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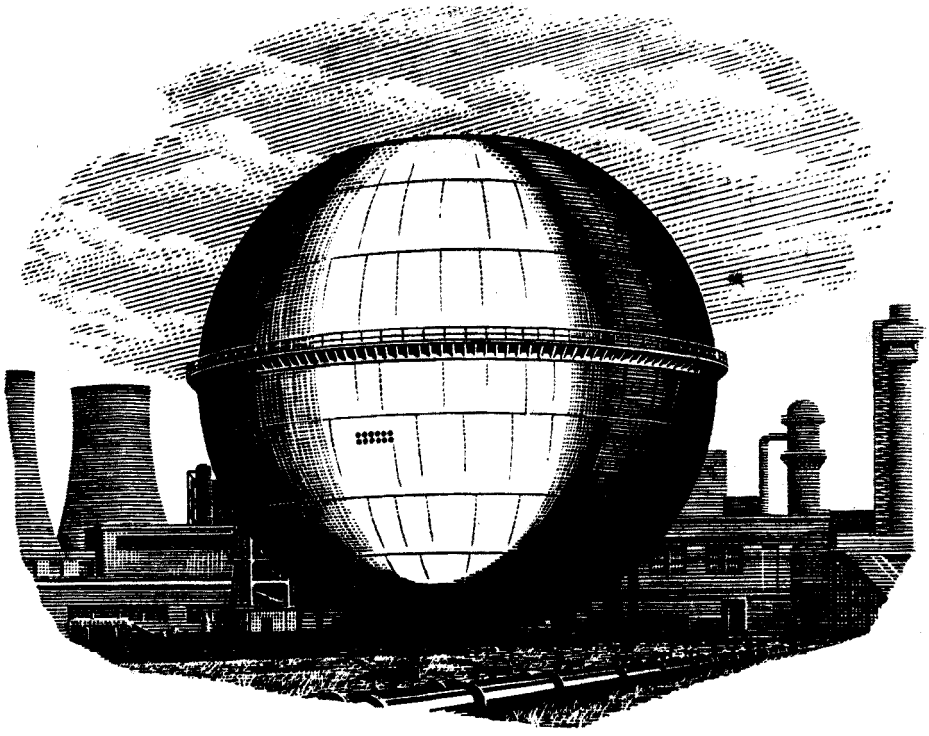

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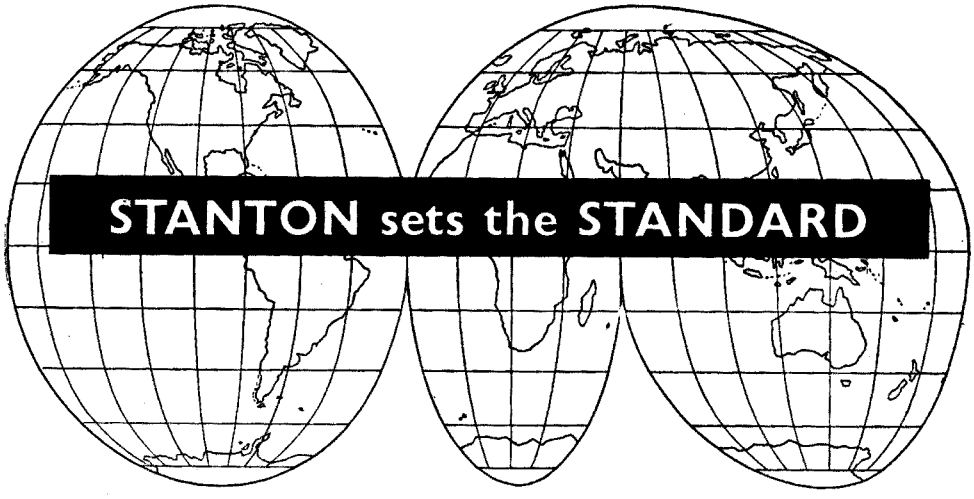

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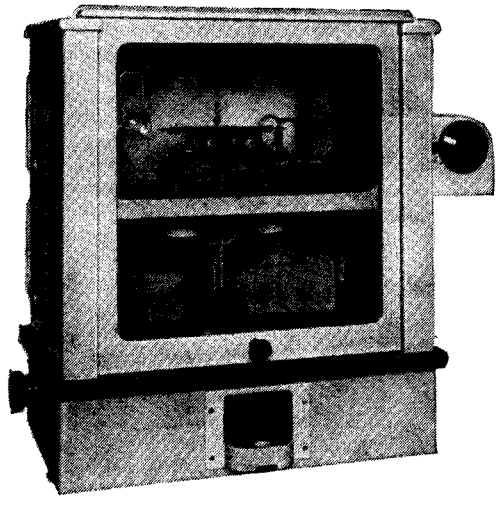

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No.13

Items of interest from our laboratory notebooks

► Most analysts know about 1,10-phenanthroline and many use it for iron determinations. Not so many people seem to know that **4,7-diphenyl-1,10-phenanthroline** is twice as sensitive as 1,10-phenanthroline in the colorimetric determination of iron. There are several papers on the subject but the latest is *Analyst,* 83 (1958) 80. The reagent is also called **Bathophenanthroline,** and we make it.

► Then, again the substitution of methyl groups in the 2,9 position has the interesting effect of making the reagent insensitive to iron and we then have a selective and sensitive reagent for copper (see *Anal. Chem.,* 28 (1956) 1158). Hopkin & Williams make **2,9-dimethyl-1,10-phenanthroline** (sometimes called **Neocuproin**).

► One does not think of sulphate as a radical one can determine absorptiometrically, but this is now possible for low concentrations. **Barium chloranilate** is the reagent and there are two papers on the subject—*Anal. Chem.,* 29 (1957) 281 and *Anal. Chem.,* 30 (1958) 202. Hopkin & Williams make it.

► Hopkin & Williams Ltd. were also early off the mark with supplies of the remarkable new colour-producing reagent for fluoride ions, **3-aminomethylalizarin-N,N-diacetic acid,** described by Belcher, Leonard and West (*Talanta,* 2 (1959) 92). This important reagent is already available from stock.

SOME THEORETICAL CONSIDERATIONS IN ANALYTICAL CHEMISTRY

IV[1]. A METHOD OF CALCULATING ASYMMETRICAL TITRATION CURVES AVOIDING THE USE OF CUBIC EQUATIONS

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(Received May 2nd, 1959)

Many ion combination reactions in analytical use result in the formation of unsymmetrical reaction products, such as,

$$2B^{+} + A^{-2} \rightleftarrows B_2A$$
$$B^{+2} + 2A^{-} \rightleftarrows BA_2$$

The product may be a sparingly soluble salt, such as silver oxalate or calcium fluoride, or it may be a sparingly ionised solute, either a simple molecule such as mercuric bromide in mercurimetry, or a complex molecule or ion such as the dicyanoargentate ion in argentimetry. Calculation of titration curves for such reactions involves in the region of the equivalence point the solution of cubic equations. This tedious procedure remains necessary when a specific point fails to be calculated, but may be avoided when constructing curves by the simple device of inverting the equations, so producing quadratic, or even linear equations.

Calculation of ion concentrations

Taking the species BA_2 as an example, and ignoring ion charges, at equilibrium,

$$\frac{[B]\,[A]^2}{[BA_2]} = K \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where K is the ionisation constant of the covalent solute, the instability (dissociation) constant of the complex or the dissolution constant of the precipitate. At points far removed from the equivalence point, the excess of B or A is sufficient to suppress the ionisation of BA_2 to such a level that the error incurred in ignoring this ionisation is negligible, and the ion concentrations can be calculated with adequate accuracy from (2) and (3),

$$[B] = \frac{K[BA_2]}{[A]^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$[A] = \left(\frac{K[BA_2]}{[B]}\right)^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Approaching or leaving the equivalence point this assumption becomes invalid, and account must be taken of the dissociation of BA_2. Since BA_2 is asymmetrical, different equations are required according to whether B or A is in excess.

1. *B in excess.* If B is in excess to an amount b calculated from the concentration of the reagent and the volume of the titration solution, and the amount of BA_2 is similarly calculated to be c, then the concentrations of the various species have to be calculated from[2]

$$[B] = b + \tfrac{1}{2}[A] \quad . . . . . (4)$$

$$[BA_2] = c - \tfrac{1}{2}[A] \quad . . . . . (5)$$

$$[A]^3 + 2b[A]^2 + K[A] - 2cK = 0 \quad . . . . . (6)$$

The point at which it becomes necessary to change over from the simple equations (2) and (3) to the more precise equations (4–6) is defined by the discriminant[3] (7) for a given maximum permissible relative error E,

$$b \leqslant \left\{\left(\frac{1-E}{2E}\right)^2 Kc\right\}^{\frac{1}{2}} \quad . . . . . (7)$$

2. *A in excess.* If the excess of A is calculated to be a, then the following equations apply[2]

$$[A] = a + 2[B] \quad . . . . . (8)$$

$$[BA_2] = c - 2[B] \quad . . . . . (9)$$

$$4[B]^3 + 4a[B]^2 + (a^2 + K)[B] - Kc = 0 \quad . . . . . (10)$$

and are required to avoid a relative error greater than E according to the discriminant[3] (11) when

$$a \leqslant \left(2\frac{(1-E)}{E} Kc\right)^{\frac{1}{2}} \quad . . . . . (11)$$

Calculation of titration curves

1. *Standard method.* By solution of the discriminants (7) and (11) for a predetermined permissible relative error, or by other means, the region on either side of equivalence within which the exact equations are required is defined. For selected increments of titrant, a or b, as appropriate is calculated, and the ion concentrations calculated from (2) and (3) outside or (4–10) inside the range defined by the discriminants. At the equivalence point, ion concentrations are calculated directly from (1)[2].

Unless the constant K has a very small value, less than 10^{-15}, the region requiring use of the exact cubic equations extends for a considerable distance on either side of the equivalence point. To avoid this tedious solution of cubics, the calculation may be reversed; instead of calculating from the volume axis to the pM axis, that is, supplying values for titrant increments and calculating ion concentrations, values may be placed on the ion concentration and the titrant volume calculated therefrom.

This may be effected by rearranging equations (6) and (10) in terms of b and a thus,

$$b = \tfrac{1}{2}\left\{\frac{K}{[A]}\left(2\frac{c}{[A]} - 1\right) - [A]\right\} \quad . . . . . (12)$$

$$a^2 + 4[B]a + 4[B]^2 + K\left(\frac{[B]-c}{[B]}\right) = 0 \quad . . . . . (13)$$

whence

$$a = \left\{\frac{K}{[B]}(c - [B])\right\}^{\frac{1}{2}} - 2[B] \quad . . . . . (13a)$$

2. *Simplified method.* Solution of the discriminants again defines the region wherein exact equations are required. Outside this region, the normal process is followed. Inside this region, the steps are as shown.

a. Place a value on [B] or [A] as appropriate.
b. Calculate a or b from (13a) or (12).
c. Calculate the true value of [A] or [B] from (8) or (4).
d. Calculate the corresponding volume of titrant (or percentage of reaction).

Calculation of titrant volume from concentrations

Step d and the corresponding conversion of the solution of the discriminant to titrant volume, may call for calculation to a high degree of precision to obtain significant results if the total titrant volume is used. It is better, therefore, to calculate v_1, the difference from 100% reaction. If V% of titrant has been added, then $V = 100 - v_1$. In a 100-ml titration, V and v_1 give the volume directly in ml, and for any other titration volume, say x, the volume in ml will be given by $V \times x/100$ and $v_1 \times x/100$. If the molarities of the two solutions are M_A and M_B, then, before the equivalence point with B as titrant,

$$v_1 = \frac{200a}{(M_A + a)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (14)$$

With A as titrant,

$$v_1 = \frac{200b}{(M_B + b)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (15)$$

After the equivalence point, at a point v_2% past the equivalence point, $V = 100 + v_2$, and with B as titrant,

$$v_2 = \frac{200b}{(M_B - b)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (16)$$

With A as titrant,

$$v_2 = \frac{200a}{(M_A - a)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (17)$$

Mercuric chloride may be used as an example with a very low dissociation constant, $6 \cdot 10^{-14}$, in illustration of the method. In the titration of 100 ml of 0.1 M chloride solution with 0.05 M mercuric perchlorate, *at the equivalence point* $c = 2.5 \cdot 10^{-2}$ and, from (1), $[Hg^{+2}] = 7.21 \cdot 10^{-6}$ and $[Cl^-] = 1.442 \cdot 10^{-5}$.

Before the equivalence point, allowing a maximum relative error of 1% ($E = 0.01$), solution of the discriminant (11) and conversion of the value of a so found into terms of volume by (14) shows that the simple equation (3) is valid up to 0.06 ml before the equivalence point, and from (2) the mercuric ion concentration at this point is $1.56 \cdot 10^{-6}$. From this point the steps are:

a. set $[Hg^{+2}]$ at values between $1.56 \cdot 10^{-6}$ and $7.21 \cdot 10^{-6}$, say $2 \cdot 10^{-6}$,
b. calculate a from (13a) which gives the value $2.34 \cdot 10^{-5}$,
c. should a pCl curve be required, calculate $[Cl^-]$ from (8), giving $2.74 \cdot 10^{-5}$,
d. calculate v_1 from (14), giving 0.047 ml, so that the titrant volume V = 99.953 ml.

After the equivalence point, the discriminant (7) gives a value of b of $1.545 \cdot 10^{-5}$, which from (16) indicates that exact equations are required until 0.62 ml of titrant has been added in excess. This point corresponds, from (3), to a chloride ion concentration of $9.85 \cdot 10^{-6}$.

a. Set $[Cl^-]$ at values between $9.85 \cdot 10^{-6}$ and $1.442 \cdot 10^{-5}$, say $1.0 \cdot 10^{-5}$,
b. Calculate b from (12), giving the value $1.0 \cdot 10^{-5}$,
c. Calculate $[Hg^{+2}]$ from (4), giving the value $1.5 \cdot 10^{-5}$,

d. Calculate v_2 from (16), giving the value 0.04 ml, so that the titrant volume $V = 100.04$ ml.

Precipitimetric titrations

The procedure and equations given apply strictly to all reactions resulting in the formation of species BA_2 or B_2A, but in the special case of sparingly soluble substances, it is customary to deal in terms of the ion product (solubility product S_{B_2A}) instead of the equilibrium constant. Thus the term $c = [B_2A]$ is absorbed into the constant and some simplification of the equations results. Taking the case of the salt B_2A in illustration, the equilibrium constant becomes

$$[B]^2[A] = S_{B_2A} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

Remote from the equivalence point, ion concentrations are calculated from

$$[B] = \left(\frac{S_{B_2A}}{[A]}\right)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (19)$$

$$[A] = \frac{S_{B_2A}}{[B]^2} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (20)$$

If the relative error allowed is E, then if *B is in excess*, when b falls below the value given by the discriminant (21),

$$b \leqslant \left(2\,\frac{(1-E)}{E}\,S_{B_2A}\right)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

the following equations are applicable,

$$[B] = b + 2[A] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

$$4[A]^3 + 4b[A]^2 + b^2[A] - S_{B_2A} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

Rearrangement of (23) in terms of b gives,

$$b = \left(\frac{S_{B_2A}}{[A]}\right)^{\frac{1}{2}} - 2[A] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (24)$$

which may be used in the simplified procedure.

When *A is in excess*, these equations become,

$$a \leqslant \left\{\left(\frac{1-2E}{2E}\right)^2 S_{B_2A}\right\}^{\frac{1}{3}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

$$[A] = a + {}^1/_2\,[B] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$

$$[B]^3 + 2\,a[B]^2 - 2\,S_{B_2A} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

Rearrangement of (27) gives

$$a = \frac{S_{B_2A}}{[B]^2} - \frac{[B]}{2} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (28)$$

for use in the simplified procedure. Titrant volumes before the equivalence point are calculated from (14) and (15), and after the equivalence point from (16) and (17).

For example, the following data were calculated by this method for drawing pAg and pC_2O_4 curves for the titration of 100 ml of 0.05 *M* oxalate with 0.1 *M* silver nitrate, using the value $1.1 \cdot 10^{-11}$ for the solubility product, and allowing an error of 1% ($E = 0.01$).

AT THE EQUIVALENCE POINT

$[Ag^+] = 2.8 \cdot 10^{-4}$; pAg = 3.553; $[C_2O_4^{-2}] = 1.4 \cdot 10^{-4}$; $pC_2O_4 = 3.854$.

BEFORE THE EQUIVALENCE POINT

$[Ag^+]$	pAg	a	$[C_2O_4^{-2}]$	pC_2O_4	*Volume of titrant added, ml*
$1.0 \cdot 10^{-4}$	4.0	$1.05 \cdot 10^{-3}$	$1.10 \cdot 10^{-3}$	2.958	95.89
$1.4 \cdot 10^{-4}$	3.854	$4.91 \cdot 10^{-4}$	$5.61 \cdot 10^{-4}$	3.251	98.065
$1.6 \cdot 10^{-4}$	3.796	$3.495 \cdot 10^{-4}$	$4.295 \cdot 10^{-4}$	3.367	98.612
$1.8 \cdot 10^{-4}$	3.745	$2.598 \cdot 10^{-4}$	$3.398 \cdot 10^{-4}$	3.469	99.008
$2.0 \cdot 10^{-4}$	3.699	$1.75 \cdot 10^{-4}$	$2.75 \cdot 10^{-4}$	3.561	99.303
$2.3 \cdot 10^{-4}$	3.638	$8.28 \cdot 10^{-5}$	$2.078 \cdot 10^{-4}$	3.682	99.629
$2.5 \cdot 10^{-4}$	3.602	$5.10 \cdot 10^{-5}$	$1.76 \cdot 10^{-4}$	3.754	99.796
$2.6 \cdot 10^{-4}$	3.585	$3.26 \cdot 10^{-5}$	$1.626 \cdot 10^{-4}$	3.789	99.87

AFTER THE EQUIVALENCE POINT

$[C_2O_4^{-2}]$	pC_2O_4	b	$[Ag^+]$	pAg	*Volume of titrant added, ml*
$1.2 \cdot 10^{-4}$	3.921	$6.275 \cdot 10^{-5}$	$3.028 \cdot 10^{-4}$	3.519	100.126
$1.0 \cdot 10^{-4}$	4.000	$1.315 \cdot 10^{-4}$	$3.315 \cdot 10^{-4}$	3.48	100.263
$8.0 \cdot 10^{-5}$	4.097	$2.105 \cdot 10^{-4}$	$3.705 \cdot 10^{-4}$	3.431	100.422
$5.0 \cdot 10^{-5}$	4.301	$3.69 \cdot 10^{-4}$	$4.690 \cdot 10^{-4}$	3.329	100.74
$3.0 \cdot 10^{-5}$	4.523	$5.455 \cdot 10^{-4}$	$6.055 \cdot 10^{-4}$	3.218	101.098
$1.0 \cdot 10^{-5}$	5.000	$1.028 \cdot 10^{-3}$	$1.048 \cdot 10^{-3}$	2.979	102.075
$5.0 \cdot 10^{-6}$	5.301	$1.473 \cdot 10^{-3}$	$1.483 \cdot 10^{-3}$	2.829	102.985

ACKNOWLEDGEMENT

The author wishes to express thanks to Dr. B. W. JEPSON for checking the mathematics and reading the paper in manuscript.

SUMMARY

A method is offered for the exact calculation of data for unsymmetrical titration curves in which the solution of cubic equations is avoided. Instead of calculating ion concentrations from the volume of titrant added in the conventional way, the calculation is reversed, and titrant volumes are calculated from supplied values of the concentration of the ion which is not in excess. The necessary precision is attained more easily by calculating the difference between the titrant volume and the equivalence point volume, so that the use of seven figure logarithms is unnecessary.

RÉSUMÉ

Une méthode est proposée pour le calcul précis dans le cas des courbes asymétriques de titrage; on évite ainsi l'emploi d'équations du troisième degré. Contrairement à la méthode habituelle, on ne calcule pas les concentrations des ions à partir du volume de la solution étalon ajoutée, mais le volume de cette dernière est calculé par la concentration de l'ion qui n'est pas en excès.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur genauen Berechnung der Daten für unsymmetrische Titrationskurven unter Vermeidung kubischer Gleichungen. Im Gegensatz zu dem üblichen Verfahren wird das Volumen der Titierflüssigkeit aus gegebenen Werten der Konzentration des nicht im Ueberschuss vorhandenen Ions berechnet.

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1 E. BISHOP, *Anal. Chim. Acta*, 22 (1960) 16.
2 E. BISHOP, *"Theory and principles of titrimetric analysis"* Volume I, Part B of *"Comprehensive Analytical Chemistry"*, Elsevier Publishing Company, Amsterdam 1959.
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ANALYSIS OF PEROXIDES

SEPARATION AND IDENTIFICATION BY PAPER CHROMATOGRAPHY

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(Received August 20th, 1959)

INTRODUCTION

The analysis of peroxides, and especially the separation and identification of the various components of mixtures of peroxides formed during the combustion of hydrocarbons in the gas-phase, has proved to be of considerable difficulty. Volumetric methods, such as the release of iodine from acidified potassium iodide or the oxidation of stannous chloride in alkaline solution, or colorimetric methods using titanous sulphate or ferrous thiocyanate are really only suitable if a single peroxide or a simple mixture of known components is to be analysed. HOLMAN *et al.*[1] have claimed that in the near infra-red there are characteristic peaks which may be used for the identification and determination of individual hydroperoxides, but even so analysis of other than simple mixtures would be difficult. The polarography of peroxides has been investigated by a number of workers[2-4], but the method appears to be best suited to the estimation of hydrogen peroxide in the presence of other peroxides, the polarograms of which are very similar.

During the last few years it has become apparent that chromatography is a powerful tool for peroxide analysis. Vapour-phase chromatography can only be used for the more volatile compounds[5], since otherwise the column temperature has to be so high that decomposition may occur. Paper chromatography, however, offers greater possibilities. TAYLOR[6] has shown that hydrogen peroxide, for instance, is separated from other peroxides on untreated paper, whilst other workers[5,7,8] using various moving and stationary phases have found that a number of peroxides have characteristic R_F values. Nevertheless the methods used have some disadvantages. The more volatile compounds, which are often of interest to the combustion chemist, tend to be lost by evaporation from the paper, and alkyl hydroperoxides are not separated one from another. Also the method for the detection of the spots is not always satisfactory. It was therefore decided to investigate the paper chromatography of peroxides in more detail, since the apparatus is simple and only small amounts of material are needed. The work has shown that the various classes of peroxide (*e.g.* hydrogen peroxide, hydroperoxides, peracids, dialkylperoxides) may be simply distinguished, and that the members of each class may be identified in most cases.

EXPERIMENTAL

The apparatus was of conventional design. A large glass tank, which could be made airtight at the top by a glass plate and rubber seal, contained the trough for the moving phase. This was

also present in a beaker so that the atmosphere was saturated during a run. The paper, Whatman No. 3, was clamped by large spring clips between two glass plates to prevent evaporation of any volatile components[9]. The plates were wide enough so that the clips did not overlap the paper, since if they did the outer spots drifted towards the edge. The length of paper between the solvent surface and the top of the plates was at least 5 cm, since otherwise the solvent front moved too fast and the spots were ill-defined. The paper was either (a) untreated, (b) treated with a 5 or 20-vol % solution of silicone oil in 80–100° petroleum ether and dried in an oven at 110° for 1 h, or (c) treated with a solution of ethylene glycol in acetone (5 or 20% by vol) and dried in a stream of air. With the treated paper it was necessary to cover the glass plates with silicone solution and allow the petroleum ether to evaporate before use, since this made them solvent repellent. With uncoated plates proper chromatograms were unobtainable. Various liquid mixtures were used as the moving phase (see next section). The samples were transferred to the paper by means of a loop of platinum wire, which was heated in a flame just before use to eliminate contamination.

When the solvent front had moved a suitable distance, the paper was removed from between the plates, and the position of the front marked. The chromatogram was dried in a stream of air for as short a time as possible (usually 1–2 min), and immediately sprayed with developer. This was either (a) a solution of ferrous thiocyanate[5], (b) a solution of *p*-phenylenediamine and acetaldehyde in aqueous acetic acid, (c) a dilute solution of *o*-tolidine and a small amount of ferrous ammonium sulphate in aqueous acetic acid, or (d) a 10-vol % solution of hydrogen iodide in glacial acetic acid. With the *o*-tolidine developer the chromatogram was enclosed between plates immediately after spraying to minimize oxidation of the ferrous salt. Characteristic derivatives of hydroperoxides were prepared by adding a sample to a solution of 0.2 g xanthydrol in 2 ml glacial acetic acid.

Concentrated solutions of hydrogen peroxide (97 and 99%), and pure specimens (98%) of *tert.*-butylhydroperoxide, di-*tert.*-butylperoxide and *tert.*-butylperbenzoate were donated by Laporte Chemicals, and *n*-, *sec.*- and *iso*butylhydroperoxides, 3-methylhexane-3-hydroperoxide, 3-methylhexyl-3-peroxytriphenylmethyl and *tert.*-amyl-9-xanthenyl were a gift from DR. A. G. DAVIES (University College, London). Di-*n*-heptylperoxide and *n*-heptyl hydroperoxide were prepared by the method of WILLIAMS AND MOSHER[10], and the other peroxides by the action of hydrogen peroxide on the appropriate alcohols and carboxylic acids or by the reaction of the appropriate aldehydes with hydroperoxides or hydrogen peroxide. The xanthydrol was from Kodak, and all other chemicals were of AnalaR quality.

RESULTS AND DISCUSSION

With the ferrous thiocyanate developer the presence of hydrogen peroxide, hydroperoxides or peracids was shown by red spots which "flashed up" immediately, whereas those due to other peroxides formed more slowly. With the diamine–aldehyde mixture the spots were pale red. The *o*-tolidine gave a blue-green colour only with compounds containing the –OOH group (or with substances which hydrolyze very readily in acid to give this group). With the hydrogen iodide solution nearly all peroxides gave a black or brown spot on a light brown background. The sensitivity of developers decreased in the above order. Two very stable peroxides, di-*tert.*-butylperoxide and 3-methylhexyl-3-peroxytriphenylmethyl, could not be detected on a chromatogram satisfactorily by any method.

A few results using untreated paper and different moving phases are shown in

TABLE I

UNTREATED PAPER

Section (a). Moving phase water–ether–*n*-butanol (1 : 10 : 10 by vol). Rate of movement of solvent front ≈ 4 cm/h.[6]		Section (b). Moving phase, ether. Rate of movement of solvent front ≈ 40 cm/h.	
Compound	R_F	*Compound*	R_F
hydrogen peroxide	0.50	hydrogen peroxide	0.14
hydroxyperoxides	0.95–0.98	hydroperoxides	1.0
hydroperoxides	1.0		

Table I. The alkyl hydroperoxides spread across the solvent front in a characteristic way. The limit of detection, *i.e.* the approximate minimum amount which, when transferred to the paper, gave a definite spot on the chromatogram on spraying with the most sensitive developer which reacted with the compound, was 20 μg for hydrogen peroxide.

Table II shows the results using the silicone treated paper. In section (a) the R_F values for various peroxides of relatively low molecular weight are given, whilst in section (b) the values are for the peroxides formed when some hydroperoxides reacted with xanthydrol.

TABLE II

SILICONE TREATED PAPER

Section (a). Stationary phase, silicone from a 5% solution. Moving phase, water–ethanol–chloroform (20 : 17 : 2 by vol). Rate of movement of solvent front $\approx$ 1.8 cm/h.[5]

Compound	R_F
di-*n*-heptylperoxide	0.018
$(Me_3COO)_2CHMe$	0.49
hydrogen peroxide	0.68
tert.-butyl, 2-hydroxyethylperoxide	0.80
cumene hydroperoxide	0.82
di-2-hydroxyethylperoxide	0.88
alkyl hydroperoxides	1.0

Section (b). Stationary phase, silicone from a 20% solution. Moving phase, 80 vol % methanol in water. Rate of movement of solvent front $\approx$ 5 cm/h.

Compound mixed with xanthydrol	R_F
tert.-butyl hydroperoxide	0.27, 0.45
tert.-amyl hydroperoxide	0.18, 0.43
n-heptyl hydroperoxide	0.0, 0.09(a), 0.19, 0.30, 0.49(b), 0.67, 0.80. Spots a and b are the strongest

The limits of detection using the silicone treated paper can only be roughly estimated. The value for hydrogen peroxide was again 20 μg, and the limits for the other peroxides must be considerably greater.

The results recorded in Section (b) varied somewhat with conditions, for instance the distance of travel of the moving phase. The *n*-heptyl hydroperoxide contained only a very small of peroxidic impurity, and it is difficult to understand why so many different peroxides were produced. There were always two main spots, a and b, and the maximum number on any chromatogram was six, the average being four. The results, however, from any one mixture of xanthydrol and heptyl hydroperoxide were reproducible. The use of the *o*-tolidine developer showed that no xanthenyl contained an –OOH group. In fact it was necessary to use the hydrogen iodide solution to detect the spots.

The results using the glycol treated paper are recorded in Table III.

It has been found that, using paper treated with a 25% glycol solution and the 5% ether solution as the moving phase, the faster moving peroxides gave more sharply defined spots, though the R_F values were not appreciably different from those in the

TABLE III

GLYCOL TREATED PAPER

Rate of movement of solvent front ≈ 15 cm/h.

Conditions: I. Stationary phase (S), glycol from a 5% solution; moving phase (M), 10 vol % *n*-butanol in 80–100° petroleum ether. II. S, glycol from a 20% solution; M, 50/50 chloroform in 80–100° petroleum ether. III. S, as for II; M, 5 vol % ether in 80–100° petroleum ether.

Compound	R_F		
	I	*II*	*III*
hydrogen peroxide	0.05	0	0
methyl hydroperoxide	0.36	0	0
ethyl hydroperoxide	0.38	0	0
tert.-butyl hydroperoxide	spreads across front	0.35	0.22
n-, *iso*-, *sec.*-butyl hydroperoxide	—	—	0.31
tert.-amyl hydroperoxide	—	—	0.43
n-heptyl hydroperoxide, 3-methylhexyl-3-hydroperoxide	—	—	0.80
cyclohexane hydroperoxide	—	—	0.30
cyclohexene hydroperoxide	—	—	0.14
n-heptan-3,3'-dihydroperoxide[a]	—	—	0.07
2,5-dimethylhexane-2,5-dihydroperoxide	—	—	0.20
cumene hydroperoxide	—	—	0.53
peracetic acid	0.26	0.05	0
perpropionic acid	0.54	0.16	0.11
per-*n*-, per-isobutyric acid	—	0.37	0.30
per-*n*-valeric acid	—	0.57	0.50
per-*n*-hexoic acid	—	0.71	—
percrotonic acid[b]	—	0.23	—
diacetylperoxide	0.37	0.18	—
hydroxymethylhydroperoxide	0.09	0	0
2-hydroxyethylhydroperoxide	0.22	0	0
dihydroxymethylperoxide	0.25	—	—
tert.-butyl, 2-hydroxyethylperoxide	—	—	0.55
n-heptyl, 2-hydroxyethylperoxide	—	—	0.92
cumyl,2-hydroxy-2-phenylethylperoxide	—	—	0.76
tert.-butylperbenzoate	—	—	1.0
di-*n*-heptylperoxide	—	—	0.95
$(Me_3COO)_2CHMe$	—	—	0.95

[a] From the reaction of heptan-3-one and hydrogen peroxide[8].

[b] Actually the main product of the gas-phase oxidation of crotonaldehyde at 150°.

final of column of Table III. The limits of detection for hydrogen peroxide, *tert.*-butyl hydroperoxide and *n*-heptyl hydroperoxide were 0.4, 10 and 20 μg respectively, and the values for the other peroxides will be of the same order.

The resolution possible with the methods described tended to vary with R_F value, since the more slowly moving spots were naturally the more sharply defined. Normally it was possible to separate compounds with low R_F values if these differed by not less than 0.03, and compounds with high R_F values if the difference was greater than 0.06. In general the R_F values were reproducible to within 5–10%.

These results amend and greatly amplify a brief preliminary report of this work[11]. Although the methods have been developed to separate and identify mainly relatively simple aliphatic peroxides, it should be easy to use them for other peroxides. The best method of tackling an unknown mixture will obviously depend on the components which may be expected to be present. However, it is recommended that the untreated paper is first used to detect any appreciable quantity of free hydrogen peroxide, and

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then as far as possible the glycol treated paper, since with this the chromatograms are obtained relatively quickly (1–2 h). Also the limits of detection are much smaller. In fact if too much peroxide was applied to the paper, smears rather than distinct spots were obtained. The silicone treated paper is best, however, for the identification of the more non-polar peroxides, *e.g.* dialkylperoxides. In fact the conditions given in Table II, Section b, may lead to better resolution in some cases. A check on any hydroperoxides present may be made by means of their xanthydrol derivatives. Also many hydroxyalkylperoxides may be split into their component hydroperoxides and carbonyl compounds by hydrolysis with dilute sulphuric acid. The former can then be identified as described, and the latter by paper chromatography of the dinitrophenylhydrazones[12]. Finally it should be mentioned that a number of synthetic and unknown mixtures have been successfully analysed using the techniques described.

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SUMMARY

The paper chromatography of hydrogen peroxide and numerous organic peroxides (mainly aliphatic) has been investigated under various conditions. By the use of several stationary and moving phases nearly the peroxides may be separated and identified in very small amounts.

RÉSUMÉ

Les auteurs ont effectué une étude par chromatographie sur papier, en vue de l'identification et de la séparation du peroxyde d'hydrogène et de divers peroxydes organiques.

ZUSAMMENFASSUNG

Es wird eine papierchromatische Methode zum Nachweis und zur Trennung von Wasserstoffperoxyd und organischen Peroxyden beschrieben.

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THE COLORIMETRIC DETERMINATION OF ORTHOPHOSPHATE IN THE PRESENCE OF CONDENSED PHOSPHATES

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During studies on the degradative changes in synthetic polymetaphosphates by ultrasonic irradiation[1], it was necessary to estimate the small amounts of orthophosphate originally present and also any orthophosphate found during the exposure. Colorimetric methods where high concentrations of sulfuric acid and molybdate are used[2,3] were inapplicable because of the labile nature of metaphosphate. The method of LOWRY AND LOPEZ[4], which is widely employed to estimate orthophosphate in the presence of acid-labile phosphate esters, was then tried. There was no color formation when fresh solutions of polymetaphosphates were tested, but after subjecting to ultrasonic vibration at 25 kc/sec for 30–60 min in a stainless steel vessel, there was a significant color development when the solutions were reanalyzed. It was found that the color formation due to orthophosphate was suppressed by high concentrations of polymetaphosphate. This inhibition was overcome partially, or nearly completely, by the addition of certain metal ions. The isobutanol extraction method of BERENBLUM AND CHAIN[5], in contrast to the direct colorimetric method of LOWRY-LOPEZ, was found to be suitable for the accurate assay of the orthophosphate in the presence of a large excess of condensed phosphates. With this method the orthophosphate content of the exposed solution was practically the same as that of the unexposed solution.

A study has been made of the influence of various condensed phosphates on the estimation of orthophosphate by the LOWRY-LOPEZ method, and of the addition of microquantities of copper and other metallic ions in preventing the inhibition.

MATERIALS AND METHODS

The technique of irradiation of the condensed phosphates used in these experiments has been reported[1]. The LOWRY-LOPEZ estimation was carried out as described by DAVISON-REYNOLD, BARRUETO AND LEMON[6]. Acidic deproteinizing agents were not present and hence sodium acetate was not added to neutralize free acid. The system contained 2 ml of the test solution, 7 ml of acetate buffer pH 4, 1 ml of 1% ammonium molybdate in 0.05 *N* sulfuric acid and 1 ml of 0.25% freshly prepared ascorbic acid. The isobutanol extraction method was carried out as modified by WEIL-MALHERBE[7]. Color comparisons were made in a KLETT-SUMMERSON photoelectric colorimeter.

RESULTS

Interference by polymetaphosphate in the estimation of orthophosphate by the isobutanol extraction method

Increasing amounts of a sample of polymetaphosphate with average molecular

weight 14,200 were added to 5 μg of orthophosphate and color was developed. There was no interference even at concentrations of metaphosphate-phosphorus as high as 600 times that of the orthophosphate.

Influence of the lower condensed phosphates on the color development of orthophosphate by the LOWRY-LOPEZ *method*

Linear condensed phosphates. Preneutralized pyrophosphate and tri-and tetrapolyphosphates were added in increasing amounts to 10 μg of orthophosphate and the color was measured after 15 and 30 min at room temperature (25–28°). All these samples contained a small proportion of orthophosphate.

TABLE I

INFLUENCE OF THE LOWER LINEAR CONDENSED PHOSPHATES ON THE COLOR DEVELOPMENT OF 10 μg ORTHOPHOSPHATE BY THE LOWRY-LOPEZ METHOD

	Phosphorus equivalent of condensed phosphate added μg	*Colorimeter reading*	
		15 min	*30 min*
Pyrophosphate	Nil	56	56
	100	56	55
	200	58	58
	400	61	61
	500	63	63
	600	62	62
	700	33	45
	800	18	28
	900	5	10
	1000	0	0
Tripolyphosphate	Nil	60	60
	1000	78	80
	2000	95	98
	3000	48	71
	4000	5	13
Tetrapolyphosphate	Nil	58	58
	200	74	78
	400	82	90
	600	86	104
	800	90	108
	1000	64	115
	2000	4	21

As seen from Table I pyrophosphate did not inhibit the color development till the ratio of pyrophosphate-phosphorus to orthophosphate was 50 : 1—60 : 1. Above this level there was progressive inhibition. When the pyrophosphate-phosphorus was 100-fold that of orthophosphate the inhibition was practically complete. Tripolyphosphate had less effect than pyrophosphate. Tetrapolyphosphate was more inhibitory than tripolyphosphate, but less so than pyrophosphate. In all cases the inhibition was partly overcome on storage of the developed color for 30 min, as indicated by the higher colorimetric readings.

Cyclic metaphosphates. Trimeta- and tetrametaphosphates at concentrations corresponding to over 5000 μg of phosphorus did not inhibit the color due to 5–20 μg of orthophosphate.

Effect of polymetaphosphates of varying molecular weights on the LOWRY-LOPEZ *estimation*

A number of polymetaphosphates, with average molecular weights ranging from 1,500 to 16,000, were tested for their influence on the color development due to orthophosphate.

TABLE II

INFLUENCE OF THE POLYMETAPHOSPHATES ON THE COLOR DEVELOPMENT OF 10 μg ORTHOPHOSPHATE BY THE LOWRY-LOPEZ METHOD

	Phosphorus equivalent of condensed phosphate added μg	*Colorimeter reading*	
		15 min	*30 min*
1. Polymetaphosphate, weight-average molecular weight 1545; orthophosphate content by the BERENBLUM-CHAIN method = 9.16% of total phosphate.	Nil	57	59
	100	117	117
	200	158	167
	300	115	220
	400	72	180
	1000	10	31
	1500	0	11
2. Polymetaphosphate, weight-average molecular weight 2045; orthophosphate content by the BERENBLUM-CHAIN method = 3.37% of total phosphate.	Nil	58	
	50	67	
	100	68	
	150	63	
	200	36	
	400	29	
	600	17	
	800	14	
	1000	11	
3. Polymetaphosphate, weight-average molecular weight 3400; orthophosphate content by the BERENBLUM-CHAIN method = 0.85% of total phosphate.	Nil	60	60
	50	62	63
	100	65	66
	150	65	70
	200	50	71
	400	38	75
	600	34	77
	800	31	81
	1000	20	76
4. Polymetaphosphate, weight-average molecular weight 5600; orthophosphate content by the BERENBLUM-CHAIN method = 0.5% of total phosphate.	Nil	60	65
	100	62	73
	200	51	78
	300	45	78
	400	42	82
	600	40	77
	800	47	87
5. Polymetaphosphate, weight-average molecular weight 8200; orthophosphate content by the BERENBLUM-CHAIN method = 0.5% of total phosphate.	Nil	57	60
	50	29	55
	100	16	26
	150	12	19
	200	10	15
	400	7	9
	600	6	8
	800	6	8
	1000	4	7

TABEL II (continued)

	Phosphorus equivalent of condensed phosphate added μg	*Colorimeter reading*	
		15 min	*30 min*
6. Polymetaphosphate, weight-average molecular weight 14,200; orthophosphate content by the BERENBLUM-CHAIN method = 0.43% of total phosphate.	Nil	63	
	50	63	
	100	63	
	150	50	
	200	38	
	300	26	
	400	19	
	500	15	
	1000	7	
7. Polymetaphosphate, weight-average molecular weight 16,700; orthophosphate content by the BERENBLUM-CHAIN method = 0.52% of total phosphate.	Nil	57	60
	50	26	50
	100	14	28
	150	15	26
	200	8	19
	400	7	10
	600	6	7
	800	5	6
	1000	3	6

As seen from Table II all the polymetaphosphates inhibited more powerfully than the lower linear condensed phosphates. There seemed to be no correlation between the degree of inhibition and the molecular weight of the samples.

Orthophosphate estimation in the dialyzate of polymetaphosphates by the LOWRY-LOPEZ *and the* BERENBLUM-CHAIN *methods*

10-ml aliquots of 1% solutions of polymetaphosphates were dialyzed against 80 ml of distilled water in the cold for 24 h. Analysis of the dialyzate indicated the presence of significant amounts of orthophosphate by the LOWRY-LOPEZ method even though the original solutions had given negative tests. The results by the BERENBLUM-CHAIN method agreed closely with those by the LOWRY-LOPEZ method. It may be pointed out that the concentration of condensed phosphate in the dialyzate was less than 30-fold that of orthophosphate.

Influence of time, temperature and ammonium molybdate concentration

A sample of polymetaphosphate with an average molecular weight of 14,200 was taken as a typical example. Ten μg of orthophosphate were mixed with 5000 and 1000 μg of metaphosphate-phosphorus. The color was developed as usual and the readings were observed at 15 min intervals. The color intensity gradually increased, reached a maximum and remained fairly constant at the end of about 90 min. This colorimeter reading corresponded to the total orthophosphate present. A solution containing 1000 μg of polymetaphosphate-phosphorus, but no added orthophosphate, yielded a color maximum after 90 min which corresponded to its orthophosphate content estimated by the isobutanol extraction method. Increasing the temperature from 27 to 37°, or the ammonium molybdate concentration from 1.0 to 1.5%, did not significantly alter the color maxima at the end of 15 min.

Influence of added copper ions

The sample of polymetaphosphate was the same as in the previous experiment. The color was developed at 25° in the presence of known amounts of copper sulfate, keeping the final volume constant.

TABLE III

INFLUENCE OF COPPER IONS ON THE COLOR DEVELOPMENT OF ORTHOPHOSPHATE IN THE PRESENCE OF METAPHOSPHATE BY THE LOWRY-LOPEZ METHOD

Final concn. of copper sulfate M	*Orthophosphate added μg*	*Phosphorus equivalent of polymetaphosphate added μg*	*Colorimeter reading*	
			15 min	*30 min*
1) $4.2 \cdot 10^{-6}$				
	10	Nil	65	65
	10	500	76	77
	10	1000	76	89
	Nil	1000	23	26
2) $8.4 \cdot 10^{-6}$				
	10	Nil	63	65
	10	500	74	76
	10	1000	75	86
	Nil	1000	21	21
3) $1.68 \cdot 10^{-5}$				
	10	Nil	70	70
	10	500	84	85
	10	1000	83	85
	Nil	1000	20	20
4) $2.94 \cdot 10^{-5}$	10	Nil	70	73
	10	500	85	87
	10	1000	84	86
	Nil	1000	21	23
5) $4.2 \cdot 10^{-5}$	10	Nil	73	76
	10	500	88	90
	10	1000	84	88
	Nil	1000	21	24

The data (Table III) show that the inhibition due to polymetaphosphate tended to be overcome by the addition of copper ions. When 500 μg of metaphosphate-phosphorus was present along with 10 μg of orthophosphate, the addition of $4.2 \cdot 10^{-6}$ *M* copper sulfate over came the inhibition. However, when 1000 μg of metaphosphate-phosphorus was present the inhibition was not completely overcome in 15 min even when the copper sulfate was increased to $4.2 \cdot 10^{-5}$ *M*. At high concentrations of copper sulfate ($4.55 \cdot 10^{-3}$ *M*) the intensity of the color of the orthophosphate standard increased abruptly and then decreased with time.

Influence of metal ions other than copper

Since the metaphosphate solution was irradiated in a stainless steel vessel, and traces of metal might be dissolved, the influence of iron, nickel and chromium on the color development of orthophosphate in the presence of polymetaphosphate was tested. Nickel ($9.1 \cdot 10^{-5}$ *M*) accelerated the color development, but the inhibition was not completely overcome. At a concentration of $4.55 \cdot 10^{-4}$ *M* of nickel, 10 μg of orthophosphate could be estimated in the presence of 500 μg polymetaphosphate-

phosphorus, but 1000 μg of metaphosphate-phosphorus interfered. Ferric ions ($9.1 \cdot 10^{-5}$ M) did not accelerate the color development but at a concentration of $1.82 \cdot 10^{-4}$ M, they also partially overcame the inhibition. Ferric ions at a concentration of $4.55 \cdot 10^{-4}$ M allowed the estimation of 10 μg of orthophosphate in the presence of 500 μg but not 1000 μg of polymetaphosphate-phosphorus. Ferrous ion ($9.1 \cdot 10^{-5}$ M) reduced the phosphomolybdate. Chromium had practically no effect even at $4.55 \cdot 10^{-4}$ M.

DISCUSSION

The presence of interfering materials in tissue extracts was noted by LOWRY AND LOPEZ[4]. Powerful inhibition has since been shown to be associated with liver, brain and bacterial extracts[8]. BRUEMMER AND O'DELL[9] and PEEL AND LOUGHMAN[8] independently and simultaneously discovered that the addition of minute quantities of copper ions helped to overcome these interferences. The former workers[9] suggested that the interference in liver extracts is due to sulfhydryl compounds and that copper ions promote their atmospheric oxidation to inactive disulfide derivates. On the other hand, PEEL AND LOUGHMAN[8] suggested that copper, which is always present as a contaminant in the reagents, is essential as an intermediate electron acceptor to bring about the reduction of phosphomolybdate by ascorbic acid. The various inhibitors function by binding the copper ions.

Results obtained by the present authors show that high concentrations of the linear lower and, more so, higher condensed phosphates inhibit the color development of small amounts of orthophosphate by the LOWRY-LOPEZ method. The cyclic metaphosphates, used in similar concentrations, do not inhibit. The fact that orthophosphate can be estimated quantitatively in the dialyzates obtained from the polymetaphosphates may be attributed to the fact that the dialyzates contain lower molecular compounds, which are less inhibitory and that the concentration of the condensed phosphate is low.

The inhibition due to polymetaphosphates is overcome, either partially or completely, by the addition of copper and other metal ions. PEEL AND LOUGHMAN[8] considered that the effect of copper is a specific one and that it cannot be replaced by other ions in comparable concentration. The present investigation shows that low concentrations of nickel and higher concentrations of iron are also effective in overcoming the inhibition due to polymetaphosphate. The addition of copper to allow the assay of orthophosphate in the presence of metaphosphate cannot be recommended as a routine practice, since the inhibition is not overcome quantitatively at higher concentrations of polymetaphosphate.

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SUMMARY

The linear condensed phosphates inhibit the development of color due to orthophosphate by the method of LOWRY AND LOPEZ. The higher members of the series (the polymetaphosphates) have more effect than the lower members (pyrophosphate, tripoly- and tetrapolyphosphates). The two cyclic metaphosphates, trimeta- and tetrametaphosphate, do not inhibit. The inhibition by

polymetaphosphate is partly overcome by storing the developed color for about 90 min. Low concentrations of copper and nickel and higher concentrations of iron(III) remove either partially or completely the inhibition due to polymetaphosphates. None of the metaphosphates interferes in the estimation of orthophosphate by the isobutanol extraction method of BERENBLUM AND CHAIN.

RÉSUMÉ

Une étude a été effectuée sur le dosage colorimétrique de l'orthophosphate en présence des phosphates condensés, par la méthode de LOWRY-LOPEZ. On a examiné également l'influence de traces de cuivre et d'autres cations sur le dosage.

ZUSAMMENFASSUNG

Der störende Einfluss von Polyphosphaten auf die colorimetische Bestimmung der Orthophosphate nach LOWRY-LOPEZ kann durch kleine Mengen Kupfer, Nickel oder Eisen(III) weitgehend ausgeschaltet werden.

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PYROMELLITEIN INDICATORS

I. ACID–BASE INDICATORS CONTAINING FOUR CONDENSED PHENOLIC GROUPS

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In 1906 SILBERRAD[1–3] reported the preparation of dyes formed by condensation of phenol and of resorcinol with pyromellitic dianhydride to give materials which he suggested could be used as dyes with mordants.

While such compounds are possible indicators, since they resemble phenolphthalein and fluorescein in their structures, no attempt seems to have been made to use them as such since that time. The dianhydride structure should permit the preparation of two very interesting groups of compounds, since it is possible to condense two or four phenolic groups. There is also the possibility of making "internally mixed" indicators by condensing two different phenols with the dianhydride.

In preparing such compounds the author has found that it is much easier to obtain

clearly defined pure compounds containing four condensed phenolic groups and the present paper is concerned with them. More work is being done on the purification of compounds containing two condensed phenolic groups, and a report on this will be presented later.

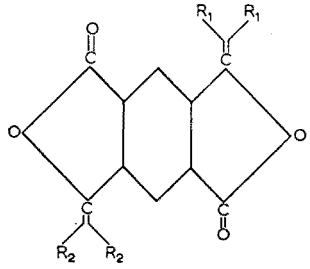

Fig. 1. Structure of pyromellitein indicators.

Indicators containing four phenolic groups of the same kind may be prepared by the usual methods for making phenolphthalein and resorcinol. When 8-hydroxy quinoline was used, it was necessary to precipitate the indicator at a pH of about 7 since it is soluble in both acids and bases, as was the case also with the condensation product with dimethyl aniline.

In testing these compounds for use as indicators, a titration was carried out using a Fisher titrimeter, the pH range and color change being noted. Samples were prepared using buffers for use in determining the transmission minima using a Beckman spectrophotometer, Model B. These results are summarized in Table I.

TABLE I

OPTICAL PROPERTIES OF PYROMELLITEIN INDICATORS (VISIBLE LIGHT)

R_1	R_2	*Color change*	*pH-range*	*Maximum absorption Å*
Phenol	Phenol	C-pink	10.3–12	5600
Oxine	Oxine	Y-Gr. (1)	10–11.5	6400 and 3700 (Green)
o-ClC_6H_4OH	*o*-ClC_6H_4OH	C-purple	9–10.9	5750
Resorcinol	Resorcinol	Pink-red	Red above 8	4600-pH 5 5000-above pH 8
Phenol	Resorcinol	Pink-red	Red above 6 (2)	4600-pH 5 4900-above pH 6
DiMeAniline	DiMeAniline	Y-Blue (3)	4–5.6	6200 (4)

1. Decrease in transmission to pH 11.5, then increase at pH 12.
2. Sudden brightness at pH 6 in visible light.
3. Sudden change at pH 4.5 going from acid to base. CO_2 interferes in base to acid.
4. Pronounced decrease in transmission below 4500 Å at all pH values.

Since some of these compounds showed fluorescence under ultraviolet light, a titration was carried out using the titrimeter along with a shielding arrangement so that the solution was exposed to ultraviolet light and shielded from daylight. Since it could be seen visually that the fluorescing indicators showed fluorescence to blue light, the maximum for the emitted fluorescent light was determined by a differential method using the Beckman. This consisted of determining the light

transmission twice by the method usually used with this instrument, except that on the first run a blue filter was placed ahead of the sample cells (including the water cell), and on the second run the same blue filter was put after the sample cells. The difference was a measure of the amount of fluorescence. The method was checked using dichlorofluorescein. What is obtained is not an absolute measure of the fluorescence but simply the maximum of the fluoresced light. Results are shown in Table II.

TABLE II

FLUORESCENT PYROMELLITEIN INDICATORS (2)

R_1	R_2	*Color change (1)*	*pH-range*	*Emission maximum*
Resorcinol	Resorcinol	Y-Fl. Gr.	0.6–2	480
Phenol	Resorcinol	Y-Fl. Gr.	1.9–4	480

1. Under near ultraviolet light.
2. The DiMeAniline and oxine compounds also showed slight blue fluorescence from pH 1–12.

The data presented in this paper were obtained in dilute solution by titrating carbonate free NaOH with HCl, both solutions being about 0.1 *N*. The polyprotic nature of these indicators make it very probable that there will be pronounced salt effects, which are yet to be determined. The oxine condensation compounds are of particular interest since they contain not only acid hydrogens but are capable of forming complexes through the nitrogen of the oxine. Particular attention will be paid to these compounds in future studies of salt effects.

ACKNOWLEDGEMENTS

The author wishes to acknowledge assistance in this project through a grant from the National Institutes of Health (P.H.S. Grant RG-5736), and also the generosity of the E. I. DuPont de Nemours Co. in supplying him with pyromellitic dianhydride.

SUMMARY

A series of indicators have been prepared by condensing pyromellitic dianhydride with various phenols. Color changes and pH ranges have been determined, both in visible light and in ultraviolet light, for those indicators that fluoresce. Of particular interest is the indicator prepared using oxine as the phenolic compound, since there is a possibility in this case of complex formation through the nitrogens of the condensed oxine groups.

RÉSUMÉ

Une série d'indicateurs ont été préparés par condensation du dianhydride pyromellitique avec divers phénols. Les pH de virage et les changements de colorations ont été déterminés.

ZUSAMMENFASSUNG

Die Kondensationsprodukte von Pyromellitsäure-dianhydrid mit verschiedenen Phenolen eignen sich als Indikatoren. Die pH Werte der Umschlagspunkte und die Farbänderungen werden angegeben.

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A RAPID METHOD FOR THE DETERMINATION OF ORGANIC CARBON IN SOIL

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INTRODUCTION

In 1927 SCHOLLENBERGER[1] proposed treating the soil with dichromate instead of permanganate and, since then, this method has tended increasingly to replace the wet combustion with permanganate. Various modifications of the dichromate oxidation have been proposed, some of which give low results compared with dry combustion and therefore require empirical conversion factors. Such unsatisfactory results must be attributed either to a desire to simplify the analysis, or to the choice of unfavourable conditions.

The modification of KURMIES[2] has been proved reliable (MEBIUS *et al.*[3]), but it would be improved if a reliable indicator could be found for direct titration of the surplus of dichromate with Mohr's salt. In the procedure of KURMIES an excess of ferrous salt is added and is titrated with permanganate, no indicator being used. It requires some experience to decide the end-point. In a recent paper SIMAKOV[4] suggests a solution of N-phenylanthranilic acid; his claim of a very sharp and clear end-point has been confirmed by us.

However SIMAKOV does not use the KURMIES modification but the simpler method of TIURIN[5], which often gives low values compared with those of the elementary analysis. We therefore decided to check the procedure of TIURIN-SIMAKOV and to try to improve it to give full agreement with the dry combustion. However, chlorides can interfere, since CrO_2Cl_2, formed as an intermediate with chloride-containing soils and boiling at 117°, can evaporate at the reaction temperature of *ca.* 150°. In such cases SIMAKOV and some others[6] add Ag_2SO_4. The KURMIES oxidation, performed at *ca.* 100°, avoids this complication. We therefore advise the KURMIES method for all saline soils, though the application of a reflux condenser, as proposed by us, might perhaps retain most of the CrO_2Cl_2 vapours.

EXPERIMENTAL

The errors of the procedure of TIURIN-SIMAKOV and other modifications of the dichromate oxidation are probably due to a. amount of sulphuric acid used, b. time of boiling, c. correctness of the blanks, or d. soil/reagent proportion. We therefore investigated these factors.

Influence of the amount of sulphuric acid

In these experiments, 0.1–0.5 g of the dried and ground soil was placed in 100-ml Erlenmeyer flasks. The required amount of conc. sulphuric acid and 10 ml of a 0.4 *N* solution of dichromate in 62% (w/v) sulphuric acid were added and, after attaching a reflux-condenser, the mixture was boiled for half an hour on an electric sand-bath. After cooling and rinsing the condenser with water, 3 to 5 drops of the N-phenylanthranilic acid were added and the titration performed with a 0.2 *N* solution of Mohr's salt at room temperature. With the addition of one drop, the colour shifted from violet to bright green. A blank determination was run simultaneously. The results with a clay soil are presented in Table I and also graphically in Fig. 1.

TABLE I

INFLUENCE OF SULFURIC ACID ON THE ORGANIC-CARBON DATA

ml conc. sulfuric acid added per determination	*ml 0.2 N Mohr's salt per determination*	*ml 0.2 N Mohr's salt per blank determination*	% C	
			in air-dry soil	*in the dry matter of the soil*
0	13.16	20.19	2.11	2.17
1	12.59	20.16	2.27	2.34
3	11.45	20.08	2.59	2.66
4	10.91	20.03	2.74	2.82
5	10.65	19.85	2.76	2.84
6	10.27	19.45	2.75	2.83
7	9.87	18.96	2.73	2.81
8	9.26	18.22	2.69	2.77

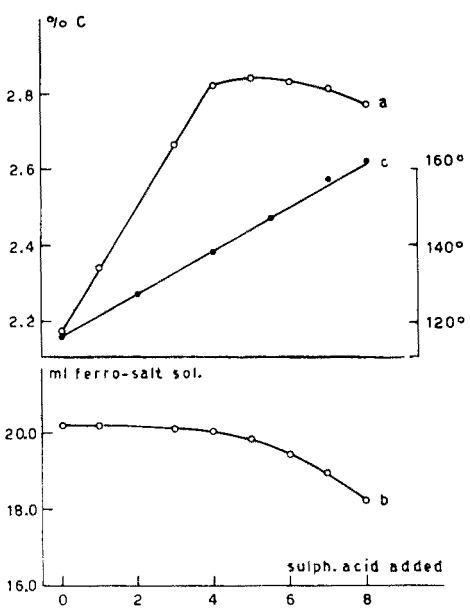

Fig. 1. Influence of quantity of sulphuric acid upon: a = % C, b = blank, c = boiling point (mean).

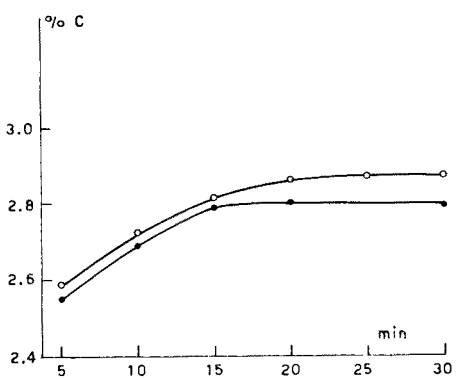

Fig. 2. Influence of the boiling time. o = clay soil, • = sandy soil.

These figures show clearly that an addition of *ca.* 5 ml of conc. sulfuric acid is the optimum for this reaction, and that the procedure proposed by TIURIN gives results that are much too low.

Influence of the time of boiling

A clay soil and a sandy soil with a rather high humus-content were used. The sand-bath had of course to be heated to the desired temperature before the start. The results are shown in Fig. 2. It is evident that 20 min boiling is sufficient but, for the sake of safety, 30 min was used in the standard procedure.

Influence of the blank determination

A difficult point with the dichromate methods is the partial decomposition of dichromate during boiling; it is thus necessary to apply a correction factor. Blanks with and without boiling were determined with 0.2 and 0.4 *N* dichromate. The concentration of sulfuric acid, the total volume, and the boiling time were the same as for all normal blanks. The results are shown in Table II.

TABLE II

INFLUENCE OF THE CONCENTRATION OF DICHROMATE IN BLANKS ON THE LOSS DURING BOILING

	ml 0.2 N Mohr's salt titrated					
	10 ml 0.4 N dichromate			*10 ml 0.2 N dichromate*		
	not boiled	*boiled*	*difference*	*not boiled*	*boiled*	*difference*
Expt. 1	20.26	19.85	0.41	10.13	9.96	0.17
Expt. 2	20.26	19.74	0.52	—	9.82	0.31
Expt. 3	—	19.83	0.43	—	9.89	0.24
mean	20.26	19.80	0.46	10.13	9.89	0.24

It is evident that the loss of dichromate depends approximately on its concentration. The correction added must therefore be proportional to the dichromate used. Since 10 ml of 0.4 *N* dichromate is used in the blanks, for each ml of dichromate consumed (expressed as ml of 0.2 *N* Mohr's salt used in the titration) a correction of 0.46/20.26 = 0.023 ml must be added.

If, for example, 10.00 ml of 0.2 *N* ferrous solution was required in the titration, while the blank required 19.80 ml, then 9.80 ml of dichromate solution was used. To this result must be added 9.80 × 0.023 = 0.22 ml; the corrected figure therefore is 10.02, and this must be used to calculate the content of organic carbon.

Influence of the soil/reagent proportion

Increasing amounts of clay and a humus-containing sandy soil, were treated with the same standard amount of reagent; the results are shown in Table III.

The influence of varying amounts was not altogether negligible. However, if the soil consumes 5–10 ml of dichromate, the results may be considered correct.

TABLE III

INFLUENCE OF SOIL/REAGENT PROPORTION

Soil taken (mg)	Sandy soil		Clay soil	
	0.2 N dichromate consumed (ml)	% C	0.2 N dichromate consumed (ml)	% C
100	4.64	2.78	2.94	1.76
200	9.18	2.75	5.84	1.75
300	13.77	2.75	8.70	1.74
400	—	—	11.46	1.72
500	—	—	14.18	1.70

Reproducibility

The standard deviation, S.D. of an individual estimate can be calculated from any series of duplicate analyses by means of the formula S.D. $= \sqrt{\Sigma\Delta^2/2n}$, where Δ denotes the difference between the duplicates and n is the number of samples in the series. From a series of 14 such determinations (whose means ranged from 1.45 to 7.68, with an average of 2.86) a value of S.D. = 0.034 was found. This represents a standard deviation of 1.2% for a single analysis.

Comparison of the method with the standard (dry combustion) method

The average carbon content of the 14 samples mentioned above was 2.861% according to the dichromate method and 2.856% according to dry combustion. The difference is clearly negligible.

Statistical analysis of the relevant data, which are assembled in Table IV, confirms this.

TABLE IV

COMPARISON OF THE RESULTS

Sample no.	% C		difference	
	dichromate method[a]	elem. analysis[a]	absolute	in %
1	2.83	2.82	+0.01	0.4
2	1.99	2.03	—0.04	2.0
3	2.94	2.97	—0.03	1.0
4	3.66	3.58	+0.08	2.2
5	2.56	2.63	—0.07	2.7
6	2.33	2.32	+0.01	0.4
7	2.45	2.48	—0.03	1.2
8	1.98	2.06	—0.08	4.0
9	2.91	2.92	—0.01	0.3
10	1.45	1.47	—0.02	1.4
11	2.67	2.60	+0.07	2.6
12	2.86	2.77	+0.09	3.2
13	7.68	7.52	+0.16	2.1
	(7.69)	—	—	—
14	1.74	1.82	—0.08	4.6
	mean 2.86	2.85		

[a] Mean of duplicate determinations.

Conclusions

The above tests justify the conclusion that the method can be used for routine

determinations of organic carbon in soil. The method is easier, quicker and cheaper than elementary analysis or the KURMIES method, and in ordinary soils of all types it is as accurate. Saline soils and those containing inorganic oxidizable substances (FeS; FeS_2; Fe^{+2}, Mn^{+2}, etc.) must be excluded; dry combustion is then the only fully reliable method.

RECOMMENDED PROCEDURE

Reagents

A 0.267 *N* solution of $K_2Cr_2O_7$ (13.072 g of $K_2Cr_2O_7$ and 550 ml of conc. sulfuric acid in distilled water, diluted to 1 l).

Sulfuric acid 95–97% ($d = 1.84$). A 0.2 *N* solution of Mohr's salt (78.390 g of $(NH_4)_2SO_4.FeSO_4.6H_2O$ and 50 ml conc. sulfuric acid in distilled water, diluted to 1 l). The indicator used by SIMAKOV[7]: 200 mg of N-phenylanthranilic acid dissolved in an 0.2% Na_2CO_3 solution.

Procedure

Weigh 0.1 to 0.5 g (depending on the estimated C-content) of the finely ground soil into an Erlenmeyer flask of 100 ml; add 15 ml (exactly) of the dichromate reagent, connect the flask by a glass joint with a reflux condenser, and boil for 30 min on an electrically heated sand-bath. Cool the flask in water, while still connected to the condenser, and then rinse the latter with water. Titrate with Mohr's salt solution at room temperature, adding 3 drops of the indicator. As the end-point is approached a few more drops of the indicator must be added. The colour change is from violet to bright green. A blank analysis must be run in exactly the same way.

The difference between the blank and the soil analysis must be corrected as explained on p. 122. From this corrected value, *A*, the content of organic carbon, expressed as % by weight of the soil, can be calculated from the expression:

$$\frac{A \times 0.2 \times 0.003 \times 100}{\text{g soil}} = \% \text{ C}$$

ACKNOWLEDGEMENT

The author is grateful to Dr. G. W. HARMSEN for his interest in this work.

SUMMARY

TIURIN's method for the determination of organic carbon in soil is modified to give results practically identical with those of the dry combustion method. The standard deviation of a single determination is only 1.2%. By using 50 mg of soil and 10 ml of 0.2 *N* dichromate solution, soils with a carbon content up to 12% can be analyzed. The method is suitable for all soils except those containing much chloride or reducing substances other than organic carbon. Carbonates do not interfere.

RÉSUMÉ

Une modification de la méthode de TIURIN est proposée pour le dosage du carbone organique dans les terres. On peut obtenir ainsi des résultats pratiquement identiques à ceux fournis par la méthode de combustion par voie sèche.

ZUSAMMENFASSUNG

Es wird eine Modifikation der Methode von TIURIN zur Bestimmung des organisch gebundenen Kohlenstoffs in Bodenproben beschrieben, die praktisch die gleichen Resultate ergibt, wie sie durch die trockene Verbrennungsmethode erhalten werden.

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DETERMINATION OF IRON IN WINE USING 2,4,6-TRIPYRIDYL-*s*-TRIAZINE

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The first use of the now well known colorimetric reagent for iron, 1,10-phenanthroline, was for the determination of iron in wine (SAYWELL AND CUNNINGHAM[1]) and its use has become more or less standard, the various procedures which have been proposed differing primarily in the method used to decompose the sample prior to color development. Usually, the organic material present is destroyed by a wet ashing with nitric and perchloric acids or with hydrogen peroxide. However, BANICK AND SMITH[2], utilizing the supersensitive iron reagent, bathophenanthroline, eliminate the usual wet ashing and extract the red iron(II) bathophenanthroline compound directly into isoamyl alcohol.

A new ferroine reagent, 2,4,6-tripyridyl-*s*-triazine (TPTZ), has recently been introduced as an extremely sensitive reagent for the colorimetric determination of iron[3]. This reagent possesses many of the advantages of bathophenanthroline and is much easier to prepare. TPTZ reacts with iron(II) to give an intensely violet colored, water soluble ion, $Fe(TPTZ)_2^{+2}$. In the presence of perchlorate, the iron(II) derivative can be extracted into nitrobenzene. The molar extinction coefficients are 22,600 at 593 mμ in aqueous solution and 24,100 at 595 mμ in nitrobenzene. The permissible pH range is 3.4 to 5.8 in aqueous solution and when the extraction into nitrobenzene is used, the pH range is increased to 2.7 to 7.0. The color conforms to Beer's law in both solvents.

The object of the present work was to develop a suitable procedure for the determination of iron in wine using TPTZ and taking advantage of the extraction feature to eliminate the wet ashing step.

RECOMMENDED PROCEDURE

Reagents

2,4,6-Tripyridyl-s-triazine (TPTZ). 0.001 *M*. Dissolve 0.312 g of TPTZ (synthesized by the method of CASE AND KOFT[4]; available from the G. Frederick Smith Chemical Co., Columbus, Ohio) in a few drops of hydrochloric acid and dilute to 1 l with water.

Hydroxylammonium chloride. 10%. Dissolve 100 g of hydroxylammonium chloride in 900 ml of water. To remove iron add 10 ml of 0.001 *M* TPTZ and 1 g of sodium perchlorate and extract with 25 ml of nitrobenzene.

Sodium acetate–acetic acid buffer. Prepare a solution 2 *M* in acetic acid and 2 *M* in sodium acetate by dissolving 115 ml of acetic acid and 164 g of sodium acetate in water and diluting to 1 l. To remove iron, add 10 ml of 10% hydroxylammonium chloride, 10 ml of 0.001 *M* TPTZ, and 1 g of sodium perchlorate and extract the solution with 25 ml of nitrobenzene.

Sodium perchlorate. 10%. Dissolve 100 g of sodium perchlorate in 900 ml of water and remove iron in the same way as with the acetate buffer.

Standard iron solution. Dissolve 0.2500 g of electrolytic iron in 20 ml of hydrochloric acid and dilute to exactly 1 l. Pipet 100.0 ml of this solution into a 1-liter volumetric flask, add 10 ml

of hydrochloric acid and dilute to volume with water. Dilute 100.0 ml of this solution and 10 ml of hydrochloric acid to exactly 1 l to give a solution containing 2.50 μg of iron per ml.

Preparation of calibration curve

Pipet various volumes from 0 to 15 ml of a solution containing 2.50 μg of iron per ml into 125-ml separatory funnels. Add 2.0 ml of 10% hydroxylammonium chloride, 5.0 ml of 0.001 *M* TPTZ, 5.0 ml of acetate buffer and 1.0 ml of 10% sodium perchlorate to each funnel. Extract each solution three times using 4.0-, 2.0- and 2.0-ml portions of nitrobenzene. Collect the extracts of each solution in a 10-ml volumetric flask, dilute to volume with ethanol and determine the absorbancy at 595 mμ using 1-cm cells.

Procedure for determination of iron with preliminary wet ashing.

Pipet a 3.00-ml sample of wine into a 250-ml conical flask fitted with a reflux head. Add 10 ml of nitric acid and 5 ml of perchloric acid and heat to fumes of perchloric acid. Cool the solution, add 20 ml of water and heat to boiling to remove any chlorine. After cooling the solution, add 2.0 ml of 10% hydroxylammonium chloride, 2.0 ml of acetate buffer and 5.0 ml of 0.001 *M* TPTZ. Neutralize the solution with ammonium hydroxide to pH 4 to 5 using a pH meter or pH indicating paper. Transfer the solution to a 125-ml separatory funnel, add 4.0 ml of nitrobenzene and shake vigorously for 1 min. Allow the phases to separate and gently swirl to remove drops of nitrobenzene clinging to the upper walls of the funnel. Repeat the extraction using two 2.0-ml portions of nitrobenzene. Collect the extracts in a 10-ml volumetric flask, dilute to volume with ethanol and measure the absorbancy at 595 mμ using 1-cm cells. Run a blank through the entire procedure and subtract its absorbancy from the absorbancy of each of the other solutions.

Procedure for the direct determination of iron

To a 3.00-ml sample of wine contained in a 100-ml beaker add 2.0 ml of 10% hydroxylammonium chloride, 5.0 ml of ethanol, 5.0 ml of acetate buffer and 5.0 ml of 0.001 *M* TPTZ. Heat the solution to boiling for 5 min, cool and transfer to a 125-ml separatory funnel. Wash the beaker with 20 ml of ethanol and 1.0 ml of 10% sodium perchlorate and add the washings to the separatory funnel. Extract the solution three times using one 4.0-ml and two 2.0-ml portions of nitrobenzene and collect the extracts in a 10-ml volumetric flask. Dilute to volume with ethanol and determine the absorbancy of the solution at 595 mμ using 1-cm cells. Run a blank through the entire procedure and subtract its absorbancy from the absorbancy of the sample solution.

RESULTS AND DISCUSSION

The iron content of several wines was determined using TPTZ in both the direct extraction and wet ashing procedures and also by 1,10-phenanthroline following wet ashing. The wine samples were obtained through retail outlets and were of U.S. and European vintage. All determinations were carried out in at least triplicate. The results are shown in Table I.

It is apparent that the determination of iron in wine by the direct TPTZ method usually, but not always, gives values which are lower than those obtained by wet ashing procedures. This is presumably due to the presence of a very stable iron compound which is not broken by TPTZ. If this is the case, it may well be that the "complexed iron" is inactive in forming a turbidity in wine and that the results obtained for "uncomplexed iron" by this direct method may be more useful to the wine producer than a knowledge of the total iron content. Various conditions, such as length of heating and ethanol concentration, were changed; but in no case was it possible to completely recover the iron from certain wines using the direct procedure. The iron which is not recovered remains in the aqueous phases as shown in one case by a wet ashing of the remaining water solution.

The iron content of the Medoc Bordeaux Red Wine was also determined by the direct method of BANICK AND SMITH and found to be 6.46 mg of iron per l, almost identical with the direct TPTZ method.

The procedure employing preliminary wet ashing followed by determination of the

iron with TPTZ does have distinct advantages over the usual 1,10-phenanthroline methods. A smaller sample may conveniently be used due to the increased sensitivity of the reagent and the extractability of the iron derivative into nitrobenzene. Iron may be easily removed from the reagents and the blank reduced to essentially zero. Also, it is unnecessary to remove the excess perchloric acid as is the case in the determination employing 1,10-phenanthroline.

TABLE I

DETERMINATION OF IRON IN WINE USING TPTZ
(Each result given is the average of at least three determinations)

Wine	*Wet ashing 1,10-phenanthroline*		*Wet ashing TPTZ*		*Direct TPTZ*	
	Fe found mg/l	*average deviation*	*Fe found mg/l*	*average deviation*	*Fe found mg/l*	*average deviation*
Italian Swiss Colony California Sherry	2.54	0.07			2.51	0.01
Meier's Ohio State Tawny-Port	3.79	0.15			3.80	0.04
Medoc Bordeaux Red Wine	7.53	0.10	7.56	0.04	6.44	0.03
Virginia Dare White Wine	4.42	0.01	4.48	0.01	4.16	0.08
Virginia Dare Red Wine	5.81	0.02	5.82	0.03	5.37	0.11
Ambassador California Burgundy	4.73	0.07	4.69	0.01	4.24	0.02
Richelieu California Port	5.15	0.01	5.12	0.02	4.68	0.01
Homestead Piestengel Rhubarb Wine	2.27	0.00	2.27	0.01	2.28	0.02

SUMMARY

Procedures are described for the determination of iron in wine using 2,4,6-tripyridyl-*s*-triazine, a new ferroine reagent. One procedure involving wet ashing with nitric and perchloric acids gives results comparable to those obtained using the usual 1,10-phenanthroline method while a direct extraction procedure often gives low but reproducible results indicating the presence of "complexed iron" in the sample.

RÉSUMÉ

Deux méthodes sont décrites pour le dosage du fer dans le vin au moyen d'un nouveau réactif, la 2,4,6-tripyridyl-*s*-triazine. On procède soit à une minéralisation par voie humide soit par extraction directe.

ZUSAMMENFASSUNG

Es werden zwei Methoden beschrieben zur Bestimmung des Eisens in Wein mit Hilfe von 2,4,6-Tripyridyl-*s*-triazin nach vorausgehender nasser Veraschung oder Extraktion.

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A SYSTEMATIC STUDY OF INSOLUBLE SUBSTANCES

V. TIME AND TEMPERATURE FACTORS IN THE PRODUCTION OF INSOLUBLE SUBSTANCES

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The effect of heat in changing the solubility of substances has been studied[1] and it has been shown that many substances which are soluble in acids under ordinary conditions are rendered difficultly soluble on heating for some time. In these studies only one temperature was taken for all substances. It was however possible that a critical temperature existed at which these substances began to be insoluble. A preliminary study of this problem has shown that substances which become difficultly soluble on heating generally begin to show changes in solubility after crossing a certain minimum temperature. Below this temperature the substance remains soluble however much it is heated. As the substances were heated at temperature intervals of 100° it is not possible to say whether there is a particular "critical temperature" but the data show conclusively that it is only after passing beyond a certain temperature range that each substance begins to change into the insoluble form.

TABLE I

Name of the compound	*Percentage left undissolved in one standard acid treatment heated for 2 h*								
	100°	*250°*	*300°*	*400°*	*500°*	*600°*	*700°*	*800°*	*900°*
1. Chromium phosphate	Soluble	26.2	52.0	75.4	78.2	85.0	90.2	95.0	95.0
2. Nickel chromite	←	Soluble	→	45.2	94.5	96.0			98.0
3. Chromium arsenate	←	Soluble		→	28.4	58.0	96.6	98.8	—
4. Cobalt antimonate	←	Soluble				→	42.0	88.6	96.0	99.3
5. Nickel antimonate	←	Soluble				→	40.2	87.0	96.0	99.8
6. Manganese antimonate	←	Soluble				→	93.2	95.0	95.0	99.7
7. Cobalt tungstate	←	Soluble					→	62.1	78.5	—

From Table I the critical temperature ranges of the following substances are:

(a) Chromium phosphate, 100–250°; (b) Nickel chromite, 300–400°; (c) Chromium arsenate, 400–500°; (d) Antimonates of nickel, cobalt and manganese, 500–600°; (e) Cobalt tungstate, 600–700°.

After the critical temperature range has been passed the percentage of the substance rendered insoluble in a given time continues to increase with increase in temperature. In the case of chromium phosphate this increase is gradual and steady but in other cases the increase is less regular. If the temperature is kept constant above the critical range the percentage of the insoluble portion increases with increase in the period of heating (Table II).

TABLE II

Name of the compound	*Percentage left undissolved in one standard acid treatment*	
	2 h at 600°	*11 h at 600°*
1. Chromium phosphate	85.0	88.0
2. Cobalt antimonate	42.0	85.0
3. Nickel antimonate	40.2	60.5
4. Manganese antimonate	93.2	93.2
5. Chromium arsenate	58.0	80.0

A few experiments were also made to see how the time factor affects the change at the maximum temperature used; at temperatures much beyond the critical range the change in solubility is very rapid (Table III). Thus on heating the antimonates of nickel, cobalt and strontium and uranium chromite for 10 min at 900°, the substance attained almost its maximum insolubility.

TABLE III

Name of the compound	*Percentage left undissolved in one standard acid treatment*	
	10 min at 900°	*2 h at 900°*
1. Cobalt antimonate	98.5	99.3
2. Strontium antimonate	98.3	99.6
3. Nickel antimonate	99.0	99.8
4. Uranium chromite	95.6	96.0

SUMMARY

It has been shown that substances which become insoluble on heating begin to change into the insoluble form after passing beyond a certain range of temperature which is characteristic for each substance. Beyond this range the percentage of insoluble portion formed increases both with time and temperature. At temperatures much beyond the critical range the change into the insoluble form is very rapid.

RÉSUMÉ

Les auteurs ont effectué une étude systématique de substances insolubles. Dans cette cinquième partie, ils ont examiné l'influence de la température et de la durée de chauffage sur leur formation.

ZUSAMMENFASSUNG

Es wurde eine systematische Untersuchung der durch Hitzeeinwirkung entstehenden unlöslichen Verbindungen vorgenommen und der Einfluss von Temperatur und Erhitzungsdauer untersucht.

REFERENCE

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GAS–LIQUID PARTITION CHROMATOGRAPHY

COLUMN BEHAVIOR AND ITS REPRODUCTION CRITERIA FOR FAVORABLE COLUMN BEHAVIOR*

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In the present paper an attempt has been made to explain the movement and the distribution of a solute in a gas–liquid partition chromatographic (GLPC) column from probability considerations. It has been shown that the mechanism of movement of the solute is analogous to a BERNOULLI trial system. Repeated independent trials are known as BERNOULLI trials if there are only two possible outcomes for each trial and their probabilities remain the same throughout[1]. If $b(k, n, p)$ be the probability that n BERNOULLI trials with probabilities p for success and $q(= 1 - p)$ for failure result in k successes and $n-k$ failures, then

$$b(k, n, p) = \binom{n}{k} p^k q^{n-k} \quad \ldots \ldots \ldots \ldots \ldots \quad (1)$$

It is possible to attribute a "probability of success" term and a total number of "trials" term to any column–solute combination. The magnitude of these two terms, however, depends upon the operating conditions of the column and the nature of the solute. Once these two terms are specified, the "behavior" of the column is exactly defined. When these two terms are reproduced, the column behavior is also reproduced. It should be noted that the emphasis is laid upon reproducing the column "behavior" instead of duplicating the operating conditions individually. Conditions responsible for producing the most favorable column behavior for normal types of solutes have also been discussed from the viewpoint of the equilibrium distribution. The compounds having moderate polarities and heats of vaporization (obeying TROUTON's relation) are considered as normal solutes. Favorable column behavior is considered to be that one which is most suitable for the separation of certain specified groups of compounds.

Column behavior

It is now well established[2-5] that whatever may be the approach to explain the mechanism in the GLPC column causing the distribution of a solute, the distribution can always be expressed by one of the conventional covergent series like the binominal, POISSON, or GAUSSIAN.

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During the chromatographic analysis the three parameters of the distribution of the solute measured are (1) the emergence time or volume, (2) the width of the distribution curve at the point of inflection, and (3) the height of the peak. It will be seen shortly that the emergence time $R_t = f(n, v, p, F)$ and $w = g(n, v, p, F)$ of the same set of variables. In the two foregoing equations, n is the number of theoretical plates which corresponds to the number of trials in a BERNOULLI system; v is the plate dimension, p is the probability of success and F is the flow rate. In this particular case, the probability of success means the probability of finding a solute molecule in the gas phase: the event of entrance or exit of a solute molecule into or out of a plate corresponds to a trial. Thus the two fundamental conditions of the BERNOULLI theorem[1], (i) that there are only two possible outcomes for each trial and (ii) that all the trials are identical and independent, are fulfilled. The solute fraction, p, stays in the gas phase and moves and is successful. The fraction, q ($q = 1-p$), stays in the stationary phase and is unsuccessful. When n is made large, using LAPLACE's approximation, the distribution can be expressed by a GAUSSIAN function and the width of the distribution curve depends on σ, the standard deviation. When p becomes infinitesimally small, *i.e.* by letting $p \to 0$ and $q \to 1$, and n is very large so that np is of moderate magnitude, the distribution becomes a POISSON function. In the case of GLPC, p is very small. In all the three types of distributions mentioned above, the height of the mean is the highest ordinate. Its value corresponds to the probability of finding the largest number of molecules at the particular trial, plate, or stage.

The situation in GLPC is a case of alternative probability (mutually exclusive events)[6] due to the constant value of partition coefficient. Mutually exclusive events mean that only one of them can happen and the happening of which automatically prevents the happening of the other. Due to the constancy of the partition coefficient, the presence of any one molecule in gas phase automatically prevents the presence of any other molecule, although the different molecules are indistinguishable. When a quantity m is introduced into the column, let p be the probability of a molecule being present in the gas phase; when introduced into the column, all the different identical molecules will have the same probability p of being present in the vapor phase, then the probability that any molecule will be in the gas phase is given by

$$P = p + p + p + \ldots\ldots\ldots\ldots\ldots \quad (2)$$

depending upon how many molecules have been introduced into the column. In other words, the largest number of probable molecules to be found at a definite stage will be double, triple, quadruple, etc., corresponding to the multiples of m introduced into the column. Therefore, it follows that the height of the mean in the distribution curve is proportional to the quantity of solute introduced into the column.

That this relation (2) is true can be proved by means of a simple analogy. Suppose one is to find out the probability of throwing an even number with an ordinary six-sided die. The events to be considered are the throwing of a 2, a 4, or a 6, each of which has a probability of 1/6. These events are mutually exclusive, that is, the appearance of any one of these automatically prevents the appearance of any others. The probability of at least one of these numbers, that is, of an even number to show up, is $1/6 + 1/6 + 1/6 = 1/2$, and therefore

$$P = p + p + p +$$

Let us now assume the following:

1. The column is composed of a large number, n, (say $n = 100$) of equivalent plates serially numbered 0, 1, 2 . . . . . n.

2. Each plate consists of a stationary phase of volume V_s and a gaseous phase of volume V_m.

3. One unit mass of the solute is introduced into the plate numbered zero within a very short time and the solute has a distribution coefficient

$$K = \left(\frac{C_s}{C_m}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

where C_s and C_m are the concentrations in the stationary and the mobile phases.

4. Subsequent portions of the gaseous phase introduced into the plate zero in installments of V_m are solute free and move from 0 to n plate.

5. At each operation (trial) V_m volume of movable phase is passed onto the next plate.

The fraction p of the solute that moves out of each plate at each operation is given by

$$p = \frac{V_m C_m}{V_m C_m + V_s C_s}$$

$$= \frac{V_m}{V_m + KV_s}$$

$$= \frac{V_m}{v} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

The quantity, $v = V_m + KV_s$, is the effective plate volume. The fraction, $q = 1 - p$ stays in the plate. The probability P_{kn} of finding a solute molecule in the gas phase after n operations (trials) at the plate number k (the $(K + 1)$th. plate) is

$$P_{kn} = \binom{n}{k} p^k q^{n-k}$$

$$= b(k, n, p) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

where $0 \leqslant k \leqslant n$. This means that as a result of n trials the solute fraction had k successes and moved to the plate number k. The expansion of the above gives the fraction of the solute in different stages. After n operations, the mean location, *i.e.* the location of the largest number of molecules on the column in terms of plate units, is given by

$$\mu = np \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

At each operation, a volume V_m of the gaseous phase is introduced into the column, so the volume throughput at the peak emergence is given by

$$\mu_v = nV_m \; (= V_R^{\cdot} \text{ in Martin's equation}^3)$$

$$= nvp \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

If the volumetric flow rate is F per unit time, then the peak emergence time is given by

$$\mu_t = \frac{nV_m}{F} \text{ units of time}$$

$$= \frac{nvp}{F} = R_t \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

Thus the statement made earlier, $R_T = f(n, v, p, F)$, is correct. In binominal distribution, the mean, that is the value of greatest probability, and the standard deviation is given by

$$\text{Mean, } \mu = np \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

$$\text{Standard deviation, } \sigma = \sqrt{npq} \quad \ldots\ldots\ldots\ldots \quad (10)$$

When n is very large, these values can be substituted in the equation for normal distribution

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{-(n-\bar{n})^2/2\sigma^2}$$

and becomes

$$P_{kn} = \frac{1}{\sqrt{2\pi npq}} e^{-(k-np)^2/2npq} \quad \ldots\ldots\ldots\ldots \quad (11)$$

So the distribution is a continuous function of the plate number, k, corresponding to the probability of finding the largest number of fractions. This is LAPLACE's approximation.

We have already expressed the emergence time in terms of the plate number, n, the probability p, the effective plate volume, v, and the volumetric flow rate, F. Similarly, the standard deviation, σ, can be expressed in terms of the above factors. If σ_v be the standard deviation in terms of effluent volume, then

$$\sigma_v = \frac{1}{p}\sigma V_m = v\sqrt{npq} \quad \ldots\ldots\ldots\ldots \quad (12)$$

because the solute in the mobile phase travels $1/p$ times faster than in the stationary phase. In units of time

$$\sigma_t = \frac{v\sqrt{npq}}{F} \text{ units of time} \quad \ldots\ldots\ldots\ldots \quad (13)$$

Letting $p \to 0$, $q \to 1$, and $n \to \infty$ whereas $np =$ constant and is of moderate magnitude, the equation (5) becomes a POISSON function. This situation approximates the type of mechanism operating in GLPC columns, whereas binominal distribution is true for GRAIG's machine[7]. It is quite obvious from the equations 5, 8, 11, 12 and 13 that the values of μ and σ completely define the distribution curve or the macroscopic behavior of the column. Also

$$\mu_t = f(n, p, v, F) \qquad (cf. \text{ eqn. } 8) \quad \ldots\ldots \quad (14)$$

and

$$\sigma_t = g(n, p, v, F) \qquad (cf. \text{ eqn. } 13) \quad \ldots\ldots \quad (15)$$

Whe shall make some *a priori* assumptions here which are substantiated by our subsequent determinations. Macroscopically and microscopically, the equations 14 and 15 are true; however, the value of p and v will be affected by such microscopic effects as the film thickness, l_f, of the coating liquid, particle diameter, d_p, of the solid support,

density and the nonuniformity of the packing. When a column is repacked it is hardly possible that such microscopic factors can be reproduced. Yet it is quite possible to compensate for the slight change in v and p by suitable adjustment of F. Sometimes even a slight variation in temperature accomplishes the desired effect because K and consequently p is temperature dependent. With proper adjustment of the variables it is possible to reproduce the probability and distribution of successes. Once the distribution is reproduced, the macroscopic column behavior is also reproduced.

In actual practice, one has to select a particular solute as standard (criteria for the selection of the standard are discussed later), inject it from time to time or when the column is repacked and measure the values of μ_v or μ_t and the width (a measure of σ_v or σ_t). These two parameters define the distribution curve. The adjustable variables are changed until the original values of the μ_v or μ_t and the width are reproduced. When the column behavior is thus reproduced for the standard, it is also reproduced for the other solutes. This has been verified for over a hundred compounds, for which the substrate to support ratio was varied between $\pm 5\%$. From the values of μ_t and σ_t it is possible to calculate the number of hypothetical plates of the column[8]. The number of plates required for a separation has been discussed by VAN DEEMTER *et al.*[4] and GLUECKAUF[9].

Using the same pressure differential and applying a pressure gradient correction to the apparent retention volume V_R does not insure that the same corrected retention volume $V_R^\cdot$ will be obtained[3,10]. Because

$$V_R^\cdot = jV_R = V_g^\cdot + KV_s \quad \ldots\ldots\ldots\ldots\ldots\ldots (16)$$

$V_R^\cdot$ = corrected retention volume, j = pressure gradient correction

$$= \frac{3(P_i/P_o)^2 - 1}{2(P_i/P_o)^3 - 1}$$

V_R = apparent retention volume, $V_g^\cdot$ = gas hold up volume, K = partition coefficient, P_i = inlet pressure, P_o = outlet pressure.

$V_R^\cdot$ is an explicit function of $V_g^\cdot$ and KV_s. It may not be possible to reproduce $V_g^\cdot$ and KV_s when the column is repacked. On operating for a long time, V_s may change slightly due to evaporation from the column. The operator generally finds it necessary to change the flow rate (pressure differential) in order to obtain the same retention volumes. It has been shown earlier that the effects of slight changes of the microscopic parameters can be corrected by varying the adjustable parameters. The operator is interested in knowing that he is operating with a column of identical behavior rather than knowing the different values of $V_R^\cdot$ due to intrinsically changed columns.

In the foregoing discussion, the transportation of material has been assumed to be entirely due to the movement of the mobile phase. In the rate theory[4,11], the contributions of the eddy diffusion and the molecular diffusion in both the phases towards the movement of the material have also been considered. It was decided to study the contribution of the diffusional terms of the equation for the height equivalent of a theoretical plate (H) deduced by VAN DEEMTER *et al.*[4,12].

$$H = 2\lambda d_p + 2\gamma \frac{D_g}{U} + \frac{8}{\pi^2} \cdot \frac{K' U l_f}{(1 + K')^2 D_l} \quad \ldots\ldots\ldots (17)$$

where γ = correction for the tortuosity of the channels and has a limiting value of 1, λ = a measure of packing irregularity and dependence upon particle diameter, D_g = molecular diffusion in gas phase, d_p = particle diameter, l_f = liquid film thickness, $K' = K \frac{\text{liquid phase}}{\text{gas phase}}$, U = linear velocity; D_l = molecular diffusion in liquid phase.

If one wants to calculate the value of H from this equation, a rather large number of assumption have to be made in computing the various terms of the equation which in itself is not a very desirable situation[13]. Therefore, it was decided to find experimentally the contribution of the diffusional terms under the chromatographic flow condition (flow rates > 20 ml/min). In the first series of experiments the column packing material was completely removed from the column, or in other words an empty tube was used. The tube was maintained at 80° and helium was flowing through it. Different types of solutes were introduced into the tube by momentarily stopping the helium flow. Figs. 1 to 3 show the distribution of the various solutes under a virtual

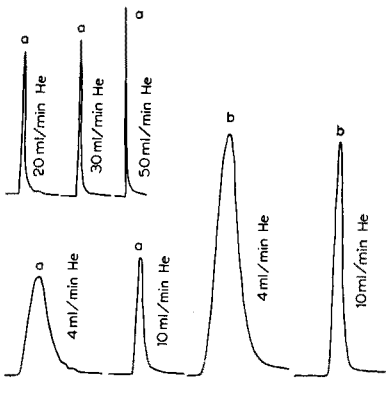

Fig. 1. Distribution of concentration of solutes in an empty tube at different helium flow rates. Length of flow tube: 1 meter; temperature of flow tube: 80°; volume of organic samples: 2 μl; volume of inorganic samples: 1 ml at 50° and 1 atm. a. carbon tetrachloride; b. sulfur dioxide.

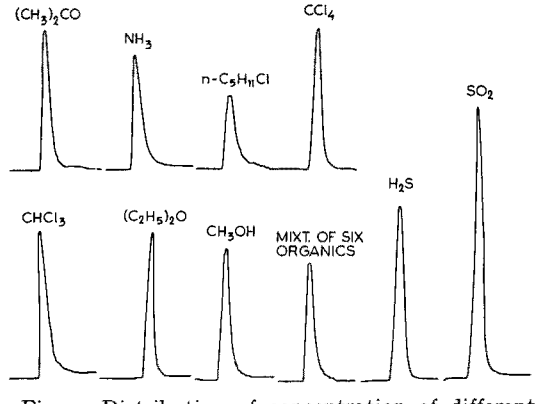

Fig. 2. Distribution of concentration of different solutes in an empty tube under identical conditions of temperature and helium flow rate. Length of flow tube: 1 meter; temperature : 80°; helium flow: 10 ml/min; volume of the organic sample: 2 μl; volume of the inorganic samples: 1 ml at 50° and 1 atm.

coefficient of diffusivity. This type of distribution was expected from TAYLOR's study[14,15] of the spread of the particles in a turbulent constant flow. Without going into the details of TAYLOR's calculations, a number of conclusions may be drawn from the experimental curves: (1) Under identical conditions of flow rate and temperature, distributions are more or less the same for all the compounds so far as their spread and the time of arrival at the exit of the tube are concerned. (2) At higher or chromatographic volumetric flow rates the virtual coefficient of diffusivity was negligible compared to the linear velocity. As a result there was no spread and all the solutes came out as plugs and at the same time. The time corresponded to the holding time

of the tube which depended upon the length of the tube. This means the signal was the same as that produced by a permanent gas in gas–liquid chromatography. (3) The effect of temperature is less important.

In the second series of experiments the tube was packed with sand, the distribution curves due to various solutes are shown in Fig. 4. Conclusions from these experiments are identical with those from the empty tube. Thus it appears that at the chromatographic flow rates the contribution of the first two terms of VAN DEEMTER's equation are negligible or at the most they may be substituted by a suitable constant.

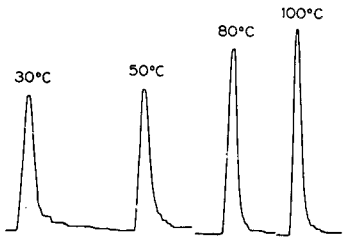

Fig. 3. The effect of temperature on the distribution of concentration of a solute in an empty tube under identical flow rates. Length of flow tube: 1 meter; helium flow: 10 ml/min; sample: 2 μl of carbon tetrachloride.

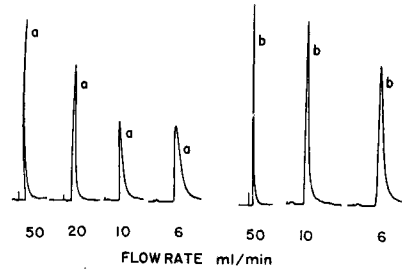

Fig. 4. Distribution of concentration of solutes in a sand packed tube at different helium flow rates. Length of sand column: 90 cm; screen fraction of sand: 40/80 mesh fraction; temperature of the sand packed tube: 80°. a. 2 μl carbon tetrachloride; b. 2 μl of a mixture of equal volumes of allyl chloride, acetone, *sec.* butyl chloride, *n*-amyl chloride, carbon tetrachloride and chloroform.

Criteria for favorable column behavior

A number of investigators have found that straight lines are obtained when logs of retention time are plotted against the boiling points of the solutes. Similar straight lines are obtained when logs of retention time are plotted against the ratio of the boiling points of the solutes and the column temperature. TENNEY[16] studied solutes like *n*-paraffins, 2-methylparaffins, cyclopentanes, cyclohexanes, 1-olefins, 2-methyl-olefins, cyclo-olefins, diolefins, alkylbenzenes, acetylenes, primary *n*-alcohols, secondary alcohols, tertiary alcohols, aldehydes, ketones, formates, acetates, ethers and acetals on columns treated with such different types of solvants as convoil-20, squalane, β, β'–oxydipropionitrile, 2-ethyl hexyl sebacate, convachlor-20. With only a few exceptions he obtained straight lines for boiling points *vs.* log retention times plots. Straight lines are also obtained when logs of partition coefficients obtained by chromatographic methods are plotted against the ratio of the boiling point of the solute to the column temperature (*cf.* Figs. 5 and 6). This is expected because the retention time changes linearly with partition coefficients (*cf.* eqns. 4, 7 and 8). It was expected and also found that similar straight line plots are obtained when the latent heats of vaporization are plotted against logs of partition coefficients (*cf.* Figs. 7 and 8). The above conclusions are true only for the compounds of the same family. Cyclopentane, being a compound of another family, did not fall in the straight line (*cf.* Figs. 5–8). It is also interesting to note that column temperature higher than the critical temperature of the solutes were never used.

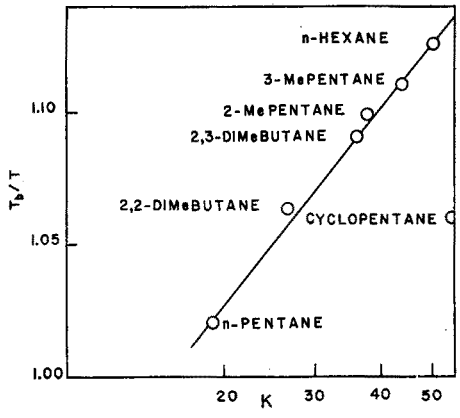

Fig. 5. Change of log partition coefficient with the ratio of boiling point to column temperature. T_b(°K) = b.p.; T(°K) = col. temp. Data taken from KEULEMANS[12]. Column: Dimethylsulfolane. Note: Cyclopentane did not fall in line with the chain hydrocarbon.

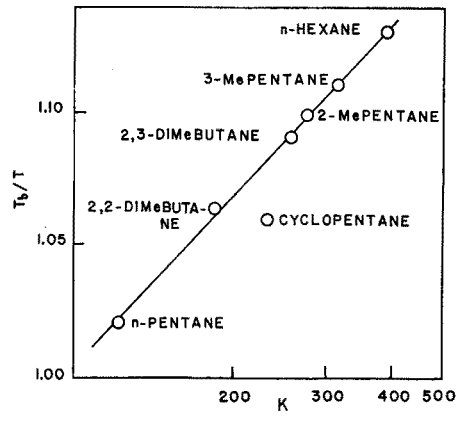

Fig. 6. Change of log partition coefficient with the ratio of boiling point to column temperature. T_b(°K) = b.p.; T(°K) = col. temp. Column: *n*-Hexadecane. Data taken from KEULEMANS[12]. Note: In this case cyclopentane is closer to the line because of the low polarity of the substrate.

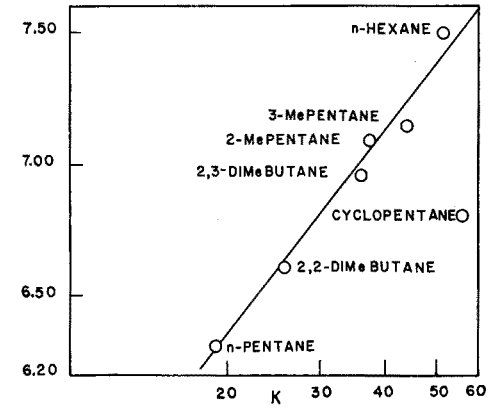

Fig. 7. Change of log partition coefficient with the molar heat of vaporization at constant pressure. Partition coefficient data taken from KEULEMANS[12] and heat of vaporization (at 25°) data taken from Chemical Engineers' Handbook. Column: Dimethylsulfolane.

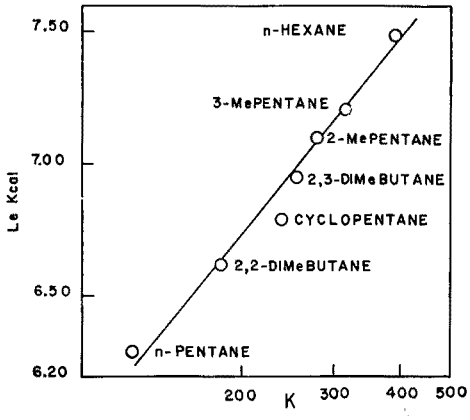

Fig. 8. Change of log partition coefficient with the molar heat of vaporization at constant pressure. Partition coefficient data taken from KEULEMANS[12] and heat of vaporization (at 25°) data taken from Chemical Engineers' Handbook. Column: *n*-Hexadecane.

Examination of data obtained by TENNEY[16] reveals yet another interesting feature (*cf.* Fig. 2 of ref.[16]). The intercept of the log retention time *vs.* boiling point curve is practically independent of temperature even for a temperature differential of 50°. The temperature coefficient of the slope is also very small. These results are expected from the general equation for the distribution coefficient in a two component–two phase assembly[17].

$$K = \frac{n_l}{n_v} = \frac{Pf_l}{Pf_v} \cdot e^{Li/RT} \quad \ldots\ldots\ldots\ldots (18)$$

where K = partition coefficient, n_l = moles of solute per unit volume of the condensed phase, n_v = moles of solute per unit volume of the vapor phase, Pf_l = partition function of the solute in the condensed phase, Pf_v = partition function of the solute in the vapor phase, Li = mean potential energy difference per mole of solute in condensed phase and vapor phase (linearly related to the molar heat of vaporization of the pure solute at constant volume), R = gas constant, T = temperature of the system.

For an one component system Pf_l/Pf_v tends to become unity. It can be shown that the Pf_l/Pf_v is practically independent of temperature for very low concentrations of the solute. Li in equation (18) may be substituted by Le/RT, where Le is the mean potential energy difference at constant pressure (Le is linearly related to molar heat of vaporization of the solute at constant pressure) and the equation (18) becomes

$$K = A' e^{(Le/RT)-1}$$
$$= A e^{Le/RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots (19)$$

Because the plot of log K against T_b/T (T_b = boiling point in °K) produces straight lines (*cf.* Figs. 5 and 6), it may be expected that a relation similar to TROUTON's rule exists between T_b and Le in the case of dilute solutions and the equation (19) may be written as

$$K = A\, e^{\alpha T_b/RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots (20)$$

where $Le/T_b = \alpha$

It has been shown earlier that the relation between retention time or retention volume and K is linear (*cf.* eqns. 7, 8, and 16), so the equation (20) may be modified to give an expression for retention time or retention volume, hence

$$R_t = fK = Be^{\alpha T_b/RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots (21)$$

where R_t = retention time

B = a constant for homologous compounds and the same substrate

The equations (19–21) may be written as

$$\ln K = \ln A + Le/RT \quad \ldots\ldots\ldots\ldots\ldots\ldots (22)$$

$$\ln K = \ln A + \alpha\, T_b/RT \quad \ldots\ldots\ldots\ldots\ldots\ldots (23)$$

$$\ln R_t = \ln B + \alpha\, T_b/RT \quad \ldots\ldots\ldots\ldots\ldots\ldots (24)$$

We can draw a number of conclusions from the equations (19–24).

1. The partition coefficient or the retention time will change expontentially with the change of the ratio Le/T or T_b/T. It is expected that for T_b/T ratio higher than unity R_t will increase very rapidly. Fig. 9 illustrates a plot n_l/n_v against T_b/T assuming $A = 1$ and $\alpha = 23$ (one component two phase system). At lower T_b/T ratios (when $T \geqslant$ critical temperature), n_l/n_v will be constant at constant pressure and the above relations are not strictly applicable. In other words, it may be safely concluded that at column tem-

peratures much lower than the boiling point of the solute ($T_b/T \gg 1$) most of the molecules will remain in the stationary (condensed) phase for most of the time and there will be fewer exchanges between the two phases, *i.e.* the column will be inefficient. At column temperatures much higher than the boiling point of the solute (say $T_b/T \leqslant 0.6$;

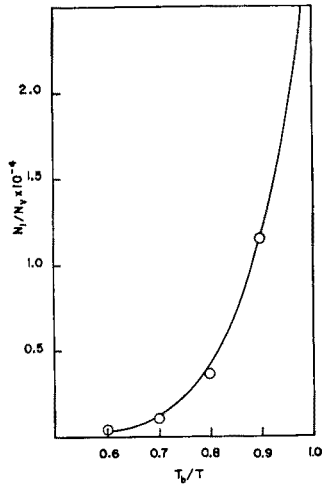

Fig. 9. Change of the ratio of number moles/unit volume of the liquid to number of moles/unit volume of the vapor in equilibrium at temperature T°K with the ratio of the boiling point to the equilibrium temperature. T_b = b.p.(°K); T = equilibrium temperature = column temperature (°K).

for pure compounds the ratio of boiling and critical temperature is approximately equal to 0.6) most of the molecules will remain in the vapor phase for most of the time and the column will be inefficient. However, the actual optimum temperature range will be determined by the slope and the intercept of the log R_t *vs.* T_b/T plot.

2. A plot of ln K or ln R_t against Le, or Le/T, or T_b or T_b/T will be a straight line and will make an intercept which will be constant and independent of temperature at least for moderate temperature ranges. This expectation has been verified by our results as well as by TENNEY's[16] data.

3. It is also expected that the slopes of the plots for different types of compounds will not be very different (*cf.* Figs. 5–8 and TENNEY[16]).

4. Combination of equations (7) and (16) and (23) should enable us to determine K and its temperature dependence in a very straight-forward manner.

Recommendation for determining the optimum column conditions

The choice of temperature of the column is the first parameter to be decided. It should be realized from the preceding discussion that any one column temperature cannot be suitable for all the solutes. The column temperature will be determined by the boiling point of the lowest boiling solute in a mixture. The optimum column temperature is given by a value of the T_b/T ratio between 1 and 0.92. The compounds boiling between the column temperature and 0.92 times the column temperature will be very nicely resolved. (*cf.* Fig. 10).

After the column temperature has been fixed, a standard solute is selected, boiling as close possible to the column temperature, preferably slightly below the column temperature. This compound should not be highly polar and should have normal heat of vaporization ($Le/T_b \approx 23$). A volume of this solute, less than the plate volume, should be injected into the column at different flow rates until the flow rate corresponding to the maximum number of plates is obtained. The optimum temperature and the flow rate are thus determined. The condition of the column can be checked

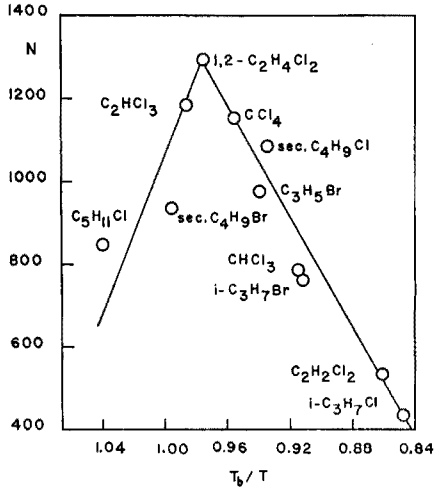

Fig. 10. Variation of the number of plates with boiling point of the solutes. Column: 20% tri-*m*-tolyl phosphate on 30/60 celite; column length: 2 meter; column temperature: 93°; He flow rates: 52 ml/min. Widely varying types of halogenated hydrocarbons were deliberately chosen to emphasize the importance of the boiling point, even when the compounds do not strictly belong to the same family.

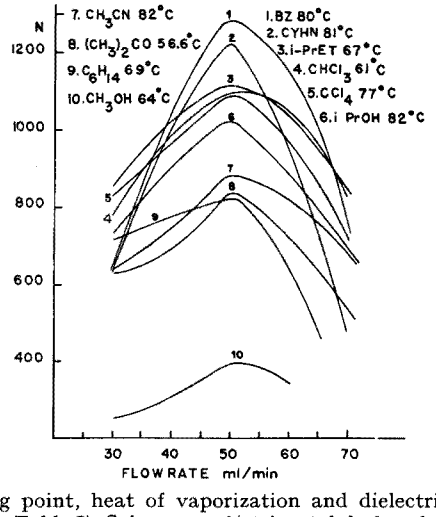

Fig. 11. Effect of boiling point, heat of vaporization and dielectric constant on the column efficiency. (Compare with Table I) Column: 20% tri-*m*-tolyl phosphate on 30/60 celite; column length: 2 meter; column temperature: 93°.

every now and then by injecting the standard, and the overall column behavior maintained uniform by adjusting the flow rate and, very rarely, the temperature. The reason that a solute having a boiling point near the column temperature is selected is that the slightest change in column condition is reflected in the number of plates. When the column is repacked with the same material, the same plate number is reproduced by adjusting the flow rate and the column temperature. The retention times may be easily expressed in terms of the standard. It was also found that for the same column, optimum flow rate was the same irrespective of T_b/T ratio, although the absolute efficiency of the column was different depending on the value of T_b/T ratio, the dielectric constant and the heat of vaporization (*cf.* Fig. 11 and Table I).

TABLE I

EFFECT OF BOILING POINT, HEAT OF VAPORIZATION, AND DIELECTRIC CONSTANT ON COLUMN EFFICIENCY

(Compare with plate number in Fig. 11)

Column: 20% tri-*m*-tolyl phosphate on 30/60 celite; column length: 2 meter; column temperature: 93°.

Solute	*b.p. (T°K) to col. temp. (T°K) ratio*	*Mol. heat of vapor const P and at b.p. K cal.*	*Dielectric constant at 20°*
Acetonitrite (7)	0.97	7.15	38.8
Isopropyl alcohol (6)	0.97	9.55	18.9
Cyclohexane (2)	0.97	6.95	2.0
Benzene (1)	0.965	7.3	2.3
Carbon tetrachloride (5)	0.955	7.1	2.2
n-Hexane (9)	0.935	6.95	1.9
Isopropyl ether (3)	0.93	—	—
Methanol (10)	0.915	8.4	32.6
Chloroform (4)	0.915	7.05	4.4
Acetone (8)	0.90	7.2	21.4

ACKNOWLEDGEMENT

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SUMMARY

The distribution of a solute in a gas–liquid chromatographic column has been discussed from the probability view point and its analogy to the BERNOULLI trial system has been shown. It has been shown that the reproduction of column behavior is possible by reproducing the probability. The criterion for the optimum column temperature has also been discussed. Recommendations have been made for determining the optimum column conditions and its reproduction.

RÉSUMÉ

L'auteur a effectué une étude, à l'aide du calcul des probabilités, sur le comportement d'une substance dans une colonne chromatographique gaz–liquide. On a déterminé également les conditions optimales et la reproductibilité.

ZUSAMMENFASSUNG

Es wurde versucht, die Bewegung und Verteilung einer Substanz in einer gas–flüssig Chromatographie-Kolonne mit Hilfe der Wahrscheinlichkeitsrechnung zu erklären. Die Kriterien für die

optimale Kolonnentemperatur werden diskutiert und Vorschläge gemacht zur Bestimmung der optimalen Kolonnen-Bedingungen und deren Reproduzierbarkeit.

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CHROMATOGRAPHIC SEPARATION OF SOME ALKALI METALS

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Numerous papers have been published[1–8] on the separation of the group I metals possibly none more successful than that of LINSTEAD[1], all however, suffer from the difficulty of identifying the position of the metal ions after development. As these metals travel as ions it occurred to the author that a combination of LINSTEAD methanol solvent to which some aqueous ammonia was added and using an acid base indicator as detector would prove simple and effective. This proved to be the case.

EXPERIMENTAL

The solvent

Pure methanol was used in early trials but redistilled commercial methanol proved successful in later experiments. To this was added one to ten percent of 15 *N* aqueous ammonia. Solvents based on ethanol *n*-proponal and *n*-butanol were used with less success.

The chromatograms

These were made by the ascending techniques using Whatman's No. 1 paper cut into sheets 20 cm by 28 cm. The spots of solution containing the alkali metal were placed on a line 3 cm from one of the longer edges and with a distance of 2.5 cm between spots. The spots nearest the vertical edges of the paper were at least 6 cm from it. The paper was coiled into a cylinder and sewn with cotton at top and bottom. This served to keep the cylinder rigid. The spots were placed on the paper with a platinum loop which held about 0.005 ml of solution, and were air dried before placing in the development jar. This latter was a 3-l Winchester jar from which the neck had been cut and the edge ground flat. The vessel was closed with a 15-cm square of plate glass. An 11-cm Petri dish carrying the solvent was placed in the bottom of the development jar and the cylinder of paper was lowered into it. No humidification period was given the paper. The time of development varied a little but none was greater than 90 min at 20 to 25° for a height of about 18 cm from the surface of the solvent pool.

Identification of the spots was obtained by spraying the partially dried paper with a 0.005 % solution of bromophenol blue in 20% alcohol which had been neutralized with 0.1 *N* NaOH solution. The paper was then treated for 15 to 30 min in an oven at 100°. The alkali spots appeared purple against a ground of yellow where the acid ions were purplish grey on most of the paper. The spots were quite easily distinguishable over the usual tenfold range of concentration used in paper chromatography.

Metal ion solution

The alkali metals, magnesium, calcium, strontium, barium, silver, cadmium were used as chlorides or nitrates at concentrations of 0.1 *M* in metal ion. When the effect of the anion was being studied, lithium, sodium or potassium were tried as sulphate, perchlorate, bromide, iodide and carbonate.

R_F values of metal ions

The R_F values of the ions were measured relative to the liquid front, no adsorption front being obtained with methanol although some of the other alcohol solutions gave one. The following results refer to nitrate solutions. The R_F values obtained for methanol with 1% and 5% of 15 *N* ammonia are the only ones given. A solution with ½% ammonia did not give satisfactory results. R_F values for concentrations over 5% had no advantages. A 2% solution gave values slightly greater than the 5% solution.

Metal ion:	*Li*	*Na*	*K*	*Rb*	*Cs*	*Mg*	*Ca*	*Sr*	*Ba*	*Ag*	*Cd*
1% 15 *N* NH_3	0.82	0.40	0.19	0.16	0.16	0	0.75*	0.75*	0.33*	0.38	0.35
5% 15 *N* NH_3	0.74	0.30	0.12	0.12	0.10	0	0.4*	0.4*	0.3*	—	—

Those values marked with an asterisk trailed from the base to the value indicated. The results for solutions containing anions other than nitrate showed no significant variation where the anion was monovalent. However, carbonate and sulphate gave considerably lower values for lithium, sodium and potassium at 0.7, 0.3 and 0.1 respectively.

DISCUSSION

As was found by LINSTEAD, methanol proved to be the best solvent to obtain separation of the ions. A butanol–water–ammonia mixture gave higher R_F values for potassium, rubidium and caesium, but no separation, and thus was of no value. Indicator solutions other than bromophenol blue were tried but were no more successful.

In theory, any indicator with a pK value of the same order as bromophenol blue should be satisfactory.

As would be expected, the position of the acid ion was revealed as a yellow spot. This usually trailed over a considerable distance and did not noticeably interfere with the cation spot. It did indicate, however, that the cation travelled independently of the anion with which it was associated. This point is borne out by radiographic identification of spots from salts in which both anion and cation are radioactive.

The alkaline earth metals from spots which are nearer the neutral colour of bromophenol blue and which trail, except magnesium which does not move, from the base line to the height indicated above. It seems that they are precipitated as carbonates during the course of the chromatogram. Silver and cadmium as the ammines give clear cut oval spots of hue similar to the alkali metal spots.

The method gives a simple means of identifying the alkali metal salts after a preliminary sulphide or basic carbonate separation of other metals and conversion to chloride or nitrate solution. Its possibilities as a means of gravimetric determination of lithium, sodium and potassium are being investigated.

SUMMARY

The chromatography of the alkali metals is described using 5% 15 *N* NH_3 in methanol as solvent and bromophenol blue to identify the spots. Interfering elements have been studied. The method gives a rapid and more positive method of separation and identification than previously possible.

RÉSUMÉ

Une séparation chromatographique des métaux alcalins est décrite. On utilise du méthanol, renfermant 5% d'ammoniaque 15 *N*, comme solvant et le bleu de bromophénol pour identifier les spots. Cette méthode est simple et rapide.

ZUSAMMENFASSUNG

Es wird eine chromatographische Trennungsmethode für die Alkalimetalle beschrieben. Als Lösungsmittel wird Methanol mit einem Zusatz von 5% 15 *N* Ammoniak verwendet. Die Identifizierung der Flecken erfolgt mit Bromphenolblau. Die Methode ist einfach und rasch.

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THE SPECTROCHEMICAL DETERMINATION OF TOTAL STRONTIUM IN BONE, MILK AND VEGETATION

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INTRODUCTION

In recent years the physiological effects produced by radioactive fission products from atomic weapons have necessitated an intensified study of the metabolism of strontium in animals and plants. The spectrographic methods outlined below were developed in response to a demand for the rapid and economic determination of total strontium in samples of ash obtained from bone, milk and vegetation.

PRELIMINARY

The spectrographic examination of bone ash was comparatively straightforward because the composition of the bone ash did not vary in its major constituents and this facilitated standardisation, but the presence of large amounts of alkali metals in the ash of milk and vegetation and the variable composition of the latter made the application of direct spectrographic methods doubtful.

In the normal course of events the calcium is determined chemically as part of the analytical scheme and it was relatively easy to modify the procedure to make the calcium and strontium oxalate precipitate available for the spectrographic analysis.

Examination of bone ash

A number of workers have examined the mineral content of bones[1-7] but their references to strontium were largely of qualitative significance. More recently quantitative estimation of strontium in bones has been carried out by emission spectroscopy[8,9] and by the neutron activation technique[10].

The spectrographic method of HODGES *et al.*[8], in which an admixture of the sample with graphite and copper sulphate is excited in a d.c.-arc, appeared to be capable of modification to permit the determination of strontium in strontium and calcium oxalate fractions separated from milk and vegetation ash. This method had one disadvantage, however, in that the coefficient of variation of 10% for a mean of triplicate exposures was inadequate and experiments were carried out with a view to improving the precision of the method.

The feasibility of using additional internal standards apart from copper was examined, calcium and barium being tried but without any improvement being obtained. Eventually improved arc stability was obtained by using a larger proportion of graphite and substituting anhydrous copper sulphate for the pentahydrate, the admixture of sample with graphite and anhydrous copper sulphate being pelleted

before burning in the arc. These modifications, combined with the microphotometry of a different copper line (at 4704.6 Å) from that advocated by HODGES *et al.*, gave an improved coefficient of variation of 6% for single exposures (3.5% for the mean of triplicate exposures) at the 90 p.p.m. level.

A calibration curve (Fig. 4) was obtained from standards made by grinding strontium carbonate into calcium phosphate. The calcium phosphate used for these standards was made from calcium carbonate which had been freed from strontium by the fractional precipitation of their compounds with 8-hydroxyquinoline[11,12].

Method (bone ash)

Copper sulphate and graphite powder are added to the bone ash in known proportions. Pellets prepared from this mixture are burnt to completion in graphite cups at 7 A d.c. Spectra are evaluated by non-recording microphotometry, calibration being carried out by means of an iron intensity pattern.

The strontium content is determined by comparison with standards made by mixing strontium carbonate with calcium phosphate in known proportions.

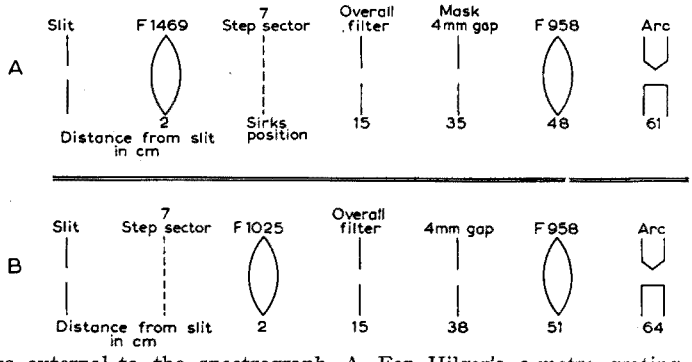

Fig. 1. Optics external to the spectrograph. A. For Hilger's 3-metre grating spectrograph. B. For large automatic quartz spectrograph.

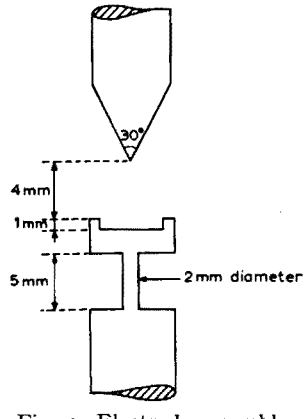

Fig. 2. Electrode assembly.

Special apparatus

Hilger 3-metre grating spectrograph *or* Hilger large automatic quartz spectrograph; de Gramont Arc and Spark stand, condensing lenses depending on spectrograph used (see Fig. 1);

Sighting lamp and screen/mask; Quartz filter (20% transmission); N.C.C. graphite electrodes 1/4″ diameter; Matthey 7-mm diameter copper electrodes; Matthey 5-mm diameter iron electrodes; Non-recording microphotometer; Hilger rotating 7-step sector.

Spectrographic conditions

Spectrograph	Hilger 3-metre grating	Hilger large automatic quartz
External optics	See Fig. 1(a)	See Fig. 1(b)
Plate mask	1.8 mm	3 mm
Slit length	20 mm	1.8 mm
Slit width	0.015 mm	
Wavelength range	3500–5000 Å(1st order)	3200–8000 Å
Optical filters	Overall neutral quartz filter 20% transmission	
Photographic plate	Ilford Ordinary	
Top electrode (—ve)	1/4″ diameter N.C.C. graphite 30° cone	
Bottom electrode (+ve)	1/4″ diameter N.C.C. graphite specially machined (See Fig. 2)	
Analytical gap	4 mm	
Sample load	Pellets prepared in special block (see Fig. 3)	
Current	7 A d.c.	
Exposure	Complete burn (indicated by the arc becoming unstable and noisy)	

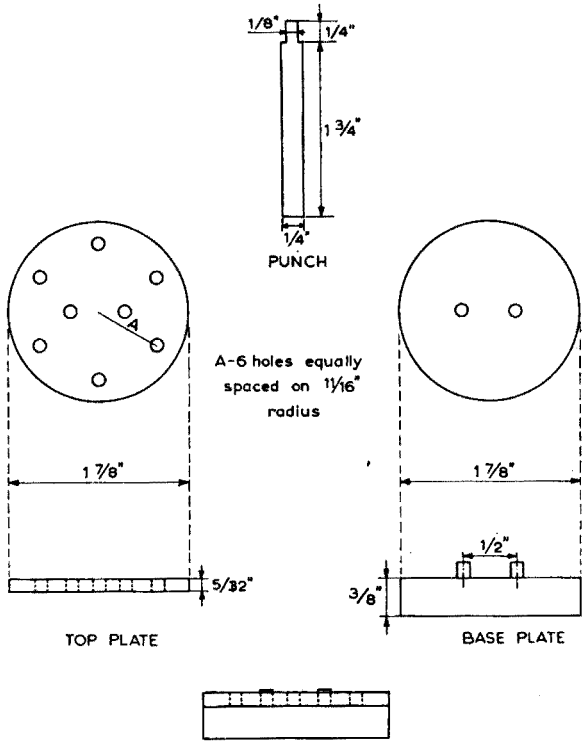

Fig. 3. Pelleting block.

Plate calibration spectrum

After completing exposure of samples or standards, photograph on the same plate an intensity pattern by means of the 7-step rotating sector (step ratio 1 : 2).

Spectrograph	Hilger 3-metre grating	Hilger large automatic quartz
Position of sector	At Sirks position	At slit
Top electrode (+ve)	Matthey 7 mm copper, 80° cone	
Bottom electrode (—ve)	Matthey 5 mm iron, flat	

Slit length	20 mm	12 mm
Plate mask	12 mm	
Analytical gap	3 mm	
Current	3.4 A d.c.	
Pre-arc	30 sec	
Exposure	25 sec	

Photographic processing

Develop in I D.2 for 4 min at 20°.
Rinse with water and fix in acid hypo until the plate is completely clear.
Wash for half an hour in an efficient washing tank and allow to dry.
Drying may be accelerated by soaking the plate in 80% methylated spirits for 2 min.

Standards

Prepare by dry grinding in an agate mortar a mixture of strontium carbonate and strontium-free calcium phosphate such that the final matrix contains 1,000 p.p.m. of strontium. By further dilution prepare standards containing 400, 300, 250, 200, 150, 100 and 50 p.p.m.

Preparation of sample

Grind in an agate mortar for five min 15 mg of sample with 135 mg of anhydrous copper sulphate and 150 mg of N.C.C. graphite powder. Prepare five pellets of each sample by loosely filling the holes in the die-plate of the pelleting block (see Fig. 3) with the powder, placing the punch in position and giving a tap with a light hammer.
Note: the anhydrous copper sulphate should be freshly prepared, by heating to constant weight at 250°, and stored in an air tight container.
If the strontium content is greater than 350 p.p.m. in the matrix dilute the sample with strontium-free calcium phosphate so that the strontium content falls within the range 50–350 p.p.m.

Spectrographic procedure

Place a pellet into the anode cup. Using an insulated graphite rod strike an arc between the electrodes. Allow to burn to completion at 7 A, the top electrode being adjusted to maintain a constant gap. Repeat three times to give quadruplicate exposures.

Interpretation of spectra

Examine the spectra qualitatively by comparison with a standard plate in a comparator to identify and mark the following lines: Sr I 4607.33 Å, Cu I 4704.59 Å, Fe 4678.85 Å (in plate calibration spectrum).

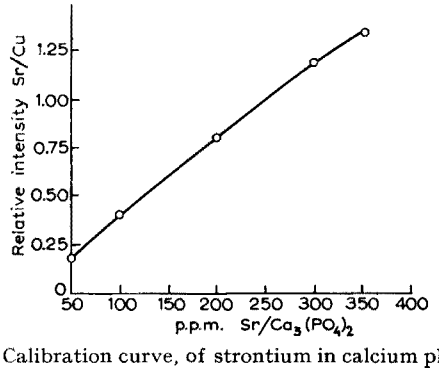

Fig. 4. Calibration curve, of strontium in calcium phosphate.

Measure the Seidel densities of the strontium and copper lines and of the iron line in the steps of the plate calibration spectrum.

Plot a curve of Seidel density of sectored iron line against log relative intensity (from the known step-ratios). By means of the curve obtained, convert Seidel densities of strontium and copper to log relative intensities, and so obtain intensity ratios of strontium/copper. Read the final concentration of strontium from the appropriate calibration curve.

Standardisation (Preparation of calibration curves)

Calibration curves are prepared as above from standards made by dry grinding of strontium carbonate with strontium-free calcium phosphate, plotting the relative intensity of the strontium line divided by the relative intensity of the copper line against the concentration of strontium (see Fig. 4).

ACCURACY AND PRECISION

The precision of the method was determined by the replication on three photographic plates of a sample of bone ash known to contain 90 p.p.m. of strontium. The coefficient of variation was 6 % for single exposures.

As a check on the accuracy, samples were examined by the neutron activation and X-ray fluorescence techniques and no significant bias was evident, as will be seen from Tables I and II below.

TABLE I

Bone ash sample No.	*p.p.m. strontium in ash*	
	Neutron activation	*Emission spectroscopy*
1	86	85
2	98	100
3	114	110
4	118	120

TABLE II

Bone ash sample No.	*p.p.m. strontium in ash*	
	X-ray fluorescence	*Emission spectroscopy*
5	134	135
6	90	90
7	198	190
8	80	80

Examination of milk ash and vegetation (Emission spectroscopy)

Initially, attempts were made to evaluate the strontium content of milk and vegetation ash by spectrographic methods involving the dilution of the sample with a large excess of ferric sulphate or barium chloride as spectrographic buffer[13–15]. The relatively high and variable sodium and potassium contents together with the presence of chloride, silica and phosphate resulted in a breakdown of the buffer effect, and erratic and unreliable results were obtained. Unsatisfactory results were also obtained by the direct application of the method already described for bone ash, for a similar reason. Neither removal of the chloride by sulphation nor admixture with salts of metals of low ionisation potential *e.g.* sodium fluoride and potassium sulphate gave any improvement. Lithium salts had been used as a spectrographic buffer and flux for the determination of various elements in biological materials[16,17], but its use in this connection was precluded owing to the interference of the most sensitive strontium line by diffuse lithium lines.

It was therefore concluded that the evolution of a direct spectrographic method appropriate to both milk ash and a wide range of vegetation ashes might necessitate a protracted investigational programme. Efforts were directed accordingly to the separation of strontium from the interfering elements and this was accomplished by precipitation of the oxalates at pH 4.0 and a temperature of 0° after removal of any siliceous matter[18].

A modification of the method used for the determination of strontium in bone ash was then applied to the determination of strontium in calcium–strontium oxalate (monohydrate) fractions separated from the ashes of milk and vegetation.

The mixed oxalate fractions were ground with graphite and anhydrous copper sulphate and the spectra recorded and assessed by the method already described for the determination of strontium in bone ash, a calibration curve (Fig. 5) being constructed from standards prepared by the grinding of strontium carbonate into calcium oxalate monohydrate.

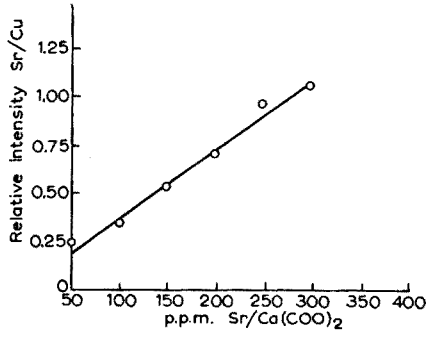

Fig. 5. Calibration curve of strontium in calcium oxalate.

Examination of milk ash and vegetation ash (flame photometry)

Once strontium could be separated easily from interfering elements flame photometry became an attractive possibility. The essential requirement was a flame photometer of adequate sensitivity, resolution and stability combined with a high flame temperature. In view of the heavy background produced an automatic background correction was considered desirable for the attainment of adequate sensitivity.

In the instrument designed and constructed at Woolwich[19] the features outlined above are incorporated, together with an integrating device; an oxy–propane flame is used with direct oxygen atomisation of the sample into the flame. A filtered air jacket is also incorporated to prevent atmospheric contamination and to smooth the edges of the flame, thus contributing to stability of the system. With this instrument 0.1 p.p.m. of strontium can be determined in the presence of 1000 p.p.m. of calcium with a precision of better than 5% per determination.

Standard solutions were made up by dissolving the appropriate amounts of strontium carbonate, calcium carbonate and oxalic acid in the minimum quantity of hydrochloric acid and the strontium/calcium ratios of the solution of the sample determined.

Reagents

Hydrochloric acid, conc., $d = 1.16$, calcium carbonate, strontium free, and dried at 100°, strontium carbonate, dried at 100° and oxalic acid.

Whenever possible all reagents should be of "AnalaR" quality and the use of redistilled or demineralized water is implied throughout.

Standards

(1) *Master solution of strontium, 1000 p.p.m.* Weigh out 0.169 g of strontium carbonate into a beaker. Cover with 10–20 ml of redistilled water and add conc. hydrochloric acid dropwise

until dissolved. Make up to 100 ml with water to give a master solution containing 1000 p.p.m. of strontium in solution. Dilute this master solution as necessary to give working standard solutions.

This solution should be bottled in polythene which has previously had prolonged washing with redistilled water.

(2) *Master solution of calcium chloride 1/8 M.* Weigh out 2.497 g of calcium carbonate and dissolve in just sufficient concentrated hydrochloric acid (about 4 ml) and 10 ml of water. Make up to 200 ml with redistilled water.

(3) *Oxalic acid solution 1/8 M.* Weigh out 3.150 g of oxalic acid ($(COOH)_2 \cdot 2H_2O$) and make up to 200 ml with redistilled water.

(4) *"Blank" solution.* Measure out 10 ml of the master solution of calcium and transfer to a 50-ml flask. Add to this 2.5 ml of conc. hydrochloric acid and 10 ml of the oxalic acid solution. Make up to 50 ml with redistilled water.

(5) *Standard for milk.* Proceed as in (4) above but before making up to 50 ml, add 5 ml of 10 p.p.m. strontium solution (prepared from successive dilution of the master solution). This gives a standard containing 1000 p.p.m. of strontium with respect to calcium.

(6) *Standard for vegetation.* Proceed as in (5) above but add 5 ml of 100 p.p.m. strontium solution. This gives a standard containing 10,000 p.p.m. of strontium with respect to calcium.

PROCEDURE

Preparation of sample

Dissolve 36.5 mg of the sample in the form of calcium oxalate monohydrate in 0.5 ml of conc. hydrochloric acid and 1 ml of redistilled water (warm if necessary). Allow to cool and make up to 10 ml with redistilled water.

Flame photometry

Switch on the instrument and allow 30 min to warm up. Light the burner and adjust if necessary for optimum atomisation. Set the internal standard gain control to give an integration time of 10–15 sec with 0.1% calcium oxalate solution. A full description of the instrument with directions for use is given in AERE C/R. 2659 (in course of publication). Adjust the gain on the line and background channels to give full scale deflection for the top standard.

Note: The top standard will contain 1 p.p.m. of strontium for milk or 10 p.p.m. for vegetation. Set the zero control for the blank solution. Repeat as necessary.

Note: The zero and gain controls are not entirely independent *i.e.* resetting of one may affect the other so that repeated adjustments may be necessary.

Obtain the readings for each sample in turn, re-calibrating as necessary.

Note: Since linear calibration is obtained in the case of the Woolwich instrument the strontium content may be read directly from the dial reading.

PRECISION AND ACCURACY (MILK ASH AND VEGETATION ASH)

The accuracy of the method was assessed by comparison between the techniques of neutron activation, X-ray fluorescence, emission spectroscopy and flame photometry, and the results are given in Table III. The coefficient of variation of the whole analytical operation (including flame photometry) is 5% per determination.

TABLE III

All results expressed as p.p.m. strontium in milk ash

Sample No.	*Flame photometry*	*Emission spectroscopy*	*Neutron activation*	*X-ray fluorescence*
M969	33	31	32	34
M971	64	64	63	65
M972	51	45	53	56
M973	41	38	39	45
M974	32	36	33	33

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SUMMARY

Samples of bone ash are mixed with anhydrous copper sulphate and graphite and pelleted. A d.c. arc is used to excite the spectra which are then evaluated by microphotometry. The effective concentration range is 50 – 350 p.p.m. of strontium in the ash and the coefficient of variation is 6% for single exposures at the 90 p.p.m. level.

In the case of milk and vegetation, strontium and calcium are separated as insoluble oxalates and the strontium content of this fraction determined by a method similar to that used for bones, with a similar range and accuracy. An alternative flame photometric method is described for the determination of strontium in the calcium-strontium oxalate fraction, the coefficient of variation of the whole analytical operation being 5% per determination.

RÉSUMÉ

Une méthode spectrochimique est proposée pour le dosage du strontium dans les os, le lait et les plantes. La cendre d'os à analyser est mélangée à du sulfate de cuivre anhydre et à du graphite; ce mélange est ensuite comprimé et prêt pour la détermination spectrochimique. Dans le cas du lait et des plantes, le strontium doit être séparé préalablement sous forme d'oxalate.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur spektralanalytischen Bestimmung von Strontium in Knochen, Milch und Pflanzen. Bei der Untersuchung von Knochen wird deren Asche mit wasserfreiem Kupfersulfat und Graphit zu Pillen gepresst. Bei Milch und Pflanzen muss das Strontium vor der Bestimmung als Oxalat abgetrennt werden.

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CATION-EXCHANGE BEHAVIOUR OF URANIUM(VI) ON AMBERLITE IR-120

SEPARATION FROM MIXTURES

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Uranium(VI) forms strong anionic complexes of the type $[UO_2(CO_3)_3]^{-4}$ in carbonate solutions and $[UO_2(SO_4)_n]^{2-2n}$ where $n = 2$ or 3 in sulphuric acid solutions. The anion exchangers, Amberlite IRA-400[1] and Dowex A-1[2] have been used to recover uranium from the carbonate and sulphate leach systems. On the same principle uranium has been separated from bismuth[3]. Uranium(VI) also forms anionic chloro- and nitrato-complexes and has thus been separated on anion exchangers from vanadium[4], iron[5] etc. DOLAR AND DRAGANIC[6] have reported the separation of traces of rare earths from uranium(VI) on the cation-exchange resin, Amberlite IR-120. Uranyl ion is eluted by 0.5 *M* oxalic acid, and the rare earths by 5 *N* hydrochloric acid.

In this paper systematic quantitative studies on the behaviour of uranium(VI) on the cation exchanger, Amberlite IR-120, are described, with hydrochloric, nitric, sulphuric, acetic, citric and perchloric acids as eluting agents. Uranium has been separated from thorium, zirconium, cerium(III), mercury, copper, nickel and phosphate.

APPARATUS AND REAGENTS

Apparatus

Hilger Quartz spectrophotometer and 1-cm cells and a Cambridge pH indicator.

Ion-exchange column: The column used was of a U-shape and consisted of a glass tube (14 mm × 30 cm), flared at the top into a bulb (24 mm diameter) and extending from the bottom into the other limb (4 mm × 27 cm). The latter had a horizontal extension (5 cm), bending vertically downwards, and fitted with a stopcock to regulate the flow of liquid down the column. A resin bed, 1.4 × 14.5 cm, was employed.

Reagents

Uranyl nitrate stock solution, approximately 1 mg per ml; 2.1 g of uranyl nitrate hexahydrate (Mallinckrodt, A.R.) were dissolved in 1 l of water containing about 1% nitric acid. The solution was standardized by the oxine precipitation method. The stock solution contained 0.85 mg of uranium per ml. A solution containing 0.17 mg of uranium per ml was prepared by dilution and used for the ion-exchange runs. 8-Quinolinol (B.D.H., A.R.): 1% solution in chloroform. Buffer solution (pH 6.0): 77 g of ammonium acetate (E. Merck, Analar) were dissolved in water and glacial acetic acid and ammonia were added to adjust the pH to 6.0 with the aid of a Cambridge pH meter. The solution was diluted to 1 l and the pH was checked. Amberlite IR-120 (Rohm and Haas Company, Philadelphia), 20 to 50 mesh, hydrogen-form cation-exchange resin. The resin was conditioned by repeated washes with 4 *N* hydrochloric acid, followed by water. After being filled, the column was washed at a flow rate of 2 ml/min with 4 *N* hydrochloric acid, and then with water till the effluent became chloride-free. At the conclusion of the runs, the resin was withdrawn from the column, air-dried, dried in an oven at 100° for 1–2 h and weighed.

Chemicals used were all of reagent grade, unless otherwise mentioned.

EXPERIMENTAL, RESULTS AND DISCUSSION

Ion-exchange behaviour studies

An aliquot of the uranyl nitrate solution containing 1.7 mg of uranium was passed through the resin bed at a rate of 2 ml per min. The resin was washed with 50 ml of water, and the uranium eluted with 200 ml of the different eluants. The latter included hydrochloric acid (1, 2, 3, 4 *N*), nitric acid (1, 2, 4 *M*), sulphuric acid (1, 2 *M*), acetic acid (2 *N*), perchloric acid (2 *M*) and citric acid (2%, 5%). In each case the elution rate was 2 ml per minute and the eluate was collected in 50-ml fractions. Each fraction was evaporated to dryness in a beaker, 10 ml of nitric acid, 5 ml of sulphuric acid and 1 to 2 ml of perchloric acid (60%) were added and the mixture evaporated again to dryness to destroy any organic matter from the resin. The residue was taken up with 5 ml of 1 *N* acetic acid, warmed and transferred to a separatory funnel. The sides of the beaker were rinsed with 10 ml of buffer solution of pH 6.0 and the washings transferred to the separatory funnel. The uranium was extracted with 8-quinolinol–chloroform and measured spectrophotometrically at 430 mμ[7]. The elution constant, E, for each eluting agent is calculated from the relation[8]:

$$E = \frac{d \cdot A}{V}$$

where V is the volume of eluant (corrected for non-effective liquid in the resin bed) which is required to displace uranium under essentially equilibrium conditions through a distance, d cm, in a column of known cross-sectional area, A cm^2.

TABLE I

BEHAVIOUR OF URANIUM(VI) TOWARDS VARIOUS ELUTING AGENTS

Weight of oven-dried resin = 7.2710 g

No.	*Eluting agents*	*Uranium recovery, % (50-ml fractions of effluent)*				*Total uranium recovery %*	*Elution constant*
		I	*II*	*III*	*IV*		
1	HCl, 1 *N*	1.2	29.4	38.2	29.4	98.2	0.1628
	HCl, 2 *N*	0.3	44.0	50.0	6.0	100.3	0.1628
	HCl, 3 *N*	58.8	29.4	11.0	1.2	100.2	0.6009
	HCl, 4 *N*	64.2	24.5	8.9	2.9	98.0	0.6009
2	HNO_3, 1 *M*	0.8	28.6	32.4	35.3	97.1	0.1192
	HNO_3, 2 *M*	38.2	47.1	8.9	5.9	100.1	0.2561
	HNO_3, 4 *M*	44.1	44.2	11.8	2.0	99.1	0.6009
3	H_2SO_4, 1 *M*	70.6	23.5	5.0	0.0	99.1	0.6009
	H_2SO_4, 2 *M*	76.8	22.0	1.0	0.0	99.8	0.6009
4	$HClO_4$, 2 *M*	—	—	—	—	63.0	—
5	CH_3COOH, 2 *N*	—	—	—	—	58.9	—
6	Citric acid, 2%	—	—	—	—	58.8	—
	Citric acid, 5%	—	—	—	—	24.7	—

The results are shown in Table I. The elution curves obtained with hydrochloric, nitric and sulphuric acids are illustrated as histograms in Fig. 1.

With 200 ml of hydrochloric acid (2–4 *N*), nitric acid (2–4 *M*) or sulphuric acid (1–2 *M*) as the eluting agent, essentially quantitative recovery of uranium was possible. It is quite evident that an increase in the eluant volume from 200 ml to

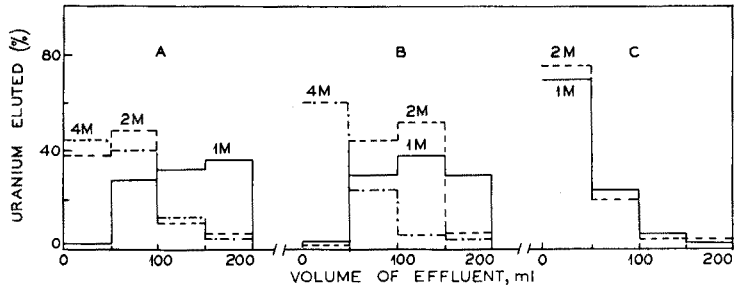

Fig. 1. Elution of uranium(VI) from Amberlite IR–120 resin. A. HNO_3, B. HCl, C. H_2SO_4.

300 ml will lead to 100% recovery of uranium (Nos. 1–3). In Fig. 1 the elution peak is gradually shifted towards the left with increased acid concentration. In Nos. 4–6 (Table I) the uranium recovery was incomplete with 200 ml of the eluting agents, and the elution peaks were not ascertained; probably they could be traced beyond an eluate volume of 200 ml. The elution constants (Table I) give a measure of the relative efficiency of the various eluants. The higher the value of the elution constant, the more efficient is the elution *i.e.*, the smaller is the volume of the eluting agent required. The eluting agents can be arranged in order of decreasing efficiency: sulphuric acid > hydrochloric acid > nitric acid > perchloric acid > acetic acid. This can be correlated with the strength of the complex formed between uranium and the eluting agent. The formation constants for UO_2SO_4, UO_2Cl^+ and $UO_2NO_3^+$ are 76, 0.88 and 0.24 respectively[9]. However, for routine work hydrochloric acid (2–4 *N*) is preferred owing to its ease of volatility in the assaying step. The whole operation takes 3 to 4 h. Organic acid eluants like citric or oxalic acid would involve time-consuming procedures.

Ion-exchange separations

Uranium is separated from various mixtures under conditions such that uranium remains as a cation and the added ion (or ions) is converted to a stable anionic complex. With such mixtures, uranium is retained on the resin bed, whereas the added ion passes through. Uranium can also be isolated from metals like thorium by selective elution with a mineral acid after adsorption on a cation-exchanger.

Separation from thorium

Thorium is more strongly adsorbed than uranium on a cation-exchanger and must be eluted with stronger acids. A mixture of uranyl and thorium nitrates in varying proportions was passed down the resin column. After washing with water (50 ml), uranium was eluted with 300 ml of 1 *N* hydrochloric acid and then thorium with 300 ml of 3 *M* sulphuric acid. The separate eluates containing uranium and thorium were diluted to 500 ml and suitable aliquots taken for analysis. Uranium

was estimated spectrophotometrically as before and thorium by the Alizarin S method[10]. Quantitative recoveries of uranium were obtained on samples with uranium to thorium ratios from 1:0.9 to 1:8.8 (Table II).

TABLE II

ION-EXCHANGE SEPARATIONS

No.	*Taken mg*		*Found mg*		*Uranium recovery %*
1	U	1.7	U	1.7	100
	Th	1.5	—		
2	U	1.7	U	1.72	101.2
	Th	7.5	Th	7.75	(Th 103.3)
3	U	1.7	U	1.75	102.9
	Th	15.0	Th	15.0	(Th 100)
4	U	17	U	16.6	97.7
	Th	1.5	—		
5	U	24.5	U	24.94	101.8
	Zr	25			
6	U	24.5	U	25.0	102.1
	Zr	100			
7	U	24.5	U	24.0	97.95
	Ce(III)	25.0			
8	U	1.7[a]	U	1.75	102.9
	Hg	13.0			
9	U	24.5	U	25.1	102.4
	Hg	50			
10	U	24.5	U	25.9	105.7
	Cu	51.0			
	Ni	51.0			
11	U	24.5	U	25.72	104.9
	Phosphate	100			
12	U	24.5	U	24.74	100.9
	Phosphate	250			

[a] Uranium estimated by colorimetric method.

The separation factors for uranium/thorium (Table III) are obtained from their elution constant ratios. Only the values with hydrochloric and sulphuric acids as eluting agents are given. With 1 *N* hydrochloric acid the separation factor is very large; thorium is not eluted and quantitative separation is feasible. This was confirmed by the experimental results. The elution constants for thorium were determined as in case of uranium except that thorium was estimated colorimetrically with Alizarin S[10].

TABLE III

SEPARATION FACTORS FOR URANIUM/THORIUM

No.	*Eluting agent*	*Elution constant (E)*		*Separation factor* $\alpha = E_U/E_{Th}$
		Uranium	*Thorium*	
1	HCl, 1 *N*	0.163	~ 0.0	∞
2	HCl, 6 *N*	0.601	0.256	2.35
3	H_2SO_4, 1 *M*	0.601	0.256	2.35
4	H_2SO_4, 3 *M*	0.601	0.601	1.00

Separations from zirconium, mercury, cerium(III), copper and nickel

Zirconium (as zirconyl oxychloride) was converted to an anionic complex with oxalic acid, mercury (mercuric chloride) with potassium iodide, and cerium(III) (as cerous chloride: cerous oxalate dissolved in hot dilute hydrochloric acid and filtered), copper (as the sulphate) and nickel (as the sulphate), with EDTA. After this pre-treatment the mixture was poured down the Amberlite IR-120 column (1.4 cm × 19.5 cm). The anionic complex passed out, the resin was washed with water till free from the excess of complexing agent, and uranium was eluted with 250 ml of 4 *N* hydrochloric acid. In each case the effluent was evaporated nearly to dryness with nitric and perchloric acids, and uranium estimated gravimetrically by the oxine method[11].

Separation from phosphate

When a mixture of uranyl nitrate and disodium hydrogen phosphate was passed through the cation exchanger, only uranium was adsorbed and could be eluted and estimated as above.

The separation of uranium from thorium is important in breeder reactors where the fissile material ^{233}U has to be separated from an intensely radioactive matrix of natural thorium. Zirconium and rare earths occur as major constituents of fission products and hence their isolation from irradiated reactor fuel (^{235}U) forms an important part of nuclear energy programmes. Phosphate is commonly associated with uranium in ores and as such its separation is significant. The proposed method can be adapted to large scale separations.

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SUMMARY

Quantitative studies are reported on the cation-exchange behaviour of uranium(VI) at the milligram level with Amberlite IR-120. Hydrochloric, nitric, sulphuric, perchloric, acetic and citric acids were tested as eluants; 200–300 ml of 2 *N* hydrochloric, nitric or sulphuric acid suffice for quantitative elution of 1.7 mg of uranium(VI) from a 1.4 cm × 14.5 cm bed. The efficiency of the eluting agents is discussed in terms of their elution constants. Uranium is separated from thorium by selective elution, from zirconium, cerium(III), copper and nickel by converting the latter into suitable anionic complexes and from phosphate just by passing the mixture through the cation exchanger.

RÉSUMÉ

Une étude quantitative a été effectuée sur le comportement de l'uranium(VI) sur une résine d'échange de cations. Il peut être alors séparé d'avec le thorium par élution sélective à l'aide d'un acide minéral; d'avec le zirconium, le cérium(III), le cuivre et le nickel en transformant ces derniers en complexes anioniques et d'avec le phosphate, simplement en faisant passer le mélange à travers l'échangeur de cations.

ZUSAMMENFASSUNG

Es wurde das Verhalten von Uran(VI) bei der Behandlung mit einem Kationenaustauscherharz quantitativ untersucht. Eine Trennung von Thorium kann durch selektive Eluierung mit einer Mineralsäure erzielt werden; zur Trennung von Zirkonium, Cer(III), Kupfer und Nickel werden diese in geeignete anionische Komplexe übergeführt; die Trennung von Phosphat erfolgt ohne weiteres beim Durchfliessen der Lösung durch die Kolonne des Kationenaustauschers.

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SPECTROPHOTOMETRIC DETERMINATION OF MOLYBDENUM WITH 9-METHYL-2,3,7-TRIHYDROXY-6-FLUORONE

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A sensitive method for the spectrophotometric determination of micro quantities of molybdenum(VI) is recommended which is based on the formation of a rose-red complex with 9-methyl-2,3,7-trihydroxy-6-fluorone.

Most of the colorimetric methods for molybdenum[1] are not sufficiently specific and preliminary separation is required. Acetyl acetone is a highly selective extractant for molybdenum, but a time-consuming wet oxidation[2] is required for its destruction before the determination of molybdenum. Extraction with α-benzoinoxime in chloroform and subsequent conversion to the quercetin complex[3] is long and tedious and vanadate and tungstate interfere seriously.

During study of phenolic derivatives as colour forming reagents[4,5], 9-methyl-2,3,7-trihydroxy-6-fluorone was found to be a very sensitive colorimetric reagent for molybdenum. The complex tends to precipitate from a slightly acid solution but this can be prevented by the presence of gelatin in 12% ethanol. The coloured compound is stable for at least 48 h and has its maximum intensity of colour at pH 1.5–2.3 with the maximum absorption at 510 mμ. The molar extinction coefficient of the complex is 24467. The system obeys Beer's law from 0.2 to 3 p.p.m. and the optimum range is from 0.8 to 3.0 p.p.m. of molybdenum, where the percent relative analysis error per

1% absolute photometric error, as calculated by Ayres method[6], is 2.8. The continuous variation and molar ratio methods indicate that the complex in solution contains the metal and the reagent in a ratio of 1:1, with an instability constant of about $2.09 \cdot 10^{-6}$ at 20°.

EXPERIMENTAL

Apparatus and solutions

The Uvispek spectrophotometer with 10.0-mm quartz transmission cells and the pH meter were as reported previously[5].

Reagent solution: Merck's reagent grade methyl fluorone (62.5 mg) was dissolved in a mixture of 210 ml of absolute alcohol and 12 ml of dilute sulphuric acid (1:6) and the solution made up to 250 ml with absolute alcohol.

Molybdenum solution: A standard molybdenum solution was prepared by dissolving a weighed amount of AnalaR ammonium molybdate in twice-distilled water and diluting to 500 ml. The molybdenum content of an aliquot portion of the solution was then determined gravimetrically with α-benzoinoxime. From this standard solution, more dilute solutions were prepared as required.

Buffer solution: A buffer of pH 2.0 was obtained by mixing solutions of *N* sodium acetate (50 ml) and *N* hydrochloric acid (52.5 ml) and diluting with water to 250 ml.

Equimolecular solutions of molybdenum and the reagent and a 0.4% (w/v) solution of gelatin were also prepared.

Solutions of various other ions were as used earlier[5].

Absorption spectra

An aliquot of standard solution containing 2 p.p.m. of molybdenum was transferred to a 25-ml volumetric flask; 1 ml of gelatin solution, 2 ml of reagent solution, 3 ml of absolute alcohol, and 3 ml of buffer solution were added. This order of addition was always maintained. A reagent blank without molybdenum was also prepared. The optical density of the test solution against both the reagent solution and distilled water and that of the blank against distilled water were measured at wavelengths from 470 to 600 mμ; the values are plotted in Fig. 1. The optical density had a maximum at 510 mμ, and all subsequent measurements were made at that wavelength.

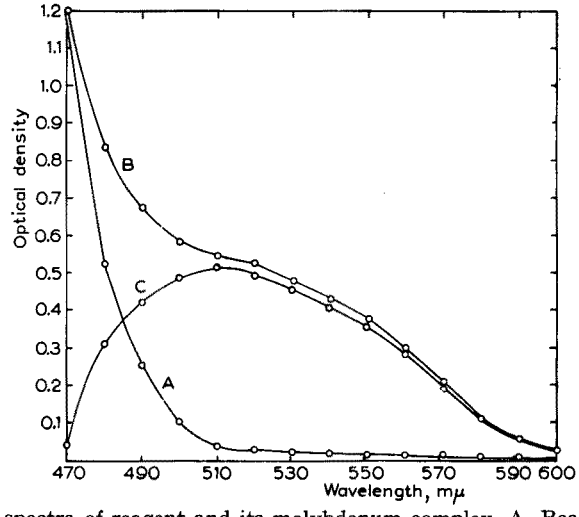

Fig. 1. Absorption spectra of reagent and its molybdenum complex. A. Reagent against water. B. Reagent plus molybdenum against water. C. Reagent plus molybdenum against reagent.

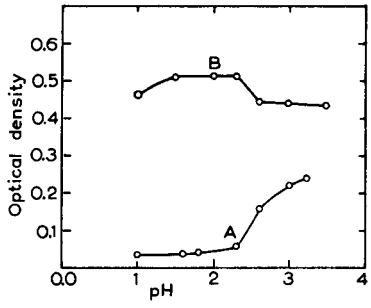

Fig. 2. A. Effect of pH on the reagent.
B. Effect of pH on the complex.

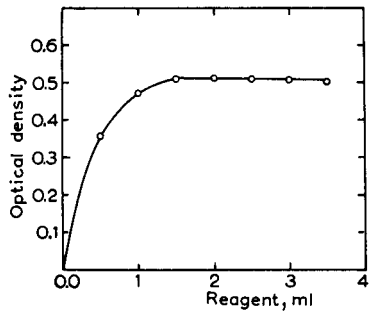

Fig. 3. Effect of reagent.

Variation of optical density with pH, reagent and time

The test and blank solutions were prepared at different pH values obtained by the addition of sulphuric acid or sodium acetate solution. The variation of the optical density of the test solutions (relative to the blank) and of the reagent solutions with pH is shown in Fig. 2 (curves A and B). The optical density is almost constant between pH 1.5 and 2.3; this range was used for all subsequent readings.

The effect of reagent concentration on optical density in presence of 2 p.p.m. of molybdenum is shown in Fig. 3. The maximum optical density is obtained only when the amount of the reagent solution is 1.5 ml or more; in all subsequent experiments, the volume of the reagent used was 2 ml. One ml of the gelatin solution was always added and the colour intensity was then found to be stable for 48 h at room temperature.

Beer's law, optimum range and photometric error

The optical density increases linearly with the concentration (0.2 to 3.0 p.p.m.) of molybdenum in accordance with Beer's law and the optimum concentration range, as obtained from the steepest slope of the curve (Fig. 4) drawn by plotting the percent absorptancy against the logarithm of concentration of molybdenum[7], is from 0.8 to

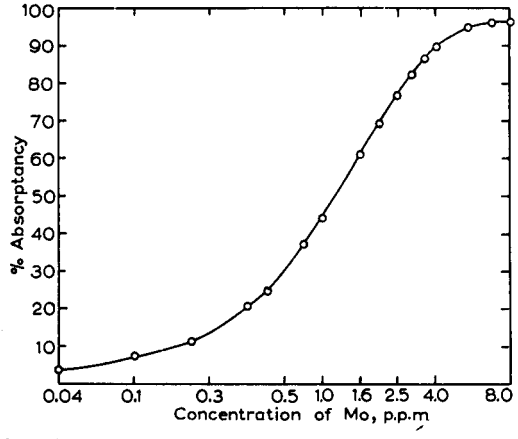

Fig. 4. Standard curve for molybdenum–methyl fluorone complex.

3 p.p.m. of molybdenum. The relative analysis error per 1% absolute photometric error for this range is 2.8%.

The ions listed in Table I do not affect the absorption of either the blank or the test solution. With ascorbic acid as a masking agent, ferric ion and vanadate can be tolerated up to 20 p.p.m. and 10 p.p.m. respectively.

Zirconium, titanium, chromate and tungstate interfere even when only 2 p.p.m. are present.

TABLE I

NON-INTERFERING IONS
Molybdenum concentration, 2 p.p.m.

Ion	*Maximum concentration investigated in p.p.m.*
Fluoride	10
Chloride	200
Nitrate	200
Perchlorate	200
Sulphate	400
Phosphate	10
Acetate	1000
Tartrate	200
Citrate	200
Oxalate	10
Ferrous (iron)	50
Uranyl	50
Aluminium	50
Sodium	500
Ammonium	200

Composition, molar extinction coefficient, degree of dissociation and instability constant

For the evaluation of composition of the complex, the absorption of mixtures of equimolecular solutions ($1 \cdot 10^{-4}$ *M*) of molybdenum and reagent were measured at

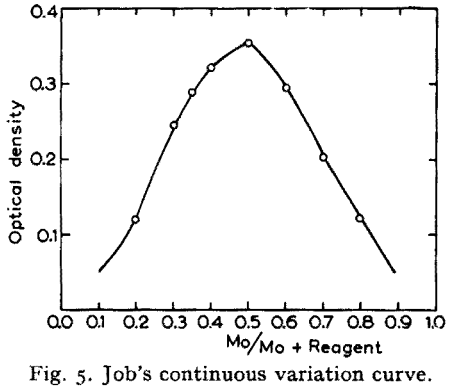

Fig. 5. Job's continuous variation curve.

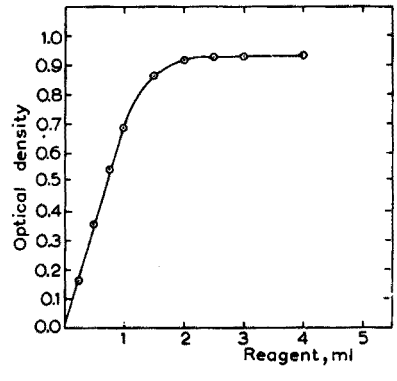

Fig. 6. Molar ratio method: $0.969 \cdot 10^{-3}$ *M* solutions of molybdenum and reagent.

510 mμ. Fig. 5 shows the absorption (after proper corrections for the absorption of the reagent) plotted against increasing amounts of molybdenum. The sharp peak

in the curve indicates definite compound formation between the molybdenum and the reagent when present in a ratio of 1:1. The nature of the curve (Fig. 6) derived from the molar ratio method confirms the observation that the molybdenum and the reagent combine in a ratio of 1:1.

The molar extinction coefficient of the complex calculated from Beer's law curve is

$$\frac{0.510 \cdot 95.95 \cdot 10^3}{2} = 24467$$

The degree of dissociation, α, is calculated from the relationship[8]

$$\alpha = \frac{E_m - E_s}{E_m}$$

The E_m and E_s values are 0.510 and 0.372 respectively at a molybdenum concentration of $2.08 \cdot 10^{-5}$ M, and the degree of dissociation, α, is thus 0.2707. The instability constant, K, from the equation

$$K = \frac{\alpha^2 C}{1-\alpha}$$

where C is the concentration of the complex in moles per l, is $2.091 \cdot 10^{-6}$.

ACKNOWLEDGEMENT

The authors wish to thank the University Grants Commission, Government of India, for providing the Hilger Uvispek spectrophotometer and the Cambridge pH meter.

SUMMARY

9-Methyl-2,3,7-trihydroxy-6-fluorone is proposed as a highly sensitive reagent for molybdenum. The rose-red complex shows maximum absorption at 510 mμ and obeys Beer's law from 0.2 to 3 p.p.m. of molybdenum, the optimum range being 0.8 to 3 p.p.m. where the percent relative analysis error is only 2.8. In solution the complex contains molybdenum and the reagent in the ratio 1:1. The instability constant of the complex is about $2.09 \cdot 10^{-6}$.

RÉSUMÉ

Une méthode spectrophotométrique pour le dosage du molybdène est proposée, utilisant un réactif nouveau et très sensible: la méthyl-9-trihydroxy-2,3,7-fluorone-6.

ZUSAMMENFASSUNG

Es wird eine spektrophotometrische Methode zur Bestimmung von Molybdän beschrieben unter Verwendung von Methyl-9-trihydroxy-2,3,7-fluoron-6 als neues und sehr empfindliches Reagenz.

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THE USE OF LANTHANUM CHLORIDE TO PREVENT INTERFERENCES IN THE FLAME PHOTOMETRIC DETERMINATION OF EXCHANGEABLE CALCIUM IN SOILS

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INTRODUCTION

Flame photometry provides a simple and rapid means for the chemical analysis of calcium. However interferences from other ions present in the solution can occur which, unless suitable steps are taken to prevent them, may seriously affect the accuracy of the results. In the determination of calcium by flame excitation techniques, ions reported to cause such interferences include aluminium[1], phosphate[2–5], sulphate[5,6], and bicarbonate[6]. In addition the author has observed that silicate can cause serious interference. Sodium and potassium may also interfere, due to some transmission of their radiation if an interference filter is used to isolate the calcium radiation[7].

If soils are leached with normal ammonium chloride for the determination of exchangeable cations[8], all of these ions may be present in the leachate although generally only in very low concentration. In leachates from acid soils however, appreciable amounts of aluminium may be present, while in the leachates from calcareous soils bicarbonate may be present in quite high concentrations.

Such interferences may be overcome either by the removal of the interfering ions by precipitation or ion exchange, by adding an excess of the interfering ion, or by the addition of some other ion which may block the interference chemically. The latter two methods, being simpler, are generally preferred. The present investigation was undertaken to examine the possible extent of likely interferences on the determination of calcium in ammonium chloride leachates of soils, and to devise a suitable means of overcoming them.

EXPERIMENTAL

The results reported were obtained using an E.E.L. flame photometer with an interference filter to isolate the calcium oxide emission band at 620 mμ. The gases used were air and a proprietary bottled gas similar to "calor" gas.

Stock solutions were prepared as follows:

Calcium: calcium carbonate was dissolved in a slight excess of hydrochloric acid. The solution was boiled to expel carbon dioxide and, after cooling, made to volume with water.

Magnesium: magnesium metal powder was dissolved in a slight excess of hydrochloric acid and the solution adjusted to volume.

Aluminium: aluminium metal was dissolved in a slight excess of hydrochloric acid as for magnesium.

Phosphate, sulphate, bicarbonate and silicate solutions were prepared from potassium dihydrogen phosphate, ammonium sulphate, sodium bicarbonate and sodium silicate, respectively.

All other cations were prepared by solution of the chlorides.

Test solutions were prepared by mixing appropriate volumes of the stock solutions and adjusting to a final volume of 20 ml.

RESULTS

(a) *The effect of ammonium chloride concentration on calcium emission*

The results of varying concentrations of ammonium chloride on the intensity of the calcium emission are given in Table I.

TABLE I

THE EFFECT OF AMMONIUM CHLORIDE ON CALCIUM EMISSION

NH_4Cl normality	Galvanometer reading		
	12.5 p.p.m. Ca	25 p.p.m. Ca	50 p.p.m. Ca
0	17	33	66
0.2	15	31	62
0.4	15	30	60
0.6			59
0.8			57
1.0	14	28	55
2.0			48

These results show that ammonium chloride partially suppressed the calcium emission. Over the range of concentration 0.2 N to 2 N this suppression was directly proportional to the ammonium chloride concentration. Since in the majority of ammonium chloride leachates of soils the calcium concentration is likely to lie within a range suitable for analysis without dilution, all subsequent investigations were made in normal ammonium chloride.

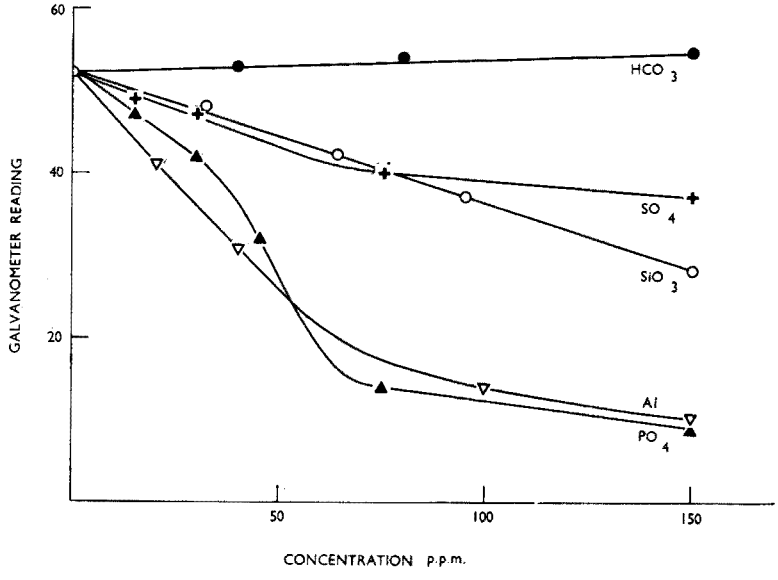

Fig. 1. The effect of aluminium, bicarbonate, phosphate, sulphate and silicate on calcium emission in normal ammonium chloride containing 50 p.p.m. Ca.

(b) The effect of extraneous ions on calcium emission in normal ammonium chloride solutions

Ions most likely to cause serious interference in the flame photometric determination of calcium include aluminium, bicarbonate, phosphate, sulphate and silicate. The results of an examination of the effect of varying concentrations of these ions on calcium emission in normal ammonium chloride solutions are shown in Fig. 1.

These results show that with the exception of bicarbonate, which showed a slight positive interference, each of the ions tested caused serious interference. With the majority of soils the concentration of each of these ions in the ammonium chloride leachate is likely to be low (generally less than 1–2 p.p.m.) so that their interference on the direct determination of calcium would be small. In very acid and alkaline soils however, greatly increased amounts of soluble aluminium are likely[9], and concentrations as high as 100 p.p.m. are possible in the ammonium chloride leachate. As is evident from Fig. 1 such concentrations of aluminium would make the direct determination of calcium on the leachate impossible.

(c) Elimination of interferences

Aluminium: As aluminium is likely to be the most serious interfering ion in ammonium chloride leachates initial investigations were directed towards overcoming interference from it.

MITCHELL AND ROBERTSON[1] found that the addition of strontium overcame the interference of aluminium with calcium in the Lundegardh technique for flame emission spectroscopy. DAVID[10] used magnesium to prevent similar interferences when using an atomic absorption flame spectrophotometric technique while YOFE AND FINKELSTEIN[5] have used lanthanum to overcome the interference of phosphate and sulphate on calcium. Tests were made to examine the possibility of using strontium, magnesium, lanthanum or barium to similarly overcome aluminium interferences under the present conditions of measurement. The results are given in Table II.

TABLE II

THE EFFECT OF BARIUM, STRONTIUM, LANTHANUM AND MAGNESIUM ON ALUMINIUM INTERFERENCE WITH CALCIUM IN NORMAL AMMONIUM CHLORIDE CONTAINING 50 p.p.m. CALCIUM

Cation added	*Galvanometer reading*				
	Aluminium added p.p.m.				
	0	*20*	*40*	*100*	*200*
Nil	52	41	31	14	9
Strontium[a] 1000 p.p.m.	85	83	80	71	64
Barium 1000 p.p.m.	62	50	42	30	26
Magnesium 1000 p.p.m.	42	44	46	20	17
Magnesium 2000 p.p.m.	44	44	44	43	21
Lanthanum 1000 p.p.m.	52	52	52	51	26
Lanthanum 2000 p.p.m.	52	52	52	52	49

[a] Sensitivity 1/3 of all other readings.

Sufficient strontium radiation, probably from the strontium oxide band at 680 mμ was transmitted by the radiation filter to cause serious interference with calcium and it was necessary to reduce the sensitivity to one third of that of the other readings in order to read these solutions. This fact alone is sufficient to exclude the use of

strontium, but it is of interest to note also, that, under the present conditions of experiment, aluminium still interfered seriously with the radiation measured. Examination of calcium-free strontium solutions showed that much of the interference measured in the presence of strontium could have been due to the effect of aluminium on strontium emission. The failure of the radiation filter to exclude strontium radiation thus prevented observation of any effect of strontium on the aluminium interference with calcium emission.

Barium only slightly reduced the aluminium interference with calcium.

Both lanthanum and magnesium, however, showed a capacity to suppress aluminium interference. Since at the concentrations used magnesium slightly suppressed the calcium emission, and, as magnesium was less effective in suppressing the aluminium interference, lanthanum was chosen for further investigation. Further, magnesium is an exchangeable cation usually determined together with calcium and the choice of an element other than one of the test elements has obvious advantages.

In order to determine the optimum lanthanum concentration required to control aluminium interference, solutions of varying calcium, aluminium and lanthanum contents were examined. Aluminium concentrations ranged from 0 to 200 p.p.m. and lanthanum from 250 to 5000 p.p.m. The results, summarized in Fig. 2, showed that irrespective of calcium, aluminium or lanthanum concentration, aluminium interference was eliminated provided the ratio of lanthanum to aluminium was equal to or greater than 12.5:1. This corresponds to an atomic ratio of approximately 2.5:1.

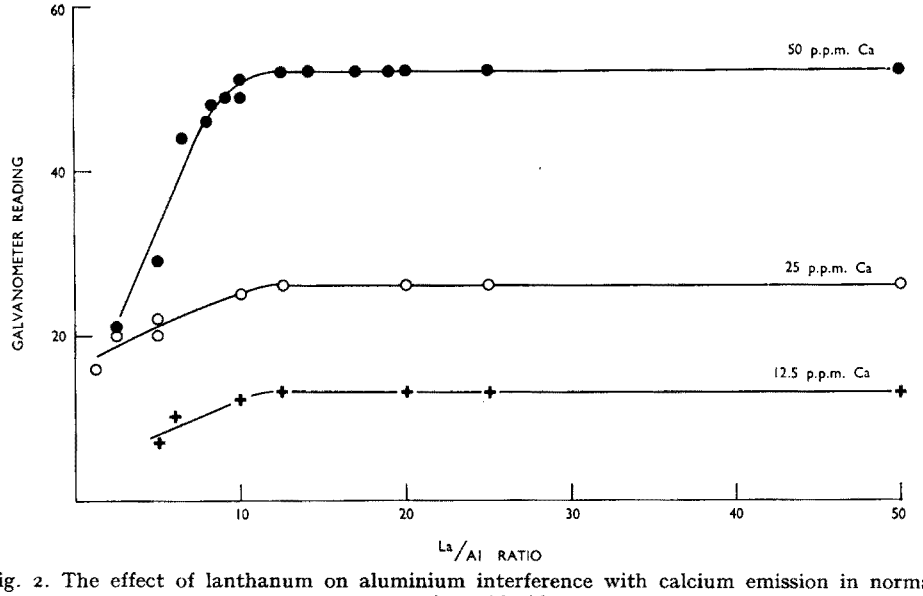

Fig. 2. The effect of lanthanum on aluminium interference with calcium emission in normal ammonium chloride.

On the basis of these results 2000 p.p.m. of lanthanum was chosen as a working concentration adequate to prevent all likely interference from aluminium in natural soil leachates. The effectiveness of 2000 p.p.m. of lanthanum in overcoming aluminium interference is shown in Table III.

TABLE III

THE EFFECT OF 2000 p.p.m. OF LANTHANUM ON ALUMINIUM INTERFERENCE WITH CALCIUM IN NORMAL AMMONIUM CHLORIDE SOLUTION

Aluminium p.p.m.	*Galvanometer readings*						*Aqueous solution 50 p.p.m. Ca*
	12.5 p.p.m. Ca		*25 p.p.m. Ca*		*50 p.p.m. Ca*		
	−La	*+La*	*−La*	*+La*	*−La*	*+La*	
0	13	13	26	26	52	52	66
20	5	13	16	26	41	52	46
40	2	13	10	26	31	52	32
100	2	13	4	26	14	52	14
160				26		52	
200	2	12	4	25	10	49	9

At each level of calcium 2000 p.p.m. of lanthanum completely prevented interference up to 160 p.p.m. of aluminium.

Bicarbonate: Calcium carbonate is slightly soluble in ammonium chloride so that bicarbonate concentrations of the order of 200–300 p.p.m. could be encountered in the leachates of calcareous soils. Table IV gives results showing the effect of varying concentrations of bicarbonate on calcium emission.

TABLE IV

THE EFFECT OF 2000 p.p.m. OF LANTHANUM ON BICARBONATE INTERFERENCE WITH CALCIUM IN NORMAL AMMONIUM CHLORIDE SOLUTION

Bicarbonate p.p.m.	*Galvanometer readings*				*Aqueous solution 50 p.p.m. Ca*
	25 p.p.m. Ca		*50 p.p.m. Ca*		
	−La	*+La*	*−La*	*+La*	
0	26	26	53	53	66
40	26	26	53	53	54
80	26	26	54	55	44
160	28	27	55	55	39
200	27	27	56	55	29
400	29	29	57	58	

These results show that whereas bicarbonate interfered quite seriously with calcium in aqueous solutions, the only effect in normal ammonium chloride solution both in the presence and absence of lanthanum was a small positive interference. This was found to be largely due to traces of calcium impurity in the bicarbonate reagent together with slight interference from sodium.

Phosphate, sulphate and silicate: Table V gives results showing the effects of phosphate, sulphate and silicate on calcium emission in the presence and absence of 2000 p.p.m. of lanthanum.

The addition of 2000 p.p.m. of lanthanum completely suppressed phosphate interference at all levels tested up to 150 p.p.m. While lanthanum greatly reduced the interference from sulphate it did not completely suppress it. Sulphate interference in the presence of lanthanum however was small and at the low concentrations likely to be present in soil leachates it would be negligible.

In aqueous solution silicate interfered seriously with calcium emission. In normal ammonium chloride solution this interference while still serious was considerably

TABLE V

THE EFFECT OF 2000 p.p.m. OF LANTHANUM ON THE INTERFERENCES OF PHOSPHATE, SULPHATE AND SILICATE WITH 50 p.p.m. OF CALCIUM IN NORMAL AMMONIUM CHLORIDE SOLUTION

Concentration of interfering ion p.p.m.	*Galvanometer reading*								
	Phosphate			*Sulphate*			*Silicate*		
	Aqueous solution	*N NH_4Cl*	*N NH_4Cl + La*	*Aqueous solution*	*N NH_4Cl*	*N NH_4Cl + La*	*Aqueous solution*	*N NH_4Cl*	*N NH_4Cl + La*
0	66	53	53	66	52	52	66	52	52
15	58	47	53	58	49	51	45	50	52
30	50	42	52	53	47	48	33	48	52
75	15	14	52	42	40	49	7	40	52
150	11	9	52	39	37	48	3	28	52
300	9	8	43				2	5	44

reduced. The addition of 2000 p.p.m. of lanthanum however completely suppressed interference from silicate at all levels tested up to 150 p.p.m. SiO_3.

Examination of solutions containing mixtures of aluminium, phosphate, sulphate, and silicate showed that in the presence of 30 p.p.m. of phosphate, 30 p.p.m. of sulphate and 12.5 p.p.m. of silicate (concentrations greatly in excess of those likely in soil leachates) aluminium concentrations could be increased to 100 p.p.m. without causing interference if 2000 p.p.m. of lanthanum were present. Thus it is apparent that the addition of 2000 p.p.m. of lanthanum to ammonium chloride leachates of soils would prevent all likely interference from these ions.

Other ions: Other ions likely to be present in ammonium chloride leachates of soils include sodium, potassium, magnesium, and, in the case of acid soils, iron and manganese. Examination of the effects of various concentrations of these ions on calcium emission in normal ammonium chloride containing 2000 p.p.m. of lanthanum showed that both sodium and potassium caused slight positive interferences with calcium. Enhancement of calcium emission was found equivalent to approximately 1 p.p.m. of calcium for each 100 p.p.m. of sodium, while the interference from potassium was found to be approximately half that of sodium. At the concentrations normally to be expected in soil leachates these interferences would be small, and, if average amounts were included as a background in the standard solutions used for preparation of the calibration lines, interference from these ions would be reduced to negligible proportions. Magnesium, which at higher concentrations in the absence of lanthanum (Table II) caused a small suppression of calcium emission, caused no interference in concentrations up to 200 p.p.m., while iron and manganese at concentrations of 100 p.p.m. also caused no interference.

(d) The effect of lanthanum on the determination of sodium, potassium and magnesium

Sodium, potassium and magnesium are usually determined in the ammonium chloride leachate in addition to calcium.

An examination of a range of normal ammonium chloride solutions containing these elements was made both in the presence and absence of 2000 p.p.m. of lanthanum. Sodium and potassium were determined using the E.E.L. flame photometer and magnesium by atomic absorption flame spectrophotometry in a manner similar to that described by DAVID[10].

This showed that addition of lanthanum had no effect on the sodium and magne-

sium readings but increased the potassium readings slightly. This increase, corresponding to 0.4 p.p.m. of potassium, was probably due to potassium impurity in the lanthanum chloride. Thus it is clear that the addition of lanthanum would have no detrimental effects on the determination of sodium, potassium or magnesium using the methods described.

(e) The effect of fungicides

If leachates are to be retained for more than one or two days before analyses are made it is generally desirable to add a preservative to prevent mould growth. An examination of a number of fungicides was therefore made for their effect on the determination of calcium, sodium and potassium.

Alcohol (1 ml per 100 ml of *N* NH_4Cl) caused an enhancement in each case while chloroform (0.1 ml per 100 ml) caused a depression. Toluene (0.1 ml per 100 ml), 36% formaldehyde (0.2 ml) and mercuric chloride (1.0 g) did not affect any of the readings.

RECOMMENDED PROCEDURE

On the basis of the preceding results, the following procedure for determination of exchangeable calcium in soils was adopted.

Leach 10 g of soil with normal ammonium chloride by the procedure described by PIPER[8], collecting the leachate in a 200-ml volumetric flask until approximately 190 ml of leachate has been collected. Add 0.4 ml of 36% formaldehyde solution and 5 ml of a 21.3% solution of a lanthanum chloride ($LaCl_3 \cdot 7H_2O$) and adjust to volume. In view of possible interference resulting from alcohol it is essential that all alcohol be removed before addition of ammonium chloride after the alcohol washing to remove soluble salts.

Prepare suitable calcium standards ranging from 0 to 100 p.p.m. of calcium in normal ammonium chloride containing 2000 p.p.m. of lanthanum, 5 p.p.m. of sodium, 10 p.p.m. of potassium and 25 p.p.m. of magnesium. Leachates can then be read on the flame photometer and calcium concentrations determined from the calibration curve. If, in the case of soils high in exchangeable calcium, a dilution is necessary, this should either be made using normal ammonium chloride containing 2000 p.p.m. of lanthanum, or if the dilution is made with water, a fresh set of standards must be prepared to correspond with the diluted samples.

Comparison with other methods

To check the reliability of the proposed method a group of soils was leached with normal ammonium chloride, but, to enable comparisons to be made, no lanthanum chloride was added before adjusting to volume. 20-ml aliquots of the leachates were taken and 1 ml of lanthanum or magnesium chloride solutions added to give final concentrations of 2000, 1000 and 500 p.p.m. of lanthanum and 2000 p.p.m. of magnesium. These were measured with the flame photometer in the usual way and calcium estimated by reading from corresponding calibration lines. In addition, calcium was estimated by direct reading of the ammonium chloride leachate without treatment.

A further series of 20-ml aliquots was taken in 50-ml centrifuge tubes, 2 drops of a ferric chloride solution containing 100 mg of iron per ml were added to each and then 5 ml of *N*/8 ammonium hydroxide. After shaking to mix they were allowed to stand overnight and then centrifuged. The supernatant liquors were then read on

the flame photometer and calcium estimated from a corresponding calibration curve.

Exchangeable calcium was also determined on a separate leachate by the oxalate-titration method of PIPER[8]. Aluminium in the leachates was determined by the method of JONES AND THURMAN[11]. These results are given in Table VI.

TABLE VI

EXCHANGEABLE CALCIUM IN SOILS AS DETERMINED BY DIFFERENT PROCEDURES

Soil No.	1	2	3	4	5	6	7
Soil pH	5.21	5.28	5.08	5.00	4.06	5.02	4.18
Aluminium in leachate p.p.m. Al	0.4	2.3	7.0	7.1	28.5	31.5	45.0
Procedure	*Calcium m.e. per 100 g*						
Oxalate-titration	3.96	4.82	0.60	1.47	1.48	1.08	1.22
Direct[a]	3.65	4.30	0.34	0.69	0.50	0.18	0.18
+ 2000 p.p.m. La	3.95	4.70	0.50	1.24	1.40	1.00	1.40
$Fe(OH)_3$ precipitation	4.19	4.56	0.50	1.20	1.42	1.00	1.20
+ 1000 p.p.m. La	3.90	4.65	0.50	1.20	1.50	1.00	1.17
+ 500 p.p.m. La	3.95	4.40	0.50	1.24	1.40	1.00	1.09
+ 2000 p.p.m. Mg	4.00	4.51	0.50	1.24	1.24	0.89	0.89

[a] Flame photometric determination directly on the ammonium chloride leachate.

These results show that the direct estimation of calcium in ammonium chloride without steps to prevent interferences can lead to highly erroneous results. The addition of lanthanum to the solution, however, yielded results which agreed satisfactorily with those obtained by the oxalate-titration method and by the method in which aluminium was co-precipitated with ferric hydroxide indicating that the lanthanum had fully prevented all interferences with calcium emission. Magnesium was less effective, particularly in the soils high in soluble aluminium.

DISCUSSION

The results show that aluminium, phosphate, sulphate and silicate can each cause serious interferences in the flame photometric determination of calcium in ammonium chloride solutions. All can be prevented by the addition of lanthanum to the solution. The mechanism of these interferences is by no means fully understood but probably a major factor in them is the formation of compounds which are stable at high temperature and which prevent the test element from reaching an excited state in the flame. LEYTON[3] showed that the formation of tricalcium phosphate in the flame was a likely reason for the interference of phosphate with calcium emission. Similar reasons can be advanced to explain a major part of the interferences resulting from aluminium, sulphate and silicate.

YOFE AND FINKELSTEIN[5] suggested that the mechanism by which lanthanum prevented the interference of phosphate and sulphate with calcium was by the formation of lanthanum phosphate and sulphate both of which had greater thermostability than the corresponding calcium compounds. Since the present results show that, irrespective of calcium, lanthanum or aluminium concentration, a minimum atomic ratio of lanthanum to aluminium of 2.5:1 is necessary to suppress aluminium interference, it seems likely that a similar mechanism operates in preventing aluminium interference.

In ammonium chloride leachates of soils the phosphate, sulphate and silicate concentrations would not often reach a level at which serious interference with calcium would result. However, in leachates from acid soils and possibly also from highly alkaline soils aluminium concentrations can reach levels which would cause serious interference. The results show that 2000 p.p.m. of lanthanum would prevent all likely interference from aluminium, phosphate, sulphate and silicate in soil leachates. For soils other than those of very low or high pH the amount of lanthanum could probably be reduced to half, or even less, of this amount.

As lanthanum chloride had no adverse effect on flame photometric determinations of sodium, potassium and magnesium it could, if desired, be added directly to the ammonium chloride leachate before adjusting to volume.

The slight interference from sodium and potassium would be negligible at concentrations normally to be expected in soil leachates and the incorporation of average concentrations in the background solutions for the standards would reduce interference to negligible proportions.

Results obtained on a group of soils show that values obtained by the proposed method agreed satisfactorily with values obtained by an oxalate-titration method.

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SUMMARY

An examination of the interferences of aluminium, bicarbonate, phosphate, sulphate and silicate in the flame photometric determination of calcium in ammonium chloride solutions has shown that, with the exception of bicarbonate, all cause serious interference. Addition of lanthanum to the solution can satisfactorily prevent each of these interferences. Provided the lanthanum to aluminium ratio is at least 12.5:1 by weight all interference from aluminium can be prevented. The use of lanthanum chloride to prevent such interferences in the determination of calcium in ammonium chloride leachates of soils is discussed and a simple flame photometric method for the determination of exchangeable calcium in soils is proposed.

RÉSUMÉ

Une méthode simple, par photométrie de flamme, est décrite pour le dosage du calcium dans les terres. L'emploi du chlorure de lanthane permet de rendre ce dosage plus sélectif.

ZUSAMMENFASSUNG

Es wird gezeigt, dass bei der flammenphotometrischen Bestimmung von Calcium in Bodenproben starke Störungen verursacht werden durch gleichzeitig vorhandenes Aluminium, Phosphat, Sulfat und Silikat. Durch Zusatz von Lanthanchlorid lassen sich die Störungen weitgehend ausschalten.

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STUDIES OF THE SOLVENT EXTRACTION OF CADMIUM AND SILVER DITHIZONATES

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INTRODUCTION

History of dithizonate extractions

Although dithizone is a very good reagent for the analytical determination of a large number of metal ions and although metal dithizonates have been studied in an empirical manner quite extensively, only in relatively recent times systematic studies of the equilibria associated with the solvent extraction of metal dithizonates have been made. WICHMAN[1] first called attention to the importance of extraction or equilibrium curves (per cent metal extracted *versus* pH) for analytical purposes. KOLTHOFF AND SANDELL[2] studied the extraction of zinc dithizonate and derived some equations to describe the extractions of divalent metal ions. IRVING AND WILLIAMS[3] and FURMAN, MASON AND PEKOLA[4] later derived some expressions which were more general and which applied to systems in which extraction proceeds as it does in the case of dithizonate extractions. Even though the equilibria involved in the solvent extraction of metal dithizonates now appear to be well understood, the amount of quantitative data for the various metal dithizonate systems remains small. Systematic studies providing pertinent constants have been made with zinc dithizonate[2,5,6], copper dithizonate[7,8], silver dithizonate[8,9], and mercury dithizonate[10].

History of extraction equations

The equation

$$E = \frac{P_r^n K_r^n (\mathrm{HR})_o^n}{P_c P_r^n K_r^n (\mathrm{HR})_o^n + P_c K_c (\mathrm{H})^n} \quad \ldots\ldots\ldots\ldots (1)$$

was derived by IRVING AND WILLIAMS[3] in 1949 to describe the solvent extraction process for two phase systems initially made up of an aqueous phase containing a metal ion and hydrogen ion and a non-aqueous phase containing a complexing agent. In equation (1) E designates the ratio of metal concentration in the non-aqueous phase to the metal concentration in the aqueous phase and is referred to as the extractability; P_r and P_c designate the partition coefficients of the complexing agent and the metal complex respectively; K_r designates the ionization constant of the complexing agent; K_c designates the dissociation constant of the metal complex; $(\mathrm{HR})_o$ designates the concentration of the complexing agent in the organic phase; (H)

designates the concentration of the hydrogen ion in the aqueous phase; and n designates the valence of the metal ion. In this paper the subscript o will be used to show that a specie is in the non-aqueous phase; other species are in the aqueous phase. In cases in which P_c is very small equation (1) may be written as

$$E = \frac{P_r^n K_r^n (\mathrm{HR})_o^n}{P_c K_c (\mathrm{H})^n} = \frac{K(\mathrm{HR})_o^n}{(\mathrm{H})^n} \quad \ldots\ldots\ldots\ldots\ldots (2)$$

where the constant K is given by

$$K = \frac{P_r^n K_r^n}{P_c K_c} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

K is thus the equilibrium constant for the extraction reaction which may be written as

$$\mathrm{M} + n\mathrm{HR}_{(o)} = \mathrm{MR}_{n(o)} + n\mathrm{H} \quad \ldots\ldots\ldots\ldots (4)$$

In equation (4) the terms M and H are the metal ion and the hydrogen ion respectively in the aqueous phase, and HR and MR_n are the complexing agent and the metal complex respectively in the non-aqueous phase. The charges of ions have been omitted so that the writing of equations might be simplified. KOLTHOFF AND SANDELL[2] in 1941 derived an equation which is equivalent to equation (2). SCHWEITZER AND HONAKER[6] made use of this equation in their study of zinc dithizonate extractions.

By taking the logarithm of equation (2) IRVING AND WILLIAMS obtained the following relation at 50% extraction

$$\mathrm{pH}_{\frac{1}{2}} = -\log K - n\log (\mathrm{HR})_o \quad \ldots\ldots\ldots\ldots (5)$$

where $\mathrm{pH}_{\frac{1}{2}}$ designates the pH at which 50% extraction is obtained.

History of masking studies

The effect on extractions of species in the aqueous phase which react with the metal ion to form water soluble complexes has been studied systematically very little. Such extractions are usually referred to as masked extractions, and a substance in the aqueous phase which forms water soluble complexes with the metal ion or ionizes to form ions which in turn form water soluble complexes with the metal ion is called a masking agent. In this work the water soluble complexes will be referred to as masking complexes.

CONNICK AND MCVEY[11] in 1949 studied the masking effect of various anions on the extraction of zirconium(IV) with thenoyltrifluoroacetone and were able to obtain the stability constants of the masking complexes. RYDBERG[12] in 1950 made a study of the complexes which are formed between thorium(IV) and acetylacetone by extracting thorium tetraacetylacetonate into benzene. RYDBERG gave a very excellent treatment of some methods for finding stability constants of masking complexes. In RYDBERG's experiments the complexes which correspond to the masking complexes were formed from the metal ion and the extracting agent. WAGGENER AND STOUGHTON[13] in 1952 used an extraction technique to study the complexes formed between thorium(IV) and halide ions. The following expression for the extractability is the reciprocal of the one used by WAGGENER AND STOUGHTON

$$E = \frac{(\mathrm{MR}_n)_o}{(\mathrm{M}) + (\mathrm{MX}) + (\mathrm{MX}_2) + (\mathrm{MX}_3) + (\mathrm{MX}_4)} \quad \ldots\ldots\ldots\ldots (6)$$

where MX, MX_2, MX_3, and MX_4 are the masking complexes in the aqueous phase.

McKAVENEY[14] and KRISHEN[15] have made some very extensive studies of the masked extractions of metal ions using acetylacetone as an extracting agent. SCHWEITZER AND HONAKER[6] appear to be the only ones who have made extensive studies of masked extractions using dithizone as an extracting agent. SCHWEITZER AND HONAKER obtained extraction curves (% metal extracted *versus* pH) for zinc dithizonate extractions in cases in which the aqueous phase contained one of several masking agents. SCHWEITZER AND HONAKER observed that masking agents cause the extraction curves to shift to higher pH values. However, they made no quantitative calculations to predict these shifts.

Statement of present problem

In the present work an extensive study was made of the extraction of silver and cadmium dithizonates from aqueous solutions into either carbon tetrachloride or chloroform containing dithizone. The concentration of dithizone in the organic solvent, $(HD)_o$, was in large excess of the concentration of metal ion in the aqueous phase. In many of the extractions the aqueous phase contained one of several masking agents. Some extractions were made without masking agent being present. In most experiments it was possible to obtain the typical S-shaped extraction curves of pH *versus* % metal extracted. From these curves the $pH_{\frac{1}{2}}$ values were obtained. These $pH_{\frac{1}{2}}$ values are the principal data given in this paper.

Equations used to treat data

It was found that for the non-masked extractions either equation (2) or equation (5) could be used to treat the data. For the treatment of the data from the masked extractions an equation was used which is a modification of equations developed by others[12,13].

By employing the stability constants of the masking complexes, equation (6) may be modified to give

$$E = \frac{(MR_n)_o}{(M) + K_1(M)(X) + K_2(M)(X)^2 + K_3(M)(X)^3 + K_4(M)(X)^4} \quad \ldots\ldots (7)$$

By writing the extractability as in equations (6) and (7) one makes the following assumptions: that no hydrolysis of the metal ion takes place, that no polymeric species are formed, that no mixed complexes are formed, that any complexes in the aqueous phase between M and R are formed to an insignificant amount, that the only significant specie in the organic phase is MR_n, that the concentration of the specie which does the masking is independent of the pH, and that the maximum number of masking ligands attached to the metal ion is four. By dividing the numerator and denominator of equation (7) by $(MR_n)_o$ and factoring (M) from the denominator one obtains

$$E = \frac{1}{\frac{(M)}{(MR_n)_o}\left[1 + K_1(X) + K_2(X)^2 + K_3(X)^3 + K_4(X)^4\right]} \quad \ldots\ldots (8)$$

It can be shown that

$$\frac{(M)}{(MR_n)_o} = \frac{(H)^n}{K(HR)_o^n} \quad \ldots\ldots\ldots\ldots (9)$$

so that

$$E = \frac{1}{\frac{(H)^n}{K(HR)_o^n}\left[1 + K_1(X) + K_2(X)^2 + K_3(X)^3 + K_4(X)^4\right]} \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

By making the definitions

$$F = \sum_{i=0}^{4} K_i(X)^i, \text{ and } K_o = 1 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

one may write

$$E = \frac{K(HR)_o^n}{(H)^n F} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

If the logarithm of equation (12) is taken there results

$$\log E = \log K + n\log (HR)_o + n\text{pH} - \log F \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

At 50% extraction equation (13) transforms to

$$o = \log K + n\log (HR)_o + n\text{pH}_{\frac{1}{2}} - \log F \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

Equation (14) is important from three aspects. If the quantities F, n, and $(HR)_o$ are known for an extraction, one may with a knowledge of the value of either one of the terms K or $\text{pH}_{\frac{1}{2}}$, calculate the other. The possibility of determining the stability constants of the masking complexes also presents itself.

EXPERIMENTAL PROCEDURES

Chemicals and apparatus

The water used to prepare solutions was purified with a column of Amberlite MB-3 resin. All glass apparatus was cleaned by washing with cleaning solution, tap water, distilled water, and then demineralized water. All chemicals were U. S. P. grade or better. Salts which contained heavy metals were purified by extracting their solutions with chloroform solutions of dithizone. Some chloroform was recovered and purified by a procedure similar to that of BIDDLE[16]. The dithizone which was Eastman White Label Grade was purified by the procedure given by WELCHER[17].

All pH measurements were made with a Beckman Model H-2 pH meter which was standardized as needed against buffers having pH values of 7.00 and 4.00. The former buffer was used in cases in which the solutions to be measured had a pH above 5.0; the latter was used for measurements below a pH of 5.0. A Tracerlab 1000 Scaler fitted with a GM tube was used to make all radioactivity measurements. Samples were stirred with a battery of magnetic stirrers mounted under a constant-temperature bath which controlled the temperature at 30° ± 0.5°.

Procedures

^{115}Cd and ^{110}Ag were used to measure the per cent of the metal extracted into the organic phase. These nuclides were obtained from the Oak Ridge National Laboratory as nitrates in solution. Both nuclides were of a high specific activity. The procedure used in the experiments was to equilibrate 10-ml portions of aqueous solution of the metal ion with 10-ml portions of the organic phase containing dithizone. The pH of the aqueous phase had been previously adjusted to approximately the desired value. It was possible to equilibrate 12 samples simultaneously and thereby cover a desired pH range. After stirring for two hours the solutions were allowed to stand for 2 h at which time a 100-lambda sample was taken from each phase, placed on a planchet, dried, and counted with the Tracerlab counter. After background counts were subtracted the counts obtained were used to calculate extractabilities and per cent extractions. In all silver extractions the initial concentration of silver in the aqueous phase was approximately $1 \cdot 10^{-7}$ M. In the cadmium extractions an initial concentration of cadmium ion of approximately $7 \cdot 10^{-5}$ M in the aqueous phase was used.

RESULTS

Cadmium extractions

Chloride, bromide and iodide ions were used as masking agents for the extractions of cadmium dithizonate into chloroform. The non-masked extractions were carried out using an aqueous solution containing the perchlorate ion. Data from the non-masked extractions were used to calculate the extraction constant, K, by the use of equation (5). After obtaining a value for K, it was possible to calculate values of $pH_{\frac{1}{2}}$ for the masked extractions by employing equation (14). To make these calculations, it was first necessary to obtain values of the stability constants for the complexes formed between the cadmium ion and the anions of the masking agents. The results of these calculations are shown in Table I along with the data necessary to make the calculations and values of $pH_{\frac{1}{2}}$ which were obtained experimentally. The value of the stability constants of the masking complexes along with the literature sources from which the values were taken are included in Table I. The

TABLE I

RESULTS OF EXTRACTION STUDIES WITH CADMIUM

Initial $(HD)_o$	*Composition of aqueous phase*	*Observed* $pH_{\frac{1}{2}}$	*Calculated*			*Stability constants masking complexes*	*Ref.*
			$pH_{\frac{1}{2}}$	K	$\log K$		
$5 \cdot 10^{-3}$	$HClO_4$, 0.1 M $NaClO_4$	2.0		4	0.6		
$2 \cdot 10^{-3}$	$HClO_4$, 0.1 M $NaClO_4$	2.5		3	0.5		
$2 \cdot 10^{-3}$	$HClO_4$, 1.0 M $NaClO_4$	2.1		10	1.0		
$5 \cdot 10^{-3}$	$HClO_4$, 0.1 M KCl	2.4	2.4			$K_1 = 30$, $K_2 = 200$ $K_3 = 300$	18
$2 \cdot 10^{-3}$	$HClO_4$, 0.1 M KCl	3.5	3.2			Same as above	18
$5 \cdot 10^{-3}$	$HClO_4$, 0.1 M KI	2.2	3.3			$K_1 = 2 \cdot 10^2$, $K_2 = 8 \cdot 10^3$ $K_3 = 1 \cdot 10^5$, $K_4 = 1 \cdot 10^6$	19
$2 \cdot 10^{-3}$	$HClO_4$, 0.1 M KBr	3.0	3.2			$K_1 = 2 \cdot 10^2$, $K_2 = 1 \cdot 10^3$ $K_3 = 3 \cdot 10^3$, $K_4 = 1 \cdot 10^4$	20
$2 \cdot 10^{-3}$	$HClO_4$, 1.0 M KBr	4.1	4.3			Same as above	20

extraction curves for the cadmium extractions had slopes at 50% extraction which were in good agreement with the value 115% cadmium extracted per pH unit which s the theoretically predicted slope for the extraction of a divalent metal.

TABLE II

RESULTS OF NON-MASKED EXTRACTION STUDIES WITH SILVER

$HClO_4$ molarity	*E*	*$K \cdot 10^{-5}$*	*log K*
1.0	6.2	6	5.8
1.7	3.4	6	5.8
2.9	1.4	4	5.6
3.4	0.9	3	5.5
4.4	0.5	2	5.3
6.0	0.2	1	5.0

Silver extractions

It was not possible to obtain extraction curves for the non-masked silver dithizonate extractions due to the fact that silver extracts at such high acid concentrations. However, some extractions were made into chloroform at know acid concentrations, and the results were used to calculate the extraction constant with the aid of equation (2). Table II gives the data and calculated values of the extraction constant for these systems.

It was found that a number of masking agents would shift the extraction curves of silver to pH values high enough so that extraction curves could be obtained. From these curves $pH_{\frac{1}{2}}$ values were obtained. By the use of equation (14) $pH_{\frac{1}{2}}$ values for extractions into chloroform were calculated for the extractions and compared with the experimental values. For the extractions into chloroform a value of the extraction constant of $6 \cdot 10^5$ as determined from the non-masked extractions was used to calculate $pH_{\frac{1}{2}}$ values. Three extraction curves were obtained for extractions into carbon tetrachloride. Since non-masked extractions were not made into carbon tetrachloride, a value of the extraction constant was not available. Therefore the values of $pH_{\frac{1}{2}}$ from the masked extractions were used to calculate a value for the extraction constant. The data and calculated results for the silver extractions are given in Table III along with the values for the stability constants of the masking complexes and the references from which the values of the constants were taken. All extraction curves for silver extractions had slopes at 50% extraction which were in good agreement with the value of 57% metal extracted per pH unit which is predicted for monovalent metal ions.

Extractions at 30° of silver dithizonate into chloroform were carried out for cases in which the aqueous phase contained one of the following substances: 1.0 *N* acetic acid, 0.1 *M* tartaric acid, 0.1 *M* citric acid, 0.1 *M* glycine, $1 \cdot 10^{-3}$ *M* ethylenediaminetetraacetic acid, $1 \cdot 10^{-3}$ *M* nitrilotriacetic acid, and 0.5 *M* sulfuric acid. No masking effect was found for any of these substances.

Enough data were obtained for extractions from potassium bromide solutions so that values for the stability constants of the silver-bromide complexes were obtained. For the systems which had a potassium bromide concentration of 0.01, 0.05, 0.1, and 0.2 molar Table III gives $pH_{\frac{1}{2}}$ values of 2.6, 4.0, 2.8, and 3.8 respectively for these data are for the systems which had an ionic strength of approximately 0.2 *M*. By the use of these data, the corresponding concentrations of dithizone, and equation (14) four simultaneous equations were written and solved for the values of K_1, K_2, K_3 and K_4. The values obtained for these constants are respectively $3 \cdot 10^4$, $3 \cdot 10^7$, $6 \cdot 10^7$, and $2 \cdot 10^9$.

DISCUSSION

The usual approach by previous investigators to the theoretical aspects of extraction studies such as this one has been to test the extraction technique as a method for determining stability constants of masking complexes. In this work the principal aim was to take values of the stability constants of complexes which could be found in the literature and predict at what pH extraction could be effected. Of all the previous extraction studies that have been made, the authors know of none which has approached the problem in this manner.

It can be seen from Tables I and III that a good degree of succes has been realized

TABLE III

DATA AND CALCULATED QUANTITIES FOR SILVER EXTRACTIONS

Organic phase	*Initial* $(HD)_0$	*Composition of aqueous phase*	*Observed* $pH_{\frac{1}{2}}$	*Calculated*			*Stability constants masking complexes*	*Ref.*
				$pH_{\frac{1}{2}}$	$K \cdot 10^{-5}$	log K		
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.1 *M* KCl	2.4	2.1			$K_1 = 7 \cdot 10^2$, $K_2 = 1 \cdot 10^4$ $K_3 = 1 \cdot 10^5$, $K_4 = 8 \cdot 10^5$	21
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.2 *M* KCl	3.1	2.8			Same as above	21
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.01 *M* KBr	2.9	2.3			$K_1 = 1 \cdot 10^4$, $K_2 = 1 \cdot 10^7$ $K_3 = 9 \cdot 10^7$, $K_4 = 8 \cdot 10^8$	22
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.01 *M* KBr, 0.2 *M* $NaClO_4$	2.6	2.3			Same as above	22
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.05 *M* KBr	4.1	3.8			Same as above	22
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.05 *M* KBr, 0.15 *M* $NaClO_4$	4.0	3.8			Same as above	22
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.08 *M* KBr, 0.12 *M* $NaClO_4$	4.7	4.3			Same as above	22
$CHCl_3$	$5 \cdot 10^{-4}$	$HClO_4$, 0.1 *M* KBr	2.9	2.9			Same as above	22
$CHCl_3$	$5 \cdot 10^{-4}$	$HClO_4$, 0.1 *M* KBr, 0.1 *M* $NaClO_4$	2.8	2.8			Same as above	22
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.1 *M* KBr	5.0	4.6			Same as above	22
$CHCl_3$	$5 \cdot 10^{-4}$	$HClO_4$, 0.2 *M* KBr	3.8	4.4			Same as above	22
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.01 *M* NaSCN	3.5	3.4			$K_1 = 4 \cdot 10^4$, $K_2 = 1 \cdot 10^8$ $K_3 = 4 \cdot 10^9$, $K_4 = 3 \cdot 10^{10}$	23
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.05 *M* NaSCN	5.4	5.1			Same as above	23
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, 0.1 *M* NaSCN	6.2	6.1			Same as above	23
$CHCl_3$	$5 \cdot 10^{-4}$	$HClO_4$, 0.1 *M* NaSCN	3.7	4.4			Same as above	23
$CHCl_3$	$2 \cdot 10^{-3}$	$HClO_4$, 0.2 *M* NaSCN	4.1	4.8			Same as above	23
$CHCl_3$	$1 \cdot 10^{-5}$	$HClO_4$, $1 \cdot 10^{-5}$ *M*, $Na_2S_2O_3$	2.6	2.7			$K_2 = 3 \cdot 10^{13}$, $K_3 = 1 \cdot 10^{14}$	24,25
CCl_4	$1 \cdot 10^{-5}$	$HClO_4$, 0.1 *M* KCl	1.3		40	6.6	Same as in other chloride extractions	21
CCl_4	$1 \cdot 10^{-5}$	$HClO_4$, 0.2 *M* KCl	1.9		50	6.7	Same as above	21
CCl_4	$5 \cdot 10^{-5}$	$HClO_4$, 0.1 *M* KBr	3.1		30	6.5	Same as in other bromide extractions	22

in predicting the pH of 50% extraction for most of the experiments. Since the extraction curves had the shape which was predicted theoretically, it would be as simple a matter to calculate any other pH on the curves as it is to calculate the pH corresponding to 50% extraction.

In the case of extractions of cadmium dithizonate from potassium iodide solutions the calculated and observed values for the $pH_{\frac{1}{2}}$ did not agree. It was thought that possibly a complex between the cadmium ion and the iodide ion such as CdI_2 was extracted into the non-aqueous phase. However, this possibility was not tested experimentally. In the case of two extraction curves for extractions of silver dithizonate from sodium thiocynate solutions, the agreement between calculated and observed values of $pH_{\frac{1}{2}}$ was not as close as expected (see Table III). The difference between the calculated and the observed $pH_{\frac{1}{2}}$ for both of these curves was found to be 0.7 pH unit. It was first thought that equilibrium had not been established for these systems. However, when much effort was made to insure that equilibrium was obtained this difference was still obtained. It should be mentioned that for most of the extractions of silver from sodium thiocyanate solutions a fairly large spread was obtained for the points making up the extraction curve, and quite a large number of extractions were necessary in order to determine the position of the extraction curves. The authors were unable to explain either of these observations for these extractions.

The values of the stability constants of the complexes between the silver ion and the bromide ions are in quite good agreement with those reported in the literature. However, it is difficult to obtain for most systems enough extraction data of sufficient accuracy to permit the determination of all of the constants by solving four equations simultaneously. Other methods[12] of a more graphical nature probably would be better suited in general for the determination of these constants.

The value $3 \cdot 10^6$ for the extraction constants which was determined for silver dithizonate extractions into carbon tetrachloride is not in agreement with a value of $4 \cdot 10^7$ previously reported by TREMILLON[27]. However TREMILLON worked at a temperature of 18° whereas this work was carried out at 30°. Some work has been completed here at this laboratory in which the effect of temperature variation on silver dithizonate extractions was observed. This work tends to show that allowing for the temperature difference the constant determined in this research and the value given by TREMILLON are in fairly close agreement. Also the effect of a change of solvent has been investigated and along with the results of some work dealing with the effects of temperature variation will be reported at a later date.

SUMMARY

An extensive study was made of the solvent extraction of cadmium and silver dithizonates into chloroform. A few extractions of silver dithizonate were made into carbon tetrachloride. In most cases extraction curves of pH *versus* % metal extracted were obtained and reported in terms of the pH of 50% extraction. The shift of the extraction curves to higher pH values due to the effect of ions in the aqueous phase which complex with the cadmium or silver ions was investigated. An equation was developed which would predict the pH of 50% extraction for the extraction from the solutions containing these complexing ions. The effects of chloride, bromide and iodide ions on the extraction of cadmium and the effects of chloride, bromide, iodide, thiocyanate and thiosulfate ions on the extraction of silver. Extraction constants for the various extractions were determined.

RÉSUMÉ

Une étude approfondie a été effectuée sur l'extraction par un solvent des dithizonates de cadmium et d'argent. On a déterminé les constantes d'extraction dans divers cas et examiné l'influence d'anions complexants.

ZUSAMMENFASSUNG

Es wurde der Verlauf der Extraktion der Dithizonate des Cadmiums und Silbers mit Chloroform in Abhängigkeit vom pH der Lösung und der Einfluss komplexbildender Anionen und Halogeniden auf die Extraktion untersucht.

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SPECTROPHOTOMETRIC DETERMINATION OF THE RARE EARTHS YTTRIUM AND CERIUM BY BROMOPYROGALLOL RED

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INTRODUCTION

The use of bromopyrogallol red as a chromogenic reagent for the spectrophotometric determination of the rare earth type elements was suggested by its use as an indicator in certain rare earth titrations[1]. This reagent forms coloured, sparingly soluble complexes with many metal ions; with the rare earths, yttrium and cerous

cerium, blue to violet-blue complexes are formed in solutions of pH greater than about 4.5. The bromopyrogallol red also exhibits acid–base indicator properties, being orange yellow in strong acid solution, claret red in nearly neutral solution and blue in alkaline solution.

The procedure to be described is suitable for pure solutions of the rare earths, yttrium and cerous cerium *e.g.* after preliminary precipitation as fluoride followed by separation on an ion-exchange column. The complex is quite stable under the conditions specified and the Beer Lambert law is obeyed at least between the range of concentration 0.5 to 4.0 p.p.m. of metal ion.

EXPERIMENTAL WORK

Preliminary experiments showed that the pH value of the solution should be maintained at an optimum value within the range 5.0–7.0. The addition of sodium acetate was found to meet this requirement, being added to stabilize the final acidity of the solution.

The colour development of the bromopyrogallol red–rare earth complex was also found to be slow at room temperature and to obtain stable conditions it was necessary to heat the sample solution for 10 min in a water bath at 80–90°.

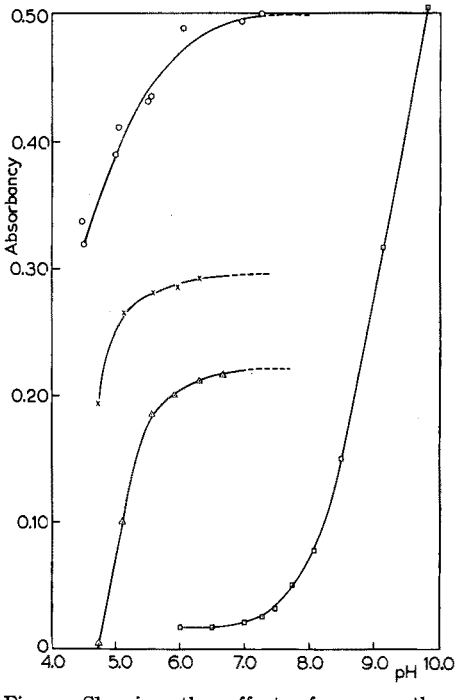

Fig. 1. Showing the effect of pH on the colour development of the bromopyrogallol red-rare earth complex. o = gadolinium and terbium curve, × = europium and neodymium curve, △ = yttrium curve, □ = bromopyrogallol red solution.

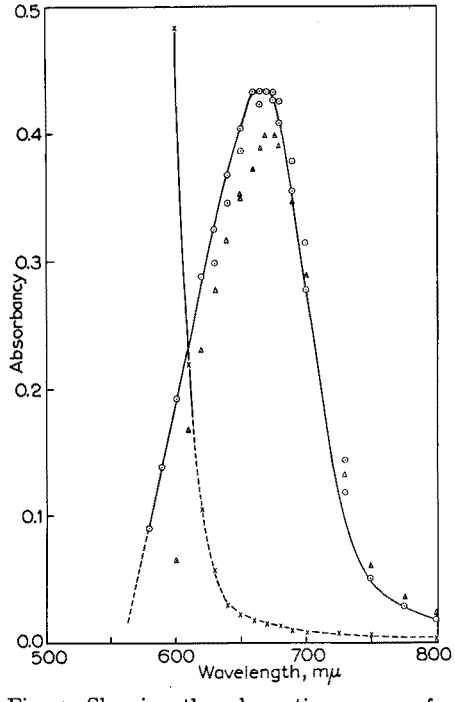

Fig. 2. Showing the absorption curves for neodymium and cerium. o = neodymium curve, △ = cerium curve, × = bromopyrogallol red solution at pH 6.5.

Effect of pH on colour development

Individual portions, each containing 20–50 μg of rare earth in dilute acid solution were adjusted to about 15 ml volume with water and 2 ml 0.05% w/v bromopyrogallol red in 50% alcohol were added, followed by the dropwise addition of 5% w/v sodium bicarbonate solution until a blue colour appeared at about pH 5. This colour was then discharged by the careful addition of 0.2 *N* hydrochloric acid, 0.10 ml of 55% w/v sodium acetate was added and the acidity adjusted with dilute hydrochloric acid or sodium hydroxide. The solution was then diluted to about 20 ml volume, heated in a water bath for 10 min, and, after cooling, transferred to a volumetric flask and diluted to a final volume of 25 ml. The absorbancy of the solution was then measured in a 1-cm cell using a Unicam SP600 spectrophotometer, at a wavelength of 665 mμ. The results are presented in Fig. 1 together with the absorbancy values for a bromopyrogallol red solution, at various acidities, without any rare earth present.

The optimum pH range for complex formation is 6.0–7.5. Above pH 7.5 the reagent blank will increase and also make the final pH value of the solution difficult to stabilize. Under the conditions of the method to be described a final pH of 6.4–6.5 is to be expected.

Absorption spectra

The absorption peaks for neodymium, europium, samarium, gadolinium, terbium and yttrium were found to be 665 mμ, whilst that for Ce was 675 mμ. Fig. 2 shows the absorption peaks for cerium and neodymium together with the curve for a bromopyrogallol red blank solution. The solutions were prepared as in the procedure to be described below, giving a final pH value of 6.5 and were measured against a blank reagent solution. At a wavelength of 665 mμ it can be seen that small variations in the concentration of bromopyrogallol red do not produce any real change in the blank reading, for which a typical figure is 0.005 to 0.010 measured in a 1-cm cell.

DETAILED METHOD

Reagents

Bromopyrogallol red: 0.05% w/v solution in 50% alcohol (indefinitely stable), sodium acetate: 55% aqueous solution of sodium acetate trihydrate, sodium bicarbonate: 5% w/v aqueous solution, hydrochloric acid: 0.2 *N* solution, ascorbic acid: 1% w/v aqueous solution, freshly prepared (for cerium determinations only), standard solutions of rare earths: prepared by accurate dilution of gravimetrically standardized solutions of the "Specpure" nitrates in dilute nitric acid.

Procedure

Rare earths and yttrium: Transfer the sample, containing 15–35 μg of yttrium or 20–50 μg of rare earth, in dilute acid solution, to a 25-ml measuring flask (Note 1). Dilute to about 15–16 ml with water. Add 2.0 ml of the bromopyrogallol red reagent and then 5% sodium bicarbonate solution, dropwise, until one drop produces a permanent blue colour. Wash down the neck of the flask with water and then add 0.2 *N* hydrochloric acid, dropwise, until the blue colour is discharged, leaving a clear red solution. Add 0.50 ml of 55% sodium acetate solution, and stand the flask in a hot water bath (80–90°) for 10 min. Cool, and dilute to the mark with water. (Note 2). Measure the absorbancy of the solution against water in 1-cm cells at 665 mμ. Carry out similar determinations on accurately measured portions of the diluted standard solutions. The Beer-Lambert law is obeyed.

Plot a calibration curve of absorbancy against concentration of rare earth. Obtain a blank reading from the interception of the calibration line with the absorbancy axis. A figure of approximately +0.005 is to be expected.

Cerium: As for rare earths and yttrium above except that 0.2 ml of 1% ascorbic acid is added before the addition of the bromopyrogallol red, to ensure reduction of any ceric cerium, and the final solution is measured at 675 mμ.

TABLE I

	Weight of solution (g)	*Absorbancy* [a]	*Absorbancy per gram of solution*
Neodymium (234.8 μg Nd/g)	0.0634	0.202	3.19
	0.0976	0.312	3.20
	0.1354	0.428	3.16
	0.1834	0.580	3.17
Gadolinium (537.0 μg Gd/g)	0.0527	0.355	6.74
	0.0742	0.490	6.61
	0.0758	0.500	6.60
	0.1057	0.705	6.67
Terbium (not standardized. Approx. 235 μg/g Tb)	0.1102	0.317	2.88
	0.1860	0.540	2.90
	0.2607	0.755	2.90
	0.3744	1.08	2.88
Yttrium (99.50 μg Y/g)	0.1159	0.255	2.20
	0.1313	0.288	2.19
	0.2248	0.490	2.18
	0.2642	0.576	2.18
	0.3386	0.738	2.18
	0.3707	0.813	2.19
Cerium (193.6 μg Ce/g)	0.0852	0.263	3.09
	0.1032	0.319	3.09
	0.1987	0.614	3.09
	0.3401	1.04	3.06

[a] A blank of +0.005 was subtracted from the absorbancy against water to give the figures recorded in this column.

Results

Table I shows a typical series of standardisations on individual rare earths, yttrium and cerium. The portions for these standardisations were dispensed from a polythene weight pipette, capacity approximately 7 g, of a type described previously by J. HERRINGTON AND D. G. VALLIS[2]. By this means, amounts of the order of 0.1 g were dispensed using a damped balance with automatic loading for all weights and with a precision of 0.2 mg. This method of dispensing small amounts of liquid has the advantage of quickness and is not influenced by temperature effects on the volume of the solutions concerned. The results are therefore quoted as absorbancies per g of solution. Results obtained on gravimetrically standardized solutions show that under the conditions of the above procedures, 20 μg of Nd give an absorbancy of 0.271, 20 μg of Y give an absorbancy of 0.439, 20 μg of Gd give an absorbancy of 0.248 and 20 μg of Ce give an absorbancy of 0.319.

Interfering ions

These procedures have been developed to deal with purified, separated solutions obtained from ion-exchange columns. The following ions are known to give complexes

at the pH of the determinations: Fe^{+2}, Cu^{+2}, Pb^{+2}, Be^{+2}, Zn^{+2}, Co^{+2}, VO_4^{-2}, Mg^{+2} (see Table II). Fe^{+3} and peroxide decolourize the solution.

TABLE II

THE INFLUENCE OF IMPURITIES ON THE COLOUR DEVELOPMENT OF THE NEODYMIUM–BROMOPYROGALLOL RED COMPLEX

Element	*Amount of impurity element added* μg	*Amount of neodymium added* μg	*Amount of neodymium found* μg
Iron (as Fe^{+2})	150	53	32
Copper (as Cu^{+2})	300	53	44
Lead (as Pb^{+2})	200	53	58
Beryllium (as Be^{+2})	200	53	53
Beryllium (as Be^{+2})	1000	53	precipitated
Zinc (as Zn^{+2})	250	53	precipitated
Cobalt (as Co^{+2})	100	53	precipitated
Vanadium (as VO_4^{-2})	100	53	46
Magnesium (as Mg^{+2})	500	53	52
Magnesium (as Mg^{+2})	3000	53	precipitated

Notes

1. (a) Excessive amounts of acid are best removed by evaporation of the sample to dryness and dissolution in the minimum of warm 0.5 *N* hydrochloric acid.
   (b) It is more convenient to place the sample in a volumetric flask in the first stage of the experiment rather than to transfer the solution at a later stage. After heating a volumetric flask to a temperature of 100° in a water bath and allowing to cool, there is no detectable change in the calibration.
2. At this stage in the procedure the pH will be 6.4–6.5 by virtue of the sodium acetate solution added.

SUMMARY

A method is described for the spectrophotometric determination of microgram quantities of rare earths, yttrium and cerous cerium ions, using the colour of the complexes formed with bromopyrogallol red. The procedure has been applied to pure solutions containing 10–50 μg of the element.

RÉSUMÉ

Une méthode est proposée pour le dosage spectrophotométrique des terres rares, de l'yttrium et du cérium(III). On utilise le rouge de bromopyrogallol comme réactif.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur spektrophotometrischen Bestimmung der seltenen Erden, des Yttriums und Cer(III) mit Hilfe von Bromopyrogallolrot.

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COLORIMETRIC DETERMINATION OF SILICON IN THE PRESENCE OF PHOSPHORUS

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During the investigation leading to the colorimetric determination of silicon in biological materials as molybdenum blue, various methods for the removal of the interference from phosphorus were investigated. Complexing agents such as oxalic[1,2] or citric acid[2,3] were used to bleach the interfering phosphomolybdate while leaving the silicomolybdate unaffected. This method is successful when only small amounts of phosphate are present to interfere, but in the presence of high concentrations of phosphate such as are found in human and rat urine, the method is not applicable unless the urine is diluted considerably. This is undesirable, for when silicate is present in only small amounts it might then escape detection and determination.

We have considered the possibility of removing most of the phosphate by a controlled precipitation method, and eliminating the small amount of remaining phosphate by solvent extraction of the heteropoly acid of phosphorus.

HURE AND ORTIS[4] have reported the extraction of phosphomolybdate from a mixture of the heteropoly acids of silicon and phosphorus by solvent extraction with ethyl acetate. WALDEN AND MELLON[5] have summarized the data on solvent extraction of phosphorus, silicon, arsenic and germanium. DE SESA AND ROGERS[6] have briefly reported the application of this solvent extraction technique to the determination of silicon and phosphorus in the presence of each other. Silicon was determined in their experiments by measurement of the total absorbance of the yellow molybdate at 332 mμ, extraction of the phosphate by isoamyl acetate and measuring the absorbance of the organic phase at 330 mμ. The silicon content was given by correcting total absorbance in the aqueous phase for phosphorus, using the previously determined absorbance index of phosphomolybdate in the aqueous phase.

Since we were interested in the determination of silicon only, the above method of determination was unnecessarily time consuming. After solvent extraction of the phosphomolybdate, silicon in the aqueous layer was determined by reduction of the silicomolybdate to molybdenum blue with sodium sulphite solution. Using this method of investigation, we have studied the efficiency of extraction of the heteropoly acids of phosphorus and silicon by several solvents. Selective solvent extraction of phosphomolybdate in the presence of silicomolybdate has been studied in detail, and a reliable and accurate method of determination of silicon in the presence of phosphorus is reported.

* I.C.I. Research Fellow.

EXPERIMENTAL

Reagents

Distilled water was used throughout. A stock solution of phosphate was prepared by dissolving 0.500 g of disodium hydrogen phosphate in water and diluting to 100 ml. Further solutions of phosphate were prepared by appropriate dilution from the stock solution.

Solutions of soluble silica were prepared by fusing 10.0 mg of finely divided quartz, 0.5 g of powdered sucrose and 1.5 g of sodium peroxide in a Parr-Bomb, and dissolving the cold melt in 100 ml of water, in a polythene beaker. Further standard solutions of silicate were prepared by appropriate dilution.

Phosphate determination

Phosphate was determined by the method of KING[7]. This involved the formation of phosphomolybdate and reduction of this complex by 1-amino-2-naphthol-4-sulphonic acid.

Silicon determination

Silicon was determined colorimetrically by a modification of the method of DIENERT AND WALDENBULKE[8]. The yellow silicomolybdate was reduced with sodium sulphite solution (30%) to molybdenum blue and the optical density was measured in a spectrophotometer at 690 mμ. The following reagents were added (in the order stated): 2.0 ml standard silicate solution (0.02 mg to 0.10 mg), 2.0 ml *N* hydrochloric acid. 4.0 ml ammonium molybdate solution (5%) (tetrahydrate), 4.0 ml sodium sulphite solution (30%) (heptahydrate).

For the blank determination the same volume of each reagent was used (2 ml water replaced the standard silicate solution) and this was used as a compensating solution. This method of determination had an accuracy of 98.9%[9].

Solvent extraction procedure

Into a 50-ml separating funnel was pipetted 2 ml of the test solution. During shaking of the funnel, 2 ml of *N* hydrochloric acid was added, then 4 ml of ammonium molybdate solution (5%) and the shaking was continued for a further 3 min. This solution had a pH of 1.0. It was then extracted once with 20 ml of an organic solvent for 30 sec. The aqueous phase was separated and 4 ml of sodium sulphite solution (30%) was added. The resulting solution was allowed to stand for 25 min for maximum colour to develop, and its optical density was then measured at 690 mμ, against the extracted blank.

TABLE I

SOLVENT EXTRACTION OF HETEROPOLY ACIDS OF PHOSPHORUS AND SILICON

Solvent	*Initial phosphate concentration 500 μg/2 ml*	*Initial silicon concentration 57 μg/2 ml*
	Phosphate found in aqueous phase after solvent extraction μg	*Silica found in aqueous phase after solvent extraction* μg
Ethyl acetate	< 5	57
n-propyl acetate	< 5	57
isopropyl acetate	< 5	55
n-butyl acetate	< 5	57
isobutyl acetate	< 5	56
n-amyl acetate	500	56
isoamyl acetate	40	57
diethyl ether	250	57
dibutyl ether	350	56
n-hexyl butyrate	270	57

RESULTS

In Table I is recorded the result of a preliminary study on the extraction of phospho- and silicomolybdate by various solvents. Phosphomolybdate was removed to some extent by all the solvents tested, except *n*-amyl acetate, but complete extraction within the limits of the experiment, was obtained with only ethyl acetate, *n*-propyl

acetate, isopropyl acetate, *n*-butyl acetate and isobutyl acetate. 92% of the phosphomolybdate was extracted by isoamyl acetate but *n*-amyl acetate gave no extraction. Silicomolybdate was not extracted by any of the solvents.

TABLE II

EXTRACTION STUDIES WITH ETHYL ACETATE AND ISOAMYL ACETATE
REMOVAL OF PHOSPHOMOLYBDATE

Phosphate present before extraction μg	*Phosphate found in aqueous phase after single ethyl acetate extraction* μg	*Phosphate found in aqueous phase after single isoamyl acetate extraction* μg	*Phosphate found in aqueous phase after double isoamyl acetate extraction* μg
200	< 5	15	< 5
500	< 5	40	11
750	< 5	60	20
1000	< 5	80	25
1500	8	115	25

The results in Table II show that the efficiency of extraction of phosphomolybdate by ethyl acetate was actually 100%, whereas only about 92% of the phosphomolybdate was removed from the aqueous phase when isoamyl acetate was used as solvent. On the other hand, silicomolybdate was not extracted by either ethyl acetate or isoamyl acetate, virtually 100% recovery of silica was obtained in the aqueous phase after solvent extraction by both reagents as shown in Table III.

TABLE III

EFFECT OF ETHYL ACETATE AND ISOAMYL ACETATE EXTRACTION ON SILICOMOLYBDATE[a]

Silica present before extraction μg	*Silica found after single ethyl acetate extraction* μg	*Silica found after single isoamyl acetate extraction* μg
38	35	38
64	64	64
70	66	69
135	135	135
157	157	160

[a] Solutions of soluble silica were treated with acid molybdate solution and the resulting yellow silicomolybdate solution extracted by the standard procedure.

To complete this study, the colorimetric determination of silica in the presence of phosphate was checked after extraction with ethyl acetate and isoamyl acetate. The results of these experiments, which are recorded in Table IV, demonstrate that after a single extraction with ethyl acetate apparent recoveries of between 100.0 and 103.1% of silica were obtained but single extractions with isoamyl acetate, in similar experiments, gave abnormally high silica recoveries. Double extraction with isoamyl acetate reduced this interference by phosphate but duplicate extraction with this solvent was less efficient than a single ethyl acetate extraction for the removal of phosphomolybdate. Double extraction with ethyl acetate was not used because the additional extraction might be expected to cause new errors. This method of silicon determination, employing ethyl acetate for the removal of phosphomolybdate, gave a mean error of +2.1% in 8 determinations (Table V).

TABLE IV

RECOVERY OF SILICA IN THE PRESENCE OF PHOSPHATE[a]

Silica present before extraction μg	*Silica found after single ethyl acetate extraction* μg	*Apparent recovery* %	*Silica found after single isoamyl acetate extraction* μg	*Apparent recovery* %	*Silica found after double isoamyl acetate extraction* μg	*Apparent recovery* %
60	61	101.7	100	166.7	72	120.0
50	50	100.0	82	164.0	61	122.0
65	67	103.1	106	163.1	78	120.0
58	58	100.0	100	172.4	68	117.2

[a] To solutions of soluble silica were added 2 ml sodium phosphate solution containing 500 μg/ml. The phosphomolybdate was removed by solvent extraction and silica was determined in the aqueous phase.

TABLE V

ACCURACY OF METHOD OF DETERMINATION[a]

Silicon present before extraction μg	*Silicon found after extraction* μg	% *error*
61	62	+ 1.7
52	52	0.0
36	38	+ 5.6
14	14	0.0
132	135	+ 2.3
58	58	0.0
26	27	+ 3.8
65	67	+ 3.1
	Mean % error = 2.1 ± 0.7	

[a] To solutions of soluble silica were added 2 ml sodium phosphate solution containing 500 μg of sodium phosphate per ml. The phosphomolybdate was removed by single extraction with ethyl acetate and silica was determined in the aqueous phase.

DISCUSSION

In a preliminary study on the selective removal of phosphomolybdate in the presence of silicomolybdate in aqueous solution (pH 1.0) ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, amyl acetate, isoamyl acetate, diethyl ether, dibutyl ether and hexyl butyrate were tested. Although phosphomolybdate was extracted to some extent by all the organic solvents tested (except amyl acetate), ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate and isoamyl acetate were the most efficient.

In a more detailed study of the efficiency of extraction of phospho- and silicomolybdate with ethyl acetate and isoamyl acetate as measured by colorimetric determination of molybdenum blue, ethyl acetate was found to have a much greater efficiency, reducing phosphate interference to tolerance level. Thus, accurate and reproducible colorimetric determinations of silicon were obtained using solutions of silicate containing 1000 μg phosphate, this being about the highest permissible concentration of phosphate which will not cause precipitation of insoluble phosphomolybdate.

ACKNOWLEDGEMENT

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SUMMARY

An accurate and reliable colorimetric method of silicon determination in the presence of phosphorus is reported. The method involves the selective extraction of phosphomolybdate with ethyl acetate and determination of the aqueous phase after reduction to molybdenum blue. Isoamyl acetate extracts phosphomolybdate to a lesser degree than ethyl acetate.

RÉSUMÉ

Une méthode colorimétrique est proposée pour le dosage du silicium en présence de phosphore. Principe: extraction du phosphomolybdate par l'acétate d'éthyle et dosage colorimétrique après réduction de la phase aqueuse en bleu de molybdène.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur colorimetrischen Bestimmung von Silizium in Gegenwart von Phosphor. Phosphormolybdat wird mit Aethylacetat selektiv extrahiert, das Silicomolybdat zu Molybdänblau reduziert und colorimetrisch bestimmt.

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ANALYSIS OF ALLOYS OF TITANIUM, NIOBIUM AND TANTALUM BY ION EXCHANGE

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In connection with studies of the oxidation behaviour of titanium alloys methods were required for the analysis of binary and ternary alloys of titanium, niobium, and tantalum. HAGUE, BROWN AND BRIGHT[1] have shown that titanium and niobium may be separated by ion exchange as chloro-fluoro complexes; niobium and tantalum may be separated similarly[2]. In the present work a method is presented in which titanium, niobium and tantalum are separated by ion exchange in hydrochloric–hydrofluoric acid media. After the separation the metals are determined spectrophotometrically.

EXPERIMENTAL

Preparation of column

The column was prepared from a 90-cm long polyethylene tube with inside diameter 1.1 cm. The bottom of the tube was closed by a polyethylene stopper. A polyethylene tube with inside diameter 0.2 cm was inserted through a hole in the stopper flush with the upper surface of the stopper. The stopper was covered with a cotton plug and the large tube was filled with a 10-cm high layer of 120–200 mesh Amberlite IRA-400 resin.

Solutions

Acid mixture 1: Mix 200 ml of hydrofluoric acid (40%, d = 1.13) and 400 ml of hydrochloric acid (d = 1.19) and dilute to 1 l with distilled water.
Acid mixture 2: Mix 50 ml of hydrofluoric acid and 250 ml of hydrochloric acid and dilute to 1 l with distilled water.
Acid mixture 3: Mix 750 ml of hydrofluoric acid with 250 ml of hydrochloric acid.

Procedure

Separation. Transfer 100 mg of the alloy to a platinum dish and add 2 ml of sulfuric acid and 2 ml of hydrofluoric acid. Add nitric acid dropwise until the sample has dissolved. Evaporate to fumes of sulfuric acid. Add 15 ml of acid mixture 1 and transfer the solution to the column. The elution of titanium is performed with 100 ml of acid mixture 1 at a flow rate of 2 ml/min. Niobium is then eluted with 100 ml of acid mixture 2. Finally, tantalum is eluted with acid mixture 3. Reject the first 100 ml of this eluate as it will contain no tantalum. Tantalum is quantitatively eluated with the next 300 ml (flow rate 2 ml/min).

Spectrophotometric determination

Titanium and niobium. Transfer the eluates containing titanium and niobium to platinum dishes, add 5 ml of concentrated sulfuric acid and evaporate to fumes. Add 2–3 drops of hydrogen peroxide and again fume to destroy organic matter from the column. Transfer the solutions to 50-ml volumetric flasks. Add 0.5 ml of hydrogen peroxide (30%) and make up to volume with conc. sulfuric acid. Mix thoroughly and read the absorbance at 430 and 365 mμ for titanium and niobium respectively. Alternatively, titanium may be determined as the hydrogen peroxide complex in 6 N sulphuric acid reading the absorption at 410 mμ.

Tantalum. (Reagent solution. Dissolve 50 g of pyrogallol in 150 ml of distilled water. Add 15 ml of sulfuric acid (1 + 9) and dilute to 250 ml in a volumetric flask.)

To the eluate containing tantalum add a few drops of sulfuric acid and evaporate to dryness in a platinum dish. Add 0.5 g potassium hydrogen sulfate and fuse. Cool and add 1 g of ammonium oxalate and 10 ml of water and warm to dissolve the salts. Transfer the solution to a 25-ml volumetric flask and make up to volume with distilled water. Transfer a 5-ml aliquot to another 25-ml volumetric flask, add 0.8 g of ammonium oxalate and 12 ml of reagent solution. Make up to volume with distilled water and read the absorbance at 400 mμ.

DISCUSSION AND RESULTS

Titanium and niobium are easily separable under a wide variety of hydrochloric and hydrofluoric acid concentrations using a large column[1]. In the proposed method a much smaller column is used in order to reduce the volumes of eluting solutions. Separation of titanium and niobium under these conditions was, however, found to be incomplete using the acid mixtures previously investigated[1]. These studies indicated, however, that the elution rate of niobium is decreased by increasing the acid concentrations, whereas the rate of titanium elution is little affected. Accordingly the separation with stronger acid mixtures was investigated. "Acid mixture 1" was found to separate the metals completely. Titanium was quantitatively washed out with 100 ml of the mixture, 150 ml being required before niobium could be detected in the eluate. This was found to be true for samples containing varying amounts of the metals (total = 100 mg).

The elution of tantalum requires a high acid concentration, the most effective acid mixture being 17.3 N HF–3 N HCl[2] (acid mixture 3). Tantalum was quantitatively eluted with 400 ml of this acid mixture using the small column. The following

mixtures of concentrated acids: 14 *N* HF–4.8 *N* HCl, 19.5 *N* HF–1.8 *N* HCl, and 11.5 *N* HF–6 *N* HCl were found to require 500, 550, and 550 ml respectively for quantitative elution.

Tantalum can be determined as peroxide in conc. sulfuric acid by a procedure similar to that used for titanium and niobium. But the absorption maximum lies in the ultraviolet region, and high and varying blanks were obtained probably owing to contaminations from the column. The pyrogallol method was therefore preferred.

Some results obtained by analyzing mixtures of standard solutions made from spectrographically pure metals are listed in Table I.

TABLE I

DETERMINATION OF TITANIUM, NIOBIUM AND TANTALUM BY ION EXCHANGE

	mg metal			*mg metal*	
	present	*found*		*present*	*found*
Ti	32.0	32.0	Ti	10.3	10.3
Nb	27.3	27.5	Nb	81.7	82.0
Ta	33.3	33.6	Ta	24.7	25.0
Ti	9.6	9.7	Ti	90.0	—
Nb	15.7	15.8	Nb	1.14	1.17
Ta	73.1	73.2			
			Nb	85.6	85.0
			Ta	12.7	12.7

ACKNOWLEDGEMENT

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SUMMARY

A procedure for the analysis of alloys of titanium, niobium and tantalum is described. After dissolution the metals are separated by ion exchange in hydrochloric–hydrofluoric acid media. Finally the metals are determined spectrophotometrically.

RÉSUMÉ

Une méthode d'analyse est décrite pour les alliages de titane, niobium et tantale. Les métaux sont séparés par échangeur d'ions et dosés finalement par spectrophotométrie.

ZUSAMMENFASSUNG

Beschreibung einer Methode zur Analyse der Legierungen von Titan, Niob und Tantal. Die Metalle werden mit einem Ionenaustauscher getrennt und spektrophotometrisch bestimmt.

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THE MICRO-DETERMINATION OF GOLD WITH *p*-DIMETHYLAMINOBENZYLIDENERHODANINE

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INTRODUCTION

An absolute measure of the amount of gold on thin films was required in order to calibrate a secondary optical method of neutron flux measurement. An examination of published methods showed that none could measure amounts of the order of 10 μg to within 1–2%. Thus the *o*-tolidine method[1] was too insensitive in our hands, the dithizone method although sensitive was irreproducible, and the rhodamine B method[2], from the published figures, too inaccurate. The *p*-diethylaminobenzylidenerhodanine method[3] in which a colloidal complex is formed in dilute acid solution was also impossible to reproduce within the required limits. A modification of the method in which the complex is extracted into a non-aqueous phase is described.

EXPERIMENTAL

Extracting solvent and reagent

The dimethylaminobenzylidenerhodanine was purified by dissolving in acetone and partly precipitating with water. An ethanolic solution of the dried product (0.04%, w/v) was used for trial extractions of 5 μg of gold from a dilute hydrochloric acid solution (about 0.12 *N*). Isoamyl acetate was the best extractant amongst chloroform, carbon tetrachloride, isoamyl alcohol, diethyl ether, benzene–chloroform (1 : 3) and isoamyl acetate. It gave the quickest extraction and had the advantages of a low volatility, and a clean separation from the aqueous phase. Most of the colour of the organic phase was caused by the yellow colour of the reagent with a maximum absorption at 455 mμ. The red-orange gold complex could be observed with more dilute solutions of the reagent and had an absorption peak at 510–515 mμ. In order to estimate small amounts of gold accurately the minimum excess of reagent compatible with complete formation of complex is required. The dilute solution of reagent can be made by extracting a portion of the ethanolic solution, diluted with water, into isoamyl acetate, or directly by diluting a stronger solution of rhodanine in isoamyl acetate. On the basis of a 1 : 1 gold-rhodanine complex, 1 ml of a 0.004% solution w/v can complex 30 μg of gold.

SOLUTIONS

Standard gold solutions: A stock solution (500 μg/ml) was prepared by dissolving gold wire in a minimum of aqua regia, evaporating almost to dryness twice with hydrochloric acid and making to a standard volume. The working solution (5 μg/ml) was prepared by dilution immediately before use. Deionized water was used for all solutions.

Constant boiling point hydrochloric acid and iso-amyl acetate: The standard laboratory grade was used without purification.

p-Dimethylaminobenzylidenerhodanine: A dilute solution in isoamyl acetate, 0.0044% (w/v) was prepared as described from a stronger stock solution. The dilute solutions are stable.

PROCEDURE

The gold film was dissolved from its terylene support in a minimum of aqua regia and evaporated almost to dryness in a water bath with some additional hydrochloric acid. The last traces of volatile acid were removed by blowing air over the surface. The residual chlor-auric

acid was diluted suitably to a standard volume and an aliquot containing between 4 and 10 μg taken. 0.10 ml $\pm$ 0.005 of constant boiling point acid was added and the total volume was adjusted to 5.00 ml; 0.30 ml of rhodanine reagent and 10.0 ml of isoamyl acetate were then added. The container, a test tube stoppered by a B14 cone covered with a "Teflon" cone to prevent solvent leakage, was shaken mechanically for 15 min; the organic layer was then poured through cotton wool directly into a 4-cm absorption cell (volume 10 ml) and the absorption of the gold solution compart with a blank (5.00 ml of water taken through the procedure) at 515 mμ after 5 min.

Effect of acidity

The acidity must be closely controlled because the colour decreases with increasing acidity. The complex is no longer formed when the acidity is greater than 1 *N*.

TABLE I

EFFECT OF ACIDITY ON EXTRACTION OF 4.6 μg OF GOLD

Normality of acid	*log I_0/I*
0.06	0.109
0.12	0.106
0.18	0.083

TABLE II

STABILITY OF AN EXTRACT OF 7.04 μg OF GOLD

Time (minutes)	*log I_0/I*
5	0.159
10	0.159
15	0.155
30	0.152
45	0.148

Effect of time on the coloured complex

The colour fades slowly and should be measured within ten minutes of extraction.

Effect of time on extraction of complex

The time for complete extraction increases with dilution of the reagent. Extraction times should not be prolonged to avoid fading of the colour. Complete extraction under the above conditions is obtained after 10–15 min.

TABLE III

TIME REQUIRED FOR COMPLETE EXTRACTION OF COMPLEX

(5.03 μg gold measured 5 min after extraction)

Extraction time (minutes)	*log I_0/I*
5	0.097
10	0.114
15	0.118
20	0.117

Effect of reagent strength

The range covered may be extended above 10 μg with a stronger reagent solution without further calibration. An excess of reagent should always be present to ensure complete extraction of gold. A 50–100% excess is advised.

Effect of temperature

No effect was observed when the ambient temperature varied from 19–29°.

The average absorbance/μg of gold for solutions whose absorbancy can be measured to 1% (*i.e.* not less than 4 μg) is 0.0231 $\pm$ 0.002. The mean deviation is therefore within $\pm$ 1% and the maximum deviation within $\pm$ 2%. The method is therefore as sensitive as the aqueous method and much more precise.

RESULTS AND DISCUSSION

TABLE IV

CALIBRATION CURVE

Gold (μg)	*log I_0/I*			*log I_0/I/μg*	*Reagent strength*
1.01		0.024		0.023(8)	
2.01		0.047		0.023(4)	
3.02		0.071		0.023(3)	
4.03	0.093,	0.094,	0.094	0.0233	1 ml ≃ 30 μg gold
5.03		0.117,	0.118	0.0234	
6.04	0.139,	0.139,	0.139	0.0230	
7.04		0.159		0.0226	
8.05		0.186		0.0231	
9.06		0.209		0.0231	
10.06		0.233		0.0231	1 ml ≃ 60 μg gold
11.07		0.257		0.0233	
12.07		0.281		0.0232	
14.09		0.321		0.0228	

The composition of the complex is reputed to be the same as that of the silver complex in which the hydrogen of the acidic imino group is replaced by univalent silver. The maximum colour obtained with a particular strength of dilute reagent indicates that only one atom of gold combined with a molecule of rhodanine (*e.g.* a reagent containing 14.4 ± 0.4 μg of rhodanine in 0.30 ml gave a maximum absorbance of 0.222, which from the calibration curve corresponds to 9.6 μg of gold. A 1:1 complex would contain 9.8 ± 0.3 μg). However, it is unlikely that the complex contains aurous gold because the reduction of auric gold would oxidize the rhodanine and the aurous chloride formed could disproportionate. Both phenomena would be expected to give irreproducible colours. The retention of trivalent gold in a complex anion is more probable.

ACKNOWLEDGEMENT

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SUMMARY

Gold can be determined in the range 4–14 μg within 2% by a simple extraction of chlorauric acid in 0.12 *N* hydrochloric acid with a solution of *p*-dimethylaminobenzylidenerhodanine in iso-amyl acetate. A 1 : 1 complex is formed with maximum absorption at 515 mμ.

RÉSUMÉ

Un microdosage de l'or est proposé au moyen de la *p*-diméthylaminobenzylidènerhodanine, avec extraction dans l'acétate d'iso-amyle.

ZUSAMMENFASSUNG

Tetrachlorogoldsäure bildet mit *p*-Dimethylaminobenzyliden-rhodanin einen Komplex, der in Isoamylacetat löslich ist. Diese Reaktion kann zur Bestimmung sehr kleiner Goldmengen nach dem Extraktionsverfahren verwendet werden.

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COLOUR REACTIONS AND PAPER CHROMATOGRAPHIC STUDIES OF YTTRIUM AND ZIRCONIUM

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INTRODUCTION

Many procedures have been reported concerning the determination of yttrium or zirconium after separation by the usual analytical methods. These include spectrometric determinations[1-7], amperometric titrations[8], gravimetric[9-13], volumetric[14], complexometric[15-20], colorimetric[21-25], and other procedures.

This paper presents a detailed account of the colour reactions of yttrium and zirconium with different organic compounds in visible and ultra-violet light, as well as the sensitivity of each reaction for the determination of these elements. Furthermore, some organic solvents are reported which may be used for their separation.

EXPERIMENTAL

Schleicher and Schüll No. 2043a paper (30 cm × 6 cm) was used for the chromatography by the downward elution method. The selected solvent mixtures were allowed to pass first through the paper and then the metal solutions were applied by means of a microapparatus (Desaga Mikrometer-Dosiergerät) after the paper had been dried by air; 0.05 ml of aqueous yttrium chloride or zirconium chloride solutions containing 20–30 μg of the element was used. The spots were dried with hot air, and the paper strip was inserted into the eluting solvent. When the solvent had percolated a sufficient distance down the paper strip, the chromatogram was removed and dried with hot air.

For the quantitative estimation of the sensitivity of each reaction, increasing amounts from 0.5 to 30 μg were plotted on the paper at 2-cm intervals and the lowest positive colour reaction was noted.

Depending on the solubility of the compounds in water or alcohol, 0.1–0.5% w/v aqueous or alcoholic solutions of the reagents were generally used for the colour reactions, and the R_F values were calculated by the method of JERCHEL AND MÖHLE (E. Merck R_F-determination compasses).

Some fluorescent reactions were also detected by means of contact photography.

RESULTS

The colour reactions observed in visible and ultra-violet light are given in Table I. With the exception of *o*-cresol-phthaleincomplexone which was dissolved in a buffer solution of ammonium hydroxide–ammonium chloride (pH = 10.5), all reagents were dissolved only in distilled water or alcohol.

The sensitivity of each reaction under the above-mentioned conditions is given in Table II. The more sensitive reagents are found at the top of the table.

The selected solvent mixtures for the separation of yttrium from zirconium and their R_F values are given in Table III.

TABLE I

Reagent	*Solvent*	*Concn. % w/v*	*Yttrium*		*Zirconium*	
			Colour		*Colour*	
			Visible	*U.V.Light*	*Visible*	*U.V.Light*
1. Pyridine(2-azo-4)-resorcinol	alc.	0.1	Purple red	(—)	Purple red	(—)
2. 1(2-Pyridyl-azo)-2-naphthol	alc.	0.1	Red	(—)	Pink	(—)
3. Chromazurol S	water	0.1	Blue	(—)	Blue-violet	(—)
4. *o*-Cresolphthalein-complexone	buffer	0.1	Blue-violet	Yellow-gr. F	Pink	Green F
5. Pyrocatechol violet	water	0.1	Violet	(—)	Blue-violet	(—)
6. Na-rhodizonate HCl vapour	water	0.1	(—)	(—)	Yellow	Yellow F
7. Alizarin	alc.	0.1	Blue-violet	(—)	Red-violet	(—)
8. Alizarinsulfonic acid	water	0.1	Red-violet	(—)	Light red	(—)
9. Quinalizarin	alc.	0.1	Blue	(—)	Red-violet	(—)
10. Purpurin	alc.	0.1	Blue-violet	(—)	Red-violet	(—)
11. Chrysacine	alc.	0.1	Light red	Red F	Pink	Pink F
12. Murexide	water	0.1	Yellow	(—)	Yellow	Yellow F
13. 8-Hydroxyquinoline	alc.	0.5	Yellow	Yellow F	Yellow	Yellow F
14. *p*-Quinonetetrakis-diisopropylphospho-nate	alc.	0.5	(—)	Yellow F	(—)	Yellow F
15. Quercetin	alc.	0.5	Yellow	Yellow-gr. F	Yellow	Yellow-gr. F
16. Morin	alc.	0.1	Yellow	Green F	Yellow	Green F
17. α,α'-Dipyridyl	alc.	0.5	(—)	Light blue F	(—)	Light blue F
18. β-Nitroso-α-naphthol	alc.	0.1	Orange	(—)	Light red	(—)
19. Aluminon	water	0.1	Red	(—)	Light red	(—)
20. Hematoxylin NH_3 vapour	alc.	0.1	Blue-violet	(—)	Red-violet	(—)
21. Diphenylcarbazone	alc.	0.1	Pink	(—)	Pink	(—)
22. Eriochrome Black T	water	0.1	Blue-violet	(—)	Red-violet	(—)

F = Fluorescence
gr = green

TABLE II

Reagent	*Yttrium*				*Zirconium*			
	1 μg	*5 μg*	*10 μg*	*15 μg*	*1 μg*	*5 μg*	*10 μg*	*15 μg*
1. Pyridine(2-azo-4)-resorcinol	+++				+++			
2. Pyrocatechol violet	+++				+++			
3. Chromazurol S	+++				+++			
4. Alizarin	++	+++			++	+++		
5. 8-Hydroxyquinoline	++	+++			++	+++		
6. Quercetin	++	+++			++	+++		
7. Hematoxylin, NH_3 vapour	++	+++			++	+++		
8. Diphenyl carbazone	++	+++			++	+++		
9. Purpurin	++	+++			++	+++		
10. Chrysacine	++	+++			++	+++		
11. Quinalizarin	++	++	+++		++	+++		
12. β-Nitroso-α-naphthol	+	++	+++		++	+++		
13. Morin	+	++	+++		++	+++		
14. Aluminon	+	++	+++		++	+++		
15. *p*-Quinonetetrakis-diisopropyl-phosphonate	++	+++			+	++	+++	
16. α,α'-Dipyridyl	+	++	+++		+	++	+++	

TABLE II (continued)

Reagent	Yttrium				Zirconium			
	1 μg	5 μg	10 μg	15 μg	1 μg	5 μg	10 μg	15 μg
17. Alizarinsulfonic acid	+	++	++	++	+	++	+++	
18. 1(2-Pyridyl-azo) 2-naphthol	++	+++			+	+	+	++
19. *o*-Cresolphthalein-complexone	+	+	++	++	++	+++		
20. Na-rhodizonate, HCl vapour	(—)	(—)	(—)	(—)	++	+++		
21. Murexide	+	+	++	+++	+	+	++	+++
22. Eriochrome Black-T	+	+	+	++	+	+	+	++

— = Negative; + = Positive; +++ = Strong positive

TABLE III

Solvents	Time	R_F values	
		Yttrium	Zirconium
1. Methanol : *n*-butanol : collidine : 6 *N*-acetic acid 2 : 1 : 1 : 1	4 h	0.76	no migration
2. Methanol : *n*-butanol : collidine : 6 *N*-acetic acid 1 : 1 : 2 : 1	5 h	0.66	no migration
3. Methanol : isopropanol : water : formic acid : ammonium formate 25 : 15 : 7.5 : 1 : 1	3 h	0.59	no migration
4. Formamide : ethanol : methanol : KSCN 10 : 10 : 10 : 0.2	$1^1/_2$ h	0.64	0.36
5. *n*-Propanol : water : hydrochloric acid : NH_4Cl 7 : 7 : 1 : 0.2	4 h	0.64	0.40
6. *n*-Propanol : water : hydrochloric acid : NH_4Cl 30 : 10 : 2 : 1	4 h	0.27	0.10

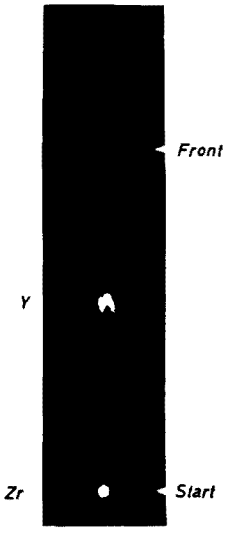

Fig. 1. Contact photography after chromatographic separation of yttrium from zirconium. Quantity 15 μg. Detecting reagent 8-hydroxyquinoline. Exposure time 20 sec. (Photographic paper Agfa-Brovira).

Fig. 1 shows a contact photograph of yttrium and zirconium after separation with a solvent mixture containing methyl alcohol, isopropyl alcohol, water, formic acid, and ammonium formate (12 : 8 : 4 : 0.6 : 0.2). The detecting reagent was 8-hydroxyquinoline. The two spots of the elements can be clearly distinguished.

DISCUSSION

Owing to the radiobiochemical importance of yttrium and zirconium as fission products or as daughter elements of strontium, our intention was to study complex formation between these two elements and different organic materials. A part of this study is reported in the present paper. For special reasons most reactions were carried out at room temperature and at a neutral pH (with the exception of *o*-cresolphthaleincomplexone); the fact that a change in the pH or an increase in the temperature could accelerate the formation of the complex was not taken into consideration. Some of the organic reagents used are well-known to form complexes with other elements, *i.e.* murexide with calcium[26-27], and *o*-cresolphthaleincomplexone[28-30], eriochrome black T[31] with barium, calcium, strontium, etc. Other reagents were selected as being likely to form complexes because of their chemical structure. Special attention was paid to alizarin derivatives. Some of the reagents were found to be very suitable for the determination of yttrium and zirconium because amounts as small as 1 μg could be detected. The colour reaction between *o*-cresol-phthaleincomplexone and yttrium or zirconium in visible and ultra-violet light was found to be more stable than that of the corresponding calcium-*o*-cresol-phthaleincomplexone derivative. The intensity of colours changed in the course of time. The colours may generally be altered by treating the paper strips with ammonia or hydrochloric acid vapour, but the fluorescence of zirconium was found to persist even after this treatment; in the case of sodium rhodizonate, the fluorescence of zirconium appeared only after exposure to hydrochloric acid vapours. The fluorescence of these two elements could also be detected by contact photography.

As solvent mixtures, two groups of solvents were selected:

I. Those in which only yttrium migrated, whereas zirconium remained at the starting point.

II. Those in which both elements migrated but with different R_F values.

The choice of solvent depends on the circumstances.

CONCLUSION

Some supplementary reactions for yttrium and zirconium are reported and their significance has been discussed. The amount which can detected varies from 1 to 30 μg according to the reagent. The colour reactions may be adapted for the determination of yttrium and zirconium.

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SUMMARY

The colour reactions of yttrium and zirconium with some organic reagents have been investigated and their sensitivity is reported. Some fluorescent reactions in ultra-violet light are also described and solvent mixtures for the separation of the two elements by paper chromatography are given.

RÉSUMÉ

Les auteurs ont examiné les réactions de coloration de l'yttrium et du zirconium obtenues avec des réactifs organiques, de même que des réactions de fluorescence en lumière ultraviolette. Ils ont effectué également une étude sur la séparation de ces deux éléments par chromatographie sur papier.

ZUSAMMENFASSUNG

Es wird über Untersuchungen von Farbreaktionen des Yttriums und des Zirkons mit einigen organischen Verbindungen im sichtbaren und U.V.-Gebiet berichtet, die papierchromatographische Nachweisempfindlichkeit dieser Reaktionen angegeben sowie die Lösungsmittel zur papierchromatographischen Trennung der beiden Elemente beschrieben.

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POLAROMETRISCHE BESTIMMUNG VON ZIRKONIUM(IV) MIT TARTRAZIN

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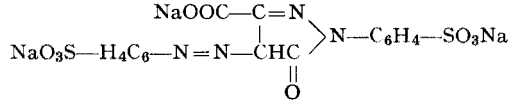
In der chemischen Fachliteratur kommt eine einzige Methode[1] zur polarometrischen Bestimmung von Zr(IV) vor, im welcher eine Cupferron-Standardlösung als Reagens herangezogen wurde.

Diese Methode wurde für Analysen von Proben mit Uranium–Niobium und Zirkoniumgehalt[2] zur Anwendung gebracht.

In der vorliegenden Arbeit wird die polarometrische Bestimmung von Zr(IV) und Zr(IV) bei Anwesenheit von U(VI) unter Anwendung von Tartrazin als Reagens für die Titrierung vorgeschlagen.



Tartrazin wurde zur gravimetrischen Bestimmung von Zirkonium[3] angewandt. Zirkonium bildet mit Tartrazin eine einheitliche Verbindung, deren Formel Zr_3 (Tartrazin) $(OOH)_3$ ist.

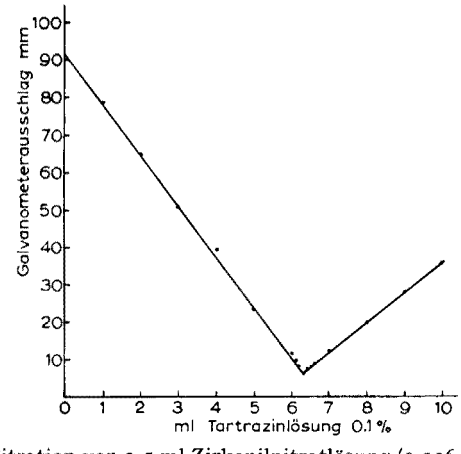


Fig. 1. Diagramm der Titration von 0.5 ml Zirkonilnitratlösung (0.006455 g Zr/ml) mit Tartrazin.

Die einheitliche Verbindung des Niederschlags hat uns dazu bewogen, für die polarometrische Titrierung von Zr(IV) Tartrazin zu benützen.

ARBEITSWEISE

Als Reagens wird eine 0.1%-ige Tartrazinlösung benützt.

Gearbeitet wird mit einer Lösung von salpetersaurem Zirkonilnitrat (RIEDL DE HÄEN) mit einem Gehalt von 0.006455 g Zr/ml.

Der Äquivalentspunkt wird an einem Polarograph-System HEYROVSKY, Type L.P. 55, Modell 1958 abgelesen. Wir arbeiten mit einer Einrichtung für polarometrische Titrationen mit einer gesonderten Bezugselektrode nach A. NEUBERGER.

Der Sauerstoff wird aus der Lösung mittels eines Wasserstoffstroms entfernt.

Am Anfang einer jeden Bestimmung wird Sauerstoff 10 Minuten lang und nach Zusatz jeder einzelnen Menge des Reagens je 30 Sekunden lang durchgeleitet.

Die Titrierung erfolgt bei einem Potential von —0.9 V.

Die Schwankung der Diffusionsstrom-Stärke während der Durchführung einer Bestimmung wird in Fig. 1 dargestellt.

Die Ergebnisse der polarometrischen Titrierung von Zr(IV) sind in der Tabelle I angeführt:

TABELLE I

Nr. der Bestimmung	*Zirkonilnitrat Lösung ml*	*Zirkoniumgehalt der Probe µg*	*Gefunden Zirkonium µg*	*Differenz %*
1	0.1	645.5	639.2	—1.01
2	0.15	968.2	957.1	—1.15
3	0.25	1613.7	1613.7	—
4	0.30	1936.5	1936.5	—
5	0.40	2682.–	2739.8	+2.15
6	0.50	3227.5	3227.5	—
7	0.50	3227.5	3201.9	—0.79
8	0.50	3227.5	3227.5	—
9	0.75	4841.3	4831.–	—0.21
10	1.00	6455.–	6501.1	+0.71

Die Fehlergrenze der Bestimmungen ist höchstens 2%.

In der Folge wurde eine Reihe von polarometrischen Bestimmungen durchgeführt von Zirkonium bei Anwesenheit von Uranium, deren Ergebnisse in der Tabelle II angeführt sind:

TABELLE II

Nr. der Bestimmung	*Zirkonilnitrat Lösung ml*	*Zirkonium-gehalt der Probe µg*	*Uranilnitrat Lösung ml*	*Uranium-gehalt der Probe µg*	*Gefunden Zirkonium µg*	*Differenz %*	*Verhältnis Zr:U*
1	0.25	1613.7	3	37.440	1603.3	—0.64	1:23
2	0.5	3227.5	3	37.440	3227.5	—	1:12
3	0.5	3227.5	3	37.440	3200.—	—0.85	1:12
4	0.5	3227.5	5	62.400	3227.5	—	1:19
5	0.5	3227.5	5	62.400	3227.5	—	1:19
6	0.5	3227.5	10	124.800	3227.5	—	1:39
7	0.5	3227.5	5	62.400	3176.3	—1.58	1:19
8	0.5	3227.5	5	62.400	3210.8	—0.52	1:19
9	0.6	3873.—	6	74.880	3806.2	—1.72	1:19
10	0.6	3873.—	6	74.880	3817.4	—1.43	1:19

Auf Grund der Untersuchung der Ergebnisse aus Tabelle II wird festgestellt, dass die von uns vorgeschlagene Methode eine polarometrische Bestimmung von Zr(IV) bei Anwesenheit von U(VI) bis Verhältnis 1:39 zulässt.

ZUSAMMENFASSUNG

Es wird eine neue Methode zur polarometrischen Bestimmung von Zr(IV) unter Anwendung von Tartrazin als Reagens vorgeschlagen.

Die neue Methode ermöglicht eine Mikrobestimmung von Zirkonium, auch bei Anwesenheit von Uran bis zum Verhältnis 1:39, wobei die Fehlergrenze der Bestimmungen höchstens 2% ausmacht.

SUMMARY

A new polarimetric procedure has been developed for the rapid determination of zirconium in the presence of tartrazin as reagent. This determination is possible in the presence of uranium.

RÉSUMÉ

Les auteurs proposent une nouvelle méthode de dosage polarométrique du zirconium, au moyen de tartrazine comme réactif. Ce dosage peut s'effectuer en présence d'uranium.

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Bemerkung zu einer Arbeit von Kovács und Tárnoky

Jüngst erschien in dieser Zeitschrift eine Arbeit der im Titel genannten Autoren, die sich mit der komplexometrischen Titration von Calcium und Magnesium befasst. Hierbei wird Plasmocorinth B als Indicator verwendet. Es sei an dieser Stelle nicht darauf eingegangen inwieweit die Autoren korrekt sind, wenn sie sagen, dass das „rote Plasmocorinth B einen farbigen Komplex mit EDTA" bildet. Es sei hier lediglich bemerkt, dass die Autoren es nicht der Mühe wert fanden in Betracht zu ziehen, dass Firmennamen bei Farbstoffen gar nichts besagen und nur die chemische Konstitution Bedeutung hat, wenn man in der Literatur forscht. So ist es ihnen entgangen, dass derselbe Farbstoff auch unter dem Namen Eriochromblau SE von der Fa. Geigy in Basel hergestellt wird und unter diesem Namen vor einiger Zeit bereits als komplexometrischer Indicator verwendet wurde. Vom Schreiber dieser Zeilen und seinen Mitarbeitern wurde die Abkürzung Erio SE eingeführt. Die Verwendung von Erio SE wurde zuerst für die Calcium und Magnesiumbestimmung (in Serum) beschrieben[2] und später auch auf andere Metallionen ausgedehnt[3]. Beide Arbeiten sowie eine eingehende Literaturübersicht[4] sind an durchaus leicht zugänglichen Stellen publiziert worden.

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Short Communication

Paper electrophoresis of inorganic anions in sodium carbonate solution

The electromigration of ionorganic anions has been studied in few electrolytes. LEDERER AND WARD[1] used normal solutions of KCl, and more recently studies were reported in which ammonium carbonate[2,3] and 0.01 *N* NaOH[4] were used as electrolytes. While this manuscript was in preparation GRASSINI AND LEDERER[5] reported the use of 0.1 *N* NaOH in the paper electrophoresis of a number of inorganic anions.

LEDERER[2] reported the successful separation of a number of halogen oxyacids but found that periodate divided into two spots which moved little, and gave a reduction comet. He also reported difficulty due to liquid flow as a result of evaporation and lack of saturation of the paper.

It was thought that sodium carbonate might be a suitable electrolyte since it is fairly alkaline, has a suitable electrolytic strength, and is not volatile. Although this latter feature would preclude the use of indicators to detect the spots it would allow the electrophoresis apparatus to be operated at a higher voltage, thus enhancing the possibility of achieving satisfactory separations. To further minimize trouble due to evaporation the paper was sandwiched with a thin sheet of polyvinylchloride between two sheets of plate glass.

EXPERIMENTAL

The electrophoresis chamber and high-voltage potentiostat were designed and built in this Department.

The solutions were prepared from "Analytical Grade" reagents in distilled water and were decimolar unless otherwise indicated. A 2% solution of sodium carbonate in water was used as the electrolyte.

The electrolyte tanks were filled with a 2% aqueous solution of sodium carbonate to a constant mark about 2 cm below the glass plate, the levels being adjusted by means of a removable siphon filled by suction through a three way T-stopcock.

Whatman No. 3MM paper was used for the results reported. The starting line and starting positions were marked with a graphite pencil. The paper strip was drawn through the electrolyte and lightly blotted by placing between sheets of blotting paper and drawing a roller (weighing 1 kg) along the strip without applying additional pressure[6]. The paper was then placed at once on the lower glass plate, centered over a line drawn across the middle of this plate. A thin sheet of polyvinylchloride was placed between the paper strip and the upper glass plate. This sheet had five holes of 0.5 cm diameter drilled in a line across the centre, starting 1.5 cm from the edge and spaced 1.2 cm apart. Thirty minutes was allowed for capillary rise of the electrolyte from the tanks. After this time inflow is very small and starting the experimental runs in the centre virtually eliminates the effect of any unsaturation of the paper since any solution inflow subsides before the ions reach the regions of more rapid inflow. The upper glass plate was then removed and the test solutions applied through the holes in the polyvinylchloride sheet, the glass plate replaced and the potential applied at once. Test solutions were applied by means of a small platinum loop. The loop used, deposited a spot which spread to a diameter of about 3–4 mm soon after application.

A potential of 300 V was applied for one hour at currents of less than 39 mA.

After electrophoresis the papers were dried and then sprayed with the appropriate detecting reagent using an atomiser operated by compressed air.

To get comparable results from run to run, chromate was placed on all sheets to act as a marker ion. Movements of chromate ions varied slightly from sheet to sheet due apparently to small variations in the potential from the supply unit and perhaps impurities in the paper. Movements reported have been corrected to the equivalent of a chromate movement of 75 mm, this being an average value for all sheets run. Where more than one result has been obtained for any ion each result is shown separately.

TABLE I

Anion	*Movement (mm)*	*Anion*	*Movement (mm)*
Ferricyanide	76	Vanadate	50
Ferrocyanide	71	Nitrite	80
Chlorate	74	Sulphate	66
Bromate	61	Thiosulphate	84
Bromate (*M*/40)	59	Thiocyanate	76
Iodate	36	Chloride (*M*)	101
Iodate (*M*/40)	36	Bromide (*M*)	103
Periodate (*M*/40)	0	Iodide (*M*)	92
Molybdate	64		

CONCLUSIONS

From the results obtained with the 15 anions examined, it would appear that similar separations to those obtained with ammonium carbonate may be achieved using sodium carbonate. A separation of molybdate and vanadate was noted, which was not obtained by LEDERER with ammonium carbonate.

Reproducibility was good, being well within 5% in all cases.

Some difficulty was experienced in attempting to use test solutions of the same molarity for comparative purposes, being limited on one hand by the comparative insolubility of some anions, and by lack of sufficiently sensitive means of detection on the other. In most cases a 0.1 *M* solution was used, being sufficiently strong to permit satisfactory detection, and not so strong as to produce an undesirably large spot or tailing.

The halides were run as molar solutions as it proved impossible to detect 0.1 *M* solutions with certainty with available methods of detection. A molar solution of chromate was used to correct the movements in these instances.

Results obtained with periodate on Whatman 1, 2, 4 and 7 papers were similar to those previously reported[3,5]. With 2% sodium carbonate, 1% sodium carbonate – 1% sodium bicarbonate, and 2% ammonium carbonate solutions as electrolytes, and with an applied potential of 150 V, periodate gave reduction comets and travelled 25 mm, 31 mm, 25 mm respectively, relative to chromate 48 mm in each case. With Whatman 3 MM paper periodate gave a single spot which scarcely moved from the starting line, possibly because of adsorption by some impurity not present in the other papers.

The use of sodium carbonate as an electrolyte allows separations comparable to those previously reported but has the defect that acid–base indicator solutions cannot be used as universal indicators.

The authors wish to thank Mr. W. T. DENHOLM for designing the high-voltage potentiostat used in this study.

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