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Chemical Reactions in Urban Atmospheres

Proceedings of the Symposium held at General Motors Research Laboratories, Warren, Michigan, October 6–7, 1969

Edited by **CHARLES S. TUESDAY,** *Fuels and Lubricants Department, General Motors Research Laboratories, Warren, Michigan*

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Discovery of "photochemical smog" created an entirely new field of atmospheric chemistry-one which studies contaminants that irritate the eyes, damage plants, and lessen visibility in cities. Examination of these pollutants has shown that, while chemical reactions in the atmosphere may remove a number or render them less harmful, different reactions may transform other pollutants into chemical forms more dangerous than the original types. In order to control and ultimately to reduce these unwanted environmental contaminants, it is necessary to understand the chemical processes which determine the level of atmospheric pollution.
This Symposium, organized to further the study of unanswered problems concerning such chemical reactions, included eleven papers by leading scientists on important developments in current research. Among the topics considered are the thermal and photochemical reactions of oxides of nitrogen with hydrocarbons, the role of singlet molecular oxygen, the photo-oxidation of alkyl nitrites and of formaldehyde, and the photochemical formation of atmospheric aerosols.

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A NEW HETEROGENEOUS SOLID-STATE COPPER(II)-SELECTIVE ELECTRODE

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(Received 15th April 1972)

Only membranes based on silver sulphide and silver halides have been used so far in these laboratories for the preparation of heterogeneous precipitate-based membrane electrodes with silicone rubber as the supporting material[1]. With the help of these electrodes pseudohalides, sulphide, silver and several metal ions can be selectively determined in addition to halide ions. Papers published in this field give information on the methods of preparation of these electrodes as well as on their application and electrochemical behaviour[2].

In the present paper, the preparation of a cation-selective copper electrode, not based on a silver salt, and its fundamental electrochemical properties, are reported.

Solid-state copper(II)-selective electrodes based on sulphide precipitates have already been prepared in different ways. The Orion Model 94–29 is an electrode of homogeneous structure based on copper sulphide and silver sulphide[3]. Mascini and Liberti[4] have prepared a similar electrode based on copper and silver sulphides but in a heterogeneous form. Hirata *et al.* reported a copper(I) sulphide-based ceramic membrane electrode of homogeneous structure[5] and a copper(II)-selective membrane electrode of heterogeneous structure[6].

EXPERIMENTAL

Reagents

All chemicals used in the measurements and preparation of the electrodes were of *pro analysi* grade (Reanal, Budapest, Hungary).

The stock solutions prepared by weight were standardized with the help of appropriate classical analytical methods.

Instrumentation and electrodes

An expanded-scale precision pH-meter (Model OP 205, Radelkis, Budapest), a conductivity meter (Model OK 102/1, Radelkis, Budapest) and a TTT1c Radiometer automatic titrator were used for the measurements.

A saturated calomel electrode and a platinum plate electrode were used as reference electrodes.

As indicator electrodes, a Radiometer glass electrode type G 200B and the copper(II)-selective membrane electrode, described below, were used. Before measurements, the latter was soaked for about 2 h in a solution containing the appropriate ions.

The microscope pictures were taken by means of a Tesla type BS 242 B electron microscope.

Preparation of membrane electrodes of heterogeneous structures

The active material used is copper sulphide, precipitated from homogeneous solution by sodium thiosulphate at pH 0. The average particle size was found to be *ca.* 1.5 μm (Fig. 1), by a statistical evaluation of electron-microscope pictures.

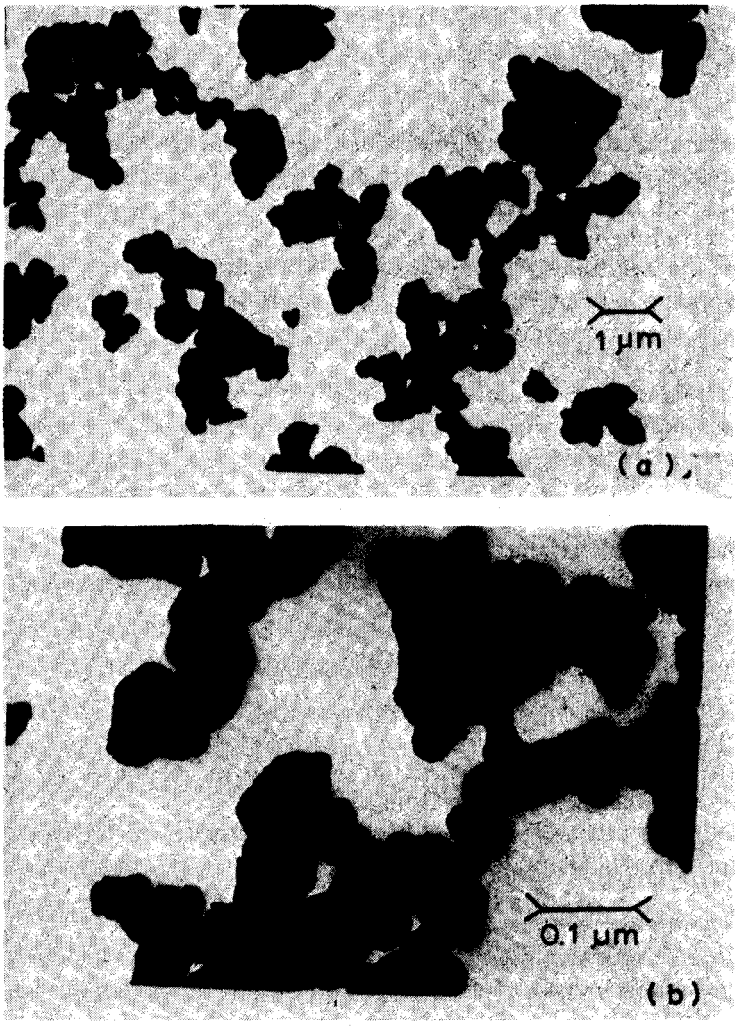


Fig. 1. Electron-microscope photographs of the electrode-active material.

After precipitation the active material was immediately embedded into silicone rubber as supporting material and the sheet prepared in this way was used for electrode membranes.

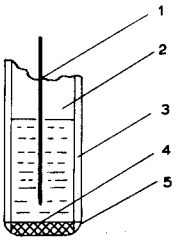
The structure of the electrode is shown in Fig. 2.

Procedures

The copper(II) ion activity was measured potentiometrically with the copper-(II)-selective electrode and a calomel reference electrode. When necessary, the calomel electrode was dipped into a 0.1 *M* potassium nitrate solution and contact with the reference and measuring half-cells was made by an agar-agar salt-bridge, containing 0.1 *M* potassium nitrate. The ionic strength was always adjusted to a constant value with potassium nitrate.

In the case of complexiметric and precipitation titrations, indirect potentiometric determination was used.

The impedance of the indicator electrode was measured in mercury with a platinum foil counter electrode.



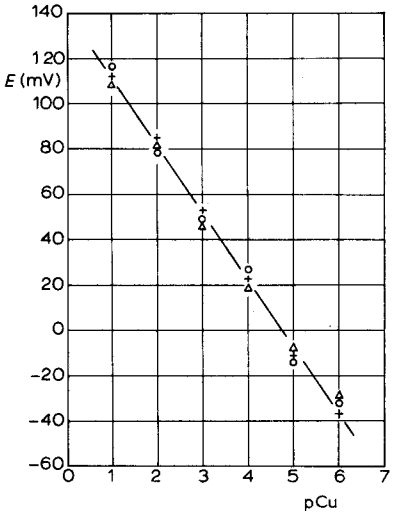


Fig. 2. Structure of the electrode. (1) Reference electrode, Ag/AgCl; (2) internal solution; (3) glass tube, 1 cm diameter; (4) membrane layer; (5) adhesive.

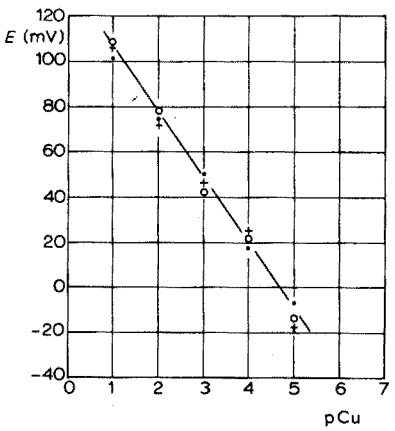
Fig. 3. Calibration curve for copper(II) ions. The points shown by ○, + and △ were obtained with three different electrodes. The line drawn represents the calculated response.

RESULTS AND DISCUSSION

Previous results obtained in studies of membrane electrodes[2] have established that the swollen surface area of the membrane takes part in precipitate-exchange reactions. Accordingly, electrodes suitably pretreated by soaking in a copper(II) solution were used for the measurements.

The calibration curves were plotted from mV readings in a range of copper(II) sulphate solutions, prepared by serial dilution. The electrodes gave a Nernstian response in the concentration range 10^{-1}–10^{-6} *M* (Fig. 3).

The cation function of the electrode was examined in the presence of various anions. Measurements were made in copper(II) sulphate, nitrate and chloride solutions; from the results obtained for the copper(II) ion activity (Fig. 4) it was concluded that the anions do not interfere and that the electrode response is due only to the copper(II) cation.



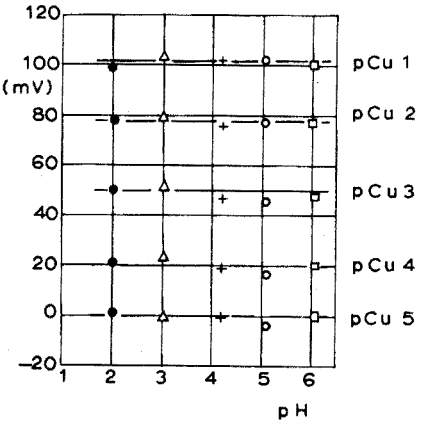


Fig. 4. Effect of various anions on the electrode response. (○) Sulphate, (+) nitrate, (●) chloride. The line drawn represents the calculated response.

Fig. 5. Effect of pH on the electrode response.

The influence exerted by the pH on the electrode function was examined. In the acidic pH range, the variation of E with pCu was examined as a function of pH, by constructing calibration curves in a range of Britton–Robinson buffer solutions. The pH of the buffer was checked with a glass–calomel electrode couple.

As can be seen from the data shown in Fig. 5, the pH does not affect the copper(II) ion function of the electrode in the range examined. Furthermore, it was established that systems containing relatively large amounts of ions do not cause measurable interference in the electrode response. At pH values of 6.4 copper(II) ions form hydroxides and the precipitates preclude direct determination. In such cases the concentration of the copper(II) ions can be determined by indirect potentiometry.

In potentiometric precipitation titrations, the concentration of the copper can be determined both in acidic and alkaline solutions. Curves obtained from precipitation titrations are shown in Figs. 6a, b and c; the pH values of the solutions were 0, 5 and 14, respectively. Aqueous solutions of sodium sulphide, or sodium sulphide

TABLE I

EXAMINATION OF THE COPPER–TETRAMINE COMPLEX WITH THE COPPER(II)-SELECTIVE ELECTRODES

c_{NH_3}	β_4' [a]	$E(mV)$			pCu [b]		
		Calc.	*Meas.*	*ΔE*	*Calc.*	*Meas.*	*ΔpCu*
—	—	51	51	—	3.00	3.00	0
0.10	10.55	−142	−151	−9	9.55	9.85	−0.30
0.25	10.23	−180	−178	+2	10.83	10.76	+0.07
0.50	10.12	−212	−206	+6	11.92	11.72	+0.20

[a] $\beta_4 = 12.6$.
[b] $\Sigma pCu = 3.00$.

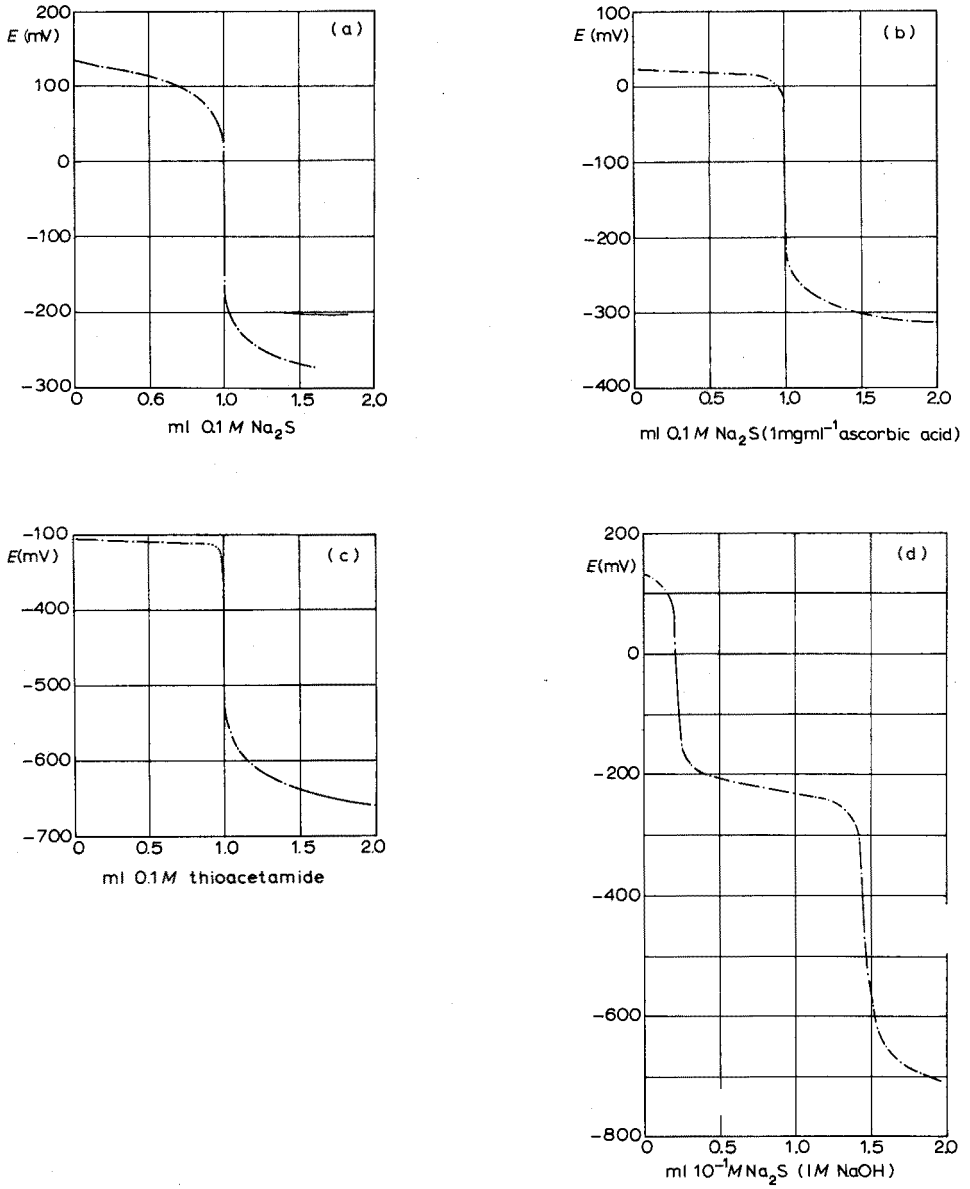


Fig. 6. Potentiometric precipitation titration curves with a copper(II)–selective electrode and an S.C.E. reference electrode. (a) Titrant Na_2S, pHO; (b) titrant Na_2S, containing 1 mg ml^{-1} ascorbic acid, pH 5; (c) titrant thioacetamide, pH 14; (d) titrant Na_2S dissolved in 1 *M* sodium hydroxide.

dissolved in ascorbic acid (1 mg ml^{-1}) solution or an aqueous solution of thioacetamide served as the titrants. When sodium sulphide dissolved in 1 *M* sodium hydroxide was used as the titrant, two inflection points were observed; the first corresponded to the formation of the hydroxide precipitate, and the second to a copper sulphide precipitate of stoichiometric composition (Fig. 6d).

If the alkaline solution also contains a complexing agent, the copper hydroxide precipitate is dissolved as a complex. The copper–tetrammine complex was examined at various pH values by the direct potentiometric method. Based on the complex equilibrium data, the conditional stability constants of the copper–tetrammine complex and the activity of the corresponding free copper(II) ions were calculated. The experimental results obtained confirmed the assumption that the electrodes measure only the free copper(II) ions. The measured and calculated data are compared in Table I; agreement between the results is satisfactory.

From the results obtained in these experiments it can be stated that the electrode is suitable for the measurement of copper(II) ions down to about pCu 12 by direct potentiometry; furthermore, the electrode can be standardized at copper(II) activities lower than pCu 6 with the use of the complex systems mentioned.

The electrode was applied as an indicator electrode in compleximetric titrations, as shown in Figs. 7a and 7b. From the titration curves given in Figs. 6 and 7,

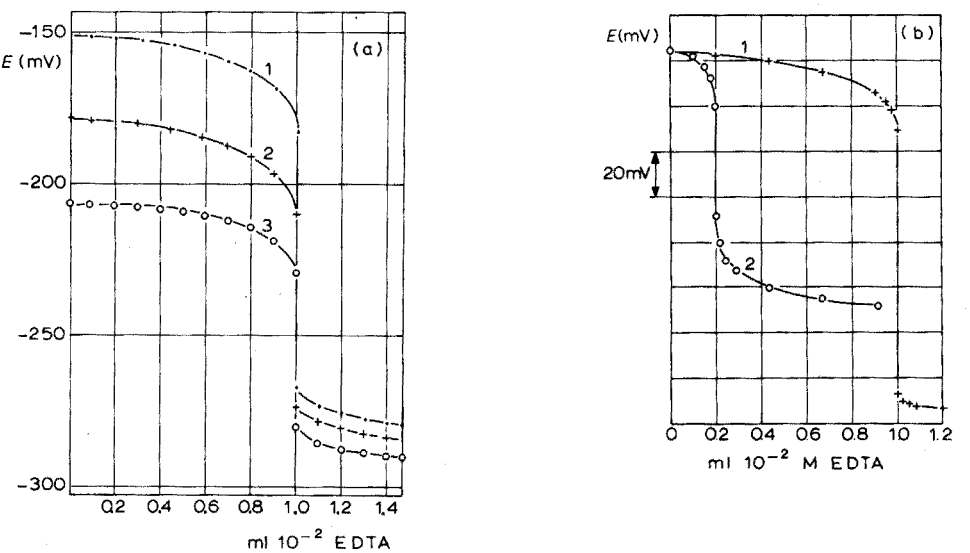


Fig. 7. Potentiometric complexiметric titration curves. (a) 10^{-3} *M* copper(II) in the presence of various ammonia concentrations: (1) 0.10 *M* NH_3; (2) 0.25 *M* NH_3; (3) 0.50 *M* NH_3. (b) 10^{-3} *M* (1) and 10^{-4} *M* (2) copper(II) in the presence of 0.25 *M* ammonia.

TABLE II

THE IMPEDANCES OF SOME COPPER(II)-SELECTIVE ELECTRODES

No.	*Conductivity (mS)*	*Impedance (kΩ)*
1	0.56	1.79
2	0.59	1.70
3	0.64	1.56
4	0.57	1.75

it is clear that the potentiometric titration of copper(II) ions could be carried out at significantly lower concentrations than those actually used in these tests.

The impedance values of some of the copper(II)-selective membrane electrodes developed are summarized in Table II.

The authors thank Dr. Éva Buzágh-Gere for taking the electron-microscope photographs.

SUMMARY

A new copper(II)-selective electrode based on copper sulphide in silicone rubber is described. Copper(II) ions in the range 10^{-1}–10^{-12} M can be measured. Various anions have no effect on the response; the electrodes can be used for direct measurements in the pH range 2–6. The impedance of the electrodes is about 1.7 kOhm. The electrodes can also be used as indicator electrodes in potentiometric titrations of copper(II) with sulphide, thioacetamide and EDTA solutions.

RÉSUMÉ

Les auteurs proposent une nouvelle électrode sélective de cuivre(II), avec sulfure de cuivre et caoutchouc silicone. On peut ainsi mesurer des concentrations de 10^{-1} à 10^{-12} M, d'un pH de 2 à un pH de 6. De nombreux ions sont sans influence sur la réponse. L'impédance de ces électrodes est d'environ 1.7 kOhm. Elles peuvent également être utilisées comme électrodes indicatrices pour des titrages potentiométriques du cuivre(II), au moyen de sulfure, de thioacétamide et d'EDTA.

ZUSAMMENFASSUNG

Es wird eine neue kupfer(II)-selektive Elektrode beschrieben, deren Wirkung auf Kupfer-sulfid in Silikonkautschuk beruht. Kupfer(II)-Ionen im Bereich 10^{-1} –10^{-12} M können gemessen werden. Verschiedene Anionen haben keinen Einfluss auf die Anzeige; die Elektroden können für direkte Messungen im pH-Bereich 2–6 verwendet werden. Die Impedanz der Elektroden beträgt etwa 1.7 kOhm. Die Elektroden können auch als Indikatorelektroden bei potentiometrischen Titrationen von Kupfer(II) mit Sulfid-Thioacetamid- und EDTA-Lösungen verwendet werden.

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VOLTAMMETRIC DETERMINATION OF URANIUM(VI) BY URANYL-PEROXODICARBONATE OXIDATION

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(Received 13th March 1972)

Uranium(VI) is frequently present in carbonate solutions as a consequence of some operations including: (*a*) carbonate separation of diverse metals[1]; (*b*) alkaline leaching of uranium ores[2]; (*c*) back-washing of uranium from the organic phases of purification processes by solvent extraction[3].

A direct determination of the uranium content in carbonate media would therefore be useful, particularly in the presence of some organic material such as dissolved tributyl phosphate.

The polarographic determination of uranium(VI) does not find wide application[4] in carbonate media because of the dependence of the limiting current of the cathodic uranium wave on solution parameters (attributed to the formation of various hydrolytic complexes of uranium(VI)).

It is well known that hydrogen peroxide, when added to an uranyl solution made alkaline with sodium carbonate, gives a yellow color[5] due to the peroxoanion[6] $[UO_2(CO_3)_2(O_2)]^{4-}$. This color is the basis of a traditional method of spectrophotometric analysis for uranium[7]; the method is selective enough but not very sensitive.

Zutic and Branica[8] have studied the peroxoanion reduction at the D.M.E. ($E_{\frac{1}{2}} = -1.15$ V), but possible analytical uses seem very limited because many substances are reduced at a lower potential than uranylperoxodicarbonate.

The present paper proposes a new voltammetric determination of uranium(VI) which differs from the usual polarographic ones in the following points:

(*a*) the anodic process concerning the oxidation of the peroxide group in the $[UO_2(CO_3)_2(O_2)]^{4-}$ anion is used for analytical purposes;

(*b*) the technique is based on the use of a platinized platinum microelectrode.

The accurate study of the oxidation wave of the uranylperoxodicarbonate complex was carried out with an electrode for which the diffusion layer was periodically renewed[9]. For analytical purposes a platinum electrode with bubbling[10] was used; this represents a simplified and more readily suitable version of the electrode with the periodically renewed diffusion layer.

The proposed method is sensitive, rapid, easy to work and gives good reproducibility. The optimal conditions for the voltammetric determination of uranium(VI) have been studied, together with the possible interferences caused by the presence of other substances.

EXPERIMENTAL

Apparatus

The voltammetric behaviour of the peroxo compound was investigated in a cell with the periodically renewed diffusion-layer electrode. The polarographic currents were recorded at $25 \pm 0.1°$ with a three-electrode system and a Polarecord model E 261 (Metrohm) modified to allow a slow change of the applied potential and connected with an IR compensator Type E446 (Metrohm). The potentiostatic current–time curves were measured on the screen of a Tektronix Type 502 dual-beam oscilloscope with a 68-TS-1 Wenking potentiostat for the control of the applied potential.

Both the reference and the auxiliary electrodes were saturated mercury(I) sulfate electrodes, connected with the polarographic cell by bridges consisting of a solid mixture of silica gel and sodium sulfate (3:2).

The voltammograms for analytical applications were measured both with the Metrohm equipment previously mentioned and with an Atlas Selector D type polarograph and a normal thermostated ($25 \pm 0.2°$) polarographic cell. A platinized platinum electrode with bubbling (surface 0.08 cm^2) was used, with periodic renewal of the solution at the electrode surface by removing the solution by 6 mm in each 5 s. A saturated mercury(I) sulfate electrode was connected to the solution in the cell by a sintered glass bridge filled with saturated sulfate solution.

The platinum microelectrodes were platinized by cathodizing them for 4 s at a current density of 1.2 A cm^{-2} in a 3% solution of chloroplatinic acid containing 0.025% of lead acetate.

Oxygen was removed by bubbling nitrogen through the solution.

All potential values are referred to the saturated calomel electrode.

Reagents

Uranium peroxide solutions were prepared from uranium peroxide dihydrate, made as described previously[11], by dissolution in carbonate solutions.

All other chemicals were reagent grade and solutions were prepared in the usual manner.

RESULTS AND DISCUSSION

Voltammetric behaviour

The current–voltage curve reported in Fig. 1 (curve a) was recorded by changing (0.47 mV s^{-1}) the voltage applied to the working electrode in the range +0.8 V to −0.7 V in a deaerated 1 *M* sodium carbonate solution containing $3 \cdot 10^{-3}$ *M* uranyl-peroxodicarbonate. This curve is characterized by a polarographic wave in the anodic zone ($E_{\frac{1}{2}} = +0.34$ V).

If the voltage scanning is performed at a relatively high sweep rate (150 mV min^{-1}) towards decreasing positive voltages, a cathodic wave ($E_{\frac{1}{2}} \cong -0.34$ V) is also displayed, whose height is equal to the anodic one. This cathodic wave appears just near the reduction peak of the surface oxide of the electrode and is strictly related to the presence of such an oxide. This wave was hardly reproducible; therefore, it was neglected.

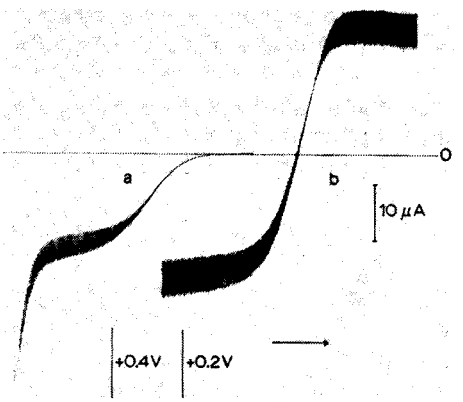

Fig. 1. Voltammetric curves of: (a) $3 \cdot 10^{-3}$ *M* uranylperoxodicarbonate; (b) $3 \cdot 10^{-3}$ *M* H_2O_2 in 1 *M* Na_2CO_3 solution.

The best shaped anodic waves were obtained in the carbonate concentration range 0.1–1 *M*. With peroxo compound solutions below pH 10.8, the wave decreased in amplitude and disappeared at pH $\leqslant$ 9.5.

It was found that the limiting current is strictly proportional to the uranylperoxodicarbonate concentration (Table I) both in aqueous 1 *M* sodium carbonate solution and in 1 *M* sodium carbonate saturated with tributyl phosphate. The amplitude of the wave can be measured down to concentrations of $1 \cdot 10^{-5}$ *M* and $2 \cdot 10^{-5}$ *M* respectively. At lower concentrations the results are uncertain.

The results of coulometric measurements showed that the uranylperoxodicarbonate oxidation process involves the global participation of two electrons per molecule according to the scheme:

$$[UO_2(CO_3)_2(O_2)]^{4-} + CO_3^{2-} \rightarrow [UO_2(CO_3)_3]^{4-} + O_2 + 2e^-$$

TABLE I

MEAN LIMITING CURRENT AS A FUNCTION OF THE URANYLPEROXODICARBONATE CONCENTRATION IN THE ABSENCE AND IN THE PRESENCE OF DISSOLVED TRIBUTYL PHOSPHATE

(Electrolyte 1 *M* Na_2CO_3)

Uranylperox. concn. (*mol/l*)	*In absence of TBP*		*In presence of TBP*	
	i_d (μA)	i_d/C ($\mu A\ l\ mmol^{-1}$)	i_d (μA)	i_d/C ($\mu A\ l\ mmol^{-1}$)
$2.00 \cdot 10^{-5}$	0.10	5.0	0.05	2.5
$5.20 \cdot 10^{-5}$	0.24	4.6	0.12	2.3
$1.16 \cdot 10^{-4}$	0.52	4.5	0.27	2.3
$2.20 \cdot 10^{-4}$	1.04	4.7	0.54	2.4
$3.50 \cdot 10^{-4}$	1.62	4.6	0.84	2.4
$4.98 \cdot 10^{-4}$	2.30	4.6	1.18	2.4
$1.00 \cdot 10^{-3}$	4.61	4.6	2.37	2.4

Logarithmic analysis of the wave showed that the ratio $\Delta E/\Delta \log i/(i_d - i)$ is equal to 55 mV, compared with the theoretical value of 29.5 mV expected for a reversible process.

Potentiostatic measurements showed that the mean limiting current, i_d, is related to the diffusion coefficient, D, by the equation:

$$i_d = a + (b/t_{tot})(2\, t_{tot}^{\frac{1}{2}} - 1.5\, t_p^{\frac{1}{2}})$$

where:
$a = nFADC/r$,
$b = nFAD^{\frac{1}{2}}\, C/\pi^{\frac{1}{2}}$,
t_p = washing period of the electrode (0.025 s),
t_{tot} = period between two subsequent washings (5 s),
n = number of Faraday per mole required by the reaction at the electrode,
r = radius of the platinum sphere (0.0937 cm),
C = molar concentration of electroactive substance,
A = effective electrode surface area, obtained by measurements of the limiting current of solutions of electroactive species with known D.

When the measured values of i_d, A and r were inserted in this expression, the diffusion coefficient value for uranylperoxodicarbonate was found to be $0.37 \cdot 10^{-5}$ $cm^2\ sec^{-1}$.

Uranyltricarbonate is not electroactive on smooth or platinized electrodes. The current–voltage curve given by a deaerated 1 M sodium carbonate solution containing $2 \cdot 10^{-3}$ M uranium is the same as that obtained with the supporting electrolyte alone (Fig. 2, curve a). When hydrogen peroxide is added to the solution an anodic wave related to the uranylperoxodicarbonate oxidation occurs; the height of this wave increases until the equivalent concentration of uranium and hydrogen peroxide is reached (Table II). The voltammetric curve given by equivalent concentrations of uranium and hydrogen peroxide appears quite similar to curve a of Fig. 1.

Further addition of hydrogen peroxide, exceeding the stoichiometric amount required for the uranylperoxodicarbonate formation, yields a voltammetric curve in which a composite wave ($E_{\frac{1}{2}} = -0.12$ V) appears, corresponding to the oxidation–

TABLE II

MEAN LIMITING CURRENT OF URANYLPEROXODICARBONATE AND FREE HYDROGEN PEROXIDE AS A FUNCTION OF THE RATIO $C_{H_2O_2}/C_U$

$C_{H_2O_2}/C_U$	$i_d(\mu A)$ *to +0.6 V*	$i_d(\mu A)$ *to +0.2 V*	$i_d(\mu A)$ *peroxo compound*
0.05	0.20		0.20
0.15	0.61		0.61
0.46	1.99		1.99
0.67	2.68		2.68
0.88	3.85		3.85
1.08	5.00	1.00	4.00
1.29	6.77	2.80	3.97
1.50	8.45	4.50	3.95
1.90	12.00	8.00	4.00

reduction process of free hydrogen peroxide (Fig. 2, wave b), in addition to the uranyl-peroxodicarbonate oxidation (Fig. 2, wave c). Potentiostatic investigation of the variation of the instantaneous current with the electrolysis time showed the cathodic and anodic limiting currents to be diffusion-controlled.

In fact hydrogen peroxide in deaerated 1 *M* sodium carbonate solution yields a composite wave at a platinized platinum electrode[12] (Fig. 1, curve b).

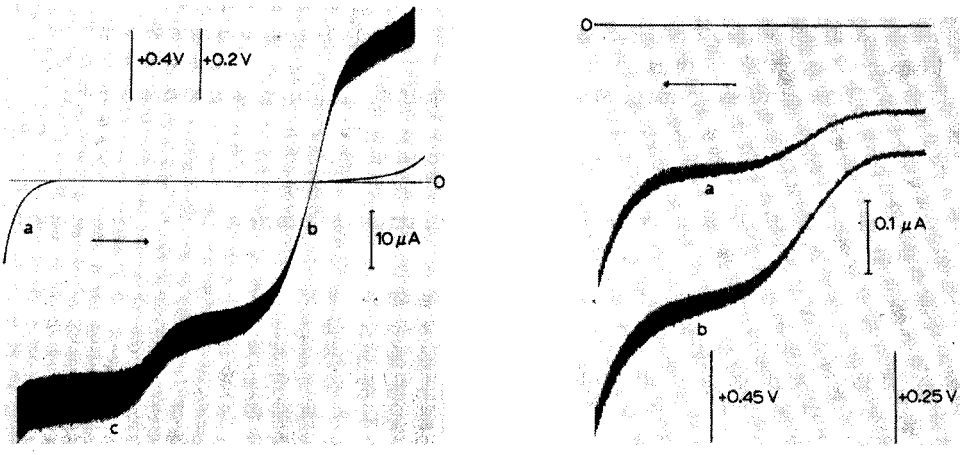

Fig. 2. Voltammetric curves of: (a) $2.7 \cdot 10^{-3}$ *M* uranium(VI) and $3.2 \cdot 10^{-3}$ *M* H_2O_2 in 1 *M* Na_2CO_3 solution; (b) supporting electrolyte alone.

Fig. 3. Application of the standard addition method. (a) Wave given by $1.8 \cdot 10^{-5}$ *M* uranium and $3 \cdot 10^{-5}$ *M* H_2O_2 in 1 *M* Na_2CO_3 solution; (b) after addition of peroxo complex standard solution.

ANALYTICAL APPLICATIONS

As shown above, the platinum microelectrode allows the study of the oxidation of the peroxo compound formed in UO_2^{2+}–H_2O_2–1 *M* Na_2CO_3 solutions. The corresponding wave is well shaped and its current is proportional to the peroxo compound concentration.

Obviously some troubles would be found in making precise current measurements where a large excess of hydrogen peroxide over uranium is present. On the other hand, in order to achieve the linearity between current intensities and uranium(VI) concentrations it is necessary that $C_{H_2O_2} \geqslant C_U$. This will be strictly realized when a hydrogen peroxide addition is made to obtain a low diffusion current, related to the free hydrogen peroxide oxidation (Fig. 3, curve a).

The determination of the uranium(VI) content can be readily made by the standard addition method. The following standard solution is required: uranyl-peroxo complex standard solution of uranium concentration C_{st} (about $5 \cdot 10^{-3}$ *M*), prepared by adding a volume A of hydrogen peroxide in excess over the stoichiometric amount required for a volume B of a uranium(VI) solution of known concentration C_U. Then $C_{st} = C_U \cdot B/A + B$. The concentration of the diluted hydrogen peroxide solution used need not be accurately known.

Procedure

To an aliquot of almost neutral uranium solution, add 1 *M* sodium carbonate solution to give a volume V_1. The base curve can be recorded from +0.1 to +0.9 V, if necessary.

In order to verify $C_{H_2O_2} > C_U$, adjust the applied voltage to +0.25 V and add a volume of hydrogen peroxide V_2 so that a low diffusion current is obtained.

Record the polarogram of the solution over the above potential range. I_1 is the observed diffusion current of the formed uranylperoxodicarbonate.

Add a volume V_{st} of the peroxo complex standard solution and record the new diffusion current I_2 in the usual way.

The uranium concentration of the unknown solution will therefore be:

$$C_1 = \frac{I_1 V_{st} C_{st}}{(I_2 - I_1)(V_1 + V_2 + V_{st}) + I_1 V_{st}}$$

This method is suitable for the determination of uranium down to a concentration of $1 \cdot 10^{-5}$ *M* (Fig. 3, curve a and b).

Effect of diverse ions

A summary of the effects of diverse ions is given in Table III. The recommended procedure was used and all samples were run in duplicate.

The choice of foreign ions was performed on the basis of: (*a*) interferences with the corresponding spectrophotometric method[13]; (*b*) elements which commonly accompany uranium; (*c*) ions which react with hydrogen peroxide.

For the sake of speed, only one carbonate washing was made when a precipitate had been formed.

Copper(II) and manganese(II) cause the most serious interference by their

TABLE III

EFFECTS OF DIVERSE IONS ON THE DETERMINATION OF URANIUM

(Initial solution: volume 25 ml, U(VI) 48 μg ml^{-1}, 1 *M* Na_2CO_3)

Cation	*Highest level studied without interference (mg added)*	*Anion*	*Highest level studied without interference (mg added)*
Aluminum	20	Acetate	150
Calcium	60	Arsenate	200
Cerium(III)	60	Chloride	90
Iron(III)	30	Fluoride	50
Magnesium	20	Chromate	150
Nickel	30	Hydrogencarbonate[b]	90
Thorium[a]	60	Nitrate	150
Titanium	25	Molybdate	200
		Sulfate	250
		Vanadate[a]	15

[a] Thorium and vanadate react with H_2O_2 in the ratio 1:1. Thorium gives an insoluble peroxo compound which does not affect the determination.

[b] A larger concentration caused the disappearance of the anodic wave.

catalytic decomposition of the peroxide.

Dissolved tributylphosphate distorts[14] polarograms at the D.M.E. and prevents a quantitative determination of uranium by conventional polarography. As shown above (Table I), tributyl phosphate depresses the diffusion current at the platinum microelectrode; but diffusion currents remain linearly related to the uranium concentration.

The method has been used in the analysis of aqueous effluents from a laboratory-scale thorium–uranium reprocessing plant for the following purposes:

(*a*) control of uranium losses in the waste stream from the tributyl phosphate co-decontamination cycle;

(*b*) determination of uranium in the thorium recovery solution from the partition cycle;

(*c*) determination of uranium in the recycled solvent, after the uranium back-extraction into aqueous phase by sodium carbonate solution.

SUMMARY

A new method for the voltammetric determination of uranium(VI) is proposed. In 1 *M* sodium carbonate solution, the oxidation of the peroxide group in the $[UO_2(CO_3)_2(O_2)]^{4-}$ ion gives a well shaped anodic wave at a platinized platinum microelectrode with bubbling. The amplitude of this wave allows the determination of uranium down to concentrations of $1 \cdot 10^{-5}$ *M* even in the presence of Ce(III), Fe(III), Ni(II), molybdates, chromates, phosphates, fluoride, dissolved oxygen and tributyl phosphate. The most serious interference arises from the ions which catalyze the peroxide decomposition.

RÉSUMÉ

On décrit une méthode de dosage de l'uranium(VI) par voltammétrie à l'aide de l'oxidation de l'ion $[UO_2(CO_3)_2(O_2)]^{4-}$ sur une électrode de platine à barbotage. Les concentrations détectables arrivent jusqu'à $1 \cdot 10^{-5}$ *M*, même en présence de Ce(III), Fe(III), Ni(II), molybdates, chromates, phosphates, fluorures, oxygen dissous et TBP.

ZUSAMMENFASSUNG

Es wird eine neue Methode für die voltammetrische Bestimmung von Uran(VI) vorgeschlagen, die auf der Oxidation des Peroxouranyldicarbonats an einer Platinelektrode beruht. Die Methode erlaubt die Bestimmung von Uran bis zu einer Konzentration von $1 \cdot 10^{-5}$ *M* herab, auch in Anwesenheit von Ce(III), Fe(III), Ni(II), Molybdaten, Chromaten, Phosphaten, Fluoriden, Sauerstoff und Tributylphosphat. Ionen, die die Peroxidzersetzung katalysieren, stören.

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A COMPUTER PROGRAM FOR THE CALCULATION OF EQUILIBRIUM CONCENTRATIONS IN COMPLEX SYSTEMS

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The calculation of the curves of acid–base titrations in non-aqueous media is difficult, because the number of equilibria involved is often quite large. Depending on the nature of the solvent and the compounds titrated, many equilibria have to be taken into account.

A general and versatile computer program for calculating complex chemical equilibria is obviously very useful in studying titrations in non-aqueous media. Its utility, however, depends very much on the efficiency of the program and the method, as computer time quickly becomes prohibitive when the number of equilibria increases. Much work has already been done on writing computer programs for the calculation of equilibrium concentrations. A literature survey of this work is given by Zeleznik and Gordon[1]. A saving in computer time in comparison to programs published already, such as HALTAFALL[2,3] and EQUIBRAT[4], can be obtained by the use of the reaction extents as main variables combined with the stoichiometric coefficients in the equilibrium constant equations. A similar approach has also been suggested by Meissner *et al.*[5] and Bugaevski[6]. Because their methods were specially developed for hand computation, these authors solved the equations successively and iterated to the equilibrium concentrations by repeating this procedure. In a computer method it is better to solve the equations simultaneously. This saves computer time and makes it possible to write the equations in any order.

The preparation of the equations for the calculations from the chemical equilibria sometimes requires rather complicated "bookkeeping". If the programming language PL1 is used, this preparation can easily be automated.

THEORY

In a mixture of compounds in which the following equilibria occur:

$$a_i\mathrm{A}+b_i\mathrm{B}+\ldots \overset{K_i}{\rightleftharpoons} p_i\mathrm{P}+q_i\mathrm{Q}+\ldots \qquad (1)$$

the equations for the equilibrium constants can be expressed as:

$$K_i\cdot[\mathrm{A}]^{a_i}[\mathrm{B}]^{b_i}\ldots=[\mathrm{P}]^{p_i}[\mathrm{Q}]^{q_i}\ldots \qquad (2)$$

where A, B, ... = reactants
P, Q, ... = products
$a, b, \ldots, p, q, \ldots$ = stoichiometric coefficients
K = equilibrium constants
i = number of reaction

If there are N equilibria and M compounds taking part in these equilibria, a matrix V with N rows and M columns can be introduced, V_{ij}, containing the stoichiometric coefficient of compound j in reaction i. V_{ij} must be taken positive if compound j is a reactant in reaction i, negative if compound j is a product in reaction i, and zero if compound j does not take part in reaction i.

The equilibrium constant equations (2) can now be written as:

$$K_i \prod_{j=1}^{M} c_j^{V_{ij}} = 1 \tag{3}$$

where c_j represents the equilibrium concentration of compound j.

With introduction of the reaction extent, x_i, defined as the concentration decrease of a reactant with stoichiometric coefficient one caused by reaction i, the equilibrium concentrations can be expressed as follows:

$$c_j = c_{0j} - \sum_{i=1}^{N} V_{ij} x_i \tag{4}$$

where c_{0j} denotes the initial concentration of compound j.

Mathematical method

The following set of N non-linear equations in the N unknown x_i has to be solved:

$$f_i(\underline{x}) = \log K_i + \sum_{j=1}^{M} V_{ij} \log c_j = 0, \qquad i = 1, 2, \ldots N$$

with

$$c_j = c_{0j} - \sum_{i=1}^{N} V_{ij} x_i, \qquad j = 1, 2, \ldots M$$

(K_i is the given equilibrium constant of equation i; c_{0j} is the given initial concentration of compound j; and the unknown x_i is the reaction extent of equation i). c_j, depending upon $\underline{x}$, represents the required equilibrium concentration of compound j, and V is the given matrix as described.

One may assume that:

$M \geqslant N$

rank of $V = M$

If this is not the case, the reaction equations should be dependent. To solve this set of equations the method of Newton is used. (N.B.: A vector is marked with an underlining.)

Linearization

Suppose a guess $\tilde{\underline{x}}$ (with corresponding $\tilde{\underline{c}} = \underline{c}_0 - V^T \tilde{\underline{x}}$) is available for the unknown $\underline{x}$. Developing $\underline{f}(\underline{x})$ around $\tilde{\underline{x}}$:

$$\underline{f}(\tilde{\underline{x}} + \mathrm{d}\underline{x}) = \underline{f}(\tilde{\underline{x}}) + Z\,\mathrm{d}\underline{x} + \ldots$$

where Z is a N by N matrix.

$$Z_{ij} = \frac{\delta f_i}{\delta x_j} = \sum_{l=1}^{M} \frac{\delta f_i}{\delta c_l} \cdot \frac{\delta c_l}{\delta x_j} = -\sum_{l=1}^{M} V_{il} \frac{1}{c_l} \cdot V_{jl}$$

or $Z = -VC^{-1}V^T$ with C a diagonal matrix ($C_{ii} = \tilde{c}_i$).

The linearized set of equations is:

$$VC^{-1}V^T \mathrm{d}\underline{x} = \underline{f}(\tilde{\underline{x}})$$

It is simple to verify that the matrix $VC^{-1}V^T$ is symmetric and positive definite for positive concentrations.

For the expression

$$(VC^{-1}V^T\underline{u}, \underline{u}) = (C^{-1}V^T\underline{u}, V^T\underline{u}) > 0$$

is valid for any $\underline{u} \neq \underline{0}$; thus the matrix $VC^{-1}V^T$ is regular in the whole region $\underline{c} > \underline{0}$, and so there exists at most one solution.

During the iterative process the concentrations should remain positive. Instead of the correction $\mathrm{d}\underline{x}$ (with the corresponding correction $\mathrm{d}\underline{c} = -V^T\mathrm{d}\underline{x}$) the correction $\alpha\mathrm{d}\underline{x}$ is made.

Initially, for α, the maximal value (α_0) is chosen with

$$\alpha \leqslant 1 \quad \text{and} \quad \tilde{\underline{c}} + \alpha\mathrm{d}\underline{c} \geqslant p_0\tilde{\underline{c}}$$

with p_0 a constant (*e.g.* $p_0 = 0.001$).

For sufficiently small positive α

$$||\underline{f}(\tilde{\underline{x}} + \alpha\mathrm{d}\underline{x})|| \leqslant ||\underline{f}(\tilde{\underline{x}})||$$

assuming $\underline{f}(\tilde{\underline{x}}) \neq \underline{0}$.

α_0 is halved as long as this equation is not satisfied. From an initial guess $\underline{x}^{(0)}$, a row of vectors $\underline{x}^{(k)}$ with $k = 1, 2, \ldots$ is obtained. The corresponding row of vectors $\underline{f}^{(k)} = \underline{f}(\underline{x}^{(k)})$ converges monotonously to zero. Consequently the row $\underline{x}^{(k)}$ converges to the solution. In the vicinity of the solution, the convergence is of the second order, *i.e.* in an iterative step k the norm of the difference between the iterand $\underline{x}^{(k-1)}$ and the solution $\underline{x}^{(\infty)}$ is squared or:

$$||\underline{x}^{(k)} - \underline{x}^{(\infty)}|| \sim ||\underline{x}^{(k-1)} - \underline{x}^{(\infty)}||^2$$

Initial guess

The only requirement for the initial guess $\underline{x}^{(0)}$ is that the corresponding concentrations $\underline{c}^{(0)} = \underline{c}_0 - V^T\underline{x}^{(0)}$ satisfy the relation $\underline{c}^{(0)} > \underline{0}$. At first $\underline{x}^{(0)}$ is set zero, and consequently $\tilde{\underline{c}}^{(0)} = \underline{c}_0$.

One can simply assume that each row of V contains both positive and negative coefficients. Then an equation is sought of which the product of the terms of the left side $= 0$ and the product of the terms of the right side $\neq 0$, or the reverse.

Suppose equation i is found.

$$\text{Put:} \quad \alpha_i = \frac{\min\limits_{j;V_{ij}>0} c_j}{\max\limits_{j;V_{ij}>0} V_{ij}}$$

$$\beta_i = \frac{\min\limits_{j;V_{ij}<0} c_j}{\max\limits_{j;V_{ij}<0} |V_{ij}|}$$

Either $\alpha_i = 0$ and $\beta_i \neq 0$, or $\alpha_i \neq 0$ and $\beta_i = 0$. By putting $x_i^{(0)} = \frac{1}{2}(\alpha_i - \beta_i)$ the concentrations are changed in the right direction. At present all the concentrations which have been met in equation i, are positive. The process is repeated until all the concentrations are positive. This is the case after maximal N steps.

Numerical aspects

It has been shown that the matrix $Z = VC^{-1}V^T$ is regular in the whole region $\underline{c} > \underline{0}$.

From a numerical point of view the matrix Z can be singular in the case of loss of significance. With the IBM 360 it is advisable to compute in double length (15 digits). Only in very extreme situations can an unacceptable loss of significance occur.

In solving the linearized set of equations $Zd\underline{x} = \underline{f}$, use is made of the particular properties of the matrix Z, *i.e.* symmetric and positive definite. The procedures used, DETSYM and SØLSYM, are very exact.

Precipitates

If compound j forms a precipitate, then this compound does not take part in the equilibrium equations, or in other words with respect to the iteration column j of matrix V is considered zero. With respect to the initial guess, the original matrix V is considered and precipitate formation is not taken into account. If precipitates are added in the starting mixture, the "formal" concentration should be given as initial concentrations for these compounds. Consequently all the concentrations, calculated from the initial guess $x^{(0)}$, are positive, including the precipitates. Finally the concentrations are calculated from the reaction extent $\underline{x}$ and the original matrix V, precipitate formation being neglected. In this case it is possible that a concentration, corresponding to a precipitate, is negative; a warning is then printed. This means, in fact, that precipitation does not occur or that a compound which is added in the initial mixture dissolves completely and the corresponding equilibrium should be omitted from the list of equilibrium equations that describe the system.

PROGRAM DESCRIPTION

The program used is shown in the Appendix.

Input

The data are put on cards, consecutively, apart from blanks. With regard to the input formats, the following rules should be respected.

Equations. The "equations" have to be closed by '*' and separated by ';'. It is advisable to take a new card for each equation.

An "equation" consists of: "left-side", '=', and "right side".

A (left or right) "side" consists of one or more "terms", separated by '+'.

A "term" is the combination of a stoichiometric coefficient and the chemical formula of a compound in the chemical equilibrium. It thus consists of a "coefficient" (a digit from 1 to 9; if the coefficient = 1, it can be omitted), and the "name of the symbol", representing a compound. In the case of a precipitate or solvent, the name is followed by the '%'-sign. A "term" can be preceded and followed by blanks. The "name of the symbol" exists of letters (A–Z), digits (1–9) and ')' or '(', but it must not begin with a digit.

Initial concentrations. The "names of the symbols" of the compounds which have an initial concentration (suppose the number = MO) are closed by ';' (or closed by blanks followed by ';') and separated by one or more blanks.

MO numbers, *i.e.* the initial concentrations, in the right order, are closed and separated by blanks and/or ','.

Equilibrium constants. N numbers, being the equilibrium constants, are closed and separated by blanks and/or ','.

Output

The input about chemical equations and formulae is printed according to exactly the same format as the actual input, and the numerical data according to a slightly modified format.

The moment an error has been detected, an error message is printed (with an error-number); and the process is stopped.

The results of the program are printed according to the format:

line 1 to M:

per line j: name of symbol of compound j

initial concentration $= c_0\ (j)$

equilibrium concentration $= c(j)$

line 1 to N:

per line i: equilibrium constant $= k(i)$

reaction extent $= x(i)$

If an equilibrium concentration is negative, which is only possible for precipitates, a warning is printed.

List of the most important symbols

N	number of equations.
M	number of compounds.
V	N by M matrix, see description of methods, declared as V (NMAX, MMAX).
VV	the same matrix as V, but with the correct upper limits.
VP	the same matrix as VV, but with the difference: $VP(I, J) = 0$ if compound j is a precipitate.
c_0	vector containing the initial concentrations.
MO	number of initial concentrations different from zero.
c	vector containing the equilibrium concentrations.
TSYMB	vector containing the names of symbols representing the compounds.
TPREC	vector; if TPREC(j) = 1 then compound j is a precipitate, otherwise TPREC(j) = 0.
LØGK	vector containing the logarithms of the equilibrium constants.
x	vector containing the reaction extents.
ØRDECO	vector; in this vector the order of the read-in initial concentrations is preserved.
Z	the (N by N) matrix of the linearized set of equations.

Program modules

The program consists of the following modules:

a. Read-in the input
entry: data on cards
exit: N,M,V,c_0, TSYMB, TPREC, LØGK
b. Initial guess of c
entry: N,M,V,c_0
exit: x,c
c. Iteration
entry: N,M,V,c,x, LØGK
exit: c,x
Finally the results are printed.

Detailed program description

Read-in the reaction equations. The procedure READC reads in a character and prints it. If NCHAR (=the number of read-in characters) is a multiple of 80 (=the number of columns on a card) a line is printed. According to the definitions of the concepts "Name of Symbols", "Term" and "Side", there are the procedures NAME, TERM and SIDE to read in these quantities.

At the entrance of NAME the first character has been read-in already, and has been put in CHAR. All the three procedures read-in upto and including the first character which does not belong to the definition; at the exit this character has been placed in CHAR. While reading, the syntactical rules are checked thoroughly.

In the procedure SIDE the matrix V and the vectors TSYMB and TPREC are filled.

Read-in the initial concentrations. The names of the symbols representing the compounds with initial concentrations are read-in. The order is preserved in ORDECO. Finally the initial concentrations are read-in and stored in the right place in c_0.

Read-in the equilibrium constants. The logarithms of these values are preserved in the array LØGK.

Initial guess of x (and c). Initially $\underline{x}^{(0)}$ is set zero. The equations are treated (one or more times) from number 1 up to and including number N. If all the concentrations, which have been found in an equation i, are positive, then the next equation is processed. All concentrations of equation i are certainly positive if $x^{(0)}(i) \neq 0$. If at least one concentration on both the left side and the right side of equation i is zero, then the $x^{(0)}(i)$ cannot be changed; processing has to be delayed until a further round and the variable READY is set zero. If in a round no $x(i)$ has been changed (in that case the variable FIRST is still zero), while only some concentrations are equal to zero, the set of reaction equations or the list of starting materials, is wrong: some equations do not take part in the equilibrium.

Iteration. Initially a test is made to ensure that the rank of $V = N$. This is the case, when the matrix VV^T (taking into account precipitates) is not singular. The process is then carried out according to the mathematical description. For $\|f\|$ the maximum norm is used. If for all j the relative correction of $c_j <$ tol c, then the process is ended. After ITMAX (=50) iterations, the process is stopped and an error message is printed.

List of errors
code
111 number of characters of a symbol > LMAX

112 number of compounds >MMAX;
113 number of equations >NMAX;
12 a compound appears in the equilibria in a soluble as well as a precipitated form;
121 '=' sign is missing in an equation;
122 the set of equations is not closed by the '*' sign;
14 a compound appears in the same equation for the second time;
15 a compound appears in the list of initial concentrations for the second time;
16 incorrect name of compound in the list of the initial concentrations;
17 an initial concentration is negative;
18 an equilibrium constant is negative;
21 in the procedure GUESS it appears that the left or right side in an equation is missing;
22 in the procedure GUESS it appears that some equations do not have a role; it is likely that there are too few starting materials;
31 the reaction equations are dependent;
32 the matrix $Z = VC^{-1}V^{T}$ proves to be singular by loss of significance;
33 after ITMAX iterations, the process has not been ended.

Restrictions and possibilities for modifications

The number of characters of a symbol, the number of equations and the number of compounds is restricted to 20. Each of these maxima can be easily changed (by modifying LMAX, NMAX and MMAX).

The maximum number of iterations (ITMAX) and the relative tolerance of c TØLC can also be easily modified.

If for one system many computations have to be made, *e.g.* with different initial concentrations and/or with different equilibrium constants, a slight modification of the program must be made. The final reaction extents of one calculation can be used to calculate the initial guess of the concentrations of the next by the use of eqn. (4).

When in the calculation of titration curves the dilution effect must be taken into account, it is necessary to correct the reaction extents by the dilution factor before using them in the calculation of the initial guess of the concentrations in the next point of the titration curve.

It is also necessary to prevent occurrence of zero concentrations in the initial guess. This can be done by replacing these zero concentrations by a very small concentration.

Some results

Tanaka and Nakagawa[7] calculated the curves for the titration of N,N-diethylaniline with perchloric acid in acetic acid which contained 0.5 *M* water.
With the program EQUIL the same titration curves were calculated, by means of the equilibria:

$HX = H^{+} + X^{-}$;	$K_{HX} = 1.4 \cdot 10^{-5}$
$B + HAc = BH^{+} + Ac^{-}$;	$K_{B} = 1.7 \cdot 10^{-6}$
$BH^{+}X^{-} = BH^{+} + X^{-}$;	$K_{d}^{BH^{+}X^{-}} = 1.7 \cdot 10^{-6}$
$HAc = H^{+} + Ac^{-}$;	$K_{s} = 3.5 \cdot 10^{-15}$
$H_2O + HAc = H_3O^{+} + Ac^{-}$;	$K_{H_2O} = 8.4 \cdot 10^{-11}$

TABLE I

EQUILIBRIUM CONCENTRATIONS IN THE TITRATION OF 0.01 *M* N,N-DIETHYLANILINE WITH PERCHLORIC ACID IN ACETIC ACID SOLVENT
(0.5 *M* water present)

Compound	*Titration degree*									
	0.90		0.95		1.00		1.05		1.10	
	Initial conc.	*Equil. conc.*	*Initial conc.*	*Equil. conc.*	*Initial conc.*	*Equil. conc.*	*Initial conc.*	*Equil. conc.*	*Initial conc.*	*Equil. conc.*
HX	$8.92 \cdot 10^{-3}$	$2.27 \cdot 10^{-9}$	$9.41 \cdot 10^{-3}$	$4.75 \cdot 10^{-9}$	$9.90 \cdot 10^{-3}$	$4.87 \cdot 10^{-8}$	$1.04 \cdot 10^{-2}$	$1.61 \cdot 10^{-6}$	$1.09 \cdot 10^{-2}$	$6.08 \cdot 10^{-6}$
H^+	0.00	$2.67 \cdot 10^{-10}$	0.00	$5.23 \cdot 10^{-10}$	0.00	$4.28 \cdot 10^{-9}$	0.00	$4.13 \cdot 10^{-8}$	0.00	$8.21 \cdot 10^{-8}$
X^-	0.00	$1.15 \cdot 10^{-4}$	0.00	$1.22 \cdot 10^{-4}$	0.00	$1.53 \cdot 10^{-4}$	0.00	$5.25 \cdot 10^{-4}$	0.00	$1.00 \cdot 10^{-3}$
B	$9.92 \cdot 10^{-3}$	$9.81 \cdot 10^{-4}$	$9.91 \cdot 10^{-3}$	$4.95 \cdot 10^{-4}$	$9.90 \cdot 10^{-3}$	$5.06 \cdot 10^{-5}$	$9.90 \cdot 10^{-3}$	$1.55 \cdot 10^{-6}$	$9.89 \cdot 10^{-3}$	$4.11 \cdot 10^{-7}$
HAc	16.50	16.50	16.50	16.50	16.50	16.50	16.50	16.50	16.50	16.50
BH^+	0.00	$1.24 \cdot 10^{-4}$	0.00	$1.23 \cdot 10^{-4}$	0.00	$1.03 \cdot 10^{-4}$	0.00	$3.04 \cdot 10^{-5}$	0.00	$1.60 \cdot 10^{-5}$
Ac^-	0.00	$1.31 \cdot 10^{-5}$	0.00	$6.69 \cdot 10^{-6}$	0.00	$8.17 \cdot 10^{-7}$	0.00	$8.48 \cdot 10^{-8}$	0.00	$4.26 \cdot 10^{-8}$
BH^+X^-	0.00	$8.80 \cdot 10^{-3}$	0.00	$9.29 \cdot 10^{-3}$	0.00	$9.75 \cdot 10^{-3}$	0.00	$9.86 \cdot 10^{-3}$	0.00	$9.87 \cdot 10^{-3}$
H_2O	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
H_3O^+	0.00	$3.21 \cdot 10^{-6}$	0.00	$6.28 \cdot 10^{-6}$	0.00	$5.14 \cdot 10^{-5}$	0.00	$4.95 \cdot 10^{-4}$	0.00	$9.83 \cdot 10^{-4}$

TABLE II

TITRATION OF 0.01 *M* DIETHYLANILINE WITH PERCHLORIC ACID IN ACETIC ACID (0.5 *M* water present)

Degree of titration	*pH*		$p[H^+ClO_4^-]$	
	Tanaka	*Present calcns.*	*Tanaka*	*Present calcns.*
0.90	9.6	9.57	8.5	8.64
0.95	9.3	9.28	8.2	8.32
1.00	6.8	8.37	5.7	7.31
1.05	5.0	7.38	4.1	5.80
1.10	4.7	7.09	3.4	5.21

The results of the calculations are given in Table I, and a comparison with the results of Tanaka and Nakagawa[7] is given in Table II.

On the basic side, there is good agreement. However, at and after the equivalence point there are differences, because the assumption of Tanaka and Nakagawa[7] that $[X^-]$ can be neglected is not valid for perchloric acid. In fact $[X^-]$ is about 10% of the total acid concentration at 1:1 neutralization.

The authors wish to thank Miss A. L. Dekkers for preparing the manuscript.

SUMMARY

A general computer program has been developed for the calculation of equilibrium concentrations in complex systems. The use of the reaction extents as master variables in the equilibrium constant equations yields a considerable saving in computer time in comparison to existing programs. The setting up of the equations for the calculations from the chemical equilibria can be automated.

RÉSUMÉ

Un programme général d'ordinateur est proposé pour le calcul des concentrations d'équilibre dans des systèmes complexes. L'établissement des équations pour les calculs des équilibres chimiques peut être automatisé.

ZUSAMMENFASSUNG

Es wurde ein allgemeines Rechenprogramm für die Berechnung von Gleichgewichtskonzentrationen in Komplexsystemen entwickelt. Die Verwendung des Reaktionsausmasses als Hauptvariable in den Gleichungen der Gleichgewichtskonstante führt zu einer beträchtlichen Einsparung an Rechenzeit im Vergleich zu bisher verwendeten Programmen. Die Aufstellung der Gleichungen für die Berechnungen aus den chemischen Gleichgewichten kann automatisiert werden.

APPENDIX

SOURCE LISTING.

```
EQUIL: PROCEDURE OPTIONS(MAIN);
DETSYM:PROCEDURE(A,N,MINPIV);
       DCL (A(*,*),MINPIV) FLOAT BIN(53),
           N FIXED BIN,
           (R,S) FLOAT BIN (53),
           (K,L,J) FIXED BIN;
       DO K=1 TO N;
         R=A(K,K);
         DO L=1 TO K-1;
           R=R-A(L,K)*A(L,K);
         END;
         IF K=1 THEN MINPIV=R;
         MINPIV=MIN(MINPIV,R);
         IF R<= 0 THEN GOTO LSYM;
         A(K,K),R=SQRT(R);
         DO J=K+1 TO N;
           S=A(K,J);
           DO L=1 TO K-1;
             S=S-A(L,J)*A(L,K);
           END;
           A(K,J)=S/R;
         END;
       END;
LSYM:
END DETSYM;
SOLSYM:PROCEDURE (A,N,B);
       DCL (A(*,*),B(*)) FLOAT BIN (53),
           N FIXED BIN,
           S FLOAT BIN (53),
           (I,K) FIXED BIN;
       DO I=1 TO N;
         S=B(I);
         DO K=1 TO I-1;
           S=S-A(K,I)*B(K);
         END;
         B(I)=S/A(I,I);
       END;
       DO I=N TO 1 BY -1;
         S=B(I);
         DO K= I+1 TO N;
           S=S-A(I,K)*B(K);
         END;
         B(I)=S/A(I,I);
       END;
END SOLSYM;
GUESS: PROCEDURE(N,M,V,C0,X,C);
       DCL (V(N,M),X(N),(C0,C) (M)) FLOAT BIN(53),
           (N,M) FIXED BIN;
       DCL (COSUM,AT,BT,AN,BN,A,B) FLOAT BIN(53),
           (I,J,FIRST,READY) FIXED BIN;
       X=0;
       C=C0;
       COSUM=SUM(C0);
LB1:   FIRST=0 ; READY=1;
```

```
        DO I=1 TO N;
          IF X(I) ¬=0 THEN GOTO LB2;
          AT,BT=COSUM;
          AN,BN=0;
          DO J=1 TO M;
            IF V(I,J)>0 THEN DO; AT=MIN(AT,C(J));
                                 AN=MAX(AN,V(I,J));
                             END;
            IF V(I,J)<0 THEN DO; BT=MIN(BT,C(J));
                                 BN=MAX(BN,-V(I,J));
                             END;
          END;
          IF AN=0 | BN=0 THEN CALL FAULT(21);
          A=AT/AN; B=BT/BN;
          IF A=0 & B=0 THEN DO; READY=0;
                                GOTO LB2;
                            END;
          IF A¬=0 & B¬=0 THEN GOTO LB2;
          FIRST=1;
          X(I)=0.5*(A-B);
          C=C-X(I)*V(I,*);
LB2:    END;
        IF READY=1 THEN RETURN;
        IF FIRST=1 THEN GOTO LB1;
        CALL FAULT(22);
        RETURN;
END GUESS;
ITER:   PROCEDURE(N,M,V,C,X,LOGK);
        DCL (V(N,M),C(M),(X,LOGK) (N)) FLOAT BIN(53),
            (N,M) FIXED BIN;
        DCL (W(N,M),Z(N,N),(F,DX) (N),(DC,LOGC) (M),
             TOLC,EPSC,P0,MINPIV,FMAX0,FMAX1,WR,ALFA) FLOAT BIN(53),
            (I,J,IT,ITMAX) FIXED BIN;
        ITMAX=150;
        TOLC=1.0E-5;
        P0=1.0E-3;
        DO I=1 TO N;
          DO J=1 TO I;
            Z(I,J),Z(J,I)=SUM(V(I,*)*V(J,*));
          END;
        END;
        CALL DETSYM(Z,N,MINPIV);
        WR=Z(1,1);
        DO I=2 TO N;
          WR=WR*Z(I,I);
        END;
        IF MINPIV <=0 | WR < 0.5 THEN CALL FAULT(31);
        FMAX0=0;
        DO J=1 TO M;
          LOGC(J)=LOG(C(J));
        END;
        DO I=1 TO N;
          F(I)=LOGK(I)+SUM(V(I,*)*LOGC);
          FMAX0=MAX(FMAX0,ABS(F(I)));
        END;
        DO IT=1 TO ITMAX;
          DO J=1 TO M;
            W(*,J)=V(*,J)/C(J);
          END;
          DO I=1 TO N;
```

```
            DO J=1 TO I;
              Z(I,J),Z(J,I)=SUM(W(I,*)*V(J,*));
            END;
          END;
          CALL DETSYM(Z,N,MINPIV);
          IF MINPIV <= 0 THEN CALL FAULT(32);
          CALL SOLSYM(Z,N,F);
          DX=F;
          ALFA=1.0E0;
          EPSC=0;
          DO J=1 TO M;
            DC(J)=-SUM(V(*,J)*DX);
            WR=DC(J)/C(J);
            EPSC=MAX(EPSC,ABS(WR));
            IF 1.0E0+ALFA*WR < P0 THEN ALFA=(P0-1.0E0)/WR;
          END;
          IF EPSC < TOLC THEN RETURN;
LC:       DO J=1 TO M;
            LOGC(J)=LOG(C(J)+ALFA*DC(J));
          END;
          FMAX1=0;
          DO I=1 TO N;
            F(I)=LOGK(I)+SUM(V(I,*)*LOGC);
            FMAX1=MAX(FMAX1,ABS(F(I)));
          END;
          IF FMAX1 > FMAX0 THEN DO; ALFA=0.5*ALFA;
                                    GOTO LC;
                                END;
          FMAX0=FMAX1;
          X=X+ALFA*DX;
          C=C+ALFA*DC;
        END;
      PUT LIST(X);
        CALL FAULT(33);
        RETURN;
END ITER;
        DCL FAULT ENTRY (FIXED BIN);
FAULT:  PROCEDURE(ERROR);
        DCL ERROR FIXED BIN;
        PUT SKIP LIST ('ERROR=',ERROR);
        GOTO LAE;
END FAULT;
        DCL (NMAX,MMAX,LMAX) FIXED BIN;
        NMAX=20;
        MMAX=20;
        LMAX=10;
BLOCK1:
BEGIN;
READC:  PROCEDURE;
        /* NON-LOCAL VARIABLES  CHAR,NCHAR; */
        GET EDIT (CHAR) (A(1));
        PUT EDIT (CHAR) (A(1));
        NCHAR=NCHAR+1;
        IF NCHAR=80 THEN DO; NCHAR=0;
                             PUT SKIP;
                         END;
        RETURN;
END READC;
NAME:   PROCEDURE;
        DCL L FIXED BIN;
```

```
        /* NON-LOCAL VARIABLES: ALF,ALFNUM,LMAX,CHAR,SYMB,PREC; */
        IF INDEX(ALF,CHAR)=0 THEN CALL FAULT(10);
        SYMB=CHAR;
        L=1;
        PREC=0;
LA11:   CALL READC;
        IF INDEX(ALFNUM,CHAR)¬=0
           THEN DO; L=L+1;
                    IF L>LMAX THEN CALL FAULT(111);
                    SYMB=SYMB||CHAR;
                    GOTO LA11;
                END;
        IF CHAR = '%' THEN DO; PREC=1;
                               CALL READC;
                           END;
        RETURN;
END NAME;
TERM:   PROCEDURE;
        DCL I FIXED BIN;
        /* NON-LOCAL VARIABLES:     CHAR,COEFF,NUM; */
LA21:   CALL READC;
        IF CHAR = ' ' THEN GOTO LA21;
        COEFF=1;
        I=INDEX(NUM,CHAR);
        IF I ¬=0 THEN DO; COEFF=I;
                          CALL READC;
                      END;
        CALL NAME;
LA22:   IF CHAR= ' ' THEN DO; CALL READC;
                              GOTO LA22;
                          END;
        RETURN;
END TERM;
SIDE:   PROCEDURE;
        DCL (J,JJ) FIXED BIN;
        /* NON-LOCAL VARIABLES:
           MMAX,M,N,SYMB,PREC,TSYMB,TPREC,SIGNUM,COEFF,V;  */

LA31:   CALL TERM;
        DO J=1 TO M;
          IF SYMB=TSYMB(J) THEN
            DO; JJ=J;
                IF TPREC(J) ¬= PREC THEN CALL FAULT(12);
                GOTO LA32;
            END;
        END;
        M,JJ=M+1;
        IF M>MMAX THEN CALL FAULT(112);
        TSYMB(M)=SYMB;
        TPREC(M)=PREC;
LA32:   IF V(N,JJ) ¬=0 THEN CALL FAULT(14);
        V(N,JJ)=SIGNUM*COEFF;
        IF CHAR='+' THEN GOTO LA31;
        RETURN;
END SIDE;
        DCL (TSYMB(MMAX),SYMB) CHAR(LMAX) VAR,
            ALF CHAR(28),NUM CHAR(9),ALFNUM CHAR(37),CHAR CHAR(1),
            (V(NMAX,MMAX),SIGNUM,COEFF) FLOAT BIN(53),
            ((TPREC,ORDEC0) (MMAX),
             PREC,NCHAR,N,M,I,J,M0) FIXED BIN;
```

```
        ALF='ABCDEFGHIJKLMNOPQRSTUVWXYZ()';
        NUM='123456789';
        ALFNUM=ALF||NUM;
        V=0;
        M=0;
        NCHAR=0;
        N=1;
        PUT LIST ('EQUIL. EQUATIONS');
        PUT SKIP(2);
LA41:   SIGNUM=1;
        CALL SIDE;
        IF CHAR ¬= '=' THEN CALL FAULT(121);
        SIGNUM=-1;
        CALL SIDE;
        IF CHAR = ';' THEN
          DO; N=N+1;
              IF N>NMAX THEN CALL FAULT(113);
              GOTO LA41;
          END;
        IF CHAR ¬= '*' THEN CALL FAULT(122);
BLOCK2:
BEGIN;
        DCL VV(N,M) FLOAT BIN(53) DEF V,
            (VP(N,M),(X,KI,LOGK) (N),(C0,C) (M)) FLOAT BIN(53);
        PUT SKIP(2) LIST ('INITIAL CONC.');
        IF NCHAR=0 THEN PUT SKIP(2);
                   ELSE PUT SKIP EDIT (' ') (COLUMN(NCHAR),A(1));
        C0=0;
        M0=0;
LA42:   CALL READC;
        IF CHAR = ' ' THEN GOTO LA42;
LA43:   CALL NAME;
        DO J=1 TO M;
          IF TSYMB(J)=SYMB THEN
            DO; IF C0(J) ¬=0 THEN CALL FAULT(15);
                C0(J)=1;
                M0=M0+1;
                ORDEC0(M0)=J;
                GOTO LA44;
            END;
        END;
        CALL FAULT(16);
LA44:   IF CHAR = ' ' THEN DO; CALL READC;
                               GOTO LA44;
                           END;
        IF CHAR ¬= ';' THEN GOTO LA43;
        PUT SKIP(2);
        DO I=1 TO M0;
          J=ORDEC0(I);
          GET LIST(C0(J));
          PUT EDIT (C0(J)) (X(2),E(11,5,5));
          IF C0(J) <=0 THEN CALL FAULT(17);
        END;
        PUT SKIP(2) LIST ('EQUIL. CONSTANTS');
        PUT SKIP(2);
        DO I=1 TO N;
          GET LIST (KI(I));
          PUT EDIT (KI(I)) (X(2),E(11,5,5));
          IF KI(I) <=0 THEN CALL FAULT(18);
          LOGK(I)=LOG(KI(I));
        END;
```

```
        CALL GUESS(N,M,VV,C0,X,C);
        VP=VV;
        DO J=1 TO M;
          IF TPREC(J)=1 THEN VP(*,J)=0;
        END;
        CALL ITER(N,M,VP,C,X,LOGK);
        PUT SKIP(8) EDIT ('NAME') (X(4),A(4));
        PUT EDIT ('INIT. CONC.') (X(LMAX),A(11));
        PUT EDIT ('EQUIL.CONC.') (X(4),A(12));
        PUT EDIT ('EQUIL. CONST.') (COLUMN(LMAX+61),A(13));
        PUT EDIT ('REACTION EXTENT') (X(5),A(15));
        PUT SKIP;
        DO J=1 TO MAX(N,M);
          PUT SKIP;
          IF J > N THEN GOTO LA46;
          IF TPREC(J)=0 THEN GOTO LA45;
          C(J)=C0(J)-SUM(VV(*,J)*X);
LA45:
          PUT EDIT (J,TSYMB(J),C0(J),C(J))
                   (F(2),X(2),A(LMAX),(2) (X(4),E(11,5,5)));
          IF C(J) <=0 THEN PUT EDIT (' NEGATIVE !') (A(11));
LA46:     IF J > N THEN GOTO LA47;
          PUT EDIT (J,KI(J),X(J))
                   (COLUMN(LMAX+56),F(2),X(4),E(11,5,5),
                                          X(4),E(21,15,15));
LA47:
        END;
END BLOCK2;
END BLOCK1;
LAE:
END EQUIL ;
```

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THE DETERMINATION OF IODINE IN MATERIALS OF BIOLOGICAL INTEREST

A COMPARATIVE EVALUATION OF NEUTRON-ACTIVATION AND AUTOMATED COLORIMETRIC METHODS*

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The iodine content of materials such as diets, excreta and tissues gives information which is often required for metabolic, nutritional and epidemiologic research. However, several analytical methods used so far have proved to be unreliable when applied to substances poor in iodine: in fact, the data available in the literature are scarce and generally rather inconsistent.

Accordingly, it seems useful to report and discuss the analytical methods based on neutron activation and colorimetry (oxidation–reduction catalysis of the As(III)–Ce(IV) system) as developed in these laboratories. The purpose of this work was to establish suitable quantitative techniques of general validity, taking into account the required sensitivity, accuracy and precision, as well as operative simplicity and applicability to routine measurements.

The methods studied are based on quite different principles, so that the comparison of the results of parallel measurements can be considered as a valuable criterion of accuracy. The high analytical sensitivity represents the most attractive feature of activation analysis, while colorimetry is easily adapted to automated procedures with a consequent high precision level.

NEUTRON-ACTIVATION ANALYSIS

The nuclear characteristics of iodine are particularly favourable to highly sensitive neutron-activation procedures based on γ-spectrometry (see Table I). Despite this sensitivity, however, a radiochemical purification of the ^{128}I activity produced is required in practically all cases, except the determination of thyroid iodine. In fact, other activatable elements present at much larger concentrations do not allow the characteristic ^{128}I photopeak to be directly resolved in the composite γ-spectrum of the irradiated samples.

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TABLE I

NUCLEAR CHARACTERISTICS OF IODINE[1,2]

^{127}I target nuclide	Isotopic abundance	100%
	(n, γ) cross-section	5.6 b
^{128}I produced nuclide	Half-life	25.0 min
	Measured γ-ray energy	0.455 MeV
	Measured γ-ray disintegration fraction	0.17

The specificity of the measurement is assured by the independent criteria of the emission energy, half-life and the chemical behaviour of the ^{128}I activity, as well as by the absence of any interfering nuclear reaction.

With regard to the analytical precision, the commonest sources of error in conventional microanalysis, arising from reagent blanks and uncontrolled losses, are avoided with this method: all the chemical treatments are carried out after the irradiation step and a chemically homogeneous yield monitor is added to the sample and submitted to the subsequent operative sequence.

The neutron-activation method, therefore, is to be regarded as highly sensitive, highly accurate and fairly precise; on the other hand, its use is limited by the requirement of specialized centres and operators, and by its unsuitability for routine measurements owing to cost and the difficulty of automation.

INSTRUMENTATION AND MATERIALS

All the irradiations were carried out in the pneumatic facility of the RS1 reactor, at the SORIN Centre, with a thermal neutron flux of $7.5 \cdot 10^{12}$ n cm^{-2} s^{-1}. The activity was measured with a 400-channel pulse-analyzer Laben A-400, and a $3 \times 3''$ well-type NaI(Tl) scintillator (Harshaw). The counting efficiency evaluated for the 0.455-MeV photopeak was 34–43% depending on counting geometry (sample volume), and the background count rate was 50 c.p.m.

All the chemicals used were of analytical grade; carrier-free ^{125}I for yield evaluations was supplied by CEA-CEN-SORIN.

METHODS

Particular analytical procedures were developed for the different materials. However the general procedure consisted of the following steps: irradiation for selected periods of samples in small polyethylene capped containers, along with comparison standards (calibrated potassium iodide solutions evaporated on filter paper disks, 0.10–10 μg per disk); radiochemical treatment of the samples after the addition of known amounts of non-interfering carrier-free ^{125}I (60-d half-life, 28-keV conversion X-ray) in the iodide form; and counting of the separated ^{128}I activity of the samples and the unprocessed standards. The chemical yield was accounted for by measuring the ^{125}I activity recovered, when necessary, after a waiting time of some hours. A correction factor was previously experimentally evaluated to account for the difference in counting conditions between standard and any final form of processed samples. The local flux variations were corrected, if necessary, by averaging the

measurements of any couple of standards between which the sample was interposed during the irradiation.

Distillation technique

The distillation technique after digestion with chromic acid and reduction of iodate to volatile iodine[3,4] is the basis of a ^{128}I separation method of general validity, that was employed here, particularly for solid samples. The simple addition of a reducing agent such as phosphorous acid, as reported in the literature[3,4], was found to be inadequate to ensure complete distillation, especially when milligram amounts of iodide were added as carrier in order to visualize the iodine evolution. A modification was therefore made, by adding a nitrite solution either simultaneously with or after the phosphite introduction; probably through the oxidation of some iodide present, quantitative iodine distillations were obtained in both cases (97% and 99.5%, respectively). The distilled iodine was recovered in an alkaline scrubber, which was more concentrated than usual to account for the acidic nitrogen oxide vapours evolved. Despite the completeness of the distillation from the flask, some losses (about 15%) were unavoidable, probably because of inefficient trapping in the scrubber. However, the actual recovery was considered satisfactory, hence no attempt was made to improve it. A strong anionic resin was added to the scrubber in order to concentrate the ^{128}I activity by adsorption; when a further purification was necessary (samples with a high sodium chloride content), the resin was washed with a nitrate solution to elute the residual ^{38}Cl activity before counting.

The chemical yields ranged from 80 to 90% (60–70% when the resin was further purified), and decontamination factors up to $5 \cdot 10^4$ were obtained for ^{24}Na (remained undistilled) and ^{38}Cl and Br radionuclides (previously volatilized during the chromic acid attack): in Fig. 1A the effectiveness of the procedure for a diet sample is shown.

A check of the method was performed with biological samples labelled *in vivo* with ^{131}I, adding ^{125}I as iodide and comparing the behaviour of the two radionuclides; an agreement within 5% resulted in all cases (Fig. 2).

Procedure. Irradiate for 5–20 min the samples corresponding to 0.1–0.5 g of dry substance. Add ^{125}I-iodide plus 5 mg of iodide carrier (1 ml of solution) and digest in a mixture of 50 ml of concentrated sulfuric acid and 5 ml of aqueous saturated chromic oxide solution, boiling for 5 min in the open distillation flask. Cool the flask with compressed air and dilute with about 50 ml of water. Connect up the condenser and add from the side-reservoir 30 ml of phosphorous acid–sodium nitrite solution (about 8 and 2 g in 25 and 5 ml, respectively). Distil until the evolution of violet iodine vapours is observed (about 1 min boiling), recovering the distillate in 10 ml of 20% sodium hydroxide containing about 3 g of Dowex 2-X8 resin (–OH form). Shake with the resin for some seconds, decant and, if a further purification is required, wash twice with 10 ml of 0.5 *M* sodium nitrate; count the decanted resin for 3–5 min.

Isotopic-exchange procedure

In order to shorten the anion-exchange procedure for iodide solutions[5], a single-step isotopic-exchange technique was developed. Elemental iodine is irreversibly fixed on strong anionic resin and the isotopic exchange between iodide ions in the solution and iodine fixed on the resin occurs very rapidly; therefore, if a resin column in the iodine form is used, drastic elution conditions for the interfering halide ions

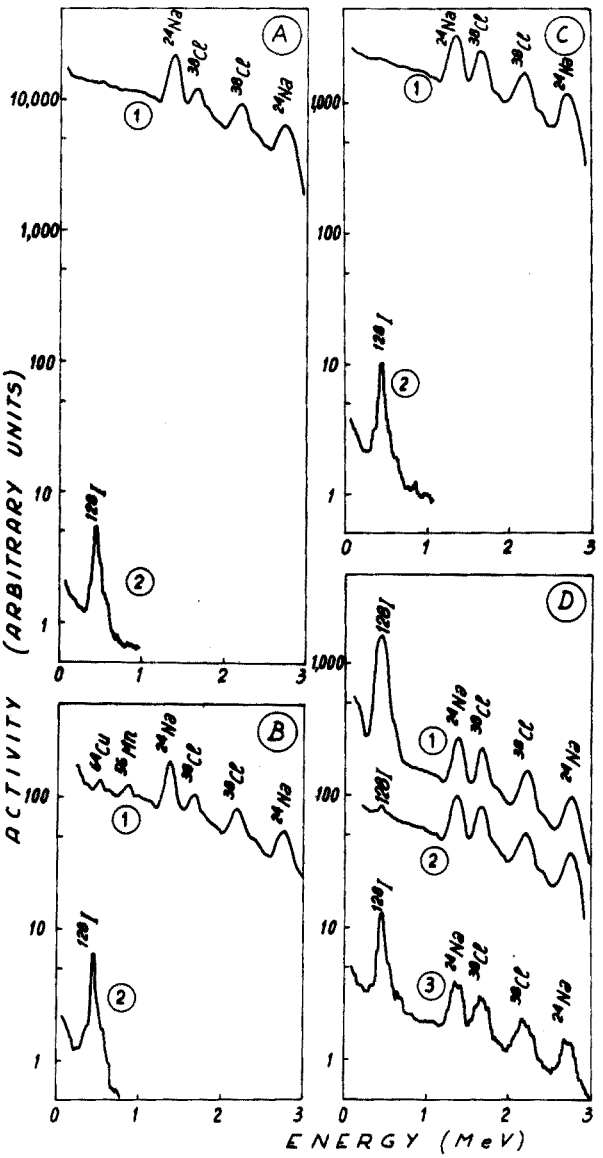
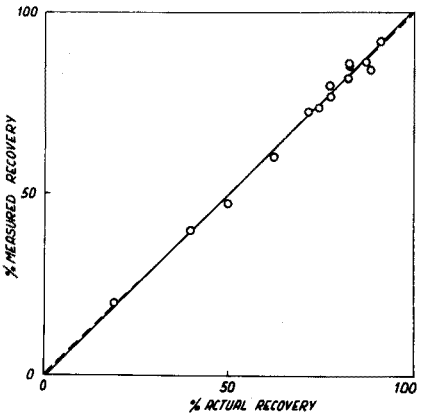


Fig. 1. Spectra of neutron-irradiated samples and of separated ^{128}I activity. (A) Homogenized food: (1) untreated sample; (2) separated ^{128}I (distillation procedure). (B) Urine: (1) untreated sample; (2) separated ^{128}I (isotopic-exchange procedure). (C) Water: (1) untreated sample; (2) separated ^{128}I (isotopic-exchange procedure). (D) Thyroid (non-destructive analysis): (1) normal gland (human); (2) iodine-deficient gland (mouse); (3) the same, irradiated under cadmium cover.

can be used without any appreciable ^{128}I loss. The decontamination attained with this procedure for chloride and bromide activities is shown in Fig. 3; among the other interfering activities, ^{24}Na and ^{56}Mn are not retained on the resin and their levels were found to be decreased to $5 \cdot 10^{-3}$ and 0.5%, respectively, after 10 min. In contrast, ^{64}Cu is retained probably through reduction to copper(I) (reductant residues on the



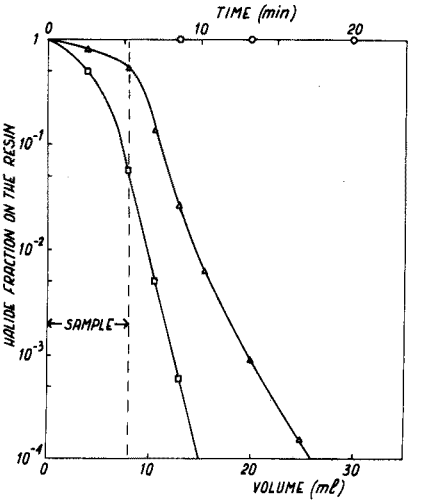


Fig. 2. Check for the distillation procedure. The actual recovery was calculated from the ^{131}I activity used for *in vivo* tracing, while the measured recovery was evaluated from the ^{125}I activity added as iodide before the chemical treatment. The high values refer to the adopted experimental conditions, the low ones refer to treatments carried out without adding nitrite. The calculated regression curve is represented by the solid line, while the theoretical equivalence is indicated by the dotted line.

Fig. 3. Iodide purification by isotopic-exchange procedure. (○) Iodide; (△) bromide; (□) chloride.

resin) and insoluble complex formation; the addition of ethylenediamine to mask copper(II) was found to be effective for a complete ^{64}Cu separation (0.2% residual activity on the resin after 10 min).

The isotopic-exchange procedure can be applied only to iodide solutions; if ion species are present which exchange slowly (*e.g.* iodate), they escape measurement. The procedure can nevertheless be used for protein-bound iodine (*e.g.* plasma iodine), since the ^{128}I activity produced is recovered quantitatively in the iodide form after suitable irradiation[5]. This possibility was experimentally confirmed as reported below.

The isotopic-exchange method led to 95–100% recoveries and was found to be effective for most of the samples in fluid form: in Figs. 1B and 1C the ^{128}I separation from irradiated urine and water samples is shown.

Batches of resin in the iodine form were prepared by iodide adsorption on Dowex 1-X10 (50–100 mesh, –OH form, up to 50 mg of iodide per g of wet resin) followed by nitrite oxidation and washing with water. Storage for several months in the dark of the resin prepared in this way did not cause deterioration of the resin. For the standard procedure, columns of 70 cm height and 0.5 cm diameter (resin bed) were used, with elution at a rate of *ca.* 1.5 ml min^{-1}.

Procedure. The technique varies somewhat according to the material involved. Irradiate 5–10 ml of water containing 0.025 ml of high-purity ammonia liquor for 20 min. Irradiate 0.5–2 ml of biological fluid (plasma samples diluted with equal volumes of deionized water to avoid possible protein coagulation) for 5 min. Add 3.5 ml of a solution containing ethylenediamine (1 ml), 4 *M* sodium nitrate (1.5 ml) and 9 *M* sulfuric acid (1 ml) plus holdback carriers (0.15 mg Cl^-, 0.075 mg Br^-, 1 mg Cu^{2+}

and 0.1 mg Mn^{2+}) and ^{125}I as iodide, for each 5 ml of irradiated water sample, and pass through resin previously conditioned with 4 *M* sodium nitrate. Pass the irradiated biological fluids directly through the resin, washing with 5 ml of water. In both cases, use 5–10 ml of 4 *M* sodium nitrate to wash the resin, and count directly on the resin bed for 2–5 min.

Particular problems connected with liquid samples

When water samples are to be analyzed, some losses can occur through adsorption on the polyethylene container walls during irradiation[5]. The experiments summarized in Fig.4 indicate a pH dependence and exclude the possibility of contribution from the Szilard–Chalmers reaction, at least for the irradiations selected. In fact a close correspondence resulted for the behaviour of the ^{128}I produced and of stable iodine, as monitored by ^{125}I added in the iodine form before the irradiation.

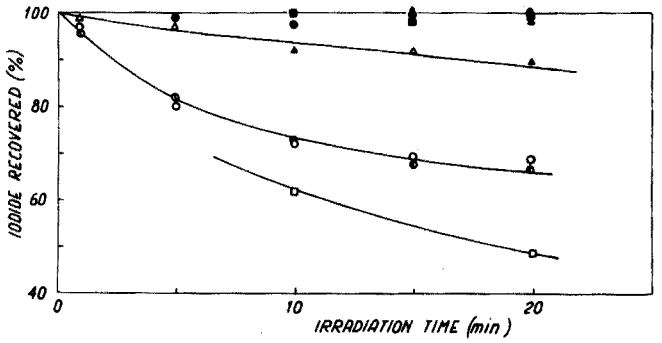


Fig. 4. Iodine losses during irradiation of water samples (adsorption on polyethylene container walls). (△) Drinking water at pH 7.5 (^{128}I activity); (○) deionized water at pH 6 (^{128}I activity); (□) deionized water at pH 5 (^{128}I activity); (▲, ●, ■) same samples containing 0.25% ammonia (^{128}I activity); (Φ) deionized water at pH 6 (stable iodine, as monitored by ^{125}I iodide tracer).

These data suggest that an iodide oxidative process takes place under irradiation, with a subsequent iodine adsorption onto the polymer. Unfavourable pH conditions for iodide oxidation were therefore adopted, by adding some ammonia to the water samples to be irradiated (see Procedure above), in order to ensure quantitative recoveries in all the cases, though satisfactory yields can be expected for the usual pH values of drinking water.

As for the reliability of the isotopic-exchange procedure for biological fluids, some experimental work was carried out to study the behaviour of protein-bound iodine (PBI) under irradiation and to establish suitable irradiation conditions for the recovery of the ^{128}I activity as iodide.

The fact that the ^{128}I produced is found as iodide can be accounted for either by a Szilard–Chalmers reaction of the "organic" iodine (thermal neutrons) or by the rupture of the iodine–protein bonds during irradiation (mainly γ-ray dose rate which was evaluated as $7.5 \cdot 10$ rad h^{-1}, or possibly fast neutrons, corresponding to a measured flux of $1.5 \cdot 10^{12}$ n (≥ 1 MeV) $cm^{-2} s^{-1}$).

The contribution of both processes was evaluated by comparing the iodide fractions of stable iodine and ^{128}I after different irradiation periods. Total ^{128}I was

obtained by means of the distillation method (see above) and ^{128}I in the iodide form by a double purification to ensure a more effective separation (trichloroacetic acid precipitation followed by ion exchange), in the presence of carrier iodide and ^{125}I-yield monitor in all cases. For stable iodine, a tracer technique was used by labelling plasma with ^{125}I-thyroxine (which accounts for about 90% PBI); labelled thyroxine was added at concentrations of 1–20 μg per 100 ml and after a 1-h incubation at room temperature, the plasma samples were submitted to exhaustive dialysis. The behaviour of stable iodine was then followed by measuring the ^{125}I activity of the irradiated samples after a suitable decay time; both electrophoresis and the chemical separations as for ^{128}I were used, and ^{131}I was added as yield monitor for the latter procedure. The two procedures were found to agree within $\pm 3\%$ and blank checks on non-irradiated labelled plasma showed negligible radiolytic contributions (less than 2%).

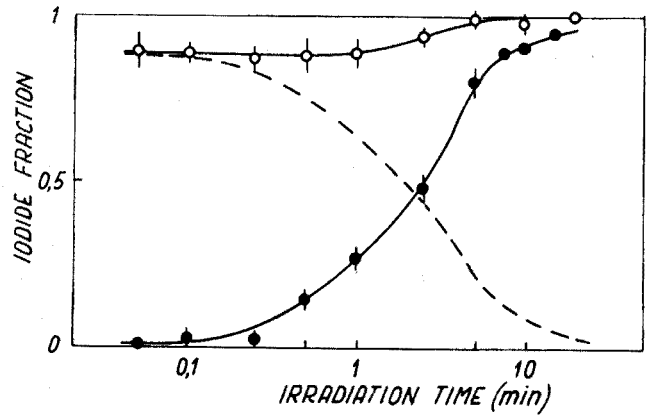


Fig. 5. Recovery of protein-bound iodine in iodide form after plasma irradiation. (○) ^{128}I activity; (●) stable iodine (as monitored by ^{125}I-thyroxine); the contribution of the Szilard–Chalmers reaction to the overall recovery is represented by the broken line.

As shown in Fig. 5 both the Szilard–Chalmers reaction and disruption of protein-bound iodine were found to take place; as expected, the contribution of the former process proved to be more important for shorter irradiations, while the latter process was found to prevail for the periods selected for the analysis. Quantitative recoveries of PBI in the iodide form were obtained, in the experimental conditions, even for irradiations of only about 5 min.

Non-destructive analysis

Owing to the particularly high iodine content, a fast and precise direct γ-spectrometric determination is feasible when thyroid gland, as whole organ, fragments or homogenates, is to be analyzed (see Fig. 1D).

In some less favourable cases, the precision of non-destructive analysis can be improved by irradiating in epithermal neutrons (flux of $9 \cdot 10^{10}$ n cm^{-2} s^{-1} in the pneumatic facility). Owing to the high resonance integral of iodine (experimentally evaluated as 185 b), in fact, a low cadmium ratio results for this element compared to the cadmium ratios of the interfering elements Cl, Na, Mn, Cu, etc. (see Table II).

TABLE II

CADMIUM RATIOS FOR IODINE AND SOME INTERFERING ELEMENTS[a]

Element (radionuclide)	R_{Cd}
Iodine (^{128}I)	4.2 ± 0.1
Chlorine (^{38}Cl)	130 ± 4
Sodium (^{24}Na)	125 ± 5
Manganese (^{56}Mn)	75 ± 4
Copper (^{64}Cu)	80 ± 3

[a] Ratios of the activities obtained by irradiation without and with cadmium lining, as experimentally evaluated for 0.05–0.5 (mg cm^{-2}) thick sources.

TABLE III

GAIN FACTORS FOR ^{128}I WITH EPITHERMAL NEUTRONS[a]

Element	*ml sample solution*			
	0	2	5	10
Chlorine	31	8.8	8.0	6.2
Sodium	29	8.	7.7	4.9

[a] Ratio between the R_{Cd} values of iodine and interfering element.

Therefore, the iodine activation may be rendered more selective when cadmium-lined samples are irradiated: even with aqueous solutions, noteworthy gain factors for the iodine activation were found, in spite of the flux rethermalization in water, as seen in Table III.

The selective epithermal-neutron activation was used occasionally for some tissue samples with abnormally high iodine contents ($\geqslant 1$ p.p.m.), but it can be regarded as a technique of more general validity for analysis of thyroid extracts and chromatograms and for iodine-deficient thyroid glands (see Fig. 1D).

In the case of normal tissues, samples corresponding to 5–15 mg wet weight were irradiated for 3–10 s and counted directly in the irradiation container after waiting for some minutes (0.5-min counting at a sample–detector distance of 10 cm). When epithermal-neutron activations were necessary, the samples and the standards were irradiated for longer periods (up to 5 min) in individual 0.2-mm thick cadmium cylindrical boxes placed in the usual polyethylene containers, transferred to the counting vials and counted in the well for 0.5–1 min.

Analysis of sodium chloride

The determination of the iodine content of sodium chloride samples (*e.g.* common salt) sets particular problems because of the matrix activation; the activated samples readily dissolved in water can be treated as iodide solutions. A combination of the epithermal-neutron activation and the isotopic-exchange separation was therefore used, in order to keep within reasonable levels the global activity produced without affecting drastically the measurement sensitivity, and to utilize a simple separative technique.

The samples (0.5–1 g) were irradiated under cadmium lining for 5 min, dissolved in 3 ml of water containing ^{125}I as iodide, passed through the column and washed with 30–40 ml of 4 *M* sodium nitrate.

METHOD EVALUATION

The minimal detectable amount of iodine can be evaluated for the activation methods by taking into account the experimental conditions for any particular procedure (build-up of the activity and decay factor corresponding to the irradiation periods selected and the operative time required for separation, counting efficiency and counting time). If a ^{128}I activity double the counting background is assumed as the minimal measurable activity, the limit of sensitivity is $3.5 \cdot 10^{-4}$, $7 \cdot 10^{-4}$ and $11 \cdot 10^{-4}$ μg of iodine for water, solid biological materials and biological fluids, respectively (for a 5 min counting time). The related minimal concentrations, accounting for sample weight and chemical losses, can be evaluated as 0.035 p.p.b. for water, 0.35 p.p.b. for biological fluids and 3 p.p.b. for solid material. It must be noted, however, that the actual sensitivity strongly depends on the degree of purification obtained in any individual assay, since the residual foreign activities affect the ^{128}I counting.

The activation method can be considered as extremely accurate, since the use of a comparative standard avoids systematic errors arising from the uncertainty related to the values of the nuclear parameters involved, and no spurious contribution results from interfering nuclear reactions.

The sources of random errors are mainly represented by the counting statistics and by the definition of the ^{128}I photopeak (up to 20% uncertainty), as low activities are often involved. As for other statistical errors affecting the method, the precision related to the operative steps was experimentally evaluated. Errors of 1–2% resulted from a series of measurements (20–50 tests) on standard preparation; with the correction factors for flux topographic inhomogeneity, adsorption on the container walls during irradiation, determination of chemical yields and normalization of counting

TABLE IV

PRECISION OF NEUTRON-ACTIVATION METHOD[a]

Material	*Mean concentration*	*Standard deviation*	*Coefficient of variation (%)*
Water	0.63 p.p.b.	0.092 p.p.b.	14.8
	1.5	0.12	7.9
	5.3	0.42	7.9
	10.2	0.71	7.1
Biological fluids	3.2 μg/100 ml	0.27 μg/100 ml	8.5
(plasma, urine)	6.3	0.25	4.0
	8.4	0.46	5.5
	13.5	0.68	5.0
Solid biological material	6.0 μg/100 g	1.41 μg/100 g	23.5
(tissues, stools, foods)	11.5	2.08	18.1
(dry weight)	46	4.9	10.6
	111	11.3	10.2
Thyroid gland	398 p.p.m.	11.5 p.p.m.	2.9
(wet weight)			

[a] Expressed as reproducibility of 10 determinations for each sample.

conditions for sample and standard, an overall uncertainty amounting to 3–5% can be ascribed to the method when the ^{128}I activity is measured without appreciable errors (high iodine concentrations). The overall precision from series of replicate determinations on the same sample is shown in Table IV.

With regard to the time required for the analysis, a single trained operator was able to perform 15–20 determinations with the isotopic-exchange procedure and 8–12 determinations with the distillation method within 8 h (irradiation, chemical treatment and counting).

AUTOMATED COLORIMETRY

The colorimetric method originally proposed by Sandell and Kolthoff[6], based on the oxidation–reduction catalysis of the arsenic(III)–cerium(IV) system, is widely used for microdeterminations of iodine. The main problem of the mineralization of biological samples has been overcome by various different procedures: among these, the digestion with the oxidizing sulfuric–nitric–perchloric acid mixture was adapted to an automatic process suggested by Technicon (Tarrytown, N.Y.) as a standard procedure for PBI measurement with the Auto-Analyzer. The method is also suitable for urinary iodine determinations, and represents a useful tool for clinical routine measurements and physiological studies. Besides the advantages given by speed and operative simplicity, the automated procedure enables a high precision level to be obtained; for the critical experimental parameters (volume delivery and reaction time) are strictly controlled, while contaminations from sample carry-over can be kept within negligible levels by interposing washing water in the sample sequence.

However, when materials poor in iodine are concerned, the sensitivity and the precision of the Technicon method are no longer adequate. Accordingly, modifications of the standard automated method were made in order to provide suitable procedures for low iodine concentrations. Owing to the operative difficulty of a whole automation for analysis of solid samples, a simple semi-automated procedure was developed for this case.

The colorimetric techniques were evaluated against neutron activation as a reference method, owing to its high accuracy.

Instrumentation and materials

Technicon Auto-Analyzer modules for PBI measurements were used. The reagent-grade chemicals and bidistilled or deionized water were checked for their iodine content when used for the determination of low-iodine contents.

Improvement of analytical sensitivity

The measured cerium(III) concentration, resulting from the catalytic action of iodine ions, depends on several parameters, such as acidity, reaction temperature and time, arsenic(III) and cerium(IV) concentrations. When iodine is present as iodate, a previous reduction is required since iodide represents the catalyzing species; therefore, the dependence of the analytical response on the chemical form of the iodine must also be considered. The influence of some experimental conditions is shown in Figs. 6 and 7.

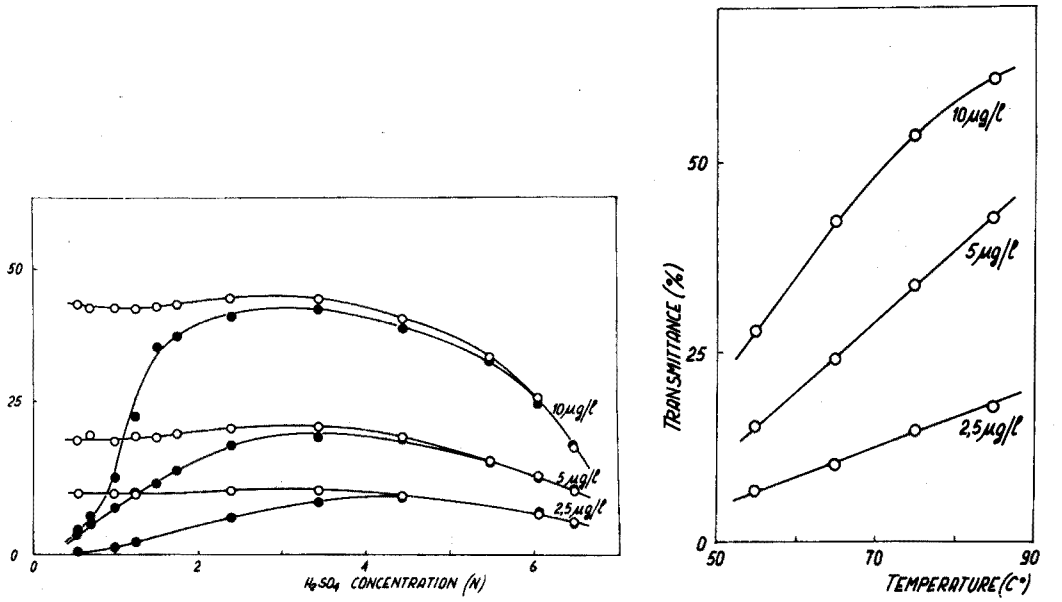


Fig. 6. Effect of acidity and iodine chemical form on the response of the arsenic(III)–cerium(IV) system (2.9-ml sample, 65°). (○) Iodide; (●) iodate. The blank value of transmittance (base-line value) has been subtracted in each case.

Fig. 7. Effect of temperature on the response of the arsenic(III)–cerium(IV) system (5-ml sample of iodide solutions, 0.3 *M* H_2SO_4). The blank value of transmittance (base-line value) has been subtracted in each case.

In order to enhance the sensitivity, *i.e.* the measured response of the system for lower iodine concentrations, the reaction parameters can be optimized. However, with regard to the acidity and iodine chemical form, the conditions are set by the preliminary digestion step. Addition of water after the sample mineralization is included in the standard Technicon scheme, in order to dilute the oxidizing attack

TABLE V

EFFECT OF INCREASING SAMPLE VOLUME, COMPARED WITH THE STANDARD PBI METHOD[a]

Material	*Sample volume (ml)*	*Number of determinations*	*Ratio with standard PBI method* (±S.D.)
Urine	0.6	8	1.01±0.04
	0.8	8	1.00±0.02
	1.2	7	0.92±0.11
Plasma	0.6	16	0.99±0.03
	0.8	10	0.88±0.13
Plasma diluted 1:5	0.6	9	0.97±0.04
	0.8	10	0.98±0.04
	1.2	10	1.00±0.04

[a] The results obtained for different sample volumes are compared with those found with parallel determinations by the PBI Technicon method (0.32 ml samples); the concentrations ranged from 2.5 to 10 µg/100 ml for both urine and undiluted plasma.

mixture and to reach more favourable conditions of acidity; of course, a parallel dilution of iodine concentration in the reaction volume is also obtained. The use of larger liquid samples, reducing accordingly the amount of water, can be useful to increase the sensitivity of the system, provided that the more diluted digestion mixture is still suitable for a complete digestion. Table V shows the results of an experimental check: 0.6 ml of plasma, 0.8 ml of urine and at least 1.2 ml of *ca.* 1.5% protein solution (1:5 diluted plasma) were found to be perfectly compatible with the resulting attack conditions. The response of the system can be further enhanced by using a larger proportion of the solution leaving the digestor for the subsequent steps.

With regard to the other parameters governing the catalytic reaction, increase of temperature was found to be effective in improving the sensitivity, compared with the standard Technicon method (55°). No attempt was therefore made to obtain a further increase in sensitivity by prolonging the reaction time. Although the base-line stability depends to some extent on the reaction temperature, the resulting increase in uncertainty is negligible compared to the sensitivity gain.

The calibration curves obtained by combining the effects of increased sample volumes and higher temperature are shown in Fig. 8 in comparison with those related to the standard Technicon method.

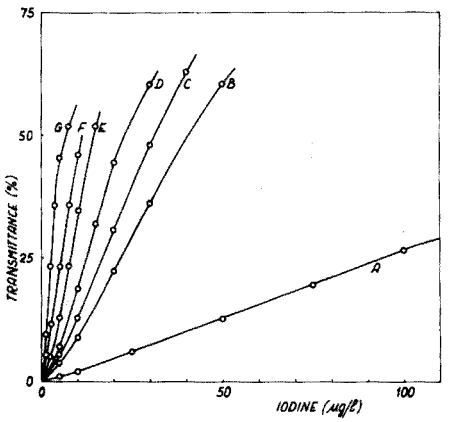


Fig. 8. Calibration curves obtained by different colorimetric procedures. (A) Standard Technicon method for PBI measurement (0.32-ml sample, digestion, 55°). (B) Procedure for plasma samples (0.6-ml sample, digestion, 85°). (C) Procedure for urine samples (0.8-ml sample, digestion, 85°). (D) Procedure for 1.5% protein solutions (1.2-ml sample, digestion, 85°). (E) Procedure for 1.5% protein solution or total iodine in water (same procedure as above, with 5 ml of the solution leaving the digestion instead of 2.9 ml). (F) solutions (5-ml lample, no digestion, 75°, 0.65 *M* H_2SO_4). The blank value of transmittance (base-line value) has been subtracted in each case.

Methods

Depending on the material to be analyzed and on the iodine concentrations involved, different procedures corresponding to the calibration curves shown in Fig. 8 were adopted. The procedures are based on the general schemes reported in Fig. 9.

Analysis of biological fluids

The standard Technicon procedure is most generally suitable for plasma and urine samples: in some cases, however, a higher sensitivity and a higher precision

	SAMPLER	PUMP n°1	DIGESTOR	PUMP n°2	HEATING BATH	COLORIMETER	RECORDER	SAMPLING SPEED CUPS PER HOUR
A		SAMPLE 0,32 ml		DIGESTED SAMPLE 2,9 ml	55°			30
B		" 0,6 ml		" 2,9 ml	85°			30
C		" 0,8 ml		" 2,9 ml	85°			30
D		" 1,2 ml		" 2,9 ml	85°			30
E		" 1,2 ml		" 5 ml	85°			30
F		NO	NO	SAMPLE 5 ml	75°	3M H_2SO_4		50
G		NO	NO	SAMPLE 5 ml	75°	0.65M H_2SO_4		50

the actual number of determinations is half the figure indicated, cups of water being alternated with sample cups.

Fig. 9. Operative schemes for automated colorimetry.

level for low concentrations can be useful. In these cases, the operative conditions corresponding to a reaction temperature of 85° and to samples of 0.6 and 0.8 ml for plasma and urine, respectively, were employed (see the calibration curves B and C of Fig. 8 and the related procedure scheme of Fig. 9). The more sensitive procedures based on the use of 1.2-ml samples and 85° reaction temperature (calibration curves D and E) were set up for particular analyses of protein solution (*e.g.* analysis of chromatographic fractions, extracts, etc.).

Analysis of drinking water

On the assumption that iodine is present in drinking water and in natural springs as iodide, very sensitive methods can be envisaged by excluding the digestion step, thus enabling the treatment of larger sample volumes and the attainment of more favourable acidity conditions without excessive dilution of samples. The techni-

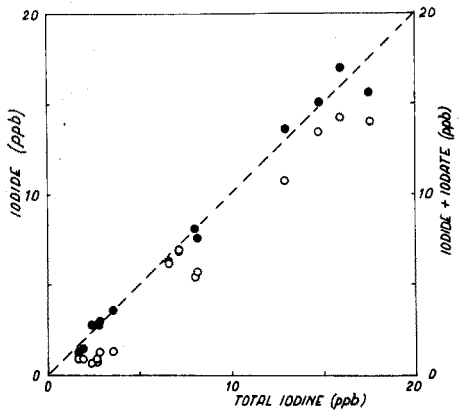


Fig. 10. Comparison of the results obtained for the analysis of drinking water by the procedure for iodide, iodide-iodate and total iodine determination. (○) Iodide; (●) iodide+iodate.

que based on this assumption, reported by Keller *et al.*[7], was somewhat modified in order to obtain the highly sensitive analytical response corresponding to the calibration curve shown in Fig. 8G.

Actually, the hypothesis made for the iodine chemical form was not confirmed by a comparison with a parallel determination of total iodine, carried out by the procedure of Fig. 9E. On the contrary, when the results obtained by operating without sample digestion at a sulfuric acid concentration of *ca.* 3 *M* (Fig. 9F and Fig. 8F) were compared, substantial agreement was found with the values of total iodine. Figure 10 shows the results of the iodine determinations performed by the three procedures; Fig. 11 shows some of the related recordings. In 3 *M* sulfuric acid, the analytical response to iodide and iodate practically coincides (Fig. 6) because of the high reduction rate of iodate to the catalytic species, hence the presence of an iodate fraction in the drinking water samples examined is strongly suggested. These un-

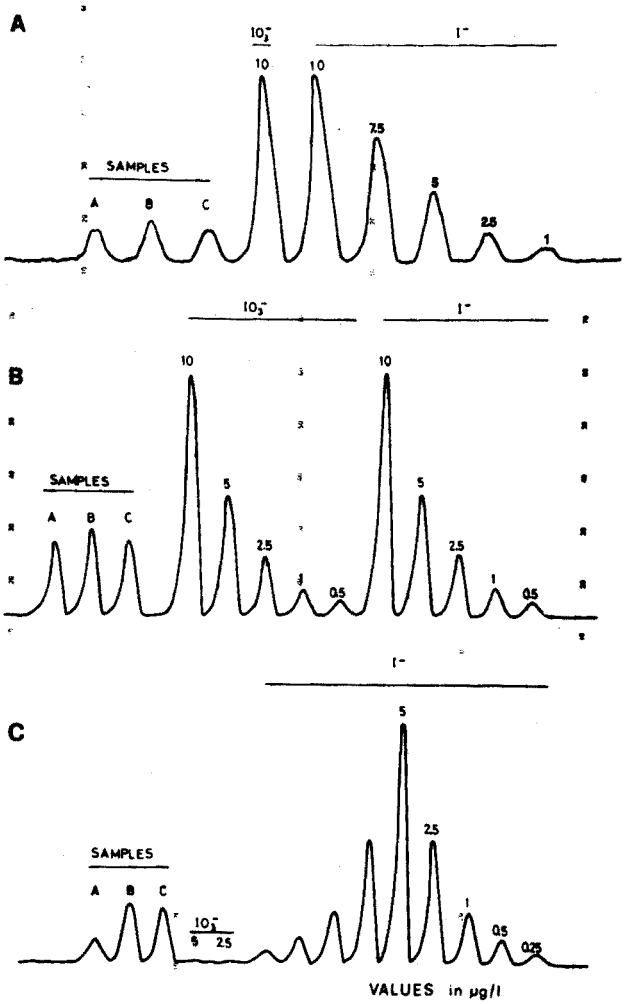


Fig. 11. Recordings obtained with automated colorimetry (water samples). (A) Iodide analysis (scheme of Fig. 9G). (B) Iodide-iodate analysis (scheme of Fig. 9F). (C) Total iodine analysis (scheme of Fig. 9E).

expected findings for water analysis suggest that the procedures corresponding to the operative schemes of Fig. 9E or 9F are advisable, despite their lower sensitivity.

Analysis of sodium chloride

Chloride ions are known to exert a weak catalytic action on the arsenic(III)–cerium(IV) system (Fig. 12, curve A) and to modify the analytical response to increasing iodide concentrations (Fig. 12, curve C compared to curves A and B). When high chloride concentrations are involved, *e.g.* in brine solutions, these effects cannot be neglected, and an equal amount of chloride must be added to the calibration iodide solution. The procedure simply consists of dissolving the sodium chloride sample at a selected concentration, preparing the calibration solutions at the same chloride content, and adopting the scheme used for iodide solutions in Fig. 9G. The main problem lies in finding an iodine-free chloride salt for preparing the calibration curve; most of the usual reagent-grade chloride salts had to be discarded. The use of a potassium chloride solution prepared from reagent-grade hydrochloric acid and potassium hydroxide (Merck) was eventually adopted (less than 0.01 μg of iodine per g of KCl, as evaluated by activation analysis).

In Fig. 12 two calibration curves for sodium chloride analysis at different concentrations are shown (curves D and E).

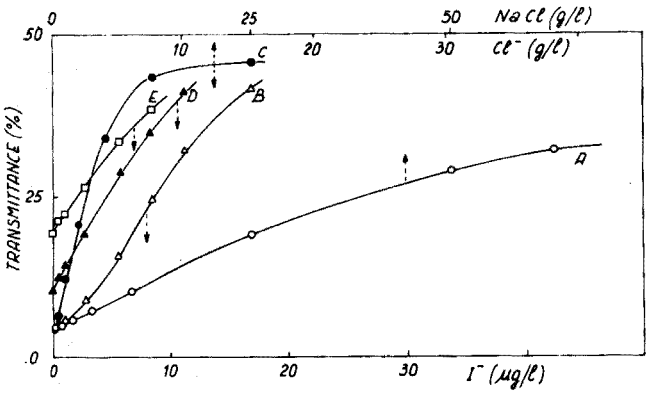


Fig. 12. Effect of chloride on the automated colorimetric system and calibration curves for sodium chloride analysis. (A) Effect of increasing Cl^- concentrations (upper abscissa scale). (B) Effect of increasing I^- concentrations (lower abscissa scale). (C) Effect of parallel increase of Cl^- and I^- concentrations (both abscissa scales). (D) Calibration curve for sodium chloride analysis: 10 g NaCl l^{-1} (lower scale), sensitivity 15 p.p.b. (E) Calibration curve for sodium chloride analysis: 25 g NaCl l^{-1} (lower scale), sensitivity 6 p.p.b.

Analysis of dried biological materials

A semi-automated procedure was studied for solid samples including an off-line digestion step in sulfuric–nitric–perchloric acid mixture and subsequent treatment of the resulting iodate solution by the scheme reported in Fig. 9F (calibration curve, Fig. 8F). Different methods were attempted for the mineralization of samples, including alkaline attack, the Mahler bomb, and a low-temperature dry asher; however, the acid mixture was found to be the most suitable technique as far as blank values, chemical yields and time were concerned. The manual mineralization procedure can affect the analytical sensitivity and precision to a larger extent than the

automated digestion, owing to the difficulty of controlling the reagent blank fluctuations. Acceptably low and constant blank values were obtained by sweeping the resulting solution with a nitrogen stream.

For the adopted procedure, samples corresponding to 0.1 g of dry weight were digested in glass tubes with 3.5 ml of concentrated sulfuric acid, 0.1 ml of concentrated nitric acid and 0.4 ml of 70% perchloric acid by heating in an electric oven at 270° for 25 min; at the end of the attack, nitrogen was bubbled into the tubes for some minutes. The clear solutions obtained were diluted to 15 ml with deionized water and transferred to the Auto-Analyzer sampler. No chemical yield correction was made, since quantitative recoveries were constantly found from a preliminary experimental check ($96 \pm 4\%$ recovery for 35 different samples using both ^{131}I-iodide and ^{125}I-thyroxine as yield monitor); a blank value corresponding to 0.2 ± 0.2 μg of iodine per 100 g of dry substance was found. The calibration solutions were processed in parallel throughout the whole procedure.

Method evaluation

The analytical sensitivity of the automated colorimetric procedures was evaluated by taking into account the precision related to the blank of the methods (base-line fluctuation and reagent blank). The minimum detectable iodine concentrations were evaluated as 5.0 p.p.b. for the standard Technicon method for PBI and, for the modified procedures, as low as 1.2 p.p.b. for plasma, 0.6 p.p.b. for urine, 0.35 p.p.b. for total iodine in water and protein solutions up to about 1.5%, 0.1 p.p.b.

TABLE VI

PRECISION OF AUTOMATED COLORIMETRY[a]

Material	*Mean concentration*	*Standard deviation*	*Coefficient of variation* (%)
Water	0.42 p.p.b.	0.033 p.p.b.	7.8
	1.8	0.10	5.4
	3.6	0.12	3.4
	6.3	0.22	3.5
	12.4	0.48	2.9
Biological fluids (plasma, urine)	1.3 μg/100 ml	0.09 μg/100 ml	7.1
	2.8	0.15	5.5
	5.3	0.20	3.8
	8.6	0.26	3.0
	15.7	0.52	3.3
Dried biological materials (tissues, stools, foods, milk)	4.5 μg/100 ml	0.77 μg/100 g	17.0
	10.9	0.83	7.6
	43	2.4	5.5
	108	3.5	3.2
Common salt	0.068 p.p.m.	0.0105 p.p.m.	14.5
	0.13	0.009	6.7
	1.01	0.017	1.7

[a] Expressed as reproducibility of 10 determinations for each sample.

and 0.05 p.p.b. for iodide-iodate and iodide solutions in water, respectively, and 5 p.p.b. for dried biological samples.

A high precision was obtained in all cases, within the concentration ranges examined, as indicated by the reproducibility tests reported in Table VI.

With regard to accuracy, various possible interferences are known, resulting in both low results (mercury, silver, cyanide, which form stable compounds with iodide) and high results (osmium, chloride, bromide which also exert a catalytic action, and organic compounds which affect the oxidation–reduction system). However, the method can be considered as highly selective, since interfering concentrations of these species can be mostly excluded or easily controlled. The comparison with neutron activation, assumed as an unbiased reference method, confirmed the absence of systematic errors (see Fig. 13).

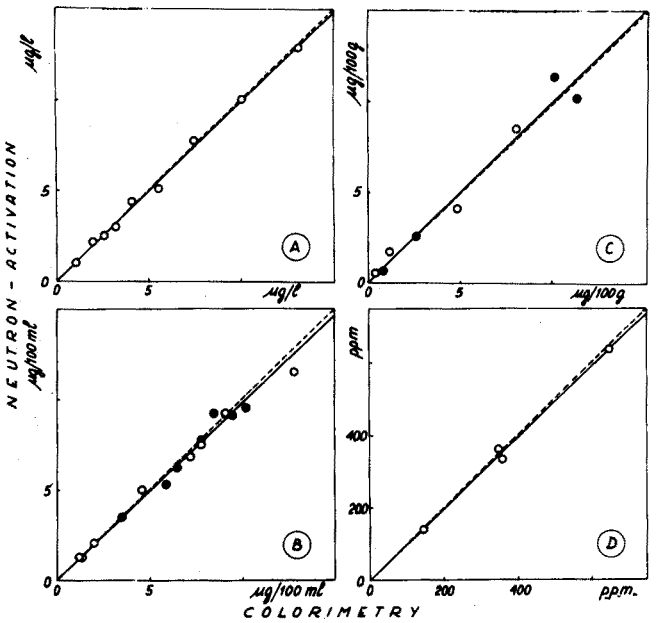


Fig. 13. Comparison of the results obtained by parallel determinations with automated colorimetry and neutron-activation analysis. (A) Water. (B) Biological fluids: (○) plasma, (●) urine. (C) Solid biological samples: (○) tissues, (●) homogenized diets. (D) Thyroid gland homogenates. The solid lines refer to the calculated regression curves while the dotted lines represent the theoretical equivalence.

DISCUSSION AND CONCLUSIONS

Both neutron-activation analysis and automated colorimetry were found to be entirely suitable methods for the materials studied and the concentrations involved. Obviously, as is usually required by any microanalytical method, care must be taken to avoid possible errors related to the successive stages of sample collection and storage and, for colorimetry, reagent selection and check.

As an example, the use of the surfactant (Brij-35), recommended by Technicon to insure a good bubble pattern, was avoided because of its high iodine content

TABLE VII

EFFECT OF STORAGE ON LIQUID SAMPLES

Material	*Storage conditions*	*Measurement method*	*No. of samples*	*Days of storage*			
				6	15	30	90
Water	*Polyethylene bottle*						
	room temperature, pH 4.5	Tracer I^-	1				82
	pH 5.5–6	Tracer I^-	3				91 ±1
	pH 7–7.5	Tracer I^-	3				94 ±3
	NH_4OH 0.25%	Tracer I^-	6				99.5±2.5
	pH 6.4–8.4	Colorimetry I^-	15	95 ±6	92 ±12	82 ±17	
		Colorimetry (total I)	15	99.5±5	96.5±10	94.5±9	
	4±2° pH 4.5	Tracer I^-	1				91
	pH 5.5–6	Tracer I^-	3				93 ±2
	pH 7–7.5	Tracer I^-	3				97 ±2
	Glass bottle						
	room temperature, pH 4.5	Tracer I^-	1				88
	pH 5.5–6	Tracer I^-	2				92
	pH 7–7.5	Tracer I^-	2				99
	pH 6.4–8.4	Colorimetry I^-	15			98.5±6	
		Colorimetry (total I)	15			96 ±5	
Plasma	Glass tubes, 4±2°	Colorimetry (total I)	18	102±2	98±5		
Urine	Glass tubes, 4±2°	Colorimetry (total I)	7	99±2	98±3		

(0.85 ± 0.10 μg ml^{-1}), when water and dried biological materials were analyzed. As for the reagents, the products supplied by Technicon were found to be acceptable for all the procedures developed, although a selection could be useful to improve the precision for the lower iodine concentrations by decreasing the blank values. A careful check of the purity of the water used for dilutions and washing between successive samples was found to be of critical importance when analysing materials of low iodine content. On the other hand, virtually no blank problem is involved in activation analysis, except for the minor ones set by plasma sample dilution or addition of ammonia to water samples before irradiation (0.02 ± 0.05 p.p.b. blank for the amount of ammonia used).

An experimental check of the iodine losses on storage was carried out for the fluid samples, under various conditions. The results reported in Table VII indicate for water samples a dependence on the pH values, the temperature and the material of the containers. For the polyethylene bottles usually employed, a slight alkalinity (*e.g.* ammonia addition) favours stability, which suggests an adsorption mechanism through iodide oxidation to iodine as confirmed by the results of the colorimetric check on iodide and iodide plus iodate concentrations.

However, despite some unexplained losses found for a few samples, acceptable recoveries were generally obtained when water samples were stored in polyethylene bottles at room temperature for periods up to one month. Biological fluids were found to be unaffected by a 2-week storage in the refrigerator.

The analytical methods discussed here were used in different investigations concerning iodine metabolism. Some miscellaneous results are reported in Tables VIII–XI.

A comparison between the neutron-activation and the automated colorimetric method can be made on the basis of the related characteristics and performance.

With regard to sensitivity, slightly lower values of minimum detectable con-

TABLE VIII

IODINE CONTENT OF HUMAN TISSUES (NEUTRON-ACTIVATION METHOD)[a]

Tissue	*Number of cases*	*Concentration (μg/100 g dry weight)*		
		Range	*Median*	*Mean ($\pm$ S.D.)*[b]
Striated muscle	21	3.5–68	16	(22)
Diaphragm	12	5.1–49	13	(18)
Heart	15	7.8–52	17	(19)
Brain	9	2.1–10	3.7	(4.9)
Lung	12	12–95	28	(33)
Liver	14	22–102	52	50 ± 23
Kidney	18	3.6–80	19	(27)
Spleen	14	7.3–37	16	(19)
Skin	8	6.9–18	12	11 ± 3.6
Thyroid	15	$(0.6–2.94) \cdot 10^5$	$1.42 \cdot 10^5$	$(1.49 \pm 0.55) \cdot 10^5$

[a] Material taken at autopsy from subjects who died by accident or, in a few cases, by cardiovascular diseases.
[b] The concentration distribution appeared to be skewed in most cases: since for abnormal distribution the mean value is scarcely indicative, the data are reported in brackets and the standard deviation is not quoted.

TABLE IX

IODINE CONTENT OF MOUSE TISSUES (NEUTRON-ACTIVATION METHOD)[a]

Tissue	*Measurement unit*	*Iodine content (wet weight)*	
		group A	*group B*
Thyroid	μg/gland	1.15 ± 0.46 (45)	0.17 ± 0.08 (33)
Muscle	μg/100 g	3.6 ± 1.5 (33)	3.2 ± 1.1 (20)
Whole plasma	μg/100 ml	10.3 ± 3.3 (32)	2.2 ± 0.6 (20)
Dialyzed plasma	μg/100 ml	5.1 ± 2.1 (26)	2.0 ± 0.5 (20)

[a] The data refer to albino mice (N.M.R.I. strain), aged from 40 to 240 days and ranging from 20 to 49 g in body weight; group A was fed with a balanced diet corresponding to an iodine intake of 1.8 ± 0.4 μg/day, while group B was kept on a low-iodine diet, corresponding to an iodine intake of about 0.13 μg/day. For the latter group the thyroid glands became macroscopically hyperplastic and their iodine concentrations were found to average about 5 p.p.m. (*i.e. ca.* 100 times lower than normal glands). The figures in parentheses refer to the number of cases examined.

TABLE X

IODINE CONTENT OF SODIUM CHLORIDE SAMPLES (COLORIMETRIC METHOD)[a]

NaCl sample		*Iodine concentration (p.p.m. ± S.D.)*
Italian common salt:	"superiore"	1.10 ± 0.02
	"raffinato"	1.01 ± 0.03
	"scelto"	0.115 ± 0.008
	stockbreeding salt	7.63 ± 0.61
	agricultural salt	0.180 ± 0.012
	pork butcher's salt	0.095 ± 0.006
	bakery salt	0.066 ± 0.007
	bay salt	0.075 ± 0.012
	Sardinian salt	0.063 ± 0.019
Reagent NaCl (Merck):	reagent grade	0.063 ± 0.013
	titrisol	1.12 ± 0.05
	suprapur	0.072 ± 0.009

[a] A single lot was analyzed for each type of sodium chloride.

TABLE XI

DAILY DIETARY INTAKE OF IODINE FOR TWO HEALTHY SUBJECTS ON UNRESTRICTED DIET (NEUTRON-ACTIVATION METHOD)[a]

Day	*Iodine intake (μg/day ± S.D.)*	
	Subject A	*Subject B*
1st	393 ± 25	26 ± 3
2nd	27 ± 4	23 ± 2
3rd	245 ± 20	32 ± 4
4th	287 ± 15	35 ± 3
5th	54 ± 5	26 ± 3
6th	32 ± 4	32 ± 4
7th		22 ± 2

[a] Data referring to homogenized duplicates of the meals.

centration were found for activation analysis in the experimental conditions adopted. However, a quite satisfactory sensitivity was obtained in both cases, and further improvements may be envisaged, *e.g.* by prolonging the irradiation and counting periods for activation analysis and by lowering the blank values (reagent selection) and increasing the reaction time for colorimetry.

The activation method can be considered as virtually free from any systematic error, but a high accuracy is also obtainable for the automated colorimetry by carefully controlling the experimental conditions, at least with the materials in the present study. The fact that no reagent check is required for activation analysis represents an advantage. Also the higher flexibility of this method must be considered, since in this case the sample weight and physical form are less restricted.

On the other hand, a higher precision was found for the automated colorimetry in all the cases examined. This technique is by far more favourable with regard to other important criteria of evaluation such as operative simplicity, speed, the need of specifically trained operators and specialized laboratories, the cost of instrumentation and the analysis cost.

Some of these advantages of the colorimetric method are due to the automation of the procedure. In principle, activation analysis could also be automated, and actually a wholly automated system for biological fluids has been described[8]. However, the automation of the colorimetric method is undoubtedly much less complicated and expensive and at least as suitable.

Apart from thyroid analysis, for which the simplest way of measurement is probably represented by the activation technique, one must conclude that automated colorimetry is the method of choice for iodine determination in material of biological interest. Activation analysis retains its validity as a useful check and reference method.

SUMMARY

Two microanalytical methods for the determination of iodine in material of biological interest were studied: neutron-activation analysis and automated colorimetry (iodide catalysis of the As(III)–Ce(IV) system). The methods were applied to the analysis of samples of water, biological fluids, common salt and solid substances such as tissues, stools and foods. The particular procedures set up and optimized for the different substances with both methods are described and discussed, and the respective advantages in terms of sensitivity, accuracy, precision and speed are compared. The sensitivity and accuracy of both techniques were found to be quite adequate. Automated colorimetry is to be preferred for its precision and operative simplicity, while activation analysis retains full validity as a reference check method.

RÉSUMÉ

On examine deux méthodes microanalytiques (activation neutronique et colorimétrie automatique) pour le dosage de l'iode dans des substances d'intérêt biologique: eau, liquides biologiques, aliments etc. Les avantages respectifs de ces deux procédés sont comparés du point de vue sensibilité, exactitude, précision et rapidité. La colorimétrie automatique semble préférable pour sa précision et sa simplicité opératoire; tandis que l'analyse par activation conserve toute sa valeur, comme méthode de référence.

ZUSAMMENFASSUNG

Es wurden zwei mikroanalytische Methoden für die Bestimmung von Jod in Stoffen von biologischem Interesse untersucht: Neutronenaktivierungsanalyse und automatisierte Kolorimetrie (Jodidkatalyse des As(III)–Ce(IV)–Systems). Die Methoden wurden angewendet auf die Analyse von Wasserproben, biologischen Flüssigkeiten, Kochsalz und Festsubstanzen wie Gewebe, Stuhlproben und Nahrungsmitteln. Die einzelnen Verfahrensschritte für die verschiedenen Substanzen werden bei beiden Methoden beschrieben und diskutiert und die jeweiligen Vorteile bezüglich Empfindlichkeit, Genauigkeit, Reproduzierbarkeit und Zeitbedarf miteinander verglichen. Empfindlichkeit und Genauigkeit waren bei beiden Verfahren gleichwertig. Die automatisierte Kolorimetrie wird wegen ihrer Reproduzierbarkeit und ihrer einfachen Handhabung bevorzugt, während die Neutronenaktivierung als Vergleichsmethode ihren Wert behält.

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A SENSITIVE METHOD FOR THE ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF CHLORIDE

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Several photometric methods for the determination of chloride have been proposed but they lack sufficient sensitivity for the evaluation of very low amounts of chloride ion[1-3]. The oldest of these photometric methods is based on the nephelometry of silver chloride; it is a very simple, but not very accurate method, and permits the determination of as little as 2 p.p.m. of chloride ion. Many if not all of the remaining methods are indirect, and are based largely on displacement reactions, involving the formation of a mercury(II) chloride complex. The most sensitive and reliable such method uses the formation of the iron(III) thiocyanate complex, after the displacement of the thiocyanate ion from mercury(II) thiocyanate by chloride. It is possible to determine as little as 0.05 p.p.m. of chloride in this way. In a recent report, precision spectrophotometry is used to decrease the amount of chloride which can be determined to 15 p.p.b. at the 95% confidence level[4]. As an alternative to the reaction with iron(III), the thiocyanate displaced by the chloride can be selectively extracted into nitrobenzene as the tris(1,10-phenanthroline) iron(II) thiocyanate complex and the absorbance of this solution can be measured at 516 nm[5]. Displacement of cyanide from mercury(II) cyanide in sulphuric acid medium allows the determination of 0.014 μg Cl^- ml^{-1}; the liberated hydrogen cyanide is distilled off and converted by means of the pyridine–pyrazolone reagent into a dye, the absorbance of which is measured at 618 nm[6].

The use of solvent-extraction gas chromatography has led to the determination of as little as 0.008 μg Cl^-ml^- with a relative mean error of 21.5–25%, by using either peak heights or peak areas[7]. The chloride ion is converted to phenylmercury(II) chloride by the reaction of the aqueous chloride solution with an aqueous solution of phenylmercury(II) nitrate in a perchloric acid medium at pH about 1.5. The phenylmercury(II) chloride is extracted into chloroform and submitted to gas chromatography after concentration of the organic phase.

It is known that organomercury(II) compounds can be determined with dithizone after conversion to the chloride form by treatment of the sample with hydrochloric acid and extraction into chloroform of the organomercury(II) chloride[8]. This has provided a basis for the development of a spectrophotometric method for the indirect determination of chloride. Dithizone has several well-known disadvantages as an analytical reagent, and in spite of its high sensitivity to mercury(II), its use was avoided in the present work. Instead, sodium diethyldithiocarbamate, which has been used for the determination of microamounts of mercury(II), by extraction of the mercury(II) diethyldithiocarbamate complex into carbon tetrachloride, and measurement of the absorbance at 278 nm[9], has been examined as a substitute for dithizone,

principally because of its increased stability in solution.

In alkaline medium the chloroform solution of phenylmercury(II) chloride reacts easily with an aqueous solution of sodium diethyldithiocarbamate to form the phenylmercury(II) diethyldithiocarbamate complex, thus displacing the chloride ion from the phenylmercury(II) compound. This complex can be extracted into chloroform and shows peak absorbances at wavelengths of 257 and 297 nm with molar absorptivities referred to chloride of $21.3 \cdot 10^3$ and $6.5 \cdot 10^3$, respectively. A sensitive method for the determination of small amounts of chloride then becomes possible.

FORMATION AND EXTRACTION OF PHENYLMERCURY(II) DIETHYLDITHIOCARBAMATE

The reaction extraction conditions for chloride and phenylmercury(II) nitrate have previously been established[7], hence it was only necessary to consider the behaviour of phenylmercury(II) chloride in chloroform solution, with an aqueous solution of sodium diethyldithiocarbamate. The absorption spectra of chloroform solutions of both sodium diethyldithiocarbamate and phenylmercury(II) diethyldithiocarbamate, extracted at different pH values, between 3.6 and 10.4, were first measured. Aliquots of the standard phenylmercury(II) chloride solution were placed in 100-ml separating funnels, the pH was adjusted to the required value, and an aqueous solution of the reagent was added to bring the final volume to about 75 ml. The funnels were shaken for 1 min after the addition of 6.0 and 3.0 ml of chloroform for the first and second extractions, respectively. The extracts were collected in 10-ml graduated flasks and the volume made up to the mark. The final concentration corresponded to a chloride concentration of 1.13 μg ml^{-1}, for the establishment of the spectrum of the complex from 400 to 200 nm, against chloroform as blank. The sodium diethyldithiocarbamate was extracted in the same way. The spectral curves in Fig. 1A, B show that the reagent absorbs slightly at pH 10, whilst the phenylmercury(II) diethyldithiocarbamate complex shows two maxima at 257 and 297 nm. A carbon tetrachloride solution of phenylmercury(II) chloride, treated with sodium diethyldithiocarbamate at pH 10, gave the spectrum of phenylmercury(II) diethyldithiocarbamate similar to that shown in Fig. 1B; but the maximum was shifted to 300 nm and the peak at 257 nm in chloroform disappeared because of the transparency limit of carbon tetrachloride. The molar absorptivity was about $7.2 \cdot 10^3$, slightly greater than in chloroform.

Chloroform solutions of phenylmercury(II) bromide, iodide, and nitrate (the last in aqueous solution) were treated in the same way as the phenylmercury(II) chloride with an aqueous solution of sodium diethyldithiocarbamate and extracted into chloroform. The spectra (Fig. 1C) were identical with that from phenylmercury(II) chloride, an indication that the anion does not affect the formation of the phenylmercury(II) diethyldithiocarbamate complex.

The wavelength of 297 nm, and chloroform as solvent, were selected as suitable conditions for subsequent investigations, although some measurements were made at 257 nm.

Effect of pH

Several aliquots of the phenylmercury(II) chloride solution in chloroform

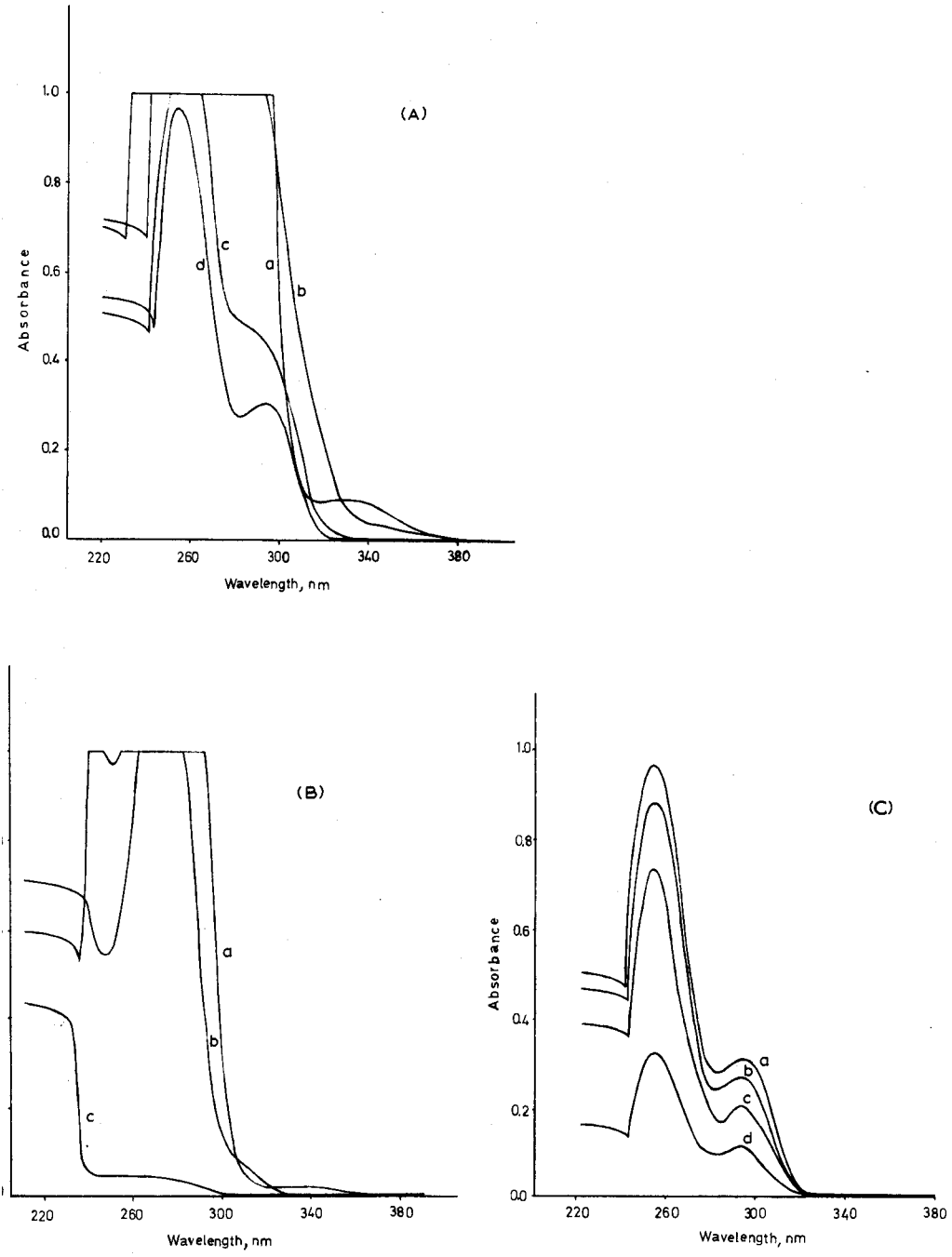

Fig. 1. (A) Absorption spectra of phenylmercury(II) diethyldithiocarbamate in chloroform. pH: (a) 3.6; (b) 6.5; (c) 8.0; (d) 10.4 at a concentration equivalent to 1.13 μg Cl ml^{-1}. (B) Absorption spectra of sodium diethyldithiocarbamate after extraction with chloroform. pH: (a) 3.6; (b) 6.5; (c) 9.2. Initial concentration in aqueous layer, 0.2%. (C) Absorption spectra of phenylmercury(II) diethyldithiocarbamate in chloroform arising from 0.001% solutions of (a) C_6H_5HgCl; (b) C_6H_5HgBr; (c) C_6H_5HgI; (d) $C_6H_5HgNO_3$.

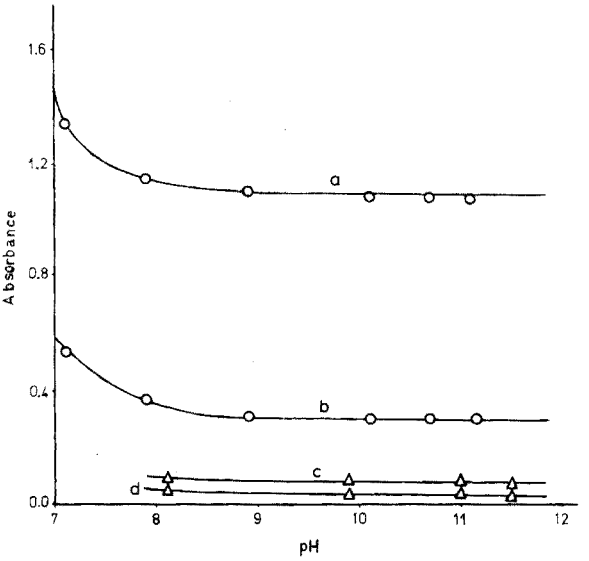

Fig. 2. Effect of pH on the extraction of phenylmercury(II) diethyldithiocarbamate at 257 nm (a) and 297 nm (b), and on extraction of sodium diethyldithiocarbamate at 257 nm (c) and 297 nm (d). Concentrations equivalent to 1.13 μg Cl^- ml^{-1}. Reagent concentration (DDTC), 0.004%.

were treated with an aqueous solution of the reagent, and extracted twice at pH values ranging from 3.5 to 11.1. The equivalent concentration of chloride was 1.13 μg ml^{-1}, and the absorbances of the solutions were measured at 257 nm and 297 nm. The results obtained showed that the absorbance remained constant in the pH interval 8.9–11.1. Similar results were obtained with the reagent solution alone; its extraction remained constant at the lowest level between pH 8.9 and 11.1. The results are illustrated in Fig. 2. A pH value of about 10.4 was chosen for the further studies.

Amount of reagent (sodium diethyldithiocarbamate)

Once the pH had been fixed, the effect of the amount of reagent was studied; 1 ml of an aqueous 0.02% solution of sodium diethyldithiocarbamate for each 10 μg of phenylmercury(II) chloride was enough to form the complex, and further additions of reagent up to 4 ml did not appreciably affect the absorbance of the system.

Time of extraction

The reaction occurred instantaneously at pH 10.4; shaking for 1 min and allowing the layers to separate for about 4 min sufficed to extract the complex. A double extraction with 6.0 and 3.0 ml of chloroform was also sufficient.

Time and temperature effects

The effects of time and temperature on the stability of the system were also studied; the absorbance of the complex remained unchanged for up to 18 h and up to 50°.

Beer's law

The relationship between absorbed radiant energy and complex concentration

was studied with the previously established conditions. Several standard solutions of phenylmercury(II) diethyldithiocarbamate were prepared from aliquots of the standard solution of phenylmercury(II) chloride treated at pH 10.4 with the aqueous solution of the reagent and extracted with chloroform. The final volume was then made up to 10 ml and the absorbances of the solution were measured at 257 and 297 nm; the results are shown in Fig. 3. The concentration is expressed in terms of the chloride ion equivalent of phenylmercury(II) diethyldithiocarbamate in the 10-ml chloroform extract. The chloride content of an original aqueous solution is then readily computed from a consideration of the appropriate dilution and extraction factors. The calibration graph is linear over the range 0.1–3.0 μg Cl^- ml^{-1} at 257 nm, and 0.1–8.0 μg Cl^- ml^{-1} at 297 nm. In the latter situation the optimal range is 1.0–8.0 μg Cl^{-1} ml^{-1}. The molar absorptivities are $21.3 \cdot 10^3$ at 257 nm and $6.5 \cdot 10^3$ at 297 nm in chloroform.

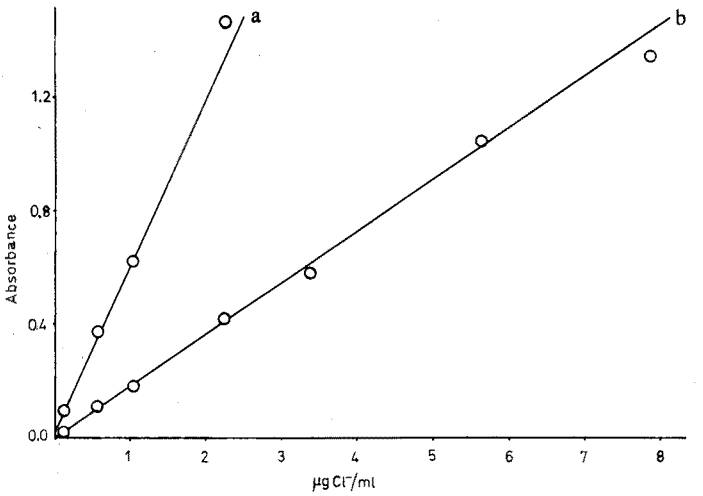

Fig. 3. Beer's law plots for phenylmercury(II) diethyldithiocarbamate. (a) 257 nm; (b) 297 nm. Concentration expressed in terms of chloride equivalent of phenylmercury(II) diethyldithiocarbamate in the 10-ml chloroform extract.

Effect of diverse ions

The effect of other species on the formation and extraction of phenylmercury(II) chloride has been studied in the previous communication[7]. Of the common ions then examined, only silver, mercury(I), mercury(II), bromide, iodide, thiocyanate, cyanide and nitrite interfered and must be absent from the solution. As the subsequent procedure depends on the reaction of a chloroform solution of phenylmercury(II) chloride with an aqueous solution of sodium diethyldithiocarbamate, there is no possibility for the normal cationic interferences of the complex-forming reaction to occur, and this part of the procedure is essentially specific for the solution of the phenylmercury(II) radical in chloroform. Undesirable effects could be produced by chloroform-soluble organic compounds and organo-metallic complexes, but these could be eliminated by previous extraction of the sample with chloroform before the formation of the phenylmercury(II) chloride.

Once the conditions of the reaction and extraction had been established, the method was applied to the determination of chloride in large volumes of aqueous solution. Solutions of different concentration of chloride were studied by the following procedure.

EXPERIMENTAL

Reagents

Phenylmercury(II) nitrate. Prepare an aqueous 0.1 % (w/v) solution, by dissolving the appropriate weight of reagent in warm distilled water. From this, prepare a 0.01 % solution by dilution.

Phenylmercury(II) chloride. Prepare a 0.1 % (w/v) solution in chloroform from the sublimed compound[7].

Chloride standard solution. Prepare a 0.1 mg ml^{-1} solution from A.R. sodium chloride. Prepare other solutions by appropriate dilution.

Sodium diethyldithiocarbamate. Prepare an aqueous 0.2 % (w/v) solution in distilled water. From this prepare a 0.02 % solution by dilution.

Chloroform and perchloric acid (60 % w/w). Analytical grade reagents were used.

Apparatus

A u.v.–visible Pye Unicam SP 800 spectrophotometer equipped with 1-cm quartz cells was used.

Recommended procedure

In 500-ml separatory funnels, place 250-ml samples of different chloride concentration and add concentrated perchloric acid in order to obtain a pH of about 1.5, followed by 1.5 ml of aqueous 0.01 % solution of phenylmercury(II) nitrate for each 10 μg of chloride. Shake the funnels vigorously for 1 min, add 10 ml of chloroform, and shake vigorously for 1 min. Allow the layers to separate, collect the organic layer and repeat the extraction with 5 ml of chloroform. Concentrate the organic solution in a pear-shaped distillation flask to about 2 ml and then transfer to a 100-ml separatory funnel which contains 1 ml of 0.02 % sodium diethyldithiocarbamate solution for each 10 μg of chloride, sufficient 0.01 *M* sodium hydroxide solution to give a pH of about 10.4, and distilled water to give a final volume of *ca.* 60 ml. Extract the complex twice with chloroform using 4 ml and 3 ml, respectively. Collect the organic layer in a 10-ml graduated flask, and dilute to the mark with chloroform. Measure the absorbance of the solution at 257 and 297 nm, respectively, in 1.0-cm quartz cells, with chloroform as reference. Carry out a blank determination on 250 ml of distilled water.

RESULTS AND APPLICATIONS

The results are shown in Table I together with data for the precision and accuracy of the procedure. Blank determinations gave an average value of 0.25 $\mu g\ Cl^{-} ml^{-1}$ in the final chloroform solution equivalent to 0.01 $\mu g\ ml^{-1}$ in the original 250-ml sample. This arises mainly from the small amount of phenylmercury(II) nitrate extracted along with the phenylmercury(II) chloride by the chloroform.

TABLE I

PRECISION AND ACCURACY DATA FOR THE U.V. SPECTROPHOTOMETRIC DETERMINATION OF CHLORIDE

	No. of detns.	*Cl^- taken*[a] ($\mu g\ ml^{-1}$)	*Cl^- found*[a,b] ($\mu g\ ml^{-1}$)	*s*	s_r(%)	*Mean error*	*Rel. mean error* (%)
A = 257 nm	8	0.020[d]	0.012	0.0021	17.50	−0.008	40.0
	8	0.040	0.031	0.0033	10.64	−0.009	25.0
	8	0.080	0.074	0.0030	4.00	−0.006	7.5
	8	0.12	0.097	0.0028	2.88	−0.023	19.2
Blank[c]	6	–	0.013	0.0017	12.87		
B = 297 nm	8	0.020[d]	0.013	0.0019	14.60	−0.007	35.0
	8	0.040	0.032	0.0039	12.18	−0.008	20.0
	8	0.080	0.072	0.0021	2.76	−0.008	10.0
	8	0.120	0.103	0.0047	4.56	−0.017	14.1
	8	0.200	0.176	0.0014	0.79	−0.024	12.0
	6	0.280	0.252	0.0079	3.02	−0.028	10.0
Blank[c]	6	–	0.014	0.0014	10.00		

[a] For a final volume of 250 ml.
[b] Average after a blank deduction.
[c] Expressed as chloride content of the solution in the 250 ml.
[d] Below optimal range.

Application of the method for the determination of the chloride content of drinking water from different sources

The method described above was used for the determination of chloride in a sample of drinking water. The sample was diluted ten times and 5-ml aliquots were placed in 500-ml separatory funnels; the final volume was made up to 250 ml, before the above procedure was applied. The gas chromatographic method[7] was also applied. The results of the analyses are shown in Table I. From these results, there appears to be good agreement between the several methods used for the determination of chloride; either of the proposed methods (gas chromatography and u.v. spectro-

TABLE II

DETERMINATION OF THE CHLORIDE CONTENT OF A SAMPLE OF TAP WATER

Method	*Cl^- found*[a] ($\mu g\ ml^{-1}$)	s_r	s_r(%)	*Mean error*	*Relative mean error* (%)
Gas chromatography	39.58	2.57	6.44	−0.42	−1.05
U.v. spectrophotometry (257 nm)	41.99	1.98	4.68	+1.99	4.98
U.v. spectrophotometry (297 nm)	39.99	2.36	5.86	−0.01	0.025
Mercury(II) thiocyanate[b]	40.0 ± 1.0	—	—	—	—
Ion-selective electrode	39.0 ± 1.0	—	—	—	—

[a] Average after blank deduction.
[b] Taken as true value.

TABLE III

DETERMINATION OF THE CHLORIDE CONTENT OF BIRMINGHAM TAP WATER

Method	*Cl^- found*[a] ($\mu g\ ml^{-1}$)
Gas chromatography	8.4
U.v. spectrophotometry (297 nm)	8.6
U.v. spectrophotometry (257 nm)	8.5

[a] After blank deduction.

photometry) compares favourably with the normally used mercury(II) thiocyanate and ion-selective electrode methods. The concentration of chloride in the final chloroform solution must be in the range 1.0–8.0 $\mu g\ Cl^-\ ml^{-1}$ which is the best interval for determining it. The procedure was applied to a sample of Birmingham tap water; the results are reported in Table III. The day-to-day average value of the chloride content given by the City Analyst's Laboratory was between 8–10 $\mu g\ Cl^-\ ml^{-1}$.

Conclusions

These investigations have led to the development of a sensitive method for the indirect determination of the chloride ion, through the formation of the phenylmercury(II) diethyldithiocarbamate complex. Although this complex in chloroform solution shows absorption maxima at 257 and 297 nm, respectively, the most reliable results are obtained at 297 nm, where the blank values are much lower than at 257 nm, in spite of the higher sensitivity achieved at the shorter wavelength. Carbon tetrachloride can also be used as solvent, but the complex then shows a maximum only at 300 nm. The limit of detection of the method can be increased by using longer cells and reducing the effect of the blank, for example, taking the blank as reference, because this remains constant for several determinations.

The method is also applicable to the determination of bromide and iodide, and in general to organomercury(II) compounds after their conversion into the chloride form. For the determination of organomercury(II) compounds the limit of detection must be lower than in the halide determinations, in which the reagent, phenylmercury(II) nitrate, is partially extracted into chloroform, and reacts with the diethyldithiocarbamate, thus contributing an amount equal to about 65% of the total blank value.

Although several ions interfere with the formation of phenylmercury(II) chloride or form other phenylmercury(II) derivatives which are soluble in chloroform, the new method is comparatively troublefree. The extraction processes confer a useful degree of selectivity on the analytical procedure which is easily and simply applied to a variety of samples. The main application remains, however, as the determination of low concentrations of chloride in water samples.

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SUMMARY

A new method for the determination of chloride ion is based on the formation of phenylmercury(II) chloride, its extraction into chloroform and reaction with sodium diethyldithiocarbamate to form phenylmercury(II) diethyldithiocarbamate. This complex has spectral maxima at 257 and 297 nm, either of which can be used for quantitative purposes. The molar absorptivities are $21.3 \cdot 10^3$ and $6.5 \cdot 10^3$ respectively, referred to the chloride ion. The method is especially suitable for the determination of trace amounts of chloride in aqueous solution and has been applied to samples of drinking water. Amounts of chloride in the range 0.04–0.32 p.p.m. can be determined in 250-ml aqueous samples with an average relative mean error of 12%. The method can be used also for bromide and iodide, and for organomercury(II) compounds. Interferences are minimal and the method compares favourably with the standard mercury(II) thiocyanate procedure.

RÉSUMÉ

Une nouvelle méthode est décrite pour le dosage des chlorures. Elle est basée sur la formation de chlorure de phénylmercure(II), de son extraction dans le chloroforme et de sa réaction avec le diéthyldithiocarbamate, pour former le diéthyldithiocarbamate de phénylmercure(II). Ce composé présente deux maxima, à 257 et 297 nm, avec des coefficients d'absorption molaire de $21.3 \cdot 10^3$ et $6.5 \cdot 10^3$ respectivement, par rapport à Cl^-. Cette méthode convient spécialement bien au dosage de traces de chlorure dans des solutions aqueuses, et en particulier dans des eaux potables. Ce procédé peut également être utilisé pour le dosage de bromures et d'iodures et pour des composés organomercuriques.

ZUSAMMENFASSUNG

Eine neue Methode für die Bestimmung von Chloridionen beruht auf der Bildung von Phenylquecksilber(II)-chlorid, dessen Extraktion mit Chloroform und Reaktion mit Natriumdiäthyldithiocarbamat unter Bildung von Phenylquecksilber-(II)diäthyldithiocarbamat. Dieser Komplex hat spektrale Maxima bei 257 und 297 nm, von denen beide für quantitative Zwecke verwendet werden können. Die molaren dekadischen Extinktionskoeffizienten sind $21.3 \cdot 10^3$ bzw. $6.5 \cdot 10^3$, bezogen auf das Chloridion. Die Methode eignet sich besonders für die Bestimmung von Spurenmengen Chlorid in wässriger Lösung und wurde auf Trinkwasserproben angewendet. Chloridmengen im Bereich 0.04–0.32 p.p.m. können in wässrigen Proben von 250 ml mit einem mittleren relativen Fehler von 12% bestimmt werden. Die Methode kann auch für Bromid und Jodid sowie für Organoquecksilber(II)-verbindungen angewendet werden. Es gibt nur minimale Störungen, und die Methode ist günstiger als das Standardverfahren mit Quecksilber(II)-thiocyanat.

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SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM WITH THIOGLYCOLIC ACID

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In 1948 König and Crowell[1] reported the use of thioglycolic acid for the detection of palladium. They found that this thioacid reacts with palladium salts in dilute aqueous solutions, an immediate yellow colour being developed over a wide range of acidities. One part of palladium in the presence of 250 parts of gold, platinum and iridium may be detected, and the yellow colour shows a maximum absorbance at 325 nm. This original work was, however, confined to the application of thioglycolic acid as a spot-test reagent, and although Yoe *et al.*[2,3] later reported some spectrophotometric methods for determining palladium by using different thioacids, only one spectrophotometric method for determining palladium with thioglycolic acid seems to have been published. This is the method of Widtman[4] who, however, developed the colour in sulphuric acid solution at pH 0.2–2.1 and surprisingly claimed an absorbance maximum at 371 nm.

It was the purpose of this investigation to clear up the above reported controversial aspects concerning the absorbance maximum, acid concentration, etc., to establish the optimal conditions, and to present a recommended method for the spectrophotometric determination of palladium with thioglycolic acid.

EXPERIMENTAL

Apparatus and solutions

Absorbance measurements were made with a Carl Zeiss spectrophotometer, model PMQII, and 1-cm quartz cells.

A Radiometer model PHM 24e pH meter was used for pH measurements.

Standard palladium solution. Dissolve 0.115 g of "Specpure" palladium wire in aqua regia and expel the last traces of nitrogen compounds by repeated small additions of, and evaporations with, hydrochloric acid. Dilute the solution to 200 ml and standardize by the dimethylglyoxime method. 1 ml of this solution contains 0.576 mg of palladium.

Reagent. Use thioglycolic acid as a 10% (v/v) solution. Merck's extra-pure 80% (w/v) solution was diluted (1 + 9) with distilled water.

Stock hydrochloric acid solutions of interfering ions, containing 0.1 mg of test ion per ml were used.

Recommended procedure

Evaporate the hydrochloric acid solution containing the palladium to inci-

pient dryness. Add 3 ml of 5 *M* hydrochloric acid and 1–2 drops of 100-vol. hydrogen peroxide and boil for 2–3 min. Cool and dilute to a suitable volume. Pipette out an aliquot, containing *ca.* 0.5 mg of palladium, into a 100-ml beaker and dilute to 70 ml with water. Adjust the solution to pH 3–6 (pH meter) by adding a 10% sodium hydroxide solution dropwise while stirring. Develop the yellow complex by adding 10 ml of thioglycolic acid solution. Transfer the solution to a 100-ml volumetric flask, dilute to the mark with water and measure the absorbance at 325 nm using a hydrogen lamp, a slit width of 0.1 mm and a 325–380 nm filter. Use a blank solution as reference.

RESULTS AND DISCUSSION

Absorbance curves

The palladium–thioglycolic acid colour was developed in two solutions. The first was a hydrochloric acid solution at pH 2.5 while the other was a sulphuric acid solution at pH 1.5. Their absorbances were measured over the wavelength range 300–500 nm. Figure 1 shows that in both solutions the absorbances are maximal at 325 nm.

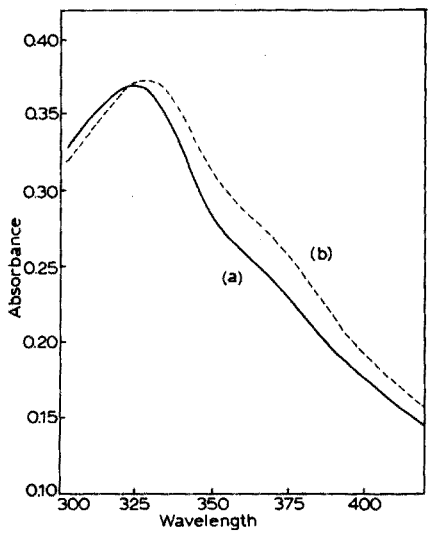

Fig. 1. Absorption spectrum of Pd–thioglycolic acid complex (a) at pH 2.5 in HCl, (b) at pH 1.5 in H_2SO_4.

This disagrees with the findings of Widtman[4] who reported a wavelength of 371 nm for maximal absorbance. However, since Widtman in his recommended procedure, used tartaric acid and thiourea as complexing and masking reagents, it was decided to investigate any possible effect of these reagents on the absorbance. It was found that the presence of tartaric acid and thiourea has no effect and again the maximum absorbance clearly occurred at 325 nm.

Effect of acid concentration

The influence of pH on the absorbance at 325 nm was studied thoroughly. The results shown in Table I indicate that the absorbances of the complex remain constant in the pH range 2.0–6.0, but gradually decrease with further increase of

TABLE I

EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON THE ABSORBANCE

(0.576 mg of Pd, 10 ml of thioglycolic acid solution, 100 ml of final volume)

Acidity	*Absorbances at 325 nm*
pH 6.0	0.426
pH 4.0	0.426
pH 3.0	0.426
pH 2.0	0.426
pH 1.5	0.421
pH 1.0	0.415
0.2 *N*	0.413
0.5 *N*	0.410

acidity, thus showing the significant effect of hydrogen-ion concentration on the intensity of the colour.

Effect of reagent concentration

Differing amounts of thioglycolic acid solution were added to a series of identical aliquots of standard palladium solution, each containing 0.288 mg of palladium at pH 2.5 and all diluted to a final volume of 100 ml. Spectrophotometric measurements were made as usual. The results shown in Table II indicate that wide variations in the amount of the reagent can be tolerated without any serious effect on the absorbance of the complex.

TABLE II

EFFECT OF REAGENT CONCENTRATION

Reagent solution (ml)	*Absorbance*
1	0.210
5	0.209
10	0.207
20	0.208
50	0.209

Stability of colour

The colours of two solutions containing 0.288 and 0.576 mg of Pd respectively, were measured immediately after formation as well as over a period of several hours. The results showed no change over a period of 24 h.

Effect of diverse ions

In the ultraviolet region, interference of foreign ions may be caused not only by their reaction with thioglycolic acid but also by their own absorbance. The effect of certain precious metals as well as of some base metals was therefore investigated, and the results are presented in Table III. It is seen that small amounts of platinum, rhodium, iridium, and gold can be tolerated but copper and moderate amounts of

TABLE III

EFFECT OF DIVERSE IONS

(0.576 mg Pd, 100 ml of final volume, pH 2.5, 10 ml of reagent solution)

Ion	*Added as*	*mg*	*Pd found (mg)*	*Difference (%)*
Pt^{4+}	H_2PtCl_6	0.01	0.576	0.0
		0.03	0.580	+0.8
		0.1	0.613	+6.4
Rh^{3+}	H_2RhCl_5	0.01	0.576	0.0
		0.03	0.613	+6.4
Ir^{4+}	H_2IrCl_6	0.03	0.576	0.0
		0.1	0.576	0.0
Au^{3+}	$HAuCl_4$	0.01	0.576	0.0
		0.05	0.576	0.0
Na^+	NaCl	20	0.576	0.0
Ni^{2+}	$NiCl_2$	0.5	0.576	0.0
		1.0	0.567	−1.6
Cu^{2+}	$CuCl_2$	0.01	0.584	+1.4
Fe^{3+}	$FeCl_3$	0.01	0.576	0.0
		0.02	0.583	+1.2
		0.1	0.611	+6.0

iron interfere significantly. Nickel in large amounts depresses the colour. Although the tolerance towards the interfering elements can be improved by using masking agents, the selective isolation of palladium, by extracting its dimethylglyoxime complex with chloroform, before its spectrophotometric determination with thioglycolic acid, is strongly recommended.

Precision

The precision of the method was estimated by applying the recommended procedure on a series of fifteen solutions, each containing 0.576 mg Pd. A mean absorbance of 0.426 at 325 nm was found. Average and maximum relative deviations were found to be 0.18% and 0.55%, respectively.

Calibration

A 25-ml aliquot of the standard palladium solution was diluted to 500 ml with water and from this working solution, containing 28.8 μg Pd ml^{-1}, aliquots of 5, 10, 15, 20, 25, 30 and 40 ml were pipetted into 100-ml beakers; the colours were developed by following the recommended procedure and the absorbances were measured at 325 nm.

For the optimal concentration range 1–10 p.p.m., a straight line was obtained which did not pass through the origin. The constants in the straight line equation, $y=bx+c$, were calculated statistically. For a final volume of 100 ml, the palladium content is represented by the equation:

$$Pd(mg) = 1.3135\,(A+0.0125)$$ where A is the absorbance.

APPLICATION OF THE METHOD TO A SYNTHETIC PRECIOUS METALS SOLUTION

A hydrochloric acid solution containing 5 mg Pt, 2.88 mg Pd, 0.5 mg Ir, 0.35

mg Au, 0.30 mg Ru as well as 50 mg each of iron, nickel and copper, was evaporated to near dryness. Hydrochloric acid (10 ml of 5 *M*) and 1–2 drops of hydrogen peroxide were added and after boiling for 1 min, the solution was transferred to a separatory funnel and diluted to *ca.* 100 ml with water. Gold was first removed by extracting with ether. To the raffinate, 5 ml of an alcoholic 1% (w/v) solution of dimethylglyoxime was added. The yellow palladium–dimethylglyoxime precipitate, after standing for 30 min, was extracted with chloroform. This solvent was evaporated off, and the residue was heated with nitric acid and finally converted to a slightly acidic chloride solution. One fifth of this solution was transferred to a 100-ml beaker and the recommended procedure was carried out.

This experiment was done in triplicate; 2.88, 2.86 and 2.87 mg of palladium were found, thus confirming a very satisfactory accuracy and reproducibility of the method in practical application.

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SUMMARY

A method is described for an accurate and precise spectrophotometric determination of palladium in hydrochloric acid medium by using thioglycolic acid. The yellow complex is developed at pH 2.5–5.0 and its absorbance is measured at 325 nm. The complex is formed very rapidly, is soluble in water and stable for 24 h. The optimal concentration range is 1–10 p.p.m.

RÉSUMÉ

On décrit une méthode de dosage spectrophotométrique pour le palladium, en milieu acide chlorhydrique, au moyen d'acide thioglycolique. Le complexe jaune se forme à un pH de 2.5 à 5.0; son absorption est mesurée à 325 nm. Il se forme très rapidement; il est soluble dans l'eau et il est stable pendant 24 h. Les concentrations optimales vont de 1 à 10 p.p.m.

ZUSAMMENFASSUNG

Es wird eine Methode für eine genaue und reproduzierbare spektrophotometrische Bestimmung von Palladium in salzsaurem Medium unter Verwendung von Thioglykolsäure beschrieben. Der gelbe Komplex wird bei pH 2.5–5.0 entwickelt und die Extinktion bei 325 nm gemessen. Der Komplex bildet sich sehr schnell, ist in Wasser löslich und 24 h lang beständig. Der optimale Konzentrationsbereich ist 1–10 p.p.m.

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ANALYTICAL SEPARATION OF RHENIUM BY EXTRACTION WITH N-BENZYLANILINE IN CHLOROFORM FROM SULPHURIC ACID MEDIA

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During the past few years, the increasing use of alloys containing molybdenum and rhenium in the electronics and other industries has magnified the need for a simple and rapid method for the determination of rhenium in a variety of materials. Molybdenum interferes with virtually all methods for the determination of rhenium, hence a preliminary separation of rhenium from molybdenum and other interfering elements is essential.

Many solvent extraction methods for rhenium have been reported in the literature. These include extraction of rhenium from acidic solutions with amyl alcohol[1], methyl isobutyl ketone[2], thenoyltrifluoroacetone[3], tributyl phosphate[4,5], thiooxine[6], mesityl oxide[7], and quaternary amines[8-11]. The ion-association complexes of rhenium with tetraphenylarsonium chloride[12] or tributylamine[13] can be extracted into chloroform or dichloromethane. Rhenium is also extracted from strongly alkaline solutions with pyridine[14], quinoline[15,16], ethyl methyl ketone[17], acetylacetone[18] and quaternary amines[19]. Other extractants can also be used for the extraction of rhenium[20-22]. However, these methods are usually unselective or have limitations which make them unsuitable for routine use.

Rhenium can also be separated from molybdenum and other elements[23], by reduction with zinc amalgam and subsequent extraction of reduced rhenium with isopentanol; but traces of molybdenum, etc. accompanying rhenium must be removed by other methods and rigid control of conditions is essential.

In this paper, N-benzylaniline in chloroform is reported as a new extraction reagent for rhenium from sulphuric acid media. Rhenium(VII) is quantitatively extracted from 3.5–4.5 *M* sulphuric acid media. The separation of the organic and aqueous phases is very rapid. Rhenium(VII) is easily stripped from the organic phase with water and determined spectrophotometrically as its thiocyanate complex[20]. Few cations interfere. Molybdenum(VI), tungsten(VI), niobium(V) and tantalum(V) are not extracted in the presence of phosphoric acid. Palladium, in traces, and platinum(II, IV) accompany rhenium, but their interference can easily be eliminated by the addition of thioglycollic acid to the rhenium thiocyanate complex and its subsequent extraction into *n*-butyl acetate. Reduction of vanadium(V), iron(III), chromium(VI), osmium(VIII), ruthenium(VII), titanium(IV) and cerium(IV) with ascorbic acid prevents the interference of these metal ions. Uranium(VI) and thorium(IV) are coextracted with rhenium, but considerable amounts of these elements can be

tolerated in the spectrophotometric determination of rhenium. Most common anions do not interfere nor does ascorbic acid. The method has been applied to the determination of rhenium in diverse synthetic mixtures.

EXPERIMENTAL

Apparatus

Absorbances were measured with a Bausch and Lomb Spectronic 20, with the accessory 1-cm diameter test tubes, and a Hilger Uvispek spectrophotometer with 1.0-cm cells.

Reagents

Analytical-grade reagents were used without purification. Niobium and tantalum solutions were prepared by fusion of their oxides with potassium pyrosulphate and dissolution of the fused mass in hot tartaric acid. All standard solutions were prepared in double distilled water.

Rhenium standard solution. This was prepared by dissolving an exactly weighed amount of potassium perrhenate (Specpure, Johnson-Matthey, Ltd., London) in water. The stock solution contained 648.7 μg Re ml^{-1}. Other solutions containing 16.22 μg Re ml^{-1} were prepared by suitable dilution.

N-Benzylaniline (B.D.H. Ltd.). 9.0 g of purified[24] reagent (m.p. 36°; b.p. 180°/12 mm) was dissolved in 100 ml of distilled chloroform. The solution on standing for some time assumed a yellow colour, but this did not affect the extraction of rhenium.

Tin(II) chloride solution. A 10% solution was prepared by dissolving 35 g of tin(II) chloride dihydrate in 100 ml of (1 + 1) hydrochloric acid and diluting to 350 ml with water. A few pieces of tin were added and the solution was filtered. The solution was prepared freshly each week.

n-Butyl acetate (E. Merck) was distilled, the 124–126° portion being used. Diisopropyl ether (Reidel) was used directly.

General procedure

To an aliquot of solution containing up to 32.5 μg of rhenium in a 75-ml separating funnel, add enough 9 *M* sulphuric acid and water to give 3.5–4.5 *M* acid in a volume of 20 ml. Shake the solution for 45 s with 10 ml of the extractant mixture containing 5 ml of the N-benzylaniline solution and 5 ml of chloroform. Swirl the separating funnel slightly and separate the chloroform layer. Re-extract the aqueous layer with 10 ml of the extractant mixture. Combine the organic layers and strip rhenium from the organic phase by shaking for 1 min with two 10-ml portions of water and then with 10 ml of 1 *M* hydrochloric acid. Transfer the aqueous back-extract to a 100-ml beaker and heat the solution to remove traces of chloroform. Cool the solution, transfer it to a 50-ml volumetric flask and make up to the mark with water. Transfer an aliquot of solution containing up to 10 μg of rhenium to a 75-ml separating funnel. Add 7.5 ml of concentrated hydrochloric acid and dilute to about 20 ml with water. Now add 3 ml of aqueous 20% potassium thiocyanate solution and 3.5 ml of the tin(II) chloride solution. Mix and allow the solution to stand for 10 min. Add 10 ml of *n*-butyl acetate and shake vigorously for 40 s. Allow the phases to separate. Discard the aqueous layer, transfer the organic layer to a 10-ml volumetric flask,

dilute to the mark if necessary, and measure the absorbance at 430 nm against a similarly treated blank.

Separation of rhenium from molybdenum(VI), tungsten(VI), niobium(V) and tantalum(V)

To an aliquot of solution containing 32.5 μg of rhenium and mg-amounts of the other elements, add 0.5 g of ascorbic acid and 3 ml of 85% phosphoric acid. Transfer the solution to a separating funnel and add enough 9 *M* sulphuric acid and water to give 3.5–4.5 *M* acid in a volume of 20 ml. Shake the solution for 45 s with 10 ml of extractant mixture containing 5 ml of N-benzylaniline solution and 5 ml of chloroform. Swirl the separating funnel slightly and separate the chloroform layer. Re-extract the aqueous layer with 10 ml of the extractant mixture. Wash the combined organic layers with 20 ml of 4 *M* sulphuric acid containing 2 ml of phosphoric acid. Separate the chloroform layer. Re-extract the acid wash with two 10-ml portions of the extractant mixture containing 3 ml of N-benzylaniline solution and 7 ml of chloroform. Combine the organic layers and strip rhenium from the organic phase by shaking for 1 min with two 10-ml portions of water and then with 10 ml of 1 *M* hydrochloric acid. Reject the organic layers and proceed with the aqueous solution as in the General procedure.

RESULTS AND DISCUSSION

Effect of acidity

The concentration of sulphuric acid was varied from 1 *M* to 4.5 *M* while other conditions remained constant, the ratio of the aqueous to organic phases being 2:1. Rhenium was quantitatively extracted from 3.5–4.5 *M* sulphuric acid, and the percentage extraction was not changed by the presence of phosphoric acid and ascorbic acid in the initial solution (see below). The results are given in Table I.

TABLE I

PERCENTAGE EXTRACTION OF RHENIUM(VII) WITH N-BENZYLANILINE IN CHLOROFORM

(Rhenium(VII) added, 32.5 μg. Conditions as in General procedure except for acidity)

M H_2SO_4	*% Extraction*	*% Extraction for* H_3PO_4*–ascorbic acid solutions*
2.0	86.6	86.2
3.0	93.3	93.0
3.5	100.0	100.0
4.0	100.0	100.0
4.5	100.0	100.0

Range of rhenium content

Rhenium(VII) could be quantitatively extracted in amounts up to 250 μg under the conditions of the above procedure. The percentage extraction for 500 μg of rhenium was 98.0%; for 5.0 mg of rhenium, 97.3%; and for 20.0 mg, 96.0%. In these tests, microgram amounts of rhenium(VII) were determined by the above

colorimetric method, and milligram amounts by the gravimetric tetraphenylarsonium chloride method.

Interference of diverse ions

Rhenium(VII) could be quantitatively separated from most of the cations tested (Table II). Under the conditions of the General procedure, molybdenum(VI), tungsten(VI), niobium(V) and tantalum(V) were partially co-extracted. However, when phosphoric acid was present, only traces of these elements accompanied rhenium in the extraction; about 0.02% of any tungsten present, and 0.2% of niobium and tantalum accompanied the rhenium, but 0.4% of any molybdenum passed into the organic phase. Scrubbing the organic phase with 4 *M* sulphuric acid containing 1.5 *M* phosphoric acid completely separated these traces from the rhenium; when the modified procedure was used, as much as 400 mg of molybdenum(VI) could be tolerated in the determination of 32.5 μg of rhenium(VII).

TABLE II

DETERMINATION OF RHENIUM IN THE PRESENCE OF DIVERSE IONS

(32.5 μg of rhenium was used)

Foreign ion	*Tolerance limit (mg)*[a]	*Foreign ion*	*Tolerance limit (mg)*
Hg(II)	20	Mg(II)	100
Ag(I)	20	Sr(II)	5
Cu(II)	30	Mn(II)	27
Cd(II)	30	Sc(III)	22
Sb(III)	24	Y(III)	30
As(III)	30	Te(IV)	25
Bi(III)	30	Se(IV)	20
Fe(II)	30	Os(VIII)[b]	10
Fe(III)[b]	30	Ru(VII)[b]	10
Cr(III)	52	Ru(III)	10
Cr(VI)[b]	52	Rh(III)	10
Co(II)	59	Pd(II)[c]	2
Ni(II)	59	Pt(II)[c]	2
Al(III)	27	Pt(IV)[c]	2
In(III)	22	Mo(VI)[d]	400
Tl(III)	20	W(VI)[d]	50
Ga(III)	23	Nb(V)[d]	7
Be(II)	18	Ta(V)[d]	7
U(VI)	10	Fluoride	10
Th(IV)	10	Nitrate	10
Zr(IV)	10	Chloride	100
Ce(III)	28	Citrate	1000
Ce(IV)[b]	28	Tartrate	1000
V(V)[b]	100	Oxalate	1000
Ca(II)	5	EDTA	100
		Ascorbic acid	1000

[a] The tolerance limit represents the amount of the ion causing a relative error of less than 2.5%.
[b] Reduced with ascorbic acid.
[c] Thioglycollic acid added to rhenium thiocyanate complex.
[d] In the presence of phosphoric acid.

Interferences of vanadium(V), iron(III), chromium(VI), osmium(VIII), ruthenium(VII), titanium(IV) and cerium(IV) could be prevented by prior reduction with ascorbic acid. Palladium(II) and platinum(II, IV) were co-extracted with rhenium, but these interferences could be avoided by addition of thioglycollic acid to the rhenium thiocyanate complex before its extraction with butyl acetate; platinum and palladium then remained in the aqueous phase. Uranium(VI) and thorium(IV) were co-extracted with rhenium, but considerable amounts of these ions could be tolerated in the spectrophotometric method for rhenium.

Phosphate, tartrate, citrate, oxalate, fluoride, chloride and EDTA could be present at mg-levels without interference.

Application to the analysis of synthetic samples

Various synthetic mixtures were prepared and analysed by the proposed method. The results are shown in Table III. It can be seen that a wide variety of diverse mixtures can be analysed with satisfactory results.

TABLE III

ANALYSIS OF SYNTHETIC MIXTURES BY THE PROPOSED METHOD

(Rhenium(VII) added, 32.5 μg; conditions as under Separation of molybdenum, etc.)

Composition of the mixture (mg)			*Re found (μg)*
Mo	*V*	*W*	
500	—	—	31.0
400	—	—	32.0
200	10	10	32.0
100	20	20	32.5
Mo(100)[a], V(10), W(10), Fe(III)(10), Co(10) Cr(VI)(10), Ni(10), Cu(10), U(VI)(10), Se(5), Te(5)			32.5
Mo(100), V(20), W(10), Fe(III)(20), Co(10), Ni(10), Cr(VI)(15), Cu(20), Pd(II)(2)[b], Pt(IV)(2)[b], U(VI)(10)			32.0
Mo(200), V(5), W(5), Fe(III)(50), Cr(VI)(5), Se(20), Te(20)			32.0
Mo(100), V(5), W(5), Os(VIII)(5), Ru(VII)(5), Rh(III)(5)			32.0
Mo(50), V(10), W(10), Nb(V)(5), Ta(V)(5)			32.5
Mo(50), V(10), W(10), Os(VIII)(5), Ru(VII)(5), Cr(VI)(10), Nb(V)(5), Ta(V)(5), Se(5), Te(5)			32.0

[a] Numbers in brackets show mg-amounts of the element.

[b] Thioglycollic acid added to rhenium thiocyanate complex.

Quantitative conversion of rhenium(VII) to the rhenium thiocyanate complex

Rhenium(VII) was quantitatively converted to yellow rhenium thiocyanate complex in 3–4 *M* hydrochloric acid in the presence of 1.2–1.4% tin(II) chloride solution. The formation of the complex was complete within 10 min in 1.9–2.2% potassium thiocyanate solution. The yellow complex could be extracted with N-benzylaniline into chloroform. This showed that the rhenium thiocyanate complex was anionic in nature and is probably[25] $ReO(SCN)_4^-$. *n*-Butyl acetate and diisopropyl ether were preferred as solvents for the extraction of the rhenium thiocyanate complex, rather than diethyl ether or isoamyl alcohol[20] because of their lesser volatility, and

because the complex was stable for several days in these solvents. The molar absorptivity of the rhenium thiocyanate complex in these solvents was $40{,}280 \pm 280$.

Conclusion

The proposed method is very simple and remarkably free from interferences. None of the experimental conditions is highly critical. The difficult separation of rhenium from molybdenum, which interferes in almost all methods for its determination, can be readily achieved by the proposed extraction method. The present method also separates rhenium from many other cations which interfere in many methods for its determination.

SUMMARY

Analytical separation of rhenium(VII) is achieved by solvent extraction with N-benzylaniline in chloroform from sulphuric acid media. Few cations interfere; common anions such as phosphate, tartrate, citrate, oxalate, fluoride, EDTA and ascorbic acid do not interfere. A simple method is described for the separation of micro amounts of rhenium from macro amounts of molybdenum, tungsten, niobium and tantalum. The method also separates rhenium from V, Cr, Se, Te, Os, Ru, Rh, Pd and Pt. The molar absorptivity of rhenium thiocyanate complex in butyl acetate and diisopropyl ether is 40280 ± 280 at 430 nm.

RÉSUMÉ

Une séparation analytique du rhénium(VII) est obtenue par extraction au moyen de N-benzylaniline dans le chloroforme, en milieu acide sulfurique. Peu de cations interfèrent; les anions courants tels que: phosphate, tartrate, citrate, oxalate, fluorure, EDTA et acide ascorbique ne gènent pas. Une méthode simple est proposée pour la séparation de microquantités de rhénium, d'avec beaucoup de molybdène, de tungstène, de niobium et de tantale; elle permet également la séparation d'avec V, Cr, Se, Te, Os, Ru, Rh, Pd et Pt. Le coefficient d'extinction molaire du complexe rhénium/thiocyanate dans l'acétate de butyle et dans l'éther diisopropylique est de 40280 ± 280, à 430 nm.

ZUSAMMENFASSUNG

Die analytische Abtrennung von Rhenium(VII) wird durch Extraktion mit N-Benzylanilin in Chloroform aus schwefelsaurem Medium erreicht. Wenige Kationen stören; übliche Anionen wie Phosphat, Tartrat, Citrat, Oxalat, Fluorid, EDTA und Ascorbinsäure stören nicht. Es wird eine einfache Methode für die Abtrennung von Mikromengen Rhenium von Makromengen Molybdän, Wolfram, Niob und Tantal beschrieben. Die Methode trennt Rhenium ebenfalls von V, Cr, Se, Te, Os, Ru, Rh, Pd und Pt. Der molare dekadische Extinktionskoeffizient des Rheniumthiocyanat-Komplexes in Butylacetat und Diisopropyläther ist 40280 ± 280 bei 430 nm.

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PARTAGE ENTRE DEUX PHASES AQUEUSE ET ORGANIQUE DE L'OXYDE DE (CARBOXY-2-ETHYL)DIPHENYLPHOSPHINE EN PRESENCE DE DERIVES BASIQUES

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(Reçu le 23 avril 1972)

Les associations en phase organique entre différents types d'agents extractants acides et d'agents neutres ont été étudiées par de nombreux auteurs tels Dyrssen[1,2] ou Liem[3,4], étant donné les possibilités de synergisme qu'offrent ces systèmes dans l'extraction des sels métalliques. Dans ce travail nous nous sommes intéressés, aux associations dans le chloroforme entre l'oxyde de (carboxy-2-éthyl) diphénylphosphine (CEDPPO ou HA) et un dérivé donneur d'électrons (B) tel la 4-(3-phénylpropyl)-pyridine (43PPP), l'oxyde de triphénylphosphine (TPPO) ou l'oxyde de trioctyl-phosphine (TOPO). Cette étude permet de mettre en évidence, par distribution liquide–liquide, la formation en phase organique de complexe mixte du type HA, B avec chacun de ces trois composés et de préciser les différentes constantes de formation $K_{1,1}$ correspondantes.

ÉTUDE THÉORIQUE DU PARTAGE

L'étude du partage du CEDPPO entre une phase aqueuse et le chloroforme a fait l'objet d'une précédente publication[5] dans laquelle sont définis les symboles K_n (avec $1 \leqslant n \leqslant 4$), K_D, ϕ, C_a et D qui sont utilisés dans les relations qui suivent.

La concentration initiale de B est constante. La concentration totale en CEDPPO varie, l'acidité et la force ionique de la phase aqueuse sont maintenues constantes

Les indices o et a désignent respectivement les phases organique et aqueuse. Nous supposons qu'à l'équilibre, en plus des différentes formes polymérisées H_nA_n (avec $1 \leqslant n \leqslant 4$), existent en phase organique des complexes mixtes du type $(HA)_x\,B_y$ dont les équilibres de formation sont:

$$x\,HA_o + y\,B_o \rightleftarrows (HA)_x\,B_{y_o}$$

et dont la constante d'équilibre de formation $K_{x,y}$ correspondante est:

$$K_{x,y} = \frac{[HA]_x[B_y]_o}{[HA]_o^x[B]_o^y}$$

Si nous appelons D' le coefficient de partage du CEDPPO entre les deux phases aqueuse et organique en présence de B, nous démontrons alors la relation:

$$D' = D + \sum_{x=1}^{x} \sum_{y=1}^{y} K_{x,y} \cdot [B]_o^y \left(\frac{K_D}{\phi}\right)^x C_a^{x-1}$$

Lorsque les complexes mixtes les plus susceptibles de se former sont de la forme (HA) B_y, cette relation peut alors se simplifier selon l'expression:

$$D' = \frac{K_D}{\phi}\left(1 + \sum_{y=1}^{y} K_{1,y}[B]_o^y\right) + 2K_2\left(\frac{K_D}{\phi}\right)^2 C_a + \ldots + 4K_4\left(\frac{K_D}{\phi}\right)^4 C_a^3 \qquad (1)$$

Ainsi lorsque $[B]_o$ est constant et que la concentration en CEDPPO varie, la représentation de log D' en fonction de log C_a est une courbe qui

(*a*) d'une part admet une asymptote horizontale dont l'ordonnée dépend de la concentration $[B]_o$,

(*b*) d'autre part tend vers la courbe de partage du CEDPPO seul dans le même système eau–chloroforme, lorsque les termes $2K_2\,(K_D/\phi)^2\,C_a + \ldots + 4K_4\,(K_D/\phi)^4\,C_a^3$ deviennent grands devant ceux

$$\sum_{y=1}^{y} K_{1,y}[B]_o^y .$$

La concentration totale du CEDPPO est faible. L'acidité et la force ionique de la phase aqueuse sont maintenues constantes. La concentration initiale de B varie

Lorsque la concentration du CEDPPO est suffisamment faible pour que, dans le chloroforme, sa forme monomère soit prédominante devant les autres formes polymères, le coefficient de partage D' peut alors s'écrire:

$$D' = \frac{K_D}{\phi}\left(1 + \sum_{y=1}^{y} K_{1,y}[B]_o^y\right)$$

et sous forme logarithmique, cette relation devient:

$$\log D' - \log\frac{K_D}{\phi} = \log\left(1 + \sum_{y=1}^{y} K_{1,y}[B]_o^y\right) \qquad (2)$$

Lorsqu'il n'existe qu'un seul complexe (HA) B_y, aux points expérimentaux obtenus en traçant log $D' - \log(K_D/\phi) = f(\log [B]_o)$, nous pouvons adapter une courbe théorique normalisée du type log $Y = \log(1 + X^y) = f(\log X)$ qui admet deux asymptotes, l'une horizontale et l'autre oblique de pente y, permettant ainsi la détermination de y et de log $K_{1,y}$.

PARTIE EXPÉRIMENTALE

Technique de partage

Le partage est étudié à 25° dans un ensemble thermostaté déjà décrit[5]. Le pH de la phase aqueuse, de force ionique $\mu = 1$, est fixé à 1.00 à l'aide de mélanges acide perchlorique–perchlorate de sodium. Les solutions dans le chloroforme du CEDPPO et des différents composés B sont préparées par pesées et ajustées ultérieurement à la concentration voulue par dilution. Les mesures de partage sont faites après mise en équilibre de 10 cm^3 de chacune des deux phases pendant 3 h suivies de 30 min de décantation.

Mesure du coefficient de partage du CEDPPO

La technique radiométrique étant, par sa facilité et sa rapidité, fort bien adaptée

à la résolution de tels systèmes, nous avons synthétisé[6] du CEDPPO marqué à l'isotope carbone-14. Pour cela nous avons utilisé de l'acide acrylique marqué au $^{14}C^*$, fourni par le Commissariat à l'Energie Atomique de Saclay (France).

Les dosages de chacune des deux phases sont faits par comptage de l'énergie β^- du $^{14}C^*$, à l'aide d'un compteur à scintillation liquide Intertechnique SL 20[6].

RÉSULTATS EXPÉRIMENTAUX

Partage du CEDPPO en présence de 4-(3-phénylpropyl)pyridine

Pour des concentrations initiales en 43PPP de $6.82 \cdot 10^{-3}$ M, $5.7 \cdot 10^{-2}$ M et $1.14 \cdot 10^{-1}$ M nous mesurons le partage du CEDPPO entre la phase aqueuse ($\mu = 1$ et pH = 1.00) et le chloroforme. La Figure 1 montre les courbes obtenues en portant log D' en fonction de log C_a. Ces courbes présentent des asymptotes horizontales dont l'ordonnée croît lorsque la concentration en 43PPP augmente, et tendent vers la courbe de partage du CEDPPO seul lorsque log C_a devient important. Comme le montre la relation (1), il existe donc en solution organique des complexes mixtes (HA)-B_y dont il faut déterminer les valeurs de y.

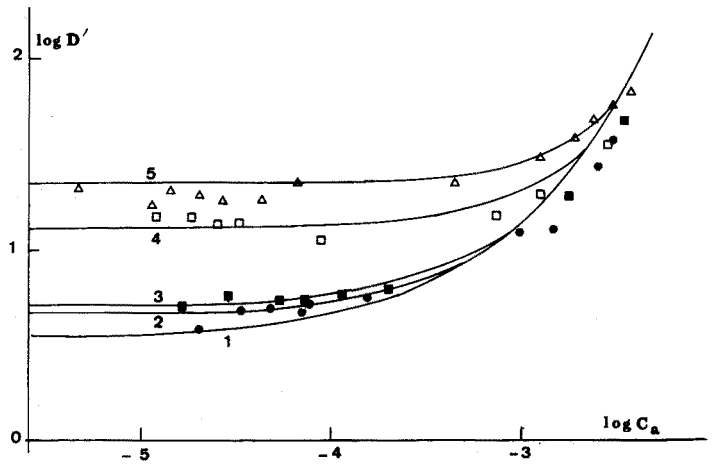

Fig. 1. Partage du CEDPPO en présence de 43PPP. (1) CEDPPO seul. Concentrations initiales en 43PPP: (2) $6.82 \cdot 10^{-3}$ M; (3) $9.68 \cdot 10^{-3}$ M; (4) $5.7 \cdot 10^{-2}$ M; (5) $1.14 \cdot 10^{-1}$ M. Courbes (2), (3), (4), (5): (−) tracé théorique calculé à partir de $K_{1,1} = 46$.

Pour des concentrations initiales en CEDPPO inférieures à 10^{-4} M, nous faisons varier la 43PPP de $3.42 \cdot 10^{-3}$ M à $2.85 \cdot 10^{-1}$ M. La concentration du CEDPPO est alors telle que sa forme monomère prédomine en phase organique. De plus la concentration de la 43PPP, toujours importante devant celle du CEDPPO, n'est pas modifiée à l'équilibre après la formation des complexes (HA) B_y. Nous portons sur la Figure 2 les résultats obtenus en traçant log $D' - \log(K_D/\phi)$ en fonction de log $[43PPP]_o$. Aux résultats expérimentaux obtenus, nous pouvons adapter une courbe théorique normalisée du type log $Y = \log(1 + X) = f(\log X)$ admettant deux asymptotes: l'une horizontale d'ordonnée nulle et l'autre oblique de pente 1. Dans ces conditions expérimentales, la relation (2) peut alors s'écrire sous la forme:

$$\log D' - \log \frac{K_D}{\phi} = \log(1 + K_{1,1}\,[43PPP]_o)$$

montrant qu'il existe un complexe mixte et un seul du type CEDPPO–43PPP entre la forme monomère du CEDPPO et la 43PPP. La constante de formation de ce complexe $K_{1,1}$ est donnée par la résolution graphique et est égale à 46 ± 2.

Nous avons recalculé à l'aide de cette constante $K_{1,1}$ les courbes de distribution du CEDPPO en présence de 43PPP. La bonne concordance entre les points expérimentaux de la Figure 1 et les courbes de partage ainsi calculées montre qu'il n'existe probablement pas d'autres complexes mixtes entre la 43PPP et le CEDPPO.

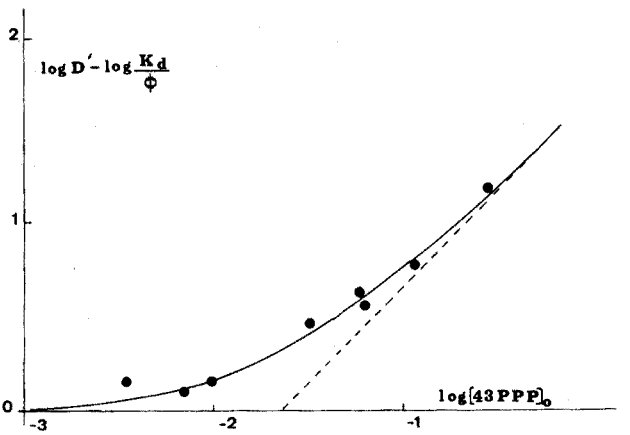

Fig. 2. Détermination de la forme du complexe mixte CEDPPO–43PPP. (–) Courbe théorique normalisée du type $\log Y = \log(1+X) = f(\log X)$.

Partage du CEDPPO en présence d'oxydes de phosphine

Une étude analogue à la précédente est menée avec l'oxyde de triphénylphosphine (TPPO) et l'oxyde de trioctylphosphine (TOPO). Nous présentons sur la Figure 3 le partage du CEDPPO en présence de ces oxydes de phosphine à des concentrations initiales de $5.07 \cdot 10^{-2}$ *M* pour le TPPO et de 10^{-1} *M* pour le TOPO.

Les courbes obtenues admettent des asymptotes horizontales montrant ainsi l'existence de complexes du type (HA) B_y. La détermination des valeurs de y est faite par le tracé des courbes $\log D' - \log(K_D/\phi)$ en fonction de $\log [B]_o$ pour des concentrations initiales en CEDPPO de $1.03 \cdot 10^{-4}$ *M*. Aux courbes 4 et 5 ainsi obtenues respectivement pour le TPPO et le TOPO, nous adaptons la courbe théorique normalisée précédemment définie (Figures 4 et 5).

Dans nos conditions expérimentales, il existe donc un seul complexe mixte CEDPPO–B qui se forme entre la forme monomère du CEDPPO et chacun des deux oxydes de phosphine. La résolution graphique donne alors des valeurs de constantes $K_{1,1}$ correspondantes égales à 71 ± 3 et 186 ± 8 respectivement pour le TPPO et le TOPO. A l'aide de ces constantes, nous avons recalculé le partage du CEDPPO en présence de TPPO ou de TOPO. La bonne concordance entre les points expérimentaux de la Figure 3 et ces courbes ainsi recalculées montre qu'il n'existe probablement pas d'autres complexes mixtes entre chacun de ces deux oxydes de phosphine et les différentes formes du CEDPPO.

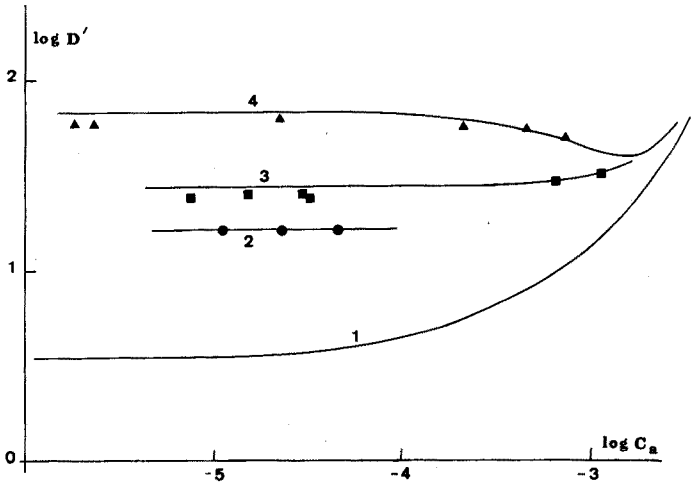

Fig. 3. Partage du CEDPPO en présence de TPPO ou de TOPO. (1) CEDPPO seul. (2), (3) Concentrations initiales de TPPO respectivement de $5.07 \cdot 10^{-2}$ M et 10^{-1} M. (4) Concentration initiale de TOPO 10^{-1} M. Courbes (2), (3), (4): (−) tracé théorique calculé à partir de $K_{1,1} = 71$ et $K_{1,1} = 186$ respectivement pour le TPPO et le TOPO.

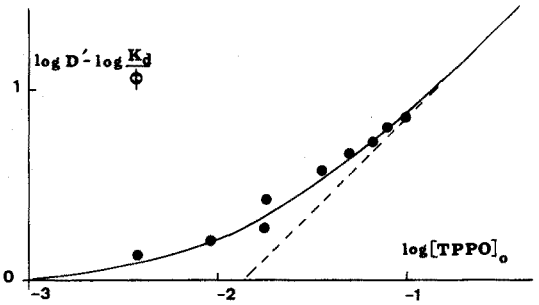

Fig. 4. Détermination de la forme du complexe mixte CEDPPO–TPPO. (−) Courbe théorique normalisée du type $\log Y = \log(1+X) = f(\log X)$.

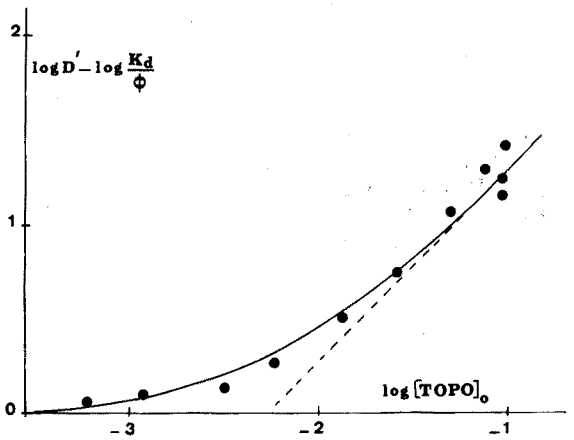

Fig. 5. Détermination de la forme du complexe mixte CEDPPO–TOPO. (−) Courbe théorique normalisée du type $\log Y = \log(1+X) = f(\log X)$.

CONCLUSION

Nous avons mis en évidence la formation de complexes mixtes CEDPPO–B dont les constantes de stabilité de 71 ± 3 pour le complexe CEDPPO–TPPO et de 186 ± 8 pour le complexe CEDPPO–TOPO sont conformes au pouvoir donneur du groupement phosphoryle de ces oxydes de phosphine. La constante de stabilité du complexe CEDPPO–43PPP égale à 46 ± 2 est légèrement moins forte que les précédentes, mais ne leur est pas directement comparable compte-tenu de la nature azotée du groupement donneur. Dans nos conditions expérimentales la formation d'autres complexes entre CEDPPO et donneurs n'a pu être mise en évidence.

RÉSUMÉ

Les auteurs ont montré par des méthodes d'extraction liquide–liquide la formation de complexes mixtes 1:1 entre l'oxyde de (carboxy-2-éthyl)diphénylphosphine et la 4-(3-phénylpropyl)pyridine, l'oxyde de triphénylphosphine ou l'oxyde de trioctylphosphine, et ont donné les valeurs des constantes de formation correspondantes.

SUMMARY

In liquid–liquid extraction, complex formation in the organic phase involves the species CEDPPO–B between (carboxy-2-ethyl)diphenylphosphine oxide and 4-(3-phenylpropyl)pyridine, triphenylphosphine oxide or trioctylphosphine oxide. The complex formation constants are given.

ZUSAMMENFASSUNG

Verteilungsversuche ergaben, dass (Carboxy-2-äthyl)diphenylphosphinoxid mit 4-(3-Phenylpropyl)pyridin, Triphenylphosphinoxid und Trioctylphosphinoxid 1:1-Komplexe bildet. Die Komplexbildungskonstanten werden angegeben.

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THE USE OF DERIVATIVES FOR THE GAS-CHROMATOGRAPHIC IDENTIFICATION OF ALCOHOLS, PRIMARY AND SECONDARY AMINES, AND THIOLS IN FOOD AROMAS

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Chemical reactions used in addition to the information obtained from gas-chromatographic retention data are a significant help with problems related to the qualitative analysis of very complex materials such as the volatile constituents of aromas and odours[1]. Some research workers have included these reactions under the common denomination of "Reaction Gas Chromatography", although this term should only be used in the restricted sense of reactions occurring inside a chromatographic column, precolumn or postcolumn, that is, in the chromatographic system as defined by Beroza[2].

The methods described in this paper involve the chromatographic separation of derivatives of the compounds to be identified. Gas-chromatographic separations of derivatives of volatile constituents of food aromas and odours show many advantages compared with the separation of the original aroma compounds, especially when the chemical reactions are specific for certain functional groups.

The derivatives studied in this paper are:

(*a*) alkyl and thioalkylbenzoates (AB, TAB) for alcohols and thiols, respectively;

(*b*) N-alkylbenzamides (B) for primary and secondary amines;

(*c*) 2,4-dinitrophenylalkylthioethers (2,4-DNPT) and 2,4-dinitrophenylalkylsulphones (2,4-DNPS) for thiols.

MacManus *et al.*[3] have separated the alkylbenzoate derivatives of alcohols, but they did not give information on column deactivation or on correlations between the retention data and molecular structure. Galetto *et al.*[4] have separated alcohol-3,5-dinitrobenzoates in columns packed with SE-30 (silicone gum rubber) operated at 250°; however, this method appears to be more suitable for alcohols of low molecular weight. Minyard *et al.*[5] have reported the separation of 3,5-dinitrobenzoates and *o*-nitrophenylurethanes formed at the column outlet and further analysed by thin-layer chromatography.

With regard to the other derivatives, N-alkylbenzamides of primary and secondary amines, and the 2,4-DNPT and 2,4-DNPS derivatives of thiols, there do not seem to be any references to their use in connection with chromatographic identification purposes.

The methods investigated in this paper have been used successfully to study the effects of γ-irradiation on alcohols in apple juice[6] and on thiols, and primary and secondary amines in hake[7].

ALKYL- AND THIOALKYLBENZOATES FROM ALCOHOLS AND THIOLS

Experimental

Reagents. The reagents used were of analytical grade, wherever possible. Standard benzoates were prepared by the conventional method of benzoylation.

Preparation of benzoyl derivatives from volatile concentrates. The volatile concentrate from flash distillation as described elsewhere[8] was acidified by addition of 10 ml of 0.4 *M* sulphuric acid, and extracted with several 5-ml portions of benzene. The benzene extracts (about 30 ml) were dried over anhydrous sodium sulphate, and then 1 ml of pyridine and 0.5 ml of benzoyl chloride were added. This mixture was vigorously shaken, and allowed to settle for several hours; after this time it was washed with 2 *M* hydrochloric acid to complete the elimination of pyridine. The excess of benzoyl chloride was hydrolyzed with water and the resulting mixture was vigorously shaken for at least 12 h. Finally the benzene phase was washed with a 10% solution of sodium carbonate, dried over anhydrous sodium sulphate and distilled down to a volume of about 0.5 ml. This solution was then injected into the gas chromatograph. Benzene and volatile constituents of aroma which were not benzoyl derivatives were removed in this distillation, whereas the benzoyl derivatives of mercaptans and alcohols remained in the residue because of their high boiling point (more than 200°).

Results and discussion

Chromatographic separation. Separations were carried out under the condi-

TABLE I

GENERAL CONDITIONS FOR THE SEPARATION OF DERIVATIVES

Equipment: Perkin Elmer F-11 gas chromatograph with dual columns
Columns: stainless steel, 1 m long, 3.2 mm external diameter, 2 mm internal diameter
Packing: 5% SE-30 silicone gum rubber on Chromosorb G-HP
Deactivation: presaturation with sample
Argon flow rate: 50–55 ml min^{-1}
Air pressure: 1.8 kg cm^{-1}
Hydrogen pressure: 1.6 kg cm^{-1}

TABLE II

CONDITIONS FOR THE SEPARATION OF DERIVATIVES OF ALCOHOLS, PRIMARY AND SECONDARY AMINES, AND THIOLS

	Benzoates	*Benzamides*	*2,4-Dinitrophenylthioethers and sulphones*
Injection port temp. (°)	325	325	325
Oven temp. (°)	125	160	185
Detector attenuation	× 500, × 100	× 1000, × 50	× 5000, × 500, × 200
Sample concentration	5 mg ml^{-1}	4 mg ml^{-1}	25 mg ml^{-1}
Standard concentration	1 mg ml^{-1}	1 mg ml^{-1}	4 mg ml^{-1}
Solvent	Benzene	Benzene	Chloroform

tions listed in Tables I and II. Total deactivation of the chromatographic system was achieved by successive injections of a standard benzoate solution into the column. Columns once deactivated must then be used exclusively for the separation of benzoyl derivatives. The reproducibility of retention data achieved in deactivated columns remained unchanged for at least one year of continuous operation.

Isothermal operation at 125° allowed separation of derivatives up to the hexylbenzoate, and with temperature programming it was possible to separate up to the decylbenzoate.

The results can only be used for semiquantitative estimations, owing to the fact that lower alcohols, such as methanol and ethanol, are not completely extracted. The efficiency of the extraction of the former was 50% and that of the latter 75%. The efficiency of the extraction of the other alcohols was better than 90%. However, by the careful control of all the extraction processes, results were reproducible.

Figure 1 shows a typical separation of alcohols as their benzoates in apple juice. Peaks corresponded to alcohol concentrations in the original juice, ranging from 0.08 p.p.m. to 13 p.p.m.

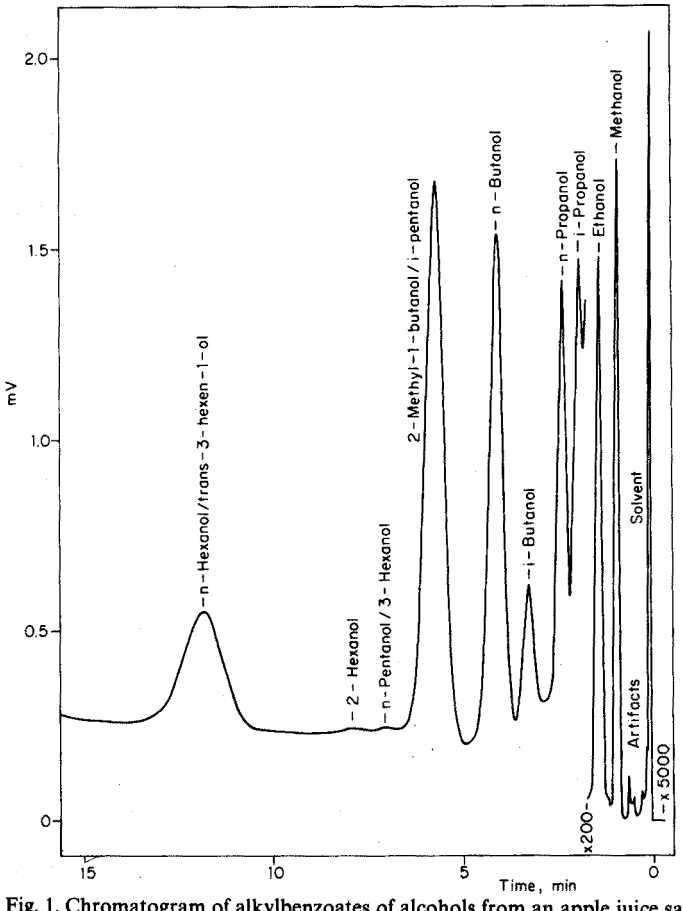

Fig. 1. Chromatogram of alkylbenzoates of alcohols from an apple juice sample.

TABLE III

RETENTION DATA OF ALKYL- AND THIOALKYLBENZOATES

(Separation conditions see Table II)

Benzoyl derivative of	*Relative retentions*	
	$r_{x.15}$	σ
Methanol	0.106	0.003
Ethanol	0.156	0.004
Isopropanol	0.185	0.000
2-Propen-1-ol	0.238	0.005
Propanol	0.262	0.002
Methylmercaptan	0.288	0.003
sec-Butanol	0.312	0.001
Isobutanol	0.358	0.002
2-Methyl-3-buten-2-ol	0.377	0.001
tert-Pentanol	0.410	0.002
Ethylmercaptan	0.418	0.003
Butanol	0.444	0.004
3-Pentanol	0.487	0.001
2-Pentanol	0.496	0.001
2-Methyl-1-butanol	0.624	0.002
Isopentanol	0.626	0.002
Propylmercaptan	0.703	0.002
1-Hexen-3-ol	0.722	0.005
3-Hexanol	0.758	0.001
Pentanol	0.766	0.003
2-Hexanol	0.824	0.003
2-Hexenol	0.846	0.001
3-Hexen-1-ol(*cis*)	1.190	0.001
3-Hexenol	1.204	0.003
Butyl mercaptan	1.208	0.004
Hexanol	1.284	0.005
3-Hexen-1-ol(*trans*)	1.292	0.008
3-Hepten-1-ol	1.958	0.002
Artifacts[a]:		
Benzoyl chloride	0.097	0.000
Benzoic acid	0.171	0.002

[a] Artifact formed in the benzoylation process.

Correlations of retention data with molecular structure. Table III shows retention data, relative to *n*-pentadecane, of lower alkyl- and thioalkylbenzoates. In Fig. 2, log $r_{x.15}$ of benzoates are plotted against the carbon number of the original alcohols and thioalcohols. From these correlations, the following information is obtainable.

(*a*) The log $r_{x.15}$ values of the ethyl- to hexylbenzoates lie on a straight line. These derivatives fulfil the linear relationship between retention time and carbon number that is characteristic of an homologous series. Mercaptan derivatives also fulfil this relationship. As both straight lines are approximately parallel, it can be deduced that the increase in molecular weight caused by the substitution of sulphur for oxygen in the molecule increases the relative retention in log $r_{x.15}$(S) by +0.42.

(*b*) The relative retentions of the 2-butanol, 2-pentanol and 2-hexanol benzoyl

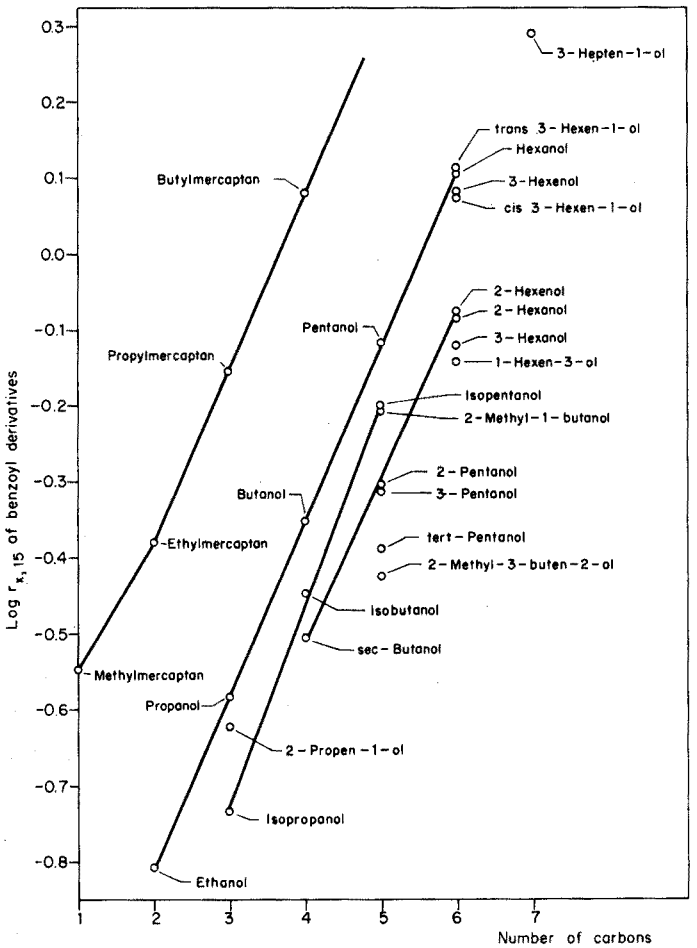

Fig. 2. Correlations between relative retentions and number of carbons for alcohols and mercaptans as their alkyl and thioalkyl benzoates, at 125°.

derivatives are markedly smaller than those of the respective *n*-alcohol benzoyl derivatives.

(*c*) Tertiary alcohol derivatives show the lowest retentions, and it is to be noted that the tertiary pentanol derivative exhibits lower retention than the *n*-butanol derivative.

(*d*) The iso-branching in alkyl chains diminishes retention; this diminution is inversely proportional to chain length and can be explained because the differences in molecular volumes between iso compounds and the corresponding normal compounds are more marked at shorter chain lengths. From the slope of the straight line for iso derivatives, it was found that for alcohol derivatives containing more than 7 carbon atoms, the relative retentions for both iso and normal alcohols were approximately equal.

(*e*) It can be observed that benzoyl derivatives showing more spherical symmetry tend to exhibit lower relative retentions.

N-ALKYLBENZAMIDES

Experimental

Reagents. Hydrochloric acid and sodium hydroxide were of analytical grade. Benzoyl chloride was twice distilled.

Chloroform was purified to remove ethanol traces, as follows. Chloroform was washed with water several times and dried over anhydrous sodium sulphate. Benzoyl chloride and pyridine (5 ml of each for 1 litre of chloroform) were added and this mixture was allowed to settle for several hours; it was then fractionally distilled in an 18-plate column. This chloroform was used freshly distilled.

Standard benzamides. Derivatives of amines were obtained from analytical-grade amines by the Schotten and Baumann method[9]. Solid derivatives were purified by fractional crystallization until constant melting points were obtained. Liquid derivatives were purified by the combination of high-vacuum distillation and column chromatography on silica gel or alumina.

Preparation of benzamides from volatile concentrates. To the aqueous volatile concentrates contained in the collection traps (as described elsewhere[8]) 5 ml of 2 *M* hydrochloric acid were added. The sample was poured into a small round-bottomed flask, and distilled under vacuum (14 mm) over a water bath. After complete removal of water, the resulting mixture (containing amine hydrochlorides and some ammonium chloride) was benzoylated by the Schotten and Baumann method[9]. After the flask had been cooled to 0°, 20 ml of aqueous 10% sodium hydroxide solution and 0.4 ml of benzoyl chloride were added. The mixture was vigorously shaken for 15 min whilst the temperature was maintained at 0°, and then shaken at room temperature until benzoyl chloride was completely hydrolyzed. Benzamides were isolated by leaching the resulting solution with five or six aliquots of chloroform (5 ml).

The chloroform extracts were dried over anhydrous sodium sulphate and distilled under vacuum to remove the solvent completely. The residue containing the benzamides was weighed and dissolved in chloroform (20 μl mg^{-1}). This solution was injected into the chromatograph.

Results and discussion

Chromatographic separation. The separations were carried out under the conditions described in Tables I and II, *n*-octadecane being used as reference for relative retentions.

Figure 3 shows a typical chromatogram of N-alkylbenzamides corresponding to the ammonia and primary and secondary amines from a 200-g sample of hake.

Correlations of retention data with molecular structure. Table IV includes the retentions of the tested benzamides relative to *n*-octadecane, and Fig. 4 the correlations of log $r_{x.18}$ of benzamides with the number of carbons of the original amines. For these derivatives, there is a linear correlation of log $r_{x.18}$ with carbon number. As usual, this linear relationship was not followed by the lower members of the series, *e.g.* ammonia and methylamine derivatives.

The log $r_{x.18}$ values of N-dialkylbenzamides lie on a different straight line and show lower retentions than the alkylbenzamides containing the same number of carbon atoms. Diisoalkylbenzamides exhibit retentions lower than those corresponding to di-*n*-alkylbenzamides.

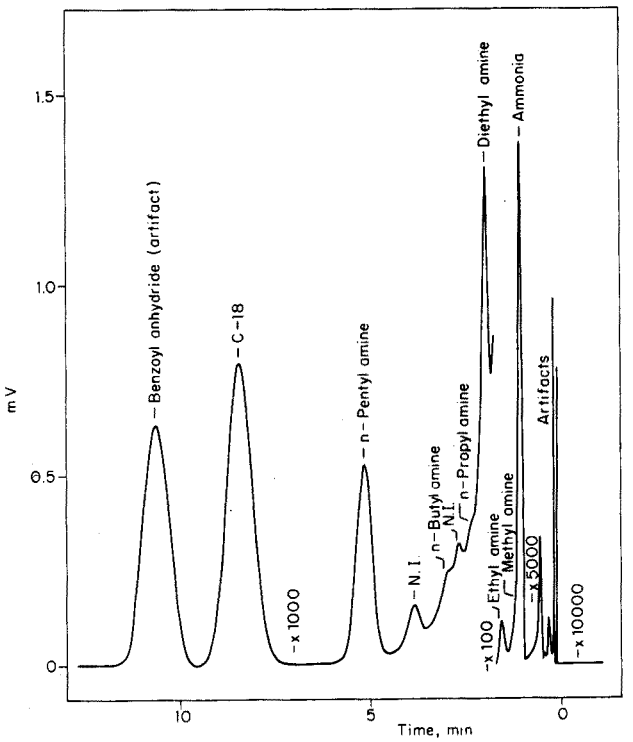

Fig. 3. Chromatogram of benzamides of amines from irradiated hake (2.04 Mrads).

TABLE IV

RETENTIONS RELATIVE TO *n*-OCTADECANE OF SOME BENZAMIDES

(Separation conditions see Table II)

Compound	$r_{x.18}$	σ
Benzamide (B)	0.112	0.002
N-Methylbenzamide (MB)	0.133	0.001
N-Ethylbenzamide (EB)	0.165	0.001
N,N'-Diethylbenzamide (DEB)	0.217	0.002
N-Alkylbenzamide (AB)	0.232	0.001
N-Propylbenzamide (PB)	0.248	0.004
N-Butylbenzamide (BB)	0.391	0.004
N-Pyrrolidinebenzamide (PRB)	0.525	0.002
N-Pentylbenzamide (PeB)	0.598	0.005
N,N'-Diisobutylbenzamide (DiBB)	0.601	0.003
n-C_{17} Secondary standard	0.642	0.000
N,N'-Dibutylbenzamide (DBB)	0.952	0.004
N-Hexylbenzamide (HB)	0.993	0.009
Benzoic anhydride[a]	1.255	0.003
N,N'-Dipentylbenzamide (DPeB)	2.00	0.003
N,N'-Dihexylbenzamide (DHB)	7.04	0.04

[a] Artifact formed in the benzoylation process.

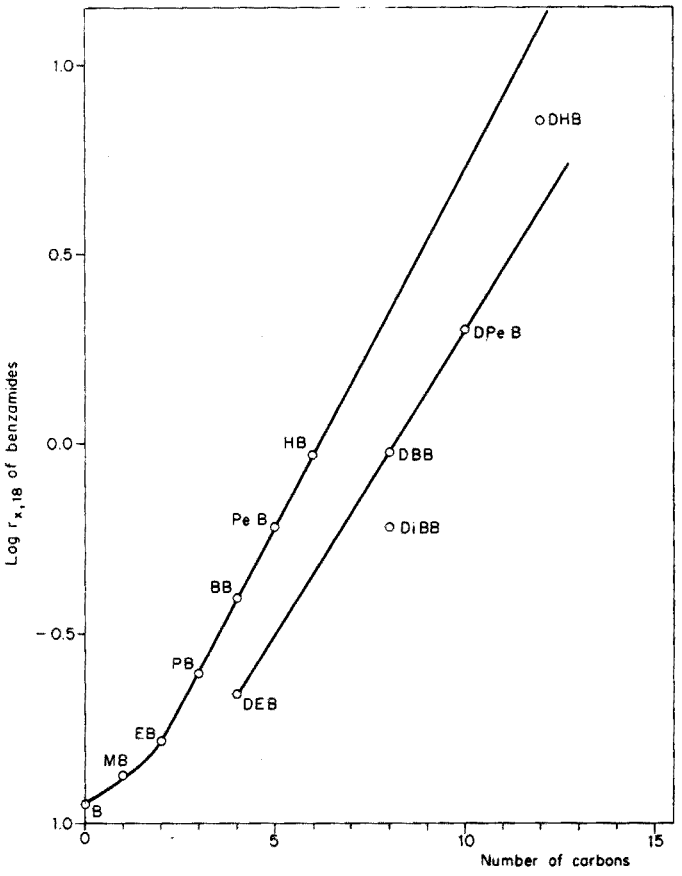

Fig. 4. Correlations between relative retentions and number of carbons for primary and secondary amines as their benzamides, at 160°.

DINITROPHENYLALKYLTHIOETHERS (2,4-DNPT) AND 2,4-DINITROPHENYLALKYLSULPHONES (2,4-DNPS) FROM THIOLS

Experimental

Reagents. 2,4-Dinitrochlorobenzene was synthesized from chlorobenzene by the conventional method[10], and purified by recrystallizing in methanol. Chloroform (analytical-grade) was fractionally distilled in an 18-plate column. Sodium hydroxide and methanol were of analytical grade.

Standard 2,4-DNPT derivatives were synthesized from pure mercaptans by the conventional method[10], and purified by repeated recrystallizations in methanol. For the standard 2,4-DNPS derivatives, the 2,4-DNPT derivatives were oxidized with potassium permanganate solution as described below.

Preparation of 2,4-DNPT derivatives from volatile concentrates. 3 ml of chloroform was added to the trap containing the frozen volatile concentrates[8], and 1 ml of 0.5 *M* sulphuric acid was added. The resulting mixture was transferred to a distillation flask, and distilled under reduced pressure until a solid residue remained which was

composed of ammonium sulphate and amine sulphates. The distillate was condensed in a trap immersed in liquid nitrogen; 4 drops of 6 *M* sodium hydroxide and 10 ml of methanol were added to the frozen distillate and the resulting liquid was transferred to a 25-ml Erlenmeyer flask containing 1 ml of a methanolic solution of 2,4-dinitrochlorobenzene (4 mg ml^{-1}). This flask was warmed to reflux for 10 min and the solvent was completely removed under vacuum. The residue was dried, and was repeatedly extracted with chloroform until the extract was not yellow. These chloroform extracts were collected, filtered and vacuum-distilled to remove all traces of chloroform. The residue was weighed and dissolved in chloroform and injected into the gas chromatograph.

Preparation of 2,4-DNPS from 2,4-DNPT derivatives. The solid residue, containing the 2,4-DNPT derivatives, was dissolved in a minimal volume of acetic acid, and a few drops of an aqueous solution of potassium permanganate were added, until the solution remained pink-coloured. Excess of permanganate was removed by adding sodium sulphite solution until the pink colour just disappeared. The resulting mixture was diluted with distilled water and extracted repeatedly with chloroform. These chloroform extracts were washed with 10% sodium carbonate solution and distilled under vacuum to remove the chloroform.

Results and discussion

Chromatographic separation and correlations of retention data with molecular structure. Separations were carried out under the conditions included in Tables I and II; the retention times of the standard 2,4-DNPT and 2,4-DNPS derivatives relative to *n*-docosane are listed in Table V. The log $r_{x\,.22}$ values of these derivatives plotted

TABLE V

RETENTIONS RELATIVE TO *n*-DOCOSANE (*n*-C_{22}) OF SOME 2,4-DINITROPHENYLALKYLTHIOETHERS AND 2,4-DINITR OPHENYLALKYLSULPHONES

(Separation conditions see Table II)

Compound	$r_{x\,.22}$	σ
2,4-Dinitrophenol[a]	0.065	0.001
2,4-Dinitroanisole[a]	0.152	0.002
2,4-Dinitrophenyl-*tert*-butylthioether	0.300	0.002
2,4-Dinitrophenylmethylsulphone	0.317	0.003
2,4-Dinitrophenylmethylthioether	0.337	0.002
2,4-Dinitrophenylisopropylthioether	0.361	0.004
2,4-Dinitrophenylethylthioether	0.379	0.005
2,4-Dinitrophenylethylsulphone	0.402	0.002
2,4-Dinitrophenylisopropylsulphone	0.461	0.005
2,4-Dinitrophenyl-*sec*-butylthioether	0.507	0.003
2,4-Dinitrophenylpropylthioether	0.518	0.001
2,4-Dinitrophenylpropylsulphone	0.525	0.001
2,4-Dinitrophenyl-*sec*-butylsulphone	0.635	0.002
2,4-Dinitrophenyl-*tert*-butylsulphone	0.639	0.000
2,4-Dinitrophenylbutylthioether	0.728	0.003
2,4-Dinitrophenylbutylsulphone	0.732	0.002

[a] Artifacts.

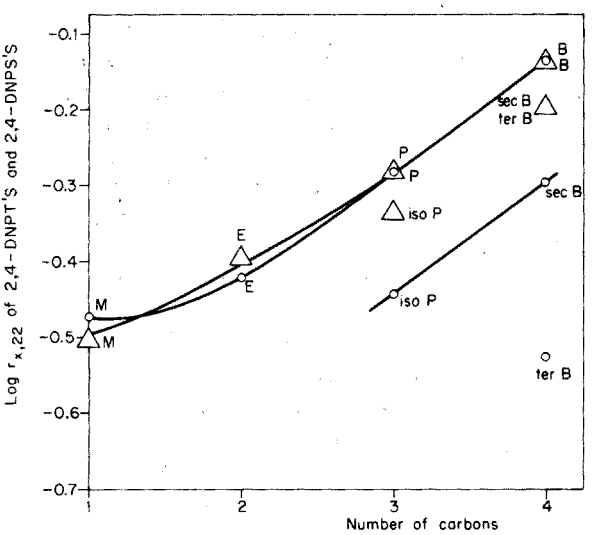

Fig. 5. Correlations between relative retentions and number of carbons for mercaptans as their 2,4-DNPT and 2,4-DNPS derivatives at 185°. M, methyl; E, ethyl; P, propyl; B, butyl. (○) Thioethers, (△) sulphones.

against the number of carbons in the original mercaptans are shown in Fig. 5. The correlations show the marked influence of the molecular structure on the relative retention of the derivatives. Chain-branching appears to be the most significant factor in determining the retention behaviour in mercaptan derivatives. Linear chains adapt better to the linear structure of the methylpolysiloxane. When branching occurs there is a steric hindrance to this adaptation which results in a marked reduction of retention volumes. This is illustrated in the following scheme in which R- is the 2,4-dinitrophenyl radical, and numbers in parentheses are the $r_{x.22}$ values.

(a) R–S–CH_2–CH_2–CH_2–CH_3 (0.728)

(b) R–S–CH_2–CH_2–CH_3 (0.518) R–S–CH(CH_3)–CH_2–CH_3 (0.507)

(c) R–S–CH_2–CH_3 (0.379) R–S–CH(CH_3)–CH_3 (0.361)

(d) R–S–CH_3 (0.337) R–S–C(CH_3)$_2$–CH_3 (0.300)

In the sulphones, chain branching does not influence the retention behaviour so markedly as in the thioethers.

The transformation of the 2,4-DNPT into the 2,4-DNPS derivatives and a further chromatographic separation improves the reliability of the qualitative identification of these compounds.

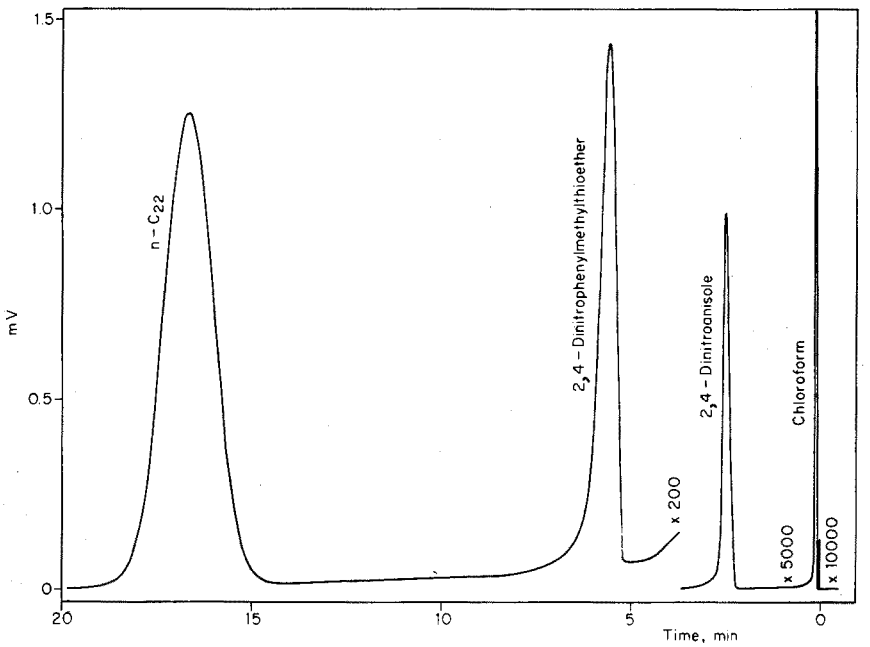

Fig. 6. Chromatogram of 2,4-DNPT derivatives from irradiated hake (4.1 Mrads).

In Fig. 6 a chromatogram of mercaptan derivatives from irradiated hake volatiles is shown. Methylmercaptan was identified; the other peak corresponds to a reaction artefact and does not interfere in the separation.

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SUMMARY

Conditions are given for the formation, from volatile concentrates of food aromas, of the following derivatives: alkyl- and thioalkylbenzoates for alcohols and thiols, N-alkylbenzamides for primary and secondary amines, and 2,4-dinitrophenylalkylthioethers and 2,4-dinitrophenylalkylsulphones for thiols. Gas-chromatographic separations were carried out on 5% SE-30 silicone gum rubber on Chromosorb G-HP in 1-m columns at temperatures ranging from 125 to 185°. Correlations between retention data and molecular structure are studied and some typical chromatograms are given. The chromatographic information from these derivatives is a significant help for the identification of some volatile constituents of food aromas.

RÉSUMÉ

On décrit les conditions de formation des dérivés suivants de concentrés

volatils d'aromes alimentaires: alcoyl- et thioalcoylbenzoates pour alcools et thiols, N-alcoylbenzamides pour amines primaires et secondaires, dinitro-2,4-phénylalcoylthioéthers et dinitro-2,4-phénylalcoylsulfones pour thiols. On effectue des séparations par chromatographie gazeuse et on examine les résultats obtenus en fonction de la structure moléculaire. Quelques chromatogrammes typiques sont donnés. Cette étude facilite l'identification de quelques constituants volatils d'aromes alimentaires.

ZUSAMMENFASSUNG

Es werden die Bedingungen angegeben, unter denen sich aus flüchtigen Konzentraten von Nahrungsmittel-Aromastoffen die folgenden Derivate bilden: Alkyl- und Thioalkylbenzoate für Alkohole und Thiole, N-Alkylbenzamide für primäre und sekundäre Amine und 2,4-Dinitrophenylalkylthioäther und 2,4-Dinitrophenylalkylsulfone für Thiole. Gaschromatographische Trennungen wurden an 5% Silikongummi SE-30 auf Chromosorb G-HP in 1 m-Säulen bei Temperaturen von 125 bis 185° ausgeführt. Beziehungen zwischen Retentionsdaten und Molekülstruktur werden untersucht und einige typische Chromatogramme vorgelegt. Die von diesen Derivaten erhaltene chromatographische Information ist eine bedeutende Hilfe bei der Identifizierung einiger flüchtiger Komponenten von Nahrungsmittel-Aromastoffen.

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REVERSED-PHASE FOAM CHROMATOGRAPHY

SEPARATION OF PALLADIUM, BISMUTH AND NICKEL IN THE TRIBUTYL PHOSPHATE–THIOUREA–PERCHLORIC ACID SYSTEM

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In a recent preliminary note[1] "foam chromatography" was proposed as a new variant of column chromatography. It was suggested that chromatographic adsorption, exchange and partition processes could be favourably influenced by giving the adsorbent a hollow spherical (cellular) form, and effecting these processes on the internal surface of the cells. This was done by using solid, rigid or flexible, foamed synthetic polymers as column fillings. Open cell-type plastic foams may be regarded as a relatively regular stack of spherical cells.

Ross and Jefferson[2], and Schnecko and Bieber[3] have already proposed the use of polyurethane foam fillings in gas chromatography.

In this laboratory, investigations have been started on the application of foamed column fillings in reversed-phase and ion-exchange[4] chromatographic separations, and on their use for redox purposes[5].

In the present paper the first detailed results on reversed-phase foam chromatography are reported.

The application of reversed-phase chromatography has recently received considerable attention because it combines the selectivity of liquid–liquid extraction and the advantages of chromatographic operation. In general, the efficiency of reversed-phase chromatographic separation depends on many interrelated parameters including the particle size of the support, surface area, flow characteristics and stationary phase. Careful control of these parameters will be of great value in increasing the potentialities of this technique.

Several papers have been published[6,7] describing various reversed-phase systems which utilize a wide variety of organic stationary phases. However, the scope of application of this technique is still limited by the relative lack of inert supports which can adsorb and retain relatively high loads of organic complexing agents and at the same time permit good flow characteristics. In that respect foamed inert supports were expected to show the advantage of good flow-rate and high loading. In the preliminary communication[1], open cell-type polyurethane foam was proposed as inert support in reversed-phase chromatography. It was found that this foam can retain a considerable amount of stationary phase more efficiently than other known reliable granular supports. The hydrodynamic properties of a foam-packed chromatographic column are extremely favourable. Relatively high flow-rates can be easily attained simply by gravity flow. The literature seems to be devoid of data on reversed-phase chromatography on foam-packed columns.

In this paper, polyurethane foam was used in a column technique where separation of palladium(II), bismuth(III) and nickel(II) in the tributyl phosphate–thiourea–perchloric acid system[1] was achieved. The effect of changing flow-rate, temperature and ratio of the separated ions on the efficiency of separation was tested.

EXPERIMENTAL

Reagents and materials

All reagents used were of analytical grade unless otherwise specified. Tri-*n*-butyl phosphate (TBP) used as stationary phase was purified as described by Hamlin *et al.*[8]. Polyurethane foam, polyether type, was an open-cell flexible polymer. This material was supplied by the North Hungarian Chemical Works, Sajóbábony, Hungary.

Palladium and nickel chloride solutions were prepared by dissolving the chloride salts in 0.25 *M* hydrochloric acid and distilled water, respectively. The two solutions were standardized gravimetrically, in the form of their dimethylglyoximate complexes[9]. Bismuth perchlorate solution was prepared by dissolving 0.1 g of bismuth metal (99.9%) in 5 ml of nitric acid and evaporating to dryness on a steam bath; 5 ml of 60% perchloric acid was then added and the evaporation was continued to about 1 ml, before the solution was diluted to 100 ml with distilled water. The resulting bismuth solution was standardized titrimetrically by EDTA[9].

Apparatus

Columns. Glass columns of 10 and 15 mm in diameter and 15 cm long were used. A separating funnel was fitted at the top of the column as a reservoir of the eluting solution.

Fraction collector. A fraction collector (type Müfém, Hungary) holding 200 tubes was employed.

Spectrophotometer. A Hungarian spectrophotometer type MOM-203 was employed; 1-cm silica cells were used.

Column preparation

The polyurethane foam was washed 3 times with acetone and then dried at 80°. The dried foam material was equilibrated with purified TBP (5 ml per 1.5 g of foam) with efficient stirring, and then allowed to remain in contact with TBP overnight to ensure complete saturation. The loaded foam was dried between two sheets of filter paper to remove excess of TBP. The foam material was then packed in the column applying gentle pressure with a glass rod. To avoid air bubbles during packing, tap (1) (Fig. 1) was connected to a suction pump while tap (2) was closed. The flat-bottomed connection of tap (1) prevented the foam material from upward movement during suction. After about 10 min of evacuation, distilled water was allowed to fill the column gradually through tap (2). Then the tap (1) stopper was replaced with a separatory funnel as a reservoir.

Experiments at controlled temperatures were performed with jacketed 15-mm i.d. colums. Water, whose temperature was kept constant at $\pm 0.1°$ by a thermostat, was circulated inside the column jacket.

The preparation of loaded foam for batch experiments was essentially the same as for column experiments (5 ml TBP per 1.5 g foam).

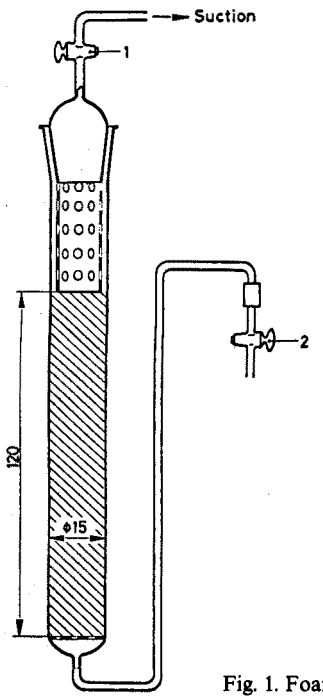


Fig. 1. Foam chromatographic column in packing step.

Procedure

Separation of palladium and nickel. The feed solution contained 1 ml of palladium chloride solution (0.618 mg Pd^{2+} ml^{-1}), 1 ml of nickel chloride solution (1 mg Ni^{2+} ml^{-1}) and 2 ml of 0.2 *M* perchloric acid containing 6% thiourea and 2% sodium perchlorate (Solution I). Sorption of this mixture took place at a flow-rate of 1 ml min^{-1}. A 0.1 *M* perchloric acid solution containing 3% thiourea and 1% sodium perchlorate (Solution II) was used to remove nickel ion. Water was then used for the elution of the palladium–thiourea complex at 2–3 ml min^{-1}.

Separation of palladium and bismuth. A mixture of 1 ml of the above palladium solution, 1 ml of bismuthyl perchlorate solution (1 mg Bi^{3+} ml^{-1}) and 2 ml of solution (I) was used as feed solution. Sorption of this mixture took place as described above; 30 ml of solution (II) was then used to wash the column. The bismuth–thiourea complex was eluted with 0.5 *M* perchloric acid, and the palladium– thiourea complex was then eluted with water.

Separation of palladium, bismuth and nickel. The feed solution contained 1 ml of each of the above palladium, nickel and bismuth solutions and 3 ml of solution (I). Nickel was eluted with solution (II), bismuth with 0.5 *M* perchloric acid and palladium with water.

The effluent from the column was collected by means of an automatic fraction collector. For overall elution each ion was collected in 100-ml measuring flasks. All the aqueous solutions used were equilibrated with TBP.

To estimate the interstitial volume of the column, TBP solution was poured on the TBP-loaded foam column; the volume of the effluent before the appearance of the TBP layer was then a measure of the interstitial volume.

Analytical methods

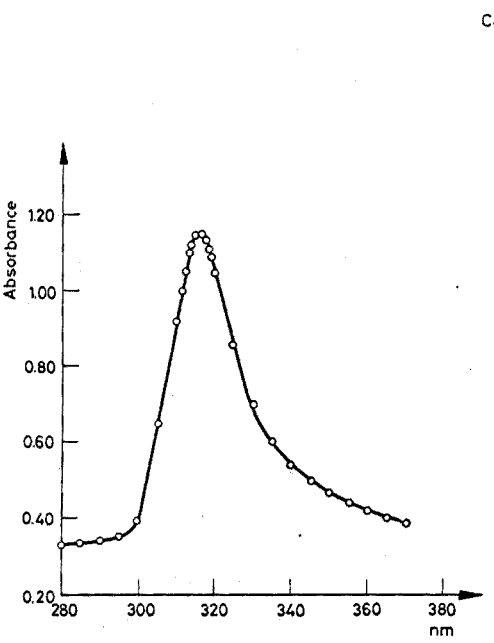
Palladium was determined spectrophotometrically as the palladium–thiourea complex at 360 nm in aqueous solution following the recommendation of Neilsch[10].

Bismuth was determined spectrophotometrically as the bismuth–thiourea complex after addition of a suitable amount of thiourea to attain a concentration of *ca.* 3%. The measurements were carried out at 470 nm[11].

Nickel was determined spectrophotometrically as the nickel–dimethylglyoxime complex at 366 nm[9].

RESULTS AND DISCUSSION

Before the column extraction of the palladium–thiourea complex was studied, the liquid–liquid extraction of the system was briefly examined. Various experiments were performed in which a fixed excess of thiourea (3%) was added to palladium (0.618 mg) at different concentrations of perchlorate ion. The optimal amount of perchlorate ion was 0.1 *M* perchloric acid and 1% sodium perchlorate; higher or lower concentrations of this ion decreased the efficiency of extraction in accordance with the results of Doležal *et al.*[12]. Excess of thiourea over palladium was used to ensure the formation of the tetracomplex[13]. It was found that the palladium–thiourea complex was quantitatively extracted with TBP from aqueous solutions containing the above concentrations of perchlorate and thiourea. The extracted species showed



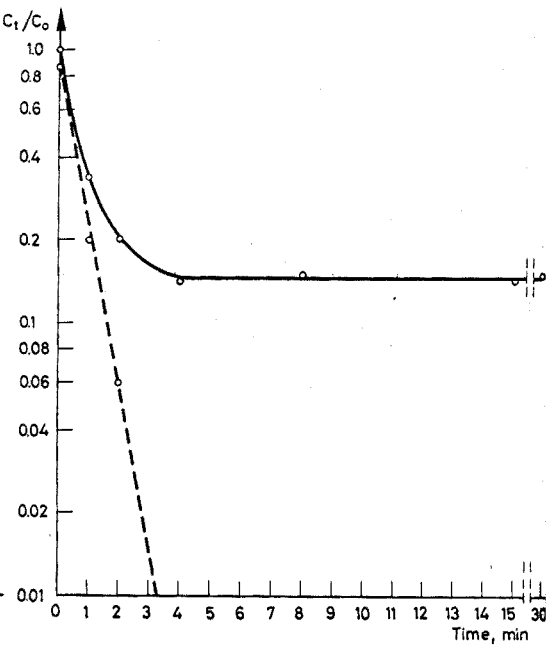


Fig. 2. Absorption spectrum of palladium–thiourea complex in TBP (equal volume of aqueous and TBP phases). Pd^{2+} $5.8 \cdot 10^{-5}$ *M*; $NaClO_4$ 1%; thiourea 3%; $HClO_4$ 0.1 *M*.

Fig. 3. Rate of adsorption of the palladium–thiourea complex on TBP-loaded polyurethane foam at room temperature. Pd^{2+} $1.44 \cdot 10^{-4}$ *M*; $NaClO_4$ 1%; thiourea 3%; $HClO_4$ 0.1 *M*.

maximum absorbance at 315 nm (Fig. 2), and a molar absorptivity of $1.0 \cdot 10^4$ mol^{-1} cm^{-1}. Beer's law was obeyed in the concentration range $1\text{–}6 \cdot 10^{-5}$ M of palladium in the initial 10-ml aqueous phase when 5 ml of TBP was used for extraction. The extracted complex was found to be stable for 8 h after extraction. Standing for 24 h caused the intensity to decrease by 2–5%.

The results obtained for liquid–liquid extraction of the palladium–thiourea complex with TBP are in good agreement with those obtained by reversed-phase chromatography with TBP as stationary phase on a column of Voltalef (polychlorotrifluoroethylene)[12].

The rate of extraction of the palladium–thiourea complex by TBP-loaded polyurethane foam was investigated by the batch technique. In separate experiments, 0.1 g of loaded foam was mixed with 10 ml of palladium–thiourea complex ($1.44 \cdot 10^{-4}$ M) in a 50-ml stoppered flask. The flasks were then shaken for different times (1–30 min) and palladium(II) was determined in the aqueous phase. It can be seen from Fig. 3 that the uptake of the complex by the loaded foam is fast. The half-life time of equilibrium adsorption was calculated from Fig. 3 and found to be 0.5 min.

The uptake of the palladium–thiourea complex by the loaded foam was found to depend on the concentration of the complex in the aqueous phase. Thus, in separate experiments palladium(II) in different concentrations was shaken with loaded foam, all other parameters being kept constant. Analysis of the aqueous phase for palladium after shaking for 30 min gave the curve shown in Fig. 4. The uptake of palladium by the foam was calculated by difference. The isotherm shows that a good linear dependency was maintained between the concentration of palladium on the foam and its concentration in aqueous solution over a relatively wide range of palladium concentration. In all cases equilibrium was approached only from one direction, that of palladium-rich aqueous phase.

Chromatographic behaviour of Pd–thiourea complex on a column of TBP-loaded polyurethane foam

Quantitative retention and elution of the palladium–thiourea complex was examined. Thus, when 0.309 mg of palladium (2 ml) in 0.1 M perchloric acid containing 3% thiourea and 1% sodium perchlorate was introduced to the column, the complex was retained as a narrow yellow band just on top of the column material. It was then washed with a solution having the same composition as that used before except for palladium(II). Elution of the complex was quantitatively effected by passing water at a rate of 1 ml min^{-1} and 25°; 10-ml fractions were collected and the absorbance of the complex was measured in the aqueous phase at 360 nm. Figure 5 shows a chromatogram indicating that all the palladium could be eluted in the first 90 ml. The curve was symmetrical with relatively sharp peak. The height equivalent to a theoretical plate was calculated from the equation given by Glueckauf[14]

$$N = 8 \frac{V_{max}^2}{W^2} = \frac{L}{HETP}$$

where N = number of plates,
V_{max} = volume of the eluate to peak maximum,
W = width of the peak at $1/e$ times the maximum solute concentration,
L = length of the bed.

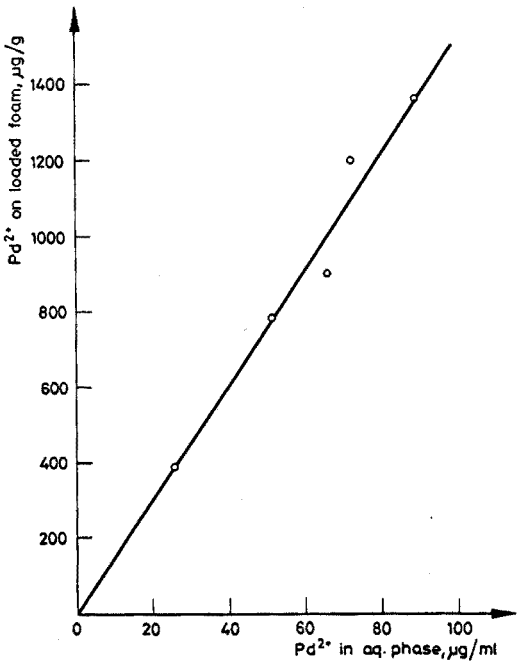


Fig. 4. Isotherm for the adsorption of the palladium–thiourea complex on TBP-loaded foam.

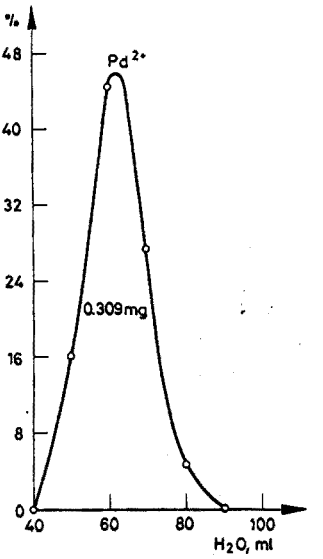


Fig. 5. Chromatogram of the palladium–thiourea complex on TBP-loaded foam at 25°. Bed height 115 mm; flow-rate 1 ml min^{-1}.

The *HETP* was found to be 1.7 mm. This value was reproducible within ±7%. The *HETP* was also estimated from the break-through capacity curve in Fig. 8[15].

$$N = \frac{\bar{v}v'}{(\bar{v}-v')^2}$$

where $\bar{v}$ is the volume of effluent at the center of the S-shaped break-through curve where the concentration is one-half of the initial concentration, and v' is the volume at which the effluent has the concentration 0.1587 of the initial concentration.

The value of the *HETP* found in this way confirmed the above value calculated from the elution curve.

Effect of temperature and flow-rate on the HETP and V_{max} of the palladium–thiourea complex elution

Flow-rates ranging between 0.9 and 6.4 ml cm^{-2} min^{-1} were applied at 25, 35 and 45°. The results are shown in Fig. 6. The increase in plate height (decrease in column performance) with increasing flow-rate was only pronounced at 25°. On the other hand, a slight increase in plate height with increased flow-rate occurred at higher temperatures, so that relatively high flow-rates could be applied without any appreciable loss in column performance.

The V_{max} value was not affected by the flow-rate (broadening of the peak). On the other hand, V_{max} decreased on increasing the temperature (Fig. 7) and at the

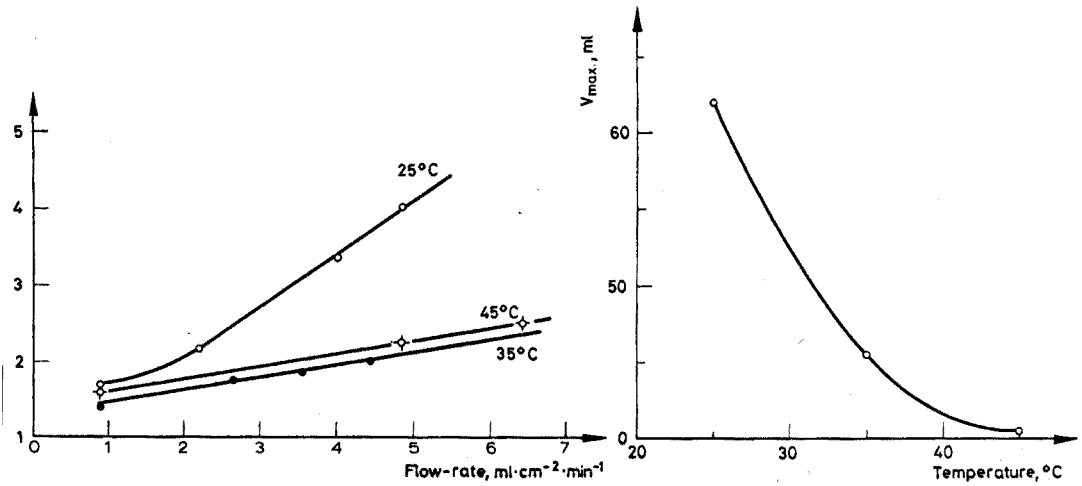


Fig. 6. Effect of temperature and flow-rate on the *HETP* of the palladium–thiourea complex.

Fig. 7. Effect of temperature on V_{max} of the palladium–thiourea complex.

same time peak width decreased, resulting in sharper peaks. In general, increasing the temperature within the above-mentioned limits seemed to improve the column performance for palladium elution.

Capacity

The practical usefulness of a column packed with TBP-loaded foam can be tested by measuring its break-through capacity. Obviously, a column with a high break-through capacity will be used in many applications. Break-through capacity was defined as the amount of palladium–thiourea complex that could be retained on the column when the complex was allowed to pass through it at a rate of 1 ml min^{-1} until the complex was first detected in the effluent solution. A solution of the following composition was used: 0.309 mg Pd^{2+} per ml of 0.1 *M* perchloric acid–3 % thiourea–1 % sodium perchlorate. Practically, this capacity was determined from the actual volume that was collected just before the appearance of the palladium–thiourea complex in the effluent solution minus the free-column volume. The resultant value was multiplied by the concentration of the original solution. After reaching the break-through volume, elution was continued until the effluent solution concentration reached that of the feed solution, namely 0.309 mg Pd^{2+} ml^{-1}. The curves of Fig. 8 represent both the break-through volume and the volume needed to reach bed saturation, for two samples of polyether open cell-type polyurethane foam, A1 and A2. It is clear from the curves that the rising portions have large slopes which indicate both a high transfer rate of the palladium–thiourea complex at the surface of the foam material and within the stationary phase, and that the adsorption equilibrium of the palladium–thiourea complex on TBP-loaded foam can be attained rapidly. These results suggest that adsorption takes place on the relatively high surface area of the foam material and that mass transfer (diffusion) does not represent an important rate-determining step, as is usual in ion-exchange resin beads. Further support for this view was the

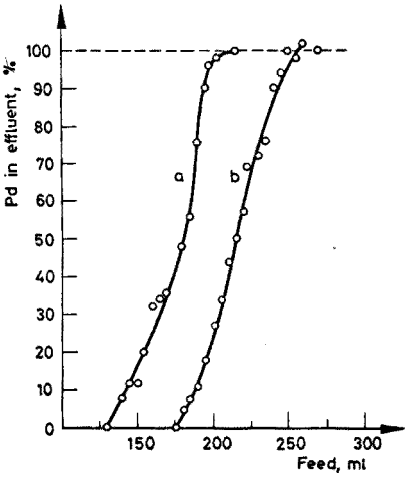


Fig. 8. Break-through curve of the palladium–thiourea complex on TBP-loaded foam. Bed height 100 and 125 mm; feed: palladium thiourea complex, 0.309 mg Pd^{2+} ml^{-1}; flow-rate 1 ml min^{-1}. (a) Polyurethane foam sample A1; (b) polyurethane foam sample A2.

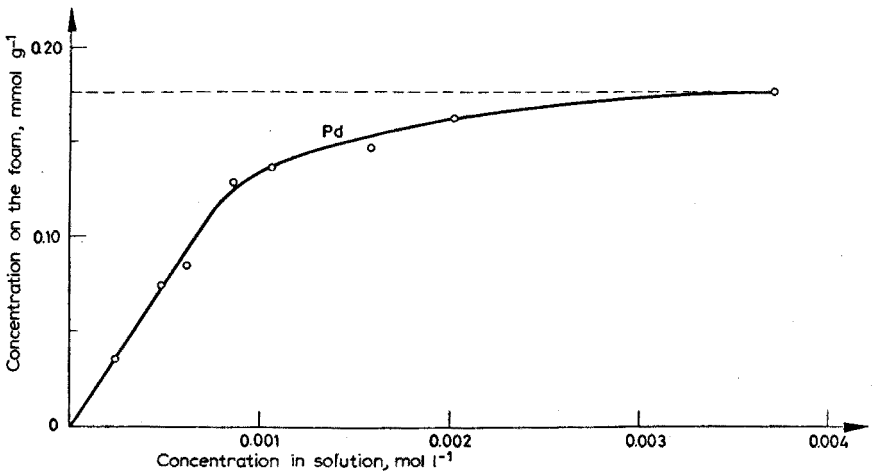


Fig. 9. Capacity determination of TBP-loaded foam (sample A2) by the batch method.

good agreement of the capacity of sample A2 when determined both in the dynamic (break-through) technique and batch (equilibrium value) technique (Fig. 9).

The break-through capacities as determined from the curves of Fig. 8 were 12.1 mg Pd^{2+} g^{-1} of loaded foam for sample A1 and 16.7 mg Pd^{2+} g^{-1} of loaded foam for sample A2.

The break-through capacity as determined above was higher for sample A2 than sample A1, although the amount of TBP adsorbed per gram of support was about the same. This difference can be attributed to structural differences in the foam samples. Factors affecting the capacity of different varieties of polymeric foams of different chemical and physical properties are under consideration.

ANALYTICAL APPLICATION

As a consequence of the above characterization of column performance for the palladium–thiourea complex, the separation of palladium(II) was tried first from nickel(II), which does not form a complex with thiourea under the experimental conditions used, and then from bismuth(III) which readily forms a thiourea complex. It has been reported[16] that the bismuth–thiourea complex is also extracted on a column packed with TBP-loaded Voltalef. Separation of the three ions was also tried.

Separation of palladium(II) from nickel(II)

Nickel(II) is known to interfere seriously in the determination of palladium by atomic absorption spectrometry or by neutron activation analysis[17,18]. By using a TBP-loaded polyurethane foam column, it was possible to separate microamounts of palladium(II) from nickel(II) as the latter does not form a complex with thiourea and hence was not retained by the bed. In fact, nickel(II) moved with the solvent front (0.1 *M* perchloric acid–3% thiourea–1% sodium perchlorate), while the palladium(II) complex was retained and subsequently eluted with water. The separation was successful and efficient, even when a large amount of nickel(II), *e.g.* 1000 mg, was present together with 0.618 mg of palladium(II) (Table I). Separation was also possible for larger amounts of palladium(II).

TABLE I

SEPARATION OF PALLADIUM OR BISMUTH FROM NICKEL

Ions present		*Ratio of*	Pd^{2+}		*Ions present*		*Ratio*	Bi^{3+}	
Pd^{2+} (*mg*)	Ni^{2+} (*mg*)	*Pd:Ni*	*Found (mg)*	*% Recovery*	Bi^{3+} (*mg*)	Ni^{2+} (*mg*)	*of Bi:Ni*	*Found (mg)*	*% Recovery*
0.618	1	1:1.62	0.600	97.1	1	1	1:1	1.008	100.8
0.618	1	1:1.62	0.612	99.0	1	1	1:1	1.008	100.8
0.618	10	1:16.18	0.612	99.0	1	10	1:10	0.975	97.5
0.618	10	1:16.18	0.630	101.9	1	10	1:10	0.980	98.0
0.618	100	1:161.81	0.620	100.0	1	100	1:100	0.982	98.2
0.618	100	1:161.81	0.625	101.1	1	100	1:100	0.983	98.3
0.618	1000	1:1618.1	0.612	99.0	1	1000	1:1000	0.982	98.2
0.618	1000	1:1618.1	0.640	103.0	1	1000	1:1000	0.995	99.5

Separation of palladium(II) from bismuth(III)

Although both bismuth(III) and palladium(II) form thiourea complexes, which are retained on a TBP-loaded foam, yet the former complex could be eluted quantitatively with 0.5 *M* perchloric acid. The palladium(II)–thiourea complex was not affected by that eluent, hence separation was achieved by eluting the bismuth(III) complex first with 0.5 *M* perchloric acid and then the palladium(II) complex was eluted as usual with water (*cf.* Table II).

Separation of nickel(II), palladium(II) and bismuth(III) from each other

Separation of the components of the mixture was made in light of the fact

TABLE II

SEPARATION OF PALLADIUM, BISMUTH AND NICKEL

Ions present			Pd^{2+}		Bi^{3+}		Ni^{2+}	
Pd^{2+} (μg)	Bi^{3+} (μg)	Ni^{2+} (μg)	*Found* (μg)	*% Recovery*	*Found* (μg)	*% Recovery*	*Found* (μg)	*% Recovery*
618			630	101.9				
618			620	100.0				
	1000				1000	100.0		
	1000				1012	101.2		
		1000					1000	100.0
		1000					1010	101.0
618		1000	610	99.0			1008	100.8
618		1000	615	99.5			1014	101.4
618	1000		620	100.0	1010	101.0		
618	1000		640	103.0	1007	100.7		
618	1000	1000	600	97.1	1008	100.8	1005	100.5
618	1000	1000	612	99.0	1008	100.8	1000	100.0

that the bismuth(III) and palladium(II) complexes could not be eluted from the column with 0.1 *M* perchloric acid–3% thiourea–1% sodium perchlorate which was the optimal eluent for nickel(II). The bismuth(III) complex was then eluted with 0.5 *M* perchloric acid and finally the palladium(II) complex was eluted quantitatively with water, at a flow-rate of 2.5 ml min^{-1} and room temperature. The elution curves are shown in Fig. 10.

The use of the TBP–polyurethane foam appears therefore, to be useful for the separation of various elements, especially in light of the fact that packed columns

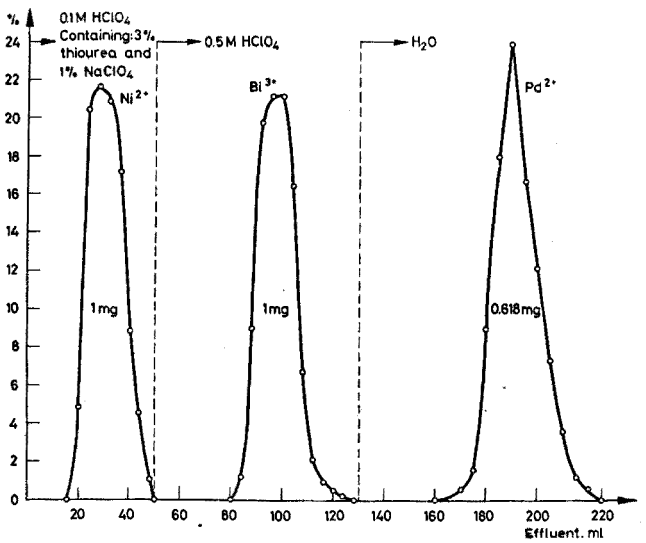


Fig. 10. Separation of Ni–Bi–Pd on TBP-loaded foam at room temperature. Bed height 100 mm; flow-rate 2.5 ml min^{-1}.

could be used over 30 cycles in elution experiments without losing their efficiency. The properties of a packed column do not change with time and separations can be achieved successfully on columns prepared 20 days before use.

SUMMARY

The feasibility of using TBP-loaded polyurethane foams as column fillings in reversed-phase chromatography was examined and their capacities were determined. Separation of nickel, bismuth, and palladium was achieved. The effect of flow-rate, temperature and ratio of the separated ions on the efficiency of separation was tested. Elution with high flow-rate affected the efficiency of the column at 25° but not at higher temperature (*e.g.* 35°). Once the column was prepared, it could be used several times without affecting its efficiency.

RÉSUMÉ

On examine les possibilités d'utilisation de mousse de polyuréthane chargée de TBP, comme remplissage de colonne pour chromatographie. On a pu ainsi réaliser la séparation nickel, bismuth et palladium, en tenant compte de l'influence de la vitesse d'écoulement, de la température et de la proportion des ions séparés. Une telle colonne peut être utilisée plusieurs fois, avant que son efficacité soit diminuée.

ZUSAMMENFASSUNG

Die Verwendbarkeit von TBP-beladenen Polyurethan-Schäumen als Säulenfüllung bei der Chromatographie mit umgekehrten Phasen wurde untersucht; die Kapazitäten wurden ermittelt. Nickel, Wismut und Palladium wurden getrennt. Der Einfluss von Fliessgeschwindigkeit, Temperatur und Verhältnis der getrennten Ionen auf die Trennwirkung wurde geprüft. Die Elution mit hoher Fliessgeschwindigkeit beeinflusste die Trennwirkung der Säule bei 25°, jedoch nicht bei höherer Temperatur, z.B. 35°. Die einmal vorbereitete Säule konnte mehrmals ohne nachlassende Trennwirkung verwendet werden.

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SEPARATION AND CHARACTERIZATION OF RUTHENIUM HYDROLYZATES BY GEL-PERMEATION CHROMATOGRAPHY

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Gel chromatography is a technique for separating solute molecules in accordance with their sizes. At first this technique was used almost exclusively for separating macromolecules in organic or biochemical systems; but in the last decade an increasing number of investigations has shown that gel chromatography can also be useful for inorganic polymeric systems.

The inorganic polymer most extensively studied so far is that of iron which, upon hydrolysis of the nitrate salt, can produce polymers having molecular weights of up to 100,000. Spiro *et al.*[1] examined these compounds at different base–iron ratios, while Henry and Rogers[2] separated the polymeric from the monomeric fraction on polydextran gels.

Ruthenium is known to form a unique series of nitrosyl nitrate compounds. Upon hydrolysis these are also capable of forming polymers of high molecular weights[3,4]. The study of these compounds is of special interest, since they occur in nitric acid solutions of irradiated fissile materials, after uranium fission.

The present paper describes an investigation of the hydrolytic behaviour of ruthenium nitrosyl nitrate in nitric acid solutions and of the properties of the fractions obtained by gel-permeation chromatography.

EXPERIMENTAL

Apparatus

Ultracentrifuge. A Beckman Model E analytical ultracentrifuge equipped with an AN-Δ rotor and photoelectric scanning system was used. Sedimentation experiments were made at a velocity of 40,000 rev min^{-1}. A double-sector KeLF 12-mm cell was used, one sector containing 0.3 ml of the ruthenium nitrosyl nitrate solution studied, and the other an equal quantity of nitric acid solution.

Spectrophotometry. A Perkin-Elmer Model 137 spectrophotometer was used with 1-cm W.F. Spectrosil cells.

Atomic absorption. A Perkin-Elmer Model 403 atomic-absorption spectrophotometer was used. Absorbances were measured at 350 nm with a ruthenium hollow-cathode lamp.

Solutions

Two stock solutions were prepared, *viz.* a 0.1 *M* solution of reagent ruthenium nitrosyl nitrate (K & K Laboratories, Plainview, N.Y.) in 0.025 *M* nitric acid (Solu-

tion 1), and a 0.1 *M* solution of a 10% ruthenium concentrate, which had been aged for several months, in 2.5 *M* nitric acid (Solution 2).

For the determination of ruthenium in the solutions, Woodhead's[5] spectrophotometric method for determining ruthenium as ruthenate–perruthenate mixtures was applied. The molar absorptivity was found to be 1047 l mole^{-1} cm^{-1} at a wavelength of 415 nm.

Gels

Sephadex (Pharmacia, Sweden) G-10 and G-25 gels were packed into glass columns (internal diameter 1.5 cm, length 13 cm) equipped with Rotaflo TF/13 stopcocks. The gels were conditioned and washed several times with dilute nitric acid.

The void volume, V_0, of a freshly prepared column was determined with Blue Dextran, and the total liquid volume ($V_0 + V_i$) by eluting a sodium chloride solution.

The chromatographic procedure for ruthenium solutions was as follows. The column was loaded with 4 ml of 0.1 *M* ruthenium nitrosyl nitrate and eluted with varying concentrations of nitric acid. The fractions obtained were then investigated by ultracentrifugation, atomic absorption, potentiometry, and u.v. spectroscopy.

RESULTS AND DISCUSSION

When a sample of 0.1 *M* ruthenium nitrosyl nitrate solution (in 0.025 *M* nitric acid) was loaded on a Sephadex G-10 column and then eluted with dilute nitric acid, two distinct bands were formed on the column. The first was black and emerged from the column at the void volume, V_0. This indicated that the fraction in question contained species of high molecular weight, which were excluded from the gel. That fraction constituted only about 4–5% of the bulk. The second fraction emerging from the column was reddish-brown in colour, and its elution was complete at the total liquid volume ($V_0 + V_i$). It was probably the monomeric form of ruthenium nitrosyl nitrate. Neither boiling the solution nor cooling it to 0° before chromatography affected the ratio between the two fractions. When, however, the pH of the original solution was raised, the percentage of the black fraction increased to 29%.

The elution curve shown in Fig. 1 was obtained by loading the column with 4 ml of Solution 1 followed by elution with nitric acid of varying concentrations: 2.5, 2, 1.5, 0.5, 0.1, and 0.025 *M*. Eluents of higher pH were obtained by adjustment with sodium hydrogencarbonate. Figure 1 shows that increasing the pH of the eluting solution raised the amount of the black (polymeric) portion. At lower pH values this portion remained practically constant, while at pH 4.5 it began to rise sharply. When these experiments were performed after the pH of the ruthenium nitrosyl nitrate solutions had been adjusted, elution taking place with a solution of the same pH, the results obtained were similar to those in Fig. 1; for under these conditions the rate of hydrolysis is high[4].

When the stock solution of ruthenium nitrosyl nitrate was loaded on Sephadex G-25, the black fraction was strongly adsorbed on the gel and could not be eluted with nitric acid. It seems that the polymeric fraction has the correct size for the pores of that particular gel. The infrared spectra of loaded G-25 did not show any substantial difference from the spectra of the pure gel, so that chemical interaction can be excluded.

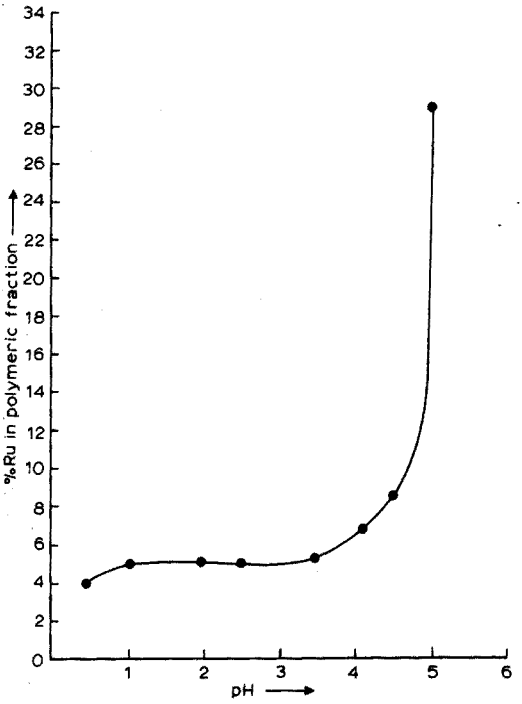

Fig. 1. Quantitative elution of the polymeric fraction of ruthenium nitrosyl nitrate as a function of pH.

The two fractions were studied by various analytical techniques, as described below.

Potentiometry

In the solid state ruthenium nitrosyl nitrate is in the trinitrate form; but on dissolution in water it loses 2 nitrate anions and one proton:

$$[RuNO(NO_3)_3(H_2O)_2] + 2\,H_2O \rightarrow [RuNO(NO_3)(H_2O)_4]^{2+} + 2\,NO_3^- \quad (1)$$

$$[RuNO(NO_3)(H_2O)_4]^{2+} \rightarrow [RuNO(NO_3)(H_2O)_3OH]^{+} + H^{+} \quad (2)$$

The cationic nature of the species obtained was proved by its strong adsorption on the sodium form of a sulfonic acid cation-exchange resin.

The reddish-brown (monomeric) fraction was more acidic when emerging from Sephadex G-10 than the stock solution which was introduced into the gel. The difference between the acidities was exactly one proton per mole ruthenium, which fits in well with eqn. (2). When this fraction was titrated with standard sodium hydroxide solution, another proton split off:

$$[RuNO(NO_3)(H_2O)_3OH]^{+} \rightarrow [RuNO(NO_3)(H_2O)_2(OH)_2] + H^{+} \quad (3)$$

This mechanism was proven by the comparative potentiometric titration of a ruthenium nitrosyl nitrate solution in 0.025 *M* nitric acid against a blank of 0.025 *M* nitric acid, both after passing through the gel. The difference exactly corresponded to the ratio of two protons per mole of ruthenium.

Ultracentrifugation

If each of the fractions obtained is run through an ultracentrifuge, the first fraction—which is polymeric—should show some sedimentation pattern. This was confirmed by the ultracentrifugation, at 40,000 rev min^{-1}, of 0.3 ml of 0.1 *M* ruthenium nitrosyl nitrate Solutions 1 and 2, which were eluted at the void volume of Sephadex G-10. In these experiments, the other sector of the cell was filled in each case with 0.3 ml of nitric acid solution at the concentration used for elution. Table I summarizes the results obtained.

It is evident that the polymeric fraction, polydisperse but nevertheless reasonably discrete, was present in Solutions 1 and 2. The acid concentration of the eluent did not appreciably influence the modal sedimentation coefficient of the polymeric fraction. The coefficient of Solution 2, which was originally more concentrated and aged, indicates a higher degree of polymerization than that of Solution 1. With regard to the second fraction obtained from the two solutions by passing them through the gel, centrifugation for 1 h did not cause any sedimentation, indicating the presence of species of low molecular weight. This confirmed expectations.

TABLE I

ULTRACENTRIFUGATION OF THE POLYMERIC FRACTIONS

Stock solution	*HNO_3 eluant*	*Sedimentation coefficient, S*
Solution 1—0.001 *M* in 0.025 *M* HNO_3	0.025 M	3.4
Solution 2—0.001 *M* in 0.9 *M* HNO_3	0.025 *M*	4.9
Solution 2—0.001 *M* in 0.9 *M* HNO_3	1 *M*	5.1

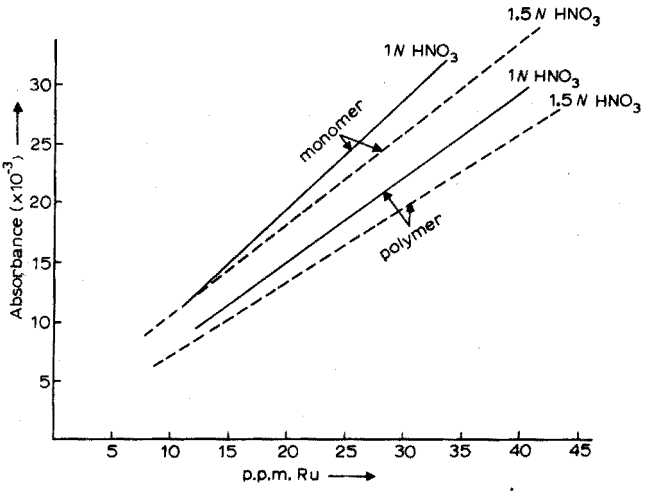

Fig. 2. Atomic-absorption calibration graphs for the monomeric and polymeric fractions of ruthenium nitrosyl nitrate.

Atomic absorption

Atomic-absorption measurements were made on the monomeric and on the polymeric fractions of Solution 1. Figure 2 shows the results obtained at two different acid concentrations. The absorbance of the polymeric fraction was invariably lower than that of the monomeric fraction, which is an indication of the lower state of atomization of the polymeric fraction. Absorbance intensity is also seen to be a function of acidity.

U.v. and visible spectrophotometry

Spectra of the two species eluted from Sephadex G-10 with 0.025 *M* nitric acid are shown in Fig. 3. Over the entire range of the ultraviolet and the visible regions of the spectrum, no difference between the monomeric and the polymeric fractions

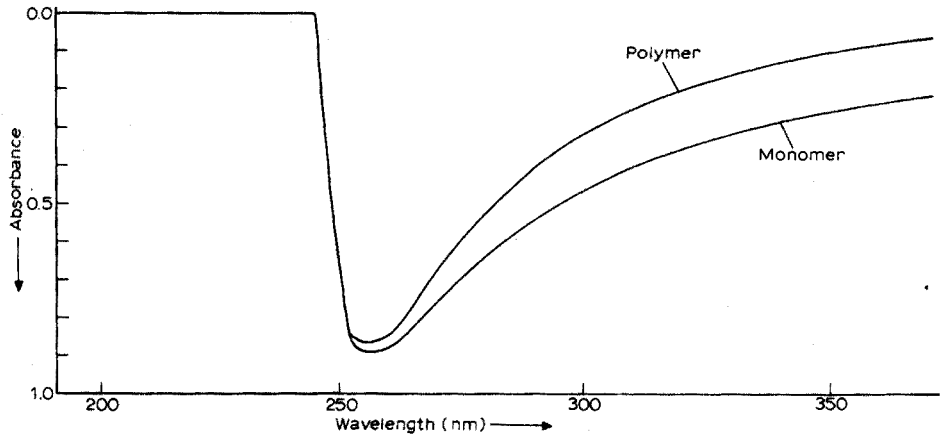

Fig. 3. U.v. spectra of monomeric and polymeric fractions of ruthenium nitrosyl nitrate.

could be observed, the maximum absorbance for both being at 255 nm; there were no signs of oxidation phenomena from ruthenium(III) to ruthenium(IV) which occasionally occur in concentrated nitric acid solutions[4]. This supports the authors' assumption that the polymer is a product of hydrolysis identical in structure to the monomeric unit. Increasing the concentration of the nitric acid solutions moves the maximum of both fractions towards the visible region. Figure 4 shows the spectra obtained when equal amounts of ruthenium nitrosyl nitrate are loaded on Sephadex G-10 and eluted with varying concentrations of nitric acid. Three groups of absorbances can be observed in these spectra:

1. For solutions eluted with 0.025 *M* and 0.1 *M* nitric acid the maxima are at 255 nm, and the colour of the eluted solutions is yellow.

2. Pinkish-yellow solutions are eluted with 0.5 *M* and 1 *M* nitric acid, and a plateau is observed in the region 260–328 nm.

3. For 1.5, 2, and 2.5 *M* nitric acid, pink solutions are eluted, and the maxima are at 337 nm.

The appearance of different colours and the shift of absorption peaks indicate changes in the degree of hydrolysis, which are caused by the changes in acid concentration.

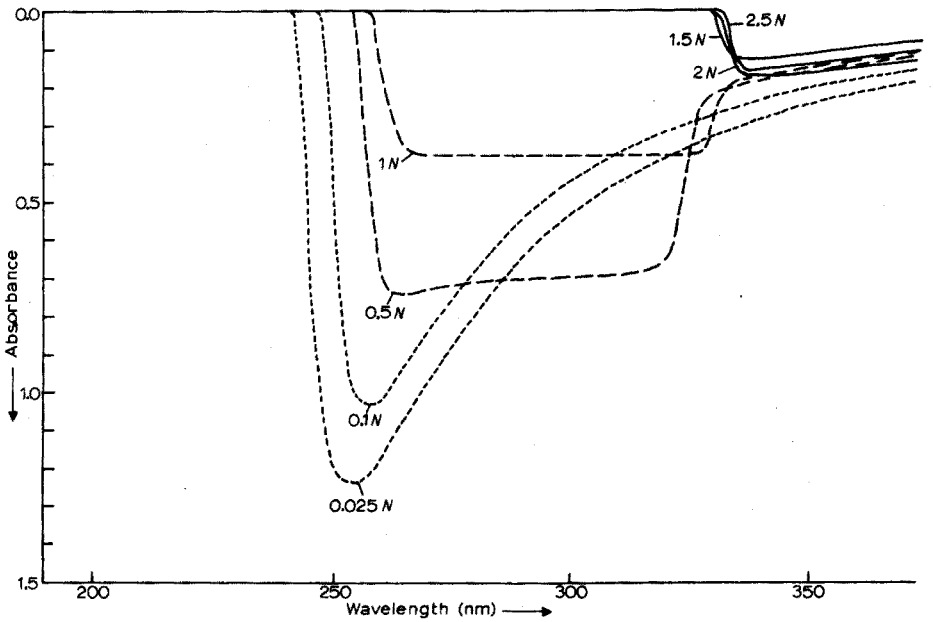

Fig. 4. U.v. spectra of the monomeric fraction of ruthenium nitrosyl nitrate eluted from Sephadex G-10 with varying nitric acid concentrations.

CONCLUSIONS

Gel-permeation chromatography has proved to be a useful tool in the separation of inorganic polymeric systems. The properties of the separated fractions could be studied by various analytical techniques. It is interesting to note that ruthenium nitrosyl nitrate forms polymeric species of high molecular weight even in relatively strong nitric acid solutions. When dissolved in an aqueous solution, reagent ruthenium nitrosyl nitrate should not be treated as comprising only one species, since hydrolysis invariably occurs and is sensitive to pH. Photometric studies showed that there is no structural difference between the monomeric and the polymeric fractions, while ion-exchange experiments indicated that both fractions are mainly cationic.

SUMMARY

Ruthenium nitrosyl nitrate hydrolyzes in nitric acid solutions and forms two fractions, a monomeric and a polymeric one. These fractions can be separated on Sephadex gels, the existence of the polymeric fraction being proved by ultracentrifuge experiments. U.v. and visible spectra of the two fractions show no difference in the absorbance patterns in a given concentration of nitric acid. Atomic absorption, however, indicated the lower state of atomization of the polymeric fraction and consequently produced lower signals than the monomeric fraction.

RÉSUMÉ

Le nitrate de ruthénium nitrosyle s'hydrolyse en milieu acide nitrique, en

donnant deux fractions: une monomérique et une polymérique. Ces fractions peuvent être séparées sur gel Sephadex. Les spectres obtenus dans l'u.v. et le visible pour les deux fractions ne présentent pas de différence d'absorption, pour une concentration donnée d'acide nitrique. Cependant, l'absorption atomique indique un degré d'atomisation plus faible pour la fraction polymérique, et par conséquent le signal produit sera plus bas que celui de la fraction monomérique.

ZUSAMMENFASSUNG

Rutheniumnitrosylnitrat hydrolysiert in salpetersauren Lösungen und bildet zwei Fraktionen, eine monomere und eine polymere. Diese Fraktionen können an Sephadex-Gelen getrennt werden, wobei die Existenz der polymeren Fraktion durch Untersuchungen mit einer Ultrazentrifuge nachgewiesen werden kann. Die beiden Fraktionen zeigen bei einer gegebenen Salpetersäurekonzentration keinen Unterschied in den Absorptionsspektren im Sichtbaren und im u.v. Die Atomabsorption zeigte jedoch den niedrigeren Atomisierungszustand der polymeren Fraktion an und ergab dementsprechend niedrigere Signale als die monomere Fraktion.

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CHOICE OF INDICATORS IN PHOTOMETRIC TITRATIONS

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In photometric titrations with visual monitoring of the end-point, the indicator is chosen to have its colour transition as close to the equivalence point as possible. This means that, for instance in acid–base titrations, the indicator should be chosen so as to have a pK_a value that is as close as possible to the pH value in the equivalence point. When a weak acid is titrated, phenolphthalein is a suitable indicator; for titration of a weak base, methyl red is appropriate. When a strong acid or base is titrated, the pK_a value of the indicator should lie somewhere between 6 and 8. In complexiметric titrations, the same principles govern the choice of indicator, *i.e.* one tries to find an indicator that has its colour transition at the equivalence point. This is naturally the correct way of choosing indicators when the end-point is monitored visually. Frequently, however, the same procedure is followed without further thought when an indicator is chosen for a photometric titration. In the present paper, it will be shown that it sometimes can prove advantageous to choose the indicator in another way. This is particularly true in those cases when the so-called Gran plots can be used to calculate the equivalence volume (Sörensen[1], Gran[2], Ingman and Still[3], Johansson[4]).

A Gran plot can briefly be described in the following way. First it is decided which reaction is the main reaction that takes place during the titration. For example, when a metal ion M is titrated with a complexing agent Y, the main reaction is

$$M + Y = MY$$

(The signs of charge are omitted for convenience.) Before the equivalence point, M is consumed in the same proportion as Y is added. After the equivalence point, the concentration of Y will increase. The construction of Gran curves involves, in principle, plotting of [M] before, and of [Y] after, the equivalence point as a function of the volume of added titrant. Two straight lines that intersect each other at the equivalence volume are then obtained. The change in concentration of M or Y should consequently be followed with the aid of an indicator. An indicator that changes its colour at the equivalence point is not suitable for this purpose, as it remains essentially unchanged during the largest part of the titration.

In the following paragraphs, some examples will be given which illustrate the choice of indicator for photometric titrations when Gran plots are used to evaluate the equivalence point.

Four typical examples will be considered:

(*a*) titration of a strong acid;

(*b*) titration of a weak acid or base;

(*c*) titration of a mixture of a weak and a strong acid; and
(*d*) titration of a metal ion.

TITRATION OF A STRONG ACID WITH A STRONG BASE

When a strong base is titrated with a strong base, the main reaction is

$$H + OH = H_2O$$

The hydrogen ion concentration will thus decrease before the equivalence point and the decrease is directly proportional to the added amount of base. If then $F_1(V) = [H]$ or, if dilution is considered,

$$F_1(V) = (V_0 + V)\,[H] \tag{1}$$

is plotted as a function of the volume of titrant the resulting straight line will intersect the V-axis at $V = V_e$ = the equivalence volume (V_0 denotes the volume of the sample at the start of the titration and V denotes the volume of added titrant).

If the course of the titration is followed by measuring the absorbance of an added acid–base indicator, the problem is thus how to calculate the hydrogen ion concentrations from these measured absorbance values. This is done in the following manner. If [HI] denotes the concentration of the acid form of the indicator and [I] the concentration of its basic form, the stability constant of the indicator reaction is

$$K_{HI} = \frac{[HI]}{[H]\,[I]} \tag{2}$$

which gives

$$[H] = [HI]/[I] \cdot K_{HI} \tag{3}$$

It can be seen from eqns. (1) and (3) that a straight line will result for

$$y = (V_0 + V)[HI]/[I] \tag{4}$$

This is well known but usually the indicator is chosen so that only a small part of the data can be used. An indicator having a colour transition that coincides with the equivalence point will not appreciably change its colour except in the immediate neighbourhood of the equivalence point (see Higuchi *et al.*[5]).

In order to make the most of a photometric titration—and particularly when titrations are done automatically—the indicator should be chosen so as to change its colour as the same pace as the (in this case) hydrogen ion concentration is changed. The best results will be obtained if half the indicator is in its acid form (and half in its basic form) at the point where half the acid titrated has been neutralized.

The logarithm of the stability constant of the indicator should consequently be equal to the pH at that point. If, for example, a 0.01 *M* solution of hydrochloric acid is titrated, the indicator should have a stability constant $\log K_{HI} = 2.3$, *i.e.* quite different from the indicators normally used in visual titrations (where $\log K_{HI}$ should be about 7 in this case). [HI]/[I] can in its turn be calculated from absorbance values according to eqn. (5), which was derived on the assumption that the base colour of the indicator is being measured

$$\frac{[HI]}{[I]} = \frac{A_I - A}{A - A_{HI}} \tag{5}$$

The equation finally arrived at will consequently be

$$F_1(V) = (V_0 + V)(A_I - A)/(A - A_{HI}) \tag{6}$$

yielding a straight line when plotted against the volume of added titrant. Here, A_{HI} denotes the absorbance when all of the indicator is in the acid form and A_I denotes the absorbance measured when all the indicator is in base form. A_I is easy to obtain in a titration, simply by continuing the titration past the equivalence point. The value of A_{HI} can be determined by adding concentrated hydrochloric acid after finishing the titration (see Ringbom[6]). An indicator having $A_{HI}=0$ seems to be the most advantageous choice for titrations of this type. In other words the indicator should be colourless or otherwise have zero absorbance at the wavelength used when all the indicator is in the acid form.

Example: *Titration of hydrochloric acid.* A 0.01 *M* solution of hydrochloric acid is to be titrated. According to the above discussion, an indicator which has log $K_{HI}=2.3$ and is colourless in acidic solution should be chosen. Quinaldine red seems suitable, its log K_{HI} being $=2.80$ (Kolthoff[7]). Since this indicator is somewhat unstable in aqueous solution, the titration should not be delayed unduly. An absorption maximum of quinaldine red is at 500 nm. The titration was performed using an instrument built from a photometer and pipettes manufactured by AGA, Lidingö, Sweden. The instrument is a filter photometer and a filter transmitting light at 500 nm was chosen.

The sample solution was 100.0 ml of hydrochloric acid and 5.0 ml of an alcoholic 0.01 % solution of the indicator. The titrant was 0.1000 *M* sodium hydroxide solution added by means of a pipette giving 1.0465 ml. The values shown in Table I were obtained.

TABLE I

PHOTOMETRIC TITRATION OF HYDROCHLORIC ACID

Added volume titrant V(ml)	*Measured absorbance*	*Corrected absorbance*	*y*
0.00	0.363	0.363	500.8
1.05	0.397	0.401	447.9
2.09	0.439	0.448	393.8
3.14	0.490	0.505	340.6
4.19	0.552	0.574	289.2
5.23	0.638	0.670	234.4
6.28	0.751	0.796	181.5
7.33	0.913	0.977	128.5
8.37	1.168	1.261	74.9
9.42	1.627	1.773	20.7
10.46	1.860	2.045	2.8
11.51	1.880	2.086	0.5
12.56	1.873	2.097	−0.2
13.60	1.859	2.100	−0.3

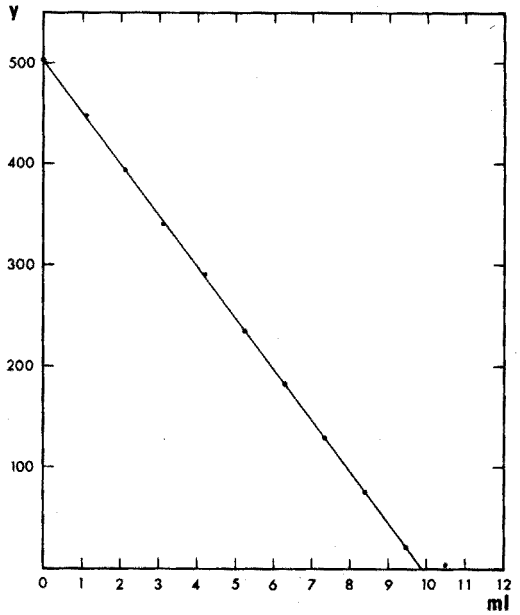


Fig. 1. A 0.01 *M* solution of hydrochloric acid is titrated with 0.1 *M* sodium hydroxide solution. Indicator quinaldine red. Wavelength 500 nm. Theoretical $V_e = 9.84$ ml.

The absorbance values are given in arbitrary units (readings of a digital voltmeter). In the third column, the absorbance values have been recalculated to $V = 0$. In the fourth column $y = (V_0 + V)(A_1 - A)/(A - A_{HI})$ has been calculated. $A_{HI} = 0$. $A_1 = 2.094$ (computed from the three last absorbance values). The course of the titration is given in Fig. 1. The result $V_e = 9.84$ ml. Another titration gave 9.85 ml and the theoretical value was $V_e = 9.84$ ml.

TITRATION OF A WEAK ACID OR BASE

When a weak acid (HA) is titrated with a strong base, the concentration of the free acid decreases in proportion to the amount of base added. The problem in this case consequently is how to compute the different concentrations of acid from the measured absorbance values. The equilibrium equation for the acid states that

$$[HA] = K_{HA}[H][A] \tag{7}$$

During the titration, A is formed in proportion to the increase in V, *i.e.* volume of base added; considering this and combining eqns. (7), (5) and (3) yields

$$[HA] = K_{HA} \cdot V \cdot (A_1 - A)/K_{HI}(A - A_{HI}) \tag{8}$$

Consequently, a plot of

$$F_2(V) = V(A_1 - A)/(A - A_{HI}) \tag{9}$$

vs. the volume of titrant results in a straight line intersecting the V-axis at the equivalence point. This equation is not quite correct.

The correct relation between V ml of titrant, V_0 ml of solution at the start of

the titration, [H], the hydrogen ion concentration, K_{HA}, the stability constant of the acid, K_w, the ionic product of water, and C_B, the concentration of the titrant, is[3,4]:

$$V_e - V = K_{HA}(V[H^+] + \frac{V_0+V}{C_B}\cdot[H^+]^2 - \frac{V_0+V}{C_B}\cdot K_w) + \frac{V_0+V}{C_B}([H^+] - K_w/[H^+]) \quad (10)$$

The simpler eqn. (9) is not valid when V approaches zero but it has the advantage that the stability constant of the indicator may be unknown and is consequently better in practical use. If the same principles as in the case when a strong acid is titrated are allowed to govern the choice of indicator, *i.e.* half the indicator should be transformed at the half-neutralization point, then K_{HI} should approximately equal K_{HA}. In the special case when K_{HA} equals K_{HI} the slope of the line is -1.

Example: *Titration of acetic acid.* A solution of acetic acid is to be titrated with sodium hydroxide. A suitable indicator for titrating this acid is bromocresol green, which has a log $K_{HI} = 4.68$. This indicator is yellow in acidic solution with an absorption maximum at 440 nm. In basic solution the colour is blue and the absorption maximum is at 610 nm. At and beyond this wavelength the acid form of the indicator does not absorb light. The same apparatus as in the example above was used and a filter transmitting at 613 nm was chosen.

The sample solution was 100.0 ml of 0.01 M acetic acid containing 20 drops of an alcoholic 0.01% solution of the indicator. The titrant was 0.1000 M sodium hydroxide solution. The values obtained are shown in Table II.

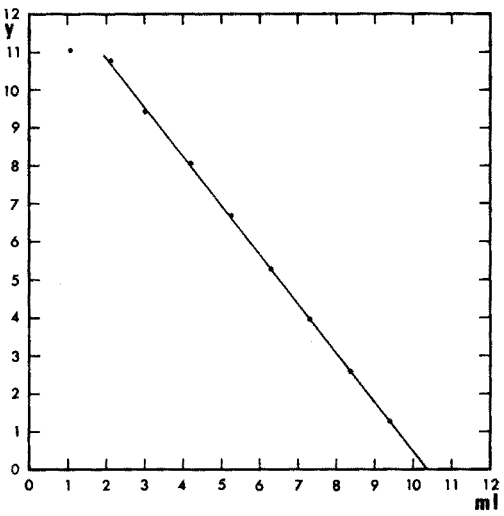
TABLE II

PHOTOMETRIC TITRATION OF ACETIC ACID

Added volume of titrant (ml)	*Measured absorbance*	*Corrected absorbance*	y
0.00	0.070	0.070	0.
1.05	0.202	0.204	11.02
2.09	0.376	0.384	10.74
3.14	0.569	0.587	9.45
4.19	0.774	0.806	8.03
5.23	0.983	1.034	6.67
6.28	1.203	1.279	5.28
7.33	1.426	1.530	3.94
8.37	1.659	1.798	2.59
9.42	1.899	2.078	1.25
10.46	2.132	2.355	−0.01
11.51	2.109	2.352	0.01
12.56	2.091	2.354	−0.00
13.60	2.071	2.353	0.00

In this case y is equal to $V(A_I - A)/(A - A_{HI})$, $A_I = 2.353$ and $A_{HI} = 0$. The course of the titration is drawn in Fig. 2. The result $V_e = 10.35$ ml, whereas the theoretical value was $V_e = 10.34$ ml.

Example: *Titration of ammonia.* A solution 0.02 M in ammonia is to be titrated with 0.1 M hydrochloric acid. One possible solution to the problem would be to choose an indicator having a stability constant $= 10^{9.5}$, *e.g.* cresolphthalein. However,



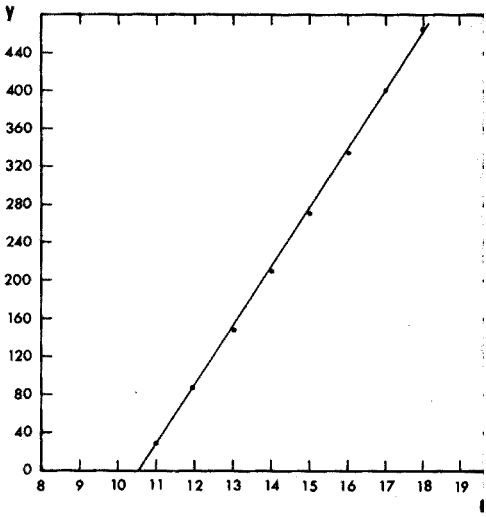


Fig. 2. A 0.01 *M* solution of acetic acid is titrated with 0.1 *M* sodium hydroxide solution. Indicator bromocresol green. Wavelength 613 nm. Theoretical $V_e = 10.35$ ml.
Fig. 3. A 0.02 *M* solution of ammonia is titrated with 0.1 *M* hydrochloric acid. Indicator quinaldine red. Wavelength 480 nm(Cd-line). Theoretical $V_e = 10.53$ ml.

phthaleins are not suitable for use in photometric titrations since they all have two colour transitions and become colourless in strongly alkaline solutions. Another solution to the problem would be to determine the equivalence volume from data obtained when an excess of hydrochloric acid has been added, quinaldine red then being equally well suited for use as indicator as in the direct titration of hydrochloric acid.

The sample solution was 45.0 ml of *ca.* 0.02 *M* ammonia and 0.4 ml of an alcoholic 0.01% solution of quinaldine red. The titrant was 0.1 *M* hydrochloric acid.

TABLE III

PHOTOMETRIC TITRATION OF AMMONIA

Added volume of titrant (ml)	*Measured absorbance*	*Corrected absorbance*	*y*
6.00	0.860	0.974	0.2
8.00	0.829	0.975	0.1
9.00	0.818	0.980	−0.2
10.00	0.803	0.980	−0.2
11.00	0.532	0.661	27.0
12.00	0.307	0.388	87.1
13.00	0.214	0.275	148.9
14.00	0.165	0.216	209.5
15.00	0.134	0.178	270.7
16.00	0.112	0.151	334.7
17.00	0.096	0.132	399.7
18.00	0.084	0.117	464.7

The titration was performed with an "Eppendorf" photometer equipped with titration attachment. This instrument utilizes the line spectrum of a cadmium or mercury lamp and in this case the cadmium line at 480 nm was chosen. The results obtained are shown in Table III.

In this case, $y = (V_0 + V)(A_1 - A)/(A - A_{HI})$; $A_1 = 0.977$; $A_{HI} = 0$. The course of this titration is given in Fig. 3. The result obtained was $V_e = 10.61$, whereas the theoretical value was $V_e = 10.53$ ml.

Example: *Titration of a mixture of a weak and a strong acid.* The components of the mixture of, for example, a weak and a strong acid can be determined in two titrations, quinaldine red being used as indicator in the first and bromocresol green being the indicator in the second titration. At low values of pH, acetic acid is dissociated to a negligible extent. The strong acid should therefore be titrated in as strong a solution as possible and data lying close to the equivalence point should not be used in the calculations.

Figure 4 shows the results of titration of about equal amounts of hydrochloric acid and acetic acid; 50 ml of a solution 0.01 *M* in hydrochloric acid and 0.01 *M* in acetic acid were titrated with 0.1 *M* sodium hydroxide solution. The equivalence volumes found were 4.81 ml and 9.82 ml; the theoretical values were 4.79 ml and 9.81 ml, respectively.

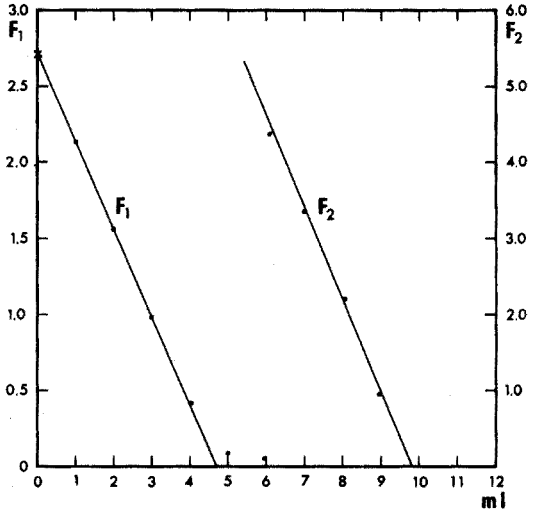


Fig. 4. A mixture of equal amounts of hydrochloric acid and acetic acid is titrated with sodium hydroxide solution. When quinaldine red is used as indicator, hydrochloric acid is determined and when bromocresol green is used, the sum is determined. Theoretical equivalence volumes 4.79 and 9.81 ml. $F_1 = (V_0 + V)[H^+]$ and $F_2 = (V - 4.81)$ [HA] are plotted; 4.81 ml is the equivalence volume of the first titration.

COMPLEXATION TITRATIONS

The following notations are used: V_0 ml of a C_M^0 molar metal ion solution is titrated by adding V ml of a C_y^0 molar solution of a complexing agent Y. Only a 1:1 complex with stability constant K_{MY} is assumed to be formed by the metal ion and the complexing agent. The stability constant for the indicator metal complex is K_{MI}.

The following equation is valid for complexation titration data taken before the equivalence point

$$V_e - V = \frac{(V_0 + V)}{C_y^0} [M] \tag{11}$$

and for data taken after the equivalence point

$$V - V_e = \frac{V_0 \cdot C_M^0}{K_{MY} \cdot C_y^0 \cdot [M]} \tag{12}$$

Combining eqn. (11) with the equilibrium expression for the indicator metal complex, written in the form

$$[M] = \frac{[MI]}{[I]} \cdot \frac{1}{K_{MI}} \tag{13}$$

and

$$\frac{[MI]}{[I]} = \frac{A_I - A}{A - A_{MI}} \tag{14}$$

we obtain

$$V_e - V = \frac{V_0 + V}{K_{MI} \cdot C_y^0} \cdot \frac{A_I - A}{A - A_{MI}} \tag{15}$$

which is valid before the equivalence point. A is the measured absorbance, A_I the absorbance of the free indicator and A_{MI} the absorbance when the indicator is fully complexed by the metal.

After the equivalence point we have

$$V - V_e = \frac{V_0 C_M^0 \cdot K_{MI}(A_{MI} - A)}{K_{MY} \cdot C_y^0 (A - A_I)} \tag{16}$$

Thus if values of

$$F_3(V) = (V_0 + V)(A_I - A)/(A - A_{MI}) \tag{17}$$

before the equivalence point or values of

$$F_4(V) = (A_{MI} - A)/(A - A_I) \tag{18}$$

after the equivalence point are plotted against V, straight lines are obtained, which intersect the V-axis at the point V_e.

Generally speaking, a complexation titration should therefore be treated in analogy with the titration of a strong acid, the only difference being that no multiplication by $(V_0 + V)$ should be made for data after the equivalence point.

The indicator should therefore be chosen in the same way as for a titration of a strong acid. However, side-reactions may interfere with the main reaction and conditional constants should therefore be used. Two more complications are frequently encountered when complexation titrations are considered. One is that single-coloured metal indicators are rare and values of both A_{MI} and A_I should therefore be known. The other complication is that rather frequently the indicator must be added in such a great concentration that it cannot be disregarded in comparison with the

concentration of a metal ion that is being determined. In this case, it is necessary to apply a correction for the amount of metal ion bound to the indicator. If C_I is the initial concentration of the indicator, the correction is

$$V_0 \cdot C_I \frac{[MI]}{[MI]+[I]}$$

Equation (15) is then replaced by

$$V_e - V = \frac{1}{C_y^0 \cdot K_{MI}} \left\{ (V_0+V)\frac{(A_I-A)}{(A-A_{MI})} + V_0 \cdot C_I K_{MI} \frac{(A_I-A)}{(A_I-A_{MI})} \right\} \quad (19)$$

If the sum of the two terms in brackets is plotted against V, the plot is a straight line that intersects the V-axis at the point V_e. Equation (19) is not fully accurate; a more accurate equation, which contains several additional terms, has been derived by Fortuin *et al.*[8].

Example: *Titration of calcium with EGTA.* To illustrate the use of the equations presented, the titration of calcium with EGTA will be discussed.

A sample solution prepared by mixing 25 ml of a 0.02000 M calcium salt solution, 100 ml of a buffer solution that was 0.1 M in both ammonia and ammonium ion, and 10 ml of a 0.001 M Calmagite solution was titrated with a 0.0979 M Na_4-EGTA solution. The titration was performed by measuring absorbance at 700 nm with a photometer equipped with an interference filter and connected to a digital recorder.

The sample solution was thus $3.70 \cdot 10^{-3}$ M in calcium ($pC_{Ca} = 2.43$). The indicator should therefore have a value of log K_{CaI} (or log $K_{CaI'}$) close to the value 2.73 (= 2.43 + 0.3). The value of log K_{CaI} for Calmagite is 6.1[9]. The conditional constant for CaI at the pH in question (9.3) is log $K_{CaI'}$ = log K_{CaI} − log $\alpha_{I(H)}$ = 6.1 − 3.1 = 3.0. Log $\alpha_{I(H)}$ was calculated with the known[10] dissociation constants of Calmagite (log $K_1 = 12.4$, log $K_2 = 8.1$). Titration data obtained under the above-mentioned conditions give a slightly lower value, $10^{2.74}$, for the conditional constant of the calcium–Calmagite complex (see below). The data obtained for the titration of calcium are shown in Table IV.

For the calculation of the values of $y = (V_e - V) \cdot C_y^0 \cdot K_{CaI'}$ (eqn. 19) given in column 4, the values of A_I, A_{CaI} and $K_{CaI'}$ must be known. The five last values in column 3 give $A_I = 1.345$; the indicator is then completely free. The value of A_{CaI} can be determined, as proposed by Ringbom[6], by adding a small volume of concentrated calcium solution ($> 1.0\,M$) after the titration and measuring the absorbance. It is, however, simpler to calculate A_{CaI} by multiplying the value of A_I by a factor determined in a separate experiment. A solution of known calcium salt content was titrated photometrically as described above.

We then have

$$K_{CaI'} = \frac{[CaI]}{[Ca]\,[I']} = \frac{(A_I-A)}{(A-A_{CaI})} \cdot \frac{1}{[Ca]} \quad (20)$$

or

$$\frac{(A_I-A)}{[Ca] \cdot K_{CaI'}} = (A-A_{CaI}) \quad (21)$$

The straight line that results when values of $(A_I - A)/[Ca]$ are plotted against A intersects the A-axis at the point A_{CaI}. The value of $K_{CaI'}$ can be calculated from the

TABLE IV

PHOTOMETRIC TITRATION OF CALCIUM

Added volume of titrant V (ml)[a]	*Measured absorbance* $A_{exp.}$	*Corrected absorbance* A	y
0	0.521	0.521	276.2
0.514	0.550	0.552	248.1
1.028	0.583	0.587	220.5
1.542	0.624	0.631	191.2
2.056	0.669	0.679	164.1
2.570	0.723	0.737	136.8
3.084	0.791	0.809	108.6
3.598	0.872	0.895	81.6
4.112	0.977	1.007	54.1
4.626	1.116	1.154	26.4
5.654	1.291	1.345	−0.03
6.168	1.287	1.346	
6.682	1.281	1.344	
7.196	1.276	1.344	
7.710	1.272	1.345	

[a] The titrant was added in increments of 0.5140 ml from an automatic pipet.

slope of the line and the value of A_I is obtained from data beyond the equivalence point.

A_{CaI} can be determined with the aid of the data obtained in the above example, since the initial calcium ion concentration ($3.70 \cdot 10^{-3}$ M) is known and the values of [Ca] at each titration point before the equivalence point can be calculated. The values obtained are shown in Table V.

Values of $(A_I - A)/[Ca]$ are plotted against A in Fig. 5. The resulting straight line intersects the A-axis at the point $A_{CaI} = 0.113$. The slope of the line gives the conditional constant $K_{CaI'} = 5.5 \cdot 10^2 = 10^{2.74}$.

The values $A_{CaI} = 0.113$ and $A_I = 1.345$ ($A_{CaI}/A_I = 0.084$) were used to evaluate

TABLE V

CALCULATION OF $(A_I - A)/[Ca]$ FOR TITRATION OF CALCIUM

V	A	$[Ca] \cdot 10^3$	$(A_I - A)/[Ca]$
0	0.521	3.67	225.0
0.514	0.552	3.28	241.8
1.028	0.587	2.90	261.4
1.542	0.631	2.52	283.1
2.056	0.679	2.15	310.1
2.570	0.737	1.78	342.3
3.084	0.809	1.41	380.1
3.598	0.895	1.05	429.7
4.112	1.007	0.69	491.9
4.626	1.154	0.33	572.2

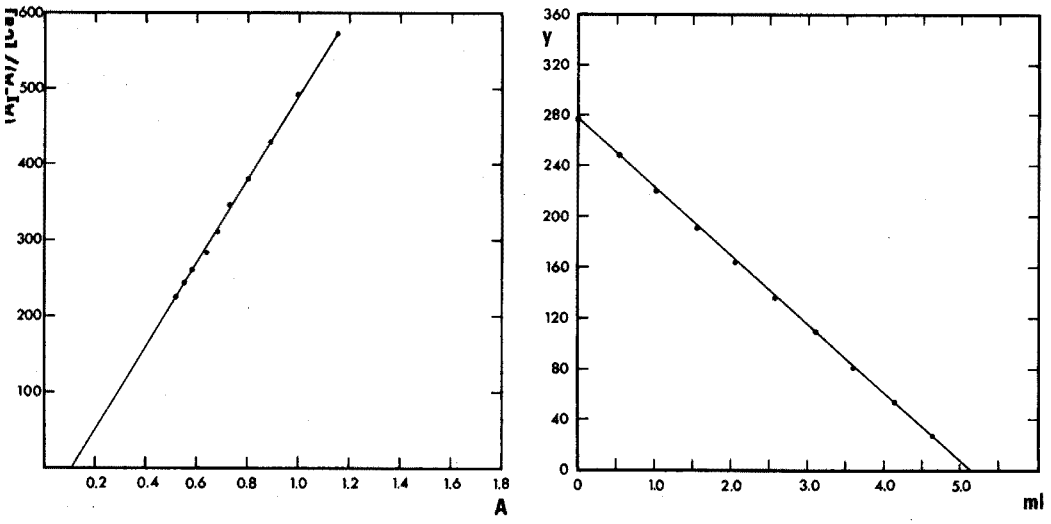


Fig. 5. Plot of $(A_1 - A)/[\mathrm{Ca}]$ for the titration of calcium with EGTA.
Fig. 6. A 0.00370 *M* calcium solution is titrated with a 0.0979 *M* EGTA solution. Indicator Calmagite, pH = 9.3. Wavelength 613 nm. Theoretical $V_e = 5.11$ ml.

V_e in the above titration. The titration curve, *y vs. V*, is reproduced in Fig. 6. The point of intersection on the *V*-axis is 5.11, which is the same as the theoretically calculated value.

I am indebted to Dr. Folke Ingman and Dr. Lennart Pehrsson for valuable discussions and comments relating the manuscript. I also thank L. Pehrsson and Mrs. K. Lindgren for carrying out the analyses. This work was supported by the Swedish Board of Technical Development.

SUMMARY

In photometric titrations with ordinary monitoring of the end-point, the indicator is usually chosen to have its colour transition as close to the equivalence point as possible. The present paper shows that it sometimes can prove advantageous to choose the indicator differently, especially when so-called Gran plots are used to calculate the equivalence volume. When an acid is titrated, the indicator should be chosen so that half the indicator is in its acid form (and half in its basic form) at the point where half the amount of acid to be titrated has been neutralized. The logarithm of the stability constant of the indicator should consequently be equal to the pH at that point. A similar concept can be used when titrations of bases or complexation titrations are considered.

RÉSUMÉ

Lors de titrages photométriques, on choisit généralement l'indicateur dont le virage est aussi près que possible du point équivalent. Il devrait cependant être

choisi, pour le titrage d'un acide, de manière à ce qu'il soit à moitié sous forme acide (et à moitié sous forme basique) au moment où le 50 % de l'acide à titrer a été neutralisé. Le logarithme de la constante de stabilité devrait par conséquent correspondre au pH, à ce point. Il en est de même pour les titrages de bases ou pour les titrages complexiométriques.

ZUSAMMENFASSUNG

Bei photometrischen Titrationen mit herkömmlicher Endpunktsbestimmung wird der Indikator gewöhnlich so gewählt, dass sein Farbumschlag so dicht wie möglich beim Äquivalenzpunkt liegt. Es wird gezeigt, dass es manchmal vorteilhaft sein kann, den Indikator nach anderen Gesichtspunkten zu wählen, besonders wenn sog. Gran-Auftragungen angewendet werden, um das Äquivalenzvolumen zu berechnen. Wenn eine Säure titriert wird, soll der Indikator so gewählt werden, dass er bei dem Punkt, bei dem die halbe Menge der zu titrierenden Säure neutralisiert worden ist, zur einen Hälfte in der sauren Form, zur anderen in der basischen Form vorliegt. Der Logarithmus der Stabilitätskonstante des Indikators soll folgerichtig gleich dem pH-Wert bei diesem Punkt sein. Entsprechendes kann auf Titrationen von Basen oder Komplexbildungstitrationen übertragen werden.

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TITRIMETRIC APPLICATIONS OF COMPLEXES WITH 1:2 METAL–LIGAND RATIOS IN THE DETERMINATION OF LIGANDS AND IN BACK-TITRATIONS

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In a previous paper[1] the theory was given for complexiметric titrations of metal ions based on a reaction with a ligand, which forms a complex containing a 2:1 metal:ligand ratio. The reaction of copper(II) with diethylenetriaminepentaacetic acid was given as an example.

This theory is also directly applicable to the complexiметric titration of a ligand with a metal ion, reacting in a metal:ligand ratio of 1:2, the only difference being that the relative concentrations used in the mathematical treatment must be taken with respect to c_L instead of to c_M. Consequently, the concentration involved in the values of Z is the analytical concentration of L and not of M.

The data for the stability constants of M_2L and ML_2 complexes show that the ratio $R = K_1/K_2$ is generally $\gg 1$ in both cases, but is usually much larger in the case of M_2L than in the case of ML_2 complexes. This means that in titrations of ligands with metal ions based on ML_2 complexes, the formation of ML_2 will play a very important role in the end-point determination.

The present paper deals with the titration of a ligand L with a metal ion M reacting in metal:ligand ratios of 1:1 and 1:2. This titration is important for the complexiметric titration of such ligands, and also forms the basis for the back-titration of metal ions.

THEORY

As already stated in the introduction the theory is completely analogous to the theory of the paper on M_2L complex formation[1]. The basic equations are

$$f = \frac{c_M}{c_L} = m + ml + ml_2 \text{ ; and } l + ml + 2ml_2 = 1$$

$$Z_1 = K_1 c_L = \frac{ml}{m \cdot l} \text{ ; and } Z_2 = K_2 c_L = \frac{ml_2}{ml \cdot l}$$

where the symbols are the same as before[1]. The relative concentrations are taken with respect to c_L, hence

$$m = \frac{[M]}{c_L} \text{ ; } l = \frac{[L]}{c_L} \text{ ; } ml = \frac{[ML]}{c_L} \text{ ; } ml_2 = \frac{[ML_2]}{c_L}$$

Transposition of the conditions found in the previous paper[1] in terms of the present problem gives the following results.

The f–l curve

$Z_2 \gg 1$ permits end-point detection at $f=0.5$. $Z_2 \ll 1$ but $Z_1 \gg 1$, which is not relevant in this paper, gives an end-point at $f=1$. Intermediate values of Z_2 give no suitable end-points.

The f–m curve

When $R \gg 1$, an end-point occurs at $f=1$. However, when R is close to, or less than, 1 and $Z_2Z_1 \gg 1$, which is not exceptional, an end-point is found at $f=0.5$.

The f–ml curve

A titration curve mainly consisting of a straight branch from $ml=0$ ($f=0.5$) to $ml=1$ ($f=1$) and two horizontal branches at either end of this straight line is found for $Z_2 \gg 1$, $Z_1 \gg 1$ and $R \gg 1$. The last condition ($R \gg 1$) is not always fulfilled in ML_2 complex formation, and then no end-point occurs at $f=1$.

The f–ml$_2$ curve

The condition $Z_2 \gg 1$ produces a sharp end-point at $f=0.5$, provided that $Z_2Z_1 \gg 1$ too. The presence of another end-point at $f=1$ is only observed when $R \gg 1$.

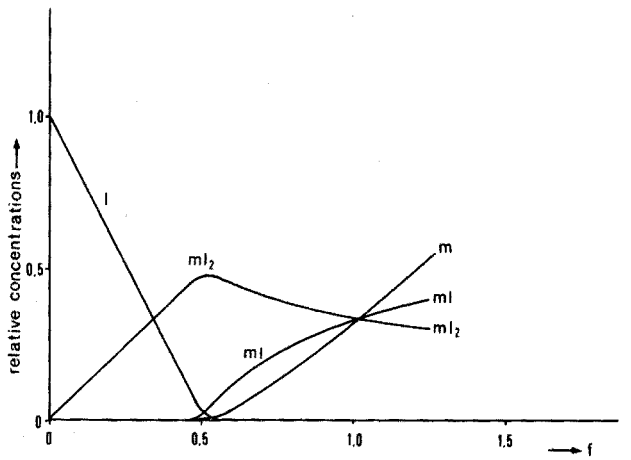

Fig. 1. *f–m*, *f–l*, *f–ml* and *f–ml*$_2$ curves for $Z_1=10^3$ and $Z_2=10^3$.

Figure 1 illustrates these results. It is obvious that end-point determination is possible only at $f=0.5$, because the condition $R \gg 1$, necessary for end-point determination at $f=1.0$ is not fulfilled.

EXPERIMENTAL

The theory was applied to some photometric titrations of ligands with metal ions.

A Zeiss PMQ II spectrophotometer provided with a 2-cm titration cell was used for all titrations.

Titration of 8-hydroxyquinoline-5-sulphonic acid

The titration of 8-hydroxyquinoline-5-sulphonic acid with copper(II) was first studied. A $9.2 \cdot 10^{-6}$ *M* solution of the ligand was titrated with copper(II) in a 0.1 *M* acetate buffer pH 5.5. Under these conditions $Z_1 = 830$ and $Z_2 = 100$. The four theoretical curves are given in Fig. 2. Again R is too small for end-point determination at $f = 1$ but at $f = 0.5$ a suitable end-point may be found.

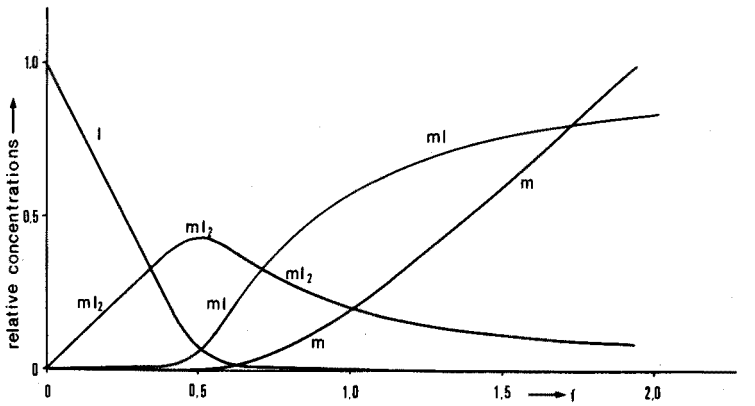

Fig. 2. *f–m*, *f–l*, *f–ml* and *f–ml*$_2$ curves for the titration of $9.2 \cdot 10^{-6}$ *M* 8-hydroxyquinoline-5-sulphonic acid with copper(II). $Z_1 = 830$ and $Z_2 = 100$.

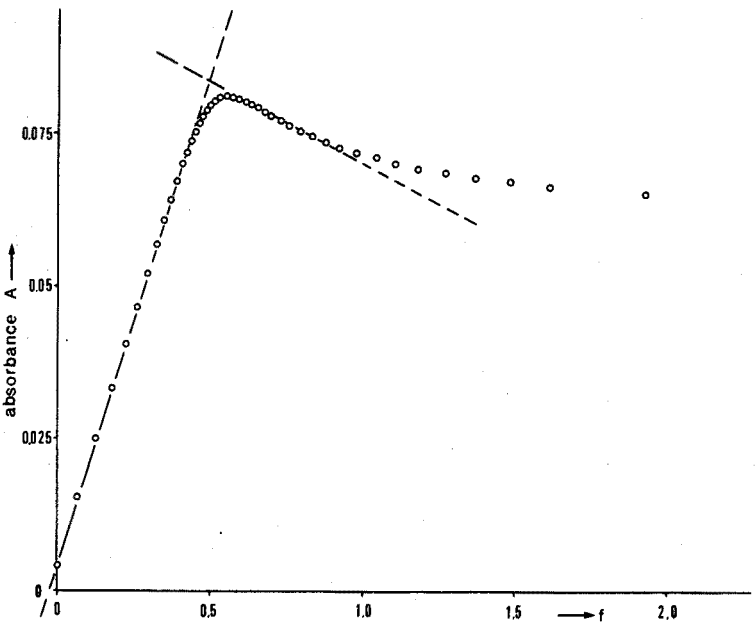

Fig. 3. Photometric titration curve of $9.2 \cdot 10^{-6}$ *M* HQS with copper(II) at 380 nm. $\varepsilon_{ML} = 3300$, $\varepsilon_{ML_2} = 9500$, $\varepsilon_L = 240$, $\varepsilon_M = 0$ (in 1 mole^{-1} cm^{-1}).

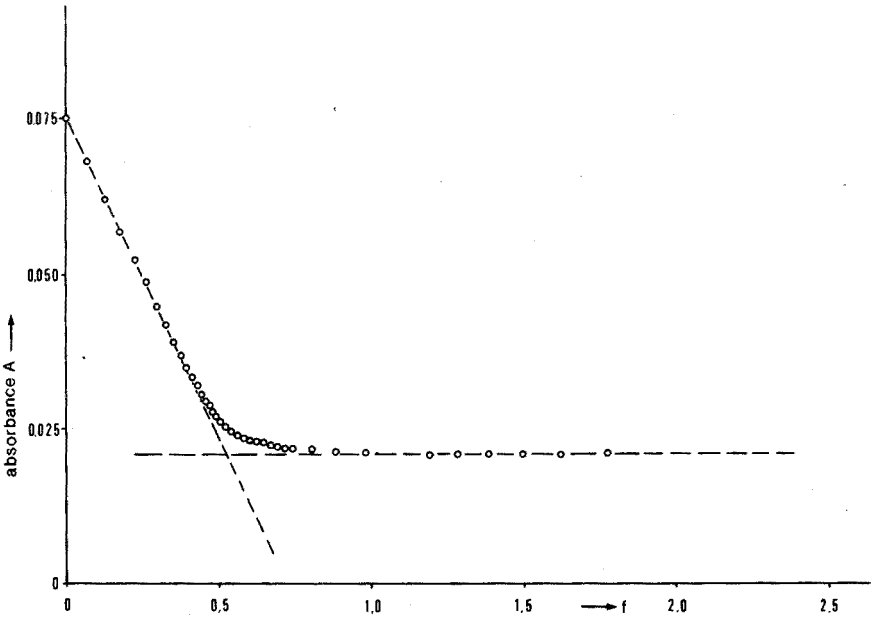

Fig. 4. Photometric titration curve of $9.2 \cdot 10^{-6}$ *M* HQS with copper(II) at 300 nm. $\varepsilon_{ML} = 1070$, $\varepsilon_{ML_2} = 2500$, $\varepsilon_L = 4150$, $\varepsilon_M = 60$ (in l mole^{-1} cm^{-1}).

The absorption spectra of the ligand and of the complexes were mentioned in a previous paper[2]. At 380 nm the chelates are mainly responsible for the absorbance, whereas at 300 nm the absorption of the free ligand prevails. The photometric titration was carried out at both wavelengths. The results are given in Figs. 3 and 4. The theoretical and the experimental curves closely agree for both wavelengths.

Back-titrations

Titration curves of similar shape are obtained in complexiметric back-titrations of metal ions involving 1:2 complex formation. It is assumed that a metal ion M is determined by the addition of a ligand L in excess and subsequent titration of the ligand with a titrant metal ion N. Both M and N form complexes of the 1:2 type, *i.e.* ML_2 and NL_2.

The mathematical treatment of such back-titrations is very complicated, as the equilibria between M and L must also be considered. It is however possible to understand these titrations qualitatively and to predict the shape of the titration curves, on the basis of the above theoretical part.

As an example, the determination of a $2.5 \cdot 10^{-4}$ *M* solution of copper(II) is considered in a 0.1 *M* acetate buffer pH 5.5. Pyridine-2,6-dicarboxylic acid is added in excess and the back-titration is carried out with cobalt(II). Under these conditions $Z_{CuL} = 1.2 \cdot 10^5$, $Z_{CuL_2} = 2.1 \cdot 10^4$, $Z_{CoL} = 1.7 \cdot 10^3$ and $Z_{CoL_2} = 10^3$. It can be predicted that in the first part of the back-titration, the excess ligand will almost quantitatively react with the titrant to form CoL_2. When the ligand has reacted, the solution contains a mixture of CuL_2 and CoL_2. Further addition of cobalt(II) will break up the weakest complex, *i.e.* CoL_2, to form CoL. The titration was carried out photometrical-

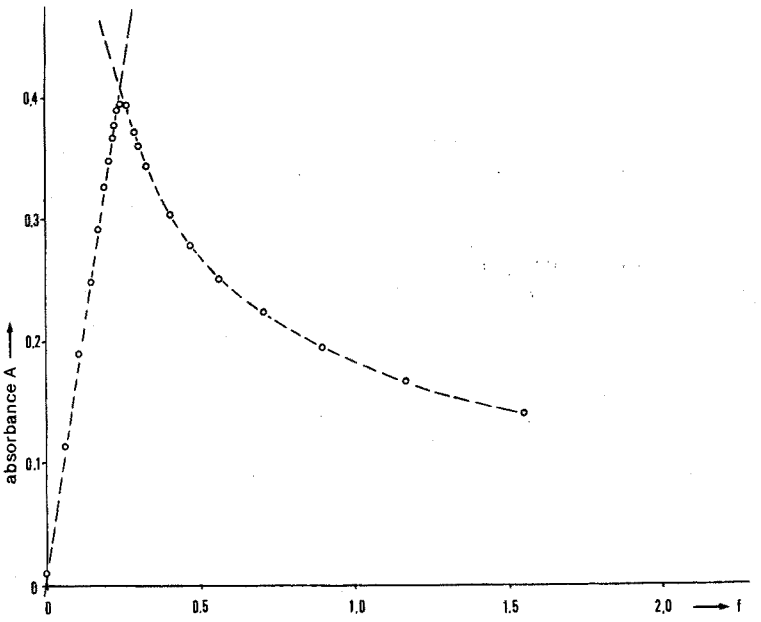

Fig. 5. Photometric back-titration of $2.5 \cdot 10^{-4}$ *M* copper(II). Ligand concentration 10^{-3} *M*; back-titrant Co(II); wavelength 360 nm. $\varepsilon_{CuL} = 15$, $\varepsilon_{CuL_2} = 22$, $\varepsilon_{CoL} = 3$, $\varepsilon_{CoL_2} = 820$ (l mole^{-1} cm^{-1}).

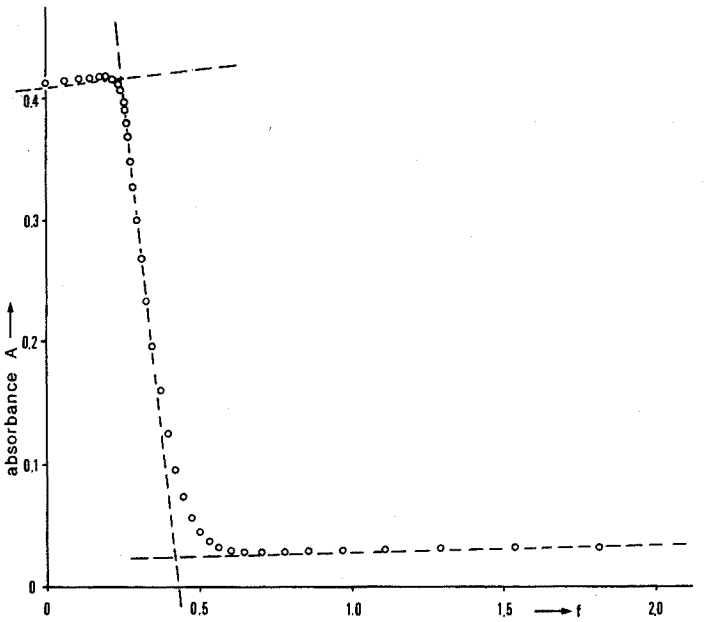

Fig. 6. Photometric back-titration of $2.5 \cdot 10^{-4}$ *M* cobalt(II). Ligand concentration 10^{-3} *M*; back-titrant Cu(II); wavelength 360 nm. $\varepsilon_{CuL} = 15$, $\varepsilon_{CuL_2} = 22$, $\varepsilon_{CoL} = 3$, $\varepsilon_{CoL_2} = 820$ (l mole^{-1} cm^{-1}).

ly. The shape of the curve depends on the wavelength; at 360 nm the absorbance of CoL_2 prevails. Figure 5 gives the titration curve. f in this case is defined as $f = c_N/c_L$. The titration curve in Fig. 5 reflects the formation of CoL_2 from $f = 0$ to $f = 0.25$, and the subsequent decomposition of CoL_2. The peak in the titration curve can be used for the determination of the excess of ligand.

Cobalt(II) can be determined similarly, with copper(II) as the back-titrant. Under the same conditions of pH, concentrations and wavelength, Fig. 6 results. The titration curve in Fig. 6 can be interpreted as follows. Af $f = 0$, cobalt(II) is quantitatively present as CoL_2. From $f = 0$ to $f = 0.25$ the excess of ligand reacts with the titrant to form CuL_2, resulting in a slight increase of the absorbance. Further addition of the titrant results in the decomposition of the weakest complex which is CoL_2, resulting in a sharp decrease of the absorbance. Near $f = 0.5$, the decomposition of CoL_2 is almost complete.

The titration curves of Figs. 5 and 6 were calculated theoretically with a desk computer. The experimental curves agree with the theoretical curves.

SUMMARY

The conditions for suitable end-point determination in linear complexometric titrations of a ligand L with a metal M, resulting in a ML_2 complex, are given. The determination of 8-hydroxyquinoline-5-sulphonic acid with copper(II), and back-titrations of copper(II) and cobalt(II) involving pyridine-2,6-dicarboxylic acid as the ligand, are given as examples.

RÉSUMÉ

Les auteurs ont défini les conditions de détermination du point final des titrages complexométriques d'un ligand L avec un métal M, donnant un complexe du type ML_2. Le dosage de l'acide 8-hydroxyquinoléine-5-sulfonique avec le cuivre(II) et le titrage indirect du cuivre(II) et du cobalt(II), au moyen de l'acide pyridine 2,6-dicarboxylique sont donnés comme exemples.

ZUSAMMENFASSUNG

Es werden die Bedingungen abgeleitet für die Endpunktsbestimmung von linearen komplexometrischen Titrationen, bei denen ein Ligand L mit einem Metall M einen Komplex ML_2 bildet. Als Beispiele werden die Bestimmung von 8-Hydroxychinolin-5-sulfonsäure mit Kupfer(II) und die Rücktitration von Kupfer(II) und Kobalt(II) mit Pyridin-2,6-dicarbonsäure als Ligand angegeben.

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SHORT COMMUNICATION

The determination of rhenium in molybdenite concentrates by instrumental neutron activation analysis with californium-252 as neutron source

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Rhenium is of very low cosmic and terrestrial abundance[1] but occurs in minor amounts in copper ores from the Western U.S. and South America. It becomes economically important because of its geochemical association with molybdenites which are concentrated as a by-product in copper milling operations. Rhenium has experienced some importance in catalytic applications in petroleum refining[2].

Previous determinations of rhenium in the presence of molybdenum have required a chemical or radiochemical separation from molybdenum, followed by spectrophotometric[3], X-ray fluorescence[4], or radiometric measurement[5,6] of the separated rhenium. Separations based on both solvent extraction[3] and ion-exchange chromatography[7-10] have been reported.

The determination of rhenium in molybdenite concentrates has often produced a spread of results among laboratories assaying the same material. An independent method not subject to chemical peculiarities of the samples is desirable to provide reliable data by which to referee the analytical discrepancies. A review of the nuclear properties of rhenium and molybdenum[11,12] indicated that instrumental neutron activation analysis (INAA) could offer a suitable method for this determination.

The recent availability of californium-252 in milligram amounts now offers laboratories the ability to utilize activation analysis at much less cost than maintaining a nuclear reactor[13-15]. The work on rhenium reported here gives some comparisons between californium-252 isotopic neutron activation and reactor activation.

Experimental

Samples of molybdenite (MoS_2) concentrates (ground to -200 mesh) were prepared by weighing 10.00 g of powdered MoS_2 into 2-dram polyethylene vials (Olympic Plastics). Standards were prepared from analytical reagent-grade molybdic oxide (MoO_3) by adding known amounts of pure potassium perrhenate ($KReO_4$) to produce concentrations of 0, 100, 200, 400, 600, 800 and 1000 p.p.m. rhenium. After grinding for 5 min to ensure thorough mixing, each standard was sealed in a clean 2-dram polyethylene vial.

Although molybdenite concentrates are primarily MoS_2, MoO_3 was used as a standard matrix because of its availability in high-purity form, and the nuclear properties of sulfur and oxygen are not significantly different with respect to the formation of γ-ray-emitting radioactive products.

Californium-252 irradiations were made for 16 h at a flux of $1.5 \cdot 10^8$ n cm^{-2} sec^{-1} (15 mg ^{252}Cf in a $91 \times 91 \times 91$ cm cube of paraffin). After a cooling period of 4.5 h, γ-ray spectra were collected using a 40-cm^3 Ge(Li) detector (Canberra Industries) coupled to a 1024-channel analyzer (TMC-1001). A biased amplifier was used to observe only that portion of the spectrum from 130 to 190 keV. Decay of the samples and standards were followed over a period of several days—counting once daily—to provide positive identification of rhenium-188 activity at a γ-ray energy of 155 keV.

The above procedure was repeated with identical samples and reactor neutrons at a flux of $2 \cdot 10^8$ n cm^{-2} sec^{-1} (5-W continuous operation), irradiating for 30 min. After 4.5-h decay, the γ-ray spectra were examined as described above.

Results and discussion

Typical spectra of the samples and standards are shown in Figs. 1 and 2. Decay curve comparisons are shown in Fig. 3 for a typical sample and standard. The experimental half-life is 17.0 h compared with the literature value of 16.7 h[12]. Gamma-ray spectral interferences were absent as shown from the clean spectra (Figs. 1 and 2) and as evidenced by the decay curve data. The only likely interferences from nuclear reactions could come from an (n, p) reaction on osmium-188, or an (n, α) reaction on iridium-191; both of which produce rhenium-188. The cross-sections for (n, p) and (n, α) reactions in the region of mass 180–190 are generally of the order of a few millibarns, therefore, the amount of rhenium-188 formed would be insignificant compared to the (n, γ) production. Both osmium and iridium are expected to be extremely low in concentration in molybdenite, and interferences from these reactions can be considered negligible.

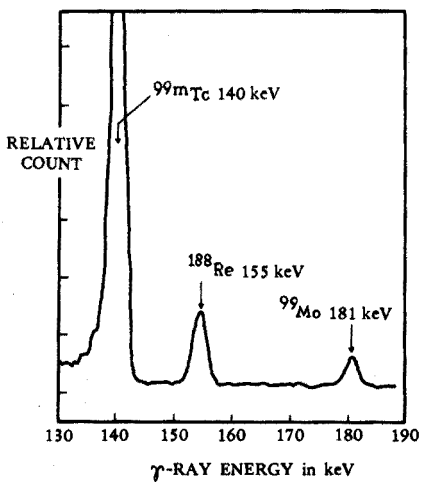

Fig. 1. Typical γ-ray spectrum of molybdenite sample from 130 to 190 keV.

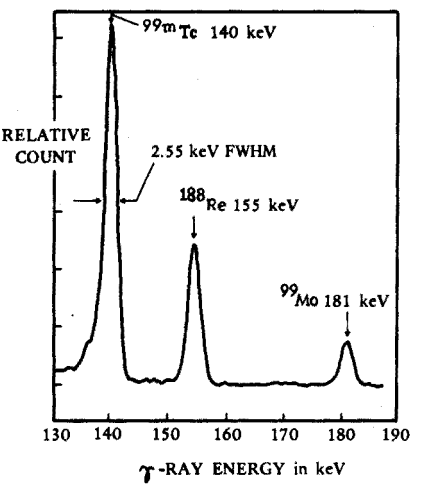

Fig. 2. Typical γ-ray spectrum of rhenium in MoO_3 standard from 130 to 190 keV.

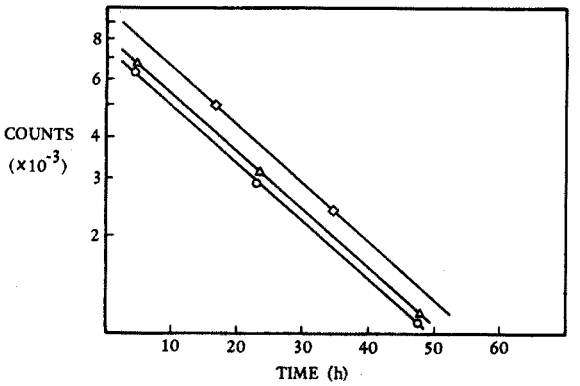

Fig. 3. Decay curves of 155-keV rhenium-188 photopeak counts. (◇) Pure 16.7-h decay; (△) typical molybdenite sample decay; (○) typical rhenium standard decay.

The corrected counting data for the standards were used for least-squares curve fitting to the best straight line, and sample concentrations were calculated from the resultant standard curve. Photopeak areas were measured by the "total peak area (TPA)" method[16]. Table I shows the analytical results from both ^{252}Cf and reactor activation compared with chemical assay data for a suite of six samples. These samples comprised a part of an inter-laboratory study on the assay of molybdenite samples from various locations. The data agree favorably with the chemical assay.

TABLE I

RHENIUM CONTENT OF MOLYBDENITE CONCENTRATES

Sample	*Rhenium concentration (p.p.m.)*		*Chemical assay[b] (p.p.m. Re)*
	^{252}Cf INAA	*Via reactor INAA*	
A	66±6[a]	60±8[a]	76
B	230±10	230±10	245
C	390±10	350±30	375
D	1160±20	1130±70	1210
E	890±20	880±70	940
F	430±10	420±30	450

[a] $\pm\sigma$ from counting statistics.

[b] F. G. Hiss and A. C. Francis, The Determination of Rhenium in Geological Samples and Rhenium Process Intermediates by Atomic Absorption Spectroscopy, *25th Annual Northwest Regional Meeting of the American Chemical Society, Seattle, Wash., June* 17–19, 1970.

Maximum deviations between activation and chemical assay appeared at the upper and lower limits of the standard curve.

The detection limit of the method (at $\phi \sim 2 \cdot 10^8$) for rhenium in molybdenite is about 15 p.p.m. rhenium (at 95% confidence).

The authors wish to acknowledge the assistance of C. C. Bertrand for the preparation of samples and standards, and of A. C. Francis for the chemical assay data.

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SHORT COMMUNICATION

Neutron activation analysis for europium in photographic film

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The neutron activation technique has only rarely been used for the analysis of photographic film, and then mostly for the determination of silver and the halogens[1,2]. The trace determination of europium was needed because this element was selected as a stable tracer for seeking the origin of flaws in photographic film during the production process. Europium was selected for this purpose because of its very high sensitivity in neutron activation analysis, its suitable half-life and its very low background concentration in normal photographic film. Other reasonable choices for a stable tracer, *e.g.* indium, were discarded because of the shorter half-life of the isotopes formed, necessitating a faster separation and measurement.

Films which were contaminated by very small quantities of tagged lubricants, were available for the analysis. Analyses had to be performed at the site of defects in the film to determine whether the europium concentration was different at these sites from that of the surrounding flawless film. Since the faults appeared as discolored spots with a diameter of 0.5–2 mm, small amounts of sample down to 10 mg had to be analysed. The high specific activity of ^{110m}Ag and ^{82}Br from the silver halide emulsion, prevented a direct instrumental determination.

Nuclear data and instrumentation

The nuclear data of europium, silver and bromine isotopes are shown in Table I. The short-lived isotopes and the long-lived ^{152}Eu and ^{154}Eu have been omitted. Neutron shadowing effects are improbable because the emulsion layer is at most 0.02 mm thick.

Irradiations were performed at a thermal neutron flux of 10^{11} n cm^{-2} sec^{-1} in the Thetis reactor for 4 h.

Chemical separation

The separation of the ^{152m}Eu activity from the matrix was based on the precipitation of the silver halides and the precipitation of europium oxalate. Both precipitations were repeated to increase the decontamination factor. A nearly quantitative separation of ^{152m}Eu was ensured, to avoid a determination of the chemical yield.

TABLE I

NUCLEAR DATA

Element	*Isotope*	*Half-life*	*Thermal neutron cross-section (barn)*	*Main γ-radiation (keV)*
Br	^{82}Br	35.3 h	3.3	554.3; 619.1; 698.3; 776.5; 1043.9; 1317.4; 1474.7
Ag	^{110m}Ag	253 d	3.2	657.7; 884.6; 937.4; 1384.0
Eu	^{152m}Eu	9.3 h	$1.4 \cdot 10^3$	121.8; 344.2; 841.6; 963.5; 1314.8

After the irradiation, wet chemical ashing of the material was performed in a digestion apparatus as described by Bethge[3], with boiling point-controlled oxidation with a mixture of perchloric, sulphuric and nitric acids[4,5]. At a temperature of about 170°, the organic material of the film, mostly polyester, was mineralized very conveniently and quickly (15–30 min).

A silver halide precipitate consisting of part of the silver activity and the bromine activity formed spontaneously during the mineralisation. The addition of a dilute hydrochloric acid solution ensured a further precipitation of the ^{110m}Ag. The coprecipitation of europium was 0.02% on the silver chloride precipitate. Although a high decontamination factor for silver and bromine was obtained in this manner (2000–5000), the silver chloride precipitation step was repeated for the determination of very low europium concentrations. A further chemical separation consisting of a classical oxalate precipitation of the rare earth ensured a further decontamination of the matrix activity and provided the rare-earth fraction in a reproducible counting geometry. The oxalate precipitate was rather slow to form; although an overnight digestion allowed the separation of about 98–99% of the europium, two rapid successive oxalate precipitations were preferred; 2–0.5% of the activity was left in the filtrate after two successive precipitations. The rare-earth activity was measured by scintillation spectrometry or high-resolution Ge(Li) spectrometry.

Procedure

Place the following in the Bethge apparatus[3]: the irradiated film (maximum weight 150 mg), 5 ml of europium carrier solution (5 mg ml^{-1}), 2 ml of 0.1 *M* silver nitrate, 1 ml of 14 *M* nitric acid, 2 ml of 70% perchloric acid, 1 ml of 18 *M* sulphuric acid and 0.5 ml of 30% hydrogen peroxide. Heat the mixture for 30 min. Cool, add 15 ml of water, and rinse the reflux funnel. Add 2 ml of 1 *M* hydrochloric acid, filter off the precipitate and wash with 0.02 *M* nitric acid. Add 2 ml of 0.1 *M* silver nitrate and repeat the precipitation. Dilute the filtrate to 150 ml. Add 6 *M* ammonia to bring the pH to 2. Heat the solution and add slowly 10 ml of oxalic acid solution (4%) with constant stirring. Heat to 70–85° for 30 min while stirring. Filter off the precipitate, add a few drops of 14 *M* nitric acid and 5 ml of europium carrier and adjust the pH to 2 again for a second oxalate precipitation. Wash the precipitates with ammonium–

oxalate wash solution (4% in 0.3 *M* nitric acid). Collect both precipitates for the measurement of the 122-keV ^{152m}Eu radiation by NaI(Tl) or Ge(Li) spectrometry.

Results and discussion

Table II shows the results of some determinations of europium in three different pieces of film. The sample size varied between 100 mg for pieces of the film to about 10 mg for the spotty defects. These were individually cut from the film and weighed 0.4–2.5 mg each. They were easily found thanks to the circular discoloration.

TABLE II

RESULTS OF EUROPIUM DETERMINATIONS

Sample	*Weight (mg)*	*Eu concentration (p.p.m.)*	*Remarks*
1	65.1	0.58	Three simultaneous determinations
	60.0	0.51	on homogenized sample of film
	53.5	0.54	containing europium chelate
2	45.0	0.0063	Three simultaneous determinations
	40.2	0.0057	on homogenized sample of film
	48.3	0.0079	
3	125	0.038	Homogenized piece of film.
	25.0	0.0056	Film with defects removed.
	13.2	6.210	Defects type I
	15.9	0.291	Defects type II

For the irradiation and measurement conditions used, namely 10^{11} n cm^{-2} sec^{-1} irradiation for 4 h and 2-h measurement with a $3 \times 3''$ NaI(Tl) detector, a concentration of about 1 p.p.b. could be determined in a 40-mg sample. The detection limit is here assumed to be 4.6 times the square root of the background radiation[6]. The radiochemical purity of the rare-earth oxalate fraction was frequently checked with a coaxial Ge(Li) detector. The 122-keV peak always appeared to be free from interference from other low-energy γ-emitters within the energy range used for the scintillation spectrometry. A small residual ^{82}Br activity was mostly responsible for the Compton background which limited the sensitivity of the europium determination. A further decontamination of the matrix activity and a higher neutron flux are thus necessary to increase significantly the sensitivity of the determination beyond the limit mentioned above.

The reproducibility of the determinations is sufficient. In the photographic material two types of spot containing a different enrichment in europium were found. Defects of type I consisted of small 1–3 mm diameter discolored spots whereas type II defects had the same dimension but were surrounded by a halo-like zone of lesser discoloration. The low europium content of the type II defects can be explained as a diffusion of the europium chelate into the surrounding area.

It appears that neutron activation analysis by the procedure mentioned allows the determination of very small amounts of europium in photographic film.

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SHORT COMMUNICATION

Improved method for automatic analysis of sulfide in water

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(Received 17th April 1972)

When the automatic colorimetric method of Casapieri *et al.*[1] was applied to the analysis of aqueous sulfide solutions, several severe problems were encountered. These were: inadequate mixing of the buffered sample before addition of the sodium nitroprusside color-forming reagent and significant fading of the developed color at the recommended sample and reagent flow rates.

The solution of these problems involved lengthening the mixing coils, adjusting the buffer and nitroprusside addition rates and eliminating the acidification of the samples before buffering. This last change gave the greatest improvement.

Apparatus

The apparatus used for automatic analysis was a single-channel "Technicon" Auto-analyzer with a sampling rate of 40 samples per h. Sample to distilled water wash ratio was 1:2. The analytical wavelength used was 520 nm.

Reagents

The reagents used for the automatic analyses were as follows. The color-forming reagent was aqueous 0.1% (w/v) sodium nitroprusside. The buffer was prepared to contain 7 g of sodium hydroxide and 58.2 g of disodium hydrogen-phosphate decahydrate in 1 l of distilled water.

The reagents for the manual methylene blue[2] reference method used to evaluate the performance of the Auto-analyzer were as commonly used in water pollution analysis. The amine–acid stock solution was prepared by adding 50 ml of concentrated sulfuric acid to 30 ml of distilled water, cooling to 25–30°, and adding 12 g of N,N-dimethyl-*p*-phenylenediamine. The resulting mixture required constant stirring for 30 min to dissolve all the amine. The working solution was prepared by diluting 25 ml of stock solution to 1 l with a (1 + 1) mixture of concentrated sulfuric acid and distilled water. The iron(III) chloride color-forming reagent was prepared by triturating 100 g of iron(III) chloride hexahydrate with distilled water and diluting to 100 ml.

The stock alkaline sulfide solution was prepared to contain 100 mg S^{2-} l^{-1} of solution by dissolving 0.750 g of sodium sulfide nonahydrate and 4 g of sodium hydroxide in 1 l of distilled water. The test and calibration solutions were then prepared at various concentrations between 0.01 and 100 μg ml^{-1} by appropriate volumetric

dilutions with distilled water. These solutions were prepared fresh daily and used within 2 h.

The reagents used in both procedures were of the highest purity available. All glassware was "Pyrex" of class A quality. All reagents were used at room temperature, *ca.* 22–25°, were kept in amber bottles and were refrigerated when not in use.

Calibration curves

Both the automatic and manual methods were calibrated with 13 points obtained in triplicate starting at 0.01 $\mu g\ S^{2-}\ ml^{-1}$ sample: 0.01, 0.025, 0.05, 0.1, etc. The Auto-analyzer calibrations returned to the baseline between samples in all cases. The manual calibration curve was obtained by adding 3 ml of amine test solution and 1 drop of the iron(III) chloride solution to 1 ml of sample with thorough mixing after each addition. These samples were allowed to stand for 30 min before reading the percent transmittance (%T) at 670 nm with a Bausch and Lomb Spectronic 20 colorimeter. The 100 %T blank for the manual method was prepared as above by substituting 1 ml of distilled water for the sample.

Discussion

The Auto-analyzer manifold was originally set up as described by Casapieri *et al.*, the distillation step being omitted to increase sensitivity. Only erratic results were obtained when hydrazine sulfate was incorporated into the stock sulfide reagent according to their directions. When fresh sulfide stock solution was made up without any hydrazine sulfate, the reproducibility improved somewhat but was still unacceptable. Several manual analyses were then attempted with the same reagent and sample volumes as would be dispensed through the manifold in 1 min. The %T was read at 520 nm on the Spectronic 20 in 1-cm cuvettes.

The acid addition rate was found to have an unexpectedly large effect on the stability of the violet color. When the acid was poured in rapidly followed by vigorous agitation, the color faded away within 30 s after addition of the iron(III) chloride.

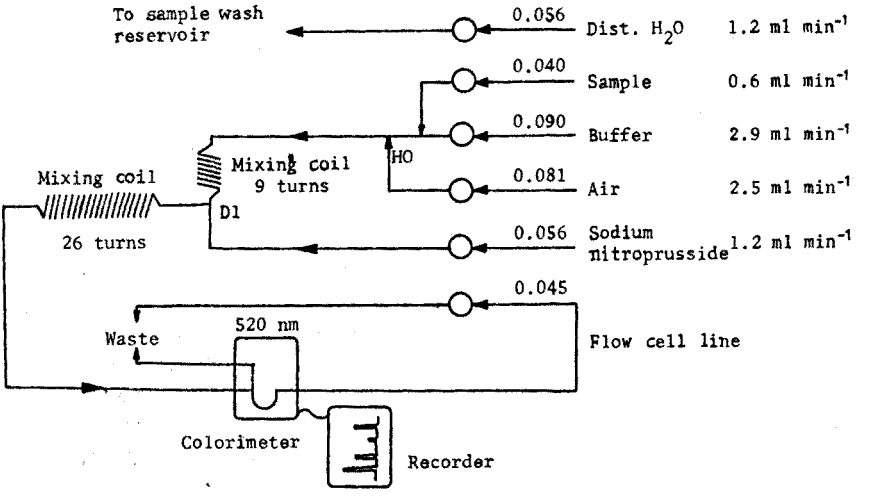

Fig. 1. Revised manifold for automatic determination of sulfide.

Addition of the chloride before adding the acid resulted in immediate bleaching. However, slow addition of acid with mixing followed by addition of 2 drops of iron(III) chloride solution produced colors stable for nearly 10 min. The acid stream was therefore deleted from the Auto-analyzer manifold. The sample and reagent flow rates and mixing coil lengths required to achieve reproducible results were then determined by trial and error. The mixing coils were made by wrapping standard transmission tubing around a 3/4-in. wooden dowel. The final version of the revised manifold is shown in Fig. 1. For routine use, these mixing coils should be replaced by glass coils of equivalent length.

With this modified manifold, the colors formed were stable for 20 min. Beer's law was followed to 40 μg S^{2-} ml^{-1} sample. Samples suspected of containing more than 50 μg S ml^{-1} should be routinely diluted 1:10 with distilled water before analysis. An estimate of the accuracy of this method may be seen by the data tabulated below for quadruplicate analyses. The maximum relative analysis error was 3.6%.

Auto-analyzer method	0.047	2.42	4.8	19.6	μg S ml^{-1}
Manual reference method	0.048	2.45	4.95	19.9	μg S ml^{-1}

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SHORT COMMUNICATION

A sensitive colorimetric method for microdetermination of gallic acid with sodium cobaltinitrite

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In an earlier communication[1], sodium cobaltinitrite was reported as a sensitive general spray reagent for detecting phenolic compounds on thin-layer chromatograms. On spraying, the phenolic compounds are converted to nitrosophenols by the action of nitrous acid and are detected as coloured cobalt(III) chelate compounds[2]. In the present communication, sodium cobaltinitrite is proposed as a colorimetric reagent for the determination of gallic acid. The cobalt chelate of the nitroso derivative of gallic acid obeys Beer's law over a reasonable range at 390–410 nm. This method has been used for the determination of free gallic acid in vegetable tannin extracts. It is more sensitive and accurate than the earlier colorimetric[3,4] and iodimetric[5] methods and other methods dependent on chemiluminescence[6] or fluorescence[7].

Reagents

All solutions were prepared from analytical-grade reagents. Methanol was distilled before use. Aqueous solutions were prepared from doubly distilled deionized water.

Stock gallic acid solution. Dissolve 100 mg of gallic acid in 100 ml of distilled methanol (0.1 % solution). (0.1 ml of this solution diluted to 100 ml contains 1 μg of gallic acid per ml.) Prepare this solution freshly.

Sodium cobaltinitrite solution. Dissolve 4.4 g of sodium nitrite in 10 ml of water and add a solution containing 2.6 g of cobalt nitrate dissolved in 2 ml of anhydrous acetic acid and 8 ml of water. Dilute this solution with 20 ml of anhydrous acetic acid and 20 ml of water.

Instrumentation

A Spectronic 20 Bausch and Lomb colorimeter with cell volume of 10 ml was used.

Calibration curve

Prepare a calibration curve by taking appropriate amounts of stock solution of gallic acid (0.1, 0.3, 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 ml) in 50-ml conical flasks. To each add 1 ml of the sodium cobaltinitrite reagent, heat the solution to boiling for 2–3 min and cool. Transfer the solutions quantitatively to 100-ml volumetric flasks and dilute to

the mark with water. Prepare the blank without gallic acid similarly and read the absorbance at 400 nm against the blank. The complex is stable for at least 8 h. Plot the absorbance against gallic acid concentration.

Results and discussion

The cobalt(III) complex of the nitroso derivative of gallic is a yellowish red compound with a broad absorption maximum at 390–410 nm. Studies at 390, 400 and 410 nm showed that this complex obeys Beer's law over the range 1–35 μg of gallic acid per ml; the mean value of the absorption maxima, *i.e.* 400 nm, was chosen for the present studies.

Effect of excess of reagent. Aliquots (1 ml) of the stock solution of gallic acid were treated with 1, 1.5, 2, 2.5, 3, 3.5, 4 ml of the sodium cobaltinitrite reagent, and the colour was developed as described above; appropriate blanks were used. It was found that the presence of excess of reagent did not interfere with the absorbance; 1 ml of the reagent, which was minimum quantity tested, is recommended for the colour development.

Effect of pH. Since gallic acid deteriorates readily in alkaline solution, owing to absorption of oxygen from atmosphere, the effect of pH was studied only below pH 7.0. The recommended procedure was followed except that different solutions were diluted to 100 ml with sodium acetate–acetic acid buffers of pH 3.6, 4.0, 4.3, 4.8 or 5.6. No change in absorbance with change in pH in this range was observed. It was, therefore, considered unnecessary to add buffer solution since the pH of the complex in acetic acid solution lies in this range.

Reproducibility. Results of replicate analyses are given in Table I. These data show that this method is quite accurate and reproducible.

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF GALLIC ACID

Concentration	15 μg ml^{-1}	20 μg ml^{-1}
Mean value of 5 determinations	15.4	19.4
s_R	0.28%	0.12%
s	0.044	0.024

Percentage recovery from thin-layer chromatograms. An aliquot (0.05 ml) of a 4% solution of a mixture of gallic acid and methyl gallate (9:1) in methanol was spotted on a 5 × 20 cm cellulose plate with a microlitre pipette. A guide spot of gallic acid was placed alongside and the chromatogram was developed in 30% acetic acid. The chromatogram was viewed under u.v. light to locate the spot of gallic acid. The corresponding area in the mixture was eluted with methanol, the solution was concentrated to near dryness and the colour was developed with 1 ml of the sodium cobaltinitrite reagent. The recovery was 94.4 ± 1.1% (4 determinations).

In separate comparison experiments, the gallic acid content was determined by the method of King and White[4], which was originally intended for the determination of pyrogallol and catechol. The recovery of gallic acid was 77.5 ± 1.7%; the rapid destruction of gallic acid under alkaline conditions in the presence of oxygen is

probably responsible for this low recovery. The method of Kind and White[4] was highly sensitive to pH changes and even the trace of acetic acid present in the cellulose removed from the developed chromatogram was found to interfere with the colour development.

Quantitative estimation of gallic acid in Eugenia jambolana seeds

Eugenia jambolana seeds have been reported to contain free gallic acid along with gallo/ellagi-tannins[8]. The present method was used to find the free gallic acid content as detailed below.

Powdered seeds of *Eugenia jambolana* (5 g) were extracted with 70% methanol for 7 h over a boiling water bath, the methanolic extract was concentrated under reduced pressure, and the volume was made up to 25 ml. An aliquot (0.1 ml) of this solution was applied to Whatman paper No. 1 and two-dimensional chromatography was done with the solvent systems: (*a*) butanol–acetic acid–water (4:1:5) upper layer in the first direction and (*b*) 2% acetic acid in the second direction. The spot corresponding to gallic acid was cut and eluted by refluxing with methanol for 4 h; the methanolic extract was evaporated to near dryness and the colour was measured as described above. The percentage of gallic acid in *Eugenia jambolana* seeds was found to be 0.52%. In repeat experiments by the method of King and White, a value of 0.36% was obtained.

Sensitivity

The minimal quantity of the gallic acid detected by this method was $1 \cdot 10^{-6}$ g ml^{-1}, hence the method is more sensitive than earlier methods[3-7]. The method is far more sensitive than the method of King and White[4] which was originally suggested for catechol and pyrogallol. It can be used for the determination of free gallic acid in tannin extracts after its separation by paper and thin-layer chromatography.

Interference from glucose

To a mixture containing 3 ml of the stock solution of gallic acid and 3 ml of 0.1% glucose solution, 1 ml of the reagent was added. The colour was developed and the absorbance read as described above. The absorbance was found to be nearly half of that required, hence glucose is a serious interference. Glucose, however, can be removed by passing the test solution through an anion-exchange column (Amberlite IRA400) as described by Brown *et al.*[9] and further elution of gallic acid. The method can thus be extended for the determination of gallic acid in tannin hydrolysate after removal of glucose.

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SHORT COMMUNICATION

The sensitivity and selectivity of the Seliwanoff test for fructose

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In 1887, Seliwanoff[1] reported that the colour reaction between resorcinol and sucrose in boiling aqueous hydrochloric acid is also given by fructose and raffinose, and a routine qualitative test for fructose based on this reaction is still in use[2]. Under the optimal conditions (5 ml of 0.05% resorcinol in (1+2) hydrochloric acid +5 drops of 1% fructose soln., boiled for 20–30 s), this test is specific for fructose provided that sucrose is absent and the amount of glucose present does not exceed 2%[2]. The sensitivity and concentration limits for fructose under the above conditions would therefore be 2500 μg and 1:20, respectively. Roe[3] has claimed that the colour intensity (sensitivity) can be increased 13-fold by using alcoholic resorcinol, raising the hydrochloric acid concentration of the medium (from 12 to 18%) and heating at 80° for 8 min, so that 10–200 mg of fructose per 100 ml may be estimated colorimetrically in the absence of pentoses, furfural, glucose (>3 mg ml^{-1}), galactose (>5 mg ml^{-1}) or xylose (>10 mg ml^{-1}). Boltz and Schenk[4] have erroneously credited Roe[3] with the development of a different spectrophotometric method for determination of 5–20 p.p.m. of fructose at 480 nm, which requires a 0.1% resorcinol–iron(III) reagent in alcohol and hydrochloric acid containing 0.00075% $FeCl_3$. Interestingly, in this procedure the tolerance limits for furfural, galactose and glucose are claimed to be exactly the same as in Roe's modification[3]. These confusing claims regarding the sensitivity and selectivity of the test prompted a re-examination in order to establish the optimal conditions for simple and rapid detection of fructose alone or in admixture with other carbohydrates.

By using essentially the same procedure as outlined in Hawk's text[2] but employing smaller volumes of test solutions, it is easily possible to detect micro amounts of fructose visually. The sensitivity and concentration limits are 20 μg and 1:2500, respectively. Upto 50 μg of fructose did not produce any precipitate, but the characteristic red colouration even with the smallest amount (20 μg) was sufficient for positive identification. Fructose in amounts below 20 μg produced a stable faint yellow colouration. The rate of the reaction (time taken for colour change) directly depended on the fructose concentration when the reagent composition and test procedure were not altered. Similar behaviour was shown by all the carbohydrates examined when the reagent described below was used (Table I). The red colour, once developed, did not fade but usually deepened somewhat on cooling. The final colour (at room temperature) was stable for more than 24 h.

TABLE I

SELIWANOFF'S TEST FOR FRUCTOSE AND OTHER CARBOHYDRATES

Compound tested	*Amount (μg)*	*Time for colour change (s)*	*Final colour*	*Remarks*
D(–) Fructose[a] (glucose-free)	50	45	Red	
	40	60	Red	
	30	90	Red	
	20	90	Red	
	10	95	Pale yellow	Colour remains unchanged upto 24 h
Dextrose[b]	3000	120	Red	
	1000	125	Red	
	500	150	Red	
	200	190	Red	
Sucrose[a]	2000	45	Red	Colour rapidly deepens on cooling
	50	85	Red	Colour slowly deepens on cooling
	30	90	Pale yellow	Yellowish colour turns red slowly
	20	120	Pale yellow	Yellowish colour turns red slowly
Mannose[c]	315	160	No colour change	Red colour develops very slowly
Maltose[a]	1000	105	Red	
	500	120	Red	
	300	180	Red	
	200	300	No colour change	Red colour develops very slowly
Galactose[a]	1040	85	Red	Colour does not deepen significantly on cooling
	780	105	Red	
	520	135	Red	
	260	165	Red	
	130	270	Red	
Raffinose[a]	50	55	Red	
	10	90	Red	
	5	125	Red	
Inulin[c]	2.75	85	Red	
	2.20	140	Red	
	1.65	240	No colour change	Red colour develops extremely slowly

[a] B. D. H. Laboratory reagent.
[b] Anhydrous, B.P., H. E. Daniels, Ltd.
[c] Koch-Light and Co., Ltd.

It was expected that upto 500 μg of glucose or galactose, 300–315 μg of maltose or mannose, 20 μg of sucrose, 5 μg of raffinose or 2.2 μg of inulin would not interfere if present with fructose, and this was experimentally verified by running the test with synthethic binary mixtures.

It is not clear why the sensitivity of the test for sucrose, raffinose and inulin

should be similar to, and even greater than, that for fructose. But there is no doubt that this colour reaction is much more sensitive and selective for fructose, especially in presence of glucose, than hitherto believed.

Method

To 5.0 ml of 0.05 % resorcinol in (1 + 2) hydrochloric acid in a pyrex test tube, add 0.01–0.05 ml of a test solution containing at least 20 μg of fructose and tolerable amounts of glucose, galactose, maltose, mannose, sucrose, raffinose or inulin. Immediately heat the mixture over a free flame with shaking. Stop heating after 100 s and compare the colour against a reagent blank similarly treated. A distinctly red colour shade will confirm presence of fructose.

The authors wish to thank Prof. M. H. Khundkar for his helpful advice and keen interest in the work.

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SHORT COMMUNICATION

Polarographic determination of hexachlorophene

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(Received 24th April 1972)

Hexachlorophene (2,2'-methylene-bis-3,4,6-trichlorophenol) is used in a wide variety of cosmetics and pharmaceuticals. The drug is usually determined in biological samples by spectrophotometry[1-3] or gas chromatography[4,5] after separation from the biological material by a series of extractions[6,7]. The present paper describes the electroreduction of hexachlorophene and the application of a.c. polarography to rapid determinations of small amounts of the drug.

Reagents and apparatus

Pharmaceutical-grade hexachlorophene was obtained from A/S Apothekernes Laboratorium for Specialpraeparater, Oslo. Stock solutions were prepared by dissolving the appropriate amount in 0.1 *M* sodium hydroxide. Alkaline solutions of hexachlorophene turn yellow upon standing. Hence, only freshly prepared stock solutions were used throughout this work and discarded after one day.

D.c. and a.c. polarograms were recorded with a Tacussel PRG 3 phase-sensitive polarograph. All a.c. polarograms were obtained at a frequency of 50 Hz and an a.c. amplitude of 10 mV. Unless otherwise stated, the demodulation phase angle was 0°. A saturated calomel electrode (S.C.E.) served as reference electrode and a platinum coil was employed as auxiliary electrode. Cyclic voltammetry experiments were performed with an instrument constructed in this laboratory to the design of Goolsby and Sawyer[8]. A Mosely 7030 AM X-Y recorder was used in conjunction with the instrument. A Metrohm E 410 hanging mercury drop electrode was used as working electrode and a platinum coil served as auxiliary electrode. All experiments were performed at $25 \pm 0.1°$. Dissolved air was removed from the solutions by bubbling oxygen-free nitrogen through the cell for 10 min and passing it over the solution during the electrolysis.

Results

Preliminary experiments showed that hexachlorophene is only slightly soluble in acidic solutions and the experiments were restricted to the pH range 6–11. D.c. polarograms recorded from phosphate and ammonia buffers exhibit a drawn-out irreversible wave (Fig. 1). The d.c. polarographic step is followed by a very well defined a.c. polarographic wave. The a.c. wave (measured at a demodulation phase angle $\phi = 0°$) is symmetrical about the summit potential (Fig. 1) and the width of the wave

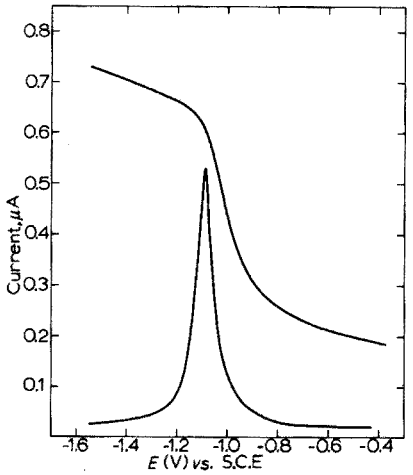

Fig. 1. D.c. and a.c. polarograms of 0.1 m*M* hexachlorophene in phosphate buffer pH 8.0. The a.c. current was recorded at demodulation phase angle $\phi = 0°$.

at half-height, ΔE_s, is 90 mV, which is close to the theoretical value for a reversible one-electron reduction[9]. The summit potential ($E_s = -1.100$ V *vs.* S.C.E.) as well as the half-wave potential ($E_{\frac{1}{2}} = -1.020$ V *vs.* S.C.E.) is independent of pH in the range 6–11, indicating that hydrogen ions are not involved in the rate-determining step. In the following experiments 0.1 *M* phosphate buffer pH 8.0 and 0.1 *M* potassium nitrate was used as supporting electrolyte.

The effect of drop time was investigated by recording polarograms of 0.1 and 0.05 m*M* hexachlorophene in phosphate buffer pH 8 at various heights of the mercury column. As indicated in Table I the height of the a.c. wave is independent of the drop time, whereas the limiting current of the d.c. wave increases linearly with the height of the mercury column, indicating that the current is controlled by an adsorption process. A.c. polarograms of the drug recorded with non-phase-sensitive demodulation showed that the base current is depressed at potentials more positive than the summit potential, which implies that it is the oxidized form of the depolarizer which is adsorbed on the electrode surface (ref. 9, p. 268).

TABLE I

EFFECT OF PRESSURE OF MERCURY ON THE HEIGHT OF THE POLAROGRAPHIC WAVE OF 0.05 m*M* HEXACHLOROPHENE IN 0.1 *M* PHOSPHATE BUFFER pH 8.0

h_{corr} (*cm*)	*D.c. current*		*A.c. current* (*μA*)
	μA	$i \cdot h_{corr}^{-\frac{1}{2}}$ ($\mu A\ cm^{-\frac{1}{2}}$)	
39.9	0.265	0.0066	0.268
44.9	0.280	0.0062	0.270
49.9	0.310	0.0062	0.268
54.9	0.350	0.0064	0.266
59.9	0.370	0.0062	0.268
64.9	0.440	0.0067	0.270

TABLE II

POLAROGRAPHIC DATA FOR THE REDUCTION OF VARIOUS AMOUNTS OF HEXACHLOROPHENE IN 0.1 *M* PHOSPHATE BUFFER pH 8.0

Concn. (*mM*)	*A.c. current* (*nA*)	i_s/C ($\mu A/mM$)
0.100	415	4.15
0.050	270	5.40
0.025	138	5.52
0.010	56.0	5.60
0.0075	41.5	5.53
0.0050	28.0	5.60
0.0010	5.6	5.60

Polarograms recorded from 0.1 *M* phosphate buffer pH 8.0 with various amounts of hexachlorophene present showed that the height of the a.c. wave increases linearly with the bulk concentration of the drug in the range 10^{-6} to $5 \cdot 10^{-5}$ *M* (Table II). As a result of the strong adsorption of the depolarizer the value i_s/C decreases at higher bulk concentrations, but the polarograms are still perfectly reproducible. When the concentration of hexachlorophene is decreased below 10^{-5} *M* reproducible waves are only obtained at relatively long drop times. The data in Table II were obtained at a mercury height of 45 cm which corresponds to a drop time of 6 sec. The d.c. polarographic step is ill-defined at low concentrations of the drug and the whole wave disappears at concentrations below $5 \cdot 10^{-5}$ *M*. Consequently, only a.c. polarography with phase demodulation $\phi = 0°$ is applicable for the determination of small amounts of hexachlorophene.

Cyclic voltammetric experiments were performed at a hanging mercury drop. Reproducible waves were obtained provided that the mercury drop was changed between each potential sweep. As indicated in Fig. 2 the cathodic wave is symmetrical

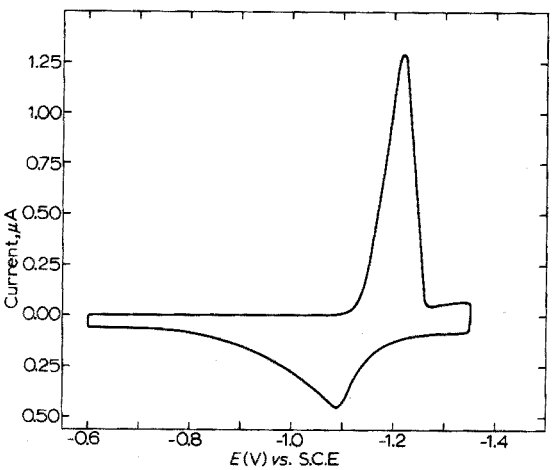

Fig. 2. Cyclic voltammogram of 0.1 m*M* hexachlorophene in phosphate buffer pH 8.0. Scan rate 0.2 V sec^{-1}.

TABLE III

VOLTAMMETRIC DATA FOR THE REDUCTION OF 0.1 m*M* HEXACHLOROPHENE IN PHOSPHATE BUFFER pH 8.0

Scan rate $v(V\ sec^{-1})$	$(i_p)_c$ (μA)	$(i_p)_a$ (μA)	$(i_p)_a/(i_p)_c$	$(i_p)_c/Cv^{\frac{1}{2}}$	$(i_p)_c/Cv$	ΔE_p (mV)
0.20	1.288	0.338	0.26	28.9	7.5	135
0.10	0.880	0.250	0.29	27.9	8.9	125
0.05	0.513	0.163	0.32	22.9	10.2	105
0.02	0.218	0.085	0.39	15.5	10.9	80
0.01	0.093	0.055	0.59	9.3	9.3	75

about the peak potential, which is a characteristic feature of an adsorption wave. Moreover, the current function $(i_p/Cv^{\frac{1}{2}})$ increases rapidly with increasing scan rate while the value i_p/Cv remains nearly constant (Table III) which also indicates an adsorption process[10]. The appearance of an anodic wave resulting from oxidation of the reduction product indicates a reversible step in the overall electrode reaction. At slow scan rates the separation of the anodic and cathodic peak, ΔE_p, approaches the theoretical value for a reversible one-electron transfer process. The variation of i_a/i_c with scan rate indicates that the charge transfer is followed by a reversible reaction[11].

Discussion

Based on the experimental results the following electrode reaction is proposed

$$RCl + e \xrightarrow{\text{slow}} (R)_{ad} + Cl^-$$

$$(R)_{ad} + e \xrightleftharpoons{\text{fast}} R^-$$

$$R^- + H^+ \longrightarrow RH$$

where RCl denotes hexachlorophene.

Hexachlorophene produces a very well defined a.c. polarographic wave at the dropping mercury electrode. By means of a phase-sensitive a.c. polarograph, by which the capacitative current is eliminated, and the faradaic response enhanced, the drug can easily be determined in the concentration range 0.4–40 μg ml^{-1}. The limit of detection is about 100 ng ml^{-1}. The peak height is independent of pH in the entire range 6–11. The proposed method for determination of the drug is very rapid and selective, and is more sensitive than the spectrophotometric methods. Unfortunately, the a.c. wave is deformed in the presence of surface-active substances like proteins. Hence, hexachlorophene must be separated from biological samples before the polarographic determination.

The authors wish to thank cand. pharm. R. Gjermundsen and cand. pharm. V. Holm, Pharmaceutical Research Laboratory, A/S Apothekernes Laboratorium for Specialpraeparater, Oslo, for their kind interest in this work and for supply of the drugs used in this investigation.

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SHORT COMMUNICATION

A chemical game of cards

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(Received 2nd February 1972)

Playing cards are nothing but symbols printed usually on cardboard. Card games represent more or less complicated systems, in which different values and "powers" (forces, activities) are given to the various "symbols" (cards) in their relation to each other. Complex systems make possible very interesting but also rather difficult games with many variations and possibilities (*e.g.* bridge).

Atomic symbols can also be used on cards. To the various elements a certain "value" can be given, *e.g.* based on the order of the electrochemical series of metals. Their chemical "behaviour" to each other—their reaction with each other—represents their various "powers". "Precipitation" may be regarded as the equivalent of "taking" a card. Here, the "system" is not a human invention, but is inherent in the chemical matter and is well known to every chemist.

Based on such considerations, several games of cards can be devised with many variations. The game which has proved most successful, and for which the name "Chem-Bridge" is proposed, is described below. It can be played for educational purposes as well as for amusement.

The game described here is played by 4 players (N, S, W, E) in pairs, as in bridge; 32 playing cards are needed on which the following symbols are drawn:

Metals	*Reagents*
Ag^+ Pb^{2+} Hg^+	KOH HCl H_2SO_4
Cu^{2+} Cd^{2+} Bi^{3+}	KOH HCl H_2SO_4
As^{3+} Sb^{3+} Sn^{2+}	NH_3 H_2S Ox
Co^{2+} Ni^{2+} Mn^{2+} Fe^{3+} Cr^{3+}	NH_3 H_2S Ox
Al^{3+} Zn^{2+}	Na_2CO_3
Ca^{2+} Ba^{2+} Sr^{2+}	(Ox = oxidation)

It is convenient to print the symbol in each corner of the card, as well as centrally, and to use different colours for the "Metal" cards and the "Reagent" cards (say red and black).

Each player receives 8 cards. The general idea of the game is that the player who produces a "precipitate" can take it at the end of the round. He can also take any other precipitates of the same kind in this particular round (*e.g.* sulphates, chlorides, sulphides, hydroxides). If an already formed "precipitate" is changed by adding a suitable reagent, then it belongs to the player who finally changes it. For example, if

the sequence is $Pb^{2+}....SO_4^{2-}....S^{2-}$, the precipitate belongs to the player who added the sulphide thus transforming $PbSO_4$ into PbS.

A precipitate can also be dissolved; in this case the metal card belongs to none of the players, but goes to the "sink", *i.e.* it is removed from play. The same thing happens with "metals" that have not been precipitated at all (*e.g.* Ag....HCl.... NH_3; or $Cu^{2+}....H_2S....Ox$; or Ni.... NH_3.... H_2S....Ox).

The player who deals the cards begins. In further play, whoever forms the last precipitate leads for the next round. If nothing has been precipitated in a particular round, the lead remains with the same player as in the previous round.

The solution in the "beaker" (as the table may be called) must be regarded as being neutral (or just sufficiently acidic to avoid hydrolysis effects). An "acid card" makes the medium acid; a "base card" (KOH, NH_3, Na_2CO_3) makes it alkaline (or ammoniacal). In all cases the last added card is responsible for the pH, *e.g.* if KOH is followed by HCl, the final solution is not "neutral" but "acid".

Some additional statements must be made: "Ox" may be able to oxidize Mn^{2+} to permanganate, S^{2-} to SO_4^{2-}, Cr^{3+} to CrO_4^{2-}, and all metals to their highest valency; KOH is regarded as a strong enough base to redissolve Pb, Zn, Al, Sb, Sn; NH_3 cannot precipitate Ag, Cu, Cd, Zn.

Some typical situations may illustrate the rules:

N	*E*	*S*	*W*	
As	Sr	H_2S	Fe	→ As belongs to S
Mn	KOH	Sn	Hg	→ Mn + Hg belongs to E
Cd	H_2S	Ox	Pb	→ Pb (as $PbSO_4$) belongs to W
Cr	KOH	Sr	Ox	→ Sr + Cr ($SrCrO_4$) belongs to W
KOH	H_2SO_4	Fe	Cu	→ no precipitate

At the end of the "game" (8 rounds) the metals won by the teams (N + S, E + W) are counted.

The following scores are suggested:

Ag, Hg	5
Cu, Bi, As, Sb	4
Pb, Cd, Sn, Co, Ni, Fe, Cr, Zn	3
Mn, Al	2
Ca, Sr, Ba	1

Additional scores are given to teams which complete a "group" of metals:

Ag, Pb, Hg	additional 7, total 20
Cu, Cd, Bi	additional 9, total 20
As, Sb, Sn	additional 9, total 20
Co, Ni, Mn, Fe, Cr	additional 11, total 25
Al, Zn	additional 5, total 10
Ca, Sr, Ba	additional 7, total 10

One "game" may be called an "analysis". The winning team is the one which has scored more points. As already stated, these rules can be varied in numerous ways.

The author would be glad to receive any comments about this game.

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BOOK REVIEWS

E. Sawicki, *Photometric Organic Analysis. Basic Principles with Applications*, Part I (Chemical Analysis, Vol. 31, Edited by I. M. Kolthoff and P. J. Elving), Wiley–Interscience, New York, 1970, xv+679 pp., price £ 15.25.

Analytically useful photometric methods are not usually studied in great depth to establish why a particular organic compound absorbs at a particular wavelength. Rather, some absorption is established and applied to detect or determine a compound in different kinds of sample by an entirely empirical process. Dr. Sawicki hopes in this text to cut through the barriers which divide biochemical, clinical, pollution, toxicological and other analysts, and to provide a uniform, more theoretical, approach to the development and application of organic spectrophotometric analysis in the ultraviolet and visible regions. If the reader can brave the jargon, and skip the rather impassioned introductory chapter, he will find a great deal of interest in this book.

Almost half of the text is concerned with the absorption spectra of zwitterionic resonance structures. The effects of solvents, dielectric constant, geometry and tautomerism are described, and structural factors which determine absorption wavelengths and absorptivities are discussed at length. Data on many hundreds of organic compounds are described and/or tabulated. Similar treatment is given to the spectra of compounds possessing cationic or anionic resonance structures.

This is indeed a praiseworthy attempt to bring reason into a rather chaotic area of organic analysis. The vast amount of information which has been assiduously collected together about the factors affecting the absorbance of organic compounds will prove most valuable to anyone seeking a deeper understanding of the subject. Part II, which covers applications, will be awaited with interest.

A. M. G. Macdonald (Birmingham)

Selected Annual Reviews of the Analytical Sciences, Vol. 1, Edited by L. S. Bark, Society for Analytical Chemistry, London, 1971, v+269 pp., price £ 5.00.

In the foreword to this first volume of a new review series, stress is laid on the need for reviews to allow analytical scientists to maintain a proper awareness of the original literature. There is now some danger that authors for the many different series of reviews and encyclopaediae will tread very similar paths, or that editors of such volumes will be led into an esoteric choice of topics to provide sufficient novelty. The editor of this series has avoided these pitfalls by selecting topics of considerable current importance and setting his authors definite time limits for the period to be covered by the reviews (usually 1967–70).

The first review (40 pp.) by Anderson, Dea and Hendrie, covers molecular sieve chromatography. The subject matter is arranged as Experimental, techniques

and materials; Applications; Applications to macromolecules; Enzymes bound to molecular sieves; Theory. This is followed by a review on photoluminescence and chemiluminescence in inorganic analysis by Bark and Wood, which is almost a monograph (90 pp.); after a discussion of instruments and quantum yield standards, the bulk of this review covers the determination of the elements.

Recent developments in activation analysis are discussed by Pierce (42 pp.) who reviews methods of activity isolation and then techniques based on reactors, accelerators, radioisotopes, charged particles and γ-photons. Atomic absorption spectroscopy is reviewed by Platt (57 pp.); this article starts with useful discussions of a.a.s. nomenclature and of radiation sources, before the main section on applications to the elements. The final review, on catalytic methods by Svehla (34 pp.), contains sections on classification of methods, and determinations of metals, non-metals and organic compounds.

All the reviews are well written and well presented. The coverage is in greater depth than that found in the A.C.S. Analytical Chemistry Annual Reviews, which have, of course, a much larger selection of topics. The two sets of annual reviews sponsored by the two Societies should coexist happily, provided that the high standard set by this first volume can be maintained.

A. M. G. Macdonald (Birmingham)

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