 minutes in a beaker sealed with Parafilm sealing tissue (Gallenkamp and Co.).

Losses of ammonia from an open beaker caused measurements to change by approximately 10 mV h^{-1}. To minimise these losses when readings were taken for more than 5 minutes, the gap between the top of the beaker and the probe was sealed with Parafilm.

TABLE II

STABILITY OF TEST SOLUTION MADE UP FROM 0·1 μg ml^{-1} AMMONIA SOLUTION

Time before immersion of probe/minutes ..	0	10	20	30	45*
mV reading	70·4	73·8	77·0	78·0	74·4

* Sealed with Parafilm.

TEMPERATURE EFFECTS—

Temperature changes affect the probe in a number of ways: through changes in the slope factor RT/F; through changes in the activity coefficients of ions; through changes in the response of the reference electrodes; through changes in the dissociation constant of the ammonium ion; and through changes in the diffusivity of the membrane. Only the last two features are not common to conventional glass electrode systems.

The effect of temperature was tested by measuring the response of the probe to changes in ammonia concentration at four temperatures (16·0, 20·5, 25·5 and 28·5 °C). At the three higher temperatures the solutions were equilibrated by immersion in a water-bath and the probe was kept in a cabinet maintained at the same temperature. Measurements were made in duplicate. The results given in Table III show that the calibration slope, Δ mV, changes only by the amount expected from consideration of RT/F, although the potentials vary by 1·5 mV °C^{-1}.

TABLE III

EFFECT OF TEMPERATURE

Concentration of ammonia/μg ml^{-1}	Millivolt readings at temperatures of 16·0 °C	20·5 °C	25·5 °C	28·5 °C
1·0	−0·7	+7·5	+13·0	+19·0
0·1	+48·0	+57·5	+64·0	+71·0
Δ mV	48·7	50·0	51·0	52·0

PRECISION—

Five batches of duplicate measurements were made with seven synthetic standards. Concentrations of ammonia solution of 0·05, 0·1, 0·2, 0·5, 1·0 and 2·0 μg ml^{-1} were used. The batches were measured on separate days over a period of 1 week, including the week-end.

The between-batch standard deviations were calculated from the results normalised within each batch with respect to the mean e.m.f. observed with 1 μg ml^{-1} ammonia solutions in that batch. The results are summarised in Table IV.

TABLE IV

PRECISION OF ANALYTICAL RESULTS

Ammonia added/μg ml^{-1}	Mean normalised e.m.f./mV	Ammonia measured/ μg ml^{-1}	Standard deviation*/μg ml^{-1} Within-batch	Between-batch	Total
2·0	−17·8	2·01	0·030	N.S.†	0·050
1·0	0	(1·00)‡	0·050	—	—
0·8	5·3	0·81	0·025	N.S.	0·028
0·5	17·1	0·50	0·010	N.S.	0·012
0·2	38·7	0·21	0·007	N.S.	0·008
0·1	53·2	0·12	0·005	0	0·005
0·05	67·2	0·06	0·004	N.S.	0·006

* Each batch of within-batch and between-batch standard deviations has 5 and 4 degrees of freedom, respectively.

† N.S. not significant.

‡ Standard.

COMPARISON AND DURABILITY OF PROBE ASSEMBLIES—

Three E.I.L. probes of the industrial type, 8002–6, and one of the laboratory type, 8002–2, were tested. A total of five glass electrodes was used in the various assemblies of the industrial probes. All the probes had essentially the same sensitivity and response characteristics.

The probes showed no deterioration in performance over a period of 3 to 4 weeks. One assembly was used intermittently for 2 months, left for 2 months in a test solution prepared from 0·1 μg ml^{-1} ammonia solution, and tested again. The probe showed no loss in sensitivity over the 2 months' use, nor after the 2 months' storage. (Although in the latter instance the probe potential drifted at a rate of 12 mV h^{-1} on being first used, 1 day later no drift was apparent.)

TABLE V

INTERFERENCE EFFECTS

Substance	Concentration of impurity/ μg ml^{-1}	Interference from ammonia (μg ml^{-1}) at ammonia concentrations of 0·1 μg ml^{-1}	Interference from ammonia (μg ml^{-1}) at ammonia concentrations of 1 μg ml^{-1}
Hydrazine	4	0·012	0·06
Cyclohexylamine	4	0·107	0·08
	1	0·023	0·03
Morpholine	10	0·002	0·03
Octadecylamine	0·4	0·026	0·14
Calcium ions	1	0	0·03
Magnesium ions	1		
Copper(II) ions	1		
Zinc ions	1	0·012	0·01
Nickel ions	1		
Carbonate ions	1	−0·004	0·02

INTERFERENCE EFFECTS—

The effects of a number of other substances that may be present in power station waters were tested at ammonia concentrations of 0·1 and 1 μg ml^{-1} by measuring the change in response when the other substances were added to the standard solution.

The results are given in Table V. The only major sources of interference were cyclohexylamine and octadecylamine, which are rarely present in power station waters; if they are present their levels are usually well below those tested.

TABLE VI

AMMONIA CONTENT OF FEED-WATER (F) AND CONDENSATE (C) SAMPLES ANALYSED BY THE AMMONIA PROBE AND AMMONIUM-SENSITIVE GLASS ELECTRODE (G.E.) METHODS

Station	Sample	Ammonia content/μg ml^{-1} Probe	Ammonia content/μg ml^{-1} G.E.	Difference, per cent.	Recovery of spike*/μg ml^{-1} Probe	Recovery of spike*/μg ml^{-1} G.E.
W	C1	0·52	0·50†	+4	0·50	0·50
	C2	0·39	0·38	+2·5	0·51	0·50
	F1	0·58	0·59†	−2	0·48	0·51
	F2	0·49	0·50†	−2	0·51	0·51
X	C1	0·70	0·74	−6	0·48	0·49
	C2	0·57	0·59	−4	0·52	0·50
	F1	0·56	0·59	−6	0·50	0·50
	F2	0·58	0·60	−4	0·52	0·50
Y	C1	0·19	0·19	0	0·20	0·20
	C2	0·19	0·19	0	0·20	0·20
	F1	0·18	0·18	0	0·19	0·20
	F2	0·18	0·17	+5	0·20	0·20
Z	C1	0·52	0·49	+6	0·53	0·51
	C2	0·47	0·46	+2	0·49	0·50
	F1	0·49	0·50	−2	0·53	0·50
	F2	0·49	0·47	+2	0·50	0·49

* Stations W, X and Z spiked with 0·50 μg ml^{-1} of ammonia; station Y spiked with 0·20 μg ml^{-1} of ammonia.

† Corrected for sodium; uncorrected values are 0·54, 0·63 and 0·53 μg ml^{-1}, for C1, F1 and F2, respectively.

ANALYSIS OF POWER STATION FEED-WATER AND CONDENSATE SAMPLES—

Samples of feed-water and condensate from two pairs of sampling points from each of four power stations were collected and duplicate determinations of the ammonia content of each sample were made by using both the ammonia probe and the ammonium-sensitive electrode[2] methods. The sodium content of the samples was determined by flame photometry with a Unicam SP900 flame photometer. Only power station W gave samples with significant sodium levels, and for this station the glass electrode results were corrected by subtracting one quarter of the sodium concentration from the apparent result. The results are summarised in Table VI.

Aliquots of the water samples were spiked with ammonium chloride solution such that the ammonia content of the sample was approximately doubled. Duplicate analyses were carried out and the recoveries calculated. The results are given in Table VI.

Analyses carried out by the two methods differed by a maximum of 6 per cent. and an average of 0·3 per cent. The results show that the probe is suitable for use in power station waters.

This work was performed at the Central Electricity Research Laboratories and the paper is published by permission of the Central Electricity Generating Board.

REFERENCES

1. Tetlow, J. A., and Wilson, A. L., *Analyst*, **1964**, **89**, 453.
2. Goodfellow, G. I., and Webber, H. M., *Ibid.*, **1972**, **97**, 95.
3. Severinghaus, J. W., and Bradley, A. F., *J. Appl. Physiol.*, 1958, **13**, 515.
4. Severinghaus, J. W., *Ann. N.Y. Acad. Sci.*, 1958, **148**, 115.
5. Smith, A. C., and Hahn, C. E. W., *Brit. J. Anaesth.*, 1969, **41**, 731.

Received *December 1st*, 1971
Accepted *March 27th*, 1972

Potentiometric Determination of *p*-Urazine with an Ion-selective Electrode

BY V. P. IZVEKOV, M. KUCSERA-PÁPAY, K. TÓTH AND E. PUNGOR

(Institute for General and Analytical Chemistry, Technical University, Budapest, Hungary)

The behaviour of *p*-urazine in 1 M sodium hydroxide solution when silver nitrate solution is added has been interpreted on the basis of potentiometric and spectroscopic measurements and elemental analysis. A potentiometric method has been developed for the quantitative determination of *p*-urazine.

p-URAZINE is used in the plastics industry as the basic starting material for the synthesis of thermostable resins[1] and in biology as a substance that has biological activity.[2]

Several workers have described methods of synthesis and the elucidation of the structure of *p*-urazine.[3–11] Their results, however, are contradictory in certain respects.

Elemental analysis and X-ray diffraction methods have been used[12] for the determination of *p*-urazine.

In this work, the behaviour of *p*-urazine in alkaline medium when silver nitrate is added has been investigated, and a potentiometric method has been developed for the quantitative determination of *p*-urazine.

EXPERIMENTAL

APPARATUS—

A Radelkis expanded-scale precision pH meter (Radelkis, Budapest, Type OP 205) was used for the potentiometric measurements. A sulphide-selective membrane electrode (OP-S-711-C, Radelkis) was used as an indicator electrode and a saturated calomel electrode (OP-810, Radelkis) as a reference electrode. The junction between the reference electrode and the solution being titrated consisted of a 0·1 M potassium nitrate solution - agar-agar salt bridge.

Ultraviolet spectra were obtained with a Spectromom 202 instrument (MOM, Hungary) and a Unicam SP700 spectrophotometer. Infrared spectra were obtained with a Zeiss UR-10 instrument (Karl Zeiss, Jena).

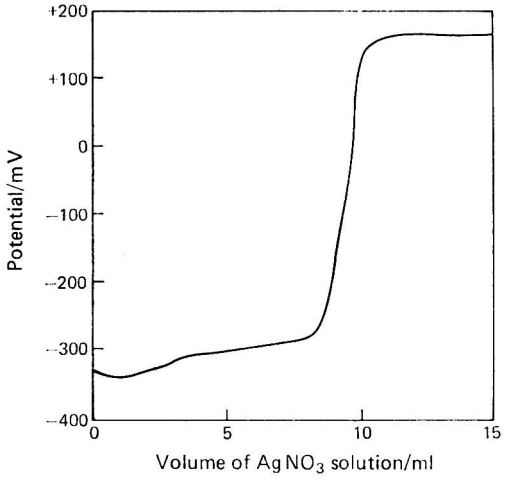


Fig. 1. Potentiometric titration curve of *p*-urazine

REAGENTS—

The reagents used were of *pro analysi* grade. *p*-Urazine was supplied by Rare and Fine Chemicals KEK Laboratories Inc., Plainview, N.Y., U.S.A.

RESULTS AND DISCUSSION

In the titration of *p*-urazine in 1 M sodium hydroxide solution with standard silver nitrate solution, one potential jump appeared in the titration graph (Fig. 1), and a black precipitate was formed. The shapes of the titration graphs were similar for various concentrations of *p*-urazine.

The black precipitate that was formed during the titration was analysed and was found to contain 97 per cent. of metallic silver. This suggested that a redox reaction proceeded during the titration. To verify this assumption, the solution *plus* the precipitate was filtered, the filtrate was cautiously evaporated to dryness and the ultraviolet and infrared spectra of the residue were measured. For comparison, the ultraviolet and infrared spectra of solutions of *p*-urazine in distilled water and in 2 M sodium hydroxide solution were also measured. All the infrared spectra were measured by using potassium bromide pellets (1 mg of *p*-urazine per 100 mg of potassium bromide) in the range 400 to 4000 cm^{-1}.

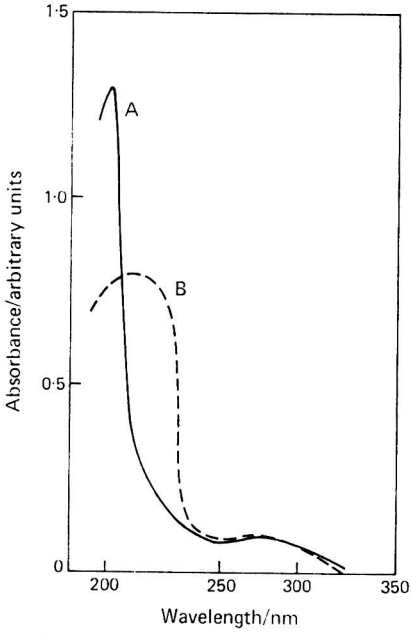


Fig. 2. Ultraviolet spectra of *p*-urazine (A) and oxidised *p*-urazine (B) in water

The ultraviolet spectrum of *p*-urazine in aqueous solution is characterised by two absorption bands at 200 and 280 nm (Fig. 2, A and B). There are σ- and π-bonds in the molecules, and on the hetero-atoms (oxygen and nitrogen) there are also non-bonding pairs (n-electrons), which can easily be excited. The above absorption bands can be ascribed to the carbonyl group in the molecule.

The low-intensity band at 280 nm is due to the forbidden n - π^* transition from the non-bonding n-orbital of the hetero-atom to the antibonding π-orbital. The band of greater intensity at 200 nm is related to the n - σ^* transition.[13]

It is known from the literature that *p*-urazine is weakly acidic, and therefore its ultraviolet spectrum was also measured in 2 M sodium hydroxide solution. The spectrum obtained (Fig. 3, B) is different from that measured in distilled water, in that there are intense bands at 241 and 366 nm, and the 280-nm band that is characteristic of the carbonyl group is absent. In alkaline medium the carbonyl group is present in its enolic form.

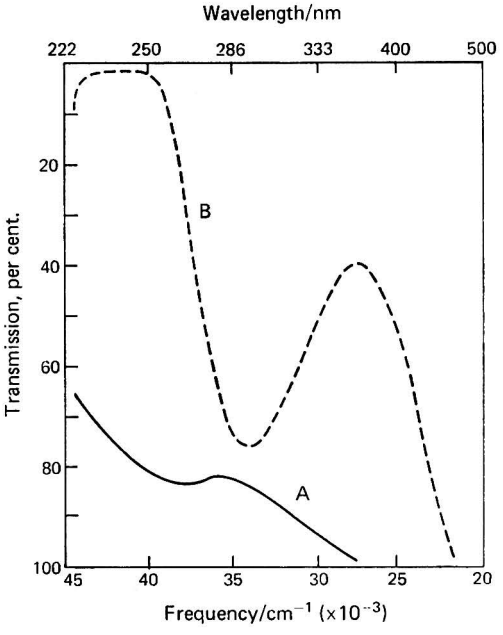
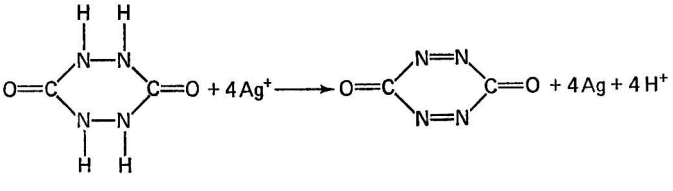


Fig. 3. Ultraviolet spectrum of *p*-urazine: A, in water; and B, in 2 M sodium hydroxide solution

In the infrared spectrum of pure *p*-urazine, we were interested in the positions of the stretching vibrations of the C=O and NH groups. In the range 1600 to 1800 cm^{-1}, three bands occur. The band at 1630 cm^{-1} is due to the deformation vibration of the N–H bond; this band is called the amide II band in the literature.[14] The stretching vibrations of the carbonyl group appear as intense bands at 1690 and 1710 cm^{-1}. Double bands of this type are characteristic of six-membered cyclic imides.[15] The stretching vibrations of the NH group appear at 3220, 3315 and 3420 cm^{-1}, which is characteristic of cyclic compounds that have a *cis* configuration.[16] The band at 3420 cm^{-1} can be interpreted as being due to the vibration of the free NH group, whereas the two other bands are due to hydrogen-bonded NH groups (Fig. 4, A). For the latter bands, the variation in the spectrum with changes in concentration could not be studied owing to the low solubility of *p*-urazine in non-polar solvents. The absence of absorption above 3450 cm^{-1} is evidence for the absence of enolic hydroxyl groups. This evidence is also supported by the very high intensity of the C=O band, which would be less intense if enolisation occurred.

In the titration of *p*-urazine with silver nitrate solution, the following reaction was assumed to take place—



To prove that this reaction occurred, the oxidation product of *p*-urazine, obtained by filtering the solution *plus* precipitate produced in the above reaction and evaporating the filtrate to dryness, was studied by spectrophotometry. In the electronic absorption spectrum of the filtrate, two bands occurred, at $\lambda_{max.} = 212$ nm and $\lambda_{max.} = 276$ nm. These maxima and the absence of other bands suggest that the compound present is different from *p*-urazine.

The fact that the long-wavelength bands of *p*-urazine and the oxidised compound in the filtrate are very close ($\lambda_{max.}$ = 280 nm for *p*-urazine and $\lambda_{max.}$ = 276 nm for the oxidised compound) indicates that carbonyl groups are present also in the oxidised compound.

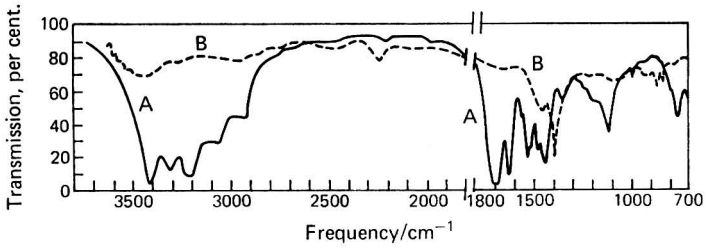


Fig. 4. Infrared spectra of *p*-urazine (A) and oxidised *p*-urazine (B) in potassium bromide pellets

It is interesting to note that the ultraviolet spectrum of the oxidised compound in 2 M sodium hydroxide solution is the same as that in distilled water, which indicates that the oxidised compound is not acidic and does not react with alkali (Fig. 5, B).

In the oxidised compound, polar groups, which could interact with alkali in a similar manner to *p*-urazine, are absent. In the infrared spectrum (Fig. 4, B) the bands due to the NH group are absent. The weak band at 1650 cm^{-1} indicates the presence of carbonyl groups, and the band at 3500 cm^{-1} is due to the moisture present in the sample; the intensity of the latter band changes with changes in the moisture content of the sample. The vibrations of the N=N bond could not be identified.

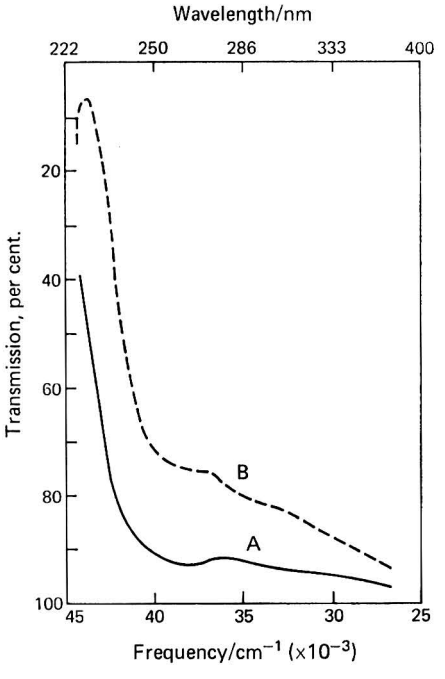


Fig. 5. Ultraviolet spectrum of oxidised *p*-urazine: A, in water; and B, in 2 M sodium hydroxide solution

The spectroscopic results, in accordance with potentiometric results, seem to support the proposed reaction mechanism given above. Furthermore, the potentiometric results can be used for the quantitative determination of *p*-urazine. The determination can be performed on the basis of the potentiometric titration graph, the equivalent weight of *p*-urazine being one quarter of its molecular weight.

The lower limit of the potentiometric determination is 10^{-3} M.

The authors thank Rare and Fine Chemicals KEK Laboratories Inc. for supplying the *p*-urazine.

References

1. Stannett, V. T., and Shibel, F. C., U.S. Patent 2 921 924, January 19th, 1960.
2. Giordano, C., Esposito, R., De Santo, N. G., and De Pascale, C., *Boll. Soc. Ital. Biol. Sper.*, 1964, **40**, 1329.
3. Curtius, T., and Heidenreich, R., *Ber. dt. chem. Ges.*, 1894, **27**, 2684.
4. ——, ——, *J. prakt. Chem.*, 1895, **52**, 454.
5. Linch, F. W., *J. Chem. Soc.*, 1912, **101**, 1755.
6. Stollé, R., *Ber. dt. chem. Ges.*, 1913, **46**, 260.
7. Diels, O., *Ibid.*, 1914, **47**, 2183.
8. Guha, P. C., and De, S. C., *J. Chem. Soc.*, 1924, **125**, 1215.
9. Buckley, G. D., and Ray, N. H., British Patent 622 955, May 10th, 1949.
10. Eley, F., and Meussebois, C., *Bull. Soc. Chim. Belg.*, 1959, **68**, 32.
11. Lutz, A. W., *J. Org. Chem.*, 1964, **29**, 1174.
12. Grove, C. S., Grillet, G. F., and Chang, R. C., *Ibid.*, 1961, **26**, 4131.
13. West, W., *Editor*, "Chemical Applications of Spectroscopy," Part I, John Wiley and Sons Inc., New York, 1968, p. 246.
14. Bellamy, L. J., "Advances in Infrared Group Frequencies," Methuen, London, 1969, p. 287.
15. Dyer, J. R., "Application of Absorption Spectroscopy of Organic Compounds," Khimiya, Moscow, 1970, p. 46.
16. Bellamy, L. J., *op.cit.*, p. 285.

Received *December 6th*, 1971
Accepted *February 10th*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 639–643

The Assay of Potassium 6-[L-(+)-α-Phenoxypropionamido]penicillanate and 6-[D-(+)-α-Phenoxypropionamido]penicillanate in Phenethicillin Potassium by Circular Dichroism Spectrometry

BY J. B. STENLAKE, G. C. WOOD, H. C. MITAL AND SHEENA STEWART

(Department of Pharmaceutical Chemistry, University of Strathclyde, George Street, Glasgow, G1 1XW)

Circular dichroism spectra of potassium 6-[L-(+)-α-phenoxypropionamido]penicillanate and 6-[D-(+)-α-phenoxypropionamido]penicillanate have been recorded, and form the basis of an assay procedure with which to determine the proportions of these two isomers in Phenethicillin Potassium. Eight commercial samples of Phenethicillin Potassium have been assayed by this method and the results are compared with those obtained by microbiological assay.

PHENETHICILLIN Potassium is a semi-synthetic penicillin, which is manufactured by acylating 6-aminopenicillanic acid with racemic α-phenoxypropionyl chloride.[1] Acylation introduces an additional centre of asymmetry into the molecule, so that two diastereoisomers that have different physical properties are produced and, as a result, the isomer composition of the product varies, depending upon the solvent-extraction and crystallisation procedures used. As a result, the product from one manufacturer can differ significantly from that from another manufacturer. The two isomers also show differences in their antibacterial spectrum, the L-isomer being more active against *Bacillus cereus*, *Sarcina lutea* and certain penicillin-resistant strains of *Staphylococcus aureus*, which not only place certain limitations on the interpretation of the results of microbiological assay, but also indicate that products from different manufacturers may vary in their antibiotic effectiveness.

The British Pharmacopoeia 1968, as amended by the B.P. Addendum 1969, requires the control of Phenethicillin Potassium by a titrimetric assay, together with the optical rotation limits $[\alpha]_D$ +215° to +240°, corresponding to isomer compositions ranging from 100 to 34 per cent. of the L-isomer. This combination of non-specific assay and determination of optical rotation is less tedious, but not as precise as the alternative of a microbiological assay for the determination of this particular mixture of isomers, as the optical rotation measurement sums the effect at all five asymmetric centres. We have therefore examined the use of optical rotatory dispersion and circular dichroism as a means of providing both assay and isomer control in a single operation.

EXPERIMENTAL

MATERIALS—

Potassium 6-[L-(+)-α-phenoxypropionamido]penicillanate [m.p. 235 to 237 °C (with decomposition), $[\alpha]_D^{20}$ +212° (1·0 per cent. w/v aqueous solution)] was supplied by Beecham Research Laboratories. Perron, Crast, Gottstein, Minor and Cheney[1] give m.p. 238 to 239 °C (with decomposition), $[\alpha]_D^{24}$ +218° (1·0 per cent. w/v aqueous solution).

Potassium 6-[D-(+)-α-phenoxypropionamido]penicillanate was synthesised from 6-aminopenicillanic acid and optically pure (+)-α-phenoxypropionic acid according to the method of Perron, Crast, Gottstein, Minor and Cheney[1] [m.p. 230 to 232 °C (with decomposition), $[\alpha]_D^{18}$ +257° (1·0 per cent. w/v aqueous solution); literature values,[1] m.p. 230 to 231 °C (with decomposition), $[\alpha]_D^{24}$ +251° (1·0 per cent. w/v aqueous solution)].

Commercial samples of Phenethicillin Potassium conformed in all respects to the requirements of the British Pharmacopoeia 1968, Monograph on Phenethicillin Potassium, as amended by the B.P. Addendum 1969.

METHODS

Absorption spectra were determined in the wavelength range from 200 to 300 nm with a Unicam SP800 recording spectrophotometer.

Optical rotatory dispersion and circular dichroism spectra were determined at 27 °C in the wavelength range from 200 to 350 nm with a Cary, Model 6001, recording spectropolarimeter fitted with a circular dichroism attachment. The slits were programmed to give a constant spectral band width of 1·5 nm, and the scan speed was 15 nm min^{-1}. The instrument was calibrated for circular dichroism with D-10-camphorsulphonic acid[2] and the optical rotatory dispersion calibration was checked with sucrose solutions.

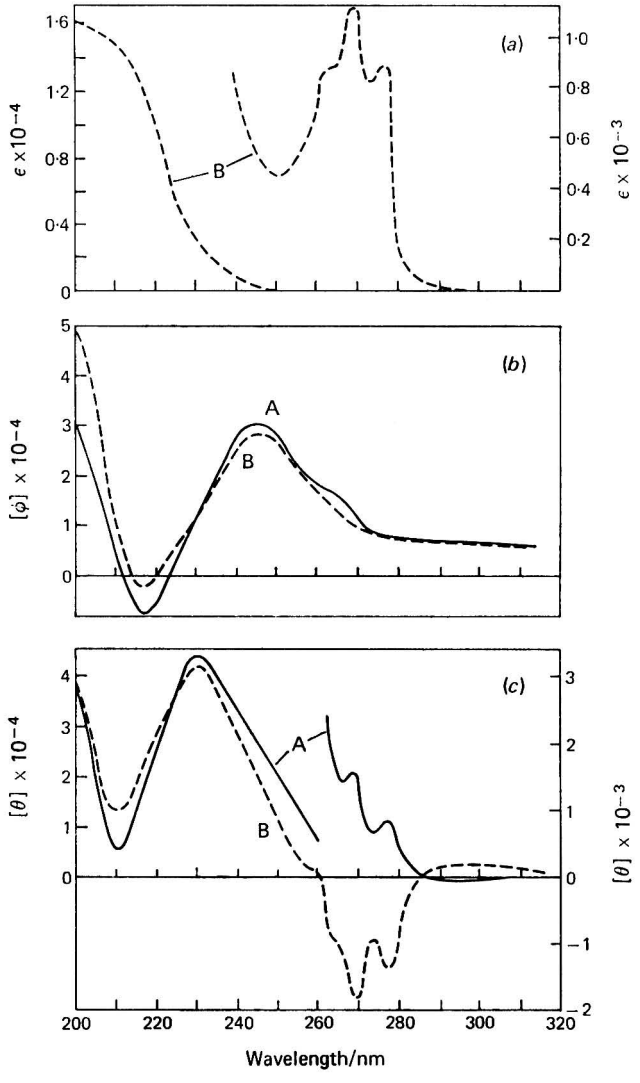


Fig. 1. (*a*) Absorption, (*b*) optical rotatory dispersion and (*c*) circular dichroism spectra. A, D-isomer and B, L-isomer of Phenethicillin Potassium. Molecular ellipticity [θ] and molecular rotation [ϕ] are in degrees cm^2 $dmol^{-1}$

Penicillin solutions were freshly prepared in water. Some samples were also examined in phosphate buffer (pH 6·2). Solution concentrations and the cell light path were chosen so that the absorbance of the solution was not greater than 1·3.

Results for microbiological assay supplied by the manufacturers were obtained by standard plate-assay procedures with *Sarcina lutea* ATCC 9341 (Manufacturer 1) and *B. subtilis* (Manufacturer 2), by using as a working reference a standard Phenethicillin Potassium sample containing a proportion of the two isomers similar to that in normal production material, the potency and L-isomer content of this material being assigned by reference to the pure L-isomer.

Results and discussion

Fig. 1 shows the absorption spectrum of the L-isomer (that of the D-isomer was not significantly different) and the optical rotatory dispersion and circular dichroism spectra of both isomers in water. Spectra in phosphate buffer were not significantly different. At wavelengths below 260 nm the shapes of the optical rotatory dispersion and circular dichroism spectra are very similar for the two isomers; the Cotton effects arise from dissymmetry in the penicillin skeleton, which is identical in both compounds. Differences in the magnitudes of the rotations and ellipticities are insufficient in the easily accessible region of this spectral range to be used as a basis for assay.

Above 260 nm the optical rotatory dispersion spectra of the two isomers are still very similar, but there is a marked difference in the circular dichroism spectra. Both isomers give peaks at wavelengths 269 and 276 nm, but of opposite sign, corresponding to the maxima at identical wavelengths in the ultraviolet absorption spectrum due to the phenoxy group, which is adjacent to the one distinguishing optical centre. The values of molecular ellipticity $[\theta]$ in degrees $cm^2\,dmol^{-1}$ are: L-isomer, $[\theta]_{269}$ $-1{\cdot}96 \times 10^3$ and $[\theta]_{276}$ $-1{\cdot}46 \times 10^3$; and D-isomer, $[\theta]_{269}$ $+1{\cdot}48 \times 10^3$ and $[\theta]_{276}$ $+0{\cdot}76 \times 10^3$. The optical rotatory dispersion Cotton effects corresponding to these circular dichroism bands are so weak as to be barely detectable when superimposed on the much larger Cotton effects corresponding to the absorption bands at lower wavelengths.

Fig. 2 shows circular dichroism spectra at wavelengths between 260 and 300 nm for the pure D- and L-isomers and three mixtures of them containing 25, 50 and 75 per cent.

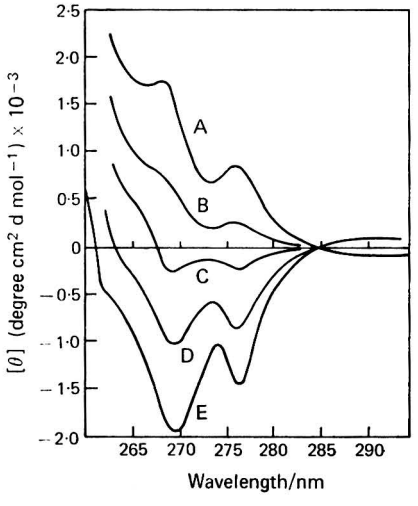


Fig. 2. Circular dichroism spectra of pure D- and L-Phenethicillin Potassium isomers and their mixtures: A, 100 per cent. D-isomer; B, C and D, 25, 50 and 75 per cent. of L-isomer, respectively; and E, 100 per cent. L-isomer

of the L-isomer. Graphs of the magnitudes of molecular ellipticities at the two main peaks at 269 and 276 nm against isomer composition (Fig. 3) show that the relationship is linear, and therefore capable of forming the basis of an assay.

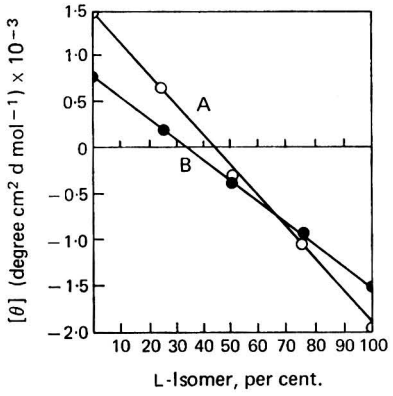


Fig. 3. Graph of molecular ellipticity [θ] at 269 nm (A) and 276 nm (B) against percentage isomer composition of Phenethicillin Potassium

Circular dichroism measurements at these two wavelengths have been used to assay, for their L-isomer contents, eight commercial batches of Phenethicillin Potassium, five from one manufacturer and three from another. In Table I the L-isomer contents, based on readings at 269 and 276 nm, are compared. Percentage contents of L-isomer calculated from the molecular ellipticity at wavelength 276 nm are higher than those calculated at 269 nm. Results based on measurements at 276 nm are probably the more accurate because, while the circular dichroism at 276 nm is less intense, the Cotton effect at 276 nm is more completely resolved from those arising at lower wavelengths in transitions of skeletal chromophores. There is, however, good correlation between the results from measurements at the two wavelengths for samples 2, 3, 4 and 5. Discrepancies between the pairs of results for the remaining samples could therefore be caused by circularly dichroic contaminants. Comparison of the results based on measurements at wavelength 276 nm with the manufacturers' figures for microbiological assay shows reasonable agreement, but that higher results are generally given by the circular dichroism method. Batches produced by the second manufacturer are, in fact, more consistent in isomer composition than the microbiological assays would suggest.

TABLE I

COMPARISON OF CIRCULAR DICHROISM AND MICROBIOLOGICAL ASSAYS OF PHENETHICILLIN POTASSIUM

Source	Sample No.	L-Isomer, per cent., from $[\theta]_{269}$	$[\theta]_{276}$	microbiological assay
Manufacturer 1	1	62	67	56
	2	53	53	56
		52	53	
	3	55·5	57	56
	4	55	57	57
		55	56	
	5	56	58	57
Manufacturer 2	6	64	68·5	63
		63	68	
	7	63	67	65
	8	64·5	72	70

The precision of the method has not been rigorously established, but the results of some replicate assays run on different solutions of the same sample shown in Table I indicate that it is of a high order. The method is rapid, and not more time consuming than the present Pharmacopoeial methods of control.

We thank Beecham Research Laboratories and Pfizer Ltd. for materials and for supplying details of the microbiological assays and results. One of us (H.C.M.) is indebted to the Royal Society for the award of a Visiting Fellowship. We also thank the Wellcome Trust for a grant to permit the purchase of the spectropolarimeter.

REFERENCES

1. Perron, Y. G., Crast, L. B., Gottstein, W. J., Minor, W. F., and Cheney, L. C., *Antibiotics A.*, 1959–60, 107.
2. Cassim, Y. J., and Yang, J. T., *Biochemistry, N.Y.*, 1969, **8**, 1947.

Received *October* 21*st*, 1971
Accepted *March* 15*th*, 1972

Determination of Riboflavine in Injections Containing Liver Extract

BY SAAD A. ISMAIEL

(*Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El Mataria St., Post El Zeitoun, Cairo, Egypt*)

Interference by the pigments of liver extract in the spectrophotometric determination of riboflavine by the method of the British Pharmacopoeia 1968 is obviated by chromatography on a column of activated acidic alumina with the mixture chloroform - ethanol (98 per cent.) - glacial acetic acid (50 + 50 + 0·3 v/v); riboflavine passes through the column while liver pigments are retained. The eluate is concentrated by evaporation, buffered at pH 4, then measured spectrophotometrically at wavelength 444 nm.

RIBOFLAVINE in injections containing liver extract could not be accurately determined by using the methods of the British Pharmacopoeia[1] or the Indian Pharmacopoeia[2] because of the great interference caused by the coloured substances present in liver extracts. This difficulty was resolved by using the method of Wahba and Fahmy,[3] which depends on the separation of riboflavine on purified talc, on which it is specifically and strongly adsorbed, but the procedure was found to be time consuming.

Attempts to separate riboflavine from the pigments of liver extract by using the paper-chromatographic method of Boeckel[4] were not successful because of incomplete separation.

A procedure is given for a quantitative method that is proposed for the determination of riboflavine in injections containing liver extract, which involves chromatography on activated acidic alumina. Liver pigments are adsorbed on to the alumina while riboflavine, accompanied by other vitamins that do not interfere in the spectrophotometric determination at wavelength 444 nm, is eluted.

EXPERIMENTAL

REAGENTS—

Activated acidic alumina (Merck 1078) is used directly from the container.

Solvent mixture—The mixture ethanol (98 per cent.) - chloroform - glacial acetic acid (50 + 50 + 0·3 v/v).

Glass-wool (Merck).

Acetate buffer, pH 4—A 10 per cent. solution of hydrated sodium acetate (B.P.) adjusted to pH 4 with glacial acetic acid.

The samples examined by the method were chosen so as to cover a wide range of injections containing liver extract with riboflavine; their formulations are given in Table I.

TABLE I

COMPOSITION OF SAMPLES

Sample	I	II	III	IV	V	VI	VII	VIII	IX
Thiamine hydrochloride/mg	50	50	100	5	10	10	10	100	10
Riboflavine/mg	0·5	1·0	2·0	1·0	2·0	1·5	1·0	1·0	0·5
Nicotinamide/mg	25	50	200	25	100	37·5	50	100	25
Pyridoxine hydrochloride/mg	—	5	10	1·5	1·0	2·5	3	10	1·0
Calcium pantothenate/mg	—	2	5	—	—	—	10	—	2
Sodium pantothenate/mg	—	—	—	—	—	2·5	—	7	—
D-Pantothenol/mg	—	—	—	—	4	—	—	—	—
Cyanocobalamin/μg	—	—	100	2·5	20	2	5	10	—
Biotin/μg	—	—	—	5	—	—	10	—	5
Choline chloride/mg	—	—	—	—	—	10·5	—	—	—
Ascorbic acid/mg	—	—	—	—	—	50	—	—	50
Benzyl alcohol/mg	20	20	20	10	20	20	20	20	10
4-Chloro-*m*-cresol/mg	—	2·5	2·5	2·5	25	2·5	2·5	2·5	2·5
Dry liver extract for parenteral use/mg	—	—	—	100	100	75	100	10	50
Water for injection	—	—	—	to 1 ml	to 1 ml	to 1 ml	to 1 ml	to 1 ml	to 1 ml
Crude liver extract (U.S.P. XV)	to 1 ml	to 1 ml	to 1 ml	—	—	—	—	—	—

PROCEDURE—

The entire procedure is conducted in subdued light. Dilute a volume of the sample, if necessary, with water to give a final concentration of riboflavine between 45 and 60 mg per 100 ml.

Prepare, in a glass tube of 2 cm diameter and 30-cm length with a stopcock, a column (2 × 8 cm) of activated acidic alumina (about 22 g) resting on a pad of glass-wool with the aid of about 25 ml of the solvent mixture. Allow the solvent to elute, then apply 2 ml of the diluted sample containing from 0·9 to 1·2 mg of riboflavine, allow it to elute again, then add 1 ml of the solvent mixture and, after it has eluted, add the solvent mixture until the yellow band approaches close to the bottom of the column. When the yellow-coloured band reaches the stopcock, collect the eluate until all the yellow colour has flushed through the stem and the eluate has become colourless (about 75 to 120 ml are collected).

Evaporate the eluate containing riboflavine on a boiling water bath so as to reduce the volume to about 10 ml, cool, add about 20 ml of water and adjust the pH to 4 with 5 ml of the acetate buffer; then transfer the solution into a 50-ml calibrated flask, make it up to volume with water, mix the solution and filter, if necessary, through a dry Whatman No. 1 filter-paper, discarding the first 20 ml of the filtrate.

Measure the extinction at wavelength 444 nm against a blank of 5 ml of the acetate buffer diluted to 50 ml with water, taking 323 as the value of $E^{1\%}_{1cm}$. The results obtained by this method and the fluorimetric method of the U.S.P.[5] are compared in Table II.

TABLE II

COMPARISON BETWEEN THE RESULTS OF THE PROPOSED METHOD AND THE FLUORIMETRIC METHOD

Sample	Stated	Amount of riboflavine/mg ml^{-1} Added*	Found by proposed method (results of 5 experiments)					Mean	Standard deviation, per cent.	Amount of riboflavine found by fluorimetric method/ mg ml^{-1}†
I	0·5	—	0·485	0·487	0·482	0·481	0·49	0·485	±0·73	0·51
		0·25	0·242	0·245	0·243	0·242	0·245	0·243	±0·63	0·248
		0·5	0·491	0·49	0·487	0·488	0·492	0·489	±0·43	0·48
II	1·0	—	0·98	0·985	0·97	0·975	0·97	0·976	±1·06	0·97
		0·5	0·49	0·485	0·492	0·487	0·485	0·488	±0·6	0·52
		1·0	0·98	0·97	0·985	0·99	0·97	0·977	±0·92	1·01
III	2·0	—	1·98	1·965	1·96	1·96	1·98	1·969	±0·51	1·925
		1·0	0·98	0·97	0·96	0·99	0·97	0·974	±1·14	1·025
		2·0	1·97	1·98	1·96	1·98	1·95	1·968	±0·64	1·94
IV	1·0	—	0·96	0·97	0·98	0·96	0·97	0·968	±0·83	0·97
		0·5	0·48	0·49	0·49	0·485	0·48	0·483	±1·09	0·485
		1·0	0·965	0·97	0·98	0·975	0·99	0·976	±0·96	1·02
V	2·0	—	1·96	1·97	1·95	1·94	1·94	1·952	±0·65	2·05
		1·0	0·96	0·97	0·98	0·965	0·97	0·969	±0·74	1·015
		2·0	1·97	1·98	1·96	1·95	1·98	1·968	±0·65	1·92
VI	1·5	—	1·46	1·47	1·44	1·46	1·45	1·456	±0·76	1·56
		0·75	0·72	0·74	0·73	0·73	0·735	0·731	±0·97	0·725
		1·5	1·47	1·46	1·45	1·48	1·47	1·466	±0·77	1·43
VII	1·0	—	0·98	0·97	0·985	0·97	0·975	0·976	±0·65	0·968
		0·5	0·48	0·49	0·485	0·487	0·49	0·486	±0·83	0·485
		1·0	0·97	0·98	0·99	0·985	0·975	0·98	±0·36	1·03
VIII	1·0	—	0·98	0·985	0·97	0·99	0·985	0·982	±0·75	0·97
		0·5	0·49	0·485	0·48	0·48	0·49	0·485	±1·0	0·515
		1·0	0·99	0·98	0·97	0·975	0·98	0·979	±0·55	0·98
IX	0·5	—	0·485	0·487	0·485	0·49	0·48	0·485	±0·54	0·505
		0·25	0·245	0·241	0·248	0·245	0·246	0·245	±1·04	0·258
		0·5	0·49	0·485	0·495	0·495	0·485	0·49	±1·0	0·482

* Added to the injection in the form of a solution containing 1 mg ml^{-1} of riboflavine and 50 mg ml^{-1} of nicotinamide.

† Mean of five experiments. The standard deviation was between ±1·48 and ±4·04 per cent., and the error of a single determination was between −7 and +10 per cent.

Several solvents were tested, *e.g.*, water, ethanol (95 and 98 per cent.), propan-1-ol, propan-2-ol and the mixtures ethanol (95 per cent.) - chloroform, ethanol (95 per cent.) - chloroform - glacial acetic acid and ethanol (98 per cent.) - chloroform - glacial acetic acid in different proportions, but the mixture ethanol (98 per cent.) - chloroform - glacial acetic acid (50 + 50 + 0·3 v/v) gave the best recovery, with sufficiently high purity for measurement at 444 nm.

The interference by the liver pigments and the other vitamin B complexes was studied by carrying out similar experiments with crude liver extract (U.S.P. XV), commercial dried liver extract for parenteral use and solutions of vitamin B complexes similar in composition to the injections but containing no riboflavine. The final solutions obtained gave readings of more than 2 at wavelength 267 nm and 0 to 0·14 at 375 nm but no extinction at 444 nm.

The elution pattern of riboflavine was studied by collecting the first 80 ml of eluate in 20-ml portions and determining the thiamine, nicotinamide and riboflavine in each portion. It was found that the first 20-ml portions contained no riboflavin but contained about half the amount of thiamine and nicotinamide, the remaining half being eluted with the riboflavine; cyanocobalamin was tenaciously adsorbed on to the column and coloured the upper third of the column pale red.

Nicotinamide in amounts ranging from 25 to 100 times that of riboflavine was effective in enhancing the elution of riboflavine, giving a recovery of between 96 and 99 per cent. Other vitamins such as thiamine, pyridoxine, pantothenol, calcium pantothenate, sodium pantothenate and ascorbic acid in amounts 50 times that of the riboflavine had no appreciable influence on enhancing the elution of riboflavine.

The method was not applicable to injections containing riboflavine-5′-phosphate (sodium salt).

Recoveries of the products studied ranged from 96·0 to 99·2 per cent.

References

1. British Pharmacopoeia 1968, The Pharmaceutical Press, London, 1968, p. 875.
2. Indian Pharmacopoeia, Second Edition, Government of India, New Delhi, 1966, p. 642.
3. Wahba, N. and Fahmy, E., *J. Pharm. Pharmac.*, 1965, **17**, 489.
4. Boeckel, C. R. C., *Semana Méd., B. Aires*, 1962, **120**, 344.
5. United States Pharmacopoeia, XVIII Revision, Mack Co., Easton, Pa., 1970, p. 910.

Received *August 2nd*, 1971
Accepted *February 18th*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 647–652

Analysis of High Purity Water by Flameless Atomic-absorption Spectroscopy

Part I. The Use of an Automated Sampling System

BY C. J. PICKFORD AND G. ROSSI

(Chemistry Division, Euratom-CCR, ISPRA (Varese), Italy)

An apparatus is described that enables fully automatic stream sampling and sample injection to be carried out when using the graphite-tube method of analysis in conjunction with atomic-absorption spectroscopy. Multiple aliquots of solution can be introduced automatically, with a drying step between each introduction. The concentration sensitivity of the method is thus increased even further, but without encountering the difficulties associated with the manual injection of multiple aliquots. In addition, a significant improvement in precision is obtained when using the automatic sampling and injection system rather than manual sampling.

The apparatus has been designed as part of a system to determine simultaneously a number of elements at levels of parts per 10^9 and below. Results for precision are presented for the determination of cobalt, chromium, copper, iron, manganese and nickel, and results for sensitivity are given for these and additional representative elements.

CORROSION studies on water-cooled nuclear power plants require the determination of metal impurities in the high-temperature water circuits at, or below, the parts per 10^9 (p.p.b.) level. The analysis of extremely dilute solutions to determine metal impurities in general represents one of the most complicated problems in analytical chemistry. In fact, the application of techniques currently in use is hindered by difficulties caused by the lack of sufficient sensitivity, and because normal classical procedures cannot always be applied. Further, any chemical pre-treatment of the sample in an attempt to isolate and concentrate the impurities is likely to introduce contamination and to result in blank values higher than the concentration levels sought. Hence, a method is needed that is both fast and accurate, and that is capable of the simultaneous determination of several elements with the required sensitivity. In addition, such a method should be capable of being automated, and have a wide applicability and flexibility, *i.e.*, it should be able to cope with samples, not only in the analytical laboratory, but also directly on the power plant, without supervision by skilled personnel. These requirements lead to the exclusion of flame atomic-absorption or atomic-fluorescence spectroscopy, as well as optical emission methods already in use for monitoring water quality.

The most recent developments in the field of flameless cells used in conjunction with atomic-absorption and atomic-fluorescence analysis suggest a possible approach to this problem. In fact, the absolute sensitivities reported in the literature compare favourably with those attained by neutron-activation methods.[1] Additionally, these techniques are very rapid and are inherently capable of automation because of the simplicity of the systems that are generally required.

Two such methods, which have been developed to the extent of being available commercially, are the carbon filament of West,[2] and the Massmann[3] furnace in its developed form; a recent review[4] has discussed the merits and disadvantages of these forms of atomisation. For the purpose of the present work, the Massmann furnace (Perkin-Elmer HGA-70) was chosen because, although less sensitive (in terms of the amount of material required to produce 1 per cent. absorbance), the sample volume can be much greater (100 μl compared with 5 μl), so that the minimum detectable concentration should be lower. In addition, it was felt that it might be easier to automate the sample introduction with a carbon-tube system rather than a carbon-rod system.

The graphite-tube atomiser has been used for the determination of trace elements in water[5]; the determinations were extremely rapid and the results showed a standard deviation of, typically, 3 to 6 per cent. The methods of sample introduction have usually been manual, with the use of an Eppendorf pipette or a microsyringe.

In order to obtain fully automatic and simultaneous determinations of up to five elements present in a flowing stream of coolant water, the authors have developed a system consisting of a central programming unit, a fully automatic stream-sampling and injection unit and a multi-channel integration and detection system.

The purpose of this paper is to describe the sampling and injection system, and to show that as well as fully automatic analyses being possible, it is also possible to inject up to 0·5 ml into the graphite tube in five aliquots, with a drying step between each addition. In this way, the sensitivity of the method is increased even further.

For the purposes of the present work, the sampling unit was used in conjunction with a commercial atomic-absorption spectrophotometer; the spectrophotometer and the detection system for five channels will be described in a later paper.

Experimental

Standard solutions—

These solutions were prepared immediately before use by the dilution of 1000 p.p.m. standards of the elements concerned with distilled water (double-distilled from quartz apparatus). Nitric acid (Merck, analytical-reagent grade) was then added to give a concentration of 0·1 per cent. Plastics reagent bottles and pipettes were used throughout.

Method—

The automatic sampling and injection unit is shown in Fig. 1. Control of this unit, the graphite-tube power supply and programmer and the read-out system is effected from a central programming unit consisting of a motor-driven 24-position switch.

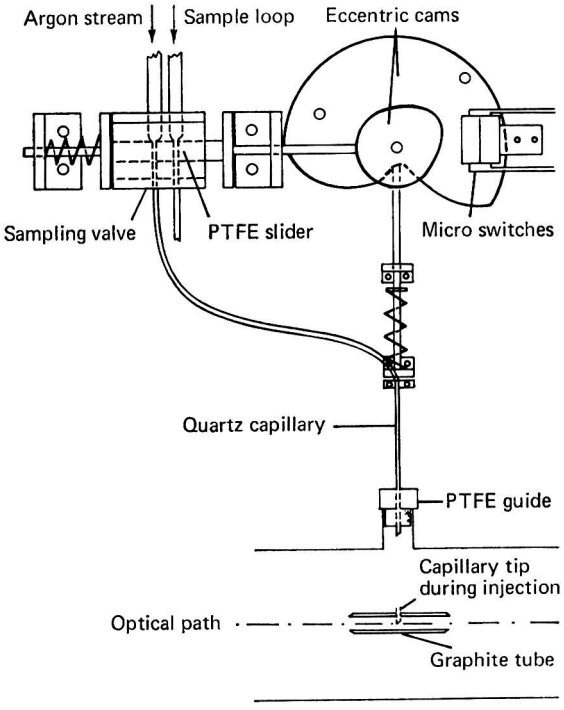


Fig. 1. Automatic sampling and injection assembly

When the programme is initiated, current is supplied to a synchronous motor (not shown in Fig. 1), which lies behind the mounting plate and rotates at 2 r.p.m. This rotates both eccentric cams, which are rigidly attached to each other. The larger (lower) cam pushes the rod connected to the quartz capillary forwards against the tension of a light spring. The quartz capillary (which is coated with the silicone Repelcoat) is flexibly connected to the push-rod so that accurate alignment of the injection assembly is not required. Alignment with the injection hole of the graphite tube is facilitated by a PTFE guide, which fits in place of the normal cap over the injection port.

When the tip of the quartz capillary has reached the centre of the graphite tube, the smaller (upper) cam acts against the push-rod connected to the sampling valve. This is a conventional type of valve made from PTFE and stainless steel, with a sampling volume of 100 μl. Movement of the central slider by 1 cm causes 100 μl from the sample stream to be transferred to the argon stream, and hence to the cell. Low sample and argon flow-rates (20 and 5 ml min^{-1}, respectively) are used to prevent possible leakage when the valve is in an intermediate position during sampling. In addition, with higher argon flow-rates, there is a tendency for the sample "slug" to break up in the tube, with some loss of precision in the absorption signal obtained. After the liquid "slug" has been transferred to the centre of the graphite tube, the spring-loaded PTFE slider of the valve returns to its former position. At no stage in the sampling operation does the sample come into contact with materials other than PTFE and quartz.

As the larger cam continues to rotate, two raised studs on its upper surface operate microswitches. The first switches on the sample liquid evaporation step of the oven unit; the second stops the motor of the sampling unit immediately after the quartz capillary has been withdrawn from the graphite tube.

After the evaporation step has been completed, there are two possible alternatives, depending on the switch settings of the central programmer. Either the charring and volatilisation steps of the graphite-tube power unit can be allowed to proceed, or a further injection of sample can take place. This last procedure can occur up to four times, with a drying step inserted after each sample injection. In this way, an effective sample volume of up to 0·5 ml can be obtained.

Although both the quartz tube and the PTFE guide were in fairly close proximity to the graphite tube during firing, neither appeared to be damaged by the intense heat. However, as a precaution against the possibility that it might increase the heat absorptivity of the PTFE, the layer of soot that accumulated on the PTFE guide after approximately two hundred firings was wiped off.

The graphite-tube power unit and programmer was modified slightly so as to be under the control of the central programmer. A relay was wired in parallel with the on - off switch of the oven unit, and several additional connections were made to the relays controlling the duration of each step of the firing sequence. Apart from these modifications, the temperature settings and general operating procedure of the oven and crucible unit have been described elsewhere.[6]

ATOMIC-ABSORPTION SPECTROPHOTOMETER—

A Beckmann 1301/DBG atomic-absorption system was used, modified slightly to provide a better signal-to-noise ratio when used with the graphite-tube atomiser. The periscope assembly and auxiliary lenses were removed, and the monochromator was raised to the same level as the hollow-cathode lamp. Two quartz lenses were introduced into the light path so as to give a light beam focused at the centre of the graphite tube. The slit width used for analyses was that necessary to ensure spectral purity, having regard to the intensity requirements.

The output of the spectrophotometer was connected to a potentiometric recorder with a zero to full-scale response time of 0·5 s (Speedomax, Leeds and Northrup). The motor switch of the recorder was controlled by the central programmer, so that recording took place only during the final volatilisation step.

The source used for obtaining the precision results was a multi-element hollow-cathode amp (Westinghouse WL 23176) containing the elements cobalt, chromium, copper, iron, manganese and nickel, and operated at 20 mA. To obtain the sensitivity figures shown in Table I, Westinghouse hollow-cathode lamps were used for each element, and were operated within the current limits recommended by the manufacturer.

Results and discussion

Sensitivity with the automatic sampling and injection unit—

Table I shows the sensitivity obtained for a number of representative elements for which hollow-cathode lamps were available. The sensitivity when using multiple injections is increased in proportion to the number of injections, compared with these figures, which are for a single injection of 100 μl.

Table I

Sensitivity of the method

Element	Wavelength/nm	Sensitivity/ μg l^{-1} per 1 per cent. absorption
Silver	328·1	0·05
Aluminium	309·3	3·0
Cadmium	228·8	0·0025
Cobalt	240·7	0·3
Chromium	357·9	1·5
Copper	324·7	0·4
Iron	248·3	0·6
Manganese	279·5	0·09
Nickel	232·0	0·7
Lead	283·3	0·4
Antimony	217·5	1·5
Vanadium	318·4	5·0

The values given in the table are comparable with other reported sensitivities[1,3,5] for the graphite-tube method when the amount of each element actually present is considered. However, because of the possibility of using a greater sample volume (up to 0·5 ml), the actual concentration sensitivities are correspondingly increased. Although multiple injection can be effected manually, it is time consuming and tedious compared with a fully automated system.

No difference was found for any of the above elements between the sensitivity (weight in grams per 1 per cent. absorption) obtained when using 100 μl of solution and that by using five 100-μl aliquots of a solution five times more dilute. However, in experiments with small injection volumes (1, 2 and 5 μl) introduced manually into the graphite cell, the weight of element *versus* absorbance graphs were not superimposable. When volumes of 10 μl or more were used, the graphs were identical within the limits of measurement. The concentration *versus* absorbance graphs obtained showed, in several instances, deviations from linearity towards the concentration axis, particularly with the more volatile elements, such as cadmium. This might have been partly due to instrument response factors.

A constant weight sensitivity was reported by Omang[7] when multiple aliquots of mineral oils were injected manually into a Perkin-Elmer HGA-70 system.

Precision of the sampling and atomisation steps—

To test the precision of the system as a whole, the standard deviation of the absorption signals from repeated measurements upon the same solution was obtained by allowing a standard solution of each element to flow through the valve in turn by siphon action. The sampling procedure was then initiated at 3-minute intervals by the central programmer, and a number of replicate absorption peaks were obtained for each element in turn, the exact number depending on the amount of solution available. In the completed experimental assembly, a peristaltic pump and multi-way valve will be used to transport the sample to the sampling valve and injection assembly, so that automatic calibration with distilled water, sample and standards can be made.

The concentration levels at which the precision measurements were made were chosen to give between 25 and 70 per cent. absorption, *i.e.*, up to the upper limit of linearity of the concentration *versus* absorbance graphs. At lower levels it was difficult to measure the peak height accurately enough (to within less than 1 per cent.) to show the maximum precision that can be attained with this system. For example, at an absorption of 10 per cent., errors associated with the precise location of the base-line, and even those occurring as a result of the thickness of the recorder trace (about 0·4 mm, corresponding to 2 per cent. of a 10 per cent.

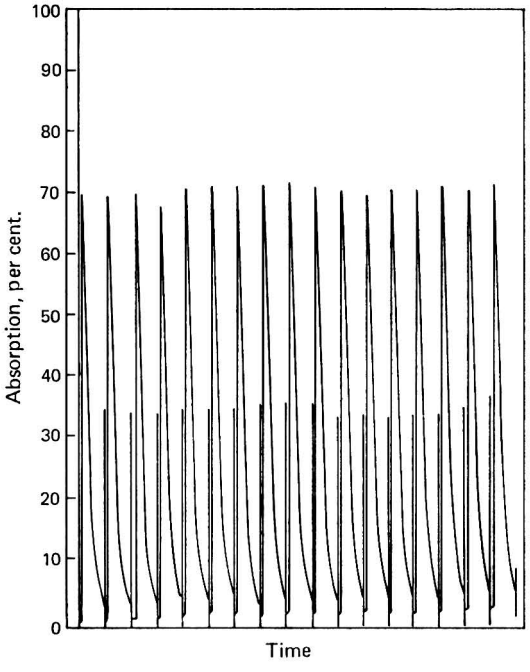


Fig. 2. Seventeen replicate absorption peaks obtained for chromium

absorption signal), can obscure the true precision of the sampling procedure. However, experiments with manganese showed that there was no variation in precision between the 12 and 65 per cent. absorption levels.

The replicate absorption peaks obtained for chromium (17 replicates at 100 p.p.b.) are shown in Fig. 2. The larger peaks are the absorption peaks due to the metal atoms, while the smaller lines between each peak correspond to the absorption of light by the injection capillary and the solvent. The standard deviation of the absorption signal from the mean (expressed as a percentage) was 1·0 per cent. In Table II are listed the results obtained for the six elements for which precision measurements were made.

TABLE II

STANDARD DEVIATIONS FOR SIX ELEMENTS

Element	Concentration/μg l^{-1}	Number of replicates	Standard deviation, per cent.
Cobalt	50	10	0·6
Chromium	100	17	1·0
Copper	50	12	1·2
Iron	30	10	1·1
Manganese	10	15	1·7
Nickel	50	10	2·4

Other general estimates of the precision that can be attained with the graphite-cell method, but using manual sample injection, are those of L'vov[8] (3 to 8 per cent.), Massmann[3] (4 to 12 per cent. with a single channel, 3 to 7 per cent. with a reference channel) and Manning and Fernandez[5] (5 per cent. at the 30 to 50 per cent. absorption level).

Our own measurements of the precision achieved when using manual injection indicate that the governing factor is the care with which the injection is made: *e.g.*, with manganese, results as high as 2·2 per cent. were obtained when extreme care was taken to position the pipette tip exactly in the same place in the graphite tube each time. When less care was taken, values as low as 11 per cent. were obtained for the reproducibility. However, an average value of 4 per cent. was obtained under normal conditions of analysis.

It can be seen that the values obtained by using the automatic sampling device represent an improvement in precision compared with manual sampling. The lower reproducibility obtained for nickel may be a result of the low intensity of the nickel 232·0-nm line from the lamp used, which, combined with the narrow slit width necessary for nickel (because of the presence of several neighbouring intense lines), resulted in a high noise level.

The difference between the precision obtained by manual and automatic sampling can best be explained in terms of the position of the sample droplet in the graphite tube. The volume reproducibility is probably not a significant factor in the reproducibility of the absorption signal, as the standard deviation of the signals obtained with manual sample injection is much greater than that due to variations in the volume delivered by the syringe (1 per cent. according to the manufacturer's handbook). Pyrometric measurements on the graphite tube during heating clearly demonstrate the presence of a temperature gradient in the tube and non-uniform heating in the region surrounding the injection hole, so that the position of the sample in the tube will clearly affect its volatilisation rate during heating. The automatic sampling system facilitates reproducible positioning of the sample in the graphite tube, whereas with manual sampling it is very difficult to deposit the sample in the same position each time.

Because these results were obtained under almost isothermal conditions, it might be expected that the reproducibility of the system would be lower when used under more realistic conditions, with temperature variations and other factors likely to influence the sampling process. However, this is also true of manual sampling, with which, in addition, large variations can be expected if sampling is carried out by different, unskilled personnel.

Conclusion

The automatic sampling system, as well as making totally automatic analyses possible, and removing operator bias effects, has the added advantage of significantly improving the precision of the entire process of sampling and volatilisation in the graphite-tube method of analysis.

It should be added that a significant improvement in the precision, especially at low absorbances, can be expected if an integration read-out system and reference channel are used to eliminate the influence of spurious absorption due to fumes or carbon particles. These developments are under investigation at present, and the results will be presented in a later paper.

We thank Euratom for the award of a postdoctoral research fellowship to one of us (C.J.P.).

References

1. *Instrum. News*, 1970, **21**, 4.
2. West, T. S., *J. Pure Appl. Chem.*, 1971, **25**, 47.
3. Massmann, H., *Spectrochim. Acta*, 1968, **23B**, 215.
4. Kirkbright, G. F., *Analyst*, 1971, **96**, 609.
5. Manning, D. C., and Fernandez, F., *Atomic Absorption Newsletter*, 1970, **9**, 65.
6. Hansen, P., "Flameless Atomic Absorption Spectrophotometry as a New Technique for Micro-analytical Trace Determinations," Bodenseewerk Perkin-Elmer & Co. GmbH, Uberlingen, 1971.
7. Omang, S. H., *Analytica Chim. Acta*, 1971, **56**, 470.
8. L'vov, B. V., *Spectrochim. Acta*, 1969, **24B**, 53.

Received *January 24th*, 1972
Accepted *March 8th*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 653–656

A Comparison of Some Methods for the Determination of Triazine Herbicides in Water

By C. E. McKone, T. H. Byast and R. J. Hance

(*Agricultural Research Council Weed Research Organization, Begbroke Hill, Yarnton, Oxford, OX5 1PF*)

By using gas chromatography with an alkali flame detector, 0·001 p.p.m. of atrazine, ametryne and terbutryne in water from two sources could be determined with recoveries close to 100 per cent. Ultraviolet spectrophotometry was adequate at the 0·01 p.p.m. level for all three compounds but was unreliable at lower levels. With cathode-ray polarography, 0·005 p.p.m. of terbutryne and ametryne and 0·01 p.p.m. of atrazine could be determined.

Until recently, little attention has been given to the use of triazines for aquatic weed control,[1–3] but currently considerable interest has arisen in the use of this group of compounds, particularly the methylthio derivatives, for this purpose (Robson, T. O., private communication, 1971).

There are several instrumental methods available for the measurement of triazines,[4] and procedures for their determination in water have been reported in which thin-layer chromatography,[5] and gas chromatography with alkali flame-ionisation detection, have been used.[6] In addition, Hayes, Stacey and Thompson[7] and Hance[8] have suggested that cathode-ray polarography can be used to determine triazine herbicides. It is of interest, however, to compare the sensitivities of such methods and to consider their relative merits. In the work reported here, a comparison was made of gas chromatography, ultraviolet spectrophotometry and cathode-ray polarography.

Materials and Methods

The herbicides studied were ametryne (2-ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine), atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and terbutryne (2-ethylamino-6-methylthio-4-t-butylamino-1,3,5-triazine). Water samples were obtained from an ornamental pond at the Weed Research Organization and from the Oxford Canal.

Fortification of water—

Stock solutions were prepared by diluting 2 ml of 1 mg ml^{-1} methanolic solutions of the herbicides to 2 litres with pond or canal water. These solutions were further diluted to give concentrations of 0·1, 0·01, 0·005 and 0·001 p.p.m. of herbicide.

Extraction procedures—

The water was not filtered and extractions were carried out on the same day as fortification. Duplicate 1-litre samples of water were shaken for 1 minute each with successive 100-ml and 50-ml portions of glass-distilled dichloromethane in a 2-litre separating funnel. The dichloromethane extracts were combined in a 250-ml separating funnel. If any emulsion was present, the funnel was shaken for 10 s, which invariably caused the emulsion to break. The dichloromethane solution was run into a 250-ml conical flask through a filter-funnel containing a small plug of glass-wool and 10 g of anhydrous sodium sulphate. The funnel and contents were washed with a further 50 ml of dichloromethane. A boiling-chip was added to the flask and the extracts were concentrated to about 0·5 ml under reduced pressure on a water-bath maintained at 35 °C. The residual solvent was removed with a stream of air.

Gas-chromatographic determination—

An alkali flame detector was used that was similar to that described by Hartmann,[9] made by modifying a standard Varian Aerograph flame-ionisation detector. The jet of the original detector was replaced with a jet of identical dimensions made from stainless steel. A salt tip of rubidium bromide was pressed with a conical indentation at the bottom so as to fit snugly on top of the jet. It was approximately 1 cm long and 0·6 cm in diameter,

with a 1 mm diameter hole. The original electrode arrangement was retained without modification other than to ensure that the polarising electrode was situated below the upper surface of the salt tip. Precise control of the hydrogen flow-rate was essential and this was achieved with a Brooks flow controller, Model 8744 (Emerson Electric Co., Hatfield, Pa., U.S.A.).

The chromatograph used was an Aerograph 1520 dual-channel instrument. The polarising potential (190 V) was taken from one electrometer channel while the signal from the detector was fed into the other channel, which was set in the electron-capture mode on attenuation 1. This arrangement gave twice the backing-off current that was available from a single channel and hence permitted greater sensitivity to be achieved.

The column, 1 m long and 4 mm i.d., was made of glass, and was packed with 2 per cent. of neopentylglycol succinate on 80 to 100-mesh Chromosorb W, High Performance. The injector temperature was 225 °C, the column temperature 195 °C and the detector temperature 230 °C. The air flow-rate was 180 ml min^{-1} and the nitrogen (carrier gas) flow-rate 70 ml min^{-1}. The hydrogen flow-rate was approximately 30 ml min^{-1}, small adjustments being made about this rate until maximum response was obtained. This system separated the three herbicides and gave retention times of 4·4 minutes for atrazine, 5·4 minutes for terbutryne and 6 minutes for ametryne.

Standard graphs for each herbicide were prepared from methanolic solutions containing 3, 6, 12, 24 and 36 ng of herbicide per 3-μl injection. Linear graphs were obtained of peak height (leading edge) *versus* nanograms of triazine injected. Although there is no theoretical justification for this procedure, it was found in this instance that the method produced linear calibrations and was considerably quicker than measuring peak heights by construction or peak areas.

The residue from the dichloromethane extracts was dissolved in 1 to 10 ml of methanol and 3-μl aliquots of the resulting solution were injected into the chromatograph. A typical chromatogram is shown in Fig. 1.

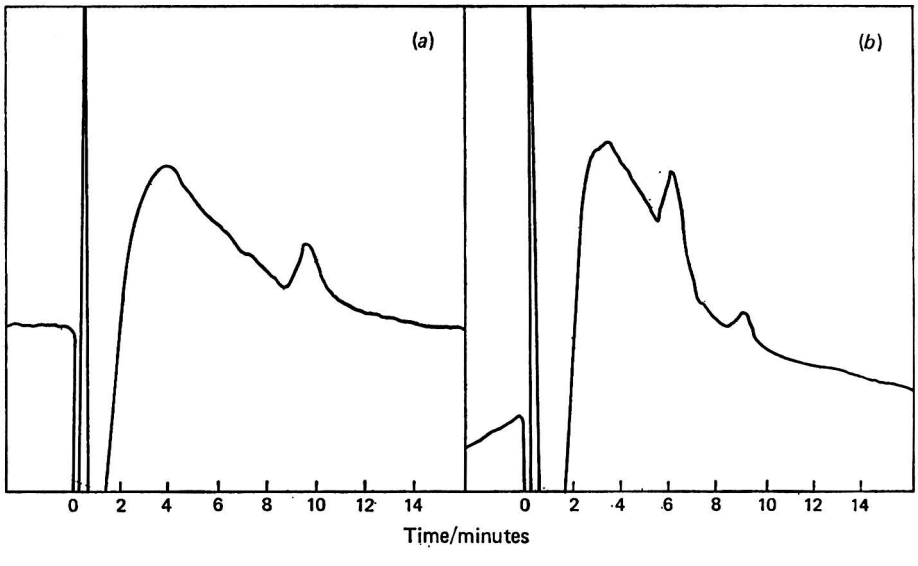


Fig. 1. Chromatograms of (*a*) blank pond water and (*b*) pond water fortified with 0·001 p.p.m. of ametryne (equivalent to 3 ng of ametryne)

ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION—

The extraction residue was hydrolysed by refluxing it for 3 hours with 10 ml of 1 N sulphuric acid. The hydroxytriazine produced was determined by measuring the absorption at 225, 240 and 255 nm, as described by Delley.[10] The same procedure was used for standards containing 2 to 100 μg of triazine.

POLAROGRAPHIC DETERMINATION—

A Southern Analytical cathode-ray polarograph, Type A 1670, was used. The residue from the dichloromethane extracts was dissolved in 1 to 10 ml of methanol, depending on the amount of residue, and a 1-ml volume of the resulting solution was diluted to 10 or 25 ml with methanol and dilute sulphuric acid so as to give a final solution that contained 50 per cent. of methanol and was 0·01 N in sulphuric acid. About 2 ml of this solution was transferred to the polarographic cell, de-oxygenated with a stream of nitrogen and a polarogram obtained by using the 100-ms R.C. derivative circuit, the sweep commencing 0·2 V before the reduction peak. Peaks occurred at about −1·05 V with respect to the mercury anode for atrazine and −1·45 V for ametryne and terbutryne. Linear calibration graphs were obtained from graphs of peak height *versus* solution concentration in the range 0·1 to 1 p.p.m. A typical polarogram is shown in Fig. 2.

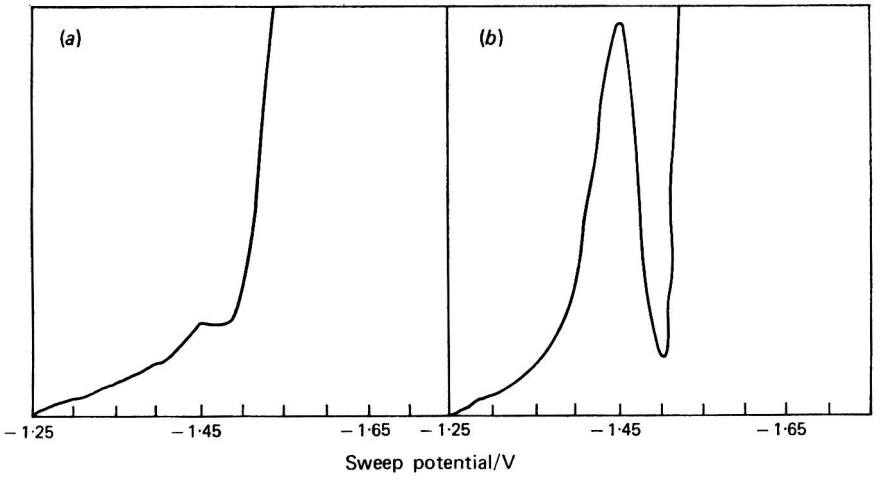


Fig. 2. Polarograms of (*a*) blank pond water and (*b*) pond water fortified with 0·01 p.p.m. of ametryne

RESULTS AND DISCUSSION

The results, corrected for blank values, are presented in Table I. At the 0·1 and 0·01 p.p.m. levels of the herbicides, the three methods gave comparable recoveries. The spectrophotometric method was unreliable at 0·005 p.p.m. and failed in all instances at 0·001 p.p.m. Good recoveries were obtained with the polarographic method down to 0·01 p.p.m. for atrazine and 0·005 p.p.m. for ametryne and terbutryne. The use of the more sensitive non-derivative mode of operation resulted in no improvement because of increased background interference. The gas-chromatographic method was successful at the 0·001 p.p.m. level for the three compounds. No precise limits of detection can be deduced from these results because of the widely different fortification levels. Taking the limit of detection as the level at which the signal is twice that of the background, then this limit for the ultraviolet spectrophotometric method would be about 0·005 p.p.m. for all three compounds, while for the polarographic method limits of detection of 0·006 p.p.m. for atrazine, 0·003 p.p.m. for ametryne and 0·002 p.p.m. for terbutryne are obtained. With the gas-chromatographic finish there was essentially no background, as even at the 0·001 p.p.m. level the signal-to-noise ratio was about 6:1 and the noise was electrical rather than that produced by co-extractives. However, the linearity of the detector response would be a limiting factor at lower levels and although this might be overcome by extracting larger volumes of water, if the volumes used are too large then manipulative losses might become significant. Although a modified flame-ionisation detector was used in this work, it seems likely that similar results could be expected from the various commercial nitrogen detectors that are now available.

TABLE I

RECOVERIES OF THREE TRIAZINE HERBICIDES FROM TWO WATERS

Herbicide	Concentration, p.p.m.	Recovery, per cent. Gas chromatography: Pond water	Gas chromatography: Canal water	Ultraviolet spectrophotometry: Pond water	Ultraviolet spectrophotometry: Canal water	Polarography: Pond water	Polarography: Canal water
Ametryne	0·1	91, 90	100, 98	89, 82	105, 86	97, 98	98, 94
	0·01	102, 100	102, 98	86, 70	103, 80	96, 88	96, 94
	0·005	104, 100	107, 103	58, 59	102, 94	88, 92	90, 84
	0·001	100, 87	100, 85	—	—	—	—
Atrazine	0·1	96, 98	98, 90	99, 94	102, 105	94, 96	91, 84
	0·01	105, 92	106, 101	100, 100	100, 105	96, 88	84, 80
	0·005	96, 103	—	25, 52	—	—	—
	0·001	90, 90	100, 100	—	—	—	—
Terbutryne	0·1	93, 95	97, 93	91, 91	93, 93	98, 98	99, 98
	0·01	100, 95	96, 100	85, 85	91, 85	99, 99	94, 99
	0·005	104, 94	103, 104	64, 58	88, 91	95, 90	95, 91
	0·001	100, 100	100, 100	—	—	—	—
Standard deviation based on differences between replicates		4·48		7·91		4·52	

Standard deviations based on differences between replicates for all determinations by each analytical method are shown in Table I. The polarographic and gas-chromatographic methods gave similar standard deviations, whereas that for the ultraviolet spectrophotometric method was greater.

The specificities of the methods differed. In the polarographic determination, atrazine could be distinguished from ametryne and terbutryne on the basis of reduction potentials, but the last two compounds were both reduced at −1·45 V. The ultraviolet spectrophotometric method was non-specific. The gas-chromatographic method adequately separated the three compounds and was therefore the most specific method of determination.

The times involved for analyses by both the gas-chromatographic and the polarographic methods were similar and the calculation of the results was simple in each instance. The ultraviolet spectrophotometric method was more time consuming because the hydrolysis stage and multiple wavelength readings were necessary and also because the calculation of the results was more complicated.

It is suggested, therefore, that gas chromatography with the use of an alkali flame detector is superior to spectrophotometry and polarography for the determination of triazines extracted from water and probably also to thin-layer chromatography, although the performance of this method at levels below 0·1 p.p.m. has not been reported.[5]

The authors thank Mr. E. G. Cotterill for valuable technical assistance.

REFERENCES

1. Schneider, E. O., *Proc. 13th NEast Weed Control Conf.*, 1959, 306.
2. Gast, A., *Symp. Int. sur les Moyens de Destruction des Plantes Aquatiques, La Rochelle*, 1964, 14.
3. Holz, W., *Ergebn. 2 European Weed Research Council Int. Symp. Wasserpflanzen, Oldenburg*, 1967, 34.
4. Mattson, A. M., Kahrs, R. A., and Murphy, R. T., *Residue Rev.*, 1970, **32**, 371.
5. Abbott, D. C., Bunting, J. A., and Thomson, J., *Analyst*, 1965, **90**, 356.
6. Tindle, R. C., Gehrke, C. W., and Aue, W. A., *J. Ass. Off. Analyt. Chem.*, 1968, **51**, 682.
7. Hayes, M. H. B., Stacey, M., and Thompson, M., *Chem. & Ind.*, 1967, 1222.
8. Hance, R. J., *Pestic. Sci.*, 1970, **1**, 112.
9. Hartmann, C. H., *J. Chromat. Sci.*, 1969, **7**, 163.
10. Delley, R., *cited by* Knüsli, E., *in* Zweig, G., *Editor*, "Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives," Volume IV, Academic Press, New York, 1964, p. 179.

Received *February 14th*, 1972
Accepted *April 7th*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 657–662

A Recommended Molybdenum-blue Procedure for the Determination of Arsenic in Steel*

By A. G. FOGG, D. R. MARRIOTT† and D. THORBURN BURNS
(*Chemistry Department, University of Technology, Loughborough, Leicestershire, LE11 3TU*)

A recent molybdenum-blue procedure suggested by Nall for the determination of arsenic in steel has been modified to give quantitative extraction of arsenic from the steel solution.

The new procedure differs from that of Nall in that reduction of arsenic in the solution of the steel is effected with tin(II) chloride instead of with copper(I) chloride and hypophosphorous acid, and arsenic(III) is extracted as the iodide rather than as the chloride. Recoveries of arsenic of greater than 95 per cent. were obtained consistently with the recommended procedure, which gives high precision and good reproducibility from day to day. Results in close agreement with the certified values, with relative standard deviations of less than 2 per cent., were obtained for the analysis of nine British Chemical Standards steel samples.

An extension of this procedure is also described in which the molybdenum blue is extracted into 1 + 4 v/v hexanol - 3-methylbutan-1-ol, giving a five-fold increase in sensitivity.

The present British Standard method for the determination of arsenic in steel[1] is based on the reduction of arsenic in solution with hypophosphite followed by iodimetric titration of precipitated elemental arsenic. The original procedure of Evans,[2,3] on which the British Standard method was based, was applied to the analysis of pig-iron and of carbon and low-alloy steels by the Methods of Analysis Committee of the British Iron and Steel Research Association.[4] In further work the application of the procedure was extended to highly alloyed steels.[5] In this latter investigation, the method was compared with a molybdenum-blue spectrophotometric procedure involving the distillation of arsenic(III) chloride. This molybdenum-blue procedure, which had been suggested previously for the determination of arsenic in electrical sheet steel,[6] was found to be critically dependent on reagent concentrations and to give erratic results. The report concluded that the use of the colorimetric procedure should be restricted to the analysis of steels containing less than 0·03 per cent. w/w of arsenic and that strict attention should be paid to the standardisation of reaction conditions. For general use, the titrimetric method was found to be more satisfactory.

The present British Standard method is considered by many analysts to give erratic results. The major errors seem to occur in the reduction and precipitation stages, and the filtration of small amounts of arsenic (the arsenic content of steels rarely exceeds 0·1 per cent. w/w) is difficult. The present investigation was made in an attempt to develop a reliable colorimetric method that would be more acceptable to the steel analyst than the present British Standard method. While making the initial survey of possible procedures, our attention was drawn to a molybdenum-blue procedure by Nall,[7] which was reported to give good reproducibility. A modified form of this procedure is recommended later in this paper for the determination of arsenic in steel.

Experimental

Molybdenum-blue procedures for the determination of arsenic in organic and inorganic matrices involve a minimum of three steps—dissolution and pre-treatment of the sample, separation of the arsenic, and formation and reduction of molybdoarsenate. After pre-treatment of the sample, arsenic is usually present as arsenic(V); if the separation is made via an arsenic(III) compound, a reduction step is required before, and an oxidation step after, the separation.

* Presented at the Third SAC Conference, Durham, July 12th to 16th, 1971.

† Present address: The Corporate Laboratories of the British Steel Corporation, 140 Battersea Park Road, London, S.W.11.

In the present investigation, it was considered essential to develop a satisfactory colorimetric finish before attempting to assess the dissolution and separation steps. The colorimetric finish by Nall was found to be very satisfactory. A rectilinear calibration graph was obtained when the procedure described later was applied directly to standard arsenic solutions without solvent extraction, *i.e.*, beginning at the iodine oxidation step. The apparent molar absorptivity based on the arsenic concentration was 25 500 l mol^{-1} cm^{-1}. Repeatability tests at the 100 μg of arsenic level (see Table I) indicated a standard deviation of less than 1 per cent. within batches, and also a consistent mean absorbance for different batches was obtained. The absorbance of the measured solutions remained unchanged for at least 18 hours.

TABLE I

REDUCTION OF MOLYBDOARSENATE WITH HYDRAZINIUM SULPHATE IN THE PRESENCE OF IODINE (NALL PROCEDURE)

Mean absorbance* of six determinations	Standard deviation, per cent.
0·680	0·5
0·682	0·6
0·688	0·4
0·677	0·4
0·680	0·3
0·684	0·5
0·674	0·6

* 2 μg ml^{-1} of arsenic.

In the present work, the dissolution procedure described by Nall[7] was also found to be very satisfactory. The steel sample was dissolved in a 2 + 1 v/v mixture of concentrated hydrochloric acid and concentrated nitric acid. The resulting solution was then evaporated to dryness and the residue was redissolved in hydrochloric acid. Care had to be taken to avoid sputtering at the drying stage. The solid residue redissolved readily in hydrochloric acid if excessive baking was avoided, but it was essential to remove all nitrogen oxide fumes.

After this stage, Nall reduced the arsenic(V) to arsenic(III) with copper(I) chloride and hypophosphorous acid, extracted the arsenic(III) chloride into chloroform and back-extracted it into water, before re-oxidising the arsenic with iodine and carrying out the colorimetric determination. The present authors found that with this procedure a recovery of arsenic of only about 65 per cent. was achieved.

The loss of arsenic undoubtedly occurs at the extraction stage, as the recovery was improved by making extractions with several portions of chloroform. The effect of making multiple extractions with chloroform at the 100 μg of arsenic(III) level is indicated in Table II. The improved recovery is in agreement with the results of Migeon and Migeon,[8] who obtained 97 per cent. recoveries by carrying out five extractions with chloroform under similar conditions.

TABLE II

EXTRACTION OF ARSENIC(III) CHLORIDE WITH CHLOROFORM

Extraction sequence	Recovery of arsenic, per cent.
1 × 15 ml	53
1 × 25 ml	69
1 × 15 ml, 1 × 10 ml	69
1 × 15 ml, 2 × 10 ml	82
1 × 15 ml, 3 × 10 ml	94

When the single extraction procedure of Nall was applied to the analysis of British Chemical Standards steels, results in close agreement with the certified values were obtained in many instances with standard deviations of the order of 4 per cent., despite the low recovery of arsenic in the procedure. On several occasions, however, values obtained for the arsenic content were extremely low—as little as 5 per cent. of the certified values—and it was considered that this was due to unsatisfactory reduction with copper(I) and hypophosphorous acid. In later work, these reductants were replaced with tin(II) chloride, with which consistently good results were obtained.

Migeon and Migeon[8] reduced solutions of steel in concentrated hydrochloric acid with either tin(II) chloride or titanium(III) chloride. Potassium iodide was then added and arsenic(III) iodide was extracted into chloroform. Complete extraction of arsenic into chloroform occurred at hydrochloric acid concentrations from 10·5 to 11 M with a single extraction step. The arsenic(III) iodide was back-extracted readily into aqueous solution by shaking the chloroform solution with water.

A modified form of this extraction procedure is incorporated into the method recommended below for determining arsenic in steel. The modifications include extraction with two portions of chloroform, as the use of a single extraction rarely results in high precision, and the use of a hydrochloric acid backwash of the combined extracts in order to minimise the entrainment of phosphorus acids from the sample solution. Sufficient tin(II) chloride was added to ensure complete reduction of the iron(III) to iron(II) and to prevent oxidation of iodide reagent to iodine. The extraction had to be carried out immediately after the reduction step. If reduced solutions were allowed to stand for periods of longer than 10 to 15 minutes, a lower recovery was obtained.

Stará and Starý[9] recommended the extraction of arsenic(III) iodide from a solution that was 3·5 to 4·5 M in sulphuric acid and 0·8 M in potassium iodide. The use of sulphuric acid was investigated in the present work, but was found to increase the rate of spontaneous re-oxidation of the arsenic(III) solution. Recoveries decreased to below 10 per cent. in 20 minutes when sulphuric acid was present. Small amounts of sulphate did not interfere in the present method, but the addition of sulphuric acid to the dissolution mixture is not recommended.

The concentration of iodide in the solution from which the arsenic(III) iodide is now extracted is only 0·1 M, but this is sufficient to prevent co-extraction of up to 250 μg of antimony (equivalent to 0·1 per cent. w/w of antimony in a steel sample). Silicon at the 1 per cent. w/w level and phosphorus and germanium at levels below 0·1 per cent. w/w in the steel did not interfere. Selenium interfered when its concentration exceeded 0·01 per cent. w/w; at this concentration decreased absorbance readings were obtained. At the 0·1 per cent. of selenium level, a 50 per cent. decrease in the absorbance was noted.

Recommended procedure for the determination of arsenic in steel

Reagents—

High purity iron powder.

Concentrated hydrochloric acid, sp. gr. 1·14—Analytical-reagent grade.

Dissolution mixture—Mix 100 ml of concentrated nitric acid (analytical-reagent grade, sp. gr. 1·42) with 200 ml of concentrated hydrochloric acid. This solution should be stored in a loosely stoppered glass bottle.

Tin(II) chloride solution, 25 per cent. w/v, in concentrated hydrochloric acid solution—Dissolve 25 g of tin(II) chloride in 75 ml of concentrated hydrochloric acid.

Potassium iodide solution, 30 per cent. w/v—Dissolve 30 g of potassium iodide and 0·5 g of sodium hydrogen carbonate in water and dilute the resulting solution to 100 ml with water. This solution should be stored in an amber glass bottle, and should be discarded when a yellow coloration appears.

Iodine solution, 1 per cent. w/v—Dissolve 0·5 g of iodine and 1 g of potassium iodide in water and dilute the resulting solution to 50 ml with water.

Ammonium molybdate solution, 1 per cent. w/v—Add 70 ml of concentrated sulphuric acid (analytical-reagent grade, sp. gr. 1·84) carefully to 400 ml of water. Dissolve 5 g of ammonium molybdate in this solution and dilute the resulting solution to 500 ml with water.

Hydrazinium sulphate solution, 0·15 per cent. w/v—Dissolve 0·15 g of hydrazinium sulphate in 100 ml of water. It is recommended that this solution be prepared fresh daily.

Standard arsenic solution, 25 μg ml^{-1} of arsenic—Dissolve 0·825 g of analytical-reagent grade arsenic(III) oxide in a small volume of 1 M sodium hydroxide solution and neutralise the resulting solution with 1 M hydrochloric acid solution with litmus paper as indicator. Dilute the neutralised solution to 500 ml with water in a calibrated flask and mix thoroughly. Transfer 10 ml of this solution by means of a pipette to a second 500-ml calibrated flask, dilute the solution to 500 ml with water and mix thoroughly. The final solution should be prepared from the stock solution immediately before use.

PREPARATION OF CALIBRATION GRAPH—

Weigh a 0·25-g amount of high purity iron powder into each of seven 100-ml conical flasks and add 0, 1, 2, 3, 4, 5 and 6 ml of the standard arsenic solution to successive flasks from a burette. Add 10 ml of the dissolution mixture to each flask, cover it with a watch-glass and warm the mixture gently on a hot-plate until the iron has dissolved. Evaporate the solution to dryness, ensuring that all nitrogen oxides are removed from the residue, while preventing the solid residue from being unduly baked. (A convenient procedure is to remove the watch-glass once dryness has been reached and to continue heating the residue until brown fumes are no longer evolved. The flask is then removed from the hot-plate and allowed to cool.) Redissolve the residue by adding 35 ml of concentrated hydrochloric acid. Swirl the solution and warm the flask gently until a completely clear solution is obtained. The following procedure is then followed for each solution in turn.

Transfer the solution to a 100-ml PTFE-stoppered separating funnel, rinsing the conical flask with 10 ml of concentrated hydrochloric acid. Add 5 ml of tin(II) chloride solution, swirl the mixture and add 3 ml of potassium iodide solution. Immediately, extract this solution with 20 ml of chloroform, shaking the mixture vigorously for 1 minute and carefully releasing the pressure that develops in the separating funnel. Allow the layers to separate and transfer the organic layer to a second 100-ml PTFE-stoppered separating funnel. Repeat the extraction with a further 10 ml of chloroform and combine the extracts in the second separating funnel. To the combined extracts, add 10 ml of concentrated hydrochloric acid and shake the mixture for 30 s. Allow the layers to separate and run the chloroform layer into a third 100-ml PTFE-stoppered separating funnel, taking care to ensure that none of the aqueous layer is transferred. To the third separating funnel add 20 ml of water and shake the mixture vigorously for 1 minute. Allow the layers to separate, discard the lower chloroform layer, avoiding losses from the aqueous layer. Run the aqueous layer into a 100-ml conical flask, rinsing the funnel with the minimum amount of water.

To this aqueous solution, add the following reagents in turn, swirling the mixture after each addition—5 drops of iodine solution, 5 ml of ammonium molybdate solution and 2 ml of hydrazinium sulphate solution. Cover the flask with a watch-glass and heat the mixture in a boiling water bath for 10 minutes (Note). Allow the solution to cool, transfer it to a 50-ml calibrated flask, dilute the solution to 50 ml with water and mix thoroughly. Measure the absorbance of this solution at 840 nm in 1-cm glass cells against water as blank.

NOTE—

The level of the solution in the flask should be below the level of the boiling water in the water-bath. In the present work, a special holder was made to prevent the flasks from overturning.

DETERMINATION OF ARSENIC IN STEEL—

Weigh an amount of the sample (0·25 g of steel containing between 0·01 and 0·06 per cent. w/w of arsenic, and 0·1 g of steel containing between 0·06 and 0·15 per cent. w/w of arsenic) into a 100-ml conical flask, add 10 ml of the dissolution mixture, cover the flask with a watch-glass and warm the mixture on a hot-plate until reaction ceases.

Proceed as for the preparation of the calibration graph, beginning at "Evaporate the solution to dryness. . . ."

TABLE III

ANALYSIS OF BRITISH CHEMICAL STANDARDS STEEL SAMPLES

British Chemical Standards steel No.	Certified arsenic content, per cent. w/w	Arsenic found, per cent. w/w	Standard deviation,* per cent.
212/1	0·020	$0·021_0$	1·2
320	0·031	$0·031_7$	0·9
322	0·012	$0·011_5$	2·4
219/3	0·032	$0·035_0$	0·7
323	0·058	$0·057_8$	0·8
324	0·084	$0·082_7$	1·7
218/3	0·035	$0·035_0$	1·2
221/1	0·032	$0·032_4$	1·6
224/1	0·030	$0·039_0$	1·6

* Six determinations.

The results of the analysis of nine British Chemical Standards steels with this procedure are given in Table III.

MODIFIED PROCEDURE FOR THE DETERMINATION OF ARSENIC IN STEELS CONTAINING LESS THAN 0·01 PER CENT. OF ARSENIC—

In the procedure described above, absorbance readings of 0·050 and 0·750, corresponding to 12 and 150 μg of arsenic, were obtained with steel samples that contained 0·005 and 0·06 per cent. w/w of arsenic, respectively, at the sample weight recommended. An attempt to increase the sensitivity of the procedure five-fold by extracting the molybdenum blue into 10 ml of an organic solvent was investigated.

The molybdenum blue was soluble in alcohols, but difficulties were encountered owing to the partial solubilities of these solvents in water and *vice versa*. The precision obtained was relatively low (see Table IV). In an attempt to compensate for the opposing solubilities, a 1 + 4 v/v hexanol - 3-methylbutan-1-ol mixture was used to extract the molybdenum blue. This proved to be very satisfactory; six determinations at the 25 μg of arsenic level gave an apparent molar absorbance of 25 100 l mol^{-1} cm^{-1} (λ = 810 nm) with a standard deviation of 0·7 per cent. The colour faded slowly on standing, but good reproducibility was obtained provided that the colour intensities of the extracts were measured within 5 minutes of carrying out the extraction.

TABLE IV

SOLVENT EXTRACTION OF MOLYBDENUM BLUE

Solvent	Apparent molar absorbance based on arsenic content*/l mol^{-1} cm^{-1}	Standard deviation, per cent.
3-Methylbutan-1-ol	26 590	3·8
Hexanol	22 620	5·0
Pentan-2-ol	28 380	7·3
Hexanol - 3-methylbutan-1-ol (1 + 4 v/v) ..	25 100	0·7

* Mean of six determinations.

ADDITIONAL REAGENTS—

Purified ammonium molybdate solution, 1 per cent. w/v—Dissolve 1 g of ammonium molybdate in 50 ml of water and transfer the resulting solution to a separating funnel. Extract the solution with successive 10-ml portions of a 1 per cent. w/v solution of diethylammonium diethyldithiocarbamate in chloroform until the extract is straw coloured. Mix the purified ammonium molybdate solution with 14 ml of concentrated sulphuric acid and dilute the resulting solution to 100 ml with water.

Dilute standard arsenic solution, 5 μg ml^{-1} of arsenic—Add 20 ml of standard arsenic solution (25 p.p.m. of arsenic) to a 100-ml calibrated flask by means of a pipette, dilute the solution to the calibration mark with water and mix the solution thoroughly.

Hexanol - 3-methylbutan-1-ol mixture (1 + 4 *v/v*).

PROCEDURE—

Prepare a calibration graph as indicated above but using 0, 1, 2, 3, 4 and 5-ml volumes of dilute standard arsenic solution. Instead of transferring the cooled molybdenum-blue solution to a 50-ml calibrated flask, transfer this solution to a 100-ml separating funnel, and use 10 ml of water to complete the transfer. Transfer by pipette exactly 10 ml of 1 + 4 v/v hexanol - 3-methylbutan-1-ol mixture into the separating funnel and shake the mixture vigorously for 1 minute. Allow the layers to separate, then run the organic layer directly into a 1-cm glass cell. Measure the absorbance of the solution immediately at 810 nm against 1 + 4 v/v hexanol - 3-methylbutan-1-ol.

The results of the analysis of two steel samples by this procedure are given in Table V. The standard deviations were higher than those obtained with standard arsenic solutions.

TABLE V

ANALYSIS OF LOW-ARSENIC STEEL SAMPLES

Steel sample	Certified arsenic content, per cent. w/w	Arsenic found, per cent. w/w	Standard deviation,* per cent.
B.C.S. steel No. 321	0·003	0·0040	6
Swedish standard steel No. JK22	0·007	0·0066	9

* Six determinations.

DISCUSSION

The recommended procedure for the determination of arsenic in steel described above has given consistently reliable results with standard deviations usually of less than 2 per cent. The apparent molar absorbance of the molybdenum blue based on the amount of arsenic taken is 24 900 l mol^{-1} cm^{-1}, which represents an arsenic recovery of more than 95 per cent.

The recommended procedure is essentially a modification of that of Nall,[7] whose sample dissolution and molybdoarsenate reduction procedures were found to be very reliable. The procedure differs from that of Nall in that reduction of arsenic in the solution of the steel is carried out with tin(II) chloride and arsenic is extracted from this solution as arsenic(III) iodide rather than as arsenic(III) chloride. The recovery of arsenic(III) iodide is greater than 95 per cent., whereas only about 70 per cent. of arsenic(III) chloride is recovered. A procedure incorporating a solvent-extraction stage that yields such a low recovery of arsenic would be expected to have a relatively poor standard deviation, to give an occasional erratic result and to be less sensitive than a procedure that gives an almost quantitative recovery. This has been shown to be the situation in the present work. Tin(II) chloride was found to be a more reliable reducing agent for the reduction of arsenic(V) to arsenic(III) than is copper(I) with hypophosphorous acid.

We thank the British Iron and Steel Research Association for financial support, and its Chemical Analysis Committee for suggesting this project. In particular, we thank Mr. P. H. Scholes for his continued interest and guidance. We also thank Mr. R. D. Hutt for carrying out part of the experimental work.

REFERENCES

1. "Determination of Arsenic, Method 1," British Standards Handbook No. 19, British Standards Institution, London, 1970.
2. Evans, B. S., *Analyst*, 1929, **54**, 523.
3. ——, *Ibid.*, 1932, **57**, 492.
4. *Restricted Rep. B.I.S.R.A.*, No. MG/D/187, 1957.
5. British Iron and Steel Research Association Methods of Analysis Committee, *J. Iron Steel Inst.*, 1958, **188**, 331.
6. *Restricted Rep. B.I.S.R.A.*, No. MG/H/120, 1956.
7. Nall, W. R., *Analyst*, 1971, **96**, 398.
8. Migeon, E., and Migeon, J., *Chim. Analyt.*, 1961, **43**, 276.
9. Stará, V., and Starý, J., *Talanta*, 1970, **17**, 341.

Received *August 2nd*, 1971
Amended *February 3rd*, 1972
Accepted *March 22nd*, 1972

Analyst, August, 1972, Vol. 97, *pp.* 663–669

A Colorimetric Method for the Determination of Phenacetin and Paracetamol

Part I.* An Automated Procedure for the Determination, in Formulations, of Phenacetin and Paracetamol Alone or in the Presence of Aspirin and Codeine Phosphate

By J. W. MURFIN

(*Analytical Research, Quality Control, The Boots Company Ltd., Pennyfoot Street, Nottingham*)

A rapid, automated, colorimetric method for the determination of phenacetin and paracetamol is described. The reaction of either compound with acidic hypochlorite followed by coupling with alkaline phenol produces a blue indophenol dye, which is measured spectrophotometrically. The method has been applied to tablets containing phenacetin or paracetamol, alone or in combination with aspirin and codeine phosphate, and to a granule formulation containing paracetamol. Standard procedures were used for the determination of aspirin and codeine phosphate.

The object of this investigation was to develop a continuous, auto-analytical system for the analysis by a colorimetric method of pharmaceutical formulations containing phenacetin (*N*-acetyl-*p*-phenetidine) or paracetamol (*p*-acetamidophenol). In some formulations, such as Aspirin, Phenacetin and Codeine Tablets B.P., the phenacetin or paracetamol was associated with aspirin (acetylsalicylic acid) and codeine phosphate; in others either was the sole analgesic present.

Most of the published colorimetric methods for the determination of phenacetin and paracetamol require their initial de-acetylation by boiling with a mineral acid for up to 2 hours, followed by application of a standard procedure[1–5] to the resulting primary aromatic amine, *p*-phenetidine (*p*-aminoethoxybenzene) or *p*-aminophenol. An automated method[6] has been described for the determination of paracetamol by hydrolysis, diazotisation of the amine produced and coupling the resulting product with 1-naphthol, but this process, which includes the need to heat and cool the solution, is rather lengthy. Two colorimetric manual methods have been suggested that do not require initial hydrolysis of the phenacetin and paracetamol; one involves heating them with 10 per cent. nitric acid[7] and the other warming with a solution of chloramine T.[8] Neither method proved sensitive enough for the desired automatic system.

Powell[9] determined phenacetin titrimetrically by hydrolysing it to *p*-phenetidine, oxidising the *p*-phenetidine to *p*-quinonechlorimide with excess of hypochlorite solution and determining the *p*-quinonechlorimide iodimetrically after removal of the excess of hypochlorite. Ninomiya[10] oxidised paracetamol directly with potassium hexacyanoferrate(III) at 0 °C, and then formed an indophenol dye by coupling the oxidation product with phenol; phenacetin does not react under these conditions.

The possibility of using Powell's reaction as a basis for a colorimetric method for phenacetin and paracetamol seemed promising and was investigated. It was found that when an aqueous alcoholic solution of phenacetin or paracetamol was mixed, at room temperature, with a dilute solution of sodium hypochlorite at a pH of about 3, a quinonechlorimide was formed within a few minutes, the initial hydrolysis to *p*-phenetidine being unnecessary. An excess of an aqueous solution of phenol was added and then, on addition of sodium hydroxide to a pH of about 10, a stable blue indophenol dye was produced. The reaction is rapid and eminently suitable for automation.

* For Part II of this series, see p. 670.

In the automated analysis of compound tablets, aspirin was determined by hydrolysis in the cold with sodium hydroxide, acidification of the resulting solution, addition of iron(III) nitrate and measurement of the resulting purple - red colour. Codeine phosphate was determined by the method described by Kuzel for tertiary amines.[11] A solution of bromocresol purple in acetic acid was added to the sample, the amine - dye complex extracted into chloroform and the yellow colour of the chloroform layer measured.

METHOD

APPARATUS—

Sampling unit.
Technicon continuous filter.
Peristaltic pump, 25-channel.
Technicon colorimeter and recorder for each channel.

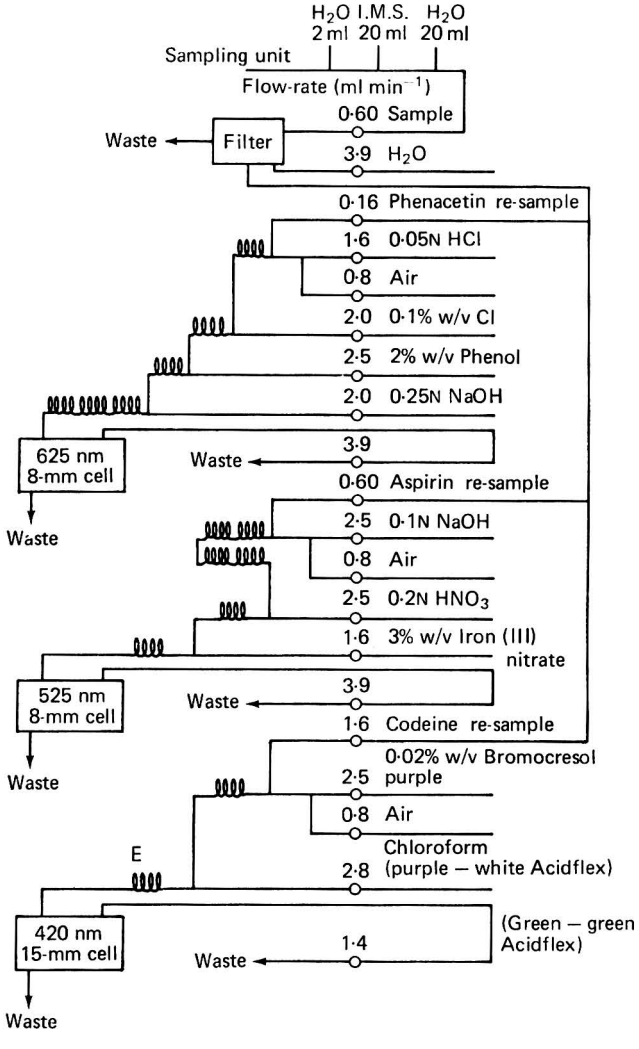


Fig. 1. Flow diagram for Aspirin, Phenacetin and Codeine Tablets B.P. All flexible tubing carrying chloroform is Acidflex, the remainder is Tygon. All coils are double mixing coils, except E, which is an extraction coil

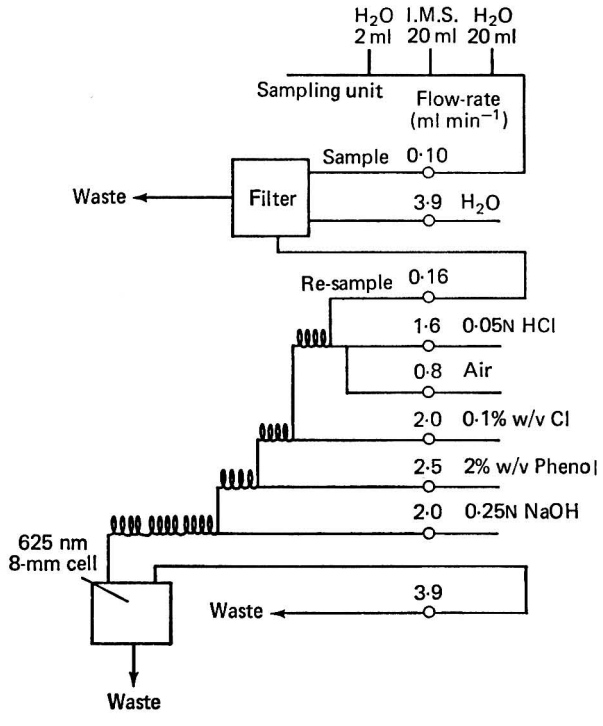


Fig. 2. Flow diagram for Paracetamol Tablets B.P. (500 mg). All tubing is Tygon. All coils are double mixing coils

REAGENTS—

It is sufficient to prepare the following solutions to an accuracy of ±5 per cent.

Hydrochloric acid, 0·05 N.

Sodium hypochlorite solution, 0·1 *per cent. w/v of available chlorine*—This solution should be prepared daily from a fresh concentrated solution.

Phenol, 2 *per cent. w/v aqueous solution.*

Sodium hydroxide solution, 0·25 *and* 0·1 N.

Nitric acid, 0·2 N.

Iron(III) nitrate solution—A 3 per cent. w/v aqueous solution of $Fe(NO_3)_3.9H_2O$.

Bromocresol purple, 0·02 *per cent. w/v in* 2 *per cent. v/v acetic acid.*

Chloroform, B.P.

Industrial methylated spirit, 48 *per cent. v/v in water*—This solution was prepared by using Industrial Methylated Spirit B.P. Other types of spirit should be checked for interference by the denaturant, or 95 per cent. ethanol used instead for preparing the reagent.

Brij 35, 10 *per cent. w/v aqueous solution*—Two drops of this solution are added per litre to all reagents except phenol and chloroform.

Aspirin, phenacetin and codeine phosphate standard solutions—Dissolve 15·00 g of aspirin, 15·00 g of phenacetin and 0·500 g of codeine phosphate hemihydrate in industrial methylated spirit, transfer the solution to a 500-ml calibrated flask, dilute to volume with industrial methylated spirit and mix. Transfer, by pipette, 5, 10, 15, 20 and 25 ml to a series of 100-ml calibrated flasks and add, in order, 43, 38, 33, 28 and 23 ml of industrial methylated spirit and dilute to volume with water. These solutions contain 1·5, 3·0, 4·5, 5·0 and 7·5 mg of aspirin and phenacetin, and 0·05, 0·10, 0·15, 0·20 and 0·25 mg of codeine phosphate per millilitre of 48 per cent. v/v industrial methylated spirit, respectively.

Aspirin, paracetamol and codeine phosphate standard solutions—Prepare as described above, but by using 15·00 g of paracetamol instead of phenacetin.

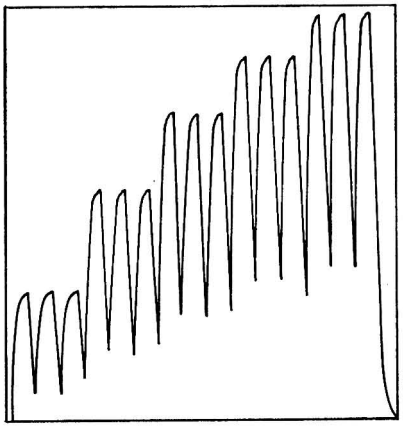


Fig. 3. Aspirin standards (1·5 to 7·5 mg ml^{-1})

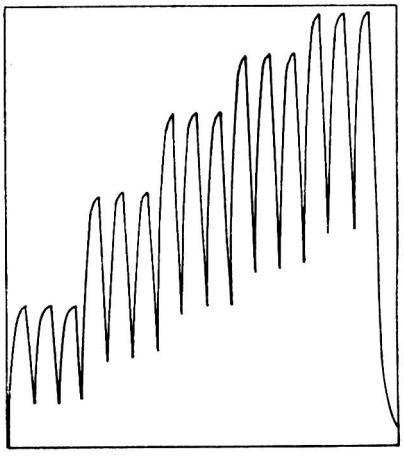


Fig. 4. Phenacetin standards (1·5 to 7·5 mg ml^{-1})

Paracetamol standard solutions—Prepare solutions containing 3·0, 6·0, 9·0, 12·0 and 15·0 mg of paracetamol per millilitre of 48 per cent. v/v industrial methylated spirit.

PROCEDURE—

Flow diagrams for aspirin, phenacetin and codeine together and for paracetamol alone are shown in Figs. 1 and 2, respectively. The manifolds illustrated are intended for tablets containing 250 mg each of aspirin and phenacetin and 8 mg of codeine phosphate (Fig. 1), and 500 mg of paracetamol (Fig. 2), but they can, of course, be adapted for tablets of different content by altering the rate of flow of the re-sample lines. The flow system of Fig. 1 can also be used for aspirin, paracetamol and codeine, but as paracetamol gives an optical density about 50 per cent. higher than that of a similar concentration of phenacetin, it may be necessary to reduce the paracetamol re-sample size from 0·16 to 0·10 ml min^{-1}.

The solvent-extraction coil for codeine was a 14-turn, 5 cm diameter coil made from tubing of 2 mm i.d.

The routine of the sampling unit, which was constructed in our workshop, was to disintegrate each tablet in 2·0 ml of water, add 20·0 ml of industrial methylated spirit and then 20 ml of water from syringe pumps, while stirring continuously with a magnetic stirrer.

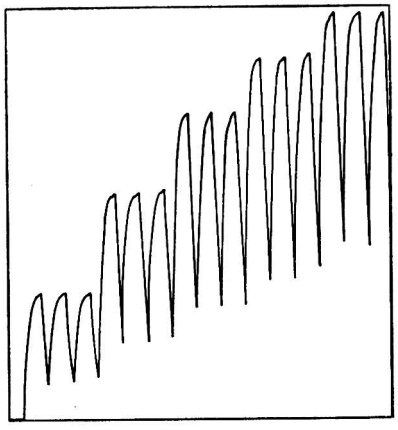


Fig. 5. Paracetamol standards (1·5 to 7·5 mg ml^{-1})

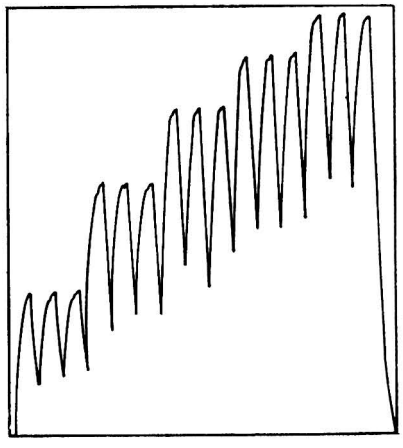


Fig. 6. Codeine phosphate standards (0·05 to 0·25 mg ml^{-1})

The time taken between each addition was adjusted so as to achieve satisfactory disintegration and dissolution. Other types of sampler are applicable, any necessary alterations being made in the manifold tubing to allow for differences in concentration of the sample solution.

For paracetamol and phenacetin, the time from commencement of sampling to recording of maximum colour was 11 minutes, for aspirin 10 minutes and for codeine phosphate 7 minutes. The sampling time was 2½ minutes and the wash time 45 s. The wash solvent used was 48 per cent. v/v industrial methylated spirit.

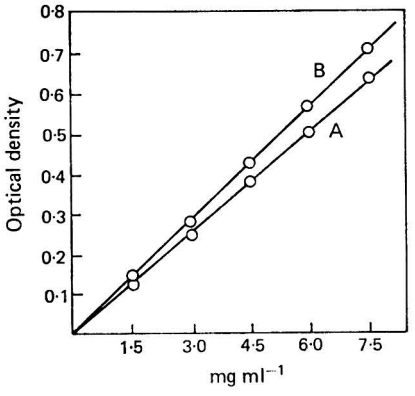


Fig. 7. Concentration *versus* optical density for phenacetin and paracetamol: A, paracetamol (0·10 ml min^{-1} sample); and B, phenacetin (0·16 ml min^{-1} sample)

In all instances suitable standards were placed at the beginning of every run and at suitable intervals during a run. For control work, standards of 90 and 110 per cent. of the theoretical values at intervals of twenty samples were used.

The following formulations were examined.

Aspirin, Phenacetin and Codeine Tablets B.P.: each tablet contains 250 mg of aspirin, 250 mg of phenacetin and 8 mg of codeine phosphate.

Aspirin, paracetamol and codeine tablets: the tablets contain 250 mg each of aspirin and paracetamol and 8 mg of codeine phosphate, and were treated as for Aspirin, Phenacetin and Codeine Tablets B.P., but with a paracetamol re-sample rate of 0·10 ml min^{-1}. Paracetamol Tablets B.P. contain 500 mg of paracetamol per tablet.

Granules containing paracetamol: these contain 13 per cent. of paracetamol together with citrates, ascorbic acid and lemon flavour. Sample solutions were prepared by hand

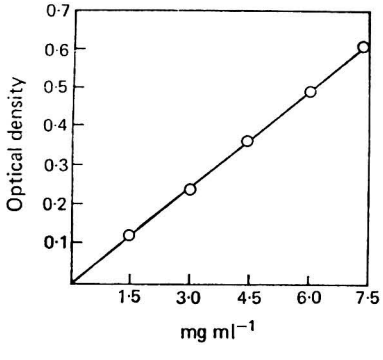


Fig. 8. Concentration *versus* optical density for aspirin

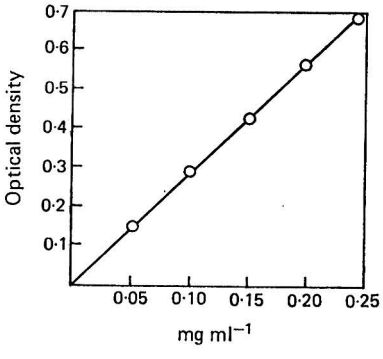


Fig. 9. Concentration *versus* optical density for codeine phosphate

(5·00 g of the granules were dissolved in 500 ml of 10 per cent. v/v industrial methylated spirit) and sampled automatically. A manifold similar to that in Fig. 2 was used, except that the sampling rate was 0·8 ml min^{-1} and the re-sample rate 0·6 ml min^{-1}.

Results and discussion

Three replicates of each of the five standards of each type were assayed. Typical recorder traces are shown in Figs. 3 to 6. Graphs of concentration against optical density are shown in Figs. 7 to 9; there is a rectilinear relationship within the range 1·5 to 7·5 mg ml^{-1} for aspirin, phenacetin and paracetamol, and 0·05 to 0·25 mg ml^{-1} for codeine phosphate.

Table I

Precision of assay of standards and compound tablets

Ten consecutive assays of the same sample

	Optical density		Coefficient of variation, per cent.
	Range	Mean	
Aspirin (standard)	0·436 to 0·445	0·443	0·9
(tablet)	0·459 to 0·464	0·461	0·3
Phenacetin (standard)	0·562 to 0·579	0·575	0·9
(tablet)	0·530 to 0·547	0·538	0·9
Paracetamol (standard)	0·501 to 0·507	0·504	0·4
(tablet)	0·498 to 0·504	0·502	0·4
Codeine phosphate (standard) ..	0·570 to 0·590	0·578	1·0
(tablet)	0·590 to 0·600	0·595	0·8

The precisions of the procedures are shown in Table I. Results of a run of ten single Aspirin, Phenacetin and Codeine Tablets B.P. are shown in Table II.

Substances normally used as tablet excipients do not interfere in the determination of phenacetin or paracetamol, nor do ingredients such as citrates, ascorbic acid (up to a concentration equal to that of the paracetamol), lemon flavour and saccharin.

Paracetamol gives a reddish blue colour when mixed with an iron(III) salt. The reaction in insensitive, but there is a slight increase in the apparent optical density of aspirin when paracetamol is present. With equal concentrations of aspirin and paracetamol, the optical density of aspirin is increased by 0·7 per cent. of its correct value. Phenacetin does not interfere in the determination of aspirin.

Table II

Assay of ten single Aspirin, Phenacetin and Codeine Tablets B.P.

600-mg tablets from the same production batch

Aspirin/ mg per tablet	Phenacetin/ mg per tablet	Codeine phosphate/ mg per tablet
265	247	8·4
264	250	8·2
260	251	8·2
250	245	8·6
253	250	8·0
260	250	8·5
260	248	8·5
260	247	8·3
255	252	8·4
260	242	8·6
Mean 259	248	8·4

The method reported for the determination of phenacetin and paracetamol is rapid and makes possible the automated continuous analysis of Aspirin, Phenacetin and Codeine Tablets B.P., and similar preparations containing phenacetin or paracetamol.

Thanks are due to Mr. D. F. C. Crawley for the design and manufacture of the sampling unit.

References

1. Devi, J. G., and Khorana, M. L., *Indian J. Pharm.*, 1953, **15**, 168.
2. Namura, N., *J. Pharm. Soc., Japan*, 1966, **86**, 331.
3. Hellevuri, E., and Seydlitz, H., *Svensk. Farm. Tidskr.*, 1946, **50**, 553.
4. Devi, J. G., and Khorana, M. L., *Indian J. Pharm.*, 1953, **15**, 193.
5. Chodakowski, A. M., and Jeske, S., *Acta Pol. Pharm.*, 1966, **23**, 47.
6. Ederma, H. L., Skerpac, J., Cotty, V. F., and Sterbenz, F., "Technicon Symposium 1966," Volume I, Mediad Inc., New York, 1967, p. 228.
7. Horn, D., *Pharm. Zentralhalle Dtl.*, 1951, **90**, 296.
8. Afanas'ev, B. N., *Aptech. Delo*, 1953, **2**, 21.
9. Powell, A. D., *Analyst*, 1919, **44**, 22.
10. Ninomiya, T., *J. Pharm. Soc., Japan*, 1965, **85**, 394.
11. Kuzel, N. R., *J. Pharm. Sci.*, 1968, **57**, 852.

Received *November 22nd*, 1971
Accepted *April 14th*, 1972

A Colorimetric Method for the Determination of Phenacetin and Paracetamol

Part II.* A Manual Procedure for the Determination of Phenacetin or Paracetamol in Formulations

By J. W. MURFIN and J. S. WRAGG

(Analytical Research, Quality Control, The Boots Company Ltd., Pennyfoot Street, Nottingham)

A manual colorimetric method for the determination of phenacetin or paracetamol in pharmaceutical preparations has been developed from the automated method described in Part I of this series. The drug (about 140 μg of paracetamol or about 200 μg of phenacetin) is added to a hydrochloric acid - sodium hypochlorite mixture at pH 3·4 and the excess of hypochlorite is removed with sodium arsenite. A solution of phenol is then added, followed by borate buffer solution (pH 9·9), and the optical density of the blue indophenol dye solution is measured at 625 nm.

The method is used to determine phenacetin in Aspirin, Phenacetin and Codeine Tablets B.P. and paracetamol in Paracetamol Tablets B.P. and paracetamol elixirs. Common tablet excipients, and drugs frequently compounded with paracetamol and phenacetin, do not significantly interfere with the reaction; the method should therefore be widely applicable to phenacetin or paracetamol formulations.

The successful application of the indophenol reaction to the automated determination of phenacetin (*N*-acetyl-*p*-phenetidine) and paracetamol (*p*-acetamidophenol) in formulations[1] led us to investigate the use of this reaction for the manual determination of these drugs.

The automated procedure requires the addition of 0·05 N hydrochloric acid and sodium hypochlorite solution (containing 0·1 per cent. of available chlorine) to a suitably diluted solution of phenacetin or paracetamol, to give a mixture with a pH of about 3. A 2 per cent. solution of phenol is then added, followed by 0·25 N sodium hydroxide solution to raise the pH to about 10, and the absorption of the solution is measured at 625 nm. When this procedure was translated directly into a manual procedure, with the same reagents, the same order of addition and the same delay periods, it was difficult to obtain reproducible results. It was concluded that, under these particular conditions, the essence of obtaining precise results was the reproducibility of the manipulations, and that these manipulations were satisfactorily performed by the automating equipment but not by hand.

To obtain good precision by a manual procedure a number of changes were found to be necessary; these included a change in the order of adding the acid, the hypochlorite and the drug solution, alterations to the concentrations of the reagents and the addition of sodium arsenite to remove the excess of hypochlorite before adding the phenol.

Experimental

Reagents—

Sodium hypochlorite solution—This solution contained 0·4 per cent. w/v of available chlorine and was prepared by diluting a concentrated sodium hypochlorite solution (containing about 16 per cent. w/v of available chlorine).

Borate buffer solution (pH 9·9)—Dissolve 20 g of boric acid, 24 g of potassium chloride and 11 g of sodium hydroxide in 2 litres of water.

Hydrochloric acid, 0·1 N.

Sodium arsenite solution, 4·0 *per cent. w/v.*

Phenol solution, 6 *per cent. w/v.*

Procedure—

Pre-determine the volume of 0·1 N hydrochloric acid that must be added to 5·0 ml of sodium hypochlorite solution and 10 ml of water for the resulting solution to have a pH of 3·4

* For Part I of this series, see p. 663.

(usually, about 3·5 ml of 0·1 N hydrochloric acid is required). The pH of the solution must be as close to pH 3·4 as possible and within the range 3·30 to 3·50.

Transfer an accurately weighed amount of powdered tablet, granule or elixir, containing about 500 mg of phenacetin or 350 mg of paracetamol, to a 100-ml calibrated flask, add 50 ml of ethanol, shake the mixture for 2 minutes and dilute to volume with water. (It is unnecessary to shake the mixture when the preparation already contains the drug in solution.) Mix thoroughly, cool to 20 °C, adjust the mixture to volume with water if required, mix and filter, if necessary. Transfer 25·0 ml of the solution to a 250-ml calibrated flask, adjust to volume with water and mix. Transfer 10·0 ml of the diluted solution to a second 250-ml calibrated flask, adjust to volume with water and mix.

To a mixture of 5·0 ml of sodium hypochlorite solution and sufficient 0·1 N hydrochloric acid to give a pH of 3·4 in a 50-ml calibrated flask, add 10·0 ml of the final sample solution. The sample solution should be delivered by pipette, with the tip of the pipette held just above the surface of the acid - hypochlorite mixture. Mix and allow the solution to stand for 5 minutes and add 2·0 ml of sodium arsenite solution. Mix and allow the solution to stand for 10 minutes and add 2·0 ml of phenol solution followed by sufficient borate buffer solution to adjust the solution to volume. Mix and allow the solution to stand for 30 minutes, then measure the extinction in a 1-cm cell at 625 nm in a suitable spectrophotometer, with water in the reference cell. Compare the extinction value with that obtained when the same procedure is carried out with 500 mg of pure phenacetin or 350 mg of pure paracetamol, as appropriate.

TABLE I

RELATIONSHIP BETWEEN COLOUR INTENSITY AND CONCENTRATION OF PHENACETIN AND PARACETAMOL

Compound	Concentration of drug/ μg per 50 ml	Optical density*	Standard deviation
Phenacetin	72·6	0·149	0·0007
	130·6	0·264	0·0006
	188·7	0·377	0·0013
	246·8	0·493	0·0013
	304·8	0·607	0·0013
	362·9	0·717	0·0015
Paracetamol	50·3	0·154	0·0007
	90·6	0·272	0·0013
	130·8	0·395	0·0014
	171·1	0·510	0·0022
	211·1	0·630	0·0013
	251·6	0·732	0·0018

* Each result is the mean of five determinations.

RESULTS

In experiments designed to examine various features of the procedure, phenacetin and paracetamol that were purified by repeated crystallisation were used. All measurements of optical density were made on a Shimadzu QV-50 spectrophotometer.

The results of investigating various factors pertaining to the procedure are presented in Tables I and II and in Figs. 1 to 4.

TABLE II

EFFECT OF VARYING THE pH OF THE FINAL SOLUTION ON THE COLOUR INTENSITY

pH of borate buffer	pH of final solution	Optical density	
		Phenacetin	Paracetamol
9·9	9·6	0·399	0·414
9·6	9·3	0·399	0·414
9·25	9·05	0·358	0·378
8·9	8·7	0·344	0·323
8·4	8·3	0·281	0·220

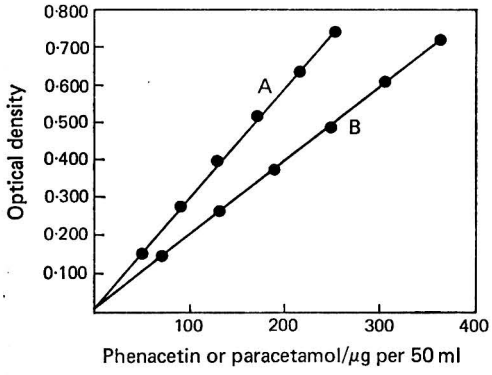


Fig. 1. Optical density *versus* concentration of phenacetin or paracetamol in final coloured solution: A, paracetamol; and B, phenacetin

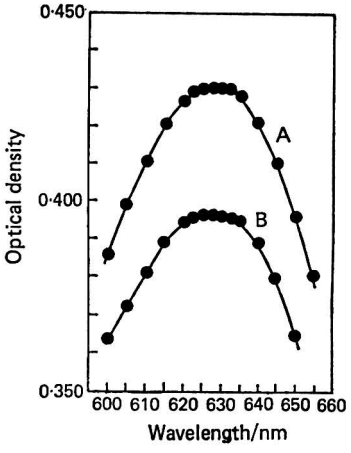


Fig. 2. Spectrum of the final coloured solutions between 600 and 660 nm: A, paracetamol; and B, phenacetin

The recovery of phenacetin from a laboratory-prepared mixture simulating Aspirin, Phenacetin and Codeine Tablets B.P. and the recoveries of paracetamol from laboratory-prepared mixtures simulating Paracetamol Tablets B.P. and Paracetamol Elixir, Paediatric, B.P.C. are shown in Table III.

The results of the determination of phenacetin in Aspirin, Phenacetin and Codeine Tablets B.P. and of paracetamol in Paracetamol Tablets B.P. and paracetamol elixirs by the colorimetric method and by the official methods are shown in Table IV. One of the elixirs examined was Paracetamol Elixir, Paediatric, B.P.C. and the other was prepared to a different formula.

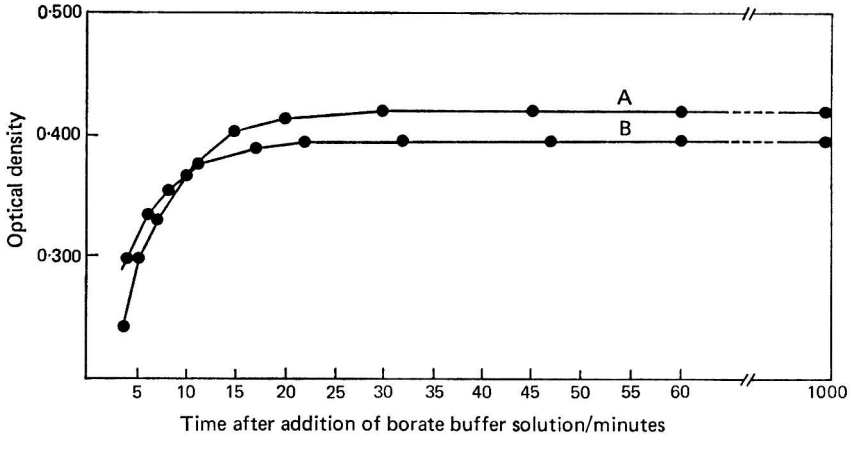


Fig. 3. Optical density of final coloured solution *versus* time after the addition of the borate buffer solution: A, paracetamol; and B, phenacetin

The effect of the presence of commonly used base ingredients of tablets and of drugs frequently associated with phenacetin and paracetamol on the optical density of the final coloured solution is shown in Table V.

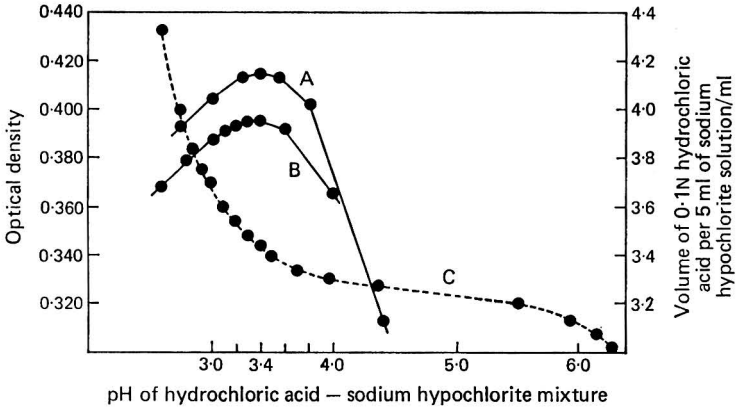


Fig. 4. pH of hydrochloric acid - sodium hypochlorite mixture *versus* optical density of final coloured solutions (solid lines, left-hand axis) and volume of 0·1 N hydrochloric acid added to 5 ml of sodium hypochlorite solution (dotted line, right-hand axis): A, paracetamol; B, phenacetin; and C, titration curve

Discussion

When the procedure was applied to samples of pure phenacetin and paracetamol, the following features were observed.

(*a*) A rectilinear relationship exists between the optical density of the final solution and the weight of phenacetin or of paracetamol contained therein (see Fig. 1 and Table I). The equation of the line of best fit is, for phenacetin, optical density = [weight (in micrograms) × 0·00196] + 0·0078 and, for paracetamol, optical density = [weight (in micrograms) × 0·00289] + 0·0117. In neither instance does the line pass through the origin, but, when assaying phenacetin or paracetamol in formulations, a single standard can be used without incurring a significant error, provided that the weight of the drug in the standard solution approximates to that in the sample solution.

Table III

Recovery of phenacetin and paracetamol from laboratory-prepared mixtures

Mixture		Recovery, per cent.
(1) *Aspirin, Phenacetin and Codeine Tablets B.P.*—		
Phenacetin	0·4990 g	100·3
Aspirin	0·500 g	
Codeine phosphate	0·016 g	
Starch	0·125 g	
Lactose	0·125 g	
Stearic acid	0·025 g	
(2) *Paracetamol Tablets B.P.*—		
Paracetamol	0·3674 g	99·9
Starch	0·035 g	
Lactose	0·035 g	
Stearic acid	0·007 g	
(3) *Paracetamol Elixir, Paediatric, B.P.C.*—		
Paracetamol	0·3651 g	99·8
Base (as B.P.C.)	to 15 ml	

(*b*) The precision of the results is reasonably satisfactory as can be seen in Table I. The mean relative standard deviation is 0·29 per cent. for phenacetin and 0·36 per cent. for paracetamol.

(*c*) The wavelength of maximum optical density of the final solution is from 625 to 630 nm (see Fig. 2). The final solution requires about 30 minutes after the addition of the borate buffer solution to reach its maximum optical density, after which it retains this value for a long period of time (see Fig. 3). For a given weight of phenacetin or paracetamol, almost the same optical density is attained from day to day.

(*d*) The pH of the hydrochloric acid - sodium hypochlorite mixture is a critical factor (see Fig. 4). It is important that the pH should be as close to 3·4 as possible and that the pH of the acid - hypochlorite mixture to which the sample solution is added is identical with that to which the standard solution is added.

As can be seen from Fig. 4, in the region of pH 3·4 relatively small variations in the volume of 0·1 N hydrochloric acid added to the sodium hypochlorite solution will significantly affect the pH of the mixture and thence the optical density of the final solution. To minimise this effect, we delivered both the acid and the hypochlorite solutions into the 50-ml calibrated flask from grade A burettes.

When paracetamol is determined, it is important that the sample and standard solutions are delivered with the tip of the pipette held close to the surface of the acid - hypochlorite mixture. If the solutions are allowed to run down the sides of the flask, low extinction values are often obtained, presumably because of the exposure of the paracetamol to chlorine vapour. This phenomenon was not observed with phenacetin.

(*e*) The pH of the final solution should not fall below about 9·3 (see Table II). With the specified borate buffer the pH of the final solution is well above this value.

(*f*) Other factors pertaining to the procedure are less critical. Variations of at least 10 per cent. can be tolerated in the volume of phenol solution added and in the lengths of the standing periods before and after the addition of the sodium arsenite solution, without affecting the final optical density. The volume of sodium arsenite solution should not fall below 2·0 ml, but this volume can be exceeded by 0·5 to 1·0 ml without any adverse effect. The temperature at which the reaction takes place is not critical; the optical density is not affected when the temperature is raised by 10 or 15 °C above room temperature.

(*g*) Light absorption resulting from the reagents is negligible.

TABLE IV

ASSAY OF PHARMACEUTICAL PREPARATIONS

Formulation	Content of phenacetin or paracetamol		
	By proposed method	By official method	Nominal
Paracetamol elixir (1)	2·44 per cent. w/v	2·46 per cent. w/v*	2·4 per cent. w/v
(2)	2·37 per cent. w/v	2·39 per cent. w/v*	2·4 per cent. w/v
Paracetamol Tablets B.P. (1) ..	498 mg per tablet	500 mg per tablet	500 mg per tablet
(2) ..	493 mg per tablet	487 mg per tablet	500 mg per tablet
Aspirin, Phenacetin and Codeine Tablets B.P. (1)	253 mg per tablet	260 mg per tablet	250 mg per tablet
Aspirin, Phenacetin and Codeine Tablets B.P. (2)	249 mg per tablet	262 mg per tablet	250 mg per tablet

* The method of the British Pharmaceutical Codex was used to determine the content of paracetamol in Paracetamol Elixir, Paediatric, B.P.C. The sample of paracetamol elixir (1) was not manufactured to the B.P.C. formula.

The procedure has given reliable results when applied to formulations; Table III shows the satisfactory recoveries obtained from laboratory-prepared mixtures. When applied to formulations from various manufacturers, the colorimetric procedure has given results similar to those obtained by the official methods (see Table IV). The absence of interference from common tablet excipients and from drugs frequently compounded with phenacetin or paracetamol indicates that the colorimetric procedure could be widely applied (see Table V).

It is interesting to note that the molar extinction coefficient for phenacetin, of about 17 900, is only 79 per cent. of that for paracetamol, which is about 22 600. If it is assumed that for both substances the coloured reaction product is phenolindophenol, then the difference in the molar extinction coefficients must be due to the less complete reaction of

TABLE V

EFFECT OF ADDING TABLET EXCIPIENTS AND OTHER DRUGS TO PHENACETIN AND PARACETAMOL ON THE OPTICAL DENSITY OF THE FINAL COLOURED SOLUTION

Substance added	Addition to phenacetin: Weight of substance added to 500 mg of phenacetin/mg	Addition to phenacetin: Optical density	Addition to paracetamol: Weight of substance added to 345 mg of paracetamol/mg	Addition to paracetamol: Optical density
No addition	—	0·400	—	0·410
Tablet base (1)	500	0·401	350	0·413
(2)	500	0·398	350	0·412
(3)	500	0·403	350	0·409
Saccharin sodium	—	—	50	0·412
Aspirin	500	0·396	350	0·410
Butobarbitone	125	0·403	90	0·408
Caffeine	500	0·399	350	0·410
Codeine phosphate	20	0·396	10	0·408
Dextropropoxyphene	175	0·397	175	0·412
Mepyramine maleate	50	0·401	35	0·409
Phenolphthalein	50	0·403	35	0·408
Phenylbutazone	50	0·400	35	0·412
Phenylephrine	10	0·398	10	0·408
Phenylpropanolamine	125	0·396	90	0·408
Vitamin C	100	0·399	70	0·414
Polyvinylpyrrolidone	100	0·403	100	0·409

Composition of the tablet bases was as follows—

Tablet base (1): Lactose (10 parts), magnesium stearate (1 part) and calcium carbonate (1 part).
(2): Sucrose (10 parts), talc (1 part) and calcium phosphate (1 part).
(3): Starch (10 parts) and stearic acid (1 part).

phenacetin compared with paracetamol. If this is so, it is surprising that a higher molar extinction coefficient is not obtained for phenacetin when the reaction conditions are changed, *e.g.*, by raising the reaction temperature or by increasing the amount of hypochlorite. The alternative explanation is that the coloured reaction products derived from phenacetin and paracetamol are not identical. We are currently investigating this aspect of the reaction and hope to report our findings at a later date.

REFERENCE

1. Murfin, J. W., *Analyst*, 1972, **97**, 663.
NOTE—Reference 1 is to Part I of this series.

Received *February 10th*, 1972
Accepted *April 14th*, 1972

Determination of Alphachloralose

Part I. In Stupefying Bait

By S. BAILEY

(*Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Hook Rise South, Tolworth, Surrey*)

A method, based on periodate oxidation, is described for the determination of alphachloralose in stupefying baits used to control bird populations.

The technique of using baits coated with alphachloralose [1,2-*O*-(2,2,2-trichloroethylidene)-α-D-glucofuranose][1] for catching birds has been previously described.[2–4] The baits consist of food coated with powdered alphachloralose at a nominal concentration of 1·5 or 2·0 per cent., with technical white oil as a "sticker". The method is used under licence from the Ministry of Agriculture, Fisheries and Food to control feral pigeons, house sparrows and wood-pigeons. The baits used to catch wood-pigeons are left exposed in the field for several days, during which time the effect of wind and rain reduces the concentration of alphachloralose. In order to determine the effective alphachloralose level on baits used for control in this fashion a suitable method of analysis is required.

The method of Lespagnol, Paris and Merville,[5] involving hydrolysis with sulphuric acid and determination of total chlorine, did not give consistent results. The colorimetric methods[6,7] were suitable for standard solutions, but gave high blank readings with the extracts from the untreated bait base.

A number of methods have been based on periodate oxidation. Griffon,[8] investigating the structure of alpha- and betachloraloses, used periodate followed by colour development with alkaline pyridine. Fleury and Jolly-Colin,[9] in determining alphachloralose, used excess of periodate and back-titration with standard iodine solution following the addition of buffered arsenic(III) oxide and potassium iodide. Hartman[10] extended the use of periodate oxidation to the analysis of technical monoglycerides in a non-aqueous chloroform - pyridine - acetic acid solvent system. After the reaction had been completed, excess of aqueous potassium iodide solution was added and the liberated iodine titrated with standard sodium thiosulphate solution. In this laboratory it has been found that while the Hartman method is ideal for analysing alphachloralose solutions, the recommended solvents are not suitable for the extraction of alphachloralose from bait. However, 74 O.P. industrial methylated spirit or absolute ethanol was found to be a suitable solvent.

The following method is used for the determination of alphachloralose in baits that do not contain other *cis*-glycollic compounds. Betachloralose, if present, will be determined by the method but the specification for the alphachloralose recommended for use in the manner approved by the Ministry of Agriculture, Fisheries and Food is such that betachloralose is not present in the bait.

Experimental

Reagents—

Industrial methylated spirit—74 O.P. (British Standard 3591:1963).

Potassium periodate reagent—Dissolve 2·8 g of AnalaR potassium periodate in 50 ml of 10 N sulphuric acid with gentle warming. After cooling the solution, add 975 ml of glacial acetic acid. Store the solution in a glass-stoppered brown bottle.

Potassium iodide solution, approximately 1·0 N.

Sodium thiosulphate solution—Make up a 0·1 N solution and standardise it accurately.

Starch indicator solution—1 *per cent.*

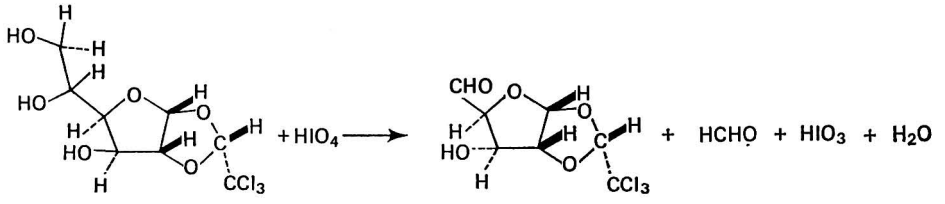
Method—

Extract a sample of bait (80 to 100 g) in a percolator[11] for 16 hours with 74 O.P. industrial methylated spirit and make the extract up to 250 ml. Transfer 25-ml aliquots by pipette into 500-ml conical flasks and add 50 ml of potassium periodate reagent. Mix the solutions by swirling the flasks and then place them in a dark cupboard for 30 minutes. At the end of this period add 20 ml of potassium iodide solution and 100 ml of distilled water and titrate

the liberated iodine with 0·1 N sodium thiosulphate solution with starch as indicator. Carry out titrations of the total iodine liberated from the periodate by using 25-ml volumes of 74 O.P. methylated spirit in place of the extract.

CALCULATION—

In the Malaprade[12,13] reaction, *cis*-glycols such as alphachloralose react with periodate as follows—



When the main reaction is complete the amount of iodine liberated on addition of the potassium iodide solution is proportional to the sum of the unchanged periodate and the iodate produced. The amount of alphachloralose is equivalent to the difference between the blank and the experimental titrations.

From the above reaction it can be seen that 1 mol of alphachloralose reacts with two equivalents of periodate. The equivalent weight of alphachloralose (molecular weight 309·54) is 154·77, so that 1 ml of 0·1 N sodium thiosulphate solution is equivalent to 15·48 mg of alphachloralose.

The method described has been used for the determination of alphachloralose in baits designed to stupefy birds. Recoveries, when using standard solutions of alphachloralose in 74 O.P. industrial methylated spirit, are complete. Blank extractions on a number of samples of all the materials commonly used as carriers, such as wheat, maize, maple peas and tic beans, gave no measurable blank values. Blank values are not obtainable from field samples. Table I shows recoveries from baits made up in the laboratory; the recovery figures reflect a loss of alphachloralose from the bait before extraction rather than during the procedure. Such a loss is unavoidable in this type of surface-coated formulation. Spiking of samples in the percolator leads to complete recoveries being obtained.

TABLE I

CONCENTRATION OF ALPHACHLORALOSE IN BAITS USED TO CONTROL BIRD POPULATIONS

Type of bait	Nominal concentration, per cent.	Actual concentration, per cent. Mean	Range
Tic bean	1·5	1·29*	1·17 to 1·35
Maple pea	1·5	1·27*	0·90 to 1·62
Tic bean	2·0	1·80*	1·73 to 1·93
Maple pea	2·0	1·81*	1·50 to 1·96
Tic bean	2·0	2·00†	1·99 to 2·00
Maple pea	2·0	2·00†	2·00

* Three samples.
† Two samples spiked in percolator.

REFERENCES

1. Forsén, S., Lindberg, B., and Silvander, B., *Acta Chem. Scand.*, 1965, **19**, 359.
2. Ridpath, M. G., Thearle, R. J. P., McCowan, D., and Jones, F. J. S., *Ann. Appl. Biol.*, 1961, **49**, 77.
3. Murton, R. K., Isaacson, A. J., and Westwood, N. J., *Ibid.*, 1963, **52**, 271.
4. Murton, R. K., Norris, J. D., and Thearle, R. J. P., *Agriculture, Lond.*, 1968, **75**, 587.
5. Lespagnol, A., Paris, J., and Merville, R., *Bull. Soc. Chim. Biol. Fr.*, 1942, **24**, 117.
6. Delvenne, J., and Barac, G., *Recl Trav. Chim. Pays-Bas Belg.*, 1947, **66**, 443.
7. Barrons, K. C., and Humner, R. W., *Agric. Chem.*, 1951, **6**, 48.
8. Griffon, H., *Annls Pharm. Fr.*, 1948, **6**, 165.
9. Fleury, M., and Jolly-Colin, J., *Ibid.*, 1950, **8**, 15.
10. Hartman, L., *Analyst*, 1956, **81**, 67.
11. D.D.T. Panel, *Ibid.*, 1960, **85**, 600.
12. Malaprade, L., *Bull. Soc. Chim. Fr.*, 1928, Series 4, **43**, 683.
13. ——, *Ibid.*, 1934, Series 5, **1**, 833.

Received *November 11th*, 1970
Amended *January 24th*, 1972
Accepted *March 17th*, 1972

Book Reviews

CHEMICAL ANALYSIS OF ADDITIVES IN PLASTICS. By T. R. CROMPTON. *International Series of Monographs in Analytical Chemistry. Volume* 46. Pp. xii + 162. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1971. Price £7.

This book is a valuable addition to the literature on the analysis of plastics materials. It is well written, rich with illustrations and references, and contains detailed descriptions of many methods that are used in everyday work in analytical plastics laboratories. Extraction, identification and quantitative determination of plasticisers, antioxidants, ultraviolet absorbers, flame retardants, organic peroxides and other additives are described, as are determinations of a number of monomers and volatile impurities. Among the techniques discussed are paper, column, thin-layer and gas chromatography, infrared and ultraviolet spectroscopy and polarography. On occasions, the author tends to go into too much detail, but that in no way detracts from the value of his work. The methods selected are sound and should prove useful to students and analysts concerned with plastics materials, although the price of the book is rather high.

In this kind of work, it is difficult to draw a line where chemical analysis ends and instrumental analysis begins, but it is nevertheless a little surprising that the author has not described some of the more recent developments in this field of analysis. Space should have been allotted to the combinations of thin-layer chromatography and of gas chromatography with mass spectrometry; similarly, nuclear magnetic resonance spectroscopy and the advances in liquid chromatography and X-ray fluorescence spectroscopy, among other techniques, deserved attention. The inclusion of these topics would have made the book more complete and up to date, and hence it is to be hoped that the deficiency will be remedied in the next edition. J. UDRIS

ULTRAMICRO ELEMENTAL ANALYSIS. By GUNTHER TÖLG. Translated by CONRAD E. THALMAYER. Pp. xiv + 200. New York, London, Sydney and Toronto: John Wiley and Sons Inc. 1970. Price £5·15.

The author presents a compilation of all available work in ultramicro elemental analysis, including the procedures pioneered and developed for samples of 30 to 100 μg by Professor Belcher and co-workers in Birmingham, and a review of the work of his own group in Stuttgart for samples of less than 30 μg.

The book is divided into General, Introduction to Weighing, Manipulation and Decomposition, and Procedures for Determination of the Elements, with chapters for carbon and hydrogen, nitrogen, oxygen, sulphur, fluorine, chlorine, bromine, iodine, phosphorus and arsenic. For each element there is a general introduction to decomposition and determination procedures, followed by principle, equipment, reagents and determination for each major method. The contents are well arranged and clearly illustrated, although the reviewer found a few diagrams with their descriptions inconveniently placed.

This admirable collection, with references to 1969, gives a wide range of methods for analytical choice, some especially designed for biochemical, biological and medical fields. If, however, the cost, availability of equipment and time are limited and a larger weight of sample is available, Professor Belcher's monograph gives more details than this volume for those methods which use simple and inexpensive equipment. For analysts with the more specialised problems with the limitation of 10^{-5}-g amounts, Professor Tölg's methods provide elegant and fascinating ultramicro techniques. B. J. STEVENSON

FLAME SPECTROSCOPY: ATLAS OF SPECTRAL LINES. By M. L. PARSONS and P. M. MCELFRESH. Pp. viii + 96. New York, Washington and London: IFI/Plenum. 1971. Price $11.50.

With increasing interest in the atomic-absorption (AAS), atomic-emission (AES) and atomic-fluorescence (AFS) spectrometric determinations of elements by using flame atomisation, the compilation of spectral lines used in all three techniques offers a valuable contribution at the present time. Most commercial atomic-absorption instruments are now capable of flame emission and many are also suitable for atomic fluorescence, but only rarely do the manuals indicate the spectral line to be used for these techniques. This is a disadvantage when, for example, the most sensitive line of an element in emission is not the same as that for absorption. Of the three tables here, that of atomic-fluorescence lines is the most useful even though data for relatively few

elements are currently available. It is possible to recognise the type of flame from the symbol used and although in many instances the relative intensity column is useless as only one fluorescence line has been observed or reported for many elements, the limits of detection given do allow some comparison to be made between the performances of different radiation sources and different flames.

Unfortunately, the coverage and presentation of information in the tables on atomic absorption and atomic emission are rarely as satisfactory as those for atomic fluorescence. The data on flame emission have been taken almost entirely from earlier compilations so that no limits of detection are given for the air - acetylene flame and no information at all is given for the nitrous oxide - acetylene flame even though the literature is covered up to the end of 1969. The table on atomic-absorption lines is at times incredible. For example, about half the data on calcium and magnesium are not from a flame at all, some of the remainder are unpublished results, and in neither case is a realistic detection limit given for any flame conditions—this for two of the most intensively studied elements. The concept of the book is excellent but although many lines are listed, data for comparing between them, between different instrumental conditions and between the three techniques could undoubtedly be improved. It would also help if the notation used in the three tables was unified and preferably in the style of the AFS table. For example, the type of flame is indicated by a different symbol in each table, thus air - acetylene is variously B (AAS table), AA (AES) and AcA (AFS).

J. M. OTTAWAY

EMISSION SPECTROCHEMICAL ANALYSIS. By MORRIS SLAVIN. *Volume 36 in Chemical Analysis: A Series of Monographs on Analytical Chemistry and its Applications.* Pp. xii + 254. New York, London, Sydney and Toronto: Wiley-Interscience. 1971. Price £7·75.

There has not been a general text on emission spectrometry for many years and the excellent texts of the period 1935 to 1955 have now been overtaken by the rapid developments in this field, particularly in instrumentation, which now allows a high degree of automation in analytical practice. This monograph will serve as an adequate modern introduction to this field, and covers all aspects from the origin of spectra, optics, sources and detectors to procedures for quantitative analysis. It will be very useful for students of analytical chemistry and to any analyst entering this field for the first time, and sufficient important references are given for any particular aspect of the subject to be studied in greater detail. The lack of any detailed discussion or examples of applications is a disadvantage and the inadequate introduction to atomic spectra is disappointing, but otherwise the coverage is satisfactory for an introductory text and the book can be recommended on this basis.

J. M. OTTAWAY

THE ANALYTICAL CHEMISTRY OF SULFUR AND ITS COMPOUNDS. Edited by J. H. KARCHMER. Part III. NUCLEAR MAGNETIC RESONANCE DATA OF SULFUR COMPOUNDS. By N. F. CHAMBERLAIN and J. J. R. REED. *Volume 29 in Chemical Analysis: A Series of Monographs on Analytical Chemistry and its Applications.* Pp. xii + 308. New York, London, Sydney and Toronto: Wiley-Interscience. 1971. Price £8·40.

This book is the third part of the volume dealing with the analytical chemistry of sulphur; previous parts have dealt with sulphur in inorganic and organic functional groups, and were intended mainly for analytical chemists. The various analytical techniques necessary were discussed at various points as they dealt with appropriate functional groups.

This particular part is intended for experienced users of nuclear magnetic resonance data, spectroscopists and others who do not necessarily use nuclear magnetic resonance spectroscopy as a tool in analytical chemistry. It is mainly a collection of data relating to named compounds. These data for approximately 1400 compounds are listed on 225 of the 308 pages of the book. For each compound, details of the chemical shifts brought about by various groups together with appropriate coupling constants are given. There are both alphabetical and structural indexes for the compounds and these, with an index of empirical formulae, are helpful in locating data for a particular compound.

It would perhaps have been more useful to list the headings used in the data tables before the tables, instead of placing them some way after the appropriate table. The present position is of use only after a determined effort has been made to memorise it and the list of headings.

This whole volume will probably serve for some time as the reference volume for sulphur; this particular part will be of great use to those people dealing with the nuclear magnetic resonance of sulphur compounds, and perhaps as more data for more compounds become available a supplement will be forthcoming.

L. S. BARK

PESTICIDES IDENTIFICATION AT THE RESIDUE LEVEL. *Advances in Chemistry Series No.* 104. By FRANCIS J. BIROS. Pp. x + 182. Washington: American Chemical Society. 1971. Price $8.50.

This book resulted from the Symposium, in May, 1970, sponsored by the Division of Pesticide Chemistry of the American Chemical Society. Eleven papers are presented and workers in the field of pesticide residue analysis will find a large amount of useful information and thought-provoking speculation in it. An introductory discussion on the limits of ultramicro methods of analysis is followed by individual papers that cover most of the instrumental techniques used in pesticide analysis. Emphasis is placed on infrared and ultraviolet spectrophotometry, gas-chromatographic detectors and combined gas chromatography - mass spectrometry. The paper by Aue, which describes flame detectors, is particularly noteworthy and contains 78 references. Reviews on techniques involving the formation of derivatives for confirmation of organochlorine residue identities, thin-layer and paper chromatography, enzymatic and immunological techniques are also included.

The style and approach of the papers indicates that they are directed at residue analysts, presupposing a basic knowledge of the techniques and methods of pesticide residue analysis. With the exception of the paper by Ercegovich on immunological techniques, none of the techniques or methods dealt with can now be considered to be novel or advanced, and most residue analysts will be familiar with them from the literature, if not from practical experience. However, the style largely adopted, somewhat between an original dissertation and a review paper, allows the authors to be expansive and speculative, giving background thinking and consideration that may be of value to many residue analysts. A particular feature, which is stressed in more than one paper, is the necessity for careful confirmation of identity of residues—a salutary reminder to those who tend to regard a gas - liquid chromatographic peak almost as sufficient confirmation of identity.

Since the symposium was held, much interesting work has been carried out on such subjects as the quantitative aspects of polychlorinated biphenyls and organomercury residues, which would merit a paper in such a publication as this. Nevertheless, this reasonably priced volume would be an interesting and useful addition to the library shelves of any pesticide residue unit and is recommended especially to those pesticide analysts who could not attend this important meeting.

J. H. RUZICKA

WANTED

Wanted—1 EEL Photosedimentometer with constant voltage transformer and galvanometer. Write to:
Morgan Crucible Co. Ltd
Thermic Division Technological Centre
Bewdley Road
Stourport-on-Severn, Worcs.

BUSINESS PROPOSITIONS

Opportunity occurs for Chemist to acquire an interest in consulting chemical practice. Applicants should state nature of previous experience in chemical, pharmaceutical or allied fields. Capital could be available for immediate purpose. Good prospects for expansion. Apply to Box No 231, c/o J. Arthur Cook, 9 Lloyd Square, London WC1X 9BA.

APPOINTMENTS VACANT

SPECTROGRAPHER

for our Chemical Laboratory. We need a young man 20+ with H.N.C., L.R.I.C., or G.R.I.C. Part I qualifications. His responsibilities will cover spectrographic analysis and development of new techniques on new equipment. The applicant must have analytical experience; instrumental experience is not essential but desirable. Salary dependent on experience. Ref. RA81.

LABORATORY ASSISTANT

16-25 with GCE 'O' Levels in Chemistry, Maths., Physics. If older would consider not qualified. Duties involve written spectographic analysis. Salary dependent on age and experience. Ref. RA82.

Benefits include good holidays, good working conditions, pension scheme, sports and social club and welfare amenities.

Please write or telephone, quoting relevant reference number, Mrs. Gudgeon, Welfare Officer, Murex Welding Processes, Hertford Road, Waltham Cross, Herts. Tel. No. Waltham Cross 33511.

APPOINTMENTS VACANT

CITY OF PORTSMOUTH

DEPUTY PUBLIC ANALYST AND SCIENTIFIC ADVISER

Post No. 102

Salary £3,054-£3,402

(75% of P.O. 2 Pts. 5-9)

The person appointed must hold the qualification of M.Chem.A. and must have had considerable experience in a senior capacity in the laboratory of a Public Analyst. The candidate should have experience in toxicological analysis, trade effluent and other environmental problems.

The Department expects to move into a new purpose-built laboratory in the Autumn.

Further details and application form from the **Public Analyst and Scientific Adviser, Trafalgar Place, Portsmouth, PO1 5JJ.**

Please quote Ref. 706.

Analytical Chemist

Weetabix Ltd. have an opening for an Analytical Chemist to be responsible for the quality control laboratory. The laboratory provides full quality control information on grocery products, speciality flour and feeding stuffs and an analytical service to the research and development laboratory.

Applicants should, therefore, have at least five years experience of analytical work in the food industry, preferably with cereal chemistry knowledge. M.Chem.A. or near equivalent would be an advantage although not essential.

The Company, which is in Northamptonshire, operates a Contributory Pension and Life Assurance scheme and will cover relocation expenses. A company car will be provided. Salary by negotiation.

Please write for further details and an application form to:-

E. R. Steiner, M.A.
Chief Chemist, Weetabix Ltd.,
Burton Latimer,
Kettering, Northants.

Completed application forms must be received at Weetabix by 11th September, 1972.

Analysis of High Purity Water by Flameless Atomic-absorption Spectroscopy

Part I. The Use of an Automated Sampling System

An apparatus is described that enables fully automatic stream sampling and sample injection to be carried out when using the graphite-tube method of analysis in conjunction with atomic-absorption spectroscopy. Multiple aliquots of solution can be introduced automatically, with a drying step between each introduction. The concentration sensitivity of the method is thus increased even further, but without encountering the difficulties associated with the manual injection of multiple aliquots. In addition, a significant improvement in precision is obtained when using the automatic sampling and injection system rather than manual sampling.

The apparatus has been designed as part of a system to determine simultaneously a number of elements at levels of parts per 10^9 and below. Results for precision are presented for the determination of cobalt, chromium, copper, iron, manganese and nickel, and results for sensitivity are given for these and additional representative elements.

C. J. PICKFORD and G. ROSSI

Chemistry Division, Euratom-CCR, ISPRA (Varese), Italy.

Analyst, 1972, **97**, 647–652.

A Comparison of Some Methods for the Determination of Triazine Herbicides in Water

By using gas chromatography with an alkali flame detector, 0·001 p.p.m. of atrazine, ametryne and terbutryne in water from two sources could be determined with recoveries close to 100 per cent. Ultraviolet spectrophotometry was adequate at the 0·01 p.p.m. level for all three compounds but was unreliable at lower levels. With cathode-ray polarography, 0·005 p.p.m. of terbutryne and ametryne and 0·01 p.p.m. of atrazine could be determined.

C. E. McKONE, T. H. BYAST and R. J. HANCE

Agricultural Research Council Weed Research Organization, Begbroke Hill, Yarnton, Oxford, OX5 1PF.

Analyst, 1972, **97**, 653–656.

A Recommended Molybdenum-blue Procedure for the Determination of Arsenic in Steel

A recent molybdenum-blue procedure suggested by Nall for the determination of arsenic in steel has been modified to give quantitative extraction of arsenic from the steel solution.

The new procedure differs from that of Nall in that reduction of arsenic in the solution of the steel is effected with tin(II) chloride instead of with copper(I) chloride and hypophosphorous acid, and arsenic(III) is extracted as the iodide rather than as the chloride. Recoveries of arsenic of greater than 95 per cent. were obtained consistently with the recommended procedure, which gives high precision and good reproducibility from day to day. Results in close agreement with the certified values, with relative standard deviations of less than 2 per cent., were obtained for the analysis of nine British Chemical Standards steel samples.

An extension of this procedure is also described in which the molybdenum blue is extracted into 1 + 4 v/v hexanol-3-methylbutan-1-ol, giving a fivefold increase in sensitivity.

A. G. FOGG, D. R. MARRIOTT and D. THORBURN BURNS

Chemistry Department, University of Technology, Loughborough, Leicestershire, LE11 3TU.

Analyst, 1972, **97**, 657–662.

A Colorimetric Method for the Determination of Phenacetin and Paracetamol

Part I. An Automated Procedure for the Determination, in Formulations, of Phenacetin and Paracetamol Alone or in the Presence of Aspirin and Codeine Phosphate

A rapid, automated, colorimetric method for the determination of phenacetin and paracetamol is described. The reaction of either compound with acidic hypochlorite followed by coupling with alkaline phenol produces a blue indophenol dye, which is measured spectrophotometrically. The method has been applied to tablets containing phenacetin or paracetamol, alone or in combination with aspirin and codeine phosphate, and to a granule formulation containing paracetamol. Standard procedures were used for the determination of aspirin and codeine phosphate.

J. W. MURFIN

Analytical Research, Quality Control, The Boots Company Ltd., Pennyfoot Street, Nottingham.

Analyst, 1972, **97**, 663–669.

A Colorimetric Method for the Determination of Phenacetin and Paracetamol

Part II. A Manual Procedure for the Determination of Phenacetin or Paracetamol in Formulations

A manual colorimetric method for the determination of phenacetin or paracetamol in pharmaceutical preparations has been developed from the automated method described in Part I of this series. The drug (about 140 μg of paracetamol or about 200 μg of phenacetin) is added to a hydrochloric acid - sodium hypochlorite mixture at pH 3·4 and the excess of hypochlorite is removed with sodium arsenite. A solution of phenol is then added, followed by borate buffer solution (pH 9·9), and the optical density of the blue indophenol dye solution is measured at 625 nm.

The method is used to determine phenacetin in Aspirin, Phenacetin and Codeine Tablets B.P. and paracetamol in Paracetamol Tablets B.P. and paracetamol elixirs. Common tablet excipients, and drugs frequently compounded with paracetamol and phenacetin, do not significantly interfere with the reaction; the method should therefore be widely applicable to phenacetin or paracetamol formulations.

J. W. MURFIN and J. S. WRAGG

Analytical Research, Quality Control, The Boots Company Ltd., Pennyfoot Street, Nottingham.

Analyst, 1972, **97**, 670–675.

Determination of Alphachloralose

Part I. In Stupefying Bait

A method, based on periodate oxidation, is described for the determination of alphachloralose in stupefying baits used to control bird populations.

S. BAILEY

Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Hook Rise South, Tolworth, Surrey.

Analyst, 1972, **97**, 676–677.

Spectroflux* materials will help you

Here's how . . . The Spectroflux* range of prefused borate fluxes provides you with a versatile aid in the rapid and accurate analysis of ores, refractories and slags.

Whether you are using instrumental or chemical methods of analysis, you will benefit from the speed and increased accuracy that are gained by first fusing your samples with the appropriate Spectroflux* material.

In the Spectroflux* range you will find borate fluxes that have been specially formulated to facilitate the analysis of a wide variety of materials. And our new publication will help you to select the flux best suited to your work. Send for your copy today.

Spectroflux is a Johnson Matthey Chemicals' trademark*

Spectroflux* prefused borate flux mixtures

JMC

Johnson Matthey Chemicals Limited
74 Hatton Garden, London EC1P 1AE, England
Telephone : 01-405 6989 Telex : 267711

THE QUEEN'S AWARD TO INDUSTRY 1968 1970

USA
Jarrell Ash Division
Fisher Scientific Co.,
590 Lincoln Street, at Route 128
Waltham, Massachusetts 02154

AUSTRALIA
Matthey Garrett Pty. Limited
160-170 Rocky Point Road,
P.O. Box 165, Kogarah, NSW 2217

SOUTH AFRICA
Johnson Matthey (Pty) Ltd
P.O. Box 14078,
Wadeville, Transvaal

FRANCE
Ets. F. C. Dannatt
68 rue du Commerce,
75 Paris 15

Volume 97 No. 1157, Pages 585-680 August, 1972

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

CONTENTS

Printed for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England
Communications to be addressed to the Editor, J. B. Attrill, 9/10 Savile Row, London, W1X 1AF
Enquiries about advertisements should be addressed to J. Arthur Cook, 9 Lloyd Square, London, WC1X 9BA

Entered as Second Class at New York, U.S.A., Post Office.