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SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 29, No. 3 September 1963

"LIGAND BUFFER": A CONCEPT USEFUL IN THE THEORETICAL CONSIDERATION OF EQUILIBRIA INVOLVING CHELATING AGENTS

PRECIPITATION AND SOLVENT EXTRACTION IN LIGAND BUFFERS

The concept "ligand buffer" is proposed for a system containing a ligand and an excess of metal. The general properties of the ligand buffer are discussed in connexion with the results of pA calculations under various conditions. The "ligand buffer" concept and pA calculations are useful in the consideration of various equilibria involved in solvent extraction, precipitation, etc., in the presence of masking agents.

M. TANAKA, *Anal. Chim. Acta*, 29 (1963) 193–201.

THE DETERMINATION OF STRONTIUM IN COAL ASH BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

An atomic absorption spectrophotometric method for the determination of 0.01%–0.40% strontium in coal ashes is proposed. After suitable dissolution of the sample, 2000 p.p.m. of lanthanum are added to overcome interferences before the solution is atomized. The results obtained were compared with those obtained by flame-emission spectrophotometry. The method is rapid and no preliminary separations are necessary.

C. B. BELCHER AND K. A. BROOKS, *Anal. Chim. Acta*, 29 (1963) 202–205.

THE DETERMINATION OF SODIUM IN PURE LIMESTONES BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

The determination of sodium in pure limestones by atomic absorption spectrophotometry is described. The interferences of chloride and calcium ions are discussed; chloride affects the dissociation equilibrium, and calcium lowers the evaporation rate of sodium, thus lowering the concentration of free sodium atoms in the flame. The sensitivity achieved with a rather rough apparatus is 0.15 p.p.m. sodium.

I. RUBEŠKA, B. MOLDAN AND Z. VALNÝ, *Anal. Chim. Acta*, 29 (1963) 206–210.

RAPID ACTIVATION ANALYSIS OF TRACE VANADIUM IN TISSUE USING 3.8-MINUTE VANADIUM-52

Submicrogram amounts of vanadium in rat liver tissue have been analyzed by rapid activation analysis. A 5-min radiochemical separation coupled with γ-ray spectrometry permitted utilization of the 3.8-min vanadium-52 radioisotope. With this procedure the lower limit of detection at a thermal neutron flux of 10^{12} n/cm^2/sec was about $3 \cdot 10^{-9}$ g of vanadium.

D. G. KAISER UND W. W. MEINKE, *Anal. Chim. Acta*, 29 (1963) 211-214.

POLAROGRAPHIC DETERMINATION OF THALLIUM

A polarographic method for the determination of thallium is described. The inclusion of DTPA and Triton X-100 in the supporting electrolyte shifts the waves of interfering metals to considerably more negative potentials while the thallium wave is completely unaffected. An acetate buffer of pH 4–5 is the preferred base electrolyte, because a Tl–DTPA complex is formed at higher pH values. The polarographic behaviour of the complex is described and the stability constant determined.

E. JACOBSEN AND G. KALLAND, *Anal. Chim. Acta*, 29 (1963) 215–219.

STRUCTURE OF THE ZIRCONIUM–ALIZARIN S COMPLEX IN RELATION TO pH CHANGES

A comparison of the absorption spectra of the zirconium–alizarin S complex with those of alizarin S at different pH values shows that the action of hydrogen ions and zirconium(IV) on alizarin S is similar. Changes caused by pH alterations are discussed. Between pH values 1 and 1.8 the zirconium–alizarin S complex has the molar ratio 1 : 1; at higher pH, suspensions of hydroxy complexes of zirconium are formed which adsorb the above complex.

G. PARISSAKIS AND J. KONTOYANNAKOS, *Anal. Chim. Acta*, 29 (1963) 220–226.

STUDIES ON THE NAPHTHORESORCINOL REACTION OF TOLLENS

PART II. THE DETERMINATION OF FREE URONIC ACID IN PRESENCE OF URONOSIDES, POLYURONOSIDES AND OTHER CARBOHYDRATES

(in German)

A micro determination for free uronic acids based on TOLLENS' reaction is proposed. Sources of error in earlier methods are avoided by the use of phosphoric acid, a reagent solution in glacial acetic acid, and extraction of the dye with toluene. Free glucuronic acid can be determined in presence of glucuronosides of the phenol–ether type up to molar ratios of *ca.* 1 : 5. Reliable results can also be obtained in the presence of other carbohydrates.

W. WAGNER, *Anal. Chim. Acta*, 29 (1963) 227–239.

SPECTROPHOTOMETRIC DETERMINATION OF SCANDIUM WITH ARSENAZO

Arsenazo is used for the spectrophotometric determination of scandium in the range 10 to 50 μg. The absorbance is measured at 570 mμ and pH 6.1. A method is proposed for the successive determination of scandium and thorium. Scandium is separated from magnesium, calcium, rare earths, zirconium, fluoride, phosphate, and some other metals by extraction with TTA in xylene. Copper, aluminum, and iron(III) are removed by 8-quinolinol–chloroform extraction. Uranium(VI) is removed by anion exchange using hydrochloric acid. Thorium is separated from scandium by anion exchange using nitric acid.

H. ONISHI AND C. V. BANKS, *Anal. Chim. Acta*, 29 (1963) 240–248.

GROWTH KINETICS OF BARIUM SULPHATE SUSPENSIONS

Growth rates of barium sulphate precipitates were followed by spectrophotometric transmission measurements. Interpretation of the results enabled the rate of precipitation to be characterized in terms of the surface area of precipitate and the concentration of reagents. Consistent rate constants were obtained which show that the rate of precipitation is not dependent upon which ion (barium or sulphate) is in excess.

A. G. WALTON AND T. HLABSE *Anal. Chim. Acta*, 29 (1963) 249–253.

DETERMINATION OF URANIUM(VI) BY TRI-*n*-OCTYL-PHOSPHINE OXIDE EXTRACTION AND COULOMETRIC TITRATION

Uranium can be determined in the usual types of dissolver solutions by extraction of uranium(VI) into a cyclohexane solution of tri-*n*-octyl-phosphine oxide (TOPO), back-extraction into an ammonium sulfate solution, and coulometric titration at controlled potential. Optimum conditions were established for the extraction and back-extraction, and the overall performance of the method was evaluated. The method is accurate, precise, and widely applicable. It should be very useful in nuclear reactor technology.

W. D. SHULTS AND L. B. DUNLAP, *Anal. Chim. Acta*, 29 (1963) 254–260.

REMOVAL OF INTERFERENCES IN ABSORPTIOMETRIC DETERMINATION OF BISMUTH WITH THORIN

Bismuth can be separated by ion-exchange adsorption on Dowex 21K resin from *ca.* 0.25 *M* HCl, after removal of volatile interferences by distillation in acid-oxidizing medium. Bismuth is eluted from the column with 1 *M* sulfuric acid. The method is useful in the absorptiometric determination of bismuth with thorin, as well as in other photometric methods.

H. A. MOTTOLA, *Anal. Chim. Acta*, 29 (1963) 216–266.

THE ELIMINATION OF THE GETTER EFFECT IN THE DETERMINATION OF GASES IN METALS BY THE VACUUM FUSION METHOD

A furnace for determinations of gases in metals is described in detail. The essential part of the equipment is the stopper, which acts as a non-gettering baffle as well as a splash shield. The stopper is constructed of two conical halves of graphite; the movement follows by means of a rotatable shaft, passing through the tube and sealed by rubber o-rings. The entire analytical equipment is outlined, and some results of analysis with and without the stopper are given.

N. LOUNAMAA, J. U. AASS, J. KÜHNE AND T. PERSSON, *Anal. Chim. Acta*, 29 (1963) 267–271.

THE DETERMINATION OF NITRATE IN SEA WATER

A heterogeneous reduction method is described for the determination of nitrate in sea water. Nitrate is reduced to nitrite with 91% efficiency by passing the water through a column of amalgamated cadmium filings. The nitrite produced is determined spectrophotometrically by the method of BENDSCHNEIDER AND ROBINSON. The method has a coefficient of variation of *ca.* 2% and is free from salt error. Temperature in the range 0°–35° has no effect on the reduction. Interference from nitrite is discussed and a method is described for its destruction if necessary. Sulphide does not interfere.

A. W. MORRIS AND J. P. RILEY, *Anal. Chim. Acta*, 29 (1963) 272–279.

POLAROGRAPHIC DETERMINATION OF MOLYBDENUM IN URANIUM–MOLYBDENUM ALLOYS

(Short Communication)

V. T. ATHAVALE, R. KALYANARAMAN AND K. A. KHASGIWALE, *Anal. Chim. Acta*, 29 (1963) 280–282.

ON THE OXIDATION STATE OF COBALT IN ITS PAN AND PAR CHELATES

(Short Communication)

T. IWAMOTO AND M. FUJIMOTO, *Anal. Chim. Acta*, 29 (1963) 282–284.

CHANGING POTENTIAL AT CHANGING STIRRING SPEED

(Short Communication, in German)

F. L. HAHN, *Anal. Chim. Acta*, 29 (1963) 285.

GRAVIMETRIC DETERMINATION AND SEPARATION OF TITANIUM WITH N-BENZOYL-N-PHENYLHYDROXYLAMINE BY DIRECT WEIGHING

(Short Communication)

V. R. M. KAIMAL AND S. C. SHOME, *Anal. Chim. Acta*, 29 (1963) 286–288.

"LIGAND BUFFER": A CONCEPT USEFUL IN THE THEORETICAL CONSIDERATION OF EQUILIBRIA INVOLVING CHELATING AGENTS

PRECIPITATION AND SOLVENT EXTRACTION IN LIGAND BUFFERS

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Many investigations have been made of the equilibria and reactions involved in the use of ethylenediaminetetraacetic acid (EDTA) and its homologues. The calculation of the metal ion concentration (pM) of a system containing a metal and a chelating agent is of great importance in the theoretical consideration of chelatometry and of various equilibria in which chelating agents participate. CHARBEREK AND MARTELL[1] explain various phenomena involving chelating agents by means of the concept of a "metal ion buffer".

For more complex systems, however, the direct calculation of the pM value is too complicated. Thus the behaviour of a metal N in a system containing a ligand and an excess of metal M is not readily understood by the direct calculation of pN. On the other hand, it is relatively easy to explain the phenomena through the calculation of the ligand concentration or pA. This has been well demonstrated in an example of chelatometry by REILLEY AND SCHMID[2].

It has also been demonstrated experimentally that a system containing a chelating agent and an excess of metal is useful in the separation of metals by precipitation[3] and by solvent extraction[4].

Chelating species being polybasic, pA changes gradually as a function of pH. Furthermore, since the ligand forms more or less stable chelates with various metals, pA is buffered at a certain level in a solution containing a ligand and an excess of metal over a wide range of pH; *i.e. pA is buffered in the presence of an excess of metal.* pM is well known to be buffered in the presence of an excess of ligand, and the term "metal ion buffer" has been proposed for a solution containing a metal and an excess of ligand. In the present paper, a new term, "ligand buffer", is proposed for a solution containing a ligand and an excess of metal, in which pA is buffered at a certain level. This concept is very convenient for the interpretation of complex equilibria involving chelating agents*.

* The following discussion is valid even for a system involving a monodentate ligand, but ligand buffers containing aminopolycarboxylic acids such as EDTA are of much greater practical importance.

DISCUSSION

Definition of a ligand buffer

A system containing a ligand and an excess of metal ion[a] is defined as a "ligand buffer". In a ligand buffer, pA is determined by C_M/C_A[b] and pH. Furthermore in the case where A forms a stable chelate MA with M, pA is determined only by the ratio C_M/C_A over a wide range of pH unless this ratio is very close to unity (see below).

Calculation of pA as functions of pH and C_M/C_A

Let us consider a system composed of a ligand A and an excess of metal M. M is assumed to form a water-soluble chelate MA, a hydroxo complex MA(OH) and a protonated complex MHA[c]. The following stoichiometric relationships hold (omitting charges for simplicity):

$$C_A = [MA] + [MHA] + [MA(OH)] + [A]' \quad (1)$$

$$C_M = [M]' + [MA] + [MHA] + [MA(OH)] \quad (2)$$

$$C_M = aC_A \quad (3)$$

where [M]′ denotes the total concentration of M not combined with A, and [A]′ the total concentration of the ligand A not combined with M. [A]′ is given by the following:

$$[A]' = [A]\alpha_{H(A)} = [A](1 + K_1[H] + \ldots + K_1 \ldots K_j[H]^j)$$

where the formation constants of proton complexes of A are denoted as $K_1 \ldots K_j$.

By appropriate substitution and rearrangement of eqns. (1), (2) and (3), we have

$$\gamma_{M',A'}[A]'^2 + \{1 + (a-1)C_A\gamma_{M',A'}\}[A]' - C_A = 0 \quad (4)$$

where $\gamma_{M',A'} = K_{M'A'} + K_{M'HA'}K_1[H] + K_{M'A'(OH)}K_{M'A'}K_w/[H]$[d]. Solving eqn. (4) for [A]′ and rejecting the negative solution which has no physical significance:

$$[A]' = \frac{\{1 + (a-1)C_A\gamma_{M',A'}\}}{2\gamma_{M,A}}\left[-1 + \sqrt{1 + \frac{4C_A\gamma_{M',A'}}{\{1 + (a-1)C_A\gamma_{M,A}\}}}\right] \quad (5)$$

By means of Taylor's expansion of the square root in eqn. (5), [A]′ is given by a series:

$$[A]' = \frac{C_A}{\{1 + (a-1)C_A\gamma_{M',A'}\}} - \frac{C_A\gamma_{M',A'}}{\{1 + (a-1)D_A\gamma_{M',A'}\}^3} + \ldots \quad (6)$$

At higher pH values only MA has to be taken into account, the series converges rapidly and [A] is given by the following:

$$[A] = [A]'/\alpha_{H(A)} = \frac{C_A}{\alpha_{H(A)}\{1 + (a-1)C_AK_{M'A'}\}} \quad (7)$$

[a] More generally, a proton can be considered as a metal ion.
[b] C denotes the total concentration of a metal or a ligand specified by the subscript.
[c] Substituting [MA] in equations (1) and (2) by [MA] + $[MA]_2$ + ... + $[MA_n]$, it is not a difficult matter to take into account successive complex formations.
[d] $K_{M'A'}$, $K_{M'HA'}$ and $K_{M'A'(OH)}$ denote the apparent stability constants of MA, MHA and MA (OH) respectively.

When [M] = [M]′ and K_{MA} is sufficiently high, eqn. (7) simplifies further, as a result of a good approximation, to:

$$[A] = \frac{1}{(a-1)K_{MA}} \tag{8}$$

It is noteworthy that [A] is independent of the total concentration of the ligand (see Fig. 1).

In the pH range where the quantities of M have to be taken into account, [A] should be calculated from eqn. (5), since the series on the right side of eqn. (6) does not converge rapidly. The general features of the pA–pH diagram of some ligand buffers are shown in Fig. 1.

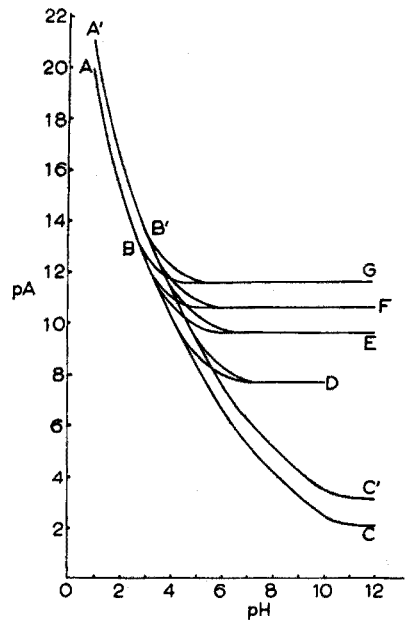

Fig. 1. pA–pH diagram for ligand buffers containing EDTA.

Curve ABC: No metal present
Curve ABD: $C_{Mg}/C_A = 1.1$
Curve ABE: $C_{Ca}/C_A = 1.1$
Curve ABF: $C_{Ca}/C_A = 2$
Curve ABG: $C_{Ca}/C_A = 11$
} $C_A = 10^{-2}\ M$

Curve A′B′C′: No metal present
Curve A′B′D: $C_{Mg}/C_A = 1.1$
Curve A′B′E: $C_{Ca}/C_A = 1.1$
Curve A′B′F: $C_{Ca}/C_A = 2$
Curve A′B′G: $C_{Ca}/C_A = 11$
} $C_A = 10^{-3}\ M$

The following formation constants of proton complexes of A were used for calculation: $\log K_1 = 10.26$; $\log K_2 = 6.16$; $\log K_3 = 2.67$; $\log K_4 = 2.00$; $\log K_5 = 1.62$; $\log K_6 = 0.92$.

It is known that the calculation of pA is a simple matter when $a \leq 1$. Thus it is possible to calculate pA in a solution containing any ligand and metals at any pH. A pA diagram constructed at a certain pH is simply a titration curve, which is easily obtained from the above expressions. A pA–pH diagram can be drawn for various ratios of C_M/C_A as given in Fig. 1. Such a diagram is useful in the theoretical consideration of various equilibria containing chelating agents.

Buffer capacity of a ligand buffer

The buffer capacity of a ligand buffer may be considered as the change in C_A/C_M required for a unit change in the pA value of the solution. This derivative is positive with respect to addition of a second metal $M_{II}(K_{M_{II}'A} > K_{M'A})$ and negative for addition of a ligand Z ($K_{MZ'} > K_{MA'}$). However, since only the absolute value is significant, the buffer capacity of a ligand buffer, π, is defined as

$$\pi = \left|\frac{d(C_A/C_M)}{dpA}\right| \tag{9}$$

When only the formation of MA has to be taken into account, from eqn. (8) we have

$$\pi = \frac{K_{MA}[A]}{2.3(K_{MA}[A] + 1)^2} \tag{10}$$

Differentiating π with respect to pA, π is found to have a maximum value of 0.109 at a point where $C_M = 2C_A$ and $pA = \log K_{MA}$. Therefore just as a proton buffer has a maximum buffer capacity when $C_{acid}/C_{base} = 2$, a maximum of buffer capacity of a ligand buffer is obtained when $C_M/C_A = 2$; that is, a solution of such composition shows a minimum change in pA when a small amount of metal or chelating agent is introduced.

Substituting eqn. (8) into (10)

$$\pi = \frac{a - 1}{2.3a^2} \tag{11}$$

Thus π is a function of a only. Some examples of π values are given in Table I. Calculation of this π value for more general cases is also useful in considering the sharpness of a chelatometric titration and will be considered in a subsequent paper.

TABLE I

BUFFER CAPACITY OF THE LIGAND BUFFER FOR VARIOUS VALUES OF C_M/C_A

$a = C_M/C_A$	*Buffer capacity*
1.1	0.036
2	0.109
5	0.070
11	0.036

Apparent stability constant of NA in a ligand buffer

From the expressions derived above, it is easy to calculate the value $C_A/[A]$ which is conveniently denoted as $\alpha_{H,M(A)}$. The apparent stability constant of a chelate NA, $K_{N'A'}$, in a system containing a ligand A and a metal M in excess is given by $K_{NA}/\alpha_{H,M(A)}\beta_N$*. The introduction of such apparent constants facilitates the theoretical consideration of a system involving chelating agents and metals. As an example, the apparent stability constants of copper–EDTA under various conditions are given in Fig. 2.

* $\beta_N = [N]'/[N]$, $[N]'$ denoting the total concentration of N not combined with A.

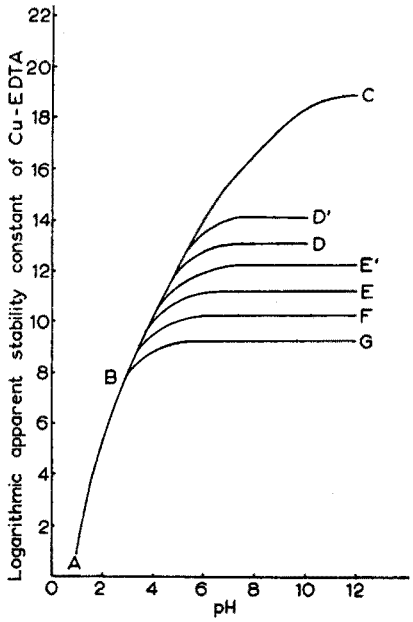

Fig. 2. Logarithmic apparent stability constants of Cu–EDTA in various ligand buffers.
Curve ABC: No metal present
Curve ABD′: $C_{Mg}/C_A = 1.1$ and $C_A = 10^{-3}\ M$
Curve ABD: $C_{Mg}/C_A = 1.1$ and $C_A = 10^{-2}\ M$
Curve ABE′: $C_{Ca}/C_A = 1.1$ and $C_A = 10^{-3}\ M$
Curve ABE: $C_{Ca}/C_A = 1.1$ and $C_A = 10^{-2}\ M$
$C_{Ca}/C_A = 2$ and $C_A = 10^{-3}\ M$
Curve ABF: $C_{Ca}/C_A = 2$ and $C_A = 10^{-2}\ M$
$C_{Ca}/C_A = 11$ and $C_A = 10^{-3}\ M$
Curve ABG: $C_{Ca}/C_A = 11$ and $C_A = 10^{-2}\ M$

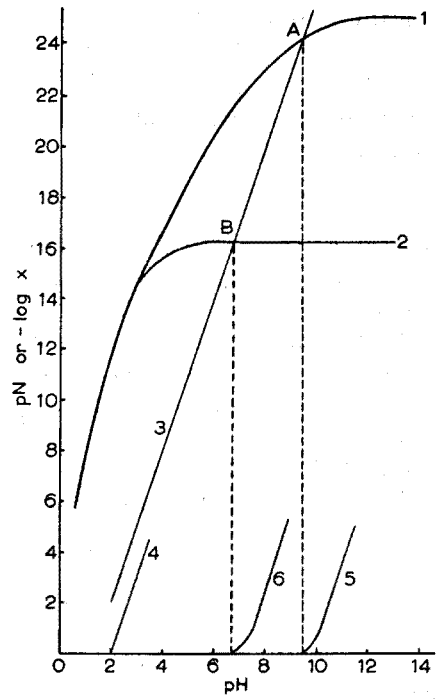

Fig. 3. pN and the fraction remaining in solution under various conditions.
Curve 1 and 5: $C_{Fe(III)} = 10^{-2}\ M$, $C_{EDTA} = 2 \cdot 10^{-2}\ M$; Curve 2 and 6: $C_{Fe(III)} = 10^{-2}\ M$, $C_{EDTA} = 2 \cdot 10^{-2}\ M$, $C_{Ca} = 2.2 \cdot 10^{-2}\ M$; Curve 3 and 4: $C_{Fe(III)} = 10^{-2}\ M$ and no complexing agent present. Curves 1, 2 and 3: pFe(III)–pH curves; Curves 4, 5 and 6: negative logarithm of the fraction of Fe(III) remaining in solution. Numerical values employed in calculation: log K_s for $Fe(OH)_3$ of −38; log $K_{Fe(III)A}$ of 25; pA values from Fig. 1.

Quantitative treatment of the precipitation in a ligand buffer

Let us consider a system containing a metal N and a ligand A in excess, in which the following stoichiometric relationships hold:

$$C_A = [NA] + [NHA] + [NA(OH)] + [A]' \quad (12)$$

$$C_N = [N]' + [NA] + [NHA] + [NA(OH)] \quad (13)$$

From eqn. (13) we obtain

$$[N]' = C_N/(1 + \gamma_{N',A'}[A]') \quad (14)^*$$

where $\gamma_{N',A'} = K_{N'A'} + K_{N'HA'}K_1[H] + K_{N'A'(OH)}K_{N'A'}K_w/[H]$. So far as no precipitation occurs

$$[A]' = C_A - C_N \quad (15)^{**}$$

* In eqns. (12), (13) and (14), [N]′ denotes the total concentration of N not combined with A. In the following, no complexing agent other than A is assumed, *i.e.* [N]′ = [N].
** A sufficiently high stability constant of NY, $K_{N'A'}$, is assumed.

Substituting eqn. (15) into (14), we have

$$[N] = C_N/\{1 + \gamma_{N,A'}(C_A - C_N)\} \quad (16)$$

In the pH range where only the formation of NY has to be taken into consideration, eqn. (16) simplifies to

$$[N] = C_N/\{1 + K_{NA}'(C_A - C_N)\} \quad (17)$$

Equation (16) or (17) allows to construct a pN–pH diagram, assuming no precipitation. Curve 1 in Fig. 3 is a pFe(III)–pH diagram in the presence of excess of EDTA and curve 2 is that in a ligand buffer composed of EDTA and excess of calcium.

On the other hand, from the solubility product principle, [N] in equilibrium with a sparingly soluble salt NR_n is given by:

$$[N] = K_s/[R]^n \quad (18)$$

where K_s denotes the solubility product of the sparingly soluble salt in question. When $N(OH)_n$ is considered, eqn. (18) can be written as:

$$[N] = K_s[H]^n/K_w^n \quad (19)$$

A pFe(III)–pH diagram in the absence of any masking agent is also given in Fig. 3 (curve 3).

In Fig. 3 the intersections of the pN curves, A and B, indicate the pH of incipient precipitation of iron(III) hydroxide under the specified conditions. Thus diagrams such as Fig. 3 enable one to predict the possible use of ligand buffers in the separation of sparingly soluble salts.

Denoting as x the fraction of N remaining in solution in the presence of A, eqn. (16) can be written as eqn. (20) in the pH range higher than the pH of the incipient precipitation:

$$[N] = C_N x/\{1 + \gamma_{N,A'}C_A - (C_N x)\} \quad (20)$$

Substituting eqn. (18) into (20), and solving for x

$$x = (1 + \gamma_{N,A'}C_A)/(\gamma_{N,A'} + [R]^n/K_s)C_N \quad (21)$$

Ordinarily, $\gamma_{N,A'}C_A$ is much greater than unity, and eqn. (21) simplifies further to

$$x = C_A/C_N(1 + [R]^n/K_s\gamma_{N,A'}) \quad (22)$$

When only the formation of NA has to be taken into account, eqn. (22) can be written as:

$$x = C_A/C_N(1 + [R]^n/K_sK_{NA'}) \quad (23)$$

If we are concerned with a hydroxide $N(OH)_n$, eqn. (23) is transformed into eqn. (24):

$$x = C_A/C_N(1 + K_w^n/K_sK_{NA'}[H]^n) \quad (24)$$

By means of any of the eqns. (21) through (24), it is possible to construct log x–pH diagrams such as curve 5 in Fig. 3.

Now, in a ligand buffer composed of a ligand A and an excess of indifferent metal M ($K_{NA} > K_{MA}$), [A] is buffered at a lower level and is given by eqn. (8) in the pH range only where MA has to be taken into consideration. In a reaction involving

precipitation, a in eqn. (8) is a function of x which is given by the following:

$$a = C_M/(C_A - xC_N)$$

Thus

$$\alpha_{H,M(A)} = C_A K_{MA}\left(\frac{C_M}{C_A - xC_N} - 1\right) \quad (25)$$

Substituting eqn. (25) into (23) and rearranging, we obtain

$$C_N^2(K - 1)x^2 + (KC_MC_N + 2C_NC_A - KC_NC_A)x - C_A^2 = 0 \quad (26)$$

where $K = [R]^n K_{MA}C_A/K_a K_{NA}$. In most cases log x–pH diagrams for a ligand buffer can be drawn by means of the quadratic eqn. (26). Curve 6 in Fig. 3 is a log x–pH diagram for iron(III) hydroxide in a ligand buffer composed of EDTA and excess of calcium. Designating the pH of incipient precipitation as pH_i, it may be interesting to note that, at pH values higher than ($pH_i + 1$), curves 5 and 6 are nearly straight lines of slope n.

Diagrams such as Fig. 3 can be drawn for other sparingly soluble salts and for other complexing agents, and enable one to predict the possible separation of metals.

Extraction of NR_n into an organic solvent from a ligand buffer

If the distribution ratio of N between aqueous and organic phases is designated as $D°$, then in the absence of any masking agent for N:

$$D° = \frac{[NR_n]_o}{[N]_w} = \frac{K_D K_{NR_n} K_a^n}{(K_{D'})^n}\left\{\frac{[HR]_o}{[H]}\right\}^n \quad (27)^*$$

where the subscripts w and o refer to the aqueous and organic phases, and K_D and $K_{D'}$ denote the distribution coefficients of NR_n and HR respectively between the two phases, K_{NR_n} the formation constant of NR_n, and K_a the acid dissociation constant of the reagent HR. Equation (27) can be rewritten in a logarithmic form:

$$\log D° = \{n \log [HR]_o - n\, pH_{\frac{1}{2}}°\} + n\, pH \quad (28)$$

where $pH_{\frac{1}{2}}°$ denotes the pH value at which $D° = 1$ and $[HR]_o = 1$ (in the absence of any masking agent).

Now, in a ligand buffer involving a ligand A which forms with N water-soluble chelates such as NHA, NA and/or NA(OH), the distribution ratio of N between the two phases, D, is given by:

$$D = \frac{K_D K_{NR_n} K_a^n}{(K_{D'})^n}\left\{\frac{[HR]_\sigma}{[H]}\right\}\frac{1}{\gamma_{N,A'}[A]'} \quad (29)$$

where $\gamma_{N,A'} = K_{NA'} + K_{NHA'}K_1 + K_{NA'(OH)}K_{NA'}K_w/[H]$ as in the preceding section. Combining eqns. (27), (28) and (29), we obtain

$$\log D = \log D° - \log \gamma_{N,A'} - \log [A]' \quad (30)^{**}$$

* Solution of the organic solvent in the aqueous phase may possibly modify the constants involved, but for most systems, such as chloroform–water, the effect of the organic solvent on the stability constants is very slight and may be neglected. Thus in the following, all constants involved in the treatment may be regarded as those obtained in pure water and pure chloroform, though most strictly these values should be determined for chloroform-saturated water and water-saturated chloroform.

** $[HR]_\sigma$ is assumed to be constant.

If only NA has to be taken into consideration,

$$\log D = \log D^{\circ} - \log K_{NA} + pA. \tag{31}$$

Of course, in eqn. (31), ($-\log K_{NA} + pA$) may be substituted by ($-\log K_{NA'} - \log [A]'$).

By means of eqn. (30) or (31), we can quantitatively account for the solvent extraction of NR_n when a ligand buffer is involved (see Fig. 4).

In Fig. 4, some examples of extraction of 8-hydroxyquinolinates in the presence of chelating agents are given. For higher values of $\log \gamma_{N,A'} + \log [A]'$ (or $\log K_{NA} - pA$), disagreement between the observed values and the theoretical ones can be seen. This discrepancy seems to be due to kinetic factors which are sometimes considerable in a system involving strong chelating agents. It may be useful to note that, owing to the kinetic factors, a pseudo equilibrium of partition of NR_n is established in favor of the organic phase when 8-hydroxyquinoline is added to the aqueous phase. In this case, there may be formation of excessive NR_n in the aqueous phase which, upon mixing with the organic solvent, is partitioned together with HR between the two phases. Thus $[NR_n]_0$ is larger than the theoretical value and is in equilibrium neither with R nor with N in the aqueous phase. However, when a chloroform solution of 8-hydroxyquinoline is used as reagent, the partition is in favor of the aqueous phase. In this case, $[R]_w$ in equilibrium with $[HR]_0$, and $[N]_w$ in equilibrium with NA, are both very low. Thus, the reaction $N + nR \rightarrow (NR_n)_w$ may proceed slowly and a pseudo equilibrium of partition of NR_n may be established in favor of the aqueous phase.

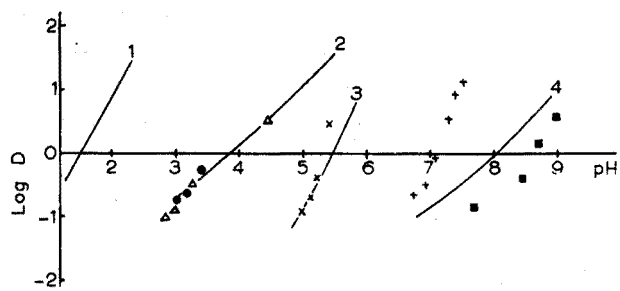

Fig. 4. Extraction of copper 8-hydroxyquinolinate from solutions involving masking agents. Curve 1: No masking agent present; Curve 2: Theoretical curve in the presence of nitrilotriacetic acid (NTA) and NTA + Ca; Curve 3: Theoretical curve in the presence of EDTA (Ca; Curve 4: Theoretical curve in the presence of EDTA.

● Experimental values for NTA+Ca; △ Experimental values for NTA; × Experimental values for EDTA + Ca; + Experimental values for EDTA; ■ Experimental values for EDTA (chloroform solution of 8-hydroxyquinoline is used as reagent).

Experimental conditions: Shaking for 3 min at about 250 shakes per min. Ionic strength was kept constant at 0.1 with $HClO_4$–$NaClO_4$. C_A: $2.5 \cdot 10^{-3}$ M; $C_{Ca} = 3 \cdot 10^{-3}$ M. Total concentration of 8-hydroxyquinoline = 10^{-3} M; $C_{Cu} = 10^{-4}$ M. Partition was made between 20 ml each of the aqueous and organic phases at 20 ±1°C. 8-Hydroxyquinoline was added to the aqueous phase as a perchloric acid solution unless otherwise noted.

Application of the concept of a "ligand buffer" and the results of pA calculations in chelatometry and polarography will be the subject of subsequent papers.

The author wishes to express his thanks to Dr. GENKICHI NAKAGAWA, Nagoya Institute of Technology, for helpful discussions.

SUMMARY

The concept "ligand buffer" is proposed for a system containing a ligand and an excess of metal. The general properties of the ligand buffer are discussed in connexion with the results of pA calculations under various conditions. The "ligand buffer" concept and pA calculations are useful in the consideration of various equilibria involved in solvent extraction, precipitation, etc., in the presence of masking agents.

RÉSUMÉ

L'auteur propose la notion de "ligand buffer" (tampon complexant) pour les systèmes renfermant un complexant et un métal en excès. Les propriétés générales du "tampon complexant" sont examinées, en tenant compte des déterminations de pA dans diverses conditions. Cette notion de "ligand buffer" et les pA calculés peuvent s'appliquer aux divers équilibres d'extraction par un solvant organique, de précipitation, etc. en présence de réactifs complexants.

ZUSAMMENFASSUNG

Der Begriff "Ligandpuffer" wird für ein System vorgeschlagen, das einen Liganden und ein Metall im Überschuss enthält. Die allgemeinen Eigenschaften des Ligandpuffers werden anhand des Ergebnisse aus den Berechnungen von pA unter verschiedenen Bedingungen diskutiert. Der Begriff "Ligandpuffer" und die pA-Berechnungen sind anwendbar bei verschiedenen Gleichgewichten, wie *z.B.* der Extraktion, der Ausfällung, usw. in Gegenwart von Maskierungsreagenzien.

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THE DETERMINATION OF STRONTIUM IN COAL ASH BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

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Strontium oxide is present in the ash of some coals and also in the fouling deposits which occur in the flues of by-product coke ovens. The strontium oxide content of certain coal ashes has been reported as: England 0.00–0.03%[1], West Virginia 0.08–1.9%[2], New South Wales 0.01–0.9%[3] and Antartica 0.04–0.2%[4], while the values reported for the lithosphere are 0.03%[5] and 0.018%[6].

The methods which have been proposed for the determination of minor concentrations of strontium include emission spectrography[1,7], flame-emission spectrophotometry[8–12], X-ray fluorescence[13] and atomic absorption spectrophotometry[14]. Flame-emission techniques have required either the separation of interfering elements as hydroxides[11] or preliminary separation of the strontium as the oxalate[10] or the use of releasing agents[9,11]. The flame-emission determination of strontium was examined by the present authors and lanthanum was found to be a satisfactory releasing agent. However, the composition of the calibration series had to be matched carefully with the composition of the samples being analysed, because spectral interferences were caused by sodium, potassium and calcium.

The use of atomic absorption spectrophotometry for analytical determinations was proposed by WALSH[15] who indicated that the technique should be free from spectral interference effects. DAVID[14] has used the atomic absorption spectrophotometric technique for the determination of strontium in soils and plants, but it was found necessary to use an anion-exchange separation to eliminate phosphate interference; however, interfering cations were not removed and the use of a standard addition technique was necessary.

A preliminary investigation in these laboratories indicated that strontium in coal ash could be determined by atomic absorption spectrophotometry, that preliminary separations were not required and that the interference of aluminium, phosphate and calcium could be suppressed by the use of lanthanum ions. DAVID[14] reported a sensivity of 0.05 p.p.m. (1% absorption) and the present authors found a sensitivity of 0.04 p.p.m. (1% absorption).

EXPERIMENTAL

The atomic absorption spectrophotometer used in this laboratory is based on the monochromator of a Hilger large quartz E492 spectrograph, a modulated strontium

hollow-cathode lamp as source, and a photomultiplier and amplifier[16] unit for detection. The low noise characteristics of the strontium lamp at the 4607 Å resonance line[17] permitted the measurement of the absorbances on an expanded scale[18].

The useful range for the determination of strontium in New South Wales coal ashes was 0.01%–0.40% strontium; 90% of the reported values fall within this range[3].

A reproducibility of ±0.01% Sr was required and adequate sensitivity was obtained by atomizing a solution containing 100 mg of sample per 100 ml into a 10-cm air-acetylene flame path, while operating the strontium hollow-cathode lamp with a current of 15 mA.

Aluminium and phosphate were found to interfere; for example, with the conditions described above and solutions which contained 2% (v/v) hydrochloric acid and 4 p.p.m. of strontium, 300 p.p.m. of aluminium caused an 80% suppression of the absorbance by strontium, and 100 p.p.m. of phosphate caused a 10% enhancement of the absorbance by strontium. Other workers[12,19,20] have suggested that lanthanum is a useful suppressing agent for this type of interference, and Fig. 1 shows the effect of varying lanthanum concentrations on the absorbance of test solutions containing 2% (v/v) hydrochloric acid, 2.0 p.p.m. or 4.0 p.p.m. strontium and 300 p.p.m. of aluminium.

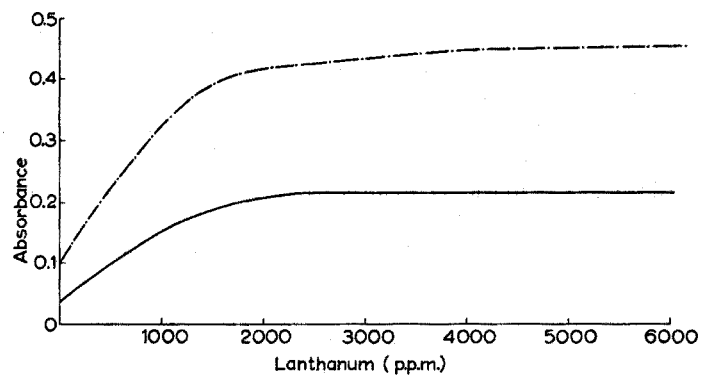

Fig. 1. The influence of lanthanum on the interference effects of aluminium. . — . — . 300 p.p.m. Al, 4.0 p.p.m. Sr; ——— 300 p.p.m. Al, 2.0 p.p.m. Sr.

A concentration of 2000 p.p.m. of lanthanum was found to be sufficient to suppress all interference from aluminium concentrations up to 300 p.p.m. Calibration test solutions which contained 2% (v/v) hydrochloric acid and 2000 p.p.m. of lanthanum were then prepared to cover the range 0.0–4.0 p.p.m. strontium. The plot of absorbance against strontium concentration was found to be linear. The highest absorbances were obtained with a rich flame, the air consumption being 10.4 l/min (S.T.P.) and the acetylene consumption being 2.6 l/min (S.T.P.).

An interference study was carried out at three strontium concentrations, namely 0.0, 2.0 and 4.0 p.p.m., under conditions similar to those used in the calibration series described above. As a reproducibility of ±0.1 p.p.m. Sr was required, any interference less than ±0.05 p.p.m. Sr was considered to be insignificant. No significant interference was encountered from 100, 200 and 300 p.p.m. Al^{3+}, 50 p.p.m. PO_4^{3-}, 100 p.p.m. Al^{3+} and 10 p.p.m. PO_4^{3-}, 200 p.p.m. Al^{3+} and 10 p.p.m. PO_4^{3-}, 300 p.p.m. Al^{3+} and 10 p.p.m. PO_4^{3-}, 20 p.p.m. Na^+, 20 p.p.m. K^+, 100 p.p.m. Fe^{3+}, 100 p.p.m. Ca^{2+},

100 p.p.m. Mg^{2+}, 2 p.p.m. Mn^{2+}, 20 p.p.m. Ti^{4+} and 90 p.p.m. SO_4^{2-} or from 50% variations in the hydrochloric acid concentration. This interference study showed that a method based on the conditions used above would be suitable for the determinations of strontium oxide in coal ash. This same solution of sample can be used to determine sodium, potassium, calcium and magnesium using flame-emission or atomic absorption spectrophotometry[21-23].

Reagents

AnalaR hydrochloric acid (s.g. 1.16) and hydrofluoric acid (s.g. 1.13), and Baker Analyzed perchloric acid (s.g. 1.70) were used.

Lanthanum solution. To 117 g of lanthanum oxide (BDH LR) and 100 ml of water, slowly add 300 ml of hydrochloric acid; boil to dissolve, cool and dilute to 1 l. EDTA titration showed the purity to be 99.5% (1 ml ≡ 100 mg lanthanum).

Strontium solution. Dissolve 1.6848 g Baker Analyzed strontium carbonate (dried at 120° for 2 h) in 100 ml of 10% (v/v) hydrochloric acid; boil to remove carbon dioxide and dilute to 1 l (1 ml ≡ 1 mg strontium).

Recommended procedure

Transfer 100 mg of 72 BS mesh coal ash to a 30-ml platinum crucible. Add 5 ml of hydrofluoric acid and heat to effect solution. Add 4 ml of perchloric acid, fume for 2 min, cool and wash down with a few drops of hydrochloric acid. Fume until salts begin to separate, cool, add 20 ml of 5% (v/v) hydrochloric acid and warm to obtain complete dissolution. Transfer to a 100-ml volumetric flask, add 2.0 ml of lanthanum solution and dilute to the mark. For calibration purposes, prepare similar solutions containing known amounts of strontium. Measure the absorbance by atomizing the solution in a 10-cm burner using a rich air–acetylene flame. The strontium content of the test samples is then read directly from the calibration graph.

RESULTS

Some New South Wales coal ashes together with a mixture prepared from NBS Argillaceous Limestone and spectrographically pure alumina, were analyzed by the proposed method and by flame-emission spectrophotometry. The compositions of the ashes studied were 14%–36% Al_2O_3, 0.09%–10.4% CaO, 0.04%–1.4% TiO_2, 0.2%–11% Fe_2O_3, 0.1%–3.5% MgO, 0.1%–1.0% Na_2O, 0.2%–1.0% K_2O and 0.1%–2.5% P_2O_5. The results are shown in Table I.

TABLE I

THE DETERMINATION OF STRONTIUM OXIDE IN SOME NEW SOUTH WALES COAL ASHES

Seam	*Colliery*	% SrO	
		Atomic absorption spectrophotometry	*Flame-emission spectrophotometry*
Bulli Bottom	Metropolitan	0.12	0.13
Bulli Top	Metropolitan	0.22	0.25
Victoria Tunnel	Burwood	0.02	0.02
Wongawilli	Berrima	0.00	0.00
NBS 1a (amended. cert. value 0.23%)		0.21	0.21

Appreciation is expressed to W. F. PICKERING for helpful discussion and to the Broken Hill Proprietary Co. Ltd. for permission to publish this work.

SUMMARY

An atomic absorption spectrophotometric method for the determination of 0.01%–0.40% strontium in coal ashes is proposed. After suitable dissolution of the sample, 2000 p.p.m. of lanthanum are added to overcome interferences before the solution is atomized. The results obtained were compared with those obtained by flame-emission spectrophotometry. The method is rapid and no preliminary separations are necessary.

RÉSUMÉ

Les auteurs décrivent une nouvelle méthode pour le dosage spectrophotométrique du strontium par absorption atomique, dans les cendres de houille (teneurs de 0.01 à 0.4%). Après dissolution appropriée de l'échantillon, on ajouts 2000 p.p.m. de lanthane. La solution est ensuite atomisée dans appareil pour spectrophotométrie par absorption atomique. Les résultats obtenus sont comparés avec ceux obtenus par spectrophotométrie de flamme. La méthode est rapide et aucune séparation préalable n'est nécessaire.

ZUSAMMENFASSUNG

Zur Bestimmung von 0.01–0.40% Strontium in Kohlenasche wird die atomare Absorptionsspektroskopie vorgeschlagen. Um Interferenzen zu unterdrücken, fügt man der Probe nach geeigneter Auflösung 2000 p.p.m. Lanthan hinzu. Die Ergebnisse wurden mit denen, die mit der Flammenspektrometrie erhalten wurden, verglichen. Die methode ist schnell und hat den Vorteil, dass eine vorherige Trennung nich erforderlich ist.

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THE DETERMINATION OF SODIUM IN PURE LIMESTONES BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

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The flame-photometric determination of sodium in the presence of large quantities of calcium is difficult because the orange molecular band system of calcium interferes with the sodium doublet, 589.0 and 589.6 mμ (Fig. 1). The interference depends on the type of flame used as well as on the spectral band width measured by the spectrophotometer. The use of light filters is not satisfactory, and with interference filters, the reported specificity factors vary between 100 and 600. When monochromators with a spectral band width of 4.5 mμ are used, the specificity factor is 380 for an acetylene–air flame and 1100 for a town gas–air flame[1]. The town gas flame has a lower temperature which favours the sodium emission, whereas the acetylene flame favours the calcium emission. We found experimentally that the emission of the CaOH band at 601 mμ is about ten times stronger in an acetylene flame than in a town gas flame.

Although the emission of the calcium band may be effectively suppressed by the addition of phosphates, aluminium salts or other substances[2] (which usually contain some sodium impurities), it is evident from the reported results that sodium contents of less than 0.001% in calcium salts cannot be determined without a prior chemical separation or unless the spectra are recorded using a monochromator to correct for interferences.

The main advantage of atomic absorption spectrophotometry is its freedom from radiative interferences even with monochromators of small resolving power. This is because the band width actually measured is not given by the slit width, but by the line width of the light source used. The determination of sodium in calcium salts by atomic absorption spectrophotometry therefore seemed very promising.

EXPERIMENTAL

Apparatus

A Zeiss Spiegel monochromator with glass prism, and an atomizer, spray chamber and town gas–air burner belonging to the Zeiss Model III flame photometer were used. The burner, which was 2.5 cm long, was adjusted along the optical axis. The air flow was about 6 l/min producing an atomization rate of 8 ml/min, of which only about 0.3 ml actually entered the flame. Compared with other atomizers this is a relatively low yield[3]. On the other hand, very concentrated solutions could be sprayed without danger of clogging the atomizer.

A sodium spectral lamp was used as a light source and run at 1.4 A with a stabilized alternating current. A FEU 18 photomultiplier was placed in the exit slit of the mono-

chromator. Although the light from the lamp was interrupted with a frequency of 100 c/s and so was the signal from the multiplier, only the effective direct current signal was measured with a scale galvanometer or a MAW recorder with a cathode tracer. The light emitted from the flame made the measurement of high sodium contents impossible.

Interferences

The effects of calcium, potassium and hydrochloric acid were studied. As expected, hydrochloric acid and chloride ions generally decreased the absorbance, *i.e.* decreased the slope of the working curves. This is evidently due to a shift in the dissociation equilibrium. The effects of calcium and potassium were therefore studied at a constant concentration of chloride in the solution. It was found, in agreement with ROBINSON[4], that up to 3000 p.p.m. of potassium had no effect on the working curves.

On the other hand, calcium, the effect of which was studied up to 60000 p.p.m., markedly lowered the slope of the working curves. The feeding rate of the atomizer was checked and found to be unaffected. The decrease can be explained by supposing that the solid salt particles are only partially evaporated and so liberate only a fraction of the sodium atoms present. The evaporated fraction diminishes as the volume of the salt particles increases with increasing concentrations of calcium chloride. This explanation is supported by the fact that the influence of calcium salts is suppressed when an acetylene–air flame with a higher temperature is used (Fig. 2).

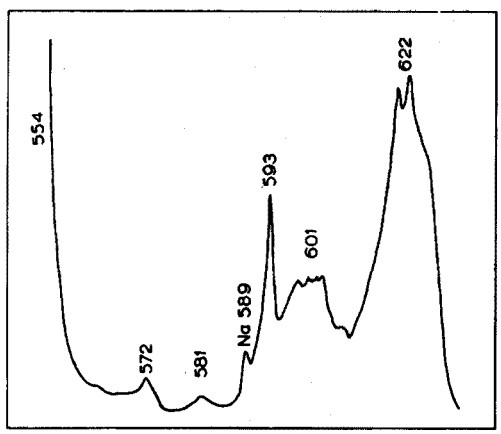

Fig. 1. Flame-emission spectrum of calcium containing 0.012% of sodium. Acetylene–air flame.

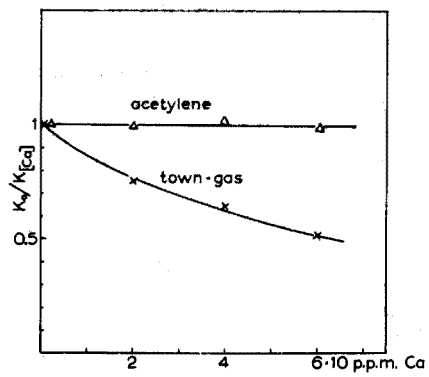

Fig. 2. The relative value of the slope of working curves for different calcium concentrations inthe solution for an acetylene–air flame and for a town gas–air flame.

According to the Langmuir formula, the evaporation rate is proportional to the diameter of the particle[5], hence the decrease in the absorbance in a town gas flame should be proportional to the cubic root of the calcium concentration ($[Ca]^{\frac{1}{3}}$). Experimentally, it was found that the slope of the working curves for different calcium con-

centrations was approximately proportional to [Ca‡]. This difference is perhaps due to the fact that the aerosol is far from being monodisperse.

The radiative interference of calcium

The addition of calcium salts not only decreases the slope of the working curves, but also shifts the curves a little upwards. It is difficult to decide whether the additional absorbance is due to the CaOH band or to sodium impurities. We found that a sample of white Carrara marble was the purest calcium salt available in sufficient quantities. The sodium content of this marble as determined spectrographically by an addition method was 0.0006%, which agrees fairly well with the content found by recording the emission flame spectra and correcting for the CaOH band emission (Fig. 3).

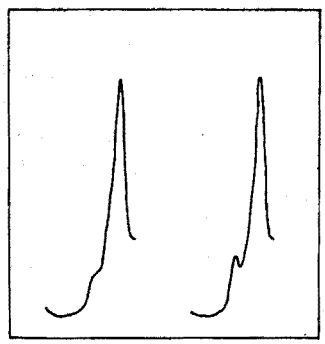

Fig. 3. Recording of a part of the CaOH band in a town gas–air flame before and after adding 0.0008% sodium. Assuming a linear relationship between the area and the concentration of sodium, the original content should be 0.00055% sodium in calcium carbonate.

The same value for the sodium content was found by linear extrapolation of the working curves for atomic absorption to the negative values of the concentration axis (Fig. 4). Thus it seems that the contribution of the CaOH band to the absorbance is so small that it can be neglected.

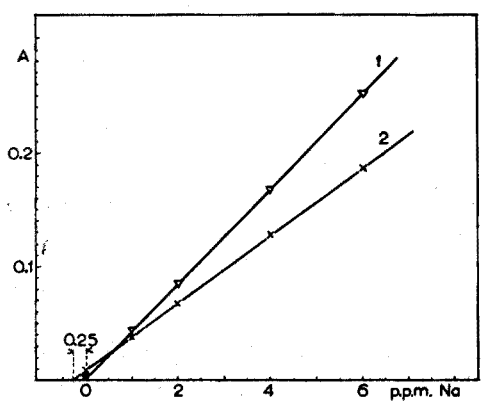

Fig. 4. Working curves for sodium without calcium and containing 20,000 p.p.m. calcium. The estimated content of 0.25 p.p.m. sodium corresponds to 0.0005% sodium in calcium carbonate.

This difference from the behaviour of the emission spectra can be explained not only by the narrowness of the measured spectral band width, but also by the fact that the intensity of the CaOH bands emitted in the flame is greater than would be expected from the equilibrium concentration of CaOH radicals. According to JAMES AND SUGDEN[6], these particles are probably generated by collision directly in the excited state. No absorbance of the CaOH band was found by scanning the continuous spectrum of a filament lamp.

RECOMMENDED PROCEDURE

Carefully dissolve 5 g of the sample in 20 ml of redistilled azeotropic hydrochloric acid. Boil and filter the solution. The volume of the filtrate should be approximately 85 ml. Incinerate the undissolved residue and treat with 0.5 ml of perchloric acid and 1 ml of hydrofluoric acid. After evaporation, dissolve the residue in 3 ml of diluted (1 : 4) hydrochloric acid and add to the filtrate. Make the volume up to 100 ml. Atomize this solution into the flame. For the determination of very pure limestones, it is necessary to clean all glass and platinum crucibles with diluted hydrochloric acid immediately before use. The acids used should be tested for sodium.

The standards used must have the same concentration of chlorides and calcium as the analysed samples. If the calcium salt used for the preparation of these standards contains some sodium, this may be determined simply by linear extrapolation of the working curves to the negative values on the concentration axis.

The use of a more efficient atomizer producing aerosols with a smaller mean drop size would probably make the use of calcium salts for the preparation of standards unneccesary.

With the rather primitive apparatus used, a sensitivity of 0.15 p.p.m. sodium could easily be achieved; this corresponds to 0.0003% sodium in limestones. Increasing the sample weight (at least up to 5-fold) correspondingly decreased the limit of detection of sodium. The accuracy of the proposed method is difficult to establish because of the lack of standards or suitable alternative procedures. The relative deviation, calculated from the results of 10 replicate analyses in the concentration range 0.001–0.07% sodium was 4.7%.

SUMMARY

The determination of sodium in pure limestones by atomic absorption spectrophotometry is described. The interferences of chloride and calcium ions are discussed; chloride affects the dissociation equilibrium, and calcium lowers the evaporation rate of sodium, thus lowering the concentration of free sodium atoms in the flame. The sensitivity achieved with a rather rough apparatus is 0.15 p.p.m. sodium.

RÉSUMÉ

Une méthode spectrophotométrique par absorption atomique est décrite pour le dosage du sodium dans la chaux. Les auteurs ont examiné l'influence de Cl^- et de Ca^{2+}. Les chlorures affectent l'équilibre de dissociation; le calcium abaisse la vitesse d'évaporation du sodium, diminuant ainsi la concentration des atomes libres de sodium dans la flamme. La sensibilité obtenue à l'aide d'un appareillage simple est de 0.15 p.p.m. sodium.

ZUSAMMENFASSUNG

Die Bestimmung des Natriums in reinem Kalkstein mit Hilfe der atomaren Absorptionsspektroskopie wird beschrieben. Es werden die Störungen, die durch Chlorid- und Calcium-Ionen hervorgerufen werden, diskutiert. Die Chlorid-Ionen beeinflussen das Dissoziationsgleichgewicht, und die

Calcium-Ionen erniedrigen den Verdampfungsgrad des Natriums und verringern damit die Konzentration von freien Natriumatomen in der Flamme. Mit einer einfachen Apparatur betrug die Empfindlichkeit 0.15 p.p.m. Natrium.

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RAPID ACTIVATION ANALYSIS OF TRACE VANADIUM IN TISSUE USING 3.8-MINUTE VANADIUM-52

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The determination of trace quantities of vanadium has been handicapped by insensitive methods of analysis. Most of the recent methods are based on the formation of a color complex and quantitative spectrophotometric determination. Activation analysis, however, which holds great promise for trace analysis of biological systems,[1] offers a procedure which is sensitive to submicrogram amounts of vanadium. This is especially helpful in biological analysis where vanadium is present in amounts undetectable by standard procedures.

Because the facilities available at this laboratory permitted short irradiations in the Ford Nuclear Reactor of the University of Michigan, rapid chemical separation in the hot lab area, and continuous γ-ray spectral analysis with a 100-channel analyzer, considerable attention has been focused on short-lived radioisotopes. Utilizing activation analysis procedures with radiochemistry, BROWNLEE AND MEINKE[2] have determined the vanadium content in crude oils, while FUKAI AND MEINKE[3] analyzed ashes of marine organisms for their vanadium content. BENSON[4] has applied this technique to vanadium compounds pre-separated on chromatographic paper, but in doing so has acquired the problems of reagent blanks which are usually eliminated in activation analysis.

Spectroscopic investigations conducted by GUELBENZU *et al.*[5] placed the vanadium content of rat livers below the microgram level. The purpose of this investigation was to establish a lower limit for vanadium content in rat liver tissue or to determine it at the submicrogram level.

EXPERIMENTAL

Apparatus

Samples were irradiated in polyethylene screw-cap rabbits in the pneumatic tube system of the Ford Nuclear Reactor of the University of Michigan. This system permitted irradiations at thermal neutron fluxes of about 10^{12} n/cm^2/sec (when the reactor was operating at full power of 1 megawatt) and delivery to a hood in the neighboring Michigan Memorial Phoenix Laboratory within 3 sec after the end of irradiation. Samples were then worked up chemically and were measured by a 3″ × 3″ NaI(Tl) crystal coupled with a special 100-channel pulse-height analyzer with duplicate memories. This equipment has been described in detail elsewhere[6-8].

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Preparation of animals and tissue

Holtzman* albino male rats weighing between 350 and 400 g were used in this study. They were maintained on Rockland Rat Diet** and had free access to drinking water. The livers were surgically removed, weighed, allowed to air-dry at room temperature for 24 h and then reweighed. They were placed in envelopes prepared from 0.1 mm thick polyethylene film, which were closed by heat sealing. The sealed sample was then irradiated in a "rabbit" along with suitable monitoring foils for a period of 10 min at full power.

Radiochemical separation

While the sample was being irradiated a nickel crucible was prepared containing vanadium-48 tracer, 10 mg of vanadium carrier, and 10 mg of copper holdback carrier. Three sodium hydroxide pellets were added and the solution was heated almost to dryness. Two minutes before the end of the irradiation, 10 g of sodium peroxide were added to the crucible and melted.

The irradiated sample was then fused in this melt for 1 min (CAUTION). A cover must be used on the crucible since the reaction may be quite violent. The outside of the crucible was then cooled by dropping it into a beaker of cold water and the melt made to solidify in a thin readily dissolvable coating by manipulation of the crucible. The melt was then dissolved by immersion into 50 ml of water followed by the addition of 42 ml of concentrated hydrochloric acid. Ten grams of tartaric acid were added and H_2S gas was bubbled into the solution. The solution was filtered and 10 ml of cupferron solution (6% aqueous) were added to the filtrate. This filtrate was extracted with 10 ml of chloroform for 1 min. The organic layer was collected and counted with the 100-

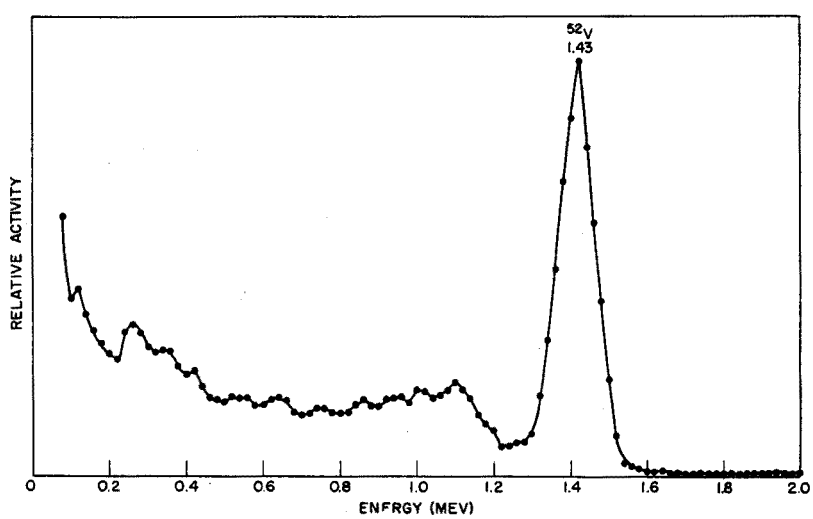

Fig. 1. γ-Ray spectrum of vanadium fraction separated from rat liver tissue (5 min after removal from reactor).

* Holtzman Company, Madison 4, Wisconsin.
** A product of Rockland Farms, New City, N.Y.; manufactured by A.E. Staley Manufacturing Company, Chicago 27, Illinois.

channel analyzer. The entire procedure could be completed in 5 min with an average vanadium recovery of about 40–45%.

Activity determination

Linearity of the measurement system was established by the use of ^{137}Cs and ^{60}Co standards. Spectra were obtained in the 0–2 MeV energy range as shown in Figs. 1 and 2. The amount of vanadium-52 was determined from the area under the 1.43 MeV photopeak, while the correction for chemical yield was made by measuring recovery of long-lived tracer vanadium-48 utilizing the 0.99 MeV photopeak after the shorter-lived peak had decayed out.

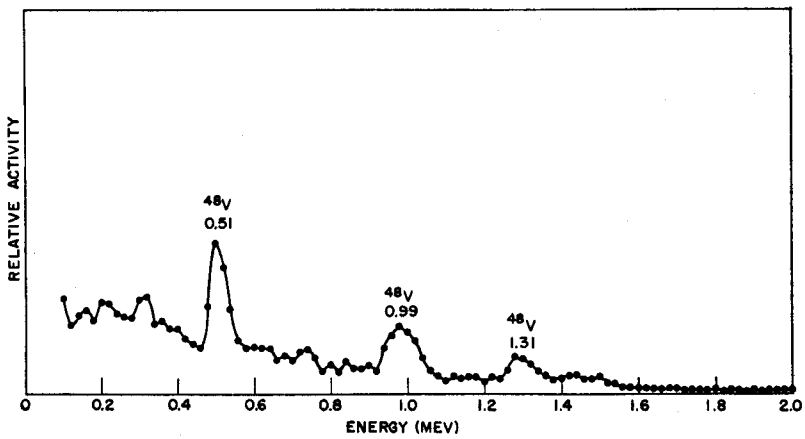

Fig. 2. γ-Ray spectrum of vanadium fraction separated from rat liver tissue (40 min after removal from reactor).

Monitoring procedures

Gold foils weighing between 0.2 and 0.3 mg were wrapped in plastic envelopes and taped to the inside of the cap of the rabbit. Following irradiation the foils were dissolved in 4 ml of aqua regia and diluted to 10 ml with distilled water. An aliquot of this solution was placed in a test tube and counted with a well-type scintillation counter. Comparison with other measurements made with calibrated gold foils permitted normalization of all irradiations to a neutron flux of $1 \cdot 10^{12}$.

RESULTS AND DISCUSSION

By this procedure, values for the normally occurring vanadium concentrations in rat liver tissue were obtained, as shown in Table I. Although these values show a variation of greater than a factor of two, they do establish an order of magnitude.

Most spectroscopic procedures are not sufficiently sensitive to detect these levels in complex biological systems. Utilization of activation analysis, *i.e.*, the short-lived vanadium-52, reduces the possibility of prolonged radioactive contamination, provides a sensitive analytical method and allows rapid analysis of numerous samples. The use of long-lived vanadium-48 for calculating chemical yield offers the added advantage of one procedure yielding two results, *e.g.*, amount of vanadium-52 and yield

TABLE I

ACTIVATION ANALYSIS FOR VANADIUM IN RAT LIVER TISSUE USING VANADIUM-52

Animal number	*Life weight (g)*	*Fresh liver weight (g)*	*Yield of separation procedure (%)*	*Vanadium found in livers 10^{-8} g/g fresh tissue*[a]
1	358.5	11.61	38.7	3.2
2	378.0	14.04	45.4	1.4
3	378.4	11.81	40.1	2.8
4	367.3	13.15	46.9	3.1
5	324.1	12.43	42.4	2.0

[a] Corrected to neutron flux of $1 \cdot 10^{12}$ n/cm²/sec.

correction from the isolated vanadium-48. Where possible it would be preferable to work with smaller samples because extreme care is required when a large irradiated sample is fused in sodium peroxide.

This work was supported in part by the Michigan Memorial Phoenix Project and the U.S. Atomic Energy Commission. Thanks are due to Professor H. J. GOMBERG, C. W. RICKER, and the staff of the Ford Nuclear Reactor for their help in making the irradiations.

SUMMARY

Submicrogram amounts of vanadium in rat liver tissue have been analyzed by rapid activation analysis. A 5-min radiochemical separation coupled with γ-ray spectrometry permitted utilization of the 3.8-min vanadium-52 radioisotope. With this procedure the lower limit of detection at a thermal neutron flux of 10^{12} n/cm²/sec was about $3 \cdot 10^{-9}$ g of vanadium.

RÉSUMÉ

De submicroquantités de vanadium ($3 \cdot 10^{-9}$ g) dans des tissus biologiques (foie de rat) ont pu être déterminées rapidement par activation. Une séparation radiochimique de 5 min, combinée avec une spectrométrie aux rayons-x, permet l'utilisation du radioisotope vanadium-52 (3.8 min).

ZUSAMMENFASSUNG

Vanadium wurde in Gewebe von Rattenlebern mit Hilfe der Neutronenaktivierungsanalyse bestimmt. Die Proben wurden kurze Zeit bestrahlt, innerhalb von 5 Minuten radiochemisch getrennt, und mit einem γ-Spektrometer die Aktivität des Vanadium-52 ($t_{1/2}$ = 3.8 min) bestimmt. Die Nachweisgrenze lag bei einem thermischen Neutronenfluss von 10^{12}n/cm²/sec bei $3 \cdot 10^{-9}$ g Vanadium.

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POLAROGRAPHIC DETERMINATION OF THALLIUM

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In practically all common supporting electrolytes thallium(I) ions produce a reversible well-defined wave at the dropping mercury electrode. The diffusion current is proportional to the concentration so that thallium may be determined polarographically in a wide range of concentrations. The position of the half-wave potential is very seldom affected by complex-forming substances, and most interfering elements may be screened by conversion to stable polarographically inactive complexes.

PŘIBIL *et al.* utilized the masking properties of EDTA and CDTA in the polarographic determination of thallium[1,2]. In the presence of EDTA, however, the wave due to bismuth coincides with that of thallium and interferes seriously in its determination. When CDTA is used as masking agent there is no interference from bismuth but the copper wave occurs at the same potential as that of thallium.

In some cases, interfering steps can be eliminated by so-called electrochemical masking[3-5] which is obtained by addition of certain surface-active substances to the supporting electrolyte. According to FUJINAGA AND IZUTZU[6], the copper–EDTA wave is completely suppressed in the presence of Triton X-100 and camphor, whereas the height of the thallium wave is not affected by these surface-active agents.

In a recent work, SHETTY *et al.*[7] utilized electrochemical masking of interfering elements in the determination of thallium. They recommend sodium triphosphate and 0.1% camphor as supporting electrolyte. In this medium the cathodic steps of most metal ions other than thallium are shifted to considerably more negative potentials. The method is very specific; even large amounts of copper, bismuth, lead and iron do not interfere. A great disadvantage of this method is, however, that the diffusion current of thallium is extremely dependent on pH and the concentration of the supporting electrolyte. When the pH is increased from 6 to 8, the diffusion current diminishes approximately 20%, and if the concentration of triphosphate is increased from 0.01 to 0.1 *M* the height of the wave decreases 36%. The authors claim, however, that quantitative determination of thallium is possible by the standard addition method.

In the course of a systematic investigation of the polarographic behaviour of the complexes formed by diethylenetriaminepentaacetic acid (DTPA) with several metal ions, we found that the cathodic waves of most metal–DTPA complexes are shifted to about −1.3 V *vs.* S.C.E. on addition of 0.01% Triton X-100. The present work was carried out in order to ascertain whether DTPA and Triton would be advantageous as masking agents in the polarographic determination of thallium.

EXPERIMENTAL

Reagents

Diethylenetriaminepentaacetic acid (DTPA) was obtained from Geigy Chemical Corp., New York. The commercial product was very pure and a 0.05 *M* stock solution was prepared by dissolving 19.667 g of DTPA and 6 g of sodium hydroxide in distilled water and diluting to 1 l. The molarity was checked by titration against dipyridylzinc thiocyanate as described by BUDĚŠINSKY[8]. Standard thallium(I) solution was prepared by dissolving the appropriate amount of thallium(I) nitrate (British Drug House Ltd.) in distilled water and standardized against potassium bromate following the procedure given by ZINTL AND RIENÄCKER[9]. Triton X-100, obtained from Rohm and Haas Co., Philadelphia, was used as surface-active agent. The remaining chemicals were of reagent grade and used without further purification.

Apparatus and technique

All polarograms were recorded with a Tast Polarograph, Selector D (Atlas Werken, Bremen, Germany). The conventional types of dropping mercury electrode (D.M.E.) and of electrolysis cell were used. The capillary characteristics measured in 0.1 *M* potassium nitrate (open circuit) at a corrected mercury height of 50.7 cm, were: $m = 2.998$ mg/sec and $t = 3.52$ sec. An external saturated calomel electrode (S.C.E.) served as reference electrode. 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. All experiments were performed at $25 \pm 0.1°$. The pH of the solutions was measured with a Beckman Zeromatic pH meter.

The reversibility of the electrode reaction was tested for each polarogram by determining the slopes of the curves of $\log i/(i_d - i)$ *vs.* the potential. Corrections were made for the residual current. Data for the plots were taken by manual operation of the polarograph, measuring the applied potential with a Hartman and Braun (No. 10018) potentiometer. Half-wave potentials were taken from the logarithmic plots and were reproducible to ± 1 mV.

RESULTS

Preliminary experiments indicated that thallium(I) in the presence of excess of DTPA gives a well-defined wave at the dropping mercury electrode, and that its half-wave potential is not affected by addition of 0.01% Triton X-100 to the supporting electrolyte.

The effect of pH on the diffusion current was investigated by recording the polarograms of 10^{-3} *M* thallium and 10^{-2} *M* DTPA using acetate, phosphate and ammonia buffers as supporting electrolytes. Triton X-100 (0.01%) was added to each solution and the pH was adjusted to the desired value by addition of potassium hydroxide or nitric acid. At pH values less than 5, the diffusion current was found to be independent of the pH of the supporting electrolyte. Above pH 5, however, the current decreased until it reached a constant value at pH 9. A further increase in pH had no influence on the diffusion current. The results, which are shown in Fig. 1, were perfectly reproducible. At a given pH value the diffusion current was constant and entirely independent of the base electrolyte used (*i.e.* acetate, phosphate or ammonia).

At pH values below 5 the half-wave potential was independent of the excess of DTPA present. The plots of $\log i(i_d - i)$ *vs.* the potentials gave straight lines and the

slopes indicated that the electrode reaction involves a reversible one-electron reduction. The observed diffusion current constant, $I = 2.68$, and the half-wave potential, $E_{1/2} = -0.460$ V *vs.* S.C.E., correspond exactly to the values obtained for thallium(I) in 0.1 *M* potassium nitrate[10], indicating that the cathodic wave below pH 5 is due to the reduction of simple thallium(I) ions.

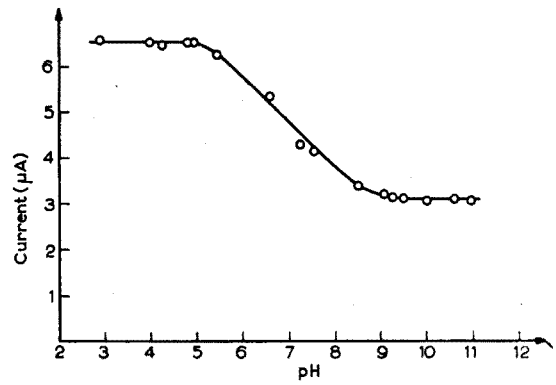

Fig. 1. Effect of pH on the wave height of thallium in the presence of excess of DTPA.

Above pH 9, the diffusion current was found to be independent of the excess of DTPA present, whereas the half-wave potential was shifted to more negative values with increasing concentrations of DTPA, indicating that a thallium–DTPA complex was formed in alkaline medium. The plots of log $i/(i_d - i)$ *vs.* the potentials indicated a reversible one-electron reduction of the complex. The plots of the values of $E_{1/2}$ *vs.* the corresponding concentrations of DTPA resulted in a straight line and the slope of the line indicated that only one ligand was coordinated in the complex. In the concentration range 0.5–10 m*M* DTPA, the half-wave potential may be expressed by the equation:

$$E_{1/2} = -0.729 - 0.060 \log C_x$$

where C_x denotes the concentration of the free DTPA ligand present.

The stability of the complex was calculated from the half-wave potentials. After appropriate correction for the ratio of the diffusion currents of the simple and complex thallium ions, the stability constant $pK_c = 4.21$ was found. The diffusion current constant in 0.2 *M* ammonia buffer, pH 9–11, with 0.01% Triton X-100 present, was $I = 1.23$.

The DTPA becomes protonated to varying degrees depending on the pH of the solution. In the pH range 5–9, the concentration of the free ligand available for complex formation increases with increasing pH and the cathodic waves obtained at these pH values are consequently due to reduction of simple thallium ions as well as of the complex. When the pH is increased from 5 to 9, the concentration of the Tl–DTPA complex increases at the expense of the simple thallium ions, and because of the great difference in the diffusion current constants, the height of the total wave decreases as indicated in Fig. 1.

Because the diffusion currents measured in the pH range 5–9 are extremely depend-

ent on the concentration of the free DTPA ligand present, thallium should not be determined within that range. Addition of 0.01–0.02% Triton X-100 has no effect on the thallium wave in acidic medium and the diffusion current only decreases slightly in alkaline medium. Given the great difference in the diffusion current constants of the thallium complex ($I = 1.23$) and thallium itself ($I = 2.68$) an acetate buffer of pH 4–5 would be advantageous as base electrolyte for the determination of thallium.

Interfering elements

Experiments showed that manganese, cobalt, nickel and zinc are polarographically inactive at all pH values in the presence of DTPA. In acetate buffer of pH 4–5, the half-wave potentials of the copper, lead, bismuth, cadmium and chromium complexes with DTPA are respectively −0.3, −0.8, −0.9, −1.0 and 1.2 V *vs.* S.C.E. When 0.01% Triton X-100 is added to the electrolyte, however, the electrode reactions are inhibited and all the waves are shifted to −1.3 V. Anions such as molybdate, vanadate and arsenite are also polarographically inactive in the presence of DTPA and Triton.

The cathodic wave of silver is not affected by the presence of DTPA and Triton and the half-wave potential of the iron(III)–DTPA complex ($E_{1/2} = 0.13$ V) is shifted only to −0.65 V *vs.* S.C.E. upon addition of Triton X-100. Very large amounts of silver and iron(III) consequently interfere in the determination of thallium and should be removed from the solution (before the addition of Triton) by controlled potential electrolysis at −0.25 V. Amounts of silver and iron less than or equal to the amount of thallium do not interfere.

Recommended procedure

The following procedure is suggested. Transfer 10 ml of the sample solution to the cell. Add 5 ml of 1 *M* acetate buffer of pH 4.6, 10 ml of 0.05 *M* DTPA and 0.01–0.2% Triton X-100, and remove dissolved air with oxygen-free nitrogen. Reord the polarogram from −0.2 to −0.8 V *vs.* S.C.E. and determine the concentration of thallium from a calibration graph.

TABLE I

DETERMINATION OF THALLIUM IN SYNTHETIC MIXTURES

Tl taken (mM)	*Added species (mM)*		*Tl found (mM)*	*Error (%)*
0.0510			0.0505	−1.0
0.1020			0.1024	+0.4
0.5100			0.5045	−1.0
1.020			1.020	0
0.0510		0.10 Cu	0.0510	0
0.0510		1.00 Cu	0.0506	−0.8
0.0510		0.10 Pb	0.0508	−0.4
0.0510		1.00 Pb	0.0512	+0.4
0.0510	0.05 Cu	0.05 Pb	0.0507	−0.6
0.0510	0.10 Cu,	0.10 Pb	0.0514	+0.8
0.0510	1.0 Cu,	1.0 Pb	0.0515	+1.0
0.0510	0.10 Cd,	0.10 Bi	0.0506	−0.8
0.0510	1.0 Cd,	1.0 Bi	0.0512	+0.4

In order to avoid too much foam during the deaeration (caused by the large amount of Triton) a minute amount of *n*-octyl alcohol should be added to the solution.

The results of a few determinations of thallium following the above procedure are reported in Table I.

DISCUSSION

The triphosphate base electrolyte recommended by SHETTY *et al.*[7] is the most specific method described in the literature for the polarographic determination of thallium. In order to avoid interference from copper and bismuth, however, it was necessary to adjust the pH of the electrolyte to 6–8, and in this pH range the height of the thallium wave is extremely dependent on pH and on the concentration of the base electrolyte. When DTPA is used as masking agent the interference from other metals is eliminated even in acidic solutions. It is thus possible to determine thallium at pH values where the diffusion current is not affected by the masking agents. The diffusion current constant of thallium in an acetate/DTPA supporting electrolyte is about twice as large as in triphosphate, hence the present method is also more accurate for the determination of small amounts of thallium.

SUMMARY

A polarographic method for the determination of thallium is described. The inclusion of DTPA and Triton X-100 in the supporting electrolyte shifts the waves of interfering metals to considerably more negative potentials while the thallium wave is completely unaffected. An acetate buffer of pH 4–5 is the preferred base electrolyte, because a Tl–DTPA complex is formed at higher pH values. The polarographic behaviour of the complex is described and the stability constant determined.

RÉSUMÉ

Une méthode polarographique est proposée pour le dosage du thallium. L'addition de DTPA (acide diéthylènetriaminopentacétique) et de triton X-100, à l'électrolyte de base, permet de déplacer les vagues des métaux gênants, alors que celle du thallium ne bouge pas. Solution de base: tampon acétique, pH 4–5. Le comportement polarographique du complexe est décrit et la constante de stabilité est déterminée.

ZUSAMMENFASSUNG

Es wird eine polarographische Methode zur Bestimmung des Thalliums beschrieben. Die Zugabe von DTPA und Triton X-100 zur Grundlösung verschiebt die Stufen störender Metalle zu beträchtlich negativeren Potentialen, während die Thalliumstufe unbeeinflusst bleibt. Als Grundlösung wird ein Acetat-Puffer mit dem pH-Wert 4–5 gewählt. Das polarographische Verhalten des Komplexes wird beschrieben und seine Stabilitätskonstant bestimmt.

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STRUCTURE OF THE ZIRCONIUM–ALIZARIN S COMPLEX IN RELATION TO pH CHANGES

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The reaction of fluoride ions with the colored complex of zirconium with alizarin S[1-7] was considered, after preliminary investigations, as the most appropriate spectrophotometric method for the determination of fluoride. However, before a satisfactory procedure for the determination could be devised, it was considered essential to make a thorough study of the zirconium–alizarin S reaction itself. A study of the experimental conditions under which the above reaction occurs gave the following results:

(*a*) The zirconium–alizarin S complex is entirely unstable in certain pH ranges.

(*b*) In other pH ranges small changes in the hydrogen ion concentration cause modification of the structure of the complex.

(*c*) The proper ratio of zirconium to alizarin S in the complex for the fluoride determination required further investigation.

The purpose of the present work was to establish the proper ratio of zirconium to alizarin S in the complex as well as to investigate the modifications which the complex undergoes even with small changes in pH.

EXPERIMENTAL

Reagents

All reagents were obtained from Merck (p. A. grade).

Zirconium solution. A 10^{-4} *M* solution of $ZrOCl_2 \cdot 8H_2O$ was prepared. The concentration of the solution was checked by gravimetric determination of zirconium as ZrO_2.

Alizarin S solution. A 10^{-4} *M* solution of sodium alizarin-3-sulfonate was prepared.

For the adjustment of pH, 1 : 1 mixtures of hydrochloric and sulfuric acids 0.3 *N*, 3 *N* and 10 *N*, and 0.1 and 1 *N* sodium hydroxide were used.

Apparatus

Spectrophotometric measurements were made with a Beckman DU spectrophotometer and 10-mm Corex cells. pH measurements were carried out with a "Radiometer 22" (Radiometer Copenhagen Co.) and a glass electrode 202 A. The filtration was carried out with platinum Neubauer Heraeus type filters.

Methods

For obtaining the curves of Fig. 1 the following procedure was used. The alizarin

S solution was adjusted to the desired pH value with a few drops of acid or alkali; the solution was left for 1 h in a thermostat at 25° and then the spectrophotometric measurements were made. For the Job curves, portions of the zirconium and alizarin S solutions were separately adjusted to the desired pH, within the range 0.5–6.0. From these solutions, x ml and $(1-x)$ ml were mixed to form the series. The mixtures were placed in a thermostat at 25° for 1 h and then measured spectrophotometrically.

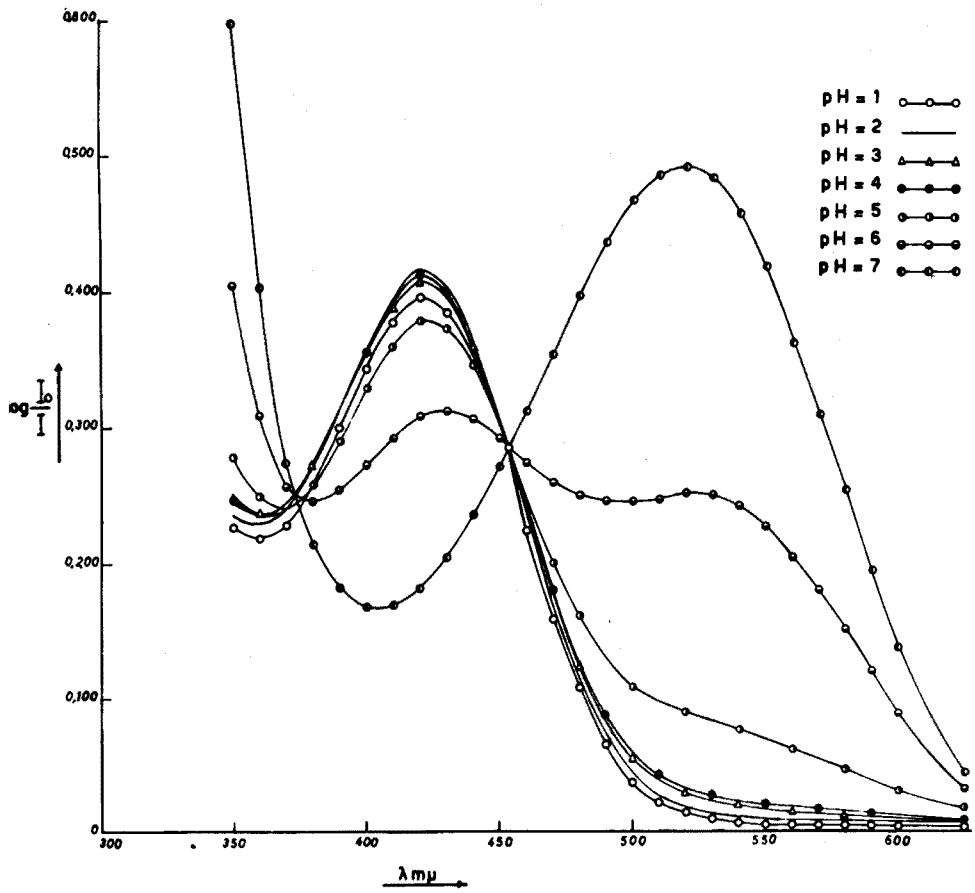


Fig. 1. Visible absorption spectra of alizarin S at different pH values.

These were the curves for the unfiltered samples. For the filtered series, the same procedure was followed except that the samples were filtered before spectrophotometry. The absence of suspensions was checked by the Tyndall effect.

RESULTS

The visible spectra (625–350 mμ) of the pure alizarin S solution (10^{-4} M) were examined over the pH range 1 to 7 (Fig. 1).

Between these pH limits the alizarin S color changes clearly from yellow to red. It can be seen from Fig. 1 that when the pH increases a shift of the maximum occurs gradually from 420 mμ to 520 mμ. This displacement of the maximum can be explained

as follows. At low pH values, alizarin S exists in the unionized yellow form (Fig. 2,a). As the pH increases, the alizarin S ionizes giving the mesomeric forms (b) and (c) which correspond to the α-phenoxyquinoid form[8] and are responsible for the red color of the alizarin S molecule.

The curves shown in Fig. 3 indicate that these mesomeric forms are also responsible for the formation of the colored complex of zirconium and alizarin S. These curves were obtained by leaving a mixture of zirconium and alizarin S at constant pH to

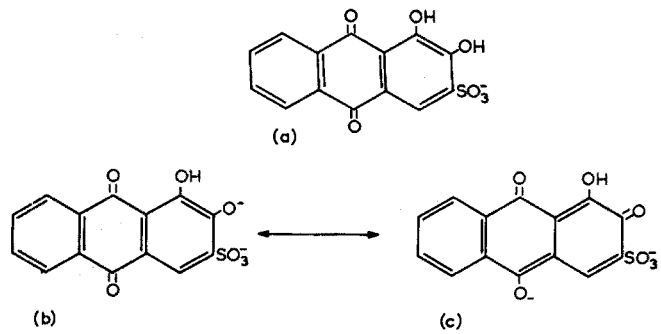


Fig. 2. (a) The unionized form of alizarin S; (b) and (c) ionized forms of (a).

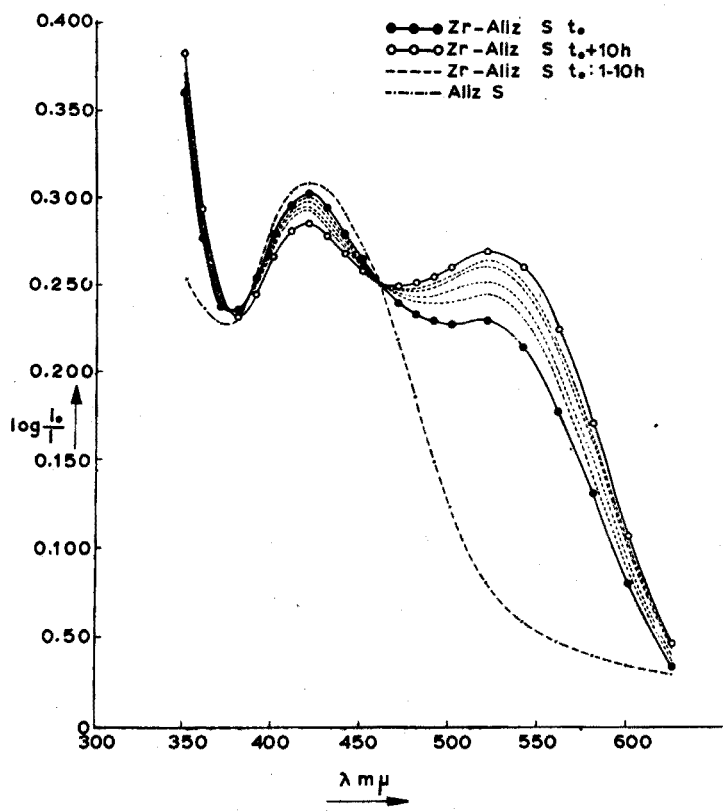
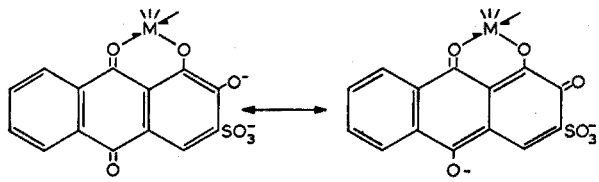


Fig. 3. Shift of the maximum absorbance of alizarin S after addition of zirconium ions.

develop for 10 h in a thermostat at 25°; the absorbance was measured every hour over the range 625–350 mμ. The first measurement (Fig. 3, curve t_0) was done 1 h after mixing the alizarin S solution with the zirconium solution. The absorption curve of alizarin S before the addition of the zirconium solution is also shown. The same shift of the maximum from 420 mμ to 520 mμ is observed with increasing time (Fig. 3, curve t_0 + 10 h) as in the case of increasing pH. Therefore, in accordance with the mesomeric forms shown in Fig. 2, the forms shown in Fig. 4 for the zirconium–alizarin S complex may be accepted.



(d) (e)

Fig. 4. Possible structures of the zirconium–alizarin S complex.

To investigate the stoichiometric ratio of the complex components, Job's method of continuous variations was applied. Measurements were made at 420 mμ and 520 mμ with pH changes from 0.5 to 6, in steps of 0.2 pH units in the range 0.5–2.0 and in steps of 1 pH unit in the range 2.0–6.0, with constant time intervals of 1 h between measurements at 25°. When the solutions of each series were prepared above a certain pH value, a suspension was observed in the solution[9]; this suspension coagulated and settled after several hours or after centrifugation, but even then the solution was not perfectly clear. Therefore, the optical densities were measured either on the solution of the complex of zirconium and alizarin S obtained after mixing the appropriate amounts of the components as Job's method requires, or after filtration of the solution through a porous platinum filter to remove the suspension formed. The results for the filtered series over the pH range 0.5–2.0 are shown in Fig. 5.

Table I shows the resulting proportions for each maximum or minimum (520 mμ and 420 mμ, respectively) for filtered or unfiltered solutions from pH 0.5 to 2.0.

DISCUSSION

From the curves shown in Fig. 5 and from Table I it is evident that the formation of the zirconium–alizarin S complex is measurable only in the pH range from 1 to 1.8. For pH values below 1, no complex formation could be observed. Between pH 1.8 and 3, the mixtures of zirconium and alizarin S formed suspensions which were checked by the Tyndall effect. Above pH 3, voluminous suspensions were formed and caused irregular measurements; for this reason the results in this pH region are not given in Table I.

Table I shows that from pH 1 to 1.8 the measurements in the filtered and unfiltered series were identical with regard to the ratio of zirconium to alizarin S. At pH 1.8 the proportionality in the Job series changed from 50:50 to 60:40 for both series. At pH 2 the measurements on the unfiltered series at 520 mμ showed a ratio of 60:40 and no result at 420 mμ; for the filtered series at 520 mμ no result was found, at 420 mμ the ratio was 60:40. The above facts can be explained as follows. Above pH 2,

insoluble hydroxy complexes of zirconium begin to be formed [10], and these complexes adsorb some of the zirconium–alizarin S complex, as well as some unreacted alizarin S. Therefore, for the unfiltered series, the difference between the optical densities of the

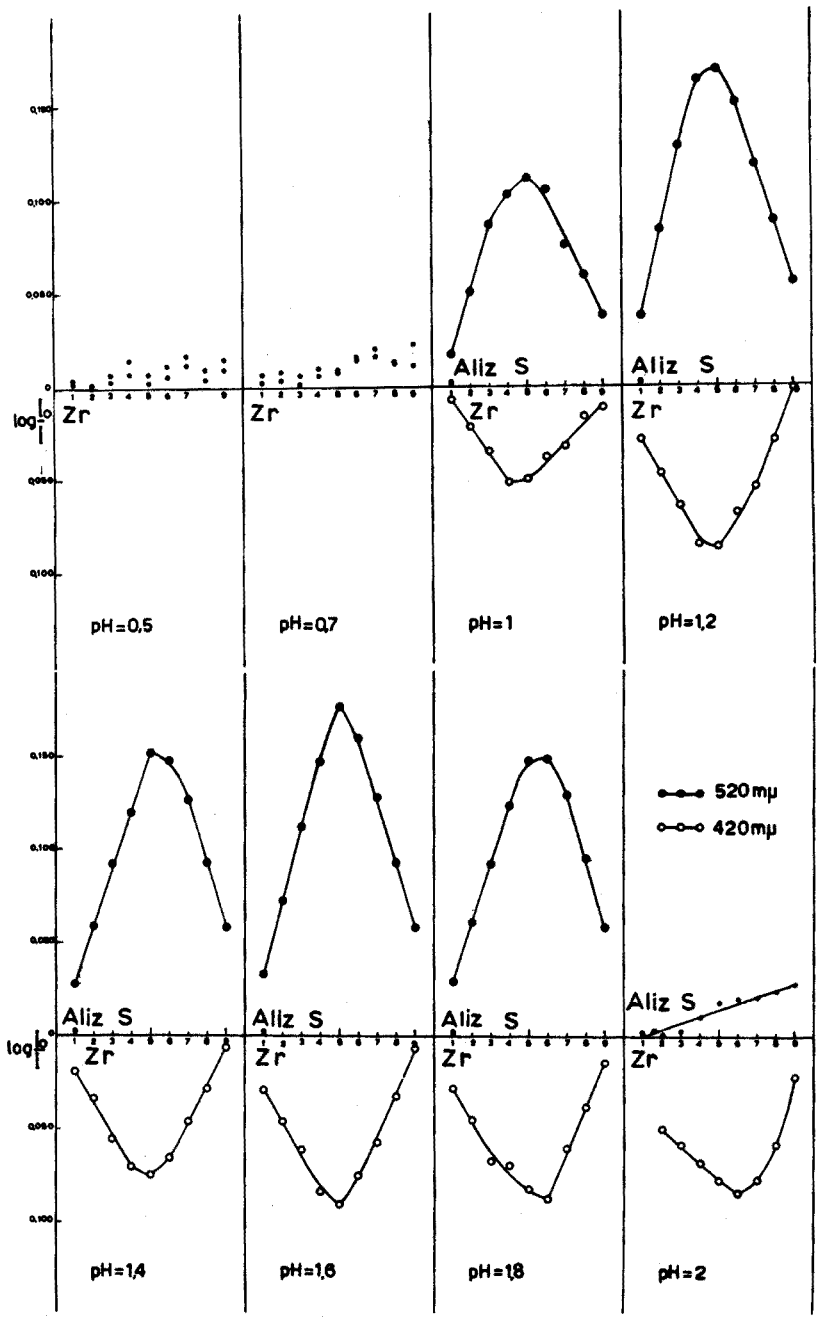


Fig. 5. Job's series for the zirconium–alizarin S compound at different pH values.

TABLE I

MOLAR RATIOS OF ZIRCONIUM TO ALIZARIN S IN pH RANGE 0.5–2

pH	*0.5*		*0.7*		*1*		*1.2*		*1.4*		*1.6*		*1.8*		2	
							Unfiltered series									
	Max.[a]	Min.[b]	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Zr : Aliz. S	(−)	(−)	(−)	(−)	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	60 : 40	60 : 40	60 : 40	(−)
							Filtered series									
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Zr : Aliz. S	(−)	(−)	(−)	(−)	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	50 : 50	60 : 40	60 : 40	(−)	60 : 40

[a] Max. = Measurements at 520 mμ.
[b] Min. = Measurements at 420 mμ.
(−) = Neither Max. nor Min. could be measured.

zirconium–alizarin S complex and the pure alizarin S is positive and a maximum is given at 520 mμ, whereas for the filtered series the values for the complex and the pure due are almost the same and cancel out. For the filtered series, owing to the diminution of the concentration of the solution with respect to the zirconium–alizarin S complex and the pure dye are quite similar at 520 mμ the difference in values is larger and therefore a minimum occurs. Thus only in the pH range 1 to 1.8 is it possible to measure a pure complex of zirconium and alizarin S in the ratio 1 : 1. Above a pH value of 1.8, some of the 1 : 1 complex is adsorbed on the surface of the suspended hydroxy complexes of zirconium.

SUMMARY

A comparison of the absorption spectra of the zirconium–alizarin S complex with those of alizarin S at different pH values shows that the action of hydrogen ions and zirconium(IV) on alizarin S is similar. Changes caused by pH alterations are discussed. Between pH 1 and 1.8 the zirconium–alizarin S complex has the molar ratio 1 : 1; at higher pH, suspensions of hydroxy complexes of zirconium are formed which adsorb the above complex.

RÉSUMÉ

Les auteurs ont effectué une étude sur la structure du complexe zirconium–alizarine S, en fonction du pH .Une comparaison des spectres s'absorption du complexe Zr–alizarine S avec ceux de l'alizarine S, à différents pH, montre que l'action des ions H^+ et ceux du zirconium sur l'alizarine S est similaire. Entre les valeurs du pH 1 et 1.8, le rapport Zr: alizarine dans le complexe est de 1 : 1. A des valeurs pH plus élevées, il y a formation d'une suspension d'hydroxo-complexes de zirconium qui absorbent le complexe Zr–alizarine S.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wird die Struktur des Zirkonium–Alizarin-S-Komplexes in Abhängigkeit vom pH-Wert untersucht. Ein Vergleich der Absorptionsspektren des Zirkonium–Alizarin-S-Komplexes mit dem Alizarin-S bei verschiedenen pH-Werten zeigt, dass der Einfluss der Wasserstoffionen und des Zirkoniums auf Alizarin-S ähnlich ist. Zwischen den pH-Werten 1 und 1.8 ist das Molverhältnis Zirkonium: Alizarin-S wie 1 : 1. Bei höheren pH-Werten werden Suspensionen von Hydroxo-Komplexen des Zirkoniums gebildet, die den o.a. Komplex absorbieren.

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STUDIEN ÜBER DIE NAPHTHORESORCIN-REAKTION VON TOLLENS

II. ZUR BESTIMMUNG VON FREIER URONSAURE NEBEN URONOSIDEN, POLYURONOSIDEN UND NEBEN ANDEREN KOHLENHYDRATEN

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Fast die Hälfte aller bekannten Polysaccharide enthält Uronsäure-Einheiten[1]. Diese sind aber auch Bestandteile natürlicher niedermolekularer Verbindungen, so mancher pflanzlicher Glykoside und Saponine, und vor allem der ausserordentlich wichtigen Gruppe der „Glukuronsäurepaarlinge", welche in den Körperflüssigkeiten der Säugetiere, besonders im Harn, aufgefunden werden.

Bis vor ungefähr einem Jahrzehnt war man allgemein der Ansicht, dass freie Uronsäuren in der Natur nicht vorkämen, nicht zuletzt deswegen, weil es an einer einfachen Nachweismöglichkeit derselben neben den gebundenen Uronsäuren mangelte. So berichten *z.B.* ARTZ UND OSMAN in ihrer Monographie[2a] über die Biochemie der Glukuronsäure: "Glucuronic acid is not known to occur free in nature". Neuere Arbeiten australischer Forscher[3] haben jedoch gezeigt, dass bei einer ganzen Reihe von Beuteltieren freie Glukuronsäure als normaler Harnbestandteil auftritt.

In einigen Fällen wurde auch ein Ausstoss von freier Glukuronsäure nach Gaben von Chemikalien beobachtet. Verabfolgt man *z.B.* Veronal an Ratten oder Meerschweinchen, so tritt im Harn dieser Tiere neben der unveränderten Substanz noch freie Glukuronsäure auf, bei den Ratten ausserdem in vermehrtem Umfang Askorbinsäure[4]. Nach Eingabe von Anilin, *o*-Anisidin, *p*-Anisidin, *p*-Phenetidin oder Phenacetin konnten aus dem Harn von Kaninchen neben verschiedenen Paarlingen ohne vorherige Hydrolyse *z.T.* beträchtliche Mengen freier Uronsäure als schwerlöslicher *p*-Toluidin-Ammoniumglukuronat-Komplex isoliert werden, wobei allerdings die Möglichkeit offenbleibt, dass es sich um ein äusserst labiles Glukuronid gehandelt hat[5].

Unkonjugierte Glukuronsäure erscheint im menschlichen oder tierischen Harn, wenn sie peroral oder parenteral in grösseren Mengen verabreicht wird. Solche Versuche dienen einmal zur Aufhellung des Schicksals der Uronsäure im Organismus[6-11], zum anderen stellen sie ein Hilfsmittel in der Differentialdiagnose von Leberkrankheiten dar[9,12,13] (sog. „Leberfunktionsprobe"). Berichte amerikanischer Autoren[14,15], wonach sich Gaben von Glukuron per os über längere Zeiträume hinweg bei rheumatischen Erkrankungen günstig ausgewirkt hätten, konnten von anderer Seite nicht bestätigt werden[9,11,16]. Dagegen sprechen viele Fälle von Hyper-bilirubinämie und Erythroblastose der Neugeborenen auf eine Behandlung mit Glukuron günstig an[17].

Das Auftreten von freier Galakturonsäure ist im Pflanzenreich zu erwarten, wo sie den wesentlichsten Baustein der Pektinstoffe in Rüben, Stengeln, Früchten, *usw.* bildet. Sie scheint sich jedoch unter natürlichen Bedingungen nirgends in grösseren Mengen anzuhäufen[18], sodass ihr Vorkommen lange Zeit umstritten war[19].

All diese teils natürlichen, teils unter bestimmten Versuchsbedingungen erzielten Vorkommen von freien Uronsäuren neben solchen im gebundenen Zustand und neben anderen Zuckern lassen ein Verfahren wünschenswert erscheinen, das die Erkennung und Bestimmung der freien Säuren in den genannten Fällen gestattet.

DIE GRUNDLAGEN DES NEUEN QUANTITATIVEN VERFAHRENS

Wahl der Säure

Den Ausführungen in Teil I dieser Studien[20] ist zu entnehmen, dass die Mineralsäure bei der TOLLENSschen Naphthoresorcin-(NR)-Reaktion eine erwünschte Doppelrolle spielt (*1* und *2*) zu der sich unvorteilhafterweise noch eine dritte Funktion (*3*) gesellt, nämlich: (*1*) Katalyse der hydrolytischen Spaltung von Oligo- und Polyuronosiden; (*2*) Kondensation von Uronsäure und NR zum Farbstoff; (*3*) Decarboxylierung der freigesetzten Uronsäure-Einheiten bei höherer Temperatur.

Unsere Untersuchungen gingen nun von folgender Überlegung aus: In der bislang ausschliesslich verwendeten mehr oder weniger starken Salzsäure bzw. Schwefelsäure sind die drei genannten Funktionen untrennbar verbunden; sie könnten jedoch bei anderen Säuren als nicht notwendig voneinander abhängige Eigenschaften durchaus in unterschiedlichem Masse vertreten sein. Diese Vermutung liess sich in mannigfachen Versuchen zunächst nicht bestätigen; endlich hat sich aber gezeigt, dass die Mineralsäure hinsichtlich Funktion (*2*) in geradezu idealer Weise durch *conc. Phosphorsäure* ersetzt werden kann, wenn zugleich der Wassergehalt des Reaktionsmediums durch wassermischbare organische Zusätze passend eingestellt wird. Die Kondensation geht dann so glatt vonstatten, dass selbst bei verhältnismässig tiefer Temperatur (70°) kurze Reaktionszeiten möglich sind. Unter diesen milden Bedingungen ist CO_2-Abspaltung nicht zu befürchten; eine bei allen bisherigen Verfahren gegebene Fehlerquelle (*vgl.*[20]) wird somit ausgeschaltet. Da die hydrolytische Aktivität der Phosphorsäure nur einen Bruchteil von derjenigen einer Mineralsäure beträgt, gelingt es nunmehr, mit Hilfe der NR-Reaktion freie Uronsäure neben bestimmten Uronosiden quantitativ zu erfassen.

Wahl des organischen Zusatzes

Nachdem bei der Mehrzahl aller bisher ausgearbeiteten quantitativen Verfahren keine maximale Farbstoffbildung angestrebt wird und Nebenreaktionen verschiedener Art eine beträchtliche Rolle spielen, ist zur Erzielung brauchbarer Werte die Zugabe einer genau bemessenen NR-Menge unerlässlich. Zur Dosierung kommt praktisch nur eine NR-Lösung in Betracht, wofür als Lösungsmittel bislang meist Wasser, vereinzelt auch Alkohol oder Aceton Verwendung fand. Die damit verbundenen Nachteile und Fehlerquellen wurden bereits geschildert[20].

Wir haben als Lösungsmittel für das NR reinsten *Eisessig* gewählt, der gegenüber den anderen Solventien ein relativ stabiles Reagens liefert. Zwar machen sich hier ebenfalls Alterungsvorgänge bemerkbar; sie verlaufen jedoch sehr viel langsamer als in der wässrigen Lösung, und die gebildeten Farbstoffe sind auch qualitativ verschie-

den[20]. Die Verwendung von Eisessig hat ferner zur Folge, dass der Wasseranteil des Reaktionsgemisches in weiten Grenzen beliebig eingestellt, vor allem stark herabgedrückt werden kann, was die Kondensation unter dem Einfluss der Säure ausserordentlich erleichtert. Das Verhältnis von 1 Teil Wasser auf 7 Teile Reaktionsgemisch (≡ 14.3% Wassergehalt) hat sich gut bewährt. Erst auf diese Weise wurde es möglich, selbst unter Einsatz der mittelstarken Phosphorsäure eine Reaktionstemperatur von 70° und eine Reaktionszeit von 75 Min (Glukuronsäure) bzw. 30 Min (Galakturonsäure) einzuhalten. Ein weiterer wesentlicher Vorteil liegt darin, dass in der überwiegend aus Eisessig bestehenden Phase die gebildeten Farbstoffe sowie die Produkte der Nebenreaktionen sämtlich in Lösung bleiben, während sie bei allen bisherigen, stets im wässrigen System arbeitenden Verfahren ganz oder doch zum grössten Teil abgeschieden werden. Damit entfallen von vorneherein alle Analysenfehler, die GRAUER UND NEUBERG[21] durch „Adsorption des NR-Pigmentes an typische Verunreinigungen" sowie durch Verharzung des Farbstoffes erklären[20]. Auch die glatte Extraktion mit Toluol ist auf das Vorliegen von *gelösten* Reaktionsprodukten zurückzuführen.

Naphthoresorcin-Konzentration

Der Mindestgehalt des Reaktionsgemisches an NR wird durch drei verschiedenartige Umstände bestimmt, nämlich: (*1*) durch die gegenseitige Abhängigkeit von (a) Reaktionstemperatur, (b) Dauer des Erhitzens und (c) NR-Konzentration in dem Sinne, dass für die Erzielung eines vorgegebenen Ausfärbungsgrades diese Variablen nicht beliebig wählbar sind. (*2*) Weiter ist zu berücksichtigen, dass, wie schon ausgeführt, das NR infolge verschiedener Nebenreaktionen der gewünschten Umsetzung entzogen werden kann. (*3*) Ausserdem macht sich bei Reihenanalysen noch der verhältnismässig hohe Preis des reinen Präparates geltend.

Die Wahl der NR-Konzentration richtet sich nun nach den jeweiligen Erfordernissen. Nachdem es uns zur Verringerung der hydrolytischen Freisetzung von gebundenen Uronsäuren auf möglichst niedrige Temperatur und kurze Erhitzungszeit ankam, haben wir die NR-Menge als abhängige Variable behandelt. 4 ml einer 0.2%-igen Lösung erwiesen sich unter den gewählten Reaktionsbedingungen als ausreichend. Das bedeutet gegenüber der quantitativ erfassbaren Höchstmenge von 100 μg Uronsäure einen ungefähr 20-fachen Reagensüberschuss, der selbst beträchtliche Verluste durch Nebenreaktionen ausgleichen dürfte. Auch der NR-Verbrauch hält sich in mässigen Grenzen.

Uronsäuregehalt

Der Gehalt der Probelösung an Uronsäure soll für die quantitative Bestimmung zwischen 20 und 100 μg pro ml liegen. Konzentriertere Lösungen sind entsprechend zu verdünnen, verdünntere unter geeigneten Vorsichtsmassnahmen anzureichern.

Regelung der Reaktionstemperatur

Die NR-Reaktion gehorcht im allgemeinen der VAN 'T HOFFschen Regel: Solange nicht das Gebiet maximaler Farbstoffbildung erreicht und überschüssiges NR zugegen ist, steigt unter sonst gleichen Bedingungen die Extinktion für je 10° Temperaturerhöhung auf etwa das Doppelte. Daraus erhellt, dass der räumlichen und zeitlichen

Konstanthaltung der Temperatur während des Erhitzens ganz besondere Sorgfalt gewidmet werden muss, wenn man vergleichbare Werte erhalten will. Für die quantitative Bestimmung ist ein guter Thermostat unbedingte Voraussetzung; er kann mit Wasser gefüllt werden, da bis etwa 80° die Verdampfungsverluste in erträglichen Grenzen bleiben.

Konstanthaltung des Volumens

Schwer verständlich erscheint es, dass bei fast allen bislang angegebenen Bestimmungsverfahren auf die Konstanthaltung der Masse des Reaktionsgemisches während des Erhitzens nicht geachtet wurde. Selbst die sorgfältige Studie von FISHMAN UND GREEN[22] macht hier keine Ausnahme.

Nach unseren Erfahrungen ergeben sich in offenen Gefässen unterschiedliche, *z.T.* beträchtliche Verdampfungsverluste (zumal beim Arbeiten im siedenden Wasserbad), wodurch die Ergebnisse stark verfälscht werden können. Nachdem der Dampfdruck unseres Reaktionsgemisches bei 70° nur gering ist, lässt sich durch Verschluss der Reagensgläser mit einem Gummistopfen die Verdampfung der niedriger siedenden Anteile völlig verhindern.

Wahl des Extraktionsmittels

Obwohl NEUBERG UND SANEYOSHI schon 1911 zum Ausschüttelen des Uronsäurefarbstoffes statt Äther das weitaus selektiver wirkende Benzol empfohlen haben, wurde in der Folgezeit noch vielfach nach der ursprünglichen Angabe von TOLLENS gearbeitet. In der quantitativen Methodik hat sich die Benzolextraktion überhaupt nicht eingebürgert. Der Grund ist wohl darin zu suchen, dass mit Benzol aus dem wässrig-mineralsauren Gemisch offenbar kein quantitativer Auszug des Farbstoffes zu erhalten ist, sei es durch Hemmung des Phasenüberganges, sei es durch einen ungünstigen Verteilungsquotienten. Über vergebliche Versuche in dieser Hinsicht berichten KAPP[29] sowie RATISH UND BULLOWA[24]; nur MEYER *et al.*[25] empfehlen gerade umgekehrt die Verwendung von Benzol und finden Äther ganz unbrauchbar, wenn reine Uronsäuren bzw. deren Salze vorliegen. Allerdings sind in der Mitteilung dieser Autoren weder Beleganalysen noch Fehlergrenzen angegeben, ja nicht einmal der Arbeitsgang wird beschrieben, sodass eine vergleichende Beurteilung unmöglich ist.

FISHMAN *et al.*[26,22] sowie neuerdings eine Reihe japanischer Autoren[27,28,12] haben das Ausschütteln mit Alkohol + Toluol vorgenommen; allein, durch den Zusatz von Alkohol wird die Selektivität des eigentlichen Extraktionsmittels ungünstig beeinflusst, wie FASHENA UND STIFF am Beispiel der Pentosenfarbstoffe aufzeigen konnten[29].

Zur Extraktion des gelösten Uronsäurefarbstoffes aus dem Reaktionsgemisch haben wir *reines Toluol* herangezogen. Es bietet anstelle der bislang üblichen Solventien (Äther, Alkohol + Äther, Amylalkohol, Benzylalkohol, Butylacetat, Chloroform) folgende wesentliche Vorteile:

(*a*) Leichte, vollständige und weitgehend selektive Aufnahme des Uronsäurefarbstoffes. Alle Verbesserungen des qualitativen Nachweises, die mit der Wahl eines aromatischen Kohlenwasserstoffes für die Extraktion verbunden sind, werden nun uneingeschränkt auch der quantitativen Analyse dienstbar gemacht.

(*b*) Sehr geringe Verdampfungsverluste infolge des relativ hohen Siedepunktes von Toluol, besonders im Vergleich zu dem bisher zumeist gebrauchten Äther.

(*c*) Ausgezeichnete Trennung der beiden Phasen, wenn man, wie vorgeschlagen,

durch Rotation in schräger Lage extrahiert. Es erübrigt sich die Verwendung eines Scheidetrichters.

(*d*) Vermeidung der Filtration des Extraktes, da sich alle unlöslichen Partikel, sofern solche überhaupt gebildet werden, in der Grenzschicht zwischen den beiden flüssigen Phasen ansammeln und beim Abheben des Toluols mit einer Pipette nicht stören.

(*e*) Fortfall der Trocknung mit einem Trockenmittel; denn die Toluolphase ist bereits vollkommen klar.

Volumen des Toluols

Die Toluolmenge wurde so bemessen, dass ein ausreichendes Volumen zur Beschikkung der Mikroküvetten des Spektralphotometers einschliesslich eines geringen Überschusses zur Verfügung steht, welcher die bequeme Abnahme der Toluolphase mittels Pipette und Gummiball ermöglicht. Das Toluol nimmt während des Extraktionsvorganges unter gleichen äusseren Bedingungen eine stets gleichbleibende Menge Essigsäure auf; so wird bei Zugabe von 2.00 ml Toluol eine Toluol-Essigsäure-Phase von 2.60 ml gebildet. Der zunächst in einem Volumen von 7.00 ml verteilte Farbstoff ist also schliesslich in 2.60 ml enthalten, und es ergibt sich demnach der günstige Anreicherungsfaktor von 2.7.

Nachdem die Konstanz des Volumens der Toluol–Essigsäure-Phase durch die ganze Versuchsreihe gewährleistet ist, erübrigt sich auch noch die Verwendung von Messkolben, sodass der gesamte Arbeitsgang bis zur Extinktionsmessung in *einem* Gefäss, und zwar einem Reagensglas durchgeführt werden kann.

Extraktionsweise

Zur Überführung des Farbstoffes von der wässrigen in die organischen Phase wurde bisher durchweg so verfahren, dass das verschlossene Gefäss kurze Zeit kräftig geschüttelt wurde. Die Folgen sind häufig eine schlechte Entmischung der Phasen sowie feinste Suspensionen wässriger Tröpfchen in der organischen Schicht, die nach der Abtrennung erst mit einem Trockenmittel geklärt werden muss.

Unter unseren Versuchsbedingungen genügt es für eine quantitative Extraktion des Farbstoffes, wenn die Reagensgläser in schräger Lage (Vergrösserung der Phasengrenzfläche) viermal je 45 Sek von Hand um die Längsachse gedreht werden. Die Toluolphase bleibt dabei klar und setzt sich sofort vollkommen scharf ab.

ARBEITSVORSCHRIFTEN

Bestimmung von Glukuronsäure

Reagentien: Conc. *o*-Phosphorsäure ($D = 1.70$) p.a., Naphthoresorcin p.a. (Hoffmann, La Roche), Eisessig (99–100%) p.a., Toluol p.a.

In graduierte, mit Gummistopfen zu verschliessende Reagensgläser von gleichen Abmessungen (Anm. *1*) pipettiert man je 1.00 ml der Probelösung, enthaltend 20–100 μg freie Uronsäure. Dann gibt man aus einer Bürette je 2.00 ml conc. Phosphorsäure in langsamer, stets gleichmässiger Tropfenfolge hinzu und vermischt sofort. Schliesslich wird (Pipette) mit je 4.00 ml einer 0.2%igen Naphthoresorcinlösung in Eisessig (Anm. *2*) überschichtet. Erst nach Beendigung dieses Arbeitsganges darf in unmittelbarer Abfolge der Inhalt jedes Glases sorgfältig gemischt werden (Anm. *3*). Nun werden die verschlossenen Gläser 75 Min lang im Thermostaten (Anm. *4*) auf 70° erwärmt. Nach dem Erkalten gibt man mit Pipette je 2.00 ml Toluol zu, verschliesst

wiederum und stellt dann die Serie gleichzeitig in Eiswasser. Etwa 15 Min später beginnt man mit der Extraktion, indem man Glas um Glas aus dem Wasser nimmt und 45 Sek lang in schräger Lage um seine Achse dreht. Als Halterung dient zweckmässig ein starkwandiges Zentrifugenglas von passender Weite. Die Temperatursteigerung des Inhalts durch die Handwärme ist möglichst gering zu halten; man stellt sofort wieder ins Eiswasser zurück.

Wenn dieser Extraktionsteilschritt viermal die ganze Serie durchlaufen hat, werden die Reagensgläser herausgenommen. Man wartet ab, bis der Inhalt auf Raumtemperatur gekommen ist und liest dann das Volumen der klaren Toluol–Essigsäure-Phase ab. Letztere wird mittels Pipette und Gummiball abgehoben und zur Extinktionsmessung bei 570 mμ in 1 cm-Mikroküvetten eingefüllt (Anm. 5).

Zur Aufstellung der Eichkurve werden drei Eichwerte mit 22, 44 und 88 μg Glukuron/ml (≡ 24.25, 48.5 und 97 μg Glukuronsäure/ml) in jeder Analysenserie mitgeführt, desgleichen ein Leerwert mit 1.00 ml Wasser.

Während des gesamten Arbeitsganges ist die Einwirkung starker Lichtquellen zu vermeiden!

Anm. 1. Wir haben Reagensgläser mit den Abmessungen 180 × 18 mm (*ca.* 45 ml Rauminhalt) verwendet, die eine 0.5 ml-Teilung besitzen.

Anm. 2. Am besten bereitet man die Lösung unmittelbar vor dem Gebrauch. Soll sie für einige Tage auf Vorrat hergestellt werden, so empfiehlt sich die Aufbewahrung in einer gut verschlossenen braunen Flasche bei Raumtemperatur (nicht im Kühlschrank).

Anm. 3. Es dürfen keine Schlieren mehr zu erkennen sein!

Anm. 4. Der Thermostat weist in seinem Deckel eine der Höchstzahl der Reagensgläser pro Analysenserie entsprechende Anzahl von Bohrungen auf, durch welche die Gläser mit geringem Spielraum eingeführt werden können. Letztere werden in der gewünschten Eintauchtiefe durch eine übergezogene konische Gummidichtung festgehalten. Durch lebhaftes Rühren wird für raschen Temperaturausgleich gesorgt.

Anm. 5. Unsere Versuchsreihen wurden teils im Beckman Spektralphotometer DU, teils im Zeiss Gerät PMQ II durchgemessen.

Bestimmung von Galakturonsäure

Die quantitative Bestimmung der Galakturonsäure erfolgt in gleicher Weise wie bei der Glukuronsäure mit der einzigen Abweichung, dass zur Farbstoffbildung nur 30 Min auf 70° erhitzt wird. Die Eichwerte enthalten hier 24.25, 48.5 und 97 μg Galakturonsäure/ml.

ERGEBNISSE UND DISKUSSION

Uronsäure ohne Zusätze

Unter den angegebenen Versuchsbedingungen ist im Bereich von 20 bis 100 μg Uronsäure das LAMBERT–BEERsche Gesetz erfüllt. Die Abweichungen der einzelnen Messpunkte von der Eichgeraden liegen fast ausnahmslos unter 2%, in keinem Falle aber über 3%.

Wie bei vielen kolorimetrischen Methoden war es auch hier nicht möglich, eine für mehrere Versuchsreihen gültige, reproduzierbare Eichgerade aufzustellen. Worauf das zurückzuführen ist, konnte trotz sorgfältiger Beobachtung aller die Reaktion be-

treffenden Umstände nicht eindeutig geklärt werden. Übereinstimmend mit diesen Erfahrungen betonen auch HOLLMANN UND WILLE[10] sowie FRETWURST UND AHLHELM[11], dass zur Erzielung höchstmöglicher Genauigkeit bei jeder Analysenserie neue Eichwerte mitgeführt werden müssen.

Uronsäure neben anderen Kohlenhydraten

Die Leistungsfähigkeit eines Analysenverfahrens für biochemische Zwecke erweist sich nur zu einem geringen Teil bei seiner Anwendung auf reine Lösungen des betr. Stoffes. Nachdem das biologische Ausgangsmaterial meist eine Unzahl der verschiedensten Begleitsubstanzen enthält und eine Abtrennung derselben oft methodisch oder vom Arbeitsaufwand her gesehen nur unvollkommen oder überhaupt nicht möglich ist, muss man den Störungen durch Begleitstoffe ganz besondere Aufmerksamkeit widmen.

Hinsichtlich der Bestimmung von Uronsäuren mittels der TOLLENSschen NR-Reaktion sind solche Untersuchungen nicht eben zahlreich. Viele Varianten wurden von ihren Autoren überhaupt nicht auf Fehler durch die Anwesenheit anderer Kohlenhydrate überprüft, obwohl aus qualitativen Arbeiten längst bekannt war, dass sie zu erheblichen Fehlschlüssen Veranlassung geben können. Nachdem bisher zur quantitativen Extraktion des Farbstoffes ausschliesslich unspezifische bzw. wenig selektive Lösungsmittel oder Lösungsmittelgemische Verwendung gefunden haben, darf es nicht wundernehmen, wenn bei Gegenwart anderer Saccharide beträchtliche Abweichungen festgestellt wurden. So haben HANSON *et al.*[30] den Einfluss von Glukose auf die Glukuronsäurebestimmung untersucht und folgende Tabelle (I) erhalten (Ausschütteln des Reaktionsgemisches mit Amylalkohol).

TABELLE I

Zugegebene Menge von		*Gefundene Menge von Glukuronsäure* (μg)
Glukuronsäure[a] (μg)	*Glukose* (μg)	
53.6	0	54.0
53.6	18	56.5
53.6	36	57.0
53.6	54	61.0

[a] In Form von L-Menthylglukuronosid.

Die Anwesenheit einer äquivalenten Menge Glukose bedingt also einen Fehler von + 13.8%!

Ausführlichere Untersuchungen über störende Einflüsse der übrigen Zucker stammen von STARY UND YUVANIDIS[31] (Ausschütteln des Reaktionsgemisches mit Alkohol + Äther). Fructose stört demnach am stärksten, eine Erfahrung, die man auch bei anderen Farbreaktionen der Kohlenhydrate immer wieder macht; 100 μg Fructose weisen ~ 16% der Extinktion von 100 μg Glukuronsäure auf! Es folgen Ribose, dann Arabinose, endlich die Aldohexosen Glukose, Mannose und Galaktose. Für 100 μg Glukose beträgt die Extinktion immer noch ~ 4% von derjenigen der gleichen Glukuronsäuremenge. STARY UND YUVANIDIS haben ferner das Verhalten der häufigsten Aminosäuren überprüft und gefunden, dass „diese sowie ihre Gemische auch bei sehr hoher Konzentration . . . völlig farblos blieben." Das steht im Einklang mit unserer Erfahrung, wonach zwecks Aufspaltung von Glukuronosiden zugesetzte β-Glukuroni-

TABELLE II

EINFLUSS VON GLUKOSE UND FRUCTOSE AUF DIE BESTIMMUNG DER GLUKURONSÄURE

Glukuron gegeben (µg)	*Zusatz von*		*Molverhältn. Hexose:Glukuron*	*Extinktion (E)*	*Glukuron gefunden (µg)*	*Abweichung (%)*
	Glukose (µg)	*Fructose (µg)*				
22.0	—	—	—	0.076	—	—
44.0	—	—	—	0.154	—	—
22.0	225	—	10 : 1	0.0775	22.4	+ 2.0
22.0	450	—	20 : 1	0.0805	23.3	+ 5.9
44.0	450	—	10 : 1	0.1555	44.4	+ 1.0
22.0	—	—	—	0.0735	—	—
44.0	—	—	—	0.1485	—	—
22.0	225	—	10 : 1	0.076	22.7	+ 3.4
22.0	450	—	20 : 1	0.080	24.0	+ 8.9
44.0	450	—	10 : 1	0.1515	44.9	+ 2.0
22.0	—	—	—	0.0695	—	—
44.0	—	—	—	0.1425	—	—
22.0	—	45.0	2 : 1	0.070	22.2	+ 0.7
22.0	—	90.0	4 : 1	0.0745	23.6	+ 7.2
44.0	—	90.0	2 : 1	0.1435	44.3	+ 0.7
22.0	—	—	—	0.070	—	—
44.0	—	—	—	0.1445	—	—
17.6	—	54.0	3 : 1	0.054	17.3	− 1.8
22.0	—	90.0	4 : 1	0.0725	22.8	+ 3.6
44.0	—	90.0	2 : 1	0.145	44.1	+ 0.3
22.0	—	—	—	0.070	—	—
44.0	—	—	—	0.144	—	—
22.0	—	45.0	2 : 1	0.0725	22.8	+ 3.6
22.0	—	90.0	4 : 1	0.076	23.9	+ 8.6
44.0	—	90.0	2 : 1	0.148	45.2	+ 2.8

TABELLE III

EINFLUSS VON GLUKOSE UND FRUCTOSE AUF DIE BESTIMMUNG DER GALAKTURONSÄURE

Galakturonsäure gegeben (µg)	*Zusatz von*		*Molverhältn. Hexose:Galakturonsäure*	*Extinktion (E)*	*Galakturonsäure gefunden (µg)*	*Abweichung (%)*
	Glukose (µg)	*Fructose (µg)*				
24.25	—	—	—	0.075	—	—
24.25	225	—	10 : 1	0.0745	24.1	—0.7
24.25	450	—	20 : 1	0.0735	23.8	−2.0
24.25	—	—	—	0.085	—	—
48.50	—	—	—	0.174	—	—
24.25	225	—	10 : 1	0.082	23.4	−3.5
24.25	450	—	20 : 1	0.0845	24.1	−0.6
48.50	450	—	10 : 1	0.168	46.9	−3.4
24.25	—	—	—	0.083	—	—
48.50	—	—	—	0.169	—	—
24.25	—	45.0	2 : 1	0.082	24.0	−1.2
24.25	—	90.0	4 : 1	0.085	24.8	+2.4
48.50	—	90.0	2 : 1	0.170	48.8	+0.6
24.25	—	—	—	0.080	—	—
48.50	—	—	—	0.1615	—	—
24.25	—	45.0	2 : 1	0.0795	24.1	−0.6
24.25	—	90.0	4 : 1	0.082	24.9	+2.5
48.50	—	90.0	2 : 1	0.1595	47.9	−1.2

dase zur Bestimmung der freigesetzten Uronsäure nicht abgetrennt zu werden braucht.

Ähnliche Verhältnisse treffen wir bei der Galakturonsäure an. KERTESZ[32] hat die Methode von RATISH UND BULLOWA[24] auf die Bestimmung von Galakturonsäure übertragen und bemerkt: "Small proportions of glucose and galactose do not seem to interfere; both arabinose and fructose in proportions exceeding the amount of galacturonic acid may completely destroy the reliability of the method".

ARTZ UND OSMAN[2c] äussern sich über die Fehlergrenze von Glukuronsäurebestimmungen im biologischen Material wie folgt: "The accuracy claimed for these methods is seldom better than $\pm 5\%$, and it is doubtful whether even this degree of accuracy is obtained consistently". DEICHMANN UND WITHERUP[33] haben gleichfalls für ihr Verfahren eine Fehlergrenze von 5% angegeben. Lassen wir demnach Abweichungen bis zu $\pm 5\%$ vom wahren Werte zu, so ermöglicht es das beschriebene Verfahren, Glukuronsäure noch neben der doppelten Menge Fructose oder der zehnfachen Menge Glukose quantitativ zu erfassen (Tabelle II). Bei der Galakturonsäure ist sogar die vierfache Menge Fructose oder die zwanzigfache Menge Glukose unerheblich (Tabelle III).

Uronsäure neben Uronosiden

Um das Verhalten der Glukuronsäure (und anderer Uronsäuren) im Organismus verfolgen zu können, ist ein Verfahren für die Bestimmung von freier neben gebundener Uronsäure im selben Substrat Voraussetzung[34].

Nähere Beschäftigung mit dieser Problematik lehrt zunächst, dass es sich hierbei um zwei methodisch recht ungleichwertige Teilprobleme handelt, je nachdem, ob die gebundene Uronsäure in Makromolekülen oder niedermolekularen Verbindungen enthalten ist.

Liegen als Begleiter hochmolekulare Polyuronoside (Chondroitin-schwefelsäure, Heparin, Hyaluronsäure, *usw.*) vor, so lässt sich durch einfache Massnahmen, *z.B.* eine Alkoholfällung, die Abtrennung von der monomeren Säure bewirken.

In weitaus den meisten Fällen wird aber die freie Uronsäure neben niedermolekularen Uronosiden zu bestimmen sein. Wenn man bedenkt, dass *z.B.* die Bindungspartner der Glukuronsäure in den Körperflüssigkeiten den verschiedensten Substanzklassen angehören und überdies mehrere Bindungstypen vorkommen, woraus sich stark unterschiedliche Eigenschaften der Paarlinge ergeben, so erscheint es begreiflich, dass den Bemühungen in dieser Richtung bisher recht bescheidene Erfolge gegenüberstehen. Im Schrifttum sind nähere Angaben nur zu den folgenden beiden Methoden zu finden:

(*1*) Entfernung der Glukuronoside durch Ätherextraktion. Dieses Verfahren diente ursprünglich zur Abtrennung von Glukuronsäurepaarlingen aus dem Harn von Versuchstieren in präparativem Massstab, wurde dann durch NEUBERG UND SCHEWKET[35] in die qualitative Analyse eingeführt und 1924 von QUICK[36] für die quantitative Bestimmung der Mentholglukuronsäure ausgearbeitet. LEVVY[37] hat 1948 die Extraktion mit Äther zur quantitativen Trennung von Mentholglukuronosid und freier Glukuronsäure vorgeschlagen. HEYNS UND KELCH[34] zeigten jedoch, dass dieses Verfahren nur bei leicht ätherlöslichen Glukuronosiden (wie Menthol- oder Borneol-glukuronsäure) zu brauchbaren Ergebnissen führt, in allen übrigen Fällen hingegen versagt.

(*2*) Fällung von freier Uronsäure als *p*-Toluidin-Komplex. SMITH UND WILLIAMS[5] fanden, dass freie Uronsäuren (dagegen nicht ihre Lactone) mit *p*-Toluidin in wässrig-alkoholischer Lösung auf Zusatz von etwas Ammoniak als schwerlösliche kristalline Komplexe der Zusammensetzung 2 *p*-Toluidin : 1 Ammonium-uronat ausfallen. In-

wieweit das auch für Uronoside zutrifft, wurde bisher nicht untersucht. Die Reaktion ermöglicht zwar halbquantitative Aussagen, wenn grössere Mengen an Uronsäuren vorliegen; ein genaues Verfahren lässt sich hierauf aber nicht aufbauen.

(3) Die Angabe bei HINSBERG UND LANG[38], dass von JARRIGE[39] eine Methode zur getrennten Bestimmung von freier Uronsäure beschrieben wurde, beruht auf einem Irrtum.

Demgegenüber gründet sich unser Vorschlag zur Bestimmung von freier neben gebundener Uronsäure auf die hydrolytische Resistenz gewisser Uronoside. Um den Anwendungsbereich des neuen Verfahrens überblicken zu können, muss folgendes vorausgeschickt werden.

Von den natürlich vorkommenden Oligo-uronosiden fällt der ganz überwiegende Teil unter die Klasse der sog. „Glukuronsäurepaarlinge". Ihr Name weist bereits auf den chemischen Aufbau hin: Es handelt sich um Verbindungen, die man sich aus einem Molekül Glukuronsäure und einem Molekül eines organischen Bausteines, der H an einem Hetero-Atom (O, N, S) gebunden enthält, durch Abspaltung von einem Molekül Wasser entstanden denken kann. Je nach dem Bindungstyp zwischen den beiden Komponenten sind vier Gruppen zu unterscheiden, die sich gegenüber hydrolytischen Einflüssen gänzlich verschieden verhalten.

(1) *Phenoläther-Typ.* Dieser stellt die glykosidische Verknüpfung eines Phenols mit dem C_1-Hydroxyl der Glukuronsäure dar. Zu diesem Typ gehört sowohl mengenmässig als auch der Zahl der Individuen nach der grösste Teil der im Säugetierkörper anzutreffenden Paarlinge. Die Phenoläther-gruppierung ist gegen Hydrolyse mit Säuren sehr widerstandsfähig, wenngleich von Fall zu Fall geringfügige Unterschiede sich geltend machen.

(2) *Alkoholäther-Typ.* Hier ist an der glykosidischen Bindung am C_1 eine alkoholische Hydroxylgruppe beteiligt. Dieser Typ umfasst mit wenigen Ausnahmen alle übrigen „gepaarten Uronsäuren". Bei den natürlich vorkommenden Vertretern ist zumeist ein sekundärer Alkohol beteiligt. Die hydrolytische Resistenz hängt von der Natur des alkoholischen Bindungspartners ab, ist aber stets viel geringer als beim Phenoläther-Typ.

(3) *Ester-Typ.* Die Glukuronsäure ist über C_1 esterartig mit der Carboxylgruppe einer (meist aromatischen) Säure verbunden. Eine solche Gruppierung setzt hydrolytischen Einwirkungen nur geringen Widerstand entgegen: Schon beim Kochen mit Wasser erfolgt rascher Zerfall in die Komponenten; desgleichen beim Stehen der schwach alkalischen Lösung bei Raumtemperatur. Paarlinge vom Ester-Typ spielen in den Körperflüssigkeiten normalerweise nur eine untergeordnete Rolle. Nach Gaben von bestimmten Arzneimitteln, wie *z.B.* Salicylsäure, erscheinen sie jedoch als eine mögliche Ausscheidungsform im Harn.

(4) *Amin-Typ.* Bei dieser eigenartigen Unterklasse, deren Konstitution noch nicht restlos gesichert erscheint, soll durch Wasseraustritt zwischen einer aromatischen Aminogruppe und dem Halbacetal-Hydroxyl der Glukuronsäure eine amin-artige Bindung zustande gekommen sein. Man vermutet, dass derartige Körper im Harn von Versuchstieren enthalten sind, die mit Anilin, Anisidin oder Phenetidin behandelt wurden. Ihre Isolierung scheitert an dem Umstand, dass sie ausserordentlich leicht hydrolysiert werden.

Diese Ausführungen machen deutlich, dass einem Verfahren zur getrennten Bestim-

TABELLE IV

EINFLUSS VON PHENOLPHTHALEIN-GLUKURONOSID (PPG) UND 8-HYDROXYCHINOLIN-GLUKURONOSID (OxG) AUF DIE BESTIMMUNG DER GLUKURONSÄURE

Glukuron gegeben (µg)	*Zusatz von*		*Molverhältn. Glukuronosid: Glukuron*	*Extinktion (E)*	*Glukuron gefunden (µg)*	*Abweichung (%)*
	PPG, ber. als Glukuron (µg)	*OxG, ber. als Glukuron (µg)*				
22.0	—	—	—	0.0695	—	—
44.0	—	—	—	0.142	—	—
22.0	88.0	—	4 : 1	0.0705	22.3	+1.4
44.0	88.0	—	2 : 1	0.141	43.7	−0.7
22.0	—	—	—	0.0675	—	—
44.0	—	—	—	0.137	—	—
22.0	132.0	—	6 : 1	0.068	22.2	+0.7
44.0	88.0	—	2 : 1	0.132	42.4	−3.6
22.0	—	—	—	0.0705	—	—
44.0	—	—	—	0.1445	—	—
22.0	88.0	—	4 : 1	0.0685	21.4	−2.8
22.0	132.0	—	6 : 1	0.075	23.4	+6.4
44.0	88.0	—	2 : 1	0.145	44.1	+0.3
22.0	—	—	—	0.064	—	—
22.0	88.0	—	4 : 1	0.065	22.4	+1.6
22.0	132.0	—	6 : 1	0.0675	23.2	+5.5
22.0	132.0	—	6 : 1	0.0645	22.2	+0.8
22.0	—	—	—	0.068	—	—
44.0	—	—	—	0.1365	—	—
22.0	—	88.0	4 : 1	0.0665	21.5	−2.2
22.0	—	132.0	6 : 1	0.067	21.7	−1.5
44.0	—	88.0	2 : 1	0.1385	44.7	+1.5
22.0	—	—	—	0.0575[a]	—	—
22.0	—	88.0	4 : 1	0.0575[a]	22.0	±0.0
22.0	—	132.0	6 : 1	0.058[a]	22.2	+0.9
22.0	—	132.0	6 : 1	0.057	21.8	−0.9

[a] Erhitzungsdauer 70 Min (statt 75 Min).

TABELLE V

EINFLUSS VON MENTHOL-GLUKURONOSID (MG) UND BORNEOL-GLUKURONOSID (BG) AUF DIE BESTIMMUNG DER GLUKURONSÄURE

Glukuron gegeben (µg)	*Zusatz von*		*Molverhältn. Glukuronosid: Glukuron*	*Extinktion (E)*	*Glukuron gefunden (µg)*	*Abweichung (%)*
	MG, ber. als Glukuron (µg)	*BG, ber. als Glukuron (µg)*				
22.0	—	—	—	0.072	—	—
44.0	—	—	—	0.146	—	—
22.0	22.0	—	1 : 1	0.0855	26.2	+19
44.0	44.0	—	1 : 1	0.175	52.8	+20
44.0	88.0	—	2 : 1	0.1945	58.5	+33
22.0	—	—	—	0.072	—	—
44.0	—	—	—	0.1445	—	—
22.0	—	22.0	1 : 1	0.090	27.5	+25
44.0	—	44.0	1 : 1	0.1785	54.3	+23.5
44.0	—	88.0	2 : 1	0.2065	62.9	+43

mung von freier neben gebundener Uronsäure, das sich auf das Hydrolyseverhalten der Uronoside stützt, prinzipielle Grenzen gezogen sind.

Unter Bedingungen, wie sie bereits den im vorhergehenden Abschnitt beschriebenen Versuchsreihen zugrundeliegen, ist die Aufspaltung der Glukuronoside mit Phenoläther-gruppierung so geringfügig, dass eine quantitative Bestimmung von freier neben derartig gebundener Uronsäure bis etwa zur fünffachen molaren Menge der letzteren möglich wird (Tabelle IV). Paarlinge vom Ester-Typ und vom Amin-Typ werden sich hingegen selbst unter den schonendsten Kondensationsbedingungen wie freie Glukuronsäure verhalten; aber auch diejenigen vom Alkoholäther-Typ können noch in beträchtlichem Ausmass hydrolysiert werden (Tabelle V).

Dennoch bedeutet dieser Umstand keine wesentliche Einschränkung der Verwendbarkeit des geschilderten Verfahrens für Reihenanalysen zur Erforschung des Glukuronsäurestoffwechsels bzw. zu Leberfunktionsprüfungen beim Menschen, und zwar aus den folgenden Gründen:

(*1*) Die unter normalen Verhältnissen im Harn ausgeschiedenen Glukuronsäurepaarlinge sind, wie gesagt, mengenmässig ganz überwiegend Verbindungen mit Phenoläther-gruppierung. Der Amin-Typ kommt praktisch nicht vor. Bei Ausschluss von Medikamenten und Drogen treten Uronoside mit Alkoholäther-bindung nur in Form der quantitativ stark zurücktretenden[40] Gruppe der Steroidkonjugate, solche mit Ester-bindung wohl nur als Bilirubin-glukuronoside in Erscheinung.

(*2*) Liegen in Sonderfällen Uronoside vom Alkoholäther-Typ in grösserer Menge vor, so lässt sich manchmal eine (zumindest teilweise) Abtrennung derselben nach LEVVY mit Äther[37] erreichen. Vielfach führt auch eine Differenzbestimmung gemäss

Gesamtglukuronsäure — gebundene Glukuronsäure = freie Glukuronsäure

zum Ziel, wobei die Gesamtglukuronsäure nach einem der bisher gebräuchlichen Verfahren mit Mineralsäure, die gebundene Glukuronsäure nach FISHMAN UND GREEN[22] ermittelt wird.

Den Herren Dr. KRÜLL und Dr. SCHLOSSHAUER von der Schering AG, Berlin, bin ich für eine grosszügige Spende von Chemikalien sehr zu Dank verpflichtet.

ZUSAMMENFASSUNG

Durch wesentliche Umgestaltungen der TOLLENSschen Naphthoresorcin-Reaktion, insbesondere: (*a*) Ersatz der Mineralsäure durch conc. Phosphorsäure; (*b*) Verwendung einer Lösung von Naphthoresorcin in Eisessig statt in Wasser; (*c*) Extraktion des gebildeten Farbstoffes mit Toluol anstelle anderer unspezifischer Lösungsmittel, wurde ein Mikrobestimmungsverfahren für freie Uronsäuren ausgearbeitet, das einige Fehlerquellen in der bisherigen Methodik prinzipiell vermeidet.

Das Verfahren ermöglicht eine einfache Bestimmung von freier Glukuronsäure neben Glukuronosiden vom Phenoläther-Typ, sofern deren molares Verhältnis einen bestimmten Grenzwert (~ 1 : 5) nicht unterschreitet. Es ergibt ferner zuverlässige Werte auch bei Anwesenheit *z.T.* beträchtlicher Mengen anderer Kohlenhydrate, wie am Beispiel der Glukose und Fructose gezeigt wird.

SUMMARY

A micro determination for free uronic acids based on TOLLENS' reaction is proposed. Sources of error in earlier methods are avoided by the use of phosphoric acid, a reagent solution in glacial acetic acid, and extraction of the dye with toluene. Free glucuronic acid can be determined in presence of glucuronosides of the phenol–ether type up to molar ratios of *ca.* 1 : 5. Reliable results can also be obtained in the presence of other carbohydrates.

RÉSUMÉ

Une méthode, basée sur la réaction de TOLLENS, est proposée pour le microdosage des acides uro-

niques libres. Les causes d'erreurs des méthodes précédentes sont évitées par l'emploi d'acide phosphorique et d'une solution du réactif dans l'acide acétique glacial, et par extraction du colorant dans le toluène. L'acide glucuronique libre peut être dosé en présence de glucuronosides du type phénol–éther, et également en présence d'autres hydrates de carbone.

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SPECTROPHOTOMETRIC DETERMINATION OF SCANDIUM WITH ARSENAZO*

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BERMAN, DUVAL AND RUSSELL[1] have reviewed spectrophotometric methods for scandium. BRUDZ *et al.*[2] have made a comparison of various spectrophotometric reagents and recommended sulfonazo for the determination of scandium. Arsenazo III[3], 4-(2-pyridylazo)resorcinol (PAR)[4], propylfluorone[5], xylenol orange[1], and 8-quinolinol derivatives[6] have been recently proposed for the spectrophotometric determination of scandium.

After a study on the determination of the rare earths with arsenazo[7], it was thought worthwhile to attempt a spectrophotometric determination of scandium with the same reagent. Although the color reaction of scandium with arsenazo has been reported by KUZNETSOV[8] and KUTEĬNIKOV[9], no procedure seems to have been developed for the determination of this element. The conditions for the determination and the separation from interfering elements are described in the present paper.

EXPERIMENTAL

Apparatus

Absorbance measurements were made with a Beckman Model DU spectrophotometer, using 1-cm cells. A Beckman Model G pH meter was used for pH measurements. A Burrell shaking machine with a time switch was used for extractions. Glass tubes (25 cm long, 1.1 cm i.d.) with coarse, sintered-glass filter discs fused into the bottom were used to hold the anion-exchange resin (Dowex 1-X8, supplied in the chloride form, 50–100 mesh). The column of resin was about 10 cm high. For the separation of uranium, the resin was washed with four 10-ml portions of water, two 10-ml portions of ethanol, four 10-ml portions of water, and four 10-ml portions of 8 *M* hydrochloric acid before use. For the separation of thorium, 8 *M* hydrochloric acid was replaced by 8 *M* nitric acid in the above treatment.

Reagents

Thenoyltrifluoroacetone (TTA) (0.5 *M*). 45 g in 400 ml of xylene.

8-Quinolinol. 5.0 g of 8-quinolinol was dissolved in 10 ml of glacial acetic acid and the solution was diluted to 100 ml with water.

* Contribution No. 1281. Work was performed in the Ames Laboratory of the U.S. Atomic Energy Commission.

Arsenazo. 0.10 g in 100 ml of water. An Eastman product, 3-(2-arsonophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid disodium salt (P 7302), was used.

Acetic acid–sodium acetate. Ten ml of 1 *M* acetic acid was mixed with 320 ml of 1 *M* sodium acetate. The pH of the mixture was 6.1.

Standard scandium solution (1.00 mg Sc/ml). 0.1534 g of Sc_2O_3 was dissolved by heating in 50 ml of concentrated hydrochloric acid. The solution was evaporated to near dryness and diluted to exactly 100 ml with water. The final concentration of acid was about 0.1 *M*. Working standard solutions (50 and 10 p.p.m. Sc) were prepared by diluting this stock solution with water.

DETERMINATION OF SCANDIUM

Absorption curve

The maximum absorption of the scandium–arsenazo complex against reagent blank was found at about 570 mμ (Fig. 1).

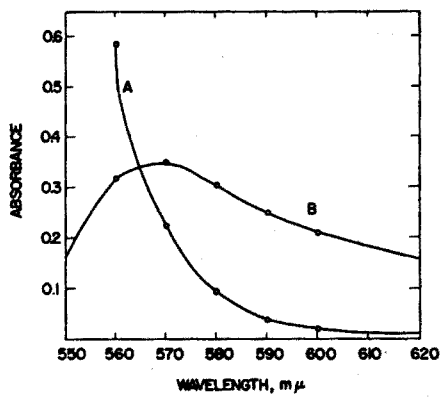

Fig. 1. Absorption curves. (A) Reagent blank against water; (B) 0.856 p.p.m. Sc against reagent blank.

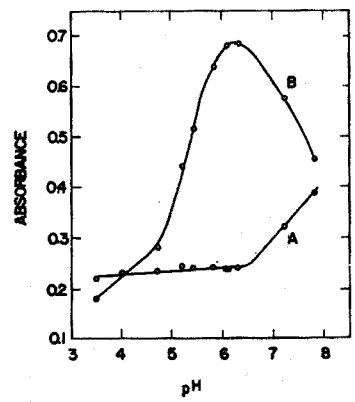

Fig. 2. Effect of pH. (A) Reagent blank against water; (B) 1.71 p.p.m. Sc against reagent blank.

Effect of pH

The maximum and nearly constant absorbance was obtained at pH 6.1–6.3 (Fig. 2). Acetic acid–sodium acetate and triethanolamine–nitric acid buffer solutions were used for the pH ranges 3.5–6.5 and 7–8, respectively.

Effect of acetate concentration

At pH 6.1, the absorbance of the complex decreased very slightly as the concentration of acetate ion increased from 0.04 to 0.14 *M*. At 0.08, 0.10, and 0.12 *M* acetate concentrations, 0.856 p.p.m. Sc gave absorbances of 0.356, 0.350, and 0.349, respectively. A concentration of 0.10 *M* was chosen.

Effect of arsenazo concentration

For 0.856 p.p.m. Sc, a constant absorbance was obtained by adding 2.0 to 5.0 ml of 0.10% arsenazo solution and diluting to 50 ml.

Stability of colored solution

Absorbances of the complex and blank solutions remained constant for 3 h. All the experiments were carried out at room temperature of about 25°.

Calibration curve

The scandium–arsenazo system conforms to Beer's law with concentrations of up to 2.0 p.p.m. Sc in the final solution. The molar absorptivity for scandium was $1.7 \cdot 10^4$.

Interference study

Table I summarizes the results of the interference study. The following ions give a color with arsenazo; aluminium, chromium, copper, iron(III), rare earths, thorium,

TABLE I

INTERFERENCE STUDY

(Final volume: 25 ml)

Addition		*Sc taken (μg)*	*Sc found (μg)*
Al(III)	0.030 mg	0	44
Ca(II)	0.10 mg	26.7	27.3
Cd(II)	0.10 mg	26.7	27.0
Co (II)	0.10 mg	0	4
Cr(III)	0.10 mg	0	35
Cu(II)	0.10 mg	0	18
Fe(III)	0.10 mg	0	25
Gd(III)	0.053 mg	0	18
Mg(II)	0.10 mg	26.7	27.5
Mn(II)	0.10 mg	26.7	27.0
Mo(VI)	0.10 mg	26.7	27.0
Ni(II)	0.10 mg	0	5
Pb(II)	0.10 mg	26.7	25.0
Th(IV)	0.10 mg	0	33
Ti(IV)	0.10 mg	0	34
U(VI)	0.10 mg	0	18
V(V)	0.10 mg	0	4
W(VI)	0.10 mg	26.7	26.7
Zn(II)	0.10 mg	26.7	27.5
Zr(IV)	0.10 mg	0	39
NaCl	0.50 mmole	26.7	26.5
$NaClO_4$	0.30 mmole	26.7	26.8
$NaNO_3$	0.50 mmole	26.7	26.8
Na_2SO_4	0.30 mmole	26.7	26.5
F (as NaF)	101 μg	26.7	21.2
P (as KH_2PO_4)	10 μg	26.7	18.3

titanium, uranium, and zirconium. Fluoride and phosphate ions decrease the absorbance of the scandium complex. An attempt to mask iron(III) with L-ascorbic acid was not successful. Hydrogen peroxide inhibits the reaction of thorium with arsenazo, but it also affects the absorbance of the scandium complex. Microgram quantities of thorium are not masked by sodium sulfate (0.30 mmole).

Determination of scandium and thorium

Although thorium interferes with the determination of scandium, scandium can be determined in the presence of small amounts of thorium by using a method similar to that which was previously used for the determination of both rare earths and thorium[10]. The present method is based on the fact that in a 0.05 *M* hydrochloric acid medium the absorbance is due to the thorium–arsenazo complex alone, while at pH 6.1 the absorbance is due to both the thorium and scandium complexes. For details of the procedure, the previous report[10] should be consulted. The results are shown in Table II. When the sample contains relatively large amounts of thorium, the separation of scandium by anion exchange is recommended.

TABLE II

DETERMINATION OF THORIUM AND SCANDIUM WITH ARSENAZO

Taken		*Found*	
Th (μg) in 25 ml	*Sc (μg) in 25 ml*	*Th (μg) in 25 ml*	*Sc (μg) in 25 ml*
0	26.7	0.0,0.0	27.8,27.5
15.2	10.6	14.5,14.5	9.8,10.5
15.2	26.7	14.5,14.5	26.5,26.0
15.2	42.7	15.5,15.5	40.5,40.5
25.3	10.6	25.0,25.0	10.0,10.0
25.3	26.7	25.0,25.0	26.5,25.3
50.7	0	50.0,50.0	0.0,0.2
50.7	10.6	50.0,50.0	9.5,9.8

SEPARATION OF SCANDIUM

TTA–xylene extraction

Extraction of scandium with TTA in benzene was studied by BRONAUGH AND SUTTLE[11]. The present work has shown that the TTA extraction at pH 1.5 is useful not only in the separation of scandium from the rare earths, but also in the separation from a considerable number of other elements. The data obtained in applying the proposed procedure are collected in Table III.

TABLE III

DETERMINATION OF SCANDIUM AFTER TTA EXTRACTION

Addition	*Sc taken (μg)*	*Sc found[a] (μg)*	*Average recovery (%)*
None	0	0.0,0.0	—
None	26.7	25.5,25.5	95.5
0.10 mg Al	0	6.5,6.5	—
0.10 mg Al	26.7	23.0,21.2	82.7
21 μg Al	0	0.0,0.0	—
21 μg Al	26.7	24.8,25.0	93.2
10 mg As(III)	0	0.5,0.4	—
10 mg As(III)	26.7	25.5,25.5	95.5
20 mg Ca	0	0.0,0.0	—
20 mg Ca	26.7	25.0,25.0	93.6
10 mg Cd	0	0.0,0.0	—
10 mg Cd	26.7	24.7,24.7	92.5

TABLE III (continued)

Addition	*Sc taken (μg)*	*Sc found*[a] *(μg)*	*Average recovery (%)*
10 mg Co	0	1.3,1.3	—
10 mg Co	26.7	25.7,25.5	95.9
10 mg Cr(III)	0	1.3,1.4	—
10 mg Cr(III)	26.7	22.5,23.0	85.4
1.0 mg Cr(III)	0	1.3,1.3	—
1.0 mg Cr(III)	26.7	24.5,24.5	91.8
1.0 mg Dy	0	0.6,0.4	—
1.0 mg Dy	26.7	26.0,26.5	98.5
1.0 mg Fe(II)[b]	0	0.8,0.7	—
1.0 mg Fe(II)[b]	26.7	25.3,25.0	94.4
20 mg Mg	0	0.0,0.0	—
20 mg Mg	26.7	25.5,25.2	95.1
10 mg Mn(II)	0	0.6,0.6	—
10 mg Mn(II)	26.7	25.7,25.2	95.5
9.8 mg Mo(VI)	0	4.5[c],2.5[c]	—
9.8 mg Mo(VI)	26.7	22.5[c],22.7[c]	85.0
0.98 mg Mo(VI)	0	0.3,0.3	—
0.98 mg Mo(VI)	26.7	25.3,25.3	94.8
10 mg Ni	0	0.2,0.1	—
10 mg Ni	26.7	24.5,24.5	91.8
10 mg Pb	0	0.0[d],0.0[d]	—
10 mg Pb	26.7	25.7[d],25.0[d]	95.1
97 μg Ti	0	8.5,8.2	—
97 μg Ti	26.7	18.5,18.5	69.3
9.7 μg Ti	0	1.3,1.3	—
9.7 μg Ti	26.7	24.0,24.3	90.6
10 μg U(VI)	0	1.9,1.7	—
10 μg U(VI)	26.7	25.0,25.2	94.0
0.50 mg V(V)	0	4.5,5.2	—
0.50 mg V(V)	26.7	24.0,24.0	89.9
0.10 mg V(V)	0	2.6,2.8	—
0.10 mg V(V)	26.7	24.8,25.2	93.3
20 μg V(V)	0	0.5,1.3	—
20 μg V(V)	26.7	25.3,25.3	94.8
10 mg W(VI)	0	0.0,0.0	—
10 mg W(VI)	26.7	24.8,24.5	92.5
0.49 mg Y	0	0.3,0.5	—
0.49 mg Y	26.7	25.2,25.5	95.1
10 mg Zn	0	0.3,0.0	—
10 mg Zn	26.7	25.7,26.5	97.8
1.0 mg Zr	0	0.0,0.0	—
1.0 mg Zr	26.7	26.0,25.8	97.0
0.10 mg F (as NaF)	0	0.0,0.0	—
0.10 mg F (as NaF)	26.7	24.7,24.6	92.5
0.10 mg P (as KH_2PO_4)	0	0.1,0.3	—
0.10 mg P (as KH_2PO_4)	26.7	25.5,25.7	95.9
0.90 mmole Na_2SO_4	0	0.0,0.0	—
0.90 mmole Na_2SO_4	26.7	25.8,26.1	97.4

[a] Values obtained with 26.7 μg Sc were corrected for respective blanks.
[b] Fe(III) reduced with hydrazine dihydrochloride prior to TTA extraction.
[c] Dilute HCl solution was filtered through a plug of glass wool prior to color development.
[d] TTA extraction from 0.03 *M* ($HClO_4$ + HNO_3) solution.

The first TTA extraction (and back-extraction) gave a recovery of 93% and the second TTA extraction 1% of scandium.

It appears that a large amount of nitrate slightly decreases the recovery of scandium. When 1.6 mmole of nitrate, as magnesium nitrate, was present, 90% of scandium was recovered. When the nitrate was replaced by chloride, 95% of scandium was recovered (Table III). It is of interest that iron(II) is completely separated from scandium, whereas iron(III) is not[7]. Molybdenum(VI), titanium, uranium(VI), and vanadium-(V) are considerably extracted with TTA. Thorium cannot be separated from scandium; 96% of thorium was recovered under the conditions described in the procedure. The presence of 0.90 mmole of sodium sulfate did not change the recovery of thorium. Zirconium is extracted with TTA, but it is not back-extracted with 2 *M* hydrochloric acid. Small amounts of fluoride and phosphate do not interfere with the extraction of scandium. This would be very valuable, because fluoride and phosphate cause a negative interference in most of the photometric methods for scandium; *e.g.* the 4-(2-pyridylazo)resorcinol[4] and xylenol orange[1] methods.

The average recovery of scandium for 24 sets of experiments is 94.6% with a range of 90.6 to 98.5%.

8-Quinolinol–chloroform extraction

Because aluminium, copper, and iron(III) cannot be separated from scandium by the TTA extraction, 8-quinolinol–chloroform extraction was studied. As shown in Table IV, these interfering elements are extracted as 8-quinolinolates, while scandium

TABLE IV

DETERMINATION OF SCANDIUM AFTER 8-QUINOLINOL–CHLOROFORM EXTRACTION

Addition	*Sc taken (μg)*	*Sc found*[a] *(μg)*	*Average recovery (%)*
None	0	0.0,0.0	—
None	26.7	25.7,25.8	96.6
1.0 mg Al, 1.0 mg Fe(III)	0	0.3,0.0	—
1.0 mg Al, 1.0 mg Fe(III)	26.7	24.5,24.5	91.8
1.0 mg Cu	0	0.2,0.2	—
1.0 mg Cu	26.7	26.0,25.3	96.3
0.98 mg Mo(VI)	0	0.4,0.4	—
0.98 mg Mo(VI)	26.7	25.7,25.3	95.5
0.97 mg Ti	0	0.0,0.0	—
0.97 mg Ti	26.7	25.5,25.0	94.8
1.0 mg V(V)	0	2.4[b],2.4[b]	—
1.0 mg V(V)	26.7	20.0[b],21.0[b]	76.8

[a] Values obtained with 26.7 μg Sc were corrected for respective blanks.
[b] Before extraction chloroform was shaken with six separate portions of water[13].

remains in the aqueous phase. Molybdenum and titanium also are extracted. Extraction of vanadium is not complete. If the sample contains much vanadium (and iron-(III), titanium, and zirconium), cupferron–chloroform extraction[12] would be applicable.

Separation of scandium from uranium

Extraction of uranium(IV) cupferrate with chloroform from 1 *M* hydrochloric acid was tried without success. Hydroxylamine hydrochloride and hydrazine dihydrochloride were used to reduce uranium(VI) to (IV).

Uranium(VI) forms anionic chloride complexes in 4–12 *M* hydrochloric acid and it is adsorbed on a strong-base anion exchange resin[14]. On the other hand, scandium is slightly adsorbed in 12 *M* hydrochloric acid[14]. These form the basis for the development of the proposed procedure. As shown in Table V, as much as 3 mg of uranium can be completely removed. Under the conditions described in the procedure, the following elements also would be adsorbed on the resin almost quantitatively[14]: Mo(VI), W(VI), Fe(III), Ru(IV), Os(IV), Co(II), Ir(IV), Pd(II), Pt(IV), Cu(II), Au (III), Zn(II), Cd(II), Hg(II), Ga(III), In(III), Tl(III), Ge(IV), Sn(IV and II), As(III), Sb(V and III), and Bi(III).

TABLE V

DETERMINATION OF SCANDIUM AFTER SEPARATION FROM URANIUM OR THORIUM

Addition	*Sc taken (μg)*	*Sc found[a] (μg)*	*Average recovery (%)*
	Anion exchange using hydrochloric acid		
None	26.7	25.8,27.0	98.9
1.0 mg U	0	0.1,0.3	—
1.0 mg U	26.7	24.8,26.2	95.5
3.0 mg U	0	0.3,0.6	—
3.0 mg U	26.7	25.7,26.5	97.8
10 mg U	0	3.8,2.8	—
10 mg U	26.7	25.3,24.8	94.0
	Anion exchange using nitric acid		
None	26.7	25.0,25.5	94.8
0.207 mg Th	0	0.0,0.0	—
0.207 mg Th	26.7	25.5,25.3	95.1
0.518 mg Th	0	0.0,0.0	—
0.518 mg Th	26.7	26.3,26.2	98.5
1.03 mg Th	0	0.2,0.1	—
1.03 mg Th	26.7	25.5,26.0	96.6

[a] Values obtained with 26.7 μg Sc were corrected for respective blanks.

Separation of scandium from thorium

Thorium(IV) forms anionic nitrate complexes in nitric acid and it is adsorbed on an anion-exchange resion (Dowex 1)[15–17]. On the other hand, scandium does not form adsorbable nitrate complexes[17,18]. As shown in Table V, 27 μg of scandium is separated from as much as 1 mg of thorium. This method is simpler than the method that involves the removal of thorium by precipitation as the iodate[12].

RECOMMENDED PROCEDURES

TTA–xylene extraction

Evaporate the sample solution which contains 10 to 50 μg of scandium in dilute hydrochloric acid to dryness. Dissolve the residue in 0.9 ml of 0.5 *M* hydrochloric acid, add 4 ml of water, and transfer the solution to a 60-ml separatory funnel. Wash

the vessel used for the evaporation with two 5-ml portions of water. If necessary, confirm that the pH of the solution is 1.5 ± 0.2. Shake the solution for 5 min with 10 ml of TTA solution. When the layers have separated, drain off the aqueous phase into a second separatory funnel. Add 10 ml of TTA solution to the second separatory funnel. Shake the system for 5 min and discard the aqueous phase. Combine the second extract with the first and wash the extracts by equilibrating them for 10 sec with 10 ml of 0.03 *M* hydrochloric acid. Discard the aqueous phase.

Shake the organic phase for 5 min with 20 ml of 2 *M* hydrochloric acid. Transfer the aqueous phase to another separatory funnel. Discard the organic phase. Shake the aqueous phase for 10 sec with 10 ml of xylene. Transfer the aqueous phase to a 50-ml beaker and evaporate to dryness. To the residue add 1 ml of 70% perchloric acid and 1 ml of concentrated nitric acid. Evaporate the solution to dryness. Wash the sides of the beaker with a minimum amount of water and evaporate the solution to dryness. Then proceed as described in *Spectrophotometric determination of scandium.*

8-Quinolinol–chloroform extraction

To a 10-ml aqueous solution (pH 2–3) add 1.0 ml of 8-quinolinol solution, 1.0 ml of 3.5 *M* ammonium acetate solution, and 3 ml of water. The pH of the solution should be in the range 4.5–4.7. Add 20 ml of chloroform and shake the system for 1 min. When the layers have separated, drain off and discard the organic phase. Add 0.3 ml of 8-quinolinol solution and 20 ml of chloroform and equilibrate the phases for 1 min. Discard the organic phase. Wash the aqueous phase by shaking it for 10 sec with 20 ml of chloroform and discard the organic phase. Transfer the aqueous phase to a 50-ml beaker and evaporate to dryness. To the residue add 3 ml of concentrated hydrochloric acid and 1 ml of concentrated nitric acid. Cover the beaker with a watch glass and heat the solution. When a vigorous reaction ends, remove and wash the watch glass and evaporate the solution to dryness. Then proceed as described in *Spectrophotometric determination of scandium.*

Separation of scandium from uranium(VI)

Evaporate the sample solution in a beaker to dryness. Dissolve the residue in 10 ml of 8 *M* hydrochloric acid. Pass the solution through the prepared resin column at a rate of about 2 ml per min. Wash the beaker and resin with four 10-ml portions of 8 *M* hydrochloric acid. Evaporate the effluent in a 100-ml beaker to dryness. Then proceed as described in *Spectrophotometric determination of scandium.* The uranium can be eluted with water.

Separation of scandium from thorium

Evaporate the sample solution in a beaker to dryness. Dissolve the residue in 10 ml of 8 *M* nitric acid. Pass the solution through the prepared resin column at a rate of about 2 ml per min. Wash the beaker and resin with four 10-ml portions of 8 *M* nitric acid. Evaporate the effluent in a 100-ml beaker to dryness. To the residue add 1 ml of 6 *M* hydrochloric acid and evaporate the solution to dryness. Then continue the determination as described below. The thorium can be eluted with 0.5 *M* nitric acid.

Spectrophotometric determination of scandium

Dissolve the residue in 1.0 ml of 0.1 *M* hydrochloric acid. Add 4 ml of water, mix,

and add 2.0 ml of arsenazo solution. Add 2.5 ml of acetic acid–sodium acetate buffer solution and 1.0 ml of 0.1 *M* ammonium hydroxide. Confirm that the pH of the solution is 6.1–6.3. Transfer the solution to a 25-ml volumetric flask and dilute to the mark with water. Measure the absorbance of the solution in a 1-cm cell at 570 mμ, using water or reagent blank as the reference.

Construct the calibration curve by taking, for example, 0, 10, 20, 30, and 50 μg of scandium, adding 1.0 ml of 0.1 *M* hydrochloric acid, and proceeding as described above.

A blank determination should accompany any separation step.

SUMMARY

Arsenazo is used for the spectrophotometric determination of scandium in the range 10 to 50 μg. The absorbance is measured at 570 mμ and pH 6.1. A method is proposed for the successive determination of scandium and thorium. Scandium is separated from magnesium, calcium, rare earths, zirconium, fluoride, phosphate, and some other metals by extraction with TTA in xylene. Copper, aluminum, and iron(III) are removed by 8-quinolinol–chloroform extraction. Uranium(VI) is removed by anion exchange using hydrochloric acid. Thorium is separated from scandium by anion exchange using nitric acid.

RÉSUMÉ

L'arsénazo est utilisé pour le dosage spectrophotométrique du scandium (teneurs de 10 à 50 μg). Une méthode est proposée pour le dosage successif du scandium et du thorium. Le scandium est séparé du magnésium, du calcium, des terres rares, du zirconium, des fluorures, des phosphates et d'autres métaux par extraction au moyen de TTA (thénoyltrifluoroacétone) dans le xylène. Cu, Al et Fe(III) sont éliminés par extraction hydroxy-8-quinoléine–chloroforme. U(VI) est séparé par échange d'anions, en milieu chlorhydrique. Th est séparé de Sc par échange d'anions en milieu nitrique.

ZUSAMMENFASSUNG

Es wird die spektralphotometrische Bestimmung des Scandiums mit Arsenazo im Bereich von 10–50 μg beschrieben. Die Absorption wird bei 570 mμ und einem pH-Wert von 6.1 gemessen. Es wird eine methode zur Bestimmung des Scandiums neben kleinen Mengen Thorium vorgeschlagen. Durch Extraktion mit einer Lösung von TTA in Xylol wird Scandium von Magnesium, Calcium, Seltenen Erden, Zirkonium, Fluorid, Phosphat und einigen anderen Metallen getrennt. Kupfer, Aluminium und Eisen(III) werden mit einer 8-Hydroxychinolin–Chloroform-Lösung extrahiert. Uran(VI) wird aus salzsaurer, Thorium aus salpetersaurer Lösung vom Scandium mit einem Anionen-Austauscher abgetrennt.

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GROWTH KINETICS OF BARIUM SULPHATE SUSPENSIONS

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The precipitation kinetics and mechanism of the formation of barium sulphate precipitates have been studied extensively over the past few years[1-6]. Unfortunately, there has been almost no agreement between various workers and as WEISS[7] has pointed out, under similar conditions surface reaction-controlled growth has been reported with reaction order ranging from zero to four and in some cases diffusion of ions to the growing crystallites has also been found to be rate-controlling. WEISS lists several factors affecting the rate of precipitation, some of which will also affect the kinetic analysis. Probably the major factor which has been overlooked in most attempts to obtain a rate law is the peculiar irregularity in shape of barium sulphate particles precipitating from aqueous solution. In addition, coagulation and sedimentation[8] may also be important under certain conditions.

Although these and many other problems render kinetic analyses difficult, the rate laws developed by NIELSEN[9] form a useful basis for such studies. Most data which are available for barium sulphate precipitation have been obtained by interpretation of the decrease in conductivity of solutions as the solid product formed. This technique is not entirely suitable because platinum electrodes have been found to exert a strong catalytic influence upon the reaction[7] and in addition the nature of the precipitate can only be inferred. The nephelometric technique has the advantage that the direct effect of the growing particles is measured, but has the disadvantage that the interpretation of such results may be extremely difficult. For spherical or improperly formed cubic particles spectrophotometric measurements appear to give results in reasonably good accord with theory[10,11]. Barium sulphate particles are irregularly shaped and consequently nephelometry affords only an estimate of the required parameters. The use of such a technique was introduced by NIELSEN[12], who obtained semi-quantitative data for the kinetics of growth of fairly large (*ca.* 5 μ) barium sulphate particles. Under these circumstances a fairly simple relationship may be used to convert the measured turbidity into suitable growth parameters. In a previous publication[13] the authors have outlined a different method of interpreting the results obtained by spectrophotometric transmission measurements and it is the intention here to present an extension of this method to a kinetic analysis of barium sulphate precipitation. In particular, attention was directed toward the determination of the effect of excess of either barium or sulphate ions on the specific rate of precipitation.

EXPERIMENTAL

Equal volumes of A.R. barium chloride and sodium sulphate solutions of the required concentration were injected into, and mixed in, a 1-cm spectrophotometer cell. The development of the suspension was followed by measuring the extinction of transmitted light ($\lambda = 5460$ Å *in vacuo*). Readings were taken every 30 sec, until the turbidity became constant. The theoretical interpretation of the data obtained by this method has been outlined elsewhere, but consists of conversion of the turbidity data to particle numbers and sizes[9]. Purification of the reactant solutions presents an interesting and apparently unique problem. If stringent steps are taken to remove as many heterogeneous nuclei as possible from the reactant solutions, then the product consists of large barium sulphate particles which sediment rapidly[7]. The theoretical analysis thus becomes extremely difficult[8]. Alternatively if the impurities are not removed, small barium sulphate particles are produced which are much more suitable for nephelometric analysis. The precautions which we have taken are intermediate. All cells and apparatus were steam cleansed and solutions filtered through 100 mμ Millipore filters. Thus the larger impurities which might impair the transmission measurements were removed. The nuclei, around which the barium sulphate crystallizes, are of unknown size but previous considerations led us to the conclusion that such nuclei are probably small enough to cause no significant errors in the interpretation of results.

THEORY AND RESULTS

It is apparent from a survey of previously published work that attempts to represent any particular run in terms of a given rate equation would probably not be particularly meaningful. Alternatively it was necessary to use some form of rate equation before a specific constant could be evaluated. For this reason the equation which would best represent the growth rate for some 28 runs was determined by the following method.

The times at which the fraction of the total solute precipitated (X) was equal to 0.4 and 0.6 and were obtained from the experimental results for each run.

$$X = \left(\frac{r}{r_F}\right)^3$$

where r = radius of average particle at time t and r_F = final radius of average particle; r was obtained from the nephelometric analysis by use of MIE theory. The main source of error at this point was the assumption that the particles were spherical. For comparative purposes, however, the assumption is not serious.

The ratio of these two times ($t_{0.6}/t_{0.4}$) averaged over all runs was then compared with the theoretical values for diffusion and surface reaction-controlled growth obtained from published Tables[9]. The results are demonstrated in Table I.

TABLE I

COMPARISON OF THE EXPERIMENTAL GROWTH RATE WITH THEORETICAL VALUES FOR REACTIONS OF VARIOUS ORDER[a]

$\frac{I_D(0.6)}{I_D(0.4)}$	$\frac{I_1(0.6)}{I_1(0.4)}$	$\frac{I_2(0.6)}{I_2(0.4)}$	$\frac{I_3(0.6)}{I_3(0.4)}$	$\frac{I_4(0.6)}{I_4(0.4)}$	$Expt.\ \frac{(0.6)}{(0.4)}$
1.52	1.26	1.45	1.79	2.35	1.39

[a] The standard deviation for the experimental quantity was 0.225.

Rate data obtained in this manner fall closest to a second order process, but both diffusion and first order surface-controlled processes yield time ratios falling within one standard deviation and in view of the approximations made, should be regarded as equally likely.

The diffusion-controlled process can, however, be investigated on a quantitative basis by using equations developed by HAM[14].

When $X < 0.5$ we may write

$$1 - X \sim \exp - \left[\frac{2D\varrho_0^{1/3}t}{r_s^2\varrho_c^{1/3}}\right]^{3/2} \qquad (1)$$

$$r_s^3 = {}^3/_4\pi N$$

where N is number of particles/ml, ϱ_0 is the total mass of precipitate as $t \to \infty$, D is the diffusion coefficient, and ϱ_c is the density of the precipitate.

Theoretically then the time at which the reaction is 40% complete is of the order of 1–10 sec. In practice a value of 1–5 min was found, indicating that the controlling factor is probably not the simple rate of diffusion of ions from solution.

Of the equations which have been investigated, the one which best fits the arbitrary situation of 0, 40% and 60% development of the precipitate, the second order relation, was therefore preferred. By choosing a specific fraction of the total precipitate formed (*i.e.* $X = 0.6$), the rate constant K_2 was obtained from

$$I_2 = \int_0^{0.6} X^{-2/3}(1 - X)^{-2}dX = K_2 m^{5/3} N^{1/3} t = 4.205 \qquad (2)$$

where m is the mass precipitated (moles/l) when $r = r_F$.

The rate constant K_2 also contains a geometrical factor. Values of K_2 are given in Tables II and III.

TABLE II

REACTION RATES IN THE PRESENCE OF EXCESS SULPHATE ION

Run	Ba^{2+}*(moles/l)*	SO_4^{2-}*(moles/l)*	$N(ml^{-1})$	$K_2 = n^{1/3}m^{5/3}$
1	$5.11 \cdot 10^{-5}$	$1.48 \cdot 10^{-3}$	$2.4 \cdot 10^8$	$1.9 \cdot 10^{-5}$
2	$5.11 \cdot 10^{-5}$	$1.48 \cdot 10^{-3}$	$2.2 \cdot 10^8$	$1.8 \cdot 10^{-5}$
3	$5.11 \cdot 10^{-5}$	$1.48 \cdot 10^{-3}$	$2.2 \cdot 10^8$	$1.9 \cdot 10^{-5}$
4	$5.11 \cdot 10^{-5}$	$1.48 \cdot 10^{-3}$	$3.3 \cdot 10^8$	$2.1 \cdot 10^{-5}$
5	$5.11 \cdot 10^{-5}$	$1.33 \cdot 10^{-2}$	$3.0 \cdot 10^8$	$2.1 \cdot 10^{-5}$
6	$5.11 \cdot 10^{-5}$	$1.33 \cdot 10^{-2}$	$1.7 \cdot 10^8$	$1.8 \cdot 10^{-5}$
7	$5.11 \cdot 10^{-5}$	$1.33 \cdot 10^{-2}$	$2.6 \cdot 10^8$	$2.0 \cdot 10^{-5}$
8	$0.99 \cdot 10^{-4}$	$1.48 \cdot 10^{-3}$	$7.1 \cdot 10^8$	$1.8 \cdot 10^{-5}$
9	$0.99 \cdot 10^{-4}$	$2.96 \cdot 10^{-4}$	$2.1 \cdot 10^8$	$1.1 \cdot 10^{-5}$
10	$0.99 \cdot 10^{-4}$	$2.76 \cdot 10^{-3}$	$4.5 \cdot 10^8$	$1.6 \cdot 10^{-5}$
11	$0.99 \cdot 10^{-4}$	$2.21 \cdot 10^{-3}$	$5.2 \cdot 10^8$	$1.6 \cdot 10^{-5}$
12	$0.99 \cdot 10^{-4}$	$9.24 \cdot 10^{-4}$	$6.2 \cdot 10^8$	$1.7 \cdot 10^{-5}$
13	$0.99 \cdot 10^{-4}$	$3.63 \cdot 10^{-3}$	$5.5 \cdot 10^8$	$1.7 \cdot 10^{-5}$
14	$0.99 \cdot 10^{-4}$	$3.26 \cdot 10^{-3}$	$1.2 \cdot 10^7$	$0.5 \cdot 10^{-5}$
28	$1.02 \cdot 10^{-4}$	$1.58 \cdot 10^{-4}$	$8.0 \cdot 10^8$	$0.9 \cdot 10^{-5}$
29	$1.02 \cdot 10^{-4}$	$1.00 \cdot 10^{-3}$	$6.9 \cdot 10^8$	$1.8 \cdot 10^{-5}$
			Average	$K_2 = 1.7 \pm 0.1 \cdot 10^{-5}$

TABLE III

REACTION RATES IN THE PRESENCE OF EXCESS BARIUM ION

Run	Ba^{2+}*(moles/l)*	SO_4^{2-}*(moles/l)*	$N(ml^{-1})$	$K_2=n^{1/3}m^{5/3}$
16	$1.33\cdot10^{-3}$	$1.00\cdot10^{-4}$	$4.5\cdot10^{8}$	$1.5\cdot10^{-5}$
17	$6.65\cdot10^{-4}$	$1.00\cdot10^{-4}$	$4.3\cdot10^{8}$	$1.4\cdot10^{-5}$
18	$1.99\cdot10^{-3}$	$0.99\cdot10^{-4}$	$5.1\cdot10^{8}$	$1.6\cdot10^{-5}$
19	$2.33\cdot10^{-3}$	$0.98\cdot10^{-4}$	$8.9\cdot10^{8}$	$1.9\cdot10^{-5}$
20	$2.53\cdot10^{-3}$	$0.95\cdot10^{-4}$	$2.1\cdot10^{8}$	$1.1\cdot10^{-5}$
21	$1.06\cdot10^{-3}$	$1.00\cdot10^{-4}$	$9.0\cdot10^{8}$	$1.9\cdot10^{-5}$
22	$3.77\cdot10^{-4}$	$1.00\cdot10^{-4}$	$1.4\cdot10^{9}$	$1.9\cdot10^{-5}$
23	$3.28\cdot10^{-3}$	$1.00\cdot10^{-4}$	$4.1\cdot10^{8}$	$1.5\cdot10^{-5}$
24	$2.78\cdot10^{-3}$	$1.00\cdot10^{-4}$	$2.1\cdot10^{9}$	$2.6\cdot10^{-5}$
25	$3.64\cdot10^{-3}$	$1.00\cdot10^{-4}$	$8.1\cdot10^{8}$	$1.9\cdot10^{-5}$
26	$2.96\cdot10^{-3}$	$1.00\cdot10^{-4}$	$1.2\cdot10^{9}$	$2.2\cdot10^{-5}$
27	$2.05\cdot10^{-3}$	$1.00\cdot10^{-4}$	$2.0\cdot10^{8}$	$2.4\cdot10^{-5}$
				Average $K_2=1.8\pm0.1\cdot10^{-5}$

DISCUSSION

Without revealing the exact order of the precipitation process the results show several important features. Except for the very early and very late stages of precipitation (which are not readily accessible by this technique), there is a strong similarity between diffusional and second order surface-controlled processes. The rate constants are remarkably consistent and suggest that the lack of reproducibility of precipitation rates, familiar to many workers in this field, is indeed due to difficulties in controlling the number of growth centres rather than inconsistencies in the kinetic process. Most significant of all was the lack of dependence of the absolute rate upon which ion was in excess. In arbitrary units the average rate with barium ions in excess was 1.78 ± 0.12 and with sulphate ions in excess was 1.65 ± 0.11.

If the reaction between surface-adsorbed ions is rate-controlling, it might have been expected that the rates in the presence of excess anion and cation would not have been equal since barium ions are preferentially adsorbed on the solid surface[15]. Alternatively, however, the form of the precipitation equations does not predict any difference between the specific rates in various excess of ions. There do not appear to be any comparable data published for the precipitation of sparingly soluble salts in the presence of strong excesses of either anion or cation, and it is not possible therefore to decide with any generality whether the precipitation equations of NIELSEN[9] are satisfactory under such conditions. The results presented here do however suggest that the precipitation is surface-controlled with order between one and two.

SUMMARY

Growth rates of barium sulphate precipitates were followed by spectrophotometric transmission measurements. Interpretation of the results enabled the rate of precipitation to be characterized in terms of the surface area of precipitate and the concentration of reagents. Consistent rate constants were obtained which show that the rate of precipitation is not dependent upon which ion (barium or suphate) is in excess.

RÉSUMÉ

Les auteurs ont suivi par des mesures spectrophotométriques les vitesses de croissance de précipités de sulfate de baryum. L'interprétation des résultats permet de caractériser la vitesse de précipi-

tation en function de la surface du précipité et de la concentration des réactifs. On constate que la vitesse de précipitation ne dépend pas d'un excès de baryum ou de sulfate.

ZUSAMMENFASSUNG

Die Wachstumsgeschwindigkeiten von Bariumsulfatniederschlägen wurden spektralphotometrisch verfolgt. Die Ergebnisse zeigen eine Abhängigkeit der Fällungsgeschwindigkeit von der Oberfläche des Niederschlages und der Konzentration der Reagenzien.

Es wurde festgestellt, dass die Fällungsgeschwindigkeit nicht von einem Überschuss an Barium- oder Sulfat-Ionen abhängig ist.

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DETERMINATION OF URANIUM(VI) BY TRI-*n*-OCTYLPHOSPHINE OXIDE EXTRACTION AND COULOMETRIC TITRATION

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The ORNL Analytical Chemistry Division has studied various schemes for the separation of uranium. A separation procedure was needed for use in conjunction with the controlled-potential coulometric titration of uranium[1,2] in a wide variety of materials, particularly in solutions of nuclear reactor fuels. Such a procedure should be quantitative, specific for uranium and easily performed, and should isolate the uranium in a final solution that is suitable for analysis by controlled-potential coulometric titration. The use of the tri-*n*-octylphosphine oxide (TOPO) extraction as a separation procedure for uranium before its determination by controlled-potential coulometry is described herein.

The TOPO extraction behavior of many elements under a variety of conditions was reported by WHITE AND ROSS[3]. The use of TOPO in the fluorimetric determination of very small amounts of uranium was also reported by WHITE[4]. This extraction procedure was extended to the colorimetric range by HORTON AND WHITE[5], who determined uranium colorimetrically in the organic phase after the addition of dibenzoylmethane. WHITE and co-workers also used the TOPO extraction for the quantitative separation of titanium[6], chromium[7], zirconium[8], cerium[9] and thorium[10].

The work of WHITE AND ROSS[3] indicated that extraction of uranium(VI) into a 0.1 *M* solution of TOPO in cyclohexane should be an excellent way of isolating it for subsequent determination by coulometric titration. They found that when either 1 *M* nitric acid or 4 *M* hydrochloric acid is used as the aqueous phase in the extraction, as much as 20 mg of uranium(VI) is extracted quantitatively in one equilibration. Therefore, 5 to 10 mg, which is the amount of uranium generally titrated by controlled-potential coulometry, should be an optimum amount to be determined in this TOPO extraction procedure. The elements that are extracted depend on whether hydrochloric acid or nitric acid is used as the extraction medium; thus, an additional degree of selectivity can be obtained by alteration of extraction conditions.

An important advantage of this extraction system is the very slight solubility of TOPO and cyclohexane in water; this allows uranium to be back-extracted and titrated directly in the stripping solution without the necessity for chemical treatment to destroy organic material. Also, TOPO extractions are characterized by rapid equilibration and good phase separation; the separation procedure should therefore be adaptable to remotely controlled operation.

* Operated by Union Carbide Nuclear Company for the U.S. Atomic Energy Commission.

EXPERIMENTAL

Instrumentation

The ORNL model Q-2005, automatic, controlled-potential coulometric titrator was used[11,12].

Extraction and titration vessels

The extraction and titration vessels used were described previously[13].

Reagents

Tri-*n*-octylphosphine oxide (Eastman Reagent No. 7440) was dissolved in cyclohexane (Eastman Reagent No. 702) to give a 0.1 *M* solution.

Mercury was washed thoroughly with 1.5 *M* nitric acid, distilled once under vacuum, and washed with 0.5 *M* sulfuric acid just before use.

Other reagents were prepared from analytical reagent grade materials.

Separation procedures

Into 5 ml of 1 *M* nitric acid, contained in the extraction vessel, pipet a test portion that is estimated to contain 5 to 10 mg of uranium(VI). Add 5 ml of a 0.1 *M* solution of TOPO in cyclohexane, stir the phases for 5 min, and discard the aqueous (bottom) phase. Add 5 ml of 3.5 *M* ammonium sulfate, stir the phases for 5 min, and drain the aqueous phase into a coulometric titration cell. Repeat the back-extraction with 3.5 *M* ammonium sulfate twice.

Some modifications to this separation procedure are required for different types of nuclear-reactor-fuel dissolver solutions. These are as follows:

Uranium–aluminum–nitric acid solutions. Place in the extraction vessel a test portion that is estimated to contain 5 to 10 mg of uranium(VI). Adjust the volume of the aqueous phase to 5 ml and the nitrate concentration to 1 *M*.

Uranium–stainless steel–aqua regia solutions. After pipetting the test portion into the cell, add 10% (w/v) hydroxylamine hydrochloride solution dropwise until the color of chromium(VI) disappears. Between the extraction and the back-extraction steps, wash the cyclohexane phase for 5 min with 5 ml of 1 *M* nitric acid; discard the aqueous phase after the wash.

Uranium–molybdenum–nitric acid solutions. Transfer 5 ml of 3 *M* nitric acid to the extraction vessel, and add a test portion that is estimated to contain 5 to 10 mg of uranium(VI). Between the extraction and back-extraction, wash the cyclohexane phase once with 5 ml of 3 *M* nitric acid. Discard the aqueous phase after the wash.

Uranium–thorium–nitric acid solutions. In a 30-ml beaker, place a test portion that is estimated to contain 5 to 10 mg of uranium(VI). Add 1 ml of 12.1 *M* hydrochloric acid; evaporate the solution to dryness. Dissolve the residue in 3 *M* hydrochloric acid, and transfer the solution to an extraction vessel. Proceed with the extraction; use 10 ml of TOPO solution.

Uranium–niobium–fluoride–nitric acid solutions. Pipet 2 ml of 12.9 *M* lactic acid into the extraction vessel and add the test portion of the sample solution. Add 5 ml of 0.1 *M* aluminum nitrate–1 *M* nitric acid solution. Proceed with the extraction; stir the phases for 10 min. Between the extraction and back-extraction, wash the cyclohexane phase with 0.5 ml of 12.9 *M* lactic acid plus 5 ml of 1 *M* nitric acid.

Uranium–zirconium–fluoride–nitric acid solutions. Extract from a 0.25 *M* sodium

fluoride–0.5 *M* nitric acic solution rather than from 1 *M* nitric acid. Between the extraction and back-extraction, wash the cylohexane phase once with 5 ml of 1 *M* nitric acid.

Titration procedure

Add 2 ml of 18 *M* sulfuric acid to the solution in the titration vessel. Place the cell in position, and deoxygenate the solution for 5 min. Prereduce the solution at +0.075 V *vs.* the S.C.E. until the current decreases to 50 μA. Coulometrically reduce uranium(VI) to uranium(IV) at −0.325 V *vs.* the S.C.E. until the current decreases to 50 μA. Record the readout voltage, Q. Then:

Uranium(VI) titrated (mg) = $Q \cdot$ coulometric factor for uranium. The derivation of the coulometric factor for uranium (for $n = 2$) is given by JONES[12].

DISCUSSION

Optimum extraction conditions

The basic separation procedure is the extraction of uranium(VI) from 1 *M* nitric acid into a 0.1 *M* solution of TOPO in cyclohexane. The extraction is essentially instantaneous but is generally carried out for 3 to 5 min to ensure quantitative extraction. Excellent phase separation is obtained almost immediately. The aqueous phase is discarded; the cylcohexane phase that remains contains uranium(VI) and is essentially free of other contaminants.

Uranium–aluminum–nitric acod solutions. The uranium(VI) in this type of sample is easily extracted provided that, in the adjustment of the nitrate content of the aqueous phase before extraction, allowance is made for the nitrate that is present in the test portion. The nitrate concentration of the aqueous phase before extraction should be about 1 *M* but may be as high as 3 *M* without significant loss of uranium in extraction. Uranium(VI) is not quantitatively extracted if the nitrate concentration exceeds 3 *M*.

Uranium–stainless steel–aqua regia solutions. Nickel(II), iron(II) and chromium(III) are not extracted by TOPO. Iron(III) is extracted by TOPO from chloride-containing media but not from nitrate containing media[3]. Chromium(VI) is extracted from both nitrate- and chloride-containing media[3]. Consequently, two modifications of the basic extraction procedure are necessary in order to accommodate dissolver solutions that are derived from the dissolution of stainless steel fuel elements in dilute aque regia (the Darex Process). First, a reducing agent, such as hydroxylamine hydrochloride, is added to ensure that chromium(VI) is reduced to chromium(III) and thus to prevent its extraction. Second, the cyclohexane phase is washed with 1 *M* nitric acid between the initial extraction and the back-extraction. Because chloride is present in this type of dissolver solution, some iron(III) is extracted along with uranium(VI). The nitric acid wash removes this iron(III) from the cyclohexane phase. Both aqueous phases are discarded; the cyclohexane phase that remains contains uranium(VI) and is free of the components of stainless steel.

Uranium–molybdenum–nitric acid solutions. Nitric acid solutions of the dejacketed molybdenum–uranium fuels generally contain small amounts of molybdenum (relative to uranium). Some iron(III) (*ca.* 0.5 *M*) may also be present. Uranium can be separated from such samples by a slightly modified basic extraction procedure.

The extractability of molybdenum(VI) into 0.1 *M* TOPO in cyclohexane from solu-

tions of the mineral acids as a function of acid concentration of the aqueous phase has been given by WHITE AND ROSS[3]. Molybdenum(VI) is readily extracted from dilute solutions (pH *ca.* 1) of hydrochloric, sulfuric, nitric and perchloric acids. However, the extractability of molybdenum from solutions of nitric and of perchloric acids decreases as the acid concentration increases. Therefore, uranium(VI) can be separated from small amounts of molybdenum(VI) by making the initial extraction from 3 *M* nitric acid and washing the cyclohexane phase once with 3 *M* nitric acid. This is the optimum nitric acid concentration that can be used in order to minimize extraction of molybdenum(VI) and at the same time to ensure quantitative extraction of uranium(VI). This separation is useful for samples in which the U : Mo weight ratio is greater than 10 : 1. When this ratio is less than 10 : 1, the results are high and the duration of the titration is extended. Since iron(III) is not extracted from the nitrate medium, its presence causes no interference.

Uranium–thorium–nitric acid solutions. Thorium is extracted from nitric acid solutions under essentially the same conditions as is uranium(VI)[3]. Accordingly, uranium(VI) cannot be separated from thorium in a nitric acid medium by TOPO extraction. Furthermore, when a nitrate medium is used, thorium may prevent the quantitative extraction of uranium(VI) by "loading" the organic phase. However, if a 3 *M* hydrochloric acid medium is used, the extraction of uranium(VI) is favored over thorium, and uranium(VI) can be separated from thorium if nitrate is absent[3]. These conditions are attained by evaporating the test portion to dryness with 12.1 *M* hydrochloric acid and redissolving the residue in 3 *M* hydrochloric acid before extraction. Small amounts of thorium are carried through this separation but do not interfere in the subsequent titration of uranium.

Uranium–niobium–fluoride–nitric acid solutions. Niobium is not extracted from a nitric acid medium into cyclohexane solutions of TOPO[3]; however, the separation procedure must be modified slightly to prevent hydrolysis of niobium during extraction. The medium that has optimum composition for separation of uranium(VI) from niobium is 1 *M* nitric acid–0.1 *M* aluminum nitrate–2.5 *M* lactic acid. Aluminum is added to complex the fluoride that is commonly present in niobium-containing solutions, and lactic acid is included to complex niobium in order to prevent its hydrolysis and/or extraction. This procedure can be used to separate uranium(VI) from solutions in which the Nb : U weight ratio is 8 : 1 or less.

Uranium–zirconium–fluoride–nitric acid solutions. Zirconium is quantitatively extracted from 7 *M* hydrochloric acid or 7 *M* nitric acid, but when the acid concentration is decreased to 1 *M*, the amount of zirconium extracted decreases greatly. The extractability of zirconium is also retarded by the presence of fluoride[3]. Therefore, the separation of uranium(VI) from dissolver solutions that contain zirconium is made from an extraction medium that is 0.5 *M* in nitric acid and 0.25 *M* in sodium fluoride. A small amount of zirconium may be carried through this separation, but it does not interfere in the subsequent titration of uranium(VI).

Optimum back-extraction conditions

A number of reagents were tested for back-extracting uranium(VI) from a 0.1 *M* solution of TOPO in cyclohexane. Generally, reagents of high pH, such as carbonates, strip uranium(VI) from the cyclohexane phase, but the phase behavior is unsatisfactory. Highly concentrated solutions of phosphate salts strip uranium almost quanti-

tatively, and the phase separations are good. However, these solutions are inconvenient for routine analytical use because uranium phosphate precipitates slowly after the back-extraction. The weaker-complexing hydroxy acids (*i.e.*, citric, tartaric and lactic) do not strip the uranium(VI). Adequate stripping is obtained by three equilibrations with 2 *M* sodium sulfate (pH = 1), and the phase separations are good; however, this reagent is inferior to ammonium sulfate. Phase separations are excellent when 3.5 *M* ammonium sulfate (pH = 2) is used; the uranium(VI) is removed quantitatively from the cyclohexane phase in three equilibrations and very nearly quantitatively in two equilibrations (Table I). The recovery can be made quantitative in two passes by making a 50% (v/v) dilution of the TOPO–cyclohexane phase with cyclohexane before back-extracting with ammonium sulfate.

TABLE I

RECOVERY OF URANIUM FROM TOPO–CYCLOHEXANE SOLUTION BY EXTRACTION INTO 3.5 *M* AMMONIUM SULFATE (pH = 2)

Number of back-extractions		*Uranium(VI) recovered (%)*[a]
2 min each	*5 min each*	
—	1	91.0
—	2	99.8
—	3	100.0
2	—	99.5
3	—	99.8

[a] The amount of uranium recovered was determined by controlled-potential coulometric titration.

Optimum titration conditions

The uranium is recovered from the cyclohexane medium with ammonium sulfate; it can then be determined directly in the stripping medium by controlled-potential coulometric titration. After the addition of 18 *M* sulfuric acid, uranium(VI) is titrated in the ammonium sulfate medium exactly as it is in the sulfuric acid medium[2].

RESULTS

Simulated dissolver-solution samples were prepared to contain known amounts of uranium(VI). From these solutions, test portions that contained 5 to 10 mg of uranium were analyzed for uranium content by the procedures described above. A number of analyses were made on each type of solution in order to measure the precision and accuracy of the complete determination. The results are summarized in Table II.

This work has shown that the extraction of uranium into cyclohexane solutions of TOPO is well suited for the separation of uranium(VI) before its determination by controlled-potential coulometric titration. The separation is quantitative, easily performed, and isolates uranium(VI) from elements that interfere in the titration. Because uranium(VI) can be titrated directly in the back-extractant, this separation may in some cases be preferred to separation by hexone extraction[14], in which traces of organic material must be destroyed by a fusion process before the titration.

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TABLE II

PRECISION AND ACCURACY OF RESULTS OF DETERMINATION OF URANIUM(VI) IN SYNTHETIC DISSOLVER SOLUTIONS

Related reactor	*Dissolver solution components*			*Results*	
	Identity	*M*	*~mg/ml*	*Uranium(VI) recovered (%)*	*S (%)*
(Standard solution)	UO_2SO_4	0.03	7	0.1	100.0
Foreign Research	U(VI)	0.03	7	0.1	100.1
	Al(III)	1.5			
	Hg(II)	0.01			
	HNO_3	1.3			
Army Package Power	U(VI)	0.02	5	0.1	100.1
	Cr(VI)	—			
	Fe(III)	0.8			
	Ni(II)	—			
	HCl	1.3			
	HNO_3	3			
N. S. Savannah	U(VI)	0.55	130	0.1	99.9
	S. steel	1.4			
	HCl	1.7			
	HNO_3	1.5			
Consolidated Edison	U(VI)	0.06	15	0.2	100.2
	Al(III)	0.1			
	Th(IV)	1.0			
	F^-	0.04			
	HNO_3	8.8			
Consumers Public Power	U(VI)	0.63	150	0.2	99.8
	Mo(VI)	0.01			
	HNO_3	4			
Fermi	U(VI)	1	240	0.2	99.7
	Fe(III)	0.5			
	Mo(VI)	0.07			
	HNO_3	3.7			
Experimental Boiling Water	U(VI)	0.07	18	0.2	99.9
	Al(III)	0.8			
	F^-	0.8			
	Zr(IV)	0.04			
	HNO_3	5			
(Experimental fuel)	U(VI)	0.02	5	0.2	99.7
	Nb(V)	0.04			
	HNO_3	5			

SUMMARY

Uranium can be determined in the usual types of dissolver solutions by extraction of uranium (VI) into a cyclohexane solution of tri-*n*-octylphosphine oxide (TOPO), back-extraction into an ammonium sulfate solution, and coulometric titration at controlled potential. Optimum conditions were established for the extraction and back-extraction, and the overall performance of the method was evaluated. The method is accurate, precise, and widely applicable. It should be very useful in nuclear reactor technology.

RÉSUMÉ

Une méthode est proposée pour le dosage de l'uranium(VI). On procède par extraction au moyen d'une solution d'oxyde de tri-*n*-octyle phosphine dans le cyclohexane, contre-extraction dans une solution de sulfate d'ammonium et titrage coulométrique à potentiel contrôlé. Cette méthode est exacte et précise et trouve de nombreuses applications. Elle pourrait être très utile dans la technologie de réacteurs nucléaires.

ZUSAMMENFASSUNG

Beschreibung einer Methode zur Bestimmung von Uran-(VI) durch Extraktion mit Tri-*n*-octylphosphinoxyd in Cyclohexan und Rückextraktion mit Ammoniumsulfatlösung. Die Bestimmung selbst erfolgt durch coulometrische Titration mit kontrolliertem Potential. Die Methode ist genau und hat viele Anwendungsmöglichkeiten, *u.a.* auch bei Kernreaktoren.

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REMOVAL OF INTERFERENCES IN ABSORPTIOMETRIC DETERMINATION OF BISMUTH WITH THORIN*

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Many elements interfere in the simple absorptiometric determination of bismuth with thorin (2-(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid or its disodium salt)[1]. Other photometric determinations of bismuth[2-4] show similar difficulties. The present report deals with separations aimed at solving such interference poblems.

EXPERIMENTAL

Apparatus

A Beckman B spectrophotometer (1-cm cell) was used for absorptiometric determinations. The ion-exchange columns were as shown in Fig. 1.

Reagents

Resin. Dowex 21K anion-exchange resin (50–100 mesh) was employed after purification by washing successively with 10% nitric acid, de-ionized water, ethanol–acetone (1 : 1), carbon tetrachloride, ethanol–acetone (1 : 1), de-ionized water, 10% sodium hydroxide and de-ionized water. The resin was finally converted to its chloride form by passing a *ca.* 0.25 *M* HCl solution through the column. The resin bed was about 9 ml in volume and 7 cm long.

Solutions were prepared from A.R. quality reagents and Specpure metals or oxides obtained from Johnson, Matthey and Co., London, as reported earlier[1].

Buffer-cyanide solution. 25 g of diammonium citrate, 5 g of sodium sulfite, and 5 g of potassium cyanide were dissolved in about 800 ml of de-ionized water. Ammonia was added to obtain a pH of 9 and lead was removed by shaking with small portions of 0.02% dithizone in carbon tetrachloride. The dithizone dissolved in the aqueous phase was removed by shaking with chloroform. The pH was finally adjusted to near 10 by adding pure ammonium hydroxide and the solution diluted to 1 l with de-ionized water. This solution was stored in a polyethylene bottle.

Procedure

The proposed course of analysis may be outlined as follows.

Dissolve the sample under acid-oxidizing conditions in an open Pyrex test tube or in a small beaker to remove volatile interferences by distillation[5]. The first distilla-

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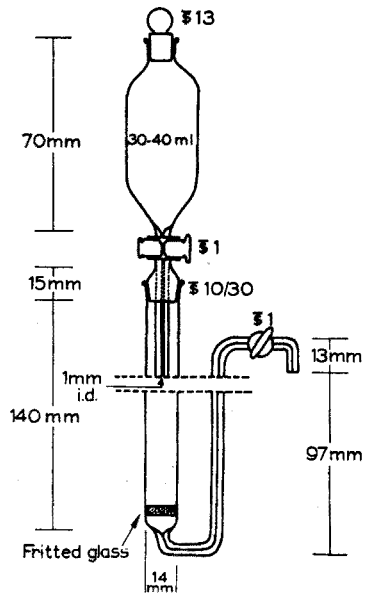

Fig. 1.

tion with 0.5–0.7 ml of each of the following acids: hydrochloric, nitric and perchloric (with or without sulfuric), with fuming to near dryness, completely eliminates osmium, ruthenium and germanium; selenium, mercury and rhenium are partially removed. A second distillation, with HCl and HBr, eliminates rhenium, selenium, mercury, arsenic, antimony and tin; the volume of the acid mixture is 2 to 3 ml. Remove the hydrochloric acid finally by fuming with perchloric acid. Each distillation must be repeated at least 3 or 4 times to remove 10 mg of any interference.

Make the residual acid solution approximately 0.25 *M* in hydrochloric acid (volume *ca.* 15 ml) and pass it through the ion-exchange column in equilibrium with *ca.* 0.25 *M* HCl at a flow rate of 1–2 ml/min.

Wash the column with at least 30 ml of 0.25 *M* hydrochloric acid. Elute the bismuth with 40 ml of 1 *M* sulfuric acid. Evaporate to near dryness. Cool the solution and neutralize the acid with pure ammonium hydroxide. Add about 20 ml of buffer-cyanide solution and extract bismuth with 5-ml portions of a 0.01% solution of dithizone in carbon tetrachloride (or chloroform). Collect the organic extracts and wash them with de-ionized water. Draw off the aqueous phase and shake the organic phase with 15 ml of 2 *M* hydrochloric acid for 3 min. Discard the organic phase. Wash the acid solution with 5 ml of organic solvent to remove droplets of dithizone. Transfer the aqueous phase to a small beaker, evaporate to near dryness and finally fume with nitric–perchloric acid mixture until a clear solution is obtained. Determine bismuth by the thorin method[1] after adjusting the medium.

Alternatively, after extracting bismuth with CCl_4–dithizone, transfer the washed organic phase to a dish and evaporate to dryness on a water bath. Destroy organic matter with 0.5 ml of concentrated nitric acid and 0.2 ml of perchloric acid added repeatedly. Finally fume with perchloric acid until clear. Determine bismuth with thorin as before.

This extraction with dithizone is incorporated in the course of analysis to remove sulfate ions which interfere in the determination of bismuth with thorin.

RESULTS AND DISCUSSION

Separation of volatile elements

Despite the good results obtained by the use of this separation technique, and the frequent use of acid attack of samples, it has seldom been applied for the elimination of interferences in the photometric determination of bismuth. An advantage is that large amounts of foreign material do not hinder the separation[6]. Bismuth may be lost in very small amounts by volatilization as bromide[7]; the results obtained for bismuth (0, 20 and 100 μg) in the present work showed that if there is any loss it is less than the experimental error associated with the thorin determination.

The use of sulfuric acid allows higher temperatures, an easier sample attack and a more rapid removal of interferences; however, the formation of insoluble substances with some samples may be a serious inconvenience, hence a final perchloric acid medium was preferred in the present work. Determination of bismuth with iodide (see Table I)

TABLE I

SEPARATION OF BISMUTH FROM INTERFERING ELEMENTS BY DISTILLATION AND ANION-EXCHANGE ON DOWEX 21K FROM *ca.* 0.25 *M* HCl

(Results are averages of three individual determinations. Bismuth determined by the thorin method, unless otherwise specified)

Foreign element	*Amount added (mg)*	*Bismuth added (μg)*	*Bismuth found (μg)*	*Foreign element*	*Amount added (mg)*	*Bismuth added (μg)*	*Bismuth found (μg)*
		0.0	0.2[a]	As (as Na_2AsO_4)	10	0.0	0.0[b]
			0.0[b]			20.0	17.9[b]
			0.0[c]			100.0	98.4[b]
		5.0	4.9[a,b]	Sn(IV)	5	0.0	0.5[b]
						20.0	20.8[b]
		50.0	49.2[a]			100.0	100.3[b]
			49.0[b]				
			50.6[c]	Sb(V)	5	0.0	0.0[b]
						20.0	19.0[b]
		100.0	99.6[a]			100.0	99.0[b]
			99.0[b]				
			98.7[c]	In	10	0.0	0.6[a]
							0.4[b]
Os (as $OsCl_6^{2-}$)	5	0.0	0.3[b]			5.0	4.9[a]
		20.0	20.2[b]			50.0	51.0[a]
		100.0	101.0[b]				48.0[b]
						100.0	99.0[a]
Ru (as $RuCl_4$)	10	0.0	0.0[b]				98.8[b]
		20.0	20.0[b]				
		100.0	100.1[b]	Pb(II)[d]	10	0.0	0.1[a]
							0.0[b]
Ge (as Na_2GeO_3)	10	0.0	0.0[b]			50.0	47.0[a]
		20.0	18.2[b]				50.5[b]
		100.0	98.4[b]			100.0	98.0[a]
							99.0[b]
Se (as Na_2SeO_3)	10	0.0	0.0[b]				
		20.0	19.0[b]	Fe(III)	10	0.0	0.1[a]
		100.0	98.0[b]				0.0[b]
						5.0	4.0[a]
Re (as ReO_4^-)	10	0.0	0.3[b]			50.0	47.2[a]
		20.0	18.9[b]				48.0[b]
		100.0	97.9[b]			100.0	97.3[a]
							98.0[b]
Hg(II)	10	0.0	0.0[b]				
		20.0	18.7[b]	Cr (as $K_2Cr_2O_7$)[e]	10	0.0	0.0[a]
		100.0	100.0[b]			50.0	50.0[a]
						100.0	98.0[a]

TABLE I (Continued)

Foreign element	*Amount added (mg)*	*Bismuth added (μg)*	*Bismuth found (μg)*
Cr(III)	10	0.0	1.5[a]
			0.0[b]
			0.0[f]
		5.0	4.9[b]
		50.0	49.0[a]
			49.4[b]
			50.0[f]
		100.0	102.0[a]
			101.0[b]
			101.3[f]
Ag[g]	10	0.0	0.5[a]
			0.7[b]
		50.0	46.5[a]
			48.0[b]
		100.0	96.5[a]
			96.7[b]
Pt(IV)	10	0.0	0.8[a]
			0.8[f]
		50.0	48.0[a]
			49.0[b]
		100.0	97.5[a]
			102.0[b]
			99.2[f]
Au	10	0.0	1.0[a]
			1.5[f]
		50.0	47.1[f]
		100.0	99.5[a]
			97.5[f]
Zr	10	0.0	0.0[a]
			0.0[b,f]
		50.0	7.0[f]
			48.5[b]
		100.0	98.2[a,b]
			42.0[f]
Ce(IV)	10	0.0	1.2[a]
			4.0[f]
			3.5[b]
		50.0	53.0[b,f]
		100.0	103.0[b]
			101.0[a]
			102.1[f]
Ce(III)	10	0.0	0.4[a]
			0.7[b]
		50.0	50.0[a]
			49.2[b]
		100.0	101.5[a]
			100.1[b]
Ir (as $(NH_4)_2IrCl_6$)	2	0.0	0.0[a]
			0.1[b]
		50.0	51.0[b]
		100.0	60.0[f]
			97.0[a]
			102.0[b]
Pd(II)	10	0.0	0.0[a,f]
		50.0	46.0[f]
		100.0	98.0[a]
			95.0[b,f]
Rh (as $RhCl_3$)	10	0.0	1.0[a]
			1.3[b]
		50.0	49.8[a]
			49.9[b]
		100.0	99.5[a]
			99.0[b]
Th	10	0.0	0.5[a]
			1.0[b]
		50.0	50.1[a]
			48.2[b]
		100.0	99.0[a]
			97.6[b]
Te (as Na_2TeO_4)	10	0.0	0.0[a]
			1.3[b]
		50.0	49.8[b]
		100.0	100.0[a]
			102.0[b]
Sc	5	0.0	0.5[a]
			1.2[b]
		50.0	47.0[b]
		100.0	98.1[a]
			97.5[b]
Be (5 mg), Al (5 mg)		0.0	0.4[b]
		50.0	48.0[b]
		100.0	98.5[b]
Mg (2 mg), Ca (2m g) Sr (2 mg) Ba (2 mg)		0.0	0.7[b]
		50.0	49.2[b]
		100.0	99.0[b]
U (as UO_2^{2+})	10	0.0	0.1[a,b]
		50.0	52.0[b]
		100.0	101.0[a]
			102.5[b]
H_2SO_4[h]	0.5 ml conc. acid	0.0	0.2[f]
		50.0	49.0[f]
		100.0	99.3[f]
	0.7 ml conc. acid	50.0	40.0[f]
		100.0	81.0[f]
PO_4^{3-}	10.6	0.0	1.0[a]
			1.5[b]
		50.0	48.0[a]
			47.0[b]
		100.0	97.5[a]
			98.0[b]

[a] The hydrochloric acid solution (*ca.* 0.25 *M*) was passed directly through the column without prior distillation.
[b] Distillation without sulfuric acid.
[c] Distillation with sulfuric acid. Determination of bismuth with iodide[2].
[d] Lead is not completely eliminated during the washing of column and an amount estimated as about 200 μg accompanies bismuth in the eluate. This amount is smaller than the interfering limit for lead in the thorin method. If the washing of the column is repeated before elution of the bismuth no lead appears in the eluate.
[e] Cr(VI) is slowly reduced to Cr(III) by the resin. Results obtained without delay.
[f] Distillation with sulfuric acid. Determination of bismuth with thorin.
[g] AgCl is precipitated and mechanically retained by the resin. It may be removed by centrifugation before passing the solution through the column.
[h] These experiments were performed in order to determine how much sulfuric acid as concentrated and may be tolerated in the acid residue of distillation.

has shown the effectiveness of separations with sulfuric acid in absence of elements which may form an insoluble residue.

The most serious interference removed in this step is ruthenium, which can only be tolerated in amounts up to 10 μg/25 ml when determining bismuth with thorin[1]. Antimony and tin are also eliminated as well as less serious interferences such as arsenic or mercury.

Separation by anion-exchange resin

KRAUS AND NELSON[8] have reported the behavior of most elements over the hydrochloric acid range 0 to 12 *M* on Dowex 1 ion-exchange resin; numerous feasible analytical separations are possible by simple control of the acid concentration. Of the various possibilities a sorption step at *ca.* 0.25 *M* hydrochloric acid appears the most promising, since the most serious interferences in the thorin determination, after the distillation step, are thus removed. For instance, Y, Sc, lanthanides, Ti(III) and Ti(IV), Zr, Hf, Th, V(IV) and V(V), U(IV) and U(VI), Fe(II) and Fe(III), Ga and In are eliminated since they are not appreciably adsorbed by strong anion-exchange resins. Lead, cobalt(II), copper and aluminum, which interfere in amounts ranging from 0.5 to 1 mg/25 ml[1], are removed in the same way.

A concentration of hydrochloric acid below *ca.* 0.25 *M* should be avoided because of the hydrolysis of bismuth. Hydrochloric acid concentrations much higher than 0.25 *M* are less desirable because of the separations obtained.

The removal of bismuth from the column with hydrochloric acid solutions is impractical with the type of resin used (with relatively high cross-linkages, the distribution coefficient for bismuth is high over the entire range of hydrochloric acid concentration). Removal in a suitable narrow band may be achieved with 1 *M* sulfuric acid solution[9]. The chloro-complexes of Ir(IV), Pd(II), Pt(IV), Au(III) and Ag, which are retained by the resin together with bismuth, remain strongly adsorbed on the resin during removal of bismuth with 1 *M* sulfuric acid.

In dealing with highly hydrolyzable elements such as niobium and tantalum, and also tungsten and molybdenum, the sorption step should be performed in a *ca.* 0.25 *M* HCl–1 *M* HF medium[10]. The presence of hydrofluoric acid does not affect the retention of bismuth as negatively charged chloride complexes. A promising medium appears to be a solution of 1 *M* HF[10], since niobium, tantalum, tungsten and molybdenum are strongly held by the resin as negative fluoride complexes while bismuth shows negligible adsorption. These possibilities need experimental confirmation.

A few determinations of bismuth as iodide[2] and with dithizone[2] indicated the utility of the ion-exchange separation for eliminating interferences in both methods.

The results summarized in Table I show that the proposed method or simple modifications of it may be applied as preliminary treatment of complex samples when bismuth is determined by any of the known absorptiometric methods.

ACKNOWLEDGEMENTS

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SUMMARY

Bismuth can be separated by ion-exchange adsorption on Dowex 21K resin from *ca.* 0.25 *M* HCl, after removal of volatile interferences by distillation in acid-oxidizing medium. Bismuth is eluted from the column with 1 *M* sulfuric acid. The method is useful in the absorptiometric determination of bismuth with thorin, as well as in other photometric methods.

RÉSUMÉ

Une méthode est proposée pour la séparation du bismuth au moyen d'un échangeur d'ions (résine Dowex 21K), après élimination des substances volatiles gênantes par distillation, en milieu acide oxydant. Le bismuth est élué de la colonne par l'acide sulfurique *M*. La méthode est utilisée lors du dosage absorptiométrique du bismuth par le thorin, de même que lors d'autres méthodes photométriques.

ZUSAMMENFASSUNG

Beschreibung einer Methode zur Abtrennung von Wismut mit Hilfe eines Ionenaustauschers (Dowex 21K) nach Entfurnung von Störenden flüchtigen Substanzen durch Destillation in Gegenwart von oxydierenden Säuren. Die Eluierung des Wismuts erfolgt mit Schwefelsäure. Die Methode eignet sich für die absorptiometrische Bestimmung des Wismuts mit Thorin oder andere photometrische Methoden.

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THE ELIMINATION OF THE GETTER EFFECT IN THE DETERMINATION OF GASES IN METALS BY THE VACUUM FUSION METHOD

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A few years ago it became necessary in our laboratory to deliver fast and accurate determinations of oxygen in metals, especially in steels. A study of the literature on this subject indicated the prevalence of the vacuum fusion method. Several apparatus were described[1-5], and also a few instruments were commercially available[6-8]. From these studies, however, it was concluded that some of the advantages of the described apparatus could probably be combined in a specially designed new apparatus. One of the main difficulties encountered in the determination of gases in metals by vacuum fusion is that of the getter effect, especially in samples with a high manganese content. Several investigations of this effect have been published[9-11] and proposals have been made to obviate this source of error[12].

In the present work, different methods of minimizing the obvious losses in oxygen yield caused by the formation of metal films were tried, without very great success, until the problem was solved by means of the equipment which is described below. The entire analytical system is merely outlined in order to give a concept of the design; the stopper funnel in the furnace is the essential part.

EXPERIMENTAL

Furnace

The details of the furnace are shown in Fig. 1. A grounded clear quartz tube is attached to a stainless steel tube by means of double rubber O-rings. In the middle of this tube there is a smaller tube with a side-arm and at the other end there is a ring, from which an alumina crucible is suspended by 3 mm stainless steel rods. The suspended tube contains the graphite crucible, and minimizes the mass of insulating powder to be outgassed at high temperature. The alumina crucible has a thickness of 2 mm, an inner diameter of 55 mm and a length of 100 mm. The graphite crucible has an external diameter of 30 mm, an external length of 93 mm, an internal diameter of 25 mm and an internal length of 80 mm.

The insulating graphite powder is of 200-mesh grade, and should be very loosely packed around the crucible, in order to obtain optimum conditions for the evolution of gases and the insulating effect.

The crucible is fitted with two funnels, of which the upper one is a stopper and condenser for evaporated metals. The movement of the stopper is carried out by means

of an O-ring sealed shaft, passing through the stainless steel tube. The stopper funnel consists of two halves, each attached to the shaft by a stainless steel rod and a chain running through the ring on the middle tube. The lower, small funnel, which is split lengthwise and fits exactly in a turned track in the crucible, directs the two halves of the stopper funnel and keeps them in position during the movement.

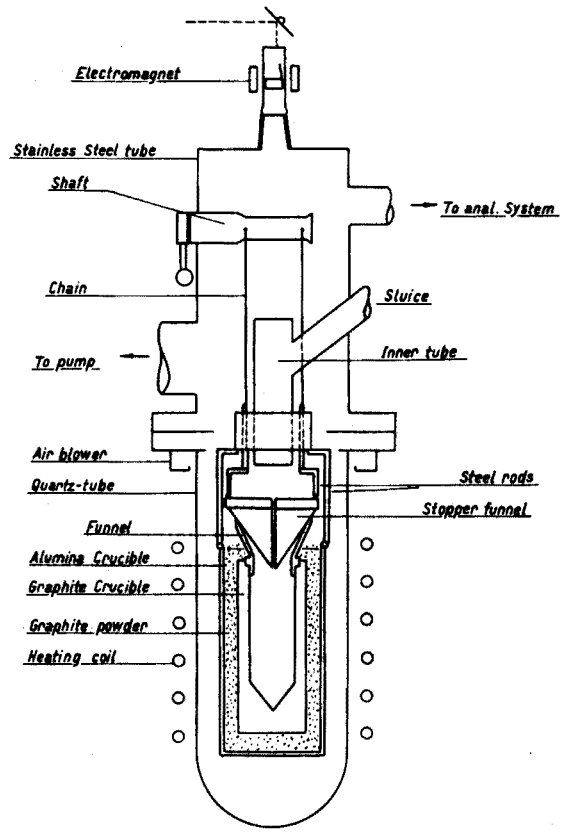

Fig. 1. Details of special furnace.

On the top of the furnace there is an optical window for measurement of temperature during the analysis. The window is protected from becoming coated with metal films by means of a magnetic directed disc. The samples are sluiced down into the crucible through a modified Ströhlein valve, which is attached to the side-arm of the middle tube of the furnace. The connection is made by an O-ring sealed standard joint. The valve is connected to the fore pump and is evacuated after each opening to atmospheric pressure.

The most important point for the functioning of the stopper as a condenser is that the two halves never reach the temperature of the crucible during analysis. The temperature, measured by means of thermocouples, is about 700° below that of the melt. Thus, the evaporated metals are condensed on the stopper funnel. The temperature, however, is high enough to evolve the absorbed gases and thus no gettering takes place.

During the outgassing of the crucible, which is carried out by heating to about 2500° for 1.5 h, the furnace is connected to an Edwards high-vacuum four-stage mercury diffusion pump with a pump speed of 73 l/sec and a backing pressure of 30 mm. During analysis, this pump connection is closed, and the transport of gases into the analytical system is taken over by a Leybold mercury vapour jet pump with a pump speed of 12 l/sec and a backing pressure of 10 mm.

The blank obtained after the outgassing is of the order of 0.000075% oxygen per gram of sample in 10 min at the working temperature of 1650°. The temperature is reached by heating with a Philips 6kW HF generator, type BC 60 B.

Analytical system

For general purposes, the analytical system was arranged to be suitable for the determination of all gases evolved by vacuum fusion. The gases are separated and their respective quantities calculated by pressure readings by means of McLeod gauges.

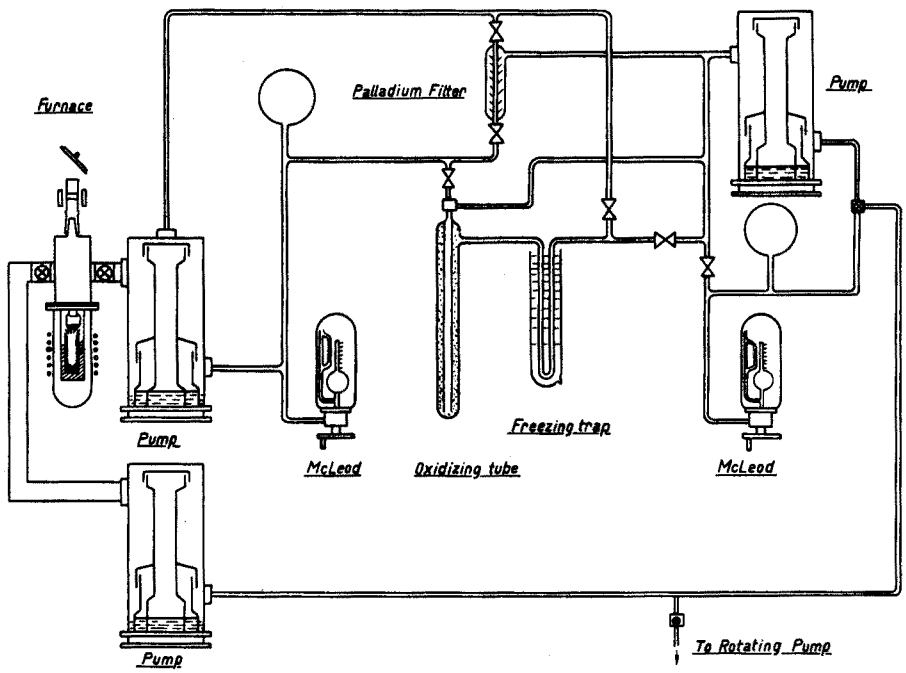

Fig. 2. Outline of the whole apparatus.

A sketch of the entire apparatus is shown in Fig. 2. The separations are obtained through a train containing a palladium filter, a tube containing Schütze Reagent, and a freezing trap. In order to cut down the time for complete separation, the gases are circulated through the train by means of a mercury diffusion pump. The evacuation of the analytical system after the analysis has been completed, is carried out by means of another mercury diffusion pump (Leybold Q-13).

The analytical system is arranged with two separated volumes. Thus, a second sample may be degassed during the analysis of the first one. Hence, the whole running

time per sample is equal to the degassing time. We have found that, for most steels, there is an evolution time for oxygen of 8–10 min, and we are now analysing about 30 samples a day with this apparatus.

RESULTS

To illustrate the function of the new stopper, some comparative results are given in table I, in which each column represents the results obtained during one day. Except for the use of the stopper funnel, the conditions were identical in all runs. The com-

TABLE I

COMPARATIVE RESULTS FOR DIFFERENT SAMPLES

Run no.	*Sample no.*	*A*[a] *g/t O_2*	*B*[b] *g/t O_2*	*Sample no.*	*A g/t O_2*	*B g/t O_2*
1	Blank	2	3	Blank	2	3
2	1	27	36	4	87	107
3	1	31	38	4	106	108
4	1	21	38	4	89	111
5	1	25	38	4	85	113
6	1	25	39	4	81	114
7	1	32	39	4	77	111
8	1	33	39	4	82	108
9	1	34	39	4	82	110
10	1	33	37	4	85	111
11	1	32	39	4	85	109
12	Blank	2	2	Blank	2	3
13	2	214	231	5	32	55
14	2	210	230	5	19	61
15	2	200	228	5	35	61
16	2	164	229	5	20	61
17	2	167	222	5	14	60
18	3	143	206	Blank	2	3
19	3	126	206	6	17	26
20	3	135	205	6	17	26
21	3	136	210	6	20	26
22	3	128	206	6	24	26
23	Blank	2	2	6	16	27
24	3	123	204	7	7	20
25	3	116	210	7	5	21
26	3	109	207	7	6	19
27	4	44	112	7	4	20
28	4	30	109	7	5	20
29	Blank	2	2	Blank	2	3

[a] A = Without stopper funnel.
[b] B = With stopper funnel.

positions of the analysed samples are listed in Table II. The average weight of the samples was 4 g. Samples 3, 6 and 7 were degassed in 10 min, the others in 8 min. The blanks were run for 10 min, and calculated as g/t oxygen per 4 g of sample.

The low results in the A-columns could, apart from the getter effect, also be caused

by the spattering of molten metal which usually occurs in furnaces heated by high frequency, when a stopper is missing. The different behaviour of samples 2 and 3, however, clearly indicates that the low results are not caused solely by the spattering of molten metal.

TABLE II

THE COMPOSITION OF THE ANALYSED SAMPLES

Sample	*C*	*Si*	*Mn*	*Cr*	*Ni*	*Mo*	*Ti*
1	0.15	0.2	0.5	0.7	3.0		
2	0.40	0.2	0.5				
3	0.06	0.3	2.0	17.5	9.0		
4	0.15	0.3	0.4	11.0		0.5	
5	0.20	0.3	1.5				
6	0.06	0.3	2.0	17.5	9.0		
7	0.06	0.5	1.5	15.0	26.0	1.5	2.0

The authors' thanks are expressed to the Directors of Aktiebolaget Bofors for permission to publish this work.

SUMMARY

A furnace for determinations of gases in metals is described in detail. The essential part of the equipment is the stopper, which acts as a non-gettering baffle as well as a splash shield. The stopper is constructed of two conical halves of graphite; the movement follows by means of a rotatable shaft, passing through the tube and sealed by rubber o-rings. The entire analytical equipment is outlined, and some results of analysis with and without the stopper are given.

ZUSAMMENFASSUNG

Ein Ofen zur Bestimmung von Gasen in Metallen wird ausführlich beschrieben. Der wesentliche Teil der Apparatur ist ein kegelförmiger Stöpsel aus Graphit oberhalb des Schmelztiegels. Die an ihm kondensierten Metalldämpfe absorbieren keine Gase. Gleichzeitig dient er als Spritzschutz. Es werden Analysenergebnisse angegeben, die mit und ohne Anwendung dieses Stöpsels gefunden wurden..

RÉSUMÉ

Un four pour le dosage des gaz dans les métaux est décrit. La partie essentielle de l'équipement est le bouchon, constitué de deux moitiés coniques en graphite, agissant comme chicanne et protection contre les projections. Quelques résultats d'analyses sont donnés, obtenus avec et sans le bouchon.

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THE DETERMINATION OF NITRATE IN SEA WATER

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Shortage of the inorganic ionic nitrogen micro-nutrients in sea water is generally one of the principal growth-limiting factors for marine phytoplankton. Of these forms of nitrogen, nitrate, besides being the form in which it is stored in the deep oceans, is quantitatively the most abundant, normally varying between about 25 and 500 μg NO_3^-–N/l. Nitrite and ammonia (ranges <4–50 μg NO_2^-–N/l and 0–10 μg NH_3–N/l, respectively) normally represent only a small fraction of the total combined nitrogen in the sea, but can occasionally, for example under anaerobic conditions, become predominant.

Two methods appear to be in current use for the determination of nitrate in sea water. In the first method[1-3], the sea water sample is treated with a solution of strychnidine in concentrated sulphuric acid and the intensity of the resulting red colour is measured spectrophotometrically. The method has been criticized on account of the difficulty of preparing a reliable reagent having an adequate sensitivity, and several workers[4-8] have attempted to prepare a more satisfactory reagent. The principal causes of variability in the determination appear to be the manner of mixing of the reagent with the sea water sample[9] and the rate of subsequent cooling; it has been claimed[10] that by careful selection of reaction flasks with similar wall thicknesses, these sources of variability can be largely eliminated.

The second method, which is widely used for the determination of nitrate in sea water, was originally described by MULLIN AND RILEY[11] and subsequently modified by AUSTIN AND STRICKLAND[12]. In these procedures, nitrate is reduced to nitrite in buffered solution by hydrazine in the presence of catalytic quantities of copper ion. After removal of excess hydrazine with acetone, the nitrite formed is determined either by the method of RIDER AND MELLON[13] or that of BENDSCHNEIDER AND ROBINSON[14]. The hydrazine reduction method suffers from a number of disadvantages:

(*1*) 24 h are required for completion of the reduction process, and this makes it difficult to use for aquarium control.

(*2*) The reduction process is inhibited at temperatures above 26°.

(*3*) The presence of particulate matter in the water produces variable results, and all samples which contain particulate matter must be filtered.

(*4*) No reduction occurs in anaerobic waters containing hydrogen sulphide, presumably owing to deactivation of the copper catalyst by conversion to copper sulphide[15].

(5) Large errors are caused if relatively much nitrite is also present in the water, as is often the case in waters depleted in oxygen. VACCARO *et al.*[16] have used ozone to oxidise nitrite quantitatively to nitrate in such waters before the determination.

(6) Unknown components of certain sea waters (*e.g.* those from around Hawaii) appear partly to inhibit the reduction process, and the yield of nitrite from a given amount of nitrate in these waters may be 25–30% lower than normal[17].

Recently a modification[18] of the method of BRAY[19] has been employed for the determination of nitrate in sea water. In this process the sea water sample is made ammoniacal, treated with a catalytic amount of manganous ion and stirred with zinc dust. After filtration the nitrite formed by reduction is determined by the method of BENDSCHNEIDER AND ROBINSON[14]. The reduction is carried out in an ice bath to minimise side reactions and a reduction yield of 85–90% is claimed. The method gave a precision somewhat inferior to AUSTIN AND STRICKLAND's hydrazine reduction procedure. A similar process has also been used by PRICE and PRIDDY[20] for the estimation of nitrate in brackish estuarine waters.

The present paper describes a simple heterogeneous reduction procedure for the determination of nitrate in sea water, which has a precision similar to that of the hydrazine method and which is free from most of its disadvantages.

The efficiency of several metals for the reduction of nitrate to nitrite was tested by shaking sea water containing 200 μg NO_3^-–N/l with the metals in the form of filings. It was found that cadmium gave satisfactory yields of nitrite particularly if it had been amalgamated by treatment with mercuric chloride solution; furthermore, cadmium showed little tendency, if the shaking was prolonged, to reduce nitrite further, as was the case with most of the other metals tested. Tests were then made using reductor columns filled with amalgamated cadmium filings and reproducible yields of nitrite of 91±1% were obtained. Reductor columns filled with unamalgamated cadmium fillings have been previously used for the determination of 0.05–3 μg nitrate/ml in slightly acidified rain water and gave a precision of ±5%[21].

Effect of column length on reduction

In order to determine the optimum column length for the reduction of nitrate in sea water, a series of glass columns of various lengths, but otherwise as described on p. 275, was filled with amalgamated cadmium filings. Aliquots (25 ml) of a natural sea water were passed through the columns and washed through with three 20-ml portions of distilled water, the rate of flow being controlled so that the whole process took 1.5 h. The combined percolate and washings were collected in 100-ml graduated flasks, and nitrite was determined by the method of BENDSCHNEIDER AND ROBINSON[14] (see p. 000). The optical densities obtained in duplicate experiments are given in Table I. They indicate that columns ranging in length from 6.5 to 21 cm gave similar amounts of reduction, but that longer columns tended to cause further reduction of the nitrite. In all subsequent work columns 20 cm in length were used, but it is probable that shorter columns would be equally satisfactory.

Effect of temperature on the reduction

The effect of temperature on the reduction process was investigated by carrying out triplicate runs on 25-ml aliquots of the same sea water using columns at room temperature (20°), and immersed in thermostat baths at 0° and 35°. The columns were

washed with three 5-ml portions of distilled water and nitrite was determined in the combined percolate and washings, as described below. The results (Table II) show, rather surprisingly, that temperature variations in the range 0°–35° have no significant effect on the yield of nitrite.

TABLE I

EFFECT OF COLUMN LENGTH ON REDUCTION OF NITRATE IN SEA WATER

Length of column of cadmium filings (cm)	*Optical density at 543 mμ in 1-cm cells*		
			Mean
6.5	0.296	0.295	0.296
12.5	0.294	0.301	0.298
21.0	0.298	0.297	0.298
28.0	0.278	0.282	0.280
36.5	0.269	0.274	0.272

TABLE II

EFFECT OF TEMPERATURE ON THE REDUCTION OF NITRATE

Temperature (°)	*Optical density at 543 mμ in 1-cm cells*			
				Mean
0	0.325	0.320	0.330	0.325
20	0.321	0.322	0.328	0.324
35	0.321	0.319	0.323	0.321

Effect of pH on the reduction

In order to investigate the effect of pH on the reduction, aliquots (25 ml) of a sea water, alone and enriched with 4 μg NO_3^-–N, were adjusted to a series of pH values by addition of 2 *N* acetic acid. They were then passed through the cadmium reductor columns, washed through with water, and the nitrite formed was determined as described below. The optical densities, which are shown in Table III, indicate that the reduction is not retarded until the pH is below 6.6, far below values normally found in sea water.

TABLE III

EFFECT OF PH ON REDUCTION OF NITRATE

pH values	8.2	7.1	6.6	4.8	3.7
Optical density difference[a]	0.278	0.276	0.276	0.268	0.261

[a] Difference between values for sea water alone, and after enrichment with 4 μg NO_3^-–N, measured at 543 mμ in 1-cm cells.

Since, owing to variations in its carbon dioxide content, the pH of distilled water is variable, it is necessary to add sufficient 1% sodium carbonate to the distilled water solutions used for calibration, in order to raise the pH into the range 6.8–8.2.

EXPERIMENTAL

Reagents

All reagents and solutions should be prepared from freshly distilled water.

Sodium carbonate solution. Prepare a 1% (w/v) solution of anhydrous sodium carbonate A.R.

Sulphanilamide reagent. Dissolve 1 g of sulphanilamide in a mixture of 90 ml of distilled water and 10 ml of concentrated hydrochloric acid.

Naphthylethylenediamine reagent. Prepare a 0.1% (w/v) solution of N-1-naphthylethylenediamine dihydrochloride. If stored in an amber glass bottle, the reagent is stable for several weeks.

Cadmium reductor.

The reductor column is constructed of Pyrex glass and consists of a 22 cm length of 0.8 cm bore tubing, which is sealed at its upper end to a 10 cm length of 2.4 cm bore tubing. At its lower end it is fused to a length of tubing with an internal diameter of 3 mm, which is bent upwards to run parallel to the main tube; level with the bottom of the 2.4 cm diameter tube it is bent over in the form of a siphon, which ends about 2.5 cm below the point of fusion of the wide tubing with the column.

Amalgamate 200 g of cadmium filings (prepared by filing cadmium sticks with a second cut hand file) by stirring with 200 ml of 1% (w/v) mercuric chloride solution for 3 min. Allow to settle and decant the supernatant liquid. Wash the amalgamated filings several times with distilled water, until the supernatant liquid is clear. Store the filings under distilled water and do not allow them to become dry. Ram a plug of glass wool into the bottom of the reductor column and fill the latter with distilled water. Pour into it sufficient cadmium filings to produce a column approximately 20 cm in length. Wash the filings thoroughly with distilled water. The average flow rate of water through the column should be *ca.* 0.6 ml per min. When not in use, the columns should be kept stoppered to prevent the filings becoming dry.

Standard nitrate solution. Prepare a solution containing 0.7220 g of potassium nitrate/l. This stock solution contains 100 μg NO_3^-–N/ml and should be used for preparing suitable working standard solutions.

Determination of nitrate

Pipette 25 ml of the sea water sample into the reductor, and wash it through with four-5-ml portions of distilled water. Collect the percolate and washings in a 50-ml graduated flask. Add 1 ml of sulphanilamide reagent and allow to stand for at least 2 and not more than 8 min. Add 1 ml of naphthylethylenediamine reagent and dilute to volume with distilled water. After not less than 10 min and not more than 2 h, measure the optical density of the solution at 543 mμ in a cell of appropriate length against a compensator cell containing distilled water. Carry out a blank determination in the same manner using 25 ml of redistilled water. Also perform a blank determination using 25 ml of the same water to which 0.2 ml of 1% sodium carbonate has been added in order to bring it to pH 7–8. Calibrate the method in the same way using 25-ml, portions of redistilled water containing 6 μg NO_3^-–N (for 1-cm cell) or 1.5 μg NO_3^-–N

(for 4-cm cell) and 0.2 ml of 1% sodium carbonate solution. The reductor column does not require any further treatment before re-use.

Determination of correction for presence of nitrite

To a 25-ml aliquot of the sample, contained in a 50-ml graduated flask, add 1 ml of sulphanilamide solution. Allow to stand for between 2 and 8 min, add 1 ml of naphthylethylenediamine reagent and dilute to volume. After 10 min, measure the optical density of the solution at 543 mμ in the same size of cell as was used for the nitrate determination. Carry out a reagent blank in the same manner using redistilled water. If a determination of nitrite in the sample has been carried out on the sample as part of the normal routine, the resulting optical density can, of course, be used, if correction is made for the differences in volume of sample taken and cell length.

CALCULATION

Correction for nitrite

Optical density from nitrite after passing through reductor (D_1) = optical density* obtained without reduction × 0.98.

Determination of nitrate

Let D_2 = optical density of sample after reduction and colour development.

Let B_2 = optical density of redistilled water after reduction and colour development.

Let S = optical density of standard (x μg NO_3^-–N) after reduction and colour development.

Let B_3 = optical density of redistilled water with 0.2 ml Na_2CO_3 after reduction and colour development.

Corrected optical density due to nitrate in sample

$$= D_3 = D_2 - B_2 - D_1$$

Concentration of nitrate in water sample

$$= \frac{40 \cdot D_3 \cdot x}{(S - B_3)}$$

If preferred, the calibration of the method can be performed by reducing and analysing sea water samples alone, and spiked with known amounts of nitrate (5 μg NO_3^-–N per 25 ml for 4-cm cell or 1.25 μg NO_3^-–N per 25 ml for 1-cm cell). The reagent blank is determined using redistilled water.

RESULTS AND DISCUSSION

Investigation of salt error and reproducibility

Since the salts present in sea water frequently reduce the sensitivity of analytical methods, tests were carried out to investigate the salt error of the method. Sea waters with a wide range of chlorinities were prepared from a water which was low in nitrate, by dilution with distilled water. Aliquots (25 ml) of distilled water and of these sea

* Less corresponding reagent blank (B_1).

waters alone, and after enrichment with 0.4, 0.8, 1.2, 2,4 and 6 μg NO_3^-–N, were analysed as described above. The results, which are shown in Table V, indicate that the method has no salt error either at high or low nitrate levels, and that the calibration graph is linear at both levels.

On the basis of the optical density increments per μg NO_3^-–N, calculated from Table V, the average yield of nitrite is 91%.

TABLE V

INVESTIGATION OF SALT ERROR

Cl (%)	*Optical density difference[a] (4-cm cell) Added nitrate (μg NO_3^-–N)*			*Optical density difference[a] (1-cm cell) Added nitrate (μg NO_3^-–N)*		
	0.4	*0.8*	*1.2*	*2*	*4*	*6*
19.0	0.106	0.214	0.322	0.138	0.275	0.409
15.0	0.110	0.222	0.328	0.142	0.272	0.401
10.0	0.109	0.212	0.321	0.141	0.281	0.416
5.0	0.109	0.219	0.315	0.140	0.279	0.423
0.0	0.107	0.217	0.322	0.139	0.284	0.411

[a] Optical density from sea water with added nitrate less optical density of sea water without added nitrate. Measured at 543 mμ.

The reproducibility of the method was checked by carrying out replicate determinations (18) on two sea waters with nitrate contents of 88 and 160 μg/l; these gave coefficients of variation of 1.9% and 1.6% respectively.

As a further test of the method, quadruplicate analyses of two sea waters alone, and spiked with known amounts of nitrate, were made by four analysts. The average results obtained (Table VI) show that the reproducibility of the method is satisfactory.

It was found that columns prepared from freshly filed cadmium gave the same yield of nitrite as columns which had been in use for 4–5 months. It is therefore probable that the columns can be used indefinitely provided that they are not allowed to become dry.

TABLE VI

COLLABORATIVE STUDY OF ANALYTICAL METHOD

(Results shown are in μg NO_3^-N/l)

Analyst	*Sea water A*		*Difference*	*Sea water B*		*Difference*
	Alone[a]	*+200 μg NO_3^-–N/l[a]*		*Alone[a]*	*+50 μg NO_3^-–N/l[a]*	
1	160	360	200	10.8	63.1	52.3
2	165	362	197	10.0	61.5	51.5
3	164	365	201	10.4	63.1	52.7
4	163	366	203	10.4	63.6	53.2

[a] Each result shown is the mean of 4 determinations.

Investigation of the interference of nitrite and its elimination

Since sea waters often contain nitrite amounting to about a tenth of the amount of nitrate present, the action of the reductor on nitrite was investigated. Aliquots (25 ml) of a nitrite and nitrate-poor sea water were passed through the columns both alone

and enriched with nitrite. The nitrite remaining was determined as described above. It was found (Table VII) that a small amount of reduction of the nitrite had in fact occurred, and that the percentage reduction increased as the amount of nitrite increased. As normally only small amounts of nitrite are present in sea water, it is sufficiently accurate to carry out a nitrite determination on a 25-ml aliquot of the sea water as described above, and to multiply the measured optical density at 543 mμ (less reagent blank) by 0.98. The resulting value is deducted from the optical density (less blank) found for the sea water after passing through the reductor, and gives the optical density due to nitrate alone.

TABLE VII

PERCENTAGE REDUCTION OF NITRITE ION ON PASSING THROUGH REDUCTOR

Weight of nitrite present (μg NO_3^-–N)	0.4	0.8	2	4
Percentage reduction	2	2	3	7

If appreciable amounts of nitrite are present in the water, nitrite should be destroyed as described below, before the reduction is carried out. Place 25 ml of the sea water in a 50-ml beaker, and add 1 ml of a 0.2% solution of hydrazine sulphate in 1 *N* hydrochloric acid, and mix well with a small glass rod. Add saturated bromine water dropwise until a pale straw colour is obtained (*ca.* 0.4 ml); add the same amount to each sample. Stir thoroughly and allow to stand for 10 min until nitrogen evolution ceases. Add 1 ml of a freshly prepared 2% (w/v) solution of phenol in 1 *N* sodium hydroxide and mix well; the solution should now have a pH of 7.5–8.5. Pass the solution through the reductor and rinse the beaker into the column with small amounts of water. Continue the determination as previously described. Carry out a blank determination in the same manner using 25 ml of distilled water.

In order to test this nitrite destruction procedure, duplicate determinations were carried out on 20-ml aliquots of sea water, very low in both nitrate and nitrite, which had been enriched with 4 μg NO_3^-–N and with various amounts of nitrite. Nitrite was destroyed as described above, the solutions were passed through the reductor, and nitrite was determined in the usual way. Similar experiments were carried out with distilled water and with sea water containing 4 μg NO_3^-–N/20 ml but no added nitrite. It was found that with 2 and 4 μg NO_2^-–N, the recoveries of nitrate were 3.9, 4.1 and 4.1, 4.1 μg NO_3^-–N respectively (4.0 μg NO_3^-–N added).

Interference of amino acids and urea

Since amino acids and urea react with nitrite in acidic solution, their possible interference in the determination of nitrate was investigated. Sea water samples, low in nitrate, containing 2 p.p.m. of added organic nitrogen compounds were analysed alone, and in the presence of added nitrate (240 μg NO_3^-–N/l). It was found that there was no interference from urea, glycine, arginine hydrochloride or methionine.

Determination of nitrate in presence of sulphide

When anaerobic waters containing sulphide are passed through the reductor, the sulphide is retained at the top of the column as a yellow layer of cadmium sulphide.

Experiments showed that if anaerobic waters containing up to 2 mg of sulphide/l and 300 μg NO_3^-–N/l were analysed as described above, the nitrate recoveries range between 98 and 100%. Since anaerobic waters rich in sulphide frequently contain considerable amounts of nitrite, tests were made on anaerobic water containing 2 mg of sulphide/l, and 300 μg of both NO_3^-–N and NO_2^-–N. After destruction of nitrite, etc., the solution was reduced and nitrite was determined colorimetrically. Again, duplicate recoveries of 98 and 99% of the nitrate originally taken were obtained.

SUMMARY

A heterogeneous reduction method is described for the determination of nitrate in sea water. Nitrate is reduced to nitrite with 91% efficiency by passing the water through a column of amalgamated cadmium filings. The nitrite produced is determined spectrophotometrically by the method of BENDSCHNEIDER AND ROBINSON. The method has a coefficient of variation of *ca.* 2% and is free from salt error. Temperature in the range 0°–35° has no effect on the reduction. Interference from nitrite is discussed and a method is described for its destruction if necessary. Sulphide does not interfere.

RÉSUMÉ

Une méthode est décrite pour le dosage des nitrates dans l'eau de mer. Les nitrates sont réduits en nitrites par passage de l'eau à travers une colonne remplie d'amalgame de cadmium. Le nitrite formé est dosé spectrophotométriquement par la méthode de BENDSCHNEIDER ET ROBINSON. L'influence des nitrites est examinée; une méthode est proposée pour leur destruction, si nécessaire. Les sulfures ne gênent pas.

ZUSAMMENFASSUNG

Es wird eine Methode zur Bestimmung von Nitrat im Meerwasser beschrieben. Das Nitrat wird zum Nitrit reduziert, indem das Wasser durch eine Kolonne mit amalgamierten Kadmiumspänen läuft. Das gebildete Nitrit wird nach der Methode von BENDSCHNEIDER UND ROBINSON spektralphotometrisch bestimmt. Die Methode hat einen Variationskoeffizienten von *ca.* 2% und ist frei von Salzfehlern. Der Einfluss des im Meerwasser enthaltenen Nitrits wird diskutiert und eine Methode für seine Zerstörung beschrieben. Sulfid stört nicht.

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Short Communications

Polarographic determination of molybdenum in uranium–molybdenum alloys

Uranium alloys are finding increasing uses in nuclear energy programmes, including those containing molybdenum which forms alloys of various composition with uranium. Some gravimetric methods are available for the determination of high contents of molybdenum in uranium–molybdenum alloys and colorimetric methods are known for trace estimations[1–3]. In the present paper, a polarographic method is described for the determination of molybdenum in the range of 0.01 to 1.0% in uranium–molybdenum alloys.

Apparatus

A "Du Bellay" polarograph was used; a mercury pool was used as anode, and the capillary had the following characteristics: $m = 1.670$ mg/sec and $t = 4.58$ sec/drop in 0.1 M potassium chloride solution (open circuit). Measurements were made at $30° \pm 0.2°$.

Reagents

Standard molybdenum solution (500 μg Mo/ml) was prepared by dissolving 1.261 g of E. Merck G.R. quality sodium molybdate in distilled water and diluting to 1 l. This solution was standardised by the lead molybdate method, and was further diluted as required.

Supporting electrolyte. 12.5 ml of glacial acetic acid, 15.42 g of ammonium acetate, 74.45 g of ethylenediaminetetraacetic acid (disodium salt) and 71.44 g of neutral sodium citrate were dissolved in water and the solution was diluted to 1 l.

30% solution of purified tri-*n*-butyl phosphate in carbon tetrachloride.

Nitric acid s.g. 1.42, C.P. grade.

Procedure

The uranium alloy turnings were washed with acetone and diethyl ether and allowed to dry. 1 g of the washed turnings was dissolved in 5 ml of diluted nitric acid (1 : 1, v/v) and evaporated nearly to dryness on a hot plate. The residue was dissolved in 5 ml of nitric acid (s.g. 1.42); 10 ml of water were added and the solution was transferred to a 100-ml separating funnel. The uranium was extracted by shaking the solution three times with 10-ml portions of equilibrated tri-*n*-butyl phosphate solution in carbon tetrachloride and separating the organic phase. The traces of extractant were removed by shaking with carbon tetrachloride and removing the organic layer. The aqueous layers were collected in a 30-ml beaker and the solution was evaporated nearly to dryness on a hot plate.

The supporting electrolyte solution (12.5 ml) was added to the beaker to dissolve the contents and the solution was then transferred to a 25-ml standard flask and

diluted to volume with distilled water. A portion of the solution was polarogrammed, after deaeration with nitrogen.

The i_d *vs.* C relationship necessary for calibration purposes is shown in Table I. Standard recoveries of molybdenum were tested using the standard molybdenum solution and pure uranium metal and following the above procedure. The results are shown in Table II.

TABLE I

RELATIONSHIP BETWEEN i_d AND CONCENTRATION OF MOLYBDENUM

Wt. of Mo in 25 ml (mg)	10	5	1	0.5	0.1
$i_d/m^{2/3}t^{1/6}$	10.470	5.285	1.036	0.528	0.104

TABLE II

RECOVERY OF MOLYBDENUM IN PRESENCE OF URANIUM

Series no.	*Mo added (mg)*	*U present (g)*	*Mo found (mean) (mg)*	*Error (%)*
1	10	1.0	9.97	−0.3
2	5.0	1.0	4.94	−1.2
3	1.0	1.0	0.99	−1.0
4	0.50	1.0	0.50	0.0
5	0.10	1.0	0.097	−3.0

Discussion

PŘIBIL AND BLAZEK[4] have reported that molybdenum gives a well defined single wave in a supporting electrolyte which is 0.1 M with respect to ammonium acetate, acetic acid and ethylenediaminetetraacetic acid (disodium salt) respectively. Vanadium does not give a reduction wave, but was found to interfere by enhancing the diffusion current of molybdenum. This was overcome by including 0.1 M sodium citrate in the supporting electrolyte.

The wave of uranium in this medium precedes that of molybdenum, hence it is necessary to remove the bulk of the uranium. This was achieved by extraction with a 30% solution of equilibrated tri-*n*-butyl phosphate in carbon tetrachloride in about 5 M nitric acid medium[5]. The small amount of uranium remaining after this extraction gave a small well defined wave which was followed by that of molybdenum.

Synthetic solutions containing 1 g of uranium and 500 μg of molybdenum with 500 μg of vanadium in one experiment, and 500 μg each of iron, cobalt, nickel, copper, lead, tin and zinc all together in another, gave molybdenum recoveries within 1% and 3%, respectively. A binary alloy of uranium and molybdenum when analysed by the proposed method gave a molybdenum result of 0.033%, the same value being obtained by a colorimetric method.

The accuracy of the determination is $\pm$ 3% over the range 0.01 to 1.0% of molybdenum in uranium–molybdenum alloys.

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On the oxidation state of cobalt in its PAN and PAR chelates

o-Hydroxypyridylazo dyes have frequently been used as metallochromic indicators and colorimetric reagents for heavy metal ions. 4-(2-Pyridylazo)-resorcinol (PAR) was suggested for the spectrophotometric determinations of cobalt(II), lead(II) and uranium(VI) by POLLARD *et al.*[1]; the solubility of the reagent and its metal chelates in water provides advantages over 1-(2-pyridylazo)-2-naphthol (PAN), which is most widely used of this class of dyes. POLLARD *et al.*[2] also reported the absorption spectra and stability constants of the metal chelates of some azo and azomethine dyes (including PAR and its cobalt complex), which have similar coordination groupings to *o*-hydroxypyridylazo dyes.[2]

CHENG AND BRAY[3] pointed out that the red cobalt(II)–PAN chelate is stable in alcoholic solution but is rapidly oxidized to a green cobalt(III) chelate in aqueous solution by atmospheric oxygen; this was utilized for the spectrophotometric determination of cobalt with PAN[4]. BAILAR *et al.*[5,6] observed the stabilization of the cobalt(III) state in several *o*-substituted azo dye complexes when cobalt(II) was used as the starting material. LIU[7] tried to prepare the cobalt(II) complex of PAN and PAR as pure crystals, but she could obtain only the PAN complex as a mixture of the cobalt(II) and cobalt(III) chelates. Recently, CORSINI, YIH, FERNANDO AND FREISER[8] estimated the stability constants of the PAN and PAR chelates with cobalt(II) as $>10^{12}$ and prepared the "cobalt(II)–PAR complex" but provided no definite evidence for the oxidation state of cobalt.

One of the present authors has discussed the acid-base properties and the absorption spectra of PAR and its metal chelates, with reference to their analytical aspects[9]. During an investigation[10] of the detection of heavy metal ions with PAN and PAR by means of resin spot tests[11], the following reactions were found with cobalt(II). A grain of swollen cation-exchange resin, which adsorbed PAN at pH 4.7 and was coloured lemon-yellow, changed colour on addition of a drop of cobalt(II) solution via reddish purple to green, the change gradually penetrating to the centre of the

resin. When PAR was used, the reddish purple colour formed in the resin phase on addition of cobalt(II) solution remained unchanged after several hours.

The absorption spectra of the cobalt–PAN and –PAR chelates were then measured in an oxidizing or reducing medium. As shown in Fig. 1, the cobalt–PAN complex showed quite different spectra depending on the medium, whereas the spectra of the cobalt–PAR complex were unaffected by the medium.

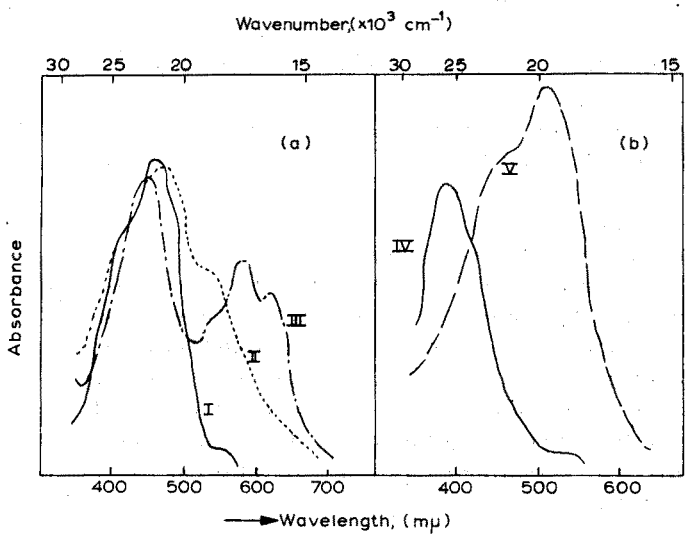

Fig. 1. Absorption spectra of PAN, PAR and their cobalt chelates in isoamyl alcohol extracted from different media. (a) I, PAN; II, Co(II)–PAN from reducing medium; III, Co(III)–PAN from oxidizing medium. (b) IV, PAR; V, Co–PAR from both media.

Magnetic susceptibility measurements showed the diamagnetism of the cobalt–PAR chelate in both media, and of the cobalt–PAN chelate in the oxidizing medium. Paramagnetism of the cobalt–PAN chelate in the reducing medium was estimated qualitatively; quantitative estimation was difficult because of the gradual atmospheric oxidation of the central cobalt atom, which caused a gradual decrease in weight of the sample under the magnetic field.

According to the ligand-field theory, the stronger the field supplied to the central cobalt atom from the ligand dye molecule, the greater is the splitting of the energy levels of the *d*-orbital electrons in the central atom; this would stabilize the octahedral configuration of the cobalt(III) state. The π-electron system of PAR (resorcinol) is smaller than that of PAN (naphthol) and in addition there is a flowing effect of electrons from the dissociated 1-hydroxyl group of PAR into the benzene ring; these effects would stabilize the trivalent state of cobalt in the PAR chelate to a greater extent than in the PAN chelate. Thus, in solutions of the cobalt–PAR chelate, the cobalt(III) state could be stabilized, even in the presence of a reducing agent such as ascorbic acid.

It seems probable, therefore, that the absorption spectra of the so-called cobalt(II)–PAR chelate reported in the literature are in fact those of the cobalt(III)–

PAR chelate, in which the molar ratio of the metal to the dye is invariably 1 : 2, by means of the continuous variations method[9].

EXPERIMENTAL

Reagents

Cobalt(II) solution. Cobalt(II) nitrate hexahydrate, $Co \cdot 6H_2O \cdot (NO_3)_2$ (Wako, analytical grade), was dissolved in water and diluted appropriately with water.

PAN and PAR solutions. 0.1% methanolic solutions of the Dotite reagents (Dojindo).

Potassium periodate. 5% aqueous solution of extra pure potassium periodate (E. Merck).

Ascorbic acid. 10% aqueous solution of extra pure L-ascorbic acid (Wako).

Buffer solution pH 4.7. 0.1 F solution of sodium acetate and acetic acid were mixed.

Procedure

In a 50-ml separatory funnel, 5 ml of 10^{-4} F cobalt(II) solution, 1 ml of the L-ascorbic acid or potassium periodate solution and 1 ml of the buffer solution were mixed thoroughly; 0.1 ml of the dye solution and 10 ml of isoamyl alcohol (analytical grade were added and shaken vigorously for a few min. The organic layer was measured spectrophotometrically with a Cary Model 11 spectrophotometer.

For the measurements of magnetic susceptibility 1 ml of 10^{-2} F cobalt(II) solution, 1 ml of the ascorbic acid or potassium periodate solution, 2 ml of the buffer solution and 1 ml of the dye solution were mixed in a 10-ml stoppered test tube. The organic layer was weighed by the Gouy method.

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Wechselndes Potential bei wechselnder Rührgeschwindigkeit

Wer eine genügende Zahl potentiometrischer Silberhalogenid-Fällungen durchgeführt hat, weiss, dass gelegentlich das Potential recht merklich von der Geschwindigkeit abhängt, mit der die titrierte Lösung gerührt wird. Meist genügt es, diese Geschwindigkeit einigermassen konstant zu halten, um analytisch brauchbare Potentialwerte abzulesen; ungewohnt starkes Auftreten dieser Störung war jetzt der Zwang, eine andersartige Untersuchung abzubrechen, um zunächst den Grund des Effektes zu finden, über den bisher kaum etwas bekannt ist.

Ein Erklärungsversuch bei KOLTHOFF UND FURMAN[1a] steht in eklatantem Widerspruch zu den Fundamenten der Thermodynamik: Je nach der Rührgeschwindigkeit sollen an der Oberfläche des suspendierten Silberhaloids andere Mengen und andere Arten von Ionen adsorbiert werden, womit sich die Gesamtkonzentration an potentialbestimmendem Ion in der bewegten Flüssigkeit ändern könne. Selbstverständlich kann nur die Geschwindigkeit, mit der sich ein Lösungs- oder Adsorptions-Gleichgewicht einstellt, nicht aber seine Lage von der Rührgeschwindigkeit abhängen. Um jedoch mathematische oder sprachliche Verständigungsschwierigkeiten zu vermeiden[1b,2], wurde überdies eine blanke, sorgfältig gereinigte Silberelektrode in niederschlagsfreien, sehr verdünnten Silberlösungen (10^{-5} bis 10^{-3} *N*) geprüft: der Effekt trat genau so auf. Richtung: die rascher gerührte Lösung erscheint konzentrierter (oder die Elektrode in ihr edler). Grösse: bis zu einer Zehnerpotenz in C_{Ag^+}, rund 50 mV in *E*, wenn die Umlaufsgeschwindigkeit von eins auf vier in der Sekunde steigt (gemessen an einem Schwimmer im Zentrum der durch einen Magnetstab gerührten Lösung). Das sieht also so aus, als ob die schwach gerührte Lösung dort, wo sie die Elektrode berührt, an den potentialbestimmenden Silberionen verarme, und das könnte *vielleicht* erklären, wieso verschiedene Elektroden den Effekt in verschiedenem Masse zeigen und wieso eine amalgamierte Silber-elektrode sich beim Durchschreiten des Endpunktes anders verhält als eine reine[1c]. Zum mindesten dürfte hierin eine brauchbare Arbeitshypothese für das Auffinden von störungsfrei arbeitenden Elektroden liegen; die entsprechenden Versuche sind im Gange.

Vollständigkeitshalber sei erwähnt: Auch die Annahme, es könne sich zwischen einer Metalloberfläche und einer gut leitenden wässrigen Lösung eine statische Potentialdifferenz ausbilden ("electricity caused by friction") wurde trotz offenkundiger physikalischer Unmöglichkeit noch durch den Versuch widerlegt, indem die Potentiale nicht statisch sondern bei Stromdurchgang, ungefähr 10^{-6} A gemessen wirden.

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Gravimetric determination and separation of titanium with N-benzoyl-N-phenylhydroxylamine by direct weighing

N-Benzoyl-N-phenylhydroxylamine has already been used for the gravimetric determination of titanium[1] and for its separation from other elements[2]. Titanium was precipitated by adding an alcoholic reagent to the cold acidic titanium solution and weighed as titanium dioxide after ignition. It has now been shown that titanium can be determined by direct weighing of the titanium–benzoylphenylhydroxylamine complex formed in hot (65–75°) acidic solution, the composition of the complex being $TiO(C_{13}H_{10}O_2N)_2$. This method can also be applied to the separation of titanium from many elements after addition of masking agents or pH adjustment.

Reagents

A standard titanium sulphate solution was prepared as described previously[2].

Magnesium–EDTA complex. 1.0 *M* EDTA (E. Merck Titriplex III) and 1.0 *M* magnesium chloride solutions were separately prepared. When required for masking, 5 ml of each solution were mixed.

Foreign ions. Standard solutions of ferric chloride, zirconium sulphate, copper nitrate, sodium vanadate, uranyl nitrate, thorium nitrate, ceric ammonium sulphate, chrome alum, cobalt nitrate, nickel sulphate, manganese chloride, zinc sulphate and disodium hydrogen phosphate were used. All the reagents were of A.R. quality.

Procedure

Heat the solution (200 ml) containing titanium to 65–75° and add benzoylphenylhydroxylamine (0.3 g dissolved in 10 ml of alcohol) dropwise with constant stirring until precipitation is complete. Add sufficient 2 *N* sulphuric acid to give a final acidity of 0.02–0.5 *N*. Digest the precipitate on a hot water bath at 65–75° for 30 min, filter on a weighed sintered glass crucible, wash with warm water (40–50°) and dry at 110° to constant weight. Calculate the titanium value on the theoretical factor (9.808% Ti in the precipitate). The results obtained by this method agreed well with those obtained by ignition of the precipitate formed under the same conditions (Table I).

Properties and composition of the complex

The titanium–benzoylphenylhydroxylamine complex when precipitated at 65–75° is yellowish white in colour, whereas a yellow precipitate is formed at room temperature. Both complexes are soluble in 50% alcohol, chloroform, benzene and ether, and decompose in presence of strong mineral acids. The titanium complex formed at 65–75° is slightly soluble in water above 80° and is practically insoluble in 15%

alcohol. Unlike the precipitate obtained at room temperature, it is of definite composition and decomposes at 167–168°C. The titanium content was established by igniting a known weight of the complex and weighing as titanium dioxide (titanium found: 9.79%; theoretical: 9.808%); nitrogen was determined by Kjeldahl's method (nitrogen found: 5.80%; theoretical: 5.73%). These results indicate that the composition of the complex is $TiO(C_{18}H_{10}O_2N)_2$.

For quantitative precipitation of titanium under the above conditions, at least three times the theoretical amount of reagent was required.

TABLE I

DETERMINATION OF TITANIUM AS OXIDE OR AS COMPLEX

Ti taken (mg)	*Weight of Ti complex (mg)*	*Weight of TiO_2 (mg)*	*Ti found (mg)*	*Error (mg)*
9.5	—	16.0	9.6	+0.100
12.0	—	20.0	12.0	—
12.0	—	20.0	12.0	—
12.0	121.2	—	11.88	−0.12
24.0	243.0	—	23.8	−0.2
9.0	90.6	—	8.87	−0.13
6.0	60.2	—	5.9	−0.1
9.5	97.5	—	9.56	+0.06
9.5	97.0	—	9.5	—

TABLE II

DETERMINATION OF TITANIUM IN PRESENCE OF FOREIGN IONS
(See text for modification required)

Foreign ion	*Amount added (mg)*	*Ti taken (mg)*	*Ti found (mg)*	*Foreign ion*	*Amount added (mg)*	*Ti taken (mg)*	*Ti found (mg)*
Fe^{3+}	15.0	11.0	11.2	Ce^{4+}	40.0	12.0	11.9
	7.5	11.0	10.9		60.0	12.0	12.0
	16.0	9.0	9.0				
				Mn^{2+}	15.0	9.0	8.95
Zr^{4+}	20.0	9.0	9.0		30.0	9.0	9.0
	40.0	9.0	9.0				
				Cr^{3+}	45.0	8.8	8.7
Cu^{2+}	22.0	9.0	9.0		90.0	8.8	8.9
	44.0	8.8	8.8				
				Th^{4+}	40.0	8.8	8.9
V^{5+}	15.0	9.0	9.0		80.0	8.8	8.8
	30.0	11.0	11.0				
				Al^{3+}	10.0	8.8	8.74
Na_2HPO_4	100.0	9.0	9.0		20.0	8.8	8.9
	150.0	9.0	8.9				
				Ni^{2+}	25.0	9.0	9.0
$(UO_2)^{2+}$	20.0	12.0	12.0		50.0	9.0	8.9
	40.0	12.0	11.9				
				Co^{2+}	27.0	9.0	9.1
					54.0	9.0	9.0

Separation of titanium after addition of masking agents

Titanium was determined in presence of iron, vanadium, zirconium or copper by complexing the foreign ions with magnesium–EDTA complex. When EDTA (disodium salt) alone was used, the titanium precipitate was found to be contaminated with EDTA (free acid). In the case of vanadium(V), the mixture was first boiled with 0.5 g of sodium sulphite and 2 ml of 18 *N* sulphuric acid to reduce it to the tetravalent state. For the separation, titanium sulphate solution and foreign ion solution were mixed with 10 ml of magnesium–EDTA complex and diluted to 200 ml; the acidity of the solution was properly adjusted and titanium was determined as described above. Tartaric acid (2 g) was added before titanium was precipitated in presence of phosphate. The results in Table II indicate that the direct weighing method was satisfactory in these cases. Fluoride, however, interfered seriously.

Separation of titanium by pH adjustment

Since uranium(VI), cerium(IV), thorium(IV), aluminium, chromium(III), cobalt, nickel, zinc and manganese(II) do not form precipitates with benzoylphenylhydroxylamine below pH 2, titanium could be separated from any of these metals by proper adjustment of the acidity. The acidity was adjusted to 0.5 *N* with respect to sulphuric acid and titanium was precipitated and determined as above. Cerium(IV) was first reduced to the cerous state with hydroxylamine hydrochloride.

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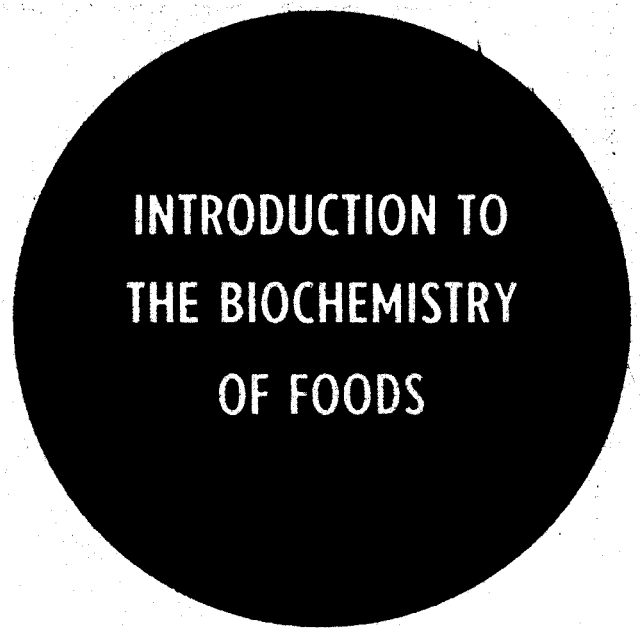

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Late Professor of Food Technology, Technion – Israel Institute of Technology, Haifa, Israel;

Visiting Professor (1960–61), Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.

Foreword by Professor S. A. Goldblith of the Massachusetts Institute of Technology

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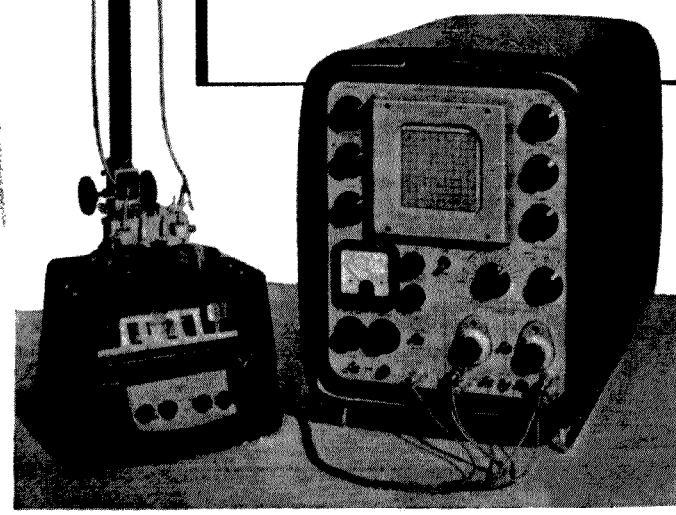

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(1) Sen, B. N., *Anal. Chim. Acta*, 1961, 24, 386-7.

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(1) British Patent No. 806,935.

(2) Schurz, J. and Stubchen, H., *Z. Elektrochem.*, 1957, 61, 754-63.

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(1) Marquet, A., *et al.*, *Bull. Soc. chim. France*, 1961, 1822-31.

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