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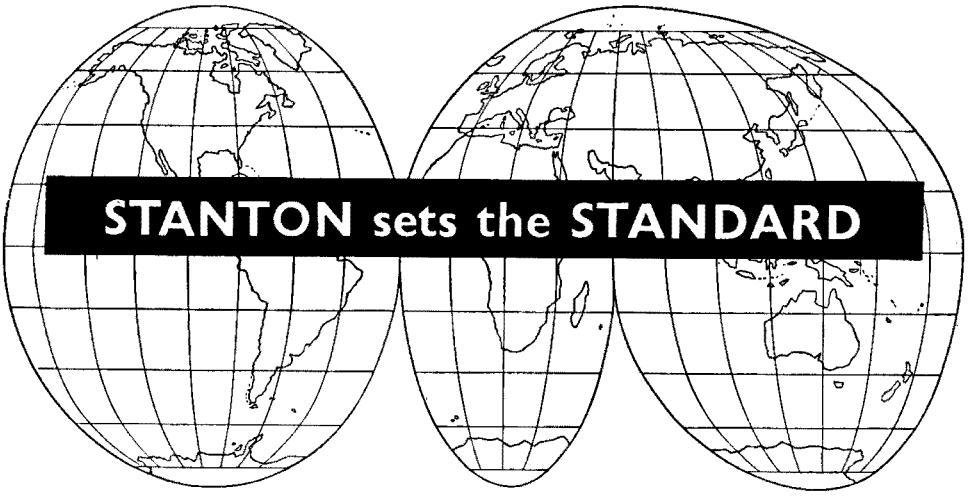

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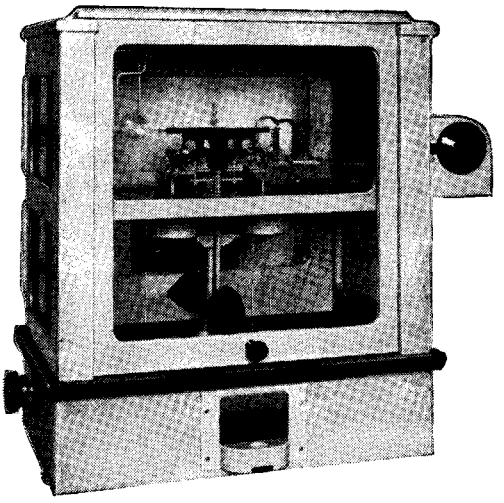

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by ETHEL BROWNING, M.D.

Formerly H.M. Medical Inspector of Factories, Ministry of Labour and National Service, London (England)

Modern civilisation is deeply concerned with the problem of ionising radiation, which is now ineradicably rooted in the present and future development of every aspect of human activity. So much material on this vital and controversial subject is available that even those who have a fairly wide knowledge of its general principles tend to be overwhelmed and confused by the spate of information. The purpose of this book is to present clearly and concisely the aspects of the problem which are of special importance to those who encounter ionising radiations in the field of industry. Also included is the important field of nuclear energy and radioactive "fall-out", because the potential injury from exposure can no longer be limited to any one source, nor can the amount considered "permissible", below which it is calculated that no injury will ensue, be estimated without taking all sources into consideration.

The author, who for many years was concerned with the clinical and haematological effects of radiation on people employed in the lighting industry and in radiography with X-rays and radioactive isotopes, has included some of her own observations on these aspects, but the bulk of the information in this book is derived from the world literature available at present. Topics dealt with include historical investigations of pioneers in this field, definitions of units of radiation, present conceptions of maximum permissible concentrations, protective measures recommended and sometimes enforceable by statutory regulation, industrial applications of ionising radiations, and their biological hazards, including the more controversial aspects of potential carcinogenesis and genetic effects.

The book will be of special interest and value to medical officers in industry, industrial welfare and safety officers, and laboratory workers, who need concise information as to the nature, safe handling and potential dangers of this powerful factor in the life of the general as well as the industrial population.

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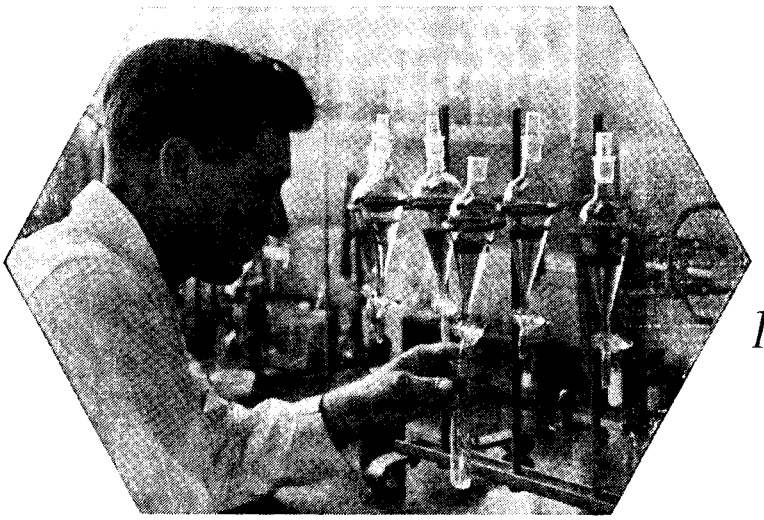

Items of interest from our laboratory notebooks

▶ 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.

SEPARATION AND GRAVIMETRIC DETERMINATION OF NIOBIUM, TANTALUM AND TITANIUM BY PRECIPITATION WITH N-BENZOYL-N-PHENYLHYDROXYLAMINE

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University of Oslo, Chemical Institute A, Blindern (Norway)

(Received September 30th, 1959)

INTRODUCTION

N-Benzoyl-N-phenylhydroxylamine, a derivative of cupferron, was originally synthesized by BAMBERGER[1]. The reagent was used by SHOME[2] for the gravimetric determination of aluminium, copper, iron and titanium, and for the colorimetric determination of vanadium. It was recommended by MOSHIER AND SCHWARBERG[3] for the gravimetric determination of tantalum in the presence of niobium, titanium and zirconium. MAJUMDAR AND MUKHERJEE[4,5] used it for the separation and determination of niobium and tantalum.

MOSHIER AND SCHWARBERG separated tantalum by two or three precipitations at pH 1, while niobium and titanium were kept in solution as the fluoride complexes. A procedure for further separation of niobium and titanium was not given by these authors.

MAJUMDAR AND MUKHERJEE precipitated niobium from a tartrate solution at a pH of 3.5–6.5. Tantalum remained in solution and was precipitated by decreasing the pH to below 1.5. Several of the more common elements were kept in solution by means of EDTA* and tartaric acid. Interfering ions were titanium, zirconium, vanadate and molybdate.

The interference caused by titanium reduces considerably the possible application of the reagent in the analysis of niobium- and tantalum-bearing ores and minerals. In these materials the earth acids are usually accompanied by considerable amounts of titanium. The value of N-benzoyl-N-phenylhydroxylamine as an analytical reagent would be greatly increased if it also could be applied in the presence of titanium.

The present paper describes a method for the separation and gravimetric determination of niobium, tantalum and titanium by precipitation with N-benzoyl-N-phenylhydroxylamine. Titanium is kept in solution with EDTA and hydrogen peroxide while the earth acids are precipitated together in 1 *N* sulphuric acid. Niobium and tantalum are then separated and determined by a modification of the method of MAJUMDAR AND MUKHERJEE[4]. After destruction of the complexing agents, titanium is also determined by precipitation with N-benzoyl-N-phenylhydroxylamine.

* Ethylenediaminetetraacetic acid, disodium salt.

EXPERIMENTAL DATA

Reagents

For the preparation of standard solutions of niobium, tantalum and titanium, known amounts of the pure metals were weighed out. Solutions of these metals tend to hydrolyse on standing; freshly prepared, clear solutions should therefore be used. In addition to these three solutions, combined standard solutions containing two or three of the components were also prepared.

N-Benzoyl-N-phenylhydroxylamine (L. Light & Co.) and other chemicals were of reagent grade quality. A 10% solution of N-benzoyl-N-phenylhydroxylamine in ethanol was used for the precipitations.

Determination of pH

The pH of the solutions was adjusted by means of pH paper indicating pH with an accuracy of ± 0.2 units.

Complexing agents for titanium

As mentioned above, the earth acids were precipitated together in 1 *N* sulphuric acid while titanium was complexed and kept in solution. Several complexing agents for titanium were tested. Among those tried unsuccessfully were EDTA, ascorbic acid, hydrogen peroxide, triethanolamine and tiron (catechol-3,5-disulphonic acid).

However, the stability of the titanium–EDTA complex may be increased by adding hydrogen peroxide. WILKINS[6] recommended peroxide to inhibit the precipitation of hydrous oxide from titanium–EDTA solutions; he suggested that one or more peroxide molecules are picked up by the titanium–EDTA complex, displacing hydroxy or oxygen ions. That the stability of this complex is increased by the addition of peroxide was demonstrated by the fact that titanium was precipitated with N-benzoyl-N-phenylhydroxylamine when the solution (1 *N* sulphuric acid) contained only EDTA, while no precipitation took place from a similar solution containing both EDTA and hydrogen peroxide.

All the titanium could not be kept in solution during the precipitation of niobium and tantalum. About 10% of the amount present was coprecipitated with the earth acids, and a double precipitation is therefore recommended. During the subsequent precipitations of niobium and tantalum, the small amount of titanium remaining with the earth acids went into solution and was finally recovered in the washings.

Procedure for the separation and determination of niobium, tantalum and titanium

(A satisfactory, preliminary separation of the three elements from other elements present in the materials to be analyzed is obtained by a double precipitation with N-benzoyl-N-phenylhydroxylamine at a pH below 1. Niobium, tantalum and titanium are thus quantitatively separated from several other elements which are kept in solution by complex formation with EDTA and tartaric acid.)

A sample (0.2–0.3 g) of the three metals (as oxides) is fused with 2–3 g of potassium pyrosulphate. The melt is dissolved by heating with 30–40 ml of 30% tartaric acid solution containing 2 ml of concentrated sulphuric acid. The resulting solution is transferred to a beaker (800-ml capacity) and diluted to about 300 ml with 2% tartaric acid solution. Sufficient sulphuric acid (1 : 1) is added carefully to make the solution 1 *N* in sulphuric acid. The solution is heated to about 60° on the boiling water bath and then removed from the bath; 1 g of EDTA and 1 ml of 30% hydrogen peroxide solution are added to complex titanium, followed by 30 ml of 10% N-benzoyl-N-phenylhydroxylamine solution added dropwise with stirring. The solution is again

placed on the boiling water bath for 45 min at 60° to coagulate the precipitate. Macerated filter paper is added* and the solution is cooled to room temperature. It is then cooled to about 5° in a refrigerator. The yellow precipitate is collected on a loose-textured filter paper and is washed free of sulphate ions with a hot wash solution prepared by dissolving 1 g of N-benzoyl-N-phenylhydroxylamine, 1 g of EDTA and 1 ml of 30% hydrogen peroxide solution in 1 l of distilled water. The washings are reserved for the determination of titanium. The filter is transferred to a porcelain crucible, and the precipitate is dried, charred and ignited at about 600° in an electric furnace.

The oxides are again brought into solution by fusion with potassium pyrosulphate, and niobium and tantalum are reprecipitated with N-benzoyl-N-phenylhydroxylamine, 0.5 g of EDTA and 0.5 ml of 30% hydrogen peroxide solution being added to complex the remaining titanium. The precipitation is done as described above. The washings are reserved for the determination of titanium. The ignited residue contains all the niobium and tantalum and a small amount of titanium. In the subsequent precipitations of niobium at pH 4.5–5.0 and of tantalum at pH about 1, coprecipitated titanium goes into solution and is recovered in the washings. When tantalum is precipitated, EDTA and hydrogen peroxide are again added to keep titanium in solution. EDTA and hydrogen peroxide should not be present during the precipitation of niobium because of the risk of incomplete precipitation.

The further procedure for the separation and determination of niobium and tantalum is as follows. The oxides of the earth acids are decomposed with potassium pyrosulphate and the melt is dissolved by heating with 30–40 ml of 30% tartaric acid solution containing 2 ml of concentrated sulphuric acid. The solution is transferred to a beaker and diluted to about 300 ml with 2% tartaric acid solution. 20 ml of 10% ammonium acetate solution are added so that a buffer action is obtained when ammonia is added. Diluted ammonia solution is added carefully while stirring until the pH is 4.5–5.0. The solution is then heated on the water bath to about 90° and 30 ml of 10% N-benzoyl-N-phenylhydroxylamine solution are added slowly with stirring. The solution is left on the water bath for 30–45 min and is stirred occasionally. Macerated filter paper is added, the beaker is removed from the water bath and cooled to room temperature. The precipitate is filtered on a loose-textured filter paper and washed free of sulphate ions with a hot 0.1% solution of N-benzoyl-N-phenylhydroxylamine in distilled water. It is then transferred to a previously weighed porcelain crucible, dried, charred and ignited to niobium pentoxide at about 900°.

To the washings are added 0.5 g of EDTA and 0.5 ml of 30% hydrogen peroxide solution. The solution is heated on the water bath to about 60° and 1 : 1 sulphuric acid is added until the pH is about 1. Tantalum is precipitated by adding 20 ml of 10% N-benzoyl-N-phenylhydroxylamine solution dropwise while stirring. The solution is left on the water bath for about an hour at 60°. Macerated filter paper is added, and the tantalum precipitate is treated as described in the preceding paragraph for the niobium precipitate.

The washings from the precipitation of tantalum are combined with all previous washings containing titanium, and the resulting solution is evaporated on the boiling water bath. Fuming nitric acid is added to destroy organic matter and excess is

* Marcerated filter paper may also be added before the addition of precipitating agent.

removed by heating on the hot plate to copious white fumes of sulphuric acid. The final, clear solution is diluted with distilled water to give a 5% solution of sulphuric acid. The solution is heated on the boiling water bath, macerated filter paper is added*, and titanium is precipitated by adding, while stirring, a 10% solution of N-benzoyl-N-phenylhydroxylamine. The solution is left on the water bath for 45–60 min and is stirred occasionally. It is cooled to room temperature and then to about 5° in a refrigerator. The precipitate is filtered, washed and ignited as in the case of niobium and is finally weighed as titanium dioxide.

Notes

The amounts of N-benzoyl-N-phenylhydroxylamine added during the different precipitations of niobium, tantalum and titanium were 8–10 times the weight of metal(s) present.

Niobium should be precipitated at a pH of 4.5–5.0. When the pH was 5–6, as prescribed by MAJUMDAR AND MUKHERJEE[6], the coagulation of the precipitate was less satisfactory. The risk of formation of hydrous oxide is also reduced at the lower pH.

The precipitations described above were all carried out in the presence of high concentrations of salts, and sulphate ions were especially liable to be carried down. The precipitates should therefore be thoroughly washed on the filter. In addition to this washing, it is also recommended that the precipitate be transferred to a beaker containing hot wash solution and kept in suspension for some time by vigorous stirring.

In the analysis of complex materials, small amounts of iron and other constituents tend to coprecipitate in the preliminary separations of niobium, tantalum and titanium. During the subsequent separations, the contaminants dissolve and may be recovered in the filtrate after the precipitation of titanium. This filtrate should therefore be reserved for the determination of other constituents.

Analysis of standard solutions

The procedure described above was tested in the analysis of solutions containing known amounts of niobium, tantalum and titanium (Table I). It can be seen that the results for titanium are somewhat high, probably because of residual amounts of sulphate.

TABLE I

SEPARATION AND DETERMINATION OF NIOBIUM, TANTALUM AND TITANIUM WITH N-BENZOYL-N-PHENYLHYDROXYLAMINE

mg Nb_2O_5 taken	*mg Ta_2O_5 taken*	*mg Nb_2O_5 + mg Ta_2O_5 taken*	*mg TiO_2 taken*	*mg Nb_2O_5 found*	*mg Ta_2O_5 found*	*mg Nb_2O_5 + mg Ta_2O_5 found*	*mg TiO_2 found*
143.4	55.2	—	none	143.4	54.2	—	—
143.4	55.2	—	none	142.4	54.6	—	—
—	—	204.1	133.4	—	—	204.3	134.8
—	—	204.1	133.4	—	—	204.6	133.4
—	—	204.1	133.4	—	—	204.0	134.4
—	—	204.1	133.4	—	—	203.2	135.9

* The addition of macerated filter paper at this stage prevents the precipitate from sticking to the walls of the beaker.

SUMMARY

A method is described for the separation and gravimetric determination of niobium, tantalum and titanium by precipitation with N-benzoyl-N-phenylhydroxylamine. Titanium is kept in solution with EDTA and hydrogen peroxide, and the earth acids are precipitated in 1*N* sulphuric acid. Niobium and tantalum are separated and determined by a modification of the method of MAJUMDAR AND MUKHERJEE. All three metals are finally precipitated with N-benzoyl-N-phenylhydroxylamine. In the analysis of complex materials niobium, tantalum and titanium are separated from other constituents by a double precipitation with N-benzoyl-N-phenylhydroxylamine in the presence of EDTA and tartaric acid.

RÉSUMÉ

Une méthode est décrite pour la séparation et le dosage gravimétrique du niobium, du tantale et du titane par précipitation au moyen de la benzoylphénylhydroxylamine. Le titane peut-être maintenu en solution par l'EDTA en présence de peroxyde d'hydrogène. Le niobium et le tantale sont dosés par la méthode proposée par MAJUMDAR ET MUKHERJEE. Le titane est également dosé par le même réactif.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur Trennung und gravimetrischen Bestimmung von Niob, Tantal und Titan durch Fällung mit Benzoylphenylhydroxylamin. Titan kann durch EDTA in Gegenwart von Wasserstoffperoxyd in Lösung gehalten werden. Niob und Tantal werden nach der von MAJUMDAR UND MUKHERJEE vorgeschlagenen Methode getrennt und bestimmt. Titan wird ebenfalls durch Fällung mit dem gleichen Reagenz bestimmt.

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1 E. BAMBERGER, *Ber.*, 52 (1919) 1116.
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3 R. W. MOSHIER AND E. SCHWARBERG, *Anal. Chem.*, 29 (1957) 947.
4 A. K. MAJUMDAR AND A. K. MUKHERJEE, *Anal. Chim. Acta*, 19 (1958) 23.
5 A. K. MAJUMDAR AND A. K. MUKHERJEE, *Anal. Chim. Acta*, 21 (1959) 245.
6 D. H. WILKINS, *Anal. Chim. Acta*, 20 (1959) 113.

Note added in proof

The procedure described in the present paper has been applied recently with satisfactory results in the complete analyses of Blomstrandine and Euxenite, two complex minerals containing essentially niobium, titanium, tantalum, uranium, thorium and yttria earths, as well as a series of minor and trace constituents.

In these analyses the use of hydrofluoric acid as decomposing agent for the minerals and for the oxides of niobium, tantalum and titanium was tried and was found to offer advantages compared with the use of potassium pyrosulphate. The coprecipitation of sulphate was considerably lowered, and in addition a quantitative separation of insoluble rare earth and alkaline earth fluorides from the soluble fluorides of earth acids and titanium was obtained. At the same time quadrivalent uranium was separated from sexivalent uranium. It was also possible to replace the initial double precipitation of niobium, tantalum and titanium (in the presence of EDTA and tartaric acid) by a single precipitation.

The analytical data obtained with the two decomposition methods compared favourably, but the method of attack by hydrofluoric acid seems to offer advantages in the analyses of minerals soluble in this acid.

Anal. Chim. Acta, 22 (1960) 301–305

SPECTROPHOTOMETRIC DETERMINATION OF OSMIUM

III. *o*-AMINOPHENOL-*p*-SULPHONIC ACID AS A REAGENT

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(Received June 10th, 1959)

In previous papers[1,2] we have discussed the spectrophotometric determinations of osmium present in different valence states by anthranilic and sulphanilic acids. In this paper the use of *o*-aminophenol-*p*-sulphonic acid as a new colorimetric reagent for osmium is described.

o-Aminophenol-*p*-sulphonic acid is a highly sensitive reagent which on reaction with osmium(VI) and (VIII) for 30 min at pH 2.5–4 and at room temperature (34°) gives an intense dark-brown colour with an absorption maximum at 440 mμ. The reagent has no action on tetra-, tri- and bivalent osmium.

EXPERIMENTAL

Apparatus

The optical density of solutions in quartz cells of 1-cm thickness was measured by a Hilger Uvispek Spectrophotometer and the pH was determined by a Cambridge pH meter.

Standard solutions and buffer

Standard solutions of osmium tetroxide and osmate (OsO_4^{-2}), solutions of other ions and the buffer were prepared as reported previously[1,2].

Reagent solution

o-Aminophenol-*p*-sulphonic acid (B.D.H.) was crystallised from water and an aqueous 1% solution of the sodium salt of the reagent was prepared.

Absorbance curves

For absorption studies, solutions were prepared by adding osmate (OsO_4^{-2}) or osmium tetroxide to the reagent solution in a 25-ml flask. The solution was made up to volume with sodium acetate–acetic acid buffer and allowed to stand for 30 min at room temperature. On plotting the optical density of the solutions, as measured against a reagent blank prepared similarly, the region of maximum absorption always occurred at 440 mμ, whether the osmium was in the octavalent or hexavalent state (*cf.* Fig. 1).

Effect of pH, reagent and time

Solutions containing fixed amounts of reagent and osmium were taken in 25-ml flasks and, after adjustment of their pH to different values, were made up to 25 ml

with water for the optical density measurements. pH values between 3.42 and 5.9 were obtained with sodium acetate–acetic acid buffers and lower or higher pH values by the addition of sulphuric acid or of sodium hydroxide. The optical density increases with increasing pH from 1 to 2.5, remains constant from pH 2.5 to 4.0 and gives low values above pH 4.0. The pH range 2.5 to 4 was found suitable for all subsequent measurements.

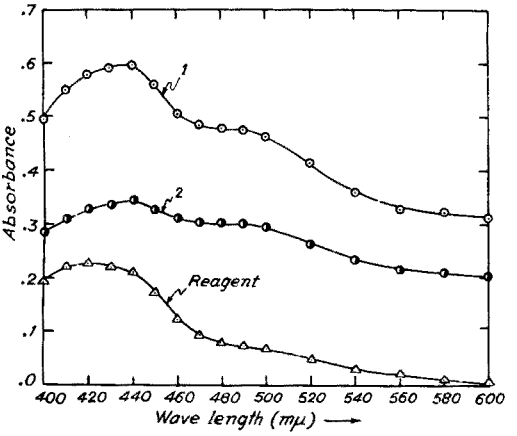

Fig. 1. Absorption curves of *o*-aminophenol-*p*-sulphonic acid (sodium salt) and its osmium complex at pH 3.4. Curve 1 6 p.p.m. osmium-(VIII) + 5 ml of 1% reagent. Curve 2. 4 p.p.m. osmium(VI) + 5 ml of 1% reagent.

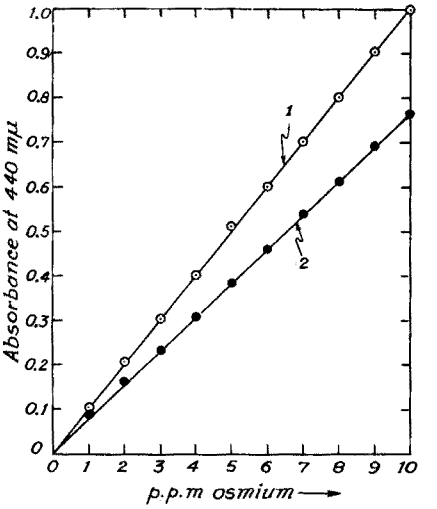

Fig. 2. Beer's law plots for (1) osmium-(VIII) and (2) osmium(VI); pH 3.4.

3 ml of the reagent was sufficient for full development of colour with 1 to 10 p.p.m. of osmium. Addition of more reagent did not alter the intensity of the colour. The colour developed immediately but did not remain constant until 30 min had elapsed at room temperature. Even overnight standing had no influence on the colour system.

Beer's law, optimum range and accuracy

Beer's law is obeyed for 1 to 10 p.p.m. of both osmium(VI) and osmium(VIII) (Fig. 2). Measurements were carried out at pH 3.42 against a reagent blank.

As in the previous case[1], the percent relative error per 1% photometric error was plotted against transmittancy to show how the analysis error varied with transmittancy (Fig. 3). Again plotting the % absorbancy at 440 mμ against log concentration (Fig. 4) shows that the optimum concentration range for both osmium(VI) and (VIII) is from 2 to 8 p.p.m. and for this range the % relative errors per 1% absolute photometric error are 3.1 and 2.98 for osmium(VI) and (VIII), respectively. This difference in the relative error is not due to any inherent defect in the method. Since alcohol, which is used to reduce the osmium(VIII) to osmium(VI), lowers the optical density of the colour system (as is evident from the sensitivity and molar extinction coefficient values given below as well from the curves in Figs. 1, 2 and 4) the percent relative error is higher with osmium(VI).

Sensitivity and molar extinction coefficient

SANDELL'S spectrophotometric sensitivities, as calculated from Beer's law curves, were found to be 0.012 $\mu g/cm^2$ for osmium(VI) and 0.01 $\mu g/cm^2$ for osmium(VIII). The molecular extinction coefficients were 15691.5 and 19495.5 for osmium(VI) and (VIII), respectively.

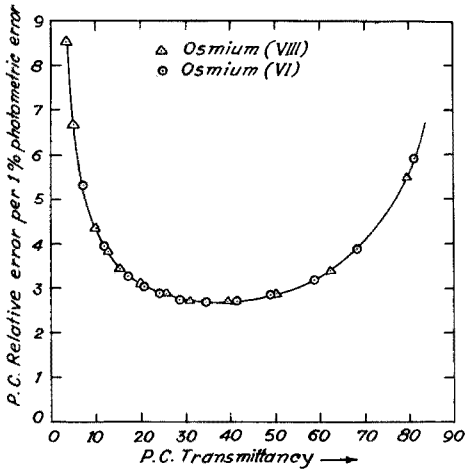

Fig. 3. Evaluation of percent relative error ($dc/c \times 100$) relative to 1% absolute photometric error.

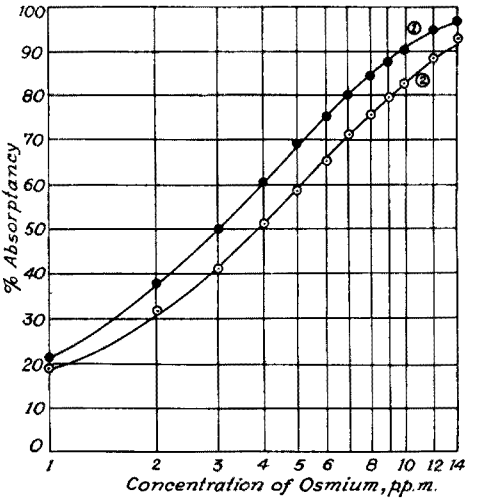

Fig. 4. Calibration curves for (1) osmium(VIII) and (2) osmium(VI) at 440 mμ.

Effect of foreign ions

Osmium(VI) can be determined only in the presence of moderate amounts of Pd^{+2}, Ir^{+4}, WO_4^{-2}, Cr^{+3}, Zn^{+2}, Zr^{+4}, Mg^{+2}, Ba^{+2} and Sr^{+2}. Ions such as Ru^{+3}, Rh^{+3}, UO_2^{+2}, Cu^{+2}, Hg^{+2}, As^{+3}, Bi^{+3}, Fe^{+3}, Al^{+3}, Co^{+2}, Ni^{+2}, Mn^{+2} and Ca^{+2} interfere even at a 1:1 ratio.

Nature of the complex in solution

The Job[5], molar ratio[6] and slope ratio[7] methods confirmed that osmium(VI) forms a 1:2 complex with the reagent.

For these methods, one drop of 6 *M* sulphuric acid was added to the mixed solution of osmium(VI) and the reagent. On the development of the colour the volume of each solution was made up to 25 ml with sodium acetate–acetic acid buffer of pH 3.42. For the slope ratio method, the first series of solutions contained a large excess of the reagent (5 ml of $0.223 \cdot 10^{-3}$ *M*) and varying amounts of osmium(VI) and in the second series the concentrations of osmium(VI) and the reagent were reversed. The optical densities of all the solutions were measured after an hour. The results are graphically represented in Figs. 5, 6 and 7.

With the molar ratio method, a sharp break was found to occur at the osmium-(VIII) to reagent ratio of 1:3 (*cf.* Fig. 6). This ratio can be explained by assuming that one molecule of the reagent is oxidised by osmium tetroxide and then a 1:2

complex formation takes place between the reduced osmium(VI) and the unoxidised excess of reagent. That osmium tetroxide and osmate (OsO_4^{-2}) yield the same complex in solution is obvious from their identical colour reaction, tolerance to pH, region of absorption maximum and adherence to Beer's law.

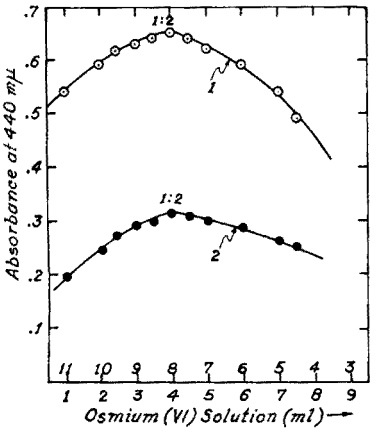

Fig. 5. Determination of the ratio of reagent to osmium(VI) by Job's method. Curve 1. Concentration of osmium(VI) = concentration of *o*-aminophenol-*p*-sulphonic acid (sodium salt) = $0.4325 \cdot 10^{-3}$ *M*; Curve 2. Concentration of osmium(VI) = concentration of *o*-aminophenol-*p*-sulphonic acid (sodium salt) = $0.223 \cdot 10^{-3}$ *M*. Total volume in each case 12 ml.

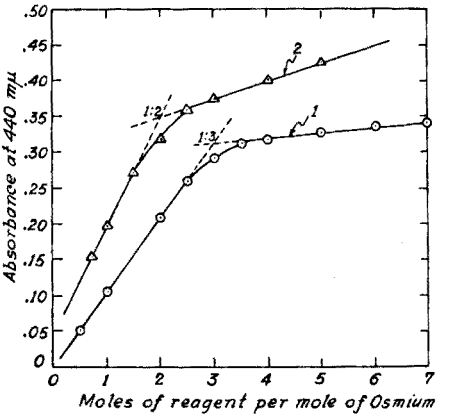

Fig. 6. Determination of the reaction ratios of reagent to (1) osmium(VIII) and (2) osmium(VI) by the molar ratio method. Concentrations of osmium(VIII) and osmium(VI) were kept constant at $2.09 \cdot 10^{-5}$ *M* and $3.46 \cdot 10^{-5}$ *M* respectively and varying amounts of reagent were added.

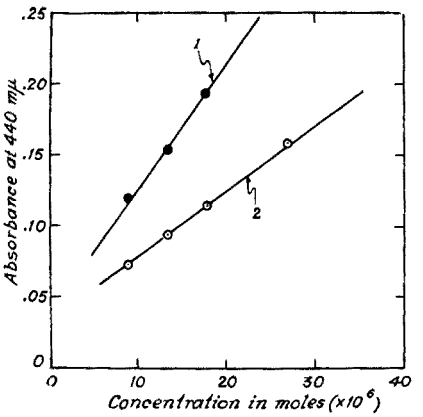

Fig. 7. Determination of the ratio of reagent to osmium(VI) by the slope ratio method. $Slope_1$ (reagent excess) = 1.44; $slope_2$ (osmium excess) = 0.73.

$\frac{Slope_1}{Slope_2}$ = 1.97, *i.e.*, a ratio of 1:2 (approx.).

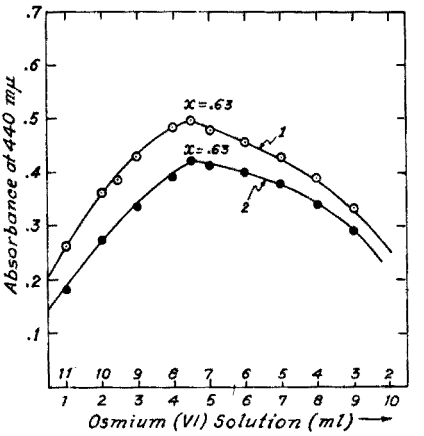

Fig. 8. Absorbance of mixtures of non-equimolecular solutions. Curve 1. $0.223 \cdot 10^{-3}$ *M* osmium(VI); $0.446 \cdot 10^{-3}$ *M* *o*-aminophenol-*p*-sulphonic acid (sodium salt). Curve 2. $0.191 \cdot 10^{-3}$ *M* osmium(VI); $0.382 \cdot 10^{-3}$ *M* *o*-aminophenol-*p*-sulphonic acid (sodium salt).

Dissociation constant of the osmium(VI)–reagent complex

The dissociation constant of the complex was evaluated from a study of the absorptions of the mixtures of non-equimolecular solutions. The colour of the solutions was developed as described above. From the data in Fig. 8, the dissociation constant, as calculated from Job's equation[1], is 1.2×10^{-7} at 34°.

Note

1-Amino-8-naphthol-3,6-disulphonic acid has been examined in exactly the same way as the monosulphonic acid for the spectrophotometric determination of osmium-(VI) and (VIII). In this case the optimum conditions are 4 ml of reagent solution, a pH range of 4.5–6 and a 2-h standing time for colour development. The absorption maximum occurs at 480 mμ; both reagents have the same optimum concentration range of osmium. The average dissociation constant with the disulphonic acid reagent is $1.2 \cdot 10^{-9}$ at 36° and the molar extinction coefficients are 14930.7 and 16642.5 for osmium(VI) and (VIII) respectively.

SUMMARY

o-Aminophenol-*p*-sulphonic acid is suggested as a very sensitive reagent for spectrophotometric determination of osmium (VI) and (VIII) at pH 2.5–4. The absorption maximum is at 440 mμ and the optimum concentration range is from 2 to 8 p.p.m. of osmium. Moderate amounts of Pd^{+2}, Ir^{+4}, WO_4^{-2}, Cr^{+3}, Zn^{+2}, Zr^{+4}, Mg^{+2}, Ba^{+2} and Sr^{+2} do not interfere.

RÉSUMÉ

L'acide *o*-aminophénol-*p*-sulfonique constitue un réactif très sensible pour le dosage spectrophotométrique de l'osmium octavalent et de l'osmium hexavalent. Absorption maximum à 440 mμ. Concentration optimum comprise entre 2 et 8 p.p.m. d'osmium.

ZUSAMMENFASSUNG

Für die spektrophotometrische Bestimmung von Osmium(VI) und Osmium(VIII) hat sich *o*-Aminophenol-*p*-sulfosäure als sehr empfindliches Reagenz erwiesen. Der günstigste Konzentrationsbereich liegt bei 2 bis 8 p.p.M. Osmium; Absorptionsmaximum bei 400 mμ.

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A SYSTEMATIC SCHEME OF QUALITATIVE ANALYSIS FOR ANIONS

V. A SIMPLIFIED SCHEME FOR THE DETECTION OF COMMON ANIONS

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A systematic and comprehensive scheme[1] of qualitative analysis for anions has been put forward from this laboratory. In this scheme a large number of anions derived both from the common and rare-elements have been included and therefore, only a fairly advanced student can be expected to tackle the problem of detecting anions in such complex mixtures. The scheme can, however, be simplified to meet the requirements of the less advanced student of chemical analysis whose knowledge with regard to the analytical behaviour of elements is limited. The following procedures and table show how the original scheme can be modified to provide for the detection of only the common anions. The detection of anions by this method will enable the student not only to do this part of the work systematically and therefore, with greater confidence, but also to tackle more easily the detection of anions in certain combinations more easily. It will be seen from the table that the detection of acids in the following combinations can be carried out with much greater ease than by the usual methods. 1. Ferro and ferricyanides. 2. Fluoride and oxalate. 3. Sulphite, sulphate and thiosulphate. 4. Arsenite, arsenate and phosphate. Tests for borate, acetate, nitrate and nitrite, which were not included in the more comprehensive scheme, have been included in this simplified scheme.

PROCEDURES

I. 2–3 g of the mixture is boiled with 8–10 g of sodium carbonate (AnalaR quality) and nearly 100 ml of water for about 10 min and then filtered. The filtrate will contain the sodium salts of practically all acids, with the exception of certain acids, which form part of insoluble salts like sulphides of copper and zinc groups, silver halides, etc.

II. The sodium carbonate filtrate is treated with excess of lead carbonate, boiled, cooled to room temperature and filtered. A black or grey precipitate shows the presence of sulphide. The sulphides of arsenic, antimony and tin dissolve in sodium carbonate to give a mixture of thio- and oxythio-salts. Lead carbonate converts thio- and oxythio-salts into oxy-salts with the precipitation of lead sulphide.

III. A small portion of the filtrate from procedure II, is treated with a strong solution of zinc nitrate in excess, the precipitate filtered off and the neutral filtrate tested for acetate with $FeCl_3$. The rest of the filtrate from procedure II is treated with zinc acetate solution (about 1–2 *M*) until no further precipitation takes place,

TABLE I

Sodium carbonate filtrate containing — S^{-2}, $Fe(CN)_6^{-4}$, $Fe(CN)_6^{-3}$, $C_2O_4^{-2}$, F^-, $B_4O_7^{-2}$, PO_4^{-3}, SO_3^{-2}, AsO_4^{-3}, AsO_3^{-3}, SO_4^{-2}, $S_2O_3^{-2}$, Cl^-, Br^-, I^-, ClO_3^-, NO_2^-, NO_3^- and CH_3COO^-. Treat with excess of $PbCO_3$ and filter

Ppt: Black or grey PbS. Confirms S^{-2}	Filt: Rest of the anions. Treat a small portion of the solution with zinc nitrate solution in excess, filter off the precipitate and test for acetate with $FeCl_3$ in the filtrate. Treat the remaining portion with $Zn(CH_3COO)_2$ solution in excess heat to boiling and filter						
	Ppt: Zinc salts of $Fe(CN)_6^{-4}$, $Fe(CN)_6^{-3}$, $C_2O_4^{-2}$, $(F)^-$, PO_4^{-3}, AsO_4^{-3}, AsO_3^{-3}, $B_4O_7^{-2}$, SO_3^{-2}. Treat with excess of NH_4OH and filter					Filt: F^-, SO_4^{-2}, $S_2O_3^{-2}$, Cl^-, Br^-, I^-, ClO_3^-, (AsO_3^{-3}), NO_2^-, NO_3^- and CH_3COO^-. Test small portions for nitrite and nitrate as shown below. Add $Ca(NO_3)_2$ solution in excess and filter	
	Ppt: $Zn_2Fe(CN)_6$ dissolve in conc. HCl and add $FeCl_3$. Blue ppt. Confirms $Fe(CN)_6^{-4}$	Filt: (F^-), PO_4^{-3}, AsO_4^{-3}, AsO_3^{-3}, $C_2O_4^{-2}$, $B_4O_7^{-2}$, SO_3^{-2}, $Fe(CN)_6^{-3}$. Add magnesia mixture in excess, 10 ml of conc. NH_4OH and filter				Ppt: CaF_2. Confirm by SiF_4 test	Filt: SO_4^{-2}, $S_2O_3^{-2}$, Cl^-, Br^-, I^-, ClO_3^-, (AsO_3^{-3}) NO_2^-, NO_3^- and CH_3COO^-. Add $Ba(NO_3)_2$ solution in excess and filter	
		Ppt: Magnesium salts of (F^-), PO_4^{-3}, AsO_4^{-3} and AsO_3^{-3}. Dissolve the precipitate in dil. CH_3COOH and add excess of $Ca(NO_3)_2$ solution and filter	Filt: $C_2O_4^{-2}$, $B_4O_7^{-2}$, SO_3^{-2} and $Fe(CN)_6^{-3}$. Add $Ca(NO_3)_2$ and $Ba(NO_3)_2$ solutions in excess and filter			Ppt: White $BaSO_4$ insoluble in conc. HCl shows SO_4^{-2}	Filt: $S_2O_3^{-2}$, Cl^-, Br^-, I^-, ClO_3^-, (AsO_3^{-3}), NO_2^-, NO_3^- and CH_3COO^-. Add $AgNO_3$ solution in excess and filter	
			Ppt: Ca and Ba salts of $C_2O_4^{-2}$, SO_3^{-2} and BO_2^-. Treat with dil. acetic acid and filter	Filt: $Fe(CN)_6^{-3}$ and BO_3^{-3} acidify the solution with acetic acid and filter			Ppt: Silver salts of S^{-2} (from $S_2O_3^{-2}$), Cl^-, Br^-, I^- and (AsO_3^{-3}). Test a portion of the ppt. with Zn + HCl for S^{-2}, then treat with 1 N HNO_3, heat and filter	Filt: ClO_3^-, NO_3^-, NO_2^- and CH_3COO^-. Test only for ClO_3^- with $NaNO_2$ as usual

	CaF_2 Confirm by SiF_4 test	AsO_4^{-3} and AsO_3^{-3}. Neutralize the acid with NaOH and add about 30 ml of 1 *N* Na_2S reagent and filter	Ba salts of $C_2O_4^{-2}$ and SO_3^{-2}. Boil with 10 ml of 10% Na_2CO_3 solution and filter off the ppt. of $CaCO_3$ and $BaCO_3$. Acidify the filtrate with CH_3COOH and add $Ca(NO_3)_2$ in excess. Filter	BO_2^-. Add $AgNO_3$ and heat. Brown ppt. of Ag_2O. Confirms $B_4O_7^{-2}$	Zinc ferricyanide dissolve in HCl and add $FeSO_4$. Blue ppt. Confirms $Fe(CN)_6^{-3}$	BO_3^{-3}. Test with $AgNO_3$ as before		Silver salts of Cl^-, Br^- and I^-. Test as usual	HNO_3 and Ag^+. Add dil. HCl and filter off AgCl. In the filtrate add Na_2S and HCl. Yellow ppt. Confirms AsO_3^{-3}
Ppt: Phosphate of Ca and Mg. Dissolve in HNO_3 and test for PO_4^{-3} by adding amm. molybdate. Yellow ppt. Confirms PO_4^{-3}	Filt: Thiosalts of AsO_3^{-3} and AsO_4^{-3}. Treat with equal volumes of 2 *N* HCl, shake and filter										
	Ppt: Yellow As_2S_3 insoluble in 6 *N* HCl shows AsO_3^{-3}	Filt: Thiosalt of AsO_4^{-3}. Add conc. HCl and heat to boiling. Yellow ppt. of As_2S_5. Confirms AsO_4^{-3}	Ppt: Ca-C_2O_4. Confirm as usual	Filt: SO_3^{-2} add bromine water, HCl and Ba-$(NO_3)_2$ white ppt. Confirms SO_3^{-2}							

Test for NO_2^-: Acidify a portion with dilute H_2SO_4 and heat. Brown fumes confirms NO_2^-.
Test for NO_3^-: Remove the halogens by Ag_2SO_4 in a portion and test for NO_3^- by the ring test as usual.

boiled for 2–3 minutes and filtered. This will precipitate $Fe(CN)_6^{-3}$, $Fe(CN)_6^{-4}$, AsO_4^{-3}, SO_3^{-2}, $C_2O_4^{-2}$, PO_4^{-3} and $B_4O_7^{-2}$ completely and F^-, AsO_3^{-3} partially. The precipitate is washed 5–6 times with distilled water, only the first one or two washings being added to the main filtrate, the rest rejected to avoid increasing the volume unnecessarily.

The precipitate is divided into 3 sub-groups as follows: a. It is treated with excess of ammonium hydroxide, warmed and filtered. For satisfactory separation the final strength of the ammonium hydroxide should be between 2–6 *N*. The undissolved portion is tested for $Fe(CN)_6^{-4}$. b. The ammoniacal filtrate from sub-group (a) is treated with magnesia mixture in slight excess and about 10 ml of concentrated ammonia is added to precipitate arsenite completely. It is heated nearly to boiling and filtered after 5–10 minutes. The precipitate will contain magnesium salts of F^-, AsO_3^{-3}, AsO_4^{-3} and PO_4^{-3}. The precipitate is washed with 2 *N* ammonium hydroxide and is tested for these acids as shown in the table. c. The filtrate from sub-group (b) is treated with excess of barium and calcium nitrate solutions, heated nearly to boiling and filtered. The precipitate contains the calcium and barium salts of $C_2O_4^{-2}$, BO_2^- and SO_3^{-2} and the filtrate soluble salts of $Fe(CN)_6^{-3}$ and borate. The precipitate and filtrate are analysed as shown in the table.

IV. Two small portions of the neutral filtrate from procedure III are tested for nitrite and nitrate as shown in the table. The rest of the filtrate is treated with calcium nitrate solution, any precipitate of calcium fluoride is filtered off and tested for fluoride as usual.

The filtrate from calcium fluoride is treated with barium nitrate solution, heated to boiling and filtered. The presence of sulphate in the precipitate is confirmed by treating it with concentrated hydrochloric acid. Barium thiosulphate is not precipitated owing to its tendency to form supersaturated solution.

V. The neutral filtrate from procedure IV is treated with excess of silver nitrate solution and filtered. The precipitate will contain the silver salts of S^{-2} (from $S_2O_3^{-2}$), Cl^-, Br^-, I^- and (AsO_3^{-3}) and the filtrate ClO_3^-. The precipitate and the filtrate are analyzed as shown in the table.

SUMMARY

The original comprehensive scheme for the detection of anions has been simplified for the systematic detection of common anions.

RÉSUMÉ

Un schéma d'analyse qualitative simplifié est proposé pour l'identification des anions courants.

ZUSAMMENFASSUNG

Es wird ein vereinfachtes Schema zum Nachweis der häufiger vorkommenden Anionen beschrieben.

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FLAME SPECTRA OF Sc, Y AND RARE-EARTH ELEMENTS*

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INTRODUCTION

The detection and determination of small quantities of rare-earth elements is of growing importance in nuclear fuel technology. Several of these elements, which accumulate in nuclear reactors as fission products, exert an inordinately detrimental effect on the neutron economy of reactors because of their extremely large capture cross section for thermal neutrons. For this reason, especially, they are undesirable as impurities in nuclear fuels or breeder materials. Since the rare-earth elements comprise a considerable portion of the fission products in spent fuels, methods for reprocessing fuels or blanket materials must be capable of reducing the concentration of rare-earth elements to tolerable limits. Reliable methods for the estimation of elements of this group are, therefore, essential in the evaluation and control of reprocessing procedures, as well as in the operation of reactors.

Unfortunately, specific and relatively sensitive methods for the determination of the rare-earth elements are lacking. Because of the similarity of chemical properties of the elements of the rare-earth group, spectrophotometric methods, utilizing chromogenic reagents, are suitable only for estimation of the total elements of this group[1]. The characteristic absorbance spectra of the rare-earth elements are not intense enough to provide the requisite sensitivity. In fact, several of these elements (yttrium, lanthanum, and ytterbium) do not absorb light in the visible region[2]. Spectrographic methods, which are used most frequently for the analysis of mixtures of rare-earth elements, suffer from the difficulty of resolving the complex spectra[3-5]. As a result of the intensified interest in the rare-earth elements, advances have recently been made in methods of analysis pertaining to this group of elements. FELDMAN AND ELLENBURG[6] have reported a method for separation of the rare-earth elements from thorium and subsequently determining them by spectrographic means. PINTA[3] studied the flame spectra of sixteen rare-earth and closely related elements. He aspirated aqueous solutions of the elements into an air–acetylene flame and used a prism spectrograph to record the emission bands and lines on photographic plates. On the basis of this study, he developed a procedure for the determination of a number of these elements.

The objective of the present investigation was to study the complex spectra of the

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rare-earth elements and the closely related elements, yttrium and scandium, more thoroughly for the purpose, primarily, of evaluating, extending and improving flame photometric methods for the estimation of the elements of this group. Chelates of the different rare-earth elements with 2-thenoyltrifluoroacetone (TTA) were extracted into an organic solvent[7], the organic extract was introduced into an oxy–hydrogen flame and the individual flame spectra were recorded by a flame photometer. Furthermore, the flame spectra of elements of this group in aqueous media were photographically recorded with a prism spectrograph to provide data for fixing the position accurately of the characteristic bands and lines. By use of an organic medium, the emissivity was greatly enhanced; in many cases, the enhancement was of the order of 100 times the emissivity attainable with aqueous solutions. By these means, as has been reported in the case of lanthanum[8], more intense, detailed spectra were obtained whereby the limits of detection of the elements can be extended. Lower concentrations of the several elements can thereby be estimated than was previously possible.

EXPERIMENTAL WORK

Apparatus

Oak Ridge National Laboratory recording flame spectrophotometer, Model No. Q-1457A[9]. This is a single-beam, high-sensitivity instrument equipped with RCA 6217 and Farnsworth 16PMI multiplier phototubes and a modified atomizer-burner from a Beckman flame photometer. Lacquer was removed from the adjusting screws used to align the palladium capillary of the burner and the screws were then silver-soldered in place. This modification was made to insure that the burner would function properly when organic solvents were aspirated into it. In addition, the flame was shielded to mask out light from the luminous cone and thus minimize interference from CH and C_2 bands when organic media are used to introduce elements into the oxy-hydrogen flame.

Reagents

4-Methyl-2-pentanone (hexone), practical grade. 2-Thenoyltrifluoroacetone (TTA), 0.1 *M* in hexone. This reagent was prepared by dissolving 22 g of technical-grade TTA in hexone, and then diluting the solution to one liter with hexone. It is stored in a cool place away from light.

Stock solutions of the rare-earth elements, 1 mg per ml. An amount of the rare-earth oxide required to give 50 mg of the element was dissolved in 10 ml of conc. HCl. This solution was transferred to a 50-ml volumetric flask and diluted to volume with 1 *N* HCl. (The oxides of rare-earth elements were obtained from Lindsay Chemical Division, American Potash and Chemical Corporation).

Acetate buffer solution, 5 *M*, pH 5.5. This reagent was prepared by dissolving 385 g of ammonium acetate in water and diluting to 600 ml with water. The pH of the solution was then adjusted to 5.5 by addition of 5 *N* acetic acid, after which it was diluted to one liter with water.

Recording of spectra

Solutions of the chelates of the rare-earth elements with 2-thenoyl-trifluoroacetone (TTA) in 4-methyl-2-pentanone (hexone) were used in recording the flame spectra of the several elements. These solutions were prepared as follows: an aliquot of the stock solution of the element which usually contained one mg of the element (exceptions: Pr, 10 mg; Nd, 2 mg) was transferred to a 60-ml separatory funnel. Eight ml of 5 *M* acetate buffer solution (pH 5.5) was added and the solution was diluted to 20 ml with water. A 20-ml portion of the 0.1 *M* solution of TTA in hexone was then added and the separatory funnel was shaken for three minutes. After the phases had separated, the organic phase was transferred to a flask and used for the determination of the flame spectrum.

The flame spectra were recorded by means of an ORNL single-beam recording flame spectrophotometer. The organic solution of the element was aspirated into the flame and the emissivity was continuously recorded over the wave length range from 380 to 900 mμ.

Operating conditions were as follows:

Sensitivity control, High; Phototube resistor, 5 MegOhms; Phototube, Blue sensitive, RCA-6217, 80 V/dynode; Red sensitive, 16-PMI, 80 V/dynode; Slit width, 0.25 mm; Spectral slit width, 1.6 mμ; Gas flow rate, Oxygen, 5100 cm^3/min; Hydrogen, 3600 cm^3/min.

Over the wave length range from 380 to 700 mμ, the blue-sensitive phototube, RCA-6217, was

used, while, at wave lengths from 700 to 900 mμ emissivity measurements were made with the red-sensitive multiplier phototube, Farnsworth 16-PMI[10]. As indicated above, all spectra were recorded with a phototube potential of 80 V per dynode, even though the phototubes will operate satisfactorily at 100 V per dynode and, at the higher voltage, the sensitivity (emissivity per unit of element per ml in test solution) is 10 times as great as at 80 V per dynode. The lower voltage was used in recording the spectra because, under the operating conditions given, at the higher voltage prominent bands or lines would be off scale. In calculating the maximum spectral intensities, which are listed in Table I, the emissivities at 100 V per dynode were used. These were computed from the data taken at 80 V per dynode with a slit width of 0.25 mm, utilizing the known constant ratio between emissivity at 100 V per dynode and emissivity at 80 V per dynode. This ratio is independent of wave length.

TABLE I

WAVE LENGTHS AND RELATIVE SPECTRAL INTENSITY OF THE RARE-EARTH ELEMENTS BY FLAME EMISSION

Conditions: ORNL Flame Spectrophotometer, Multiplier phototube, blue, RCA — 6217, red, Farnsworth — 16-PMI; Voltage per dynode — 100; Slit width, 0.25 mm; Gas flow rate: O_2, 5100 cm^3/min, H_2, 3600 cm^3/min; Medium, TTA in hexone, 0.1 *M*;
Hilger, large Littrow Spectrograph, Photograph plates: Kodak (3) 103a-F; Kodak I—N; Exposure time, 4–10 minutes; Medium, HCl, 1 *N*.

Element	*Spectrographic line and band maxima, mμ*	*Spectrophotometric*		
		Line and band maxima, mμ	*Width of band, mμ*	*Relative spectral intensity scale div./μg/ml*
Scandium	450.3	450	448–485	1
	453.6	453	448–485	2
	457.1	457	448–485	2
	460.7	462	448–485	2
	467.3	466	448–485	7
	470.7	n	—	—
	n	473	448–485	7
	474.2	475	448–485	5
	485.8	486	485–500	20
	489.3 NL	n	—	—
	509.7	n	—	—
	513.4	n	—	—
	517.1 NL	n	—	—
	573.7	574	570–635	5
	577.4	578	570–635	14
	581.2	582	570–635	17
	584.9	586	570–635	16
	592.3	592	570–635	10
	596.5	595	570–635	6
	603.2	602	570–635	88
	607.2	607	570–635	170
	610.9	610	570–635	130
	614.8	n	—	—
	619.3	n	—	—
	644.3	642	640–675	1
	648.2	647	640–675	2
	649.6	650	640–675	2
	656.7	657	640–675	2
Yttrium	470.7	468	464–480	4
	481.8	484	480–500	12
	505.1	506	502–560	3
	507.9	509	502–560	3
	527.5	527	502–560	1
	573.0	n	—	—
	575.6	575	565–630	4

TABLE I (Continued)

Element	Spectrographic line and band maxima, mμ	Spectrophotometric		
		Line and band maxima, mμ	Width of band, mμ	Relative spectral intensity scale div./μg/ml
Yttrium	593.9	n	—	—
	595.6	n	—	—
	597.8	598	565–630	80
	600.4	n	—	—
	603.7	n	—	—
	613.7	614	565–630	80
	616.5	n	—	—
	618.2	n	—	—
	620.0	n	—	—
Lanthanum	437.4	437	435–450	8
	441.8	442	435–450	9
	443.6	n	—	—
	538.0	n	—	—
	540.7	n	—	—
	543.2	n	—	—
	545.8	545	537–556	3
	560.2	560	557–585	11
	562.8	562	557–585	11
	586.8	n	—	—
	701.1	n	—	—
	702.4	n	—	—
	704.1	n	—	—
	705.5	n	—	—
	707.0	n	—	—
	708.5	n	—	—
	710.1	n	—	—
	713.2	n	—	—
	716.3 NL	n	—	—
	719.4	n	—	—
	738.0	n	—	—
	740.6	741	735–765	8
	743.7	744	735–765	8
	762.5	n	—	—
	787.7	n	—	—
	791.1	791	785–820	8
	794.5	795	785–820	7
	808.6	n	—	—
	812.2	n	—	—
	815.9	n	—	—
	845.4	n	—	—
	849.0	n	—	—
	852.7	n	—	—
	856.4	n	—	—
Praseodymium	n	416	410–750	0.1
	n	438	410–750	0.3
	n	452	410–750	0.3
	n	460	410–750	0.3
	n	467	410–750	0.3
	n	473	410–750	0.2
	n	482	410–750	0.3
	n	492	410–750	0.3
	n	502	410–750	0.2
	n	512	410–750	0.3
	n	518	410–750	0.4
	n	530	410–750	0.3

TABLE I (Continued)

Element	*Spectrographic line and band maxima, mμ*	*Spectrophotometric*		
		Line and band maxima, mμ	*Width of band, mμ*	*Relative spectral intensity scale div.μg/ml*
Praseodymium	535.1	n	—	—
	n	540	410–750	0.5
	n	562	410–750	0.6
	569.2	569	410–750	0.8
	576.4	577	410–750	0.8
	n	594	410–750	0.3
	602.0	n	—	—
	603.8	604	410–750	3
	n	614	410–750	0.6
	n	618	410–750	0.5
	628.0	628	410–750	0.4
	629.8	630	410–750	0.7
	636.0	637	410–750	0.6
	644.0	n	—	—
	647.6	649	410–750	0.5
	n	655	410–750	0.4
	n	664	410–750	0.3
	n	672	410–750	0.2
	681.8	n	—	—
	688.7	n	—	—
	n	695	410–750	0.4
	709.7	709	410–750	0.4
	732.2	735	410–750	0.3
	n	748	410–750	0.2
	n	830	820–840	<0.1
	849.0	n	—	—
	n	930	920–940	<0.1
Neodymium	462.5	465	460–470	0.5
	495.9	n	—	—
	513.8	515	500–585	0.5
	532.4	533	500–585	0.5
	568.0	n	—	—
	572.6	574	500–585	0.5
	597.5	n	—	—
	599.7	600	595–610	1
	621.9	622	610–730	1
	635.2	n	—	—
	637.0	n	—	—
	638.6	638	610–730	2
	644.5	645	610–730	2
	662.5	662	610–730	4
	n	700	610–730	3
	n	712	610–730	3
	741.5 NL	n	—	—
Samarium	441.9*	n	—	—
	444.2*	n	—	—
	458.2*	n	—	—
	461.5*	462	450–500	1
	467.1*	n	—	—
	472.9*	473	450–500	1
	476.0*	n	—	—
	484.2*	n	—	—
	488.4*	n	—	—
	520.1*	n	—	—

TABLE I (Continued)

Element	Spectrographic line and band maxima, mμ	Spectrophotometric		
		Line and band maxima, mμ	Width of band, mμ	Relative spectral intensity scale div./μg/ml
Samarium	527.1*	n	—	—
	603.4	n	—	—
	607.2	n	—	—
	613.7	613	570–700	8
	624.1	623	570–700	9
	639.6	638	570–700	8
	652.3	652	570–700	8
Europium	413.0*	412	410–422	1
	420.5*	420	410–422	1
	443.6*	n	—	—
	459.4*	459*	—	22
	462.7*	463*	—	18
	466.2*	466*	—	15
	564.8*	n	—	—
	576.5*	575	570–700	2
	601.8	598	570–700	13
	C	625	570–700	11
	C	648	570–700	9
	C	655	570–700	8
	C	684	570–700	3
Gadolinium	446.3	n	—	—
	448.1	n	—	—
	449.9	450	445–500	2
	461.6	n	—	—
	464.3	464	445–500	8
	479.9	n	—	—
	481.7	n	—	—
	484.4	484	445–500	2
	489.2	n	—	—
	492.8	492	445–500	4
	513.4	514	510–640	1
	540.5 NL	n	—	—
	545.0 NL	546	510–640	3
	566.5 NL	n	—	—
	568.1	n	—	—
	569.9	570	510–640	7
	580.7	n	—	—
	582.1	582	510–640	15
	591.8	n	—	—
	593.3	n	—	—
	598.7	599	510–640	27
	600.7	n	—	—
	609.1	n	—	—
	612.4	612	510–640	27
	614.3	n	—	—
	616.7	n	—	—
	619.4 NL	n	—	—
	622.4	622	510–640	32
	624.2	n	—	—
Terbium	447.2	448	445–490	1
	457.3	457	445–490	1
	460.7	460	445–490	2
	472.7*	n	—	—

TABLE I (Continued)

Element	*Spectrographic line and band maxima, mμ*	*Spectrophotometric*		
		Line and band maxima, mμ	*Width of band, mμ*	*Relative spectral intensity scale div./μg/ml*
Terbium	478.5	478	445–490	1
	514.1	515	505–640	1
	527.0	525	505–640	2
	534.7*	535	505–640	10
	560.3	560	505–640	6
	564.0*	565	505–640	7
	572.7	574	505–640	11
	585.8	n	—	—
	592.1	n	—	—
	594.1	n	—	—
	598.0	598	505–640	13
	605.7	605	505–640	8
	607.3	n	—	—
	607.9	n	—	—
	616.1	615	505–640	3
	635.1	n	—	—
Dysprosium	418.7*	n	—	—
	421.2*	n	—	—
	453.4	454	445–465	1
	456.9	457	445–465	1
	514.0	515	500–640	5
	524.7	n	—	—
	526.3*	n	—	—
	528.0	527	500–640	15
	540.0	541	500–640	11
	549.3	550	500–640	10
	569.5	n	—	—
	572.9	575	500–640	18
	583.3	584	500–640	15
	600.7	n	—	—
	605.1	608	500–640	3
Holmium	446.5 NL	n	—	—
	449.0 NL	n	—	—
	510.5	510	500–545	7
	514.3	n	—	—
	515.7*	515	500–545	10
	527.6	527	500–545	7
	532.0	532	500–545	6
	556.4	555	550–600	5
	560.9	560	550–600	9
	565.9	566	550–600	25
	569.6*	n	—	—
	572.8	n	—	—
	585.1	585	550–600	2
Erbium	498.6	n	—	—
	504.0	503	485–585	12
	506.6	n	—	—
	514.7	514	485–585	5
	545.1	546	485–585	8
	552.0	552	485–585	13
	560.8	560	485–585	8
	566.3	566	485–585	6

TABLE I (Continued)

Element	Spectrographic line and band maxima, mμ	Spectrophotometric		
		Line and band maxima, mμ	Width of band, mμ	Relative spectral intensity scale div./μg/ml
Thulium	409.4*	409	407–425	1
	410.6*	n	—	—
	418.8*	418	407–425	1
	420.4*	n	—	—
	436.0*	n	—	—
	438.6*	n	—	—
	473.4*	n	—	—
	481.5 NL	483	470–580	7
	489.3 NL	n	—	—
	493.9 NL	492	470–580	10
	522.6 NL	523	470–580	4
	532.9 NL	n	—	—
	534.8 NL	n	—	—
	539.5 NL	538	470–580	9
	541.5 NL	542	470–580	8
	553.0 NL	553	470–580	7
	558.8 NL	n	—	—
	567.6*	n	—	—
Ytterbium	398.8*	399*	—	60
	451.5 NL	n	—	—
	474.2	474	465–562	5
	475.6	n	—	—
	476.5	n	—	—
	477.4	478	465–562	7
	484.1	484	465–562	6
	496.6	497	465–562	9
	518.0	516	465–562	6
	529.6	530	465–562	12
	544.0	545	465–562	5
	555.6*	556	465–562	11
	573.3 NL	573	565–580	9
Lutetium	409.5	411	407–420	2
	468.4	468	465–480	25
	517.0	518	505–530	25
	540.5 NL	n	—	—
	599.3 (597.1–601.4) NL	600	595–605	2
	674.9 (668.8–681.0) NL	675	665–685	1

* — Line; C — bands masked by continuum; n — not observed; NL — not reported in literature.

Spectra of the same elements were also recorded photographically by means of a Hilger, large Littrow spectrograph, to take advantage of the greater resolving power of this instrument in accurately locating the position of the characteristic lines and bands. Light from the flame of the Beckman burner was focused on the entrance slit of the spectrograph by means of a lens. Strong solutions of the rare-earth elements containing 10 to 25 mg of the element per ml dissolved in 1 *N* HCl were aspirated into the flame. Kodak 1030-F(3) and 1-N plates were used to photograph the spectra. The period of exposure ranged from 4 to 10 min, depending upon the concentration and sensitivity of the element. The spectral data obtained were used in fixing the wave lengths assigned to the band maxima and lines in Tables I and II. These data were compared with literature values[11–16]. Several lines and bands which were not previously reported are indicated in Table I.

TABLE II

PRINCIPAL EMISSION BANDS AND LINES OF Sc, Y, AND RARE-EARTH ELEMENTS: RELATIVE SPECTRAL INTENSITY AND INTERFERENCES

Element	*Wave length, mμ*	*½ Band width, mμ*	*Sensitivity, scale div./μg/ml*	*Interference scale div./μg/ml*
Scandium	607	10	170	(a)
Yttrium	482	5	12	Tm-7; Yb-6; Gd-2
	598	8	80	Gd-27; Eu-13; Tb-13; Sm-6; Dy-2; Lu-2; Pr-1
	614	8	80	Gd-27; Sm-8; Eu-6; Tb-3; Pr-1
Lanthanum	442	5	9	None
	741	10	8	None
	791	10	8	None
Praseodymium	604	5	3	Y-40; Gd-20; Tb-8; Sm-6; Dy-3; Nd-1
Neodymium	663	8	4	Eu-4; Sm-4
	700	15	3	None
	712	8	3	None
Samarium	614	18	8	Y-80; Gd-27; Eu-5; Tb-3; Dy-2
	624	10	9	Y-40; Gd-32; Eu-11; Tb-2
	640	10	8	Eu-8; Gd-2; Nd-1
	652	10	8	Eu-8; Nd-1
Europium	459	Line	22	Tb-2; Sm-1
	463	Line	18	Gd-8; Tb-2; Sm-1
	466	Line	15	Gd-8; Tb-2
Gadolinium	464	5	8	Eu-18; Sm-1
	622	20	32	Y-40; Eu-11; Sm-9; Dy-2; Tb-2; Nd-1
Terbium	535	5	10	Yb-10; Tm-5; Ho-3; La-3; Y-1
	573	5	11	Dy-18; Yb-9; Gd-7; Er-2; Tm-2; Y-2
	598	5	13	Y-80; Gd-27; Eu-13; Sm-4; Pr-1
Dysprosium	528	5	15	Yb-10; Ho-6; Tm-2
	540	5	11	Tm-9; Tb-8; Gd-2; Yb-2
	549	5	10	Er-8; Tm-8; La-3; Gd-2; Tb-2
	573	10	18	Ho-10; Yb-9; Tb-8; Gd-5
	583	5	15	Gd-15; Tb-8; Ho-4; Y-4
Holmium	516	8	10	Lu-20; Er-5; Dy-5; Tm-2; Y-2; Yb-2
	532	5	6	Yb-12; Tm-7; Dy-5; Tb-4; Gd-1
	566	10	25	La-8; Gd-7; Tb-7; Er-6; Tm-5; Yb-4

(a) Easily separated; therefore, mutual interferences are not listed.

TABLE II (Continued)

Element	*Wave length, mμ*	*½ Band width, mμ*	*Sensitivity scale div./μg/ml*	*Interference scale div./μg/ml*
Erbium	504	12	12	Y-3; Yb-2
	552	18	13	Dy-8; Tb-5; Yb-5; Gd-3; La-3
Thulium	482	5	7	Y-12; Yb-6; Gd-1
	494	10	10	Gd-4; Yb-2
	540	12	9	Dy-11; Gd-3; La-3; Ho-2; Tb-2; Yb-2
Ytterbium	399	Line	60	None
Lutetium	468	5	25	Eu-10; Gd-4; Y-4; Tb-1
	517	5	25	Ho-10; Er-6; Dy-5; Y-2

DISCUSSION

Flame spectra of scandium, yttrium and all rare-earth elements except cerium and promethium are presented in Fig. 1. These spectra were recorded with an Oak Ridge National Laboratory grating spectrophotometer[9]. Hexone solutions containing 50 μg per ml of the elements were, with two exceptions, used in obtaining all spectra; for neodymium and praseodymium, solutions containing 100 and 500 μg per ml of the element, respectively, were utilized. For several of the elements, the spectra of which contain exceptionally strong bands or lines, spectra were also recorded with less concentrated solutions in order to retain the intense lines or bands on scale. Broken lines are used in plotting these spectra. The same wave length scale is used in plotting all spectra, in order to facilitate comparison between spectra.

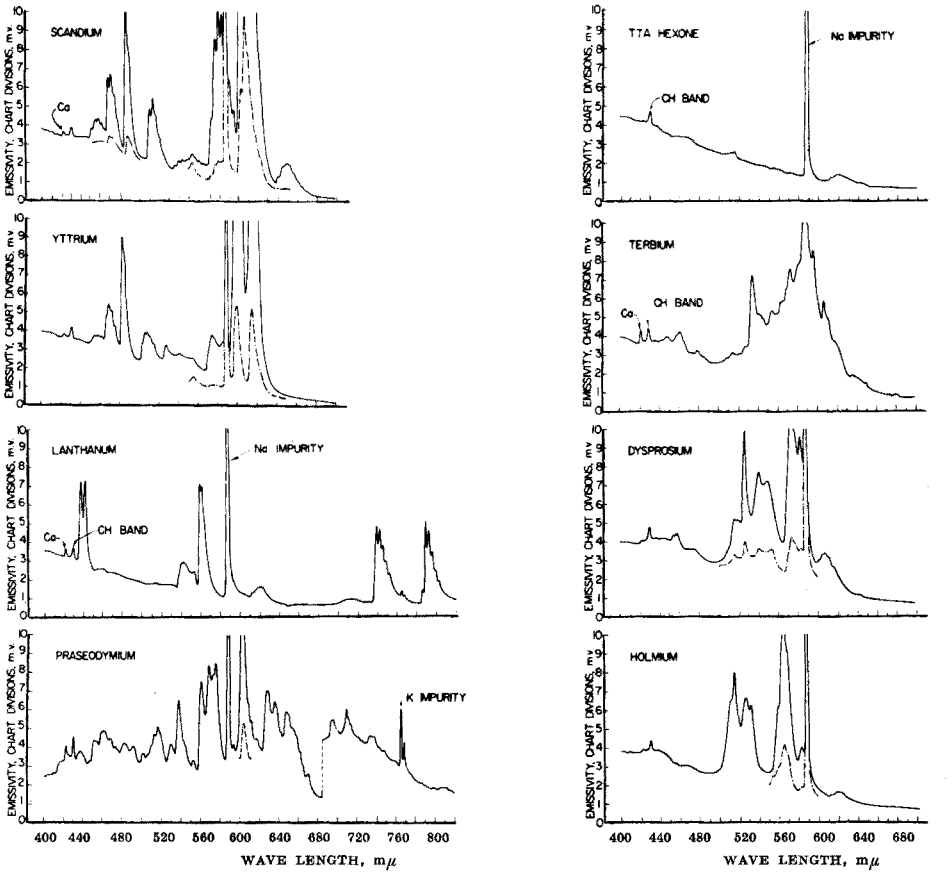
The background spectrum is also shown in Fig. 1. In recording this spectrum, a solution of TTA in hexone was aspirated into the flame. Hexone rather than aqueous solutions were used in making spectral measurements with the flame spectrophotometer because of the pronounced enhancement of emissivity attainable with this medium. When hexone is used, however, the flame spectrum contains a CH band at 431 mμ and C_2 bands at 473, 517, 564, and 619 mμ which will obscure portions of the desired elemental spectra unless they are eliminated. It was found that this could be accomplished either by lowering the flame or by shielding the flame to exclude light from the luminous inner cone. The latter method proved to be the most practical and essentially elimitated the CH and C_2 bands from the back-ground spectrum without decreasing the sensitivity of spectral measurements appreciably. Although the band structure is practically eliminated, a certain amount of continuous background radiation is observed. The intensity of this background radiation is a maximum in the near ultraviolet region and is degraded toward the red region of the spectrum. A sodium line is also observed due to sodium as an impurity in the hexone. Many of the spectra of the rare-earth elements also exhibit a calcium line at 423 mμ, due, evidently, to traces of calcium in some of their oxides.

The wave length maximum of each line or band of the spectra together with the band widths and the relative spectral intensity of each line or band are tabulated in

Table I. The positions of lines or of band crests were accurately fixed from spectral data recorded photographically with a prism spectrograph, whereas spectra obtained by the use of the flame spectrophotometer were used to establish the band widths. The relative spectral intensity was determined by measuring the peak height in scale divisions, and then dividing this value by the concentration of the element in μg per ml. The peak height was measured by averaging the background height on either side of the band and then using this average height as the base line from which to measure the height of the bandcrest.

Although organic solutions were found to be advantageous in making flame spectrophotometric measurements, aqueous solutions are to be preferred in spectral measurements with a prism spectrograph because of the period of time required, from 4 to 10 min, to record the spectra photographically. When an organic solution is burned for extended periods of time, difficulties are encountered due to clogging of the burner which are avoided if an aqueous solution is used. The spectrographic measurements were, therefore, made with aqueous solutions.

By comparison of the spectral data appearing in Table I, deductions can be made relative to the interference of various elements of this group with the estimation of a particular element at a specific wave length. The band widths are included because



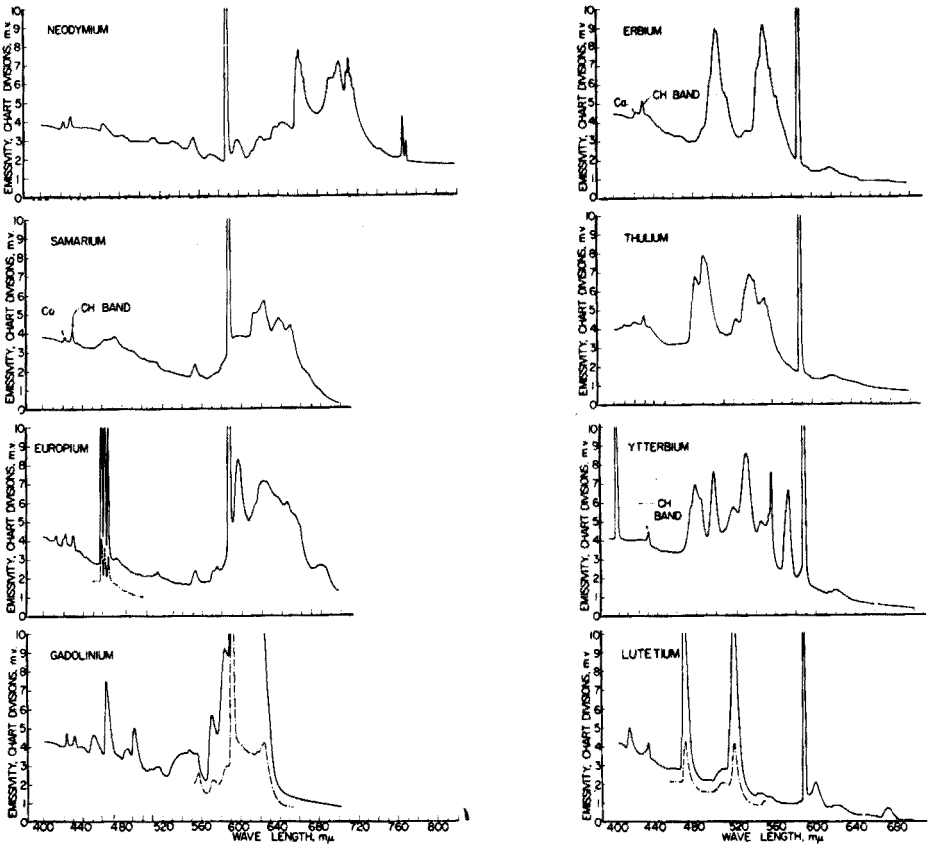


Fig. 1. Flame emission spectra of scandium, yttrium and the rare-earth elements:

ORNL Flame spectrophotometer; Multiplier phctotube: Blue, RCA — 6217; Red, Farnsworth — 16PMI; Voltage per dynode — 80; Slit width, 0.26 mm; Spectra slit width, 1.6 mμ; Gas flow rate: Oxygen, 5100 cm^3/min; Hydrogen, 3600 cm^3/min; Medium, TTA in hexone, 0.1 *M*. Concentration, μg/ml (Plotted as solid lines), Sc, Y, La, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu — 50; Nd — 100; Pr — 500. (Plotted as broken line): Sc, Y — 5; Eu, Gd, Dy, Ho, Lu — 10; Pr — 100.

of their usefulness in the evaluation of interferences. In Table II, the major line or bands of the different elements, the relative spectral intensity of each, the 1/2-band widths and the relative spectral interference of other elements of the group studied are presented. No spectral interference by other elements of the group was observed for lanthanum, ytterbium, or two bands of neodymium. In so far as overlapping spectra are concerned, each of these three elements can be estimated by flame photometry in mixtures of the other rare-earth elements without recourse to separation techniques. Although some spectral interference is encountered in all other cases, it is possible, by judicious selection of the wave length, to estimate some of the other rare-earth elements without serious interference. For instance, europium could be determined by measuring the emissivity at 459 mμ in mixtures containing any other rare-earth elements except terbium and samarium. Even if these two interfering elements were present to the extent of 10% each in a sample which was 40% europium, the error

due to spectral interference would be of the order of 3% of the true value for europium. Only europium and neodymium will interfere with the estimation of samarium at 652 mμ. If the ratio of the interfering elements and samarium does not exceed 1 to 20, the error will not exceed 5% of the true value. A study of methods for the estimation of individual elements in this group is in progress.

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SUMMARY

Flame spectra are presented of scandium, yttrium and the rare-earth elements, except cerium and promethium. These elements were extracted with a solution of 2-thenoyltrifluoroacetone (TTA) in 4-methyl-2-pentanone (hexone) and their spectra in the organic medium were subsequently recorded by means of a flame spectrophotometer. The precise wave length and relative spectral intensity of each line and band are tabulated. Included, also, are the 1/2-band widths and the relative spectral interferences of other elements of the group with the emission of measurement of the bands and lines listed. Operating conditions, applicability and limitations of the method in the estimation of some of the rare-earth elements are given.

RÉSUMÉ

Les auteurs ont effectué une étude sur les spectres de flamme du scandium, de l'yttrium et des terres rares, sauf ceux du cérium et du prométhéum. Les chélates de ces éléments avec la thénoyl-2-trifluoroacétone sont extraits dans la méthyl-4-pentanone-2; leurs spectres sont enregistrés au moyen d'un spectrophotomètre de flamme. Des recherches sur le dosage de ces éléments sont en cours.

ZUSAMMENFASSUNG

Es werden die Flammenspektren von Scandium, Yttrium und der seltenen Erden mit der Ausnahme von Cer und Promethium beschrieben. Die Chelate dieser Elemente mit Thenoyl-2-trifluoraceton werden mit Methyl-4-pentanon-2 extrahiert und die Spektren mit einem Flammenspektrophotometer registriert. Die Anwendungsmöglichkeit dieser Methode zur Bestimmung einiger Elemente der seltenen Erden wird beschrieben.

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REACTIONS BETWEEN ALKALOIDS AND TETRAPHENYLBORON AND THEIR ANALYTICAL APPLICATION

A HETEROMETRIC STUDY

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INTRODUCTION

A heterometric study of the reaction of alkaloids and other large nitrogen-containing compounds in potassium iodide solution with bismuth nitrate has already been carried out[1]. The reaction could be studied only at very low pH values. Similar reactions could be investigated at higher pH values if tetraphenylboron (TPB) was used as titrant instead of bismuth. We present here a study of reactions of large nitrogen-containing compounds (A) with TPB at pH values 1–9. The results are interesting because they throw light on the behaviour of these compounds at higher pH values. The use of TPB has an additional advantage: only very low concentrations of bismuth could be used as titrant, because at higher concentrations bismuth iodide was precipitated. No such limitations existed in the case of TPB; also, high concentrations of TPB permitted the inclusion of the less sensitive compounds.

During the last seven years, different methods have been suggested for the analysis of basic heterocyclic nitrogen compounds of smaller molecules with TPB[2], *e.g.* choline and its derivatives have been determined as well as many alkaloids. The TPB-N-salt compounds appeared to be insoluble in ether, $CHCl_3$ and CCl_4, but were soluble in acetone. Generally the salt extract was evaporated and the salt identified by its melting point. In another method the salt was dissolved in perchloric acid and then the TPB was titrated with silver nitrate potentiometrically; often 100 mg or more of the insoluble compound was needed for the analysis.

We found that the sensitivity of TPB towards the nitrogen-containing compounds was about three times less than that of bismuth. The individual character and structure of the different compounds was also more pronounced with bismuth. Nevertheless, some aspects could be better studied with TPB. We were able to establish that the order of sensitivity of the different compounds towards TPB was sometimes entirely different than in the case of bismuth iodide. Thus acriflavine and rivanol, which were the least sensitive with bismuth iodide, were the most sensitive with TPB. This is of course caused by the different character of the anions used. A comparison of TPB with bismuth iodide therefore gives information on the structural requirements in both cases.

EXPERIMENTAL

Instrumentation

The same instrumentation and the same working conditions were observed as in previous heterometric investigations[3].

Reagents

Altogether 13 nitrogen-containing compounds were studied. The same reagents were used as in the similar work with bismuth iodide.

Sodium tetraphenyl boron, Hopkin & Williams, Ltd. A 0.01 *M* aqueous standard solution was prepared, from which more dilute solutions were obtained.

8-Hydroxyquinoline, "Baker Analyzed".

RESULTS AND DISCUSSION

The results of the experiments carried out are presented in Tables I and II. The course of the reactions in some of these experiments are presented in Fig. 1 (Table I) and in Fig. 2 (Table II). The tables show the final compounds (↓↓) as well as the intermediate compounds (↓).

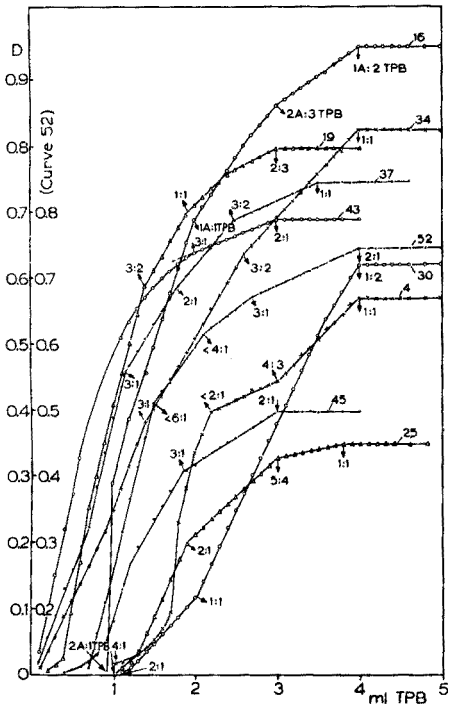

Fig. 1. Titrations of nitrogen compounds with sodium-tetraphenylboron (Table I).

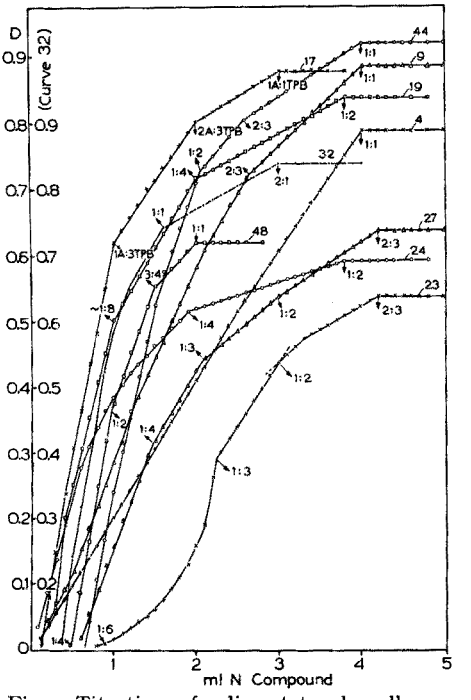

Fig. 2. Titrations of sodium-tetraphenylboron with nitrogen compounds (Table II).

Sensitivity

In the tables the nitrogen-containing compounds are shown according to decrease in molar sensitivity. The order of sensitivity in mg does not always agree with the order of molar sensitivity. Acriflavine, which appears first in Table I, was among the least sensitive with bismuth iodide. Approximately one mg was necessary for the determination of the most sensitive compounds, while in the case of the least sensitive oxine, about 5 mg were needed. On the other hand, nitron, cinchonine and quinine were among the most sensitive compounds, both with TPB and bismuth iodide. Antipyrine and atropine belong to the less sensitive alkaloids with both reagents.

TABLE I

General composition: a ml K M nitrogen compound (= A) + b ml supplements + ad 20 ml (or 10 ml) H_2O + x ml mM sodium-tetraphenylboron (TPB) T = 20°

Expt. No.	Solution of nitrogen compound	Initial ppt. at ml	Vol. ml	Titrant (= TPB) Molarity	End-point (ml) Calcul.	End-point (ml) Found	Optical density at the end-point	Molar ratio [A]:[TPB]	% error	Titration time in min	Intermediate compounds
1	3 ml 0.001 M acriflavine + 1 ml N HCl	0.1	20	0.001	3.00	i 3.00 h	0.72	1 : 1	0.0	25	3 : 2↓
2	3 ml 0.001 M acriflavine + 1 ml M Na-acetate	0.1	20	0.001	3.00	i 3.00 h	0.8	1 : 1	0.0	22	2 : 1↓
3	3 ml 0.001 M acriflavine + 5 ml 0.2 M Na_2HPO_4	0.1	20	0.001	3.00	i 3.00 h	0.66	1 : 1	0.0	25	2 : 1↓
4	4 ml 0.001 M rivanol + 1 ml N HCl	1.0	20	0.001	4.00	i 4.00 h	0.57	1 : 1	0.0	28	4 : 1↑
5	4 ml 0.001 M rivanol + 1 ml M Na-acetate	1.0	20	0.001	4.00	i 4.00 h	0.62	1 : 1	0.0	29	4 : 1↑
6	4 ml 0.001 M rivanol + 5 ml 0.2 M Na_2HPO_4	1.2	20	0.001	4.00	i 4.00 h	0.54	1 : 1	0.0	38	4 : 1↑
7	4 ml 0.001 M nitron + 1 ml N HCl	0.1	20	0.001	4.00	i 4.00 h	0.79	1 : 1	0.0	12	
8	4 ml 0.001 M nitron + 2 ml N CH_3COOH	0.1	20	0.001	4.00	i 3.99 h	0.82	1 : 1	0.2	11	
9	4 ml 0.001 M nitron + 2 ml M Na-acetate	0.1	20	0.001	4.00	i 4.00 h	0.80	1 : 1	0.0	11	
10	4 ml 0.001 M nitron + 5 ml 0.2 M Na_2HPO_4	0.1	20	0.001	4.00	i 3.98 h	0.74	1 : 1	0.5	12	
11	4 ml 0.001 M cinchonine + 1 ml N HCl	0.3	20	0.0025	3.20	i 3.20 h	0.73	1 : 2	0.0	14	1 : 1↓ → 2 : 3↓
12	4 ml 0.001 M cinchonine + 2 ml N CH_3COOH	0.3	20	0.0025	3.20	i 3.18 h	0.86	1 : 2	0.5	14	1 : 1↓ → 2 : 3↓
13	4 ml 0.001 M cinchonine + 2 ml N CH_3COOH + + 2 ml M Na-acetate	0.3	20	0.0025	3.20	i 3.20 h	0.81	1 : 2	0.0	13	1 : 1↓ → 2 : 3↓
14	6 ml 0.001 M cinchonine + 2 ml M Na-acetate	0.1	20	0.0025	3.60	i 3.58 h	0.89	2 : 3	0.5	16	3 : 2↓ → 1 : 1↓
15	6 ml 0.001 M cinchonine + 5 ml 0.2 M Na_2HPO_4	0.1	20	0.0025	3.60	i 3.60 h	0.88	2 : 3	0.0	16	3 : 2↓ → 1 : 1↓
16	5 ml 0.001 M quinine + 1 ml N HCl	1.0	20	0.0025	4.00	i 4.00 h	0.95	1 : 2	0.0	11	2 : 1↑ → 1 : 1↓ → 2 : 3↓
17	5 ml 0.001 M quinine + 2 ml N CH_3COOH	0.3	20	0.0025	4.00	i 4.00 h	1.01	1 : 2	0.0	11	1 : 1↓
18	5 ml 0.001 M quinine + 2 ml N CH_3COOH + + 2 ml M Na-acetate	0.2	20	0.0025	4.00	i 3.98 h	0.90	1 : 2	0.5	12	1 : 1↓

19	5 ml 0.001 *M* quinine + 2 ml *M* Na-acetate	0.3	20	0.0025	3.00	i 3.00 h	0.79	2 : 3	0.0	13	3 : 2↓ → 1 : 1↓
20	5 ml 0.001 *M* quinine + 5 ml 0.2 *M* Na_2HPO_4	0.4	20	0.0025	3.00	i 3.00 h	0.70	2 : 3	0.0	13	3 : 2↓ → 1 : 1↓
21	5 ml 0.001 *M* strychnine + 1 ml *N* HCl	0.4	20	0.00133	3.75	i 3.72 h	0.67	1 : 1	1.0	32	~2 : 1↓ → 3 : 2↓
22	5 ml 0.001 *M* strychnine + 2 ml *N* CH_3COOH	0.3	20	0.00133	3.75	i 3.80 h	0.74	1 : 1	1.0	32	~2 : 1↓ → 4 : 3↓
23	5 ml 0.001 *M* strychnine + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	0.4	20	0.00133	3.75	i 3.75 h	0.70	1 : 1	0.0	29	~2 : 1↓ → 4 : 3↓
24	5 ml 0.001 *M* strychnine + 2 ml *M* Na-acetate	0.4	20	0.00133	3.75	i 3.82 h	0.61	1 : 1	1.4	30	3 : 1↓ → 2 : 1↓
25	5 ml 0.001 *M* strychnine + 5 ml 0.2 *M* Na_2HPO_4	1.2	20	0.00133	3.75	i 3.78 h	0.35	1 : 1	0.5	33	~4 : 1↑ → 2 : 1↓
26	5 ml 0.001 *M* papaverine + 1 ml *N* HCl	0.1	20	0.0015	3.33	i 3.38 h	0.73	1 : 1	1.4	16	3 : 1↓ → ~3 : 2↓ (?)
27	5 ml 0.001 *M* papaverine + 2 ml *N* CH_3COOH	0.1	20	0.0015	3.33	i 3.36 h	0.88	1 : 1	0.8	17	2 : 1↓ → 3 : 2↓
28	5 ml 0.001 *M* papaverine + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	0.1	20	0.0015	3.33	i 3.33 h	0.81	1 : 1	0.0	16	2 : 1↓ → ~3 : 2↓
29	5 ml 0.001 *M* papaverine + 2 ml *M* Na-acetate	0.1	20	0.0015	3.33	i 3.37 h	0.82	1 : 1	1.2	17	3 : 1↓ → 2 : 1↓ → 3 : 2↓
30	5 ml 0.001 *M* sparteine + 1 ml *N* HCl	1.1	20	0.0025	4.00	i 4.00 h	0.62	1 : 2	0.0	17	2 : 1↑ → (1 : 1↓)
31	6 ml 0.001 *M* sparteine + 2 ml *N* CH_3COOH	0.1	20	0.0015	4.00	i 4.00 h	0.73	1 : 1	0.0	16	2 : 1↓ → 3 : 2↓
32	6 ml 0.001 *M* sparteine + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	0.1	20	0.0015	4.00	i 4.00 h	0.74	1 : 1	0.0	16	4 : 3↓
33	6 ml 0.001 *M* sparteine + 2 ml *M* Na-acetate	0.1	20	0.0015	4.00	i 4.00 h	0.74	1 : 1	0.0	16	2 : 1↓ → ~4 : 3↓
34	6 ml 0.001 *M* sparteine + 5 ml 0.2 *M* Na_2HPO_4	0.1	20	0.0015	4.00	i 4.00 h	0.82	1 : 1	0.0	17	~3 : 1↓ → ~3 : 2↓
35	7 ml 0.001 *M* phenanthroline + 1 ml *N* HCl	0.1	20	0.002	3.50	i 3.50 h	0.86	1 : 1	0.0	11	~2 : 1↓ → 3 : 2↓
36	7 ml 0.001 *M* phenanthroline + 2 ml *N* CH_3COOH	0.1	20	0.002	3.50	i 3.50 h	0.90	1 : 1	0.0	11	~2 : 1↓ → 3 : 2↓
37	7 ml 0.001 *M* phenanthroline + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	0.1	20	0.002	3.50	i 3.50 h	0.74	1 : 1	0.0	12	3 : 1↓ → 2 : 1↓ → 3 : 2↓
38	7 ml 0.001 *M* phenanthroline + 2 ml *M* Na-acetate		20	0.002		no ppt.					

continued

TABLE I (continued)

Expt. No.	Solution of nitrogen compound	Initial ppt. at ml	Vol. ml	Titrant (= TPB) Molarity	End-point (ml) Calcul.	End-point (ml) Found	Optical density at the end-point	Molar ratio [A]:[TPB]	% error	Titration time in min	Intermediate compounds
39	3 ml 0.005 *M* pyramidone + 1 ml *N* HCl	0.4	10	0.005	3.00	i 3.00 h	0.80	1 : 1	0.0	26	2 : 1↓
40	3 ml 0.005 *M* pyramidone + 2 ml *N* CH_3COOH	0.5	10	0.005	3.00	i 3.00 h	0.97	1 : 1	0.0	25	2 : 1↓ → 3 : 2↓
41	3 ml 0.005 *M* pyramidone + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	0.4	10	0.005	3.00	i 3.00 h	0.85	1 : 1	0.0	23	2 : 1↓ → 3 : 2↓
42	3 ml 0.005 *M* pyramidone + 2 ml *M* Na-acetate		10	0.005		no ppt.					
43	3 ml 0.005 *M* antipyrine + 1 ml *N* HCl		10	0.0025	3.00	i 3.00 h	0.69	2 : 1	0.0	17	3 : 1↓
44	3 ml 0.005 *M* antipyrine + 5 ml *N* HCl	0.2	10	0.0025	3.00	i 3.00 h	0.64	2 : 1	0.0	14	5 : 1↓ → 3 : 1↓
45	3 ml 0.005 *M* antipyrine + 2 ml *N* CH_3COOH	0.6	10	0.0025	3.00	i 3.00 h	0.40	2 : 1	0.0	19	3 : 1↓
46	3 ml 0.005 *M* antipyrine + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate					no ppt.					
47	4 ml 0.004 *M* atropine + 1 ml *N* HCl	0.6	20	0.004	4.00	i 3.97 h	0.60	1 : 1	0.8	10	<3 : 2↓ → 4 : 3↓
48	4 ml 0.004 *M* atropine + 2 ml *N* CH_3COOH	0.7	20	0.004	4.00	i 4.00 h	0.72	1 : 1	0.0	10	2 : 1↓ → ~4 : 3↓
49	4 ml 0.004 *M* atropine + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	0.7	20	0.004	4.00	i 4.01 h	0.95	1 : 1	0.2	7	2 : 1↓ → 3 : 2↓
50	4 ml 0.004 *M* atropine + 2 ml *M* Na-acetate	0.6	20	0.004	4.00	i 4.00 h	0.92	1 : 1	0.0	10	<2 : 1↓ → 4 : 3↓
51	4 ml 0.004 *M* atropine + 5 ml 0.2 *M* Na_2HPO_4	0.7	20	0.004	4.00	i 4.00 h	0.94	1 : 1	0.0	8	<2 : 1↓ → 3 : 2↓
52	4 ml 0.01 *M* oxine + 1 ml *N* HCl	0.5	10	0.005	4.00	i 4.00 h	0.74	2 : 1	0.0	14	~6 : 1↓ → ~4 : 1↓ → 3 : 1↓
53	4 ml 0.01 *M* oxine + 2 ml *M* Na-acetate	0.2	10	0.005	4.00	i 4.00 sh. h	0.34	2 : 1	0.0	10	~8 : 1↑ → ~4 : 1↓ → 3 : 1↓

i = intersection point; h = horizontal maximum density line; ↑ = soluble intermediate; ↓ = insoluble intermediate; ↓↓ = final compound at the first optical maximum density point.

The striking difference in sensitivity towards each titrant in the case of acriflavine and rivanol must be caused by the "ribboned" structure of these compounds when acting as cations. Generally the sensitivity of the titrations with TPB is only a third of that with bismuth iodide. But in the case of acriflavine and rivanol, almost equal amounts of compound were required for the analysis in each cases. Although the sensitivities with TPB and bismuth iodide were different, the compounds could be determined with both reagents with the same precision; the error was usually zero. Generally the titration time was about twice as long with TPB as with bismuth iodide.

COMPOUNDS

Final compounds

Probably all the final compounds obtained in this work are composed of large regular salts without complex formation. *Four* final compounds were obtained:

$$A_2[TPB]\downarrow\downarrow,\ A[TPB]\downarrow\downarrow,\ A_2[TPB]_3\downarrow\downarrow \text{ and } A[TPB]_2\downarrow\downarrow.$$

In all titrations the first maximum density point coincided exactly with the quantitative formation of one of these compounds. Therefore, all the above compounds could be used equally successfully for the analysis of the nitrogen-containing compounds. The following may be stated regarding the composition of the final compound. The compound $A[TPB]_2\downarrow\downarrow$ was only obtained at pH values of 1–5 with two alkaloids, namely, quinine and cinchonine. Both these compounds contain two ring systems with one nitrogen in each ring. The N-atoms lie apart from each another and act separately and independently.

In addition, sparteine (Table I, Expt. 30 and Table II, Expts. 11 and 12) also gave a final compound $A[TPB]_2\downarrow\downarrow$ at low pH values. With bismuth iodide, the activity of the N-atoms was more pronounced. Thus, both nitron and antipyrine gave final compounds $A[BiI_n]_2\downarrow\downarrow$ if titrated with bismuth. But if titrated with TPB, the second nitrogen always remained inactive. In the reverse titrations of bismuth iodide with nitron, only one nitrogen was used in the titration and the final compound $A[BiI_n]\downarrow\downarrow$ was obtained, just as in all titrations with TPB, $A[TPB]\downarrow\downarrow$ was obtained. Thus parallel heterometric titrations with both titrants may reveal fine differences in the electronic and the stereochemical structure of the complex cyclic nitrogen compound.

In two cases, the composition of the final compounds depended on the pH value of the titrated solution, *i.e.* with cinchonine and quinine (Table I, Expts. 11–20 and Table II, Expts. 19–28). The compound $A_2[TPB]_3\downarrow\downarrow$ obtained in both cases at pH values 7–9 must be considered as having the structure of a stoechiometric mixture of $A[TPB] + A[TPB]_2\downarrow\downarrow$.

The final compound $A_2[TPB]\downarrow\downarrow$ obtained with antipyrine and with oxine (Table I, Expts. 43–46, 52–53 and Table II, Expts. 32–34, 49) presents a special problem. In the reverse titration oxine also gave a final compound $A[TPB]\downarrow\downarrow$ at pH value $\sim$1, (Table II, Expt. 48), while antipyrine always gave the compound $A_2[TPB]\downarrow\downarrow$ only. It is hardly conceivable that TPB can act as a doubly charged anion $[TPB]^{-2}$. On the other hand, the assumption that with both the latter nitrogen compounds an additional molecule of the compound was necessary for the formation of the solid compound is not readily comprehensible. It is interesting to note that each of these compounds contains two attraction points in the molecule, *i.e.* either two N-atoms

TABLE II

General composition: a ml KM tetraphenylboron (TPB) + b ml supplements + ad 20 ml (or 10 ml) water + x ml mM N-compound (= A) T = 20°

Expt. No.	Solution titrated	Vol. ml	Initial ppt. at ml	Titrant (= A) Name	Molarity	End-point Calcul.	End-point Found	Optical density at the end-point	Molar ratio [A]:[TPB]	% error	Titration time in min	Intermediate compounds
1	3 ml 0.001 M TPB + 1 ml N HCl	20	0.1	Acriflavine	0.001	3.0	i 3.0 h	0.79	1 : 1	0.0	33	1 : 3↓
2	3 ml 0.001 M TPB + 2 ml N CH_3COOH	20	0.1	Acriflavine	0.001	3.0	i 3.0 h	0.78	1 : 1	0.0	20	
3	3 ml 0.001 M TPB + 2 ml M Na-acetate	20	0.1	Acriflavine	0.001	3.0	i 3.0 h	0.62	1 : 1	0.0	24	
4	4 ml 0.001 M TPB + 1 ml N HCl	20	0.1	Nitron	0.001	4.0	i 4.00 h	0.79	1 : 1	0.0	11	
5	4 ml 0.001 M TPB + 2 ml N CH_3COOH	20	0.1	Nitron	0.001	4.0	i 4.01 h	0.98	1 : 1	0.2	13	
6	4 ml 0.001 M TPB + 2 ml M Na-acetate	20	0.1	Nitron	0.001	4.0	i 4.02 h	0.90	1 : 1	0.5	12	
7	4 ml 0.001 M TPB + 5 ml 0.2 M Na_2HPO_4	20	0.1	Nitron	0.001	4.0	i 4.01 h	0.84	1 : 1	0.2	13	
8	4 ml 0.001 M TPB + 1 ml N HCl	20	0.1	Papaverine	0.001	4.0	i 4.00 h	0.82	1 : 1	0.0	18	1 : 2↓ → 2 : 3↓
9	4 ml 0.001 M TPB + 2 ml N CH_3COOH	20	0.1	Papaverine	0.001	4.0	i 4.02 h	0.88	1 : 1	0.5	18	1 : 2↓ → 2 : 3↓
10	4 ml 0.001 M TPB + 2ml M Na-acetate	20	0.2	Papaverine	0.001	4.0	i 4.02 h	0.83	1 : 1	0.5	19	1 : 2↓ → 2 : 3↓
11	8 ml 0.0025 M TPB + 1 ml N HCl	20	0.0	Sparteine	0.0025	4.0	i 4.00 h	0.77	1 : 2	0.0	14	(1 : 8↓) → 1 : 6↓ → 1 : 4↓
12	4 ml 0.0025 M TPB + 1 ml N HCl	20	2.0	Sparteine	0.00125	4.0	i 4.00 h	0.21	1 : 2	0.0	22	1 : 4↑ → ~ 1 : 3↓
13	4 ml 0.0015 M TPB + 2 ml N CH_3COOH	20	0.3	Sparteine	0.0015	4.0	i 4.02 h	0.78	1 : 1	0.5	15	1 : 2↓
14	4 ml 0.0015 M TPB + 2 ml N CH_3COOH + + 2 ml M Na-acetate	20	0.1	Sparteine	0.0015	4.0	i 4.00 h	0.89	1 : 1	0.0	16	1 : 2↓ → 3 : 4↓
15	4 ml 0.0015 M TPB + 2 ml M Na-acetate	20	0.1	Sparteine	0.0015	4.0	i 4.00 h	0.90	1 : 1	0.0	16	1 : 2↓ → 3 : 4↓
16	4 ml 0.0015 M TPB + 5 ml 0.2 M Na_2HPO_4	20	0.1	Sparteine	0.0015	4.0	i 4.00 h	0.93	1 : 1	0.0	17	1 : 2↓ → 3 : 4↓
17	3 ml 0.002 M TPB + 1 ml N HCl	20	0.1	Rivanol	0.002	3.0	i 3.00 h	0.88	1 : 1	0.0	38	1 : 3↓ → 2 : 3↓
18	3 ml 0.002 M TPB + 2 ml M Na-acetate	20	0.1	Rivanol	0.002	3.0	i 3.00 h	0.95	1 : 1	0.0	40	2 : 3↓
19	3 ml 0.0025 M TPB + 1 ml N HCl	20	0.2	Quinine	0.001	3.75	i 3.8 h	0.84	1 : 2	1.3	12	~ 1 : 8↓ → 1 : 4↓
20	3 ml 0.0025 M TPB + 2 ml N CH_3COOH	20	0.2	Quinine	0.001	3.75	i 3.8 h	0.90	1 : 2	1.3	12	1 : 4↓
21	3 ml 0.0025 M TPB + 2 ml N CH_3COOH + + 2 ml M Na-acetate	20	0.2	Quinine	0.001	3.75	i 3.8 h	0.75	1 : 2	1.3	12	1 : 4↓ → 1 : 3↓
22	5 ml 0.00125 M TPB + 2 ml M Na-acetate	20	1.0	Quinine	0.001	4.16	i 4.2 h	0.52	2 : 3	1.0	18	1 : 6↑ → 1 : 3↓ → 1 : 2↓
23	5 ml 0.00125 M TPB + 5 ml 0.2 M Na_2HPO_4	20	0.9	Quinine	0.001	4.16	i 4.2 h	0.54	2 : 3	1.0	20	1 : 6↑ → 1 : 3↓ → 1 : 2↓
24	6 ml 0.00125 M TPB + 1 ml N HCl	20	0.1	Cinchonine	0.001	3.75	i 3.80 h	0.59	1 : 2	1.3	14	1 : 4↓
25	6 ml 0.00125 M TPB + 2 ml N CH_3COOH	20	0.1	Cinchonine	0.001	3.75	i 3.80 h	0.93	1 : 2	1.3	14	~ 1 : 3↓

26	6 ml 0.00125 *M* TPB + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	20	0.1	Cinchonine	0.001	3.75	i 3.80 h 0.66	1 : 2	1.3	13	1 : 4↓
27	5 ml 0.00125 *M* TPB + 2 ml *M* Na-acetate	20	0.6	Cinchonine	0.001	4.16	i 4.20 h 0.64	2 : 3	1.0	17	1 : 4↓ → 1 : 3↓ → 1 : 2↓
28	5 ml 0.00125 *M* TPB + 5 ml 0.2 *M* Na_2HPO_4	20	1.1	Cinchonine	0.001	4.16	i 4.18 h 0.36	2 : 3	0.5	17	1 : 6↑ → ~ 1 : 4↓ → 1 : 2↓
29	7.5 ml 0.001 *M* TPB + 1 ml *N* HCl	20	0.2	Strychnine	0.002	3.75	i 3.8 h 0.94	1 : 1	1.3	48	1 : 2↓ → 2 : 3↓
30	7.5 ml 0.001 *M* TPB + 2 m *N* CH_3COOH + + 2 ml *M* Na-acetate	20	0.1	Strychnine	0.0025	3.0	i 3.0 h 0.95	1 : 1	0.0	52	1 : 2↓ → 2 : 3↓
31	10 ml 0.001 *M* TPB + 5 ml 0.2 *M* Na_2HPO_4	20	0.3	Strychnine	0.0033	3.33	i 3.38 h 0.85	1 : 1	1.3	52	~ 1 : 2↓
32	3 ml 0.0025 *M* TPB + 1 ml *N* HCl	10	0.1	Antipyrine	0.005	3.0	i 3.00 h 0.84	2 : 1	0.0	20	1 : 1↓
33	3 ml 0.0025 *M* TPB + 2 ml *N* CH_3COOH	10	1.4	Antipyrine	0.005	3.0	i 3.00 h 0.43	2 : 1	0.0	22	1 : 1↑
34	3 ml 0.0025 *M* TPB + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	10		Antipyrine	0.005		no ppt.				
35	4 ml 0.002 *M* TPB + 1 ml *N* HCl	20	0.1	Phenanthroline	0.002	4.0	i 4.00 h 0.87	1 : 1	0.0	13	1 : 4↓ → 1 : 2↓ → 3 : 4↓
36	4 ml 0.002 *M* TPB + 2 ml *N* CH_3COOH	20	0.1	Phenanthroline	0.002	4.0	i 4.00 h 1.03	1 : 1	0.0	12	1 : 4↓ → 2 : 3↓
37	4 ml 0.002 *M* TPB + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	20	0.1	Phenanthroline	0.002	4.0	i 4.00 h 0.83	1 : 1	0.0	12	1 : 4↓ → 1 : 2↓ → 2 : 3↓
38	4 ml 0.002 *M* TPB + 2 ml *M* Na-acetate	20		Phenanthroline	0.002		no ppt.				
39	3 ml 0.005 *M* TPB + 1 ml *N* HCl	10	0.4	Pyramidon	0.005	3.0	i 3.0 h 0.88	1 : 1	0.0	22	1 : 3↓
40	3 ml 0.005 *M* TPB + 2 ml *N* CH_3COOH	10	0.4	Pyramidon	0.005	3.0	i 3.0 h 0.95	1 : 1	0.0	23	1 : 3↓ → 1 : 2↓
41	3 ml 0.005 *M* TPB + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	10	0.5	Pyramidon	0.005	3.0	i 3.0 h 0.70	1 : 1	0.0	20	1 : 3↓ → 1 : 2↓
42	3 ml 0.005 *M* PTB + 2 ml *M* Na-acetate	10		Pyramidon	0.005		no ppt.				
43	4 ml 0.004 *M* TPB + 1 ml *N* HCl	20	0.8	Atropine	0.004	4.0	i 4.00 h 0.79	1 : 1	0.0	9	3 : 4↓
44	4 ml 0.004 *M* TPB + 2 ml *N* CH_3COOH	20	0.7	Atropine	0.004	4.0	i 4.00 h 0.92	1 : 1	0.0	9	1 : 2↓ → 2 : 3↓
45	4 ml 0.004 *M* TPB + 2 ml *N* CH_3COOH + + 2 ml *M* Na-acetate	20	0.9	Atropine	0.004	4.0	i 4.00 h 0.84	1 : 1	0.0	8	~ 1 : 4↑ → 3 : 4↓
46	4 ml 0.004 *M* TPB + 2 ml *M* Na-acetate	20	0.8	Atropine	0.004	4.0	i 4.00 h 0.82	1 : 1	0.0	8	~ 1 : 4↑ → 2 : 3↓
47	4 ml 0.004 *M* TPB + 5 ml 0.2 *M* Na_2HPO_4	20	0.8	Atropine	0.004	4.0	i 4.00 h 0.77	1 : 1	0.0	8	~ 1 : 4↑ → 1 : 2↓ → 2 : 3↓
48	4 ml 0.005 *M* TPB + 1 ml *N* HCl	10	0.5	Oxine	0.01	2.00	i 2.00 h 0.62	1 : 1	0.0	12	1 : 4↑ → 1 : 2↓ → 3 : 4↓
49	4 ml 0.01 *M* TPB + 2 ml *M* Na-acetate	10	0.6	Oxine	0.02	4.00	i 4.00 h 0.59	2 : 1	0.0	16	1 : 4↑ → 1 : 1↓ → 3 : 2↓

i = intersection point; h = horizontal maximum density line; ↑ = soluble intermediate; ↓ = insoluble intermediate; ↓↓ = final compound at the first optical maximum density point.

or one N-atom and one OH-group. In other studies with TPB we have found intermediates in which the $[TPB]^-$ acted with four attraction points; the latter may only be the four phenyl groups. If so, both the stereochemical and the electronic moments are responsible for the formation of the stable final compound $A_2[TPB]\downarrow\downarrow$.

It is interesting to note that CRANE[2], who also analysed the compound formed between TPB and oxine, found the bimolecular compound $A_2[TPB]$ only.

Because of the reduced sensitivity of TPB towards N-atoms, the final compound $A[TPB]\downarrow\downarrow$ with one active nitrogen atom was usually obtained in this work. This was also the case when nitron, pyramidone, phenanthroline or atropine was titrated with TPB.

In contrast to the reaction between nitrogen-containing compounds and bismuth iodide, final compounds of the same composition were obtained in all titrations whether the compounds were titrated with TPB or vice versa. Oxine was the only exception, giving in the reverse titrations $A[TPB]\downarrow\downarrow$ at pH $\sim$1, and $A_2[TPB]\downarrow\downarrow$ at pH $\sim$7.

Intermediate compounds

The different sequences of intermediate compounds traced depended on the final compounds obtained. The sequences were different in straight titrations of alkaloids with TPB and in the reverse titrations.

Thus the following sequences of intermediate compounds, at the molar ratio of [A] : [TPB], were obtained if the nitrogen compounds were titrated with TPB:

(1) $4:1\uparrow \rightarrow 3:1\downarrow\uparrow^* \rightarrow 2:1\downarrow\uparrow \rightarrow 3:2\downarrow \rightarrow 1:1\downarrow\downarrow$

(2) $1:1\downarrow \rightarrow 2:3\downarrow \rightarrow 1:2\downarrow\downarrow$ (pH's 1–5)

(3) $3:2\downarrow \rightarrow 1:1\downarrow \rightarrow 2:3\downarrow\downarrow$ (pH's 7–9)

(4) $4:1\downarrow \rightarrow 3:1\downarrow \rightarrow 2:1\downarrow\downarrow$

In the reverse titrations of TPB with alkaloids, the following sequences of compounds at the molar ratio of [A] : [TPB] were obtained:

(5) $(1:4\downarrow) \rightarrow 1:2\downarrow \rightarrow 2:3\downarrow \rightarrow 1:1\downarrow\downarrow$

(6) $(1:6\downarrow) \rightarrow 1:4\downarrow \rightarrow 1:2\downarrow\downarrow$ (pH's 1–5)

(7) $1:6\downarrow \rightarrow 1:3\downarrow \rightarrow 1:2\downarrow \rightarrow 2:3\downarrow\downarrow$ (pH's 7–9)

(8) $1:1\downarrow \rightarrow 3:2\downarrow \rightarrow 2:1\downarrow\downarrow$

The pH dependence of the reactions

With the exception of cinchonine and quinine, the same final compounds were obtained at all pH values between $\sim$1 and $\sim$9. This is very striking, as it must be assumed that the nitrogen compounds always acted as cations. Generally the highest maximum density values were obtained at pH $\sim$3. In some cases the high pH values acted favourably on the maximum density values obtained (*e.g.* cinchonine, sparteine and atropine). In other cases, there was an optimum pH value above which the

* $\downarrow\uparrow$ = the compound may be either insoluble or soluble.

maximum density value decreased gradually with rising pH (*e.g.* acriflavine, rivanol. quinine, strychnine, phenanthroline, antipyrine and oxine).

No precipitation at all occurred at pH ~5 with antipyrine or at pH ~7 with phenanthroline or pyramidone. The sensitivity of the precipitation towards increase in pH was very pronounced in ring compounds containing two nitrogen atoms bound to one another (*e.g.* pyramidone and antipyrine) or two nitrogen atoms stereochemically near one another (*e.g.* phenanthroline).

The pH of the solution probably plays a double role; low pH values favour the function of the N-atoms as cations, but also increase the solubility of the large salt. If so, the most basic compounds should give the best results at higher pH values. Two N-atoms in close vicinity weaken the basic character of the compound and make it more sensitive to rising pH values and more soluble.

ANALYTICAL ASPECTS

TPB is about three times less sensitive towards large nitrogen-containing compounds than bismuth as iodide, but it is possible to carry out titrations at pH values of 1–9. Because of the solubility of TPB this reagent can be used in any desired concentration. TPB can thus be used for the determination, or for the study, of less sensitive compounds, which was impossible with bismuth. Less complex molecules such as hexamethylenetetramine, caffeine, etc. can be studied with TPB in larger concentrations. On the other hand, the sensitivity towards TPB is high in special cases such as the "ribboned" acriflavine or rivanol. At pH ~7 complex nitrogen compounds can be studied and analysed in the presence of bi- or polyvalent metals, which can be kept in solution by the addition of masking agents. All these aspects will be considered in future reports, more nitrogen compounds will be included and single compounds will be determined in mixtures. The following may be stated regarding the sensitivity of the determinations with TPB. Approximately one mg of compound in 20 ml of solution was necessary for the determination of the most sensitive compounds such as acriflavine, nitron, cinchonine and phenanthroline. About two mg were necessary in the case of quinine, strychnine, papaverine and sparteine. About double the amount was necessary in the case of the less sensitive alkaloids such as antipyrine and atropine. These latter remained in solution when the titration was carried out at pH ~7, and thus the determination could be extended to mixtures.

GENERAL METHOD

1–2 mg of the large nitrogen-containing compound in 20 ml of aqueous solution, containing 2 ml of *N* acetic acid (or 1 ml of *N* hydrochloric acid) are titrated with a 0.001–0.005 *M* aqueous solution of sodium tetraphenylboron.

Note: In the case of smaller compounds the amount of sample and the concentration of the TPB must be increased.

SUMMARY

The reactions between tetraphenylboron (TPB) and acriflavine, rivanol, nitron, cinchonine, quinine, strychnine, papaverine, sparteine, phenanthroline, pyramidon, antipyrine, atropine and oxine were studied heterometrically at pH values 1–9. The reactions were about one third as sensitive as with bismuth nitrate in iodide solution. The sensitivity in the above series decreases from acriflavine to oxine. Precise titrations could always be carried out with TPB. Compounds with two adjacent N-atoms gave no precipitation at pH 5–7. The structures of the compounds and the analytical aspects are discussed and a method of determination is presented.

RÉSUMÉ

Les auteurs ont effectué une étude hétérométrique des réactions entre l'anion tétraphénylborique et les composés suivants: acriflavine, rivanol, nitron, cinchonine, quinine, strychnine, papavérine, spartéine, phénanthroline, pyramidon, antipyrine, atropine et oxine. La structure des composés obtenus est discutée; une méthode de dosage est proposée.

ZUSAMMENFASSUNG

Die Reaktionen des Tetraphenylbor-anions mit Acriflavin, Rivanol, Nitron, Cinchonin, Chinin, Strychnin, Papaverin, Spartein, Phenanthrolin, Pyramidon, Antipyrin, Atropin und Oxin wurden heterometrisch untersucht. Die Strukturen der Reaktionsprodukte werden diskutiert und eine Methode zur Bestimmung dieser Verbindungen beschrieben.

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INFRARED DETERMINATION OF TRACES OF SULFATE IN REAGENT CHEMICALS

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Although the infrared spectra of a large number of inorganic compounds have accumulated in the literature, there have been relatively few applications of infrared spectrophotometry to inorganic analytical problems. This is partly because of the solvent problem, but the alkali halide pressed disk technique[1–3], coupled with freeze-drying[4,5] actually makes it relatively easy to obtain the infrared spectra of materials present originally in aqueous solution. It is particularly interesting to consider the possibility of developing infrared methods for the determination of small quantities of inorganic anions because it is precisely in this area that other modern analytical techniques have been most deficient. An investigation of the infrared spectrophotometry of the sulfate ion was reported recently[5] which emphasized the techniques and results but did not deal with applications. In this paper, it is shown that these techniques can be combined with a chromatographic concentration and isolation step to provide a method for determining small quantities of sulfate in samples containing massive quantities of other substances. The method is well suited to the determination of sulfate impurities in reagent chemicals. It is as accurate as is ordinarily required at the trace level, and while it is not rapid from the standpoint of total elapsed time,

it requires a very small amount of operator time per sample because a large number of samples can be processed together with a minimum of attention.

SCHWAB AND DATTLER[6] were apparently the first to observe the retention of anions on columns of acid-washed alumina, and they worked out a selectivity series for about a dozen anions. KUBLI[7] later added a few more ions to the series. Sulfate stood high in its tendency to be retained but was easily displaced by hydroxyl ion. NYDAHL[8,9] then showed that analytical separations of sulfate from many other anions were feasible in practice, and developed methods for sulfur in steels, biological materials, and other types of samples, in which the chromatographic separation of sulfate on alumina was the key step. In addition, he measured distribution coefficients for sulfate and several other anions under various conditions, pointed out the important effect of pH upon sulfate retention, and emphasized that alumina was very much more selective for sulfate than were synthetic anion-exchange resins. FRITZ *et al.*[10] studied the process further in developing a separation designed to precede a titrimetric sulfate determination, and noted the remarkable facts that sulfate was quantitatively adsorbed even from 5 *M* perchloric acid or 2 *N* hydrochloric acid solutions, even at dilutions as low as 0.5 p.p.m., and even at ratios of chloride to sulfate of at least 4500 : 1. At the same time, it was relatively easy to elute the sulfate at will by means of hydroxyl ion, *e.g.*, with a dilute ammonia solution.

It was reasonable to try to combine this excellent separation technique with the infrared determination of sulfate. The coupling occurs through the freeze-drying step: potassium bromide is added to the column eluate, the solution is freeze-dried, and the resulting powder is pressed into a disk for infrared examination. The column operation takes only a few minutes, and the disk pressing and infrared recording likewise are not time-consuming. The freeze-drying operation unfortunately is slow, but once set in operation, it requires no attention. The work can conveniently be planned so that samples freeze-dry overnight. Furthermore, as many samples as desired can be freeze-dried at one time. Thus in practice the method is fairly rapid in terms of operator time per sample.

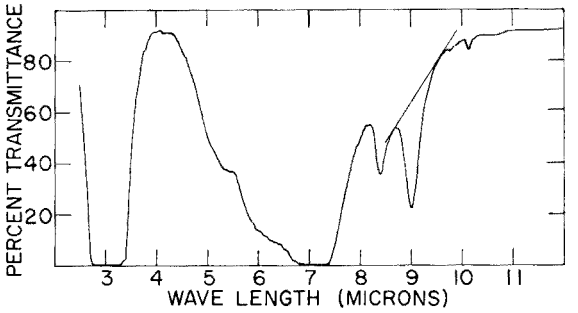

Fig. 1. Spectrum of freeze-dried column eluate.

In the previous report[5], it was noted that the shape and intensity of the sulfate band near 9μ varied with the cation accompanying the sulfate, and potassium sulfate was recommended as best for quantitative measurements. However, the sulfate in the column eluate appears as ammonium sulfate if, as seems best, ammonia is the

eluting agent. The spectrum of a freeze-dried column eluate is shown in Fig. 1. This spectrum is not so good as that of potassium sulfate because it is more difficult to establish the base-line, but experience has shown that reliable results can be obtained using a base-line like that indicated in the figure. Preliminary attempts to convert the column eluate into potassium sulfate by means of ion-exchange columns were unsuccessful because, although the cation exchange of potassium for ammonium did occur, extraneous absorption bands, some of which interfered with the sulfate band, were introduced by the action of the ammoniacal solution upon the resins. No amount of prior washing of the resins prevented this.

EXPERIMENTAL

Apparatus and reagents

Infrared spectra were recorded with a Perkin-Elmer Model 137 "Infracord" spectrophotometer. Only the spectrum from about 7 to 11 μ was required. Solutions were freeze-dried in tubes about 10 cm long made from standard taper 19/38 inner joints, using a 24-port VirTis freeze-dryer. Disks were prepared with a Hilger H920 die obtained from the Jarrell-Ash Co., Newtonville, Mass., using a Loomis 20-ton hydraulic press.

The chromatographic column was similar to those described by NYDAHL[9] and FRITZ[10], about 10 cm long and 1 cm in diameter. The treatment of the alumina has also been described[9,10]. The alumina used in this work was Alcoa activated alumina, grade F-20; this material was free of elutable sulfate, whereas some samples of chromatographic alumina from other sources contained large quantities of sulfate which slowly came off the column for an indefinite time.

A special grade of potassium bromide for infrared work, manufactured by E. Merck, Darmstadt, Germany, was obtained from Terra Chemicals, Inc., New York, N.Y. Ordinary reagent-grade potassium bromide often shows a very slight absorption in the sulfate region, whereas the E. Merck preparation does not. All other chemicals used were of the usual reagent-grade; the principal ones, hydrochloric acid and ammonia, fortunately were found to be sulfate-free.

Preliminary experiments

Since ammonium sulfate is not a suitable standard material, a calibration curve was prepared based upon oven-dried potassium sulfate. Aliquots of a standard solution were run through the entire procedure including the chromatographic step

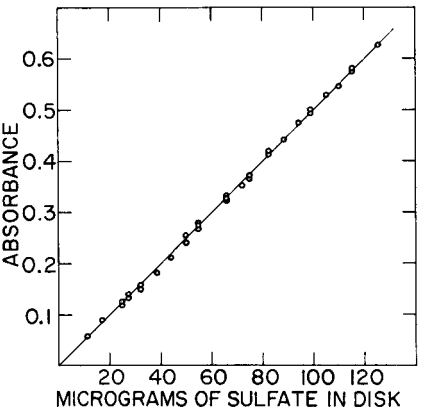

Fig. 2. Calibration curve.

(see procedure below) to yield the data shown in Fig. 2. This calibration curve appears to be reproducible but of course it is good practice to run standards through the procedure along with each batch of unknown samples.

Because the potassium bromide which was on hand was known to be sulfate-free, it was the first salt to be studied for interference in the procedure. It was found, in the most extreme case tested, that bromide in weight ratio to sulfate of 12,000 : 1 did not interfere in any way. According to KUBLI's selectivity series[7], chloride, nitrate, permanganate, perchlorate, acetate and sulfide are all less strongly retained on alumina than is bromide, and hence interference from these ions would not be expected. Iodide, nitrite and thiocyanate are held only slightly more strongly than bromide and likewise could be tolerated in fairly large amounts.

It was believed that phosphate might be the most troublesome ion because it is often present as an impurity in the same reagents containing traces of sulfate, it exhibits absorption bands near 9 μ, and a clean separation of phosphate from sulfate on the alumina column was not anticipated. The results of some experiments with sulfate–phosphate mixtures are shown in Table I. First, it was found that even the smallest quantity of phosphate tested interfered with the sulfate determination if it was added to the sulfate solution *after* the chromatographic step. The interference was eliminated by the alumina column at this level. But as the quantity of phosphate was increased, the column separation broke down. The highest ratio of phosphate

TABLE I

EFFECT OF PHOSPHATE ON THE DETERMINATION OF 2.76 mg OF SULFATE

mg of PO_4^{-3}	*weight ratio* PO_4^{-3}/SO_4^{-2}	*mg of* SO_4^{-2} *found*	*error mg*	*% relative error*
1.38[a]	1 : 2	3.10	0.34	12.3
2.76[a]	1 : 1	3.42	0.66	23.9
5.52[a]	2 : 1	4.69	1.93	70.0
1.38	1 : 2	2.82	0.06	2.2
2.76	1 : 1	2.71	0.05	1.8
5.52	2 : 1	2.72	0.04	1.4
5.52	2 : 1	2.76	0.00	0.0
5.52	2 : 1	2.76	0.00	0.0
8.28	3 : 1	2.70	0.06	2.2
8.28	3 : 1	2.69	0.07	2.5
11.04	4 : 1	2.71	0.05	1.8
11.04	4 : 1	2.82	0.06	2.2
13.80	5 : 1	2.84	0.08	2.9
13.80	5 : 1	2.98	0.22	8.0
16.56	6 : 1	3.32	0.56	20.2
16.56	6 : 1	3.41	0.65	23.5
27.60	10 : 1	3.48	0.72	26.0
27.60	10 : 1	4.17	1.41	51.0

[a] In these three cases, phosphate was added *after* the chromatographic step in the procedure.

to sulfate which could be tolerated under the conditions of the procedure presented here was about 4 : 1 (on a weight basis as PO_4^{-3} and SO_4^{-2}). This sort of behavior was expected because previous workers had stated[8-10] that only a partial separation of phosphate and sulfate was obtainable on alumina columns. However, in most reagent chemicals, phosphate impurities are present in smaller quantities than sulfate impurities[11], so the applicability of the method is actually not badly jeopardized by phosphate.

Procedure

To prepare the sample, simply dissolve a weighed quantity of the reagent-grade chemical in dilute hydrochloric acid. According to FRITZ[10], the acid concentration should not exceed about 2 *N*, but aside from this, it is not critical. The authors have used 2 to 5% by weight hydrochloric acid, which corresponds to about 0.6 to 1.6 *N*. In cases where an insoluble chloride might form, perchloric acid could be substituted for hydrochloric. The quantity of sample, and the size of the aliquot taken later for freeze-drying, can be varied, of course, depending upon the percentage of sulfate in the sample, the solubility of the sample, and other factors. In most cases, the authors dissolved a 50-g sample in 100 ml of hydrochloric acid, because it seemed preferable to use a small aliquot of a large sample in order to minimize sampling errors due to possible inhomogeneity.

Prepare an alumina column according to the directions of NYDAHL[9]. Pretreat the column and test for elutable sulfate as described[9]. Prepare the column to receive the sample by a final washing with about 10 ml of 2 to 5% hydrochloric acid. Then pass the sample through the column. A fairly rapid flow rate of 10 to 15 ml per minute is satisfactory for the column operation. Wash the column with about 50 ml of 2 to 5% hydrochloric acid solution, and then with about 30 ml of water. Now elute the sulfate by passing about 5 ml of 1 *M* ammonia followed by about 40 ml of 0.1 *M* ammonia through the column. Dilute the eluate to 50 ml in a volumetric flask.

Pipet a 1-ml aliquot of the column eluate into the bottom of a freeze-drying tube which already contains 300 mg of potassium bromide. Swirl the tube gently to dissolve the potassium bromide. Then dip the tube into a dry ice–acetone mixture with a swirling motion so that the liquid freezes in a thin layer extending partway up the wall of the tube. With the VirTis freeze-dryer, samples can be placed in the evacuated system without breaking the vacuum; in some freeze-dryers, all the samples are introduced at the same time and the system is then evacuated. In any case, keep the sample in the freezing mixture until it is to be evacuated, and then freeze-dry it overnight. Once the freeze-drying begins, the sample remains frozen without further external cooling and the process requires no attention.

Scrape the light, voluminous powder from the sides of the freeze-drying tube with a spatula, and transfer it to the die. Form the powder into an even layer, and press the disk. Various directions have been recommended for the pressing operation. The authors evacuated the die for about a minute and then pressed with a total applied force of about 20,000 lb. for 8 to 10 minutes. This force corresponds to a pressure of slightly over 100,000 lb. per sq. in. on a plunger 0.5 in. in diameter. (Several writers have apparently confused force and pressure in describing their own conditions.) Remove the disk from the die. It should be clear and unblemished, but actually slight cloudiness and surface irregularities do not seriously affect the measurement of the sulfate band at 9 μ.

Record the spectrum of the disk from about 7 to 11 μ, using a disk of 300 mg of pure potassium bromide in the reference beam. Draw the base-line on the chart, measure I and I_0, compute the transmittance, and convert this into the absorbance. Weigh the disk, and correct the absorbance value to a 300 mg basis by multiplying it by the factor (300/actual weight of disk). Compare this corrected absorbance value with a calibration curve obtained with standard sulfate samples run through the same

procedure to obtain the quantity of sulfate in the freeze-dried aliquot, and then, using the appropriate dilution factors, calculate the quantity of sulfate in the original sample.

RESULTS

Pure sulfate solutions: Twenty replicate solutions of potassium sulfate were run through the entire procedure. The concentrations were such that 55.0 μg of sulfate should have been found in the disks prepared from the freeze-dried aliquots of column eluates. The actual results ranged from 53.8 to 57.0 μg, with an average value of 55.0 μg, an average deviation of 0.85 μg or 1.5%, and a standard deviation of 1.0 μg or 1.9%.

Reagent-grade chemicals: The percentages of sulfate (as SO_4^{-2}) in several reagent-grade chemicals obtained through ordinary commercial channels were determined using the procedure described above. Results are shown in Table II. Since the reliability of the analyses shown on the reagent labels was not known, it was decided to compare infrared sulfate determinations with determinations made by another method on the same samples in the same laboratory. For this comparison, the barium chloranilate colori-

TABLE II

SULFATE DETERMINATION IN SOME REAGENT-GRADE SALTS

Reagent	*Sulfate stated on label, %*	*Sulfate found, %*
KBr	0.0037	0.0034
		0.0034
		0.0032
		0.0032
$CaCl_2 \cdot 2H_2O$	0.003	0.0062
		0.0062
		0.0063
		0.0062
$NaNO_2$	0.004	0.0015
		0.0014
		0.0013
		0.0014
$Th(NO_3)_4 \cdot 4H_2O$	0.001	0.0011
		0.0011
		0.0010
		0.0011
K_2CO_3	0.002	0.0020
		0.0016
		0.0020
		0.0018
$KMnO_4$	0.005	0.0043
		0.0048
		0.0041
		0.0057
$CuCl_2 \cdot 2H_2O$	0.003	0.0031
		0.0029
		0.0030
		0.0029
$Na_2MoO_4 \cdot 2H_2O$	0.015	0.0140
		0.0148
		0.0139
		0.0155

metric method[12] was selected. Because the sulfate level in most reagents is too low for optimal results with this colorimetric method, the comparison was made in this way: 0.01 *M* solutions of the salts shown in Table III were "spiked" with additional sulfate, and aliquots of the solutions were analyzed by the two methods. The results in Table III show that the infrared method is consistent with the barium chloranilate method; the latter, although fairly new, is a recognized method for determining sulfate.

TABLE III

COMPARISON OF INFRARED AND BARIUM CHLORANILATE METHODS IN DETERMINING SULFATE ADDED TO REAGENT SOLUTIONS

Reagent	*Sulfate concentration in original solution, μg/ml*[a]	
	Infrared	*Ba-chloranilate*
KNO_3	424	422
$CaCl_2 \cdot 2H_2O$	594	594
$Na_2C_2O_4$	429	433
$NaHCO_3$	354	361
$CoCl_2 \cdot 6H_2O$	617	616

[a] Figures given are averages of 3 measurements.

ACKNOWLEDGEMENT

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SUMMARY

A method for determining traces of sulfate in reagent-grade chemicals is presented. The sample is passed through an alumina column in acid solution to isolate the sulfate, which is then eluted with ammonia. Potassium bromide is added to an aliquot of the eluate, and the solution is freeze-dried. The resulting powder is pressed into a disk whose infrared spectrum is recorded. The method is reasonably accurate and requires a minimum of operator time per sample because a large number of samples can be processed together.

RÉSUMÉ

Une méthode est proposée pour le dosage de traces de sulfate dans divers produits chimiques ,,pro analysi''. Le sulfate est séparé par chromatographie et dosé finalement par spectrophotométrie infrarouge.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur Bestimmung von Spuren von Sulfat in ,,pro analysi'' Chemikalien. Das Sulfat wird chromatographisch abgetrennt und infrarot-spektrophotometrisch bestimmt.

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COULOMETRISCHE PERMANGANATOMETRISCHE BESTIMMUNG DER FERROCYANID- UND JODIDIONEN

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Durch bisherige analytische Anwendung der elektrolytisch quantitativ gebildeten Permanganationen[1], wurden coulometrische Methoden zur quantitativen Bestimmung des Oxalat-, Ferro- und Arsenitions[2], sowie des Wasserstoffperoxyds[3], mit konstanter Stromstärke, ausgearbeitet.

Auf Grund der Reaktionen des Ferrocyanid- und Jodidions mit Permanganationen konnten wir die coulometrische Permanganatometrie mit konstanter Stromstärke auch auf Ferrocyanid- und Jodidionen ausbreiten und Methoden für ihre quantitative Mikro- und Halbmikrobestimmung ausarbeiten.

Bei bisher ausgearbeiteten Methoden erfolgt die Endpunktbestimmung der coulometrischen Titration durch Redox-Indikatoren[1,2], sowie potentiometrisch[4]. Bei der coulometrischen permanganatometrischen Bestimmung der Ferrocyanid- und Jodidionen ist die potentiometrische Methode die geeignetere.

Es wurde die klassische diskontinuierliche Methode, sowie diejenige mit polarisierten Indikatorelektroden aus Platin angewandt. Dabei befindet sich die Indikatorelektrode ausserhalb des Kräftefeldes, welches zwischen den Generatorelektroden herrscht[5]. Ausser Gebrauch werden Indikator- und Generatorelektroden in der Anolytenlösung aufbewahrt. Vor Ausführung der Bestimmung soll an den zu gebrauchenden Elektroden einige Minuten die Elektrolyse im System in dem die Bestimmung ausgeführt wird, durchgeführt werden[5,6].

Vor Zugabe der zu bestimmenden Substanz wird der Anolyt, durch elektrolytische Erzeugung der Permanganationen, auf das Potential des Endpunktes der coulometrischen Titration eingestellt[7,8]. In bestimmten Fällen kann die Titration auch ohne vorherige Einstellung des Anolyten ausgeführt werden, was aber nicht genügend zuverlässig ist, wobei der Erfolg sehr stark von der Reinheit und Herkunft der Indikatorelektrode aus Platin[9], sowie von der Reinheit der angewandten Chemikalien abhängt.

Unter unseren Arbeitsbedingungen, bei Anwendung der klassischen diskontinuierlichen potentiometrischen Methode, sowie der polarisierten Indikatorelektrode, betragen, in der Nähe des Endpunktes, die Zeitinkremente für elektrolytische Erzeugung der Permanganationen durch Stromstärken von 5 und 10 mA etwa 6 Sekunden. In den Systemen, mit denen wir gearbeitet haben, tritt in der Nähe des Endpunktes eine Potentialverschiebung der Indikatorelektrode nach positiveren Werten ein, besonders bei Bestimmungen von Jodidionen, und weniger bei Arbeiten mit

Ferrocyanidionen[10-12]. In der Nähe des Endpunktes sind zwei bis drei Minuten für die Potentialeinstellung nötig.

Während der Bestimmungen mit polarisierter Indikatorelektrode[8,13] wurden die Ablesungen am Mikroampèremeter 30 Sekunden nach Stromunterbrechung im coulometrischen Stromkreis vorgenommen. Das mechanische Rühren des Anolyten wurde dabei nicht unterbrochen. Als Referenzelektrode, Indikatorkathode, diente eine gesättigte Kalomelelektrode und bei einer Spannung am Potentiometer von 2 V bei 10 Ω, betrug der Widerstand, in Serie geschaltet mit der Indikatoranode, 150,000 bis 190,000 Ω.

Der Endpunkt wurde aus $E = f(t)$, $\frac{\Delta E}{\Delta t}$ und $\frac{\Delta^2 E}{\Delta t^2}$ rechnerisch nach der Methode von HOSTETTER – ROBERTS[14,15], oder graphisch festgestellt. Im System mit polarisierter Indikatorelektrode wurde der Endpunkt der Titration graphisch als Schnittpunkt der Funktion $i = f(t)$ und der Geraden $i = 0$ im Koordinatensystem $i—t$ bestimmt.

Wie auch bei anderen coulometrischen permanganatometrischen Bestimmungen[2-4] werden auch diese Bestimmungen im getrennten Anolyten unter mässigem Rühren ausgeführt.

BESTIMMUNG DER FERROCYANIDIONEN

Die Reaktionsbedingungen

$$5\,[Fe(CN)_6]^{-4} + MnO_4^- + 8\,H^+ \rightleftarrows Mn^{+2} + 5\,[Fe(CN)_6]^{-3} + 4H_2O$$

und die Bedingungen der elektrolytischen Erzeugung der Permanganationen ermöglichen die Ausführung dieser Titration mit elektrolytisch erzeugten Permanganationen, mit genügender Genauigkeit.

Wegen der teilweisen, unmittelbaren Oxydation des Ferrocyanids an der Anode, überzieht sich diese im Laufe der coulometrischen Titration mit einer dunklen Schicht von Ferricyanid. Beim Arbeiten im Anolyten mit 4 bis 6 *N* Schwefelsäure ist dieser Überzug an der Anode beträchtlich und die erzielten Resultate weisen einen unzulässig grossen positiven Fehler auf. In 6 *N* Schwefelsäure, bei kleineren Stromdichten und in 8 *N* Schwefelsäure, bei grösseren Stromdichten, ist die Anodenschicht bedeutend geringer und die Resultate sind befriedigend. Nach jeder Bestimmung wird die Anode in konz. Chlorwasserstoffsäure gewaschen.

Ausgeführt wurden Bestimmungen von 4 bis 33 mg Ferrocyanid, mit Stromstärken von 5.00 bis 10.08 mA, Stromdichten von 1.3 bzw. 2.5 mA/cm², bei 25 bis 30°, in 50 ml des Anolyten folgender Zusammensetzung:

(a) bei Stromdichte von 1.3 mA/cm²: 8 *N* H_2SO_4 und 0.54 *M* oder 0.27 *M* $MnSO_4 \cdot 4H_2O$, sowie 6 *N* H_2SO_4 und 0.27 *M* $MnSO_4 \cdot 4H_2O$;

(b) bei Stromdichte von 2.5 mA/cm²: 8 *N* H_2SO_4 und 0.54 *M* oder 0.27 *M* $MnSO_4 \cdot 4H_2O$.

Die Anode ist aus Platinblech von 4 cm² Oberfläche.

Das Potential der Indikatorelektrode aus Platin (P = 4 cm²) im Anolyten der angegeben Zusammensetzung hat den Wert $e_h = 0.75$ V (± 0.03). Durch elektrolytische Erzeugung der Permanganationen im Grundelektrolyten, unter den angeführten Bedingungen, bekommt das Potential der Indikatorelektrode einen positiveren Wert des Potentials des Endpunktes der Titration des Ferrocyanids mit Permanganationen,

$e_h = 0.89$ V. Die Zeitinkremente für die Erzeugung von Permanganationen bei dieser Einstellung betragen 0.1 bis 3 Sekunden, beim Ablesen des Potentials 30 Sekunden nach Unterbrechung des Stroms im coulometrischen Stromkreis. Nach Zugabe der zu untersuchenden Ferrocyanidlösung (1–6 ml 0.05 N $K_4[Fe(CN)_6] \cdot 3H_2O$, erreicht das Potential der Indikatorelektrode den negativeren Wert $e_h = 0.71$ V. Die coulometrische Titration wird dann wie eine potentiometrische Titration, durch Verfolgung des Potentials der Indikatorelektrode aus Platin in Bezug auf eine gesättigte Kalomelelektrode, weitergeführt, mit dem Unterschied, dass die benötigten Permanganationen elektrolytisch erzeugt werden.

Bei dem System mit polarisierter Indikatorelektrode wird, nach der angegebenen Einstellung des Anolyten und nach Zugabe der zu titrierenden Lösung, das Potentiometer auf 0.89 V eingestellt und weitertitriert.

TABELLE I

50 ml des Anolyten		*Genommen*	*Dauer*	*Gefunden*	*Fehler*	
H_2SO_4 *N*	$MnSO_4 \cdot 4H_2O$ *M*	$[Fe(CN)_6]^{-4}$ *mg*	*sec*	$[Fe(CN)_6]^{-4}$ *mg*	*mg*	%
$i = 5.00\ mA$, $D = 1.3\ mA/cm^2$, $t = 27°$ ($\pm$ 2)						
8	0.54	3.890	357.1	3.923	0.033	0.9
		6.483	590.9	6.490	0.007	0.1
		8.644	785.2	8.624	—0.020	—0.2
		10.81	984.7	10.81	0.00	0.0
		10.81	981.9	10.78	—0.03	—0.3
		11.16	1019.2	11.16	0.00	0.0
		12.97	996.4	12.92	—0.05	—0.4
		13.40	1226.9	13.48	0.08	0.6
8	0.27	3.890	355.9	4.908	0.018	0.5
		6.483	588.1	6.458	—0.025	—0.4
		10.81	990.7	10.88	0.07	0.6
		12.97	1191.1	13.08	0.11	0.8
6	0.27	3.890	357.1	3.922	0.032	0.8
		6.483	590.3	6.483	0.000	0.0
		10.81	987.3	10.84	0.03	0.3
		12.97	1186.2	13.03	0.06	0.5
$i = 10.08\ mA$, $D = 2.5\ mA/cm^2$, $t = 28°$ ($\pm$ 1)						
8	0.54	6.915	312.8	6.926	0.011	0.2
		8.644	390.4	8.643	—0.001	—0.01
		12.97	585.7	12.97	0.00	0.0
		17.29	781.4	17.30	0.01	0.1
		21.61	979.1	21.68	0.07	0.3
		27.66	1255.6	27.80	0.14	0.5
		32.42	1470.1	32.55	0.13	0.4
8	0.27	8.644	392.1	8.680	0.036	0.4
		12.97	591.1	13.09	0.12	0.9
		21.61	982.6	21.76	0.15	0.7
		32.42	1475.9	32.68	0.27	0.8

Die erzielten Resultate sind in der Tabelle I zusammengestellt und den Verlauf der Potentialänderung $E = f(t)$, bei einer coulometrischen Titration von 6.483 mg $[Fe(CN)_6]^{-4}$, mit der Stromstärke von 5.00 mA zeigt die Fig. 1.

Unter den angegebenen Bedingungen beträgt der Potentialsprung im Endpunkt, für 6 Sekunden 50–100 mV. Die Beziehung i–t bei einer coulometrischen Titration von

6.698 mg $[Fe(CN)_6]^{-4}$ mit einer Stromstärke von 5.00 mA, mit polarisierter Indikatorelektrode ist aus der Fig. 2 zu ersehen.

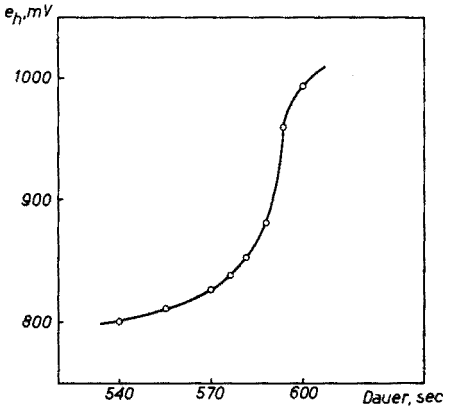

Fig. 1. $E = f(t)$. Verlauf der Potentialänderung der Indikatorelektrode aus Platin bei Titration von 6.483 mg $[Fe(CN)_6]^{-4}$ mit einer Stromstärke von 5.00 mA im Grundelektrolyten mit 8 N H_2SO_4 und 0.54 M $MnSO_4 \cdot 4\,H_2O$.

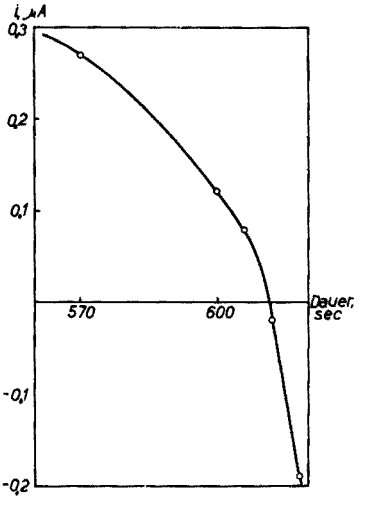

Fig. 2. Funktion i–t bei Titration von 6.698 mg $[Fe(CN)_6]^{-4}$ mit der Stromstärke von 5.00 mA an einer polarisierten Indikatorelektrode.

BESTIMMUNG DES JODIDIONS

In Anwesenheit von Schwefelsäure oxydiert Kaliumpermanganat das Jodidion bis zu elementarem Jod

$$5J^- + MnO_4^- + 8\,H^+ \rightleftarrows Mn^{+2} + 4\,H_2O + 2\tfrac{1}{2}\,J_2$$

Nach der Oxydation des gesamten Jodids oxydiert das überschüssige Permanganat das befreite Jod bis zum Jodat.

Die Bedingungen der quantitativen Reaktion der Jodid- und Permanganationen, sowie die Bedingungen der elektrolytischen Erzeugung der Permanganationen erlauben die Ausführung dieser Reaktion auch mit elektrolytisch erzeugten Permanganationen. Die Konzentration der Schwefelsäure im Anolyten, sowie die Temperatur sind auf kleinere Werte beschränkt, als bei der Bestimmung der Ferrocyanidionen, wegen der Zersetzbarkeit der Alkalijodide unter dem Einfluss stärkerer Schwefelsäure bei höherer Temperatur.

Das erzeugte elementare Jod färbt die Lösung, weswegen in diesem Falle das Reaktiv nicht als Indikator dienen kann. Direkte Anwendung eines Redox-Indikators ist ebenfalls unzulässig, sondern erst nach Überführung des Jods in eine farblose Verbindung[16,17] oder nach Extraktion des Jods während der Reaktion durch ein Lösungsmittel, wie z.B. durch Chloroform, Kohlentetrachlorid oder Benzol[18], das sich mit Wasser nicht mischt. Der Endpunkt kann ausserdem auch potentiometrisch bestimmt werden[19,20].

Wir konnten für die Endpunktbestimmung einen Indikator, sowie die potentiometrische Methode gebrauchen. Wie bei der volumetrischen Bestimmung des Jodids

mit Permanganat, haben wir auch bei der coulometrischen permanganatometrischen Bestimmung die potentiometrische Methode der Endpunktbestimmung als die praktischere und zuverlässigere vorgezogen.

Es wurden 3 bis 10 mg Jodidionen, mit Stromstärken von 5.02 und 10.08 mA und Stromdichten von 0.7 bis 1.3 mA/cm² in 50 ml des Anolyten mit 4.5 N H_2SO_4 und 0.27 M $MnSO_4 \cdot 4H_2O$ bei 25° bis 30° an der Anode aus Platinspirale mit einer Oberfläche von 7.6 cm², ausgeführt.

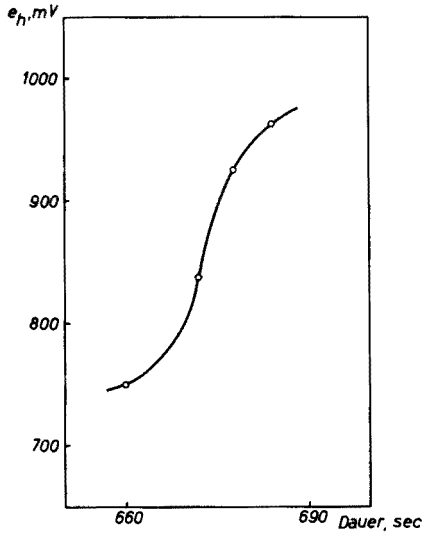

Fig. 3. $E = f(t)$. Verlauf der Potentialänderung der Indikatorelektrode aus Platin bei Titration von 4.464 mg J^- mit einer Stromstärke von 5.02 mA, ohne Anolyteinstellung.

TABELLE II

50 ml des Anolyten: 4.5 N H_2SO_4, 0.27 M $MnSO_4 \cdot 4H_2O$

Genommen J^- *mg*	*Dauer sec*	*Gefunden* J^- *mg*	*Fehler*	
			mg	%
$i = 5.02\ mA,\ D = 0.7\ mA/cm^2$				
3.041	458.9	3.030	—0.011	—0.4
3.144	473.7	3.128	—0.016	—0.5
3.703	563.1	3.718	0.015	0.4
4.117	624.5	4.123	0.006	0.2
4.117	623.6	4.117	0.000	0.0
4.828	729.8	4.818	—0.010	—0.2
5.517	833.7	5.505	—0.012	—0.2
6.082	922.0	6.088	0.006	0.1
6.082	914.2	6.036	—0.046	—0.8
$i = 10.08\ mA,\ D = 1.3\ mA/cm^2$				
6.222	469.9	6.223	0.001	0.02
7.857	596.9	7.914	0.057	0.8
7.857	591.7	7.842	—0.015	—0.2
8.494	635.5	8.426	—0.068	—0.8
9.335	699.7	9.277	—0.058	—0.6
10.04	759.6	10.07	0.03	0.3
10.04	755.3	10.01	—0.03	—0.3

Die erzielten Resultate enthält die Tabelle II. Die Funktion $E = f(t)$ bei der Titration von 4.464 mg Jodidionen mit einer Stromstärke von 5.02 mA zeigt die Fig. 3. Für einen Anolyten der angeführten Zusammensetzung und für die angegebenen Jodidmengen beträgt der Potentialsprung im Endpunkt, für 6 Sekunden, etwa 100–150 mV.

Das Potential der Indikatorelektrode aus Platin ($P = 4$ cm^2) im angewandten Anolyten hat den Wert $e_h = 0.73$ V. Durch elektrolytische Erzeugung der Permanganationen nimmt das Potential einen positiveren Wert, $e_h = 0.85$–0.88 V, an, wie dies schon bei der Ferrocyanidbestimmung angeführt wurde. Nach Zugabe der zu untersuchenden Lösung mit Jodidionen (1 bis 5 ml 0.05 *N* KJ) erreicht das Potential der Indikatorelektrode den Wert $e_h = 0.57$ V.

Durch diese coulometrischen permanganatometrischen Bestimmungen der Ferrocyanid- und Jodidionen wurde das Anwendungsgebiet der Grundmethode erweitert und es wurden auch weitere Möglichkeiten für die Anwendung des Coulombs als Urtitersubstanz geschaffen.

ZUSAMMENFASSUNG

Die coulometrische Permanganatometrie wurde zur quantitativen Bestimmung von Ferrocyanid- und Jodidionen angewandt.

Es wurden Mikromethoden zur coulometrischen quantitativen permanganatometrischen Bestimmung des Ferrocyanid- und Jodidiones mit konstanter Stromstärke und mit potentiometrischer Endpunktbestimmung ausgearbeitet.

SUMMARY

Micromethods have been developed for the quantitative coulometric permanganatometric determination of ferrocyanide and iodide ions at constant current strength; the end-point is determined by potentiometry.

RÉSUMÉ

Des méthodes ont été établies pour le microdosage coulométrique par permanganométrie des ions ferrocyanures et iodures, à courant constant et détermination potentiométrique du point final.

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VOLTAMMETRIC, POTENTIOMETRIC AND AMPEROMETRIC STUDIES WITH A ROTATED ALUMINUM WIRE ELECTRODE

V. AMPEROMETRIC TITRATION OF FLUORIDE WITH ALUMINUM

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INTRODUCTION

The rotated aluminum electrode (RAlE) should be a suitable indicator electrode for the direct amperometric measurement of fluoride. Experimental results in the third paper of this series[1] showed that in the proper medium the anodic depolarization current of fluoride at the RAlE is proportional to the fluoride concentration provided the latter is smaller than about $3 \cdot 10^{-4}$ *M*. Therefore, it is reasonable to expect that this electrode will be suitable for the amperometric titration of fluoride. The present paper deals with the amperometric titration of fluoride with an aluminum salt. Subsequent papers will discuss the direct amperometric titration of fluoride with thorium and zirconium, and the literature on these amperometric titrations will be discussed in these papers. Considering the great practical importance of the determination of fluoride at high dilutions we have concentrated mainly on the titration in the concentration range between 10^{-4} and 10^{-3} *M*.

Agents like aluminum and thorium ions form a series of complexes with fluoride. During the titration the composition of the mixtures of complexes formed changes with the concentration of fluoride. From the known stability constants of the various complexes it is possible to calculate the concentration of "free" fluoride (not bound to aluminum) during a titration. On this basis it is possible to calculate theoretically the amperometric titration lines. This has been done in section IV, and the calculated curves are compared with the experimental curves.

Particularly in solutions dilute in fluoride and aluminum there is no simple (stoichiometric) ratio between fluoride and aluminum at the end-point; moreover this ratio changes with the concentration of fluoride titrated. From the stability constants of the various complexes it is possible to calculate the over-all composition of the reaction product at the end-point. In Table I experimental and calculated reaction ratios are compared.

Considering the varying ratio and therefore the empirical nature of the titration in very dilute solutions in aqueous media, titrations have also been carried out in 50% ethanol in the presence and absence of varying concentrations of alkali nitrates. It has been possible to find conditions under which the composition of the reaction product was reasonably constant at the end-point. Results are given in the experimental part.

EXPERIMENTAL

Materials

Sodium fluoride solution: A stock solution was prepared which contained 2.206 g of the salt per l. This was standardized gravimetrically using the lead-chlorofluoride method[2]; the concentration was 0.0528 *M*. Dilute solutions were prepared by proper dilutions of aliquots.

Aluminum nitrate solution: A stock solution was prepared which contained 6.252 g of $Al(NO_3)_3 \cdot 9H_2O$ per l. It was standardized by the oxine gravimetric method[3] and was found to be 0.0167 *M* in Al^{+3}.

All other reagents were C. P. chemical products.

Apparatus

The electrode (RAIE) and titrating equipment have been already described[4]. For the measurement of current in the amperometric titrations a Leeds and Northrup Electrochemograph was used.

The pH of all solutions was measured with a Beckmann glass electrode pH meter. For the measurement of pH in 50% ethanol solutions the glass electrode system was used with the saturated calomel electrode in water as a reference electrode. The pH values reported have no exact significance. The glass electrode was calibrated with a phosphate buffer of known pH in water. The pH of this buffer is different in 50% ethanol. No correction for the medium effect was made since we were interested only in relative values of pH.

Amperometric titrations

Aqueous medium. Titrations of fluoride in concentrations ranging from $1 \cdot 10^{-4}$ *M* to $2 \cdot 10^{-3}$ *M* were carried out in acetate buffers of varying pH. The compositions of the buffers in the titration mixtures were: a. 0.17 *N* acetic acid + 0.02 *M* sodium acetate, pH = 3.6; b. 0.1 *N* acetic acid + 0.1 *M* sodium acetate, pH = 4.6; c. 0.1 *N* acetic acid + 0.1 *M* sodium acetate + 0.5 *M* potassium nitrate, pH = 4.5.

The values of the formation constants of the various aluminum–fluoride complexes reported by BROSSET AND ORRING[5] refer to acid solutions 0.5 *M* in potassium nitrate. In order to compare calculated with experimental titration curves, a number of experiments was carried out in the presence of 0.5 *M* potassium nitrate.

In order to test the rate of attainment of equilibrium during the titration, experiments were carried out in acetate buffers at pH 3.6 and 4.6 in which known concentrations of aluminum were added to a given fluoride mixture. After measuring the fluoride current, these mixtures were allowed to stand for varying periods of time, up to 72 h, after which the fluoride current was measured.

50% Ethanol as medium. The composition of the titration mixture was: a. 0.35 *N* acetic acid + 0.01 *M* sodium acetate, (apparent) pH = 4.0; b. 0.17 *N* acetic acid + 0.035 *M* sodium acetate, (apparent) pH = 4.8. Several experiments were done in the buffers in the presence of 0.25 to 0.5 *M* alkali nitrate (sodium, potassium or lithium nitrate).

Titration procedure

Determine the residual current in the buffer. Place 50 ml of the fluoride containing solution (adjusted to pH with buffer) into the electrolysis cell and remove oxygen by bubbling nitrogen for at least 5 min. Introduce the RAIE, start its rotation at 600 r.p.m. and connect with the SCE. Apply a potential of —0.75 V and record the fluoride current after it has attained a constant value. Start the addition of the aluminum solution from a microburet and wait until the current is constant after each increment addition of the reagent. Pass nitrogen through after each addition and over the solution during the titration. Subtract the residual current (see note) and plot the results after correcting readings for the volume increase: $(V + v)/V$ where V denotes the original volume of solution and v the volume of reagent added at any point.

Notes: (1) The residual current must be determined first in a separate experiment by recording the current in the buffer solution containing no fluoride. With our electrode it was 0.2 to 0.3 μA (anodic) in acetate buffers of pH 3.6 to 4.6, but higher values were found in unbuffered solutions of KNO_3 (0.8 μA).

(2) Immediately after the addition of aluminum the current shows a sharp decrease and then slowly decreases with time. A steady value at each point during the titration is reached in no more than 15 min, depending on the concentration of fluoride. For fluoride concentrations of the order of $1 \cdot 10^{-3}$ *M* the steady current is attained in about 5 min, but in more dilute solutions it is necessary to wait 10 to 15 min to attain a constant value.

(3) In solutions initially 10^{-3} *M* in fluoride or higher, the plot of current *versus* titrant added is not linear at the beginning of the titration, see Fig. 2, curves (1) and (2). This is mainly due to the fact that the proportionality between current and fluoride concentration at the RAlE only holds up to about $3 \cdot 10^{-4}$ *M* solutions[4]. When the initial fluoride concentration was 0.001 *M* reasonably straight lines could be plotted after addition of about 40% of the aluminum found at the end-point.

Location of end-point. In the plot of fluoride current *versus* added aluminum connect the experimental points by drawing the best fitting straight line.

False end-point (FEP). This corresponds to the point of intersection between the two straight lines drawn through the experimental points (see points A in Figs. 1 and 2).

True end-point (TEP): This corresponds to the point of intersection of the reaction line with the abscissa (point B in Figs. 1 and 2).

Note: When a reaction is complete at the end-point, the current – after correction for the residual current – should be zero. As a rule this is not the case. In general

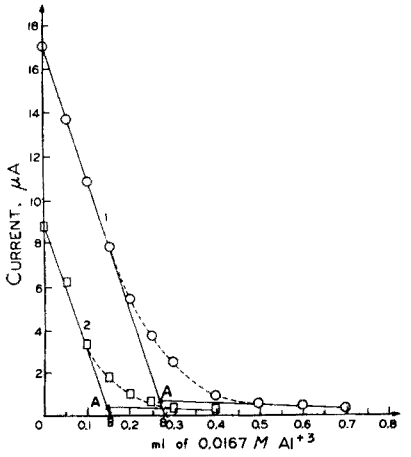

Fig. 1. Amperometric titration curves at pH 3.6. 1, $2 \cdot 10^{-4}$ *M* NaF; 2, $1 \cdot 10^{-4}$ *M* NaF. A, false end-point (FEP); B, true end-point (TEP).

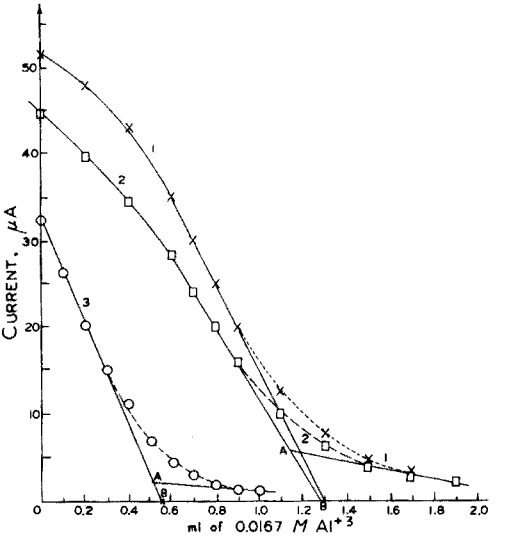

Fig. 2. Titration curves in acetate buffer of varying pH. 1, $1 \cdot 10^{-3}$ *M* NaF at pH 3.6; 2, $1 \cdot 10^{-3}$ *M* NaF at pH 4.6; 3, $5 \cdot 10^{-4}$ *M* NaF at pH 4.6. A, FEP; B, TEP.

the slopes of the reaction line and of the excess reagent line are found by using only those experimental points which are far enough removed from the end-point that according to the mass action law the reaction may be considered complete. This means that the excess reagent line should be horizontal. From Figs. 1, 2 and 3 it

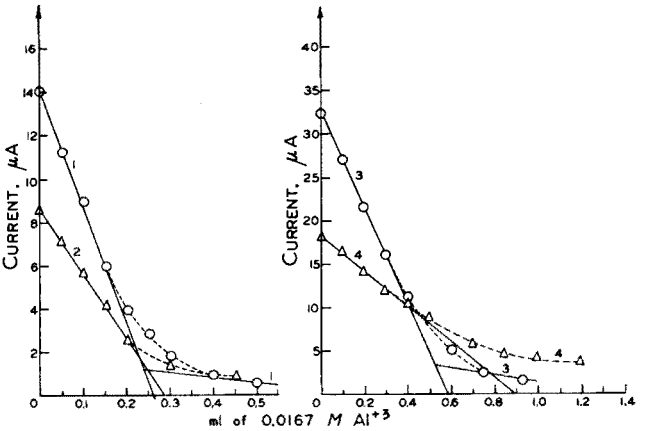

Fig. 3. Comparison of titration curves in buffered and unbuffered media of 0.5 M KNO_3. 1, $2 \cdot 10^{-4}$ M NaF in 0.1 N HAc + 0.1 M NaAc + 0.5 M KNO_3, pH 4.5; 2, $2 \cdot 10^{-4}$ M NaF in 0.5 M KNO_3, initial pH 6.5, final 5.6; 3, $5 \cdot 10^{-4}$ M NaF in 0.1 N HAc + 0.1 M NaAc + 0.5 M KNO_3, pH 4.5; $5 \cdot 10^{-4}$ M NaF in 0.5 M KNO_3, initial pH 6.5, final 5.4.

is seen that the excess reagent line (corrected for i_r) has quite a pronounced slope indicating that the reaction is not complete even with a large excess of reagent. Therefore, the point of intersection of the two lines (FEP) cannot correspond to a stoichiometrically defined end-point. The larger the current at the FEP the larger the concentration of unreacted fluoride. Since the reaction is not complete even with a relatively large excess of reagent, (see excess reagent lines) the TEP also cannot correspond to a simple stoichiometric composition. As a matter of fact, the "reaction line" should not be straight under these conditions, not only because of incompleteness of the reaction, but also because the composition of the complex formed is changing as the titration proceeds. Thus, the ratio of F/Al reacted at the TEP (and also at FEP) may be expected to vary with the concentration of fluoride titrated, as has been found to be the case in Table I (see DISCUSSION).

EXPERIMENTAL RESULTS

Aqueous media

Examples: Some plots at various fluoride concentrations in acetate buffers of pH 3.6 and 4.6 are shown in Figs. 1, 2 and 3. At the TEP the currents were 1.8, 2.5, 6.5 and 8.5 μA for initial fluoride concentrations of 1, 2, 5, 10 and $20 \cdot 10^{-4}$ M.
Assuming that the over-all composition at the TEP corresponds to AlF_3, the calculated end-points for $1 \cdot 10^{-4}$ M, $2 \cdot 10^{-4}$ M, $5 \cdot 10^{-4}$ M and $1 \cdot 10^{-3}$ M fluoride should be 0.106 ml, 0.212 ml, 0.529 ml, and 1.058 ml of 0.0167 M Al^{+3} as compared to the experimental values 0.152 ml, 0.275 ml, 0.54 ml and 1.28 ml respectively.

In Table I are reported the experimental fluoride/aluminum ratios at the FEP and TEP. At initial fluoride concentrations of 1 and $2 \cdot 10^{-4}$ M the values at the TEP

TABLE I

Comparison between the experimental F/Al ratio at end-point and the theoretical value.

NaF concentration *M*	*Fluoride–aluminum ratio*			
	FEP	*TEP*	*Calculated at TEP*[a]	
a. in 0.17 *N* HAc + 0.02 *M* NaAc	pH = 3.6			
$1 \cdot 10^{-4}$	2.11	2.09	2.17	
$2 \cdot 10^{-4}$	2.44	2.36	2.42	
$1 \cdot 10^{-3}$	2.62	2.40	3.05	2.60[b]
$2 \cdot 10^{-3}$	2.62	2.35	3.30	2.8 [b]
b. in 0.1 *N* HAC + 0.1 *M* NaAc	pH = 4.6			
$1 \cdot 10^{-4}$	2.21	2.10	2.22	
$2 \cdot 10^{-4}$	2.79	2.61	2.50	
$5 \cdot 10^{-4}$	3.05	2.94	2.85	
$1 \cdot 10^{-3}$	2.88	2.48	3.10	2.7 [b]
c. as b + 0 5 *M* KNO_3	pH = 4.5			
$2 \cdot 10^{-4}$	2.65	2.44	2.50	
$5 \cdot 10^{-4}$	2.93	2.72	2.85	
$1 \cdot 10^{-3}$	2.99	2.54	3.10	2.7 [b]

[a] Calculated values correspond to solutions of ionic strength $\mu = 0.5$.
[b] See discussion.

and FEP do not differ much (Table I). In more concentrated solutions ($1 \cdot 10^{-3}$, $2 \cdot 10^{-3}$ *M* fluoride) there is much greater uncertainty in the determination of the end-points, because of the more pronounced curvature of the titration lines, the high currents at the end-point(s) and the pronounced slope of the excess reagent lines.

Titrations were also carried out at different concentrations of fluoride in acetate

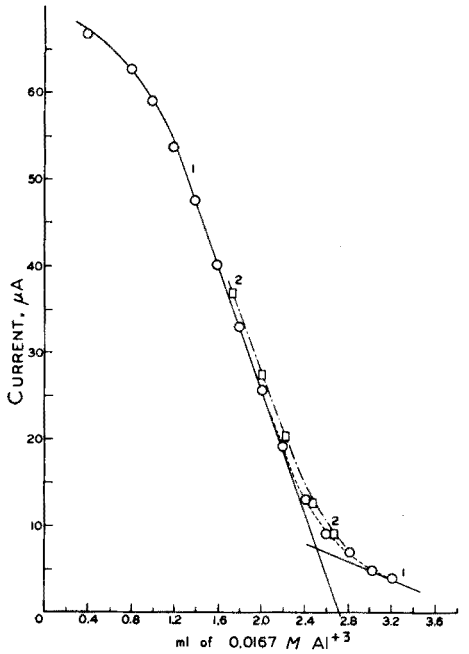

Fig. 4. Effect of time of waiting upon titration curve after addition of aluminum. 1, titration curve of $2 \cdot 10^{-3}$ *M* NaF at pH 3.6; 2, current readings after standing for 72 h.

buffers which were 0.5 *M* in potassium nitrate and also in 0.5 *M* potassium nitrate solutions containing no buffer. Examples of the titration lines are presented in Fig. 3. In the acetate buffer (pH 4.6) the presence of 0.5 *M* potassium nitrate has hardly any effect on the results (Table I). In an unbuffered 0.5 *M* potassium nitrate the results are irregular and poorly reproducible. This may be accounted for in part by the fact that the pH during the titration decreased from 6.5 to about 5.5.

Time of attainment of equilibrium during the titration

Two sets of titrations were carried out of a $2 \cdot 10^{-3}$ *M* fluoride solution both in acetate buffers of pH 3.6 and of pH 4.6. One titration was carried out according to the procedure described above. In the other "titration" mixtures were made containing 80, 90, 100, 111 and 120% respectively of aluminum, the percentage based on the formation of AlF_3. The mixtures (at pH 3.6 and 4.6) were allowed to stand for up to 72 h. The mixtures were then transferred to the electrolysis cell and the fluoride current was measured. In most of the mixtures no precipitate was observed. If a precipitate was visible, the mixture was centrifuged and the fluoride current was measured again. The fluoride concentration was calculated using a calibration curve determined in the same supporting electrolyte as in the mixtures. As is illustrated in Fig. 4 the results by the direct titration were the same (within 3%) as those obtained from titration lines constructed after a waiting period of 72 h. Titrations were also carried out at pH 2 (0.01 *N* perchloric acid). In such a medium the reaction between fluoride and aluminum was found highly incomplete, but equilibrium was established within 40 min.

Titrations in 50% ethanol

From Fig. 5 it is clear that fluoride ions depolarize the aluminum electrode in a 50% ethanol medium but that the limiting current is reduced to about one half the value found in aqueous medium. Results of fluoride titrations in an acetate buffer of pH 4.0 in 50% ethanol are given in Table II. In the absence of added sodium

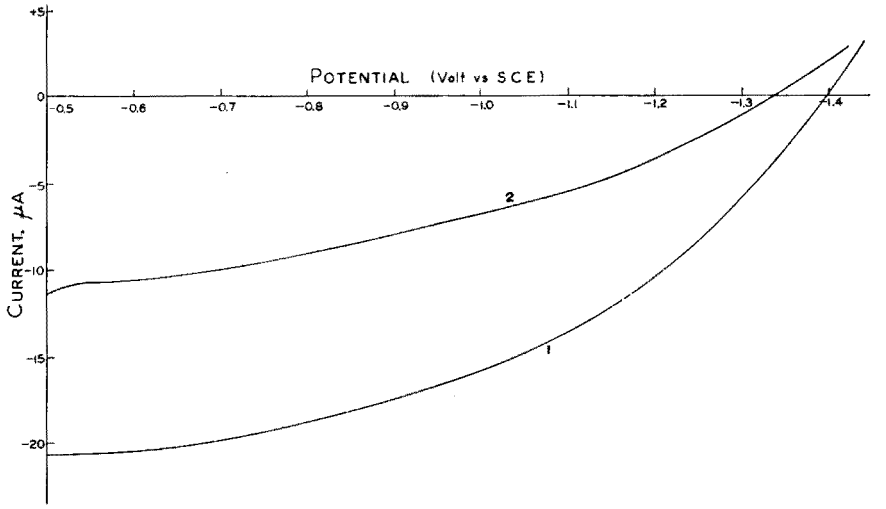

Fig. 5. Comparison of current–potential curves of fluoride in water and 50% ethanol. 1, $2 \cdot 10^{-4}$ *M* NaF in acetate buffer pH 4.0, aqueous medium; 2, same as 1, in 50% ethanol, pH 4.0.

TABLE II

Effect of sodium nitrate on fluoride/aluminum ratios at the end-point in 50% ethanol solutions buffered with 0.35 *N* HAc + 0.01 *M* NaAc, pH 4.0.

NaF concentration *M*	*$NaNO_3$ concentration* *M*	*F/Al ratio*	
		FEP	*TEP*
$2 \cdot 10^{-4}$	0	3.0	2.75
	0.2	5.0	4.1
	0.5	6.2	5.7
$5 \cdot 10^{-4}$	0	3.3	3.0
	0.2	6.0	5.8
	0.5	6.1	5.9
$1 \cdot 10^{-3}$	0	5.5	4.7
	0.2	6.1	5.8
	0.5	6.1	6.0

nitrate the F/Al ratio at the TEP increases from 2.75 with $2 \cdot 10^{-4}$ *M* fluoride to 4.7 with $1 \cdot 10^{-3}$ *M* fluoride. Addition of sodium nitrate in a concentration of 0.5 *M* greatly improves the situation and in the range of fluoride concentrations studied the F/Al ratio at the end-point was found to be 5.9 $\pm$ 0.1. Examples of titration lines are given in Figs. 6 and 7.

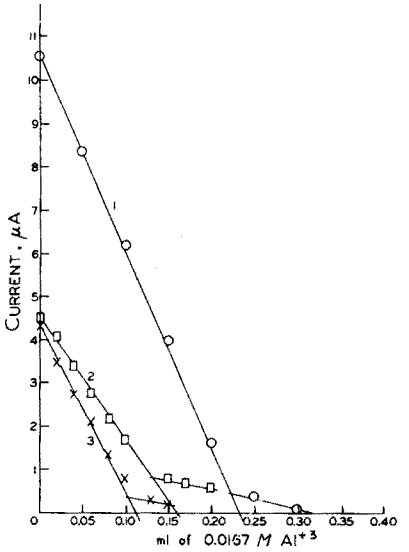

Fig. 6. Effect of $NaNO_3$ concentration on titration of $2 \cdot 10^{-4}$ *M* NaF in 50% ethanol, pH 4.0. 1, no $NaNO_3$; 2, 0.2 *M* in $NaNO_3$; 3, 0.5 *M* in $NaNO_3$.

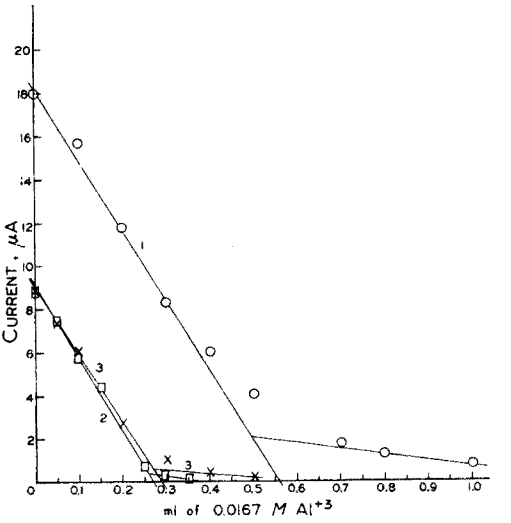

Fig. 7. Effect of $NaNO_3$ concentration on titration of $5 \cdot 10^{-4}$ *M* NaF in 50% ethanol, pH 4.0. 1, no $NaNO_3$; 2, 0.2 *M* in $NaNO_3$; 3, 0.5 *M* in $NaNO_3$.

From results in Table III it is interesting to note that at the same concentration (0.5 *M*) potassium nitrate has the same effect on the F/Al ratio at the TEP as sodium nitrate has, while lithium nitrate hardly exerts any effect. It would appear that Na_3AlF_6 and K_3AlF_6 are considerably more stable or less soluble in the salt containing buffer in 50% ethanol than Li_3AlF_6 is.

TABLE III

Effect of kind of alkali nitrate (0.5 *M*) on the F/Al ratio in 50% ethanol buffered with 0.35 *N* HAc + 0.01 *M* NaAc, pH 4.0

NaF concentration M	*Salt present*	*F/Al ratio*	
		FEP	*TEP*
$2 \cdot 10^{-4}$	$NaNO_3$	6.2	5.7
	KNO_3	6.6	5.7
$5 \cdot 10^{-4}$	$NaNO_3$	6.1	5.9
	KNO_3	5.9	5.8
	$LiNO_3$	5.5	3.5
$1 \cdot 10^{-3}$	$NaNO_3$	6.1	5.9
	KNO_3	6.1	5.9
	$LiNO_3$	4.2	3.7

Although the reproducibility of the experiments in Table III is no better than 5%, it appears possible to titrate fluoride in the buffer (pH 4.0) in 50% ethanol in the presence of 0.5 *M* sodium or potassium nitrate with an accuracy of about 10%. Three unknowns (to the operator) were titrated. Taking a reaction ratio of F/Al of 6, the following results were obtained:

	fluoride present (M)	*fluoride found (M)*
solution 1	$2.5 \cdot 10^{-4}$	$2.2 \cdot 10^{-4}$
solution 2	$5.5 \cdot 10^{-4}$	$5.4 \cdot 10^{-4}$
solution 3	$8.3 \cdot 10^{-4}$	$8.9 \cdot 10^{-4}$

DISCUSSION

It is of interest to compare the experimental titration curves with those calculated from the various equilibrium constants and also to compare at the end-point the experimental reaction ratio with that calculated from equilibrium constants.

Using the customary notation[6] Al^{+3} and F^- are designated as M and L, respectively, and electrical charges are omitted. M and L form a series of mononuclear complexes ML_n, with consecutive equilibrium constants K_n and cumulative equilibrium constants β_n:

$$K_n = [ML_n]/[ML_{n-1}]\,[L] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$\beta_n = [ML_n]/[M]\,[L]^n \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$$\beta_n = K_1 \ldots . K_n \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

When the ligand may be protonated the ordinary acid dissociation K_a is used:

$$K_a = [H^+]\,[L]/[HL] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

The total concentrations of M and L, respectively, in all forms are:

$$C_M = [M] + [ML] + [ML_2] + \ldots .$$
$$= [M]\,\{1 + \beta_1[L] + \beta_2[L]^2 + \ldots .\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

$$C_L = [L] + [HL] + [ML] + 2[ML_2] + \ldots .$$
$$= [L]\left(1 + \frac{[H^+]}{K_a}\right) + [M]\,\{\beta_1[L] + 2\beta_2[L]^2 + \ldots .\} \quad . \quad (6)$$

In the titration of L with M let the initial total concentration of L, *i.e.*, [L] + [HL], be C_L, and let the number of moles of M added per total mole of L be r, *i.e.*, $C_M = r \cdot C_L$. From the equations above it follows directly that

$$r = \left(1 - \frac{[L]\,(1 + [H^+]/K_a)}{C_L}\right) \frac{1}{\bar{n}} \quad \ldots\ldots\ldots\ldots \quad (7)$$

where $\bar{n}$ is the average ligand number, *i.e.*, moles of L bound per mole of M, defined by (8):

$$\bar{n} = \frac{\beta_1[L] + 2\,\beta_2[L]^2 + \ldots}{1 + \beta_1[L] + \beta_2[L]^2 + \ldots} \quad \ldots\ldots\ldots\ldots \quad (8)$$

When a physical measurement (*e.g.*, current in an amperometric titration) is made which is directly proportional to [L], and if pH and volume are constant during the titration, equation (7) becomes

$$r = \left(1 - \frac{i}{i_0}\right) \frac{1}{\bar{n}} \quad \ldots\ldots\ldots\ldots \quad (9)$$

where i_0 and i are respectively the initial current and current after the addition of r moles of M per mole of L.

Equation (9) permits the simple and exact calculation of titration curves, given the values of K_n. It is emphasized particularly that while the calculation of r in terms of i/i_0 is simple and exact, requiring no approximation, the reverse calculation of i/i_0 in terms of r in general cannot be carried out exactly. There is an entirely analogous situation (generally overlooked) in acid–base calculations. Given a polybasic acid, the calculation of pH after the addition of a given amount of base generally must be carried out approximately, whereas the reverse calculation of the amount of base required to reach a given pH is easy and exact.

The calculation of the titration curve is carried out as follows. The initial value of [L] is calculated from the known C_L and pH. Using the known K_n, $\bar{n}$ is calculated from equation (8) for [L] equal to 1.0, 0.8, 0.6, ... etc. times the initial value, corresponding to i/i_0 equal to 1.0, 0.8, 0.6, ... Substitution in (9) gives r. As an example, the calculated titration curves of $2 \cdot 10^{-4}$ M and $2 \cdot 10^{-3}$ M F^- with Al^{+3}, at pH such that [HF] is small compared to [F^-], are given in Table IV.

TABLE IV

Calculated titration curves of F^- with Al^{+3}; [HF] $\ll$ [F^-] ; $\mu = 0.53$

i/i_0	$C_F = 2 \cdot 10^{-4}$ M		$C_F = 2 \cdot 10^{-3}$ M	
	$\bar{n}$	r	$\bar{n}$	r
1.0	2.63	0	3.52	0
0.8	2.54	0.079	3.44	0.058
0.6	2.43	0.165	3.33	0.120
0.4	2.28	0.263	3.18	0.189
0.2	2.02	0.396	2.90	0.276
0.1	1.77	0.508	2.63	0.342
0.01	0.90	1.10	1.77	0.560

The following equilibrium constants are those reported by BROSSET AND ORRING[5]: $\log K_1 = 6.13$, $\log K_2 = 5.02$, $\log K_3 = 3.85$, $\log K_4 = 2.74$, $\log K_5 = 1.63$, $\log K_6 = 0.47$, which apply at 25° and an ionic strength of 0.53. A series of such calculated titration curves for C_F from $2 \cdot 10^{-5}$ M to $5 \cdot 10^{-3}$ M is shown in Fig. 8.

In practice it is advisable to calculate exact values of $\bar{n}$ covering the range of [L] of interest and plot $\bar{n}$ *vs.* -log [L]. (This also facilitates calculation when the concentration of HL must be considered, *vide infra*). Values of $\bar{n}$ for the Al^{+3}–F^- system are given in Table V. Many of these values have been given previously by BROSSET.

TABLE V

Average ligand number at various [F^-] (25°, $\mu = 0.53$)

$-\log [F^-]$	$\bar{n}$	$-\log [F^-]$	$\bar{n}$
8	0.013	2.5	3.72
7	0.121	2	4.16
6	0.66	1.5	4.61
5.5	1.07	1.0	5.04
5	1.51	0.5	5.43
4.5	1.94	0.0	5.73
4	2.38	−0.5	5.90
3.5	2.82	−1.0	5.98
3	3.26		

Calculation of end-points

If the decrease in current (or other quantity directly proportional to concentration) upon addition of reagent is linear before the equivalence point, the latter is found by extrapolation of the straight line to zero current. Equation (9) shows that a linear decrease occurs only if $\bar{n}$ is constant over the range of [L] covered by the titration. This situation does not exist in the system Al^{+3}–F^- in aqueous medium. Actually

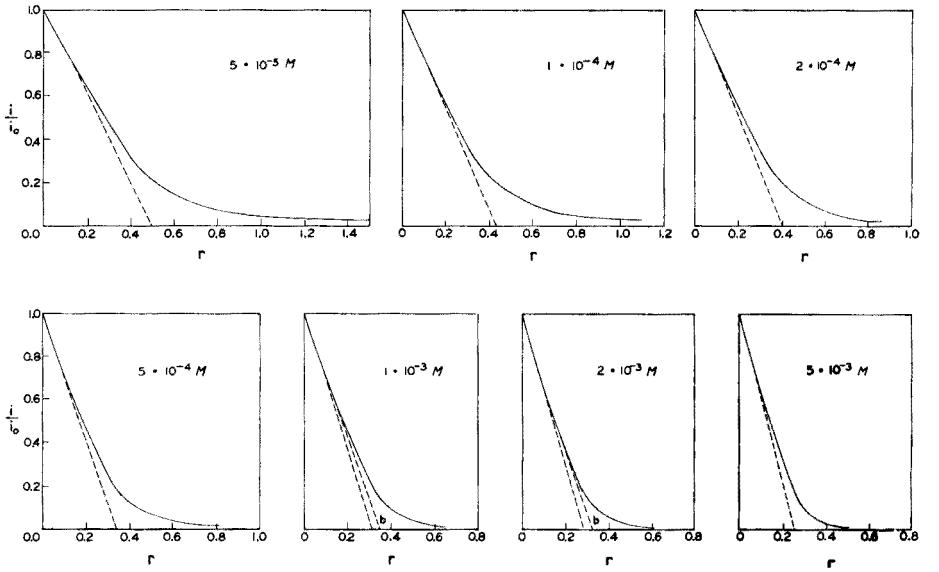

Fig. 8. Calculated titration curves. Titration of fluoride with Al^{+3} at various initial concentrations of F^-. $\mu = 0.53$, 25°; [HF] ≪ [F^-]; r = moles Al^{+3} added per mole F^-.

over the range $[F^-]$ from 10^{-6} M to 10^{-1} M, $\bar{n}$ varies practically linearly with log $[F^-]$, from 0.66 to 5.04. (Table V). Therefore all the titration lines must be curved (Fig. 8), in agreement with the experimental results (Figs. 1–3).

The extrapolation of curves (Fig. 8) to the end-point is not objective but involves personal judgment. If the curve approximates a straight line for the first half of the titration, it appears reasonable to assume that the straight line drawn through the points would have a slope about equal to the average of the slopes at 0% and 50% of the titration. Equation (9) shows that the mole ratio of L to M at the extrapolated end-point (TEP) would be $\frac{1}{2}(\bar{n}_0 + \bar{n}_{\frac{1}{2}})$, where $\bar{n}_0$ and $\bar{n}_{\frac{1}{2}}$ are the average ligand numbers corresponding to the initial *free* fluoride concentration and to half the initial concentration, respectively. Values derived in this way are given in Table VI. A plot of $\bar{n}_{TEP}$ *versus* log of initial fluoride concentration yields a straight line.

TABLE VI

Calculated mole ratios at TEP, titration of F^- with Al ($\mu = 0.53$; 25°)

Initial F^- *M*	$\bar{n}_{TEP}$
$5 \cdot 10^{-5}$	1.98
$1 \cdot 10^{-4}$	2.25
$2 \cdot 10^{-4}$	2.51
$5 \cdot 10^{-4}$	2.86
$1 \cdot 10^{-3}$	3.13
$2 \cdot 10^{-3}$	3.39
$5 \cdot 10^{-3}$	3.75

Finally, if the pH is such that [HF] is not negligible compared to $[F^-]$, the necessary correction is simple. For example, in the titration of $4 \cdot 10^{-3}$ M fluoride at pH $= pK_a = 2.94$, one half of the fluoride not bound to aluminum is present as HF. Therefore this titration should be identical with that of $2 \cdot 10^{-3}$ M fluoride at pH $> pK_a$. In general, the ratio $[F^-]/C_F$ is calculated from the evident relation

$$\frac{[F^-]}{C_F} = \frac{K_a}{K_a + [H^+]}$$

and the value of $\bar{n}_{TEP}$ read from a plot of Table VI.

In this way the calculated values of n_{TEP} given in Table I have been derived. It will be noted that the calculated mole ratios in the titration of $1-5 \cdot 10^{-4}$ M fluoride are in remarkably good agreement with the experimental ratios found at the TEP. The deviation becomes greater at fluoride concentrations of $1-2 \cdot 10^{-3}$ M. It has been mentioned – and it is illustrated again in Fig. 2 – that at these high fluoride concentrations the current is not linearly proportional to fluoride concentration. In the titration of $1 \cdot 10^{-3}$ M fluoride (curves 1 and 2, Fig. 2) the current *vs.* reagent curve became approximately linear after about 30% of the free fluoride had disappeared and remained virtually straight until about 65% had reacted. Using this portion of the calculated curve (Fig. 8) the extrapolated F/Al ratios at the TEP are approximately 2.7 (pH 4.6) and 2.6 (pH 3.6) which, as was to be expected, are in much better agreement with the experimental values (2.48 and 2.40, respectively) than the values obtained by extrapolation of the initial slope of the line. In a similar way the values have been found for the titration of $2 \cdot 10^{-3}$ M fluoride. These have been added to Table I with the designation (b).

As a further test of agreement between the experimental and calculated titration curves the experimental values of i/i_0 at the true end-point were compared with the theoretical values read from Fig. 8. In $1 \cdot 10^{-3}$ *M* fluoride solution the (initial) current i_0 is less than proportional with fluoride concentration. Therefore, in the calculation of i/i_0 from curve 2 in Fig. 2 in the titration of $1 \cdot 10^{-3}$ *M* fluoride, i_0 was taken equal to twice the value of i_0 in the titration of $5 \cdot 10^{-4}$ *M* fluoride (curve 3, Fig. 2). Also, in the titration of $1 \cdot 10^{-3}$ *M* fluoride the calculated end-point was taken equal to that given in the last column of Table I (with designation (b)). It is evident from Table VII that the experimental and calculated values of i/i_0 at the TEP are in excellent agreement.

TABLE VII

Comparison of theoretical and experimental values of i/i_0 at the TEP

Initial fluoride concentration *M*	i/i_0 *found*	i/i_0 *calculated*
$1 \cdot 10^{-4}$	0.20	0.20
$2 \cdot 10^{-4}$	0.18	0.19
$5 \cdot 10^{-4}$	0.17	0.18
$1 \cdot 10^{-3}$	0.10	0.10
$2 \cdot 10^{-3}$	0.07	0.09

The agreement between calculated and experimental titration curves is very gratifying. However, from the practical point of view the amperometric titration of fluoride with aluminum in aqueous acetate buffers is not attractive because the ratio of fluoride to aluminum reacted at the end-point increases with increasing initial concentrations of fluoride (Table I). Thus a calibration curve determined under the same experimental conditions must be constructed. Addition of much potassium nitrate hardly affects the results.

When the titration is carried out in an acetate buffer (pH 4.0) in a medium of 50% ethanol, the reaction ratio at the true end-point is greater than in water, but again increases considerably with increasing initial fluoride concentration (Table II). When the buffered medium of 50% ethanol is made 0.5 *M* in potassium or sodium nitrate, a constant reaction ratio of 5.9 ± 0.1 is found at the true end-point in a concentration range of fluoride between $2 \cdot 10^{-4}$ *M* and $2 \cdot 10^{-3}$ *M*. Thus no calibration curve is necessary and this reaction medium can be recommended for the amperometric titration of fluoride.

The average value of the reaction ratio is 5.9, slightly less than is necessary for the formation of Na_3AlF_6 or K_3AlF_6. In this connection it is of interest to mention that BROSSET AND WAHLBERG[7] in the precipitation of potassium aluminum fluoride from aqueous solutions, which were 0.5 *M* in potassium nitrate, found a composition corresponding to $K_3AlF_{5.9}$. When sodium instead of potassium nitrate was used, the composition corresponded to about $Na_3AlF_{5.6}$. The X-ray pattern of these precipitates closely resembled that of cryolite.

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SUMMARY

The rotated aluminum electrode is a suitable indicator electrode in the amperometric titration of fluoride. Fluoride in concentrations varying between $1 \cdot 10^{-4}$ and $2 \cdot 10^{-3}$ *M* was titrated with

a standard aluminum nitrate solution in aqueous buffer solutions (pH between 3.6 and 4.6). Equilibrium was established within 15 min. The fluoride–aluminum ratio at the (true) end-point was found to increase from 2.2 in 10^{-4} *M* fluoride to 2.8 in $2 \cdot 10^{-3}$ *M* fluoride. The titration calculated from the stability constants of the various aluminum–fluoride complexes were found to be in excellent agreement with the experimental curves.

From a practical point of view titration in an acetate buffer in 50% alcohol in the presence of 0.5 *M* potassium or sodium nitrate is recommended. The fluoride–aluminum ratio at the end-point was found to be 5.9 ± 0.1 and independent of the fluoride concentration.

RÉSUMÉ

L'électrode rotative d'aluminium peut être utilisée pour le titrage ampérométrique des fluorures, au moyen d'une solution étalon de nitrate d'aluminium. Les courbes de titrage, calculées à partir des constantes de stabilité des divers complexes aluminium–fluorure, correspondent très bien aux courbes expérimentales.

ZUSAMMENFASSUNG

Es wird die Eignung der rotierenden Aluminiumelektrode zur amperometrischen Bestimmung von Fluorid mit einer Aluminiumnitratlösung beschrieben. Die auf der Basis der Stabilitätskonstanten der verschiedenen Aluminium–Fluorid Komplexen berechneten Titrationskurven stimmen sehr gut mit den experimentell erhaltenen Kurven überein.

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THE DETERMINATION OF CARBOXYL GROUPS IN POLYETHYLENE TEREPHTHALATE

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INTRODUCTION

The carboxyl content of polyethylene terephthalate can be used for the characterisation of this polymer. This content may be considered as a measure of the thermal degradation[1,2], and from it and from the hydroxyl content the average molecular weight of the polymer can be calculated[3].

WARD[4] describes an IR-spectrophotometric method. POHL[5] discusses a volumetric method applied by CONIX[3] in work on the determination of the average molecular

weight of polyethylene terephthalate. POHL's method has the advantage over that of WARD[4] that it does not need an IR-spectrophotometer. The method involves dissolution of a 100–200-mg sample in benzyl alcohol at 203°, mixing the solution with chloroform whereby a metastable solution is formed, and titration with benzyl-alcoholic sodium hydroxide solution to phenol red indicator. The value thus found must be corrected for the amount of carboxyl groups that are formed during the dissolution at this high temperature. This correction is proportional to the time necessary for complete dissolution and is independent of the initial carboxyl content of the sample.

POHL[5] prefers dissolution in hot benzyl alcohol to that in acidic, phenolic solvents, in which the sample can be dissolved at much lower temperatures, because in the latter case the end-point indication is obscured.

Although the method yields precise results and requires less time for a determination, it still has some disadvantages. First, it is always necessary to apply the correction mentioned. Second, the solvent used, *viz.* benzyl alcohol, must be of high purity and free from water, lest hydrolysis at the high temperature during dissolution should take place. It was therefore decided to study the possibility of a determination in another solvent that would not involve the disadvantages mentioned.

EXPERIMENTAL

Two requirements must be met by the solvent used, *viz.* the sample should dissolve at such a temperature that no thermal degradation occurs, and its acidity should be much lower than that of the carboxyl group to be titrated.

Preliminary tests showed that 2 g of the polymer dissolve in 50 ml of a mixture of *o*-cresol and chloroform (70 + 30 g) by heating on a hot plate for 20 min. In this way solution could be effected at about 90°. As the results of the determinations were found to be independent of the dissolution time, it may be concluded that no thermal degradation occurs. After cooling to room temperature the solution remained clear. These solvents were purified in order to reduce the blank value as far as possible. The solution thus obtained was titrated potentiometrically with 0.1 *N* ethanolic potassium hydroxide solution.

The difference in potential at the equivalence point was about 100 mV. In this titration a model H 2 Beckman pH-meter and an S.C.E. and a glass electrode were used. Precise results could be obtained in this way.

In order to check the method, the determination of the carboxyl groups of polyethylene terephthalate in the above-mentioned mixture of *o*-cresol and chloroform by H.F. titration with 0.05 *N* ethanolic sodium hydroxide solution was also examined. It proved necessary to increase the conductivity of the solution by the addition of 10 ml of acetone; the solution again remained clear. The H.F. apparatus used was constructed in collaboration with the physical department of the N.V. Research. During the titration the frequency was kept constant at 0.6 MHz and the grid current was plotted against the amount of titrant added. From the resulting graph the equivalence point was determined. During the titration the solution was stirred mechanically. The stirrer was only stopped when the grid current was read.

The results obtained by these two methods were compared with those obtained according to POHL's method. This method was slightly modified in this company's Polyester Department; chloroform was added to the benzyl alcoholic solution instead

of the reverse, so that the titration could be carried out in the vessel used for dissolution. The correction factor was found to be 2.4 mequivs. per kg per min. It was determined on several samples and found to be independent of the initial carboxyl content of the sample, which agrees with POHL's statement.

Seven samples of polyethylene terephthalate were analysed in triplicate by the three methods mentioned, *viz.* visually according to POHL, potentiometrically, and by H.F. titration. The samples used were ground in a Wiley mill. The potentiometric procedure, which is recommended, is given in detail at the end of this paper. The equivalence points in these potentiometric titrations were calculated by interpolation according to HAHN[6]. The results obtained are listed in Table I, which also gives the differences between the mean results of the three methods for each sample, d_i, the mean values of these differences, $\bar{d}_i$, and the standard deviations of single determinations by the respective methods.

The potentiometric method required 2-g samples and the visual method 0.2-g samples, hence it was investigated whether the sample size in the first method could be decreased, so that a better comparison between the two methods would be obtained.

When 200-mg portions of samples 3 and 7 (Table I) were dissolved in the *o*-cresol–chloroform mixture and titrated with 0.02 *N* ethanolic potassium hydroxide solution, the results were somewhat high, compared with the *y*-values of Table I. Better results

TABLE I

CARBOXYL CONTENT OF POLYETHYLENE TEREPHTHALATE IN MEQUIVS. PER KG

Sample	*Visually in benzyl alcohol and chloroform* x	*Potentiometrically in o-cresol and chloroform* y	*HF-titration in o-cresol, chloroform and acetone* z	$d_1 = \bar{x} - \bar{y}$	$d_2 = \bar{x} - \bar{z}$	$d_3 = \bar{y} - \bar{z}$
1	22.5	22.3	23.7			
	22.4	21.9	21.3	—0.1	—0.1	0
	22.1	23.0	22.2			
2	21.9	22.8	20.9			
	23.0	22.4	21.6	—0.5	+1.0	+1.5
	21.0	22.2	20.5			
3	79.8	81.0	81.1			
	80.3	80.6	80.2	—0.1	—0.6	—0.5
	80.8	79.7	81.3			
4	25.8	25.1	25.0			
	22.5	24.0	23.7	—0.8	—0.2	+0.6
	24.2	25.9	24.5			
5	32.0	31.0	30.6			
	29.5	30.4	31.1	+0.2	+0.6	+0.4
	31.5	31.0	29.6			
6	23.2	23.3	23.2			
	25.1	22.5	22.6	+1.7	+1.2	—0.5
	25.6	23.0	24.3			
7	58.3	60.0	59.2			
	59.6	59.8	57.3	—0.7	+0.9	+1.6
	58.8	58.9	57.5			
mean $\bar{d}_i$				0	+0.4	+0.4
st.dev.	$s_x = 1.05$	$s_y = 0.58$	$s_z = 0.86$	$s_{d_1} = 0.85$	$s_{d_2} = 0.70$	$s_{d_3} = 0.86$

were obtained when 5 ml of 0.02 N ethanolic potassium hydroxide solution was added to the polymer solution and the excess was back-titrated potentiometrically with 0.02 N ethanolic hydrochloric acid. The potential drop around the equivalence point was about 45 mV. When, however, samples with carboxyl contents of 20–30 mequivs./kg were analysed, it was necessary to take at least 600 mg for analysis to obtain reproducible results. The minimum sample size as a function of the carboxyl content is given in Table II.

TABLE II

COOH content mequivs./kg	*minimum sample size mg*
50–90	200
30–50	400
20–30	600
15–20	800

Final results for some samples are given in Table III, in which the potentiometric results, y', are compared with the visual ones from Table I.

An increase in the sample size in the visual method was also examined; when 10 ml of benzyl alcohol is used not more than 400 mg of the polymer can be dissolved.

DISCUSSION

Application of the t-test to the $\bar{d}_i$ values of Table I reveals that they do not differ significantly from zero. This means that no systematic differences between the results of the respective methods can be proved. It must, therefore, be assumed that all three methods yield the same results when applied to one sample. The methods investigated are independent of each other, since different solvents are used and the equivalence points in the titrations are indicated in different ways. Because of this

TABLE III

CARBOXYL CONTENT OF POLYETHYLENE TEREPHTHALATE IN MEQUIVS. PER KG

Sample	*Visually* x	*Potentiometrically* y'	*Sample size in the potentiometric method*	$d_4 = \bar{x} - \bar{y}'$
1	22.5	21.5	600 mg	
	22.4	20.9		+1.4
	22.1	20.4		
3	79.8	81.9	200 mg	
	80.3	82.8		—0.9
	80.8	80.3		
4	25.8	23.4	600 mg	
	22.5	24.3		+0.4
	24.2	23.6		
7	58.3	59.7	200 mg	
	59.6	59.2		—0.4
	58.8	59.1		
mean, $\bar{d}_4$				+0.1
st. dev.	$s_x = 0.93$	$s_{y'} = 0.82$		$s_{d_4} = 1.01$

independence and the fact that the results obtained by the three methods do not differ significantly, the results found must be assumed to be accurate.

When the standard deviations of single determinations, *viz.* s_x, s_y and s_z are compared with the aid of the F-test, a significant difference can be detected on the 0.05-level only between s_z and s_y. It must therefore be assumed that the results of the potentiometric method, when applied to 2-g samples, are more precise than those of the visual method.

The potentiometric method can also be applied to smaller samples. From Table III it can be seen that when small samples of 200 to 600 mg are used in the potentiometric method, no systematic differences between the results of this method and those of the visual one can be detected. The standard deviation, $s_{y'}$, is somewhat higher than s_y, but does not differ significantly from the latter value.

The correction factor in the visual method was found to be 2.4 mequivs. per kg per min. This value does not agree with that mentioned by POHL[5], who found 0.88 mequivs. per kg per min. When a sample contains 23 mequivs. of carboxyl groups per kg (Table I, samples 1, 2, 4 and 6) and the dissolution time is 2 min, the correction amounts to 4.8 mequivs. per kg, which is more than 20% of the amount to be determined. Such a correction must be considered inadmissibly large.

It should be remarked that the standard deviation s_x is of the same order of magnitude as that mentioned by POHL[5], *viz.* 0.86 mequivs. per kg.

The potentiometric method has the advantage over the visual method that larger samples can be analyzed, so that the results are more precise and that no correction is required; however, it takes longer than the visual method. This disadvantage can be overcome by dissolving several samples simultaneously. In contrast to the HF titration, no special apparatus is required in the potentiometric method. For these reasons the latter is preferred to the visual and HF titration. In this investigation the HF titration was only used as a comparison method.

Finally, it may be remarked that the carboxyl groups in polyethylene terephthalate are dissociated to a relatively high extent. This is in accordance with the position of these groups in the molecules, bound as they are to the aromatic nuclei. Experimental evidence for this fact is obtained from the present results. The carboxyl groups can be sharply titrated to a visual end-point in a neutral solvent, *viz.* benzyl alcohol, whereas in the potentiometric titration in an acidic solvent a potential difference around the equivalence point of about 100 mV was observed at a concentration of $0.8 \cdot 10^{-3}$ to $3.2 \cdot 10^{-3}$ equivs./l.

RECOMMENDED PROCEDURE

Reagents

o-Cresol, c.p., (British Drug Houses) purified by distillation. Chloroform, c.p., purified by shaking with sodium carbonate solution, drying over anhydrous sodium sulphate, and distillation. Ethanolic potassium hydroxide solution, 0.1 (t_1) N, standardized against potassium hydrogen phthalate. Ethanolic potassium hydroxide solution, 0.02 N. Ethanolic hydrochloric acid, 0.02 (t_2) N.

Procedure

Weigh out 2 (p_1) g of ground polyethylene terephthalate in a 150-ml beaker to the nearest 0.005 g. Add 50 ml of a mixture of 70 g of *o*-cresol and 30 g of chloroform and heat to 90° until the sample has dissolved. This takes at most 30 min. Cool to room temperature, immerse the electrodes in the solution, stir and titrate potentiometrically with $t_1 N$ ethanolic potassium hydroxide solution (v_1 ml). Carry out a blank by titrating 50 ml of the solvent with this titrant (v_0 ml).

If small samples are to be analyzed, weigh out 0.2–0.6 (p_2) g of the ground polymer to the

nearest 0.001 g and dissolve as described above. After cooling add 5 ml of 0.02 N ethanolic potassium hydroxide solution, immerse the electrodes in the solution, stir and titrate potentiometrically with t_2 N ethanolic hydrochloric acid and record the first equivalence point (v_2 ml). Carry out a blank by adding 5 ml of 0.02 N ethanolic potassium hydroxide solution to 50 ml of the solvent and titrating potentiometrically with t_2 N ethanolic hydrochloric acid (v_3 ml).

Calculation

The sample contains:

$$\frac{1000(v_1 - v_0')t_1}{p_1} \text{ mequivs. of COOH per kg or } \frac{1000(v_3 - v_2)t_2}{p_2} \text{ mequivs. of COOH per kg.}$$

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SUMMARY

Three methods for the determination of carboxyl groups in polyethylene terephthalate are investigated, *viz.* (a) dissolution in hot benzyl alcohol, addition of chloroform, and titration with benzyl alcoholic potassium hydroxide solution to the phenol red end-point, (b) dissolution in a mixture of *o*-cresol and chloroform, and potentiometric titration with ethanolic potassium hydroxide solution, and (c) dissolution in a mixture of *o*-cresol, chloroform and acetone, and titration with ethanolic potassium hydroxide solution with HF-indication of the end-point.

The three methods yield the same results when applied to several samples of different carboxyl content. For several reasons the potentiometric method is preferred to the other two methods.

RÉSUMÉ

Trois méthodes pour le dosage des groupes carboxyles dans le téréphtalate polyaethylène ont été étudié, c'est à dire (a) par dissolution dans l'alcool benzylique, et addition de chloroforme suivi par une titration avec une solution benzylalcoolique de l'hydroxyde de potassium avec l'indicateur phenolrouge, (b) par dissolution dans une mélange de *o*-crésole et chloroforme suivi par une titration potentiométrique avec une solution aethanolique de l'hydroxyde de potassium et (c) par dissolution dans une mélange de *o*-crésole, chloroforme et acétone suivi par une titration ,,H.F." avec une solution aethanolique de l'hydroxyde de potassium.

Les trois méthodes donnent des résultats identiques quand ils sont appliquées aux échantillons avec un teneur carboxyle variable. La méthode potentiométrique est recommendée.

ZUSAMMENFASSUNG

Drei Methoden zur Bestimmung der Carboxylgruppen in Polyaethylenterephtalat wurden untersucht, und zwar (a) Lösen in heissem Benzylalkohol, Zugabe von Chloroform und Titration mit benzylalkoholischer Kaliumhydroxydlösung auf Phenolrot, (b) Losen in einem Gemisch von *o*-Kresol und Chloroform und potentiometrische Titration mit aethanolischer Kaliumhydroxydlösung und (c) Lösen in einem Gemisch von *o*-Kresol, Chloroform und Aceton, und H.F.-Titration mit aethanolischer Kaliumhydroxydlosung.

Die drei Methoden geben dieselben Ergebnisse, wenn sie auf mehrere Muster mit verschiedenem Carboxylgehalt angewandt wurden. Von den genannten Methoden wird die potentiometrische Methode bevorzugt.

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DOSAGE POLAROGRAPHIQUE DE LA TYROSINE, DU TRYPTOPHANE ET DE LA PHÉNYLALANINE EN PRÉSENCE LES UNS DES AUTRES

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Nous avons proposé dans diverses publications[1], des méthodes de dosage polarographique de ces trois acides aminés. Ces derniers se nitrent facilement, à l'encontre de la plupart des autres et les dérivés que l'on obtient donnent des sauts polarographiques parfaitement dessinés dont le potentiel $E_{\frac{1}{2}}$ est toujours inférieur à 1 V. Dans ce travail, pour rendre la méthode encore plus spécifique, nous avons cherché à doser chacun d'eux en présence des deux autres. La plupart des autres acides aminés ne se nitrent pas dans ces conditions et par conséquent ne gênent pas. Dans chaque cas nous donnerons la précision et la sensibilité de la méthode.

I. Dosage de la tyrosine en présence de tryptophane et de phénylalanine

La tyrosine traitée par l'acide nitrique 0.15 *N*, à l'ébullition pendant 2 h, donne un dérivé nitré qui se réduit sur la goutte de mercure et donne un polarogramme dont le $E_{\frac{1}{2}}$ vaut 0.64 V et dont la hauteur est, dans des limites déterminées, rigoureusement proportionnelle à la concentration. La présence de dérivés sulfurés de la forme –SH (cystéine) de même que celle du tryptophane rend la nitration impossible dans les conditions indiquées. Tous les essais que nous avons effectués pour induire la réaction de nitration, sans augmenter la concentration d'acide nitrique sont restés vains. L'addition d'un cristal de nitrite de sodium provoque bien une réaction mais les sauts polarographiques qui en résultent ne correspondent plus à la concentration de tyrosine. Une séparation s'avère donc indispensable. Or il se trouve que l'acétate de mercure précipite l'un et l'autre de ces deux composés. Cette réaction est lente mais quantitative, les produits de solubilité étant très faibles; la méthode s'applique aussi au dosage de très petites quantités de tyrosine. Pour accélérer la précipitation, il est bon de travailler à 50–60°, ce qui a l'avantage de donner un précipité plus aisément filtrable. Les traces de mercure qui restent en solution ne gênent pas la détermination. Une difficulté se présente lorsque le précipité tryptophane–mercure est trop abondant. En effet il adsorbe des quantités non négligeables de tyrosine. C'est pourquoi nous proposons d'utiliser un back-carrier qui sera adsorbé à la place de la tyrosine. Nous avons montré que la phénylalanine convient particulièrement bien car elle ne gêne pas le dosage de la tyrosine, même en grand excès et qu'elle est plus fortement adsorbée que cette dernière sur le précipité tryptophane–mercure. En effet nous avons pu montrer que la phénylalanine était capable de remettre en

solution la tyrosine préalablement adsorbée par le précipité. Nous avons donc procédé de la façon suivante:

Une solution contenant 0.0119 g de tyrosine et 0.080 g de tryptophane a été divisée en deux portions et la précipitation a été faite. On a ensuite ajouté 0.070 g de phénylalanine dans l'une des solutions qui a été chauffée et bien agitée. Les polarogrammes effectués après nitration ont montré que la solution ayant reçu la phénylalanine donne un saut de 7% plus élevé que la solution témoin, il semble donc bien se produire en présence de phénylalanine une certaine remise en liberté de la tyrosine.

Nous avons cherché la quantité minimum de phénylalanine à ajouter à une solution renfermant des quantités déterminées de tyrosine et de tryptophane, pour que ce dernier ne gêne pas le dosage de la tyrosine.

TABLEAU I

Tyrosine (mg)	*Tryptophane (mg)*	*Phénylalanine (mg)*	*Saut μA*	*Mol. Phényl. / Mol. Trypt.*
10.8	80	40	10.9	0.62
10.8	80	60	11.5	0.93
10.8	80	80	11.4	1.24
10.8	80	120	11.4	1.85

Nous remarquons que la première prise présente un saut un peu faible, mais que les suivantes donnent par contre des hauteurs pratiquement identiques. Ce résultat est parfaitement plausible, car les poids moléculaires de la phénylalanine et du tryptophane étant respectivement de 165.19 et 204.2, le rapport moléculaire 1/1 correspond à 64.7 mg de phénylalanine pour 80 mg de tryptophane. En dessous de cette limite, la quantité de phénylalanine ne semble pas être suffisante pour empêcher une certaine adsorption de la tyrosine.

MODE OPÉRATOIRE

a. On place la prise contenant environ 10 à 20 mg de tyrosine dans un tube à centrifuger et on la dissout dans 30 ml d'acide nitrique 0.15 *N*. On ajoute à celle-ci une quantité de phénylalanine correspondant aux 3/4 au moins du poids de trytophane. Si celui-ci n'est pas connu, mettre un bon excès (on peut contrôler en effectuant les mêmes opérations sur une prise parallèle contenant davantage de phénylalanine). Tiédir à 50–60° pour faciliter la mise en solution.

b. Ajouter une quantité de solution d'acétate mercurique (à 2% dans l'acide nitrique 0.15 *N*) telle, que la solution devienne totalement opaque au moment de l'adjonction, plus un petit excès de 0.5 ml environ.

c. Placer les tubes à centrifuger dans un bécher d'eau maintenue à environ 60° et laisser reposer ainsi à chaud pendant une à deux heures.

d. Centrifuger environ 1/4 d'heure et, si nécessaire, filtrer sur double filtre pour enlever le dernier trouble éventuel. Transvaser dans un cylindre gradué ou dans un flacon jaugé pour amener à un volume connu et prélever une partie aliquote de cette solution (20 ou 25 ml).

e. Placer cette prise dans un erlenmeyer rodé avec réfrigérant à reflux et ajouter 20 ml d'acide nitrique 0.15 *N* ainsi que quelques cristaux de nitrite de sodium. Porter à ébullition durant deux heures, puis refroidir sous l'eau courante.

f. Neutraliser ensuite par l'hydroxyde de potassium à 20% en contrôlant au moyen de papier indicateur universel. Placer dans un flacon jaugé de 50 ml et compléter par de l'eau bidistillée.

g. Prélever 5 à 7 ml de cette solution, ajouter 2 ml de solution tampon de pH 5* et compléter à 10 ml. Chasser l'oxygène et polarographier à la sensibilité convenable.

On effectue les mêmes opérations sur une prise à laquelle on a ajouté une quantité connue de tyrosine témoin (étalon interne).

* 51.5 ml de Na_2HPO_4 0.2 *M* + 48.5 ml d'acide citrique 0.1 *M*.

Des essais de contrôle ont été faits et les résultats suivants ont été enregistrés au moyen d'un polarographe Sargent XXI et à la sensibilité de 0.080 μA/mm.

TABLEAU II

Tyrosine (mg)	*Tyrosine ajoutée*	*Tryptophane (mg)*	*Phénylalanine (mg)*	*Saut µA*	*Tyrosine trouvée (mg)*
9.3	—	80	60	5.76	9.5
9.3	4.4	80	60	8.43	
16.2	—	50	40	10.58	16.05
16.2	5.2	50	40	14.01	

APPLICATIONS

Dosage de la tyrosine dans l'albumine du sang

Nous avons dosé la tyrosine sans hydrolyse préalable de l'albumine, afin d'obtenir la teneur en tyrosine „libre", puis nous avons effectué la même détermination sur la tyrosine liée aux chaines peptidiques. Dans ce dernier cas, elle est libérée par l'un des procédés d'hydrolyse classique.

On utilise principalement le traitement par ébullition à reflux en présence d'hydroxyde de sodium, d'hydroxyde de potassium ou d'acide sulfurique avec adjonction éventuelle de certains sels minéraux tels que le chlorure d'étain.

La littérature donne les chiffres suivants, concernant les divers procédés d'hydrolyse:

Traitements et pertes moyennes indiquées par les auteurs:

BLOCK ET BOLLING (1943)[2]. Traitement par NaOH 5 N; Durée 5 h; Température entre 115 et 125°; Pertes de tyrosine: 11 à 17%.

LUGG (1938)[2]: NaOH 5 N; Durée de 20 à 30 h; Température 100°; Pertes nulles de tyrosine.

JORPS (1932)[2]: NaOH 5 N; Durée de 14 à 18 h; Température 100°; Pertes de tyrosine = 0 à 9%.

La majorité des auteurs considèrent l'hydrolyse alcaline par l'hydroxyde sodium, eventuellement l'hydroxyde de potassium, comme étant la meilleure pour le dosage de la tyrosine. Nous conformant à cette manière de voir, nous avons appliqué la méthode de BOLLING ET BLOCK. C'est la meilleure bien que la tyrosine ne soit pas entièrement libérée. L'erreur due aux pertes se maintient dans une limite raisonnable, ce qui permet l'utilisation d'un facteur correctif (1.18) déterminé sur un grand nombre d'analyses. La durée de ce traitement n'est pas exagérée.

a. Dosage de la tyrosine totale. Une prise d'albumine d'environ 30 mg est placée dans un erlenmeyer rodé en présence de 20 ml d'hydroxyde de sodium 5 N. On chauffe à reflux entre 115 et 125° pendant 5 h au moyen d'un bain d'huile ou sur une plaque chauffante électrique.

Après refroidissement, on neutralise par de l'acide nitrique concentré et transvase dans un cylindre gradué. On ajoute ensuite une quantité d'acide nitrique telle que la concentration finale de l'acide soit de 0.15 N. (Il est inutile de faire cette acidification avec grande précision, les essais ont montré que de petits écarts n'influencent pas la hauteur des vagues polarographiques).

On place à nouveau la solution dans l'erlenmeyer rodé et on fait bouillir à reflux

durant deux heures. Les essais qualitatifs préliminaires ont montré qu'il ne se produit aucune précipitation en présence d'acétate mercurique, il a donc été inutile dans ce cas, d'utiliser ce réactif.

Après refroidissement, on porte la solution au volume de 50 ml dans un flacon jaugé, on en prélève 7 ml auxquels on ajoute 2 ml de la solution tampon de pH 5 et on complète à 10 ml. On polarographie après avoir chassé l'oxygène.

On procède de manière analogue avec des prises contenant une quantité connue de tyrosine pure servant d'étalonnage interne.

Nous avons enregistré les valeurs suivantes:

TABLEAU III

Albumine (Prise en mg)	*Tyrosine ajoutée (mg)*	*Saut μA*	*Tyrosine trouvée (mg)*	*Résultat %*
28	—	0.62	1.24	4.4
28	8.0	5.52		
23	—	0.58	0.985	4.3
23	7.0	4.90		

Les chiffres de la colonne 4 ont été calculés en tenant compte du facteur correctif 1.18.
Les chiffres de la colonne 5 indiquent la teneur de l'albumine en tyrosine.

La qualité des sauts est très bonne et il est possible de travailler à une sensibilité encore plus grande que celle de 0.090 μA/mm qui a été utilisée, ce qui facilite la mesure des hauteurs.

b. Dosage de la tyrosine libre. Comme il a été dit plus haut, nous avons fait parallèlement un essai de nitration sur l'albumine non hydrolysée, de manière à obtenir une courbe comparative. Nous constatons que dans ce cas, il ne se produit pratiquement pas de saut. Seule une faible vague, à peine dessinée, marque l'emplacement du saut de la tyrosine. Cette légère ondulation, parfaitement reproductible, est très probablement due à une petite quantité de tyrosine rendue libre par une faible hydrolyse lors de la préparation de l'albumine.

Remarque: Les valeurs que nous avons obtenues pour la teneur de l'albumine en tyrosine sont en accord avec celle que l'on peut trouver dans la littérature.

(Documenta Geigy, Tables scientifiques[3] = 4.66%; BLOCK ET BOLLING[2] = entre 3.9 et 7.3%; D. E. DUGGAN ET S. UNDENFRIEND[4] = 5.5% pour l'albumine de sang de bovidés).

Ces essais nous ont permis de contrôler l'application de la méthode de dosage à un mélange complexe, en éprouvant la technique opératoire. Pour les mesures quantitatives rigoureuses, nous utiliserons un produit de synthèse dont la composition est exactement connue.

Dosage de la tyrosine dans la N-carbobenzoxy-S-benzyl-L-cystéinyl-L-tyrosine

Nous avons procédé à l'analyse d'un produit de synthèse aimablement mis à notre disposition par MM. BOISSONNAS *et al.*[5] dont la composition est exactement connue. Il s'agit de la N-carbobenzoxy-S-benzyl-L-cystéinyl-L-tyrosine; la molécule de ce composé peptidique a un poids de 508.57. La tyrosine s'y trouve donc dans le rapport 181/508.6 = 0.356.

Les dosages ont été conduits de la manière suivante:

On effectue deux prises traitées de façon identique, sauf que l'une d'elle reçoit une quantité étalon de tyrosine pure. On ajoute 10 ml d'hydroxyde de sodium 5 *N* et on chauffe pendant 5 h à environ 120°. Après refroidissement, on neutralise par de l'acide nitrique concentré, on amène à 25 ml dans un cylindre gradué, tout en ajoutant 2.5 ml d'acide nitrique 1.5 *N* et 1 ml de solution d'acétate mercurique à 2% dans l'acide nitrique 0.15 *N*. On transvase dans une éprouvette à centrifuger et on centrifuge jusqu'à obtention d'une solution tout à fait limpide, ce qui ne nécessite d'ailleurs que quelques minutes. On décante soigneusement et on prélève 20 ml que l'on place dans un erlenmeyer rodé. On ajoute encore 10 ml d'acide nitrique 0.15 *N* et un ou deux cristaux de nitrite de sodium, puis on fait bouillir à reflux durant 2 h. L'analyse se termine ensuite de la manière habituelle (voir page 2).

Les résultats suivants ont été obtenus:

TABLEAU IV

Prises d'hydrolysat (mg)	*Tyrosine (mg) valeur théorique*	*Tyrosine étalon (mg)*	*Saut µA*	*Tyrosine trouvée (mg)*	*Ecarts %*
6.5	1.97	—	0.755	1.88	−4.6
6.5	1.97	5.2	2.84		
6.5	1.97	—	0.80	2.03	+3
6.5	1.97	5.2	2.84		
8.9	2.70	—	0.91	2.75	+1.85
8.9	2.70	5.3	2.67		
5.5	1.67	—	0.578	1.63	−2.5
5.5	1.67	5.9	2.675		

Nous voyons d'après ces chiffres que les écarts se maintiennent dans une limite raisonnable, compte tenu de la petitesse des prises, rendue nécessaire par la faible quantité de produit disponible.

II. Dosage du tryptophane en présence de tyrosine et de phénylalanine

a. Dosage du tryptophane seul ou en présence de phénylalanine. Le tryptophane ne se nitre pas avec l'acide nitrique 0.15 *N* utilisé pour le dosage de la tyrosine. Par contre l'acide 1.5 *N* donne un composé qui se réduit au polarographe au pH 5, comme dans le cas de la tyrosine. Les sauts sont parfaitement reproductibles et bien dessinés. La phénylalanine ne gêne en aucune façon; il faut, pour la nitrer, des conditions beaucoup plus énergiques.

La vague du tryptophane, au pH 5, se situe dans le voisinage de celle de la tyrosine à 0.75 V (par rapport à la surface mercure/solution). Le mode opératoire est semblable à celui mis en oeuvre pour le dosage de la tyrosine:

On utilise 40 ml d'acide nitrique 1.5 *N* pour une prise de l'ordre de 10 à 30 mg de tryptophane.

On nitre par ébullition à reflux durant deux heures.

On neutralise par l'hydroxyde de potassium à 20%.

On amène à 50 ml. On prélève une partie aliquote que l'on additionne de solution tampon de pH 5 (phosphate disodique/acide citrique). On polarographie cette solution.

Les valeurs suivantes ont été obtenues en travaillant à la sensibilité de 0.080 µA/mm.

TABLEAU V

Tryptophane (mg)	*Concentration (mg/l)*	*Saut µA*	*K mg/l/µA*
11.0	77	5.68	13.55
15.2	106.4	7.44	14.3
19.3	135.2	9.12	14.8
22.4	156.8	10.16	15.65

Note. La valeur de K est régulièrement croissante car la droite d'étalonnage tracée à l'aide de ces points ne passe pas tout à fait par l'origine.

Les résultats sont identiques que le tryptophane soit ou non en présence de phénylalanine.

b. Dosage du tryptophane en présence de tyrosine. Le dosage différentiel n'est pas possible, il y a interaction de ces composés au cours de la réaction qui fait que les sauts polarographiques ne sont ni reproductibles, ni proportionnels aux concentrations de ces composés. L'augmentation de la concentration de l'acide nitrique ne donne pas de résultats meilleurs. Nous avons tenté de détruire la tyrosine, plus sensible que le tryptophane à l'oxydation. On y parvient avec le permanganate de potassium par exemple, mais cet oxydant détruit partiellement le tryptophane, il n'est par conséquent pas utilisable.

Ceci a d'autre part été montré dans la thèse de R. GUERNE[6] qui indique que le saut du tryptophane est diminué d'environ 70% (il est probable que le saut est alors donné par les produits résultant de l'oxydation). Nous avons pensé pouvoir utiliser, pour cette destruction, l'oxygène par barbotage dans la solution chaude. Même après plus d'une heure, il nous a été impossible de faire disparaître le saut de la tyrosine. L'emploi de l'eau oxygénée n'a pas donné de résultat. En conclusion, il ne nous a pas été possible de détruire la tyrosine en présence de tryptophane sans provoquer des interférences.

Etant donné ces résultats, une séparation s'imposait. Pour cela, nous avons utilisé l'acétate de mercure(II) qui nous a déjà permis d'éliminer l'effet gênant du tryptophane (voir page 1).

Comme il a été dit précédemment, le précipité tryptophane–mercure est insoluble dans l'acide nitrique dilué, même à chaud. Mais si on le met en suspension dans l'acide nitrique 1.5 *M* et qu'on fasse bouillir à reflux, le précipité prend une teinte jaune plus foncée, puis se dissout en quelques minutes. On obtient finalement une solution jaune tout à fait limpide dans laquelle on peut doser le tryptophane. Des essais quantitatifs, conduits avec soin, ont été entrepris sur des solutions de cet acide aminé.

On place dans un tube à centrifuger l'échantillon de tryptophane, on le dissout dans l'acide nitrique 0.15 *N*. On introduit une solution d'acétate mercurique à 2% (dans NO_3H 0.15 *N*) en quantité telle qu'il se produise presque immédiatement une précipitation jaune pâle opaque (un excès est sans importance). On place ensuite le tube à centrifuger dans un bécher d'eau chaude servant de bain-marie qu'on maintient à environ 60° pendant 2 h, de manière à obtenir une précipitation totale.

L'addition d'une nouvelle quantité d'acétate mercurique ne doit plus provoquer de trouble, on s'assure ainsi que la précipitation a été quantitative. Dans ces conditions,

la solution surnageante n'est pas colorée en jaune et sa polarographie ne présente pas de saut, même si on le porte à ébullition à reflux.

Le précipité obtenu est centrifugé durant 5 à 10 min jusqu'à ce que la solution soit tout à fait limpide.

On l'introduit ensuite dans un erlenmeyer rodé en ajoutant 20 ml d'acide nitrique 1.5 *N* et on fait bouillir à reflux durant 2 h. Après refroidissement, on neutralise par une solution d'hydroxyde de potassium à 20%, on transvase dans un flacon jaugé pour compléter à 50 ml par de l'eau bidistillée et on prélève 7 ml de cette solution. La prise est additionnée de 3 ml de solution tempon de pH 5 (phosphate disodique–acide citrique), on en élimine l'oxygène et on polarographie.

Nous donnons ci-dessous les valeurs obtenues pour une série d'analyses:

TABLEAU VI

Tryptophane (mg)	*Concentration (mg/l)*	*Saut μA*	*Ecarts %*
7.2	72	4.90	0
12.6	126	8.36	+5
18.8	188	11.4	−0.8
21.7	217	13.0	0

On peut voir d'après le tableau ci-dessus que l'utilisation du précipité tryptophane–mercure(II) comme moyen de séparation se présente de manière favorable.

Remarque: Au cours de la nitration, le mercure qui a servi à la précipitation du tryptophane est libéré, mais au moment de la neutralisation par l'hydroxyde de potassium, il précipite à nouveau de façon pratiquement quantitative de sorte qu'il ne gêne pas la détermination polarographique.

c. Dosage du tryptophane en présence de tyrosine et de phénylalanine. Nous allons donner maintenant le mode opératoire d'un dosage du tryptophane en présence de tyrosine et de phénylalanine.

La présence de phénylalanine ne nécessite aucune précaution spéciale lors des opérations de dosage. Par contre, il est nécessaire que la séparation de la tyrosine soit faite avec grand soin.

MODE OPÉRATOIRE

a. Placer la prise contenant de 5 à 30 mg de tryptophane et une quantité suffisante de phénylalanine (en ajouter si nécessaire; voir mode opératoire page 2) dans un tube à centrifuger; y ajouter 20 à 30 ml d'acide nitrique 0.15 *N*. Dissoudre par léger chauffage.

b. Introduire une solution d'acétate mercurique à 2% (dans l'acide nitrique 0.15 *N*) jusqu'à obtention d'un trouble jaune pâle opaque.

c. Maintenir au bain-marie à environ 60° pendant 1 à 2 h, de manière à assurer une précipitation complète.

d. Centrifuger le précipité formé et décanter la solution lorsque celle-ci est tout à fait limpide. Effectuer un lavage au moyen d'acide nitrique 0.15 *N* (environ 20 ml) en remettant le précipité en suspension, puis centrifuger à nouveau.

e. Placer le composé tryptophane–mercure(II) dans un erlenmeyer rodé de 100 ml en ajoutant 30 ml d'acide nitrique 1.5 *N*. Chauffer à ébullition à reflux durant 2 h.

f. Après refroidissement, neutraliser par l'hydroxyde de potassium à 20% et centrifuger le léger trouble qui se forme.

g. Compléter à 50 ml par de l'eau bidistillée, prélever 5 à 7 ml que l'on amène à 10 ml au moyen de la solution tampon de pH 5.

h. Chasser l'oxygène par un courant d'azote pur pendant 8 à 10 min, puis polarographier.

Remarque : Après la seconde décantation (opération d), il est préférable, afin d'éviter un transvasage du précipité, d'introduire l'acide nitrique 1.5 *N* directement dans le tube à centrifuger contenant le composé tryptophane–mercure(II) propre. On y commence le chauffage au moyen d'un bain-marie bouillant de manière à ce que le précipité se dissolve. La solution est ensuite transvasée quantitativement dans l'erlenmeyer rodé pour poursuivre la nitration (opération e). De cette manière on évite toute perte de produit; celui-ci est en effet assez adhérent aux parois et sa récupération quantitative nécessite des soins.

Nous donnons ci-dessous, les résultats de différentes analyses faites sur des prises contenant des quantités variables de tyrosine et de phénylalanine.

TABLEAU VII

Prises (mg)			*Tryptophane ajouté (mg)*	*Saut µA*	*Tryptophane trouvé (mg)*	*Ecarts %*
Tryptophane	*Tyrosine*	*Phénylalanine*				
9.0	15	15	—	5.69	8.8	—2.2
9.0	15	15	4.2	8.41		
7.9	30	30	—	4.98	7.6	—3.8
7.9	30	30	2.2	6.42		
8.0	20	20	—	5.42	8.4	+5
8.0	20	20	5.1	8.70		

Nous voyons d'après ces résultats que la marge d'erreur que l'on observe est de ±5%. Elle est supérieure à celle du dosage de la tyrosine (±3%). Mais il faut tenir compte du fait que l'on a maintenant un dosage comprenant une précipitation suivie de lavage et de transvasage. Les opérations effectuées sur un précipité sont presque toujours moins précises que celles ne nécessitant que des manipulations de solutions

III. Dosage de la phénylalanine en présence de tyrosine et de tryptophane

Le dosage de la phénylalanine a déjà été décrit[6]. Rappelons dans les grandes lignes la méthode utilisée.

Les prises sont traitées dans de petites éprouvettes à fond plat, au moyen de 0.5 ml d'acide nitrique 10 *N*. On porte à ébullition sur une plaque chauffante électrique durant 1 h 30 min tout en assurant un reflux convenable au moyen d'un récipient contenant de l'eau froide. On neutralise en présence de rouge de méthyle, substance qui intervient ensuite au cours du dosage polarographique comme suppresseur de maximum. On polarographie la solution tamponnée au pH 6 (phosphate disodique–acide citrique). Les écarts sont de l'ordre de 2.5% pour les concentrations de l'ordre du milligramme.

Remarque : Lors de l'application de cette méthode, nous avons préféré utiliser un bain d'huile pour assurer le chauffage des éprouvettes. La température de 120° environ donne des résultats comparables à ceux obtenus en travaillant directement sur la plaque électrique à la température d'ébullition de la solution. Comme cette température est relativement élevée, il se produit fréquemment des projections qui risquent de causer des pertes de substances (l'emploi de pierres à ébullition pour d'aussi faibles volumes n'est pas pratique). A 120°, la solution n'est pas encore en ébullition et il n'y a par conséquent pas de projections possibles. Le bain d'huile nous assure d'autre part une température uniforme entre les divers tubes à essai en cours de traitement.

Le dosage n'est évidemment pas possible en présence de tyrosine et de tryptophane

qui sont partiellement détruits par cette nitration et dont les résidus perturbent la mesure polarographique.

Une solution de tyrosine traitée par le permanganate de potassium jusqu'à coloration rose persistante puis décoloration par l'eau oxygénée peut être soumise deux heures à l'action de l'acide nitrique sans présenter le moindre saut polarographique. On peut donc en conclure que cet acide aminé est entièrement oxydé et que les produits d'oxydation qui en résultent ne peuvent être nitrés dans ces conditions.

Nous avons traité de la même manière une solution de phénylalanine et nous avons pu voir que la hauteur du saut polarographique produit après traitement par HNO_3 10 *N* n'est pas modifiée. Nous préférons utiliser pour l'oxydation une solution concentrée de permanganate (la solution saturée est à 6.4% environ) plutôt que des cristaux dont l'action est plus lente.

Le tryptophane soumis à l'action du permanganate, même à chaud et en présence de nitrate d'argent laisse subsister un saut, il ne peut donc être éliminé de cette manière. Cependant, nous avons vu qu'il est facile de le précipiter et c'est la combinaison d'une oxydation et d'une précipitation qui rendra possible le dosage de la phénylalanine en présence des deux autres acides amiéns. Pour l'élimination du tryptophane, la méthode qui consiste à le précipiter directement par l'acétate de mercure est longue et délicate et les résultats manquent de précision. Nous avons constaté qu'il est préférable de procéder d'abord à la nitration du mélange et d'éliminer ensuite seulement le tryptophane.

Elimination de la tyrosine et du tryptophane après nitration

Nous avons vu précédemment que le précipité que donne le tryptophane en présence de mercure commence à se dissoudre au moment de l'ébullition de l'acide nitrique 1.5 *N*. Mais si l'on amène une solution de tryptophane nitré à un pH légèrement acide (pH compris entre 2 et 3), on s'aperçoit qu'il se forme, en présence d'un excès de mercure, un précipité jaune orange de tryptophane nitré. (La solution ne doit être ni trop acide, ni neutre). Ce qui montre que la précipitation au tryptophane peut se faire aussi bien après qu'avant la nitration.

MODE OPÉRATOIRE

On place la prise contenant un mélange des trois amino-acides dans un petit tube à fond plat (la pesée se fait directement dans le tube). On ajoute 1 ml d'acide nitrique 10 *N*. On place dans un bain d'huile à 120° pendant 1 h 30 min. Le reflux est assuré par un récipient contenant de l'eau froide, placé sur l'ouverture du tube.

Après refroidissement, on ajoute de l'hydroxyde de potassium à 20% au moyen d'un compte-gouttes très effilé, jusqu'à neutralisation (visible par le virage de la teinte à l'orange). On transvase ensuite dans un tube à centrifuger en ajoutant environ 10 ml d'eau bidistillée.

On réacidifie légèrement avec de l'acide nitrique dilué de manière à obtenir un pH compris entre 2 et 3, puis on ajoute 5 ml de solution d'acétate mercurique à 2%. On place dans un bain-marie à environ 60° pendant 1 à 2 heures; on centrifuge ensuite pendant quelques minutes, jusqu'à obtention d'une solution limpide.

On décante soigneusement dans un deuxième tube à centrifuger, on ajoute 2 ml d'acide nitrique 0.1 *N* à la solution ainsi que 2 ou 3 gouttes de nitrate d'argent à 10%, puis une solution concentrée de permanganate de potassium jusqu'à coloration rose persistante (de 2 à 3 ml environ). On place au bain-marie à 60–80° pendant 15 minutes. (La présence de nitrate d'argent est nécessaire pour que l'oxydation se fasse dans le temps indiqué; la tyrosine subsiste bien plus longtemps en l'absence de ce réactif. La coloration rose finale doit être bien nette et dominer la couleur brune due au bioxyde de manganèse; si elle tend à disparaître pendant le chauffage, rajouter un peu de permanganate de potassium).

Après oxydation, on ajoute quelques gouttes d'eau oxygénée pour détruire l'excès de permanganate.

La solution est ensuite transvasée dans un cylindre gradué et neutralisée par de l'hydroxyde de potassium à 20%; le mercure, le manganèse et l'argent précipitent. On amène ensuite par de l'eau bidistillée à un volume bien déterminé et on verse dans un nouveau tube le liquide contenant le dépôt. On centrifuge quelques minutes jusqu'à obtention d'une solution limpide de teinte légèrement jaune (la teinte du dérivé nitré de la phénylalanine est faible comparée à celle que donnent les autres amino-acides).

On prélève une partie aliquote aussi grande que possible, que l'on amène à 50 ml dans un flacon jaugé. On prend 7 ml de cette solution et on complète à 10 ml au moyen de la solution tampon de pH 5.

On chasse l'oxygène par un courant d'azote pur pendant 8 à 10 min, puis on polarographie.

Les essais ont montré qu'il ne se produit pas de pertes de phénylalanine au cours du traitement. Il se produit en fait une faible perte par adsorption sur le tryptophane précipité, mais comme nous l'indiquons plus loin, nous avons constaté, dans ces conditions, une nitration plus forte de la phénylalanine, ces deux phénomènes semblent se compenser partiellement.

Nous avons obtenu par exemple les chiffres suivants, avec des prises contenant des quantités variables de phénylalanine.

TABLEAU VIII

Prises (mg)			*Saut* μA	*Phénylalanine trouvée (mg)*	*Ecarts* %
Phénylalanine	*Tyrosine*	*Tryptophane*			
7.9	20	20	3.16	8.1	+2.5
9.0	20	20	3.52	8.9	—1.1
9.4	20	20	3.61	9.2	—2.1
3.5	30	30	1.30	3.3	—6
6.6	30	30	2.91	6.75	+2.3
10.1	30	30	4.06	10.3	+2

Nous voyons que les erreurs que l'on commet dans le dosage de la phénylalanine en présence de tyrosine et de tryptophane sont acceptables, elles ne dépassent pas $\pm 5\%$.

Mais nous avons fait une constatation importante. Si l'on effectue une nitration de la phénylalanine pure dans les mêmes conditions de température et de durée, on obtient régulièrement un saut d'environ 10% plus petit que dans le cas où l'on est en présence de tyrosine et de tryptophane. Tout ce passe comme si la phénylalanine, en présence de ces deux acides aminés, subissait une nitration induite plus énergique que lorsqu'elle est seule.

Le même phénomène se produit en présence de nitrite de sodium. Ce sel, que l'on n'ajoute pas lorsqu'on travaille avec des concentrations acides aussi fortes, a cependant, une influence certaine sur le déroulement de la nitration. Nous avons observé en effet que:

a. En présence de nitrite, la réaction s'amorce rapidement et la coloration jaune apparaît en quelques minutes. La coloration finale est un peu plus intense que celle que l'on obtient lors du traitement de phénylalanine pure. Le saut polarographique est également un peu plus élevé.

b. Dans le cas de la phénylalanine nitrée par l'acide nitrique 10 N pur, la réaction se produit très progressivement et la coloration finale n'est atteinte qu'insensiblement.

Nous ne pouvons affirmer cependant que, quantitativement, l'induction produite

par le nitrite est comparable à celle due à la tyrosine ou au tryptophane. Il est donc préférable de procéder à un étalonnage interne de la manière habituelle.

Remarque: Si, au cours des opérations, l'oxydation de la tyrosine ou la précipitation du tryptophane n'a pas été faite correctement. On s'en aperçoit par le fait que les sauts se trouvent déformés.

Dosage par étalonnage interne

En procédant de la manière qui a été décrite plus haut (voir page 9), nous avons effectué quelques dosages par étalonnage interne de la phénylalanine en présence de quantités cinq fois plus fortes de tyrosine et de tryptophane.

Nous avons obtenu les valeurs suivantes

TABLEAU IX

Phénylalanine (mg)	*Phénylalanine ajoutée (mg)*	*Saut µA*	*Phénylalanine trouvée (mg)*	*Ecarts %*
5.7	—	1.38	5.9	+3.5
5.7	5.6	2.79		
4.0	—	0.90	3.8	−5
4.0	5.9	2.30		
4.1	—	0.945	4.0	−2.5
4.1	5.7	2.29		

Pour que le dosage soit exact, il est bien évident qu'il est nécessaire d'éliminer soigneusement les deux amino-acides gênants. Avec un excès de mercure et si l'on a attendu un temps suffisant, l'élimination du tryptophane est complète. En ce qui concerne l'oxydation de la tyrosine, on doit, si l'on a des doutes, faire une nouvelle oxydation à chaud de quelques minutes. Si la première oxydation a été totale, on retrouve un saut exactement identique, compte tenu de la petite dilution faite par l'introduction des différents réactifs nécessaires.

IV. Dosage du tryptophane, de la tyrosine et de la phénylalanine en présence les uns des autres

a. La prise contenant les substances à doser est mise en solution dans l'acide nitrique 0.15 *M*. On effectue la précipitation du tryptophane et on dose la tyrosine dans la solution résultante (voir page 2).

b. Le précipité renfermant le tryptophane est dissous dans l'acide nitrique 1. 5 *M*. On continue ensuite le dosage selon la méthode habituelle (voir page 7).

c. Sur une deuxième prise de substance, on procède à la nitration directe avec l'acide nitrique 10 *M*; on précipite le tryptophane et on oxyde la tyrosine de sorte que le saut résultant donne la teneur en phénylalanine (voir page 9).

Chaque polarogramme doit avoir la forme correspondante à celle des produits purs. Les interférences se signalent immédiatement par des courbes irrégulières, des inflexions dans le tracé des sauts etc. Elles sont la preuve d'une mauvaise séparation ou de la présence de substances gênantes.

Application à l'analyse d'une peptone

Nous avons pu nous procurer auprès de la maison Hoffmann-La Roche, un produit dont la teneur en acides aminés qui nous intéresse est approximativement connue. Cette maison a utilisé pour ses dosages des méthodes biologiques.

Le produit est un hydrolysat de caséine (Peptone „Roche") dont les teneurs suivantes nous ont été indiquées: Tyrosine = 2.4%; Tryptophane = 2.1%; Phénylalanine = 10.9%. (L'ordre de grandeur de la précision de ces valeurs n'a pu nous être donné).

Cette peptone contient en outre les acides aminés suivants (en %): Alanine = 2.5, Arginine = 3.1, Acide aspartique = 12.5, Cystine = 1.2, Acide glutamique = 16.5, Histidine = 2.5, Isoleucine = 8.7, Leucine = 8, Méthionine = 5.5, Proline = 1.9, Sérine = 8, Thréonine = 6.3, Valine = 10.5, Lysine = 8.

L'analyse a été conduite comme il a été indiqué plus haut. Malgré la complexité de la substance étudiée, les réactions se déroulent normalement et l'on obtient des courbes polarographiques de très bonne qualité.

a. Dosage du tryptophane. Les dosages en ce qui concerne le tryptophane ont donné les résultats suivants:

TABLEAU X

Peptone (mg)	*Tryptophane témoin (mg)*	*Saut μA*	*Valeur trouvée (mg)*	%
200	—	0.945		
200	6.2	2.36	4.05	2.02
200	9.3	3.11		
200	—	0.90		
200	8.0	2.61	4.1	2.05
			Valeur indiquée	2.1

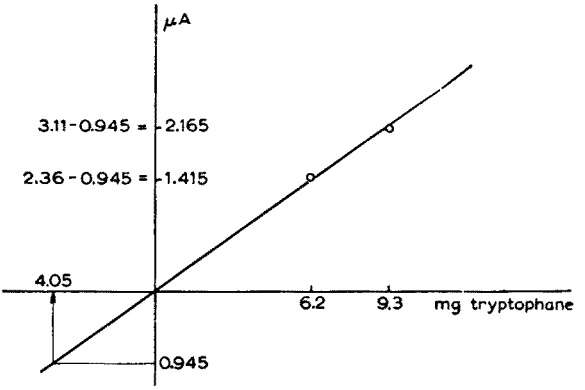

Fig. 1. Dosage du tryptophane; étalonnage interne double.

Les calculs ont été faits par résolution graphique, le procédé est très pratique pour obtenir le résultat d'analyses par étalonnage double. On effectue le tracé de la manière suivante: on porte en ordonnée positive les augmentations de hauteur de saut dues à l'introduction des témoins et en ordonnée négative le saut dû au produit seul. On porte ensuite en abscisse positive le poids des témoins. On trace la droite moyenne

passant par les deux points témoins et l'origine; l'endroit où elle coupe l'ordonnée du saut du produit pur détermine en abscisse négative la teneur en produit cherché (voir Fig. 1).

Les résultats trouvés concordent bien avec la valeur indiquée par la maison Hoffmann-La Roche.

b. Dosage de la tyrosine. L'application directe de la méthode mise au point pour le dosage de la tyrosine n'a pas donné immédiatement de bons résultats dans le cas qui nous intéresse. Les sauts étaient de mauvaise qualité et les résultats non reproductibles.

Nous nous sommes donc proposés de reconstituer la peptone étudiée à partir des acides aminés qui la constituent.

Nous nous sommes procuré ces amino-acides et les avons étudiés séparément du point de vue de leur comportement vis-à-vis des divers réactifs que nous utilisons au cours de l'analyse.

Nous pouvons constater qu'aucun de ces produits ne se nitre dans les conditions de travail utilisées pour la tyrosine. L'adjonction de quelques cristaux de nitrite de sodium demeure sans effet. La tyrosine se nitre facilement et correctement en présence de tous ces acides aminés et aucun d'entre eux ne subit lui-même de nitration induite.

A la suite de ces essais, nous avons repris l'étude du dosage et il a pu être constaté que l'erreur provenait de l'emploi d'un trop grand excès de mercure.

(Ceci ne s'était pratiquement jamais produit lors des essais de mise au point sur des substances de composition connue. Certaines perturbations occasionnelles, dues probablement à cette cause, avaient été attribuées à des erreurs de manipulation).

En présence d'une quantité d'acétate mercurique trop considérable et de cristaux de nitrite de sodium, la tyrosine forme une combinaison (de teinte légèrement rougeâtre) qui n'est plus entièrement détruite pendant les deux heures de traitement par l'acide nitrique bouillant.

Pour être dans les meilleures conditions possibles, nous avons modifié un point de la méthode de la manière suivante:

a. On précipite le tryptophane en milieu acide nitrique 0.15 *N* de la manière habituelle. La précipitation étant une réaction lente, il faut que les ions Hg^{+2} restent en solution durant ce temps;

b. Au lieu de procéder ensuite directement à la nitration à partir de cette solution, nous neutralisons par l'hydroxyde de potassium à 20% de manière à éliminer l'excès de mercure sous forme de HgO que l'on centrifuge. On ramène ensuite à la normalité de 0.15 au moyen d'une quantité calculée d'acide nitrique 1 *N*. On ajoute alors seulement le nitrite de sodium et on porte à ébullition à reflux durant 2 h.

TABLEAU XI

Peptone (mg)	*Tyrosine témoin (mg)*	*Saut µA*	*Valeur trouvée (mg)*	%
200	—	0.810	4.7	2.35
200	9.5	2.43		
200	—	0.788	4.75	2.38
200	5.7	1.73		
			Valeur indiquée	2.4

Le reste des opérations demeure sans changement.

En procédant de cette manière, nous avons obtenu des sauts parfaitement réguliers et reproductibles. Les résultats ont été enregistrés dans le Tableau XI.

Les résultats trouvés concordent donc sensiblement avec ceux indiqués par la maison Hoffmann-La Roche.

c. Dosage de la phénylalanine. Les résultats que nous avons obtenus sont les suivants:

TABLEAU XII

Peptone (mg)	*Phénylalanine témoin (mg)*	*Saut µA*	*Valeur trouvée (mg)*	%
100	—	1.035		
100	6.5	2.68	4.1	4.1
100	9.3	3.45		
100	—	1.057		
100	4.3	2.14	4.2	4.2
100	8.4	3.17		
			Valeur indiquée	10.9

Les chiffres trouvés sont nettement inférieurs à celui qui nous a été donné. Il est possible qu'une partie de l'amino-acide soit encore lié et ne peut, par conséquent, pas être mise en évidence par notre méthode. Afin de vérifier si l'hydrolyse peut être poussée davantage, nous avons traité une prise de peptone par l'hydroxyde de sodium 5 *N* à 120–125° pendant 5 h, selon le procédé indiqué par BLOCK ET BOLLING[2].

Les analyses ont été ensuite refaites et nous trouvons les chiffres suivants:

TABLEAU XIII

Hydrolysat prise (g)	*Teneur en peptone (mg)*	*Phénylalanine ajoutée (mg)*	*Saut µA*	*Valeur trouvée (mg)*	%
2.13	100	—	1.24	4.8	4.8
2.13	100	9.0	3.56		
2.13	100	—	1.26	4.85	4.85
2.13	100	9.2	3.65		

Les résultats sont plus élevés que ceux enregistrés précédemment, il y a donc eu libération supplémentaire de phénylalanine. Mais des hydrolyses plus poussées nous donnent ensuite des valeurs pratiquement constantes allant de 4.8 à 4.9%, il faut donc admettre que la phénylalanine a été entièrement libérée.

La maison Hoffmann-La Roche ne pouvant garantir le chiffre qu'elle nous indique, la constance de nos résultats nous permet de croire qu'ils sont voisins de la teneur réelle en phénylalanine.

En résumé:

Acide aminé	*Valeur indiquée par Hoffmann-La Roche*	*Valeur obtenue*
Tryptophane	2.1	2.02–2.05
Tyrosine	2.4	2.35–2.38
Phénylalanine	10.9	4.80–4.85

Selon les constatations faites au cours des essais de mise au point, la précision des chiffres indiqués est de l'ordre de $\pm 5\%$ environ.

RÉSUMÉ

Il est proposé une méthode polarographique de dosage de la tyrosine, du tryptophane et de la phénylalanine en présence les uns des autres. La méthode est spécifique car les autres acides aminés ne gênent pas la détermination ainsi qu'en témoigne le dosage de la peptone Hoffmann-La Roche. La méthode consiste à nitrer les acides aminés, puis à polarographier le dérivé obtenu. La méthode est sensible et précise car les sauts sont parfaitement bien dessinés. En jouant sur la concentration de l'acide, sur la stabilité différente de ces substances vis-à-vis de l'oxydation et sur la formation de précipité en présence de mercure, il est possible de doser chacun de ces acides aminés en présence des deux autres.

SUMMARY

A polarographic method is described for the determination of tyrosine, tryptophan and phenylalanine in mixtures, in which the animo acids are first converted to their nitro derivatives. Under suitable conditions each of these animo acids can be determined in the presence of the others.

ZUSAMMENFASSUNG

Es wird eine polarographische Methode beschrieben zur Bestimmung von Tyrosin, Tryptophan und Phenylalanin in deren Gemisch. Zur Messung werden die Nitroderivate verwendet. Durch Einhaltung bestimmter Bedingungen können die einzelnen Aminosäuren in Gegenwart der anderen bestimmt werden.

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GRAVIMETRIC DETERMINATION OF ALUMINIUM IN BRONZES AND BRASSES

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The preparation and properties of thioglycolic acid have long been known[1], but significant analytical applications of this acid have been made only recently; an example is the photometric determination of iron[2].

With the ions of some metals, *e.g.* Cu, Ag, Au, thioglycolic acid forms salts which are insoluble in water and in dilute mineral acids[3]; with many other metal ions it

possesses a reducing and complexing action which masks the ion. In the latter case, thioglycolic acid is often used along with ammonium benzoate which was introduced by KOLTHOFF *et al.*[4] for the gravimetric determination of aluminium. Its main advantages are that it gives a precipitate, consisting of a mixture of aluminium benzoate and hydroxide, which can easily be filtered and washed; moreover, it prevents almost completely the co-precipitation of the metals of group IV. Precipitation is carried out at pH 3–4. Under the conditions of precipitation of aluminium, Fe^{+3}, Cr^{+3}, Sn^{+4}, Bi^{+3}, Ce^{+4}, Ti^{+4}, Zr^{+4} and Th^{+4} also precipitate[5]. In the presence of thioglycolic acid, which reduces some ions and complexes others, only Al^{+3}, Ti^{+4}, Zr^{+4}, Th^{+4} precipitate[5].

The separation of aluminium with ammonium benzoate has been applied to copper[6] and magnesium[7] base alloys. In both cases the aluminium is subsequently determined as 8-hydroxyquinolate. For copper alloys a sample containing about 10 mg of aluminium is taken; separation of tin and antimony and reduction of the copper with hydroxylamine at pH = 4.2 must be done before the precipitation with benzoate.

Reactions of thioglycolic acid with copper

Thioglycolic acid forms a precipitate with copper, and we have examined the possibility of carrying out a preliminary separation of copper before precipitating aluminium with ammonium benzoate. BERG AND ROEBLING[3] report that thioglycolic acid in acidic solution forms a precipitate with copper; the colour changes from black-blue to pure yellow and one part of the precipitate is soluble in 2 million parts of 0.2 *N* mineral acid solution.

We first prepared two copper precipitates with thioglycolic acid by two different methods.

a. A solution of metallic copper in about 0.4 *N* hydrochloric acid (obtained by adding hydrogen peroxide, which was subsequently destroyed by boiling) was treated with a large excess of thioglycolic acid solution; a whitish precipitate was formed.

b. One volume of 1 *M* copper sulfate solution in about 0.4 *N* hydrochloric acid was treated with two volumes of 1 *M* thioglycolic acid solution; a yellow-whitish precipitate was formed.

The precipitates were filtered, washed with water, vacuum-dried at 50° and submitted to elemental analysis. The results were:

Method	*Cu* %	*S* %	*C* %	*H* %
a.	41.01	20.66	15.40	2.01
b.	40.98	20.68	15.45	1.98

Evidently the two precipitates are identical and consist of cuprous thioglycolate ($CuSCH_2COOH$). Similar salts of thioglycolic acid with Ba, As, Sb and Pt have been described by KLASON AND CARLSON[8].

When 1 volume of 1 *M* cupric sulfate or nitrate solution in about 0.4 *N* hydrochloric acid is mixed with 1 volume of 1 *M* thioglycolic acid solution, the solution is discolored, *i.e.* copper is reduced, but no precipitate is obtained; on adding another volume of 1 *M* thioglycolic acid solution, cuprous thioglycolate is formed.

When equal volumes of 1 *M* cuprous chloride solution in about 0.4 *N* hydrochloric acid and 1 *M* thioglycolic acid solution are mixed, a precipitate is immediately formed; elemental analysis indicates that it is cuprous thioglycolate. Therefore, in hydrochloric acid solution the copper is first reduced and dithiodiglycolic acid is formed:

$$2\,Cu^{+2} + 2\,HS-CH_2-COOH = 2\,Cu^{+} + \begin{matrix} S-CH_2-COOH \\ | \\ S-CH_2-COOH \end{matrix} + 2H^{+}$$

Subsequently, cuprous thioglycolate precipitates:

$$2\,Cu^{+} + 2HS-CH_2-COOH = 2\,CuS-CH_2-COOH + 2H^{+}$$

If the 1 *M* cupric sulfate or nitrate solution is prepared in dilute sulphuric or nitric acid rather than in dilute hydrochloric acid, and an equal volume of 1 *M* thioglycolic acid solution is added, cuprous thioglycolate is immediately formed but copper is still present in the filtrate. This copper is completely precipitated by adding another volume of the thioglycolic acid solution. This difference may be explained by the fact that in hydrochloric solution copper forms the chloro-cuprous ion, $CuCl_3^{-2}$; therefore, it may be assumed that the cupric ion is first reduced to chloro-cuprous ion and then precipitated by the thioglycolic acid. However, in sulphuric or nitric solution, as soon as the Cu^{+2} ion is reduced to Cu^{+} ion, the latter precipitates as cuprous thioglycolate and some unreduced or unprecipitated copper remains if the amount of thioglycolic acid is inadequate.

With aqueous solutions of cupric sulfate or nitrate in the absence of mineral acids, the thioglycolic acid forms a precipitate which changes from black-blue to yellow. This last precipitate also proved to be cuprous thioglycolate on elemental analysis.

Copper separation and aluminium precipitation

The preliminary separation of copper as cuprous thioglycolate in alloys where it is the base metal is not easy by filtration because the precipitate is very bulky. It is preferable to bring to volume and then filter a portion of the solution before the precipitation of aluminium. In analyses which do not require extreme accuracy, a single precipitation with benzoate is sufficient. When accurate results are desired, double precipitation is essential. With a single precipitation, small amounts of the other elements present in the alloy remain occluded.

The solution may contain up to 0.10–0.12 g of aluminium, but an amount of less than 0.05 g is preferable; its volume must not exceed 100 ml if the aluminium content is less than 10 mg, or 150 ml in other cases. The precipitate must be filtered and washed when warm, otherwise some ammonium benzoate crystallizes in the funnel spout. On cooling, the filtrate becomes cloudy owing to ammonium benzoate, but this dissolves on heating. If it does not, some precipitate has passed into the filtrate. For the double precipitation, the first precipitate can be redissolved on the filter with dilute hydrochloric acid, or the filter may be destroyed by wet combustion. The second method is preferred in order to ensure that all the precipitated aluminium is dissolved; furthermore, if perchloric acid is used, any silica from the beakers or possibly present in the sample can be separated by filtering.

The determination of the aluminium as oxide[9] requires ignition in a platinum

crucible and up to 1200° in order to obtain a non-hygroscopic oxide; otherwise, high results are obtained.

The precipitation of aluminium can be carried out also on the nitric acid solution of the alloy from which copper has been removed by electrolysis. However, if the separation of copper is not complete, which may happen if the rectified current is not well-filtered the aluminium oxide obtained by calcining has a feeble blue instead of white color.

EXPERIMENTAL

Reagents (Merck, pro analysi)

Dilute hydrochloric acid (1+1), concentrated sulphuric acid, $d = 1.83$, concentrated nitric acid, $d = 1.38$, concentrated ammonia, $d = 0.88$, dilute ammonia (1+1), perchloric acid (60% solution), hydrogen peroxide (30% solution), thioglycolic acid (80% solution).

Ammonium benzoate solution: dissolve 100 g of ammonium benzoate in 700 ml of hot distilled water and dilute to 1000 ml with distilled water (if the benzoate precipitates when the solution is cold, heat before use).

Benzoate wash solution: dissolve 10 g of ammonium benzoate in 800 ml of distilled water, add 20 ml of glacial acetic acid and dilute to 1000 ml with distilled water.

Bromophenol blue indicator: triturate 0.40 g of bromophenol blue with 8.5 ml of 0.1 *N* NaOH; dilute to 100 ml with distilled water.

PROCEDURE

In a 500-ml flask, dissolve 1 or 2 g of alloy (depending on whether aluminium is more or less than 5%) with 40 ml of 1:1 hydrochloric acid and add slowly 30% hydrogen peroxide solution. When the alloy does not respond to hydrogen peroxide, *e.g.* manganese bronzes, use dilute nitric acid instead of hydrogen peroxide. To complete the dissolution of metal heat moderately, dilute with 150 ml of distilled water and boil for about 10 min to remove hydrogen peroxide.

At 60°–80° add gradually while stirring, 20 ml of 80% thioglycolic acid solution, cool under the tap and bring to volume with distilled water. Through a dry Whatman No. 40 paper, filter 250 ml of the solution into a graduated flask. Transfer the contents into a tall-form 400-ml beaker, washing the flask with distilled water. Concentrate to 100 or 150 ml depending on whether the aluminium content is more or less than 10 mg. At 70° add 20 ml of the ammonium benzoate solution and stir with a glass rod to dissolve any precipitate formed. Add a few drops of bromophenol blue indicator and then dilute 1:1 ammonia until the indicator starts to change color. The solution must have a pH of 3.5 to 4 which may be checked by narrow-range pH paper. Boil for 5 min while stirring. Allow to settle for a few minutes and filter on a Whatman No. 40 paper, washing at least three times with the hot benzoate wash solution. Transfer the filter and precipitate back to the original beaker, add 10 ml of sulfuric acid ($d = 1.83$), let the paper carbonize, add 15 ml of nitric acid ($d = 1.38$), cover with a watchglass and heat moderately until all nitrous vapors are removed.

Then wash the watchglass and beaker walls carefully with distilled water. Remove the watch glass and heat to fumes of sulphuric acid. Remove the beaker from the plate, add immediately 5 ml of 60% perchloric acid and then reheat to fumes of perchloric acid.

As the perchloric acid fumes thicken, the residual organic substances are destroyed and the solution becomes colorless.

Allow to cool and dilute with distilled water to about 80 ml rinsing the glass walls; boil for a few minutes, allow to cool down to below 50°, and filter on Whatman No. 40 paper into a tall-form 400-ml neutral glass beaker washing with distilled water. This filtration may be omitted if the solution is perfectly clear, but it is generally advisable because some silica, though almost invisible, is always present.

Concentrate the filtrate to less than 100 ml, if the aluminium present is less than 10 mg; then, at about 70°, add 2 ml of the thioglycolic acid solution and re-precipitate the aluminium with ammonium benzoate as described above.

Filter on Whatman No. 40 paper and wash several times with hot benzoate wash solution. Place the filter and the precipitate in a weighed platinum crucible, dry and burn the filter, calcine in a muffle at 1200° for 2 h, cool in a desiccator and weigh.

$$\text{Al } \% = \frac{\text{weight of } Al_2O_3 \times 52.91}{\text{sample weight}}$$

RESULTS

The method described has been used to determine aluminium in synthetic solutions and in N.B.S. standards. The synthetic solutions were prepared from suitable weighed amounts of metallic copper and aluminium, to which were added convenient aliquots of solutions of known composition containing the other elements. These solutions were obtained by dissolving the metal concerned in dilute hydrochloric acid and a little dilute nitric acid.

The composition of the synthetic solutions was varied to simulate determinations on different percentages of aluminium (from 0.5% to 10%) and in the presence of different amounts of the elements generally found in copper alloys.

The results obtained (Tables I, II and III) are mostly satisfactory; the highest probability of errors appears when the aluminium content is high (about 10%) and low results are obtained. It is therefore advisable to use samples that contain less than about 0.05 g of aluminium. Table IV shows the results obtained by a single precipitation of the aluminium. The values found are rather high; the spectrographic examination indicates that the aluminium precipitate is contaminated by small amounts of the other alloying elements. Hence a double precipitation is necessary to ensure pure precipitates.

Table V shows the results obtained with some N.B.S. standards. Some of these samples lacked aluminium which was then added in varying percentages. On standard 164, determinations were made on both 1-g and 2-g samples; the results obtained agree in all cases with the certified values.

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TABLE I

DETERMINATIONS ON SYNTHETIC SOLUTIONS

No.	Weight (g)		Al %	
	Cu	Al	calculated	found
1	1.9820	0.0258	1.28	1.28
2	1.9798	0.0228	1.13	1.16
3	1.9200	0.0844	4.21	4.25
4	1.8416	0.1614	8.05	8.06
5	1.8404	0.1650	8.22	8.24
6	1.8012	0.2070	10.31	10.28
7	1.8022	0.2058	10.25	10.29
8	1.8000	0.2020	10.09	10.09
9	1.9990	0.0072	0.31	0.34
10	2.0008	0.0068	0.30	0.29
11	1.9908	0.0122	0.54	0.59
12	1.9812	0.0248	1.09	1.12
13	1.9816	0.0212	0.93	0.96

continued

TABLE I (continued)

No.	Weight (g)		Al %	
	Cu	Al	calculated	found
14	1.9216	0.0870	3.84	3.87
15	1.9226	0.0855	3.77	3.78
16	1.9218	0.1606	6.87	6.86
17	1.9216	0.1684	7.18	7.15
18	1.7618	0.2446	10.81	10.79
19	1.7620	0.2438	10.79	10.78
20	1.7656	0.2470	10.85	10.62
21	1.7606	0.2396	10.60	10.51
22	1.7623	0.2380	10.53	10.28
23	1.7638	0.2450	10.78	10.78
24	1.7660	0.2461	10.82	10.71
25	1.7648	0.2412	10.65	10.53

Other elements present (g)

		Fe	Mn	Ni	Zn	Pb
Tests	1–8	—	—	—	—	—
Tests	9–25	0.05	0.05	0.05	0.10	0.005

TABLE II

DETERMINATIONS ON SYNTHETIC SOLUTIONS

No.	Weight (g)		Al %	
	Cu	Al	calculated	found
1	1.7600	0.0977	4.32	4.37
2	1.7600	0.0955	4.29	4.27
3	1.7600	0.1107	4.91	4.90
4	1.7600	0.1252	5.48	5.50
5	1.7600	0.1031	4.58	4.58
6	1.7600	0.1032	4.58	4.58
7	1.7600	0.1015	4.51	4.52
8	1.7600	0.1073	4.75	4.73
9	1.9220	0.0840	3.67	3.68
10	1.9217	0.0818	3.58	3.56
11	1.8405	0.1668	7.29	7.32
12	1.9612	0.0433	1.97	2.00
13	2.0002	0.2495	9.75	9.73
14	1.7597	0.2448	10.62	10.58
15	1.7613	0.2514	10.82	10.78
16	1.7600	0.2460	10.62	10.61
17	1.7600	0.2433	10.65	10.56
18	1.7600	0.2432	10.65	10.57
19	1.7600	0.2461	10.76	10.67
20	1.7600	0.2403	10.53	10.56

Other elements present (g)

		Fe	Mn	Ni	Zn	Pb
Tests	1–4	0.05	0.05	0.05	0.10	0.006
Tests	5–8	0.05	0.05	0.05	0.10	0.012
Tests	9–20	0.05	0.05	0.05	0.10	0.030

TABLE III

DETERMINATIONS ON SYNTHETIC SOLUTIONS

No.	Weight (g)		Al %	
	Cu	Al	calculated	found
1	1.9230	0.0860	4.15	4.16
2	1.9212	0.0872	4.21	4.24
3	1.8412	0.1633	7.90	7.88
4	1.8436	0.1656	7.99	8.04
5	1.7390	0.2639	12.79	12.66
6	1.7426	0.2670	12.89	12.83
7	1.9936	0.0100	0.42	0.46
8	1.9936	0.0108	0.45	0.48
9	1.9932	0.0140	0.58	0.61
10	1.9808	0.0204	0.84	0.88
11	1.9816	0.0221	0.93	0.94
12	1.9798	0.0226	0.95	0.95
13	1.9224	0.0852	3.57	3.53
14	1.9036	0.1041	4.37	4.34
15	1.9046	0.1070	4.49	4.42
16	1.8634	0.1410	5.93	5.89
17	1.8602	0.1454	6.12	6.18
18	1.8420	0.1666	10.00	10.10
19	1.7420	0.2649	11.14	11.14

Other elements present (g)

		Fe	Mn	Ni	Zn	Pb	Sn
Tests	1–6	—	—	—	—	—	0.06
Tests	7–9	0.05	0.05	0.05	0.10	0.06	0.06

TABLE IV

RESULTS OBTAINED BY A SINGLE PRECIPITATION OF ALUMINIUM

No.	Weight (g)		Al %	
	Cu	Al	calculated	found
1	1.7604	0.2402	10.61	10.71
2	1.7614	0.2454	10.81	10.99
3	1.7622	0.2398	10.59	10.73
4	1.7676	0.2517	11.03	11.11

Other elements present (g)

		Fe	Mn	Ni	Zn	Pb
Tests	1–4	0.05	0.05	0.05	0.10	0.0125

TABLE V

DETERMINATIONS ON N.B.S. STANDARDS

Composition			Weighed sample g	Al added g	Al %	
					calculated	found
N. 63c Phosphor bronze			1.9905	0.0132	0.65	0.64
			1.9925	0.0186	0.92	0.93
Cu = 80.46	Sb = 0.52		1.9821	0.0235	1.17	1.17
Pb = 9.35	Ni = 0.32	S = 0.06	1.9032	0.1025	5.11	5.13
Sn = 9.03	P = 0.15	As = 0.02	1.9027	0.1053	5.24	5.30
Fe = 0.002	Zn = 0.09					

continued

TABLE V (continued)

Composition	Weighe sampled g	Al added g	Al % calculated	Al % found
N. 52c Cast bronze	1.9895	0.0127	0.63	0.66
	1.9914	0.0169	0.84	0.86
Cu = 89.25 Ni = 0.76 P = <0.001	1.9831	0.0208	1.03	1.06
Sn = 7.85 Pb = 0.011 S = 0.002	1.9824	0.0220	1.09	1.08
Zn = 2.12 Fe = 0.004	1.9006	0.1040	5.19	5.22
	1.9010	0.1054	5.25	5.28
N. 164 Al–Mn bronze	2	—	6.21	6.18
	2	—	6.21	6.17
	2	—	6.21	6.16
Cu = 63.76 Sn = 0.63	2	—	6.21	6.19
Zn = 21.89 Pb = 0.22	2	—	6.21	6.17
Mn = 4.68 Ni = 0.046	2	—	6.21	6.19
Fe = 2.52 Si = 0.038	2	—	6.21	6.16
Al = 6.21	1	—	6.21	6.22
	1	—	6.21	6.25
	1	—	6.21	6.18
	1	—	6.21	6.21
N. 62c Manganese bronze	2	—	1.22	1.20
	2	—	1.22	1.25
Cu = 59.16 Fe = 0.74 Ni = 0.28	2	—	1.22	1.20
Zn = 37.24 Mn = 0.66 Pb = 0.24	2	—	1.22	1.21
Al = 1.22 Sn = 0.39 Si = 0.068	2	—	1.22	1.22
N. 158 Silicon bronze	2	—	0.54	0.52
Cu = 90.86 Fe = 1.48 *Al = 0.54*	2	—	0.54	0.52
Si = 2.72 Mn = 1.31 Ni = 0.006	2	—	0.54	0.53
Zn = 2.07 Sn = 0.97 Pb = 0.004	2	—	0.54	0.52

SUMMARY

The gravimetric determination of aluminium in bronzes and brasses is described. After separation of copper by thioglycolic acid, aluminium is determined with ammonium benzoate.

RÉSUMÉ

Une méthode est proposée pour le dosage gravimétrique de l'aluminium dans des bronzes et des laitons. On procède par précipitation de l'aluminium au moyen de benzoate d'ammonium, après séparation du cuivre par l'acide thioglycolique.

ZUSAMMENFASSUNG

Beschreibung einer Methode zur gravimetrischen Bestimmung von Aluminium in Bronzen und Messing. Kupfer wird mit Thioglycolsäure abgetrennt und das Aluminium mit Ammoniumbenzoat gefällt.

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A SIMPLE SATURATED CALOMEL REFERENCE ELECTRODE FOR POLAROGRAPHY

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INTRODUCTION

All reference electrodes[1,2] used in polarography are sensitive to movement except the LADISCH AND KNESBACH[3] design; in general their construction requires a platinum to glass seal, or glass blowing experience. The half-cells described in the present paper have the following advantages. Their shape permits maximum interphase contact with a minimum amount of reagents in an electrode body that fits into an H-cell; this contributes to the more efficient use of the latter. They have a low

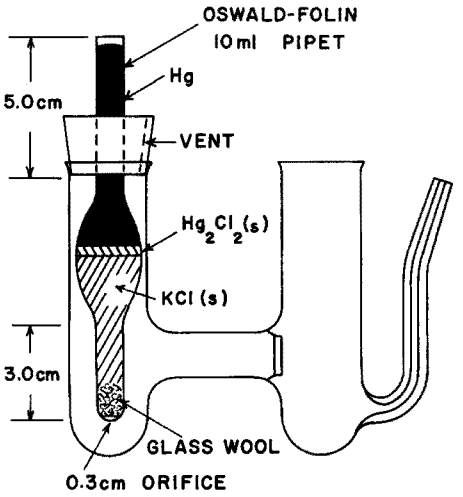

Fig. 1. Construction details of a saturated calomel reference electrode body that fits into an H-cell, test tube with side-arm, or other container.

resistance and the resulting saturated calomel electrodes can be transported without alteration of their 'potential' characteristics.

METHOD

The simplest fabrication technique is to cut a 10-ml Oswald-Folin pipet to the dimensions indicated in Fig. 1 and fire-polish the delivery end until an orifice of approximately 0.3 cm is formed to hold a glass wool plug; the resulting half-cell has a slightly higher resistance than one with a sintered glass tip. The sintered glass tip, which is an alternative choice, is made by sealing the delivery tip and filling it to a depth of approximately 3 cm with 60–80 mesh ground glass obtained by pounding, rather than grinding, the sections cut from the pipets. The body is evacuated (and kept under vacuum) while the tip is slowly heated and rotated in the upper part of a Meker burner flame until the extreme end fuses and more than half of the inner wall area of the tip appears frosted.

The vacuum line is disconnected when the tip has cooled just sufficiently to permit handling, and any excess unsintered glass is removed. With a thin file, such as is used to open ampoules, the tip is deeply scored completely around at the point where the break is to be made so as to form a 0.5-cm sintered glass plug.

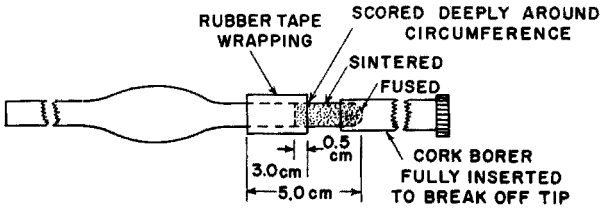

Fig. 2. Mode of breaking off fused glass extremity of pipet without shattering the sintered glass tip.

To protect the latter from shattering, a 10-cm, length of rubber tape is wrapped round the tip so that the lower edge of the wrapping coincides with the scoring in the glass tubing (Fig. 2). The rubber covered tubing is held firmly (avoiding application of pressure to the bulb) while inserting the fused glass end into a snugly fitting cork borer which acts as a lever in effecting the break. The suitability of the sintered glass tip or glass wool plug should be checked by filling the pipet with distilled water and observing the drainage; the pipet should be empty in 35 to 50 sec.

The resulting body is immersed in a large vertically mounted test tube containing enough saturated C.P. potassium chloride solution to half-fill the bulb of the pipet. Finely ground solid potassium chloride is introduced through a stemless glass funnel in small portions (with simultaneous rotation to avoid stratification) until the bulb of the pipet is filled to the half-way mark.

The saturated liquid level within the bulb is maintained at least 0.5 cm above the solid, and the salt is allowed to settle for 15 min. Then 3 to 5 g of electrolytic mercurous chloride are added in small amounts with occasional swirling of the pipet, to wet the calomel. After standing undisturbed for 30 min, the body is removed from the test tube to allow complete drainage of excess solution. A few small drops of mercury are poured gently down the sides of the tube into the bulb (without directly touching the calomel surface insofar as possible) and the pipet is rotated to cause packing of the salts. The remaining free space is filled with mercury and the body is reimmersed in an H-cell or test tube which is three-quarters filled with saturated potassium chloride.

Twenty-four hours later, the mercury is removed and if there is evidence of channeling or large drops are embedded, these are removed, and the calomel surface must be reconditioned by adding a small amount of saturated potassium chloride from a capillary pipet, swirling, draining, and refilling with mercury. The body is then immersed in an appropriate container, such as an H-cell (Sargent #S-29400 with medium porosity fritted glass disc and 4.5-cm agar plug) or test tube with side-arm (A. H. Thomas #6243-A) and flexible salt bridge. In any case, the stopper and inside lip of the vessel should be lightly coated with vaseline to limit creeping of the potassium chloride.

DISCUSSION

Half-cells which had bodies containing glass wool plugs or sintered glass tips, and were inserted into the arm of an H-cell, had resistances of 540 to 680 ohms (measured by the PESCE, KNESBACH AND LADISCH method)[4]; if the container was a test tube with side-arm and flexible salt bridge, the respective resistances were 890 to 1070 Ω. The bridge had a diameter of 0.5 cm, and was 36.2 cm long of which 15 cm was an 0.8-cm outside diameter gas washing tube filled to a depth of 13.7 cm with agar–potassium chloride. The coarse 1.7-cm fritted glass tip was kept in saturated potassium chloride when not in use.

The resistances of the suggested half-cell designs compared favorably with that of the large-area saturated calomel electrode, prepared in a Heyrovsky vessel with a side-arm bent to 180° and connected to the same salt bridge, which had an impedance of 720 Ω. The reproducibility of replicate limiting current measurements, regardless of the type of reference half-cell used, did not vary by more than 1.0%. The average potential of these electrodes *versus* a quinhydrone half-cell was 0.228_0 V with extreme values of 0.227_4 and 0.229_0, and a standard deviation of 0.000_6, as compared to 0.227_4, 0.228_2, and 0.228_9 for the large area saturated calomel electrode.

Saturated calomel electrodes, prepared by the method indicated above, have been used continually for the past two years in the Occupational Health laboratories of Bolivia, Colombia and Perú because it is considerably easier to change the solution to be analyzed and rinse the other arm of the H-cell. These half-cells have maintained their characteristics and are expected to last indefinitely.

The mode of making the sintered glass tip is described because it is much simpler than previously published information.

SUMMARY

A saturated calomel reference electrode which can be readily assembled is described. This half cell has a low resistance, is easily transportable, and materially increases the efficiency of the H-cell in polarography.

RÉSUMÉ

Une nouvelle électrode de référence au calomel est proposée; elle peut être utilisée en analyse polarographique et elle présente certains avantages.

ZUSAMMENFASSUNG

Es wird eine neuartige Kalomel Bezugselektrode beschrieben, die sich bei der polarographischen Analyse verwenden lässt und gewisse Vorteile besitzt.

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THE SIMULTANEOUS DETERMINATION OF COPPER AND IRON IN HIGH PURITY ALUMINIUM USING THE K 1000 CATHODE RAY POLAROGRAPH

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(Received August 20th, 1959)

A method is described for the rapid simultaneous determination of copper and iron in high purity aluminium (>99.99%). It is based on the solubility of high purity aluminium in sodium hydroxide solutions. It is only required to dissolve a weight of the sample in a 20% solution of sodium hydroxide, to add a solution of sucrose[1] and to analyze polarographically.

A polarographic method for the analysis of high purity aluminium was first carried out by SEMERANO[2] applying preliminary separation of copper, lead, cadmium, nickel, zinc and iron as sulphides and re-dissolving the sulphides in nitric acid. The method is very tedious and requires extremely high analytical skill.

Copper present in aluminium in amounts less than 50 μg is not reduced by hydrogen to the metallic state in the process of dissolving the aluminium in sodium hydroxide. The iron is converted into ferrous hydroxide, which is soluble in sodium hydroxide. This behaviour of copper and iron in connection with the high sensitivity of the Cathode Ray Polarograph[3-5] was the basis for the development of the method.

The present method is very rapid, fully eliminating chemical manipulations, such as filtering, extraction, etc., and enables the simultaneous estimation of copper and iron within 15 to 20 min, with a tolerance of $\pm$0.0001% for copper contents <0.005%, $\pm$0.00001% for iron contents <0.0001% and $\pm$0.0001% for iron contents between 0.0001% and 0.005%.

EXPERIMENTAL

To observe the linearity of the peak heights of copper and iron with the actual concentrations of these metals in aluminium, the following investigations were carried out.

0.1 g of super purity aluminium were introduced into five 5-ml flasks. By means of a precision microburette, 0.01–0.04 ml of standard solutions of copper and iron were added to each flask to increase the concentration by 0, 10, 20, 30 and 40 p.p.m. for copper, and 0, 0.1, 0.2, 0.3 and 0.4 p.p.m. for iron. 1.5 ml of a 20% solution of sodium hydroxide were added to each flask and the samples brought into solution by placing them on a low heated hot plate and carefully controlling the violence of the reaction by lifting the flask when necessary. When the samples were completely dissolved, the flasks were cooled, 0.5 ml of *M* sucrose were added, and the solutions made up to the mark with water. This technique was used due to small quantity of

super purity aluminium available at the time. By having larger samples (0.5 to 1 g), placing them in 50-ml tall pyrex beakers covered by watch glasses, and adding a corresponding volume of sodium hydroxide, the process of dissolving the aluminium is simplified by placing the beaker on a hot plate, and if the reaction becomes too violent, arresting it by injecting water from a wash bottle. After cooling, the solutions are transferred into 10–20 ml flasks, sucrose added in the rate stated above, and made up to the mark with water. The polarograms are recorded on 5 ml of the solutions after deaerating with nitrogen for 3 min. In the experiment, the polarograms were recorded on start potential —0.1 V for copper, scale factor 0.04 and start potential —0.6 V for iron, scale factor 0.06. The concentrations of each element and corresponding peak heights are tabulated below.

TABLE 1

Sample	*Copper added p.p.m.*	*Peak height divisions*	*Iron added p.p.m.*	*Peak height divisions*
1	0	0	0	16
2	10	10	0.1	20
3	20	21	0.2	24
4	30	30	0.3	27
5	40	40	0.4	31

No copper was detected in the original sample. Iron was present in the sample and the concentration is easily calculated. As the addition of 0.1 p.p.m. has an increase in wave-height of 4 divisions, the peak height (16 divisions) on the original sample corresponds to 0.4 p.p.m. The polarogram on 5 ml of the blank showed no presence of iron. Typical polarograms of copper and iron are shown below (Figs. 1 and 2).

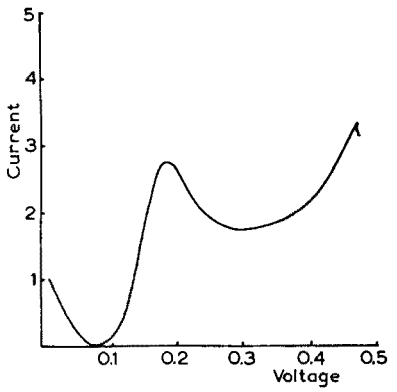

Fig. 1. Typical reduction wave of copper in aluminium.

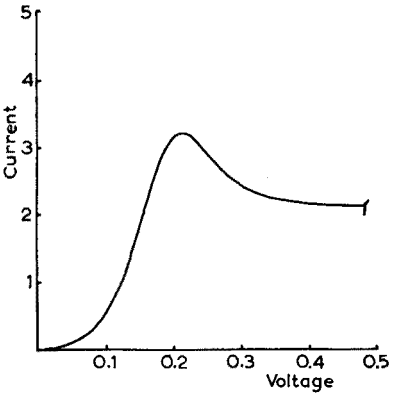

Fig. 2. Typical reduction wave of iron in aluminium.

During the investigation, the effect of the presence of lead on the reduction waves of copper and iron was studied, and also the effect of the addition of various concentrations of EDTA to the basic electrolyte. The addition of lead in various concentrations showed no interference with either the copper or iron peak heights. Lead is reduced with the formation of a well shaped wave preceding Fe-wave, hence indicating

the possibility of determining all three metals simultaneously (Figs. 3 and 4). The addition of a saturated solution of EDTA from 0.5 to 2% results in no significant alteration in the shape of the waves or in the peak heights. An increase in EDTA to

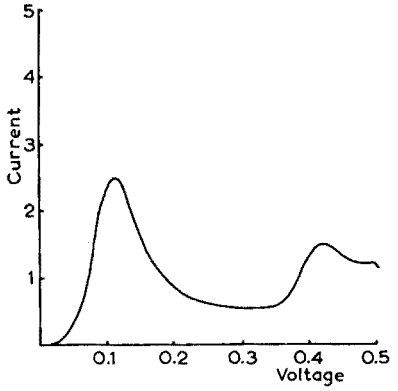

Fig. 3. Simultaneous determination of Cu and Pb in aluminium (20 p.p.m. Cu, 20 p.p.m. Pb).

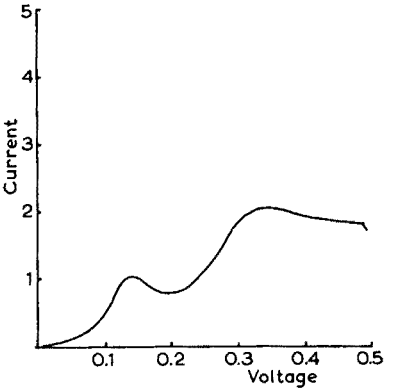

Fig. 4. Simultaneous determination of Pb and Fe in aluminium (20 p.p.m. Pb, 1 p.p.m. Fe).

over 3% depresses the copper wave, and increases the height of the iron wave. An addition of 6% EDTA completely removes the copper wave, and increases the peak height for iron by 25%. EDTA must be used in the basic electrolyte when the manganese content is considerably higher than the iron content. Manganese interferes with the determination of iron, but this can be overcome by the addition of 2% of a saturated solution of EDTA, as manganese is not reduced in its presence.

To show that the method could be applied for the routine control of the analysis of high purity aluminium, a series of seven samples was used, with copper and iron contents previously found by classical methods, by the British Aluminium Co. Ltd. 0.5 g of sample were placed in 50-ml beakers, dissolved in 5 ml of 20% sodium hydroxide, transferred into 10-ml flasks, 2 ml of *M* sucrose added and the solution made up to the mark with water. The polarograms were recorded on a 5-ml aliquot of each solution as stated above. The copper waves were observed on start potential —0.1 V and scale factor 0.04 using direct current, and the iron waves on start potential —0.6 V and scale factor 0.004 using derivative current. The concentrations of each metal were calculated by comparison, taking sample 3 as the "standard". The results obtained are tabulated below.

TABLE II

Sample	*Cu found by classical method p.p.m.*	*Cu found by polarographical method p.p.m.*	*Fe found by classical method p.p.m.*	*Fe found by polarographical method p.p.m.*
1	5	3	5	8
2	5	3	5	9
3	10	10	5	5
4	10	10	5	9
5	15	18	10	9
6	15	19	20	15
7	35	30	10	10

The copper content of each sample was also estimated by standard addition using a precision microburette and introducing directly into the cell 0.01 ml of a standard copper solution (0.3 mg/ml). Figs. 5 and 6 illustrate the copper wave in sample 1 before and after the addition of 3 μg of copper. Wave height on original sample is 10 divisions and after addition is 45 divisions. Hence 3 μg of copper corresponds to a wave height of 35 divisions, and

$$10 \text{ divs.} = \frac{3}{35} \times 10\ \mu g = 0.86\ \mu g$$

Weight of sample in the cell = 250 mg, thus concentration of copper

$$= \frac{0.86 \times 10^6}{250 \times 10^3} \text{ p.p.m.}$$
$$= 3 \text{ p.p.m.}$$

Standard addition cannot be applied for iron determination due to the slow reaction of iron with sucrose, to form a reducible iron complex. The iron content could be obtained from a calibration graph prepared from high purity aluminium as described above.

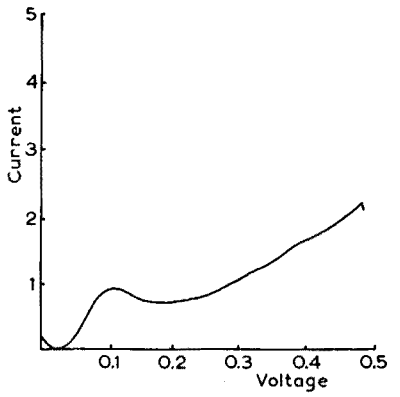

Fig. 5. Reduction wave of copper in sample 1.

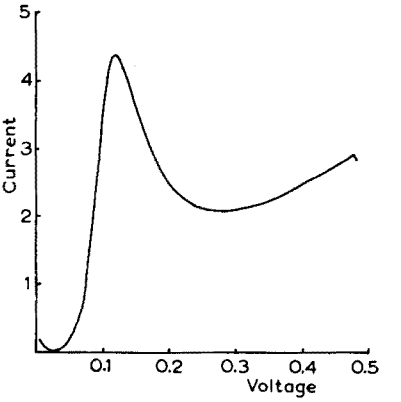

Fig. 6. Reduction wave of copper in sample 1 after the addition of 3 μg of Cu.

DISCUSSION OF RESULTS

The method is applicable only for the analysis of high purity aluminium. Its simplicity, speed and accuracy is demonstrated, and it will be of high practical value in laboratories where a large number of determinations are required. This method could not be used on conventional polarographs as the quantity of the element under consideration is very small (<50 μg). The saving of time and reagents in the analysis is enormous compared with existing chemical[7] or polarographic[2,6,7] methods.

All measurements have been carried out at a temperature of 25°, using a mercury-pool as reference electrode throughout the investigation. All potentials are given on the European sign convention. On this convention the potential of the saturated calomel electrode (S.C.E.) has been taken as $+0.246$ V *versus* the normal hydrogen electrode (N.H.E.).

ACKNOWLEDGEMENTS

The author wishes to express his thanks to the Directors of Southern Instruments Limited for permission to publish this work, and to the British Aluminium Company Limited for supplying the necessary samples.

SUMMARY

An accurate simultaneous determination of copper and iron in 99.99% aluminium can be carried out in 10–15 min, using the linear sweep polarograph. Lead can also be determined in the same solution. The basic electrolyte used is sodium hydroxide and sucrose, but the addition of ethylenediaminotetraacetic acid (EDTA) is necessary if manganese is present. The effect of EDTA on the reduction wave of copper and iron has been investigated.

RÉSUMÉ

Une méthode rapide est décrite pour le dosage polarographique du cuivre et du fer dans un aluminium très pur (> 99.99%). Le plomb peut être dosé dans la même solution. En présence de manganèse, l'addition d'acide éthylènediaminotétracétique (EDTA) est nécessaire. L'influence de ce dernier (EDTA) sur la réduction du cuivre et du fer a été examinée.

ZUSAMMENFASSUNG

Es wird eine Schnell-methode beschrieben zur polarographischen Bestimmung von Kupfer und Eisen in Aluminium von 99.99% Reinheit. Blei kann ebenfalls in der gleichen Lösung bestimmt werden. Die Gegenwart von Mangan erfordert einen Zusatz von Aethylendiaminotetraessigsäure (EDTA). Der Einfluss des EDTA Zusatzes auf die Reduktion des Kupfers und Eisens wurde untersucht.

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SPECTROCHEMICAL DETERMINATION OF LITHIUM, SODIUM, POTASSIUM AND RUBIDIUM IN ROCKS AND MINERALS USING THE STALLWOOD JET

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(Received August 29th, 1959)

The STALLWOOD jet[1] has been used extensively during the last five years at McMaster University for analysis of silicate rocks and minerals. Two methods developed for some major, minor and trace-elements have already been described[2,3], but in neither of these was it feasible to determine the alkali metals, especially lithium and rubidium.

* Present address: École Nationale Supérieure de Géologie, Nancy (France).

Experiments were therefore carried out to develop a method suitable for lithium, sodium, potassium and rubidium in their usual concentration ranges. The yellow-infrared spectral region affords the most sensitive lines. Thallium and caesium were tried as internal standards, and the latter was chosen because of more satisfactory lines and sensitivity.

The spectrograph was a Jarrel-Ash Co. 21-foot grating instrument, Wadsworth mount, using the first-order spectrum with dispersion of 5.2 A/mm. The optical train was standard and comprised a spherical lens, a diaphragm of 1-cm aperture serving as a secondary source, and a pair of crossed cylindrical lenses. Further details of the method are given in Table I.

TABLE I

SPECTROCHEMICAL PROCEDURE

Slit	11 mm long; 30 μ wide.
Arc-gap	5 mm
Intensity reduction	Rotating step-sector with log intensity-ratio 0.2 adjacent to the slit.
Filter	Corning Glass Co. No. 3486 (yellow), to absorb UV.
Voltage	230 V on open-circuit.
Current	5 A
Gas-flow	4 l air/min
Exposure	100 sec
Electrodes	Lower (anode), 1/8 inch graphite rod with plain crater 1.5 by 6 mm; upper, 1/8 inch rod: both of National Carbon Co. "Special" Grade.
Emulsion and range	Eastman-Kodak Type I-N, 5750–8250 Å.
Processing	4 min in D-19 developer at 20°, stop-bath, acid fix and wash.
Sample	2 parts mineral, 1 part Cs_2CO_3, 1 part graphite.

Line transmission was measured with an Applied Research Laboratories densitometer and plate calibration curves were constructed in the usual manner. Calculations of the ratio of log intensity analysis line to internal standard line (corrected for background) were made on a calculating-board[4]. Samples were analysed in triplicate, usually with one spectrum on each of three pairs of plates.

Working-curves were established by arcing standards. For lithium and rubidium these were artificial, prepared from Johnson-Matthey "Specpure" chemicals, using a matrix close to granite in composition. For sodium and potassium both artificial standards and National Bureau of Standards clays and feldspars were used. The materials were mixed by hand in agate mortars and some trouble was experienced with the caesium carbonate, which is hygroscopic and must be weighed and mixed as soon as possible after removing it from the dessicator, in which it should be stored.

TABLE II

SPECTRAL LINES AND PRECISION DATA

Element	*A*	*E* %	*Determined*		*Recommended*	
			G–1	*W–1*	*G–1*	*W–1*
Li	6707.844	5.1	26 p.p.m.	12 p.p.m.	21 p.p.m.	13 p.p.m.
Li	6103.642					
Na	8183.270					
Na	6160.760	9.1	3.27%		3.25%	
K	7698.979					
K	6938.980	4.6	7.4 %		5.42%	
Rb	7800.227	5.2	283 p.p.m.	24 p.p.m.	214–254 p.p.m.	21–29 p.p.m.
Cs	6983.488		Internal standard			

Spectral lines are listed in Table II. All are free of line interference of the kind expected in normal silicate minerals, but Li 6103 and Na 6160 have troublesome interference from a calcium oxide band spectrum in Ca-rich samples such as W-1. The Cs line is rather diffuse but can be measured satisfactorily over a wide range of transmission values. K 7698 shows self-absorption at concentrations above 0.4% K_2O and K 6938 could only be measured down to 1.7%: samples falling within the gap must be diluted and measured on K 7698, or must have potassium added so that they can be measured on K 6938. Wherever possible the standard rocks G-1 and W-1 were analysed (usually 12 times) and the results are included in Table II, along with representative values from the literature: the latter include lithium and rubidium[5], and sodium and potassium[6].

The working-curves were computed statistically following a method which has been described elsewhere[7]. This procedure also gives a value for the standard deviation for triplicate analysis, which is divided by the amount present and converted to per cent to obtain the relative error E, also given in Table II. E was not evaluated for several of the lines used, because few of the samples to be analysed fell in the ranges in which they could be used, but by visual inspection appears to be from 5–10%.

The analyses of G-1 and W-1 are satisfactory except for Rb in G-1, which is somewhat high, and for K 6938 which gave the high value of 7.4 % K_2O for G-1. The disagreement is serious and the working-curve for K 6938 needs re-calibration.

Owing to the one hundred per cent dilution of the sample and the buffering action of both diluents, combined with the features of the STALLWOOD jet, matrix effects are at a minimum and the method appears suitable for a wide variety of silicates.

ACKNOWLEDGEMENTS

The authors wish to acknowledge financial assistance from the National Research Council of Canada and the Geological Survey of Canada.

SUMMARY

A D.C. spectrochemical method using the STALLWOOD jet has been developed for the determination of Li, Na, K and Rb in silicate minerals and rocks. Samples are mixed with graphite and Cs_2CO_3 in the ratio 2 : 1 : 1, and Cs serves as internal standard. Precision and accuracy is satisfactory.

RÉSUMÉ

Une méthode spectrochimique est proposée pour le dosage de Li, Na, K et Rb dans des minéraux et des roches silicatées. On mélange les échantillons à analyser, en poudre, avec du graphite et du carbonate de césium (Cs étant utilisé comme étalon interne).

ZUSAMMENFASSUNG

Es wird eine spektrochemische Methode beschrieben zur Bestimmung von Li, Na, K und Rb in Mineralien und Silikatgesteinen, unter Verwendung des „STALLWOOD jet". Die zu analysierende Substanz wird mit Graphit und Cäsiumcarbonat vermischt, wobei das Cäsium als interner Standardsubstanz dient.

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DETERMINATION OF LYSINE IN PROTEIN HYDROLYZATES USING LYSINE DECARBOXYLASE FROM *SALMONELLA HADAR*

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(Received August 13th, 1959)

Chemical, microbiological and enzymatic methods for the estimation of lysine in proteins have been described[1-3]. The chemical methods are rather cumbersome, while the bacteriological methods are time-consuming. The enzymatic assay involves the use of lysine decarboxylase in acetone–ether-dried preparations from *Bacterium cadaveris*[4-6]. The arginine decarboxylase which is also contained in these preparations is destroyed by keeping them at 0° for several days. Checking the preparations for arginine decarboxylase activity before use has been recommended[5].

Our studies on amino acid decarboxylases of *Salmonella hadar* revealed that when this microorganism was grown under suitable conditions, its cell-free extracts contained only lysine decarboxylase. A method for the determination of lysine in protein hydrolyzates based on these findings is described in the present paper.

EXPERIMENTAL

Growth of bacteria and preparation of cell-free extracts

Salmonella hadar, strain 1281[7]*, was grown in the following medium: trypticase soy broth, 1%; NaCl, 0.5%; Na_2HPO_4, 0.25%; NaH_2PO_4, 0.25%; Bovril, 0.3%; L-lysine monohydrochloride, 1%**. The pH was adjusted to 6.2–6.5.

Three successive passages of *Salmonella hadar* were made in tubes containing 5 ml of the medium. The tubes were incubated for 24 h at 25° under a layer of liquid parraffin[8]. After the third passage, the bacteria were inoculated into Erlenmeyer flasks containing 100 ml of the medium and were incubated for 24 h at 25° under liquid paraffin. The cells were centrifuged, washed twice and resuspended in 15 ml of distilled water. For the preparation of cell-free extracts, 15 ml of such suspensions were held in a 9 kc Raytheon sonic oscillator for 60 min. Bacterial debris was removed by centrifugation at 18,000 g for 15 min and the supernates were kept at 3–5°.

Protein hydrolysis

One gram of protein was heated under reflux with 50 ml of 6 *N* hydrochloric acid in a stirred oil bath at 120–125° for 35 h. The hydrolyzates were concentrated in vacuo at 50°, some water was added and the solution was again evaporated in vacuo, this procedure being repeated three times in all. The residue was dissolved in a

* Obtained from the Central Laboratories, The Ministry of Health, Jerusalem.

** If lysine is omitted from the medium, the cell-free extracts contain arginine decarboxylase in addition to lysine decarboxylase.

small amount of 0.1 *M* citrate buffer, pH 5, the pH was adjusted to 5 by addition of NaOH and the solution was diluted to 25–100 ml by addition of 0.1 *M* citrate buffer, pH 5.

Determination of lysine

The lysine content of the hydrolyzates was estimated in a Warburg apparatus at 37°. The main compartment of the vessels contained 2 ml of hydrolyzate or 1 ml of hydrolyzate and 1 ml of citrate buffer, pH 5. The side arm contained 0.5 ml of cell-free extract. Maximum output of CO_2 was obtained after about 45 min. Control experiments with KOH in the center well showed that no oxygen uptake occurred.

TABLE I

LYSINE CONTENT OF PROTEINS (PER CENT)[a]

Protein[b]	*Found in 3 separate hydrolyzates*	*Average*	*Previous findings*[c]
Human serum albumin	11.7; 11.6; 11.8	11.7	11.5–13.7
Casein	9.3; 9.4; 9.7	9.5	7.0– 9.8
Edestin	2.7; 2.3; 2.8	2.6	1.7– 2.8
Zein	0.07; 0.06; 0.07	0.07	0 – 0.2
Human hair	2.7; 2.8; 2.5	2.7	1.9– 4.2

[a] The values found are given for the dry protein. Moisture was estimated by drying the proteins in vacuo over phosphorus pentoxide at 100°.

[b] Human serum albumin was kindly supplied by the Blood Fractionation and Plasma Drying Institute, Tel-Aviv-Jaffa. Casein, edestin and zein were purchased from the Nutritional Biochemicals Corporation, Cleveland, Ohio. Human hair was obtained from a barber's shop.

[c] Figures taken from BLOCK AND WEISS[2] (pp. 252; 266; 278; 308; 316).

RESULTS

The results obtained with 5 proteins are given in Table I. They are well within the range of values previously obtained by other methods.

Preliminary experiments with solutions of pure L-lysine gave the expected theoretical values, while the following amino acids were not decarboxylated: glycine, L-alanine, L-serine, DL-valine, L-leucine, DL-isoleucine, L-cysteine, L-cystine, L-threonine, L-methionine, L-glutamic acid, L-aspartic acid, L-tyrosine, L-phenylalanine, L-tryptophan, L-histidine, L-proline, L-hydroxyproline, L-arginine, DL-citrulline, and DL-ornithine.

Additional evidence for the accuracy of the method was given by the following experiment. A part of the hydrolyzates of 2 proteins (human hair and human serum albumin) was run through a Decalso column which adsorbs only arginine and lysine[9]. The 2 amino acids were subsequently eluted. The lysine values for the 2 proteins, as estimated in the eluates, were 2.7 and 12.0%. The corresponding values found in the untreated parts of the hydrolyzates were 2.5 and 11.6%.

SUMMARY

Lysine may be determined in protein hydrolyzates with the aid of lysine decarboxylase contained in cell-free extracts of *Salmonella hadar* grown under suitable conditions.

RÉSUMÉ

La lysine peut être dosée dans des hydrolysats de protéines à l'aide de la lysine-décarboxylase, contenue dans des extraits de *Salmonella hadar*, cultivés dans des conditions appropriées.

ZUSAMMENFASSUNG

Lysin kann in Proteinhydrolysaten mit Hilfe einer Lysin-decarboxylase bestimmt werden, die in Extrakten von geeignet gezüchteten *Salmonella hadar*-Bakterien enthalten ist.

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REVUES DE LIVRE

Les réactions électrochimiques (Les méthodes électrochimiques d'analyse) par G. CHARLOT, MME. J. BADOZ-LAMBLING et B. TRÉMILLON, Masson et Cie, édit. Paris 1959, 396 p., 407 figures, Broché 6.000 fr., Cartonné toile 6.800 fr.

Le Professeur CHARLOT vient de faire paraître un ouvrage qui aura — nous en sommes certains — un très grand retentissement parmi les analystes. Il nous avait déjà donné, dans le même genre, il y a quelques années: ,,L'analyse des réactions en solution''; on sait que de nombreux chercheurs se sont inspirés de ce livre pour leurs travaux. Cette fois, le Professeur CHARLOT et ses collaborateurs ont choisi comme thème les réactions électrochimiques et les méthodes d'électroanalyse, sujet combien redoutable pour ceux qui sont chargés de cet enseignement. Mais leur tâche sera désormais grandement facilitée s'ils veulent se donner la peine d'étudier la substance de cet ouvrage. Les chimistes d'autre part pourront acquérir des bases solides et véritablement générales qui leur permettront de résoudre sans difficulté la plupart des problèmes qui se poseront à eux. En effet, les plus grandes difficultés que l'on rencontre dans ce domaine proviennent du manque de coordination entre les différents chapitres qui le constituent, ce qui empêche d'en avoir une vue d'ensemble. Cette lacune vient d'être comblée. Quelques esprits chagrins reprocheront peut-être aux auteurs un excès de concision, mais avec un peu d'habitude, ce fait n'enlève rien à la clarté de l'exposé.

Citons les chapitres les plus importants de cet ouvrage: les réactions électrochimiques qualitatives et quantitatives et les courbes potentiel–courant au cours des réactions chimiques, le cas des réactions rapides et celui des réactions lentes, l'influence des facteurs physiques sur les phénomènes électrochimiques, la détermination expérimentale des courbes intensité–potentiel. Puis viennent les applications à la potentiométrie, à l'ampérométrie, à la coulométrie. Un chapitre spécial est consacré aux méthodes électrochimiques les plus récentes telles que la chronopotentiométrie, la chronoampérométrie, les diverses méthodes polarographiques oscillographiques et alternatives et la polarographie par redissolution anodique. Enfin, dans un dernier chapitre intitulé: ,,solvants variés'', les auteurs examinent le comportement des propriétés électrochimiques dans divers milieux et appliquent ces considérations aux méthodes physicochimiques d'analyse.

Dans ce livre, tout est neuf et on ne sait ce qu'on doit le plus admirer, la manière toute nouvelle de concevoir le problème ou la façon remarquable de présenter l'exposé. Des considérations générales, découlent le principe et l'interprétation de toutes les méthodes d'électroanalyse. Le Professeur CHARLOT et ses collaborateurs ont fait un travail considérable. On ne saurait trop recommander ce livre à tous ceux qui, de près ou de loin, s'intéressent à cette discipline.

D. MONNIER (Genève)

Anorganische qualitative Mikroanalyse, von HANS MALISSA, Düsseldorf und A. A. BENEDETTI-PICHLER, New York. Mit 55 Textabbildungen. VIII, 333 Seiten, (Monographien aus dem Gebiete der qualitativen Mikroanalyse) Herausgegeben von A. A. BENEDETTI-PICHLER, New York 1959, Ganzleinen $ 11,65.

Ainsi que M. BENEDETTI-PICHLER l'annonce dans sa préface, ce livre inaugure une nouvelle série de monographies, destinée aux chimistes analystes et dont les trois premiers volumes seront consacrés à la microanalyse qualitative en général.

L'ouvrage que nous avons en mains concerne plus spécialement l'analyse minérale. Le suivant traitera de la microanalyse organique, tandis-que le troisième s'intitulera: ,,*Manipulateurs mécaniques en analyse chimique. Modes opératoires à l'échelle du microgramme*".

Le but principal de ces livres est de donner aux lecteurs une vue d'ensemble des microméthodes actuellement utilisées, en développant suffisamment les détails des modes opératoires proposés, ce qui — selon l'auteur — n'est pas le cas des nombreux traités existant déjà.

Sans pouvoir partager entièrement cet avis, nous tenons néanmoins à saluer ici cette initiative, car nous estimons que toute oeuvre de récapitulation est fort utile de nos jours, en raison du développement prodigieux de la science.

Une brève analyse du contenu permettra à nos lecteurs d'apprécier les nombreuses qualités de ce livre.

L'introduction comprend un aperçu historique succint, les définitions actuelles de la sensibilité, spécificité et sélectivité des réactions, ainsi que quelques recommandations concernant la pureté des réactifs et le choix des ustensiles.

Le second chapitre, intitulé ,,Méthodes générales de travail", traite successivement toutes les réactions effectuées au cours d'une analyse qualitative, sans en omettre les moins usuelles. Le lecteur trouvera suffisamment de détails pour qu'il puisse les appliquer sans trop de difficultés.

Le troisième et le quatrième chapitre sont tout spécialement consacrés à la technique des réactions d'identification. Le problème est examiné d'une manière que nous n'hésitons pas à qualifier de magistrale, dans ses généralités et dans ses détails. Les tables des sensibilités des réactions, telles qu'elles ont été dressées par WENGER, DUCKERT ET RUSCONI, sont complétées par quelques réactions plus récentes. Suit, à la fin, une longue partie décrivant minutieusement les réactions d'identification principales de chaque élément.

L'ouvrage se termine par un grand chapitre comprenant l'exécution d'une analyse qualitative. Après une énumération des essais préliminaires les plus variés, les auteurs exposent deux schémas d'analyse (l'un pour les cations, l'autre pour les anions) basés sur les modes opératoires de NOYES ET BRAY. Ici encore les possibilités d'application de la méthode sont discutées sur une base scientifique, incitant l'analyste à travailler d'une façon intelligente et éclairée et non pas à effectuer des opérations de ,,cuisine" en se basant aveuglement sur des ,,recettes" sacrées.

Le chapitre se termine avec quelques prescriptions relatives à l'analyse des aciers, des peintures (tableaux), des verres, des glaçures, des objets en céramique et des silicates. Quelques pages, enfin, sont encore consacrées à l'analyse des poussières et à la recherche des éléments dans les produits organiques.

Bien que la réputation des auteurs se passe de tout commentaire, nous tenons à faire ici l'éloge de cet excellent ouvrage, dans lequel la théorie s'allie à la pratique d'une manière originale, propre à M. BENEDETTI-PICHLER, et nous nous faisons un plaisir de reconnaître que ce livre jette une nouvelle lumière dans le domaine de la microanalyse. Persuadé du succès qu'il emportera, nous souhaitons tout simplement que les volumes suivants de cette série soient à la même hauteur.

I. KAPÉTANIDIS (Genève)

Communication

Mr. W. J. PARKER has resigned his position as Secretary of the Polarographic Society. Mr. J. H. GLOVER has been co-opted as Secretary and all communications to the Society including subscriptions should be sent direct to him at:

75, Craven Gardens, Wimbledon, S.W. 19. Great Britain.

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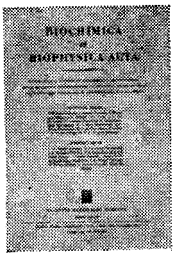

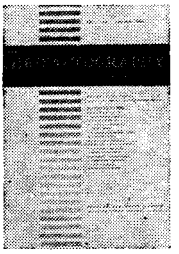

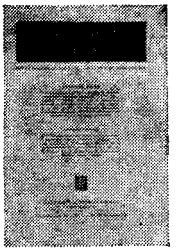

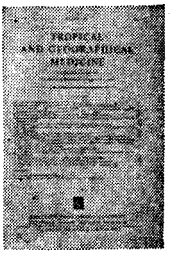

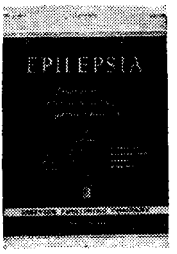

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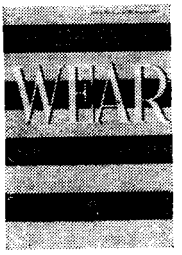

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