

DETERMINATION OF SMALL AMOUNTS OF FLUORIDE
BY THE FORMATION OF TERNARY COMPLEXES

by

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(i)

ABSTRACT

Four anions have been determined by spectrophotometry.

The determination is based upon an entirely new type of reaction, which has been called "kinetochromic" because the four anions here determined have the common characteristic of producing, under certain conditions, an extraordinary enhancement in the speed of the formation of a complex between Xylenol Orange and Zirconium (IV), but no formation of any new ternary complexes takes place as the spectra show.

The method which is highly sensitive, gives apparent molecular extinction coefficients of 200,000 for fluoride, 400,000 for phosphate, 344,000 for arsenate and 22,000 for sulphate and is virtually free from the interferences of the most common cations.

ACKNOWLEDGEMENTS

The work recorded in this thesis was carried out in the Chemistry Department of the Imperial College of Science and Technology between October, 1964, and September, 1966. It is entirely original except where due reference is made and no part of the work has been previously submitted for any other degree.

I would like to express my gratitude to my supervisor, Professor T.S. West, for his unfailing assistance, and ever-encouraging guidance and advice.

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(iii)

FOREWORD

It is a well known fact that the determination of traces of elements is a necessity of today's chemistry. This is the reason why the use of new techniques is so important.

Although fortunately some new and rather sensitive methods have been recently developed for the determination of fluoride and phosphate, the determination of sulphate ion is a case of a very important anion to determine and in which field no colorimetric or spectrophotometric methods have appeared in the literature.

Therefore, it can be said that there is still much to be done on the determination of anions and that the need of further research in this field is a very acute one.

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INTRODUCTION

The present work has been undertaken to develop a spectrophotometric method for the determination of traces of fluoride ion.

The determination of small amounts of fluoride presents difficulties because the two classical techniques of analytical chemistry, gravimetric and titrimetric, are not very suitable for applications on the micro or submicro-scale.

More recently, colorimetric or spectrophotometric methods have been used, but most of them are based on the bleaching effect of the fluoride ion on coloured metal complexes. These have the disadvantage of suffering from many interferences of different ions; besides which we can consider these methods as negative ones¹, since they make use of a quenching effect, instead of the development of a colour.

In 1958, Belcher, Leonard and West² described a completely new method for the determination of fluoride, based on the fact that alizarin-complexan (1,2-dihydroxy-anthraquinon-3-ylmethylaniline-NN-diacetic acid), forms a red chelate with cerium (III), which in the presence of fluoride ion gives a new blue complex.

Therefore, we can classify the most widely used methods for the determination of fluoride as follows:

- 1) Gravimetric
- 2) Titrimetric
- 3) Colorimetric and spectrophotometric.

Let us review, briefly, the main advantages and disadvantages of each group.

GRAVIMETRIC METHODS

Forchhammer³ determined the fluoride ion by precipitating it as calcium fluoride. Sulphuric acid was added to the precipitate and the extent to which the liberated hydrochloric acid etched glass was used as a measure of the amount actually present. Carles⁴ used the same method but he precipitated the fluoride as barium fluoride instead of calcium fluoride.

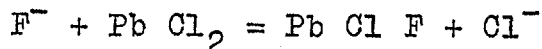
Carnot⁵ evaporated large quantities of water to which calcium hydroxide had been added. After washing the calcium fluoride precipitate free from soluble salts and treating it with acetic acid to eliminate calcium carbonate, silica and sulphuric acid were added. The liberated silicon tetrafluoride was passed through a solution of a potassium salt and the potassium fluoro-silicate was precipitated and afterwards weighed.

Gautier and Claussmann⁶ precipitated the fluoride as barium fluoride by the addition of barium chloride; barium sulphate would precipitate too, in the determination of fluoride in waters, retaining a portion of the fluoride, but by an elaborate treatment of the whole precipitate in special apparatus, lead fluoride was eventually obtained and converted to colloidal lead sulphide, which was measured colorimetrically against known standards. The amount of fluoride present was deduced from the quantity of lead sulphide.

Some other authors^{7,8} have determined the fluorine in soluble fluorides by precipitation as thorium fluoride. They ignited the precipitate to thorium oxide under suitable conditions and calculated the amount of fluoride present.

Investigation of these methods showed that a relatively large portion of the fluorides was not precipitated because it was found in the filtrate.

More recently, fluoride has been determined as lead chlorofluoride,⁹ using lead chloride as precipitant. The reaction is:



This method has been modified by Belcher and Tatlow,¹⁰

giving much better results. These authors have found that the optimum pH range for the precipitation is between 3.6-5.6,¹¹ and it can be obtained by adding the requisite amount of acetic acid to the neutral fluoride solution.

The lead chlorofluoride is precipitated by adding the hot fluoride solution to the hot lead chloride solution, and allowing it to stand overnight although satisfactory results may be obtained after standing for only four hours. Drying is effected at a temperature of 110°C, but lead chlorofluoride can be heated up to a temperature of 538°C without suffering any change.

But in all cases the solubility of the compounds is too great for the convenient application of any of these methods on the micro or submicro-scale. It is, therefore, preferable to use a titrimetric method in spite of its inherent difficulties.

TITRIMETRIC METHODS

The classical titrimetric method for the determination of fluoride was given by Willard and Winter.¹² They described it for titrating soluble fluorides and silico-fluoride solutions with standard thorium nitrate using a zirconium-sodium alizarin sulphonate lake solution as indicator.

The bleaching effect of the fluoride on the zirconium-alizarin mixture is well known. When a very dilute solution of fluoride is added to a small amount of the lake the colour fades very slowly until the end-point of the formation of a zirconium-fluoride complex is reached. The lake then decolorizes quite suddenly. This suggested to the authors the possibility of using the lake as an indicator to show when the precipitation of the fluoride is completed. Using this lake as indicator, when all the fluoride to be determined is present in the beaker, the lake does not give any colour, because of the bleaching effect of the fluoride. If in these conditions the standard thorium nitrate solution is added slowly, the fluoride precipitates because thorium fluoride has been formed and therefore the zirconium-fluoride complex starts to break. When all the fluoride has been precipitated the end-point of the titration has been reached and it can be seen by a permanent reappearance of the violet-red colour of the zirconium-alizarin lake. Since thorium fluoride is quite insoluble in ethyl alcohol the titration is carried out in a 50% ethyl alcohol solution which allows more accurate results.

When interfering elements are present in the soluble fluoride solutions, the fluoride must be separated from

these other elements before the titrations can be made. This may be done by volatilizing it as hydrofluorosilicic acid by adding perchloric or sulphuric acid, water and several pieces of glass to the sample in a distillation flask and heating to distil. When only small amounts of fluoride are present in the sample and the temperature is not allowed to rise above approximately 125°C the pieces of glass appear to supply the silica necessary to combine with the fluoride to form hydrofluorosilicic acid, and there is no noticeable etching of the flask. If more fluoride is present or the temperature is allowed to rise higher, silica is taken from the flask, and etching is noticeable. In either case, however, the fluorine is recovered quantitatively.

Insoluble fluorides which can be decomposed by perchloric acid at the concentration used in the above procedure may be placed in the distillation flask and the fluoride determined as described.

This method gives better results than the previous one of volatilization as silicon tetrafluoride used by Reynolds and Jacob¹³ and others, and also better than the improved one given by Casares,¹⁴ but in spite of this, Willard and Winter's method has been modified by Gilkey, Rohs and Hansen,¹⁵ and by Huckabay, Welch and Metler,¹⁶

to give better and simpler control of the distillation temperature and thus permit more accurate determinations.

More recently, several other authors have suggested improvements to Willard and Winter's method. For instance, Armstrong¹⁷ determined fluoride by titration with thorium nitrate in aqueous solution using sodium alizarin sulphonate, which gives a pink lake with an excess of titrant as indicator. The use of aqueous solution rather than the alcoholic one is claimed to give better results because the equivalence of the thorium nitrate solution for the fluoride over the range 0.5 to 10 μ g. is exact, a situation which does not hold when alcoholic solutions are employed.

Other modifications have been described for the determination of fluoride in wines, waters, etc. Thus a sharper end-point was obtained using Armstrong's method, but adjusting carefully the acidity of the solutions prior to the titration with thorium nitrate,¹⁸ or by a back-titration (compensation) method.

The back-titration (compensation) method is normally used for determining less than 0.02 mg. of fluoride per 50 ml. of titration medium.¹⁹ An aliquot of the sample is added to a Nessler tube, adjusted to a definite acidity with hydrochloric acid, and titrated with standard thorium nitrate to the first definite pink colour, using alizarin-

sulphonate as indicator. A blank aliquot of water is treated with the same volume of thorium nitrate and titrated with standard sodium fluoride until the lake colour matches that of the sample. The degree of acidity is very important in the back-titration, because at pH greater than 3.5,²⁰ there may be errors due to the red colour of free alizarin and at pH less than 3.5 the thorium lake does not show its full colour. Therefore, the thorium must be standardized with fluoride at exactly the same pH as that at which the titration is made.

The back-titration method has been applied successfully by some authors²¹ for determination of fluoride in foods and biological materials.

However, zirconium-alizarin lake or sodium alizarin sulphonate are not the only indicators used in titrimetric methods for the determination of fluoride. Milton and coworkers²² employed Solochrome Brilliant Blue B.S. as indicator. Their method is based upon titration of the fluoride solution with thorium nitrate until excess is shown by the formation of a lake with Solochrome Brilliant Blue B.S. (sodium salt of sulpho-dichlorohydroxydimethyl-fuscin dicarboxylic acid).

The method has the following advantages over the existing alizarin technique:

a) The end-point is more definite; the colour change is sharp from pink to blue, and easily judged, and therefore allows greater sensitivity.

b) The end-point is immediate.

c) The colour does not fade.

A systematic study was made by Willard and Horton³³ of colorimetric and fluorimetric indicators for the titration of fluoride with thorium nitrate. From the results of these studies the best two-colour indicators in visible light in order of decreasing effectiveness appear to be:

- 1) Purpurin sulphonate
- 2) Alizarin Red S
- 3) Eriochromcyanine R
- 4) 2,3-Dicyanoquinizarin
- 5) Chrome Azurol S.

The best one-colour visual indicators are:

- 1) Quercetin
- 2) Morin.

The best fluorescent indicators are:

- 1) Morin
- 2) Quercetin
- 3) Ferron.

Although thorium nitrate has been the most studied reagent for titrimetric methods for the determination of fluoride, other reagents have been used, e.g. cerous nitrate.²⁴ Also Kolthoff,²⁵ gave a procedure for the titration of fluoride using zirconium oxychloride as reagent and purpurin as indicator, which was accurate to $\pm 2\%$. Another of the reagents used for titrating fluoride was aluminium chloride. This titration depends on the formation, in nearly neutral solution, of the stable complex ion AlF_6^{-3} , the sodium salt of which is only slightly soluble in the presence of an excess of sodium salt or in alcoholic solution.

Published methods propose conductometric titrations in alcoholic solution with aluminium chloride,²⁶ titration in aqueous solution with basic aluminium chloride to a phenolphthalein end-point,²⁷ and titration in neutral (phenolphthalein) aqueous solution to a methyl red end-point.²⁸ Geyer,²⁹ who reviewed most of the methods for determining fluorides found that this last one gave the best results. However, Saylor and Larkin³⁰ have described a better method, using titration of fluoride in aqueous solution with aluminium chloride using Eriochromocyanine R as an internal indicator. A solution of this dye is yellow-orange at pH 5.4-6.0, yellow at higher pH and

red-orange at lower pH. The colour also varies with the time of standing of the solution. For using it as an indicator, it is necessary to adjust the pH so that the Eriochromcyanine is yellow before the addition of the aluminium solution.

Nevertheless in titrating small amounts of fluoride as suggested by all the above authors, it is difficult to determine the end-point of the titration with sufficient precision. Much, as it can be seen, depends on the individual analyst's colour perception and, therefore, these methods do not give good inter-laboratory precision.

COLORIMETRIC AND SPECTROPHOTOMETRIC METHODS

Until recently, methods for the determination of traces of fluoride were almost all based on the ability of the ion to destroy coloured organic complexes of certain metals such as zirconium, thorium, titanium, aluminium or iron, through the formation of the very stable fluoride complex of the metal. The decrease in colour of the metal complex was a measure of the fluoride concentration.

Steiger's method³¹ is based on the powerful bleaching effect that the fluoride ion has on the yellow colour produced by the treatment of a titanium solution with hydrogen peroxide. A solution of definite volume is

prepared containing the fluoride to be estimated, also having a known amount of titanium present; another one is prepared with the same amount of titanium present but without any fluoride. The two solutions should be of the same depth of colour because they contain the same amount of titanium per milliliter, but the one having the fluoride will be found to be of a lighter shade, owing to the bleaching effect which that element has on a solution of titanium complexed by hydrogen peroxide. The bleached solution can be compared in a colorimeter with a second one containing an equivalent amount of titanium per milliliter and the bleaching effect recorded. The extent of this bleaching is not directly proportional to the amount of fluoride present, but it is possible to obtain a curve, and the quantity corresponding to a given bleaching can be found.

The zirconium-alizarin method, so often used in titrimetry, seemed adequate for colorimetry. Zirconium nitrate and sodium alizarin sulphonate react to form a brilliant reddish violet lake which is stable in acid solution. De Boer has shown that a number of metallic salts form a lake with this particular dye but only two, zirconium and hafnium, are stable in the presence of hydrochloric acid. The stable reddish violet colour

produced by zirconium nitrate and sodium alizarin sulphonate in acid solution, however, is destroyed by the addition of fluoride, and the pale yellow colour of the alizarin sulphonate reappears. These conditions may be created where various amounts of fluoride will react with the same amount of the lake under identical conditions and cause the formation of a colour series ranging from a red to a yellow.

The method is carried out under the following conditions: the samples to be determined are transferred to Nessler tubes; an exact amount of the indicator is added to each sample, well mixed, and compared with standards in one hour, or better still, after both samples and standards have stood overnight. Comparisons are conveniently made in a three-hole colorimeter in which each sample is compared with the two closest standards.

The application of the method to the determination of fluoride in sea waters suffers from several disadvantages, such as the marked slowness of the reaction and the effect of various salt concentrations upon the indicator; for instance, sulphate ion bleaches also the organic complexes owing to the formation of stable complex sulphates $[\text{ZrO}(\text{SO}_4)_2]^{-2}$.³² But the interference of the sulphate can be overcome,^{33,34} by carrying out the reaction in the

presence of a great excess of sulphuric acid which produces a more stable reagent,³⁵ but with considerable loss in sensitivity.

Because fluoride also destroys the colour of the aluminium lake of Eriochromcyanine, this makes a good reagent for the colorimetric determination of the fluoride.³⁶ The comparisons in colour are made in a colorimeter ten or fifteen minutes after mixing. As the reaction is affected by pH, all very alkaline samples should be made slightly acid with a few drops of dilute hydrochloric acid.

The rate of colour decrease varies with the temperature, therefore, to obtain accurate results it is necessary that the sample, standard solutions and reagent are at the same temperature (room temperature). As the amount of fluoride increases, the colour changes from a red-purple to red, cherry-red, peach-pink and orange. In the lower range colour differences corresponding to 0.05 p.p.m. can easily be distinguished. In the upper range the colour changes continue to occur when more than 6 p.p.m. of fluoride are present, but the deep orange colours are not easily distinguished. Because the colour change is rapid in the beginning, a time interval is required for the contents of the tubes to which the reagent was added to reach the colour of the first tube.

Ferron (7-iodo-8-hydroxyquinoline-5-sulphonic acid) was described by Yoe,³⁷ as a colorimetric reagent for ferric iron. When using this compound as a reagent for fluoride,³⁸ the green colour of the ferron-iron complex, assumes a yellowish hue when fluoride is present. Matching this colour with a solution of known fluoride **content makes possible** a measurement of the amount of fluoride in the sample.

The proportions of ferron, ferric chloride and hydrochloric acid must be controlled in order to have a stable fluoride reagent, because when the iron content is too low, the green colour is too weak, and on the contrary, when more iron than necessary is in solution the colour is darker than desired. Hydrochloric acid has been found to be the best of the three acids tried in the reagent; sulphuric acid causes a partial discharge of the colour and nitric acid a complete decolorization. But if the concentration of hydrochloric acid is increased beyond the desired point, the colour becomes paler, whereas a muddy green results from a deficiency of the acid. Therefore the composition of the ferron-iron reagent used was as follows:

Saturated water solution of ferron..... 90 ml.

Water solution 2N to HCl and 0.1N to
FeCl₃..... 10 ml.

Distilled water.....100 ml.

More recently, very similar methods have been applied, but using a spectrophotometer instead of a colorimeter. The colorimetric reagents investigated were iron thiocyanate,^{38,39} titanium-hydrogen peroxide,^{31,40,41} zirconium-alizarin sulphonate,^{23,42,43,44} and thorium-alizarin sulphonate.

The zirconium-alizarin sulphonate method was supposed to offer the best possibilities for the spectrophotometric determination of small amounts of fluoride. During the investigation of the various published zirconium-alizarin sulphonate reagents, it was observed³⁵ that as the ratio of alizarin sulphonate to zirconium was increased, the final colour developed in the solution was intensified. In selecting the composition of the reagent, the following factors were considered:

1. Intensity of the colour developed by the reagent.
2. Concentration of fluoride ion to be determined.
3. Sensitivity of the reagent to slight changes in the acidity of the sample.

After consideration of these factors the reagent chosen was:

Zirconium oxychloride.....	0.30 g.
Alizarin sodium sulphonate.....	0.35 g.
Sulphuric acid (concentrated).....	40 ml.

Hydrochloric acid (concentrated)..... 120 ml.

Diluted to 1000 ml. with distilled water.

Several attempts have been made, more recently, to improve the method, either by extracting the liberated alizarin reagent into pentanol,⁴⁵ or into iso-butanol containing hydroxylamine hydrochloride.⁴⁶

Furthermore, zirconium-alizarin lakes usually give colours too faint for good spectrophotometric measurements and the red and yellow colour mixture produced by the zirconium-alizarin reagent is different for each fluoride content.

Thus, Icken and Blank⁴⁷ proposed a method based on the principle of the bleaching effect that the fluoride ions have on the thorium-alizarin lake, but replacing the visual detection of the end-point in the titrimetric methods, by the spectrophotometer, which permits reproducible estimation of fluoride in much lower concentrations.

The colour reagent was prepared in the following way:

Hydrochloric acid 0.1N..... 10 ml.

Hydroxylamine hydrochloride 1% aqueous
solution..... 10 ml.

Alizarin sodium monosulphonate 0.1%..... 40 ml.

Thorium nitrate tetrahydrate
[Th(NO₃)₄·4H₂O] 0.05%..... 40 ml.

This colour reagent must be freshly prepared.

The components are added in the sequence listed.

The hydroxylamine is included in order to discharge traces of fluorine which might be found in the distilled samples due to decomposition of the perchloric acid used in distillation.⁴⁸ The amount of hydrochloric acid is chosen so as to give a pH of 2.75 in the final solution. Dahle and coworkers⁴⁹ found that at this pH small changes do not influence the titre of the thorium nitrate solution and hence they do not affect the intensity of the colour.

The same authors, Ioken and Blank, modified the composition of the colour reagent for determinations with a photoelectric colorimeter. They claim that the colorimetric procedure allows accurate determination of fluoride in lower concentrations.

Other reagents such as zirconium-Eriochrome cyanin R lake,^{50,51,52} Chrome Azurol S,⁵³ ferric thiocyanate,⁵⁴ etc. have also been studied.

However, in all these methods, Beer's law is frequently not obeyed, owing to the diversity of complex fluorides which can coexist in the solution and usually they are subject to interferences by many anions.

Fortunately, studies in another field revealed a new reaction of the fluoride ion. Alizarin complexan

(1,2-dihydroxy-antraquinon-3-ylmethylaniline-NN-diacetic acid),⁵⁵ forms a red chelate with cerium (III); in an acetate buffer the red colour of the cerium (III)-alizarin complexan changes to the lilac-blue colour of the ternary complex² on addition of fluoride. As we can see this is the first reaction in which the fluoride ion itself produces a new coloured compound.

The fluoride test is carried out in an acetate buffer at pH 4.3, because at this pH, alizarin complexan, which behaves as an acid-base indicator, begins its transition from yellow to red. In these conditions the lilac-blue colour of the complex may be compared with the scarlet-red of the alizarin complexan-cerrous chelate in a spectrophotometer at a wavelength of 610 mμ, which was seen to be the most suitable for the measurement of the fluoride complex.

The order of addition of reagents is important in that a much quicker colour response is obtained if the alizarin complexan and the cerium (III) ion are added separately to the fluoride ion, rather than as a composite solution of the cerium (III) chelate.

The calibration curve is linear over the range 8 to 35 μg. of fluoride, but its projection does not pass through the origin.

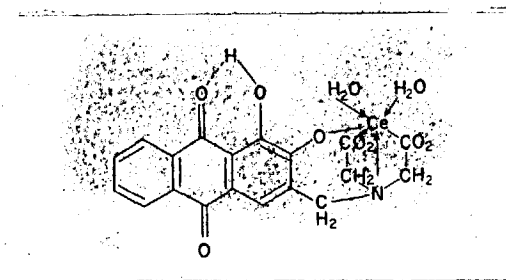
The test is free from interferences of the most common anions and, within the range 15-50 $\mu\text{g.}$, an absolute accuracy of $\pm 0.5\%$ can be achieved. This method has been applied to the determination of fluoride in the analysis of some organic compounds,⁵⁶ and the results obtained were very satisfactory.

More recently, the nature of the metal chelate has been investigated,⁵⁷ and from the results of their experiments the authors concluded:

1. The chelates formed by alizarin complexan are considerably more stable than those formed by alizarin itself, owing to the more powerfully chelating complexan group in the substituted reagent.

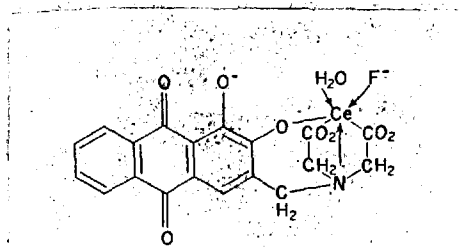
2. Most of the cations that react with alizarin complexan are hexaco-ordinate, but a few can be only tetraco-ordinate.

3. The cerium (III) alizarin complexan chelate has the following structure:



In the same paper, the authors studied the nature of the ternary complex, applying Job's method of continuous variations⁵⁸ between the 1:1 cerium (III) alizarin complexan chelate and the fluoride ion, and it was concluded that a 1:1 complex is formed. A large excess of fluoride not only breaks down any existing ternary complex, but also causes precipitation of cerium (III) as its insoluble fluoride, so that such a system shows only the yellow colour of the metal-free reagent.

From these results they concluded that the fluoride ion displaces one of the co-ordinated water molecules remaining on the cerium (III) atom; also the entry of the fluoride ion within the co-ordination sphere of the cerium (III) ion causes the deprotonation of the remaining 1-hydroxyl group. Therefore, the structure of the ternary complex is:



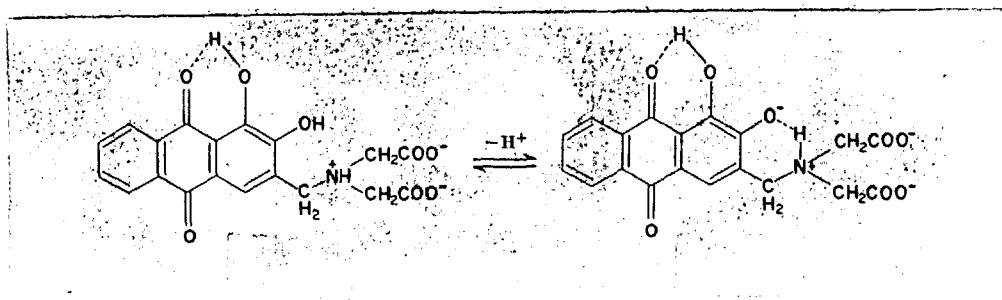
As there is no steric factor involved, the specificity of the fluoride action is due to the unusually strong electrophilic properties of this atom.

Studies have also been made of the effect of pH and time of development of the colour and also into the mechanism of the cerium (III)-alizarin complexan-fluoride reaction at the optimum pH.⁵⁹

These experiments, extending over the pH range 3.5-12, indicated that the highest sensitivity of the fluoride reaction, measured against the cerium (III) chelate, occurs at pH 5.0-5.2. At pH 3.5, both the cerium (III) chelate and the fluoride complex are largely dissociated, as is shown by the presence in the spectrum of the free alizarin complexan of an absorption band which disappears at pH 4.0, whilst over the range pH 6-12 a change of wavelength of maximum absorption is observed. All the investigations show clearly that the method should not be carried out beyond the range pH 4-6 and that the optimum sensitivity occurs at pH 5.0-5.2. At this pH, the alizarin complexan exists as a mixture of the two ionisation states a) and b):

a)

b)



The fact that at pH 6 there is a change of wavelength of maximum absorption made these workers think that the hydrolysis of the ternary fluoride complex is occurring with replacement of the fluoride ion by the hydroxyl ion.

Attempts have been made to enlarge the range of determination of fluoride. In earlier papers the authors recommended amounts of $5 \cdot 10^{-4} M$ solutions of cerium (III) nitrate and alizarin complexan. But they saw⁵⁹ that using $5 \cdot 10^{-3} M$ solutions of cerium (III) nitrate and alizarin complexan, and a standard fluoride solution containing 50 $\mu g/ml$. calibration curves were obtained up to 500 μg . obeying Beer's law from 50-275 μg ., and when larger amounts of cerium (III) and reagent were used, a linear calibration curve was obtained over the range 100-550 μg .

Some more experiments were carried out with $10^{-2}M$ solutions of cerium (III) and reagent at pH 5.2. The wavelength of maximum absorption for the fluoride complex measured against the cerium (III) chelate changed to 630 mμ and the calibration curve followed the same pattern, being linear up to 800 μg.

As it can be seen the pH and permissible concentration ranges are inter-related, because whilst the method operated satisfactorily at pH 4.3 with $5.10^{-4}M$ solutions, precipitation occurs at this pH with $5.10^{-3}M$ solutions. At pH 5.0-5.2 this difficulty is not encountered even with $10^{-2}M$ solutions.

The minimum reaction time employed throughout these studies was 60-90 minutes though qualitatively the reaction appeared to be quicker in the more concentrated solutions.

Considering the practical applicability of the cerium (III) alizarin complexan method for the determination of fluoride, it has been shown⁶⁰ that two ratios of cerium (III) to alizarin complexan can be used. A 1:1 mole ratio gives high tolerance for common anions that do not complex cerium (III), as well as for many cations. A 1:2.5 ratio gives increasing tolerance for complexing anions such as sulphate and phosphate.

Use of 20% acetonitrile or acetone medium rather than water alone increases both the sensitivity of the reaction and the stability of the complexes.

It has been suggested⁶¹ that lanthanum-reagent complex has advantages over the cerium (III) complex in sensitivity. A narrower pH range was then studied⁶² for both lanthanum and cerium (III) chelates with the alizarin complexan. Repeated experiments revealed that at pH 4.3 and 610 mμ, the cerium (III) reagent was slightly more sensitive than the reaction based on lanthanum. At pH 4.5 and 610 mμ the difference in sensitivity between the two reagents is very small while at pH 5.1 and 620 mμ the lanthanum reagent is 4% more sensitive than the cerium (III) one.

An enhancement of the sensitivity was obtained for both reagents at pH 4.3 by addition of acetone to 25% v/v, but the most sensitive means of determination is to use the lanthanum reagent in aqueous solution at pH 5.2 with measurements at 281 mμ. This procedure is 200% more sensitive than the standard method at 620 mμ.

Other authors²⁵ studied exhaustively the behaviour of the fluoride ion with most of the lanthanides, and found that lanthanum, cerium, praseodymium, neodymium

and samarium, formed the fluoride complex (the sensitivity to fluoride falling with increasing atomic number), and terbium, erbium, ytterbium, scandium and yttrium failed to do so. The intermediate element europium and gadolinium formed extremely weakly coloured fluoride complexes. Elements 61, 66, 67, 69 and 71 were not examined. This proves once more that the lanthanum based system gives the most sensitive method for the determination of fluoride known at present.

CHAPTER ONE

DETERMINATION OF FLUORIDE ION BY THE FORMATION OF TERNARY COMPLEXES BETWEEN SEVERAL NN+DICARBOXY-METHYLAMINOMETHYL DERIVATIVES OF SOME HYDROXYANTHRAQUINONES, A METAL AND THE FLUORIDE ION

Owing to the difficulties that the accurate determination of fluoride ion has usually involved and the successful results obtained by the formation of a ternary complex between an organic reagent (Alizarin Complexan), a metal (lanthanum or cerium) and the fluoride,² it was thought that better results (higher sensitivity), might perhaps be obtained using another NN-di(carboxy-methyl)aminomethyl derivatives of some hydroxyanthraquinones, instead of the Alizarin Complexan.

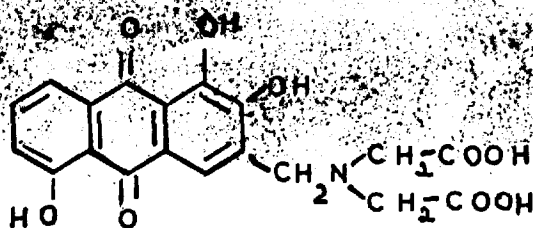
The NN-di(carboxy-methyl)aminomethyl derivatives used were the ones prepared by Belcher, Leonard and West,⁵⁵ by Mannich-type condensation of hydroxyanthraquinones with formaldehyde and iminodiacetic acid. Although an excess of the aldehyde and aminoacid was used, a monosubstituted product was always obtained. All the products were analysed after the condensation in order to know their degree of purity and the results were very satisfactory.

The free acids are usually yellow or orange-brown powders, which in dilute solution are yellow or yellow-pink below pH 4.5, red between pH 4.5-7 and violet at higher pH. This behaviour shows clearly that all these compounds can be used as complexometric indicators, and some experiments have shown that 1,2-dihydroxyanthraquinone-3-ylmethylamine-NN-diacetic acid gives, under certain conditions, better results for the titration of Pb^{2+} , Zn^{2+} and Co^{2+} than Xylenol Orange, which was the best complexometric indicator hitherto proposed for these titrations.

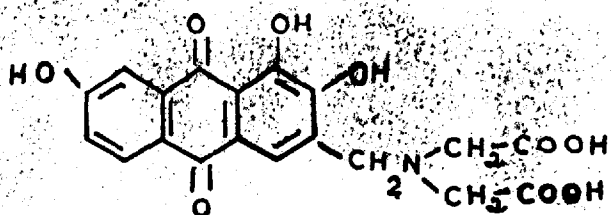
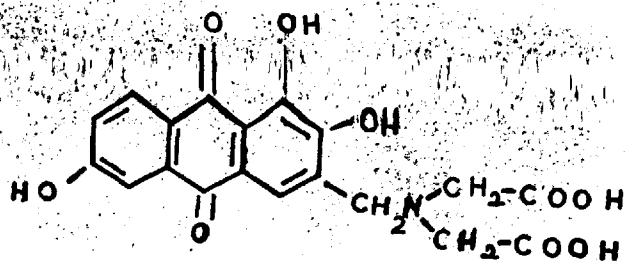
In the present work, everyone of these compounds has been used as an organic reagent to form a ternary complex with a metal (lanthanum and cerium are the experimented ones) and the fluoride ion, whose spectrum has been run with the spectrum of the correlative binary complex formed by the organic compound and the metal in order to compare their sensitivity for the determination of fluoride with the similar and earlier technique in which the Alizarin Complexan was used.

The NN-di(carboxymethyl)aminomethyl derivatives of the hydroxyanthraquinones investigated are:

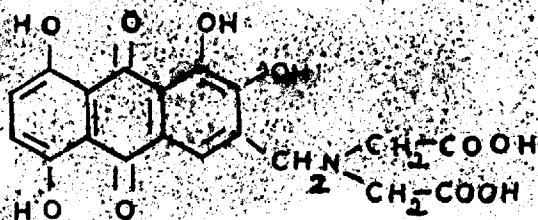
1. Alizarin Brilliant Bordeaux Complexan



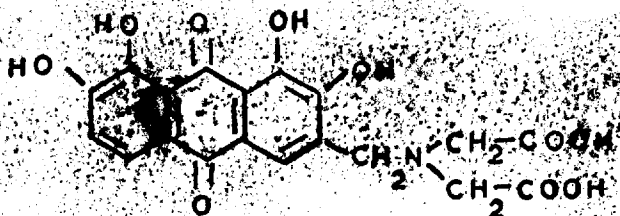
2. Flavopurpurin Complexan



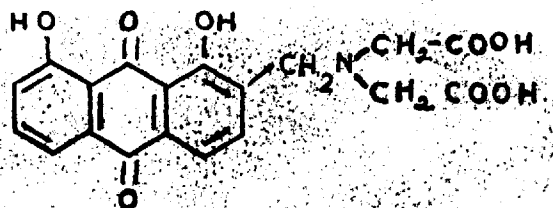
4. Quinizarin Complexan



5. Dihydroxy Chrysazin Complexan



6. Chrysazin Complexan



In all cases the concentration of the solutions used was $5 \cdot 10^{-4} M.$, and for every reagent the procedure followed was:

1. a) Spectrum of the reagent at pH 4.3
b) Spectrum of the reagent + cerium (III) at pH 4.3
c) Spectrum of the reagent + cerium (III) + fluoride
pH 4.3
2. Repeat of a), b), c) at pH 5.2
3. Repeat of a), b), c) at pH 4.3 but using lanthanum (III) as metal instead of cerium (III)
4. Repeat of a), b), c) at pH 5.2, using La(III) instead of Ce(III).

EXPERIMENTAL

Apparatus. Beckman DB recording spectrophotometer with 1 cm. cuvettes ~~operated~~.

Reagents.

$5 \cdot 10^{-4} M$ Alizarin Brilliant Bordeaux Complexan.

Suspend 200.5 mg. of Alizarin Brilliant Bordeaux Complexan (A.B.B.C.) in distilled water and add the minimum amount of 2N NaOH solution to assure complete solution of solid. Add HCl diluted, carefully till pH 5.0-6.0. Dilute with distilled water ~~to~~ 1 liter.

$5.10^{-4}M$ Flavopurpurin Complexan. Suspend 200.5 mg. of Flavopurpurin Complexan (F.P.C.) in distilled water and operate as above.

$5.10^{-4}M$ Anthrapurpurin Complexan. Suspend 200.5 mg. of Anthrapurpurin Complexan (A.P.C.) in distilled water and operate as above.

$5.10^{-4}M$ Quinalizarin Complexan. Suspend 208.5 mg. of Quinalizarin Complexan (Q.A.C.) in distilled water and operate as above.

$5.10^{-4}M$ Dihydroxy Chrysazin Complexan. Suspend 208.5 mg. of Dihydroxy Chrysazin Complexan (D.C.C.) in distilled water and operate as above.

$5.10^{-4}M$ Chrysazin Complexan. Suspend 192.5 mg. of Chrysazin Complexan (C.C.) in distilled water and operate as above.

$5.10^{-4}M$ Ce(III) nitrate and $5.10^{-4}M$ La(III) nitrate solutions. Stocks solutions of pure cerium (III) nitrate and lanthanum (III) nitrate were standardised against 0.02M EDTA at pH 5.2 using Xylenol Orange as indicator, and an aliquot was diluted to provide exactly $5.10^{-4}M$ reagents.

PH 5.2 Buffer. Dissolve 100 g. of AnalaR sodium acetate trihydrate in distilled water and add 11 ml. of glacial acetic acid. Dilute to 1 liter.

PH 4.3 Buffer. Dissolve 105 g. of AnalaR sodium acetate trihydrate in distilled water and add 100 ml. of glacial acetic acid. Dilute to 1 liter.

5.10^{-4} M sodium fluoride solution. A stock 5.10^{-3} M solution was prepared ^{by} dissolving 210 mg. of AnalaR sodium fluoride and diluting to 1 liter. 100 ML. of this solution were taken and diluted to exactly 1 liter with distilled water.

Procedure

In every case three spectra were run. A first one of the reagent chosen at a fixed pH. A second one of the binary complex formed by the reagent and the metal and a third one of the ternary complex formed between the organic reagent, the metal and the fluoride.

a) Pipette 5 ml. of $5 \cdot 10^{-4}M$ organic reagent into a 50-ml. standard flask, add 2 ml. of buffer solution (pH 4.3) and dilute the content of the flask to 50 ml. with distilled water.

b) Pipette 5 ml. of $5 \cdot 10^{-4}M$ organic reagent into a 50 ml. standard flask and 2 ml. of buffer solution (pH 4.3), followed by 5 ml. of $5 \cdot 10^{-4}M$ cerium (III) solution and dilute the contents of the flask to 50 ml. with distilled water.

c) Pipette 5 ml. of $5 \cdot 10^{-4}M$ organic reagent into a 50 ml. standard flask and 2 ml. of buffer solution (pH 4.3) followed by 5 ml. of $5 \cdot 10^{-4}M$ cerium (III) solution, and 5 ml. of $5 \cdot 10^{-4}M$ sodium fluoride solution; dilute the contents of the flask to 50 ml. with distilled water.

Set the solution aside for one and a half hours and run the spectra of the three solutions between 700 m μ and 220 m μ against distilled water in 1 cm. cuvettes.

Procedure B. Repeat exactly procedure A, but using in a), b), c) 2 ml. of buffer solution (pH 5.2) instead of 2 ml. of buffer solution (pH 4.3).

Procedure C. Repeat exactly procedure A, but pipetting in b) and c) 5 ml. of 5.10^{-4} M lanthanum (III) nitrate solution instead of cerium (III) nitrate solution.

Procedure D. Repeat exactly procedure C but using in a), b), c) 2 ml. of buffer solution (pH 5.2) instead of 2 ml. of buffer solution (pH 4.3).

When the spectra had to be obtained in U.V. light, the concentration of all reagents was reduced to ~~their~~ half, due to the fact that the recorder of the Beckman D.B. spectrophotometer does not work for optical densities higher than 1.0

RESULTS AND DISCUSSION

In every case a yellow colour was given by the solution of the reagents, a lilac-blue colour by the binary complexes and a scarlet-red colour was given by the ternary complexes where the latter were obtained.

The A.B.B.C. gave the highest sensitivity of all the reagents investigated especially when cerium (III) was used as chelating metal. pH 4.3 and 620 mμ would be the

optimum conditions for the determination of fluoride in the visible range as can be seen in Fig.1. Slightly lower sensitivity was obtained at pH 5.2 and 640 mμ. In ultraviolet light, the best results were obtained when using cerium (III) as chelating metal at pH 5.2 and 275 mμ (Fig.2). This system gave the highest sensitivity for the determination of fluoride in all the derivatives of hydroxyanthraquinones investigated. But in all instances the sensitivity was lower than that obtained with alizarin complexan.

Only with the first three organic reagents investigated, A.B.B.C., F.P.C., and A.P.C., were spectra similar to the A.C. ones obtained although in all three cases the sensitivity was lower than with A.C. and decreased from the A.B.B.C. to the A.P.C.

When Q.A.C. was used as organic reagent a slight precipitate appeared for the ternary complexes of both cerium (III) and lanthanum (III) at pH 4.3, but the precipitate became much larger for the ternary complexes of both metals at pH 5.2. In spite of these precipitates the spectra could be obtained normally.

When using D.C.C. as organic reagent a big precipitate was obtained for the ternary complexes of both cerium (III) and lanthanum (III) at both pH 4.3 and 5.2, and it was not possible to run any spectra.

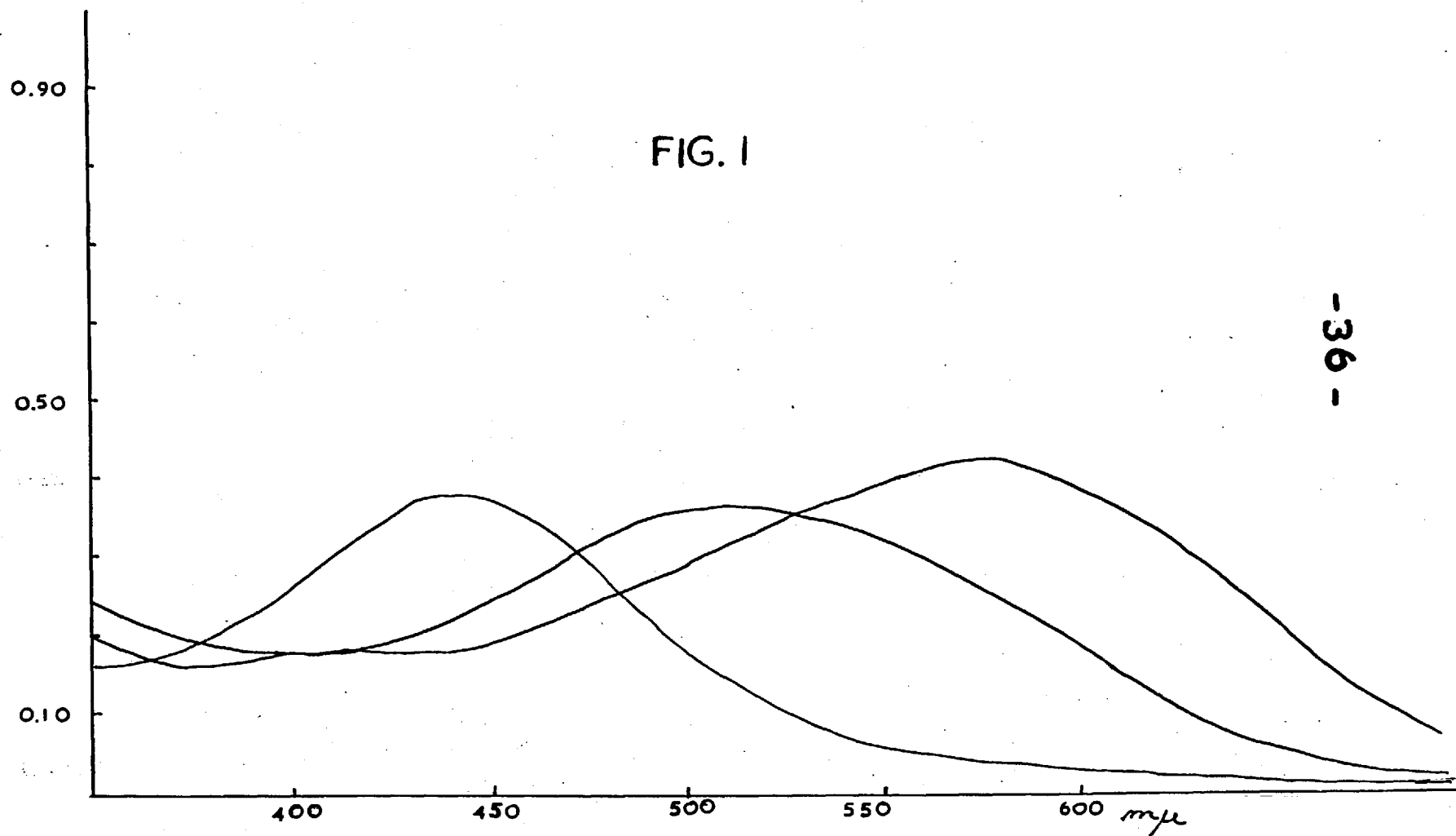
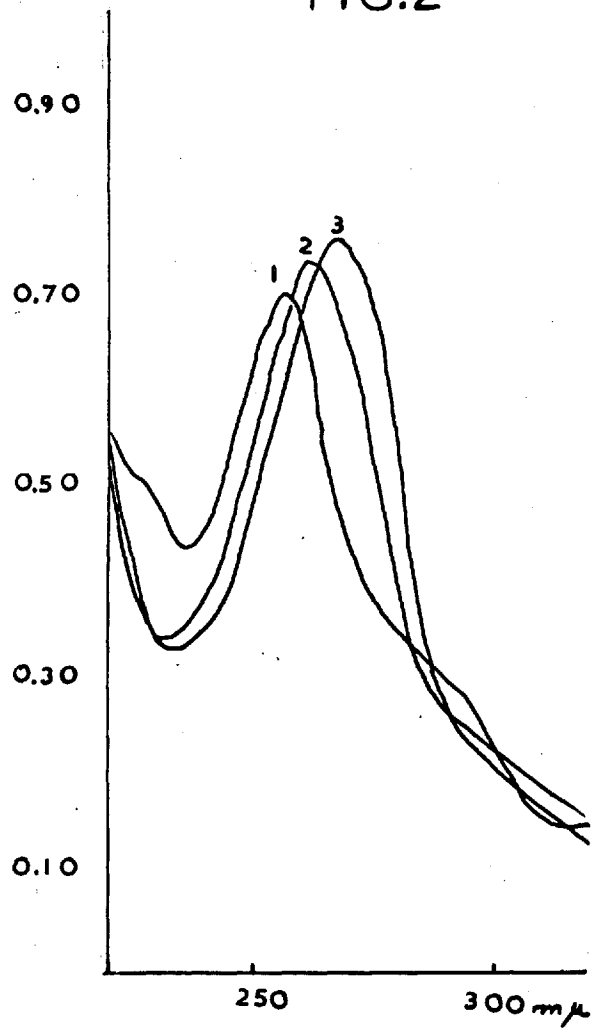


FIG.2

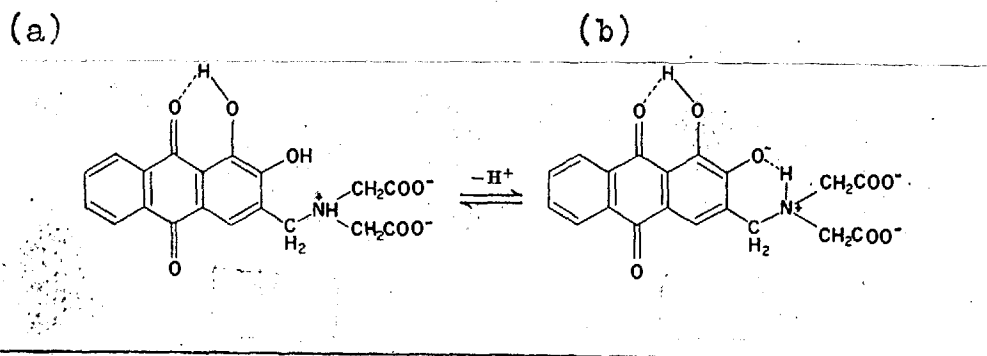


1: ABBC
2: ABBC/Ce
3: ABBC/Ce/F

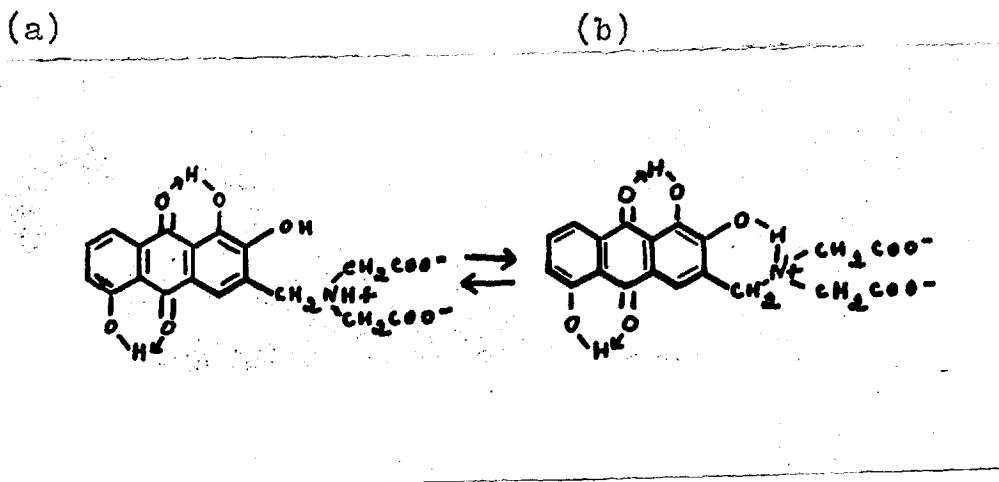
With C.C. no ternary complexes were formed in either case, because no difference between the spectra of the binary complexes and the supposed ternary ones could be observed.

The above facts show clearly the great influence that the hydroxyl groups play in the formation of binary and ternary complexes. A possible explanation of the results obtained could be as follows:

It was said previously⁵⁹ that alizarin complexan in a weakly acid solution coexists as a mixture of the two ionization stages (a) and (b)

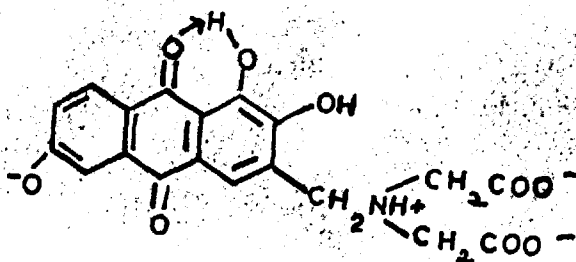


In the case of A.B.B.C. the structure of the reagent in weakly acid solution would be:



As can be seen, both reagents A.C. and A.B.B.C. are very similar and the anthraquinone group in the 5 position is remote from the reaction centre. It is not surprising that very little difference in the results has been obtained in either case.

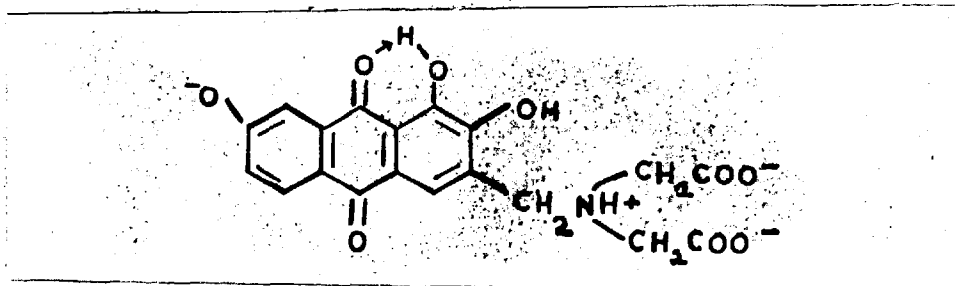
If F.P.C.'s structure is considered now:



it can be seen that there may be an electrophilic effect of the O^- of the easily ionised 6-hydroxy group. As a result the F.P.C. molecule may be richer in electrons than A.C. at pH 4.3. When the cerium (III) complex is formed the stability of the cerium (III) complex will probably be greater because of this. However, when the fluoride ion reacts with the cerium (III) complex, then the availability of electrons in the aromatic ring will be such that there will not be so great a withdrawal of electrons from the 1-9 hydroxyquinone linkage. Therefore the reaction will be poorer. All this suggests that the

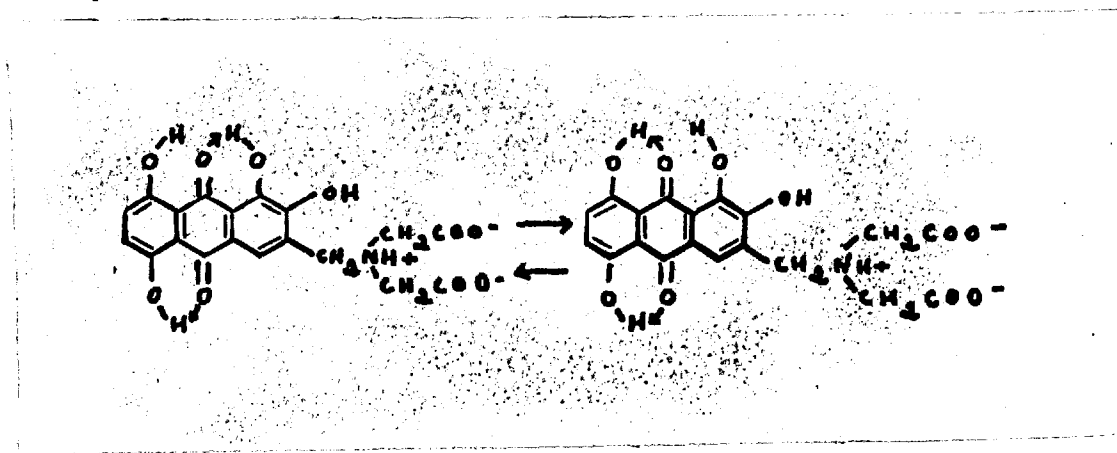
cerium (III) complex of F.P.C. will be stronger than the cerium (III) complex of A.C., and will probably form at a lower pH.

The case of A.P.C. is similar to the F.P.C. one:



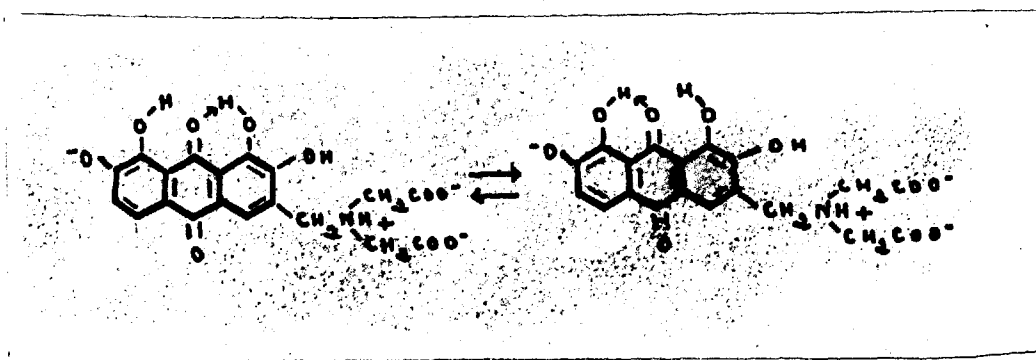
but the hydroxyl group is now in a more favourable position to influence the complexan group electrophilically. It will ionise at much the same pH as F.P.C., but the O^- will now supply even more electrons to the nitrogen atom than in F.P.C. reagent. Therefore, the cerium (III) complex will be stronger, but the poorness of the fluoride reaction is even more marked.

If Q.A.C. is considered now:



probably the fluoride effect will be observed at a lower pH than for A.C. Because the two ionisation forms may not be so widely separated as in A.C., the colour reaction might not be so deep. It is observed that the bands due to the cerium (III) complex (equivalent to the red ionised form of the indicator considered as an acid) and the ternary complex (equivalent to the blue ionised form of the indicator considered as an acid) are very close together and have considerable overlap. The reagent could be compared more closely to A.B.B.C. than to A.C.

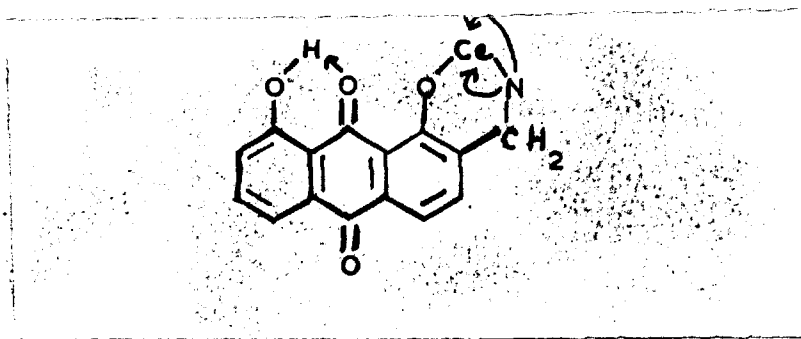
In D.C.C. there is a combination of the principles



involved in Q.A.C. and A.P.C. The position of the 7-hydroxyl

group will feed more electrons into the complexan group in the three position that in the A.B.B.C.; therefore, colour reaction will be even less marked.

Because only one hydroxyl group is adjacent to the complexan centre in the C.C., this hydroxyl will be involved in the formation of the binary complex and when fluoride ion reacts, there is no second adjacent hydroxyl group available to give a chromogenic effect, by a second ionisation.



The only hydroxyl group available is in the 8 position which is too far away. If it had been in the 9 position it might have shown some reaction.

CHAPTER TWO

DETERMINATION OF FLUORIDE ION BY XYLENOL ORANGE + ZIRCONIUM (IV) METHOD

When the present work was undertaken, the formation of a binary complex between Xylenol Orange and Zr(IV), and of a ternary complex between Xylenol Orange, Zr (IV) and fluoride ion, which could allow the spectrophotometric determination of fluoride ion, was expected.

Very serious difficulties were found in the development of the method, because of the well known hydrolysis~~isim~~ of the zirconium salts which made it difficult to find the conditions in which the results were reproducible. This early part of the work consequently occupied an inordinately long period of time, particularly since the expected ternary complex was not eventually obtained.

The best results were found with zirconyl chloride in hydrochloric acid solution, but the spectra showed that, surprisingly, there is not apparently formation of a new complex when the fluoride ion is present, but there is an extraordinary enhancement of the intensity of the colour, which allows the determination of amounts of fluoride as small as 0.5 $\mu\text{g.}$ in 100 ml. of solution, and

gives an "apparent" molecular extinction coefficient of 200,000.

As the method has been developed in rather strongly acid solution (0.35 M HCl), serious interferences from cations were not observed, although rather serious ones were found for some anions.

EXPERIMENTAL

APPARATUS. Beckman DB recording spectrophotometer with 1 cm. quartz cuvettes.

REAGENTS.

5.10⁻⁴M Xylenol Orange solution. ^{Dissolve} ~~Dilute~~ 336.3 mg. of Hopkin and Williams Xylenol Orange [Batch No. 019011] in freshly distilled water and dilute to exactly 1 liter (pH 4.8).

5.10⁻⁴M Zirconyl Chloride solution. Prepared by dilution from an accurately standardized (EDTA, 1M HNO₃, X.O., at 90°C), 0.01 M solution of Hopkins and Williams zirconyl chloride (ZrOCl₂.8H₂O) in 0.0125 M HCl which has been allowed to stand overnight before making the dilution. The 5.10⁻⁴M solution must only be used between 4 hours and five days after its preparation and should be kept in a cool place.

An improvement can be obtained if the 0.01 M zirconyl chloride solution is heated up to 50°C after its preparation, 5N HCl is added to make the solution 0.1 M to HCl and it is then cooled down immediately in order to avoid further hydrolisation. After standardisation of the 0.01M solution, a $5 \cdot 10^{-4}$ M one is made taking the exact amount of the 0.01 M solution and diluting it with distilled water until approximately 1 liter, heating it up to 40°C, adding HCl to make the solution 0.06 M to HCl and cooling it down at once.

$5 \cdot 10^{-4}$ M and $2.5 \cdot 10^{-5}$ M sodium fluoride solution. A stock $5 \cdot 10^{-3}$ M solution was prepared dissolving 210 mg. of AnalaR sodium fluoride and diluting to exactly 1 liter. 100 ml. and 5 ml. of this solution were taken and diluted to 1 liter with distilled water to make respectively $5 \cdot 10^{-4}$ M and $2.5 \cdot 10^{-5}$ M sodium fluoride solutions.

Calibration Curve. Pipette 1-12 ml. of $2.5 \cdot 10^{-5}$ M sodium fluoride solution into 100-ml. standard flasks, add 5 ml. of $5 \cdot 10^{-4}$ M Xylenol Orange and 7 ml. of 5 M HCl, followed by 10 ml. of cool (15°C) $5 \cdot 10^{-4}$ M Zr(IV) to each flask. Dilute the contents of the flasks to 100 ml. with cool (15°C) distilled water and set the solutions aside during 90 minutes in a cool place. Prepare, simultaneously

with the other solutions, a control solution containing no fluoride. Measure the optical density (0.045-0.55) of the fluoride solutions against the control solution at 550 m μ . in 1 cm. cells within 60 minutes. The curve is linear from 0.475 μ g. to 5 μ g., but falls off at higher concentrations.

ABSORPTION SPECTRA OF XYLENOL ORANGE, X.O.-ZIRCONIUM COMPLEX AND X.O.-ZIRCONIUM-FLUORIDE COMPLEX

Although the main purpose of the present work has been the determination of fluoride ion, it was necessary to know previously the nature of the complex formed by X.O. and Zr(IV), in order to develop the method in the best way. Therefore, three solutions were prepared: a 1:1 reagent-zirconium solution (5 ml. X.O. and 5 ml. Zr), a 1:2 reagent-zirconium solution (5 ml. X.O. and 10 ml. Zr) and a third one for the reagent itself (5 ml. X.O.). 7 ml. of 5 N HCl were added to each solution and they were diluted to 100 ml. with distilled water.

The spectra were run against distilled water, in 1 cm. cells, after the solution had been set aside for 90 minutes in a cool place.

From the spectra it can be seen that a 1:2 complex is formed. Although Cheng⁶³ claims the formation of a 1:1

complex, the spectra, in Fig.3, show a large excess of reagent in the 1:1 complex. Moreover, the presence of two aminocarboxy^{groups} in the X.O. molecule, makes quite understandable the formation of the 1:2 complex. Cheng reports the maximum wavelength as 535 mμ., whereas in fig.3 it is 550 mμ., but this difference may be due to the X.O., because it has been observed that different manufacturers or even different batch numbers lead to slightly different spectra.

The X.O. in acid solution is light yellow, the X.O.-Zr complex light red and its spectrum shows that the maximum absorption takes place at 550 mμ, as ~~it~~ has been said above.

To establish the nature of the suspected X.O.-Zr-fluoride complex, a 1:2:1 spectrum was run by preparing a solution containing 2.5 ml. of X.O. sol., 5 ml. of Zr(IV) sol., 2.5 ml. of $5 \cdot 10^{-4}$ M fluoride solution and 7 ml. of 5N HCl, a second one with 2.5 ml. of X.O. sol., 5 ml. of Zr(IV) sol. and 7 ml. of 5N HCl and a third one with 2.5 ml. of X.O. sol. and 7 ml. of 5N HCl. The three solutions were diluted to 100 ml. with distilled water and the spectra were run against distilled water, in 1-cm. cells, after the solutions had been set aside for ninety minutes.

FIG. 3

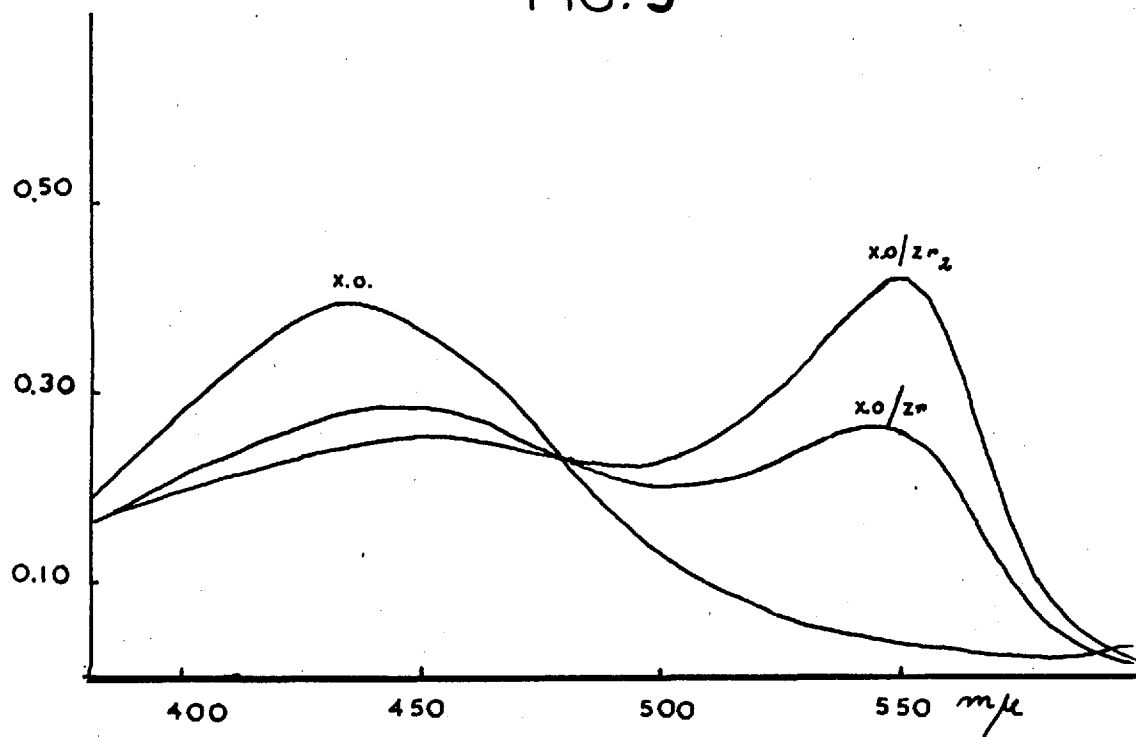
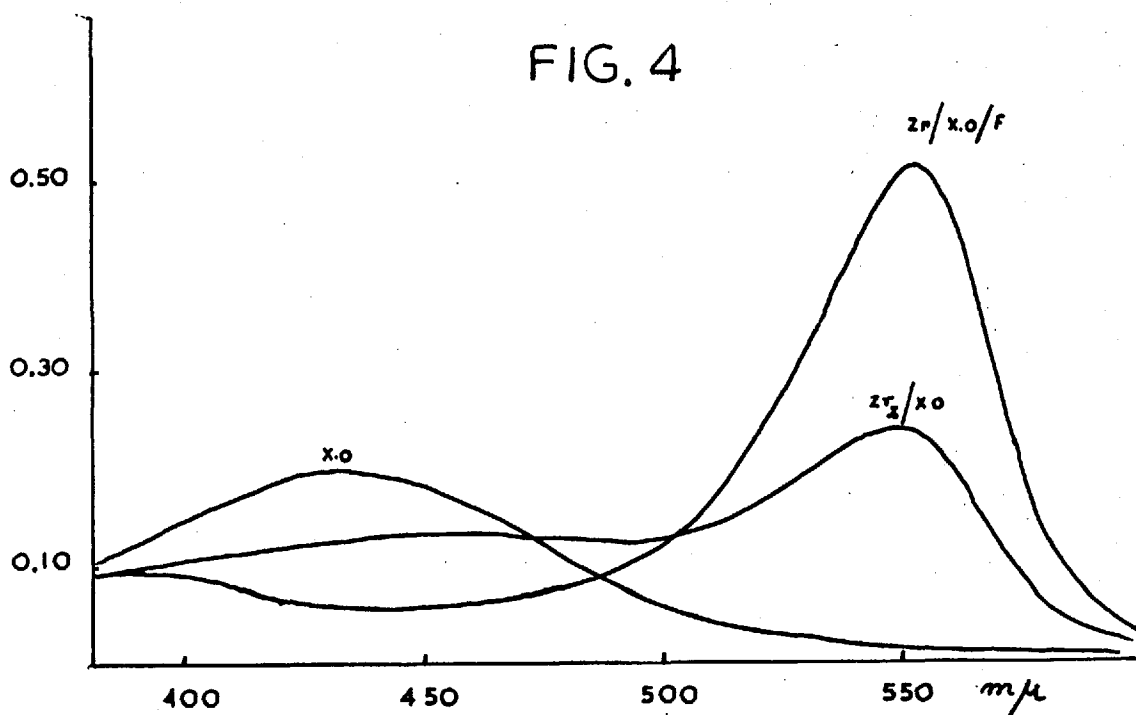


FIG. 4



It can be seen from Fig.4, that in the presence of fluoride, no formation of a new complex appears to occur, but there is a great increase in the intensity of the colour and, therefore, of the absorption. This suggests the existence of what West⁶⁴ has called a kinetochromic effect of fluoride ion on the formation of the X.O.-Zr complex, and which allows the determination of small amounts of fluoride ion by a highly sensitive method. This is dealt with under "Discussion" .

The optimum wavelength for the measurements is 550 mμ.

OPTIMUM ACIDITY FOR THE DEVELOPMENT OF THE COMPLEX

In order to find the optimum acidity for the development of the method, several solutions were prepared by transferring 5 ml. of $5 \cdot 10^{-4}M$ X.O. solution to each volumetric flask, 10 ml. of $5 \cdot 10^{-4}M$ $ZrOCl_2$ solution and 1 ml. of $2.5 \cdot 10^{-4}M$ sodium fluoride to each one of the flasks as well as different amounts of 5M HCl. The contents of the flasks were diluted to 100 ml. with distilled water and measured against a blank containing no fluoride, at 550 mμ., in 1 cm. cells after the solution had been set aside for ninety minutes.

TABLE I

<u>MOLARITY</u> <u>H to HClV of the solution</u>	<u>Optical Density</u>
0.035.....	0.095
0.070.....	0.100
0.110.....	0.110
0.140.....	0.200
0.180.....	0.300
0.220.....	0.340
0.250.....	0.400
0.280.....	0.480
0.320.....	0.480
0.360.....	0.500
0.390.....	0.490
0.450.....	0.490
0.540.....	0.460
0.720.....	0.390
0.900.....	0.310
1.100.....	0.250

The results, in Table I, show that the highest sensitivity occurs when the acidity of the final solution is in the range 0.28-0.45M ~~to~~ HCl. The value of the optical density does not change much in this range and therefore the acidity chosen to carry out the experiments has been 0.35M ⁱⁿ ~~to~~ HCl.

STABILITY OF THE COMPLEX

As a consequence of the kinetochromic action of the fluoride ion on the formation of the X.O.-Zr(IV) complex the increment in the optical density between X.O.-Zr(IV)-F system and the binary complex is not stable for a long time, because the depth of the colour of the X.O.-Zr(IV) complex increases with time and after several hours reaches the colour of the X.O.-Zr(IV)-F ^{SYSTEM} ~~complex~~.

In order to find out the range of time in which the readings can be made with accuracy and reproducibility, a solution was prepared by pipetting into a 100 ml. flask, 1 ml. of $2.5 \cdot 10^{-4}$ M sodium fluoride solution followed by 5 ml. of X.O., 7 ml. of 5M HCl. and 10 ml. of Zr(IV) solution. The contents of the flask were diluted to 100 ml. with cool distilled water and the optical density of the solution was measured over a period of time against a blank prepared at the same time and containing no fluoride, at 550 mμ. and in 1 cm. cells.

TABLE II

EFFECT OF THE TIME UPON THE STABILITY OF THE COMPLEX

<u>TIME</u>	<u>OPTICAL DENSITY</u>
0 minutes.....	0.000
5 minutes.....	0.100
15 minutes.....	0.140
30 minutes.....	0.250
45 minutes.....	0.340
60 minutes.....	0.395
75 minutes.....	0.440
90 minutes.....	0.495
105 minutes.....	0.490
120 minutes.....	0.500
135 minutes.....	0.500
150 minutes.....	0.500
180 minutes.....	0.420

The results, in Table II, show that the highest reading can be obtained ninety minutes after the preparation of the solutions and that the value of the optical density against the blank remains constant during 1 hour. After a period of $2\frac{1}{2}$ hours following the preparation of the solutions the value of the readings starts to decrease.

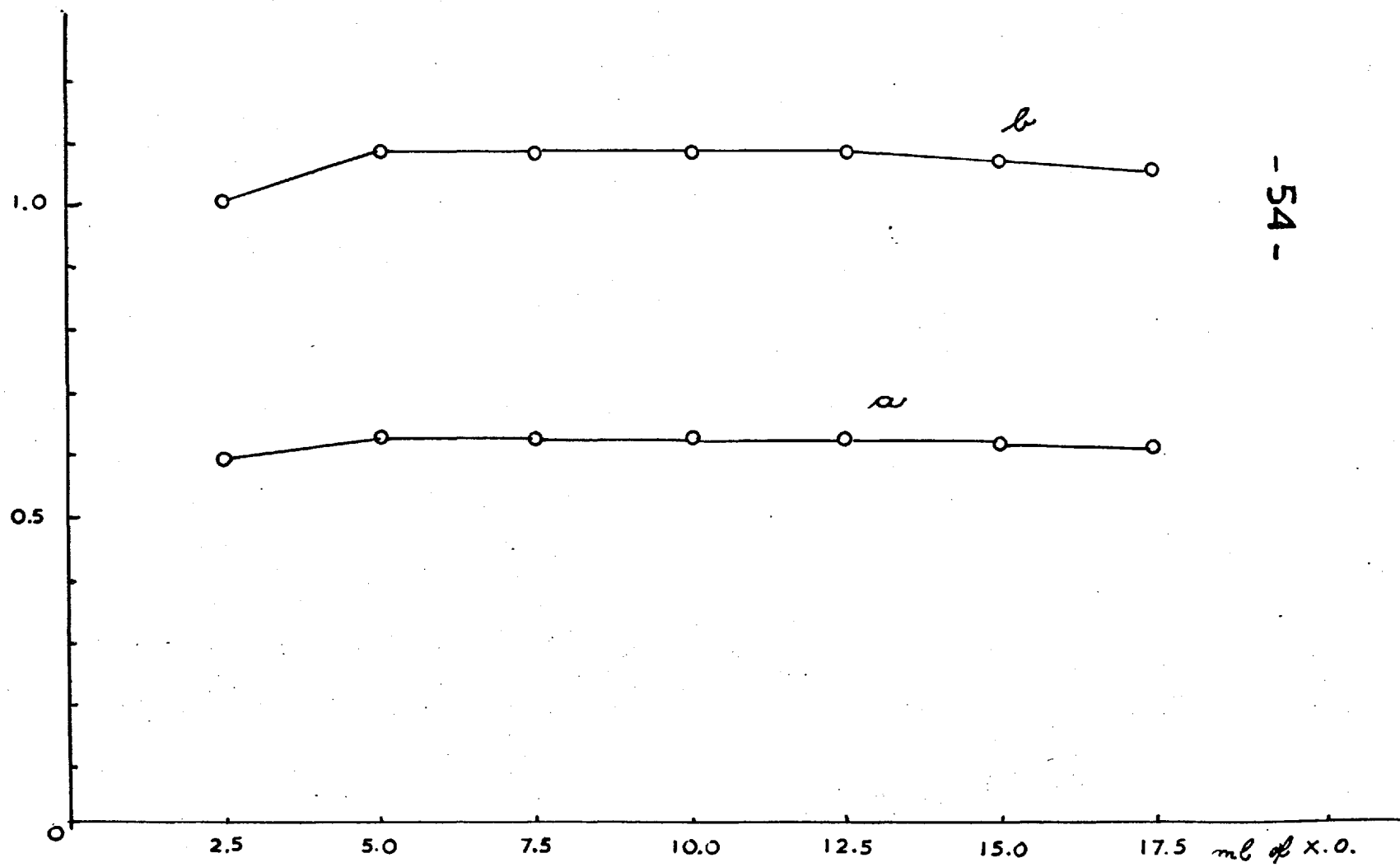
EFFECT OF THE REAGENT CONCENTRATION

Two experiments were carried out to investigate the influence of the reagent concentration on the development of the colour of the system.

A first set of solutions was prepared by pipetting into 100-ml. flasks, 1 ml. of $2.5 \cdot 10^{-4}M$ sodium fluoride solution, followed by 5 ml. of Zr(IV) sol., 7 ml. of 5N HCl and by 2.5, 5 or 7.5.....ml. of X.O. sol. The contents of the flasks were diluted to 100 ml. with distilled water. A second set of solutions was prepared in a similar way, but in this case the amount of Zr(IV) solution added was 10 ml. All the solutions were set aside for ninety minutes in a cool place and then measured against distilled water in 1-cm. cells.

The results of the first experiment are shown in the curve a in Fig.5, the results of the second in curve b of the same figure and as it can be seen, the value of the

FIG. 5



optical density is much higher in the second experiment, but in both cases the optical density starts to be constant when the amount of X.O. is 5 ml., remains constant for higher concentrations and decreases very slightly when a great excess of reagent is present.

THE NATURE OF THE COMPLEX

To confirm the composition of the binary complex X.O.-Zr(IV), the method of continuous variations was next applied, and as Fig.6 shows, X.O. and Zr(IV) form a 1:2 complex.

Once the composition of the X.O.-Zr(IV) was established, new experiments were carried out, in order to study the nature of the "apparent" complex that the fluoride ion forms with the 1:2 X.O.-Zr(IV) complex. Three methods were applied: the method of the continuous variations or Job's plot, the mole ratio method or Yoe-Jones's plot and the slope ratio method or Harvey-Manning's plot.

In all three cases (Fig.7, Fig.8, Fig.9), the results indicate that the composition of the complex is: $\text{X.O.}_4\text{Zr}_8\text{F}_{11}$.

CALIBRATION CURVE

1-10 ml. of $2.5 \cdot 10^{-5}\text{M}$ solution of sodium fluoride were pipetted into 100 ml. standard flasks and 5 ml. of $5 \cdot 10^{-4}\text{M}$ X.O. solution were added, followed by 7 ml. of

FIG. 6

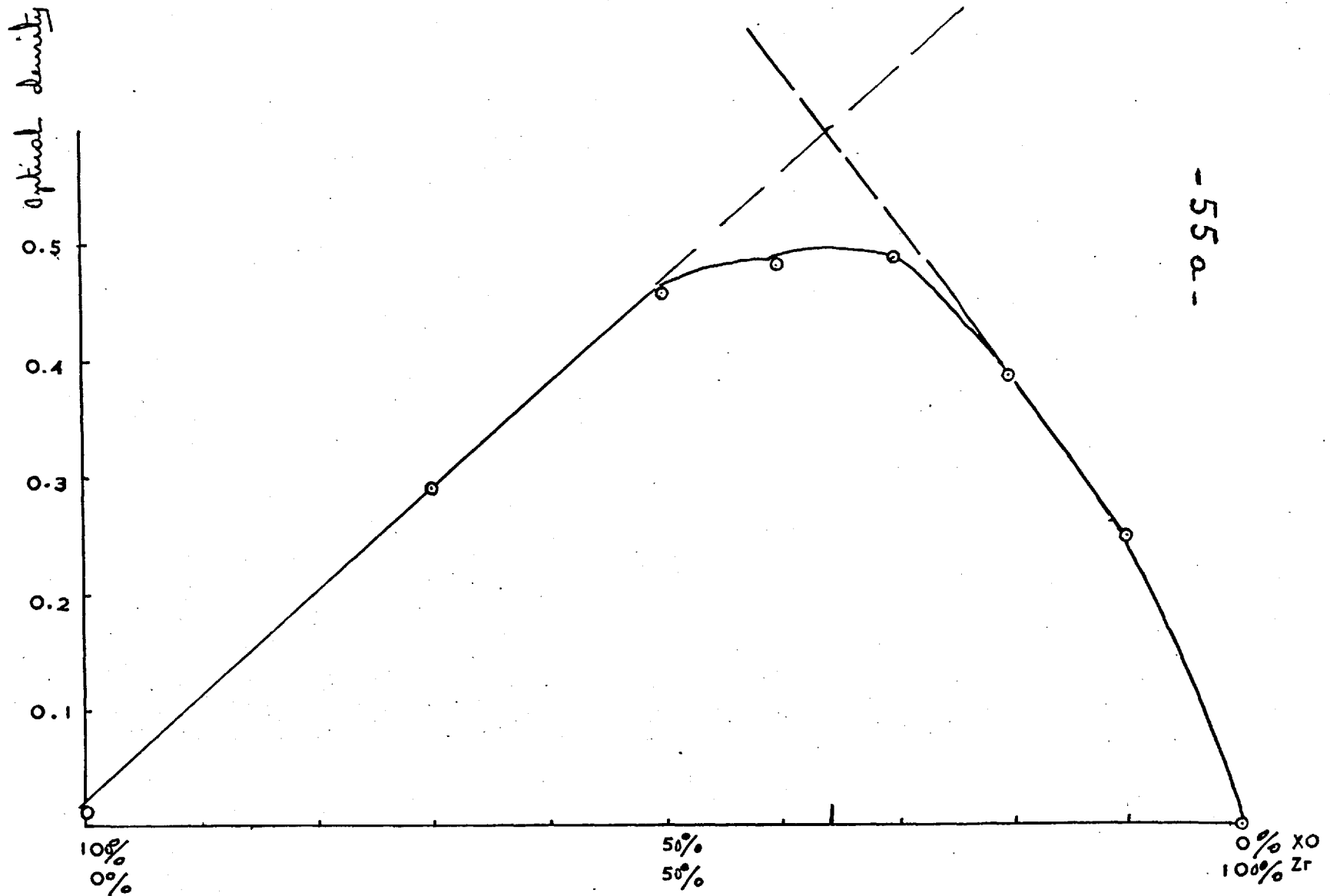


FIG. 7

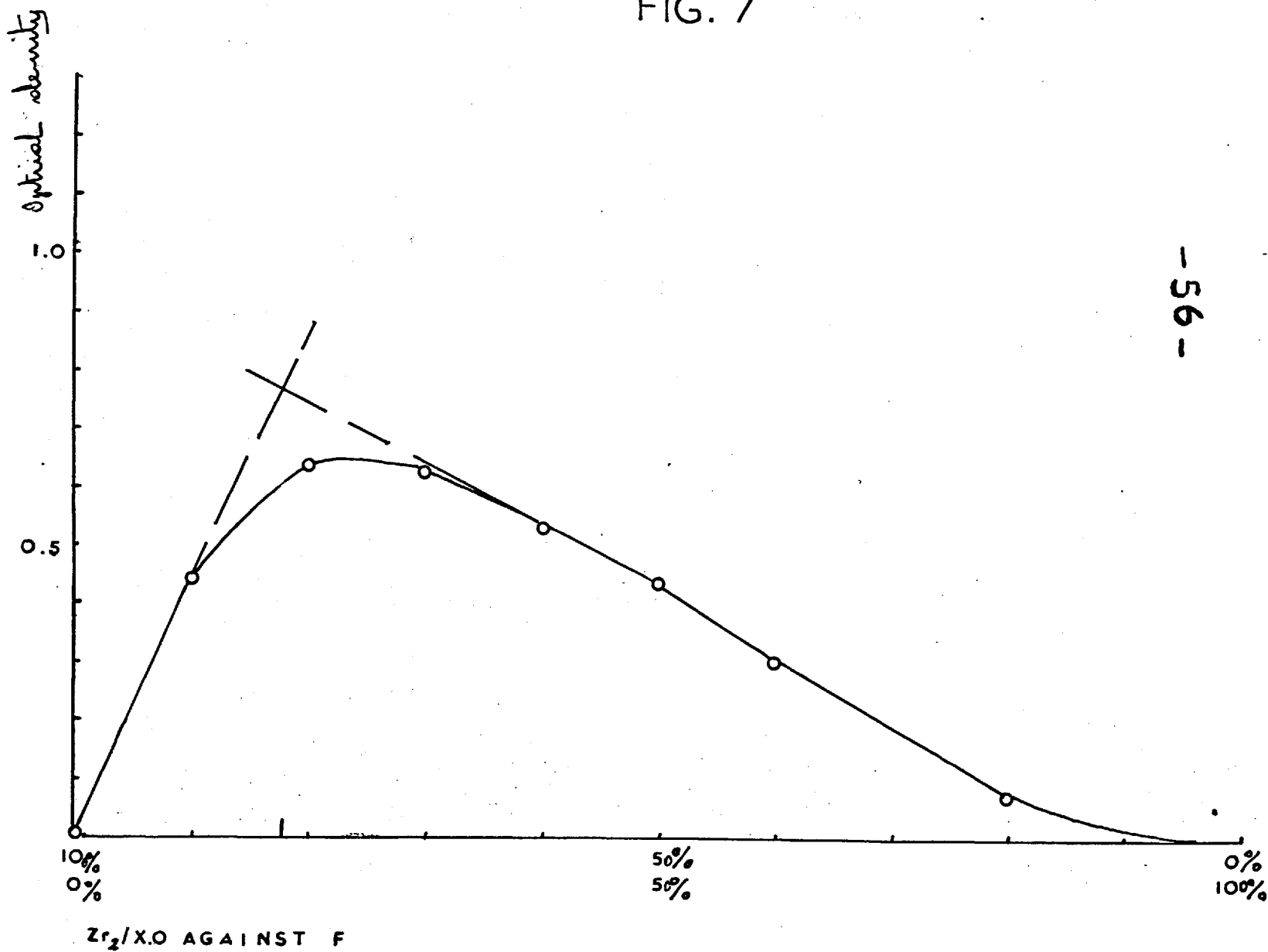
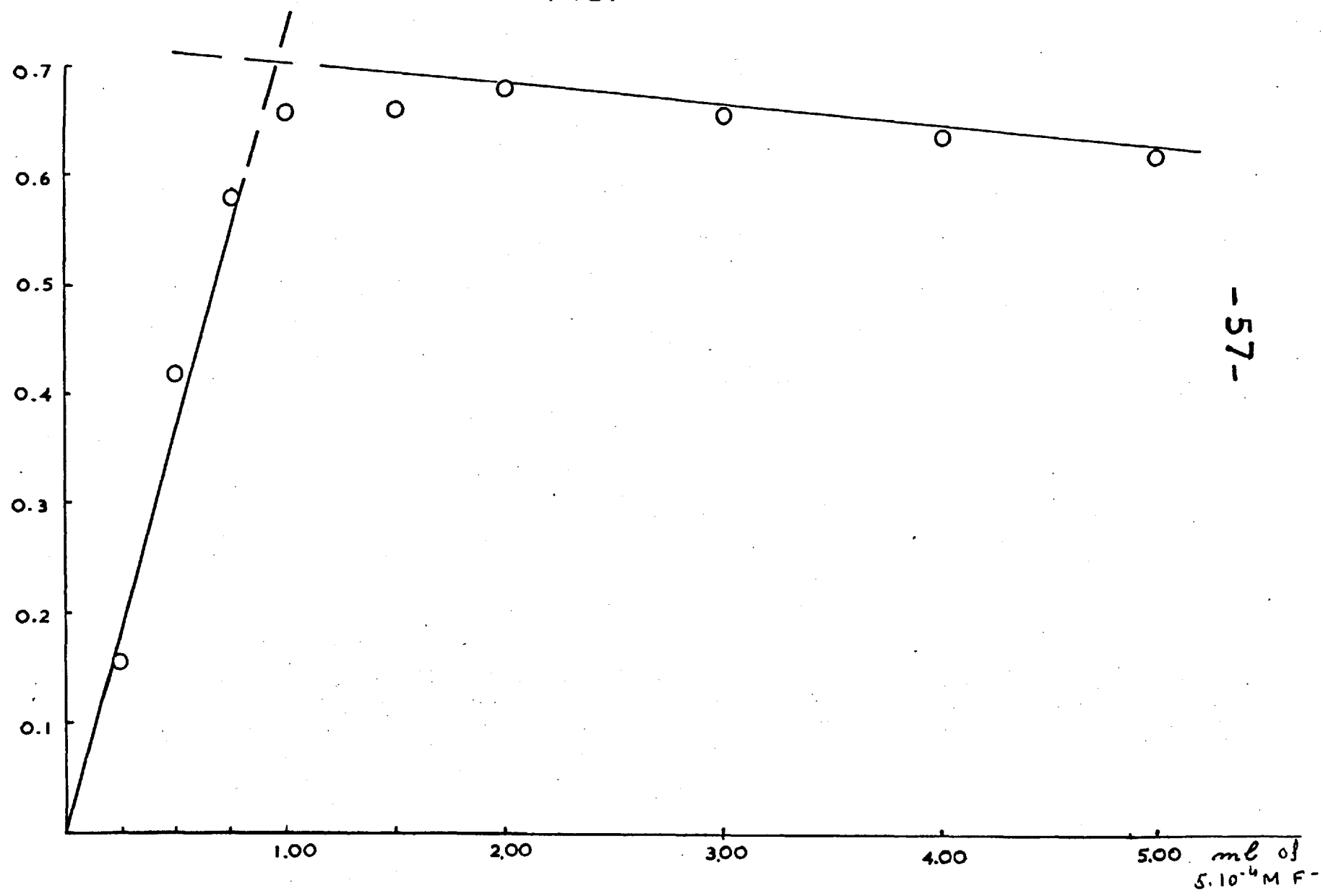
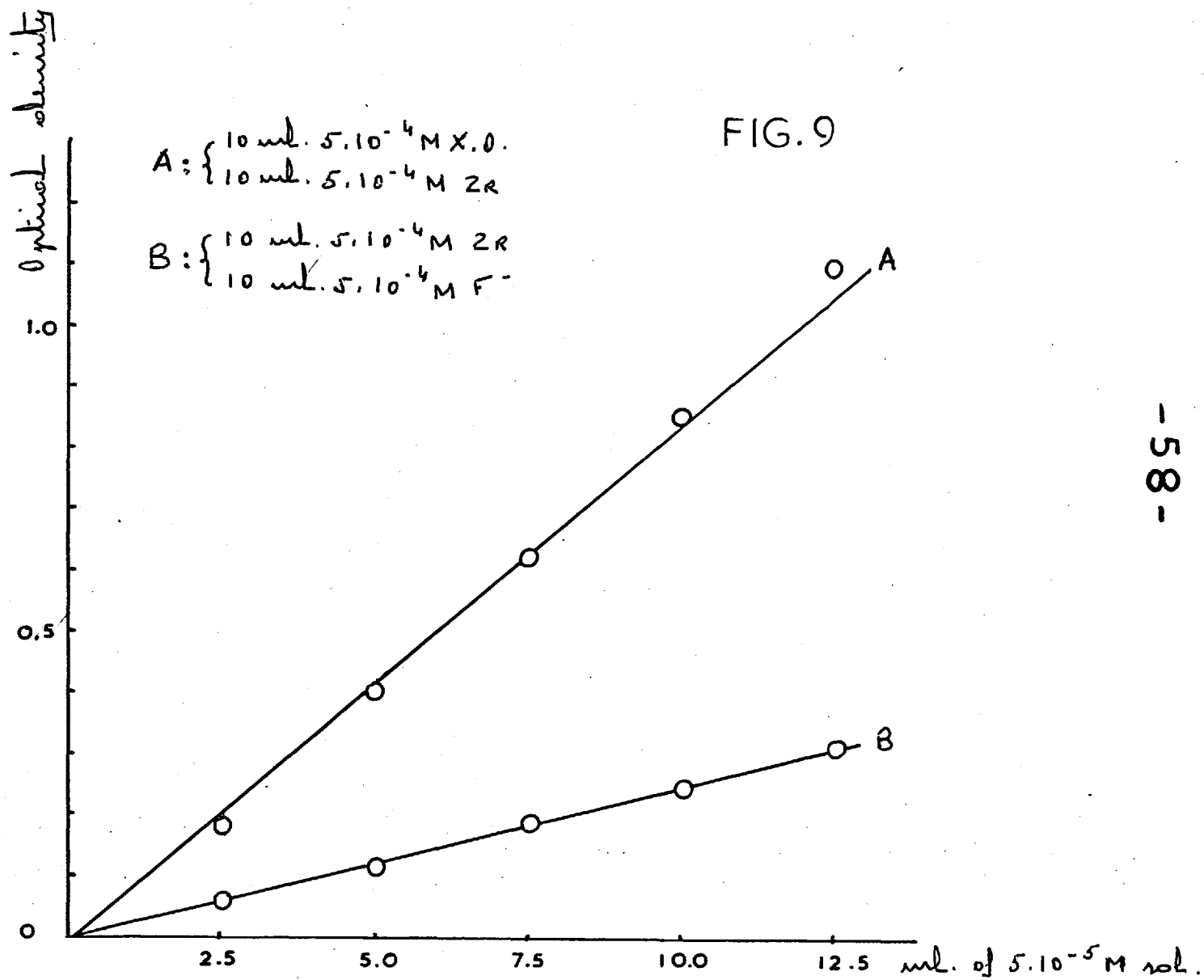


FIG. 8





5M HCl and 10 ml. of 5.10^{-4} M Zr(IV) solution. The contents of each flask were diluted to 100 ml. with cool distilled water and the solutions were measured after $1\frac{1}{2}$ hours, against a blank, prepared at the same time and containing no fluoride, at 550 m μ ., in 1 cm. cells.

The calibration curve (Fig.10), was found to follow Lambert-Beer's law from 0.5 μ g. to 5 μ g. of fluoride, and the effective molecular extinction coefficient is 200.000.

DETERMINATION OF FLUORIDE ION IN THE PRESENCE OF FOREIGN IONS

To study the effect of foreign ions on the spectrophotometric determination of fluoride, 32 ions (cations and anions), were investigated adding a 100-fold excess of them to the solution containing fluoride; the most common ions, such as Na, K, NO_3^- and ClO_4^- , were added in 1000-fold excess.

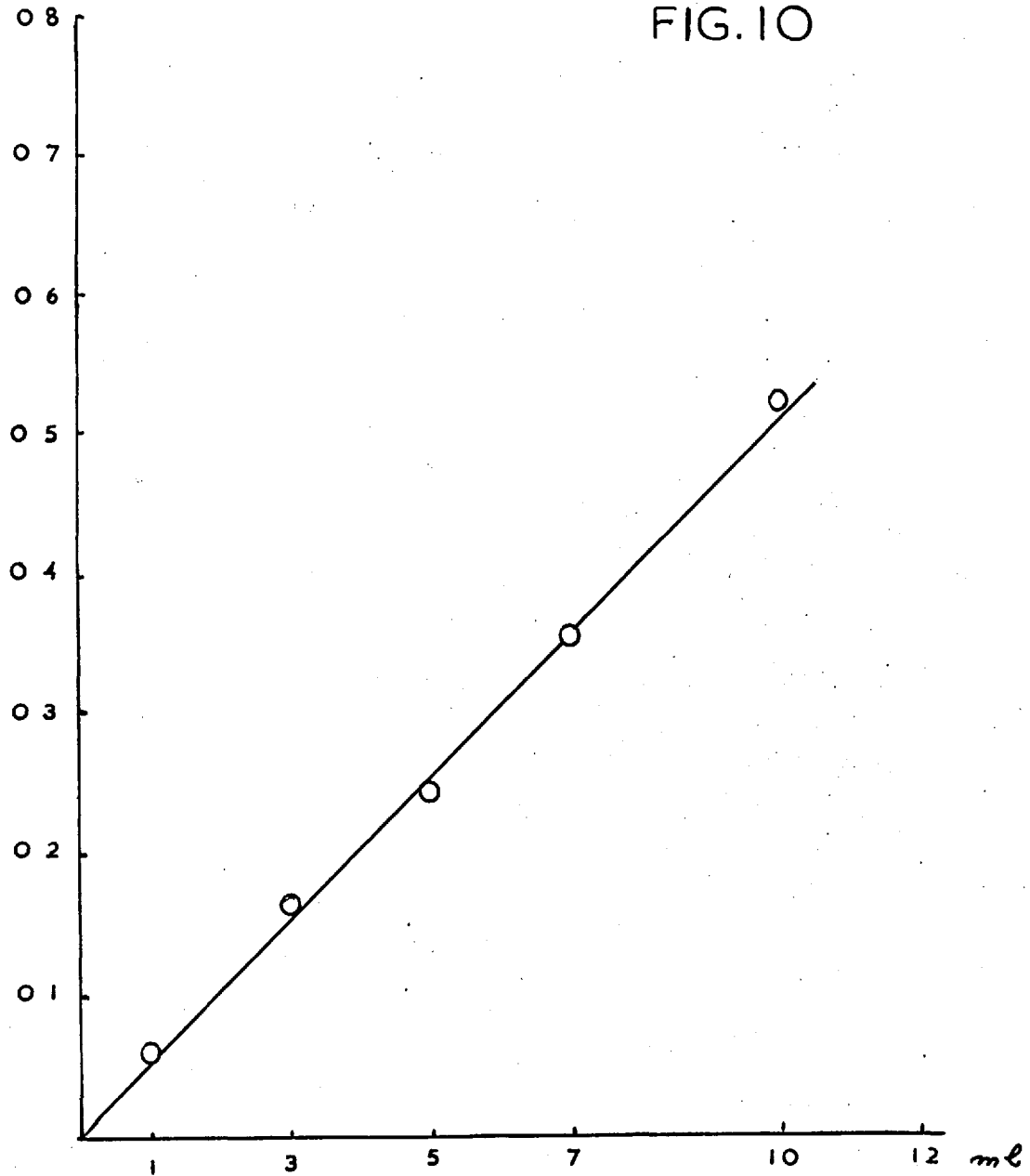
The only serious interference found among the cations was that due to Al^{+3} which gives very low results, but generally, cations were not found to give serious interferences, because in most instances, the interference disappears when the cation is in 10-fold excess in the solution.

Rather serious interferences were found among the anions, such as sulphate, phosphate and arsenate, which

-60-

Optical density

FIG. 10



yielded high results when present in small amounts and low results when present in a large excess, apart from sulphate, but it is believed that these anions can be separated by distillation before the determination of fluoride starts.

Procedure. 2.5, 25, or 0.25 ml. of $10^{-2}M$ solutions of foreign ion were pipetted into 100-ml. standard flasks, followed by 1 ml. of $2.5 \cdot 10^{-4}M$ sodium fluoride solution, 5 ml. of $5 \cdot 10^{-4}M$ X.O. solution, 7 ml. of 5M HCl solution and by 10 ml. of $5 \cdot 10^{-4}M$ Zr(IV) solution. The contents of the flask were diluted to 100 ml. with cool distilled water, and the solutions were measured against a blank containing no fluoride, after ninety minutes, at 550 m μ ., in 1 cm. cells.

The results are shown in Table III.

TABLE III

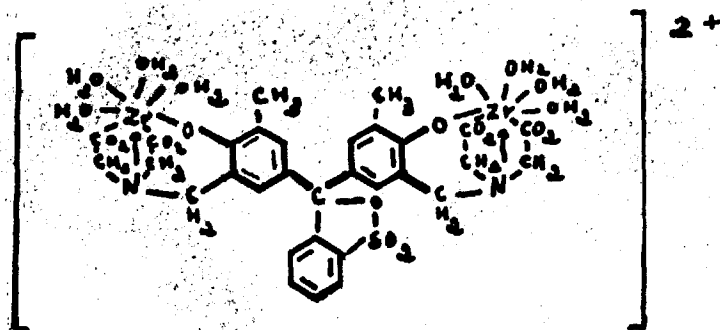
<u>ION</u>	<u>OPTICAL DENSITY</u>	<u>ION</u>	<u>OPTICAL DENSITY</u>
F	0.490	Pb ⁺² (100)	0.495
Al(100)	0.100	Sr(100)	0.490
Al(10)	0.200	Tl ⁺¹ (100)	0.495
Ba(100)	0.495	V ⁺⁵ (100)	0.490
Be(100)	0.490	Zn(100)	0.490
Ca(100)	0.490	NO ₃ ⁻ (1000)	0.490
Cd(100)	0.485	ClO ₄ ⁻ (1000)	0.490
Co(100)	0.680	B ₄ O ₇ ⁻³ (100)	0.490
Co(10)	0.490	PO ₄ ⁻³ (100)	0.200
Cr ⁺³ (100)	0.485	PO ₄ ⁻³ (10)	0.580
Cu ⁺² (100)	0.580	AsO ₄ ⁻³ (100)	0.600
Cu ⁺² (10)	0.490	AsO ₄ ⁻³ (10)	0.600
Fe ⁺² (100)	Bleached	SO ₄ ⁻² (100)	0.690
Fe ⁺² (10)	0.110	SO ₄ (10)	0.600
Fe ⁺³ (100)	0.700	Acet.(100)	0.490
Fe ⁺³ (10)	0.520	Cit.(100)	0.660
Hg ⁺² (100)	0.490	Cit.(10)	0.660
K(1000)	0.495	Oxa.(100)	Bleached
Mg(100)	0.495	Oxa.(10)	0.550
Mn ⁺² (100)	0.485	Succ.(100)	0.610
Na(1000)	0.490	Succ.(10)	0.490
Ni(100)	0.560	Tart.(100)	0.485
Ni(10)	0.500		

DISCUSSION

It can be seen from the experimental results obtained in this study, that the method proposed here for the determination of fluoride ion is more highly sensitive than any other known spectrophotometric method by as much as ca. one order of magnitude and that it is relatively free from cation~~h~~ interferences. Obviously one of the best ways of assessing its practical merits is to discuss its performance relation to that of the system based on cerium (III)-alizarin complexan or lanthanum-alizarin complexan which appears, from the literature survey, to be the best available method at the present time.

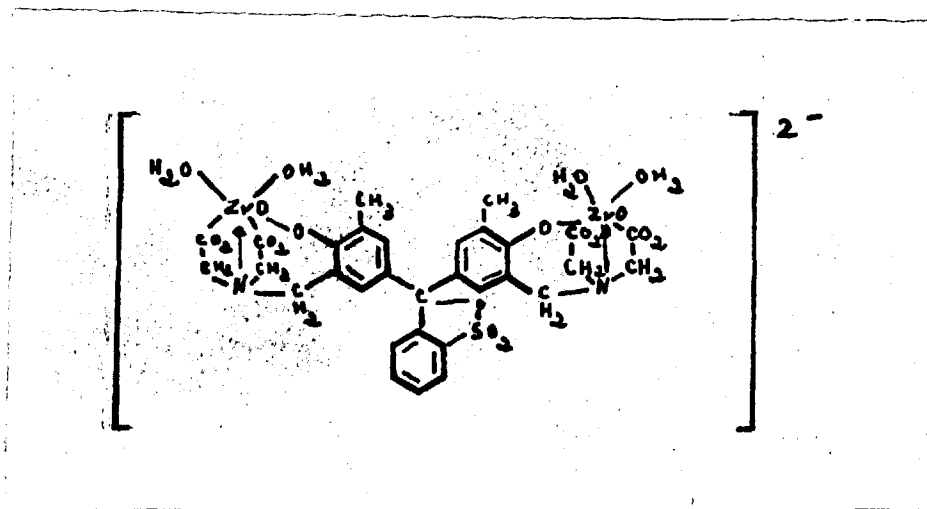
This work was well in hand before the publication by Wilson and Cooke⁶⁵ of a spot-test method for fluoride ion based on the same reaction and was in fact instigated by observations by West,⁶⁶ following his earlier work on the alizarin complexan reagent. When the present investigation was started, it was envisaged that the nature of the colour system was similar to that of the alizarin complexan system, i.e. it was expected that a ternary complex between fluoride, zirconium and Xylenol Orange was involved. However, it soon became apparent from the unusual ($X.O._4:Zr_8:F_1$) stoichiometry of the complex that

this was unlikely to be the case, since the fluoride ion did not appear to be occupying spare coordination positions on the zirconium-metal to the ligand in the $X.O.:Zr_2$ complex. If this had been the case, the ternary complex would have been expected to correspond to $X.O.:Zr_2:F_x$, where x is an integer between 1 and 8, as a result of the replacement of water molecules coordinated to the two zirconium ions in the $X.O.:Zr_2$ to a maximum number of eight. This assumption arises from the hypothesis that the $zirconium_2$:xylenol orange complex involves zirconium rather than zirconyl ions and that the zirconium exhibits a coordination number of eight



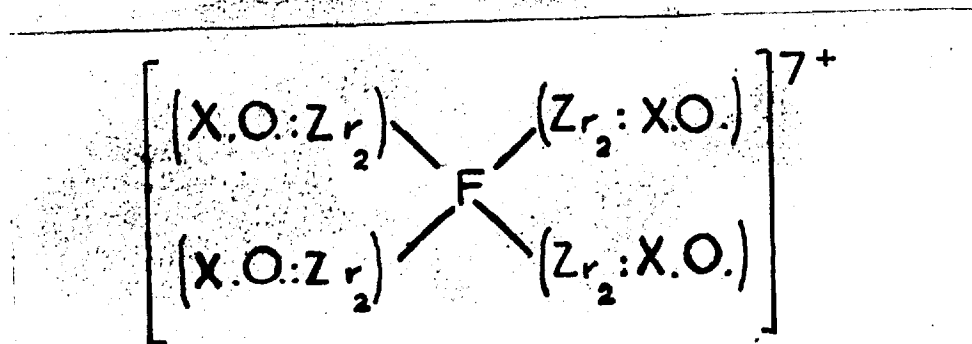
The most probable stoichiometry would be $X.O.:Zr_2:F_2$. However, if this assumption were extended to cover the situation where zirconyl ion, rather than zirconium ion,

were involved, a similar situation would still arise except that the expected stoichiometry of a ternary complex would be $X.O.: (ZrO)_2:F_y$ where y is an integer between 1 and 4 and is more probably 2.

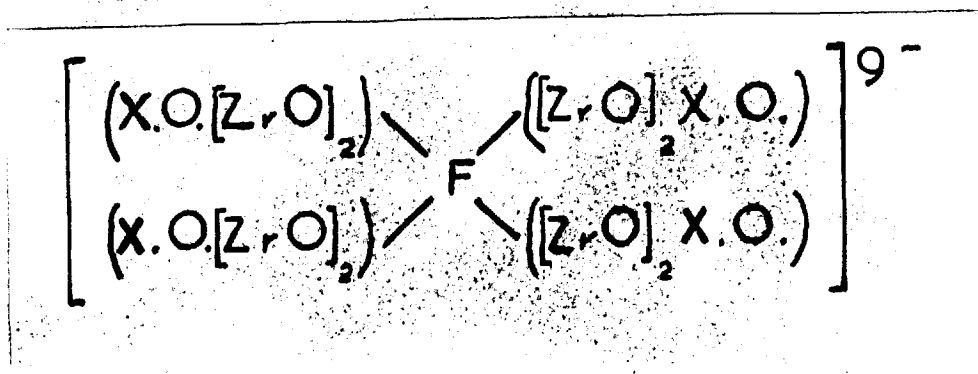


However, whether one assumes the $(X.O.:Zr_2)^{2+}$ or the much less probable $(X.O.: (ZrO)_2)^{2-}$ constitution for the primary complex it will be seen that the observed stoichiometry, which involves a multiplicity of $(X.O.:Zr_2)$ species per fluoride ion, cannot be predicted in either case. Indeed one has to postulate that the only possibility for the existence of a ternary complex in this instance is that four $(X.O.:Zr_2)$ primary complexes have entered into the coordination sphere of the fluoride ion. This would then demand that the ternary complex (ignoring water

molecules) should be of the nature,



or alternatively

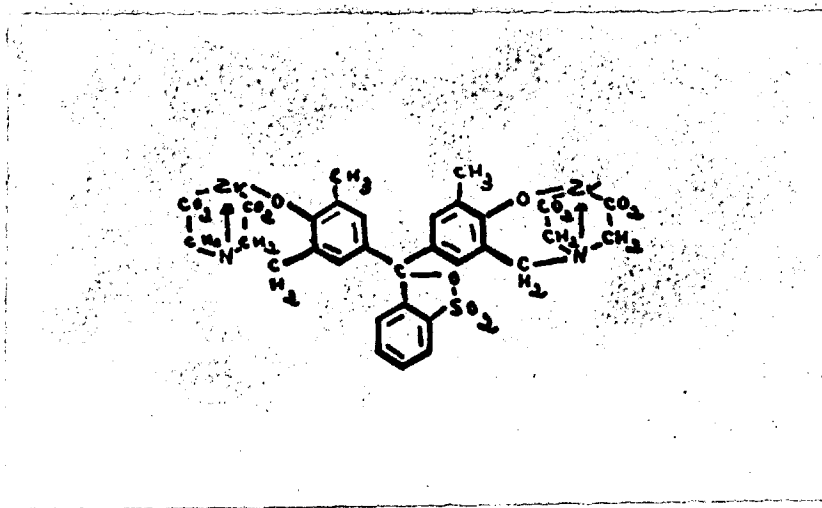


in the yet more unlikely eventuality of the union of four doubly charged anions with one central anion to form a coordination complex.

It is virtually impossible to build up such a massive complex around a small central body such as the fluoride ion and the short range electrostatic forces involved would obviously discourage such a formation.

In the case of the alizarin complexan-cerium (III) (or lanthanum) system the formation of the ternary complex

resulted in a very considerable shift in the wavelength of maximum absorption, so that the reaction was usually a very obvious one. In the initial stages of this work it was observed that whereas the mixed solution of xylenol orange and zirconium exhibited a yellow colour, the addition of fluoride ion led to the rapid formation of a red coloured complex and this led to postulation of a similar mechanism for this system also. A similar line of thought may indeed be deduced from the spot-test work of Wilson and Cooke⁶⁵ who describe a yellow to red colour change. However, an examination of the structure of xylenol orange shows that the electron-induction theory proposed by Leonard and West⁵⁷ for the alizarin complexan-cerium (III)-fluoride complex may not be held here; the hydroxyl groups of the xylenol orange are fully occupied by the primary complex formation with zirconium



and the positions vicinal to the two hydroxyl groups are occupied by methyl groups rather than by hydroxyl groups as in alizarin complexan. Consequently, there is no possibility that a further ionisation of protons from such groups can be promoted by the entry of the strongly electrophilic fluoride ion into the coordination sphere of the zirconium ion.

This immediately leads to the deduction that no colour change is theoretically possible upon reaction of the fluoride ion with the binary complex ($X.O.:Zr_2$). The existence of such a colour change mechanism becomes even more untenable, however, when one examines the value of the molecular extinction coefficient for the suspected ternary complex. In the instance of cerium (III)-alizarin complexan-fluoride system a value of 30.000 was obtained whereas in the present work an apparent molecular extinction coefficient value of 200.000 was obtained. This value is well beyond the maximum obtained for any other known absorbancy species in solution and is double the theoretical value of 100.000 calculated by Braude⁶⁷ on the basis of the finite light capture cross-section of an organic molecule.

Thus, three experimentally improbable facts militate against the preconceived idea of the existence of a

ternary complex system between xylenol orange, zirconium and fluoride ions:

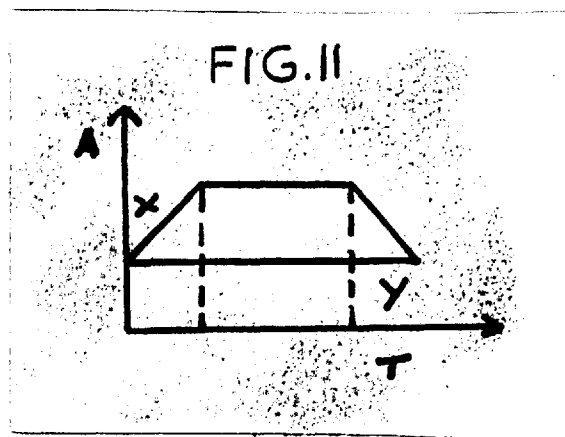
a) The unusual and sterically improbable $X.O._4:Zr_8:F_1$ stoichiometry.

b) The existence of an apparent colour change upon the addition of fluoride ion where theoretically such a change is impossible.

c) The (theoretically) improbably high value of the molecular extinction coefficient (200.000) for this sterically improbable and theoretically impossible colour change.

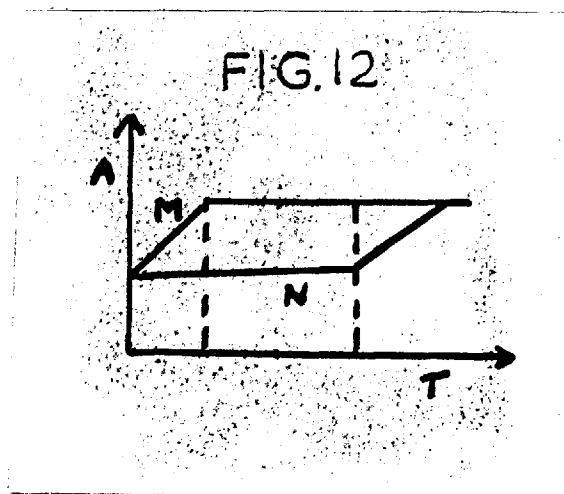
With these observations in mind several significant features slowly became apparent from experimental data. The first of these is that the validity of a Lambert-Beer plot relationship between the optical density of the solution and the amount of added fluoride exhibits a linear relationship only over a finite time range. This is, however, not uncommon in colour systems, for one normally accepts that a colour body requires a time of development (ie. formation) and, since intense colour and instability are common partners, not uncommonly coloured complexes frequently decompose upon standing for a given time. A plot of absorbance (A) for an absorbing

complex against time (T), therefore, frequently follows a path such as X in Fig.11 whereas the reagent blank commonly follows path Y. In the present instance, however, a different behaviour



emerged which is shown (schematically only) in Fig.12.

After an initial development time, following the addition of fluoride ion the absorbance of the solution at 550 mμ. increases to a maximum value as shown in curve M. This is not unusual for a stable complex, but the path, N, followed by the "reagent" blank is unusual.



It clearly shows that whether or not fluoride ion is present in the system the same net absorbance will eventually be produced. This behaviour is unusual and is of course very significant in retrospect, though it was rather puzzling and highly inconvenient in the early stages of the work when an analytical method was in the

process of being established.

The fact that the final absorbance of the solution was the same irrespective of the presence or absence of the fluoride ion suggested, though it did not necessarily imply, that the same colour-body was developed by path M or N. This was readily verified by running the absorption spectra of both solutions after the full development time had elapsed to allow paths M and N to come together.

This last observation of the coincidence of absorption spectra and the fact that the stoichiometry of the reaction $\text{X.O.}_4\text{:Zr}_8\text{:F}_1$ could equally well be written as $\text{X.O.:Zr}_2\text{:F}_{1/4}$ reveals that in all probability the colour body is simply the $(\text{X.O.:Zr}_2)^{2+}$ complex and the fluoride ion is not directly incorporated in the complex, but as is suggested by the time plot curves M and N in fig.12, merely promotes or catalyses the formation of the zirconium complex of xylenol orange.

A slow reaction between zirconium and xylenol orange solution is not unexpected because of the well known complexity of the aqueous chemistry of zirconium. It is generally recognised that the existence of free Zr(IV) ions in such media is only remotely possible and that the simplest species to exist in acid solution is most probably the ZrO^{2+} ion. However, in most instances there

can be little doubt that the slow reaction of Zr in aqueous solution is due to the presence of polymeric species involving extensive cross bridging between zirconium and oxygen atoms, e.g. -Zr-O-Zr-O-Zr-O- in acid solution or between zirconium and hydroxyl groups in less acid media. Not unnaturally the reaction between such a zirconium species and a bulky organic molecule such as xylenol orange would be expected to be slow.

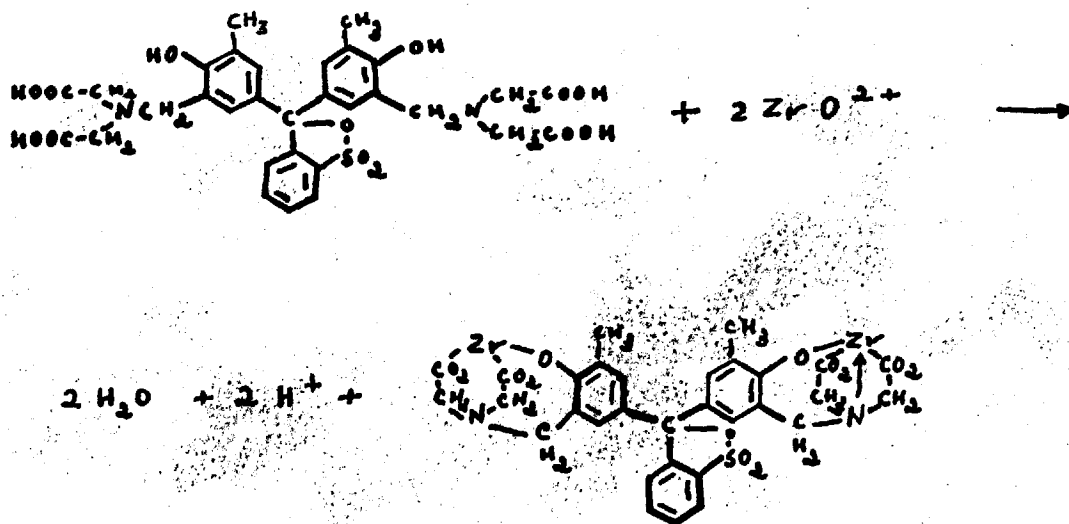
In the present study, it was found that there was considerable difference in the behaviour of solutions of differing zirconium salts, e.g. sulphate, nitrate, perchlorate, oxychloride, etc. The sulphate could not be used at all ^{IN THE ORIGINAL TESTING PROCEDURE} because no yellow colour could be obtained and with the perchlorate the blank (X.O.:Zr_2) system developed rather too quickly so that the net effect produced by the fluoride ion was considerably less than for the oxychloride. With nitrate solution the development of colour was very slow, even upon the addition of fluoride ion so that the oxychloride produced the optimum yield of colour and was used for all future work.

Yet again, it was found that the age of the solution of the oxychloride had a very profound effect. When it is freshly prepared the solution cannot be used because the blank itself develops a red colour instantly. After

standing overnight, however, the oxychloride solution becomes satisfactory. After the solution has stood for a total of five or six days, it again cannot be used satisfactorily, because the fluoride induced colour reaction gradually becomes weaker and requires a longer development time.

This behaviour can be explained in the following terms:

a) A freshly prepared solution of zirconium oxychloride contains a very high proportion of free zirconyl ions which reacts quickly with xylenol orange:

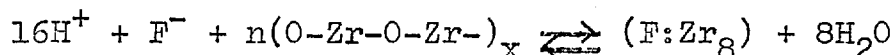


As a result of this rapid reaction between zirconium and xylenol orange the fluoride ion in small concentrations has no effect at all as the colour reaction.

b) After standing overnight the zirconium oxychloride solution undergoes partial polymerization to form simple polymeric species such as $-\text{Zr}-\text{O}-\text{Zr}-\text{O}-\text{Zr}-\text{O}-\text{Zr}-\text{O}-\text{Zr}-$. These would be expected to react only slowly with xylenol orange and indeed it is well known in EDTA titrations of zirconium solutions with EDTA that the solution should be boiled ~~or~~ warmed to 90°C to yield a satisfactory method with xylenol orange indicator. In the boiling process the polymeric species break down to yield zirconyl ions, which can react quickly with the xylenol orange. It is thought that in the present instance fluoride ion which has a high affinity for zirconium ions behaves in a similar manner in breaking up the polymer chains.

Under the conditions of this procedure in which a substoichiometric amount of fluorideⁱⁿ relation to zirconyl ions is present there is no danger of formation of the strongly bound $(\text{ZrF}_6)^{2-}$ species or other higher complexes. Consequently, since zirconium species vastly predominate over fluoride ions in the solution it is possible to postulate that several zirconyl ions will coordinate loosely with the fluoride ion to form a body which is labile towards reaction with xylenol orange. Since the stoichiometry of the colour reaction follows the $\text{X.O.}_4\text{Zr}_8:\text{F}_1$

pattern it is logical to envisage such a reaction as:

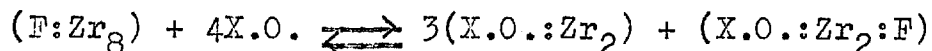


and quick reaction of the labile (F:Zr_8) aggregates, which would tend to be in equilibrium with free Zr(IV) ion or at least ~~three~~ zirconyl ions with ~~xylenol~~ orange



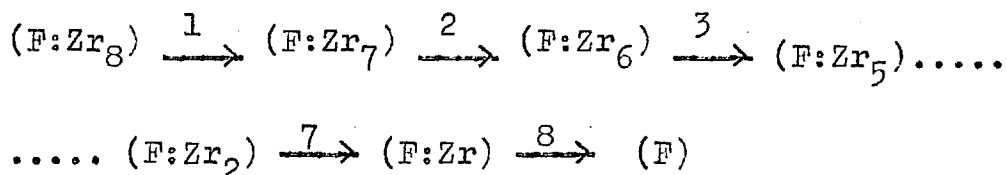
However, a difficulty arises in this last equation; in that it would then be logical to assume that the liberated fluoride ion should then continue its "catalytic" action as the rapid production of four more (X.O.:Zr_2) species and so on, till all the zirconium ions have reacted.

However, this does not happen in practice because there is a definite (i.e. finite) pattern in the stoichiometry of the colour production. Under these circumstances it is logical to assume that the fluoride ion remains attached to one of the zirconium ions so that the overall reaction would better be represented as:



In this way it is possible to suppose that each fluoride ion can yield four (X.O.:Zr_2) species only and cannot act as a conventional catalyst. There is a certain logical merit in this argument since one can see that whereas

the stepwise breakdown of the substoichiometric zirconium complex or aggregate can readily proceed through the first seven stages:

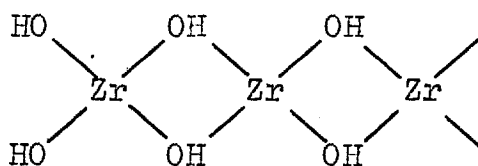


The last stage means breaking down a very powerful bond between fluoride and zirconium in what might be termed the first stage of formation of the stepwise build up of a normal $(\text{ZrF}_6)^{2-}$ complex. As has been explained previously there is no reason to suppose that the presence of a fluoride ion in a $(\text{X.O.}:\text{Zr}_2)$ complex should ^{LEAD} ~~be~~ to a colour different from the normal fully aquated complex.

One would tend to think of the $(\text{F}:\text{Zr})$ body as a normal $(\text{ZrF})^{3+}$ complex ion, but of the postulated substoichiometric $(\text{F}:\text{Zr}_8)$, $(\text{F}:\text{Zr}_7)$ bodies as dynamic aggregates rather than true complexes. Indeed it might be more logical to argue that the aggregates are more of the nature $(\text{F}:(\text{ZrO})_8)$ rather than $(\text{F}:\text{Zr}_8)$.

However, until further studies can be undertaken to elucidate a more firmly based hypothesis for the reaction mechanism it is not profitable to introduce further discussion at this stage other than the above theory.

Lastly (c) it is possible to explain the slow re-
action of an oxychloride solution which has aged for
longer than five days ~~on~~ the basis that further polymeris-
ation or hydrolysis of the polymeric species renders the
zirconium present in the solution increasingly inactive
as resultant to the formation of ^{HYDROXYDE} ~~fluoride~~ aggregates, e.g.



so that the activity towards fluoride ion gradually falls
off.

The above "kinetochromic" theory will also account
for the activity of other ions such as phosphate, sulphate,
etc., in the Zr:X.O. system and these will be discussed
more briefly at the appropriate places in the thesis.

CHAPTER THREE

DETERMINATION OF PHOSPHOROUS AND ARSENIC

AS PHOSPHATE AND ARSENATE

When sub-micro amounts of an element have to be determined, methods other than classical gravimetric and titrimetric ones have to be applied. This is the case for sub-micro determination of phosphorous and arsenic. Beyond doubt, the most widely used method for determining small amounts of these elements, by colorimetry and spectrophotometry, is the molybdenum blue method.

Although there is a wide literature on the topic, the method, as will be seen in the present discussion, presents serious difficulties.

The molybdenum blue method, is based on the formation of a heteropoly complex, which, upon reduction, produces a blue colour, the intensity of which is proportional to the amount of phosphorous or arsenic incorporated in the complex. The method, although better than all the classical methods, has, however, many uncertainties, because the condensations to give heteropolyacids may take place in several proportions and the products of reduction have not a very constant composition. According to Wu,⁶⁸

only the complexes which contain either nine or twelve molybdenum atoms to one of phosphorus or arsenic are reducible. As it has been said, the reduction of the heteropolyacid produces a blue coloured compound, but the constitution of this reduction product is uncertain, because the absorption spectra for these reduction products vary with several factors, such as:

- 1) Molybdate concentration
- 2) Reductant used and conditions of reduction
- 3) Acid concentration
- 4) Time of standing and temperature of heating.

Amongst many others, Boltz and Mellon⁶⁹ have studied the effect of all the above variables in the development of the colour and they conclude:

1) When the molybdate concentration, originally present, is too high, there is such a marked increase in the blue colour, and, therefore, in the final absorptivity, that measurements are not possible.

2) The best results seem to be obtained using hydrazine sulphate as ~~reductant~~^{reducing} agent and measuring the absorption at 830 mμ., because if chlorostannous acid is used as a reductant,⁷⁰ the maximum absorption is obtained at a wavelength of 820 mμ., and using hydroquinone and controlling the pH between 4 and 4.7, absorption

spectra with ~~absorption of~~ ^{A MAXIMUM AT} approximately 700 mμ., were obtained.

3) The acid concentration seems to have a great importance too, in the formation of molybdenum blue, because at low acidity, an increase in the intensity of the blue colour is observed, probably due to the reduction of the excess of molybdate solution.

4) The final temperature of the sample for maximum colour development was found to be 90°C - 93°C, and the colour obtained under those conditions was stable for twelve hours, the molecular extinction coefficient for the phosphorus being 26,800.

In the case of arsenic, the maximum absorption band occurs at about 840 mμ., but it must be said that it was observed that for low concentrations of arsenic, the nature of the curve at the blue end of the spectrum differs from that for higher concentrations. This is due to the presence of quinquivalent molybdenum ions which are formed by the reduction of the excess molybdate reagent. When the arsenic concentration is higher, the amount of excess of molybdate reduced is less, and this is shown in the bands of the spectrum.

Boltz and Mellon's method was applied to the determination of phosphates in biological materials,⁷¹ with

the only modification of using aminonaphthosulphonic acid as reducing agent instead of hydrazine sulphate, because it was claimed⁷² that the ~~first one~~ ^{FORMER} gave better results than the latter.

As phosphoreous and arsenic commonly occur together, and they are determined by the same methods, the presence of one of them interferes seriously with the determination of the others. Therefore, several attempts have been made to find a method to separate them. The best one seems to be liquid-liquid extraction, using a 20%, by volume, solution of 1-butanol in chloroform,⁷³ which enables the measurement of 25 µg. of phosphoreous in the presence of 4 mg. of arsenic and gives a molecular extinction coefficient of 24,400 at 310 mµ.

There are many other authors who have tried to improve the conditions of the determination of phosphorus and arsenic, usually by changing the nature of the reducing agent and using ferrous ammonium sulphate,⁷⁴ stannous chloride or 1-amino-2-naphthol-4-sulphonic acid⁷⁵ and many others as reductant; but the results were never entirely satisfactory. A valuable contribution to the determination of phosphorus is the method given by Lueck and Mellon,⁷⁶ who measured spectrophotometrically, in the ultraviolet region, the absorptivity of the decomposition

products of molybdo-phosphoric acid. After extraction of the acid with an ether-isobutyl alcohol solution, a retrograde extraction with a basic buffer solution was used and the molybdate was transferred to the aqueous phase. The absorption was measured at pH 9, at 230 m μ . and in 1-cm. cells. The molecular extinction coefficient for phosphorus is then 57,400.

But, far from any doubt, the most sensitive method, known at present for the determination of phosphorus is the one giving molecular extinction coefficients of 96,900 or 359,000 depending on the procedure used.⁷⁷ In this technique, phosphate is converted to phosphomolybdic acid and then extracted into a mixture of chloroform and butanol (4 + 1) by volume. If the phosphomolybdic acid is broken down by an alkali, the twelve molybdate ions corresponding to each phosphate ion are set free and then the molybdenum is determined spectrophotometrically by the green colour which develops at 710 m μ . with 2-amino-4-chlorobenzenethiol hydrochloride in chloroform, as described by earlier authors.⁷⁸ A molecular extinction coefficient of 96,900 is obtained and the procedure is as follows:

1,2,3 and 4 ml. of a phosphorus solution containing 1 μ g. per ml., are pipetted into 50-ml. separating funnels,

followed by 10 ml. of molybdate reagent solution. The contents of each flask are diluted to 25 ml. with distilled water; 10 ml. of chloroform-butanol mixture are then added to each funnel and the funnels are shaken during two minutes. The non-aqueous phase is then transferred to a new 50-ml. funnel, 10 ml. of chloroform-butanol mixture are added again, and all the funnels shaken for another two minutes. The non-aqueous extracts are washed by shaking during one minute with 10 ml. of 2N HCl. The aqueous solution is discarded, 10 ml. of 2N ammonia solution are added and the solutions are shaken for one minute. After separating the ammoniacal aqueous solutions, washing the funnels with 2N HCl and collecting all the washings the pH is adjusted to 2.2 with 2N HCl, the solutions transferred to 100 ml. graduated flasks and the contents of the flasks diluted to 100 ml. with distilled water. 25 ml. of these solutions are then pipetted into 50-ml. separating funnels and 0.25 ml. of 4% 2-amino-4-chloro-benzenethiol are added. The solutions are set aside during 15 minutes, after mixing, and then 10 ml. of chloroform added. The funnels are shaken for one minute and the chloroform extracts measured spectrophotometrically, in 1-cm. cells, at 710 mμ. against a blank similarly prepared but containing no phosphorus.

If the whole extract is taken instead of only a 25-ml. aliquot, there is an extraordinary enhancement of the sensitivity due to the determination of the total molybdenum recovered. The coefficient of molecular extinction is then 359,000, but unfortunately the precision in this case is not so high as in the above. Phosphorus can be determined following this procedure in the presence of 100-fold excess of germanium, antimony, silicon or arsenic.

Another method for the determination of phosphorus based on what can be called an amplification method, is that due to Umland and Wunsch.⁷⁹ ^{Like} ~~As~~ Djurkin, Kirkbright and West, they make use of the multiplication factor twelve, resulting from the determination of molybdenum in the dodecamolybdophosphoric acid, instead of the direct determination of phosphorus. In this method, the molybdophosphoric acid is extracted from its 0.4N HCl solution by an ether isobutanol mixture (5+1), which is freed from excess molybdic acid by washing the organic layer with 0.4N HCl. It is then re-extracted and the phosphomolybdate is broken down by 0.1N NaOH solution. The molybdenum is determined photometrically as its thiocyanate according to the method given by Reynik.^{80,81}

The authors claim that 1-10 $\mu\text{g.}$ of phosphorus can thus be determined with a formal extinction coefficient of 150,600.

From the methods described above, it can be seen that the determination of phosphorus and arsenic is either inaccurate because the molybdenum blue has an uncertain composition, or tedious, in the amplification processes, because they require a large number of solvent extractions.

The method described in this thesis, is based upon an entirely new reaction, which gives highly sensitive results.

As in the case of the fluoride ion, the presence of either phosphate or arsenate, produces, under certain conditions of acidity, temperature, hydrolysis of zirconium solution, etc., a rapid formation of the X.O.-Zr(IV) complex, while the solution not containing phosphate or arsenate, develops its colour much more slowly.

The coefficients of molar extinction obtained by this method are of 400,000 for the phosphate and 344,000 for the arsenate.

PART II: EXPERIMENTAL

APPARATUS.

Beckmann D.B. recording spectrophotometer with
1-cm. cells.

REAGENTS.

$2.5 \cdot 10^{-5} \text{M}$ phosphate solution.

Weigh exactly 0.7801 g. of AnalaR Na_2HPO_4 and dilute to 500 ml. with distilled water, in order to make a 10^{-2}M solution. Take 25 ml. of this solution and dilute to 1000 ml. to have a $2.5 \cdot 10^{-4} \text{M}$ solution. To prepare a $2.5 \cdot 10^{-5} \text{M}$ solution, 100 ml. from the $2.5 \cdot 10^{-4} \text{M}$ solution must be taken and diluted to exactly 1000 ml.

$2.5 \cdot 10^{-5} \text{M}$ arsenate solution

Prepared by weighing 0.9891 g. of AnalaR As_2O_3 suspending it in a small amount of distilled water, adding a few drops of nitric acid and heating until the solution is completely clear; bromine water is then added in order to oxidize As(III) to As(V) and the excess of bromine is removed from the solution by addition of formic acid until the yellow colour of the solution disappears. The As(V) solution is then transferred to a graduated flask and diluted to 500 ml. with distilled water to make a

$10^{-2}M$ solution. 25 ml. of this solution are taken and diluted to 1000 ml. with distilled water and 100 ml. of the new solution are again diluted to 1000 ml. to have a $2.5 \cdot 10^{-5}M$ arsenate solution.

The other reagents are prepared as described in the fluoride determination.

ABSORPTION SPECTRA OF THE COMPLEXES

It was already discussed in the fluoride determination that the absorption spectra and the Job's Plot, clearly show that the complex formed between X.O. and Zr(IV) is a 1:2 complex; therefore, in the phosphate and arsenate determinations, the experiments have been carried out assuming that X.O. and Zr(IV) form a 1:2 complex.

To find out the kind of complex that phosphate and arsenate form with X.O. Zr_2 a set of four solutions was prepared: a first solution containing 2.5 ml. of $5 \cdot 10^{-4}M$ X.O. which would show the spectrum of the reagent itself, a second one containing 2.5 ml. of $5 \cdot 10^{-4}M$ X.O. and 5 ml. of $5 \cdot 10^{-4}M$ Zr(IV), to show the complex formed between reagent and metal ion and a third and fourth solutions containing 2.5 ml. of the X.O. sol., 5 ml. of the Zr(IV) sol. and 2.5 ml. of $5 \cdot 10^{-4}M$ phosphate and arsenate solutions, respectively, to see the ternary complexes formed

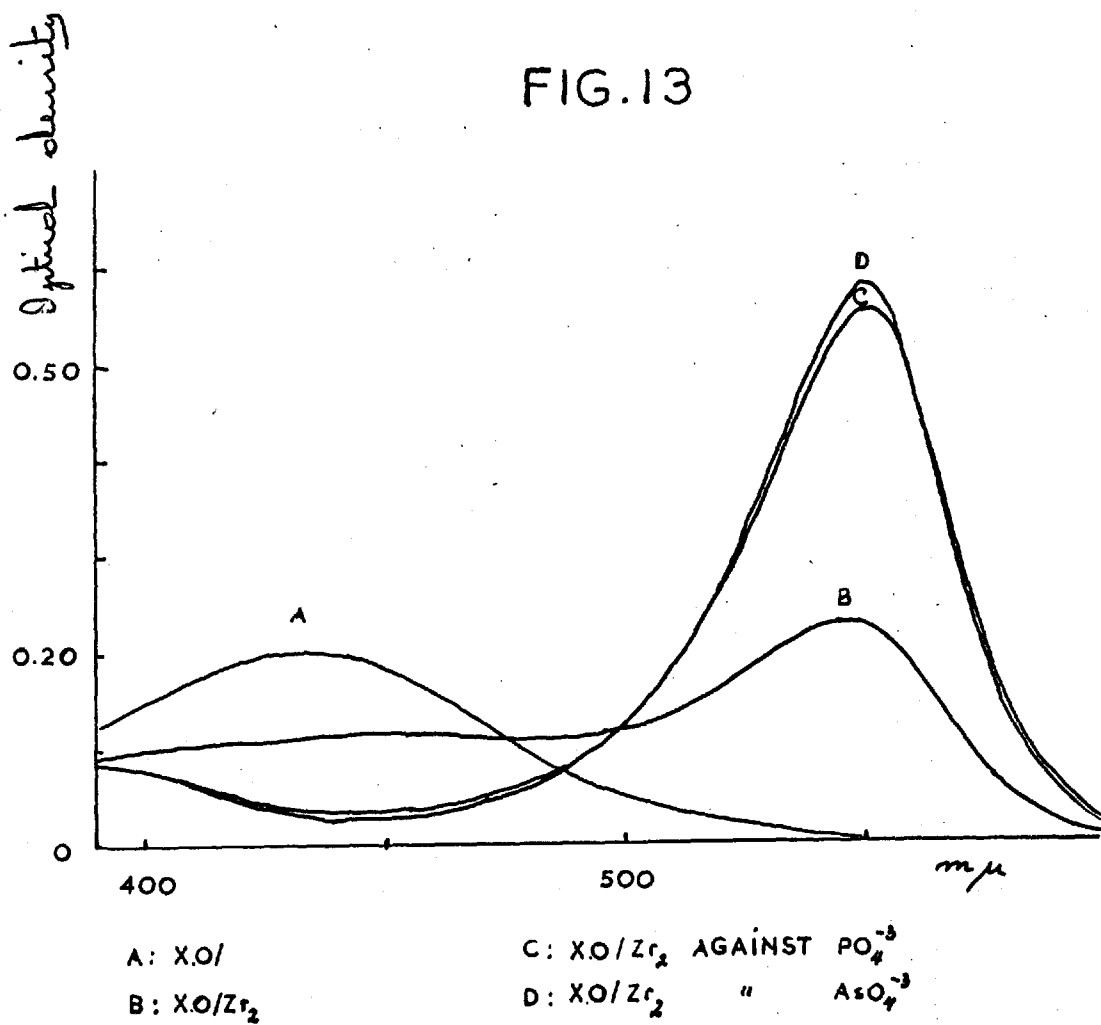
between X.O., Zr(IV), and phosphate or arsenate. Seven milliliters of 5N HCl were added to each flask, and the contents of the four flasks were diluted to 100 ml. with distilled water.

The spectra were run in the Beckmann D.B. recording spectrophotometer, in 1-cm. cells, against distilled water, after keeping the solutions in a cool place (15°C) during ninety minutes.

The results in fig.13 show that as in the case of the fluoride, there is no evidence by the spectra of the formation of any kind of new ternary complexes between X.O., Zr(IV) and phosphorus or arsenic because the wavelength of maximum absorption is the same, 550 mμ., for the binary complex and the supposed ternary ones. However, when phosphorus or arsenic are present, an extraordinary increase in the value of the optical density can be observed at the wavelength of maximum absorption. This fact ensured the possibility of the determination of phosphate and arsenate by a highly sensitive method.

It must be pointed out that fig.13 shows a higher absorption when arsenic is present than when phosphorus is present, whereas the sensitivity of the reaction is actually higher for phosphate, as will be seen by the calibration curves and by the molecular extinction

FIG.13



coefficients. This is probably due to the fact that in the spectra, phosphate and arsenate are in a rather large excess according to the upper limit of the calibration curves and therefore the same behaviour cannot be expected. Furthermore, it must be said that the presence of small excesses of phosphorus above the region in which Beer's Law is followed, produces a very rapid fall off in the straight line relationship. This effect is not so evident in the case of the arsenic. This can be attributed to the strength of the complex formed between phosphate ion and zirconium above the substoichiometric level.

It must be added, finally, that when the determination of fluoride ion was carried out in the presence of a large excess of phosphorus (100-fold excess), a completely bleached solution was obtained whereas when a 100-fold excess of arsenic was present an optical density higher than that due to fluoride ion alone was obtained.

The above facts lead to the conclusion that phosphate forms a very strong complex with Zr(IV) , and that this complex formation breaks down the X.O. Zr_2 complex, the formation of which is promoted by the presence of substoichiometric amounts of phosphate. The arsenate, on the other hand, forms a weaker complex with Zr(IV) which, therefore, does not break down the X.O. Zr_2 complex.

EFFECT OF THE ACID CONCENTRATION

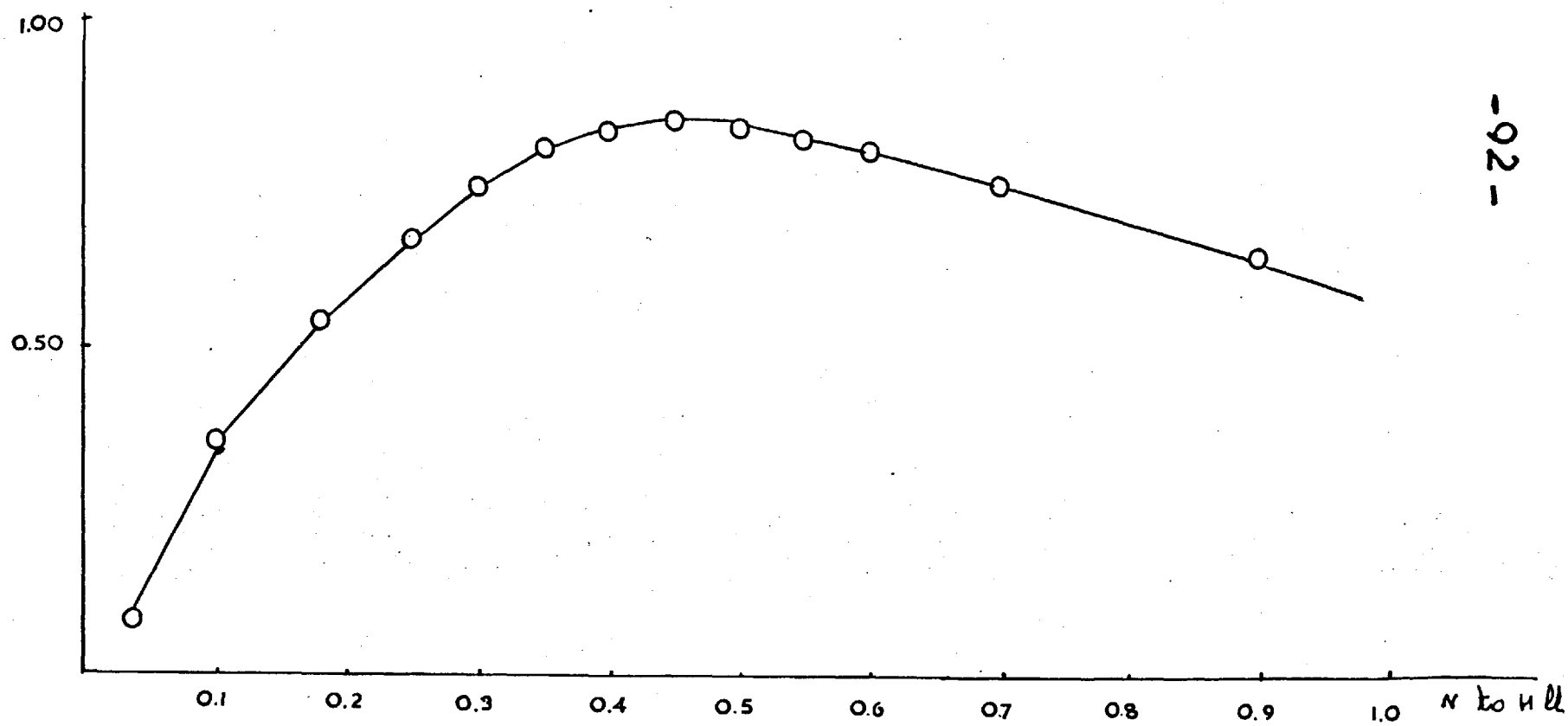
a) PHOSPHATE

In order to find out the optimum acidity for the development of the phosphate determination, the following experiment was carried out:

Several sets of two solutions were prepared, one of them containing 5 ml. of X.O. solution and 10 ml. of Zr(IV) sol., and another containing 5 ml. of X.O. sol., 10 ml. of Zr(IV) sol. and 10 ml. of $2.5 \cdot 10^{-5} M$ phosphate solution. Different amounts of 5N HCl were added to make the solutions 0.05N to HCl, 0.1N, 0.15, 0.20, etc. The contents of each flask were diluted to 100 ml. with distilled water, and all the solutions were set aside for ninety minutes in a cool place. Each solution was measured against its corresponding blank (containing no phosphate), in 1-cm. cells, at 550 m μ .

Fig.14 shows the result of the experiment, and it can be seen that there is a rather wide interval of acidity (from 0.35 N to 0.60 N), in which the value of the optical density does not change much. It was thought, therefore, that the most convenient acidity to operate was the highest possible, because the higher the acidity of the solution, the less interferences the method will present for cations; however, it was necessary to work at the lowest acidity

FIG. 14



(0.35N) because, unfortunately, Beer's Law was not completely followed at higher acidities. It seems quite probable that due to the fact that this is a clear case of catalytic reaction, high acidities produce a very rapid development of the colour for low concentrations of phosphate, and a not so rapid one for higher concentrations.

Therefore, it was established that the optimum acidity for the development of the method, is the one in which the solution is 0.35 N to HCl.

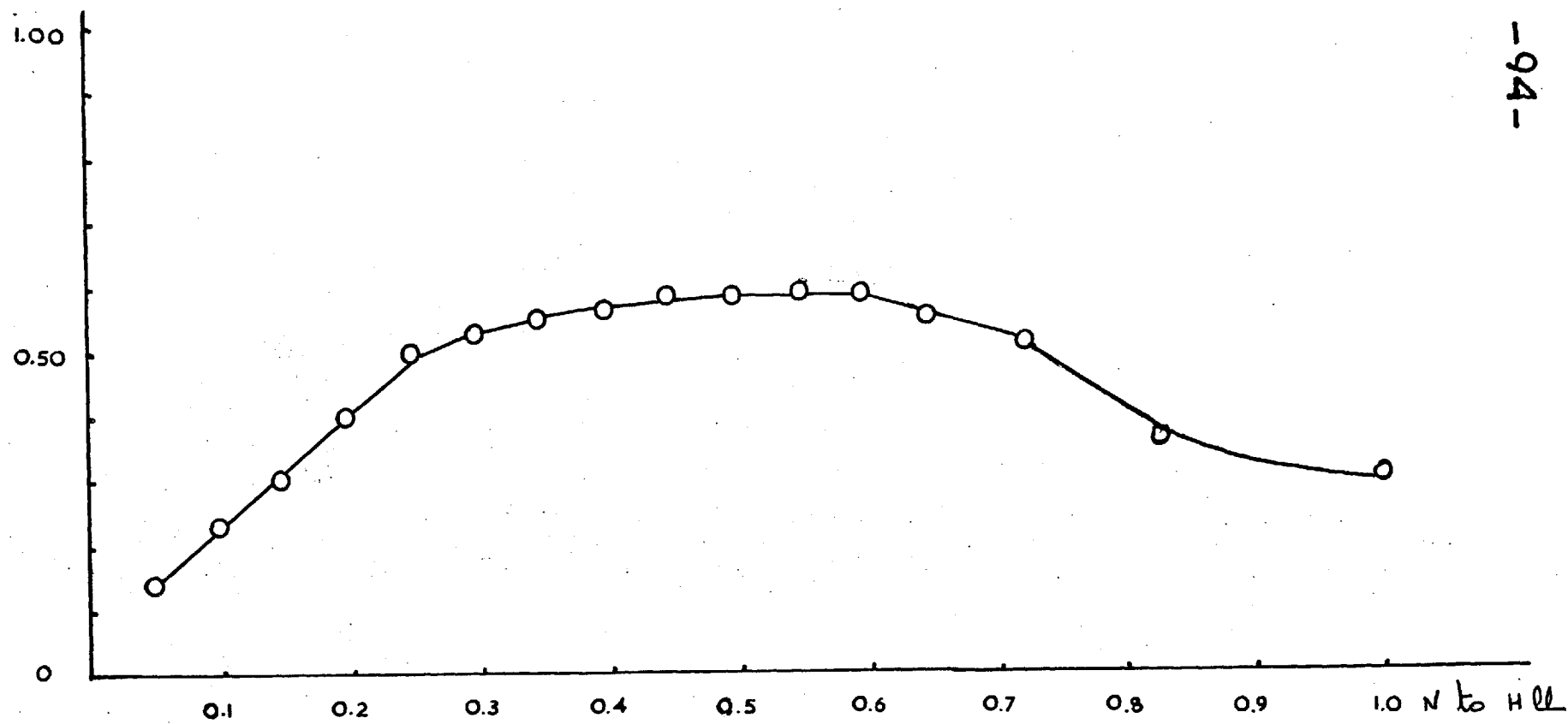
b) ARSENATE

Experiments similar to the ones carried out for the phosphate were prepared for the arsenate and it was found again, that although the value of the optical density remains practically constant between the range of acidities 0.30 N to 0.60 N, fig.15; for a concentration of 10 ml. of $2.5 \cdot 10^{-5}$ M arsenate, Beer's Law is best followed when the solutions are 0.35 N to HCl.

EFFECT OF THE TIME

The time of development of the colour seems to have a great importance in the reaction under study. It must be considered that just because there is no formation of a new complex, but increase in the speed of the formation of the colour, the colour of the blank is not fully

FIG. 15



developed when the measurements are carried out, but it develops with time and when this happens there is no possibility of determination of either phosphate or arsenate.

To study the effect of time on the reactions, a similar experiment was prepared for phosphate and for arsenate:

a) PHOSPHATE

A solution was prepared containing 5 ml. of X.O. sol., 10 ml. of $2.5 \cdot 10^{-5} \text{M}$ phosphate sol., 10 ml. of Zr(IV) sol., and 7 ml. of 5 N HCl; the contents of the flask were diluted to 100 ml. with distilled water, and its optical density was measured over a period of time, in 1-cm. cells, at 550 m μ . against a blank prepared at the same time and containing no phosphate. The results of the experiment are shown in Table IV, and as can be seen, the optical density reaches its maximum value 75 minutes after the solutions have been prepared and it remains constant for 60 minutes afterwards. The value of the differential optical density then progressively decreases and after several hours, no difference between the colour of the blank and the colour of the solution containing phosphate can be detected.

TABLE IV

VARIATION OF THE OPTICAL DENSITY OF THE PHOS-
PHATE WITH THE TIME

<u>TIME</u>	<u>OPTICAL DENSITY</u>
0 minutes	0.000
5 minutes	0.200
15 minutes	0.400
30 minutes	0.550
45 minutes	0.640
60 minutes	0.760
75 minutes	0.820
90 minutes	0.820
105 minutes	0.815
120 minutes	0.825
135 minutes	0.820
150 minutes	0.790
180 minutes	0.750

b) ARSENATE

A solution containing 5 ml. of X.O. sol., 10 ml. of $2.5 \cdot 10^{-5} \text{M}$ arsenate sol., 10 ml. of Zr(IV) sol. and 7 ml. of 5 N HCl was prepared. The contents of the flask were diluted to 100 ml. with distilled water and the optical density of the solution was measured over a period of time as in the case of the phosphate, against a blank containing no arsenate, in 1-cm. cells and at a wavelength of 550 m μ .

The results, in Table V, are similar to those obtained for the phosphate, although the value of the optical density is always lower than that observed for phosphate at similar times.

Therefore, it was decided that the optimum time at which the measurements must be carried out is after 75 minutes and within 105 minutes after the solutions have been prepared.

EFFECT OF THE TEMPERATURE

Although quantitative experiments have not been done to show the influence of temperature on the reaction under study, it has been observed that the temperature must not be higher than 17-18°C to obtain successful results. By the process recommended here the solutions have been allowed to develop their colours in a cool place (usually

TABLE V

VARIATION OF THE OPTICAL DENSITY OF THE
ARSENATE WITH THE TIME

<u>TIME</u>	<u>OPTICAL DENSITY</u>
0 minutes	0.000
5 minutes	0.150
15 minutes	0.260
30 minutes	0.380
45 minutes	0.490
60 minutes	0.590
75 minutes	0.680
90 minutes	0.685
105 minutes	0.680
120 minutes	0.680
135 minutes	0.650
150 minutes	0.620
180 minutes	0.550

about 15°C), but it must be said that all the results have been obtained without the help of a thermostat and that fluctuations of two or three degrees centigrade do not affect the results.

NATURE OF THE COMPLEXES

As has been seen above, the study of the absorption spectra of the solutions does not give evidence of the formation of a new ternary complex between X.O., Zr(IV) and either phosphate or arsenate. The absorption spectra are identical to those formed between X.O. and Zr(IV) but are formed much more quickly. Nevertheless, the different behaviour of the two anions, inasmuch as sensitivity and speed of formation of the colours are concerned, suggested the possibility of some degree of formation of a complex or association. Therefore, the different known methods for the determination of the nature of a complex were applied to both phosphate and arsenate, and the results obtained were different.

a) PHOSPHATE

The method of continuous variations, the Yoe-Jones Plot and the Harvey-Manning Plot were applied to the phosphate, assuming, as it has already been discussed, that X.O. and Zr(IV) form a 1:2 complex.

FIG. 16

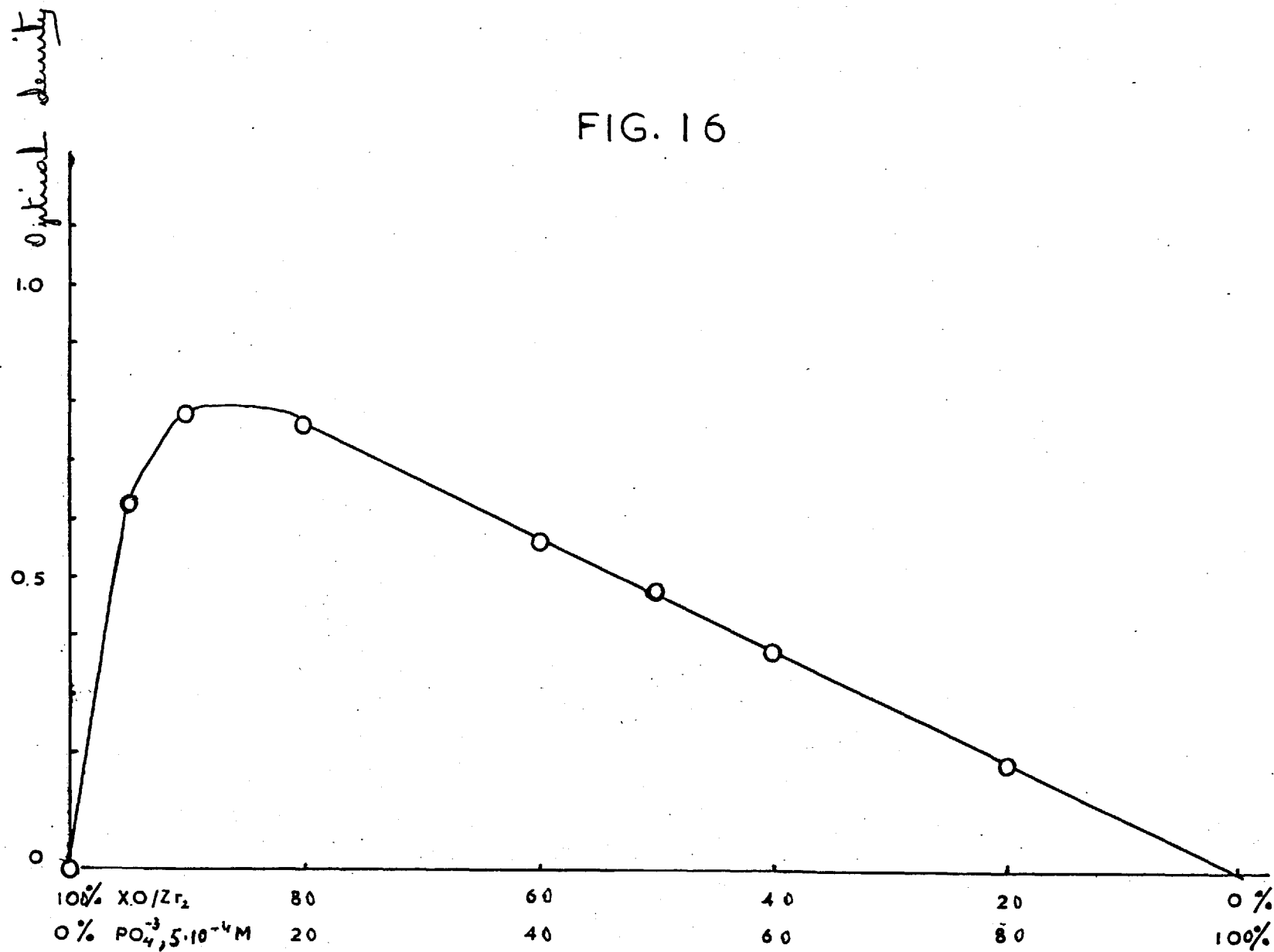


FIG. 17

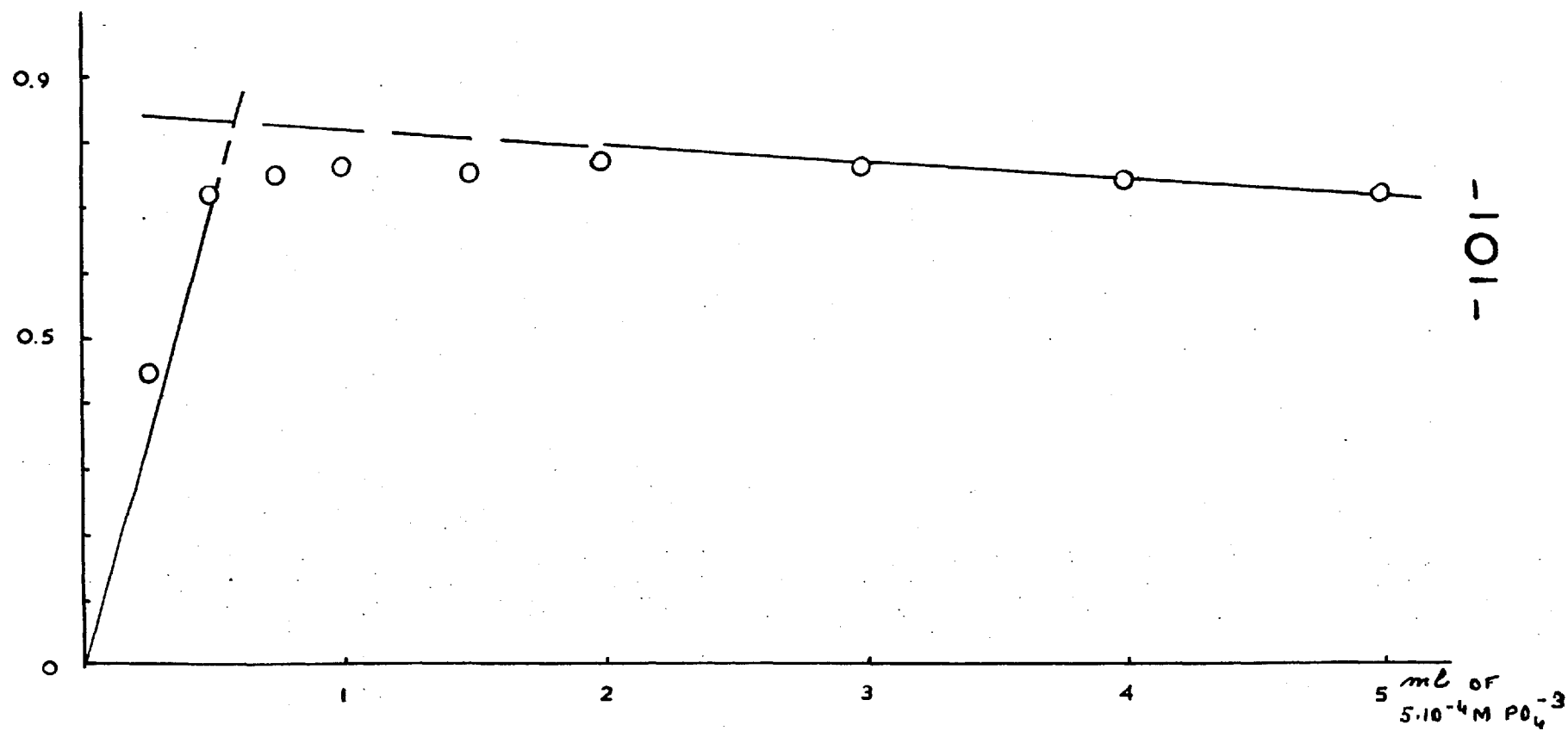
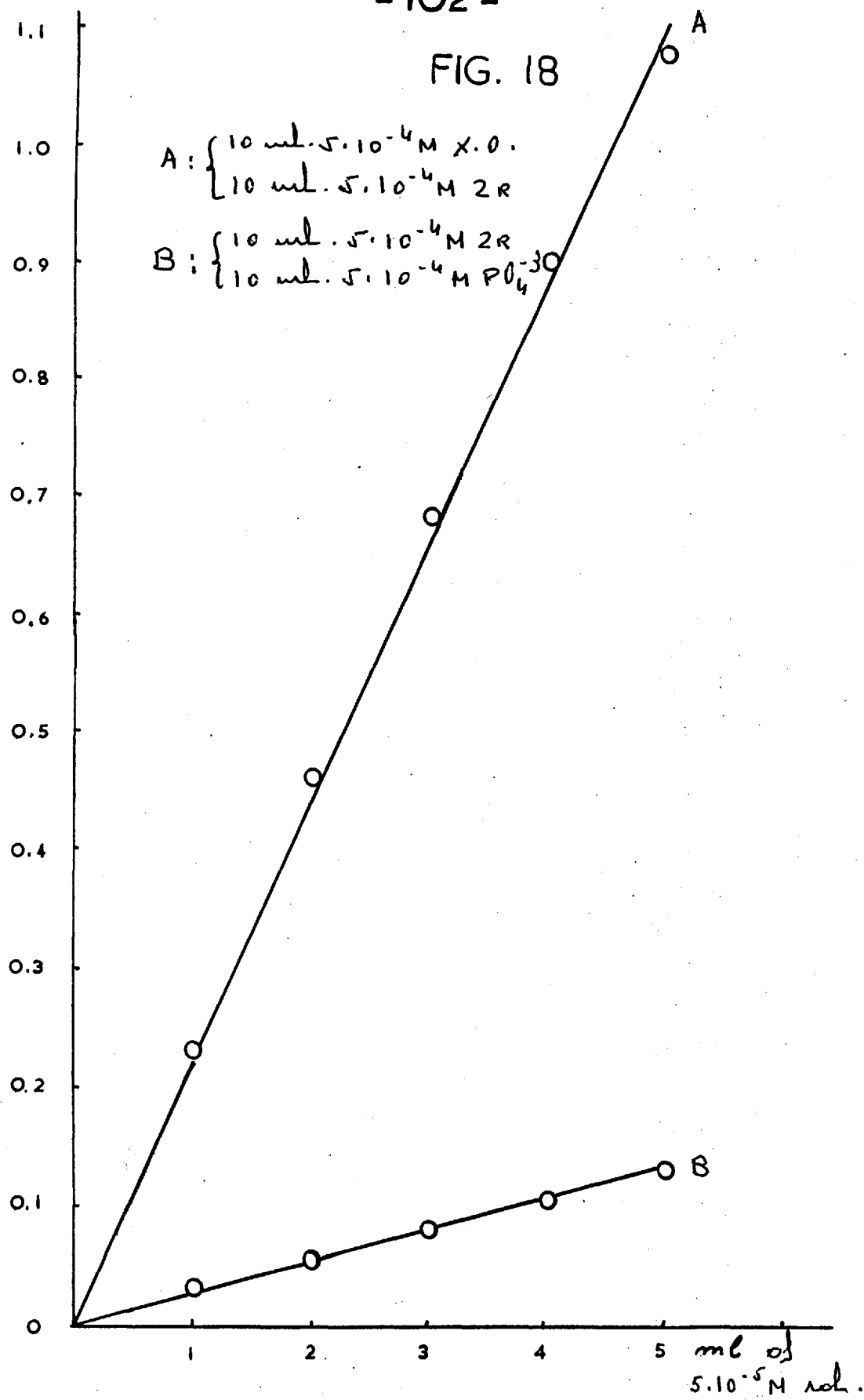


FIG. 18



All the three plots, fig.16, fig.17, fig.18, give evidence at first sight of the formation of a complex with a rather unusual stoichiometry: $X.O.8Zr_{16}P_1$.

Perhaps the least convincing of the three plots, in this case, is the mole-ratio method, but this is due to the high sensitivity of the method for low concentrations of phosphate and the rapid decrease of the sensitivity for slightly higher concentrations.

b) ARSENATE

The above mentioned three methods for the determination of the nature of complexes, were applied to the arsenate and fig.19, fig.20 and fig.21, show that the apparent complex formed has the composition: $X.O.4Zr_8As_1$.

CALIBRATION CURVES

a) PHOSPHATE

A calibration curve was prepared by pipetting 1, 3, 5, ... ml. of $2.5 \cdot 10^{-5} M$ phosphate solution into 100-ml. graduated flasks, followed by 5 ml. of X.O. solution, 7 ml. of 5N HCl and 10 ml. of Zr(IV) solution. The contents of the flasks were diluted to 100 ml. with distilled water and kept in a cool place ($15^{\circ}C$) during ninety minutes. The optical density was then measured at 550 μ . in 1-cm. cells against a blank prepared at the same

FIG. 19

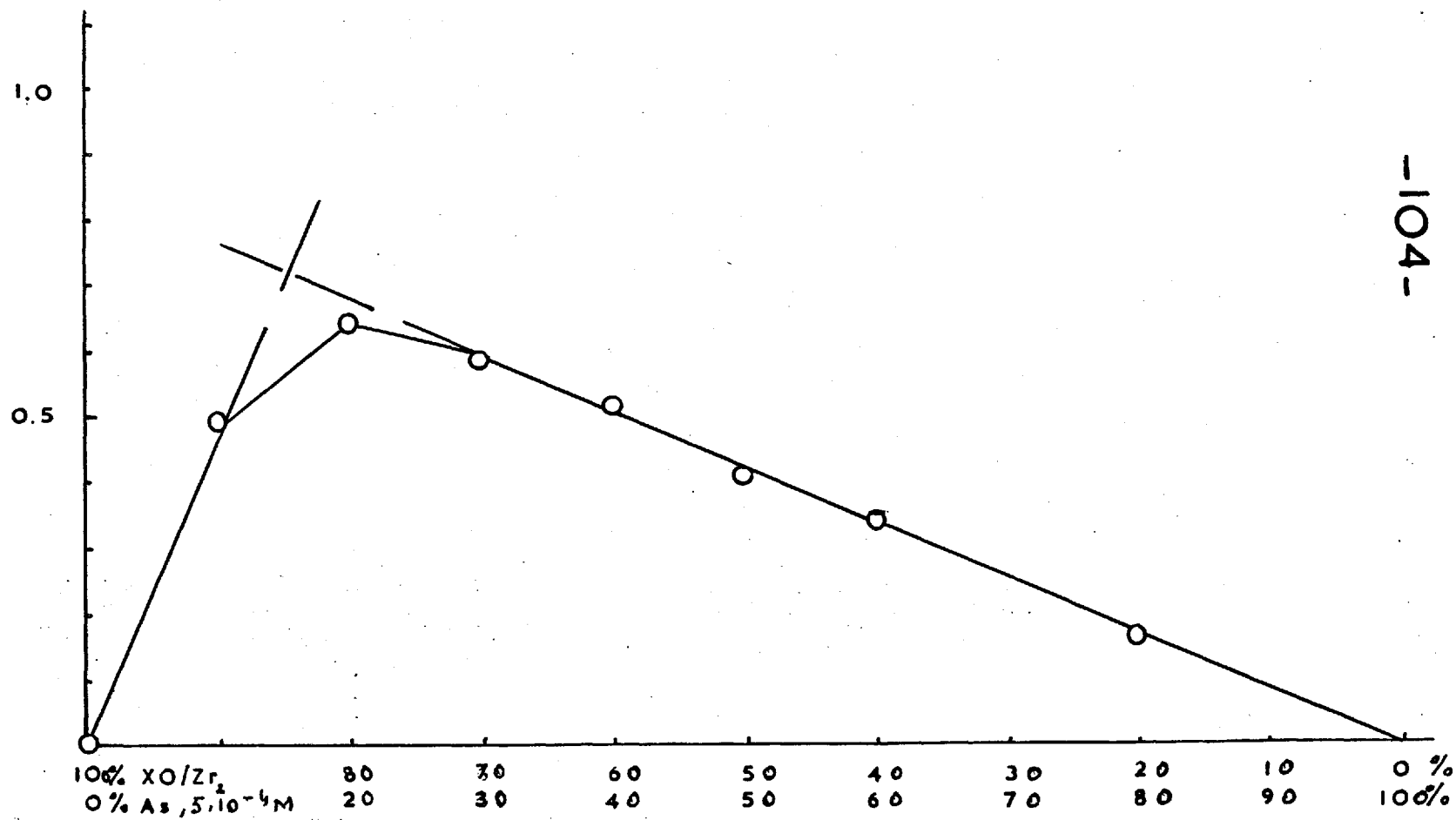


FIG. 20

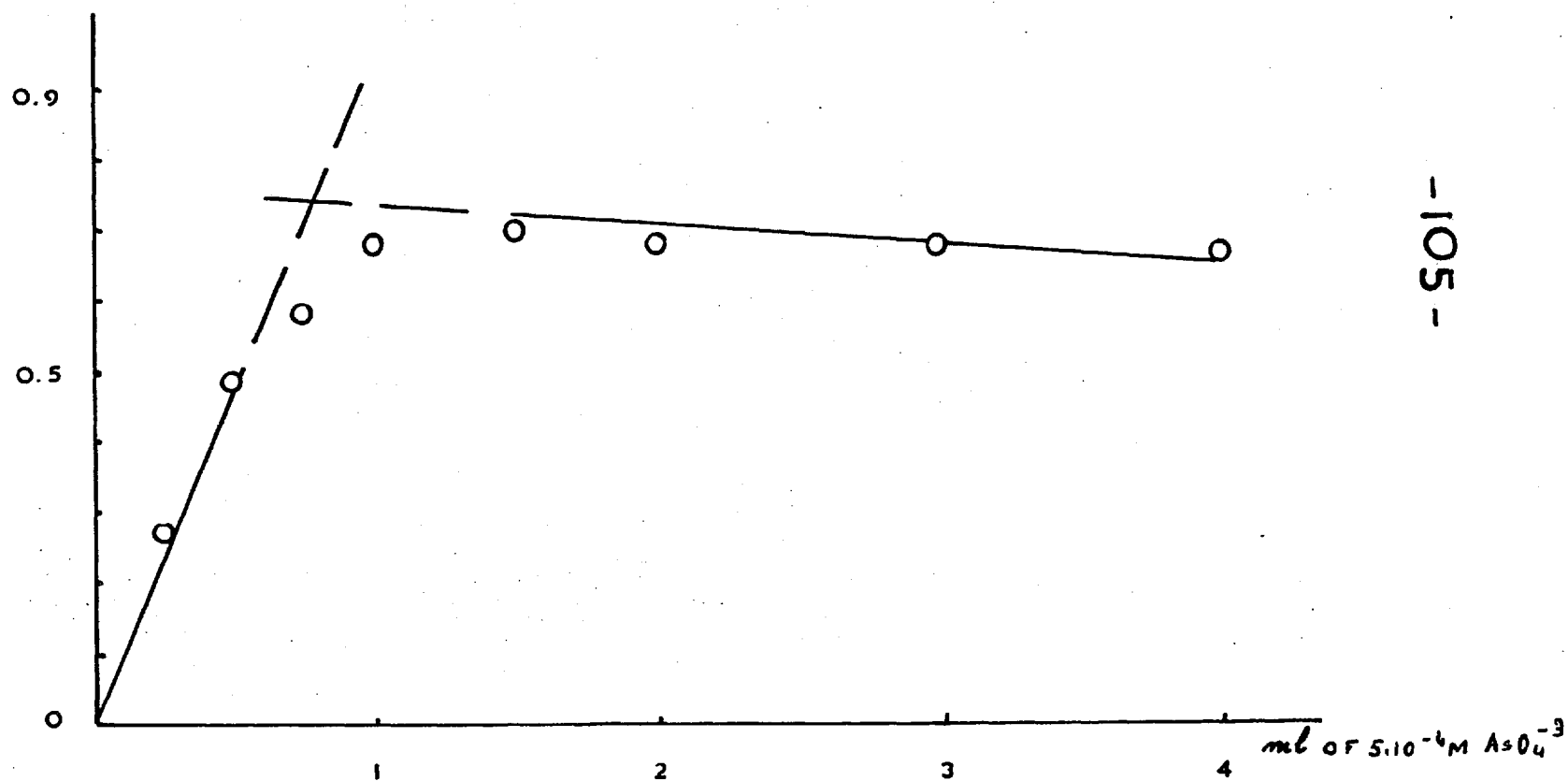
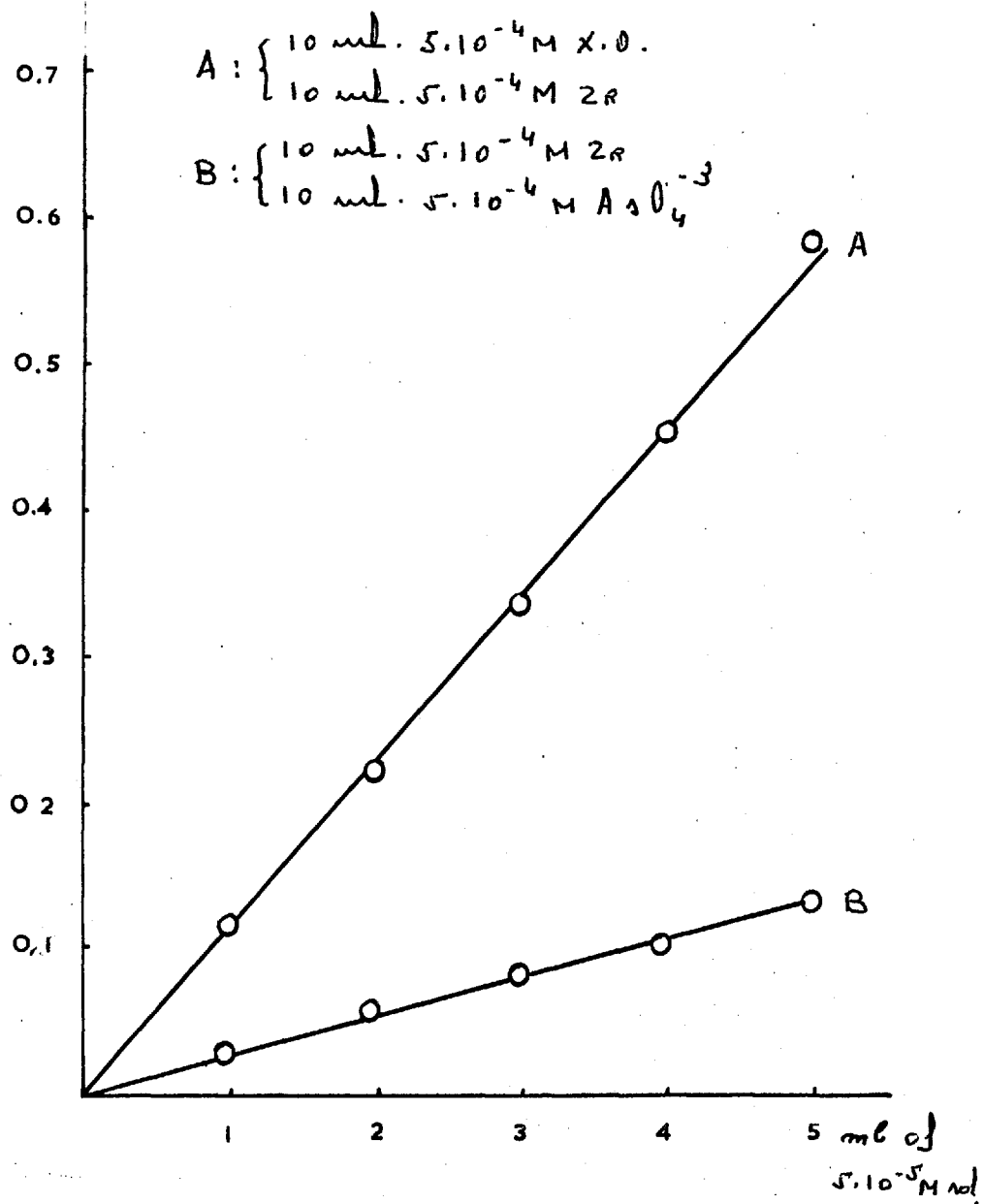


FIG. 21



time and in the same way, but containing no phosphate.

The curve, fig.22, follows Beer's Law from 1 to 6 ml. of $2.5 \cdot 10^{-5} \text{M}$ phosphate, but falls off rapidly for higher concentrations.

b) ARSENATE

1, 3, 5, ... ml. of $2.5 \cdot 10^{-5} \text{M}$ arsenate solution were pipetted into 100-ml. graduated flasks; 5 ml. of X.O. solution, 7 ml. of 5N HCl and 10 ml. of Zr(IV) solution were added; the contents of each flask were then diluted to 100 ml. with distilled water, the solutions were set aside in a cool place during ninety minutes and their optical densities measured at 550 m μ . in 1-cm. cells, against a blank containing no arsenate.

The calibration curve, fig.23, is linear from 1 to 7 ml. and falls off for higher concentrations.

EFFECT OF FOREIGN IONS

The most common cations and anions have been added, generally in 100-fold excess and sometimes in 1000-fold excess, to solutions containing either phosphate or arsenate; the results have been quite satisfactory because it has been found that very few cations interfere and although some anions do, this is not surprising, because these anions are fluoride, sulphate and arsenate or phosphate in the phosphate or arsenate determinations.

FIG. 22

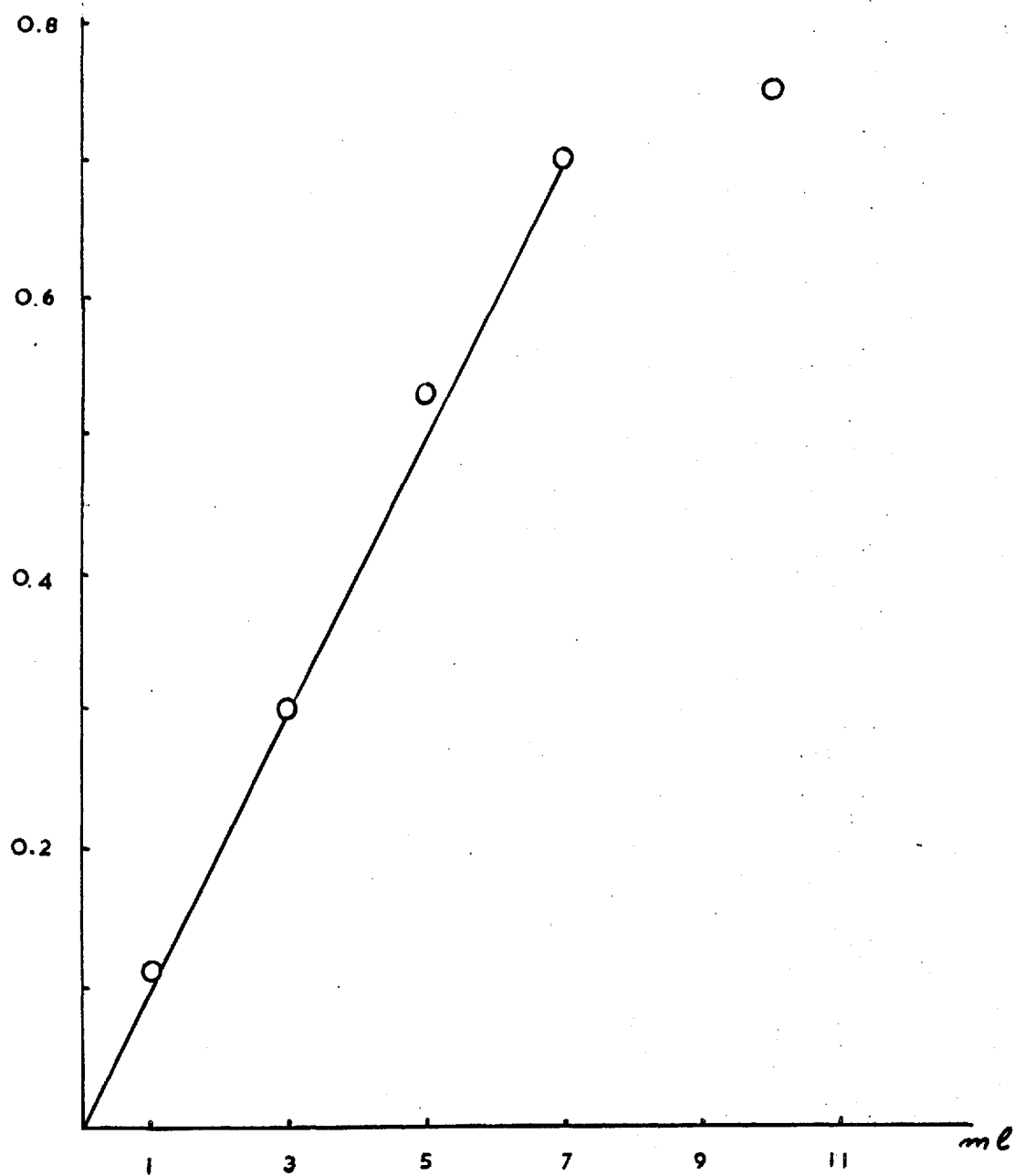
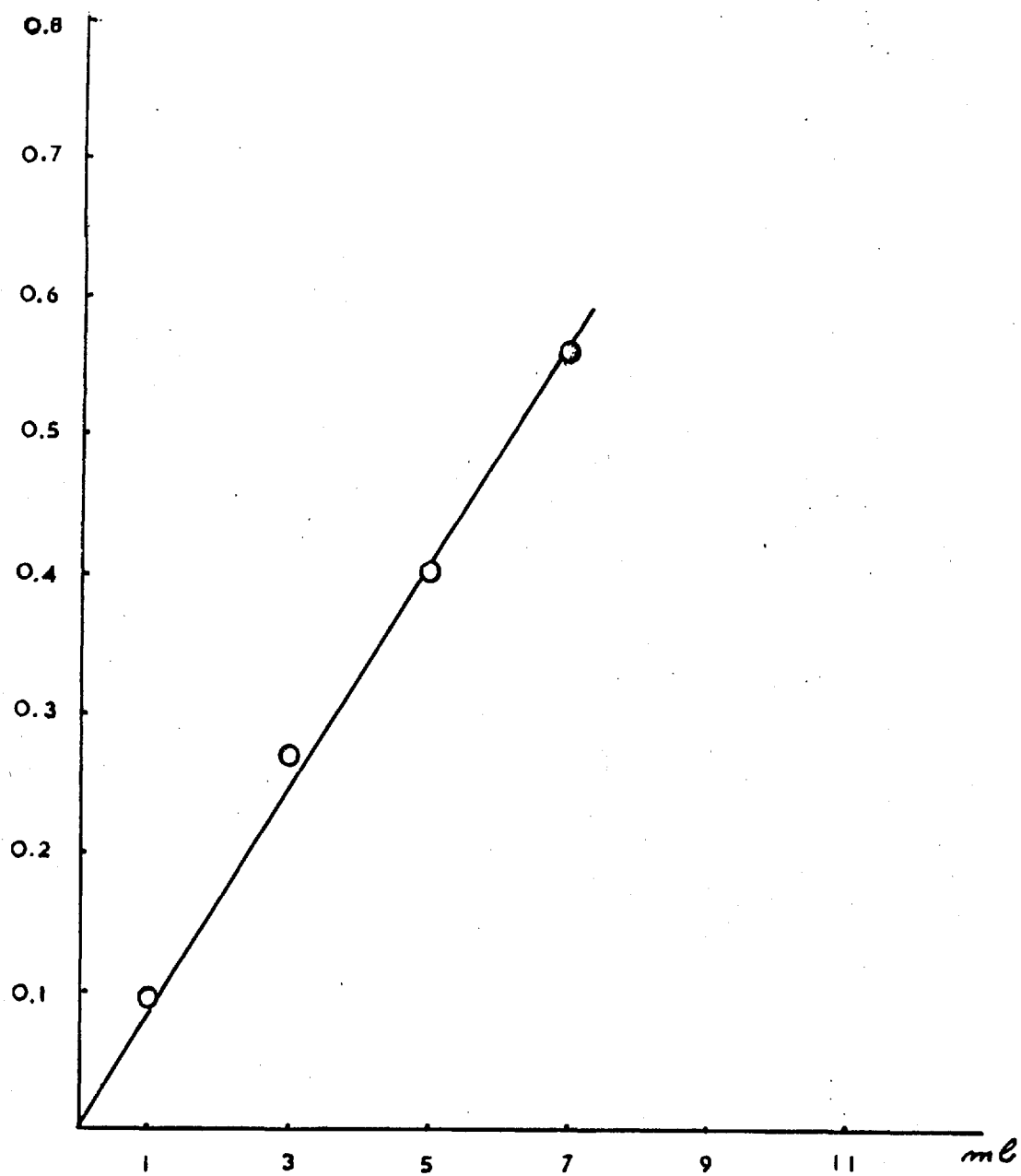


FIG. 23



a) PHOSPHATE

The behaviour of twenty cations and twelve anions has been tested by preparing solutions containing either 100-fold excess, 1000-fold excess or 10-fold excess (as specified in Table VI), of the foreign cation or anion, 10 ml. of $2.5 \cdot 10^{-5} \text{M}$ phosphate solution, 5 ml. of X.O. solution, 7 ml. of 5N HCl and 10 ml. of Zr(IV) solution. The solutions were all diluted to 100 ml. with distilled water. At the same time, a solution not containing any cation or anion, but only phosphate, was prepared in the same way, as well as a blank containing no phosphate. All the solutions were set aside during ninety minutes in a cool place (15°), and the optical density measured against the blank, in 1-cm. cells, at 550 m μ .

The results, in Table VI, show that Fe(II) and Fe(III) are the only serious interferences among the cations, because the slight interference of the Co(II) disappears when Co(II) is present in 10-fold excess.

b) ARSENATE

An analogous experiment to that described above was prepared for the arsenate and the results obtained (Table VII) are very similar.

TABLE VI
EFFECT OF FOREIGN IONS IN THE DETERMINATION
OF PHOSPHATE

<u>ION</u>	<u>OPTICAL DENSITY</u>	<u>ION</u>	<u>OPTICAL DENSITY</u>
PO_4^{-3}	0.820	K(1000).....	0.820
Al(100).....	0.820	$\text{B}_4\text{O}_7^{-3}$ (100).....	0.820
Ba(100).....	0.820	F^- (100).....	bleached
Ca(100).....	0.820	AsO_4^{-3} (100).....	0.850
Cd(100).....	0.815	SO_4^{-2} (100).....	0.850
Co(100).....	0.850	NO_3^- (1000).....	0.820
Co(10).....	0.820	Cl_4^- (1000).....	0.820
Cr(100).....	0.820	Tart.(100).....	0.700
Cu(100).....	0.815	Tart.(10).....	0.820
Fe^{+2} (100).....	0.155	Cit.(100).....	0.740
Fe^{+3} (100).....	0.100	Cit.(10).....	0.820
Hg^{+2} (100).....	0.820	Oxal.(100).....	bleached
Be(100).....	0.820	Oxal.(10).....	0.700
Mg(100).....	0.820	Succ.(100).....	0.820
Ni(100).....	0.815	Acet.(100).....	0.820
Pb(100).....	0.820		
Sr(100).....	0.820		
Tl^{+1} (100).....	0.820		
V^{+5} (100).....	0.815		
Zn(100).....	0.820		
Na(1000).....	0.820		

TABLE VII
EFFECT OF FOREIGN IONS IN THE DETERMINATION
OF ARSENATE

<u>ION</u>	<u>OPTICAL DENSITY</u>	<u>ION</u>	<u>OPTICAL DENSITY</u>
AsO ₄ ⁻³	0.520	V ⁺⁵ (100).....	0.580
Al(100).....	0.520	V ⁺⁵ (10).....	0.520
Ba(100).....	0.520	Zn(100).....	0.520
Ca(100).....	0.525	Na(1000).....	0.520
Cd(100).....	0.520	K(1000).....	0.515
Co(100).....	0.740	B ₄ O ₇ ⁻³ (100).....	0.520
Cr(100).....	0.520	F ⁻ (10).....	0.770
Cu(100).....	0.520	PO ₄ ⁻³ (10).....	0.900
Fe ⁺² (100).....	bleached	SO ₄ ⁻² (100).....	0.850
Fe ⁺³ (100).....	0.580	NO ₃ ⁻ (1000).....	0.520
Hg ⁺² (100).....	0.525	ClO ₄ ⁻ (1000).....	0.520
Be(100).....	0.520	Tart.(100).....	0.390
Mg(100).....	0.525	Cit.(100).....	0.640
Ni(100).....	0.520	Oxal.(100).....	bleached
Pb(100).....	0.520	Succ.(100).....	0.515
Sr(100).....	0.520	Acet.(100).....	0.520
Tl(100).....	0.520		

DISCUSSION

The method here proposed for the determination of phosphate and arsenate is a highly sensitive one, because as ~~it~~ has been said above, the molecular extinction coefficients obtained are 400,000 for the phosphate and 344,000 for the arsenate.

The main advantage of the present phosphate method compared with the amplification methods, recently described in the literature, is its simplicity, because there is no need for solvent extractions, and a determination can be carried out in a very simple way, as has been said above.

Due to the conditions of high acidity in which the X.O.-Zr method has been developed, the determinations can be done in the presence of 100-fold excess of the most common cations. On the other hand, it can be seen that the presence of certain anions leads to wrong results when phosphate or arsenate have to be determined.

However, although the interferences of fluoride, sulphate and arsenate or phosphate are very serious ones, their separation is frequently described in the literature. The best way seems to be by chromatography using as

solvent for the separation of the anions a mixture of 80 ml. of n-butanol, 40 ml. of pyridine, 80 ml. of water and 5 ml. of ammonia (d. 0.88). The values of R_f are then: Sulphate, 0.02; Phosphate, 0.04; Arsenate, 0.07; Fluoride, 0.12.⁸²

The main disadvantage of the presently discussed method is the limited time range in which the optical density of the solutions requires to be measured after their preparation, but the reason for this limited colour stability is that as with the fluoride ion, this is an obvious case of a kinetochromic reaction in which the reagent blank develops the same colour, although much more slowly, under the conditions in which the reaction is carried out, than the solution containing phosphate or arsenate. It seems, therefore, that the reactions are kinetic ones, and that the evidence of the Job's Plots, slope ratio plots and mole ratio plots, must be interpreted in this way rather than in the conventional manner which would tend to interpret them as due to complex formation.

One of the most striking things is the different behaviour of such ~~similar~~ anions as phosphate and arsenate. This different behaviour can be observed in the following facts, that must be pointed out:

a) Whereas the stoichiometry of the apparent complex formed by the phosphate seems to be: $X.O._8Zr_{16}P_1$, the stoichiometry of the complex formed by the arsenate is $X.O._4Zr_8As_1$.

b) The colour starts to develop much more rapidly in the presence of phosphate than in the presence of arsenate.

c) The apparent molecular extinction coefficient for the phosphate is of 400,000 and for the arsenate is of 344,000.

d) When the phosphate is present in a slightly superior amount to the upper limit of the calibration curve, the value of the optical density falls off very quickly whereas this effect is not so remarkable for the arsenate.

e) When large amounts of phosphate are present (100-fold over the upper limit of the calibration curve), the reaction does not take place, the resulting yellow solution being of the same colour as free X.O. in acid solution; but if large amounts of arsenate are present, the reaction still takes place, although with much lower sensitivity than that for submicroamounts.

All the above facts show a profound difference in the behaviour of the two anions and although the "kineto-chromic" theory discussed for the fluoride can be applied here, the different behaviour of the two anions seems a phenomenon rather difficult to explain until further work is done, though the explanation probably relates to the strength of the substoichiometric complexes formed between zirconium and these anions and to their coordinating abilities.

CHAPTER FOUR

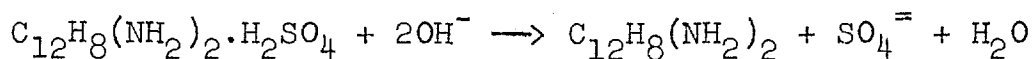
DETERMINATION OF SULPHATE ION BY THE XYLENOL-ORANGE-ZIRCONIUM METHOD.-

In spite of the great importance that the determination of sulphate anion involves, most of the methods described, until now, are not satisfactory for small amounts. They are usually either gravimetric or titrimetric and both of them have many disadvantages especially those involving barium sulphate which is a very insoluble salt and well known for its coprecipitation of cations and anions. Most titrimetric methods are based either on the precipitation of sulphates such as benzidine sulphate or others, which are rather soluble, or which have very difficult end-points. There is only one spectrophotometric method for the determination of sulphate, known at present, and it is an indirect one. More indirect methods based on reduction of the sulphate to sulphide will not be discussed here. But to be able to point out the need for new methods for the determination of sulphate it is necessary, first of all, to review carefully some of the latest methods described in the literature and it will be seen then that further research must be done in this field.

Due to the many sources of error that exist in the

gravimetric method of determining sulphate as barium sulphate many workers have thought that perhaps better results might be obtained by titrimetric techniques.

A rather old method although not very widely used, is the one which determines sulphate by its precipitation as benzidine sulphate, filtration and titration at about 50-75°C with sodium hydroxide using phenolphthalein as indicator⁸³:



As the accuracy of the method depends mainly upon the solubility of the benzidine sulphate and this is rather high (0.098g. per litre at 25°C⁸⁴) the method has not enjoyed much popularity.

The most widely used methods for the determination of sulphate have been those in which the sulphate ion has been titrated with barium chloride using either sodium or potassium rhodizonate or tetrahydroxyquinone as indicator.

The method is based on the fact that if sulphate ion is in the solution, and a barium salt is added, barium sulphate is formed and rhodizonate or tetrahydroxyquinone remains as such, giving to the solution a yellow colour until all the sulphate has been precipitated. When that happens, the excess of barium chloride added forms the

barium salt of the indicator which is orange-red and the end-point of the reaction has been reached. There are a great deal of workers who have used this method^{85,86,87,88} and many others, but the method has not been generally accepted due to the difficulty in indentifying the true end-point, because as the end-point is approached, but before it is reached, there is a localised formation of the red barium salt of the indicator which is dispersed through the solution by stirring. Because of the low concentration of the sulphate when the end-point is approached, the reaction becomes very slow and because there is a gradual change in the colour from yellow to orange throughout the titration the end-point is not indicated by a sudden change and therefore is difficult to recognize unless the solution which is being titrated can be compared with a standard which has the orange-red hue that should be seen when the end-point has just been passed. With this purpose a titration technique has been developed⁸⁹ which not only identifies the end-point colour, but permits the continuous comparison of the colour of the solution with a standard colour filter. This method was applied rather successfully to the determination of sulphur⁹⁰, but as has been said above these methods have neither a sharp end-point nor a great accuracy, therefore, some other indicators

have been investigated such as Alizarin Red S (sodium-3-alizarin sulphonate) and o-(2-hydroxy-3,6-disulpho-1-naphthylazo)-benzene arsonic acid disodium salt most commonly known as Thorin⁹¹.

Alizarin Red S is an acid-base indicator, yellow in acid form and red in the basic form. The first transition range is from pH 3.7 to 5.2. It forms a weak complex with barium, because if diluted Alizarin Red S and barium are mixed and pH is raised, a red colour appears about 0.8 pH unit lower than it would be in the absence of barium. If more concentrated Alizarin Red S is employed, a red precipitate is formed with barium above pH 1.5.

In the sulphate titration Alizarin Red S functions as an adsorption indicator. During the titration barium sulphate is presumably precipitated with sulphate ions adsorbed on the surface, giving the precipitate a negative charge. When the first excess of barium has been added, the precipitate acquires a positive charge due to the barium ions adsorbed on the surface. When this happens the negatively charged indicator ion is attached to the surface, forming a pink barium complex. The result of this mechanism is that when the precipitate is allowed to settle at the end-point the pink colour is all in the precipitate while the liquid is yellow.

In the case of Thorin the colour change in the end-point is from yellow to pink and works in the same way as Alizarin Red S, although the colour change is sharper in the case of the Thorin especially at lower pH values. However, no end-point is observed unless some water-miscible organic solvent is present in the titration. The best solvents seem to be methanol or ethanol and the optimum concentration about 30 to 40%. With less alcohol the end-point becomes less distinctive and if the alcohol concentration is too high, equilibrium is slow, causing false end-points which appear and then slowly fade. The presence of alcohol produces a light, flocculent and gelatinous precipitate in contrast to the fine, crystalline precipitate obtained from aqueous solutions. The optimum pH range for the titration under these conditions is about 2.3 to 3.7, because at lower pH values the end-point is less distinctive and therefore the results are slightly high. It is not convenient to heat the titration solution because this may reduce the surface area of the precipitate, so that the adsorption of the indicator is reduced, or it may "age" the precipitate altering the adsorptive forces at the surface.

For the determination of microamounts of sulphate it is better to titrate it with 0.005M barium perchlorate

using Thorin as indicator. The titration is carried out in 80% alcohol within the pH range 2.5 to 4.0⁹².

The coprecipitation of other ions is one of the main sources of errors in this method; for this reason when the sulphate ion is in the presence of other ions it must be separated from them before the titration, in order to avoid coprecipitation phenomena. The best way seems to be the use of a column of activated alumina in acid solution which will retain the sulphate. The sulphate is then eluted from the column with diluted ammonium hydroxide, passed through a small cation exchange column and then titrated⁹³.

But when Pribil and Maricova⁹⁴ showed that the coprecipitation is prevented in most of the cases if barium sulphate is precipitated in the presence of EDTA, it was thought⁹⁵, that a better and more rapid method for the determination of sulphate might be the dissolution of the precipitate in an excess of standard EDTA, and the back-titration of the excess of EDTA with standard magnesium chloride using Solochrome Black as indicator.

Experiments were made first adding ammoniacal EDTA to standard barium chloride solution and titrating the excess, and as the results were satisfactory, new experiments were then carried out treating the sulphate solution with barium

chloride, filtering the precipitate, dissolving it in an excess of EDTA and titrating the excess as it has been said above, and the results were equally satisfactory. But in further works⁹⁶, the same authors show that even the necessity of dissolving the barium sulphate precipitate can be avoided by titrating the barium remaining in the solution after the precipitation of barium sulphate. As it was known that the titration of EDTA with magnesium gives better results than the reverse, attempts were made to titrate the excess of barium with EDTA so that the end-point was just only passed, and then to determine the slight excess of EDTA by titration with standard magnesium chloride. Unfortunately, unsatisfactory results were obtained unless a great excess of EDTA was added after the end-point of the barium titration and this excess caused the dissolution of part of the barium sulphate precipitate, so that inaccurate results were obtained. It was decided therefore that the barium sulphate precipitate should be removed from the solution, before the titration was performed and in this case, satisfactory and accurate results were obtained. This last procedure has been applied by the same authors to the determination of sulphur in steels⁹⁷, in organic compounds⁹⁸, and by Lewis⁹⁹ with slight modifications to the determination of inorganic and total sulphate in urine, and it has given good results.

Belcher, Nutten and Stephen¹⁰⁰ prepared the sulphates of the 4-amino-4'-halogenodiphenyls, determined their solubilities in water and discovered that 4-amino-4'-chlorodiphenyl had the least soluble sulphate, it was thought that this could be a new reagent for the alkali-metric determination of sulphate. The determination was carried out in the following way: sulphate was precipitated with 4-amino-4'-diphenylhydrochloride, filtered, washed and transferred to the precipitation vessel. The contents of the vessel were boiled for one minute, three or four drops of a mixed indicator formed by aqueous phenol-red (sodium salt) and aqueous bromothymol-blue (sodium salt), were added and the solution was titrated with standard alkali to the first purple colour in the solution. The solution was then re-heated and the titration continued until the first permanent purple tinge was obtained. The first false end-point is due to the fact that filter paper retains a small amount of precipitate and therefore, the first end-point is false; however, it is a good guide to show the nearness of the real end-point.

In more recent work¹⁰¹, the same authors study the possibility of using some salts other than the hydrochloride of 4-amino-4'-chlorodiphenyl, as reagents for the determination of sulphate but none of them had any advantage.

In the same paper, the authors study the optimum excess of reagent necessary for the best precipitation of sulphate and the optimal conditions for the determination of higher amounts of sulphate and the elimination of the most undesirable interferences.

The 4-amino-4'-chlorodiphenyl has also been studied from the spectrophotometric~~a~~ point of view¹⁰² and it has shown that there is a strong absorption band in the ultra-violet region exhibiting maximum absorption at 254 mμ with a molecular extinction coefficient of 22.090. This property suggested the idea of the spectrophotometric determination of sulphate avoiding the errors due to the titrimetric methods and achieving an enhancement in the sensitivity because it would allow the determination of 30 to 120 μg. of sulphate. In order to obviate errors due to the washing of the precipitate, a difference method was used in which the amount of reagent remaining in the solution after the precipitation of the sulphate was determined.

To determine inorganic sulphate, an excess (at least 100%) of 4-amino-4'-chlorodiphenyl in 0.1 N HCl was added to the sulphate solution at pH 2 to 7, and the precipitate was removed by centrifugation; the reagent remaining in the solution was determined spectrophotometrically and the amount of sulphate deduc~~ed~~ed by difference.

This last method has the advantage of being the only spectrophotometric one, until now, but the results cannot be of a great accuracy considering the fact that the 4-amino-4'-chlorodiphenyl sulphate has an appreciable solubility and therefore, the errors due to the partial solubility are higher when lower concentrations have to be determined.

The method described in the present chapter, is based on the same principle as the determination of fluoride, phosphate and arsenate described in previous chapters.

Although the method applied to the sulphate does not have such a high sensitivity as it has for the other anions, it is most interesting because as has been said above the only spectrophotometric~~ai~~ method available, at present, is a differential one. Therefore, it can be said that the X.O.-Zr(IV) method is the first one in which sulphate ion gives a positive coloured reaction which can be used for its spectrophotometric determination, giving a molecular extinction coefficient of 22.000 and being relatively free from cationic interferences.

EXPERIMENTAL:-

APPARATUS:-

Beckmann DB recording spectrophotometer operated with 1-cm. cells.

REAGENTS:-

5.10^{-4} M sodium sulphate solution:-

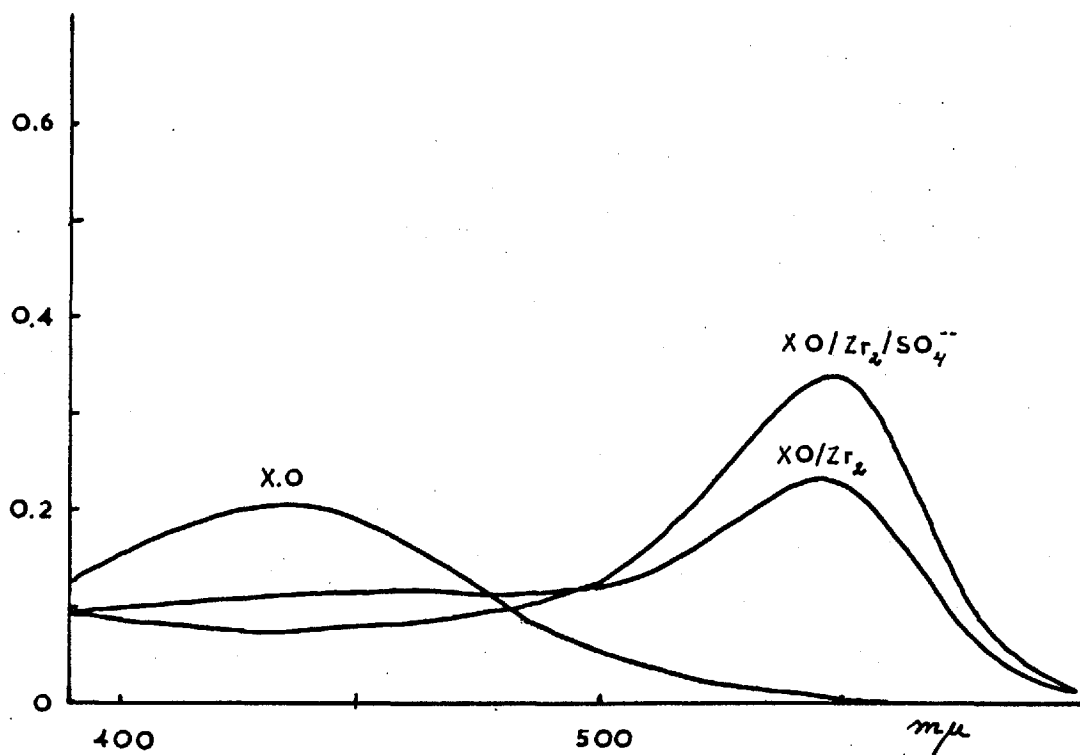
Weigh and dissolve 0.7103 g. of AnalaR sodium sulphate and dilute to 500 ml. with distilled water in order to make a 10^{-2} M solution. Take 50 ml. of this solution and dilute them to exactly 1 liter to make a 5.10^{-4} M solution.

The other reagents used are the same that have been used in the fluoride, phosphate and arsenate determinations.

ABSORPTION SPECTRA:-

After the results obtained for fluoride, phosphate and arsenate, which have already been described in chapters II and III of the present thesis, no complex formation between X.O.-Zr₂ complex and sulphate ion was expected. To ensure this, three solutions were prepared, as in former cases: A first one containing 2.5 ml. of X.O. sol. and 7 ml. of 5 N HCl, a second solution containing 2.5 ml. of X.O. sol., 5 ml. of Zr(IV) sol. and 7 ml. of 5N HCl and a third one

FIG. 24



with 2.5 ml. of X.O. sol., 5 ml. of Zr(IV) sol., 2.5 ml. of 5.10^{-4} M sulphate sol. and 7 ml. of 5N HCl. The contents of all three flasks were diluted to 100ml. with distilled water, and the spectra run, against distilled water, in 1-cm. cells, after the solutions had been set aside for ninety minutes in a cool place.

As was expected and can be seen in fig. 24, the spectra show that no new complex is formed and although the colour of the $X.O.Zr_2$ complex is developed more rapidly when sulphate ion is present, the kinetic effect of this anion is not so marked as the kinetic effects of fluoride, phosphate and arsenate. This fact will be confirmed when by the calibration curve can be seen that the molecular extinction coefficient is only 22.000 compared with the previously obtained values of 200.000, 400.000 and 344.000, respectively.

EFFECT OF THE HYDROCHLORIC ACID CONCENTRATION IN THE SOLUTIONS.

In previous parts of this thesis it was said that although the optical density of a solution containing $X.O.Zr_2$ complex and 10 ml. of $2.5.10^{-5}$ M sol. of phosphate or arsenate, remains constant for a rather wide range of acidity, the calibration curves could only be successfully obtained when the solutions were 0.35 N to HCl. Therefore,

TABLE VIII
EFFECT OF HCl CONCENTRATION

<u>N of Acid</u>	<u>ml. of sol.</u>	<u>Optical Density</u>
0.1.....	1	0.18
0.1.....	2	0.25
0.1.....	3	0.25
0.1.....	4	0.25
0.1.....	5	0.25
0.2.....	1	0.145
0.2.....	2	0.310
0.2.....	3	0.390
0.2.....	4	0.470
0.2.....	5	0.490
0.3.....	1	0.100
0.3.....	2	0.205
0.3.....	3	0.310
0.3.....	4	0.420
0.3.....	5	0.550
0.4.....	1	0.115
0.4.....	2	0.245
0.4.....	3	0.350
0.4.....	4	0.460
0.4.....	5	0.550
0.5.....	1	0.190
0.5.....	2	0.350
0.5.....	3	0.400
0.5.....	4	0.490
0.5.....	5	0.520

to avoid possible errors, it was decided that an experiment should be prepared in which the variation of the optical density of the calibration curve with the acidity could be observed instead of the variation of the optical density of one solution with a determined concentration.

Several sets of solutions were prepared, therefore, by pipetting 1,2,3..... ml. of $5 \cdot 10^{-4}$ M sulphate solution into 100-ml. graduated flasks, followed by 5 ml. of X.O. sol., 10 ml. of Zr(IV) sol. and different amounts of 5 N HCl sol. in each set of solutions in order to make the sets 0.1, 0.2..... N to HCl. The contents of all the flasks were diluted to 100 ml. with distilled water, and the optical density of the solutions was measured in 1-cm. cells, at 550 m μ ., against a blank of the same acidity but containing no sulphate, after the solutions had been set aside during ninety minutes in a cool place.

The results in Table VIII, show that the optimum acidity for the development of the method is, as it was in the determination of the other anions, 0.35-0.40 N to HCl.

EFFECT OF TIME.

A calibration curve was prepared containing 1,2,3..... ml. of $5 \cdot 10^{-4}$ M sulphate solution, 5 ml. of X.O. sol., 10 ml. of Zr(IV) sol. and 7 ml. of 5 NH HCl sol. The contents of

TABLE IX
EFFECT OF TIME

<u>TIME</u>	<u>ml. of sol.</u>	<u>OPTICAL DENSITY</u>
30 min.	1	0.06
30 "	2	0.115
30 "	3	0.12
30 "	4	0.15
30 "	5	0.19
60 "	1	0.09
60 "	2	0.19
60 "	3	0.28
60 "	4	0.39
60 "	5	0.53
75 "	1	0.105
75 "	2	0.210
75 "	3	0.335
75 "	4	0.450
75 "	5	0.550
90 "	1	0.100
90 "	2	0.220
90 "	3	0.340
90 "	4	0.440
90 "	5	0.550

the flasks were diluted to 100 ml. with distilled water and the optical density of the solutions measured over a period of time, in 1-cm. cells, at 550 mμ., against a blank prepared in the same way but containing no sulphate.

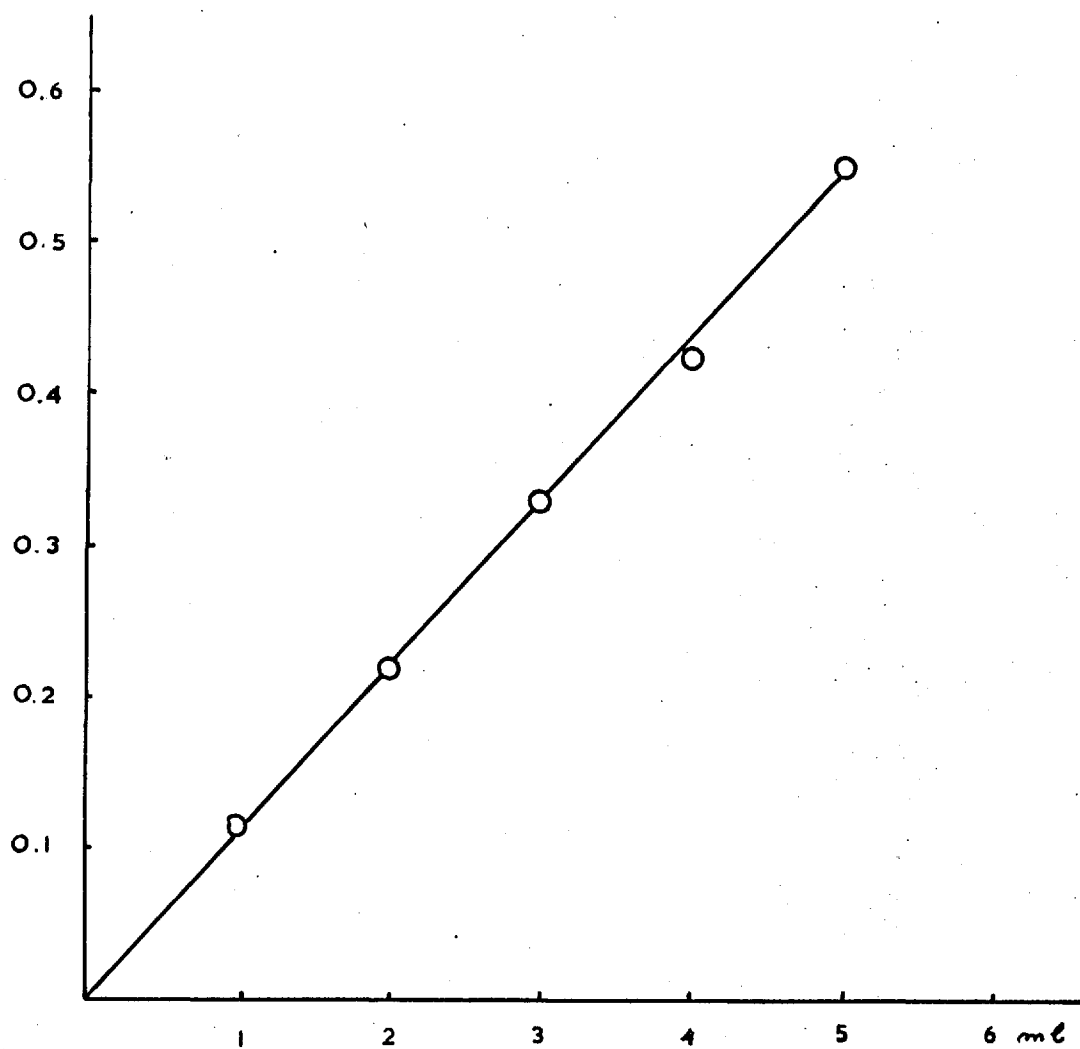
It can be seen, in Table IX, that the calibration curve reaches its optimum value 75 minutes after the solutions have been prepared and although no measurements have been done after 105 and 120 minutes, it can be predicted by the similar behaviour, in this respect, of sulphate and phosphate and arsenate, that the measurements can be carried out between 75 and 120 minutes after the preparation of the solutions.

CALIBRATION CURVES.

Although it can be seen in Table VIII, that the optimum acidity for the development of the method is 0.35 N, it must be pointed out that it seemed, in Table VIII too, that for an acidity of 0.2 N, Beer's law is followed for the lower values of the prepared calibration curve, with a molecular extinction coefficient (31.500) higher than for the acidity of 0.35 N.

Therefore, two calibration curves were prepared. In the first one, 1, 2, 3 ml. of 5.10^{-4} M sulphate sol. were pipetted into 100-ml. graduated flasks, followed by

FIG. 25



5 ml. of X.O. sol., 10 ml. of Zr(IV) sol. and 7 ml. of 5N HCl. The contents of the flasks were diluted to 100 ml. with distilled water and the optical density measured in 1-cm. cells, at 550 mμ. after the solutions had been set aside in a cool place, for 75 minutes. Beer's law is followed from 1 to 5 ml. of the sulphate solution and the molecular extinction coefficient is of 22.000. (Fig. 25).

A second calibration curve was prepared, by pipetting 0.5, 1, 1.5 ml. of 5.10^{-4} M sulphate solution into 100-ml. graduated flasks, followed by 5 ml. of X.O. sol., 10 ml. of Zr(IV) sol. and 4 ml. of 5 N HCl sol. All the solutions were diluted to 100 ml. and the value of the optical density measured, as usual, 75 minutes after the preparation of the solutions. The results, in Table X, show that Beer's law is followed from 0.5 to 2.5 ml. of sulphate solution and that the molecular extinction coefficient is 31.500.

Although the value of the molecular extinction coefficient is higher in the second calibration curve, the limited range of concentration in which sulphate can be measured and the lower acid concentration makes preferable to use the first calibration curve for the determination of sulphate.

TABLE X
CALIBRATION CURVE FOR LOW AMOUNTS OF SULPHATE

<u>ml. of solution</u>	<u>Optical density</u>
0.5	0.075
1.0	0.155
1.5	0.230
2.0	0.315
2.5	0.370
3.0	0.410

EFFECT OF FOREIGN IONS.

As was done in the determination of the other anions, the behaviour of several cations and anions was observed when they were in the presence of a solution containing X.O.Zr₂ complex and 3 ml. of 5.10⁻⁴ M sulphate sol., prepared as usual. The ions were usually in 100-fold molar excess over the amount of sulphate and, as shown in Table XI, Ca, Co, Fe(III), Be, Ni, Tl(I), V(V) cause positive interferences among cations, with Fe(II) yielding the only negative one. The interferences of Be and Ni disappear when they are present in 10-fold excess.

It can be observed, that among the anions, apart from the expected interferences of fluoride, phosphate and arsenate, the only interferences are the negative ones due to the organic anions tartrate, citrate and oxalate.

TABLE XI
EFFECT OF FOREIGN IONS

<u>Ion</u>	<u>OD</u>	<u>Ion</u>	<u>OD</u>
SO ₄ ⁼	0.35	V ⁺⁵ (100)	0.54
Al(100)	0.345	Zn(100)	0.35
Ca(100)	0.540	Na(1000)	0.35
Cd(100)	0.350	K(1000)	0.35
Co(100)	0.520	B ₄ O ₇ ⁻³ (100)	0.35
Co(10)	0.400	F ⁻ (10)	0.15
Cr(100)	0.350	AsO ₄ ⁻³ (10)	0.55
Cu(100)	0.345	PO ₄ ⁻³ (10)	0.55
Fe ⁺² (100)	Bleached	NO ₃ ⁻ (1000)	0.35
Fe ⁺² (10)	0.25	ClO ₄ ⁻ (1000)	0.35
Fe ⁺³ (100)	0.46	Tart.(100)	0.00
Fe ⁺³ (10)	0.38	Ci.(100)	0.15
Hg ⁺² (100)	0.35	Oxa.(100)	Bleached
Be(100)	0.46	Succ.(100)	0.35
Be(10)	0.35	Acet.(100)	0.35
Mg(100)	0.35		
Ni(100)	0.49		
Ni(10)	0.35		
Sr(100)	0.35		
Tl ⁺¹ (100)	0.40		

DISCUSSION

The main merit of the method described above for the determination of sulphate is that it appears to be the first positive spectrophotometric one which has been found so far. When it is said that this method is the first positive spectrophotometric ^{to} ~~appeared~~, one bears in mind the one in which an excess of 4-amino-4'-chlorodiphenyl hydrochloride is used to precipitate sulphate, and the excess of reagent (not precipitated by the sulphate) is measured spectrophotometrically in the ultra-violet region. As was already said, this latter method has the disadvantage of the solubility of the sulphate of 4-amino-4'-chlorodiphenyl which can lead to errors when submicroamounts of sulphate have to be determined.

It was rather disappointing to find that the $X.O.Zr_2$ method, is much less sensitive for sulphate than for all the other anions previously determined. This fact was first noticed, when the spectra of $X.O.Zr_2$ complex in the presence of all the anions were run and was confirmed when the values of the different molecular extinction coefficients were compared.

However, if one considers the need for spectrophotometric methods for the determination of sulphate, it can

be said that the presently described method is a valuable contribution.

When a Job's plot, slope-ratio plot and mole-ratio plot were applied to show that as in the other cases, the kinetic reaction follows a definite pattern, rather confusing results were obtained. It seems by Job's plot that the pattern followed is $X.O.Zr_2(SO_4)_2$ which agrees with the low sensitivity of the present reaction compared with the high ones obtained for the other anions and with the high value of the optical density when large amounts of sulphate ion are present in the $X.O.Zr_2$ complex.

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