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SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA*

Vol. 31, No. 3, September 1964

THE DETERMINATION OF SILICA BY CONWAY'S MICRO-DIFFUSION TECHNIQUE

A new approach is suggested to the determination of silica. Silica is converted to volatile silicon-fluoride compounds by the action of hydrofluoric acid and is quantitatively isolated by Conway's micro-diffusion technique in a polypropylene cell. To promote diffusion, a sulphuric acid medium is used; ethylene glycol serves as absorbent. Quantities of 20–70 μg SiO_2 per 0.5 ml were tested, with quantitative recovery. The silica was determined spectrophotometrically by the molybdenum blue method.

A. ALON, B. BERNAS AND M. FRENKEL

Anal. Chim. Acta., 31 (1964) 197–205.

A STUDY OF FLUX MONITORING FOR INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

The accuracy of instrumental neutron activation analysis is dependent upon measurement of the neutron flux to which samples are submitted. Flux monitoring techniques described and evaluated include: target cooling water activity, BF_3 counters, plastic scintillators, and a simple reference sample system. Evaluation is made on the basis of results obtained for oxygen analysis using primary standard materials. The superiority of the reference sample system for flux measurement during irregular neutron flux production is shown.

F. A. IDDINGS,

Anal. Chim. Acta, 31 (1964) 206–212.

PRECISE AND ACCURATE DETERMINATION OF CHROMIUM IN STEEL

Determination of chromium(VI) by addition of weighed amounts of solid ammonium ferrous sulfate in excess and back-titration with permanganate, using spectrophotometric end-point detection, is applied to the determination of chromium in steel. Except in the case of vanadium, and especially of vanadium + tungsten, very high selectivity and precision are claimed. Relative standard deviations of the order of 0.05% to 0.15% were found. Recoveries in excess of 99.95% were obtained in most cases. Results for the determination of chromium in standard samples of steel are given.

N. LOUNAMAA,

Anal. Chim. Acta, 31 (1964) 213–223.

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* In the summaries of papers of August 1964, the volume no. 30 has to be changed in 31.

THE SPECTROPHOTOMETRIC DETERMINATION OF VARIOUS METALS WITH FORMALDOXIME

(in French)

Formaldoxime forms highly coloured complexes (ε = 4000–18000) with manganese, cerium, vanadium, nickel and iron in alkaline media. On the basis of a study of reaction mechanisms and of the optimum conditions for the formation of the different complexes, new spectrophotometric methods are described for determination of cerium and iron, as well as improved methods for determination of manganese, nickel and vanadium. The use of formaldoxime for Mn, Ce and V is particularly beneficial in trace analysis and the methods suggested are specific.

Z. MARCZENKO,

Anal. Chim. Acta, 31 (1964) 224–232.

SPECTROPHOTOMETRIC DETERMINATION OF FORMALDEHYDE, FURFURAL, AND VANILLIN IN BISULFITE SOLUTIONS

Formaldehyde, furfural, and vanillin are determined in solutions containing bisulfite. The interference of bisulfite is eliminated by oxidation with iodine before furfural and vanillin are determined spectrophotometrically in ultraviolet light. Formaldehyde is determined with chromotropic acid by a slightly modified procedure; bisulfite is not destroyed previously.

K. CHRISTOFFERSON,

Anal. Chim. Acta, 31 (1964) 233–238.

THE USE OF RIGID ETHANOLIC SOLUTIONS FOR THE PHOSPHORIMETRIC INVESTIGATION OF ORGANIC COMPOUNDS OF PHARMACOLOGICAL INTEREST

Phosphorescence excitation and emission spectral peaks, lifetimes, working curves and limits of detection of 22 organic compounds of pharmacological importance in rigid (77°K) ethanolic solution are given. Ethanol can be easily prepared in a high degree of purity, and most drugs are much more soluble in ethanol than in most solvents previously used for phosphorimetric studies. The possible application of phosphorimetry to the trace analysis of drugs in biological fluids is discussed.

J. D. WINEFORDNER AND M. TIN,

Anal. Chim. Acta, 31 (1964) 239–245.

THE USE OF 2,6-DICHLOROPHENOLINDOPHENOL AS INDICATOR IN ACID–BASE TITRATIONS

2,6-Dichlorophenolindophenol shows acid–base indicator properties. Its colour is red in acidic media and blue in alkaline media. The transitional pH value is 5.7 and the colour change is sharp and easy to observe. The use of the indicator in various acid–base titrations, as well as the effects of neutral salts, alcohol and temperatures were checked. The absorption spectra of the acidic and basic forms of the indicator are presented.

K. ERÖSS, G. SVEHLA AND L. ERDEY,

Anal. Chim. Acta, 31 (1964) 246–250.

THE DETERMINATION OF THE STABILITY CONSTANTS OF THE LANTHANIDE–α-HYDROXYISOBUTYRATE AND LACTATE COMPLEXES BY POTENTIOMETRIC TITRATION

The stability constants of the lanthanide and yttrium complexes with α-hydroxyisobutyrate and lactate, have been determined by potentiometric titration. The average number of ligands bound per metal ion has been calculated by the method of CALVIN–WILSON and indicates the formation of uninegative tetra-ligand complexes of the form ML_4^-. The 4 formation constants have been derived by 4 procedures: BJERRUM's half-$\bar{n}$-method, FRONAEUS' and POULSEN's extrapolation methods, and by least squares calculation, using an IBM 1620 digital computer.

H. DEELSTRA AND F. VERBEEK,

Anal. Chim. Acta, 31 (1964) 251–257.

A NON-DISTILLATION KJELDAHL METHOD FOR NITROGEN BASED ON PRECIPITATION AS TETRAPHENYLBORATE

A simple, accurate method for the determination of protein nitrogen by a modification of the Kjeldahl procedure, eliminating the distillation step and weighing ammonium tetraphenylborate (TPB) precipitate directly, is described. The gravimetric factor is favorable, and no standard solutions are required. Interference from mercury catalyst was prevented by conversion to the soluble tetraiodomercurate(II) complex (method 1), removal of the tetraiodomercurate via anion-exchange resin (method 2), or removal of mercury as mercuric sulfide (method 3). These 3 TPB methods gave results as accurate and precise as the usual distillation approach. Values by method 1, averaged 100.5% recovery with a standard deviation of 1.8; and by method 2, which is used when large concentrations of mercury are present, they averaged 100.4% with 1.8 std. dev.

F. E. CRANE, JR. AND E. A. SMITH,

Anal. Chim. Acta, 31 (1964) 258–267.

GRAVIMETRIC DETERMINATION OF TUNGSTEN(VI) WITH N-BENZOYL-N-PHENYLHYDROXYLAMINE

Tungsten can be quantitatively precipitated with N-benzoyl-N-phenylhydroxylamine in presence of hydrochloric acid (0.5–1 *N*). The precipitate can be weighed as $WO_2(C_{13}H_{10}O_2N)_2$ or WO_3. Molybdenum, vanadium, titanium, and iron can be separated by prior precipitation of these metals with the above reagent in presence of tartrate ions; tungsten is then determined in the filtrate. Uranium does not interfere, but chromium(VI), fluoride and phosphate do.

V. R. M. KAIMAL AND S. C. SHOME,

Anal. Chim. Acta, 31 (1964) 268–271.

AN EMPIRICAL CORRELATION OF TWO METHODS FOR PHENOLS IN CIGARETTE SMOKE

A specific gas-chromatographic and a non-specific colorimetric method for the determination of phenols in cigarette smoke are described. The rapid, non-specific method has been correlated with the specific procedure so that the amount of phenol in smoke can be predicted with $\pm 15\%$ at the 95% confidence level. The trapping efficiency of phenols by Cambridge filter pads is also presented.

E. T. OAKLEY, J. O. MILLHAM AND L. WEISSBECKER,
Anal. Chim. Acta, 31 (1964) 272–278.

THE DETERMINATION OF PALLADIUM BY ATOMIC ABSORPTION SPECTROSCOPY

A commercial atomic absorption spectrophotometer was used without modification to establish the most suitable operating conditions for the determination of palladium. A study of the effect of organic solvents miscible with water, and of acids was then carried out. In general, organic solvents led to increased sensitivity. With a complex of palladium, $Pd(py)_2Cl_2$, in 50% ethanol, the sensitivity was also enhanced and, with a hexone solution of the complex, $Pd(py)_2(SCN)_2$, amounts of palladium below 2 p.p.m. could be determined.

G. ERINC AND R. J. MAGEE,
Anal. Chim. Acta, 30 (1964) 279–284.

COLORIMETRIC DETERMINATION OF MANGANESE IN AMBER GLASS

(Short Communication)

T. S. HERMANN,
Anal. Chim. Acta, 31 (1964) 284–286.

MALACHITE GREEN: A NEW IODIMETRIC INDICATOR

(Short Communication)

J. O. MEDITSCH,
Anal. Chim. Acta, 31 (1964) 286–289.

DETERMINATION OF OXYGEN IN HELIUM BY WINKLER'S METHOD

(Short Communication)

K. G. DIVEKAR, K. A. KHASGIWALE AND M. SUNDARESAN,
Anal. Chim. Acta, 31 (1964) 289–292.

ULTRAMICRO TITRATION OF FATTY ACIDS IN NON-AQUEOUS SINGLE-PHASE SYSTEMS

(Short Communication)

C. PRIES AND C. J. F. BÖTTCHER,
Anal. Chim. Acta, 31 (1964) 293–294.

IRREGULARITIES IN THE POTENTIOMETRIC TITRATION OF KETIMINES

(Short Communication)

F. A. IDDINGS,
Anal. Chim. Acta, 31 (1964) 294–296.

THE DETERMINATION OF PALLADIUM BY ATOMIC ABSORPTION SPECTROSCOPY

G. ERINC* AND R. J. MAGEE

Department of Chemistry, Queen's University, Belfast (Northern Ireland)

(Received January 30th, 1964)

Atomic absorption spectroscopy first suggested by WALSH[1,2] has become in recent years a well-established analytical technique. For the determination of small amounts of elements it has been very successful and a considerable volume of literature has appeared on the subject. ELWELL AND GIDLEY[3], who discuss the theory and technique, indicate that although atomic absorption spectroscopy is suitable for the determination of many elements, little if any information has been published for some elements. For palladium this is particularly true. The literature on atomic absorption spectrophotometry has 3 references relating to the determination of palladium[4–6]. Two of these references, however, merely restate the work of LOCKYER AND HAMES[4], who determined a number of noble metals, including palladium, by this technique. The smallest amount of palladium determinable was 2 p.p.m.

The present investigation arose out of a desire to determine palladium in some platinum metal alloys for which it was hoped the atomic absorption technique would be satisfactory. However, as the conditions of determination and many essential details were not available, it was decided to establish these. The results of the investigation are set out below.

EXPERIMENTAL

A standard Uvispek H700 spectrophotometer, fitted with a Hilger H1100 atomic absorption attachment, was used. A standard Hilger and Watts palladium hollow-cathode lamp was employed for the line source. Although some trends in the results indicated that modification of the spraying chamber might be an advantage, no alterations were made to the standard commercial equipment.

The arrangement of the equipment and the method of operation have been described many times, elsewhere, and will not be repeated here. No modifications to the usual procedure were made.

RESULTS

The effect of lamp current

In the earlier work on palladium[4] the slit-width used was not stated. Therefore,

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with a wide slit-width of 1.00 mm, the lamp current conditions were first investigated. The results obtained on spraying a 50 p.p.m. Pd solution (as $PdCl_2$) are shown in Table I. From a series of results the best lamp current was concluded to be 44.5 mA.

TABLE I

EFFECT OF LAMP CURRENT

(Slit-width: 1.00 mm; air pressure: 20 lbs/sq. in.)

O.D.	*Lamp current (mA)*
0.017	39.0
0.016	30.0
0.009	20.0
0.019–0.020	44.5
0.020–0.021	60.0

TABLE II

CHOICE OF WAVELENGTH

(Solution sprayed: Pd, 50 p.p.m. in water; slit-width: 1.00 mm; air pressure: 20 lbs/sq. in.; lamp current: 44.5 mA)

Wavelength (Å)	*Response (optical density)*
2430	—
2435	0.010
2440	0.021
2445	0.034
2448	0.039
2450	0.037
2455	0.022
2460	0.012
2465	0.010
2470	0.015
2476	0.024
2480	0.021
2485	0.012
2490	0.005
2495	—

Wavelength

LOCKYER AND HAMES[4] in their determination of palladium used the wavelength of 2476 Å. In the present work it was decided to investigate the response at different wavelengths in the region 2430–2490 Å. The results are shown in Table II. All the results obtained indicated, as shown, that the best response occurred at 2448 Å. In subsequent determinations this wavelength was always used. Outside the range mentioned in Table II, the sensitivity fell off rapidly.

Slit-width

It became clear in the preliminary investigations that slit-width had a considerable effect on the response and, as the earlier publication on palladium did not indicate the slit-width used, the other conditions mentioned in Table II were maintained

and the slit-width was varied over the range 0.05 to 2 mm. Of the values investigated slit-widths of 0.225, 0.100 and 0.075 mm showed the best response. However, values for a slit-width of 0.075 mm were not easily reproducible. The reproducibility of values at the other 2 slit-widths was examined (Table III). It will be seen that while the reproducibility with a slit-width of 0.100 mm is quite good, it shows, particularly for higher concentrations, a bigger spread than a slit-width of 0.225 mm. Because of these considerations the latter slit-width was used in subsequent work.

TABLE III

REPRODUCIBILITY AT DIFFERENT SLIT-WIDTHS

Pd solution sprayed (p.p.m.)	*Response (optical density)*							
	Slit-width 0.225 mm					*Slit-width 0.100 mm*		
2.0	0.002	0.002	0.002	—	—	—	—	—
5.0	0.009	0.008	0.009	0.010	0.008	0.011	0.012	0.011
10.0	0.017	0.019	0.020	0.019	0.018	0.031	0.024	0.028
25.0	0.042	0.041	0.045	0.043	0.042	0.062	0.066	0.076
50.0	0.062	0.070	0.071	0.072	0.069	0.112	0.126	0.119
75.0	0.082	0.089	0.088	0.089	0.085	0.144	0.142	0.157
100.0	0.093	0.100	0.096	—	—	0.160	0.185	—
125.0	0.101	—	—	—	—	—	—	—
250.0	0.114	—	—	—	—	—	—	—

Air pressure

In the above determinations the air pressure was held at 20 lbs/sq. in. The effect of air pressure was investigated with a slit-width of 0.3 mm, a wavelength of 2448 Å and a lamp current of 44.5 mA, a 50 p.p.m. solution of palladium being sprayed. Pressures of 22, 24 and 26 lbs/sq. in. were tested and the best response and reproducibility were obtained at 24 lbs/sq. in. (optical density 0.053). This pressure was used in subsequent investigations.

Propane gas pressure

With the burner an air–propane flame was used throughout the work. The pressure of the propane was about 2.5 lbs/sq. in. According to ELWELL AND GIDLEY[3] the time for a droplet to pass through 1 cm of flame is about 10 msec. Since a part of the time is required for the droplet to evaporate to dryness and the metal atoms to vaporise, it would appear that lowering the propane pressure would decrease the velocity of gases and consequently the velocity at which the droplets pass through the flame, so that an enhancement of sensitivity might result. Evidence that this does occur has been given[7]. In the present work with palladium, evidence was also found that reduction of the propane gas pressure produced an enhancement of sensitivity, but pressures could not be accurately altered and determined with the apparatus available since, below 2.5 lbs/sq. in., 'blow-back' conditions arose too easily for continued use.

The effect of organic solvents

In flame photometry the use of organic solvents miscible with water has frequently been used to increase sensitivity[8].

In atomic absorption spectroscopy an interesting investigation of solvents, miscible and immiscible with water, has been reported by ALLAN[9]. This author found that in the determination of Cu, Fe, Mn, Zn and Mg, certain organic solvents when added to aqueous solutions, produced small increases in sensitivity. Further, when the element is extracted into an organic solvent immiscible with water, gains in sensitivity up to 7 times were obtained.

In an attempt to improve the sensitivity in the determination of palladium, the influence of several organic solvents was investigated. The results obtained are shown in Table IV.

TABLE IV

EFFECT OF ORGANIC SOLVENTS

(Solution sprayed: Pd, 50 p.p.m.; lamp current: 44.5 mA; wavelength used: 2448 Å; slit-width: 0.225 mm; air pressure: 24 lbs/sq. in.; propane pressure: 2.5 lbs/sq. in.)

Solvent	*Response (optical density)*	*Remarks*
Water	0.072	
30% (v/v) Methanol	0.097	
50% (v/v) Methanol	0.104	
30% (v/v) Ethanol	0.096	
50% (v/v) Ethanol	0.109	
30% (v/v) *n*-Propyl alcohol	0.078	
50% (v/v) *n*-Propyl alcohol	0.079	Upper part of flame is yellowish in colour
30% (v/v) Isopropyl alcohol	0.100	
50% (v/v) Isopropyl alcohol	0.103	Upper part of flame is yellowish in colour
30% (v/v) *tert.*-Butyl alcohol	0.106	Upper part of flame is yellowish in colour
50% (v/v) *tert.*-Butyl alcohol	0.109	
30% (v/v) Acetone	0.090	Upper part of flame is yellowish in colour
50% (v/v) Acetone	0.097	

It can be seen from these results that, generally, mixing organic solvents with water does increase the sensitivity of the determination of palladium. The best result was obtained with 50% (v/v) *tert.*-butyl alcohol. Higher concentrations of solvent were examined, up to 80%, but no improvement over the 50% concentration was obtained.

In some of the flames as indicated, the upper part was yellowish in colour but the flame was steady and reproducibility was not affected 'Background' values, *i.e.* in the absence of palladium, were low, the highest being 0.013 for 50% isopropyl and *tert.*-butyl alcohols.

When an element is sprayed into a flame the factors that the use of an organic solvent are likely to influence are the concentration of the atoms, or the flame temperature. The flame temperature for a number of organic solvents has been measured[9]; it was found that, while the temperature is lowered, the effect is very small.

As far as the concentration of atoms is concerned, it is not supposed that the use of organic solvents will result in an increase in the rate of vaporisation of the palladium compound[9], or in the degree of dissociation into atoms[9,10]. The principal cause

of the increase in sensitivity is thought, therefore, to be an increase in the amount of palladium reaching the flame. This arises because, with an organic solvent present droplets are smaller, so that more are sprayed into the flame in a given time than with water alone.

Mineral acids

Hydrochloric and nitric acids are normally used in palladium solutions to prevent hydrolysis. The influence of different concentrations of these acids, and sulphuric acid, on the response of 50 p.p.m. palladium was investigated as shown in Table V. The conditions established above for lamp current, etc., were maintained. The optical densities reported are an average of a series of readings.

TABLE V

EFFECT OF MINERAL ACIDS

Acidity (M)	*Optical density*		
	HCl	*HNO_3*	*H_2SO_4*
0.1	0.073	0.067	0.061
0.5	0.071	0.067	0.059
1.0	0.068	0.066	0.055
2.0	0.063	0.065	0.045
5.0	0.056	0.062	0.032

From these results it can be seen that up to 5 *M* concentration, except in the case of sulphuric acid, there is only a small decrease in response. Thus, to prevent hydrolysis in palladium solutions which are to be sprayed into a burner, hydrochloric or nitric acid up to a concentration of 5 *M* is satisfactory. Above 5 *M* in all 3 acids there is a rapid decrease in response. This effect is not considered to be an anion effect, but rather to be caused by a change in density of the solution, so that less solution, and hence less palladium, reaches the burner. Another factor may be the rate of evaporation of the acid. This is confirmed by the results with sulphuric acid, where a fall-off in response is observed at much lower concentrations than for the other more easily evaporated acids. In connection with the investigations on the effect of acids, acetic acid was also considered. Apart from other factors, the reaction of this acid in the flame, as shown by the equation

$$CH_3COOH = CH_3OH + CO - 34 \text{ kcal}$$

is endothermic.

It appeared interesting, therefore, to discover if, by a lowering of flame temperature, rate of evaporation, or other factors, the use of acetic acid would lead to an enhancement of the sensitivity for palladium. To test this a solution containing 50 p.p.m. of Pd under the usual operating conditions was sprayed with the results shown in Table VI. It can be seen that a marked increase in sensitivity occurs up to 90% acetic acid.

The behaviour of palladium complexes

The applications of metal complexes in atomic absorption spectrophotometry are few in number. ALLAN[11] used ammonium pyrrolidine dithiocarbamate to complex

copper before extraction into an organic solvent which was then sprayed into the flame with interesting results.

In the present investigation the first complex chosen was palladium pyridine chloride (Pd (py_2) Cl_2), which was prepared by the method of DREW *et al.*[12]. An appropriate amount of the dried compound was dissolved in ethanol and diluted with water to the required concentration, *i.e.*, 50% (v/v) ethanol. Results obtained are shown in Table VII with those for an aqueous solution of $PdCl_2$ and $PdCl_2$ in 50% (v/v) ethanol for comparison. All the usual conditions of operation were maintained.

TABLE VI

EFFECT OF ACETIC ACID

Solvent	*Response*	*Background (without Pd)*
Water	0.074	0.00
30% Acetic acid	0.087	0.00
50% Acetic acid	0.091	0.002
90% Acetic acid	0.104	0.005

TABLE VII

EFFECT OF ORGANO-PALLADIUM COMPLEX

Concn. (p.p.m.)	*Response*		
	$PdCl_2$ in water	*$PdCl_2$ in 50% ethanol*	*Pd $(py)_2$ Cl_2 in 50% ethanol*
5	0.008	0.023	0.021
10	0.019	0.036	0.034
25	0.041	0.063	0.067
50	0.070	0.100	0.100
75	0.089	0.118	0.118
100	0.100	0.125	0.118

TABLE VIII

ANALYTICAL RESULTS

Pd present (p.p.m.)	*Found from Curve I, Fig. 1 (p.p.m.)*	*Found from Curve II, Fig. 1 (p.p.m.)*
Unknowns 40	39	41
60	58	61
6	7	7
Alloy 1 Pd −79.7% Pt −20.0%	Pd −80.1%	(Average of 3 determinations)
Alloy 2 Pd −73.9% Ag −26.1%	Pd −71.8%	(Average of 3 determinations)

It can be seen that, while the results for $PdCl_2$ in 50% ethanol and for the palladium complex in 50% ethanol are superior to those for $PdCl_2$ in water alone, especially at lower concentrations, there is little to choose between the two sets of results in 50% ethanol.

Analytical results

With palladium chloride in water, a calibration curve was prepared for amounts of palladium up to 100 p.p.m. at 3 different slit-widths: 0.3 mm, 0.225 mm and 0.1 mm. The curves for the first 2 slit-widths are reproduced in Fig. 1.

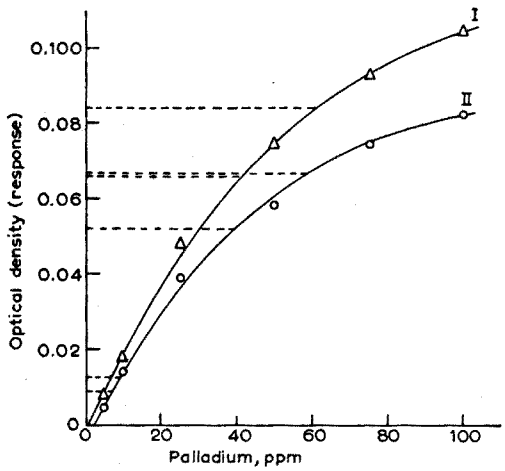

Fig. 1. Calibration curves for amounts of palladium up to 100 p.p.m., at 2 slit-widths: I, 0.22 mm; II, 0.3 mm.

To test the value of the procedure analytically, 3 "unknown" solutions and alloys were examined by one of us (G.E.). One of the alloys contained palladium and platinum and the other palladium and silver. No special treatment of the alloys was required. They were dissolved in aqua regia, the acid was evaporated and the residue taken up in hydrochloric acid. The results for the "unknowns" and the alloys are shown in Table VIII.

The extraction of palladium

In the use of the palladium complex above, the complex was dissolved in a mixed solvent. The possibility of extracting a palladium complex was then investigated. The complex $Pd(py)_2Cl_2$, however, is not readily soluble in many organic solvents. Investigations were, therefore, undertaken with the complex $Pd(py)_2(SCN)_2$ and the solvent hexone in which the complex readily dissolves. This complex was prepared by the method of FORSYTHE *et al.*[13]. To a solution containing palladium chloride, pyridine solution was added until the pH reached a value between 6 and 6.5. The solution was then transferred to a separation funnel and 1 ml of 40% potassium thiocyanate solution added. After 2–3 min, 8 ml of hexone were added and the mixture was shaken. The hexone phase was removed, transferred to a volumetric flask and made up to the mark. The solution obtained in this manner was sprayed into the flame.

A calibration curve for amounts of Pd from 1–100 p.p.m., using the usual operating conditions, was prepared as shown in Table IX. It can be seen that the response in hexone is higher than that in aqueous solutions, *e.g.*, 10 p.p.m. Pd in hexone—0.039;

10 p.p.m. Pd in water—0.020. However, against this must be set the high background value of 0.020 for hexone alone.

One feature of the hexone extraction method is however advantageous. In aqueous solutions the lower limit for the determination of palladium is 2 p.p.m. With palla-

TABLE IX

CALIBRATION CURVE FOR EXTRACTED PALLADIUM COMPLEX

Pd present (p.p.m.)	*Optical density*[a]
1	0.022
3	0.026
5	0.031
8	0.036
10	0.039
25	0.058
50	0.083
75	0.103
100	0.106

[a] Background: hexone alone 0.020.

TABLE X

RESULTS OBTAINED BY EXTRACTION METHOD

Pd present (p.p.m.)	*Pd found (p.p.m.)*	*Optical density*[a]
0.10	0.08	0.022
0.20	0.22	0.025
0.50	0.50	0.030

[a] Not corrected for background.

dium extracted by hexone, on the other hand, it was found that amounts below 2 p.p.m. Pd could be determined quite readily. The results for 3 solutions of complex in hexone, containing amounts of palladium below 1 p.p.m. are shown in Table X. The method used was exactly the same as that outlined above for amounts exceeding 1 p.p.m. Pd. The determination of palladium by atomic absorption spectrophotometry is both convenient and rapid, and suitable for the determination of the element. The method recommended is either, with the use of a simple salt or complex in a mixed solvent, *e.g.* 50% ethanol, or by means of a complex soluble in an organic solvent. For very small amounts of palladium the latter is particularly suitable.

SUMMARY

A commercial atomic absorption spectrophotometer was used without modification to establish the most suitable operating conditions for the determination of palladium. A study of the effect of organic solvents miscible with water, and of acids was then carried out. In general, organic solvents led to increased sensitivity. With a complex of palladium, $Pd(py)_2Cl_2$, in 50% ethanol, the sensitivity was also enhanced and, with a hexone solution of the complex, $Pd(py)_2(SCN)_2$, amounts of palladium below 2 p.p.m. could be determined.

RÉSUMÉ

Les auteurs ont établi les conditions opératoires pour le dosage du palladium, en utilisant un spectrophotomètre par absorption atomique. Ils ont examiné l'influence des solvents organiques et des acides. Avec un complexe de palladium $Pd(py)_2(SCN)_2$, en solution dans l'hexone, des teneurs en palladium inférieures à 2 p.p.m. ont pu être déterminées.

ZUSAMMENFASSUNG

Für ein handelsübliches, nicht verändertes, atomares Absorptions-spektralphotometer wurden die günstigsten Arbeitsbedingungen zur Bestimmung von Palladium festgelegt. Der Einfluss von Säuren und mit Wasser gemischter, organischer Lösungsmittel wurde untersucht. Im allgemeinen führen organische Lösungsmittel zur Steigerung der Empfindlichkeit. Der Komplex $Pd(py)_2Cl_2$ in 50%igen Äthanol verbessert die Empfindlichkeit ebenfalls. Mit einer Hexonlösung des Komplexes $Pd(py)_2(SCN)_2$ konnten weniger als 2 p.p.m. Palladium bestimmt werden.

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A STUDY OF FLUX MONITORING FOR INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS*

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The accuracy and precision of neutron activation analysis are as dependent upon the measurement of the neutron flux during irradiation of the sample as on the weighing and counting of the sample. Since the neutron output of instrumental sources may be irregular and is often prone to change rapidly, the measurement of neutron flux incident upon each sample is necessary for analytical accuracy.

Measurement of neutron flux is accomplished by detection of secondary radiation produced by an interaction of the neutrons with a specific element. For practical purposes, either the activity of a reference material simultaneously exposed to the neutrons with the sample is counted or a counter responsive to a neutron interaction is calibrated against a standard sample. A popular technique, carried over from activation in nuclear reactors, is the irradiation of a sample containing the element(s) of interest in a position identical to that of the sample. Rotating tables, which place the samples and standards consecutively in front of an instrumental neutron source, have been used. Equivalent or "calibrated" positions around the neutron source are common[1,2]. In some cases, a known quantity of an element with a half-life similar to that of the desired element can be mixed in with the sample to act as an internal standard. Elements with well-known cross-sections, but different half-lives, are also used as internal standards[3].

Since reference samples and internal standards are tedious or impractical in many cases, counting devices that give a response proportional to neutron flux are usually used. These include BF_3 counters, organic scintillators, solid state devices, and various monitors such as target cooling water. In each case, the measured response is proportional to the total number of neutrons produced. Such measurements are useful only if the neutron output of the source is steady. Should the neutron output vary during irradiation of the sample, simple integral counts will yield erroneous results. The instantaneous rate of neutron production can be recorded but is difficult to use in calculations. Reference samples are normally used when the neutron output may not be constant during the activation period.

This paper describes a simple and effective reference sample system and compares

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several commonly used flux monitoring systems with the reference sample system. Important advantages and disadvantages of the various systems are noted. Data supporting the superiority of the reference sample system over the others in instances in which neutron production is irregular are presented. Precision data for each system studied are shown for analyses in which the neutron flux was relatively steady. All data were accumulated for oxygen analyses using a single standard oxygen sample. Most of the systems were evaluated simultaneously to provide valid intercomparison of the data. A brief description of each system used is given; also included is an estimate of the total cost of setting up each technique.

EXPERIMENTAL

Sample and reference sample systems

The sample and reference sample systems used at Esso Research Laboratories were identical except that the sample was counted between two 3″ × 3″ NaI(Tl) crystals and the reference sample in front of a ½″ × 1½″ NaI(Tl) crystal. Two parallel pneumatic tubes made from ½″ diameter plastic water pipe carried the sample and reference between the counting and the activation positions. The pneumatic system is similar to that described by RHODES AND MOTT[4]. Figure 1 depicts the dual transfer system.

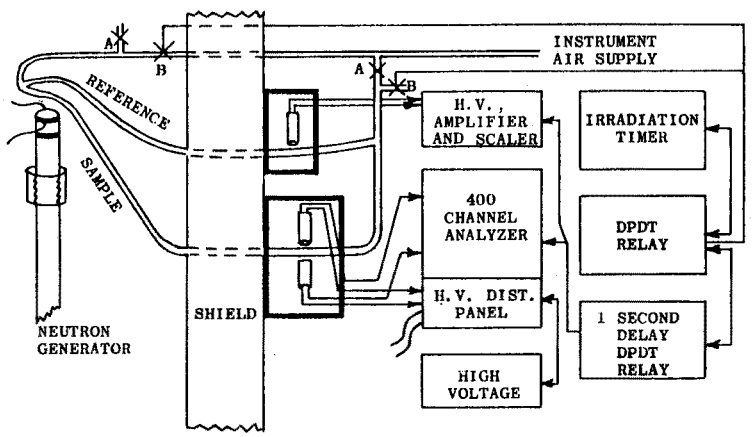

Fig. 1. Reference sample flux monitor system.

Transfer time was less than 1 sec. A system of relays was required to control the neutron generator, turn on the air return valves, provide a time delay for sample transfer, and trigger the multichannel analyzer and reference system scaler simultaneously. A clock timer controls the operation of the neutron generator and a DPDT relay. The relay controls the 2 air valves returning the standard and reference samples and a time delay relay. The time delay relay triggers the multichannel analyzer and reference sample counting periods after a delay of 1 sec to allow the samples to reach the detectors.

The reference sample for these studies was 1 ml of water. The short (7.35 sec) half-life of the nitrogen-16 from the ^{16}O (n,p) reaction permits the same reference sample to be recycled in the transfer system after a one-min count. When the

reference material without elements producing interfering radiation can be selected, such as the water reference for oxygen analysis, a simple counting system consisting of either a GM tube or small scintillation crystal connected to an inexpensive scaler is satisfactory. Elements producing long half-life products require that a set of calibrated standards be used.

Organic scintillators

A plastic scintillator such as Pilot Chemicals Inc. scintillator "B" is commonly used for fast neutron flux monitoring. Fast neutrons produce recoil protons in hydrogenous materials. The recoil protons are produced in and detected by the Pilot "B". A disc, $\frac{1}{8}''$ to $\frac{1}{2}''$ thick, mounted on the face of a photomultiplier tube makes a satisfactory detector. Other organic scintillatois such as anthracene work very well. Weaker pulses from the detector arising from gamma radiation should be discriminated against for good results. Figure 2 depicts the system used for Pilot "B" monitor including the timing controls. The timing controls are designed to turn the scaling equipment on and off with the neutron generator.

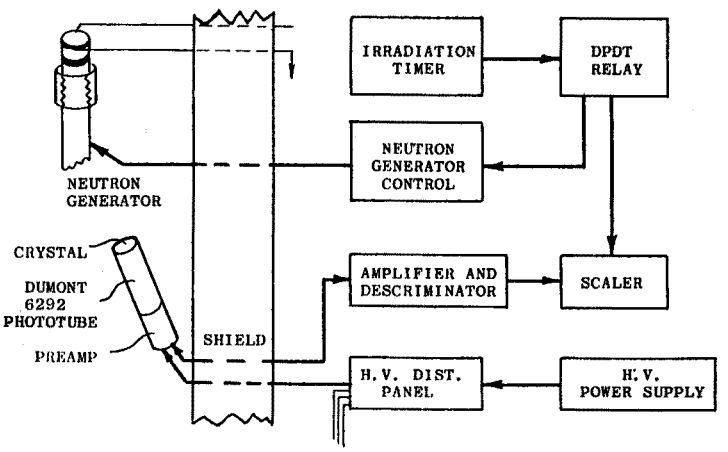

Fig. 2. Pilot "B" and N.E. proton recoil flux monitors.

Another proton recoil detector used in this study is often used in fast neutron counting. Again a hydrogenous plastic is used to produce the protons[5]. The protons are detected by a ZnS(Ag) scintillator. A layer of ZnS(Ag) scintillator is packed against the plastic. The surface between the plastic and scintillator is increased by concentric grooves cut into the plastic. The detector was a Nuclear Enterprises 404 crystal mounted on a DuMont 6292 phototube. The same electronic system as shown in Fig. 2 was used for the NE 404 detector.

BF_3 detector

Thermal neutrons interact with boron-10 to produce an α-particle. This reaction coupled with the use of boron-10 enriched BF_3 as a filling gas in a proportional counter makes a simple and sensitive detector for thermal neutrons. By filtering out thermal neutrons with a cadmium foil and then thermalizing fast neutrons with

paraffin, the BF_3 proportional detector can be made to count fast neutrons[6]. The Nuclear-Chicago DN_3 neutron probe and a similar home-made version using a cadmium-covered paraffin shield around a BF_3 detector were used as fast neutron

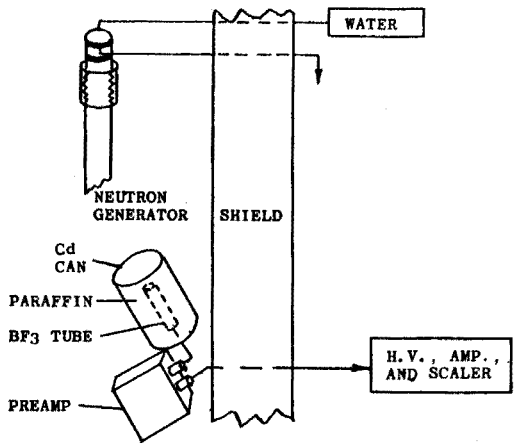

Fig. 3. BF_3 flux monitor.

monitors. Figure 3 shows the system necessary for the monitor. No timing devices were found to be necessary since the background before or after irradiations was essentially zero.

Cooling water monitor

In neutron generators using the (d,t) reaction to produce neutrons, the fast neutrons produce considerable nitrogen-16 activity in target-cooling fluids containing oxygen. Water flowing through a series of pressure and flow regulators cools the target on the Esso Research Laboratory neutron generator. The water then flows

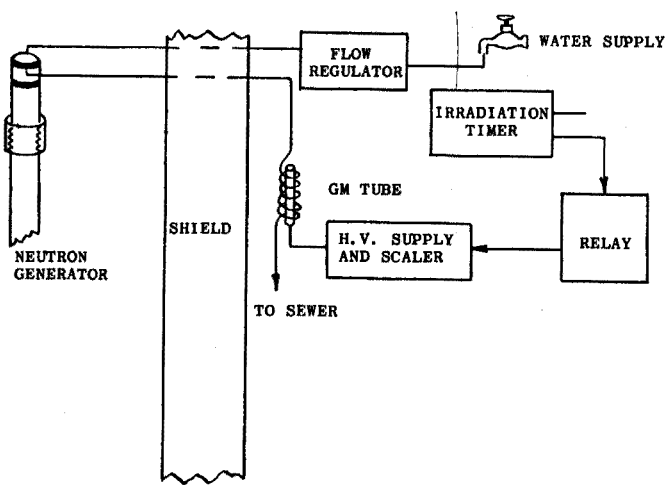

Fig. 4. Cooling water flux monitor.

through a coil outside the generator shielding wall. The coil is wrapped around either a GM tube or scintillation counter as shown in Fig. 4. STEELE AND MEINKE have described similar systems[7].

RESULTS

The precision of the flux monitoring systems studied is shown by the constancy of the ratio between the counts measured by the system and the nitrogen-16 activity produced in the standard oxygen sample being irradiated. Table I is a collection of

TABLE I

PRECISION OBTAINED WITH FLUX MONITORING SYSTEMS IN TERMS OF SYSTEM COUNTS/OXYGEN STANDARD ACTIVITY

	BF_3	*Pilot "B"*	*N.E.p-recoil*	*Cooling water*	*Reference system*
Average ratio	0.1117	1.514	0.2894	0.1874	0.0133
Standard deviation	0.0032	0.063	0.0067	0.0187	0.00082
No. of data points	18[a]	10	10	10	25[b]

[a] Includes data from 3 different days.
[b] Includes data with irregular neutron output.

typical data. The number of counts made, the average value for the ratio of system counts to nitrogen-16 activity, and the standard deviation for each system are shown.

All of the systems shown in Table I give about the same precision except for the cooling water monitor. The poorer precision obtained with the cooling water monitor system is the result of fluctuations in water flow rate during some of the measurements. Even with a series of pressure and flow regulators on the water supply flow, irregularities were observed. Use of a captive water supply with a pump-around has been sug-

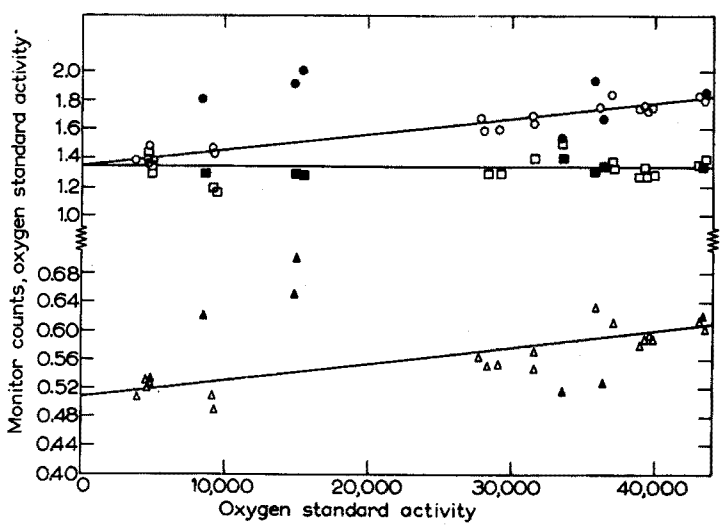

Fig. 5. Comparison of relative effect of irregular neutron flux on neutron flux monitoring systems. ○, Pilot "B" monitor system; △, BF_3 monitor system; □, reference sample system. Solid points indicate neutron flux output changes during irradiation.

gested by STEELE to alleviate the problem of flow-rate irregularities[8]. Another disadvantage to the water monitor is that very high backgrounds must be contended with during long irradiations. The high background results from the water activity being counted at the same time that neutrons are being produced.

The data for the reference sample flux monitoring system included measurements made in which large variations in neutron output of the neutron generator were produced during irradiations. The effect of neutron flux variation during irradiation on the 3 monitoring systems is shown in Fig. 5. Solid points in Fig. 5 represent measurements made when the neutron flux was manually altered during the irradiation. Figure 5 shows that only the reference sample monitoring sytem and not the integral count systems (represented by a plastic scintillator monitor and the BF_3 monitor systems) can cope with irregular neutron flux irradiations. This is the main advantage in the use of the reference sample system. Also of note in Fig. 5 is the fact that the ratio of reference sample activity to standard sample activity is constant at all flux levels used.

TABLE II

COMPARISON OF FLUX MONITORING EQUIPMENT COSTS

	BF_3	*Pilot "B"*	*N.E.p-recoil*	*Cooling water*	*Reference system*
Approximate cost		$1400			$900

Comparing the precision obtained (Table I) and the relative costs of the systems as tabulated in Table II, the reference sample system seems to be the most desirable monitoring system. For cases in which the reference sample system is inconvenient and an integral count monitor can be used, the cooling water monitor is the cheapest and the BF_3 monitor the simplest to set up since it requires no switching or timing to start and stop counts. In some instances, the plastic scintillators can be set up without the external switching and would be preferred.

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SUMMARY

The accuracy of instrumental neutron activation analysis is dependent upon measurement of the neutron flux to which samples are submitted. Flux monitoring techniques described and evaluated include: target cooling water activity, BF_3 counters, plastic scintillators, and a simple reference sample system. Evaluation is made on the basis of results obtained for oxygen analysis using primary standard materials. The superiority of the reference sample system for flux measurement during irregular neutron flux production is shown.

RÉSUMÉ

L'exactitude de l'analyse par activation au moyen de neutrons dépend de la mesure du flux de neutrons auquel sont soumis les échantillons. Les techniques de contrôle du flux, décrites et évaluées, comprennent: activité de l'eau de refroidissement de la cible, compteur BF_3, scintillateurs plastiques et système de référence. L'évaluation est basée sur les résultats obtenus pour l'analyse de l'oxygène. L'auteur montre l'avantage d'un système de référence pour la mesure du flux, lors d'une production irrégulière de flux de neutrons.

ZUSAMMENFASSUNG

Die Genauigkeit der Neutronenaktivierungsanalyse mit einem Generator ist von der Messung des Neutronenflusses, welcher die Probe trifft, abhängig. Es werden Monitor-Techniken beschrieben und berechnet, die die Aktivität des Target-Kühlwassers, einen BF_3-Zähler, einen Plastikszintillator und die Anwendung einfacher Vergleichsproben berücksichtigen. Auf der Grundlage der Ergebnisse, die mit Sauerstoffanalysen unter Anwendung von Standardproben erhalten wurden, werden Berechnungen durchgeführt, die die Überlegenheit der Anwendung von Vergleichsproben für Flussmessungen bei unregelmässigem Neutronenfluss zeigen.

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PRECISE AND ACCURATE DETERMINATION OF CHROMIUM IN STEEL

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When direct-reading optical spectrometers and X-ray spectrometers are calibrated and checked with independent chemical methods, very high requirements are set for the chemical method. For the determination of chromium in high-alloy steels, optical spectroscopy can give a precision of 0.5% (relative standard deviation) at a 20% level of chromium and X-ray fluorescence is about twice as good. In our experience, none of the usual wet-chemical methods is continuously capable of giving the same precision and also accuracy that is required. In the absorptiometric determination of chromium after oxidation with perchloric acid, complete recoveries of chromium seem to be difficult to maintain over any length of time. The visual titration with permanganate to measure the excess of iron(II) sulfate involves such difficulties as the need for a close control of the iron(II) solution and especially the visual detection of the end-point in strongly colored solutions. Exceptional precision was claimed in a paper by KEILY *et al.*[1] in the determination of chromium in pure chromium metal and in chromium oxide. These authors employed solid ammonium ferrous sulfate in the reduction of chromate and the excess of iron(II) was measured by titration with permanganate or dichromate, using an amperometric indication. The advantages of using solid ammonium ferrous sulfate are obviously the higher precision of a weighing operation and the stability of the solid reagent. Our aim was thus to utilize these advantages for steel analysis but with another indication of the end-point.

Earlier experience with the use of *o*-phenanthroline was very satisfactory, and a method was consequently developed in which ammonium persulfate–silver nitrate oxidation of chromium, solid ammonium ferrous sulfate and *o*-phenanthroline as an indicator in the back-titration with permanganate were utilized. However, the method was not applicable to the analysis of steels containing tungsten. Preliminary tests of reproducibility for a titration based on a photometric detection of the permanganate gave very satisfactory results. It was therefore decided to carry out a thorough study of the whole method of determination. The results of this study are described in the present paper.

According to the general scheme at our laboratory, the following requirements were stipulated for the method to be developed.

Range. 0.5–30% chromium in all steels.

Precision. Chromium percentages of 1, 5 and 20 should give a relative standard deviation ($n = 10$) of 0.5, 0.20 and 0.10, respectively.

Interferences. No interference from elements commonly present in high-alloy steels.

Especially the following elements should be checked: Fe, Mn, V and W (present 5000 mg, 3(15)%, 3% and 20%).

Accuracy (= recovery). $100 \pm 2\delta\%$.

Development of the method

Dissolution of the sample. The following points had to be considered when choosing the acids and their amounts for dissolving samples: (*a*) the resistance of material to be dissolved; (*b*) the difficulty in dissolving anhydrous chromic sulfate; (*c*) the need for phosphoric acid to dissolve tungstic acid, to prevent the formation of insoluble chromic sulfate, and to form the colorless iron(III) complex in the titration; (*d*) the effect of acidity on the oxidation (see p. 215); (*e*) the precipitation of iron(III) phosphate in weakly acid solutions; and (*f*) the formation of manganese(III) phosphate, which is difficult to reduce with hydrochloric acid and thus introduces too high results for chromium.

Space does not permit complete description of all the numerous experiments which were carried out in order to decide on the acids and their amounts chosen for the final method. The correct choice of these amounts is, however, considered to be one of the critical points of the method proposed. This is especially true as regards the amount of phosphoric acid, which in amounts larger than those recommended gives rise to positive errors, owing to manganese(III). The precipitation of iron(III) phosphate also becomes critical if the acidity is too low.

Oxidation. Successful and generally recommended methods[2-4] are based on the oxidation of chromium and manganese with ammonium persulfate in the presence of silver ions, and the reduction of permanganate is carried out with chloride ions. The use of silver nitrate in combination with chloride ions was not desirable in the present case, since the resulting turbidity interfered with the photometric titration. A filtration would have been a complication in a method intended for high precision. The use of azide[5] as a reductant for permanganate was tested, but led to definitely lower

TABLE I

THE EFFECT OF THE ACIDITY ON THE OXIDATION RECOVERY

(Present: 500 mg Fe, 5 mg Mn, 100 mg Cr and 5 ml H_3PO_4 (d = 1.69) in 300 ml. Method: see p. 214)

ml H_2SO_4 (d = 1.84) added	*Recovery Cr (%)*
5[a]	99.97
6	99.93
7	99.92
8	99.93
9	99.88
10	99.83
12	99.85
15	99.82
20[b]	—

[a] Solution faintly turbid ($FePO_4$).

[b] Oxidation not possible (solution too acid).

recoveries; a reduction of 0.3 to 0.6% of the amount of chromium was always found. No distinct improvement in the oxidation itself or in the recovery, was obtained by using cobalt and/or copper as a catalyst[6]. Since the almost obsolete method of using only persulfate was found to give good recoveries under carefully controlled conditions, its use was accepted. The influence of the acidity on the oxidation recovery is shown by the results presented in Table I. The results listed in Table II indicate that the low recovery really depends on the oxidation and not on the reduction with hydrochloric acid or on any influence of hydrochloric acid on the titration. The amount of hydrochloric acid was found not to be critical (Table III).

Titration. No special experimental work was required when solutions containing only chromium or chromium + manganese were titrated. As was to be expected, difficulties arose in the case of chromium + vanadium and especially in that of chromium + vanadium + tungsten. The basic reason for these difficulties is the close resemblance of the oxidation potentials of the systems Cr(III)–Cr(VI) and V(IV)–V(V) in acid solutions as shown in a study by DUCRET[7]. In practice, this means that it is hardly possible to find any conditions where vanadium can be oxidized completely without some chromium also being oxidized. In addition, the reaction kinetics is disadvantageous; the oxidation of vanadium by permanganate is slow compared with, for example, the oxidation of iron(II).

TABLE II

THE EFFECT OF THE ACIDITY ON THE TITRATION RECOVERY AND OF HYDROCHLORIC ACID ON CHROMIUM(VI)

(Present: 500 mg Fe, 5 mg Mn, 100 mg Cr and 5 ml H_3PO_4 (d = 1.69))

ml H_2SO_4 (d = 1.84) added	*Recovery Cr[a] (%)*	*Recovery Cr[b] (%)*
5	100.02[c]	100.00
	100.03[c]	100.01
10	99.99	100.00
	100.02	99.99
15	100.00	100.01
	100.00	99.98

[a] Method as described, but $K_2Cr_2O_7$ (NBS 136B) added immediately before titration.
[b] Method as described, but $K_2Cr_2O_7$ added before reduction of Mn(VII) with HCl.
[c] Solution faintly turbid ($FePO_4$).

TABLE III

THE INFLUENCE OF VARYING AMOUNTS OF HYDROCHLORIC ACID[a]

(Present: 500 mg Fe, 100 mg Cr, 7 ml H_2SO_4 and 5 ml H_3PO_4 (d = 1.69). Method: see p. 214)

mg Mn added	*ml HCl (1 + 4) added*	*Recovery Cr (%)*
5	40	99.98
10	50	99.96
15	60	99.97
20	80	99.94
25	100	99.96

[a] Varying amounts of hydrochloric acid must be added because of the wide range of manganese.

The strong complex which vanadium forms with tungsten and phosphoric acid is particularly troublesome in the present case, since very marked changes of color result. Before reduction with iron(II), the solution is orange to yellow (Cr(VI) and V(V) + W(VI) + PO_4^{3-}) and becomes dark brown-green (Cr(III) and V(IV)) during the reduction. During oxidation with permanganate, the absorption at 530 mμ followed with a spectrophotometer is essentially unchanged as long as the excess of iron(II) sulfate only is oxidized. When the oxidation of the vanadium starts, the absorption decreases, the brown component being destroyed. The addition of the permanganate must be continued until an increase in the absorption can again be observed as a result of an excess of permanganate. This operation performed with the precision required in the present work, was found to be very critical. A routine procedure with strictly regulated time intervals and amounts of titrant added, was achieved after long series of experiments.

As a result of the development work partly related above, the following procedure has been accepted. It is described in detail, since it is felt that the precision later claimed for it can only be obtained by rigorous adherence to the given procedure.

EXPERIMENTAL

Apparatus

A Beckman B-photometer equipped for titrations with an assembly which includes a housing for a 500-ml beaker, a stirrer and a 20-ml syringe-buret (Metrohm).

Reagents (all reagent grade)

Sulfuric acid conc. (d = 1.84), phosphoric acid conc. (d = 1.69), nitric acid conc. (d = 1.41), and hydrochloric acid conc. (d = 1.19).

Procedure

For less than 10% chromium, weigh in 1 g (± 0.5 mg) and for more than 10% chromium, 0.5 g (± 0.2 mg) of the steel. Dissolve low-alloy steel in 10 ml of sulfuric acid, 5 ml of phosphoric acid and about 100 ml of water. Dissolve tool steel (containing tungsten) in 7 ml of sulfuric acid, 10 ml of phosphoric acid and about 100 ml of water. Dissolve high-alloy steel in 20 ml of hydrochloric acid and 5 ml of nitric acid followed by treatment with 7 ml of sulfuric acid and 5 ml of phosphoric acid, the hydrochloric acid and nitric acid being expelled by evaporation. After dissolution of low-alloy steel and tool steel, oxidize with nitric acid. Check *very* carefully that no carbides remain undissolved before continuing.

Dilute the solution to about 300 ml with water, mix carefully and add some glass beads. If no manganese is present, add about 5 mg of manganese (as manganese(II) sulfate solution). Then add 1 g of solid ammonium persulfate and heat to boiling. If the solution does not become red (permanganate) carefully add some more persulfate and continue with these additions until the solution becomes red. (If very large amounts of manganese are present, some of it is precipitated as manganese dioxide which, however, is brought into solution later on by the addition of hydrochloric acid.) Then boil the solution for at least 10 min, *i.e.* until all the excess of persulfate is destroyed, which can readily be checked by stirring with a glass rod (no evolution of gas bubbles).

Thereafter add diluted hydrochloric acid (1 + 4), until all the manganese is reduced, *i.e.* the solution becomes orange to yellow without any further change. Boil gently for 3–5 min and cool immediately in running water. If more than 1 mg of vanadium is present, add solid sodium acetate, until the pH of the solution is 1; if less than 1 mg is present, the acidity need not be adjusted.

Transfer the solution carefully into the titration beaker. Add to the solution an exactly weighed amount (Note 1) of the solid ammonium ferrous sulfate which has been calculated according to the content of Cr and V. Start the stirrer. Adjust the zero point of the buret and insert the tip into the solution to be titrated. Adjust the wavelength to 530 mμ and check carefully that a constant absorbance is achieved in the solution, *i.e.* the solution is homogeneous after the reduction has been completed.

If less than 1 mg of vanadium is present, start the titration by adding the 0.05 *N* permanganate solution cautiously until an increase of more than 0.003 absorbance units is observed. If this increase disappears during 2 min, add a further 0.05 ml of permanganate and continue until the increase of the absorbance is stable for 2 min. Note the amount of permanganate added from the buret (Note 2).

If the solution contains more than 1 mg of vanadium, add the permanganate initially at a rate of about 0.5–1 ml/30 sec, depending on the expected amount of vanadium, until a distinct enhancement is noticed with every addition and the basic absorbance no longer decreases. Then add the titrant in portions of 0.1 ml/2 min. Continue very carefully until a distinct increase of absorbance (0.003 units) can be observed as compared with the earlier lowest value after 2 min. This point is critical and requires training. Calculate the amount of chromium with due attention to the reduction grade of the ammonium ferrous sulfate (Note 3) and the titer of the permanganate (Note 2). No correction for the volume of the solution is required.

Notes

(*1*) To the weight of ammonium ferrous sulfate required to reduce the expected amounts of chromium and vanadium, an excess of 5 to 10 mg should be added.

(*2*) Checking of the normality of the permanganate. Add to the titration cell 10 ml of sulfuric acid, 5 ml of phosphoric acid and 300 ml of water and about 200 mg of ferrous ammonium sulfate. Titrate to the first permanent increase of absorbance of the solution at the wavelength 530 mμ. This increase must not be greater than 0.001 to 0.002 absorbance units. Repeat the procedure with an exactly weighed amount of ferrous ammonium sulfate (200 mg).

(*3*) Checking of the reduction grade of the ferrous sulfate. Add to the titration cell 10 ml of sulfuric acid, 5 ml of phosphoric acid, 300 ml of water and an exactly weighed amount of about 500 mg of potassium dichromate (NBS 136) and 22.69 × mg Cr + 2 mg ammonium ferrous sulfate. Titrate with permanganate as above (Note 2).

RESULTS

Interferences

In order to investigate the effect of the elements Mn, Ni, Co, Mo, V, W and V + W on the determination of chromium, varying amounts of these elements were added to solutions containing exactly known amounts of chromium (added as $K_2Cr_2O_7$, NBS 136B and reduced by sulfur dioxide). The recoveries including the whole procedure are given in Table IV. The effect of iron is shown by the results given in Table V.

TABLE IV

RECOVERY OF CHROMIUM IN THE PRESENCE OF INTERFERING ELEMENTS

(Present in all samples: 100 mg Cr, 1000 mg Fe, 5 mg Mn[a], 10 ml H_2SO_4 (d = 1.84), 5 ml H_3PO_4 (d = 1.69))

Mn		*Ni*		*Co*		*Mo*		*V*		*W*[b]		*V* + *W*[b]	
Added (mg)	*Cr Recovery (%)*	*Added (mg)*	*Cr Recovery (%)*	*Added (mg)*	*Cr Recovery (%)*	*Added (mg)*	*Cr Recovery (%)*	*Added (mg)*	*Cr Recovery (%)*	*Added (mg)*	*Cr Recovery (%)*	*Added (mg)*	*Cr Recovery (%)*
5	99.96	0	99.94	0	99.92	0	99.94	0	99.92	0	99.93	2+100	99.95
20	99.94	20	99.94	10	99.93	10	99.94	1	99.94	20	99.96	5+100	99.93
50	99.93	50	99.97	50	99.91	20	99.93	2	99.87	50	99.95	10+100	99.93
100	99.91	100	99.96	100	99.93	50	99.92	5	100.02	100	99.95	10+200	99.88
150	99.94	200	99.93	200	99.94	100	99.94	10	99.90	200	99.98	20+200	99.84
300	99.80	500	99.90					20	99.92				

[a] Except in case of Mn added.
[b] 7 ml H_2SO_4 + 10 ml H_3PO_4.

TABLE V

THE EFFECT OF IRON ON THE RECOVERY OF CHROMIUM

(Present: 5 mg Mn, 100 mg Cr, 5 ml H_3PO_4. H_2SO_4 varied only to give about the same acidity in all cases)

mg Fe added	*ml H_2SO_4 added*	*Recovery Cr (%)*
0	6	99.97
500	8	99.98
1000	10	99.92
2000	11	99.90
3000	12	99.86
4000	13	99.76
5000	14	99.71

TABLE VI

REPRODUCIBILITY OF TITRATION WITH VARIOUS AMOUNTS OF CHROMIUM

(Unreduced $K_2Cr_2O_7$ as sample, $n = 20$)

Range of sample weight (mg)	30–36	130–140	280–290
Range of Cr taken (mg)	10.5–12.5	46–49	99–105
Corresponding % Cr in steel	1	5	20
Absolute standard deviation (mg Cr)	0.019	0.020	0.019
Relative standard deviation (%)	0.19	0.040	0.019
Mean recovery	99.45	99.86	99.96

TABLE VII

REPRODUCIBILITY OF THE DETERMINATION OF CHROMIUM IN STEEL ($n = 10$)

Sample no.	BC 9L[a]	NBS 50C[a]	NBS 160A[a]
Weight of sample (mg)	970–1050	1000–1100	540–550
Chromium (%)	1.03	4.13	18.74
Range of Cr (mg)	10–11	41–45	101–105
Absolute standard deviation (mg Cr)	0.020	0.050	0.020
Relative standard deviation (%)	0.19	0.11	0.02
Mean	1.032	4.127	18.720

[a] Composition of samples: BC 9L: 0.50 C, 0.44 Mn, 2.8 Ni, 0.22 Mo, 0.04 V, — W; NBS 50 C: 0.72 C, 0.34 Mn, 0.07 Ni, 0.08 Mo, 1.16 V, 18.4 W; NBS 160 A: 0.06 C, 1.6 Mn, 14.1 Ni, 2.8 Mo, 0.05 V, — W.

Precision

Unreduced potassium dichromate (NBS 136B assay 99.98%) was used as a sample to check the precision of the titration; the results are given in Table VI.

An analytical balance was used for weighing samples, which gives rise to an uncertainty of 0.1 mg in the sample weight. This is probably the reason why the relative standard deviation is much larger with smaller amounts of sample.

To judge the precision of the whole procedure, 3 standard samples were analyzed repeatedly; the results are given in Table VII.

Recovery

Assuming that the potassium dichromate used as reference (NBS 136B) has the

TABLE VIII

RECOVERY OF CHROMIUM WITH SYNTHETIC SAMPLES

Added other than Cr (mg)	*Corresp. Cr in a steel (%)*	*$K_2Cr_2O_7$ weighed(mg)* [a]	*Cr thus weighed(mg)*	*Iron(II) sulfate weighed(mg)*	*$KMnO_4$ (ml)*	*Cr found (mg)*	*Cr recovery (%)*	*Mean recovery (%)*
1000 Fe	1	30.04	10.618	246.01	0.29	10.603	99.86	99.80[b]
10 Mn		27.96	9.883	231.8	0.29	9.871	99.88	
		32.16	11.368	262.8	0.41	11.339	99.74	
		28.38	10.032	233.5	0.34	10.005	99.73	
1000 Fe	5	139.62	49.354	1281.3	8.55	49.332	99.96	99.96
5 Mn		140.68	49.729	1289.1	8.50	49.713	99.97	
20 V		139.38	49.269	1280.0	8.55	49.275	100.01	
200 W		139.10	49.170	1276.6	8.55	49.125	99.91	
1000 Fe	10	280.08	99.005	2250.8	0.34	98.920	99.91	99.97
10 Mn		282.14	99.734	2270.2	0.32	99.741	100.00	
		279.43	98.775	2246.3	0.38	98.732	99.96	
		280.38	99.111	2254.4	0.33	99.088	99.98	
500 Fe	20	283.37	100.168	2280.0	0.39	100.165	100.00	99.99
7 Mn		288.34	101.925	2319.5	0.36	101.906	99.98	
		280.91	99.298	2259.9	0.39	99.304	100.00	
		283.00	100.037	2274.5	0.25	100.041	100.00	

[a] Since Fe and W are added as metal, Cr in $K_2Cr_2O_7$ is reduced.

[b] A subsequent checking of the semi-micro balance used indicated an error of 0.06 mg at the 30 mg level, which would enhance the recovery to about 100.00%.

TABLE IX

DETERMINATION OF CHROMIUM IN SOME STANDARD SAMPLES

Sample no.	Composition of the sample (%)											Certificate values			Values obtained	
	C	Mn	Ni	Mo	V	W	Co	Cu	Ti	Nb +Ta	Al	Lowest	Highest	Mean		
NBS 30E	0.55	0.8	0.03		0.15							0.929	0.939	0.934	0.932	0.932
NBS 36A	0.1	0.4	0.24	0.92	0.006							2.39	2.44	2.41	2.397	2.395
NBS 51B	1.2	0.6	0.05		0.002							0.442	0.467	0.455	0.447	0.452
NBS 72F	0.3	0.6	0.05	0.18	0.005							0.881	0.90	0.891	0.889	0.889
NBS 106B	0.3	0.5	0.2	0.2	0.003						1.0	1.16	1.21	1.18	1.186	1.180
NBS 107A	2.7	0.6	1.0	0.8	0.03							0.469	0.491	0.479	0.479	0.479
NBS 139A	0.4	0.8	0.5	0.2	0.003							0.481	0.490	0.486	0.488	0.489
NBS 159	0.5	0.8	0.1	0.4	0.05							0.99	1.03	1.00	1.014	1.014
BCS 225/1	0.4	0.7	1.5	0.3	<0.01							1.13	1.15	1.14	1.140	1.139
JK 7	0.5	0.4	3.4	0.3	0.002							0.51	0.545	0.53	0.542	0.545
JK 7A	0.3	0.5	3.3	0.25	0.005							1.13	1.16	1.15	1.152	1.151
BCS 251/1		1.5	1.2	1.5	0.65							0.50	0.52	0.51	0.526	0.524
BCS 253/1		0.8	1.0	0.7	0.51							0.98	1.01	0.99	1.003	1.004
BCS 256/1		1.0	0.2	0.5	0.18							2.29	2.35	2.33	2.346	2.344
BCS 257/1		0.6	0.3	0.2	0.12							2.95	3.01	2.97	2.996	2.985
BCS 258/1		0.4	0.5	0.8	0.35							1.27	1.31	1.29	1.318	1.314
NBS 73B	0.35	0.3	0.2	0.01	0.03							12.79	12.85	12.82	12.774	12.774
															12.762	12.765
NBS 133A	0.12	1.0	0.2	0.3	0.03							12.86	12.94	12.89	12.867	12.878
NBS 101E	0.05	1.8	9.5	0.4	0.04							17.93	18.03	17.98	17.969	17.965
NBS 160A	0.06	1.6	14.1	2.8	0.05							18.70	18.79	18.74	18.716	18.718
															18.725	18.717
BCS 211/1	0.2	0.3	0.2									12.70	12.85	12.80	12.776	12.788
BCS 261	0.08	0.7	13.1							0.71		17.11	17.25	17.20	17.232	17.224
BCS 235	0.14	0.5	8.7	0.04		0.68		0.98	0.62			18.95	19.12	19.01	19.093	19.104
JK 8A	0.05	1.1	10.9	2.4	0.2							17.4	17.5	17.5	17.540	17.543
JK 8B	0.04	1.2	11.2	2.7	0.03							17.60	17.9	17.80	17.791	17.800
NBS 50C	0.7	0.3	0.07	0.08	1.16	18.4						4.11	4.15	4.13	4.119	4.113
NBS 132A	0.8	0.3	0.1	4.5	1.94	6.2						4.18	4.23	4.21	4.225	4.222
NBS 153A	0.9	0.2	0.2	8.8	2.06	1.8	8.5					3.69	3.76	3.72	3.717	3.725
BCS 241/1	0.8	0.3	0.07	0.5	1.57	19.6	5.6					5.00	5.05	5.03	5.045	5.040
JK 12	0.8	0.2		0.5	1.1	18.5	5.0					5.03	5.19	5.1	5.128	5.136

purity claimed of 99.98%, it has been shown by the results given earlier in Table II that the recovery of the titration, which of course includes any errors in the standardization of the iron(II) sulfate and the potassium permanganate solution, is within the limits of the precision. To test the recovery of the whole procedure, synthetic samples were analyzed with the results shown in Table VIII.

To obtain a verification of the usefulness of the method described, several standard samples of different origin were analyzed; the results are given in Table IX.

DISCUSSION

Interferences

Of the elements tested, only vanadium, especially together with tungsten, is a considerable interference. The basic advantages of the present method cannot be utilized to the same extent as in the absence of vanadium. Manganese, too, if present in very large amounts (more than 25 mg) interferes somewhat, but recoveries of 99.5 to 100.5% Cr were still obtained in the case of special steels containing 15% manganese and 1% chromium. Of all the other elements not tested here, only elements which may give precipitates, such as titanium and niobium, are likely to interfere in the determination of chromium. Samples included in the comparative tests (Table IX), however, indicated that this interference is not serious, at least as long as the amounts of the elements mentioned are within the range which occurs in steels.

Precision

As shown by the reproducibility tests (Tables VI and VII), the precision is very satisfactory in all cases where the basic features of the method can be utilized. Essentially the same order of reproducibility is achieved, which can be attributed to the weighing operations in the present case.

The most critical points as regards the precision are the *dissolution* of samples containing high contents of carbides, and the *oxidation* of chromium, including especially the complete destruction of persulfate before the addition of hydrochloric acid[8].

A satisfactory precision in the presence of vanadium can only be obtained with very careful standardization of the titration procedure and a certain training of the analyst is then required. With this exception, the precision of the method fulfils all the requirements of a checking method.

Recovery

In most respects, what has been said above about the precision is also true as regards the recovery. Generally, this means that errors which cause low recoveries are even difficult to keep constant. Normally, again with the exception of vanadium, a recovery of between 99.8 and 100.2% should be obtainable with rather little training in the use of the method presented. Recoveries of between 99.95 and 100.05%, however, require very careful work throughout the procedure. It is recommended that the recovery be checked with reference dichromate; the ease of this operation is one of the basic advantages of this method.

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SUMMARY

Determination of chromium(VI) by addition of weighed amounts of solid ammonium ferrous sulfate in excess and back-titration with permanganate, using spectrophotometric end-point detection, is applied to the determination of chromium in steel. Except in the case of vanadium, and especially of vanadium + tungsten, very high selectivity and precision are claimed. Relative standard deviations of the order of 0.05% to 0.15% were found. Recoveries in excess of 99.95% were obtained in most cases. Results for the determination of chromium in standard samples of steel are given.

RÉSUMÉ

L'auteur propose une méthode de dosage du chrome dans les aciers, par addition d'une quantité pesée de sulfate double de fer(II) et d'ammonium, en excès, et titrage en retour au moyen de permanganate, avec détection spectrophotométrique du point final. Sauf dans le cas du vanadium, et spécialement du vanadium + tungstène, la méthode est très sélective et très précise.

ZUSAMMENFASSUNG

Eine sehr selektive und genaue Methode zur Bestimmung von Chrom in Stählen wird beschrieben und eingehend untersucht. Die Methode beruht auf der Zugabe von Mohrschem Salz zur Chrom(VI) enthaltenden Lösung und der spektralphotometrischen Rücktitration mit Permanganat. Es ergaben sich relative Standardabweichungen von 0.05 bis 0.15% entsprechend Chromgehalten von 20 bis 1% und in den meisten Fällen Ausbeuten von mehr als 99.95%. Bei Anwesenheit von Vanadin und besonders von Vanadin + Wolfram sind die Ergebnisse schwieriger zu erzielen. Andere im Stahl vorkommende Elemente stören nicht.

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DOSAGE SPECTROPHOTOMETRIQUE DE DIFFERENTS METAUX (Mn, Ce, V, Ni, Fe) AU MOYEN DE LA FORMALDOXIME

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La formaldoxime est utilisée depuis longtemps pour le dosage colorimétrique du manganèse[1-11]. On peut citer également quelques travaux concernant l'emploi de ce réactif pour le dosage du nickel[11,12] et du vanadium[13].

Dans quelques travaux, effectués partiellement avec MINCZEWSKI ET KASIURA[14-16], nous avons mis en évidence certaines propriétés de la formaldoxime et déterminé la formule des complexes que forme la formaldoxime avec les métaux mentionnés ci-dessus. Celà a permis de choisir et de préciser les conditions optimales pour le dosage spectrophotométrique du manganèse, du vanadium et du nickel, et par ailleurs de proposer des méthodes nouvelles de dosage du cérium et du fer au moyen de la formaldoxime[15-20].

Le comportement de la formaldoxime en solution et les résultats des études sur la formation des complexes avec Mn, Fe, Ni, Ce, V font l'objet d'une publication particulière[21]. Nous nous bornons à présenter ici les applications analytiques issues de nos travaux fondamentaux et relatifs au dosage spectrophotométrique des métaux précités. Nous rappelons simplement les conclusions essentielles de l'étude générale.

Après alcalinisation d'une solution contenant la formaldoxime et l'ion métallique, on obtient un complexe soluble. Les métaux mentionnés (ainsi que Cu et Co) fournissent des complexes très colorés, permettant la réalisation d'un dosage spectrophotométrique.

On peut utiliser la formaldoxime sous forme d'une solution acide ou sous forme d'un chlorhydrate stable (voir ci-dessous). La formaldoxime en solution acide perd progressivement, au fur et à mesure de la dilution, sa capacité de réagir avec les métaux, en se transformant en forme "inactive". Par exemple, une solution 1 M contient 55% de la forme "active", la solution 0,1 M, seulement 5%. Cette transformation a lieu rapidement. Cela oblige, lors du dosage des métaux, à utiliser le réactif en assez grand excès et à alcaliniser rapidement la solution pour éviter que la transformation du réactif sous forme "inactive" ne précède la réaction avec les ions métalliques; réaction qui n'a lieu qu'en milieu alcalin.

D'autre part, la formaldoxime peut se colorer après alcalinisation de la solution, en présence d'oxydants (*p. ex.* $S_2O_8^{2-}$, H_2O_2, Br_2aq.) et en l'absence de tout ion métallique. La présence d'oxydants est donc à éviter.

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En milieu alcalin, la formaldoxime a tendance à l'autoxydation. C'est une raison supplémentaire d'utiliser le réactif en excès. Malgré cette tendance à former des composés peroxydés, la formaldoxime, surtout en milieu acide, possède des propriétés réductrices.

PARTIE EXPÉRIMENTALE

Réactifs et appareillage

(*1*) Formaldoxime, solution 1 *M*. On mélange 79.0 g d'aldéhyde formique (solution à 38% de CH_2O) avec une solution de 70.0 g de chlorhydrate d'hydroxylamine et on dilue avec de l'eau jusqu'à 1 l. La solution est acide (HCl 1 *N*).

(*2*) Chlorhydrate de formaldoxime, $(CH_2NOH)_3 \cdot HCl$. On dissout 105 g de chlorhydrate d'hydroxylamine dans 110 ml d'eau, on y ajoute 45 g de paraformaldéhyde et on agite à 40° jusqu'à l'obtention d'une solution limpide. Celle-ci est chauffée à 40°, sous une pression de *ca.* 20 mm de mercure. Lorsque des cristaux commencent à se former, en quantité importante, on ajoute 80 ml d'éthanol anhydre. Le lendemain, les cristaux sont filtrés, recristallisés dans l'alcool anhydre et séchés dans une étude à vide (*ca.* 100 mm de Hg à 35°). La teneur théorique du produit en chlorhydrate est de 20.67%; en pratique, on obtient 20.8–20.7%.

(*3*) Solutions étalon (0.1 mg Me/ml et plus diluées) de manganèse, cérium, vanadium, nickel et fer.

(*4*) Spectrophotomètre Unicam SP 500.

Quelques remarques sur l'exécution des dosages

Dans les modes opératoires donnés ci-dessous, on utilise la formaldoxime sous forme de solution 1 *M*. On peut également utiliser le chlorhydrate cristallisé; 0.1 g de $(CH_2NOH)_3 \cdot HCl$ correspond à environ 4 ml de la solution 1 *M*, quant à la teneur en forme "active" de formaldoxime.

Pour chaque dosage décrit ci-dessous, on prépare une courbe d'étalonnage, en utilisant une solution étalon du métal à doser. Pour l'étalonnage, le mode opératoire est le même que celui indiqué pour le dosage.

Pour des quantités de métaux très inférieures à celles citées dans les modes opératoires, il est bon d'effectuer un essai à blanc comme référence. Dans le cas du dosage de traces de métaux, on peut utiliser des méthodes de comparaison visuelle avec des microtubes, si on ne dispose pas de microspectrophotomètre ou microcolorimètre.

Dosage du manganèse

Lorsqu'on alcalinise une solution contenant des ions manganèse et de la formaldoxime, il se forme très rapidement, sous l'influence de l'oxygène, un complexe brun-rouge, indépendamment de la concentration du réactif utilisé pour alcaliniser (NaOH ou NH_3). Le complexe est stable et les quantités de formaldoxime en excès et de NaOH (NH_3) sont sans influence. La courbe 1 de la Fig. 1 représente le spectre d'absorption du complexe formé, dont la formule est $[Mn(CH_2NO)_6]^{2-}$. Le coefficient d'absorption molaire (ε) à une longueur d'onde $\lambda_{max} = 455$ mμ est égal à 11200.

La sensibilité de la méthode de dosage du manganèse avec la formaldoxime est donc quatre fois plus grande que celle de la méthode classique, basée sur la coloration des ions MnO_4^- ($\varepsilon = 2400$).

En milieu faiblement alcalin (NaOH 0.04 *N*), on peut chauffer le complexe 15 min

à 90° sans observer une diminution de l'intensité de la coloration. Les concentrations plus faibles et plus fortes en soude sont moins favorables. La stabilité du complexe à 90°, en milieu ammoniacal, est assez faible. Parmi les complexes de différents métaux avec la formaldoxime, c'est celui du manganèse, qui résiste le mieux aux températures élevées.

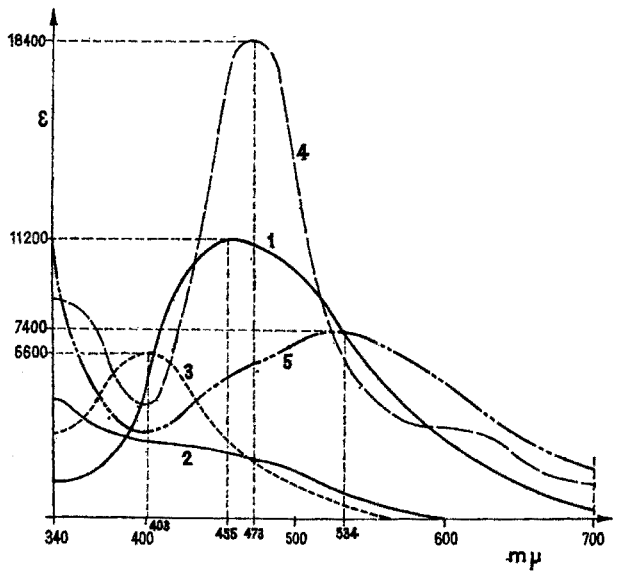

Fig. 1. Courbes d'absorption des complexes de la formaldoxime avec le manganèse (1), le cérium (2), le vanadium (3), le nickel (4) et le fer (5).

Le système coloré Mn(IV)–formaldoxime (0–3 μg Mn/ml) suit assez bien la loi de Beer.

Le complexe Mn(IV)–formaldoxime présente une stabilité exceptionellement grande. Les citrates, tartrates, oxalates, phosphates, pyrophosphates, sulfures, cyanures et EDTA en grand excès, ne gênent pas. Il est à souligner aussi que des réducteurs, comme l'acide ascorbique, NH_2OH, SO_3^{2-}, n'empêchent pas la formation rapide du complexe de Mn(IV).

En revanche le nickel, le cobalt et le cuivre donnent des complexes très colorés, facilement détruits par les cyanures. Il en est de même de petites quantités de fer ferreux. En raison de la coloration de $[Fe(CN)_6]^{4-}$ cet élément doit être séparé par extraction ou précipitation. On peut séparer Al, Ti, Fe, Ce, etc., par l'hydroxyde de zinc, le précipité de $Zn(OH)_2$ ne retenant pas les traces de manganèse de la solution.

Avec les autres métaux, la formaldoxime forme des complexes incolores ou des complexes faiblement colorés (*p.ex.* Ti, U, Mo, Cr, métaux du platine) avec un coefficient d'absorption molaire de 500 à 40. Ces complexes sont relativement peu stables; Al, Ti et U peuvent être maintenus en solution sous forme de complexes tartriques.

La méthode de dosage du manganèse par la formaldoxime en présence de cyanures et à chaud (90°) pour détruire les complexes colorés du vanadium et du cérium, rend la méthode tout à fait spécifique pour cet élément.

Mode opératoire. La solution, contenant moins de 50 μg de manganèse est débarrassée de la majeure partie du fer (des quantités inférieures à celles de Mn ne gênent pas) au moyen d'une extraction sous forme de chlorure ou par précipitation avec $Zn(OH)_2$. A la solution faiblement acide, ajouter du tartrate de Na-K (en présence de Al, Ti, Cr(III), U, etc.), de l'acide ascorbique (en présence de Fe(III)) et du cyanure de potassium (en présence de Ni, Co, Cu). Ajouter ensuite 2 ml de formaldoxime 1 *M* et immédiatement la solution de NaOH 1 *N* jusqu'à neutralisation; ajouter encore 2 ml de NaOH 1 *N*. Compléter la solution dans une fiole jaugée, avec de l'eau, jusqu'à 50 ml. Après 10 min, mesurer la densité optique de la solution (à $\lambda_{max} = 455$ mμ ou avec un filtre bleu), en utilisant l'eau comme solution de référence.

Si on soupçonne la présence de vanadium ou de cérium il faut chauffer la solution colorée pendant 5 min à 90°. Si après refroidissement la solution est trouble, il faut la filtrer.

Dosage du cérium

Après alcalinisation de la solution contenant des ions cérium et de la formaldoxime en grand excès, on obtient sous l'influence de l'oxygène une coloration orange due avant tout au complexe $[Ce(CH_2NO)_6]^{2-}$. Le système renferme aussi des complexes hydroxoformaldoximiques en quantités insignifiantes. La concentration de NaOH 0.05–0.1 *N* est la plus favorable. Pendant 5 à 15 min la coloration est stable.

La courbe 2 sur la Fig. 1 représente un spectre d'absorption du complexe Ce(IV)–formaldoxime. Le maximum d'absorption est situé dans le proche ultraviolet (à $\lambda_{max} = 340$ mμ le coefficient d'absorption molaire $\varepsilon \sim 4700$). A la longueur d'onde 400 mμ, $\varepsilon \sim 3200$.

La méthode colorimétrique de dosage du cérium par la formaldoxime est environ 50 fois plus sensible que la méthode basée sur la coloration jaune du cérium(IV) et 3 fois plus sensible que la méthode au carbonate de potassium.

La solution du complexe formaldoxime–cérium(IV) suit la loi de Beer (0–20 μg Ce/ml).

La réaction entre la formaldoxime et le cérium n'est pas gênée par les citrates, tartrates, sulfosalicylates et oxalates. Par contre, fluorures, phosphates et EDTA empêchent cette réaction. En raison de ses propriétés réductrices, l'acide ascorbique gêne la réaction, en empêchant le cérium(III) de se transformer en complexe coloré du cérium(IV).

Une séparation du cérium de la plupart des métaux peut s'effectuer en le précipitant sous forme d'oxalate ou de fluorure. Les cyanures, sans influence sur la réaction du cérium avec la formaldoxime, masquent Ni, Co, Cu et Fe(II). Les autres terres rares, ainsi que le scandium, l'yttrium et le thorium, forment avec la formaldoxime des complexes incolores sans influence sur le dosage du cérium. La présence de ces éléments peut être avantageuse dans le cas d'une faible teneur en cérium; ils jouent alors le rôle d'entraîneur, *p. ex.* lors de la séparation du cérium sous forme d'oxalate.

Mode opératoire. A la solution (*p. ex.* précipité d'oxalates bien lavé et dissout dans HCl dilué ou précipité de fluorures lavé, chauffé avec H_2SO_4 ($HClO_4$) jusqu'à élimination de HF et le reste dilué avec de l'eau), qui ne contient plus que 0.5 mg de Ce, ajouter 5 ml de la formaldoxime 1 *M*. Alcaliniser tout de suite la solution avec NaOH 1 *N*, en ajoutant 4 ml en excès après la neutralisation. Amener la solution colorée dans une fiole jaugée avec de l'eau jusqu'à 50 ml et après 10 min mesurer la densité

optique de la solution (à $\lambda = 400$ mμ ou avec un filtre bleu) en utilisant l'eau comme solution de référence.

Dosage du vanadium

Au cours de la réaction du vanadium avec la formaldoxime, en milieu alcalin, et en présence d'un excès convenable de réactif, il se forme un complexe brun-orange, dont la formule est probablement $[V(CH_2NO)_6]^-$. Si la formaldoxime n'est pas en excès, on obtient un complexe de même couleur, mais d'intensité plus faible et dans lequel le rapport de V/CH_2NO^- est égal 1 : 3. On suppose que dans ce cas il se forme l'hydroxocomplexe $[V(OH)_3(CH_2NO)_3]^-$ dans lequel les groupes OH^- sont remplacés par CH_2NO^-, lorsque la concentration en formaldoxime augmente. Avec un rapport $V : CH_2NO^-$ d'environ 1 : 200–600, l'intensité de la coloration est maximum. La courbe 3 sur la Fig. 1 montre le spectre d'absorption ($\lambda_{max} = 403$ mμ, $\varepsilon = 6600$). Pour réagir avec la formaldoxime, le vanadium doit être au degré d'oxydation IV. Dans le complexe le vanadium(IV) se transforme ensuite assez lentement, sous l'action de l'oxygène, en vanadium(V).

Le milieu ammoniacal (>1 *N* NH_3) est le plus favorable. Dans des solutions de soude, la coloration se forme plus lentement, ce qui s'explique par la plus grande stabilité des hydroxocomplexes dans ce milieu. La présence d'hydroxylamine accélère la formation du complexe en facilitant la réduction du vanadium(V). Mais en trop forte quantité, elle empêche la transformation ultérieure en vanadium(V).

La coloration est maximum au bout de 30 à 45 min et reste stable au moins pendant 2 jours. Elle diminue de moitié après 10 min de chauffage à 60°.

Le système coloré ne suit pas bien la loi de Beer et il faut éviter de mesurer des absorptions de solutions dont la concentration est supérieure à 5 μg V/ml.

La méthode à la formaldoxime est supérieure aux autres méthodes de dosage colorimétrique du vanadium. Le coefficient d'absorption molaire (ε) dans la méthode à l'eau oxygénée est ~ 300; dans la méthode à l'acide phosphovanadotungstique ~ 2000 ($\lambda = 400$ mμ) et dans la méthode à l'hydroxy-8-quinoléine ~ 4000.

En présence de H_2O_2 et d'EDTA, la réaction est plus lente; mais les tartrates, citrates, oxalates, fluorures, cyanures et phosphates sont sans influence.

Pour rendre spécifique la méthode de dosage du vanadium à la formaldoxime, il faut utiliser des séparations ainsi que des "masquages" des métaux gênants. On sépare d'abord les traces de vanadium à l'aide de Fe(III), Al, Ti, etc., précipités par l'ammoniaque à pH 6–7. Après fusion du précipité calciné avec le carbonate de sodium, on traite par l'eau, le vanadium passe en solution (de même que Al, Zn). On peut réduire des traces éventuelles de MnO_4^- par addition d'éthanol. L'expérience montre que les pertes en vanadium au cours de ces opérations ne dépassent pas 10%. L'addition du tartrate a pour but de complexer l'aluminium. On ajoute aussi un peu de cyanure de potassium pour complexer des traces possibles de nickel, cobalt et cuivre.

Mode opératoire. (*a*) *Séparation du vanadium.* Chauffer jusqu'à 70° la solution à analyser contenant moins de 0.1 mg de V et ajouter de l'ammoniaque goutte à goutte afin d'atteindre un pH de 6–7. S'il n'y a pas de fer, d'aluminium ou de titane dans la solution, ajouter *ca.* 2 mg de Fe(III) comme entraîneur. Chauffer légèrement jusqu'à coagulation du précipité, puis filtrer et laver avec une solution de NH_4NO_3 chaude et diluée. Sécher et calciner le filtre avec le précipité dans un creuset de platine. Faire une fusion au carbonate (2 g de Na_2CO_3, ou moins selon le besoin). Reprendre le résidu

de fusion par 25 ml d'eau contenant 3 gouttes d'éthanol. Maintenir la suspension à 60° pendant 30 min et filtrer le précipité, qui, lavé avec de l'eau chaude, est à rejeter. Acidifier faiblement le filtrat avec HCl (1 + 1) et chauffer pour chasser CO_2.

(*b*) *Dosage du vanadium.* A la solution refroidie, ajouter 0.5 g de tartrate Na-K, 0.1 g de $NH_2OH \cdot HCl$, *ca.* 10 mg de KCN, 3 ml de formaldoxime 1 *M* et alcaliniser immédiatement avec de l'ammoniaque (1 + 4), puis amener à 50 ml dans une fiole jaugée. Après une heure mesurer la densité optique de la solution ($\lambda = 403$ mμ ou avec un filtre bleu) en utilisant l'eau comme solution de référence.

Dosage du nickel

Après alcalinisation de la solution contenant des ions nickel et de la formaldoxime, on observe une coloration verte qui se transforme en brun avec le temps. En milieu alcalin (pH > 12) il existe en solution exclusivement un complexe brun du nickel(IV), dont la formule est $[Ni(CH_2NO)_6]^{2-}$. A un pH inférieur à 12 (solutions ammoniacales et solutions diluées de NaOH (< 0.01 *N*)), il y a formation du complexe brun et d'un complexe vert. Dans des conditions particulières, il peut se former également d'autres complexes de la formaldoxime avec le nickel. Le complexe brun est le plus stable et sa coloration est la plus intense. Pour l'analyse colorimétrique, ce complexe est intéressant.

Il faut alcaliniser par NaOH, dont la concentration dans une solution à analyser doit être de 0.02 à 0.1 *N*. Lorsque l'excès de formaldoxime est assez grand et l'alcalinité de la solution supérieure à 0.1 *N*, on n'obtient pas une coloration brune maximum probablement à cause de l'influence de grandes quantités de composés peroxydés, formés dans ces conditions par autoxydation de la formaldoxime.

La courbe 4 sur la Fig. 1 représente le spectre d'absorption du complexe brun. A $\lambda_{max} = 473$ mμ le coefficient d'absorption molaire $\varepsilon = 18400$. La sensibilité de cette méthode est donc supérieure à celle de la méthode à la diméthylglyoxime ($\varepsilon = 15000$) et à l'α-furyldioxime ($\varepsilon = 17000$). Les solutions brunes suivent la loi de Beer jusqu'à une concentration de 2 μg Ni/ml, puis on observe quelques déviations.

Le complexe brun ne résiste pas à des températures supérieures à 50°. A la température normale, la stabilité du complexe est grande. La présence de tartrates, citrates, oxalates, sulfures en grand excès n'influence pas la réaction du nickel avec la formaldoxime. Cyanures et EDTA forment avec le nickel des complexes plus stables qu'avec la formaldoxime. La diméthylglyoxime (sans excès) ne gêne pas la formation du complexe brun de la formaldoxime. Cette propriété permet une séparation du nickel de tous les métaux gênants (*p. ex.* Mn, Co, Fe, Cu, Ce, V, etc.) avant sa réaction avec la formaldoxime. Lors du dosage de traces de nickel, on ne peut pas le séparer par précipitation à la diméthylglyoxime; on utilise alors l'extraction avec succès. Nos expériences ont démontré que la séparation par extraction de 10 μg de nickel d'avec de très grandes quantités de métaux gênants était quantitative.

Mode opératoire. Séparer d'abord le nickel avec la diméthylglyoxime par extraction dans le chloroforme. Agiter l'extrait chloroformique pendant une minute avec deux portions de 5 ml HCl 0.1 *N*. A la solution aqueuse contenant moins de 50 μg de Ni, ajouter 1 ml de formaldoxime 1 *M* et immédiatement 5 ml de NaOH 1 *N*. Amener la solution colorée à 50 ml avec de l'eau, dans une fiole jaugée. Après 15 min mesurer la densité optique de la solution brune (à $\lambda = 473$ mμ ou avec un filtre bleu) en utilisant l'eau comme solution de référence.

Dosage du fer

La formaldoxime, ajoutée à la solution contenant des ions ferriques, les réduit à l'état ferreux; il se forme ensuite après alcalinisation un complexe jaune orange du fer(II), se transformant, sous l'action de l'oxygène, en complexe violet de la formaldoxime avec le fer ferrique $[Fe(CH_2NO)_6]^{3-}$. Cette transformation est plus rapide en solution ammoniacale (suffisamment forte) qu'en solution de NaOH (probablement à cause de la formation des hydroxocomplexes transitoires peu labiles). Seul le complexe violet présente un intérêt en analyse colorimétrique; il est le plus intensément coloré et très stable.

La courbe 5 sur la Fig. 1 représente le spectre d'absorption de ce complexe. Le coefficient d'absorption molaire $\varepsilon = 7400$ à $\lambda_{max} = 534$ mμ. L'accord avec la loi de Beer est satisfaisant jusqu'à une concentration de 5 μg Fe/ml.

Le complexe violet une fois formé (durée: 10–15 min) n'évolue plus en fonction du temps. Les températures supérieures à 50° le détruisent.

La réaction colorée n'est pas gênée par l'excès de tartrates, citrates, oxalates, fluorures, phosphates, sulfosalicylates, EDTA. La formaldoxime ajoutée à la solution d'EDTA provoque après alcalinisation à l'ammoniaque la coloration, due à la présence de traces de fer dans ce réactif. Les cyanures (surtout en présence de réducteurs), les sulfures et l'oxine empêchent la réaction entre la formaldoxime et le fer.

Avant le dosage du fer au moyen de la formaldoxime il faut séparer les métaux gênants. Par double précipitation avec de l'ammoniaque on peut séparer le fer d'avec le cuivre, le cobalt et le nickel. Le nickel est séparé plus exactement par extraction en présence de diméthylglyoxime. Le cérium est complexé par les fluorures. On sépare le fer d'avec le manganèse par précipitation avec $Zn(OH)_2$. Dans la plupart des cas la quantité du fer à doser dépasse celle du manganèse qui l'acompagne. Au lieu de séparer le manganèse on peut alors doser dans un prélèvement la somme Fe + Mn et dans un autre le manganèse seulement après le masquage du fer au moyen de cyanures, en présence d'acide ascorbique.

La méthode de dosage du fer au moyen de la formaldoxime permet de doser le fer total sans tenir compte du degré d'oxydation du fer dans la solution.

Mode opératoire. Séparer le fer d'avec Ni, Co, Cu par une double précipitation sous forme de $Fe(OH)_3$, au moyen de NH_3. S'il n'y a pas d'aluminium dans la solution, il faut en ajouter (~ 2 mg) comme un entraîneur. Ensuite séparer le fer d'avec le manganèse par précipitation de $Fe(OH)_3$, avec $Zn(OH)_2$.

A la solution acide, contenant moins de 50 μg de Fe, ajouter 2 ml de formaldoxime 1 *M*, 10 ml d'ammoniaque concentrée et compléter la solution avec de l'eau jusqu'à 50 ml dans une fiole jaugée. Après 15 min mesurer la densité optique de la solution violette (à $\lambda = 534$ mμ ou avec un filtre vert) en utilisant l'eau comme solution de référence.

CONCLUSIONS

On a présenté ci-dessus 5 méthodes spectrophotométriques de dosage de métaux sous forme de complexes colorés de la formaldoxime. Trois parmi elles (méthodes de dosage pour Mn, Ce et V) méritent d'être utilisées largement dans les laboratoires de chimie analytique. Les méthodes de dosage du nickel et du fer peuvent être utilisées dans des cas particuliers; l'α-furyldioxime et la diméthylglyoxime (avec oxydant) pour le nickel et le thiocyanate (avec extraction), la phénanthroline ou la bathophénanthroline

pour le fer, ne donnent en effet pas de meilleurs résultats.

La méthode à la formaldoxime est très avantageuse pour le dosage du manganèse, en comparaison avec la méthode bien connue, utilisant la coloration des ions MnO_4^-, après oxydation (periodate ou persulfate). La formaldoxime permet de doser le manganèse avec une sensibilité 4–5 fois supérieure à celle de la méthode au permanganate. Ceci est particulièrement appréciable dans l'analyse de traces.

L'importance de la méthode à la formaldoxime pour le cérium est due à deux faits: (*1*) elle rend possible le dosage du cérium en présence de tous les éléments des terres rares et du thorium; (*2*) c'est la méthode la plus sensible parmi les méthodes spécifiques pour le cérium. Il faut souligner encore ici la facilité de séparation du cérium de tous les métaux gênants.

Quant au vanadium on ne dispose pas de bonnes méthodes. La méthode exigeant le moins de séparations, est la méthode à l'eau oxygénée, mais sa sensibilité est très faible. La méthode à la formaldoxime, comme d'autres méthodes plus connues (*p. ex.* à l'acide phosphotungstique, à l'acide benzohydroxamique, à l'oxine), exige de nombreuses séparations, mais sa sensibilité est supérieure.

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RÉSUMÉ

La formaldoxime forme en milieu alcalin avec les ions manganèse, cérium, vanadium, nickel et fer des complexes très colorés (coefficients d'absorption molaires de 18000 à 4000). Après explication du mécanisme des réactions et détermination des conditions exactes de formation des complexes particuliers de la formaldoxime, on a élaboré de nouvelles méthodes spectrophotométriques de dosage du cérium et du fer, amélioré et précisé les conditions optimales pour les méthodes de dosage du manganèse, du nickel et du vanadium. L'emploi de formaldoxime pour le dosage du manganèse, du cérium et du vanadium a une importance particulière dans l'analyse des traces.

SUMMARY

Formaldoxime forms highly coloured complexes (ε = 4000–18000) with manganese, cerium, vanadium, nickel and iron in alkaline media. On the basis of a study of reaction mechanisms and of the optimum conditions for the formation of the different complexes, new spectrophotometric methods are described for determination of cerium and iron, as well as improved methods for determination of manganese, nickel and vanadium. The use of formaldoxime for Mn, Ce and V is particularly beneficial in trace analysis and the methods suggested are specific.

ZUSAMMENFASSUNG

Formaldoxim bildet stark gefärbte Komplexe (ε = 4000–18000) mit Mangan, Cer, Vanadin, Nickel und Eisen in alkalischem Medium. Neue spektralphotometrische Methoden zur Bestimmung von Cer und Eisen ebenso wie verbesserte Methoden zur Bestimmung von Mangan, Nickel und Vanadin werden beschrieben auf grund von Untersuchungen über die Reaktionsmechanismen und die optimalen Bedingungen für die Bildung der unterschiedlichen Komplexe. Die Anwendung des Formaldoxims zur Bestimmung von Mn, Ce und V eignet sich besonders bei der Spurenanalyse. Die vorgeschlagenen Methoden sind spezifisch.

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SPECTROPHOTOMETRIC DETERMINATION OF FORMALDEHYDE, FURFURAL, AND VANILLIN IN BISULFITE SOLUTIONS

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In trying to isolate and separate carbonyl compounds from sulfite waste liquor by means of an ion-exchange column in the bisulfite form, bisulfite solutions of increasing concentration were used as the eluant. Therefore, it was desirable to find methods of determining micromolar amounts of the compounds in bisulfite solutions which were several orders of magnitude stronger. In this study only formaldehyde, furfural, and vanillin were investigated.

Aldehydes react with bisulfite solutions to form addition compounds, salts of α-hydroxysulfonic acids. The determination of very small amounts of aldehydes in concentrated bisulfite solutions is rather troublesome. Titration with iodine, which has often been utilized for similar tasks, requires for quantitative work both a relatively strong bisulfite complex and not too low an aldehyde concentration. Among the aldehydes investigated only formaldehyde forms a sufficiently stable complex. Furthermore, the amounts of aldehydes in the liquors are very small. If it is desired to make quantitative determinations of the aldehydes in such solutions, the aldehydes must be fully released from the complexes and the bisulfite ions destroyed. Otherwise, the extent of the interfering effect of the bisulfite ions, estimated by means of equilibrium data, must be taken into consideration and a correlation equation must be worked out[1].

Several accurate methods exist for the spectrophotometric determination of aldehydes, but the interfering effect of the bisulfite ions makes their utilization impossible. Thus, Schiff's reagent cannot be used successfully[2].

In a method worked out by LAPPIN AND CLARK[3], the aldehydes are converted to 2,4-dinitrophenylhydrazones, which on addition of a solution of alkali hydroxide yield a wine-red color. The precipitation with 2,4-dinitrophenylhydrazine can be performed in the presence of bisulfite, but during the dissolution of the hydrazone in alcoholic alkali, the bisulfite precipitates owing to its lower solubility in alcoholic solution. The color normally produced in this treatment is noticeably reduced as a result of the precipitated bisulfite.

The search for a general method for the determination of small amounts of aldehydes in concentrated bisulfite solutions was therefore abandoned. In the present work an attempt was made to split the bisulfite complex and to oxidize the bisulfite with iodine followed by suitable pH adjustment. This appeared to give extremely

good results for furfural and vanillin when analyzed in ultraviolet light. The determination of formaldehyde is based on the reaction with chromotropic acid[4]. In this case, the analysis could be performed directly on the bisulfite solution, although the bisulfite content had a certain effect. Less reproducible values were obtained after the oxidation of the bisulfite with iodine.

EXPERIMENTAL

Apparatus

A Beckman Spectrophotometer, Model DU, with 1-cm cells was used throughout the measurements.

The melting points were determined using Kofler's hot stage microscope RCH with variable resistance.

A B-D Cornwall Luer-Lok Syringe, (1.000 ± 0.001) ml, with a metal pipet holder was used to feed in the aldehyde.

Reagents and solutions

Formaldehyde. A weighed amount of about 10 ml of an aqueous formaldehyde solution (about 35%, Merck), the strength of which was determined according to Tappi Standard T600 m-45, was made up to 1 l with distilled water. This solution was standardized by precipitation of the compound with dimedone[5]. The melting point of the derivative was 188–189°. The formaldehyde content determined by the two methods agreed very well.

Furfural. About 1 g of redistilled furfural (Merck, p.a.) was weighed out and diluted to 1 l with distilled water. The furfural content of the solution was determined via the 2,4-dinitrophenylhydrazone derivative[6]. The value agreed well with that calculated from the added amount of furfural.

On determining the melting point of the 2,4-dinitrophenylhydrazone, a value of 185° was obtained when a heating rate of 2–4°/min was used. If, however, the temperature was held at 175–180° for at least 5 min, the substance underwent a conversion, which resulted in a crystalline material (m.p. 220–221°). After recrystallization from ethanol, the 2,4-dinitrophenylhydrazone melted at 185°, and no alteration of the melting point occurred on prolonged heating.

Vanillin. A weighed quantity of about 1.5 g of vanillin (B.D.H. Laboratory Reagent) was dissolved in 100 ml of ethanol in a 1-l volumetric flask and filled up to the mark with distilled water. On precipitating with 2,4-dinitrophenylhydrazine, a supplementary determination of the strength of the solution was achieved. The derivative melted at 262–263°.

The *bisulfite solutions* were prepared from sodium pyrosulfite (Merck, p.a.).

Recommended procedures and discussion

Formaldehyde. The directions given by BRICKER AND VAIL[4] have been slightly modified so as to give more reproducible values.

Weigh (100 ± 10) mg of chromotropic acid and transfer to a 50-ml beaker, followed by 1 ml of the test solution. The formaldehyde content ought not to exceed 100 μg; if necessary, dilute the solution before addition. In order to avoid spattering, evaporate the solution to dryness in a drying-oven at 110°. Subsequently, heat the residue on a hot plate at about 200° for 10 min. After cooling the residue, add 5 ml of concentrat-

ed sulfuric acid to the beaker, and then heat in boiling water for 30 min. Allow the colored solution to cool and then dilute to 50 ml with distilled water (in a volumetric flask). Measure the absorbance against a reagent blank at 570 mμ after 5–44 h have elapsed from the dilution. Within this time interval, only slight variations in the reading values were observed. The absorption maximum at 570 mμ did not shift when bisulfite was present in the test solution.

The effect on the absorbance value when varying amounts of bisulfite were present in the test solution was studied in 5 series with bisulfite concentrations ranging from 0 to 0.8 *M*. The measured values of the absorbance were plotted against the formal dehyde concentrations in the final solutions and a linear relationship was found. As

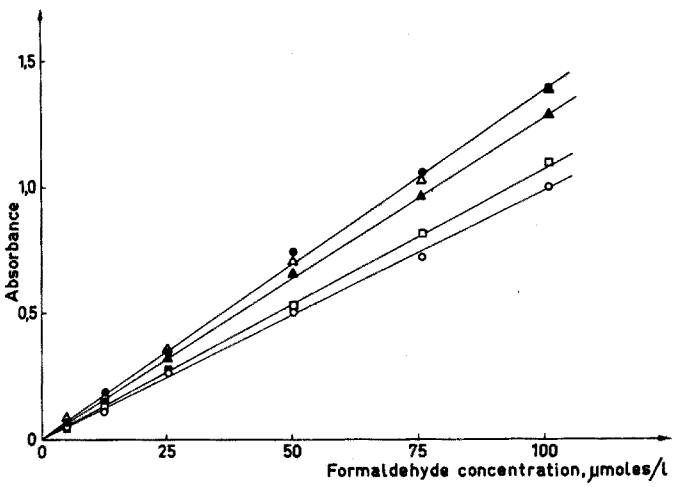

Fig. 1. Absorbance at different bisulfite concentrations of the test solution *vs.* concentration of formaldehyde in the final solution. ○, 0 *M* $NaHSO_3$; ●, 0.1 *M* $NaHSO_3$; △, 0.2 *M* $NaHSO_3$; ▲, 0.4 *M* $NaHSO_3$; □, 0.8 *M* $NaHSO_3$.

can be seen from Fig. 1, a multitude of straight lines is obtained, the slope of which is a function of the bisulfite concentration.

The molar absorptivities for formaldehyde in aqueous and bisulfite solutions are shown in Table I.

Furfural. Introduce 1.00 ml of the test solution into a 50-ml volumetric flask. Add some water and oxidize the excess of bisulfite with 0.1 *N* iodine. In order to release the bound bisulfite[7,8], raise the pH to 7–8 with solid sodium bicarbonate, and add

TABLE I

SPECTROPHOTOMETRIC DETERMINATION OF FORMALDEHYDE

Concn. of bisulfite in the test soln.	*Molar absorptivity*
0 *M* $NaHSO_3$	$0.99 \cdot 10^4$
0.1 *M* $NaHSO_3$	$1.38 \cdot 10^4$
0.2 *M* $NaHSO_3$	$1.38 \cdot 10^4$
0.4 *M* $NaHSO_3$	$1.29 \cdot 10^4$
0.8 *M* $NaHSO_3$	$1.09 \cdot 10^4$

more iodine cautiously so as to produce a faint yellow color which lasts for 10–15 min. Remove the excess of iodine with a few drops of 0.1 *N* sodium thiosulfate solution and, finally, dilute the solution to the mark with distilled water. Measure the absorbance of the solution against a suitable reagent blank at 277.5 mμ[9].

The stability of the solution was studied by making readings during a week, but no change was observed.

The sodium bicarbonate can also be added to the test solution before the oxidation with iodine. In this connection, however, it ought to be mentioned that the addition of too large an excess of iodine has no detrimental effect upon the furfural in acid solution, but the excess must be reduced (with bisulfite) before the solution is made alkaline.

If the solution has already been adjusted to pH 7–8, caution must be exercised at the end of the titration, so that only a slight excess of iodine remains. If this is not done, there is a risk of converting some furfural to furoic acid[10], resulting in a corresponding decrease in the absorbance value. Another reason why a large excess of iodine should be avoided is that the tetrathionate formed in the reaction with the added thiosulfate exhibits an absorption at the wavelength used in the determination of furfural.

The investigated concentration range for furfural covered 7–100 μmoles/l in the final solution. Sample solutions were made in water (pH 6 and 8) and in 0.2–0.8 *M* sodium bisulfite (pH 3–4). In the bisulfite solutions the furfural was allowed to react with the bisulfite for about 30 min in order to reach equilibrium. Thereafter the bisulfite was destroyed as described above.

After plotting the values of the absorbance *vs.* the concentration of furfural, it was clear that Beer's law was obeyed. The discrepancies in the slope of the lines, caused by different bisulfite content of the solution, were slight or nonexistent and hardly discernible on a plot (Fig. 2).

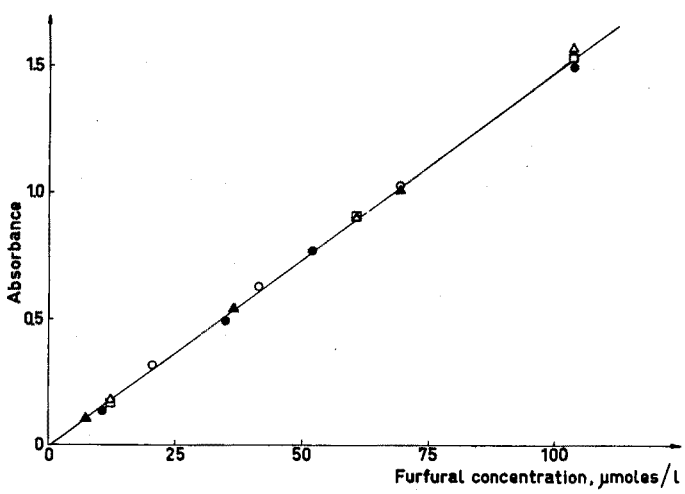

Fig. 2. Absorbance at different bisulfite concentrations of the test solution *vs.* concentration of furfural in the final solution. ○, o *M* $NaHSO_3$ (pH 6); ●, o *M* $NaHSO_3$ (pH 8); △, 0.2 *M* $NaHSO_3$; ▲, 0.4 *M* $NaHSO_3$; □, 0.8 *M* $NaHSO_3$.

In Table II are given the molar absorptivities for furfural in water (pH 6 and 8) and bisulfite solutions.

Vanillin. Pipet 1.00 ml of the test solution into a 50-ml volumetric flask. Add some water and oxidize the bisulfite with 0.1 *N* iodine. Maintain a slight excess of iodine for 10–15 min, and then remove it with a few drops of thiosulfate. Add solid sodium

TABLE II

SPECTROPHOTOMETRIC DETERMINATION OF FURFURAL

Concn. of bisulfite in the test soln.	*Molar absorptivity*
0 *M* $NaHSO_3$ (pH 6)	$1.50 \cdot 10^4$
0 *M* $NaHSO_3$ (pH 8)	$1.50 \cdot 10^4$
0.2 *M* $NaHSO_3$	$1.49 \cdot 10^4$
0.4 *M* $NaHSO_3$	$1.47 \cdot 10^4$
0.8 *M* $NaHSO_3$	$1.50 \cdot 10^4$

carbonate until a pH of 10–11 is reached[11]. After adjustment of the volume to the mark with distilled water, measure the absorbance of the solution at 347 mμ. The solution remained stable for weeks.

Owing to the low stability constant of the vanillin bisulfite compound, both free and bound bisulfite could be titrated with iodine in acid medium. This is of great importance in quantitative determinations, as vanillin is easily oxidized to vanillic acid in alkaline solution (*cf.* PEARL[12]).

Sample solutions were made by diluting the stock solution to obtain vanillin in water or 0.2–0.8 *M* sodium bisulfite. The bisulfite was oxidized as described in the analysis method. The vanillin concentration of the solution measured in the spectrophotometer lay within the interval 5–65 μmoles/l.

Variations in the bisulfite concentration had no influence upon the absorbance, which was directly proportional to the vanillin concentration (see Table III).

From the data in Table III the molar absorptivities were found to be $2.54 \cdot 10^4$ for vanillin in water, and $2.58 \cdot 10^4$ for vanillin in 0.2–0.8 *M* bisulfite.

TABLE III

SPECTROPHOTOMETRIC DETERMINATION OF VANILLIN

Concn. of vanillin in the final soln. (μmoles/l)	*Absorbance of the final soln. at various bisulfite concns.*[a]			
	0	*0.2*	*0.4*	*0.8*
5.0	0.126	0.127	0.128	0.128
10.0	0.254	0.259	0.258	0.260
19.9	0.508	0.516	0.512	0.516
33.2	0.849	0.858	0.857	0.858
39.9	1.02	1.03	1.03	1.02
49.8	1.26	1.28	1.28	1.30
66.4	1.65	1.67	1.69	1.70

[a] The bisulfite concentration of the test solution in moles/l.

SUMMARY

Formaldehyde, furfural, and vanillin are determined in solutions containing bisulfite. The interference of bisulfite is eliminated by oxidation with iodine before furfural and vanillin are determined spectrophotometrically in ultraviolet light. Formaldehyde is determined with chromotropic acid by a slightly modified procedure; bisulfite is not destroyed previously.

RÉSUMÉ

Le formaldéhyde, le furfural et la vanilline ont été dosés dans des solutions renfermant du bisulfite. Ce dernier est éliminé par oxydation au moyen d'iode. Le furfural et la vanilline peuvent ensuite être dosés spectrophotométriquement en lumière ultra-violette. Le formaldéhyde est dosé au moyen d'acide chromotropique (sans destruction préalable du bisulfite).

ZUSAMMENFASSUNG

Formaldehyd, Furfurol und Vanillin werden in Lösungen, die Bisulfit enthalten, spektralphotometrisch bestimmt. Bevor Furfurol und Vanillin spektralphotometrisch mit ultraviolettem Licht bestimmt werden, werden die Störungen durch das Bisulfit durch vorherige Oxydation mit Jod eliminiert. Formaldehyd wird mit einem modifizierten Chromotropsäure-Verfahren bestimmt. Das Bisulfit wird dabei vorher nicht zerstört.

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THE USE OF RIGID ETHANOLIC SOLUTIONS FOR THE PHOSPHORIMETRIC INVESTIGATION OF ORGANIC COMPOUNDS OF PHARMACOLOGICAL INTEREST

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Phosphorimetry as a means of chemical analysis was first suggested by KEIRS, BRITT AND WENTWORTH[1]. Later, PARKER AND HATCHARD[2] reviewed the possibilities of using phosphorescence measurements for the analysis of chemical constituents. MCGLYNN, NEELY AND NEELY[3] studied the total luminescence of naphthalene, phenanthrene and 1,2,4,5-tetramethylbenzene and gave spectra and working curves for each compound. WINEFORDNER AND LATZ[4] applied phosphorimetry to the analysis of aspirin in blood serum and plasma. Because of the great selectivity and sensitivity of the aspirin analysis, WINEFORDNER AND LATZ[4] discussed the possibilities of applying phosphorimetry to the analysis of trace amounts of drugs in biological fluids as well as the analysis of trace constituents in other areas of science. The molecular requirements for phosphorescence of organic compounds are not well understood, although the presence of a conjugated ring system seems essential. The present status of phosphorescence theory has been reviewed by several authors[1,2,5].

In this manuscript, further evidence of the great potential of phosphorimetry as a means of drug analysis is presented. In most previous phosphorimetric studies, E.P.A. (a mixture of ethyl ether, isopentane and ethyl alcohol in a volume ratio of 5:5:2) was used as the solvent. The low solubility of most drugs in E.P.A. prevents its general use in phosphorimetry. In addition, the fluorimetric grade E.P.A. is quite expensive. A solvent study by WINEFORDNER AND ST. JOHN[6] demonstrated that ethanol provides a clear, rigid solvent at liquid nitrogen temperature. Because most drugs are quite soluble in ethanol, it was used as the solvent for the studies described. The wavelengths for maximum phosphorescence excitation and emission, the phosphorescence lifetimes, the limits of detectability and the working curves of 22 organic compounds of pharmacological importance are given. The extremely low limits of detectability (about 0.01 μg/ml ethanol for most drugs) indicate the possibility of applying phosphorimetry to the analysis of certain trace drugs in biological fluids.

EXPERIMENTAL

Apparatus

An Aminco-Bowman spectrofluorimeter (American Instrument Company, Silver Spring, Maryland) with an Aminco-Keirs phosphoroscope, a potted RCA 1P28

photomultiplier tube and a Model 135 Moseley X-Y recorder were used to obtain all excitation and emission spectra as well as all decay times. All studies were performed with the slit arrangement recommended by the manufacturer (slit arrangement number 4). All working curves were obtained by taking the intensity readings directly from the photomultiplier microphotometer meter. Samples to be measured were held in the special Aminco fused quartz micro sample tubes.

Materials

The solvent for all measurements was specially purified technical grade ethanol. Technical grade ethanol was placed in a 3-l round-bottom flask connected to a 120 cm long by 2.5 cm I.D. vacuum column (containing borosilicate glass helices—$^3/_{32}''$ coil I.D.) and a separable distilling head (No. LG 7220, No. LG 5705 and No. LG 6290, respectively, Labglass, Inc., Vineland, New Jersey). The flask was heated, and the distilling head was adjusted to give a reflux ratio of 10:1 (*i.e.*, about 2 l of pure ethanol could be prepared in 10 h). The first 500 ml of ethanol were discarded and the next 2 l were kept for use. The resulting ethanol always had a phosphorescence background as small as or smaller than the commercially available fluorimetric grade ethanol. Stock solutions containing 10^{-2} mole of each of the organic compounds listed in Table I per liter of purified ethanol were prepared. By successive dilution, standard solutions having concentrations from 10^{-2} *M* to 10^{-8} *M* were prepared. In the preparation of dilute solutions, pickup of phosphorescent impurities from the glassware resulted in an intolerable phosphorescence background unless all glassware was always cleaned thoroughly according to the directions specified by the American Instrument Company[7].

Procedure

A solution containing 10^{-4} *M* of the organic compound of interest was placed in the sample tube, and the sample tube was then aligned in the quartz Dewar flask containing liquid nitrogen[7]. Excitation and emission phosphorescence spectra were obtained according to specified directions[7]. From the spectra, the wavelengths giving maximum excitation and emission were then set on the spectrofluorimeter, and the relative intensity readings from the photomultiplier microphotometer meter were recorded for a series of standard solutions (10^{-8} to 10^{-2} *M*) of the desired compound. The relative intensities were corrected for the background phosphorescence[4]. Plots of the corrected relative intensity readings *vs.* sample concentration were then constructed. The above procedure was repeated for each of the 22 compounds listed in Table I. The lifetimes of each of the compounds in Table I were measured using the X-Y recorder. The shutter on the excitation monochromator was closed manually and simultaneously the recorder was allowed to scan the decay at the rate of 1 sec/inch.

After each of the above measurements are taken, the sample tube is removed and viewed carefully. If the sample is clear and uncracked, the data are acceptable. If the sample is clear and cracked, the sample is allowed to liquefy, the sample tube dried on the outside with a soft tissue and the measurement procedure repeated. Often the experimenter is given a warning of the cracking while the sample tube is immersed in the liquid nitrogen. If the photometer signal drifts slowly and decreases or increases abruptly several sec after immersion in the liquid nitrogen, the sample has most likely cracked and the measurement process must be repeated.

RESULTS AND DISCUSSION

In Table I wavelengths are given for phosphorescence excitation and emission peaks with the experimental set-up described. The phosphorescence lifetimes in sec for each compound are also listed in Table I. No spectra are listed for any of the compounds because the spectra would be characteristic of the experimental set-up used. Several methods of correcting emission and excitation spectra in order to be independent of the experimental set-up have been previously given[8-10]. However, corrected spectra or corrected spectral peaks would give no more useful data to the analyst than the data listed in Table I. From the analytical standpoint, the parameters of major importance are the wavelengths for which the instrument in concern gives the maximum phosphorescence excitation and emission for the compound in concern. The analyst must always determine the excitation and emission spectra for the desired compound on his own instrument prior to selecting the optimum wavelengths, although for similar experimental conditions the optimum wavelengths will not be too different from those given in Table I. All lifetimes were determined by measuring the time for the phosphorescence intensity to decrease from a certain intensity to e^{-1} of that value. The excitation and emission wavelengths used in the lifetime studies

TABLE I

WAVELENGTHS OF PHOSPHORESCENCE EXCITATION AND EMISSION PEAKS, PHOSPHORESCENCE LIFETIMES AND PHOSPHORIMETRIC LIMITS OF DETECTION

Compound[a]	*Excitation maximum for phosphorescence*[b] *(mμ)*		*Emission maximum for phosphorescence*[b] *(mμ)*			*Lifetime*[b] *(sec)*[c]	*Limit of detection (μg/ml)*
Phenobarbital	240		380			1.8	0.1
Mebaral	240		380			2.2	0.01
Rutonal	240		380			2.5	0.02
Atropine	240		380			2.1	0.1
Benzocaine	310		430	420	440	5.3	0.007
Procaine hydrochloride	310		430	420	440	3.5	0.01
p-Aminobenzoic acid	310		430	420	440	3.2	0.004
Butacaine sulfate	310		430	420	440	5.7	0.05
Cyclaine hydrochloride	240	290	400	410	370	2.4	0.006
Metycaine hydrochloride	240	290	400	410	370	2.7	0.006
Benzoic acid	240	290	400	410	370	2.3	0.005
Cocaine hydrochloride	240	290	400	410	370	2.7	0.01
Quinidine sulfate	340	250	500	470		1.3	0.05
Quinine hydrochloride	340	250	500	470		1.3	0.04
Lidocaine	265	240	400			1.1	1.2
Caffeine	285	245	440			2.0	0.2
Ephedrine	225	410	390			3.6	0.2
Phenylephrine hydrochloride	290	240	390			2.4	0.01
Tronothane hydrochloride	300	240	410			1.2	0.02
Cinchophen	350	270	520	490		0.8	0.02
Physostigmine sulfate	315	260	420			3.6	0.03
Chlortetracyline	280		410			2.7	0.05

[a] Compounds are arranged according to similar structural and spectral characteristics.
[b] All working curves and lifetimes determined using the first peak listed and using a sample concentration of 10^{-4} mole/l.
[c] All lifetimes are measured with an accuracy of ± 0.2 sec.

are designated in Table I. Solutions containing 10^{-4} moles of the drug per liter of ethanol were used for all lifetime measurements.

In Figs. 1–4, working curves of phosphorescence intensity in relative units (photometer deflection units) *vs.* concentration in moles of compound per liter of ethanol are given. However, it is not convenient to represent the limiting detectable concentrations for each drug on the working curve plots. In addition, a more useful unit for

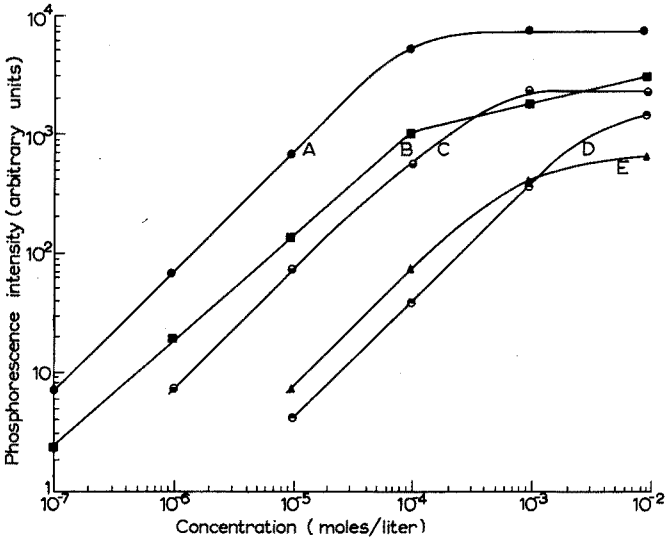

Fig. 1. Phosphorescence working curves for: (A) *p*-aminobenzoic acid; (B) cyclaine hydrochloride; (C) mebaral; (D) atropine; (E) ephedrine in rigid ethanol solvent (77°K).

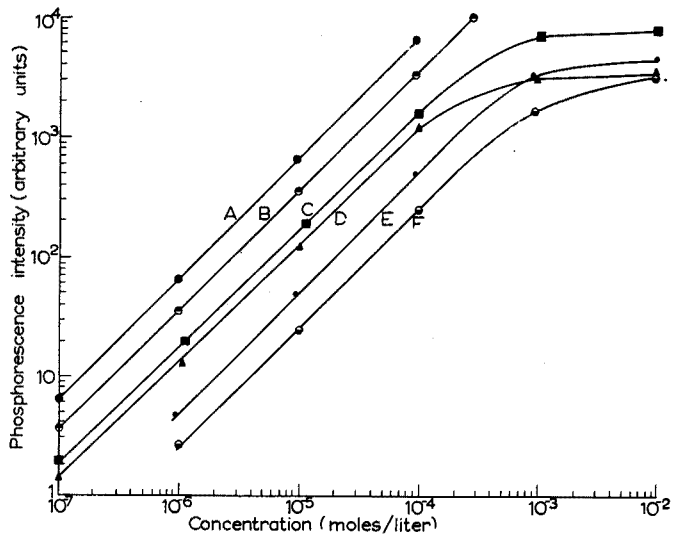

Fig. 2. Phosphorescence working curves for: (A) benzocaine; (B) physostigmine sulfate; (C) butacaine sulfate; (D) cocaine hydrochloride; (E) phenylephrine hydrochloride; (F) rutonal in rigid ethanol solvent (77°K).

limiting detectable concentration than moles per liter of ethanol is micrograms of the drug per milliliter of ethanol. The limits of detection for each drug are listed in Table I. Because the sample volume required for analysis is less than 1 ml, the magnitudes of the limiting detectable concentrations are also the same as or less than the limiting detectable amounts in μg. The limit of detectability was taken as that concentration (limits of detectabilities in mole/l were converted to μg/ml for Table I) which produces a detector signal twice the signal due to the background

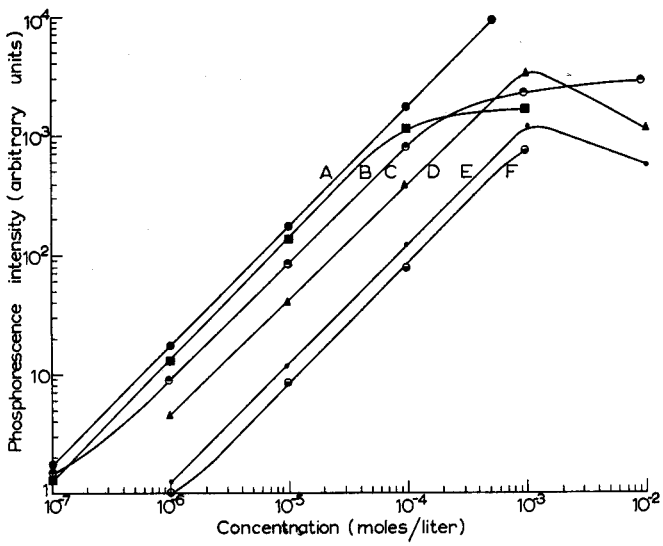

Fig. 3. Phosphorescence working curves for: (A) tronothane hydrochloride; (B) benzoic acid; (C) chlortetracycline; (D) cinchophen; (E) caffeine; (F) lidocaine in rigid ethanol solvent (77°K).

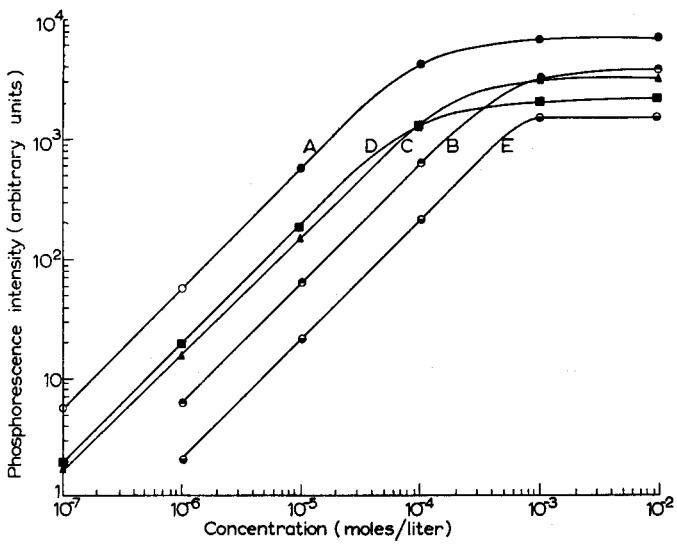

Fig. 4. Phosphorescence working curves for: (A) procaine; (B) quinine hydrochloride; (C) m caine hydrochloride; (D) quinidine sulfate; (E) phenobarbital in rigid ethanol solvent (77°K).

phosphorescence (*i.e.*, the phosphorescence due to pickup of impurities by the glassware and the impurities in the ethanol). Glassware, when exposed to the atmosphere, may adsorb significant amounts of organic vapors which may phosphoresce upon cooling. Even after a number of washings of glassware, quantities of organic materials may still be adsorbed in small and variable amounts. This results in slightly less than a two-fold uncertainty for the phosphorescence background. If the phosphorescence background could be maintained constant, *i.e.*, due only to the impurities in the ethanol, then the limit of detection would be determined primarily by the minimum signal detectable over the background fluctuation (noise). The background fluctuation would be determined mainly by noise in the photomultiplier amplifier, instability in the xenon light source, mechanical instability in the phosphoroscope and inaccuracy in alignment of the Dewar flask and the sample tube. With the set-up and procedures described, the limits of detection of most drugs in Table I are of the order of 0.01 μg/ml of ethanol which is comparable to the sensitivities obtained by fluorimetry[11]. With refinements in the sampling technique, the limits of detection can probably be decreased to 0.001 μg/ml or lower. For many organic compounds phosphorimetry may well be the most sensitive of all techniques. Phosphorimetric measurements possess the additional advantages of great selectivity, small sample size and good speed of analysis because of relatively few physical separations[1,4,12] which outweigh the mechanical disadvantages of using liquid nitrogen and of critical sample tube alignment[4].

Working curves and limits of detectability comparable to those listed in Figs. 1–4 and Table I, respectively, were obtained by using the experimental set-up described by WINEFORDNER AND LATZ[4]. Because of the convenience and greater accuracy of the commercial apparatus, only results obtained on it are given.

According to the phosphorescence excitation and emission wavelengths and the phosphorescence lifetimes given in Table I, it should be possible to analyze simultaneously 2 or more components by judicious choice of emission and excitation wavelengths and phosphoroscope speed. Compounds with similar excitation and emission wavelengths will certainly give poor results unless physically separated (liquid–liquid extraction, chromatography, etc.) prior to analysis, whereas compounds differing in spectral characteristics should give good results by using simultaneous equations[1]. The accuracy of results as well as the limit of detection will be determined primarily by the constancy and the small value of the phosphorescence background. In this manuscript the background was determined by the impurities in the ethanol and the pickup of phosphorescence impurities on the glassware used for solution preparation. In an actual application as in the analysis of drugs in blood, urine and other biological fluids, the background will be determined by the phosphorescent impurities extracted from the biological fluid as well as by those listed above. Biological fluids cannot be analyzed directly by phosphorimetry because of the problem of solvent cracking[4,12], and therefore the compound of interest must be removed before analysis by liquid–liquid extraction or by a similar technique[12]. LATZ[12] has shown that the phosphorescence background resulting when blood is extracted from an acidic or basic solution is not much greater than that due only to the phosphorescent impurities in the solvent (E.P.A. in that case—the purified ethanol used in this paper had a slightly smaller phosphorescence background than the E.P.A. used by LATZ). LATZ[12] also showed that the phosphorescence background resulting when urine is extracted with ether at pH values above 5 is no greater than the background

resulting from blood extraction. However, at low pH values the phosphorescence background from urine is considerably larger. The low background from the extract of the biological fluid, the great sensitivity and selectivity of analysis and the speed and ease of performing the analysis should certainly result in a number of useful clinical procedures. The authors are now in the process of analyzing blood for several of the drugs listed in Table I. These results will be presented at a later date. Only studies of the latter nature will determine the exact advantages and disadvantages of phosphorimetry compared to other analytical methods.

This research was carried out as a part of a study on the phosphorimetric analysis of drugs in blood and urine, supported by a grant from the U. S. Public Health Service (GM 11373-01). The authors would also like to thank Professor P. A. FOOTE, Dean of the College of Pharmacy, for the donation of small amounts of a number of the drugs used in this study.

SUMMARY

Phosphorescence excitation and emission spectral peaks, lifetimes, working curves and limits of detection of 22 organic compounds of pharmacological importance in rigid (77°K) ethanolic solution are given. Ethanol can be easily prepared in a high degree of purity, and most drugs are much more soluble in ethanol than in most solvents previously used for phosphorimetric studies. The possible application of phosphorimetry to the trace analysis of drugs in biological fluids is discussed.

RÉSUMÉ

Les auteurs ont effectué une recherche phosphorimétrique sur 22 composés organiques présentant un intérêt pharmacologique, en utilisant des solutions éthanoliques rigides (77°K). L'éthanol peut en effet être obtenu à un haut degré de pureté; d'autre part, la plupart des drogues y sont plus solubles que dans de nombreux solvants précédemment utilisés pour des études phosphorimétriques. On examine également les possibilités d'application de cette méthode pour le dosage de traces de drogues dans des liquides biologiques.

ZUSAMMENFASSUNG

In unterkühlter (77°K) äthanolischer Lösung werden Phosphoreszenzanregung, Emissionsspektrallinien, Lebensdauer, Arbeitskurven und Nachweisgrenzen von 22 pharmakologisch wichtigen organischen Verbindungen angegeben. Äthanol kann leicht in hoher Reinheit hergestellt werden, und die meisten Arzneimittel sind in Äthanol besser löslich als in den meisten Lösungsmitteln, die bisher für phosphorimetrische Untersuchungen benutzt wurden. Eine mögliche Anwendung der Phosphorimetrie auf die Spurenanalyse von Medikamenten in biologischen Flüssigkeiten wird diskutiert.

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THE USE OF 2,6-DICHLOROPHENOLINDOPHENOL AS INDICATOR IN ACID–BASE TITRATIONS

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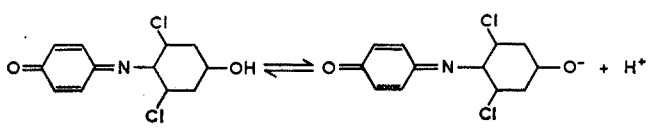
2,6-Dichlorophenolindophenol is widely used as a titrant for the determination of ascorbic acid. It can be used also as an indicator for a number of titrations with ascorbic acid as a titrant[1–3]. As has been pointed out already[3], the oxidized form of the indicator changes its colour according to the pH of the solution. In acidic medium the colour is red, while in alkaline medium it changes to blue. The colour change is very sharp and easy to observe. Thus 2,6-dichlorophenolindophenol has been examined as an acid–base indicator.

Indicator exponent

The colour change of the indicator is probably due to the splitting off of hydrogen ions:



acidic form (red) alkaline form (blue)

To establish the exact pH value of the colour change, a 1% aqueous solution of the indicator was prepared and its absorption spectra were measured in acidic and in alkaline medium. The spectra are shown in Fig. 1; the essential difference between

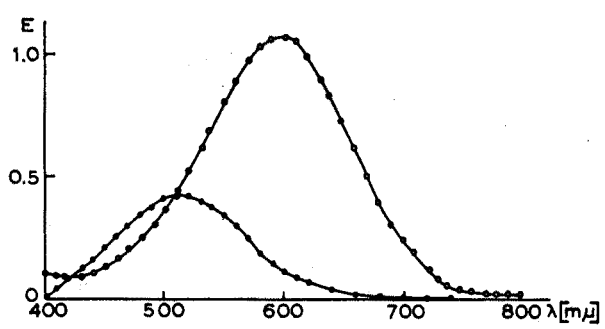


Fig. 1. Absorption spectrum of 2,6-dichlorophenolindophenol (0.02 g/l): ●–●, in acidic solution (pH 4.0); o–o, in alkaline solution (pH 6.5).

the two curves is the difference of absorbance at a wavelength of 600 mμ. In a further series of experiments solutions were prepared containing the same amounts of the indicator at various pH values, and the optical densities of the peak at 600 mμ were measured. The obtained results are to be seen on Fig. 2. The obtained curve is similar

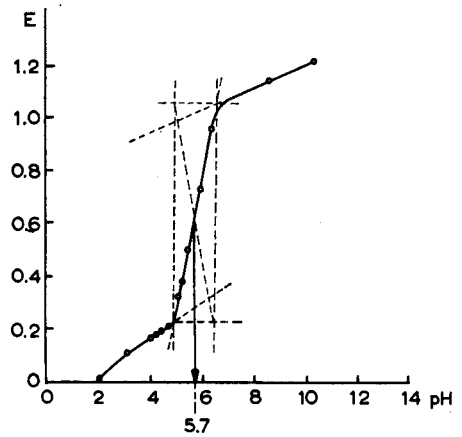


Fig. 2. Variation of optical density of 2,6-dichlorophenolindophenol at 600 mμ with pH.

to a polarographic wave, and can be evaluated for the transitional pH value, similarly to the method of determination of half-wave potentials. This method shows that the transitional pH value is 5.7, thus the indicator serves for the same purposes as methyl red. It must be emphasized, however, that the colour change of 2,6-dichlorophenolindophenol is much sharper than that of methyl red, and therefore the standard deviations obtained by titrations using the proposed indicator are generally lower than those obtained with methyl red.

Effect of neutral salts

It is generally known that some indicators (especially those which change from coloured to colourless) are sensitive to the neutral salt concentration of the solution. To investigate whether this is true also for 2,6-dichlorophenolindophenol, titrations of 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid were carried out in the presence of various amounts of sodium chloride. The results obtained in the titrations with 0.1 *N* acid, are presented in Table I. It can be seen from these results that

TABLE I

EFFECT OF NEUTRAL SALTS

(Titration of 20.00 ml of 0.1 *N* NaOH with 0.1 *N* HCl)

NaCl added (g)	*Number of parallel runs*	*Consumed acid (ml)*	*Note*
0	3	20.00	
1	3	20.00	
2	3	19.98	
6	3	19.97	
10	3	19.76	Unsharp end-point

unless practically saturated salt solutions (10 g in a 40-ml end volume) are used, the end-points are not sensitive to neutral salt concentrations. Entirely analogous results were obtained in the reverse titration.

Effect of alcohol

There are numerous acid–base titrations which are best carried out in the presence of alcohol. Therefore, the effect of added alcohol on the accuracy of the end-point determination was examined. It was found that the presence of very large quantities of alcohol has almost no effect on the accuracy of the determinations. For example, the titration of 20 ml of 0.1 *N* acid with alkali, or *vice versa*, in presence of up to 30 ml of ethanol showed an error of only 0.02 ml.

Effect of temperature

In some cases, acid–base titrations must be carried out at higher temperatures. Some indicators, such as methyl red itself, are not suitable in these cases. To investigate whether 2,6-dichlorophenolindophenol is usable at higher temperatures titrations were carried out with 0.1 *N* hydrochloric acid as well as with 0.1 *N* sodium hydroxide at various temperatures. It was found that 2,6-dichlorophenolindophenol can be used up to 80° with no diminution in accuracy.

EXPERIMENTAL

Reagents

2,6-Dichlorophenolindophenol indicator. A filtered aqueous 0.1% solution of the reagent was used. It was, however, advantageous to prepare a ground mixture with pure sodium chloride in a 1 : 500 (w/w) ratio; about 0.2–0.5 g of the solid mixture was used for each titration.

Acid and alkali standard solutions. These were prepared from analytically pure reagents. They were standardized by the usual methods, using methyl red as indicator.

The absorption spectra of the indicator were obtained with a Hilger Uvispec spectrophotometer using 1-cm silica cells.

Procedures

For various acid–base titrations using 2,6-dichlorophenolindophenol as indicator, the following procedures were worked out.

Determination of alkali and alkaline earth hydroxides and ammonium hydroxide. To the solution to be determined, add some drops of the 2,6-dichlorophenolindophenol solution (or 0.2–0.5 g of the ground mixture) and titrate with 0.1 or 0.01 *N* hydrochloric acid until the bluish red transitional colour of the indicator appears.

Determination of strong acids. To the sample solution add indicator as above and titrate with 0.1 or 0.01 *N* sodium hydroxide to the bluish red transition point.

Determination of alkali carbonates. To the sample solution add indicator as above and titrate with 0.1 or 0.01 *N* hydrochloric acid to the bluish red colour. Heat the solution to boiling, boil out carbon dioxide, cool the solution and finish the titration.

Determination of alkali tetraborate. To the sample solution, add indicator as above and titrate the solution with 0.1 or 0.01 *N* hydrochloric acid until the bluish red colour appears.

TABLE II

ACCURACY AND PRECISION OF THE METHODS

Titrated	*Titrant*	*Deviation from the value*		*Standard deviation*		*Standard deviation of the true*	
		ml	%	*ml*	%	*ml*	%
20 ml 0.1 *N* NaOH	0.1 *N* HCl	−0.04	−0.2	±0.03	±0.16	±0.01	±0.05
10 ml 0.1 *N* NaOH	0.1 *N* HCl	−0.02	−0.2				
5 ml 0.1 *N* NaOH	0.1 *N* HCl	+0.04	+0.8				
20 ml 0.01 *N* NaOH	0.01 *N* HCl	+0.22	+1.1	±0.08	±0.38	±0.03	±0.17
10 ml 0.01 *N* NaOH	0.01 *N* HCl	−0.10	−1.0				
5 ml 0.01 *N* NaOH	0.01 *N* HCl	−0.05	−1.0				
20 ml 0.1 *N* KOH	0.1 *N* HCl	−0.01	−0.05	±0.02	±0.12	±0.07	±0.04
10 ml 0.1 *N* KOH	0.1 *N* HCl	+0.02	+0.2				
5 ml 0.1 *N* KOH	0.1 *N* HCl	0.00	0.00				
20 ml 0.01 *N* KOH	0.01 *N* HCl	−0.12	−0.6	±0.08	±0.38	±0.02	±0.11
10 ml 0.01 *N* KOH	0.01 *N* HCl	−0.07	−0.7				
5 ml 0.01 *N* KOH	0.01 *N* HCl	−0.09	−1.8				
20 ml sat. $Ca(OH)_2$	0.01 *N* HCl	−0.02	−0.1	±0.06	±0.31	±0.03	±0.13
10 ml sat. $Ca(OH)_2$	0.01 *N* HCl	−0.14	−1.4				
5 ml sat. $Ca(OH)_2$	0.01 *N* HCl	−0.02	−0.4				
20 ml 0.1 *N* NH_4OH	0.1 *N* HCl	+0.19	+0.95	±0.21	±1.06	±0.06	±0.33
10 ml 0.1 *N* NH_4OH	0.1 *N* HCl	+0.11	+1.21				
5 ml 0.1 *N* NH_4OH	0.1 *N* HCl	+0.22	+4.4				
20 ml 0.01 *N* NH_4OH	0.01 *N* HCl	+0.17	+0.85	±0.15	±0.72	±0.06	±0.30
10 ml 0.01 *N* NH_4OH	0.01 *N* HCl	+0.01	+0.10				
5 ml 0.01 *N* NH_4OH	0.01 *N* HCl	−0.43	−8.6				
20 ml 0.1 *N* $Na_2B_4O_7$	0.1 *N* HCl	−0.09	−0.4	±0.01	±0.07	±0.004	±0.02
10 ml 0.1 *N* $Na_2B_4O_7$	0.1 *N* HCl	0.00	0.00				
5 ml 0.1 *N* $Na_2B_4O_7$	0.1 *N* HCl	+0.03	+0.6				
20 ml 0.01 *N* $Na_2B_4O_7$	0.01 *N* HCl	−0.31	−1.5	±0.36	±1.7	±0.13	±0.60
10 ml 0.01 *N* $Na_2B_4O_7$	0.01 *N* HCl	−0.17	−1.7				
5 ml 0.01 *N* $Na_2B_4O_7$	0.01 *N* HCl	−0.15	−3.0				
20 ml 0.1 *N* Na_2CO_3	0.1 *N* HCl	−0.01	−0.05	±0.02	±0.11	±0.006	±0.03
10 ml 0.1 *N* Na_2CO_3	0.1 *N* HCl	+0.05	+0.05				
5 ml 0.1 *N* Na_2CO_3	0.1 *N* HCl	−0.01	−0.2				
20 ml 0.01 *N* Na_2CO_3	0.01 *N* HCl	−0.17	−0.85	±0.05	±0.26	±0.02	±0.10
10 ml 0.01 *N* Na_2CO_3	0.01 *N* HCl	−0.14	−1.4				
5 ml 0.01 *N* Na_2CO_3	0.01 *N* HCl	−0.07	−1.4				
20 ml 0.1 *N* HCl	0.1 *N* NaOH	+0.01	−0.05	±0.03	±0.17	±0.01	±0.05
10 ml 0.1 *N* HCl	0.1 *N* NaOH	+0.06	+0.6				
5 ml 0.1 *N* HCl	0.1 *N* NaOH	+0.05	+1.0				
20 ml 0.01 *N* HCl	0.01 *N* NaOH	+0.20	+1.0	±0.10	±0.50	±0.04	±0.20
10 ml 0.01 *N* HCl	0.01 *N* NaOH	+0.10	+1.0				
5 ml 0.01 *N* HCl	0.01 *N* NaOH	+0.16	+3.5				
20 ml 0.1 *N* H_2SO_4	0.1 *N* NaOH	+0.04	+0.2	±0.02	±0.07	±0.004	±0.02
10 ml 0.1 *N* H_2SO_4	0.1 *N* NaOH	+0.02	+0.2				
5 ml 0.1 *N* H_2SO_4	0.1 *N* NaOH	+0.02	+0.4				
20 ml 0.01 *N* H_2SO_4	0.01 *N* NaOH	+0.15	+0.7	±0.08	±0.40	±0.03	±0.16
10 ml 0.01 *N* H_2SO_4	0.01 *N* NaOH	+0.18	+1.8				
5 ml 0.01 *N* H_2SO_4	0.01 *N* NaOH	+0.16	+3.2				
20 ml 0.1 *N* HNO_3	0.1 *N* NaOH	−0.04	−0.2	±0.03	±0.16	±0.009	±0.05
10 ml 0.1 *N* HNO_3	0.1 *N* NaOH	+0.04	+0.4				
5 ml 0.1 *N* HNO_3	0.1 *N* NaOH	+0.11	+2.2				
20 ml 0.01 *N* HNO_3	0.01 *N* NaOH	+0.18	+0.9	±0.14	±0.71	±0.06	±0.29
10 ml 0.01 *N* HNO_3	0.01 *N* NaOH	+0.13	+1.3				
5 ml 0.01 *N* HNO_3	0.01 *N* NaOH	+0.12	+2.4				

RESULTS

The accuracies and precisions obtainable by these methods are summarized in Table II. It is apparent from these data that 2,6-dichlorophenolindophenol can be used as an indicator in acid–base titrations with quite satisfactory results.

SUMMARY

2,6-Dichlorophenolindophenol shows acid–base indicator properties. Its colour is red in acidic media and blue in alkaline media. The transitional pH value is 5.7 and the colour change is sharp and easy to observe. The use of the indicator in various acid–base titrations, as well as the effects of neutral salts, alcohol and temperatures, were checked. The absorption spectra of the acidic and basic forms of the indicator are presented.

RÉSUMÉ

Le 2,6-dichlorophénolindophénol est proposé comme indicateur acide–base: rouge en milieu acide et bleu en milieu alcalin (pH de virage: 5.7). Les auteurs l'ont utilisé pour divers titrages acides–bases et ils ont examiné l'influence de sels neutres, de l'alcool et de la température.

ZUSAMMENFASSUNG

2,6-Dichlorophenol-indophenol zeigt Eigenschaften eines Säure–basenindikators. Seine Farbe ist rot im sauren und blau im alkalischen Medium. Der Umschlag erfolgt beim pH-Wert von 5.7 mit scharfem Farbwechsel und ist leicht zu beobachten. Die Anwendung des Indikators bei verschiedenen Säure–Basen-Titrationen als auch die Einflüsse von Neutralsalzen, Alkohol und der Temperatur wurden kontrolliert. Die Absorptionsspektren des Indikators werden angegeben in seiner sauren und alkalischen Form.

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THE DETERMINATION OF THE STABILITY CONSTANTS OF THE LANTHANIDE α-HYDROXYISOBUTYRATE AND LACTATE COMPLEXES BY POTENTIOMETRIC TITRATION

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The α-hydroxyisobutyrate and lactate ions are known to form stable soluble complexes with the lanthanides. CHOPPIN AND CHOPOORIAN[1] reported values of the stability constants for the first 3 complexes of 9 lanthanide elements and yttrium. However, their, as well as other, investigations on the same subject[2,3] provided evidence that the isobutyrate and lactate anions form uninegative tetra-ligand complexes with the lanthanides. It was also shown by EECKHAUT *et al.*[4] that other α-hydroxycarboxylate ligands as methylethyl- and methylpropyl glycolate form analogous complexes of the type ML_4^- (M = trivalent lanthanide ion; L = organic ligand).

In the work described here, the determination of the 4 stability constants of the lanthanides and yttrium has been performed potentiometrically, using a glass–calomel electrode system and the CALVIN–WILSON titration method[5], while CHOPPIN AND CHOPOORIAN used quinhydrone electrodes and FRONAEUS' titration technique[6].

The calculation of $\bar{n}$, the average number of L^- bound to M^{3+}, has been carried out by a modification of the method used by CALVIN AND WILSON[5] for M^{2+} and complexes ML_2.$[L^-]$ has been calculated from the apparent dissociation constant of the acid[7].

The stability constants are derived graphically from corresponding $\bar{n}$ and $[L^-]$ values by BJERRUM's half-$\bar{n}$ method[8], FRONAEUS'[6] and POULSEN'S[9] extrapolation methods, and by means of a computer program, using the IBM 1620 digital computer[4,10].

EXPERIMENTAL

Reagents

Lactic acid, analytical grade from BDH, was dedimerized by completely neutralizing with sodium hydroxide and then removing the sodium ions with a Dowex 50 column in the hydrogen form. The lactic acid used gave a negative dimer test. Other reagents were the same as in a previous investigation[4].

Apparatus and procedure

A Radiometer pH M-4C potentiometer with a G 202 B glass electrode and a K 100

saturated calomel electrode were used. The glass electrode was standardized against NBS standard buffers at 25°.

All titrations and pH measurements were made in a thermostated sample cell at 25.0 ± 0.1°. Rapid mixing was achieved by magnetic stirring which was stopped during the measurements.

Titration series of the perchlorates of the lanthanides were performed once in 25 ml of 0.1 *M* α-hydroxyisobutyric acid and 2 ml of 0.320 *M* perchloric acid (in the presence of 0.2 *M* sodium perchlorate) with 1.910 *M* sodium hydroxide solution. The metal–acid ratio was 1/20. Other titration series were carried out in 25 ml of 0.3 *M* α-hydroxyisobutyric acid (lactic acid) and 2 ml of 1.960 *M* perchloric acid (also in the presence of 0.2 *M* sodium perchlorate), with 2.981 *M* sodium hydroxide solution. The metal–acid ratio was 1/45.

Each titration series was at least duplicated.

RESULTS

FRONAEUS[6] has proved that when formation of complexes of the type M_yL_n ($y > 1$) occurs, the formation curve is dependent on the metal ion concentration. Preliminary experiments at different metal ion concentration for some lanthanide–α-hydroxyisobutyrate complex systems showed that polynuclear complexes are not present in measurable amounts. This agrees with similar observations of CHOPPIN AND CHOPOORIAN[1], and SONESSON for the lanthanide–glycolate complexes[11].

Special attention was also paid to the effect of ionic strength. The results for yttrium and ytterbium, using increasing amounts of sodium perchlorate, are listed in Tables I and II. The total ionic strength (column 2) was calculated from the con-

TABLE I

STABILITY CONSTANTS OF THE YTTRIUM–α-HYDROXYISOBUTYRATE COMPLEXES AS A FUNCTION OF THE IONIC STRENGTH

Mol $NaClO_4$	I_{total}	$\log K_1$	$\log K_2$	$\log K_3$	$\log K_4$	$\log \beta_4$
0[a]	0.075	3.21	2.60	2.00	1.39	9.20
0[b]	0.081	3.21	2.595	1.98	1.375	9.16
0[c]	0.091	3.23	2.60	1.97	1.34	9.14
0.05[a]	0.119	3.13	2.55	1.97	1.40	9.05
0.05[b]	0.126	3.13	2.55	1.96	1.38	9.02
0.1[b]	0.170	3.11	2.50	1.87	1.25	8.73
0.1[c]	0.179	3.13	2.48	1.84	1.19	8.64
0.2[a]	0.252	3.12	2.47	1.83	1.19	8.61
0.2[b]	0.262	3.14	2.43	1.79	1.24	8.60
0.2[c]	0.273	3.13	2.42	1.80	1.16	8.51
0.5[a]	0.520	3.06	2.49	1.86	1.27	8.68
0.5[b]	0.525	3.03	2.41	1.79	1.18	8.41
1.0[a]	0.964	3.09	2.46	1.83	1.20	8.58
1.0[c]	0.973	3.06	2.41	1.78	1.13	8.38
2.0[b]	1.861	3.11	2.43	1.74	1.06	8.34

[a] $C_M/C_{HL} = 1/20$.
[b] $C_M/C_{HL} = 1/10$.
[c] $C_M/C_{HL} = 1/5$.

centration of the different metal salts in solution at pH = pK_A of the acid. By plotting the log β_4 values against the square root of the ionic strength, it can be seen that the stability constants remain approximately constant from an ionic strength of 0.2. All other titrations were performed in 0.2 M sodium perchlorate.

TABLE II

STABILITY CONSTANTS OF THE YTTERBIUM–α-HYDROXYISOBUTYRATE COMPLEXES AS A FUNCTION OF THE IONIC STRENGTH

Mol $NaClO_4$	I_{total}	log K_1	log K_2	log K_3	log K_4	log β_4
0[b]	0.079	3.40	2.83	2.26	1.69	10.18
0.05[a]	0.119	3.26	2.76	2.27	1.77	10.06
0.05[b]	0.123	3.385	2.81	2.235	1.66	10.09
0.1[b]	0.168	3.345	2.745	2.14	1.54	9.77
0.2[a]	0.252	3.32	2.73	2.15	1.55	9.75
0.5[a]	0.520	3.30	2.71	2.12	1.53	9.66
0.5[b]	0.523	3.27	2.71	2.14	1.58	9.70

[a] C_M/C_{HL} = 1/20.
[b] C_M/C_{HL} = 1/10.

Some titration curves of the series using 0.3 M α-hydroxyisobutyric acid together with a blank titration in the absence of metal (curve A) are given in Fig. 1.

The stability constants derived from the experimental $\bar{n}$ and [L] values by means of the 4 procedures, are represented in Table III. The results of the lanthanide–lactate complexes are given in Table IV.

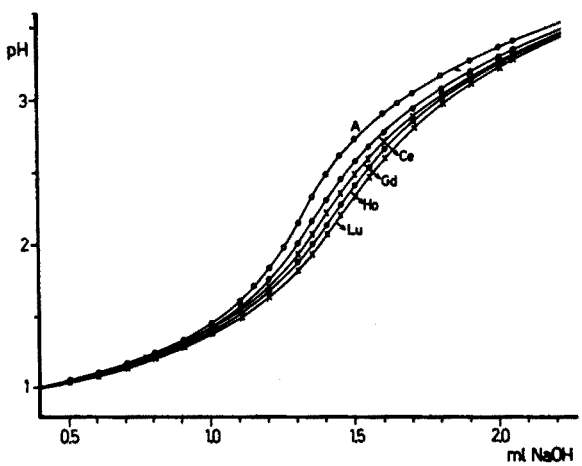

Fig. 1. Titration curves of some lanthanide–α-hydroxyisobutyrate complex systems.

When allowance is made for the differences in titration technique, calculation methods and ionic strength, the results given in Tables III and IV agree well with those obtained by CHOPPIN AND CHOPOORIAN[1] for the first 3 constants. Also the fourth constant log K_4 of the complex ML_4^- is calculated with an acceptable deviation (column 6).

TABLE III

STABILITY CONSTANTS OF THE LANTHANIDE AND YTTRIUM–α-HYDROXYISOBUTYRATE COMPLEX SYSTEMS, DETERMINED BY 3 GRAPHICAL METHODS AND BY COMPUTER CALCULATION FROM THE SAME SETS OF $\bar{n}$ AND $[L^-]$ DATA

Element	*Stability constant*	*Bjerrum*	*Fronaeus*	*Poulsen*	*Computer*
Lu	$\log K_1$	3.38	3.21	3.21	3.18 ± 0.055
	$\log K_2$	2.80	2.71	2.80	2.865 ± 0.06
	$\log K_3$	2.21	2.13	2.40	2.025 ± 0.055
	$\log K_4$	1.62	1.94	1.37	1.92 ± 0.045
	$\log \beta_4$	10.01	9.99	9.78	9.99 ± 0.11
Yb	$\log K_1$	3.32	3.12	3.12	3.13 ± 0.10
	$\log K_2$	2.73	2.70	2.68	2.74 ± 0.12
	$\log K_3$	2.15	2.03	2.20	2.07 ± 0.115
	$\log K_4$	1.55	1.85	—	1.78 ± 0.09
	$\log \beta_4$	9.75	9.70	—	9.72 ± 0.20
Tm	$\log K_1$	3.25	3.08	3.08	3.10 ± 0.08
	$\log K_2$	2.65	2.72	2.68	2.69 ± 0.095
	$\log K_3$	2.04	1.92	2.32	1.92 ± 0.08
	$\log K_4$	1.43	1.75	—	1.62 ± 0.09
	$\log \beta_4$	9.37	9.47	—	9.33 ± 0.19
Er	$\log K_1$	3.20	3.02	3.02	3.01 ± 0.06
	$\log K_2$	2.57	2.69	2.61	2.69 ± 0.075
	$\log K_3$	1.94	1.78	2.23	1.875 ± 0.065
	$\log K_4$	1.32	1.62	1.38	1.45 ± 0.055
	$\log \beta_4$	9.03	9.11	9.24	9.025 ± 0.11
Ho	$\log K_1$	3.18	3.00	3.00	2.98 ± 0.08
	$\log K_2$	2.53	2.58	2.58	2.56 ± 0.09
	$\log K_3$	1.88	2.00	2.17	1.90 ± 0.11
	$\log K_4$	1.23	1.20	—	1.30 ± 0.13
	$\log \beta_4$	8.82	8.78	—	8.74 ± 0.20
Dy	$\log K_1$	3.10	2.95	2.95	2.94 ± 0.07
	$\log K_2$	2.44	2.39	2.52	2.51 ± 0.09
	$\log K_3$	1.80	2.13	2.07	1.84 ± 0.10
	$\log K_4$	1.15	—	—	1.21 ± 0.14
	$\log \beta_4$	8.49	—	—	8.50 ± 0.20
Tb	$\log K_1$	3.10	2.90	2.90	2.92 ± 0.03
	$\log K_2$	2.30	2.32	2.44	2.32 ± 0.02
	$\log K_3$	1.68	1.92	1.88	1.62 ± 0.03
	$\log K_4$	1.00	—	—	1.23 ± 0.03
	$\log \beta_4$	8.08	—	—	8.09 ± 0.04
Gd	$\log K_1$	3.00	2.85	2.85	2.79 ± 0.06
	$\log K_2$	2.21	2.19	2.37	2.19 ± 0.11
	$\log K_3$	1.58	1.90	1.44	1.52 ± 0.22
	$\log K_4$	0.91	—	—	1.15 ± 0.24
	$\log \beta_4$	7.70	—	—	7.65 ± 0.35
Eu	$\log K_1$	2.92	2.82	2.82	2.79 ± 0.035
	$\log K_2$	2.14	2.01	2.27	2.07 ± 0.065
	$\log K_3$	1.56	1.75	1.20	1.48 ± 0.15
	$\log K_4$	0.88	—	—	1.25 ± 0.20
	$\log \beta_4$	7.50	—	—	7.59 ± 0.25
Sm	$\log K_1$	2.87	2.80	2.80	2.75 ± 0.015
	$\log K_2$	2.07	1.98	2.12	2.02 ± 0.03
	$\log K_3$	1.50	1.58	1.19	1.40 ± 0.07
	$\log K_4$	0.84	—	—	1.21 ± 0.10
	$\log \beta_4$	7.28	—	—	7.38 ± 0.14

TABLE III (Continued)

Element	*Stability constant*	*Bjerrum*	*Fronaeus*	*Poulsen*	*Computer*
Nd	$\log K_1$	2.86	2.79	2.79	2.74 ± 0.04
	$\log K_2$	1.95	1.74	2.00	1.68 ± 0.075
	$\log K_3$	1.35	1.17	1.42	1.56 ± 0.10
	$\log K_4$	0.52[a]	—	—	0.60 ± 0.19
	$\log \beta_4$	6.68	—	—	6.58 ± 0.25
Pr	$\log K_1$	2.67	2.64	2.64	2.59 ± 0.015
	$\log K_2$	1.85	1.66	1.62	1.78 ± 0.025
	$\log K_3$	1.25	1.51	1.19	1.23 ± 0.05
	$\log K_4$	0.55[a]	—	—	0.78 ± 0.07
	$\log \beta_4$	6.32	—	—	6.38 ± 0.10
Ce	$\log K_1$	2.60	2.60	2.60	2.55 ± 0.035
	$\log K_2$	1.74	1.41	1.40	1.53 ± 0.09
	$\log K_3$	1.05	1.60	1.48	1.41 ± 0.15
	$\log K_4$	0.41[a]	—	—	—
	$\log \beta_4$	5.80	—	—	5.49[c] ± 0.18
La	$\log K_1$	2.48	2.32	2.32	2.30 ± 0.02
	$\log K_2$	1.60	1.46	1.40	1.74 ± 0.06
	$\log K_3$	0.92	1.52	1.20	—
	$\log K_4$	—	—	—	—
	$\log \beta_4$	5.00[c]	—	—	4.04[b] ± 0.065
Y	$\log K_1$	3.12	2.90	2.90	2.92 ± 0.13
	$\log K_2$	2.47	2.44	2.49	2.70 ± 0.14
	$\log K_3$	1.83	2.04	1.94	1.72 ± 0.17
	$\log K_4$	1.19	—	—	1.44 ± 0.18
	$\log \beta_4$	8.61	—	—	8.78 ± 0.30

[a] Extrapolated value ($\bar{n} > 3$).
[b] $\log \beta_2$.
[c] $\log \beta_3$.

TABLE IV

STABILITY CONSTANTS OF THE LANTHANIDE– AND YTTRIUM–LACTATE COMPLEX SYSTEMS DETERMINED BY THE METHOD OF BJERRUM[a] AND BY COMPUTER CALCULATION[b]

Element		*log K_1*	*log K_2*	*log K_3*	*log K_4*	*log β_4*
Lu	a	3.23	2.38	1.55	0.72	7.88
	b	3.05 ± 0.03	2.51 ± 0.03	1.41 ± 0.03	0.86 ± 0.04	7.83 ± 0.07
Yb	a	3.18	2.31	1.44	0.57	7.50
	b	3.03 ± 0.025	2.42 ± 0.025	1.34 ± 0.03	0.69 ± 0.035	7.48 ± 0.06
Tm	a	3.10	2.25	1.40	0.53	7.28
	b	2.92 ± 0.03	2.34 ± 0.03	1.34 ± 0.035	0.65 ± 0.05	7.25 ± 0.07
Er	a	3.07	2.23	1.40	0.52	7.22
	b	2.86 ± 0.035	2.33 ± 0.03	1.33 ± 0.035	0.66 ± 0.05	7.18 ± 0.075
Ho	a	2.98	2.16	1.33	0.51	6.98
	b	2.73 ± 0.04	2.30 ± 0.05	1.27 ± 0.06	0.67 ± 0.075	6.97 ± 0.10
Dy	a	2.86	2.04	1.24	0.44[c]	6.58
	b	2.59 ± 0.045	2.23 ± 0.045	1.09 ± 0.065	0.63 ± 0.09	6.54 ± 0.15
Tb	a	2.88	2.03	1.18	0.31[c]	6.40
	b	2.65 ± 0.045	2.17 ± 0.05	1.07 ± 0.07	0.44 ± 0.10	6.33 ± 0.16
Gd	a	2.84	1.99	1.15	0.26[c]	6.24
	b	2.57 ± 0.05	2.15 ± 0.05	1.02 ± 0.08	0.47 ± 0.15	6.21 ± 0.17

TABLE IV (Continued)

Element		*log* K_1	*log* K_2	*log* K_3	*log* K_4	*log* β_4
Eu	a	2.83	1.94	1.06	0.17[c]	6.00
	b	2.55 ± 0.05	2.12 ± 0.055	0.88 ± 0.10	0.51 ± 0.18	6.06 ± 0.20
Sm	a	2.81	1.92	1.04	0.13[c]	5.90
	b	2.56 ±0.055	2.04 ± 0.065	0.90 ± 0.12	0.41 ± 0.25	5.91 ± 0.30
Nd	a	2.65	1.79	0.93	0.08[c]	5.45
Pr	a	2.58	1.70	0.85	—	5.13[e]
Ce	a	2.49	1.57	—	—	4.06[d]
La	a	2.44	1.49	—	—	3.93[d]
Y	a	2.92	2.10	1.27	0.43[c]	6.72
	b	2.80 ± 0.06	2.14 ± 0.065	1.22 ± 0.10	0.55 ± 0.14	6.71 ± 0.20

[c] Extrapolated value ($\bar{n} \geqslant 3$).
[d] log β_2.
[e] log β_3.

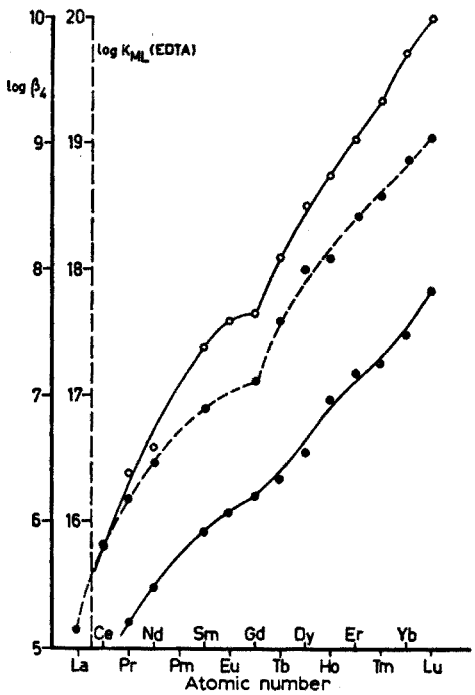

Fig. 2. Log β_4 for the lanthanide–lactate (⊙) and α-hydroxyisobutyrate (○) complex systems, and log K_{ML} for the lanthanide–EDTA (●) complex systems as a function of the atomic number.

The logarithms of the over-all stability constants of the lanthanides with α-hydroxyisobutyrate and lactate ligands were plotted against the atomic number (Fig. 2). The log K_{ML} values of the ethylenediaminetetraacetate–lanthanide complexes found by SCHWARZENBACH and co-workers[12,13], are also given in Fig. 2. The cation-exchange elution data give the same pattern[14], showing that the separation is mainly determined by the ratio of the stability constants of the complexes involved.

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SUMMARY

The stability constants of the lanthanide and yttrium complexes with α-hydroxyisobutyrate and lactate, have been determined by potentiometric titration. The average number of ligands bound per metal ion has been calculated by the method of CALVIN–WILSON and indicates the formation of uninegative tetra-ligand complexes of the form ML_4^-. The 4 formation constants have been derived by 4 procedures: BJERRUM's half-$\bar{n}$-method, FRONAEUS' and POULSEN's extrapolation methods, and by least squares calculation, using an IBM 1620 digital computer.

RÉSUMÉ

Les auteurs ont déterminé par titrage potentiométrique les constantes de stabilité de complexes de lanthanides et de l'yttrium (α-hydroxyisobutyrates et lactates). Les calculs effectués par la méthode de CALVIN–WILSON indiquent la formation de complexes du type ML_4^-.

ZUSAMMENFASSUNG

Die Stabilitätskonstanten von Komplexen der Lanthanide und des Yttriums mit α-Hydroxyisobutyrat und Lactat wurden mittels potentiometrischer Titration bestimmt. Die mittlere Zahl der Liganden die pro Metallion gebunden werden, wurde nach der Methode von CALVIN–WILSON berechnet und die Bildung von ML_4^- festgestellt. Die Bildungskonstanten wurden nach 4 verschiedenen Verfahren ermittelt.

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A NON-DISTILLATION KJELDAHL METHOD FOR NITROGEN BASED ON PRECIPITATION AS TETRAPHENYLBORATE*

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Organic nitrogen (especially in proteins) is often determined by some variation of the standard Kjeldahl procedure; some variations, which avoid the distillation step, have been recommended. The more successful of these usually fall into 2 general categories. The first involves a titration with a standardized solution, the ammonia being reacted with substances like formaldehyde or hypobromite ion. Many procedures in this category require careful temperature and pH control, or some other difficult step, although some authors[1] claim that the hypobromite method is straightforward. The second category involves the formation of a color by ammonia with Nessler's reagent, ninhydrin, phenol or similar substances. Subsequent measurement of the intensity is often complicated by the non-reproducibility and fading of the color on standing. The accuracy has been improved by extraction of the color before measurement[2,3], and constancy in the microgram region obtained for the phenol–hypochlorite reaction[4], but the extra steps lengthen the time required to obtain final results. The *Annual Reviews of Analytical Chemistry* provide comprehensive surveys of this subject, the most recent being that of SMITH *et al.*[5].

Previously we had found[6] that ammonium ion could be determined gravimetrically by precipitation as ammonium tetraphenylborate, $NH_4B(C_6H_5)_4$ or simply NH_4TPB. The gravimetric factor for nitrogen is the favorably low value of only 0.04154, and no distillation equipment or standardized solution is necessary. Since organic nitrogen is converted quantitatively to ammonium ion during digestion with hot sulfuric acid in the Kjeldahl procedure, the possibility of a gravimetric determination of nitrogen was indicated. However, mercury(II), which is a preferred catalyst in Kjeldahl procedures, would interfere seriously by reacting with TPB ion in a non-stoichiometric manner. WITTIG *et al.*[7] suggested that the reaction with mercuric(II) chloride may be formulated:

$$B(C_6H_5)_4^- + 4\ HgCl_2 + 3\ H_2O \rightarrow 4\ C_6H_5HgCl + 3\ H^+ + 4\ Cl^- + H_3BO_3$$

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and MONTEQUI[8] established that a second reaction occurs to a partial degree:

$$B(C_6H_5)_4^- + 2\ HgCl_2 + 3\ H_2O \rightarrow 2\ (C_6H_5)_2Hg + 3\ H^+ + 4\ Cl^- + H_3BO_3$$

Thus, because the composition of the mercury-containing precipitate is variable, its weight cannot be subtracted from the total to give the weight of ammonium-TPB. Also, it was found[9] that TPB ion gives precipitates with potassium, rubidium, cesium, silver, thallium and protonated amine ions. Of these, only potassium is sometimes found in biological samples and fortunately the formation of potassium-TPB is stoichiometric. So, by running aliquot samples both with and without volatilization of the ammonia, the nitrogen contents can be evaluated.

In this paper a method for eliminating interference from the mercury catalyst is recommended. This method involved complexation of the mercury as tetraiodomercurate(II) ion at a pH of about 2, followed by precipitation of ammonium-TPB in the resulting solution. The results obtained compared favorably with those of 2 longer procedures, which involved removal of HgI_4^{2-} by anion-exchange frontal chromatography or of HgS via filtration, before precipitation of NH_4TPB. Also, for comparative purposes, some results were obtained by the usual distillation procedure.

EXPERIMENTAL

Reagents

Unless otherwise stated the water was either purified by distillation or deionization, and always boiled shortly before use to render it free of ammonia. The inorganic solids used were always of analytical-reagent purity, and it was especially important that their potassium contents be nil.

The organic compounds used for procedure testing, with their accompanying specifications were as follows: acetanilide (m.p. 115–117°) from Eastman Chemicals; glycine (d.p. 234–235°) 99.5% minimum assay from Matheson, Coleman and Bell; L(+)-glutamic acid (d.p. 224–225°) and DL-lysine monohydrochloride (d.p. 233–235.5°) both from Nutritional Biochemicals Corp., Cleveland.

The anion-exchange resins used were either Dowex 1-X8 (50–100 mesh) or Amberlite CG-400 (100–200 mesh) both of the strongly basic type. The exchange tubes were 15–20 cm long and 10–12 mm diameter.

Sodium tetraphenylborate (TPB) was J. T. Baker's reagent grade (99.7% assay) used without further purification. Aqueous solutions (prepared by weighing and diluting) were clarified with aluminum oxide and filtered as described by GLOSS AND OLSON[10]. The solution was stable for several weeks if stored in the refrigerator, and did not require refiltering before use.

Mercuric sulfate catalyst was prepared by dissolving 50 g of mercuric(II) oxide in 250 ml of 6 *N* sulfuric acid with heating, followed by dilution to 500 ml with ammonia-free water.

The saturated wash solution was prepared similarly to that of FLASCHKA[11] by dissolving about 500 mg of ammonium-TPB and 300 mg of potassium-TPB in a few drops of acetone, and then adding slowly with stirring about 2 l of water. After equilibration, the mixture was filtered.

Complexation procedures

(*a*) *Method 1* (recommended method). Transfer a dry, weighed sample containing 0.1–0.5 mequiv of organic nitrogen to a small Kjeldahl flask. Add *ca.* 0.25 g of

sodium sulfate, 0.3 ml of mercuric(II) sulfate solution and 1–1.5 ml of concentrated sulfuric acid. Add the liquids down the sides to insure that all sample is washed to the bottom. Heat the mixture gently with a low flame under a hood until frothing ceases. Gradually increase the flame, taking care to avoid spattering and to keep the flame below the liquid surface, so as not to volatilize (burn) any dry solid with loss of nitrogen. After all the black carbon has disappeared, boil the solution. Incipient boiling for 20 min (to 30 min for refractory substances) was usually found sufficient.

After cooling, dissolve the resulting digest in a minimum of freshly boiled (ammonia-free) water, and transfer it quantitatively to a 50- or 100-ml Erlenmeyer flask or beaker. Add a 2 *M* sodium iodide solution dropwise with swirling until the red mercury precipitate dissolves completely, and then a 1-ml excess to complex the mercury quantitatively. Dilute the solution with water to 60–70 ml and mix well.

Add 3 drops of 3 *M* sodium acetate, and adjust to pH 2–3 with 2 *N* sodium hydroxide or 2 *N* sulfuric acid, as required, using either methyl orange or a pH meter as indicator. Pour a volume of 0.05–0.08 *M* sodium tetraphenylborate solution, sufficient to provide a 25–50% excess of TPB, slowly down the side of the vessel with stirring. Allow the resulting white precipitate to stand for 15–30 min at room temperature, and then filter quantitatively through a previously weighed glass suction crucible (medium porosity). Test the first portion of the filtrate for complete precipitation by addition of a small volume of dilute ammonium sulfate. If cloudiness results, then complete precipitation of ammonium is indicated. Wash the precipitate with 2 or 3 20-ml portions of the special wash solution. Dry the precipitate for 3–6 h at 50–60° under vacuum. Since indicator is adsorbed on the precipitate, its dryness is evident by the fading of color. For precipitates larger than 75 mg, it is advisable to rewash until the weight loss between successive weighings is less than 0.3 mg.

Before calculating the nitrogen content, it is necessary to deduct any potassium-TPB or other precipitatable impurities in the NH_4TPB precipitate. To do so, dissolve all, or a known portion of the precipitate, in a minimum (10 ml or less) of ethylene glycol, add 2 pellets of sodium hydroxide, and warm gently on a hot plate until all ammonia has escaped. About 10 min of heating should suffice to give a negative litmus test. Add a 6-fold excess of water to the cool solution with swirling, let the cloudy precipitate (KTPB), if any, stand for 15–20 min, filter through a weighed glass crucible, wash with 5–10 ml of saturated wash solution, and dry to constant weight at 60°. Subtract the weight (adjusted for the portion taken) of KTPB residue from that for the total precipitate to give the weight of NH_4TPB.

The blank value of KTPB to be subtracted, may be obtained alternatively as follows. Digest a duplicate sample of the organic nitrogen-containing substance and complex the mercury as before, or more simply use an aliquot of the previous eluant. Add 2 drops of phenolphthalein and pellets of sodium hydroxide (one in excess of neutralization). Warm the alkaline solution cautiously until all ammonia has been expelled (litmus test), adding water to keep the volume near 25 ml. Adjust the pH to 2–3 with dilute sulfuric acid, using methyl orange or a pH meter, and if necessary add more iodide. Add 5–10 ml of TPB reagent to the clear solution (filter if necessary). If any precipitate forms after standing for 20 min, filter, wash, dry and weigh as before. Subtract the weight, adjusted for the size of the aliquot, from the total to give the corrected weight of NH_4TPB. Multiplication by the gravimetric factor, 0.04154, gives the weight of nitrogen present.

(*b*) *Method 2.* This involves the same procedure as method 1 through the addition of excess sodium iodide. Then dilute the solution in a volumetric flask and pass through an anion-exchange resin in the acetate form. After discard of the forerun, take a suitable aliquot of the eluate. Next, add sodium acetate, adjust the solution to pH 2–3 and follow the procedure given for method 1.

(*c*) *Sulfide method* (Method 3). This employs the same initial steps as method 1 through the dissolution of the digest in ammonia-free water. Then, instead of adding sodium iodide, partially neutralize with dilute sodium hydroxide to incipient formation of cloudy mercuric oxide, and bubble hydrogen sulfide into the solution to precipitate mercuric sulfide. Expel excess hydrogen sulfide by warming on a steam bath and remove the mercuric sulfide by atmospheric filtration. Collect the filtrate plus washings in a 100-ml volumetric flask. Neutralize an aliquot (25-ml) to methyl orange (pH about 3) with dilute sodium hydroxide, and add a 25% excess of TPB reagent. After standing for 1 h filter the precipitate, wash and dry at 60° as before.

(*d*) *Distillation method.* To a 25-ml aliquot of a Kjeldahl digest, add 6 *N* sodium hydroxide via the funnel in the all-glass distillation assembly until the phenolphthalein (red) color change, and add 1 ml in excess. Then add 1 ml of 1 *M* sodium sulfide to free the ammonia from mercury complexes. Steam-distil the ammonia into 20 ml of 4% boric acid solution until the final volume is around 75 ml. Add 2 drops of methyl purple to the distillate and titrate with standard 0.05 *N* sulfuric acid to the first appearance of a green color, or else potentiometrically with a pH meter and glass-calomel electrodes.

RESULTS AND DISCUSSION

Data presented in Tables I and II make possible a comparison of the 2 proposed non-distillation TPB methods (1 and 3) for nitrogen with the usual distillation method. The recommended method (1) requires one less time-consuming step than the other two, because the mercury catalyst is left in solution as tetraiodomercurate(II) during

TABLE I

COMPARISON OF RECOMMENDED METHOD 1[a] AND SULFIDE METHOD 3[b] FOR DETERMINATION OF NITROGEN AS AMMONIUM-TPB[c]

Nitrogen taken (mequiv/aliquot)[d]	*No. of aliquots*	*Ammonium-TPB (Theory, mg)*	*Ammonium-TPB found (% recovery)*	
			Recommended method	*Method 3*
0.3867	3	130.40	100.0–100.7	101.8–103.2
0.3932	3	132.62	101.3–102.5	99.8–101.0
0.4010	3	134.94	99.9–100.4	101.5–102.8
0.4303	3	145.15	101.5–103.2	99.6–102.3
0.4349	3	146.69	101.1–105.2	99.8–100.3
		Average	101.4%	101.3%
		Standard deviation	1.5	1.1

[a] Method 1 (recommended) involved complexing mercury in solution as tetraiodomercurate(II). Blanks averaged 3.42 ± 0.13 mg.

[b] Method 3 involved removal of mercury as mercuric sulfide. Blanks averaged 0.11 ± 0.06 mg.

[c] Ammonium sulfate was used as test material. All precipitates were produced with 25% excess TPB at pH 2.

[d] 25-ml (one-tenth) aliquots were precipitated in all cases.

TABLE II

COMPARISON OF RECOMMENDED METHOD 1 WITH NORMAL DISTILLATION METHOD FOR NITROGEN AS AMMONIA

Nitrogen taken (mequiv/aliquot)	*No. of aliquots*	*Ammonium-TPB found by Method 1 (% recovery)*	*Ammonia distilled and titrated (% recovery)*
Acetanilide[a]			
0.3761	4	99.3–100.8	96.0– 98.6
0.3875	3	99.8–101.0	96.4–100.5
0.4019	4	101.0–103.9	94.6– 97.6
0.4542	4	101.6–104.0	97.4– 99.5
0.4854	4	100.0–104.8	97.3– 98.8
0.5179	4	99.6–102.3	100.5–101.5
0.5413	4	98.0–102.3	97.9–101.0
	Average	101.3%	98.3%
	Standard deviation	1.7	1.9
Glycine			
0.3979[a]	3	101.9–103.1	97.9– 98.2
0.4089[a]	3	101.6–104.6	96.9–100.8
0.6530[a]	3	101.4–104.3	97.2– 98.3
0.7007[a]	3	103.8–106.2	97.7– 98.3
0.4797–0.8888[b]	9	99.1–100.4	96.0–100.1
	Average	101.8%	98.3%
	Standard deviation	2.3	1.2
L(+)-Glutamic acid			
0.4131[a]	3	100.8–102.2	95.9– 99.6
0.4235[a]	3	100.5–105.4	97.5– 99.3
0.5395[a]	3	100.6–100.9	98.6–100.3
0.5421[a]	3	99.8–101.4	98.9– 99.8
0.4232–0.6843[b]	16	100.0–102.0	98.8–101.4
	Average	101.2%	99.4%
	Standard deviation	1.1	1.5

[a] 25-ml aliquots (one-tenth portions) were used for comparison.
[b] 25-ml aliquots (one-fourth portions) were used for comparison.

precipitation of ammonium-TPB. The results in Table I for ammonium sulfate are just as accurate and nearly as precise by method 1 as by method 3 (average 101.4% recovery and std. dev. 1.5 *vs.* 101.3% and 1.1). As expected, the blank values were somewhat higher and more variable by method 1 than by method 3 when the mercury was removed as sulfide by the extra filtration step (3.42 ± 0.13 mg *vs.* 0.11 ± 0.06 mg). The results in Table II indicate that, for acetanilide, method 1 was slightly more accurate (higher values) and precise than the usual distillation method in our hands (101.3% recovery and std. dev. 1.7 *vs.* 98.3% and 1.9). The results for 2 amino acids (glycine and glutamic acid) are higher and slightly less accurate but just as precise by method 1 as by the distillation procedure.

In Table III a larger number (59) of results obtained with standard powdered blood samples by 3 different TPB methods are given, for comparison with the usual distillation one. In method 2, the mercury is complexed with excess iodide, as in the recommended method 1, but it is then removed by anion-exchange chromatography

TABLE III

COMPARISON OF 3 TETRAPHENYLBORATE METHODS AND A DISTILLATION METHOD FOR THE DETERMINATION OF NITROGEN IN BLOOD SAMPLES

Nitrogen in sample[a] *(%)*	*Recovery as ammonium tetraphenylborate precipitate*			*Recovery by distillation*[e] *(% recovery)*
	Method 1[b] *(Range (mg) and % recovery)*	*Method 2*[c] *(Range (mg) and % recovery)*	*Method 3*[d] *(Range (mg) and % recovery)*	
1.882	101.2–128.3(3) 98.6–104.3	62.6– 99.8(3) 98.9–103.6		102.3–103.7(2)[f]
2.584	44.2– 83.1(6) 98.0–103.4		35.8– 90.8(4)[f] 99.2–103.1	
3.35	62.7– 91.1(3) 98.8–101.3	51.2–134.7(7) 99.0–102.9	73.8–112.0(2) 100.6–101.2	96.0–103.2(4)
4.07	101.0–222.8(6) 97.0–102.5	67.9–156.7(15) 97.8–103.9	69.0–131.1(9) 99.2–100.8	97.3–104.1(9)
4.65	95.2–169.1(6) 100.2–103.2	88.0–161.7(7) 97.8–101.9		96.2(1)
6.72	126.3–270.3(6) 98.1–101.6	142.8–265.1(6) 97.4–103.7	73.9–192.4(5) 98.2–102.6	97.6–103.0(4)
7.24	84.4–237.3(4) 98.9– 99.7	33.1–335.7(12) 96.6–103.8		96.1–104.2(10)
11.90	128.7–365.2(5) 98.4–102.5	249.4–438.1(9) 98.0–103.3		97.4–102.8(6)
Average	100.5%	100.4%	100.4%	99.9%
Standard deviation	1.2	1.8	1.8	2.5

[a] Dried animal blood samples and percentages were provided by Thorn Smith Laboratories (Royal Oak, Michigan).
[b] In Method 1 (recommended method), the mercury was left in solution as the complex.
[c] In Method 2, mercury was removed by anion-exchange resin.
[d] In Method 3, the mercury was removed as mercuric sulfide.
[e] Distillation into boric acid followed by titration with acid to methyl purple end-point.
[f] The figures in brackets indicate the number of replicate determinations.

before precipitation of ammonium-TPB. The samples were used after drying over calcium chloride at room temperature. Because of the low nitrogen contents (2–12%) and the use of aliquoting (one-fourth) all sample weights could be kept above 100 mg. Thorn Smith provides nitrogen percentages, obtained by the usual Kjeldahl distillation followed by titration with standard sulfuric acid to a methyl red end-point. These values are listed in Table III as theoretical values. Although the average of our ammonia recovery values by distillation agrees quite well (99.9% recovery) with Thorn Smith's, it is felt that the extensive variation among results (std. dev. 2.50) is due partly to the inhomogeneity and deliquescent nature of the samples, especially the 11.90% one. Also Thorn Smith recommends that some samples be used without drying and others be dried at 100°. This may help to explain some of the variations recorded by PARSONS *et al.*[12], who used these blood samples in evaluating a method for nitrogen utilizing gas chromatography. The precision by the 3 non-distillation TPB methods was considerably better than 2.50, because the extremely low gravimetric factor (0.04154) magnified the final weight of precipitate compared to that of the original sample. As before, results slightly higher than theory (100.4–100.5) were obtained by all TPB methods, and method 3 gave the best precision (std. dev. of

1.2). The precision shown was about the same (std. dev. 1.8) by both the recommended method and method 2, but the expected range and 99% confidence limit by method 2 was slightly better, and the blanks were lower (averaging 1.62 mg). In some instances, when the quantities of TPB precipitates weighed more than 200 mg, rather high results were obtained by the recommended method 1, and were discarded. Also method 2 gave lower, more consistent results and is the preferred procedure when the quantity of complexed mercury in solution is greater than 2.1 mg/ml. If bromide is used as complexing agent instead of iodide, the concentration of mercury which can be tolerated in solution is much lower. Low results were often obtained by all TPB procedures, when the weight of precipitate was less than about 35 mg, and it was especially important in these instances to use a minimum volume of wash solution, thoroughly saturated with precipitate. Experiments performed with Dowex 1-X4 and Dowex 1-X8 resins as the tetraiodomercurate remover in method 2 showed only a slightly better efficiency for the more extensively cross-linked 1-X8 resin. The progress of removal can be followed visually, since the adsorbed ion is yellowish brown in color. If the column is packed with coarse resin (100 mesh) on top and fine (200 mesh) in the bottom half, the recommended volume of resin should suffice for the treatment of about 5 samples before it needs to be replaced. It was found quite important that the ionic strength of the conditioning solution for the resin be as close as possible to that of the sample solution for exchange. The eluate should come through colorless.

In the complexation procedures (method 1 or 2) if considerable concentrated sulfuric acid was used in the initial Kjeldahl decomposition step, then the excess acid should be partially neutralized with sodium hydroxide to incipient formation of mercuric oxide, before adding the sodium iodide. Sodium acetate should be added after the iodide but before the sodium-TPB precipitant, to provide a buffering effect and insure that loss of ammonia would not occur by the solution accidentally becoming more alkaline than pH 5. However, the sodium acetate can be eliminated, if caution is observed in the dropwise addition of sodium hydroxide (dilute) solution. If appreciable iron is present in the original sample, it is important that the solution be quite acidic in order to prevent its interference upon addition of TPB solution. RUDORFF AND ZANNIER[13] found that sodium-TPB produced dark precipitates from weakly acidic solutions which contained iron(III). On the other hand, SPOREK AND WILLIAMS[14] as well as WENDLANDT[15] obtained no precipitation on addition of iron to a more acidic sodium-TPB solution. It is not recommended that iron be masked in weakly acidic medium as fluoride or EDTA complex, as has been suggested[16]. It is safer to adjust the sample solution at least as acidic as pH 2.5. We found that as much as 0.75 mg of iron(III) per ml of solution could be tolerated, even when the concentration of excess TPB ion was as great as 0.01 *M*. Some analysts prefer to use copper (which is less efficient) instead of mercury as the Kjeldahl catalyst. We found that as much as 0.90 mg of copper(II) per ml could be tolerated, without producing high results with a 0.01 *M* TPB concentration.

We had found previously[6] that an optimum concentration of excess TPB was 5–6 mequiv per liter of solution, in the absence of interfering ions. Depending on the nitrogen content, this is usually a 25–50% excess. However, if the quantity of tetraiodomercurate left in solution is greater than recommended, an appropriate reduction in the concentration of excess TPB should be made. Although the sodium-

TPB solution should be as concentrated as possible to maintain small volumes, concentrations greater than 0.08 *M* might give high results and require frequent refiltering before use. The total volume of mother liquor should be greater as the quantities of precipitate increase, in order to minimize the coprecipitation of soluble salts. Since the excess TPB reagent tends to deteriorate on long standing and at elevated temperatures, a relatively short standing period at room temperature is recommended before filtration. The precipitate is rather soluble in water, having a molar solubility[6] of $2.9 \cdot 10^{-4}$. Thus, following the practice with potassium-TPB precipitates, it is necessary to wash with water pre-saturated with the precipitate in order to minimize solubility losses. Low temperatures are specified for drying the precipitates because ammonium-TPB begins to decompose at 130°. Alternatives for drying at 60° under vacuum for 3 h are 100° for 1 h or 65° for 15–17 h at atmospheric pressure. Fortunately the dry residue exhibits no hygroscopic tendency, even in a humid atmosphere.

Since some biological samples may contain potassium as well as nitrogen, 4 methods were investigated extensively for evaluating the amount of ammonium-TPB in the mixed precipitate; or the quantity of potassium-TPB to be subtracted as a blank. The first was a method by RUDORFF AND ZANNIER[17] involving the addition of formaldehyde to prevent the precipitation of ammonia from 1 *N* alkaline solution. Our weights of potassium-TPB precipitated in the range 100–300 mg averaged about 1.7% (relative) low. The second method proposed by FLASCHKA *et al.*[18] involved the ignition of a mixture of ammonium- and potassium-TPB. The former volatilizes and the latter changes into potassium metaborate, which is neutralized with excess standard acid and then titrated with base. The method proved rather difficult to follow, and our results for nitrogen were about 2.8% (relative) low and those for potassium were about 3.2% high. The third approach tried was KOHLER'S[19], in which the mixed TPB precipitate is dissolved in a solution of acetone, followed by addition of aqueous sodium hydroxide. Upon heating, ammonia is volatilized, and addition of water reprecipitates the potassium-TPB. We obtained erratic results, mainly because acetone was a poor solvent, and proved to be too volatile. Several other possible solvents were investigated and ethylene glycol was found to be very satisfactory for both TPB and sodium hydroxide. Experiments were performed to measure the stability of TPB ion to hot, alkaline glycol solution. Weighed quantities (100–200 mg) of ammonium-TPB were heated for 20 min (more than sufficient to expel all ammonia) with 10 ml of glycol just under the boiling point, followed by cooling, dilution with 30 ml of water and addition of excess ammonium chloride. The TPB ion proved quite stable to this treatment, an average of 83% remaining, which is sufficient to precipitate a considerable amount of potassium quantitatively. To determine the optimum ratio of water to glycol for minimum loss of potassium-TPB due to solubility, the U.V.-absorption of TPB ion at 266 mμ was measured; the minimum loss of 0.14 mg/ml occurred at a ratio of 6:1. At a ratio of 3:1, the loss was 0.21 mg/ml. Of course, the excess TPB present in actual test samples considerably reduces this value. Most of our test runs gave low values for potassium-TPB (about 1.0 mg) and correspondingly high values for ammonia. The last (fourth) method involved simply addition of a slight excess of sodium hydroxide to an aliquot portion of the digest, and then cautious heating until all ammonia was expelled. Addition of TPB precipitates the potassium remaining in the aqueous solution. Results of test samples fell within $\pm 0.8\%$ relative.

Because sodium-TPB is expensive, analysts may wish to recover the reagent from the ammonium- and potassium-TPB precipitates. An ion-exchange conversion of potassium-TPB from aqueous acetone solutions has been published by REIMERS[20]. A recovery of better than 90% of sodium-TPB, after recrystallization from acetone, was claimed. Unfortunately we were not able to apply this method to the exchange of ammonium for sodium, mainly because the presence of even small amounts of water in the acetone threw the ammonium-TPB out of solution, and the presence of water was necessary in order to realize a reasonable conversion yield. Of various alkaline treatments investigated, best results were obtained with a variation of WITTIG's procedure[21] in which ammonium-TPB was heated in methanol with sodium methoxide just under the boiling point at 400 mm pressure for several hours, followed by recrystallization from acetone or chloroform. Yields of crude product as high as 60% were obtained.

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SUMMARY

A simple, accurate method for the determination of protein nitrogen by a modification of the Kjeldahl procedure, eliminating the distillation step and weighing ammonium tetraphenylborate (TPB) precipitate directly, is described. The gravimetric factor is favorable, and no standard solutions are required. Interference from mercury catalyst was prevented by conversion to the soluble tetraiodomercurate(II) complex (method 1), removal of the tetraiodomercurate via anion-exchange resin (method 2), or removal of mercury as mercuric sulfide (method 3). These 3 TPB methods gave results as accurate and precise as the usual distillation approach. Values by method 1, averaged 100.5% recovery with a standard deviation of 1.8; and by method 2, which is used when large concentrations of mercury are present, they averaged 100.4% with 1.8 std. dev.

RÉSUMÉ

Les auteurs proposent une méthode simple et exacte pour le dosage de l'azote des protéines. Elle consiste en une modification du procédé de Kjeldahl, sans distillation, avec pesée du tétraphénylborate d'ammonium, directement précipité. Le facteur gravimétrique est favorable; on évite l'emploi de solutions titrées.

ZUSAMMENFASSUNG

Es wird eine einfache, genaue Methode zur Bestimmung des Stickstoffs im Protein beschrieben, bei der ein modifiziertes Kjeldahl-Verfahren angewandt wird. Anstelle der Destillation mit anschliessender Titration wird Ammoniumtetraphenylborat, das einen günstigen gravimetrischen Faktor besitzt, gefällt und gewogen. Es werden verschiedene Methoden angegeben, um Störungen durch das als Katalysator verwendete Quecksilber zu vermeiden.

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GRAVIMETRIC DETERMINATION OF TUNGSTEN(VI) WITH N-BENZOYL-N-PHENYLHYDROXYLAMINE

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Several organic reagents have been used for the gravimetric determination of tungsten[1-3] and its separation from other elements but their scope is limited, especially in the separation and determination of tungsten in presence of molybdenum.

Benzoylphenylhydroxylamine has been employed by SHOME[4], and subsequently by other workers[5,6], as an organic precipitant for various metal ions. In the present investigation it was shown that tungsten can be determined by direct weighing of the metal complex with benzoylphenylhydroxylamine and can be separated from molybdenum, vanadium, titanium and iron, by precipitating the foreign metals with benzoylphenylhydroxylamine in acid solution in presence of tartrate ions. Tungsten is then precipitated from the filtrate by further addition of the organic reagent followed by increasing the acidity of the solution.

When an alcoholic solution of benzoylphenylhydroxylamine is added to a solution of sodium tungstate and then acidified a yellow-coloured precipitate is formed; on digestion on a boiling water bath, this changes to a white crystalline precipitate. The yellow precipitate is stable and partially soluble in alcohol and chloroform, and is of indefinite composition. The white precipitate is, however, completely soluble in 95% alcohol and chloroform; analysis showed its composition to be: $WO_2(C_{13}H_{10}O_2N)_2$. The decomposition temperature of the white complex is 194–195°. It is interesting to note that when tungsten is precipitated in presence of tartrate ions, the white complex is formed even in the cold.

EXPERIMENTAL

Reagents

Tungsten solution. Standard tungsten solutions were prepared by dissolving weighed amounts of sodium tungstate in water and determining the tungsten contents by the oxine method.

Benzoylphenylhydroxylamine. A 3% solution of the organic reagent in 95% ethanol was used.

Foreign ions. Standard solutions of ammonium molybdate, sodium vanadate, uranyl nitrate, titanium sulphate and ferric ammonium sulphate were employed in the study of separations.

Sodium potassium tartrate solution. A 10% solution of sodium potassium tartrate was used for masking tungsten during the precipitation of foreign metals.

All the reagents used were of A.R. grade.

Determination of tungsten

A solution containing a known amount of tungsten was diluted to 200 ml and heated to 80°; 10 ml of the organic reagent was added dropwise with stirring and the yellow-coloured complex was precipitated when the acidity of the solution was adjusted to 0.1 *N* with 3 *N* hydrochloric acid. The white crystalline complex obtained on digestion of the yellow precipitate over a boiling water bath was allowed to settle, filtered on a glass sintered crucible, washed with hot water at 60° (containing 2 ml of 3 *N* hydrochloric acid per 100 ml of wash solution), dried at 115° and weighed to constant weight. The metal content was calculated on the basis that the precipitate contained 28.72% of tungsten.

In another series of experiments the precipitate was filtered through a quantitative filter paper, washed, ignited at 900° and weighed as WO_3. The results are given in Table I.

Effect of reagent concentration. The precipitation of tungsten was carried out using different amounts of the reagent, the other conditions remaining the same. For complete precipitation of the metal the least amount of the reagent required was about thrice the theoretical amount.

Effect of acid concentration. Solutions containing tungsten(VI) and benzoylphenyl-

TABLE I

DETERMINATION OF TUNGSTEN AS OXIDE OR BY DIRECT WEIGHING

W (taken) (mg)	*Wt. of WO_3 (mg)*	*Wt. of precipitate (mg)*	*W (found) (mg)*	*Error (mg)*
27.0	34.0	—	26.92	—0.08
54.0	67.8	—	53.76	—0.24
35.0	44.0	—	34.89	—0.11
27.0	—	93.2	26.76	—0.24
54.0	—	187.4	53.8	—0.2
27.0	—	94.2	27.06	+0.06
35.0	—	121.7	34.94	—0.06
35.0	—	121.8	34.94	—0.06
34.0	—	117.6	33.78	—0.12
34.0	—	119.0	34.18	+0.18

TABLE II

ESTIMATION OF TUNGSTEN IN PRESENCE OF TARTRATE
(10 ml of 10% sodium tartrate)

HCl (6N) added (ml)	*W (taken) (mg)*	*Wt. of precipitate (mg)*	*W (found) (mg)*	*Error (mg)*
10	35.0	119.0	34.18	—0.82
10	35.0	117.0	33.7	—1.3
15	35.0	121.0	34.6	—0.4
20	35.0	122.0	35.04	+0.04
25	35.0	121.8	34.8	—0.2

hydroxylamine (thrice the theoretical amount) were adjusted to different acid concentrations with 3 *N* hydrochloric acid. The precipitates were filtered, washed, and dried to constant weight. The precipitation of tungsten was complete when the acid concentrations of the solutions were between 0.05 *N* and 1 *N* with respect to hydrochloric acid.

Determination of tungsten(VI) in presence of tartrate ions

The precipitation of tungsten with benzoylphenylhydroxylamine was conducted in presence of various amounts of sodium tartrate. It was observed that in presence of tartrate the precipitation of tungsten started in 0.12 *N* acid solution and was complete when the acidity was increased to 0.5 *N* with respect to hydrochloric acid (Table II).

The following procedure was developed for the determination of tungsten in presence of tartrate. Known amounts of tungstate and 10 ml of 10% sodium tartrate solution were mixed and diluted to 200 ml; 0.3 g of benzoylphenylhydroxylamine (dissolved in 20 ml of ethanol) was then added with stirring and the acidity was adjusted to 0.5–1 *N* with 6 *N* hydrochloric aid. The precipitate formed was stirred well, allowed to settle, heated over a boiling water bath for 1 h, filtered, washed and weighed as before.

Separation of tungsten from molybdenum, vanadium, titanium and iron

Since in presence of tartrate tungsten does not form a precipitate with benzoylphenylhydroxylamine when the acid concentration is less than 0.12 *N*, the metal can be easily separated from molybdenum, vanadium, titanium or iron by the prior precipitation of these ions with the above reagent from solutions of low acidity. Tungsten

TABLE III

SEPARATION OF TUNGSTEN FROM MOLYBDENUM, TITANIUM, VANADIUM, IRON OR URANIUM

Foreign ion added (mg)	*W (taken) (mg)*	*Wt. of precipitate (mg)*	*W (found) (mg)*	*Error (mg)*
Mo^{6+} 33	17.0	59.6	17.12	+0.12
22	17.0	58.7	16.86	—0.14
22	34.0	118.8	34.13	+0.13
11	34.0	119.0	34.14	+0.14
11	35.0	121.0	34.75	—0.25
V^{5+} 18	35.0	122.0	35.04	+0.04
9	34.0	119.0	34.18	+0.18
27.0	35	121.0	34.75	—0.25
Ti^{4+} 10.0	34.0	118.6	34.07	+0.07
15.0	34.0	118.8	34.14	+0.14
10.0	17.0	59.0	16.94	—0.06
Fe^{3+} 12.0	17.0	59.2	17.00	—0.06
18.0	17.0	59.4	17.06	+0.06
12.0	35.0	121.0	34.75	—0.25
UO_2^{2+} 31.0	38.0	132.0	37.91	—0.09
62.0	38.0	131.5	37.77	—0.13
31.0	35.0	122.0	35.04	+0.04

can be subsequently precipitated from the filtrate containing the tartrate ions as described above.

Procedure. A solution containing a known amount of tungsten and the foreign ions was diluted to 200 ml and 10 ml of 10 % sodium tartrate was added. Titanium or vanadium was precipitated from cold solution with benzoylphenylhydroxylamine at pH 1 to 1.5, allowed to settle, filtered and washed. Molybdenum or iron was precipitated from hot solution. The pH for molybdenum was adjusted to 1 to 1.5 and that of iron at 4. The precipitates were allowed to settle over hot water bath (80°), filtered and washed. In all the above cases, the wash liquid contained 1% (w/v) of the reagent and the acidity of the wash liquid was the same as that of the solution from which the precipitation was carried out.

The filtrate and the washings were then concentrated to about 200 ml and filtered if any organic matter appeared during the prolonged boiling. Tungsten was then precipitated in cold by further addition of the reagent as described before.

As uranium is not precipitated with benzoylphenylhydroxylamine below pH 3 the metal does not interfere with the estimation of tungsten with benzoylphenylhydroxylamine. The results of the separations are given in Table III.

Interferences. Since chromium(VI) oxidises the organic reagent in acid solutions, tungsten cannot be determined in presence of this ion; fluoride and phosphate ions prevent the complete precipitation of tungsten.

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SUMMARY

Tungsten can be quantitatively precipitated with N-benzoylphenyl-N-hydroxylamine in presence of hydrochloric acid (0.5–1 *N*). The precipitate can be weighed as $WO_2(C_{13}H_{10}O_2N)_2$ or WO_3. Molybdenum, vanadium, titanium, and iron can be separated by prior precipitation of these metals with the above reagent in presence of tartrate ions; tungsten is then determined in the filtrate. Uranium does not interfere, but chromium(VI), fluoride and phosphate do.

RÉSUMÉ

Le tungstène peut être précipité quantitativement avec la N-benzoylphénylhydroxylamine, en présence d'acide chlorhydrique. Le précipité est sous forme de $WO_2(C_{13}H_{10}O_2N)_2$ ou de WO_3. Mo, V, Ti et Fe peuvent être séparés par précipitation préalable avec ce réactif, en présence d'acide tartrique. Le tungstène est ensuite dosé dans le filtrat. L'uranium ne gêne pas; mais chrome(VI), fluorure et phosphate gênent.

ZUSAMMENFASSUNG

Wolfram lässt sich quantitativ mit N-Benzoyl-phenylhydroxylamin in Gegenwart von Salzsäure (0.5 bis 1*N*) fällen. Der Niederschlag kann als $WO_2(C_{13}H_{10}O_2N)_2$ oder WO_3 gewogen werden. Molybdän, Vanadin, Titan und Eisen können durch vorhergehende Fällung mit dem genannten Reagens in Gegenwart von Tartrationen abgetrennt werden; Wolfram wird anschliessend im Filtrat bestimmt. Uran stört nicht, jedoch Chrom(VI), Fluorid und Phosphat.

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AN EMPIRICAL CORRELATION OF TWO METHODS FOR PHENOLS IN CIGARETTE SMOKE

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The gas-chromatographic method for the determination of phenol in cigarette smoke suggested by HOFFMANN AND WYNDER[1] is complicated and lengthy, and 200 cigarettes are required per determination. By using a flame ionization detector in the gas chromatograph, trapping on Cambridge filter pads as described by WARTMAN *et al.*[2] and by using a less complicated clean-up procedure based on that of BURGAN as reported by ROE *et al.*[3], it was possible to determine phenol, *o*-cresol and *m*- + *p*-cresol in smoke from 12 filtered or 8 non-filtered cigarettes. Phenol-^{14}C was added to the extract as an internal standard. The loss of radioactivity was used to correct for losses of phenol during concentration. We found that the percentage loss of substituted phenols as internal standards was different from that of phenol.

A colorimetric procedure, presented by LORENTZEN AND NEURATH[4] at the Third World Tobacco Scientific Congress in Salisbury, Southern Rhodesia, was adapted to the steam-distillate from the Cambridge filter pads. This method proved simple enough for routine determination of phenols in cigarette smoke. However, a number of phenols react with the 4-aminoantipyrene reagent to form colored products. Some selectivity was achieved by extraction of the products into chloroform followed by spectrophotometric analysis, but the method still gave a "phenol number" which included *o*-cresol, *m*-cresol, guaiacol, 2,5-xylenol, and 2,6-xylenol as well as phenol. Phenol, however, has the highest absorptivity and is present in smoke in the highest concentration, so that all calculations were based on the absorptivity of pure phenol.

A correlation was made of the cigarette smoke results of the gas-chromatographic procedure for phenol with those of the colorimetric method for certain phenols and excellent agreement was found.

The total particulate matter, phenols, and nicotine in smoke can all be determined on the same cigarettes with the methods described here. The Cambridge filter pads can first be weighed to determine total particulate matter in the smoke. The phenols can then be distilled from the pads in an acidic medium, followed by distillation of the nicotine in an alkaline medium.

EXPERIMENTAL

Gas chromatographic procedure

Reagents

Freshly distilled phenol, *o*-cresol, *m*-cresol, *p*-cresol, and phenol-^{14}C.

Scintillating solution: 4 g of 2,5-diphenyloxazole and 50 mg of *p*-bis[2-(5-phenyloxazolyl)]benzene per liter of toluene (Pilot Chemicals Inc., Watertown, Mass.).

Apparatus

Automatic smoking machine[5]; Griffith still[6]; F & M 609 Flame Ionization Gas Chromatograph with 1 mV recorder and 3/16″ × 9′ copper column packed with 10% (w/w) Castorwax on 60–80 mesh Chromosorb W; Model LSC-10 Tracerlab Liquid Scintillation Counter.

Procedure

Cigarettes were smoked on the automatic smoking machine under standard conditions (a 35-ml puff of 2-sec duration at 1-min intervals) to a 25-mm butt length. The phenols from 6 filtered or 4 non-filtered cigarettes were trapped on Cambridge filter pads. The pads were immediately sealed in rubber-stoppered test tubes to prevent loss of phenol. Two filter pads were combined in a Griffith still, 5 ml of 0.2 *N* hydrochloric acid were added and the phenols were steam-distilled. 250 ml of distillate was collected. 55 mμC of phenol-^{14}C (specific activity = 16.6 mμC/μg phenol) was added to the distillate.

Two ml of 2 *N* sodium hydroxide was added to the distillate which was then extracted with 100 ml of anhydrous diethyl ether. The aqueous layer was acidified with concentrated hydrochloric acid, then neutralized with solid sodium bicarbonate, and saturated with sodium chloride. The aqueous layer was extracted with 3 50-ml portions of anhydrous diethyl ether. The combined ether extract was dried over

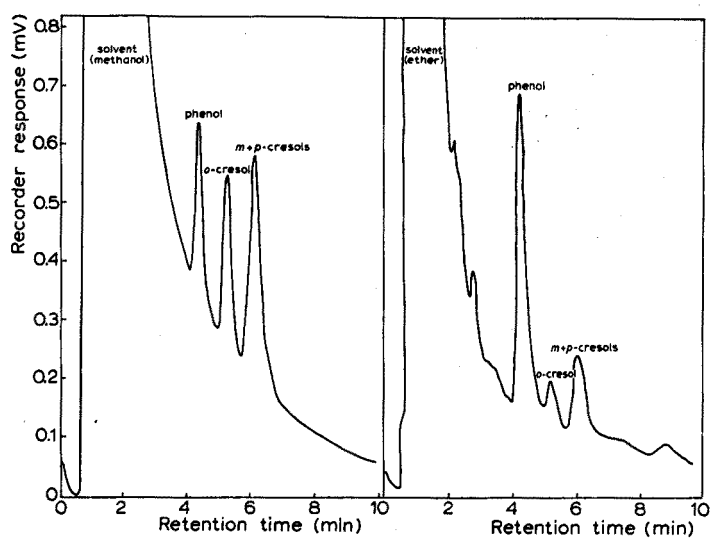

Fig. 1. Typical chromatograms of standard phenols (left) and phenols from smoke (right).

anhydrous magnesium sulphate, filtered after 2 h, cooled in a refrigerator and rapidly evaporated to 10 ml at room temperature by blowing dry nitrogen gas over the solutions.

A 5-μl aliquot was chromatographed on the F & M 609 Flame Ionization Gas Chromatograph. The column temperature used was 175° (isothermal), the injection port temperature was 275°, the detector block temperature was 310°, and the nitrogen carrier gas flow was 35 ml/min.

A typical chromatogram of phenols in cigarette smoke is shown in Fig. 1. Standards of phenol, *o*-cresol, *m*-cresol and *p*-cresol were prepared to contain 0.01–0.1 μg/μl of each compound in methanol. It was necessary to use methanol in the preparation of standard solutions rather than diethyl ether so that the standards would be stable over a period of several weeks. However, it was found that the net peak heights of the phenols in either solvent were the same. A linear relationship between net peak height

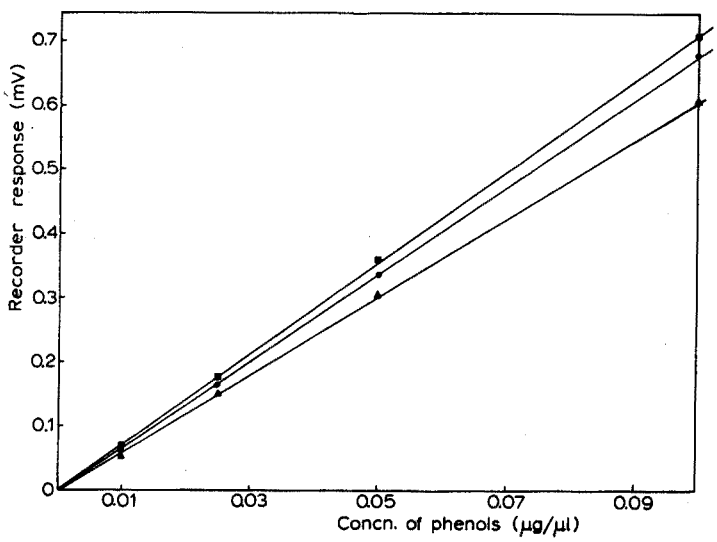

Fig. 2. Calibration curve for phenol by the gas-chromatographic procedure. Peak height (recorder response in mV) is plotted *vs.* concentration of each phenol (μg) per μl of solution. ● Phenol, ▲ *o*-cresol, ■ *m*- + *p*-cresol.

and concentration was obtained (Fig. 2). However, the peak heights increased during the first part of each day so that 5 μl of a standard was run before the chromatographing of each of 2 consecutive samples. The ratio of peak heights was used to calculate the amount of phenol, *o*-cresol, and *m*- + *p*-cresol in the sample.

Recovery correction

A Model LSC-10 Tracerlab Liquid Scintillation Counter was used for the determination of phenol recovery. A 200-μl aliquot of the sample solution was added to 20 ml of scintillating solution. Counting efficiency of each sample was determined by adding a

toluene-^{14}C internal standard. The phenol recovery was determined by comparing the sample activity with the activity for an aliquot of the spiking solution taken at the time of spiking. The recovery data were used to correct each result. The average recovery was 85%.

Colorimetric procedure

Reagents

4-Aminoantipyrene: aqueous 3% solution (Matheson, Coleman and Bell).
Potassium ferricyanide: aqueous 10% solution (Baker).
Chloroform: reagent grade (Mallinckrodt).

Apparatus

Cary Model 11 Spectrophotometer.

Procedure

LORENTZEN AND NEURATH's colorimetric procedure for phenols was adapted for use with the steam distillate of the Cambridge filter pads. Cigarettes were smoked and filter pads steam-distilled as described above, except that only 4 cigarettes were smoked and only a single filter pad was used for each distillation.

A 100-ml aliquot of the distillate was pipetted into a 250-ml separatory funnel for the smoke from filtered cigarettes. For the smoke from non-filtered cigarettes a 50-ml aliquot plus 50 ml of distilled water was used. Three-tenths ml of 3 *N* ammonia solution was added and the solution was mixed by swirling; 0.7 ml of 3% 4-aminoantipyrene was then added and the solution was swirled; 0.7 ml of 10% $K_3Fe(CN)_6$ was added again followed by swirling. The order in which the reagents were added was critical. The red colored product was extracted with 4 10-ml portions of chloroform. The chloroform extracts were filtered through a small wad of cotton into a 50-ml volumetric flask and made to volume with chloroform. The absorbance at 454 mμ was measured against a blank in the reference beam of the spectrophotometer. The blank was

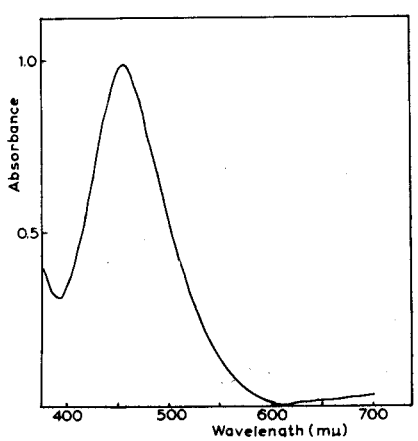

Fig. 3. Typical spectrum of red colored complex formed by the reaction of certain phenols with 4-aminoantipyrene.

treated in the same manner as the sample. The micrograms of phenols/cigarette as phenol, based on a standard curve for pure phenol were calculated.

The absorption spectrum is shown in Fig. 3 and the Beer's law plot in Fig. 4. The absorbance of the developed color conformed to Beer's law in the region of 0–500 μg of phenol per 50 ml of chloroform solution. The color was also found to be stable for at least several hours.

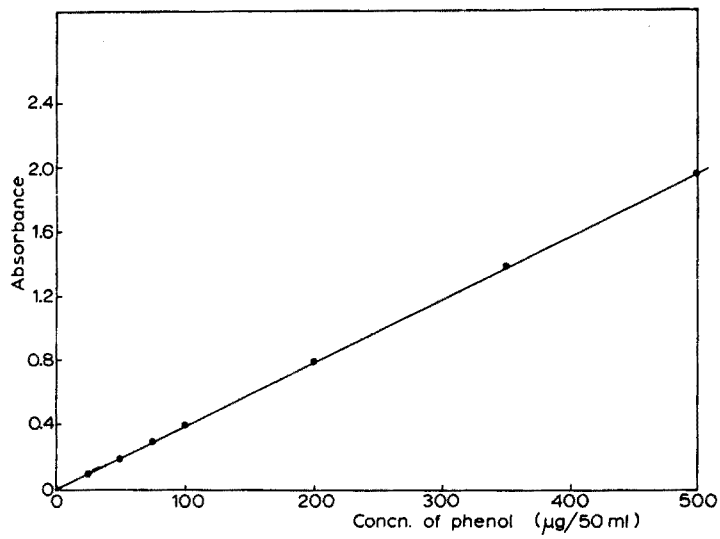

Fig. 4. Calibration curve for phenol by the colorimetric procedure. Absorbance at 454 mμ (using Cary Model 11 Spectrophotometer) is plotted *vs.* concentration of phenol (μg) in 50 ml of $CHCl_3$ solution.

DISCUSSION

It should be mentioned here that the Cambridge filter pads proved to be very efficient phenol collectors. A back-up trap containing cellulose powder and cellulose acetate placed behind the Cambridge filter pad contained less than one μg of phenol per cigarette, or less than 1% of the total phenol delivered, when 85-mm non-filtered cigarettes were smoked. The cellulose–cellulose acetate trap was used in our laboratory during the early part of our investigation and gave trapping efficiencies equal to those obtained with the HOFFMANN AND WYNDER system. There was no loss of phenol-^{14}C from the cellulose–cellulose acetate trap during smoking.

Various amounts of pure phenol from 50 to 250 μg were added to clean Cambridge filter pads, then distilled and analyzed by the colorimetric procedure as described and gave recoveries of 95–105%. Pure phenol at the 200-μg level added to smoke pads gave average recoveries of 101% based on 4 determinations. An average value for phenol in smoke from similar cigarettes was used as a "smoke blank".

There was a 15% loss of phenol if the pads were left out in the open for 20 min. The pads were therefore sealed in test tubes immediately after weighing for TPM and processed as soon as possible.

Correlation of the two procedures

Aliquots (50 ml) of the steam-distillate from filter pads containing the phenols of 12 filtered cigarettes or 8 non-filtered cigarettes were analyzed by the colorimetric procedure. The remaining 200 ml were spiked with phenol-^{14}C and analyzed by the gas-chromatographic procedure. A straight line was obtained from a log–log plot of the micrograms of phenol/cigarette by the gas-chromatographic procedure *vs.* the micrograms of phenols as phenol/cigarette by the colorimetric procedure over the range of 10–300 μg/cigarette. The graph is shown in Fig. 5; the dotted lines represent the 95% confidence limit.

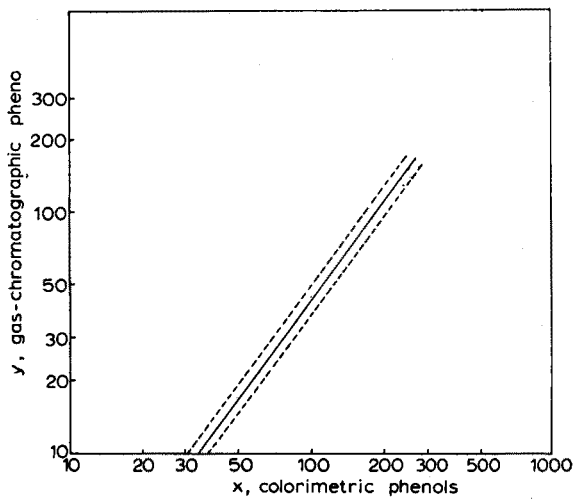

Fig. 5. Correlation of gas-chromatographic phenol (y) *vs.* colorimetric phenols (x).

TABLE I

COLORIMETRIC PHENOLS *vs.* GAS-CHROMATOGRAPHIC PHENOL

Sample	*Colorimetric (µg/cigarette)*	*Gas-chromatographic (µg/cigarette)*
Filtered cigarettes		
Brand A – 80 mm	50.1	17.1
Brand B – 85 mm	42.9	13.8
Brand C – 85 mm	49.4	16.5
Brand D – 85 mm	72.3	29.5
Non-filtered cigarettes		
Brand E – 70 mm	133.0	62.8
Brand F – 85 mm	181.6	98.9

The data shown in Table I were analyzed in an IBM 1620 computer, using a polynomial approximation of the least squares procedure. The equation that was used was:

$$\log y = A_0 + A_1 \log x \pm 2\sigma$$

where y = gas-chromatographic phenol/cigarette and x = colorimetric phenols as phenol/cigarette.

The derived equations for the correlation were:
(*1*) for individual determinations:

$$\log y = -1.05 + 1.34 \log x \pm 0.08$$

and (*2*) for brand averages of 3 to 10 determinations:

$$\log y = -1.07 + 1.35 \log x \pm 0.06$$

The correlation error at the 95% confidence level was 20% for the individual determinations and 15% for the averages of 3–10 determinations. The error by either procedure for individual values was 16% at the 95% confidence level for cigarettes purchased on the open market and randomly selected by brands.

The authors wish to express their appreciation for the technical assistance of Dr. H. D. MERWIN, Messrs. G. SEGURA and J. P. BELL and Mrs. M. CHAVIS.

SUMMARY

A specific gas-chromatographic and a non-specific colorimetric method for the determination of phenols in cigarette smoke are described. The rapid, non-specific method has been correlated with the specific procedure so that the amount of phenol in smoke can be predicted with ±15% at the 95% confidence level. The trapping efficiency of phenols by Cambridge filter pads is also presented.

RÉSUMÉ

Les auteurs décrivent une méthode spécifique par chromatographie gazeuse et une méthode non spécifique par colorimétrie, pour le dosage des phénols dans la fumée de cigarettes. Ces deux procédés ont été utilisés parallèlement et comparés. L'efficacité des filtres Cambridge pour retenir les phénols a été examinée.

ZUSAMMENFASSUNG

Eine spezifische, gaschromatographische und eine unspezifische, kolorimetrische Methode zur Bestimmung von Phenolen im Zigarettenrauch werden beschrieben. Durch Verknüpfung beider Verfahren konnte der Gehalt an Phenol im Rauch mit einem Fehler von ±15% bei einem Vertrauensbereich von 95% bestimmt werden. Die Wirksamkeit von Cambridge-Filtern wird angegeben.

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THE DETERMINATION OF SILICA BY CONWAY'S MICRODIFFUSION TECHNIQUE*

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Silica is traditionally determined in the macro range by direct or indirect gravimetry, or by titrimetry; in the micro range colorimetry is usually employed. In the latter, the problems of interferences, mainly of the inhibiting type, are overcome either by the use of corrective graphs or by a study of each system separately. The results of any of these approaches are more or less dependent on the possible presence of undesirable ions or compounds. Consequently, much effort has been expended to develop separative techniques for silica before its determination. Those most extensively used require considerable prior information on the composition of the sample, and demand manipulative skill and time.

It is the aim of this communication to describe a new approach to the separation of silica and thus to complement the existing methods by one which leads to general simplification.

The volatility of silicon-fluoride compounds was utilized in the development of the proposed method for the determination of silica. Use is made of the principle of diffusion of gases in applying Conway's microdiffusion technique. The addition of hydrofluoric acid to an aqueous acidic silica-containing solution converts the silica to its fluoride compounds; it is assumed that silica volatilizes as silicon tetrafluoride, this view being supported by the investigations of FOX AND JACKSON[1], who studied the factors affecting the volatilization of fluorine by distillation. Furthermore, WHYNES AND DEE[2] report that silicon tetrafluoride is the only form of fluorine found in the gas phase above fluosilicic acid. As an absorbing medium ethylene glycol was chosen because of its favorable absorption characteristics, and its chemical inertness with respect to hydrofluoric acid, silicon tetrafluoride and hydrofluosilicic acid. Its presence did not affect the subsequent colorimetric determination using the molybdenum blue method[3]. Both the reaction and the subsequent absorption of the silicon tetrafluoride were found to be quantitative.

Thus only simple, inexpensive equipment and a minimum of manual operations are required to isolate and recover silica quantitatively.

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In order to establish the stoichiometry and the variables controlling the diffusion of the silicon fluoride species in the Conway technique, the following factors were investigated: (*a*) the media for reaction and absorption; (*b*) the effect of excess of hydrofluoric acid over stoichiometric requirements; (*c*) the rate of diffusion; and (*d*) the temperature.

EXPERIMENTAL

Apparatus

The experiments were carried out in polypropylene microdiffusion cells of 44 mm diameter. These cells (Aloe Scientific Div., Brunswick Corp., St. Louis, Mo., U.S.A.) are a modification of the original Conway unit. Such a cell consists of: (*a*) a closing (outer) compartment which functions as a sealing area; (*b*) a reaction (middle) compartment containing the acidified sample and the reactant; and (*c*) an absorption (inner) compartment containing the absorbing agent. The closing compartment has proved to be particularly useful since this innovation of liquid trap sealing completely excluded the otherwise troublesome possibility of gas leakage. The risk of absorption in this chamber is eliminated as the sealing liquid is the same as that which is used as the reaction medium.

Reagents

Tergitol wetting agent. Aqueous 0.01% solution.

Hydrofluoric acid. 300 μg F^-/ml, kept in a polyethylene container.

Procedure

Place 0.3 ml of pure ethylene glycol into the inner compartment of a polypropylene Conway microdiffusion cell. Since aqueous solutions do not wet polyethylene and the sample solution would thus occupy only a small part of the surface area, add 3 drops of the diluted wetting agent into the reaction compartment. This ensures complete spreading of the sample over the maximum surface area and thus permits more rapid diffusion. Place the sample aliquot—not more than 0.5 ml—containing up to 70 μg of SiO_2 into the reaction compartment. Add at the opposite side of the same compartment 0.5 ml of 80% (w/v) sulphuric acid and 0.4 ml of hydrofluoric acid solution. Close the cell immediately after the addition of hydrofluoric acid. The sealing chamber should contain the 80% sulphuric acid in sufficient quantity (approximately 0.5 ml) for satisfactory sealing conditions. Rotate very carefully to achieve mixing between sample and reagent solutions. Let the diffusion proceed for 6 h at ambient temperature. Transfer quantitatively the contents of the inner compartment by rapid successive water washings using a Pasteur pipet into a 50-ml polystyrene container and develop the molybdenum blue color as described by MULLIN AND RILEY[3]. Transfer to a 50-ml volumetric flask, dilute to volume, and read the transmittance. Carry a blank through all stages of the procedure.

Notes

(*1*) The cleaning of the diffusion cells is carried out by soaking both bottoms and tops in a warm aqueous solution of a commercial detergent for 1 h and then thoroughly rinsing with distilled water.

(*2*) Despite the use of the glass-made Pasteur transfer pipet, no contamination

problems with respect to silica were encountered. It is advisable to avoid prolonged contact with the sample material.

RESULTS AND DISCUSSION

Choice of reaction and absorption media

Perchloric and sulphuric acids were investigated as reaction media from which gaseous diffusion, after addition of hydrofluoric acid, takes place. Perchloric acid led to unsatisfactory results both at elevated and ambient temperatures. Sulphuric acid, on the other hand, permitted quantitative diffusion even at ambient temperature and was, therefore, used in all further work.

Two reagents were examined as possible absorbing media: aqueous sodium hydroxide and ethylene glycol. Sodium hydroxide was not found suitable because the heat of reaction (and possibly of the ambient surroundings) caused evaporation of water from the small volume in the inner chamber and thus conditions for absorbing the silicon tetrafluoride were far from satisfactory.

Ethylene glycol absorbs silicon tetrafluoride and hydrofluoric acid strongly, is chemically inert with respect to them and was found to be a satisfactory absorbent. Moreover, it did not interfere in the subsequent colorimetric determination. Experimental results are summarized in Table I.

TABLE I

EFFICIENCY OF REACTION AND ABSORPTION MEDIA[a]

Reaction medium[b]	*Absorption medium*	*Temperature* (°)	SiO_2 *added* (μg)	SiO_2 *found* (μg)	% *Recovery*
$HClO_4$	1.3 *N* NaOH	60	20	0	0
$HClO_4$	1.3 *N* NaOH	25	20	0	0
H_2SO_4	1.3 *N* NaOH	25	20	0	0
H_2SO_4	1.3 *N* NaOH	25	20	12	60.0
H_2SO_4	Ethylene glycol	25	20	20	100
H_2SO_4	Ethylene glycol	25	50	48.3	96.5
H_2SO_4	Ethylene glycol	25	70	71.8	102.5
$HClO_4$	Ethylene glycol	25	20	16.5	82.5
$HClO_4$	Ethylene glycol	60	50	39	78.0
$HClO_4$	Ethylene glycol	25	70	70	100

[a] Diffusion time was 6 h in all experiments.
[b] The concentration of perchloric acid was 72% and that of sulphuric acid 80% in all experiments.

Effect of excess of hydrofluoric acid

A large excess of hydrofluoric acid is known to inhibit the color development of molybdenum blue. The possible effect of its presence in the reaction compartment up to 200% over stoichiometric requirements was therefore investigated. Sulphuric acid was used as reaction medium and ethylene glycol as absorbent. The quantitative silica recoveries obtained (see Table II) show that the presence of fluoride within the above limit had no detrimental effects upon the color development. It was also

TABLE II

THE RECOVERY OF SILICA IN THE PRESENCE OF EXCESS OF HYDROFLUORIC ACID

SiO_2 present (μg)	F^- added as HF (μg)	Stoichiometric ratio Si : F[a]	SiO_2 found (μg)	% Recovery
20	25	1 : 1	19.5	97.5
20	37	1 : 1.5	19.6	98.0
20	50	1 : 2	20.0	100
50	64	1 : 1	47.5	95.0
50	96	1 : 1.5	50.0	100
50	128	1 : 2	47.5	95.0
70	89	1 : 1	68.5	97.8
70	133	1 : 1.5	67.0	95.8
70	178	1 : 2	68.5	97.8

[a] Assuming the SiF_4 compound.

established that when a fluoride excess of 500% was present in the absorption compartment, the color development of molybdenum blue was satisfactory and consistent results were obtained.

Rate and extent of diffusion

These were investigated in sulphuric acid reaction medium using ethylene glycol as absorbent. An excess of hydrofluoric acid of 200% over stoichiometry was used

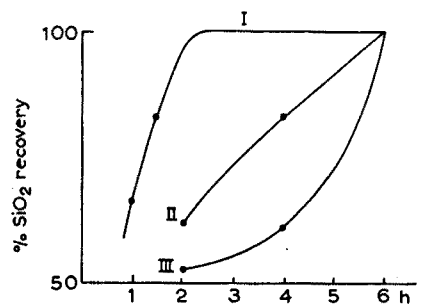

Fig. 1. Rate and extent of diffusion. I, 20 μg SiO_2; II, 50 μg SiO_2; III, 70 μg SiO_2.

in all experiments. The rate and extent of diffusion for 20, 50, and 70 μg of silicon dioxide at ambient temperature (which is not necessarily the cell temperature) are given in Fig. 1. It can be seen that quantitative diffusion is achieved after 2 to 6 h for 20 to 70 μg SiO_2.

Applications and comparative data

The method was originally developed for determining minute amounts of silica in concentrated phosphoric acid and potassium dihydrogen phosphate, since no suitable separative method for these ions was available. It was successfully applied also to systems where silica is a major component.

Tabulated in Table III are comparative data for phosphoric acid, potassium dihydrogen phosphate, National Bureau of Standards sample Burned Magnesite No. 104 and andalusite ($Al_2O_3 \cdot SiO_2$). The results show the overall reliability of the

TABLE III

APPLICATIONS AND COMPARATIVE DATA

System	*% SiO_2 found by*		*Remark*
	Classical method	*Microdiffusion method*	
Phosphoric acid (85%)			
sample a	0.023[a]	0.022	
sample b	0.075[a]	0.073	
sample c	0.075[a]	0.079	
Potassium dihydrogen phosphate	0.022[a]	0.018[d]	
		0.019[d]	
NBS Burned Magnesite No. 104	Certified 2.54[b]	2.36[d]	NaOH fusion and
		2.40[d]	H_2O leaching
Andalusite ($Al_2O_3 \cdot SiO_2$)			
sample a	46.6[c]	46.6	NaOH fusion and
sample b	54.0[c]	55.5	H_2O leaching

[a] As K_2SiF_6 precipitation and titrimetry.
[b] Mean of 9 determinations (extreme values: 2.49–2.62).
[c] By gravimetry.
[d] Independent duplicate determinations.

method. In the analyses involving separation from phosphoric acid or phosphate, no phosphate ion could be detected in the absorption compartment (the limit of detection of the test used was 0.4 μg/ml).

The permission of the Management of the Israel Mining Industries Laboratories, Haifa, to publish this communication is gratefully acknowledged. Mrs. S. AVIV and Mrs. S. HAKKERT carried out parts of the experimental work.

SUMMARY

A new approach is suggested to the determination of silica. Silica is converted to volatile silicon-fluoride compounds by the action of hydrofluoric acid and is quantitatively isolated by Conway's microdiffusion technique in a polypropylene cell. To promote diffusion, a sulphuric acid medium is used; ethylene glycol serves as absorbent. Quantities of 20–70 μg SiO_2 per 0.5 ml were tested, with quantitative recovery. The silica was determined spectrophotometrically by the molybdenum blue method.

RÉSUMÉ

Les auteurs proposent une méthode de dosage de la silice: formation de composés volatils par action de l'acide fluorhydrique et séparation quantitative par microdiffusion selon Conway, dans une cellule de polypropylène. On utilise l'acide sulfurique comme milieu et l'éthylèneglycol comme absorbant. La silice est dosée spectrophotométriquement par la méthode au bleu de molybdène.

ZUSAMMENFASSUNG

Zur Bestimmung von Siliciumdioxid wird ein neuer Weg vorgeschlagen. Das SiO_2 wird durch Umsetzen mit Fluorwasserstoffsäure in flüchtige Siliciumfluoridverbindungen überführt und quantitativ durch die Conwaysche Mikrodiffusionstechnik in einer Polypropylenzelle isoliert. Um die Diffusion zu fördern, wird ein schwefelsaures Medium benutzt; Äthylenglykol dient als Absorptionsmittel. Das SiO_2 (20–70 μg) wurde spektralphotometrisch mit der Molybdänblau-Methode bestimmt.

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Short Communications

Colorimetric determination of manganese in amber glass

In studies connected with the analysis of amber glass, the determination of manganese was necessary. Most of the samples contained small amounts of manganese and larger amounts of other elements as oxides. The latter may interfere in colorimetric methods by producing turbidity or by forming colored complexes. A conventional procedure[1] was found to be inadequate for the determination of manganese in these glasses because of the relatively large amounts of turbidity in the treated samples. Filtration of the samples did not increase the precision of the method.

Interference caused by turbidity can be eliminated by adding hydroquinone to the absorption cell after measurement of the absorbance[2]. The hydroquinone destroys the color of permanganate so that only scattering is measured. However, hydroquinone could not be employed in the present study owing to formation of colored complexes of molybdenum–hydroquinone. Therefore, other reagents were sought which would reduce permanganate, without yielding colored complexes with Mo, Fe, Al, Zn or Mg, and without precipitating these metals from solution.

Of the reagents studied, the di-sodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) met these requirements. In an independent study, Nordling[3] used a similar technique for the selective reduction of permanganate in solutions obtained from chromium steels.

Reagents

Standard solutions of manganese were prepared from pure electrolytic manganese. The solutions used for the interfering ion studies were prepared from reagent-grade salts or from metals dissolved in nitric acid.

Recommended method

To the sample (0.1–1.0 g) of ground glass in a platinum dish, add 10 ml of hydrofluoric acid (40%), heat for 15 min, add 3 ml of perchloric acid (72%), and evaporate to fumes; then add 2 ml of perchloric acid and evaporate again to fumes. Add 10 ml

of concentrated sulphuric acid and 5 ml of phosphoric acid (85%), and dilute the mixture to 90 ml with water. Add 0.2–0.5 g of potassium metaperiodate to the mixture, heat it to boiling and keep it at or just below the boiling point for at least 10 min. Cool the mixture, dilute it to 100 ml, and measure the absorbance of the suspension at 525 mμ. For minimum reading error, the absorbance should lie between 0.25 and 0.65. If the absorbance is much out of this range, a suitable sample size should be taken or a more appropriate dilution should be made. A Beckman Model DU spectrophotometer using 1-cm quartz cells was used in the present work.

After the latter measurement, add 25–30 mg of powdered Na_2EDTA to the absorption cell to render the suspension colorless and measure the absorbance again. The difference is proportional to the amount of manganese present.

Construct a working curve from standard solutions containing 0, 0.25, 0.50, 0.75 and 1.0 mg of manganese per 100 ml.

Results and discussion

Na_2EDTA was added to the absorption cell as a solid rather than as an aqueous

TABLE I

ANALYSIS OF STANDARD SAMPLES FOR MANGANESE EMPLOYING THE NEW METHOD

Mn added (μg)	*Mn found (μg)*	*% Deviation*
0[a]	0.0	—
10[a]	9.7	3
20[a]	19.8	1
30[a]	30.0	0
50[a]	49.9	0.2
50[b]	50.1	0.2
50[c]	50.0	0
100[a]	101	1
500[a]	496	0.8
1000[a]	1011	1.1
0[d]	0.2	—
0[e]	1.1	—

[a] These samples contained 0.5% Fe, Al, Zn and Mg, 9.5% K, 0.1% Mo, 16% Na and 72–73% Si as oxides.
[b] This sample contained 0.5% Fe, Zn, Al, Mg, K and Na as oxides; it also contained 1% Mo and 95–98% SiO_2.
[c] This solution did not contain diverse ions.
[d] This sample contained 1.0% iron as Fe_2O_3, 5% of the other metal oxides, and 69% silicon as SiO_2.
[e] This sample contained 5.0% iron as Fe_2O_3, 5% of the other metal oxides, and 65% silicon as SiO_2.

solution[3] so that the volume of the suspension in the cell would remain constant.

The procedure was tested on synthetic samples containing known amounts of Mn, Fe, Zn, Al, Mg, K, Si, Mo and Na, and gave satisfactory results (Table I). Of the elements studied, only unusually large amounts of iron interfered. Four glass samples were analyzed by the new method and by an alternative method[2]. These results are shown in Table II. The alternative method employs a scanning procedure (550–470 mμ) which is intended to correct for the turbidity of the suspension.

TABLE II

ANALYSIS OF AMBER GLASS FOR MANGANESE

Sample number	Mn found (%)		Mn found (%)	
	New method	Precision[a]	Periodate method	Precision[a]
1	0.180		0.156	
	0.183	0.89	0.163	3.70
	0.181		0.168	
2	0.251		0.240	
	0.249	1.47	0.236	2.90
	0.256		0.239	
3	0.117		0.147[b]	
	0.119	0.87	0.152[b]	5.59
	0.115		0.136[b]	
4	0.029	—	0.013	—
	0.028			

[a] The precision is given as the relative standard deviation.
[b] These solutions were filtered with Whatman 40 filter paper before development of color.

Although the accuracy of the new method cannot be estimated from these results, it should be more accurate than the periodate method because it is more precise.

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Malachite green: a new iodometric indicator

GAUTIER[1] has shown that iodine decolorizes methylene blue, forming the hydriodide of tetraiodomethylene blue, a brown precipitate, which reacts with reducing agents such as arsenite, sulfite and thiosulfate, with regeneration of methylene blue. He has suggested the use of the dye as an iodimetric indicator, as easy to use as starch and more reliable with dilute solutions (0.01 *N* or less).

We have observed that a precipitate is also given by the reaction of iodine with other dyes containing the quaternary ammonium group, such as malachite green, methyl green, crystal violet, methyl violet, acridine orange and rhodamine B, and that the precipitates also react with reducing agents restoring the respective dyes. These other dyes were therefore investigated as iodimetric indicators.

Reagents

Aqueous 0.05% solutions of methylene blue, malachite green, methyl green, crystal violet, methyl violet, acridine orange, and rhodamine B were prepared. Aqueous 0.2% starch solution was also prepared.[2]

Preliminary tests

To about 50 ml of 0.1 *N* sodium thiosulfate solution was added 0.20 ml of the aqueous 0.05% dye solutions, and the whole was titrated with 0.1 *N* iodine solution. Table I shows the observed changes of color. Sharp changes of color were observed in the solutions containing methylene blue, malachite green and methyl green; the best color change was found with malachite green.

TABLE I

OBSERVED CHANGES OF COLOR

Dye	*From*	*To*
Methylene blue	Blue	Brown
Malachite green	Blue	Green
Methyl green	Blue	Greenish-brown
Crystal violet	Violet	Reddish-brown
Methyl violet	Violet	Reddish-brown
Acridine orange	Yellow	Orange
Rhodamine B	Rose	Red

The behavior of malachite green was therefore compared with the previously suggested methylene blue and the classical starch indicator.

Titration procedures

(a) Titration of thiosulfate solutions with iodine solutions. Approximately 50 g of 0.1 *N* (or 0.01 *N*) thiosulfate solution was weighed out into a 250-ml Erlenmeyer flask and 0.20 ml of methylene blue or malachite green indicator (or 2.50 ml of starch solution) was added. The 0.1 *N* (or 0.01 *N*) iodine solution was added from a weight buret, with continuous magnetic stirring until near the end-point. Then an 0.01 *N*

TABLE II

TITRATION OF THIOSULFATE SOLUTION WITH IODINE SOLUTION

	Indicators		
	Malachite green	*Methylene blue*	*Starch*
0.1002 N iodine solution	0.10256	0.10259	0.10258
	0.10257	0.10256	0.10257
	0.10255	0.10256	0.10255
	0.10256	0.10254	0.10253
Average	0.10256	0.10256	0.10256
Average deviation (%)	0.005	0.013	0.018
Maximum deviation (%)	0.01	0.03	0.03
0.010035 N iodine solution	0.010054	0.010062	0.010083
	0.010051	0.010068	0.010085
	0.010048	0.010064	0.010082
	0.010049	0.010062	0.010088
Average	0.010051	0.010064	0.010085
Average deviation (%)	0.020	0.020	0.020
Maximum deviation (%)	0.03	0.04	0.03

(or 0.001 N) iodine solution, prepared by a ten-fold dilution, was added from a 1-ml volume buret until the change of color was detected.

Blank solutions were prepared and titrated, and the obtained values subtracted. The results of the titrations are shown in Table II. The values shown in all the Tables are the weight normalities found for the thiosulfate solution.

(b) Titration of iodine solutions with thiosulfate solutions. The same procedure was used, but 250-ml iodine flasks were used instead of Erlenmeyer ones; the indicators were added when the solutions were pale yellow, and no blanks were determined.

The results of the titrations are shown in Table III

TABLE III

TITRATION OF IODINE SOLUTION WITH THIOSULFATE SOLUTION

	Indicators		
	Malachite green	*Methylene blue*	*Starch*
0.1002 N iodine solution	0.10254	0.10259	0.10258
	0.10258	0.10257	0.10255
	0.10256	0.10256	0.10254
	0.10257	0.10254	0.10257
Average	0.10256	0.10256	0.10256
Average deviation (%)	0.013	0.015	0.015
Maximum deviation (%)	0.02	0.03	0.02
0.010128 N iodine solution	0.010113	0.010129	0.010175
	0.010119	0.010134	0.010179
	0.010116	0.010130	0.010180
	0.010118	0.010136	0.010175
Average	0.010117	0.010132	0.010178
Average deviation (%)	0.020	0.028	0.023
Maximum deviation (%)	0.04	0.04	0.03

Results

Examination of the results shows that in the titration of 0.1 N solutions of thiosulfate with iodine or in the reverse titration, malachite green, methylene blue and starch behave similarly.

In the titration of 0.01 N solutions, however, the use of malachite green indicator leads to lower values of normality than those determined with the use of starch indicator. The same occurs, to a smaller extent, with methylene blue indicator.

This agrees with the observation of KOLTHOFF AND BELCHER[3] that in the titration of iodine with thiosulfate in presence of starch, the blue color disappears a little too soon, and in titrations with iodine the blue color appears a little late.

The lower values found for the normality of 0.01 N solutions of thiosulfate with the use of the dyes, seem to confirm this error. It is obvious that, from this aspect, methylene blue and particularly malachite green, are better indicators than starch for dilute solutions.

Starch in the presence of 50% ethanol, fails to give a satisfactory end-point. It was found that methylene blue and malachite green functioned as efficiently in 50% ethanol as in aqueous solutions. In the presence of 20% sodium chloride solution, starch does not give sharp iodometric end-points but both dyes behave satisfactorily. It should be noted, however, that the dyes are not suitable as indicators in reactions

where precipitates are formed, *e.g.* in the standardization of thiosulfate solutions against potassium ferrocyanide or copper, because the dyes are adsorbed by the precipitates.

In titrations in acidic solutions, as in the determinations of sulfur in steels, the color change of malachite green is different from that shown in neutral solutions, because the dye is also a pH indicator.

Discussion

Malachite green and methylene blue may find application in the iodimetric procedure for the determination of weak acids. For example, KAMATH AND MAINKAR[4] found difficulties with the sensitivity of the starch end-point in the determination of carboxylic acids in lac, since lac is insoluble in water and soluble in alcohol.

Applications may also appear in the iodimetric titration of cyanide in carbonate–bicarbonate medium, where it is not advisable to use starch as indicator, for the reaction between cyanide and iodine is measurably slow, and the addition of starch makes it even slower[3].

Methylene blue and especially malachite green seem preferable to starch in titrations with dilute solutions, or in the presence of large amounts of electrolytes or ethanol. In addition to methylene blue and malachite green, methyl green has also shown good possibilities as an iodimetric indicator.

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Anal. Chim. Acta, 31 (1964) 286–289

Determination of oxygen in helium by Winkler's method

A knowledge of the oxygen contents in the range of microliters per liter of blanket gases in reactors is an important requirement. Amongst the chemical methods, WINKLER's[1] method has been applied by many workers[2–5] for determination of very low amounts of oxygen in gases. However, a review of the application of this method revealed many ambiguities and contradictory data. This paper reports the results of reinvestigation of the optimum experimental conditions to be followed in WINKLER's method based primarily on the work reported by SILVERMAN AND BRADSHAW[5].

The method is based on the liberation of iodine from potassium iodide by the manganic salt produced when manganese(II) hydroxide is oxidized by the oxygen in the sample; the iodine liberated is then determined spectrophotometrically. One of the primary requirements of such a determination is the preparation of reagents with reproducible blanks.

Blank values

Manganous chloride solution was prepared by SILVERMAN AND BRADSHAW'S procedure[5]. In agreement with these authors, we observed that manganese chloride solution prepared by PEPKOWITZ AND SHIRLEY'S method[4] gave high blanks. On the other hand, PEPKOWITZ AND SHIRLEY'S procedure for preparation of alkaline potassium iodide solution was found to be more suitable. SILVERMAN AND BRADSHAW prepared alkali with helium purging, iodide with carbon dioxide purging, and mixed them just before use. We found that iodide gave some yellowish colour due to iodine when purged with carbon dioxide. Again the use of a large excess of iodide as reported by previous workers[4,5] was found to produce high blanks; the aqueous phase after extraction of iodine invariably developed a yellow colour after some standing. The blank values with this large excess of iodide were high and variable. A smaller concentration of iodide (2 mg/ml) was found to be suitable. This gave a low and reproducible blank and a colourless aqueous phase for sufficiently long periods.

Extraction experiments

A perusal of the calibration graphs given by SILVERMAN AND BRADSHAW[5] (optical densities against iodine concentrations) showed a serious discrepancy in that the optical densities for the same amount of iodine extracted into different volumes of *o*-xylene did not show any linear relationship with the volume of the solvent. It was therefore thought necessary to reinvestigate the extraction of iodine in order to obtain more reliable and reproducible data.

For this purpose, standard dichromate solution was used to liberate the desired micro amounts of oxygen. To preadjusted volumes of dichromate solution in separating funnels, 15 ml of potassium iodide (2 mg/ml) and 5 ml of 1 *N* sulfuric acid were added. Iodine was extracted with 25 ml of *o*-xylene. In another set, standard iodine

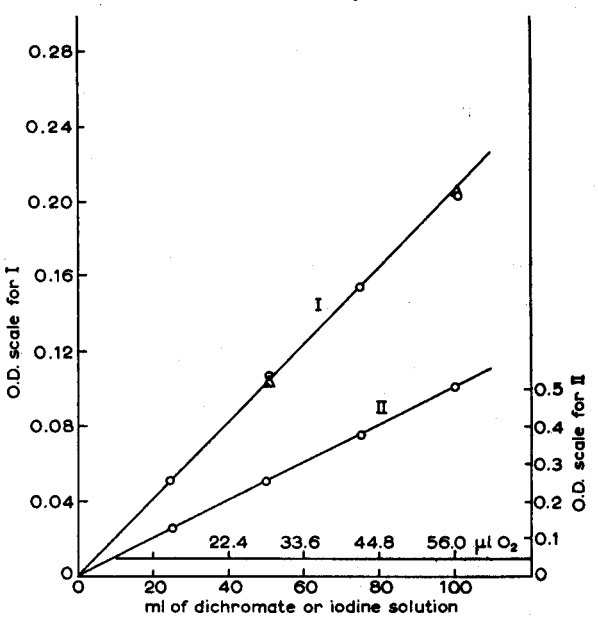

Fig. 1. Calibration curves for small amounts of iodine. (I) 25 ml *o*-xylene used; o = 0.0001 *N* dichromate solution used, Δ = 0.0001 *N* iodine solution used. (II) 10 ml *o*-xylene used.

solution in potassium iodide was used. Aliquots of iodine equivalent to the amoutns of dichromate solution in the previous set were taken in separating funnels and iodine was extracted with 25 ml of *o*-xylene. Figure 1 shows that the optical densities obtained for both sets at 495 mμ were identical within experimental error. This shows that under these conditions, extraction of iodine was quantitative. Another set of experiments using 10 ml of xylene for extraction gave optical density values 2.5 times higher than the previous values, as would be expected. In both series, Beer's law was obeyed throughout the range studied, *i.e.* up to 60 μl O_2, in contrast to the findings of SILVERMAN AND BRADSHAW.

Apparatus

The following two changes were made in the apparatus and procedure of SILVERMAN AND BRADSHAW[5]. They suggested that the extraction of iodine should be carried out under vacuum. It was found that *o*-xylene dissolved some grease, making the iodine extract turbid. In the present work PEPKOWITZ AND SHIRLEY's procedure[4] of extraction into a separating funnel after acidification was followed, and this in no way vitiated the results.

A small change was made in the design of the reaction flask as shown in Fig. 2. The reaction flask was separated from the remaining train by fusing a vacuum stopcock at the top, while the removable side-arm was permanently fused into the flask. This served two purposes, the reaction flask could be safely rotated, and during the period of shaking, the rest of the train could be washed, dried and kept ready for the further acidification step.

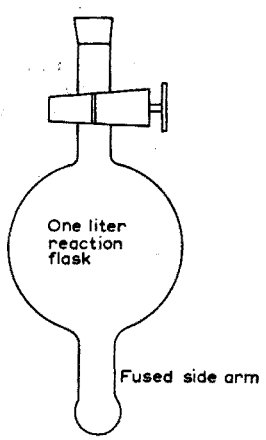

Fig. 2. Design of the reaction flask.

Analysis of helium

SILVERMAN AND BRADSHAW[5] gave the manganous chloride blank as 5.27 $\pm$ 1.9 μl O_2/l, the alkaline iodide blank as 2.5 $\pm$ 0.2 μl O_2/l and the *o*-xylene blank as 3. But there was no further clarification as to how these blanks were taken into account in working with sample gases, where amounts down to about 2 p.p.m. with a precision of $\pm$ 0.3 p.p.m. were reported.

We found after several experiments that a cumulative blank of 11 ± 5 μl O_2 was always inherent with the procedure described above and that was the lowest limit for analysis.

A summary of the helium gas analysis is given in Table I. In another case helium was partially purified by passing over heated uranium turnings at 600°; the results obtained for this gas sample are given in Table II.

TABLE I

DETERMINATION OF OXYGEN IN HELIUM

Pressure of helium (cm Hg)	*Optical density at 495 mμ*	*Average O.D.*	*μl O_2/l at N.T.P.*
	0.240		
80	0.260	0.253 ± 0.013	57.8
	0.261		
	0.185		
60	0.180	0.182 ± 0.003	53.0
	0.180		
	0.135		
38	0.140	0.138 ± 0.003	61.0
	0.140		
		Average value:	57.3 ± 4.3

TABLE II

ANALYSIS OF PARTLY PURIFIED HELIUM GAS FOR OXYGEN

Pressure of helium (cm Hg)	*O.D. at 495 mμ*	*μl O_2/l at N.T.P.*
	Untreated helium	
76	0.280, 0.260, 0.260	60.4
	Partially purified helium	
70	0.130	29.7
60	0.125	33.1
	Average value:	31.4 ± 1.7

The lower limit of usefulness of the method falls accordingly at about 15 μl O_2/l with 1 l gas sample at N.T.P.

The authors wish to thank Dr. V.T. ATHAVALE for his keen interest and helpful discussions during the course of this work.

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Ultramicro titration of fatty acids in non-aqueous single-phase systems

A procedure is described by which trace amounts of fatty acids can be determined by titration in a non-aqueous single-phase system. Compared with the methods involving titration in a two-phase system of which one is aqueous, as used in the well known and widely employed method of DOLE[1], the new method is more accurate and has the advantage of being simpler, with respect to both procedure and titration assembly. During titration no complicated mixing device is required and a vibrator which mixes the introduced titrant with the sample solvent is sufficient. No extensive precautions have to be taken to avoid the uptake of carbon dioxide. The end-point of the titration is quite sharp and is reproducible to better than 0.1 μl.

A solution of potassium methylate in anhydrous methanol is used as the titrant, and chloroform, chloroform-methanol or benzene is used as the solvent. For the determination of fatty acids in biological material the use of these solvents has an additional advantage, in that the fatty acids, which are isolated from the sample by extraction with one of them, need not be transferred to another solvent such as heptane.

The chloroform used for the determinations was stabilized with 1% methanol. The addition of methanol was very effective in preventing decomposition, and the blank value found was entirely due to consumption by the indicator.

Experimental

The titration was carried out with an ultramicro titrator (Beckman) which allows direct readings to 0.01 μl, with visual estimations to 0.001 μl. Glass titration cups, holding about 250 μl were used.

The titrant was prepared by dissolving about 4 g of freshly cut potassium in 1 l of absolute methanol to give an approximately 0.1 *N* solution of CH_3OK. The indicator was a 0.2% solution of Nile blue (Geigy) in methanol.

Procedure

A solution of the sample of fatty acid was made in chloroform or benzene so as to be about 0.01 *N*. Indicator solution (5 μl) was added to 100 μl of the sample and the mixture was titrated with the potassium methylate solution under constant stirring. A suitable rate of addition of the titrating solution proved to be about 1 μl/5 sec. If the titrant was added too quickly errors of up to 10% could be observed owing to incomplete mixing. Best mixing was observed when the stirring vibrator tip was placed almost at the bottom of the titrating cup.

Results

To check the reproducibility, 0.01 *N* solutions of stearic acid, elaidic acid and linoleic acid in chloroform and benzene were prepared. Samples of these acids were titrated at least 10 times, by the procedure outlined above. The mean values of the observed consumption of titrant and standard errors are given in Table I, together with the values of the reagent blank and the calculated titre of the titrant.

TABLE I

DETERMINATION OF THE TITRE OF A POTASSIUM METHYLATE SOLUTION IN ANHYDROUS METHANOL WITH DIFFERENT PURE FATTY ACIDS IN CHLOROFORM AND BENZENE

Fatty acid and solvent 1 μmol in 100 μl solvent	*Consumption of potassium methylate solution*		*Reagent blank value*		*Titre of the potassium methylate*
	Mean of 10 observ. (μl)	*Δ s*	*Mean of 6 observ. (μl)*	*Δ s*	
Stearic acid/chloroform	12.16	0.04	0.54	0.02	0.0861
Stearic acid/benzene	12.13	0.03	0.54	0.02	0.0863
Elaidic acid/chloroform	11.94	0.03	0.54	0.02	0.0877
Elaidic acid/benzene	11.97	0.04	0.54	0.02	0.0875
Linoleic acid/chloroform	12.12	0.04	0.54	0.02	0.0864
Linoleic acid/benzene	12.15	0.02	0.54	0.02	0.0861

From the data it can be seen that the reproducibility is better than 0.05 μl. There were no differences between the reagent blank values obtained with chloroform and benzene as sample solvents.

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Anal. Chim. Acta, 31 (1964) 293–294

Irregularities in the potentiometric titration of ketimines

Ketimines (R–C(=N–H)–R′) can be analyzed by potentiometric titration with perchloric acid in glacial acetic acid[1]. The platinum–glass electrode system can produce variations from the usual S-shaped potentiometric curves with some ketimines. If the imine carbon is adjacent to a tertiary carbon, the ketimine reacts in the acetic acid. The reaction produces a bright orange solution and a change in the shape of the titration curve. The reaction requires only minutes with ω-cyclohexylpentyl *s*-butyl ketimine but progresses for several days with isopropyl *m*-tolyl ketimine.

Figure 1 shows the change in titration curve for aliquots of an acetic acid solution of isopropyl *m*-tolyl ketimine over a period of 11 days. A voltage spike is obtained after 3 days. The voltage spike is similar to that described by YAKUBIK, SAFRANSKI AND MITCHELL[2] for potentiometric titration of organic acids in pyridine using a silver–glass electrode system. After 10 days, there is no more change in color or in the titration curve.

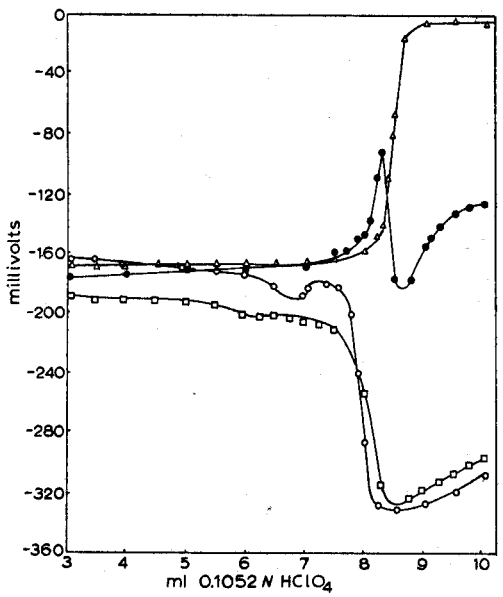

Fig. 1. Change in the shape of the potentiometric titration curve for isopropyl *m*-tolyl ketimine in glacial acetic acid. △ Solution 1 day old; ● 3 days; □ 8 days; ○ 11 days.

Alteration of the potentiometric titration curve is partially explained by a study of the behavior of the glass and the platinum electrodes separately in the titration of fresh and old ketimine solutions. To isolate the behavior of the electrodes, titrations are made with each electrode *vs.* a simple reference electrode. A platinum wire inside the buret tip, which is in contact with the solution during titration, makes an excellent reference. A solution of pyridine simulates the results obtained with fresh ketimine.

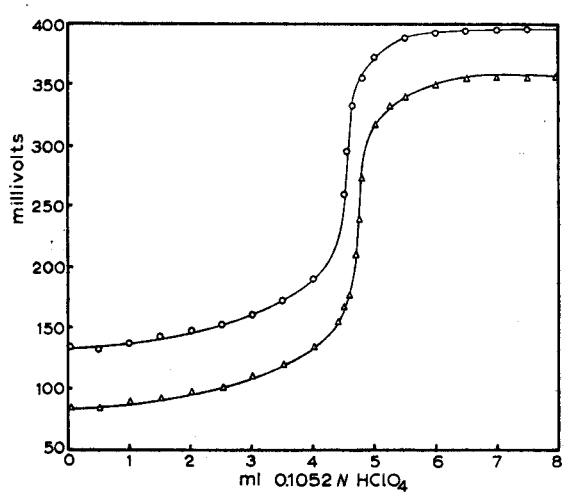

Fig. 2. Potentiometric titration of glacial acetic acid solutions of: ○ pyridine, △ old isopropyl *m*-tolyl ketimine, with a glass electrode *vs.* the buret tip internal reference electrode.

Aliquots of the pyridine and an old isopropyl *m*-tolyl ketimine solution titrate to give the results shown in Figs. 2 and 3.

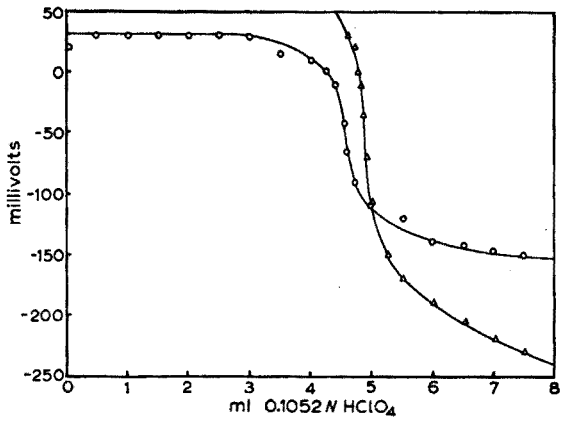

Fig. 3. Potentiometric titration of glacial acetic acid solutions of: o pyridine, △ old isopropyl *m*-tolyl ketimine, with a platinum electrode *vs.* the buret tip internal reference electrode.

Titrations made with the glass electrode *vs.* the internal reference electrode show essentially no differences between the pyridine and old ketimine solutions (Fig. 2). The difference in starting potential of the curves is not necessarily real. Such variations are a function of the preconditioning of the electrodes. In Fig. 3, the platinum–reference electrode curves show that a larger change in potential is obtained in the titration of the old ketimine than for fresh ketimine (pyridine). Also, the slope for the potential change is greater.

If the results for the glass and platinum *vs.* reference electrode curves are algebraically combined, the resultant curves resemble the first and last curves in Fig. 1. As the ketimine reacts in the acetic acid, the role of the platinum electrode changes from pseudo-reference to indicating electrode.

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Corrigendum

Theory of Titration Curves. Part I. The locations of inflection points on acid-base and related titration curves.

Equation (16) of our paper described the location of the point of maximum slope on the potentiometric titration curve for the titration of a monobasic weak acid with a strong base, but the quantities D and G appearing in it were incorrectly defined by equations (17) and (18). The errors arose because the definition of one of the fundamental parameters was changed after deriving the equation but before publishing it. The equations should read

$$D = f\varrho C_a^0 K_w K_a \tag{17}$$

$$G = K_w + (1 - f)\varrho C_a^0 K_a \tag{18}$$

The values in Table III are correct as given. We are greatly indebted to M. Y. LE DUIGOU for calling the errors to our attention and for confirming the correctness of equation (16) and of a typical value from Table III.

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Anal. Chim. Acta, 31 (1964) 297–298

Book Reviews

H. PURNELL, *Gas Chromatography*, John Wiley & Sons, New York and London, 1963, vii + 441 pp., price 90 s.

Of a number of books that have been published recently on gas chromatography, Dr. PURNELL's is one of the more interesting and useful. Dr. PURNELL has for many years worked in the field of gas chromatography, and has made many contributions to the subject, based on systematic appraisal of the use of the factors involved in making columns for effective rapid analysis of gases.

The book is well devised. It contains an introductory section on those properties, such as solution and vaporisation properties, and on the behaviour of gases, which are involved in gas chromatography. There follows a treatment of the theory of gas chromatography, both with regard to the underlying thermodynamics and to the kinetic aspects underlying the method and design of the equipment needed. The final section reviews the factors of importance in the design and use of columns and of detectors for the monitoring of the effluent gas stream. The coverage in the section on different techniques is very comprehensive.

The literature of gas chromatography is vast, and continues to grow. In the trivia of some of the contributions to the literature, and amidst so great a number of contributions, it is sometimes difficult to grasp the important principles. For all who use, or intend to use, gas chromatography, the book will be extremely valuable. Indeed so concise a treatment of so many aspects must often be indispensable.

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Anal. Chim. Acta, 31 (1964) 298

Metodi di Separazione nella Chimica Inorganica, Vol. 1, Edited by M. LEDERER, Consiglio Nazionale delle Ricerche Fondazione "F. Giordani" Corsi e Seminari di Chimica, No. 2, Rome, 1963, 328 pp., price $12.

This is the second volume of a series devoted to postgraduate summer courses organized by the Italian National Research Council on specialized fields of chemistry. The 1962 summer course on separation methods in inorganic chemistry directed by M. LEDERER is now published in two volumes containing the lectures given by speakers of various nationalities.

The papers in this volume are as follows. Liquid–liquid extraction, by H. IRVING AND R. J. C. WILLIAMS (in Italian); Liquid–ion exchangers: separations on inert supports impregnated with ion exchangers, by E. CERRAI (in Italian); Adsorption chromatography in inorganic chemistry, by L. SACCONI (in Italian); Adsorption of inorganic substances on paper, by M. LEDERER (in English); Chromatographic

separations on paper impregnated with synthetic inorganic exchangers, by G. ALBERTI (in Italian); Gas–liquid chromatography, by A. T. JAMES (in English); The application of high-voltage electrophoresis in inorganic chemistry, by D. GROSS (in English); Chromatographie und Komplexchemie — ein Übersichtsreferat unter spezieller Berücksichtigung eigener Ergebnisse, by E. BLASIUS (in German); Chromatographie des phosphates condensés et autres polyanions, by P. EBEL (in French); Techniques et applications de la radio-chromatographie en phase gazeuse, by J. P. ADLOFF (in French).

This volume thus comprises an up-to-date survey of some of the most important separation methods and techniques in inorganic chemistry; there are numerous examples and a good bibliography on each topic. The book should be very useful to everybody working in the field of inorganic chemistry. It is a very well produced volume, the printing and reproductions being of a high standard.

G. MILAZZO (Rome)

Anal. Chim. Acta, 31 (1964) 298–299

C. J. VAN NIEUWENBURG AND J. W. L. VAN LIGTEN, *Quantitative Chemical Micro-Analysis*, Elsevier Publishing Company, Amsterdam–London–New York, 1963, viii + 181 pp., price D.fl. 17.50/D.M. 19.50/£ 1.15.0.

Das vorliegende Buch ist eine Zusammenfassung des Unterrichtsprogrammes des Mikroanalytischen Laboratoriums der Technischen Universität Delft und enthält zum grössten Teil, nämlich in insgesamt 6 von 7 Kapiteln, Methoden der anorganischen quantitativen Mikroanalyse. Die Autoren geben einleitend einen kurzen Überblick über die geschichtliche Entwicklung und betonen, dass vor allem in letzter Zeit durch die Einführung von asymmetrischen, nach dem Substitutionsprinzip arbeitenden Mikrowaagen und durch die Entwicklung der Kolbenbüretten noch bestehende arbeitstechnische Schwierigkeiten beseitigt wurden. Im 2. Kapitel werden Geräte für die Mikrogravimetrie, sowie die anzuwendende Trocken- und Wägetechnik detailliert beschrieben. Die beiden nächsten Kapitel enthalten ausschliesslich Arbeitsvorschriften. Zunächst werden 108 gravimetrische Einzelbestimmungen von Elementen beschrieben, die als Kation bzw. Anion in gelöster Form vorliegen. Die Angaben bei den einzelnen Methoden sind genau, sodass ohne weiteres nach diesen Vorschriften gearbeitet werden kann. Anschliessend geben die Autoren Beispiele für die Trennung und gravimetrische Bestimmung von zwei nebeneinander vorliegenden Elementen. In den Kapiteln 5 und 6 wird die Mikromassanalyse behandelt, wobei vor allem Vorschriften für die komplexometrische Halbmikrobestimmung mit EDTA gegeben werden. Abschliessend folgen einige Beispiele für jodometrische Titrationen. Leider sind in diesen Kapiteln bei der Angabe der Faktoren bzw. Atomgewichte nicht die Atomgewichte 1961 verwendet worden. Im letzten Kapitel werden in gedrängter Form Methoden der organischen Elementaranalyse im Halbmikromassstab angegeben, wobei bei der Wahl der einzelnen Verfahren vor allem deren didaktischer Wert für den Lehrbetrieb berücksichtigt wurde. Zusammenfassend kann gesagt werden, dass das Buch von VAN NIEUWENBURG UND VAN LIGTEN für den Unterrichtsbetrieb empfohlen werden kann.

W. SCHÖNIGER (Basel)

Announcement

INTERNATIONAL SYMPOSIUM ON MICROCHEMICAL TECHNIQUES — 1965

AUGUST 22–27, 1965

The Pennsylvania State University will conduct this 1965 Symposium at University Park, Pennsylvania, U.S.A. The program is being organized by the American Microchemical Society (formerly the Metropolitan Microchemical Society) with the sponsorship of the International Union of Pure and Applied Chemistry. The technical sessions and social events will parallel in organization and conduct those of the corresponding 1961 Symposium held under similar auspices and sponsorship. The University will provide adequate accommodations and facilities for individuals or families. The lectures will be held in conveniently located buildings. Details on registration and local arrangements will become available early in 1965. If you attended the 1961 Symposium, such information will be sent to you routinely; otherwise, the Organizing Committee should be informed of your possible interest.

A call is now being made for research papers directed toward small scale operations, techniques, and methods in all phases of chemistry, including clinical chemistry and biochemistry. Papers centered on history, speculation, or review will not be considered. Although the technical sessions eventually organized will depend on the papers offered, it is hoped to have sessions on the following subjects: (1) micro methods in structural elucidation; (2) micro techniques in peptide studies; (3) clinical and forensic analysis; (4) novel micro and ultramicro approaches in organic elemental analysis; (5) micro techniques with high-energy materials; (6) novel micro separation methods; (7) inorganic microanalysis and trace analysis; (8) micro methods in air and water pollution studies; (9) determination of physical properties with small samples; (10) education for instrumentation; (11) general papers.

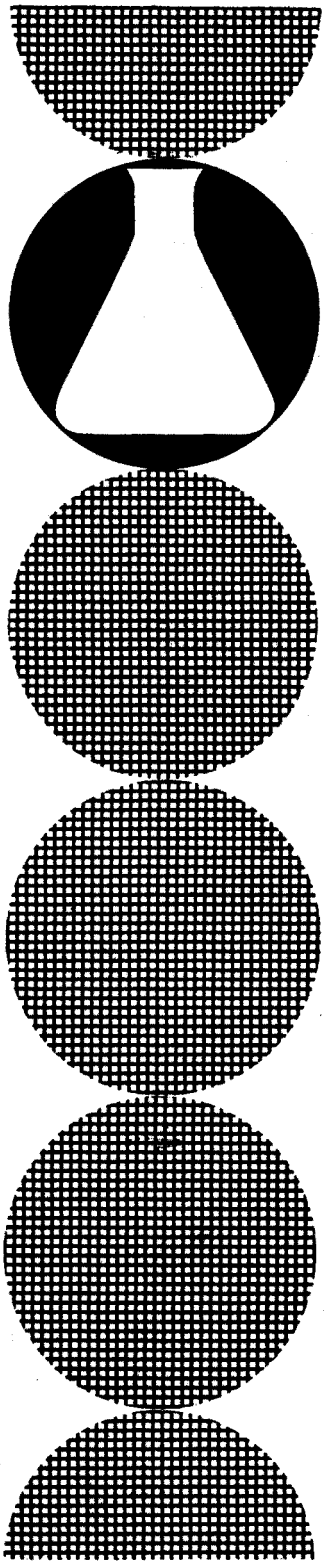
All correspondence regarding papers (or requests for addition to the Symposium mailing list) should be directed to: Mr. Howard Francis, Jr., Vice-Chairman, Intern. Sym. Microchem. Techniques – 1965, c/o Pennsalt Chemicals Corp., 900 First Ave., King of Prussia, Pennsylvania, U.S.A.

A descriptive title and an abstract (3 copies) must be received by January 31, 1965, and the text of the paper by April 31, 1965. Papers will not be considered for presentation "by title" or in absentia. The abstract of not more than 300 words, preferably in English, should provide a definite description of the nature, scope and novelty of the paper. Unusual symbols and complex mathematical formulae should not be used. The edited abstracts will appear in the Symposium program.

The letter of transmittal of the abstract should contain the following information: (a) the name of the person who will present the paper in the case of multiple authors; (b) a statement that the paper in substance or essence has not or will not be submitted for publication elsewhere; (c) the language of the oral presentation (English being preferred even if a manuscript must be closely followed); (d) the estimated presentation time (not to exceed 25 minutes with 5 additional minutes for discussion); (e) the size of lantern slides to be shown; (f) any special facilities that may be required.

The right of original publication of all research papers contributed to the Symposium is reserved by the *Microchemical Journal*, published by Academic Press under the auspices of the American Microchemical Society. A release to publish elsewhere cannot be entertained. The exception to this rule is that the plenary lectures will be published in the International Union of Pure and Applied Chemistry's journal, *Pure and Applied Chemistry*.

The full text of the paper with illustrations must be submitted in duplicate. Each manuscript will be acknowledged on receipt and will be placed in type for appearance in issues of the *Microchemical Journal* to be mailed directly following the Symposium. Papers should conform in organization to the practices of that *Journal* as described in recent issues on the page entitled "Information to Authors"; copies of that page are available on request.



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