THE DETERMINATION OF TRACES

OF INORGANIC ANIONS BY

KINETOCHROMIC SPECTROPHOTOMETRY

by

R.V. HEMS, B.Sc., (LONDON)

A thesis submitted for the degree of

Doctor of Philosophy of the University of

London

Department of Chemistry,
Imperial College of Science & Technology,
London, S.W.7.   August, 1969

Abstract

The effect of several inorganic anions on the reaction between polymerised zirconium solutions and various colorimetric and fluorimetric reagents has been investigated, and is shown to be purely kinetic. The nature of the reaction, termed "kinetochromic spectrophotometry", is explained, and compared with conventional absorption spectrophotometry.

The effect of sulphate on the zirconium-Methylthymol Blue reaction has been used as the basis for a direct spectrophotometric procedure for the determination of sulphate. The calibration graph is linear over the range 0.1-2.4 ppm (10-240 µg) of sulphate, with an effective molar absorptivity at 586 nm of 2.0×10^4. The development time is 60 minutes.

A procedure for the determination of fluoride has been developed based on the kinetochromic effect of this anion on the zirconium-Methylthymol Blue reaction. The method is highly sensitive for fluoride ion, the calibration range extending from 0.25-4.75 µg (0.005-0.095 ppm) of fluoride. The effective molar absorptivity for fluoride at 586 nm after 60 minutes development is 3.2×10^5.

The use of the fluorimetric indicator Morin in kinetochromic spectrophotometry has been studied, with sulphate as the catalyst. A procedure for the fluorimetric determination of sulphate was

developed, with a linear calibration curve extending from 2×10^{-5} $- 10^{-4}$M sulphate (2-9.6 ppm) after 60 minutes development.

Calcein Blue has been shown to be a highly sensitive fluorimetric reagent for the determination of zirconium in aqueous solution. Application of the recommended procedure gives a linear calibration curve extending from 20-100 ng of zirconium (0.0002-0.001 ppm).

<u>ACKNOWLEDGEMENTS</u>

The work described in this thesis is entirely original except where due reference has been made. No part of the work has been previously submitted for any other degree. The work was carried out in the Chemistry Department, Imperial College of Science and Technology, between October 1966 and August 1969.

I wish to express my thanks to my supervisors, Professor T.S. West and Dr. G.F. Kirkbright, for their invaluable assistance, guidance and encouragement during the course of this work, and my research colleagues for helpful discussions.

I am deeply indebted to the Ministry of Technology for their financial support and for the award of a special grant (to Professor T.S. West) for the purchase of the recording spectrophotometer used in this work.

R.V. Hems

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Preface

The methods described in this thesis for the spectrophotometric determination of sulphate and fluoride ions are significant in that they are direct. They are based on the effect of these anions on the slow reaction which normally exists between slightly aged aqueous solutions of zirconium (IV) and the metallochrome Methylthymol Blue.

The vast majority of spectrophotometric methods which already exist for the determination of trace amounts of fluoride or sulphate ion in aqueous solution, however, are indirect. They frequently depend on the bleaching action of these anions on a soluble coloured complex of an organic reagent with a metal ion which forms a stable sulphate or fluoride complex or insoluble salt.

These procedures are well known and often show appreciable selectivity and sensitivity. The methods to be described in this thesis, however, show considerably better sensitivity, particularly that for fluoride ion, while still retaining appreciable selectivity. In order that these procedures may be more easily compared with existing methods, a brief review of these methods is presented here.

1.2 Analytical methods for the determination of traces of sulphate

Even though great importance is attached to the determination of

sulphate anion in aqueous solution, most of the available methods are not satisfactory for small amounts. They are usually gravimetric or titrimetric techniques which have many disadvantages, particularly those which involve the precipitation of barium sulphate. Barium sulphate is an appreciably soluble salt and known to coprecipitate other cations and anions.

Several spectrophotometric procedures have been reported which, although still having the disadvantages of the precipitation of (usually) barium sulphate, enable much smaller quantities of sulphate to be determined.

We can classify the most widely used methods for the determination of sulphate as follows: (1) Gravimetric (2) Turbidimetric and Nephelometric (3) Titrimetric and (4) Spectrophotometric.

(i) Gravimetric Methods

Precipitation as barium sulphate is used extensively for the gravimetric determination of sulphate.[1] The precipitant is generally a solution of barium chloride and the precipitation is effected from a solution which is about 0.05N with respect to hydrochloric acid. The presence of this small amount of hydrochloric acid prevents the precipitation of the barium salts (notably carbonate and phosphate), and the coprecipitation of barium hydroxide, and assists the formation of a more easily filtered precipitate. The white crystalline precip-

itate of barium sulphate is filtered, washed, ignited and weighed.

The greatest source of error in this procedure is the tendency of barium sulphate to carry down and retain the constituents of both the solvent and precipitant, and the recommended experimental conditions must be carefully followed. The most common anionic interference is the nitrate ion, which must be removed by repeated evaporation with hydrochloric acid. Other anions such as bromate, ferrocyanide and chlorate also interfere by coprecipitation. [2,3] Ethylenediamine-tetra-acetic acid has found widespread use [4,5] as a complexing agent for the removal of interference from many cations in the determination.

Several methods have been proposed for facilitating the filtration of barium sulphate precipitates [6,7] while filtration with organic colloids such as starch [8] or an ethereal solution of pyroxylin [9] have also proved useful. More recently, several attempts [10-14] have been made to obtain a constant and accurate weight of barium sulphate precipitate without resource to ignition, i.e. by drying the precipitate with alcohol or at temperatures of 110°C. or higher. The most recent work is that of Nishimura [15] who demonstrated that barium sulphate can be weighed accurately after air drying the precipitate after washing with alcohol and ether at room temperature.

As an illustration of the continued viability of the gravimetric procedure for the determination of sulphate, Azeem [16] recently deter-

mined sulphate in the presence of soluble silicate gravimetrically as barium sulphate after the addition of dimethylformamide to complex the silicate.

Other methods for the gravimetric determination of sulphate include the use of the complex hexammino cobaltic bromide [17] and of octammino-μ-amino-μ-nitro-dicobaltic nitrate [18]; these reagents have not come into general use.

In general, gravimetric methods, though very accurate for large amounts of sulphate, are tedious and time-consuming, and are of limited use when trace amounts are to be determined.

(ii) <u>Turbidimetric and Nephelometric Methods</u>

Small amounts of some insoluble compounds may be prepared in a state of aggregation such that moderately stable suspensions are obtained. When light is passed through the suspension, part of the incident beam is dissipated by absorption, reflection and refraction. Measurement of the intensity of the remaining transmitted light can be used for determining the concentration of the suspension, and is the basis for turbidimetric analysis. In nephelometry, the light scattered by the particles of the suspension is measured directly at an angle (usually $90°$) to the incident beam.

The rather low solubility of barium sulphate, particularly in mixed solvent systems of water and polyhydric alcohols, has an obvious

application in turbidimetry and nephelometry, and numerous such
procedures are to be found in the literature.

The basic method was given by Denis and Reed [19] who determined
1-10 ppm of sulphate ion in a 25 ml. sample solution. This method has
been modified by later workers, such as Toennies and Bakay [20] who
precipitated the barium sulphate in 18 per cent ethanol – 22 per cent
propylene glycol. Their method is adaptable to sulphate concentrations
ranging from 0.01 to 100 ppm. Other workers who employed partial non-
aqueous media in order to obtain uniformity of particle size include
Takiyama and Suito [21] who employed an ethanol-gelatine mixture, Omiti
[22] (glycerol-ethanol) and Volmer and Frohlich [23] (glycine-gum arabic).
Barium acetate is an appropriate reagent for development of turbidity
[24] while an appropriate wavelength for reading turbidity is 380 nm [25].
The turbidimetric method has recently been applied to the determination
of sulphur in plastics [26] and in air particles [22] as sulphate. The
tradional use of additives in order to stabilise the suspension formed
has. recently been deprecated by Wimberley [27] who was able to determine
10-100 ppm of sulphate with a linear calibration without the use of
any additives.

Until quite recently, very little attention had been given to the
replacement of barium by other precipitants for the sulphate ion in
these turbidimetric and nephelometric procedures. Benzidine and its
substitution products are not as sensitive a precipitant for the

sulphate ion as is barium chloride. However, further work by Belcher and his co-workers [28] found that the precipitant 4-amino-4'-chloro-diphenyl (CAD) was an excellent reagent for sulphate and it has since found considerable application. Haslam, Hamilton and Squirrel [26] recommended a turbidimetric CAD-hydrochloride method as an alternative to the standard barium chloride method, particularly when only trace amounts of sulphur are to be detected. The suspension of CAD sulphate is stabilised by the presence of gum ghatti and peptone in the reagent solutions, and the absorbance of the suspension is measured after 30 minutes at 700 nm, a considerable improvement on the development times of two hours or more often employed. Martin and Stephen [29] developed a nephelometric procedure by measurement of CAD sulphate suspensions, stabilised by gum ghatti. They were able to determine as little as 0.025-0.25 mg. of sulphate in 10 ml. volumes of test solution (i.e. 2.5-25 ppm of sulphate), and by the use of a modified gum ghatti solution extended the lower limit of the calibration range to 1-25 ppm [30].

In general, however, nephelometric and turbidimetric methods for the determination of sulphate are time-consuming, tedious and insensitive and very prone to errors from various sources. By far the most widely used techniques for sulphate are titrimetric procedures, involving either a visual end-point or a colorimetric one.

(iii) <u>Titrimetric Methods</u>

Titrimetric procedures almost invariably involve titration of the sulphate ion by a soluble barium salt. When a standard solution of barium chloride is used, the indicator employed is either sodium or potassium rhodizonate or tetrahydroxy-quinone.

The method depends on the fact that when a barium salt titrant is added to the sample solution containing sulphate, barium sulphate is formed and rhodizonate or tetrahydroxyquinone remain as such, imparting to the solution a yellow colour until all the sulphate has been precipitated. At the end-point, the excess of barium chloride added forms the barium salt of the indicator, so that the colour of the solution changes to orange-red. Many workers have used this method [31-37] but it has not generally been accepted due to the difficulty in identifying the true end-point. 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 becomes dispersed through the solution by stirring. Consequently there is a very gradual change in the colour of the solution from yellow to orange throughout the titration. The end-point is thus difficult to recognise 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. In order to overcome this problem, a titration technique has been developed [38] which not only identifies the end-point colour, but allows

the continuous comparison of the colour of the solution with a standard colour filter. This method has been applied successfully to the determination of sulphur [39] but as has been said above, these methods give neither a sharp end-point nor great accuracy, and many attempts have been made to improve the procedure. Handa [40] proposed the use of the disodium salt of tetrahydroxy-p-benzoquinone as indicator, which gives the same colour change at the end-point (yellow⟶ orange) as potassium rhodizonate. He suggested, however, that if an inert dye such as xylene cyanole, indigo carmine or methylene blue is added, the colour change at the end point is more easily detected, being from greyish-brown to greyish-violet.

Fritz and Freeland [41] investigated the use of Alizarin Red S (sodium-3-alizarin sulphonate) and Thorin (0-(2-hydroxy-3,6-disulpho-1-naphthylazo)-benzene arsonic acid, disosodium salt) as indicators.

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. In the sulphate titration, Alizarin Red S functions as an adsorption indicator. During the titration, barium sulphate is presumably precipitated with 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 attracted to the surface, forming a pink barium complex.

As a result, when the precipitate is allowed to settle following a titration, the pink colour is all in the precipitate while the supernatant liquid is yellow.

In the case of Thorin the colour change at the end-point is from yellow to pink. The mode of action of Thorin is the same as that of Alizarin Red S, although the colour change is sharper in the case of Thorin, especially at lower pH values. However, no end-point is observed unless some water-miscibleorganic solvent is present in the titration. The best solvent appeares to be the lower alcohols, with optimum concentration of about 30-40%. With less alcohol the end-point becomes less distinctive and if the alcohol concentration is too high, then equilibrium is slow, causing false end-points which appear and then slowly fade.

Although the use of these indicators appreciably improved the reproducibility and accuracy of the titration, probably the greatest improvement in the reliability of titrimetric procedures for the determination of sulphate came when a 0.005 M. barium perchlorate solution was used as titrant, usually with Thorin as the indicator. Since its introduction [42] the method has been widely adapted [43-47].

Budesinsky [48] showed that the end-point was sharper in the barium perchlorate titration if Thorin was replaced as indicator by Sulphonazo III (4,5-dihydroxy-3,6-bis(O-sulphophenylazo)-2,7-naphtha-

lenedisulphonic acid). There has recently [49] been a comparison of the various indicators used for this titration, which concluded that dimethylsulphonazo III (4,5-dihydroxy-3,6-bis(p-methyl-0-sulphophenyl-azo)-2,7-naphthalenedisulphonic acid) was the best indicator to use. The titration of sulphate using barium perchlorate solution is carried out in 80% alcohol within the pH range 2.5 to 4.0, and reproducible determinations of reasonable accuracy can be obtained. The co-precipitation of other ions, however, causes the biggest drawback of this method, and sulphate ion must be separated from virtually all interferences before titration in order to avoid co-precipitation.

A major improvement in this method was when Pribil and Maricova [4] showed that the co-precipitation is prevented in most of the cases if barium sulphate is precipitated in the presence of EDTA. This led Belcher and his co-workers [50] to develop a satisfactory procedure that involved the dissolution of the barium sulphate precipitate in excess of a standard solution of EDTA, and then back-titrating the unused EDTA with a standard magnesium chloride solution using Solochrome Black as indicator. This procedure has successfully been applied to the determination of sulphur in steels [51], in organic compounds [52], and with slight modifications, to the determination of inorganic and total sulphate in urine [53]. It has also been shown [54, 55] that 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 by EDTA, but the results were not very satisfactory.

A radically new titrimetric procedure resulted from the work of Belcher, Nutten and Stephen [28] who showed that 4-amino-4'-chlorodiphenyl (CAD) was an admirable precipitant for sulphate ion. The determination consists of precipitation of CAD sulphate followed by filtration and washing. The precipitate is then transferred to the precipitation vessel and 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 the filter paper retains a small amount of precipitate. This titrimetric method was, however, unsatisfactory in that CAD sulphate does have an appreciable solubility. A major improvement, however, resulted when the procedure was adapted for spectrophotometry [56].

(iv) Spectrophotometric Methods

Solution spectrophotometric methods of analysis allow the sensitive determination of small amounts of sulphate with good accuracy.

Jones and Letham [56] showed that CAD had a strong absorption peak at 254 nm. and subsequently developed a procedure for the determination of

30–120 μg of sulphate. In order to obviate errors due to washing of the CAD sulphate precipitate a difference method was used, in which the amount of CAD remaining in solution after precipitation of the sulphate was determined. An excess of CAD (at least 100%) in 0.1N HCl was added to the sulphate solution at pH 2 to 7, and the precipitate removed by centrifugation before the excess CAD remaining was determined spectrophotometrically at 254 nm.

An excellent colorimetric method for the determination of sulphate has been reported by Bertolacini and Barney [57]. The method is based on the reaction of solid barium chloranilate with the sulphate ion at pH 4 in 50% ethanol solution to liberate the highly coloured acid-chloranilate ion. The broad absorption peak of the ion at 530 nm is used for the determination. Ethanol decreases the solubilities of barium sulphate and barium chloranilate and increases the sensitivity of the method so that 2 ppm (1 μg) of sulphate can be determined.

In a later paper [58] the same authors showed that the sensitivity could be increased by a factor of 30, to give a limit of detection of 0.06 ppm based on an absorbance of 0.005 absorbance units, by measuring the absorbance at 330 nm. instead of 530 nm.

The latest modifications of this indirect determination includes that of Carlson, Rosell and Vallejos [59] who used a concentrated phosphate buffer to improve the sensitivity by a factor of four when

absorbances were measured at 530 nm., and Schafer [60] who used 80%
isopropyl alcohol as the solvent medium. Several other workers have
found this procedure satisfactory [61-63].

Several other indirect colorimetric methods have been developed
which also depend on the ability of the sulphate ion to decompose a
coloured metal-dye lake. The amount of decomposed complex is propor-
tional to the concentration of sulphate ion in the sample solution.
Examples of this method are the action of sulphate on the Thorium-
Xylenyl Orange lake [64], or the insoluble Thorium borate – Amaranth
dye [65]. An adaption of the bleaching action of sulphate to spectro-
fluorimetry has recently been given by Guyon and Lorah [66]. Their
procedure depends on the effect of sulphate on the fluorescence of
the Thorium – Morin complex in 80% Ethanolic solution at pH 2.35.
The concentration of sulphate (0-40 μg in 50 ml.) is determined from
the decrease in fluorescence measured after 20 minutes.

An important colorimetric procedure for the determination of sulphur
is based on the formation of Methylene Blue from the reaction of
sulfide with p-amino-N,N-dimethylaniline in the presence of ferric
chloride. This reaction may be represented as shown in Fig. 1.1.

This reaction is very sensitive for sulphides and many applications
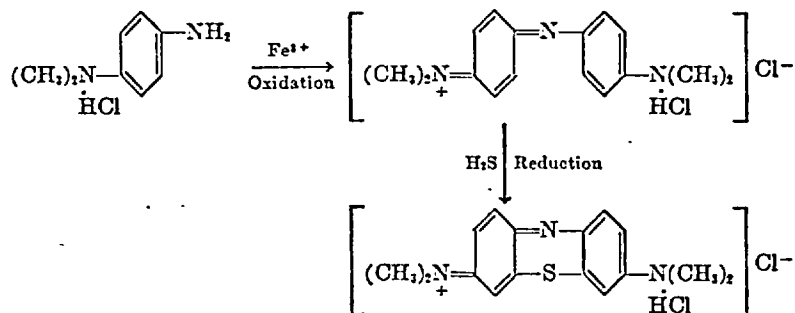of the procedure are to be found in the literature.

Fig. 1.1 Methylene Blue reaction mechanism

One of the earliest studies for the proper conditions for

quantitative use of the methylene blue method was that of Mecklenburg

and Rosenkranzer [67] for determining hydrogen sulphide. As low as

0.01 ppm of hydrogen sulphide in the sample solution was detectable,

but 3 hours were required for full colour development when the

concentration was 0.2 ppm.

Sand et al [68] developed the methylene blue method as an ultra-

sensitive technique for hydrogen sulphide in gases. The sulphide was

absorbed in 2 per cent acidic zinc acetate solution, and reacted with

the sulphate of p-amino-N,N-dimethylaniline at 10°C.

The methylene blue procedure has been applied extensively to the

determination of sulphur as sulphate. Of the various methods which have been used to convert the sulphate samples to aqueous solutions of sulphide, the reduction accomplished through the use of a mixture of hydriodic acid, formic acid and red phosphorus [69] was particularly successful. By application of this procedure to the determination of sulphate in diverse plant materials, 5-50 µg. of sulphate could be determined with a molar absorptivity of 34,500 at 670 nm.

The methylene blue procedure is thus undoubtedly sensitive. However, experimental conditions must be very carefully controlled if the method is to yield good precision and accuracy.

It may be concluded from this review of the methods available for the determination of sulphate that no entirely satisfactory method for the determination of traces of sulphate exists at present. The most accurate, reliable and sensitive of the indirect procedures are undoubtedly those that use spectrophotometry or spectrofluorescence. For trace quantities of sulphate it is clear that a direct, highly sensitive method is required. The application of kinetochromic spectrophotometry can be seen from the following chapters to go some way towards satisfying this requirement. The technique first investigated by Cabello-Tomas [70] has been adapted for the direct spectrophotometric determination of sulphate, giving a molar extinction coefficient of 2×10^3, and a calibration curve extending over the range 0.1-2.4 ppm

(10-240 µg) of sulphate.

1.3 Analytical Methods for the determination of traces of Fluoride

Until fairly recently, the methods available for the determination of trace quantities of fluoride ion were not very satisfactory. The classical techniques of gravimetry and titrimetry are not very suitable for applications on the micro or submicro scale. With one exception, the available colorimetric procedures were indirect, and could be considered negative since they were based on the bleaching effect of the fluoride ion on coloured metal complexes.

The most widely used methods for fluoride can be classified as follows: (i) Gravimetric (ii) Titrimetric, and (iii) Spectrophotometric.

(i) Gravimetric Methods

There are many useful reagents for the precipitation of insoluble fluorides. Some of the better known precipitants are calcium [71], thorium [72], barium [73], and lead chloride [74]. This latter precipitant causes the precipitation of lead chlorofluoride by the reaction:

$$F^- + PbCl_2 \longrightarrow PbClF + Cl^-$$
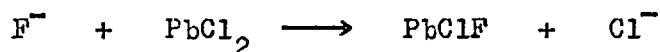
This method has been modified by Belcher and Tatlow [75] to give much better results. The lead chlorofluoride is precipitated by adding the hot fluoride solution to the hot lead chloride solution and allowing

it to stand for at least four hours. Drying is effected at a temperature of 110°C.

None of the precipitants are adequately insoluble, however, for application of gravimetry to trace quantities, and titrimetric methods are to be preferred.

(ii) Titrimetric Methods

One of the most widely used titrants for the determination of fluoride is thorium nitrate, and one of the first indicators to gain wide acceptance was a zirconium-sodium alizarin sulphonate lake solution which was introduced by Willard and Winter [76].

The action of fluoride ion on bleaching the violet-red colour of the zirconium-alizarin mixture to form stable zirconium fluoride complexes was well known, so that a sample solution containing fluoride and the indicator gave no colour. On titration with thorium nitrate solution, the insoluble thorium fluoride is formed, causing the zirconium fluoride complex to decompose. At the end-point, all the fluoride has been precipitated, and the liberated zirconium once again complexes with the alizarin, and the colour of the solution changes to violet-red. A 50% ethanol solution is generally employed to depress the solubility of thorium fluoride.

Other indicators have been widely accepted for the thorium nitrate titration of fluoride such as sodium alizarin sulphonate [77-79],

including the latest modification which uses a fluoride specific electrode to monitor the end-point [80], Solochrome Brilliant Blue B.S. [81] and Methylthymol Blue [82], the latter being claimed to give the sharpest end-point compared with all other indicators used.

A comprehensive study was made by Willard and Horton [83] of colorimetric and fluorimetric indicators for the titration of fluoride with thorium. They concluded that the best colorimetric indicators were, in order of decreasing effectiveness, the two-colour indicators purpurin sulfonate, Alizarin Red S, Eriochromcyanin R, dicyanoquinizarin and Chrome Azurol S; and the best fluorescent indicators were pure sublimed morin and quercetin.

Other titrants such as cerous nitrate [84] or aluminium chloride [85] have been used instead of thorium nitrate, but they are usually inferior.

In the titration of small amounts of fluoride by any of the methods listed above, it is difficult to detect the end-point with sufficient precision. The application of instrumental techniques, such as spectrophotometry, facilitates the reliable determination of trace quantities.

(iii) <u>Spectrophotometric Methods.</u>

Until recently, methods for the determination of traces of fluoride

by spectrophotometry were almost all based on the ability of the ion to destroy coloured organic complexes of certain metals such as zirconium, thorium, titanium, lanthanum and aluminium, 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. Examples of the many varieties of metal complexes used for the determination of fluoride in this way are Thorium – SPADNS [86], thorium – Amaranth [87], aluminium – hematoxylin [88], the zirconium complexes with SPADNS [89], Xylenyl Orange [90], and Alizarin Red S [91], and the chloranilate complexes with thorium [92] and lanthanum [93]. There are similarly many instances of the bleaching action of fluoride on fluorescent metal complexes such as Eriochrome Red B [94]. Perhaps the most widely accepted metal–complex system however, is that of zirconium – Eriochrome Cyanine R which was first introduced by Megregian [95]. It is very sensitive to small fluoride concentration differences; a concentration difference of 0.02 μg. fluoride per ml. can be accurately measured in the range 5.0 – 120 μg. per 100 ml., over which concentration range Beer's Law is obeyed. The sensitivity is 0.500 absorbance unit per centimeter of light path per μg. per ml. of fluoride. Many applications of this method are reported in the literature [96-99], and the continued appearance of new modifications indicates that its application to routine determination of fluoride in a wide range of materials requires careful adherence to recommended operating conditions established for each type of sample.

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 completely new, specific reaction of the fluoride ion. Investigations [100] into the properties of NN-di-(carboxymethyl)aminomethyl derivatives of some hydroxy-anthraquinones revealed that alizarin complexan (1,2-dihydroxy-anthraquinone-3-ylmethylamine-NN-diacetic acid) forms a red chelate with cerium (III); in an acetate buffer at pH 4.3, the addition of fluoride ion causes the red colour of the cerium (III) - alizarin complexan complex to change to a lilac-blue colour due to the formation of a ternary complex with the fluoride ion. [101] This was the first reaction to be developed in which the fluoride ion itself produces a new coloured compound.

Initially, [101-103] the fluoride test was carried out at pH 4.3, because at this pH the yellow to red acid-base transition of alizarin complexan just becomes apparent. The lilac-blue colour of the complex could thus be compared with the scarlet-red colour of the alizarin complexan-cerium (III) chelate. The most suitable wavelength for measurement of the fluoride complex was determined to be 610 nm, which gave a calibration curve which was linear over the range 8-35 μg of fluoride, but did not pass through the origin on extrapolation. The

test is free from interferences of the most common anions and, within

the range 15-50 μg. , an absolute accuracy of $\pm$ 0.5% can be achieved.

Subsequent investigations [103] elucidated the mechanism of this

unique reaction. From their experiments the authors found that the

chelates formed by alizarin complexan are considerably more stable

than those formed by alizarin itself, undoubtedly due to the more

powerfully chelating complexan group in the substituted reagent. The

structure of the cerium (III) -alizarin complexan chelate was consid-
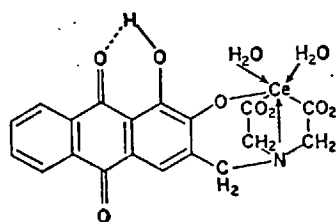
ered to be as shown in Fig. 1.2:

Fig. 1.2

The authors then applied Job's method of continuous variations [104]

to the ternary complex with fluoride and concluded that a 1:1 complex

between the cerium chelate and fluoride was formed. A large excess

of fluoride not only breaks down any existing triple 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. The authors thus concluded that the structure of the

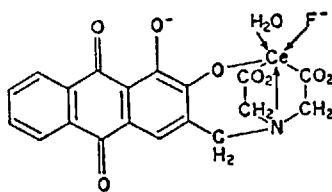ternary complex was of the type shown in Fig. 1.3.

Fig. 1.3

The fluoride ion displaces one of the co-ordinated water molecules

remaining on the cerium (III) atom. The entry of the fluoride ion

within the co-ordination sphere of the cerium (III) ion causes

deprotonation of the remaining 1-hydroxyl group (cf Fig. 1.2). The

specificity of the fluoride action was assumed to be due to the

unusually strong electrophilic properties of the ion, since there are

no steric factors involved.

The optimum conditions for the determination of fluoride ion by

this method were subsequently more thoroughly investigated [105]. The

results indicated clearly that the method should not be operated

beyond the range pH 4-6 and that optimum sensitivity occurs at pH 5.0-

5.2. Maximum colour for the fluoride complex develops within 15 minutes, but the cerium (III) chelate reference solution requires 60 minutes development time in which to attain a stable colour. Consequently a safe development time of 90 minutes was recommended.

The range of calibration for fluoride was seen by the same authors to be dependent upon the concentrations of the cerium (III) and alizarin complexan solutions employed. In earlier papers [100, 102, 103] the authors recommended amounts of 5.10^{-4}M solutions of cerium (III) nitrate and alizarin complexan, which gave a calibration range of 5-50 µg. fluoride, linear from 5-35 µg. They later found [105] however, that using 5.10^{-3}M solutions of the reagents gave calibration for 50-500 µg. of fluoride, linear from 50-275 µg. and that when larger amounts of cerium (III) and reagent were employed the calibration range could be extended for 100-1000 µg. of fluoride, linear from 100-550 µg. or for 200-1600 µg. of fluoride, linear from 200-800 µg.

Although the alizarin complexan method for fluoride is reasonably free from anionic interference, particularly that from sulphate, it suffers seriously from interference from many of the more common cations.

It has been shown [106] that a 1:1 mole ratio of cerium (III) to alizarin complexan gives high tolerance for common anions that do not complex cerium (III), as well as for many cations, while a 1:2.5 ratio

gives increasing tolerance for complexing anions such as sulphate and phosphate. It was also seen [106] that the use of a 20% acetone or 20% acetonitrile medium increased the sensitivity and the stability of the complexes.

The behaviour of fluoride ion with the complexes formed between alizarin complexan and lanthanides other than cerium has also been investigated [107]. The cerium (III) reagent is most sensitive at pH < 4.5 and the lanthanum reagent at pH > 5.0. An enhancement of sensitivity may be obtained for both reagents at pH 4.3 by the addition of acetone to 25% V/V, but the authors concluded that the most sensitive means of determination is to use the lanthanum reagent in aqueous solution at pH 5.2 with measurement at 281 nm. This procedure is 200% more sensitive than the standard method at 620 nm.

Further improvements of the method were obtained [108] when the cerium (III)-alizarin-fluoride complex was extracted into a solvent comprising tribenzylamine in a mixture of pentyl and secondary butyl alcohols.

It can be seen from this review of methods available for the determination of fluoride that the application of kinetochromic spectrophotometry provides, to the best of the author's knowledge, only the second direct reaction for the fluoride ion that exists. The effect of fluoride on the zirconium-Xylenol Orange system has already been

investigated [109] as a method for the determination of fluoride ion, providing an effective molar absorptivity of 2.0×10^5.

The analytical methods for fluoride and sulphate described as part of this thesis are based on the effect of these anions on the zirconium-Methylthymol Blue system. The action of fluoride yields an effective molar absorptivity of 3.0×10^5 while for sulphate the sensitivity is more conventional at 2.0×10^4. Because of the acidity of the reaction, no other cations interfere by forming coloured complexes with the Methylthymol blue. None of the common anions interfere, except those which exhibit a strong complexing action on zirconium such as oxalate and citrate, together with the two other kinetochromic catalysts, phosphate and arsenate.

CHAPTER II

The Nature of the Reaction

2.1 The Aqueous Chemistry of Zirconium

It is well known that aqueous solutions of zirconium (IV) tend to polymerise to form rather inactive species, and that the rate of formation of these polymeric species is dependent on the concentration of zirconium ions, the anion associated with the zirconium, the acidity of the solution, the temperature and the time of standing after dissolution. In the determination of zirconium by colorimetric or titrimetric procedures, care must be taken to ensure that the zirconium solutions used are completely depolymerised prior to analysis, otherwise erroneous results are obtained.

Babko and Gridchina [110] studied the rate of establishment of equilibrium between zirconium and Xylenol Orange in acidified zirconium chloride solutions and concluded that maximum colour develops immediately only in solutions 1–2N in hydrochloric acid containing 10^{-4} or 10^{-3}M zirconium which they surmised did not contain polymeric species.

Pilkington and Wilson [111] studied the depolymerisation of zirconium in sulphate systems under the conditions required for a direct titration with EDTA and found that 0.1M zirconium sulphate solutions could be depolymerised by boiling for 15 minutes with 5N sulphuric acid.

Sinha and Das Gupta [112] and Pakalns [113] have both recently studied

systematically and comprehensively the polymerisation and depoly-

merisation of zirconium on boiling and ageing in various concent-

rations of nitric, hydrochloric, sulphuric and perchloric acids;

Sinha and Das Gupta found that 3N nitric acid can be used to depoly-

merise zirconium solutions for titration with EDTA while Pakalns, in

the spectrophotometric reaction with Arsenazo III, also recommended

boiling the zirconium solution for 15 minutes in acid, the nature and

strength of which depended upon the composition and zirconium concent-

ration of the sample to be analysed.

The nature of the polymeric species which exist in aqueous solution

is uncertain. It is known, however, that a complex equilibrium exists

between various simple and polymeric species, the principal ions being

$[ZrO]^{2+}$, $[ZrO(OH)]^{+}$, $[Zr(OH)_3]^{+}$, $[Zr_2O_3]^{2+}$ and $[Zr_4(OH)_8]^{8+}$ [111].

For simplicity in writing reactions, the zirconyl ion, ZrO^{2+} is usually

taken as the principal ionic species in aqueous solution.

2.2 The Evolution of Kinetochromic Spectrophotometry

In an attempt to discover new ternary systems for the determination

of fluoride ion similar to the cerium (III) – alizarin complexan –

fluoride system discovered by Belcher, Leonard and West [101], Cabello-

Tomas investigated [70] the reaction between fluoride, zirconium and

Xylenol Orange (Fig. 2.1) and discovered an apparently similar ternary

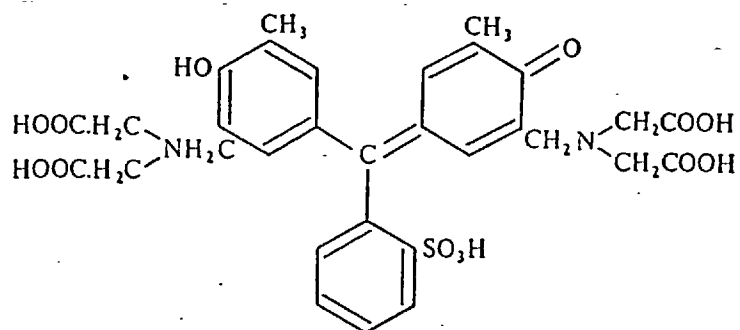system. In the absence of fluoride a yellow colour was obtained on
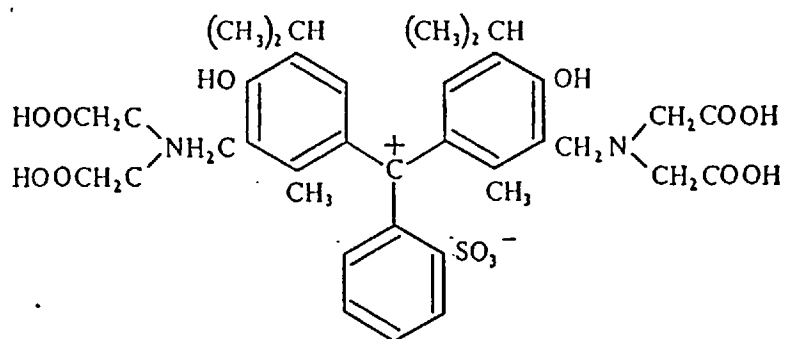
Fig. 2.1   Xylenol Orange

Fig. 2.2   Methylthymol Blue

mixing the zirconium with Xylenol Orange, but in the presence of
fluoride a red colour resulted. A spot test for fluoride based upon
the same reaction was also published by Wilson and Cooke. [115]
Subsequent investigation revealed, however, that the reaction could
not be due to ternary complexation for two principal reasons:-

a) In the original work, when a ternary system was suspected,
zirconium solutions of unknown age and acidity were employed. When
subsequently fresh zirconium solutions were prepared and used, it was
found that the formation of the red colour occurred immediately upon
mixing of the zirconium solution with the Xylenol Orange, even in the
absence of fluoride ion. After the fresh zirconium solution had stood
for several days, however, the apparent ternary system was once again
observed, viz. yellow in the absence and red in the presence of
substoichiometric amounts of fluoride.

b) Examination of the absorption spectra revealed another anomaly.
In the cerium-alizarin complexan-fluoride system, the addition of
fluoride resulted in a considerable shift in the wavelength of maximum
absorption. Thus the peak wavelength at pH 4.3 was 423 nm for the
alizarin complexan solution only, 510 nm for the cerium chelate and
567 nm for the ternary complex with fluoride. The wavelength of
maximum absorption is 430 nm for acidic Xylenol Orange solutions and
550 nm for the 2:1 Xylenol Orange : zirconium complex. The addition

of substoichiometric amounts of fluoride did not produce any shift in the peak wavelength however, but did show a considerable hyperchromic effect at 550 nm.

The conclusion to be drawn, therefore, was that the effect of fluoride was in some way a kinetic one based on its catalytic action on the slow reaction between partly polymerized zirconium species and Xylenol Orange. The term "kinetochromic spectrophotometry" was suggested for this analytical technique. [116]

It was also found that when fluoride ion was added in greater than substoichiometric quantities relative to the zirconium, the reaction was completely bleached. This bleaching action has been described for the determination of fluoride [90] and is in accordance with the modus operandi of the many indirect spectrophotometric methods for the determination of fluoride given in Chapter 1. Further investigation showed that the catalytic action of fluoride was not unique; substoichiometric amounts of sulphate, arsenate and phosphate were also found to give the same reaction, arsenate and phosphate being comparable with fluoride in their effectiveness, but sulphate much less effective.

Chromophores other than Xylenol Orange were not investigated in the original work. During the investigations into the analytical application of kinetochromic spectrophotometry for this thesis, it was found that Methylthymol Blue (Fig. 2.2) also gave the same reaction and

was preferable in that a colour change of greater contrast (yellow to blue) was produced than that given with Xylenol Orange (yellow to red).

2.3 The Kinetic Nature of the System

Freshly prepared aqueous solutions of zirconium (IV) will react very rapidly with an aqueous solution of Methylthymol Blue to form an intense blue soluble chelate. Owing to the tendency of zirconium solutions of intermediate acidity to polymerise on standing, however, a solution of zirconium which has aged for several days will react much more slowly with the Methylthymol Blue solution. Under these conditions, the rate of attainment of equilibrium between zirconium and Methylthymol Blue can be increased by the addition of substoichiometric amounts of fluoride, sulphate, arsenate or phosphate. Experimental conditions may be arranged so that, for example, the sulphate-catalysed reaction proceeds at a reasonable speed whilst the uncatalysed reaction, or blank, proceeds only to a limited extent.

If the absorbance values of the blank and catalysed solutions are recorded at various development times, at 586 nm, the peak wavelength for the binary zirconium-Methylthymol Blue complex, against distilled water as a reference, then a series of curves can be constructed as shown in Fig. 2.3. The initial rate of reaction is thus directly proportional to the quantity of added sulphate.

There are three ways in which data from this system could be used for the determination of sulphate:-
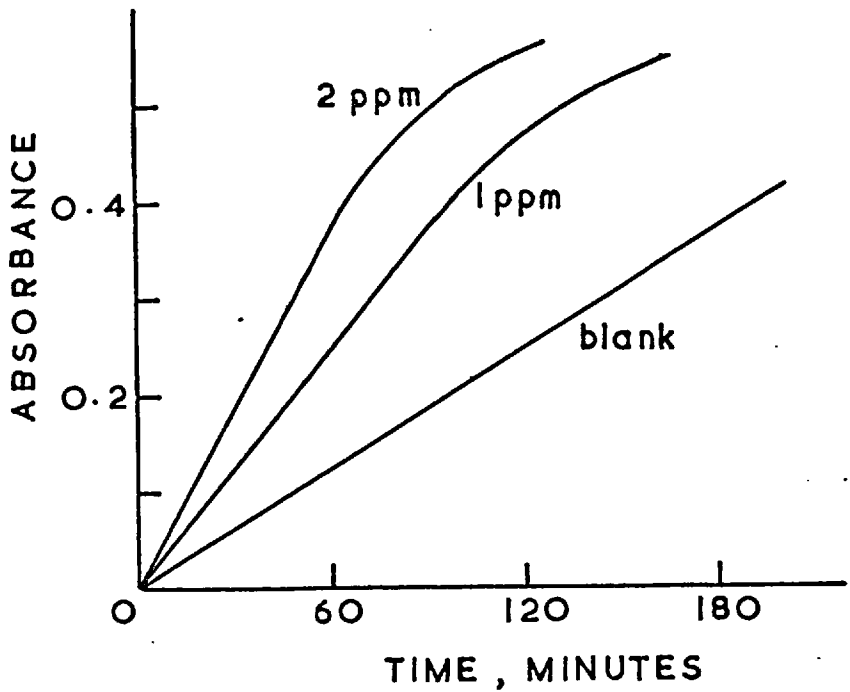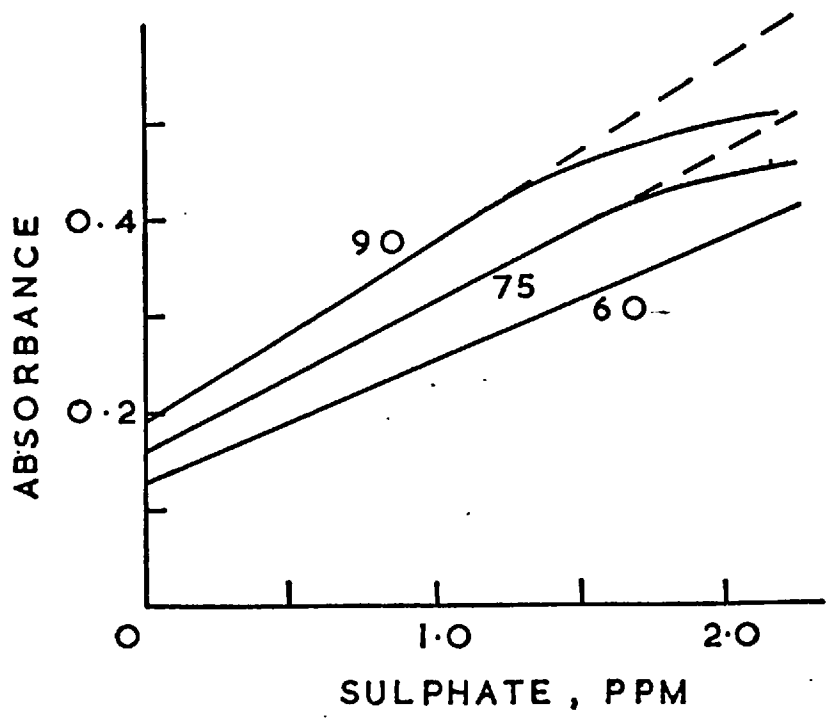
FIG 2.3

FIG 2.4

a) The initial rate of reaction

In this method, curves such as figure 2.3. are first constructed. The initial rate of reaction, expressed as the change in absorbance with time, dA/dT, is then obtained by drawing a straight line at the initial slope of the curve. A plot of dA/dT vs. concentration of sulphate should then be linear.

b) The fixed absorbance method

In this method, the time required for the absorbance to reach a set level is recorded. This time would be inversely proportional to the concentration, and a plot of $1/t$ vs. concentration of sulphate would also be linear.

c) The fixed time method

In this method the absorbance of the solution is measured after a pre-set development time. The absorbance is directly proportional to the concentration of the zirconium-Methylthymol Blue complex, which in turn is directly proportional to the sulphate concentration. This last method was the one used throughout the investigations into kinetochromic spectrophotometry. Thus, from Fig. 2.3. the absorbance produced by the sulphate at a set time of (say) 60 minutes is recorded and a calibration plot of absorbance (at t = 60 minutes) vs. concentration of sulphate is linear, as shown in Fig. 2.4.

2.4 Choice of Development Time

Since the sulphate merely increases the rate at which the zirconium-Methylthymol Blue complex is formed, the final absorbance of all solutions, including the blank, is ultimately the same. Care must be taken therefore, to choose a development time such that linear calibration curves result over the desired range of sulphate. As is evident from Fig. 2.3, and is shown in Fig. 2.4, calibration curves constructed using absorbance values obtained at development times in excess of 60 minutes deviate from linearity for higher levels of sulphate concentration.

The choice of development time is further influenced by other factors, such as the sensitivity obtained. This can be illustrated by representing the data from Fig. 2.3 in another way. If the absorbance values due to the blank are algebraically subtracted from the reagent solution for all equivalent development times, then curves such as those shown in Fig. 2.5 result.

The resulting net absorbance due to the presence of the sulphate catalyst increases to a maximum value but then decreases, eventually becoming zero when all solutions have reached final equilibrium. From Fig. 2.5 it can be seen that after 90 minutes of development, 2 ppm of sulphate produces a maximum net absorbance of 0.300 absorbance units, while after 140 minutes, 1 ppm of sulphate produces a maximum net absorbance of 0.225 absorbance units.

FIG 2.5
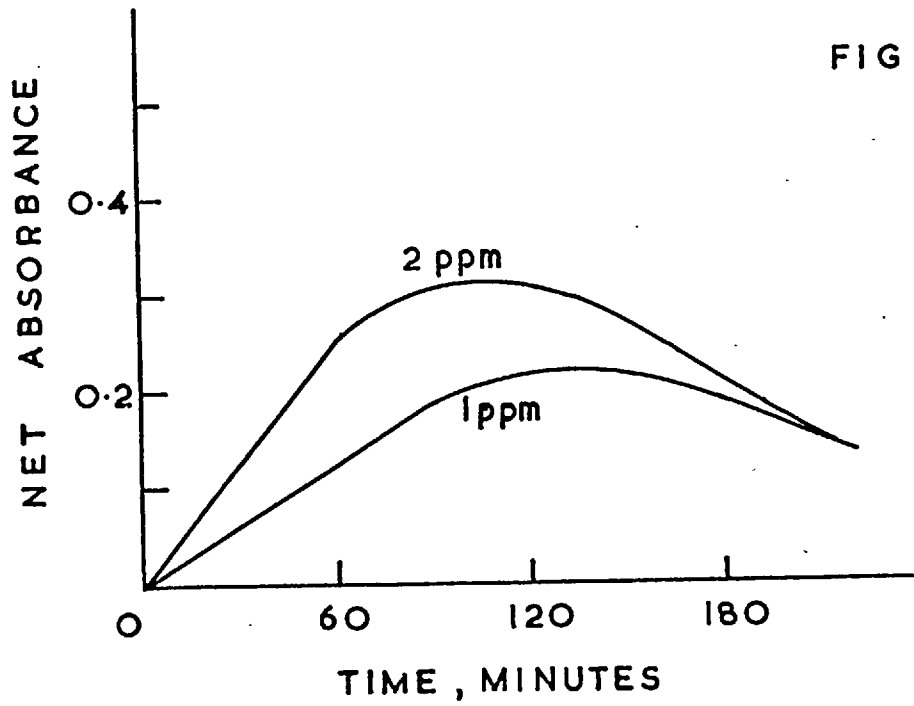
Thus, although 90 minutes is the best time for determining 2 ppm

of sulphate ion, this time would not yield the highest sensitivity

for the determination of 1 ppm, since maximum net absorbance for 1

ppm of sulphate does not occur until 140 minutes have elapsed.

The range over which linear calibration is required also affects

the choice of development time. From Fig. 2.5. it might appear obvious

that, if 2 ppm was to be the upper limit of the calibration curve,

then 90 minutes should be the development time. It is clear, however,

from fig. 2.4 that linear calibration curves do not extend over the

range 0-2 ppm of sulphate after 90 minutes, but only after 60 minutes

development. The reason is evident from Fig. 2.3.

There are consequently three interrelated variables of considerable

importance.

a) The development time

b) The sensitivity obtained

c) The extent of the useful calibration range.

As an illustration of the effect of these parameters, it can be said

that long development times give rise to highly sensitive calibration

curves extending over very narrow ranges, while short development

times give wider calibration ranges of lower sensitivity. Thus, for

example, in the determination of fluoride ion, an effective molar

absorptivity greater than 5.0×10^5 could be obtained with a development time of 2 hours, over the small calibration range of 0-19 ppb (0-19 nanogram per ml.) With a development time of just 45 minutes 0-95 ppb (0-0.095 ppm) could be determined with an effective molar absorptivity of 2.0×10^5.

In the development of the procedures for the determination of fluoride and sulphate, a convenient development time (usually 60 minutes) was chosen at the outset, and all other variables were optimised so that smooth calibration curves compatible with the highest sensitivity were obtained when absorbances were measured after this time.

2.5 Comparison of kinetochromic spectrophotometry with conventional Spectrophotometry

It is clear from the description of kinetochromic spectrophotometry given above that this technique shows marked differences from the procedures normally encountered in spectrophotometric methods of analysis.

In conventional spectrophotometry a plot of absorbance vs. development time for the blank and a reagent solution can be represented as shown in Fig. 2.6. In the absence of the species to be determined, the absorbance of the reagent solution only (blank) will remain constant (A_B). On the addition of the determinable species, the

FIG 2.6

Conventional spectrophotometry

FIG 2.7
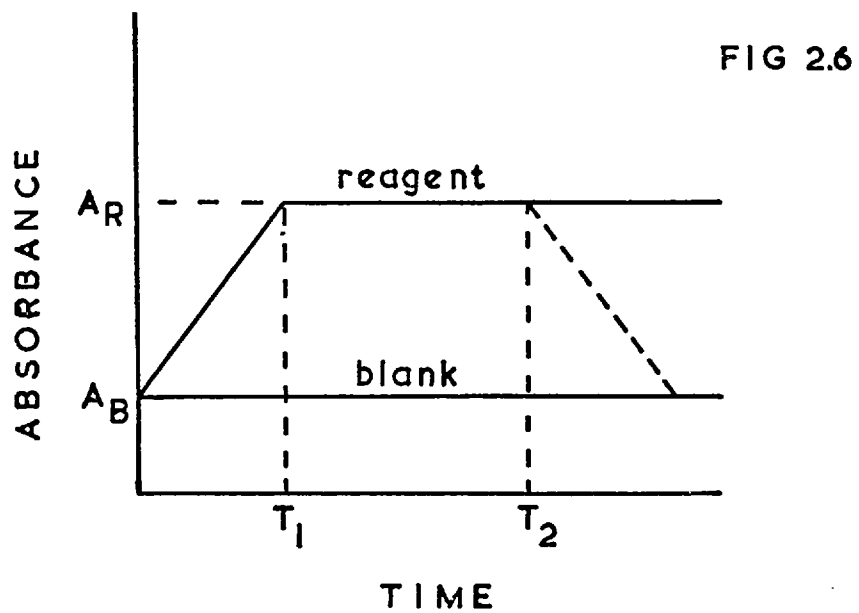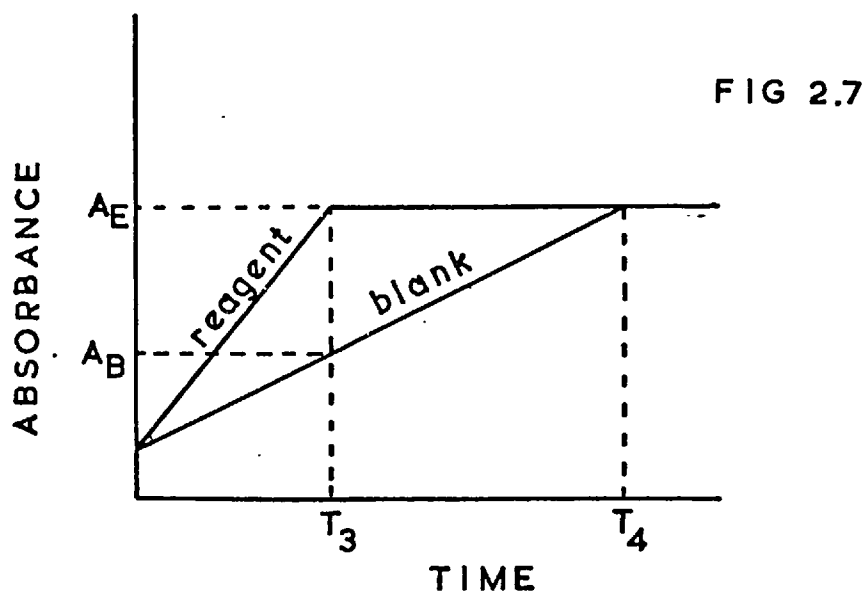
Kinetochromic spectrophotometry

absorbance increases to a maximum, remaining at this level (A_R) for a considerable time after which the absorbance occasionally decreases to that of the reagent alone, usually as a result of decomposition of the complex formed. After the initial development period, absorbance measurements of the solutions can be made at any time between, e.g., T_1 and T_2, and reproducible values for the net absorbance A_n $(= A_B - A_R)$ obtained.

In kinetochromic spectrophotometry, however, a similar plot of absorbance vs. development time can be represented as shown in Fig. 2.7. The fundamental difference clearly is that the absorbance of the blank does not remain constant with time, but increases slowly to reach the equilibrium value A_E after a time T_4, and remains at this level thereafter. The reagent solutions also slowly increases in absorbance but at a faster rate than the blank, reaching the equilibrium value A_E before the blank, at T_3. There is thus no long period of time in which absorbance measurements can be made. The maximum net absorbance A_n $(= A_E - A_B)$ occurs only at time T_3.
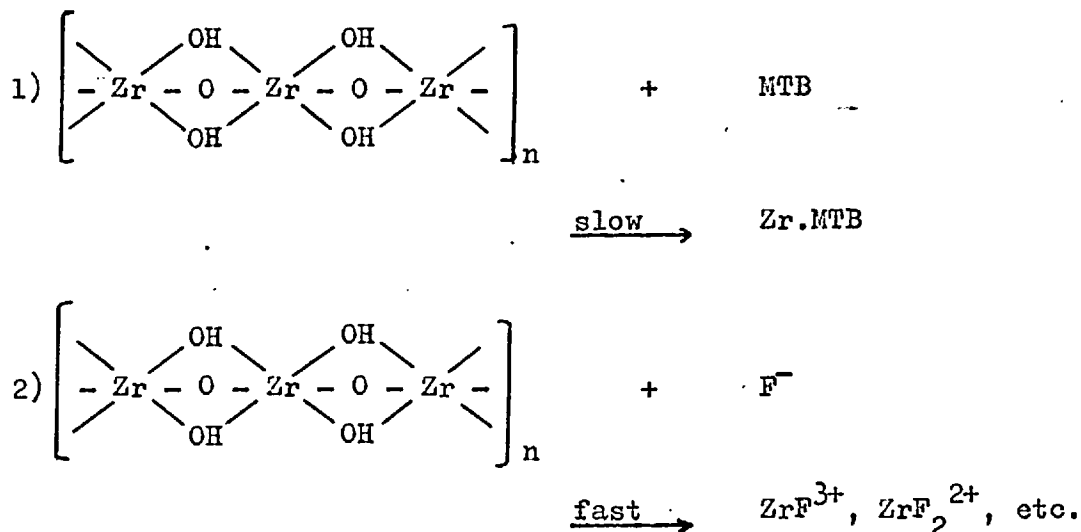
In order that reproducible results are obtained, it is therefore essential that the procedures described for the determination of fluoride and sulphate by kinetochromic spectrophotometry are strictly adhered to, particularly with respect to the development time of the solution. The absorbances of all solutions must be recorded after

each solution has developed for exactly 60 minutes after mixing of the reagents.

Owing to the stability of the blank in conventional spectrophotometry (Fig. 2.6) it is usual to measure the net absorbance (A_n) directly by using the blank as reference solution. In kinetochromic spectrophotometry this clearly is not possible. All absorbance measurements are therefore made against distilled water as a reference. Calibration curves plotted directly using these values will not, of course, pass through the origin (Fig. 2.4)

2.6 Mechanism of the Reaction

No special study of the mechanism of kinetochromic reactions have been made, but speculative explanations have been given [109] which may be represented as follows:-

1)

$$\left[-Zr \overset{OH}{\underset{OH}{\diagup}} O - Zr \overset{OH}{\underset{OH}{\diagup}} O - Zr - \right]_n \qquad + \qquad MTB$$

$$\xrightarrow{\text{slow}} \qquad Zr.MTB$$

2)

$$\left[-Zr \overset{OH}{\underset{OH}{\diagup}} O - Zr \overset{OH}{\underset{OH}{\diagup}} O - Zr - \right]_n \qquad + \qquad F^-$$

$$\xrightarrow{\text{fast}} \qquad ZrF^{3+}, ZrF_2^{2+}, \text{etc.}$$

3) ZrF^{3+} + MTB $\xrightarrow{\text{fast}}$ Zr.MTB + F^-

4) $\left[\begin{array}{c} \diagdown \\ -Zr \\ \diagup \end{array} \begin{array}{c} OH \\ \diagup \quad \diagdown \\ -O- \\ \diagdown \quad \diagup \\ OH \end{array} \begin{array}{c} \\ Zr \\ \end{array} \begin{array}{c} OH \\ \diagup \quad \diagdown \\ -O- \\ \diagdown \quad \diagup \\ OH \end{array} \begin{array}{c} \\ Zr- \\ \diagup \end{array} \right]_n$ + F^-(excess)

$\xrightarrow{\text{fast}}$ $\left[ZrF_6 \right]^{2-}$
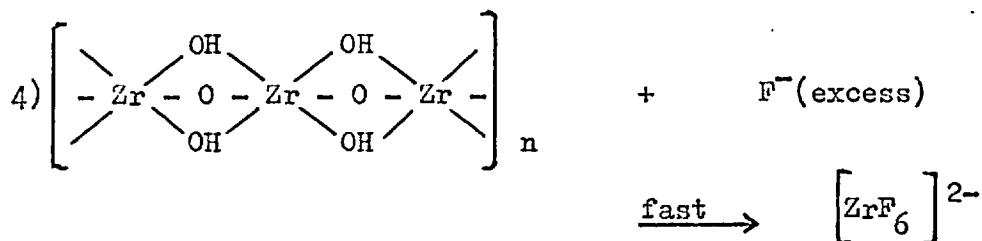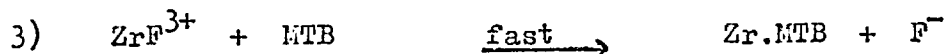
The blank reaction, represented by equation 1, contains polymeric zirconyl species which break down and react with the Methylthymol Blue very slowly. When fluoride is added in substoichiometric amounts it rapidly breaks down the polymeric species in some way to form intermediate labile complexes such as ZrF^{3+} (equation 2), which can then react rapidly with the Methylthymol Blue to form the binary zirconium – Methylthymol Blue complex (equation 3) and reliberate the fluoride ion. If larger than substoichiometric amounts of fluoride are added to the system, the very stable complex $\left[ZrF_6 \right]^{2-}$ is formed (equation 4), which will not react with the Methylthymol Blue, so that catalysis of the system is not observed, and the solution remains yellow.

The relative magnitude of the catalytic effect of the various anions can perhaps be predicted on the basis of the relative stabilities of the anionic complexes formed with zirconium. For example, the most stable complex of zirconium in aqueous solution is the hexafluorozirconate (IV) ion $\left[ZrF_6 \right]^{2-}$ [117], formed simply by the addition of a soluble

fluoride to aqueous zirconium solution, while some of the least stable complexes are the tetrasulphatozirconate (IV) ion, $\left[\text{Zr}(\text{SO}_4)_4\right]^{4-}$, and the oxodisulphatozirconate (IV) ion, $\left[\text{ZrO}(\text{SO}_4)_2\right]^{2-}$. These latter ions are formed only when the solutions are strongly acidic in sulphuric acid. It would consequently be expected that the catalytic action of fluoride would be far greater than that due to sulphate, and this is borne out by experiment.

CHAPTER III

Preliminary Investigations into the Reaction

between Xylenol Orange and Zirconium

3.1 Preface

At the outset of these investigations the initial task was to

reproduce the results obtained by Cabello-Tomas [70] in her earlier

investigation of the Xylenol Orange-zirconium-fluoride system.

Subsequent difficulties which were encountered in obtaining similar

results led to the necessity of re-investigating many of the

experimental parameters.

3.2 Preparation of Zirconium Solutions

The kinetochromic reactions which are dealt with in this study

depend upon the fact that the zirconium solutions employed are

polymerised. The preparation of zirconium solutions which had poly-

merised to a convenient extent was consequently of paramount importance

and merited considerable attention.

The effect of boiling the solution to control the polymerisation

and depolymerisation of zirconium solutions has already been studied

[70, 110, 112, 113] and found to be unsatisfactory, [70] and so was not

further investigated.

The recommended [70] standardised ageing procedure for the preparation

of zirconium solutions was initially adopted. A 10^{-2}M stock solution

of zirconium was prepared by dissolution of $ZrOCl_2.8H_2O$ (GPR grade)

and allowed to stand for between 15 hours and 14 days. Suitable

aliquots were then diluted to give a working 5×10^{-4}M zirconium

solution, which could then be used after 4 hours and up to 5 days

before being discarded. The variability of the results obtained,

however, indicated that the use of these recommended conditions was

not satisfactory, and that the age of the zirconium solution should be

more strictly controlled. Consequently, a 10^{-2}M zirconium solution was

prepared and allowed to stand for exactly 17.0 hours before suitable

portions were taken for dilution. The resultant 5.10^{-4}M zirconium

solution was then allowed to stand for exactly 5.0 hours before use.

The recommended acidity of 0.0125M in hydrochloric acid for the

stock 10^{-2}M zirconium solution was used without further investigation.

The acidity of the dilute 5.10^{-4}M zirconium solution was studied,

however, and the results obtained are shown in Fig. 3.1. The highest

sensitivity was obtained when the 5.10^{-4}M zirconium solution was 0.05M

in hydrochloric acid (curve 4). Solutions of lower acidity were too

extensively polymerised so that less effective catalytic action of the

fluoride ion was observed (e.g. curves 1, 2, 3). Solutions of higher

acidity were little polymerised, so that both reagent and blank reacted

rapidly, and the net absorbance due to the fluoride was small, and

occurred rapidly (curves 5, 6).

FIG 3.1
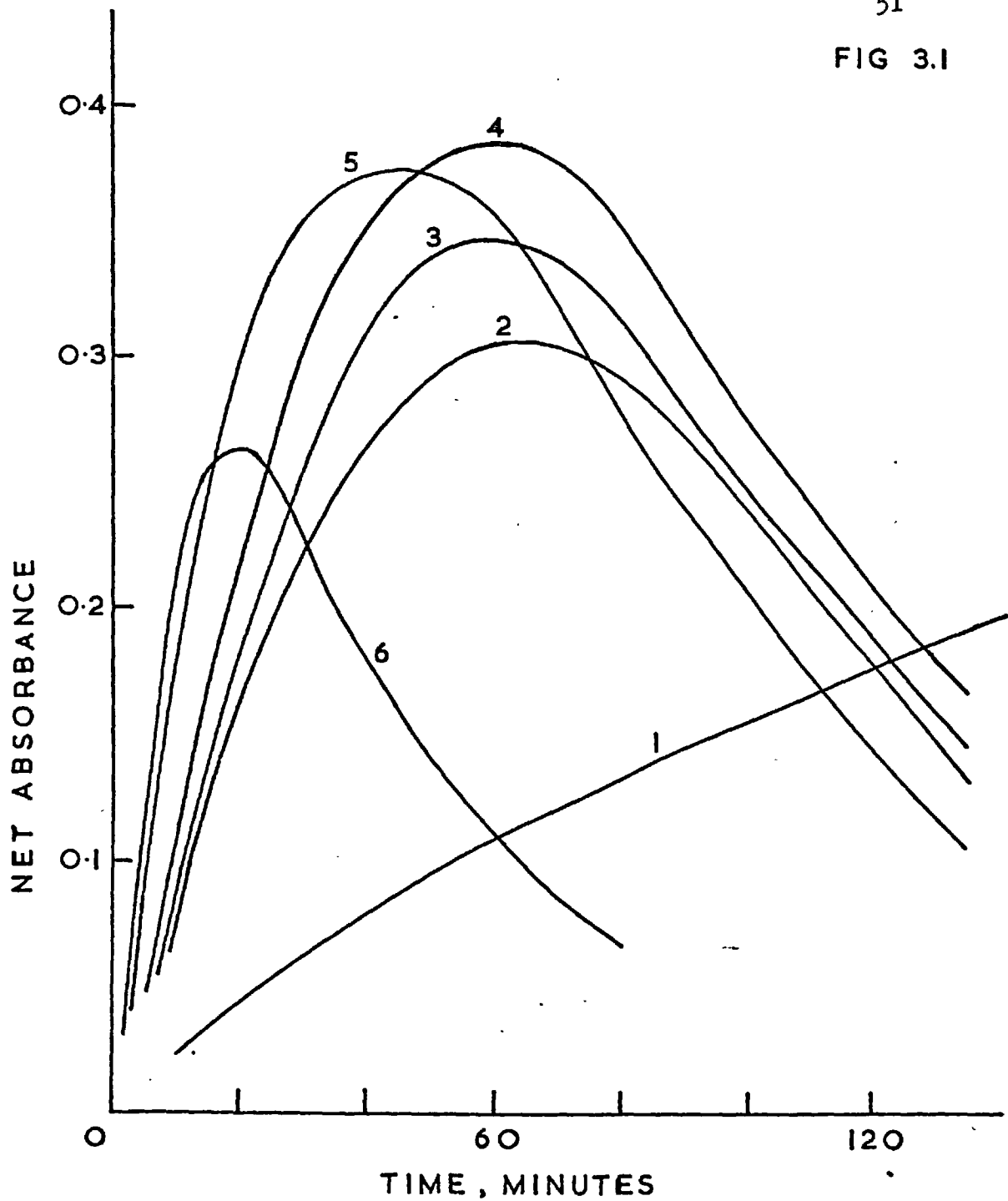
CURVE	1	2	3	4	5	6
ACIDITY OF 5.10^{-4}M $ZrOCl_2$	0.0006M	0.006M	0.026M	0.050M	0.10M	0.20M

The experiment was conducted as follows:

50.0 ml. of 10^{-2}M zirconium solution (17 hrs. old, 0.0125 M in hydrochloric acid) were transferred into a series of six 1 litre volumetric flasks containing 0, 1.0, 5.0, 10.0, 20.0, 40.0 ml. respectively of 5N hydrochloric acid. Subsequent dilution to the mark gave 5.10^{-4}M zirconium solutions which were 0.0006M, 0.006M, 0.026M, 0.05M, 0.10M and 0.20M in hydrochloric acid respectively. These solutions were used after they had each aged for exactly five hours.

Into two 100 ml. volumetric flasks was added 5.0 ml. of 5.10^{-4}M Xylenol Orange and 7.0 ml. of 5N hydrochloric acid. To one solution was added 1.0 ml. of 2.5×10^{-4}M sodium fluoride. Both flasks were thoroughly shaken. At zero time, 10.0 ml. of the 5.10^{-4}M zirconium solution 0.0006M in hydrochloric acid was added in turn to both flasks followed by immediate shaking and dilution to volume with distilled water.

The absorbance of the fluoride-containing solution was measured in a 1 cm. cell at 550 nm. against the blank solution as a reference at various development times. The procedure was repeated in an identical manner, but using the 5.10^{-4}M zirconium solutions of other acidities.

3.3 Effect of Temperature

The temperature at which the kinetic reaction should proceed was

next investigated. 5.0 ml. of 5.10^{-4}M Xylenol Orange and 7.0 ml. 5N hydrochloric acid were added to two 100 ml. volumetric flasks immersed in a water-bath maintained at $50°C$. To one flask was added 1.0 ml. 2.5×10^{-4}M sodium fluoride and both solutions thoroughly mixed. At zero time, 10.0 ml. of 5.10^{-4}M zirconium solution (5 hrs. old, prepared by dilution from a 10^{-2}M zirconium solution 17 hrs. old) were added to both flasks, followed by immediate shaking and dilution to the mark. Absorbance measurements were made on the fluoride-containing solution in 1 cm. cells at 550 nm against the blank (no fluoride) at various development times. The procedure was repeated with the water-bath maintained at $21°$, $16°$, $11°$, and $0°C$.

The results are shown in Fig. 3.2. The highest ultimate sensitivities were obtained when the reaction was allowed to proceed at $0°C$, but the development time required was very long, ca. 7 hours. A convenient operating temperature, which could be maintained with only minimal thermostatic control, was $16°C$. This gave maximal sensitivity after ca. 90 minutes development time.

3.4 Effect of Age of the Zirconium Solution

A series of experiments was conducted a) maintaining the age of the 10^{-2}M zirconium solution at 17.0 hours before use for dilution, and varying the time after which the resulting 5.10^{-4}M zirconium solution was used, and b) maintaining the age of the 5.10^{-4}M zirconium solution at 5.0 hours, while varying the age of the parent 10^{-2}M zirconium solution.
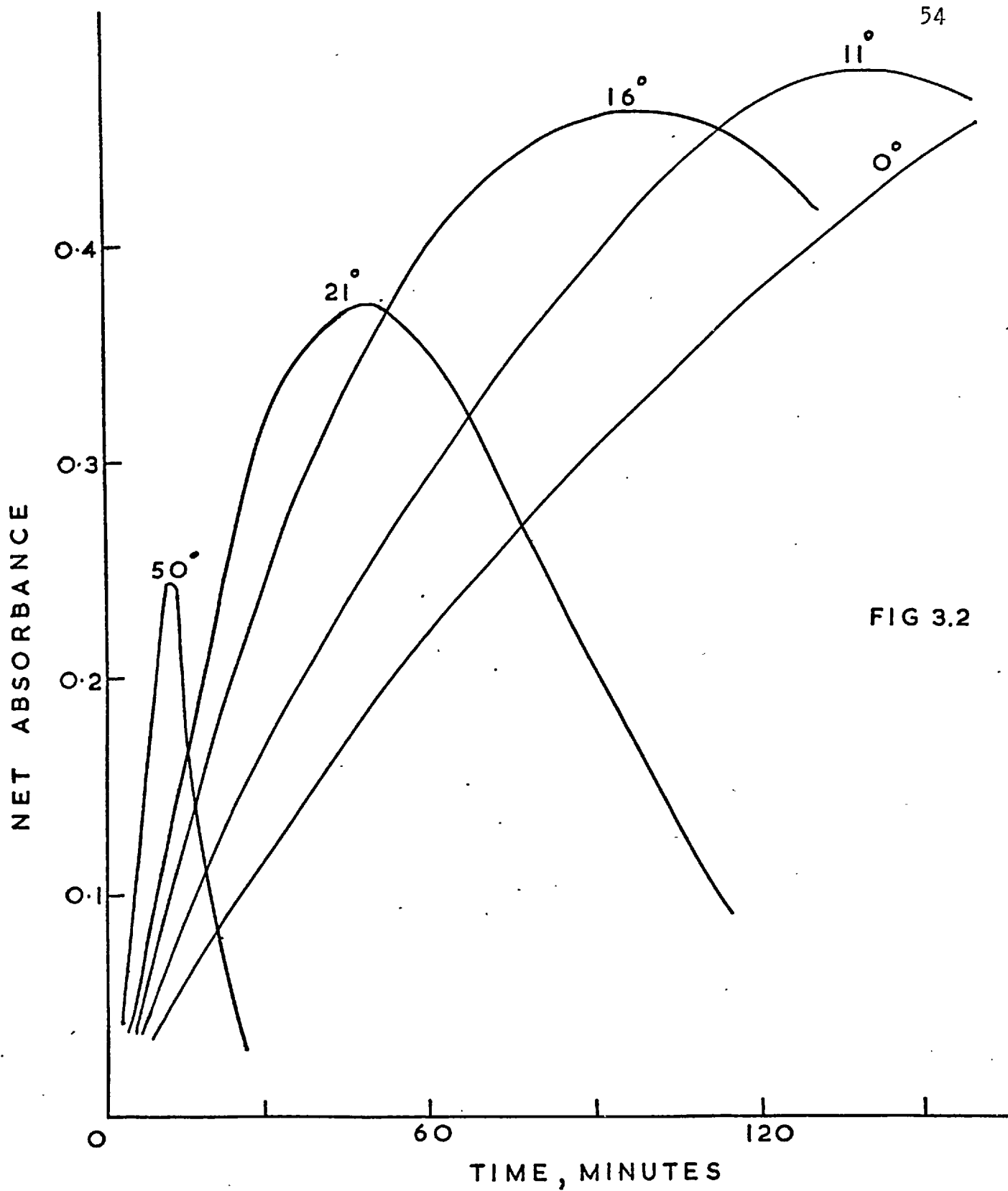
FIG 3.2

For each experiment, two 100 ml. flasks were prepared, containing 5.0 ml. 5.10^{-4}M Xylenol Orange and 7.0 ml. 5N hydrochloric acid, and placed in a water-bath maintained at 16^0C. To one flask was added 1.0 ml. of $2.5.10^{-4}$M sodium fluoride. At zero time 10.0 ml. of 5.10^{-4}M zirconium solution of the appropriate age characteristics was added to both flasks, followed by mixing and dilution to the mark for both solutions. Absorbance measurements were made for the fluoride-containing solution in 1 cm. cells at 550 nm. against the blank (no fluoride) at various development times. The results are shown in Fig. 3.3. Optimum sensitivity was obtained at a development time ca. 90 minutes when a 10^{-2}M zirconium solution 17 hours old is used to provide aliquots for dilution to 5.10^{-4}M and when this dilute (5.10^{-4}M) solution is used after it has aged for 5 hours.

Although highest sensitivity is obtained under these conditions, adherence to this rather rigid time schedule was considered inconvenient. It was therefore decided that a range of ages for the stock 10^{-2}M and working 5.10^{-4}M zirconium solutions was necessary as, in fact, had been originally recommended [70]. The extent of polymerisation of the dilute (5.10^{-4}M) solution varied too widely however when the original age ranges were followed. Analysis of the results of many further experiments showed that the 10^{-2}M zirconium solution should be allowed to age 2-7 days at room temperature before dilution, and that the working 5.10^{-4}M solution should be used 2 hours-4days after preparation.

FIG 3.3

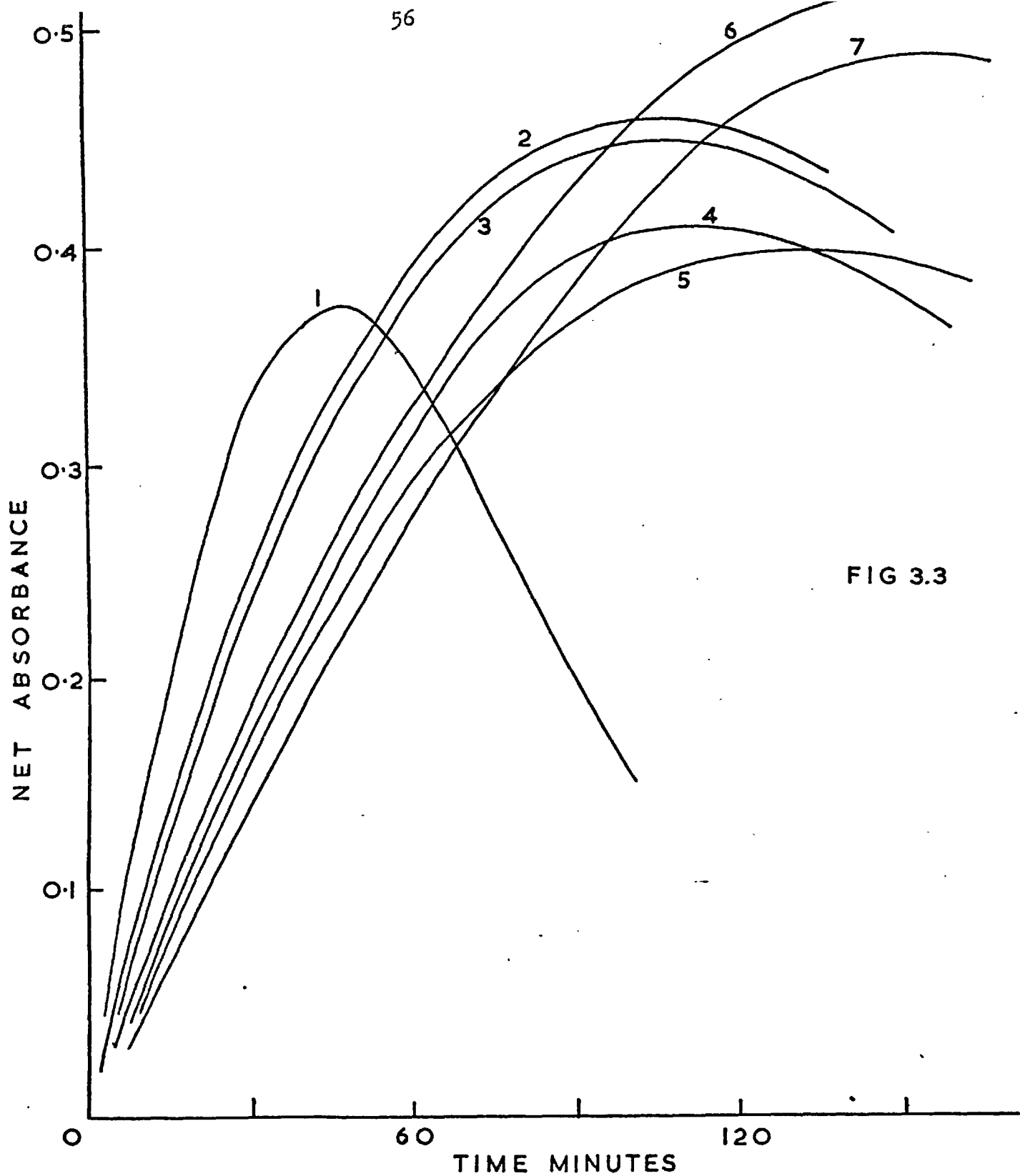NET ABSORBANCE (y-axis)

TIME MINUTES (x-axis)

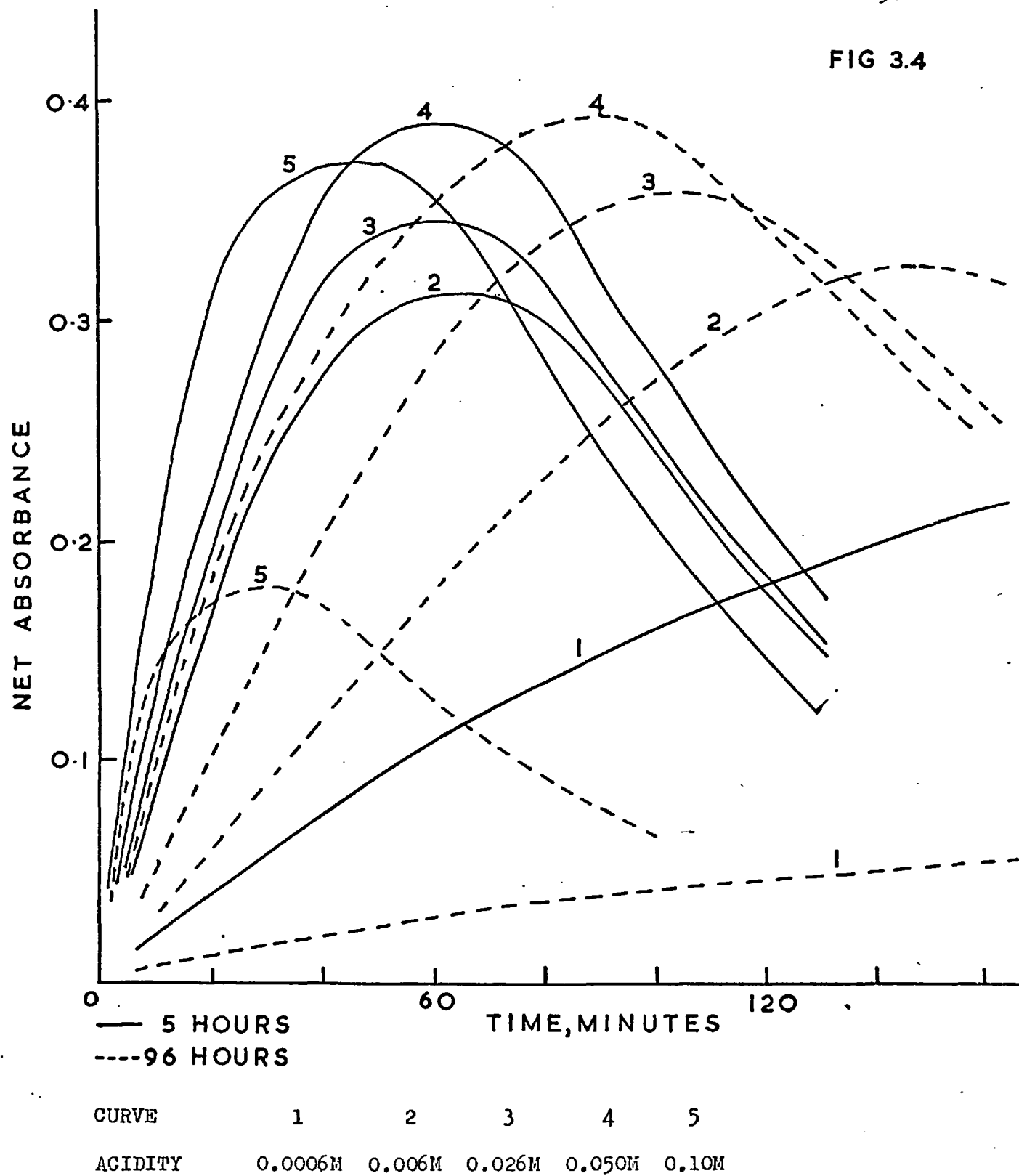| CURVE	1	2	3	4	5	6	7	
Age 10^{-2}M Zr	17	17	17	17	17	113	185	HOURS
Age 5.10^{-4}N Zr	·1	5	28	77	124	5	5	HOURS

Full details will be given at a later stage.

3.5   Effect of other variables

A study was made of the effect of the acidity of the 5.10^{-4}M zirconium solution upon the degree of polymerisation of the dilute (5.10^{-4}M) solution as this solution aged. Experimental conditions were identical to those used in the previous study of acidity (page 52) except that the reacting solutions were maintained at 16°C. 5.10^{-4}M zirconium solutions of varying acidities which had aged for 5 hours and then 96 hours, were used. The results (fig. 3.4) show that an acidity of 0.05M in hydrochloric acid for the dilute (5.10^{-4}M) solution produced the least change in the degree of polymerisation as the dilute solution ages. Less acidic (5.10^{-4}M) solutions rapidly polymerise further, causing the reaction rate to become too slow, while more acidic solutions depolymerise and the blank reaction proceeds too rapidly.

The optimised acidity at which the catalysed and uncatalysed reaction should proceed was 0.35N in hydrochloric acid [70] and was not further studied.

It was necessary, however, to devise a standardised procedure for the preparation of the reagent solutions. The original procedure did not follow a rigid time schedule, but it soon became apparent that this was undoubtedly necessary, as indeed it would be in any kinetic method of analysis. A time schedule was therefore devised, and

FIG 3.4

Curve labels and axes: NET ABSORBANCE (vertical), TIME, MINUTES (horizontal)

—— 5 HOURS
----96 HOURS

CURVE	1	2	3	4	5
ACIDITY	0.0006M	0.006M	0.026M	0.050M	0.10M

strictly adhered to, in the preparation and measurement of all reacting solutions.

3.6 The determination of sulphate

The optimised procedure, similar to that originally developed [70], was applied to the determination of sulphate ion. Over the range 0.1-2.4 ppm (10-240 μg) of sulphate, linear calibration curves were obtained, but the sensitivity was very low after conventional development times (ϵ = 6000 after 90 minutes). Higher sensitivities (ϵ = 15,000) were obtainable but only at protracted development times (ca. 3 hours).

There was no obvious reason why the values of sensitivity should not have been equal to that claimed [70] ($\epsilon \approx$ 22,000 after 90 minutes). There appeared to be several ways in which the system Xylenol Orange-zirconium-sulphate might be altered in order that greater sensitivity might be obtained: (a) replacement of the zirconium solution by metals which were known to hydrolyse in a manner similar to that of zirconium and which might evoke the same kinetochromic response; (b) continuing to use zirconium solution, but replace the Xylenol Orange by another metallochromic dyestuff; and (c) further modification of the existing system.

3.7 Replacement of Zirconium by other metals

Three metals were investigated. All of them were known to hydrolyse and give complexes with Xylenol Orange. They were titanium, thorium,

and aluminium.

(i) Titanium

5.10^{-3}M solutions of titanium tetrachloride were prepared by dilution from the standard reagent (15% w/v $TiCl_4$, 15% w/v HCl, technical grade). The spectrophotometric determination of titanium with Xylenol Orange has been shown [118] to occur at an optimum pH of 3-4.5. In an acetate buffer of pH 3.7, freshly prepared titanium solutions reacted immediately with the Xylenol Orange to give a red chelate. Aged titanium solutions reacted similarly. There was no difference in the rates of reaction for either fresh or hydrolysed titanium solutions when trace amounts of fluoride or sulphate were present.

Measurement of the absorbance at 550 nm showed that there was an initial large increase in absorbance, for both fast and hydrolysed titanium solutions, followed by an appreciable further increase in the next 24 hours. This second, slow, increase was not affected either by the presence of trace amounts of fluoride – though larger amounts tended to bleach the reaction – nor by whether the titanium solution was freshly prepared or hydrolysed.

On this evidence, replacement of zirconium by titanium could not be employed.

(ii) <u>Thorium</u>

A 5.10^{-4}M solution of thorium nitrate $(Th(NO_3)_4.6H_2O$, GPR) was used.
Thorium solutions of pH 3.5 reacted immediately with Xylenol Orange
in an acetate buffer of pH 4.3, even after standing for several days,
with no further increase in absorbance with time. Thorium solutions
which were adjusted to pH 7 with sodium hydroxide, however, gave
much slower reactions with Xylenol Orange at pH 4.3, decreasing in
rate as the thorium solution aged. Under all conditions, the addition
of trace amounts of sulphate or fluoride had a slight bleaching effect
but no catalytic action was observed. Consequently no further studies
were made.

(iii) <u>Aluminium</u>

The spectrophotometric determination of aluminium with Xylenol
Orange has been described by Otomo [119] at a pH 3.5. 5.10^{-4}M solutions
of aluminium were prepared by dissolution of aluminium chloride (GPR)
in 0.025M hydrochloric acid. Freshly prepared solutions of aluminium
gave reasonably slow reactions with Xylenol Orange at pH 3.7, while
the reaction of hydrolysed (aged) solutions was noticeably slower.
No kinetochromic reactions were observed, however, with either fresh
or hydrolysed aluminium solutions when varying amounts of fluoride and
sulphate were added. Further studies were therefore not made.

3.8 <u>The use of other metallochromic indicators</u>

A preliminary investigation was conducted on the following indicators:

Alizarin Complexan, Bromopyrogallol Red, catechol violet, SPADNS, Pyrogallol Red, Alizarin Black SN, Pyrocatechol violet and Methyl-thymol Blue.

A series of 5.10^{-4}M solutions of the indicators were prepared. For each indicator, two 5 ml. portions were taken, and to each portion were added 0.5 ml. of 5N hydrochloric acid and 10 ml. of 5.10^{-4}M. zirconium solution. The zirconium solution was 4 days old, and was derived from a 10^{-2}M stock solution 10 days old. To one of the metal-indicator solutions was added 1 ml. of 5.10^{-4}M fluoride solution. The rates of reaction were carefully observed.

Alizarin complexan and catechol violet were not observed to react with zirconium alone, nor in the presence of fluoride. SPADNS reacted slowly with zirconium, but there was no observable difference when fluoride was present. All other indicators studied reacted immediately with zirconium, with no detectable difference in the presence of fluoride, save one. When fluoride was absent, methylthymol blue reacted slowly with zirconium to give a yellow to blue colour change. In the presence of fluoride, the rate of this reaction was increased.

As a result of this preliminary study, methylthymol blue was selected for further investigation.

3.9 Further modifications of the Xylenol Orange-Zirconium system

One of the major disadvantages of kinetochromic spectrophotometry

compared with conventional spectrophotometry is that absorbance measurements of all solutions must be made after each solution has been allowed to stand for exactly the same length of time (Chapter II). One way of overcoming this problem would be to stop the kinetic reaction in some way, after the required development time, so that the absorbance of the solutions could be measured at leisure, without strict regard to time. With this in mind, several techniques were investigated.

(i) <u>Solvent extraction</u>

If, after a certain development time, the unreacted Xylenol Orange present in the solution could be extracted into an organic phase, further formation of the complex with zirconium should cease. Many solvents were tried, in various combinations, including chloroform, iso-butyl methyl ketone, benzene, xylene, carbon tetrachloride, di-n-butylamine, ethylacetate, acetylacetone, amyl alcohol, amyl acetate, n-butanol, tribenzylamine, pentyl alcohol and sec-butyl alcohol.

No one solvent was found as an extractant for Xylenol Orange, but a mixture of amyl alcohol and di-n-butylamine appeared to do so. A ratio of approx. 5:1 pentanol:di-n-butylamine was required, and the optimum pH was <u>ca</u>. pH 8-9. When the solvent was added to a normal "blank" solution of Xylenol Orange-zirconium complex, 0.35N in hydrochloric acid, no extraction occurred at all. Adjustment of the solution to pH 8 resulted in complete extraction to give a colourless

aqueous layer and a purple organic layer contaminated with a slight flocculent red precipitate. Addition of the solvent mixture to a series of calibration standards prepared under optimised conditions with sulphate as catalyst did not give any extraction whatever.

It was concluded that Xylenol Orange was only extracted in its basic form, and that the complex was not extracted at all. The flocculent red precipitate observed in the organic phase on extraction of zirconium-xylenol orange solutions which had been adjusted to pH 8, was presumed to be solvated zirconyl species which had adsorbed molecules of the dye. No other solvent systems were found that extracted either xylenol orange, in acid or basic form, or the zirconium complex.

(ii) Other techniques

It was considered possible that if the reaction between Xylenol Orange and zirconium were to be swamped with alcohol after proceeding for a certain time, the zirconyl species may become solvated so that further reaction with Xylenol Orange would not occur. Investigations showed that the reaction definitely slowed when swamped with 75% ethanol, but did not stop.

The possibility of allowing the reaction to proceed for a certain time, and immersing the reacting solutions in an ice-bath or similar freezing mixture was also investigated, with qualified success. The

blank and catalysed reactions did slow to a remarkable extent. There were inherent experimental difficulties, however, in subsequently measuring the absorbance of these solutions, without allowing the temperature to rise and the rate of reaction to increase once more. Very erratic and irreproducible results were obtained so that no further studies were made.

CHAPTER IV

The Determination of Sulphate by Catalysis

of the Zirconium-Methylthymol Blue reaction

4.1 Introduction

The direct spectrophotometric determination of sulphate reported

in this chapter depends on the action of sulphate in aqueous solution

on the reaction between zirconium and Methylthymol Blue (MTB). The

effect of anions on this system, and on the similar reaction between

zirconium and Xylenol Orange, has been described in Chapter II of

this thesis, and has been shown to be purely kinetic (Section 2.3).

Although this catalytic effect also provides the basis of sensitive

methods for the determination of fluoride and phosphate, owing to the

dearth of direct methods for the spectrophotometric determination of

sulphate, this aspect was investigated first.

4.2 Investigations of Experimental parameters
Spectral characteristics

The absorption spectra of Methylthymol Blue and of its zirconium

complex in acidic medium are shown in Fig. 4.1. The blank (MTB)

solution was 4×10^{-5}M in MTB and 0.2M in hydrochloric acid. The

complex (MTB-Zr) solution was 4×10^{-5}M in MTB, 10^{-4}M in zirconium

and 0.2M in hydrochloric acid. The wavelength of maximal absorption

for the reagent and complex are 436 nm and 586 nm respectively. For

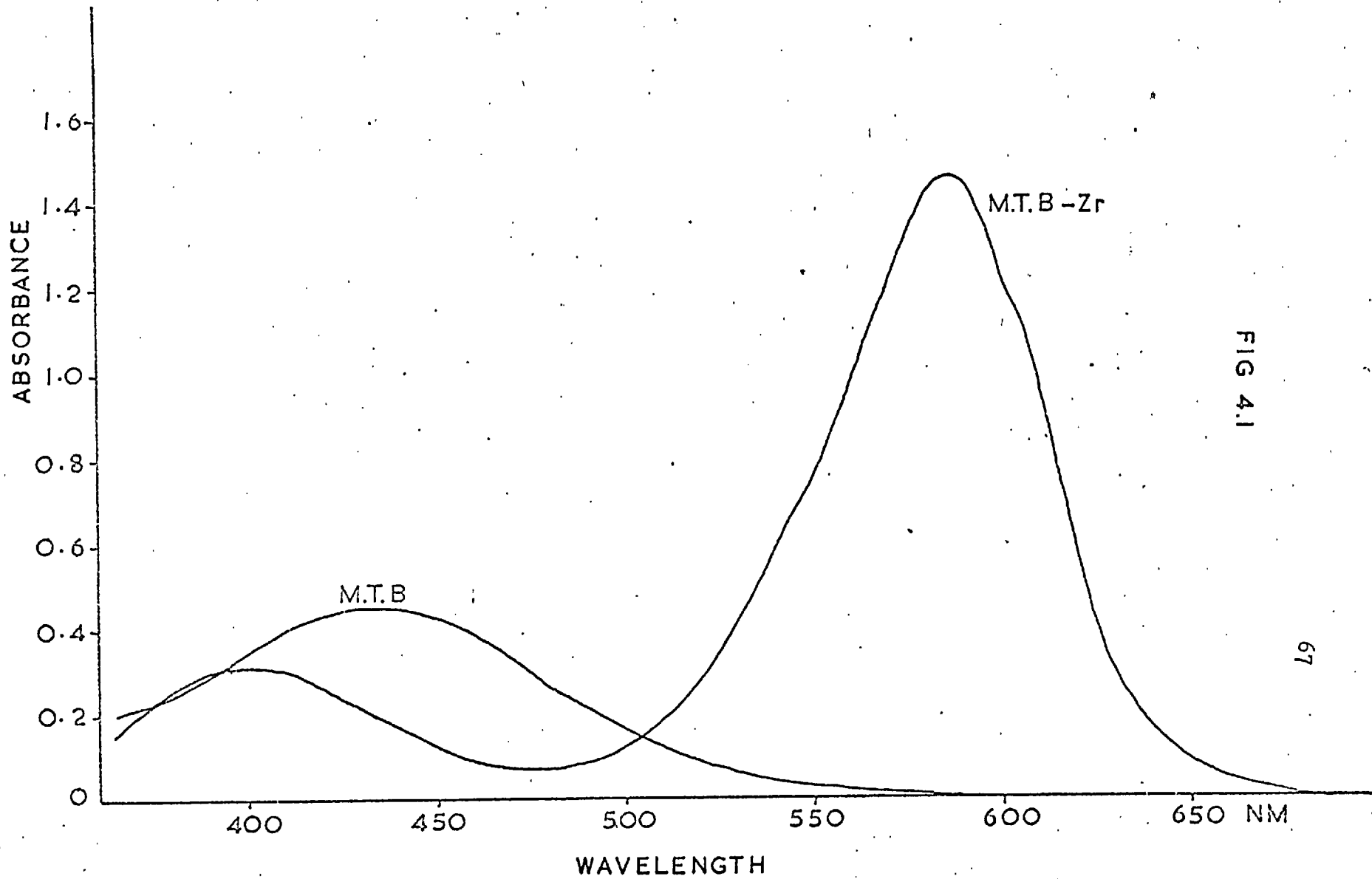the reasons given in Chapter II, all measurements on sulphate samples

FIG 4.1

67

were made at 586 nm.

Preparation of Zirconium Solutions.

As in the reaction of zirconium with Xylenol Orange (Chapter III), freshly prepared zirconium solutions react rapidly with Methylthymol Blue to form a soluble blue 1:1 chelate, and the rate of reaction decreases with increasing age of the zirconium solution. The highest sensitivity for the determination of sulphate is obtained when the experimental conditions are arranged so that the difference between the rates of the catalysed and uncatalysed (blank) reactions is greatest.

Freshly prepared or very old solutions cannot be used, for the reasons already given (Chapter III). It has been found that a 10^{-2}M zirconium solution in 0.0125M hydrochloric acid polymerizes at a convenient rate at room temperature, fast enough for the solution to be used after two days, but slow enough for the solution to be usable for up to seven days. When the 10^{-2}M stock solution is diluted to the 10^{-3}M working solution the acidity should be adjusted to 0.05M in the diluted solution. This optimum acidity was found experimentally (Chapter III) to produce the least change in the degree of polymerization as the dilute solution ages.

The effect of the age of the 10^{-2}M and 10^{-3}M zirconium solutions on the rate of the catalysed reaction with sulphate is shown in Fig. 4.2. This shows the net difference in absorbance between sulphate-catalysed
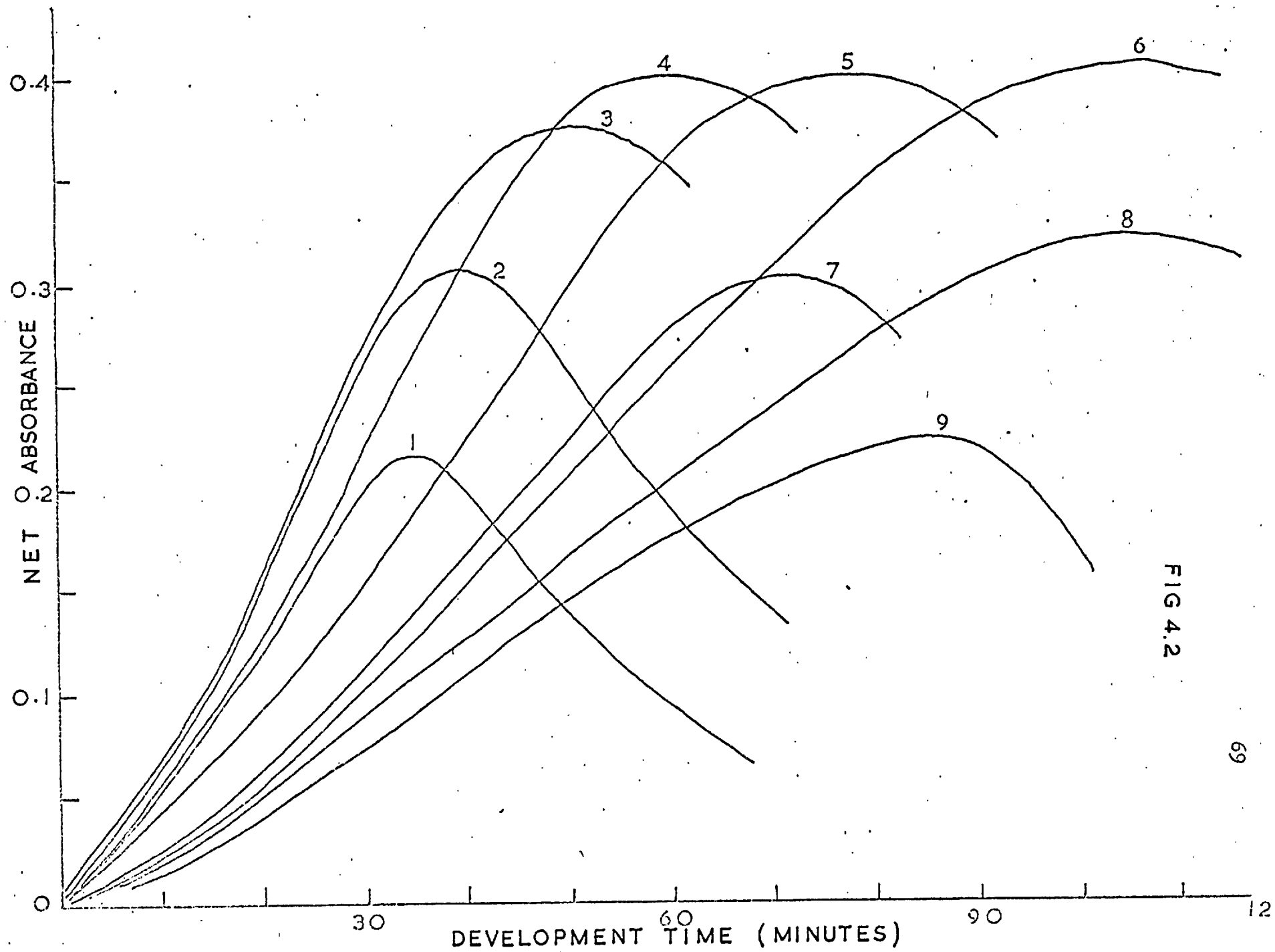
FIG 4.2

NET ABSORBANCE

DEVELOPMENT TIME (MINUTES)

and uncatalysed reactions for various ages of zirconium solutions. All solutions were 2.5×10^{-5}M in MTB, 10^{-4}M in zirconium and 0.20M in hydrochloric acid. In addition, the reagent solutions contained 240 μg of sulphate. Absorbances were measured in 10 mm cells against a water blank at 586 nm.

The ages of the zirconium solutions for the various curves are given in table 4.1.

Table 4.1

Curve	Age 10^{-3}M Zr	Age 10^{-2}M Zr	Curve	Age 10^{-3}M Zr	Age 10^{-2}M Zr
1	22 hours	0.9 days	6	24 hours	16.9 days
2	15 minutes	1.8 days	7	7.2 days	7.0 days
3	1 hour	2.0 days	8	3.0 days	14.0 days
4	4 hours	2.8 days	9	22.0 days	5.0 days
5	1.3 hours	8.7 days			

A 10^{-3}M solution derived from a freshly prepared 10^{-2}M solution (or prepared directly by dissolving $ZrOCl_2 \cdot 8H_2O$) is polymerized only to a small extent so that even with further ageing of the dilute (10^{-3}M) solution both blank and sample solutions react rapidly. The net absorbance for sulphate is therefore small, and reaches a maximum after 30-45 minutes (cf curves 1 and 2). However, 10^{-3}M zirconium solutions derived from stock 10^{-2}M solutions which have aged for 2-10

days are satisfactory (cf. curves 3-5), but the best sensitivity at 60 minutes development time is obtainable for up to four days. The highest sensitivities, all other conditions being optimal, were obtained with a 10^{-3}M solution from 2 hours to 1 day old which is derived from 10^{-2}M solution which is 2-7 days old (cf. curve 4).

Effect of Zirconium Concentration

Figure 4.3 shows a plot of net absorbance vs. time, for a fixed constant concentration of Methylthymol Blue and sulphate, and varying amounts of zirconium. A series of six solutions were prepared, 2.5×10^{-5}M in MTB, 0.20M in hydrochloric acid, with 2, 4, 6, 8, 10-fold molar excess of zirconium over MTB. Another six solutions were prepared similarly, but contained 240 µg of sulphate. All solutions were measured at 586 nm in 10 mm cells against a water blank.

To give maximum sensitivity an excess of zirconium is required over that required to form the 1:1 reagent-zirconium complex. For high sensitivity to be obtained with only a moderate development time, the overall concentration of zirconium in the final solution of which the absorbance is to be measured, should be 10^{-4}M.

Effect of Methylthymol Blue Concentration

Figure 4.4 shows the net difference in absorbance of the catalysed reaction for varying Methylthymol Blue concentrations at constant zirconium concentration. Six solutions were prepared, 10^{-4}M in
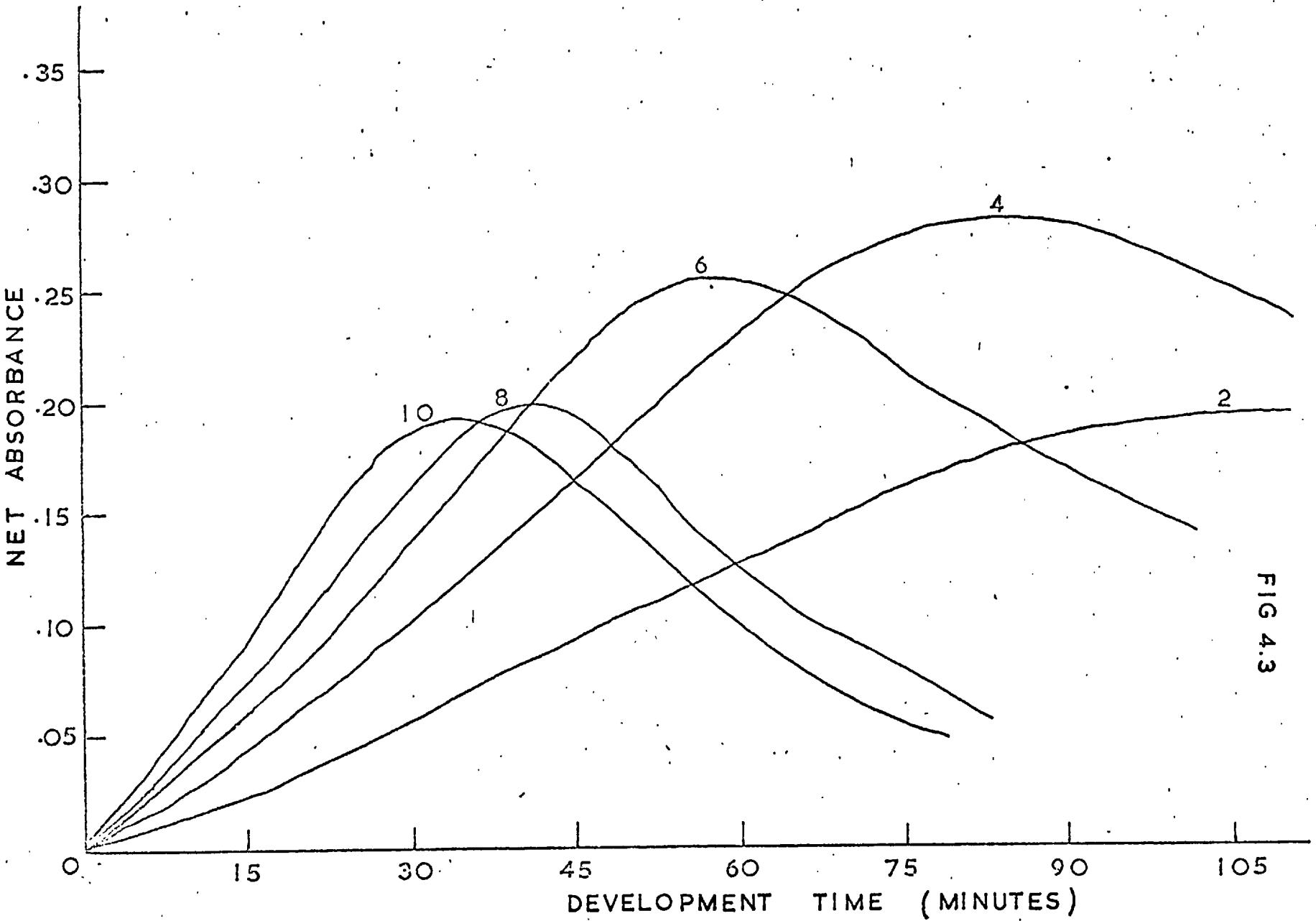
FIG 4.3

FIG 4.4
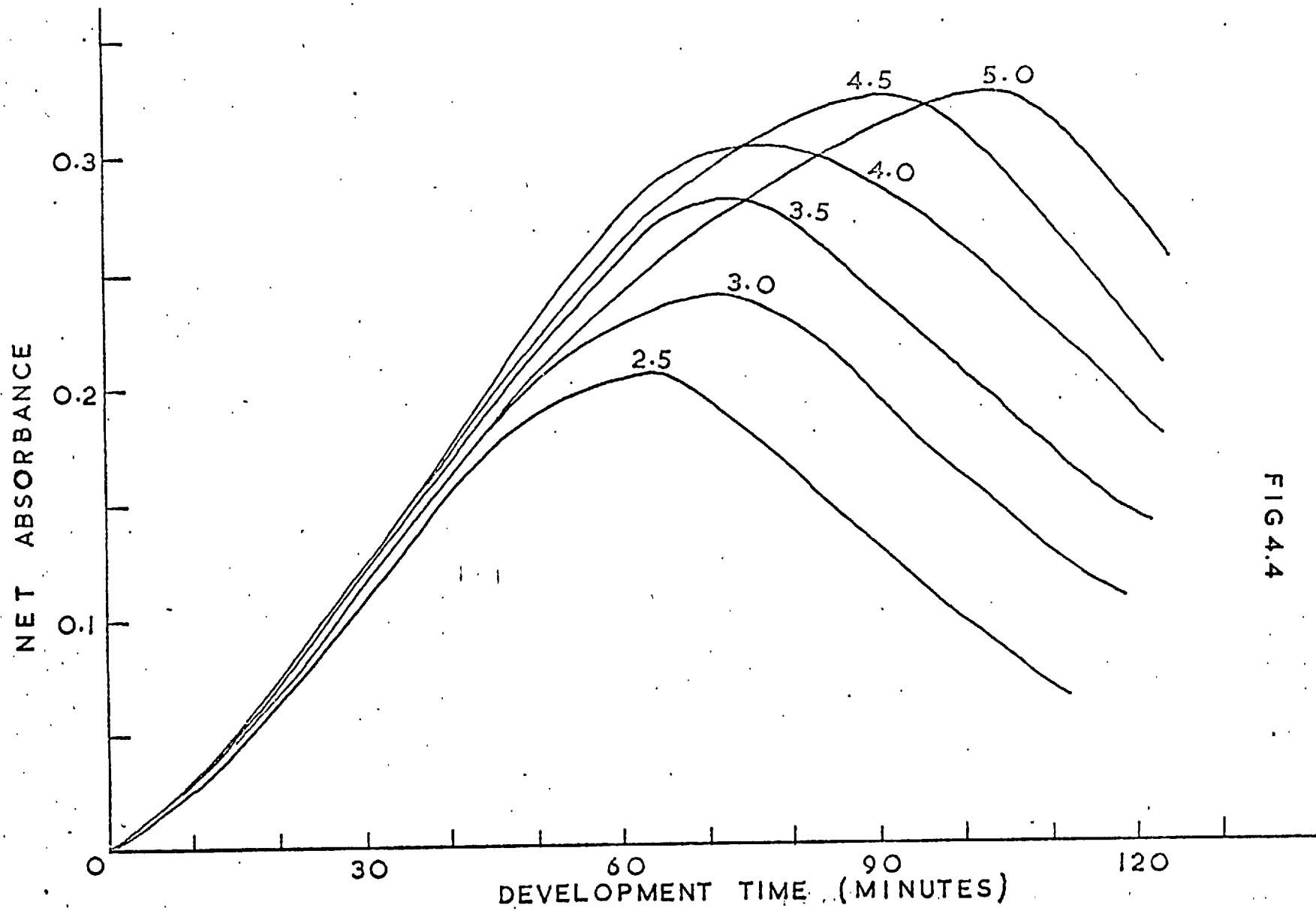
73

zirconium, 0.20M in hydrochloric acid and 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 x 10^{-5}M in MTB. Six other solutions were prepared similarly, but also contained 240 μg of sulphate. Absorbances were measured at 586 nm in 5 mm cells against a water blank.

The net absorbance increases rapidly with increase in the MTB concentration up to 4 x 10^{-5}M with only a small increase in development time for maximum absorbance. At higher reagent concentrations the development time becomes impractically long, with little further increase in sensitivity. For a 60 minute development time, the optimal MTB concentration is therefore 4 x 10^{-5}M.

Effect of Acidity

Figure 4.5 shows the effect of acidity on the rate of colour development with plots of the net difference in absorbance due to the catalysed reaction at varying acidities of the final solution. Blank solutions were 4 x 10^{-5}M in MTB, 10^{-4}M in zirconium and 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40M in hydrochloric acid. Reagent solutions were similar, but contained in addition 240 μg of sulphate. Absorbances were recorded at 586 nm in 10 mm cells against a water blank.

The plots show that sensitivity for sulphate is highest when the catalysed reaction takes place in 0.2M hydrochloric acid.

Effect of Time

For reasons already given (Section 2.4) it was necessary to choose an arbitrary development time in order that the experimental parameters
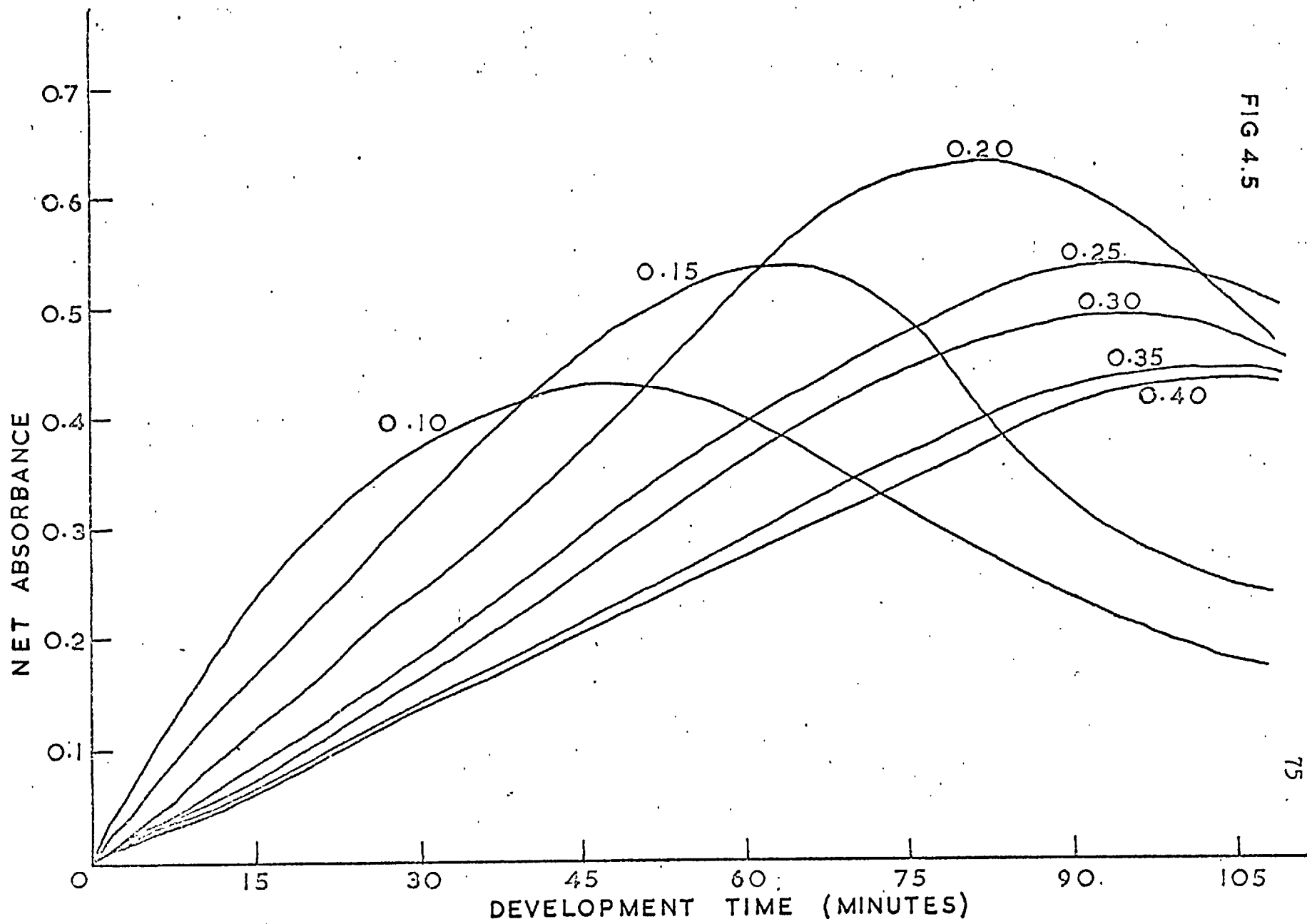
FIG 4.5

0.20

0.25

0.30

0.35

0.40

0.15

0.10

NET ABSORBANCE

DEVELOPMENT TIME (MINUTES)

75

could be optimised.   A development time of 60 minutes was considered convenient.

Effect of Temperature

The rate of the reaction with or without added sulphate is somewhat temperature dependent.   Under optimal experimental conditions this dependence was not found to be critical enough to warrant special control of temperature.   The parameters were consequently optimised at room temperature $(23 \pm 2^{o}C)$.

Precision

The recommended procedure was applied to the determination of 240 µg of sulphate.   The average net absorbance at 586 nm (10 results, 5 mm cell) after 60 minutes was 0.300, and the relative standard deviation was 2.2%.

Calibration Curve and Sensitivity

Calibration curves prepared by the recommended procedure are shown in Fig. 4.6.   As explained previously (Section 2.4) the development time determines the extent of linearity of the calibration curves. Thus the curves are linear between 10 and 240 µg of sulphate for 60 minutes development time $(\varepsilon = 2.02 \times 10^{4})$, from 10 to 144 µg for 72 mins., $(\varepsilon = 2.35 \times 10^{4})$ and from 10 – 96 µg for 84 minutes $(\varepsilon = 2.69 \times 10^{4})$.

Thus, under the recommended conditions, maximum sensitivity for the determination of up to 100 µg of sulphate occurs after 84 minutes development, and accurate   determination of 240 µg cannot be made with more than   60 minutes development time.   The

FIG 4.6

84

72

60

CONCENTRATION OF SULPHATE , PPM
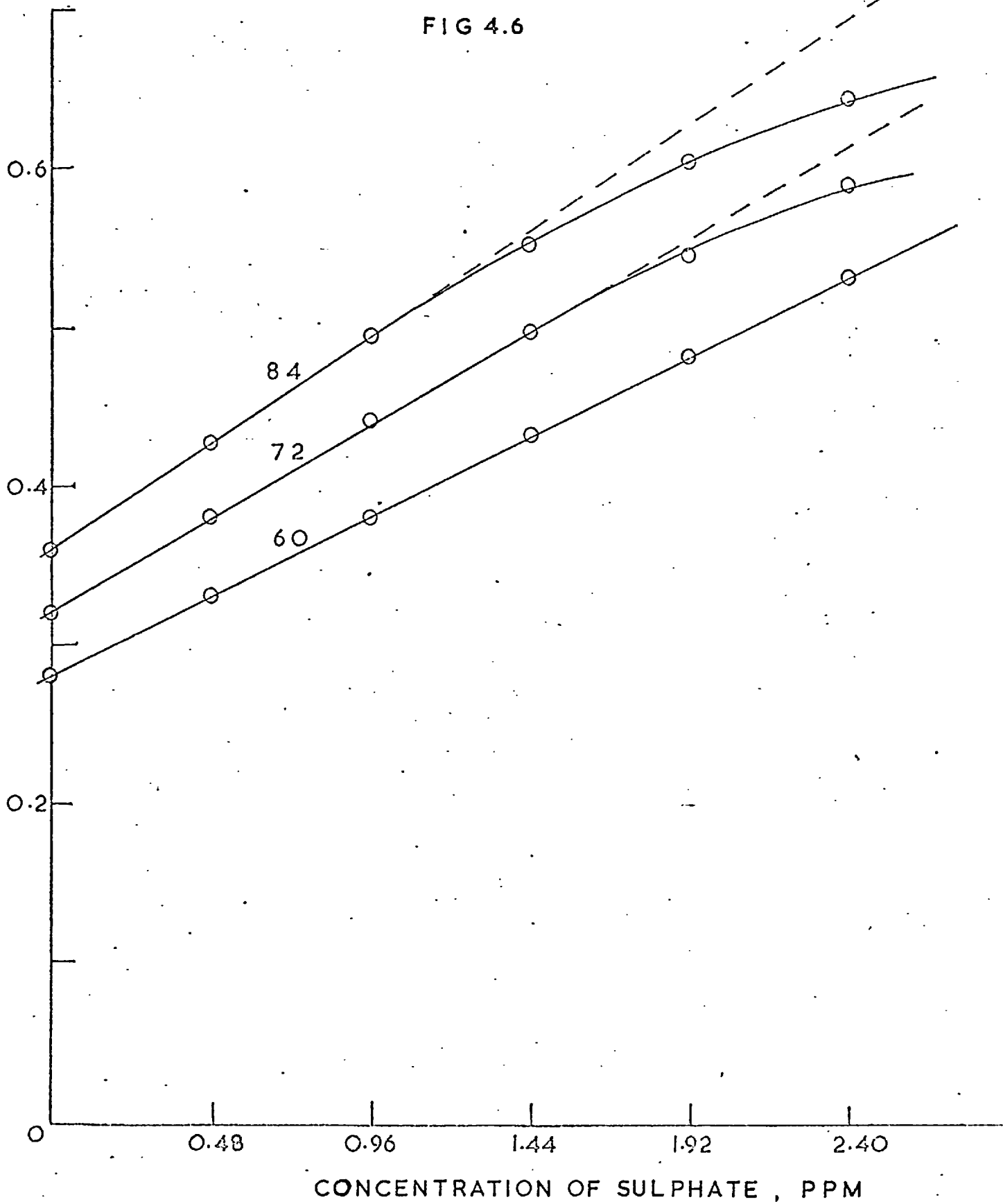
highest molar absorptivity available under the recommended conditions
is approximately 2.0×10^4 and is obtained with 60 minutes development.
It is evident that in practical analysis two standard sulphate
solutions at the limits of the required concentration range should
be developed along with the unknown sulphate samples.

Interferences

The effect of diverse ions on the determination of 240 μg of
sulphate was investigated. Solutions were prepared according to the
recommended procedure, but contained, in addition, the interference
in 100-fold molar excess over the sulphate. For the serious anionic
interference due to fluoride, phosphate and arsenate, the effect of
0.1, 1.0 and 10-fold molar excesses of these anions were also
investigated. The results are shown in Table 4.2.

An ion was considered to interfere if it produced an error greater
than twice the relative standard deviation of sulphate alone in pure
solution (i.e., > 4.4%). Fluoride, phosphate and arsenate were found
to interfere even at trace concentrations (0.1 - fold molar excess).

4.3 Experimental

Reagents

Methylthymol Blue, 5×10^{-4}M. Prepared by dissolving 0.0946 g. of
reagent (Hopkin and Williams Ltd.) in 250 ml. of freshly distilled
water; this solution was discarded after 4-5 days.

Table 4.2

Interference Effect of various ions in 100 fold Molar

Excess over 240 µg SO_4^{2-}

Ion	Absorbance	Ion	Absorbance	Ion	Absorbance
–	0.295	Li	0.300	AsO_4^{3-}*	0.402
Al	0.290	Mg	0.297	$B_4O_7^{2-}$	0.299
Bi	BiOCl pptd	Mn	0.449	Br^-	0.298
Ca	0.301	Na	0.295	Cl^-	0.295
Cd	0.295	Ni	0.294	ClO_4^-	0.297
Ce	0.325	Sb	SbOCl pptd	CO_3^{2-}	0.300
Co	0.292	Se	0.415	F^-*	0.410
Cr	0.288	Si	0.300	I^-	0.298
Cu	0.300	Sn	0.176	NO_3^-	0.295
Fe	0.135	Sr	0.439	PO_4^{3-}*	0.410
Hg	0.290	Te	bleached	S^{2-}	0.405
K	0.295	Th	0.352	SO_3^{2-}	0.410
La	0.295	V	0.136	acetate	0.295
		Zn	0.295	oxalate	bleached
				tartrate	0.154

* 0.1 Molar Excess.

<u>Stock sulphate solution</u>, 10^{-2}M. Prepared by dissolving 1.420 g. of sodium sulphate (Na_2SO_4, analytical reagent grade) in distilled water, and diluting to 1 litre. Working standards (5×10^{-4}M, 48 ppm) were obtained by appropriate dilution.

<u>Zirconium Reagent Solution</u>, 10^{-2}M. Prepared by dissolving 1.611 g. of zirconium oxychloride ($ZrOCl_2 \cdot 8H_2O$, GPR, Hopkin and Williams Ltd.) in 0.0125M hydrochloric acid, and diluting to 500 ml. with the same acid. This stock solution was allowed to age for between 2 and 7 days at room temperature. A 10^{-3}M solution was prepared by diluting 50 ml. 10-fold and 5 ml. of 5M hydrochloric acid were added to make the final acidity 0.05M. This 10^{-3}M solution may be used for up to 4 days, but best sensitivities are obtained when it is used between 2 hours and 1 day after preparation.

All solutions were brought to room temperature before use.

All other reagents were of analytical reagent grade, unless otherwise stated.

Apparatus

Absorption spectra were recorded on a Unicam SP800A double-beam spectrophotometer. Absorbance measurements were made with a Unicam SP500 spectrophotometer fitted with matched 5 and 10 mm silica cells.

Preparation of Calibration Curve

To a series of six 100 ml. volumetric flasks each containing 8 ml. of 5×10^{-4}M Methylthymol Blue, add 4 ml. of 5M hydrochloric acid and

0, 1, 2, 3, 4 or 5 ml. of 5×10^{-4}M sulphate solution respectively.
At zero time, pipette 10 ml. of 10^{-3}M zirconium solution into the
first flask. After 30 seconds dilute the solution to 100 ml. with
distilled water, mix thoroughly and leave to stand. Repeat the
addition of 10^{-3}M zirconium solution to the other flasks at 2 minute
intervals. Allow the solutions to stand for 60 minutes. Measure the
absorbance of each solution at 586 nm against a water blank, using 5
mm silica cells, permitting 2 minutes to elapse between the measure-
ment of each solution.

Run a blank and a standard (5 ml. of 48 μg/ml. sulphate solution)
with each group of samples. The calibration graph is linear over the
range 0.1 - 2.4 ppm (10-240 μg). The effective molar absorptivity for
sulphate at 586 nm is 2.0×10^4.

Sample pretreatment for removal of fluoride, phosphate and arsenate

Transfer 5 ml. of the sample solution containing less than 240 μg.
of sulphate and equimolar amounts of the interfering anions to a 10
ml. conical flask. Add 50 mg. of magnesium oxide (AnalaR), together
with several drops of 2M sodium hydroxide to ensure that the solution
is alkaline. Boil the solution gently for 5 minutes, with occasional
shaking, and then cool the solution in ice-water for 1-2 hours.
Filter the solution through a sintered-glass filter (porosity No. 3)
and wash the conical flask and precipitate with three 2.5 ml. portions
of ice-cold 10^{-4}M sodium hydroxide (pH 10). Transfer this interference
free sulphate solution to a 100 ml. volumetric flask with a further two

2.5 ml. portions of 10^{-4}M sodium hydroxide. Determine the sulphate

content of the solution by the recommended procedure.

Analysis of Organic Compounds

The recommended procedure was applied to the determination of the

sulphur content of organic compounds. The samples ($\sim$ 10 mg., Micro-

Analytical Reagent standards) were decomposed by the oxygen flask

technique with 5 ml. of 0.3% hydrogen peroxide solution as absorbent.

After destruction of the peroxide and carbonate by boiling with

alkali and acid respectively, the absorbing solution was diluted to

250 ml. A 5 ml. portion of the final solution, containing $\sim$ 120 μg.

of sulphate, was analysed in triplicate, and the mean taken.

For one determination, the sulphate standard was mixed with triphenyl-

phospine (MAR standard) before decomposition, such that, after

decomposition and dilution, a 5 ml. portion contained $\sim$120 μg. of

sulphate and $\sim$ 100 μg. of phosphate. The interfering phosphate was

removed by the recommended magnesium oxide separation procedure before

analysis for the sulphate content. The results are shown in Table. 4.3.

4.4 Additional Experimental Investigations

Investigations into the use of metallochromes other than Xylenol

Orange for the kinetochromic effect with zirconium led to the

discovery that Methylthymol Blue gave a similar reaction (Section

3.8).

The reaction between MTB and zirconium has been comprehensively

TABLE 4.3

Analysis of Sulphur Content of Organic Compounds

by Recommended Method

Compound	μg S		% S in compound	
	Actual	Found	Actual	Found
S–Benzyl thiouronium chloride	41.1	40.8	15.8	15.7
	41.1	41.4	15.8	15.9
Benzyl Disulphide	56.3	56.5	26.0	26.1
Benzyl Disulphide + Triphenyl Phosphine	41.0	41.2*	26.0	26.1*

*After removal of P by MgO procedure.

investigated by Cheng [121] who showed that a 1:1 complex was formed in 0.3-1.2N perchloric acid, with a peak of maximal absorption at 580 nm. For this study, the maximal peak was found to occur at 586 nm (Fig. 4.1). A Job continuous variation plot (Fig. 4.7) was prepared. 0-10 ml. of 5.10^{-4}M MTB and 5-0 ml. of 10^{-3}M zirconium solution respectively were added to a series of 100 ml. volumetric flasks, together with 3 ml. of 5N hydrochloric acid. The solutions were diluted to the mark, and allowed to stand for two days, before the absorbance of the solutions was measured against a water blank at 586 nm. The formation of a 1:1 complex is clearly indicated.

A mole-ratio plot (Fig. 4.8) was also prepared, using 5 ml. 5.10^{-4}M MTB, 0-5 ml. 10^{-3}M zirconium solution and 3 ml. 5N hydrochloric acid, diluted to 100 ml. A 1:1 complex is again indicated.

Initially, the kinetochromic action of zirconium with MTB with sulphate anion as catalyst was studied under the same experimental conditions as those developed when Xylenol Orange was the chromophore. It soon became apparent however, that the reaction conditions were far less critical than had been the case with Xylenol Orange. There was less temperature dependence, so that experiments could be conducted at the more convenient level of 23°C (room temperature), rather than 15°C. The development time was also considerably shorter and the sensitivity higher. Thus, with MTB, the molar absorptivity was $\approx$ 20,000 after 60 minutes development time, while with Xylenol Orange the molar absorptivity $\approx$ 15,000 after 3 hours development. Methyl-

FIG 4.7

ABSORBANCE

$[Zr] / [MTB] + [Zr]$

FIG 4.8

ABSORBANCE

$[Zr] / [MTB]$
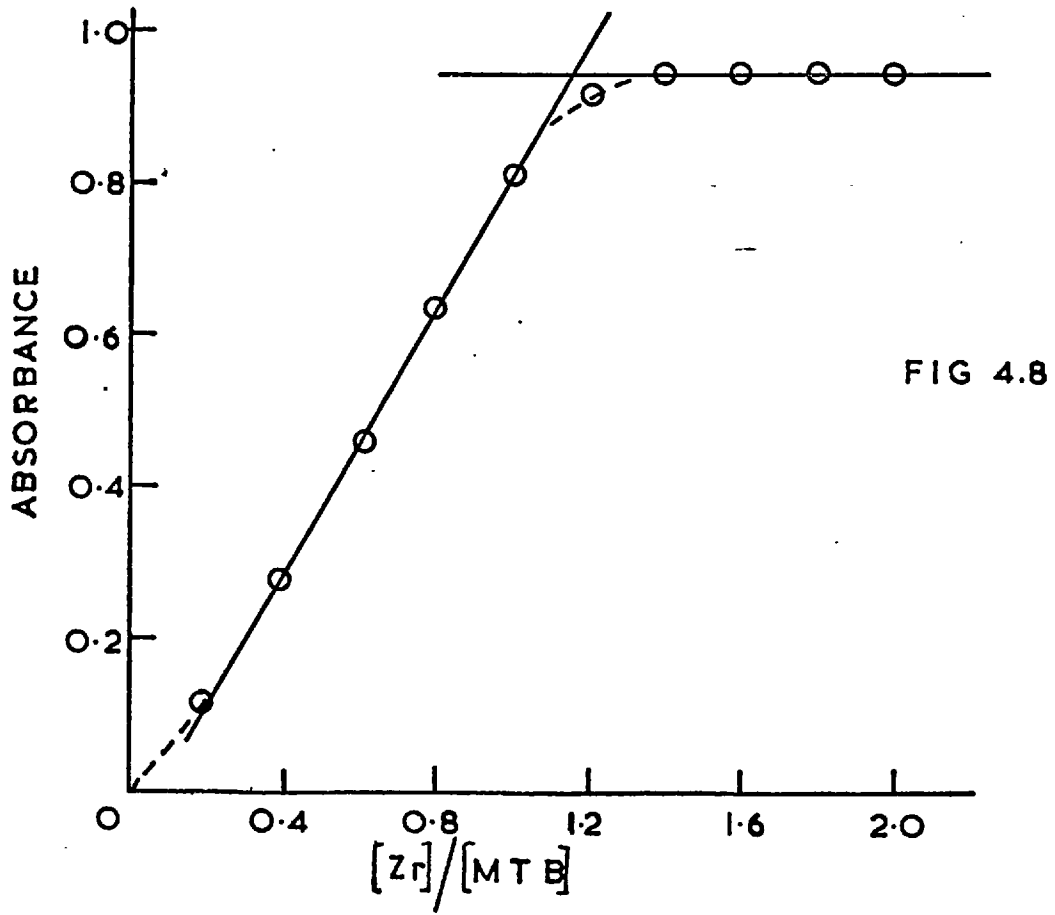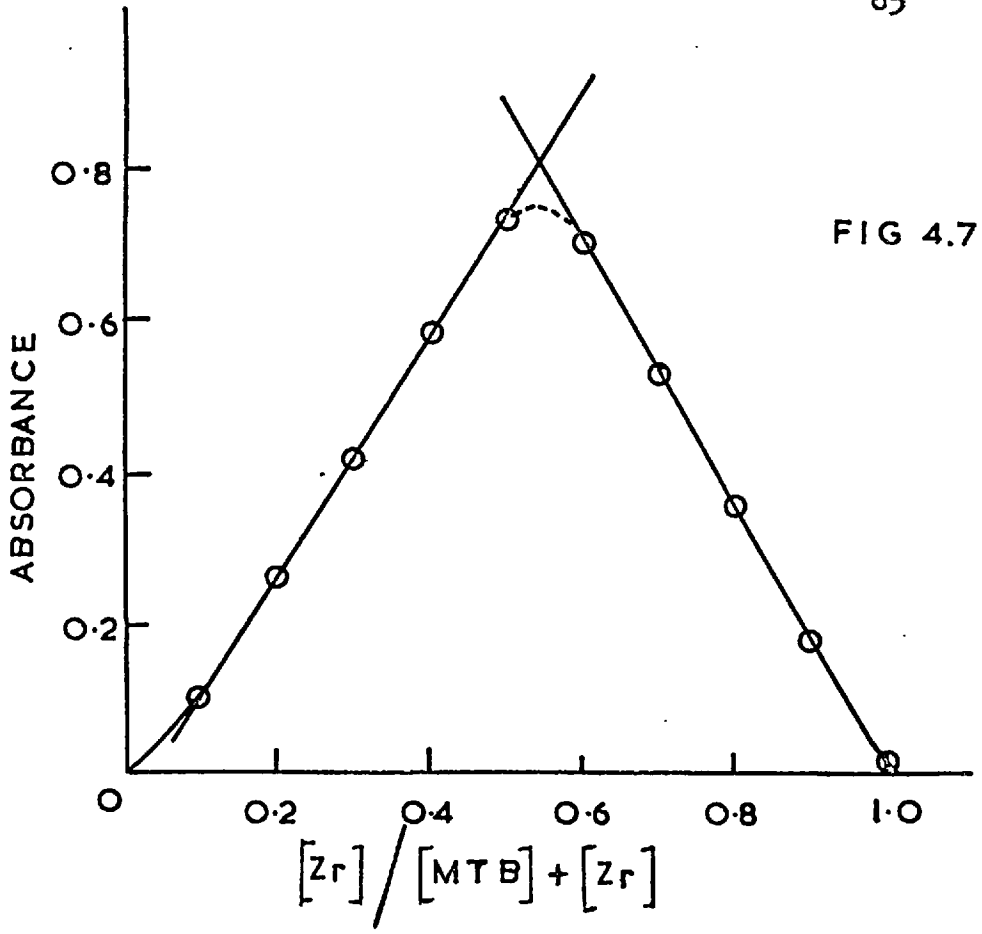
thymol Blue was thus found to be superior to Xylenol Orange for kinetochromic spectrophotometry. A procedure for the determination of sulphate was subsequently developed.

Removal of Interference

Methods for the removal of cationic interference were not investigated, since satisfactory methods using cation exchange have been given elsewhere [120]. The removal of anionic interference, particularly that due to trace quantities of phosphate, fluoride and arsenate was, however, extensively investigated.

The application of solvent extraction was initially studied. Kirkbright, Smith and West [122] have described how phosphomolybdic acid $H_3PO_4(MoO_3)_{12}$, silicomolybdic acid $H_3SiO_4(MoO_3)_{12}$ and arseno-molybdic acid $H_3AsO_4(MoO_3)_{12}$ may be formed, and extracted into organic media. The method was applied to the removal of interference due to phosphate and arsenate.

The heteropolyacids are formed in the presence of a large excess of molybdate reagent in 0.96M hydrochloric acid, and may be extracted into n-butanol. It was found that molybdenum interfered seriously in the determination of sulphate. This interference could be removed by extraction of the molybdenum with amyl acetate from a solution 5M in hydrochloric acid. The procedure developed was as follows:

5 ml. of the sample under investigation was made 1M in hydrochloric acid, and the requisite quantity (1000-fold excess) of molybdate

reagent added to form the heteropolyacids. The solution was extracted twice with 20 ml. portions of n-butanol (presaturated in 1M hydrochloric acid). The acidity of the aqueous phase was adjusted to 5M by the addition of concentrated hydrochloric acid. The solution was then extracted twice with 20 ml. portions of amyl acetate presaturated in 5M hydrochloric acid.

The aqueous phase was heated on a steam bath for 10 minutes to remove organic matter, neutralized by the addition of concentrated ammonium solution, and transferred to a 100 ml. volumetric flask. The sulphate content of the sample was then determined by the recommended procedure.

A water blank taken through the extraction procedure was ~40% higher in absorbance compared to an untreated water sample. Sulphate samples also gave higher absorbance values (~ 60%) after extraction compared to untreated sulphate standards. Phosphate and arsenate, present in 5-fold excess over the sulphate, gave absorbance readings ~15% greater than the extracted sulphate sample, indicating that their interference had been largely removed.

The large increase in the rate of reaction for the extracted samples was considered to be due to impurities in the reagents used, and the presence of excess organic reagent in the final sample solution. It was verified by the use of atomic absorption spectrophotometry that only trace quantities of molybdenum remained in the aqueous phase after the second amyl acetate extraction so that the increase in

reaction rate was not caused by molybdenum interference.

No further studies were made with solvent extraction, owing to the high rates of reaction encountered, coupled with the fact that the final sample volume was high ($\sim$ 60 ml.) and was of very high ionic strength. In addition, considerable time was required for the separation.

The application of ion-exchange was next investigated. Nielsen [96] has described a procedure for the concentration of fluoride ion on an anionic exchanger. All anions are initially adsorbed. Elution with 0.2M sodium acetate elutes the fluoride first (20 ml.), then phosphate (50-70 ml.), while 100 ml. fails to elute the sulphate present. This can only be removed by elution with 25 ml. of 1M acetate solution. This procedure was studied for the removal of interference due to phosphate, arsenate and fluoride.

The anion exchanger used was Amberlite IRA-400 (14-52 mesh), chloride form. This was converted to the acetate form before use. A resin column 5 cm. high and 11 mm. in diameter was used. Results in agreement with those given [96] were established. 25 ml. of 1M sodium acetate successfully desorbed sulphate from the resin, but very little catalytic activity was observed when the recommended procedure was applied to the eluate. The decrease in rate was shown to be due to the very high acetate concentration of the sample solutions. Removal of the excess acetate as acetic acid by acidification and boiling was

unsuccessful. The use of sodium hydroxide, hydrochloric acid and nitric acid as eluents was studied, without success.

Honda and Tadano [123] have described the adsorption and desorption of sulphate on the weakly basic phenolic anion exchanger Amberlite IR -4B. The removal of anions other than sulphate is accomplished with hydrochloric acid, and sulphate is removed with 0.2N sodium hydroxide. Application of this procedure showed that phosphate interference was appreciably reduced, but that the desorption of sulphate was irreproducible under the conditions used.

The use of collectors in removing the anionic interferences was investigated. Palaty [64] and Lambert, Yasada and Grotheer [65] have described how the interference of fluoride and phosphate in the determination of sulphate may be eliminated by the removal of the anions as the insoluble lanthanum salts. According to the level of interference, either a solution of lanthanum chloride, or a suspension of lanthanum hydroxide was used, in slight excess. Application of the procedure was, however, unsuccessful. Qualified success was obtained, however, when the lanthanum hydroxide was precipitated _in situ_, in that the removal of the interference due to amounts of phosphate and arsenate equimolar with sulphate was accomplished. The interference due to fluoride was reduced, but not removed. The procedure developed was as follows:-

5 ml. of the sample solution containing less than 240 μg. of sulphate and equimolar amounts of phosphate and/or arsenate were added to a 10

ml. centrifuge tube. 2 ml. of 5.10^{-2}M lanthanum chloride were added, followed by 0.5 ml. of 2N sodium hydroxide. The solution was thoroughly mixed, and centrifuged for 10 minutes. The supernatant liquid was transferred to a 100 ml. volumetric flask. 5 ml. of 10^{-2}M lanthanum chloride and 0.5 ml. 2N sodium hydroxide were added to the centrifugate, the solution thoroughly mixed and then centrifuged again. The supernatant wash liquid was added to the 100 ml. flask as before. The sulphate of the solution then was determined by the recommended procedure. It was necessary to take a water blank and the sulphate standards of the calibration curve through the centrifugion procedure, whence there was a reduction of sensitivity obtained. The presence of trace impurities in the reagent used caused the absorbance of the centrifuged water blank to increase by $\sim$7% compared to an untreated pure water sample. There was also adsorption of trace amounts of sulphate by the lanthanum hydroxide, so that the centrifuged standards were reduced in absorbance by $\sim$8%. The overall sensitivity was consequently reduced by $\sim$15%.

The use of solid zinc oxide as a collector was unsuccessful. Precipitation of zinc hydroxide in situ was also unsuccessful. The use of magnesium oxide as a collector was next studied. Several workers 46, 120 have removed the interference of phosphate in sulphate determinations by this technique. A procedure was developed whereby the interference due to arsenate and fluoride, as well as phosphate, could be removed, when these three anions were present in amounts less than equimolar to sulphate. Details have already been given (Section 4.3).

Filtration of the magnesium oxide was carried out at $0^{\circ}C$ since other workers [42] have found that at room temperature the collection of phosphate is not so effective. The magnesium salts of phosphate, arsenate and fluoride are all slighly soluble in acid, so that the procedure must be carried out in an alkaline medium.

CHAPTER V

The determination of fluoride by catalysis

of the Zirconium - Methylthymol Blue reaction

5.1 Introduction

The slow reaction between polymerised zirconium solutions and

Methylthymol Blue is catalysed by sulphate, fluoride, aresenate and

phosphate anions.   In the previous chapter, the kinetochromic action

of sulphate was investigated, and forms the basis for a direct

spectrophotometric method for the determination of sulphate anion

[124].   This chapter reports a study made of the effect of fluoride

ion on the reaction between zirconium and Methylthymol Blue.

5.2 Investigation of Experimental Parameters

Spectral characteristics

The wavelength of maximum absorbance for the zirconium - Methyl-

thymol Blue complex occurs at 586 nm (Fig. 4.1).   All absorbance

measurements were made at this peak wavelength.

Preparation of zirconium solutions

The conditions optimised for the preparation of zirconium

solutions which were developed for the determination of sulphate

were also used in this study.   Stock 10^{-2}M zirconium solutions were

prepared in 0.0125M hydrochloric acid, and allowed to stand 2-7

days. 10^{-3}M working solutions 0.05M in hydrochloric acid were

prepared by dilution, and allowed to age between 1 hour and 4 days

before use.

Development Time

For the reasons already given, the development time was artificially fixed as 60 minutes, and all experimental parameters were optimised so that smooth calibration curves were obtained when absorbances were measured after this time.

Temperature

All experiments were conducted at room temperature ($23^{\circ} \pm 2^{\circ}C$).

Effect of Acidity

The effect of acidity on the rate of colour development is shown is Fig. 5.1. Graphs which show the variation of the net difference in absorbance due to the catalysed reaction with development time, at varying hydrochloric acid molarities of the final solutions, are given. The MTB concentration was $10^{-4}M$ with a zirconium concentration of $2 \times 10^{-4}M$. Reagent solutions contained in addition 4.75 μg of fluoride ($5 \times 10^{-6}M$). Absorbances were measured in 2 mm. cuvettes at 586 nm against a water blank. With a 60 minute development time, highest sensitivities are obtained when the reaction is allowed to proceed in 0.7 M hydrochloric acid.

Effect of Methylthymol Blue concentration

The effect of the MTB concentration on the net absorbance due to the catalysed reaction obtained at development times of 30, 60 and 90 minutes is shown in Fig. 5.2. The solutions were $2 \times 10^{-4}M$ in zirconium, 0.7M in hydrochloric acid and 0.8, 0.9, 1.0, 1.1, 1.2 x

FIG 5.1
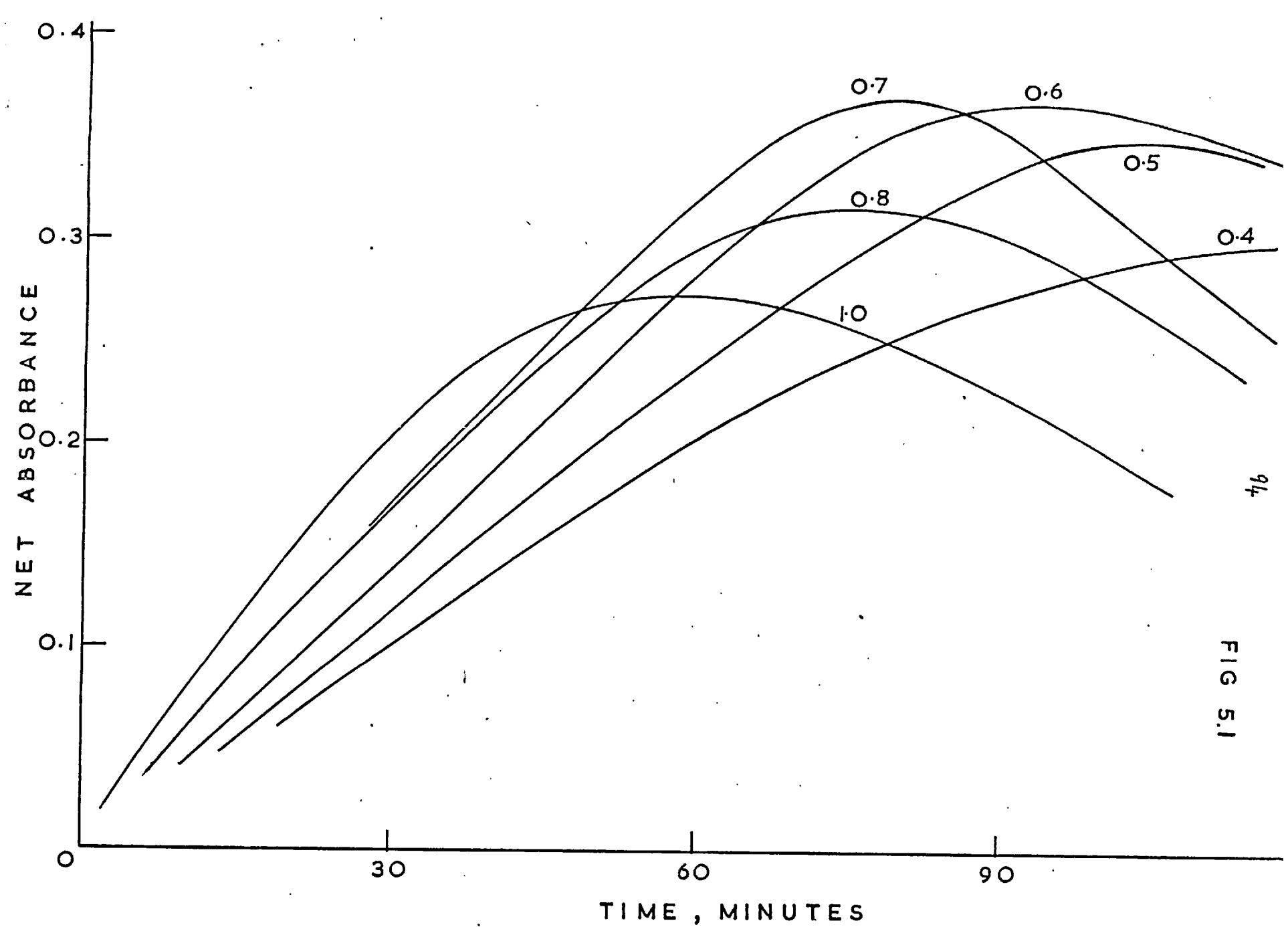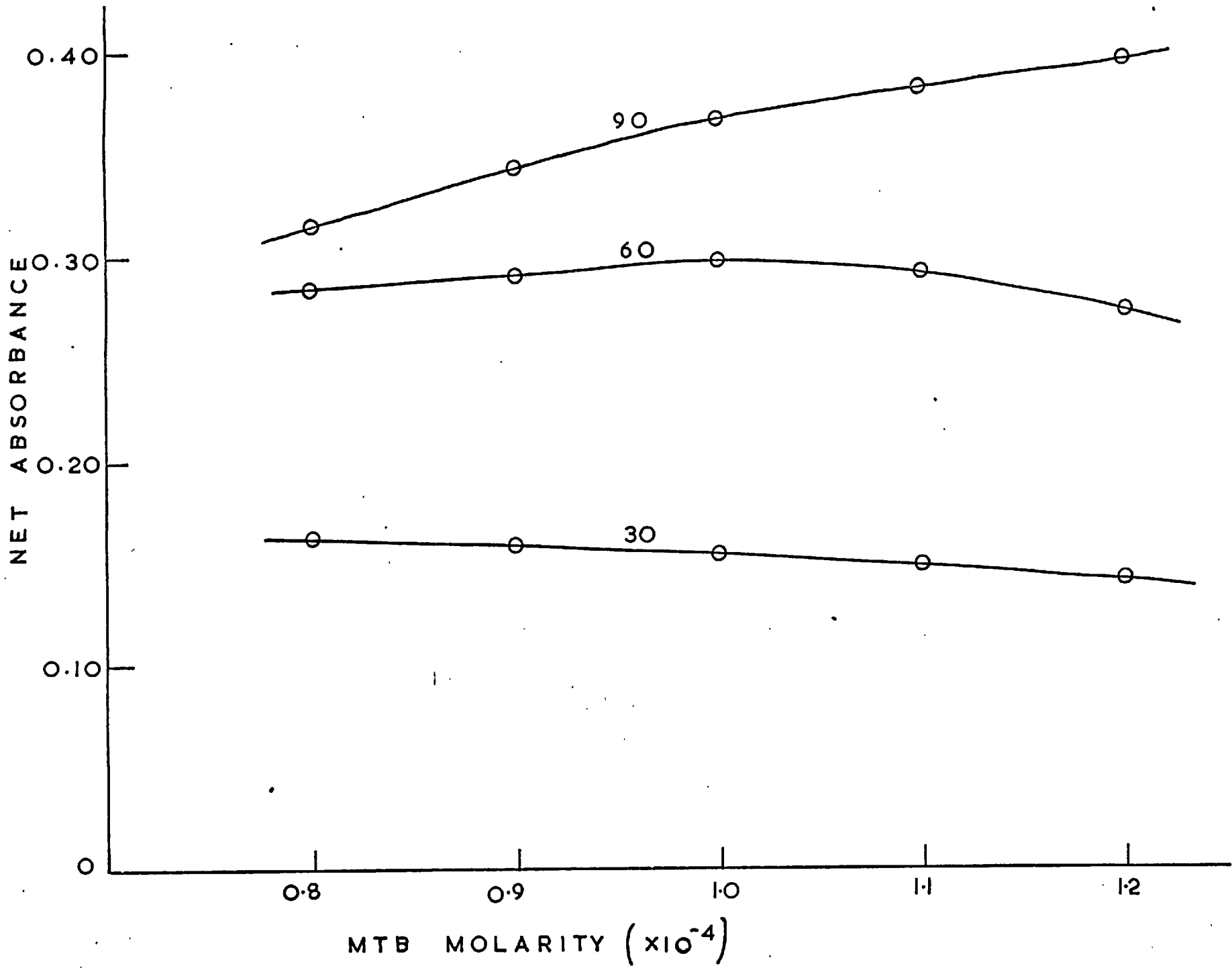
FIG 5.2

Y-axis: NET ABSORBANCE (0.10, 0.20, 0.30, 0.40)

X-axis: MTB MOLARITY $(\times 10^{-4})$ (0.8, 0.9, 1.0, 1.1, 1.2)

Curves labelled: 90, 60, 30

10^{-4}M in Methylthymol Blue. The reaction was catalysed by 0.095 ppm (4.75 μg) fluoride. Absorbance measurements were made at 586 nm against a water blank in 2 mm cells.

When the MTB concentration was increased the maximum net absorbance value obtained also increased, but with consequent increase also of the development time necessary to achieve the maximum. For a development time of 60 minutes, figure 5.2. shows clearly that a MTB concentration of 10^{-4}M yields the highest sensitivity. It is necessary to use 2 mm. cuvettes to enable absorbance measurements to be made within the optimum range of 0.2 - 0.8 absorbance units.

Effect of Zirconium concentration

The effect of the Methylthymol Blue:zirconium concentration ratio upon the net absorbance obtained at development times of 45, 60 and 75 minutes is shown in Fig. 5.3. The solutions were 0.7M in hydrochloric acid, 10^{-4}M in MTB and 1.0, 1.5, 2.0, 2.5, 3.0 x 10^{-4}M in zirconium. Reagent solutions were catalysed by 0.095 ppm (4.75 μg) of fluoride. Absorbances were measured at 586 nm against distilled water in 2 mm cells.

For a 60 minute development time, a Methylthymol Blue:zirconium ratio of 1:2 gave the highest net absorbance value.

Calibration Curve and Sensitivity

Calibration curves were prepared by the recommended procedure
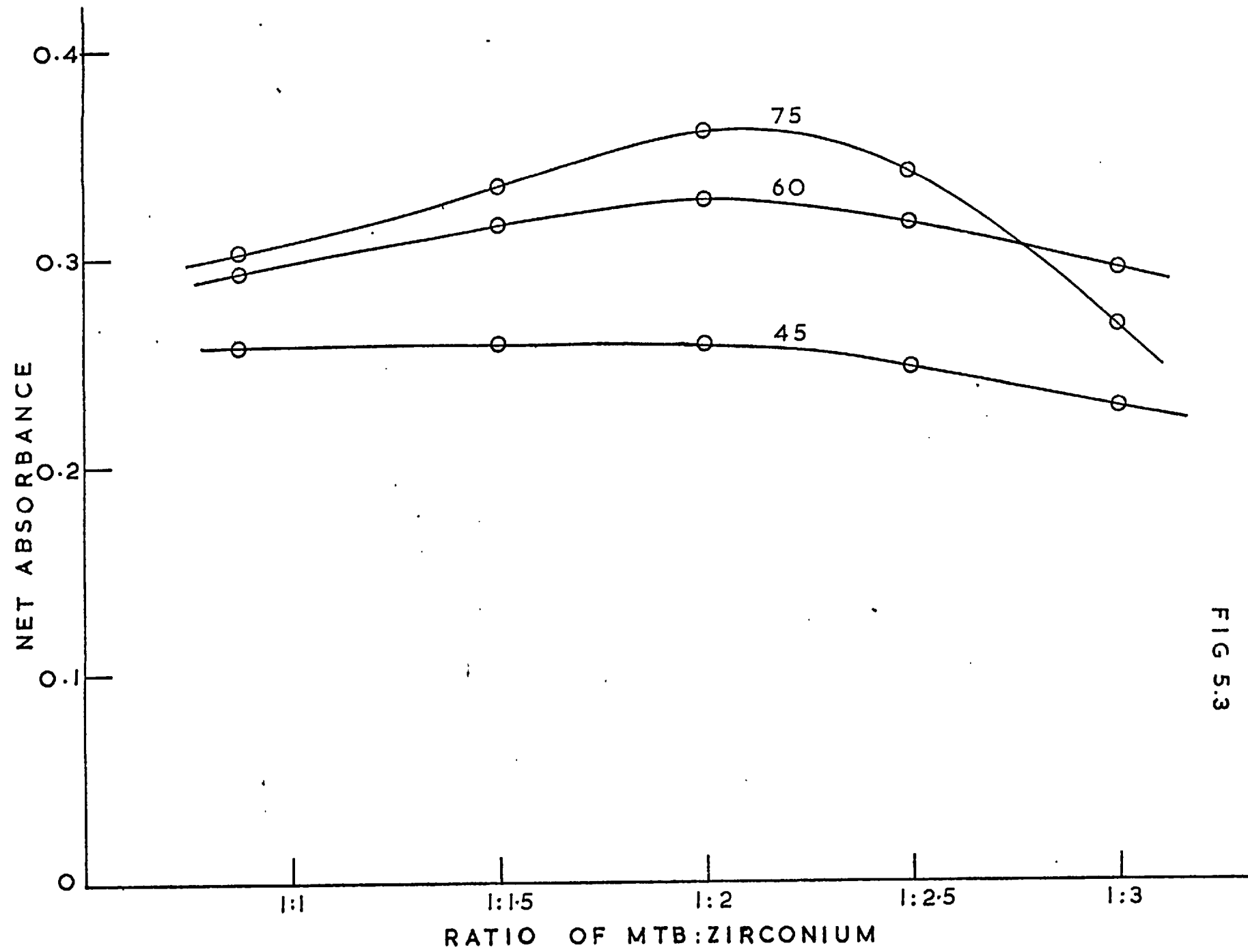
FIG 5.3

and the absorbances measured at 586 nm against a water blank at various development times. The solutions were 10^{-4}M in MTB, 2×10^{-4}M in zirconium, 0.7M in hydrochloric acid and 0, 1, 2, 3, 4, 5 $\times 10^{-6}$M in fluoride (0-4.75 µg; 0-095 ppm fluoride).

Unlike the linear calibration curves obtained in the determination of sulphate (Chapter IV), smooth curves result from the action of fluoride ion on the Methylthymol Blue reaction. The effect of development times of 36, 60 and 84 minutes upon the shape of the calibration curve is shown in Fig. 5.4. After 60 minutes, solutions containing larger quantities of fluoride begin to approach the maximum absorbance possible under these conditions, causing the shape of the curve to change.

From the calibration curve, 4.75 µg of fluoride (0.095 ppm) gives an effective molar absorptivity of 330,000 at 586 nm after 60 minutes development.

Precision

The recommended procedure was applied to the repetitive determination of 4.75 µg of fluoride over a period of several months. The average net absorbance at 586 nm (10 results, 2 mm cells) after 60 minutes was 0.323 and the relative standard deviation was 2.2%.

Interferences

Those cations which interfered in the determination of sulphate (Table 4.2) were considered to interfere in the determination of

FIG 5.4
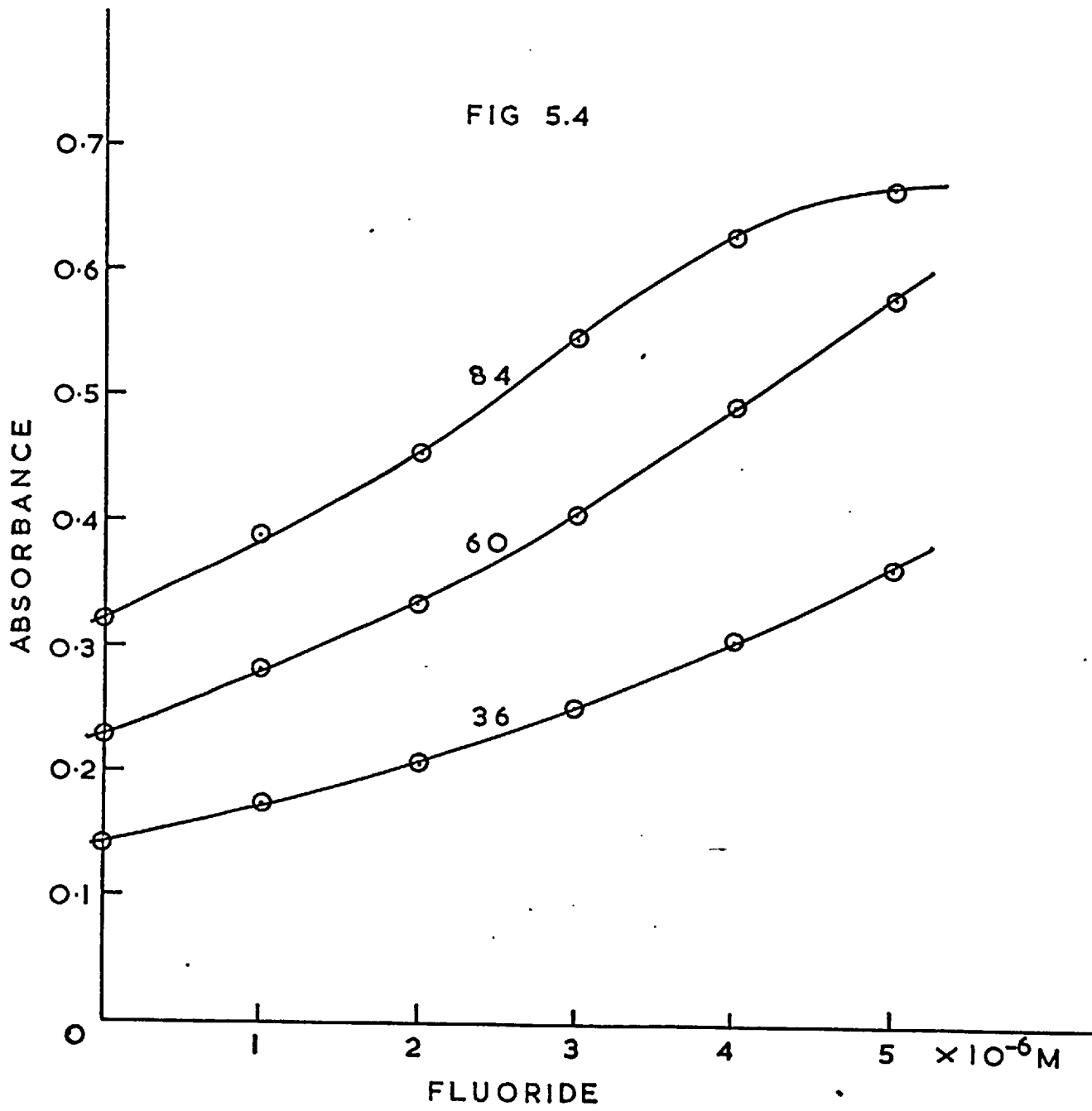
fluoride also, and no further cationic interference studies were made. The effect of anionic interference on the determination of fluoride by the recommended procedure was, however, investigated. Table 5.1 shows the interference effect of phosphate, arsenate and sulphate when these anions are present in 0.1, 1, 10 and 100-fold molar excess over 4.75 µg of fluoride. Trace quantities of phosphate and arsenate cause serious interference and must be removed before the determination of fluoride. Sulphate, however, does not interfere when present in amounts less than equimolar with fluoride.

<u>Table 5.1</u>

<u>Interference effect of anions in varying molar</u>

<u>excess over 4.75 µg fluoride</u>

	Net absorbance			
Molar Excess	0.1	1	10	100
Arsenate	0.346	0.450	0.510	0.509
Phosphate	0.415	0.510	0.511	0.510
Sulphate	0.320	0.328	0.425	0.505

Net absorbance for fluoride = 0.321

5.3 <u>Experimental</u>

<u>Apparatus</u>

Spectra were recorded with a Unicam SP.800A double-beam spectrophotometer. Absorbance measurements were made with a Unicam SP600

spectrophotometer. Matched 2 mm quartz cuvettes were used.

Reagents.

Methylthymol Blue, 5×10^{-4}M. 0.0976 g. of reagent were dissolved in freshly distilled water and diluted to 250 ml. The solution was discarded after 4-5 days.

Stock fluoride solution, 10^{-2}M. 0.2100 g. of sodium fluoride (AnalaR) were dissolved in distilled water and diluted to 500 ml. Working standards (5×10^{-5}M, 0.95 ppm) were prepared by appropriate dilution. All fluoride solutions were stored in silica-free (polythene) containers.

Zirconium reagent solution, 10^{-2}M. 1.611 g. of zirconyl chloride were dissolved in 0.0125M hydrochloric acid, and diluted to 500 ml. with the same acid. This stock solution was allowed to age for 2-7 days at room temperature. A 10^{-3}M solution was prepared by adding 50 ml. of stock solution to 5 ml. of 5M hydrochloric acid and diluting to 500 ml. The final acidity was 0.05M. This 10^{-3}M solution was used 1 hour-4 days after preparation.

Distilled water from an all-glass still was used throughout. All other reagents were of analytical reagent grade unless otherwise stated. All solutions were brought to room temperature before use.

Preparation of Calibration Curve

To a series of 50 ml. volumetric flasks each containing 10 ml.

of 5×10^{-4}M Methylthymol Blue, add 7 ml. of 5M hydrochloric acid and 0, 1.0, 2.0, 3.0, 4.0 and 5.0 ml. of 5×10^{-5}M sodium fluoride. At zero time, pipette 10 ml. of 10^{-3}M zirconium solution into the first flask. After 30 seconds dilute the solution to 50 ml. with distilled water, mix thoroughly and leave to stand. Repeat the procedure for the other flasks at two-minute intervals. Allow the solutions to stand 60 minutes. Measure the absorbance of each solution at 586 nm against a water blank, using 2 mm cells, permitting two minutes to elapse between the measurement of each solution.

Run a blank and several standards with each group of samples. The calibration range extends from 0.25-4.75 μg of fluoride (0.005-0.095 ppm). The effective molar absorptivity for fluoride at 586 nm is 3.2×10^{5}.

Effective molar absorptivities greater than 4.0×10^{5} are obtainable if the calibration range is restricted to 0.045-0.095 ppm (2.25-4.75 μg) fluoride. The procedure for the preparation of calibration curves is identical to that described above, except that the aliquots of fluoride solution added are 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml. of 5×10^{-5}M fluoride.

5.4 Additional Experimental Investigations

Order of Addition of Reagents

The reagent solutions of Methylthymol Blue, hydrochloric acid and standard fluoride (or sample) may be mixed in any order. It is

essential, however, that the recommended procedure for the final addition of zirconium to the 50 ml. flasks is rigidly adhered to, if meaningful results are to be obtained.

Effect of flask volume before dilution

It was found that the volume of the standard solutions, before final addition of zirconium solutions and dilution to 50 ml. had a definite effect upon the rate at which the subsequent reaction proceeded. It is necessary to ensure that the volumes of calibration standards and sample solutions agree to within a few ml. before the zirconium solution is finally added according to the recommended procedure.

Removal of interferences

The removal of cationic interference was not investigated, since satisfactory methods have already been given [120]. The removal of the serious anionic interference due to phosphate, arsenate and sulphate was, however, investigated.

The anion exchange procedure of Nielsen [96] described previously (Section 4.4) was again studied. In this procedure all anions are initially adsorbed on an anion exchanger. The fluoride can then be eluted with 20 ml. of 0.2M sodium acetate solution, with the other (interfering) anions remaining on the column.

Experiments were conducted using 25 ml. of 0.2M acetate solution as eluant for fluoride. It was shown that this concentration of

acetate in the final solution (0.1M acetate) caused an acceleration in the rate of reaction between zirconium and Methylthymol Blue. This was caused by the buffer action of the acetate so that the pH of the solution was raised. The increase in the rate of reaction was counteracted by the use of 5 ml. of 5M hydrochloric acid in the preparation of the standard solutions, instead of the recommended 7 ml. Under these conditions, with final concentrations of 0.5M hydrochloric acid and 0.1M sodium acetate, the reaction between zirconium and Methylthymol Blue was catalysed by fluoride ion under the recommended conditions, but with a slight decrease in sensitivity of 5%.

The use of the strongly basic anion exchanger Amberlite IRA 400 was investigated, with a resin column of 5 cm. in height and 11 mm. in diameter. It was verified that all anions were completely adsorbed on the the column in the acetate form. When 25 ml. of 0.2M acetate solution was used as eluant, fluoride could be reproducibly removed from the column, but with a recovery of only 45%. The use of 25 ml. of 0.25M acetate solution increased the recovery from 4.75 μg of fluoride to 75%. When 25 ml. quantities of 0.30M and 0.35M acetate solution were used as eluants, the recovery of fluoride remained at ~75% for both concentrations.

In view of the unsatisfactory inefficiency of the desorption of fluoride from the anion exchanger, no further studies were made on the removal of interferences by anion exchange.

The interference of phosphate in the determination of fluoride can be eliminated by the removal of phosphate as the insoluble silver salt. [90, 125-127] The separation procedure described by Fennel [125], using silver nitrate solution as precipitant, was initially investigated, but without success.

Collection of interfering phosphate with solid silver oxide has been successful in several cases [126, 127] and this was next studied. The experimental procedure employed for the study was identical to that already described for the sample pretreatment of solutions in the determination of sulphate (Section 4.3), except that silver oxide was used instead of magnesium oxide. The removal of up to 2-fold molar excess of phosphate (i.e. 48 μg) over 4.75 μg of fluoride was attempted, using up to 700 mg. of silver oxide, but without success. Various modifications, including the use of alcohol to depress the solubility, were tried, but very inefficient separations resulted, together with high blank readings.

The use of lead nitrate solution as precipitant was also studied. Lead phosphate was successfully precipitated, but the fluoride also present in the solution was efficiently collected by the precipitate, so that no catalytic action was observed. All attempts to avoid co-precipitation, or to recover the adsorbed fluoride quantitatively were not successful.

The removal of phosphate interference thus presented considerable

difficulty. In order that 4.75 µg of fluoride can be determined within the limits of experimental error, it is essential that the sample solution contains less than 1 µg. of phosphate. Published separation procedures [90, 96, 125-127] are effective when mg. quantities of fluoride and phosphate are present, but they do not appear to be effective when only trace quantities are to be separated.

Many workers [78, 87, 94, 96] have separated fluoride from interfering ions by distillation of hydrofluorosilicic acid, H_2SiF_6, usually by adoption or modification of the method first proposed by Willard and Winter [76]. More recently, micro-diffusion techniques have been developed, [89, 128, 129] enabling much smaller quantities of fluoride to be accurately determined. These techniques could not be investigated however, owing to the lack of the specialised equipment necessary. Further investigations into the removal of anionic interference were therefore postponed.

CHAPTER VI

The Determination of Sulphate by catalysis

of the zirconium – Morin reaction

6.1. Introduction

The kinetochromic procedures for the determination of sulphate and fluoride described in previous chapters are sensitive, particularly that for fluoride. It was considered that the sensitivity could be improved further if the spectrophotometric system could be replaced by a more sensitive spectrofluorimetric technique. Attempts were therefore made to replace Methylthymol Blue with a fluorescent indicator, which reacted with polymerised zirconium solutions in a similar manner to that of MTB.

Three reagents are commonly employed for the fluorimetric determination of zirconium; flavanol [130], Quercetin [131] and Morin [132]. No samples of flavanol or Quercetin were available so that their kinetochromic action could not be investigated. The reaction between zirconium and Morin was, however, studied and a favourable kinetochromic response was obtained. This chapter reports the findings of a study made using sulphate as the catalyst.

6.2 Investigation of Experimental Parameters.

Preparation of zirconium solutions

Zirconium solutions were prepared and used in a manner identical to that previously described (Section 4.2). 10^{-2}M zirconium solutions

were prepared in 0.0125M hydrochloric acid and allowed to stand 2-7 days. 10^{-3}M zirconium solution, 0.05M in hydrochloric acid, were prepared by appropriate dilution and used 1 hour-4 days after preparation.

Spectral characteristics

The excitation and emission spectra of the zirconium-Morin complex in acidic medium are shown in Fig. 6.1. The solution was 10^{-4}M in zirconium, 10^{-3}M in Morin, and 2M in hydrochloric acid. The excitation spectrum was obtained by measuring the emission at 505 nm while the emission spectrum was obtained by excitation at 417 nm. These spectra are uncorrected for variations in the emission characteristics of the lamp, the transmission of the two monochromators and the response characteristics of the photomultiplier. The relevant correction curves have been given elsewhere [133].

The excitation and emission maxima occur at 417 nm and 505 nm respectively. All subsequent fluorescence measurements were made with the excitation and emission monochromators set at 417 and 505 nm respectively. The fluorescence of morin solutions alone was found to be negligible under these conditions.

Effect of Acidity

The effect of acidity on the fluorescence intensity of the zirconium-Morin reaction has been studied [132]. The fluorescence reaches a maximum in approximately 1.8M hydrochloric acid. At this

FIG 6.1

EXCITATION   EMISSION

A   B

FLUORESCENCE

350   400   450   500   550 NM

WAVELENGTH
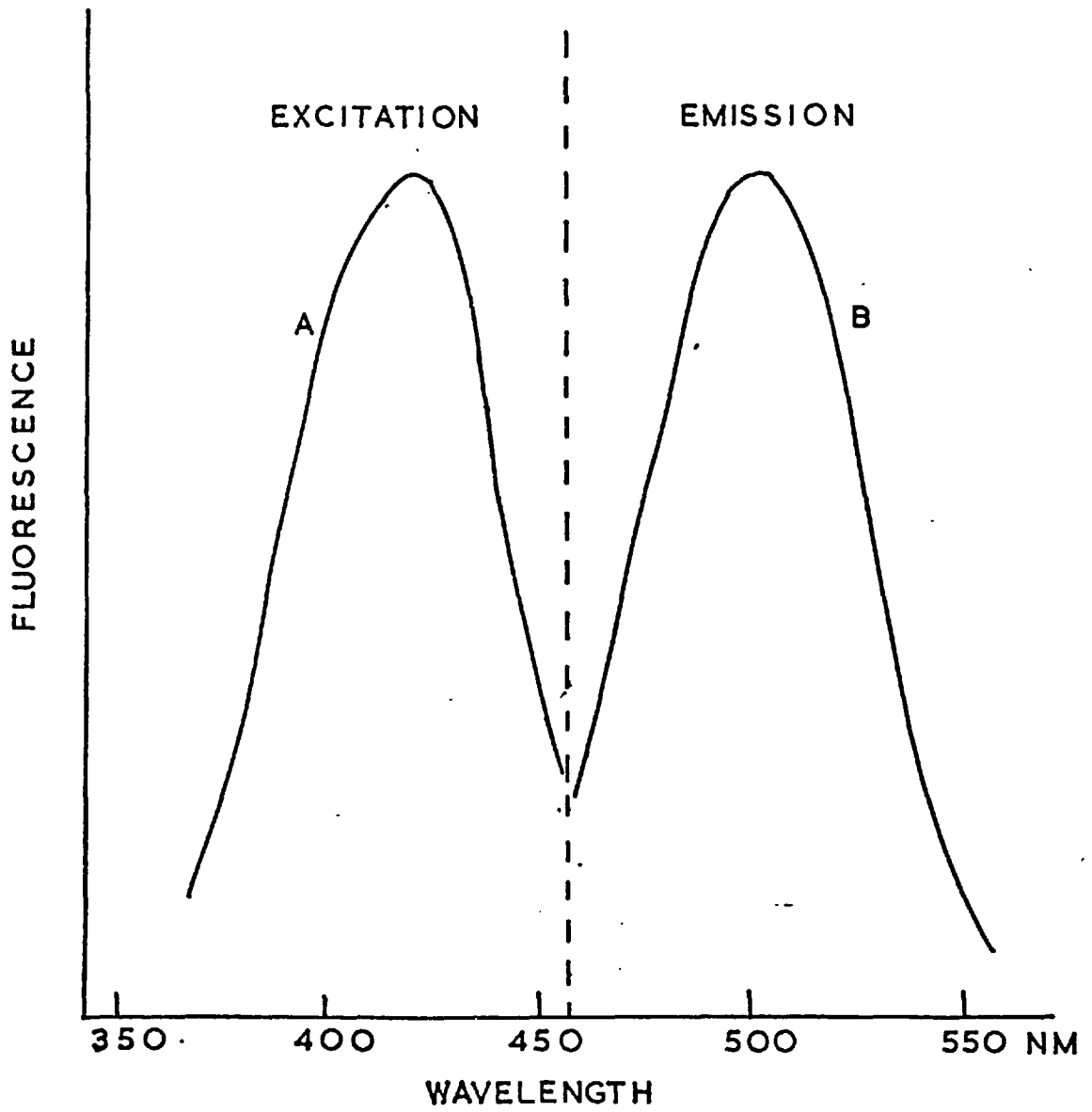
acidity maximal fluorescence is developed within five minutes after the mixing of the solutions, even when polymerised zirconium solutions are employed. This rate of reaction is clearly too rapid for satisfactory kinetochromic measurements to be made. It was found that the rate of the blank reaction between zirconium and Morin became conveniently slow when the solutions were 0.6M in hydrochloric acid, and that satisfactory enhancement in the rate was effected by the presence of sulphate in the solution. In agreement with previous workers [132], the maximum fluorescence signal obtained at 0.6M acidity was 55% lower than that obtained when solutions were 1.8M in hydrochloric acid.

Effect of Morin Concentration

The effect of morin concentration on the net fluorescence (uncorrected) due to the sulphate catalysed reaction is shown in Fig. 6.2. The solutions were 10^{-4}M in zirconium and 0.6M in hydrochloric acid. The Morin concentration was varied from 0.7 - 1.3 x 10^{-4}M. Reagent solutions were catalysed by 10^{-4}M sulphate. All fluorescence measurements were made after 60 minutes development time, exciting at 417 nm and emitting at 505 nm. A Morin concentration of 10^{-4}M gave optimum results.

Effect of zirconium concentration

The effect of zirconium concentration on the net fluorescence (uncorrected) due to sulphate catalysis is shown in Fig. 6.3. The solutions were 10^{-4}M in Morin, 0.6M in hydrochloric acid, 0.6-1.4M
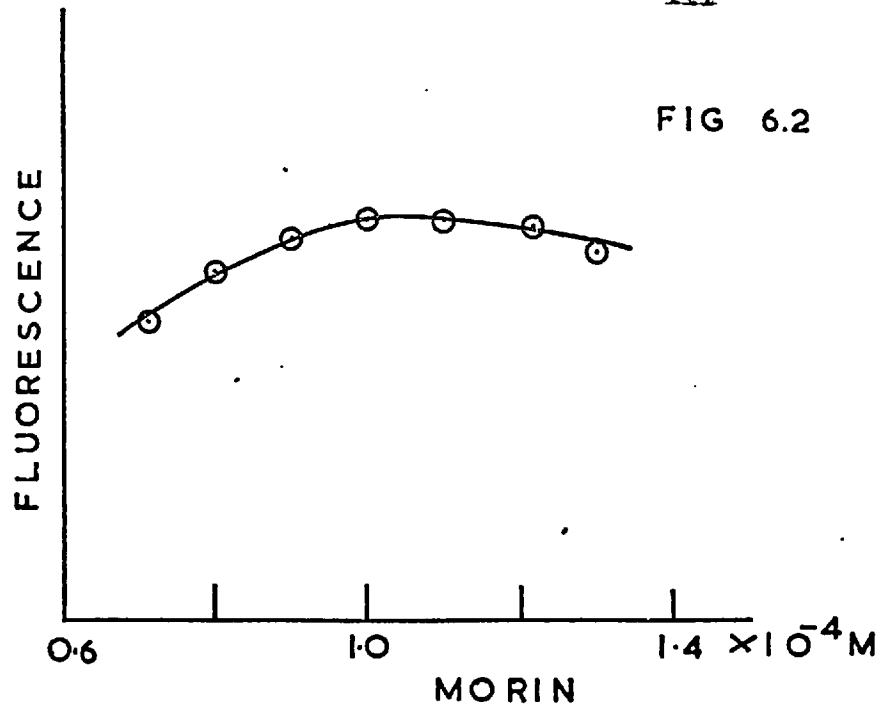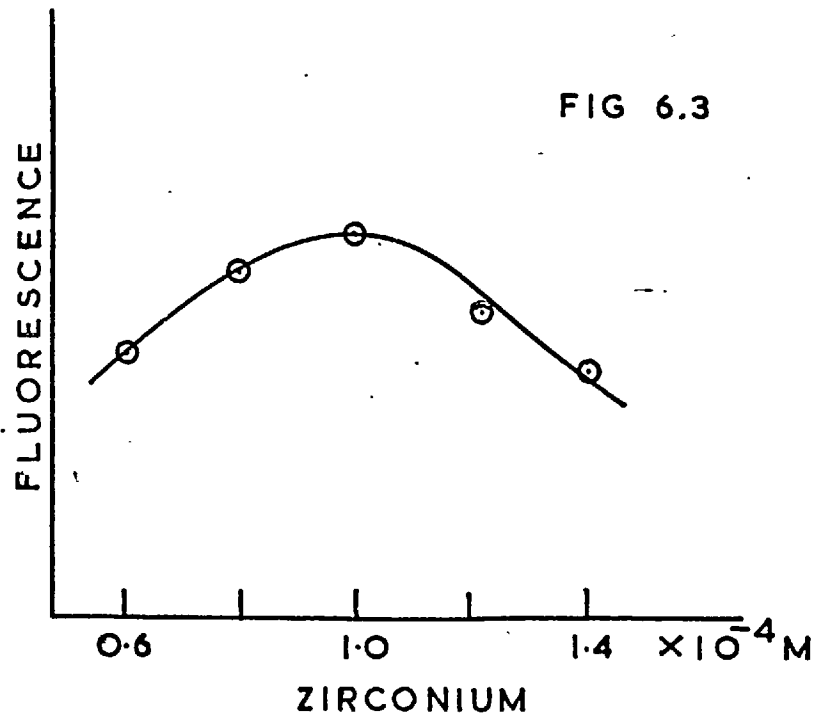
FIG 6.2

FLUORESCENCE

0·6   1·0   1·4 ×10⁻⁴M

MORIN

FIG 6.3

FLUORESCENCE

0·6   1·0   1·4   ×10⁻⁴M

ZIRCONIUM

in zirconium and catalysed by 10^{-4}M sulphate. Fluorescence measurements were made after 60 minutes at 505 nm, exciting at 417 nm. An amount of zirconium equimolar with Morin gives optimum net fluorescence.

Calibration Curve

A calibration curve was prepared under the optimised conditions over the range 2×10^{-5} - 1.8×10^{-4}M sulphate (2-17 ppm). The calibration curve (Fig. 6.4) is linear up to 10^{-4}M sulphate (9.6 ppm, 480 μg) but deviates from linearity at higher sulphate concentrations.

This non-linearity may be explained in a manner similar to that previously given to account for the curvature of the calibration curves obtained for sulphate by its effect on the zirconium - Methyl-thymol Blue reaction, at development times in excess of 60 minutes (Section 4.2). The reason is that the solutions containing greater than 10^{-4}M sulphate are at, or are approaching, the maximum fluorescence possible under the conditions used. The fluorescence values are consequently no longer directly proportional to the sulphate concentration, as is the fluorescence of solutions containing less than 10^{-4}M sulphate.

When the fluorescence was measured after 20 minutes development time only, the resultant calibration curve was linear over the complete range (2×10^{-5} - 1.8×10^{4}M sulphate) but was also of much lower sensitivity than that obtained ater 60 minutes. This is

FIG 6.4
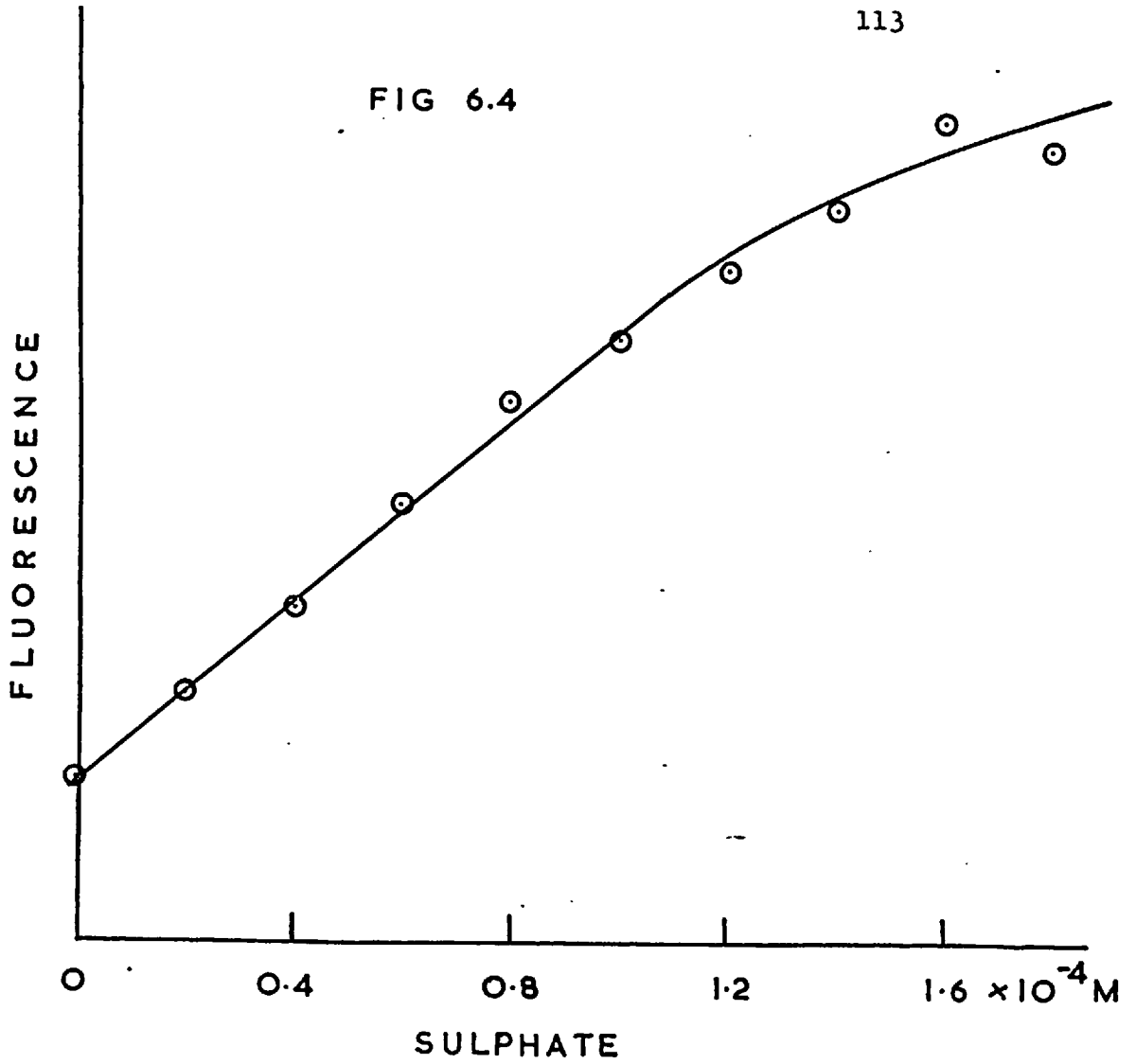
in accordance with Section 2.4.

Effect of Time

As in all kinetochromic reactions, the development time was
artificially fixed at the outset. 60 minutes development was
considered convenient, and all fluorescence measurements were made
after each solution had stood for this length of time.

Effect of Temperature

No particular study was made of the effect of temperature, and
all experiments were conducted at room temperature $(23 \pm 2^{\circ}C)$.
Large variations in temperature would be expected to affect the
procedure in a manner analagous to that observed for the zirconium-
xylenol orange reaction (Section 3.3).

6.3 Discussion

The results of this study show that the determination of sulphate,
fluoride, phosphate and arsenate by the catalytic action of the
anions on the reaction between polymerised zirconium solutions and
metal-chelating reagents need not be restricted to absorption
spectrophotometric measurement. Under the experimental conditions
optimised above, the determination of sulphate by kinetochromic
spectrofluorimetry with Morin is feasible, but the procedure does
not appear to be as sensitive as that developed using Methylthymol
Blue.

Usually it is true that the analytical sensitivity of fluorescence

spectrophotometry is inherently greater than that of absorption spectrophotometry. It does not necessarily follow, however, that better sensitivity for kinetochromic reactions will automatically result if the absorption spectrophotometric methods for zirconium used hitherto were to be replaced by a more sensitive fluorimetric method for zirconium.

The predominant factor affecting the sensitivity of the kineto-chromic determination of anions is the effectiveness of the anions in catalysing the formation of the zirconium complex. The sensitivity of the complex once formed is of minor importance. Until further work has been done on elucidating the mechanism of the reaction, it cannot easily be predicted which zirconium complex will give rise to the greatest degree of catalysis.

Interference of foreign ions

Owing to the discouraging sensitivity of the procedure for sulphate compared with the spectrophotometric method (Chapter IV) it was not considered necessary to undertake a comprehensive study of interfering ions.

Interference in the determination of sulphate by the recommended procedure may, however, be expected from two groups of ions. Those elements which interfere in the fluorimetric determination of zirconium with Morin [132] would be expected to interfere here also. These include aluminium, beryllium, gallium, germanium, antimony,

scandium, tin and thorium. Interference can also be expected from those anions which interfered in previous kinetochromic procedures, particularly phosphate, arsenate and fluoride.

6.4 Experimental

Apparatus

The instrument used in this study was a double monochromator spectrofluorimeter (Farrand Optical Co., catalogue No. 104244) fitted with a 150 watt D.C. Xenon arc lamp and an R.C.A. IP 28 photomultiplier, and equipped with a Honeywell chart recorder. Fused quartz cells (10 x 20 x 50 mm) were used throughout. 20 nm bandwidth slits were used in both the exciting and analyzing mono-chromators and no filters were used during these experiments.

Reagents

Morin, 10^{-3}M 0.0756 g. of Morin reagent (Hopkin and Williams Ltd.) were dissolved in absolute alcohol, and diluted to 250 ml. with alcohol. The solution is stable for weeks [132].

Stock sulphate solution 10^{-2}M 1.420 g. of sodium sulphate (AnalaR) were dissolved in distilled water, and diluted to 1 litre. Working standards (10^{-3}M) were prepared from this stock solution by appropriate dilution.

Zirconium reagent solution 10^{-2}M 1.611 g. of zirconyl chloride were dissolved in 0.0125M. hydrochloric acid, and diluted to 500 ml. with the same acid. This stock solution was allowed to age for 2-7 days.

A 10^{-3}M solution was prepared by mixing 50 ml. of stock 10^{-2}M

solution with 5 ml. of 5M hydrochloric acid and diluting to 500

ml. with distilled water. The final acidity was 0.05M. This 10^{-3}M

solution was used 1 hour – 4 days after preparation.

All other reagents employed were of analytical reagent grade.

Preparation of Calibration Curve

To a series of 50 ml. volumetric flasks containing 5 ml. of 10^{-3}M

Morin and 6 ml. of 5M hydrochloric acid, 0–9 ml. of 10^{-3}M sulphate

solution were added. At zero time, 5 ml. of 10^{-3}M zirconium

solution were pipetted into the first flask. After 30 seconds, the

solution was diluted to 50 ml., mixed thoroughly, and allowed to

stand. The addition of zirconium was repeated for the other flasks

at two-minute intervals. The solutions were allowed to stand 60

minutes before the fluorescence of each solution was measured at

505 nm. with excitation at 417 nm. at two minute intervals.

CHAPTER VII

The Spectrofluorimetric Determination of

Zirconium with Calcein Blue

7.1 Introduction

Solution spectrofluorimetry provides very sensitive methods for the determination of zirconium in aqueous solution. Of the three reagents which are commonly recommended, viz. Flavonol [130], Quercetin [131] and Morin [132], the reaction of the latter compound, Morin, with Zirconium gives the most sensitive, and generally preferred method. The procedure described by Geiger and Sandell [132] enables as little as 0.025 μg of zirconium to be determined in a sample volume of 25 ml.

During investigations into the use of fluorescent reagents in kinetochromic spectrophotometry (Chapter VI), the indicator Calcein Blue [3-aminomethyl-4-methylumbelliferone-NN-diacetic acid] (Fig. 7.1) was considered. Comparison of its structure with that of Methylthymol Blue (Fig. 2.1) showed that both reagents contain an imino-diacetic acid group vicinal to a phenolic hydroxyl group. It

FIG 7.1
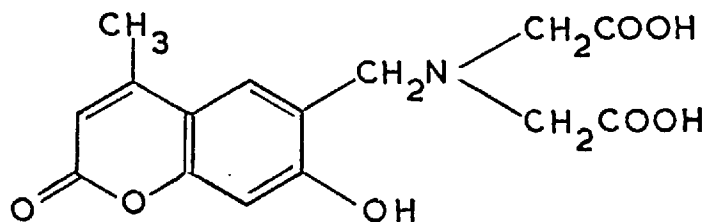
was possible therefore that zirconium may have complexed with
Calcein Blue in a manner similar to that with Methylthymol Blue.
Subsequent qualitative and quantitative studies showed that trace
amounts of zirconium did have an analytically useful effect on
solutions of Calcein Blue.

Since its introduction by Wilkins [134], Calcein Blue has been used
extensively as a metallofluorescent indicator for the complexometric
titration of several metal ions with EDTA. In the range pH 4-10,
the brilliant blue fluorescence of the indicator is quenched by the
addition of copper ions. Nickel and Chromium may thus be determined
[134] at between pH 4-10 by the addition of excess of a standard
solution of EDTA, then back-titrating the excess with a standard
copper solution. The end point occurs when all the EDTA has been
consumed so that the next addition of copper solution quenches the
blue fluorescence of the indicator.

At pH > 12, Calcein Blue itself does not fluoresce but forms
fluorescent complexes with calcium, strontium and barium [134].
These elements can thus be titrated with standard EDTA at pH 13-14
in the presence of Calcein Blue indicator. The end point occurs
when all the metal has been preferentially complexed with the EDTA
so that the metal-Calcein Blue complex is completely destroyed and
the fluorescence of the solution is quenched.

A study of the literature revealed, however, that Calcein Blue

does not appear to have been used as a direct fluorimetric reagent for the determination of any metal ion and no previous record of a reaction between Calcein Blue and zirconium has been found. The possibility that the reaction of Calcein Blue with zirconium might provide a sensitive method for the direct fluorimetric determination of zirconium is therefore of interest. As little as 0.2 nanogram of zirconium per ml. can be determined within 15 minutes development time by the procedure described here.

7.2 Investigation of Experimental Parameters

To counteract long-term variations in the intensity of the Xenon arc source, the transmission characteristics of the monochromators and the sensitivity of the photomultiplier, all fluorescence intensities were compared with a solution of quinine bisulphate in 0.1M sulphuric acid. This solution is widely used as a fluorescence standard and exhibits reproducible fluorescence intensities over a long period of time.

Preparation of Zirconium Reagent solutions

It was obviously not necessary to adopt the ageing procedure for the zirconium solutions which had hitherto been used, as zirconium solutions that had not polymerised at all were required. It has been shown [112] that zirconium solutions 3M in hydrochloric acid do not polymerise on standing, and so stock 10^{-3}M zirconium solutions were prepared at this acidity. Dilute (10^{-6}M) solutions were prepared daily by appropriate dilution. This dilute solution could

be expected to polymerise during the course of the day, but there
was no observable decrease in sensitivity as a result of this.

Spectral Characteristics

Figure 7.2 shows the excitation and emission spectra of an aqueous
solution 10^{-5}M in Calcein Blue at pH 5.5. Curve A was obtained by
measuring the fluorescent intensity at 450 nm, while curve B was
obtained with the excitation wavelength set at 330 nm. These spectra
are uncorrected for variations in the emission characteristics of
the lamp and the response characteristics of the photo-multiplier.
The relevant correction curves have been given by Chen [135]. The
blue fluorescence of Calcein Blue exhibits its excitation maxima
at 330 nm and its fluorescence emission maximum at 450 nm.

Also shown in Fig. 7.2, at the same instrumental settings as used
for the reagent alone, are the excitation and emission spectra of
solution 10^{-5}M in Calcein Blue and 5×10^{-5}M in zirconium at pH 5.5.
Curve C was obtained by measuring the fluorescence emission at 415
nm and curve D with the excitation wavelength set at 340 nm. The
addition of zirconium has an apparent quenching effect of the
fluorescence due to Calcein Blue, with a wide broadening of the
resulting emission spectra.

Excitation and emission spectra of much more dilute solutions of
the reagent and its zirconium complex at pH 5.5 are shown (uncorrected)
in figure 7.3. The fluorescence of solutions 10^{-8}M in Calcein Blue
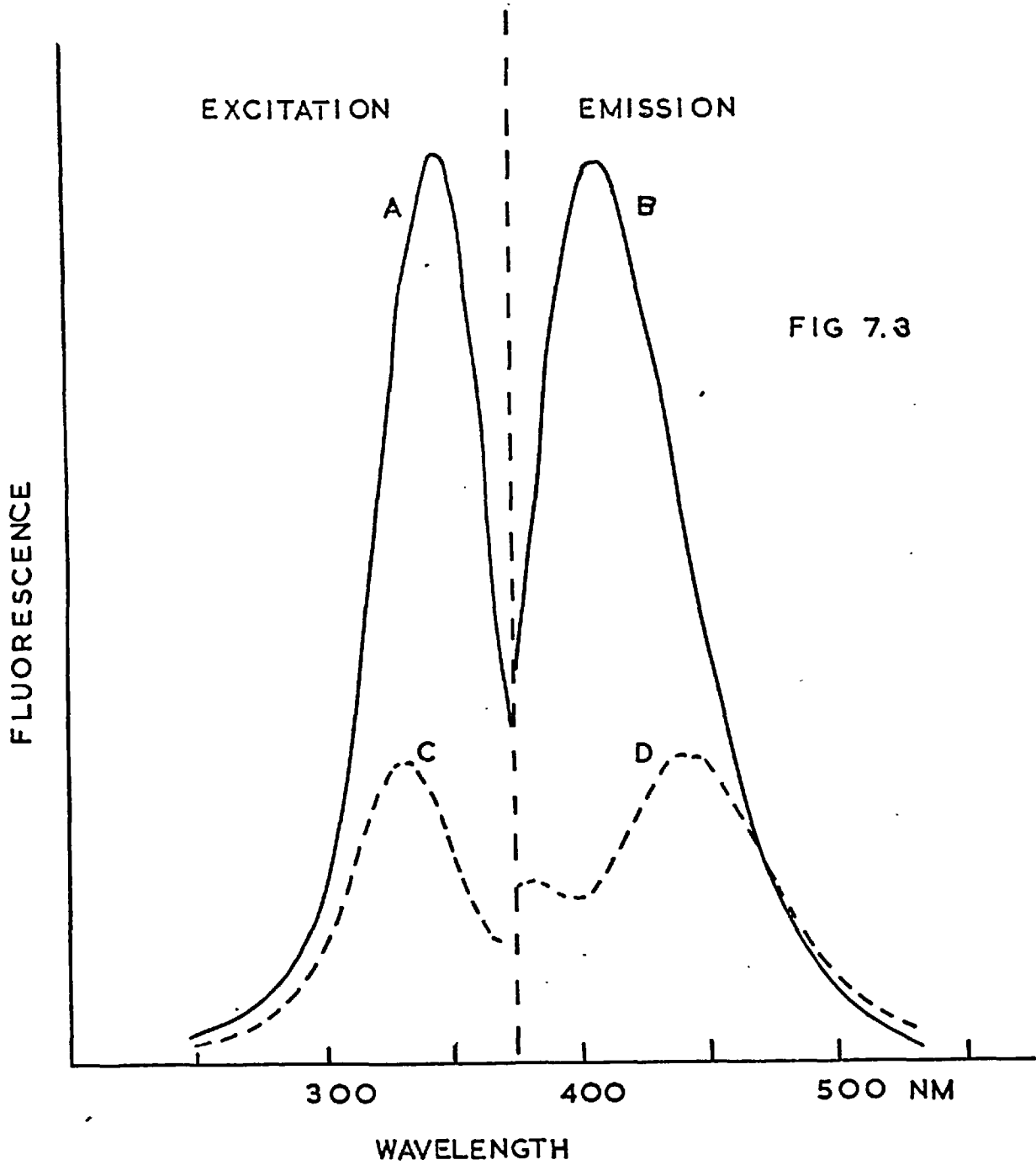
FIG 7.2

EXCITATION   EMISSION

A   B

FIG 7.3

FLUORESCENCE

C   D

300   400   500 NM
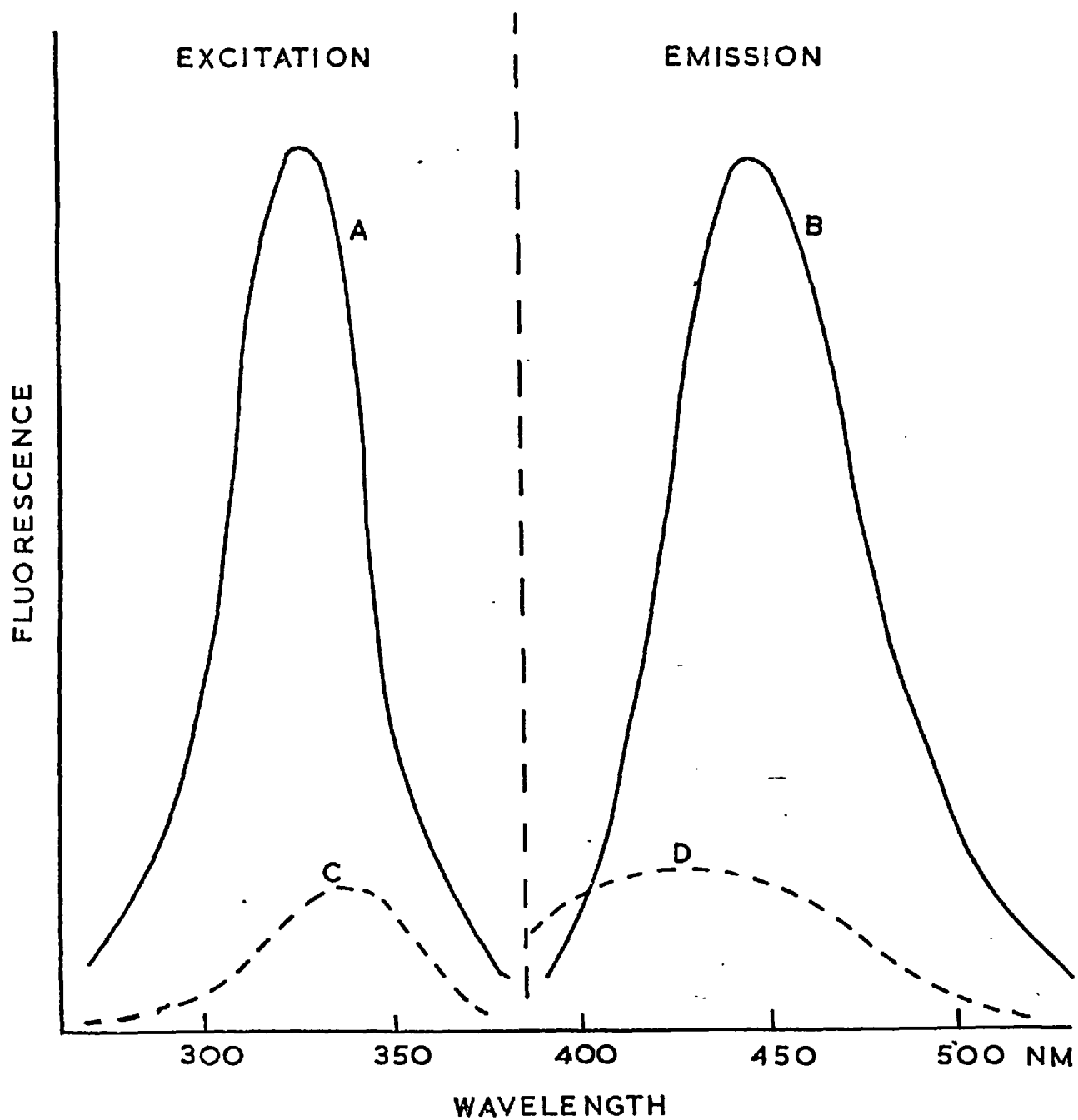
WAVELENGTH

were measured at 442 nm (Curve C) with the excitation wavelength set at 332 nm (Curve D). Spectra of solutions 10^{-8}M in Calcein Blue and 2×10^{-8}M in zirconium were recorded with an excitation wavelength set at 342 (Curve B) and measuring the fluorescence emission at 406 nm (Curve A). At these low concentrations, the presence of zirconium causes a large increase in the fluorescence emission at 406 nm, and this increase is directly proportional to the concentration of zirconium present.

In order to effect the largest difference in fluorescence emission between the zirconium complex and Calcein Blue alone at pH 5.5, it is necessary that the fluorescence of the complex is measured at 405 nm with excitation at 350 nm.

Effect of pH

The intensity of the fluorescence of reagent solutions 10^{-8}M in Calcein Blue and of its zirconium complex 10^{-8}M in zirconium was measured over a range of pH values obtained by adjusting the pH with ammonia or acetic acid. Fluorescent intensities were measured at 405 nm with excitation at 350 nm, within 15 minutes after mixing of the solutions. The results are shown in Fig. 7.4.

The fluorescence of the reagent alone (Curve A) increases slowly with increasing pH until ca. pH 6, after which much larger increases in fluorescence intensity with increasing pH are obtained. The zirconium complex (Curve B) shows maximum fluorescence at ca. pH 7.
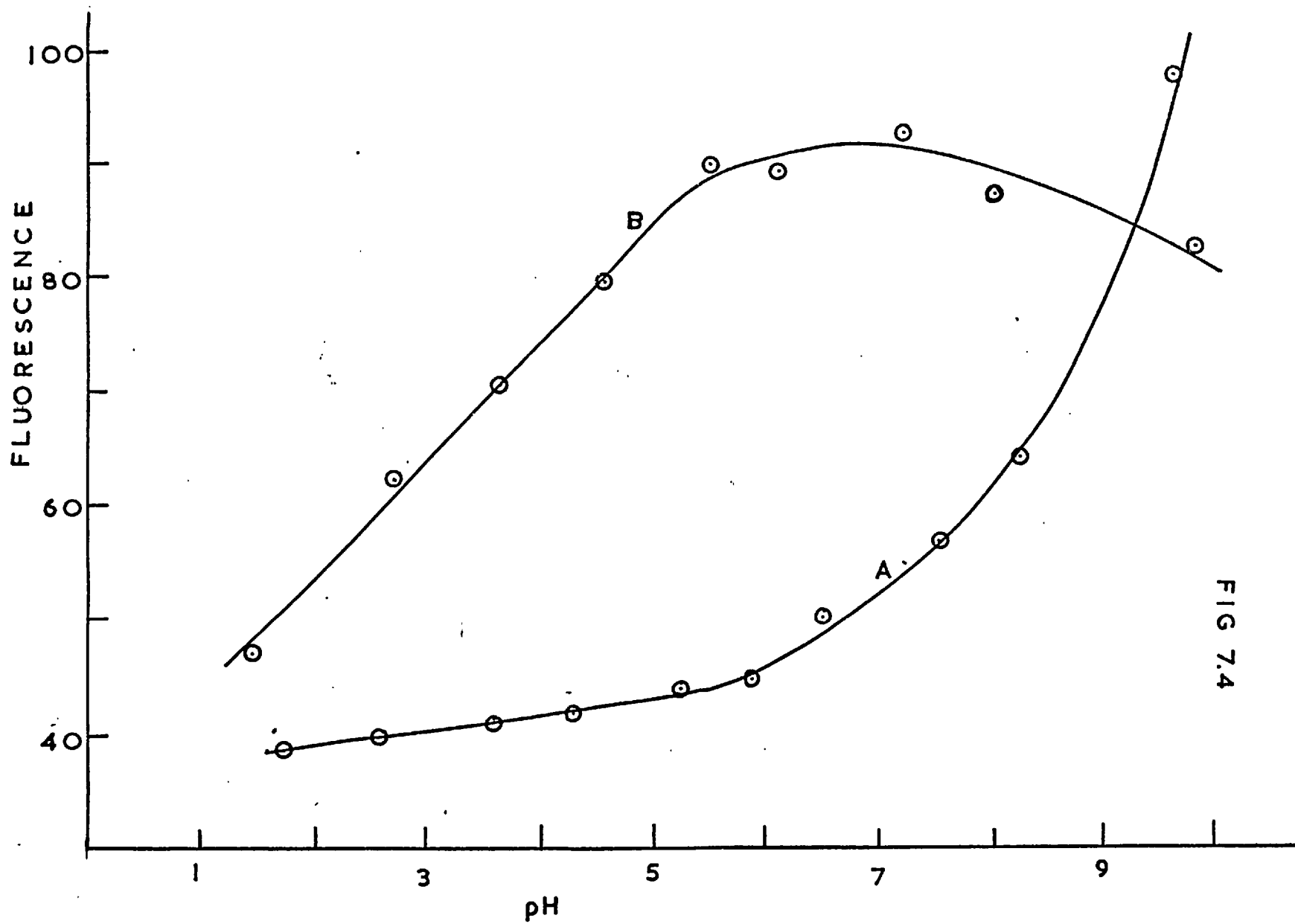
FIG 7.4

Maximum difference in fluorescence intensity due to the zirconium complex is observed when the solutions are pH 5.5 - 6.

Initially, a buffer solution of pH 5.8 was used, consisting of appropriate quantities of sodium acetate and acetic acid, and results of reasonable sensitivity and reproducibility were obtained. It was later found, however, that higher sensitivity coupled with much better reproducibility was obtained if an acetic acid/ammonium hydroxide buffer of pH 5.5 was used. This latter buffer was subsequently used throughout the study described here.

Effect of Reagent Concentration

The effect of Calcein Blue concentration was studied using 1 ml. of 10^{-6}M zirconium solution and varying amounts of 10^{-6}M Calcein Blue solution, diluted to 100 ml. at pH 5.5. The exciting wavelength was set at 350 nm and the emission measured at 405 nm. Fig. 7.5 shows the effect of varying excess of reagent on the fluorescence emission of the zirconium complex (blank subtracted). An amount of reagent equimolar with the zirconium present gives optimum results. For the determination of 20-100 nanograms of zirconium, 1 ml. of 10^{-6}M Calcein Blue was used, equimolar with the maximum zirconium concentration present.

When the concentration of Calcein Blue is greater than (approximately) 6.5 times the zirconium concentration, the fluorescence of the blank (no zirconium) is greater than that observed for the

FIG 7.5

FLUORESCENCE

EXCESS CALCEIN BLUE
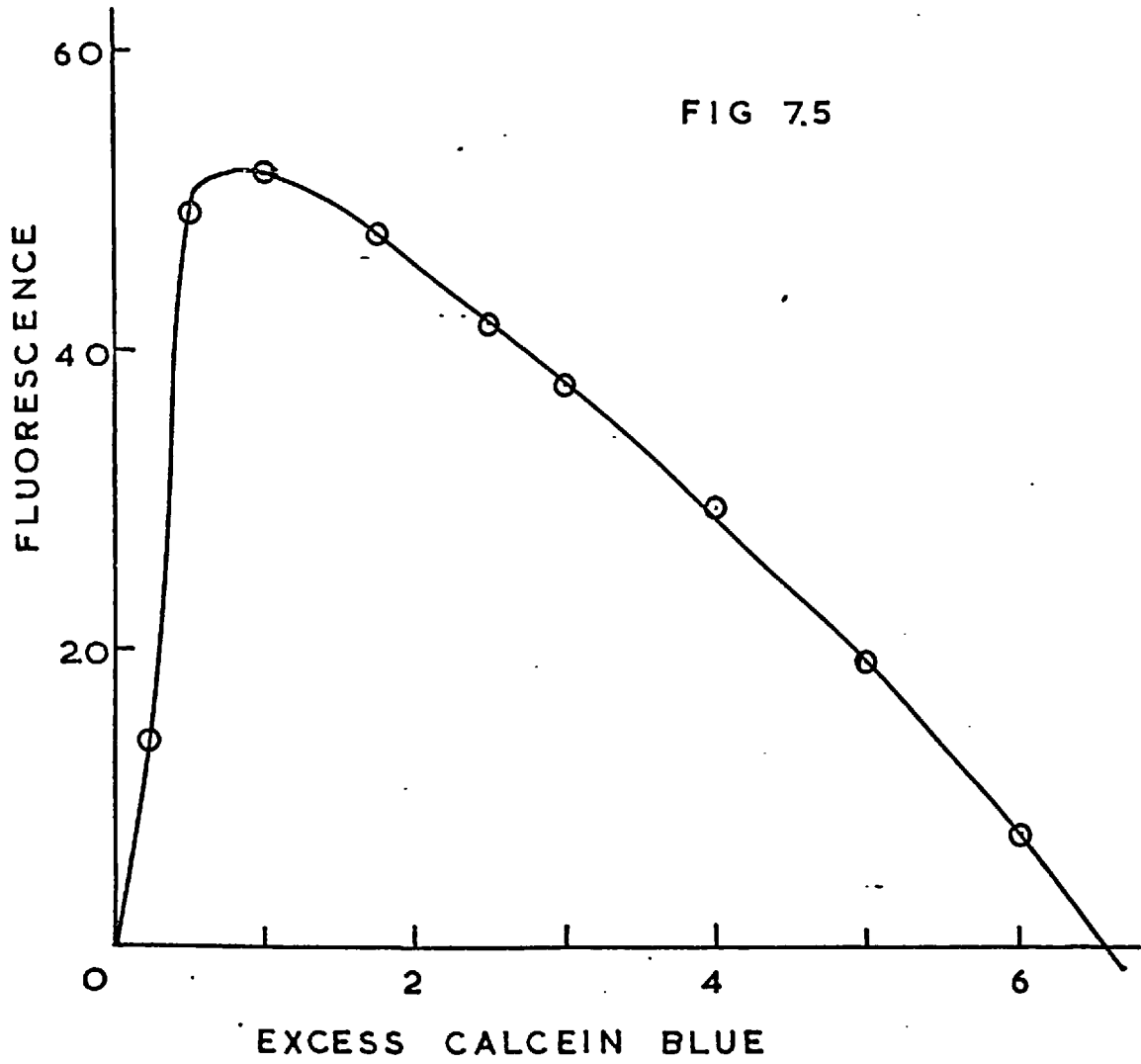
complex, measured at 405 nm. The extent of linearity of the calibration curve is consequently limited by this factor.

Effect of Time

The variation in fluorescence intensity of dilute zirconium solutions with time was studied. A standard solution 10^{-8}M in zirconium prepared by the recommended procedure showed a gradual reduction in fluorescence over a period of two hours when allowed to stand in the darkness, after which the fluorescence had fallen by 10% from the time of measurement immediately after mixing. A similar reduction in intensity was observed after standing the solution for two hours in normal laboratory conditions, i.e., under fluorescent tube lighting. Continuous irradiation of the solution at 350 nm by the Xenon Arc in the spectrofluorimeter for a similar period caused a decrease in fluorescence of 32%.

All fluorescence measurements in this study were made within 15 minutes after mixing of the solution, whence reproducible results could be obtained.

Order of Addition of reagents

The effect of the order of mixing of the solutions on the fluorescent intensity obtained by the recommended procedure was investigated. It was found that the preferred order of addition of reagents to the volumetric flasks was: Calcein Blue or zirconium (any order), the buffer solution, followed by dilution to volume

with distilled water. Under these conditions, maximum fluorescence due to the zirconium complex is obtained immediately, with a gradual decrease in intensity with time.

When the order of addition was: Calcein Blue or buffer (any order), the zirconium solution, followed by dilution to volume, very little fluorescence intensity due to the zirconium complex was initially observed. The emission of the complex slowly increased with time, however, reaching a maximum after an undetermined period of at least one hour.

In all experiments reported in this study, the zirconium solution was allowed to react with the Calcein Blue for at least five minutes before the addition of the buffer solution and dilution to volume. No particular study was made of the effects of allowing the zirconium and reagent to react for periods of time longer than five minutes before addition of the buffer.

Effect of Temperature

In all experiments, the temperature of the solutions was $21 \pm 3^{\circ}C$. No significant variation of fluorescence intensity with temperature was noted, but no specific study of temperature effects was made.

Precision

The recommended procedure was applied to the repetitive

determination of 0.1 µg. of zirconium over several days. The average net fluorescence (8 readings) for the complex was 58.8 with a relative standard deviation of ± 1.8%.

Effect of Foreign Ions

The effect of 30 cations and 12 anions on the determination of zirconium by the recommended procedure was investigated. Initially the effect of a 500-fold molar excess of the foreign ion over 0.1 µg of zirconium was studied. An ion was considered to interfere at this level when it caused an error greater than ± 5% in the determination of zirconium. Those ions which were found to interfere were subsequently reinvestigated at lower concentrations, viz. 50-fold and 5-fold molar excess over 0.1 µg of zirconium. The results are shown in Table 7.1.

Table 7.1

Effect of various ions on the determination of 0.1 µg. Zr

Ion	Molar Excess	Interference %	Ion	Molar Excess	Interference %
Ag	500	+128	Be	50	−2.5
	50	+5.8	Bi	500	quenched
	5	−		50	quenched
Al	500	+100		5	−5
	50	+82	Ca	500	−
	5	+22	Cd	500	+25
Ba	500	−		50	+12.5
Be	500	−9.2		5	−

Table 7.1 (Continued)

Ion	Molar Excess	Interference %	Ion	Molar Excess	Interference %
Ce(III)	500	quenched	Sn(IV)	50	-4
	50	quenched	Sr	500	+25
	5	+5		50	-4.2
Co	500	-5	Te	500	-56
Cr(III)	500	quenched		50	-14
	50	-41		5	-5
	5	-4.5	Th	500	+112
Cu(II)	500	quenched		50	+83
	50	quenched		5	+7.5
	5	-2	Ti	500	-16
Fe(III)	500	quenched		50	-4.5
	50	quenched	Tl	500	+47
	5	quenched		50	+10.8
Hg(I)	500	+5		5	+4.5
K	500	+2	UO_2^{2+}	500	+6.5
La	500	-3		50	+1
Li	500	-	Zn	500	-4
Mg	500	-9	AsO_4^{3-}	500	-41.6
	50	-1		50	-4
Mn	500	-55	Br^-	500	-1
	50	+1	Cl^-	500	-
Na	500	-	CO_3^{2-}	500	+1
NH_4^+	500	-2	F^-	500	+15
Ni	500	-19.2		50	+83
	50	-5		5	+20
Pb	500	+16	I^-	500	-2
	50	+3	MoO_4^{2-}	500	-78
Sn(IV)	500	-13.3		50	-25

Table 7.1 (Continued)

Ion	Molar Excess	Interference %	Ion	Molar Excess	Interference %
MoO_4^{2-}	5	-5	VO_3^-	500	quenched
NO_3^-	500	-		50	-18.3
PO_4^{3-}	500	quenched		5	-4.5
	50	quenched	WO_4^{2-}	500	quenched
	5	-62		50	quenched
SO_4^{2-}	500	+2.5		5	quenched

Those ions which interfere even at the 5-fold molar excess level obviously constitute the most serious interference. At this level, thorium, aluminium and fluoride ions cause positive interference, while iron and tungstate completely quench the fluorescence of the zirconium complex. Phosphate also constitutes a serious negative interference at the 5-fold excess level. Perhaps the most unexpected interfering effect is that of fluoride. In many other methods for the determination of zirconium, fluoride and phosphate cause serious interference by the action of these anions in forming very stable complexes with the zirconium. In this spectrofluorimetric procedure, phosphate does bleach the reaction in the expected manner. Fluoride, however, gives a positive interference, particularly at the 50-fold molar excess level.

The separation of interfering ions in the determination of trace amounts of zirconium has received considerable attention [117],

particularly using solvent extraction and ion exchange. A highly selective extractant for micro amounts of zirconium is thenoyltrifluoroacetone (TTA). Zirconium has been separated from aluminium, iron, the rare earths, thorium and uranium in 6M hydrochloric acid by a 10-minute extraction with an equal volume of TTA in Xylene [136].

Freund and Miner [137] have also successfully separated zirconium from iron and aluminium by the use of anion exchange.

Structure of the complex

The nature of the complex formed between zirconium and Calcein Blue was investigated at pH 5.5 by application of the mole-ratio procedure. 1.0 ml. of 10^{-6}M Calcein Blue solution was reacted with 0-2.0 ml. of 10^{-6}M zirconium solution in a series of 100 ml. flasks, 5 ml. of pH 5.5 buffer added, and the solution diluted to volume. The fluorescence of the solution was measured immediately at 405 nm with excitation at 350 nm. The (uncorrected) plot (Fig. 7.6) would appear to indicate the formation of a 1.3:1 complex between zirconium and Calcein Blue. In view of the probable low assay of the Calcein Blue used, however, this figure is almost certainly inaccurate, and the truer value is probably higher. Further studies are required into the purification of Calcein Blue for use as a reagent for the quantitative determination of Zirconium. Up to the present, relatively impure Calcein Blue has been available, as this is adequate for its "qualitative" use as a metallofluorescent indicator.

FIG 7.6
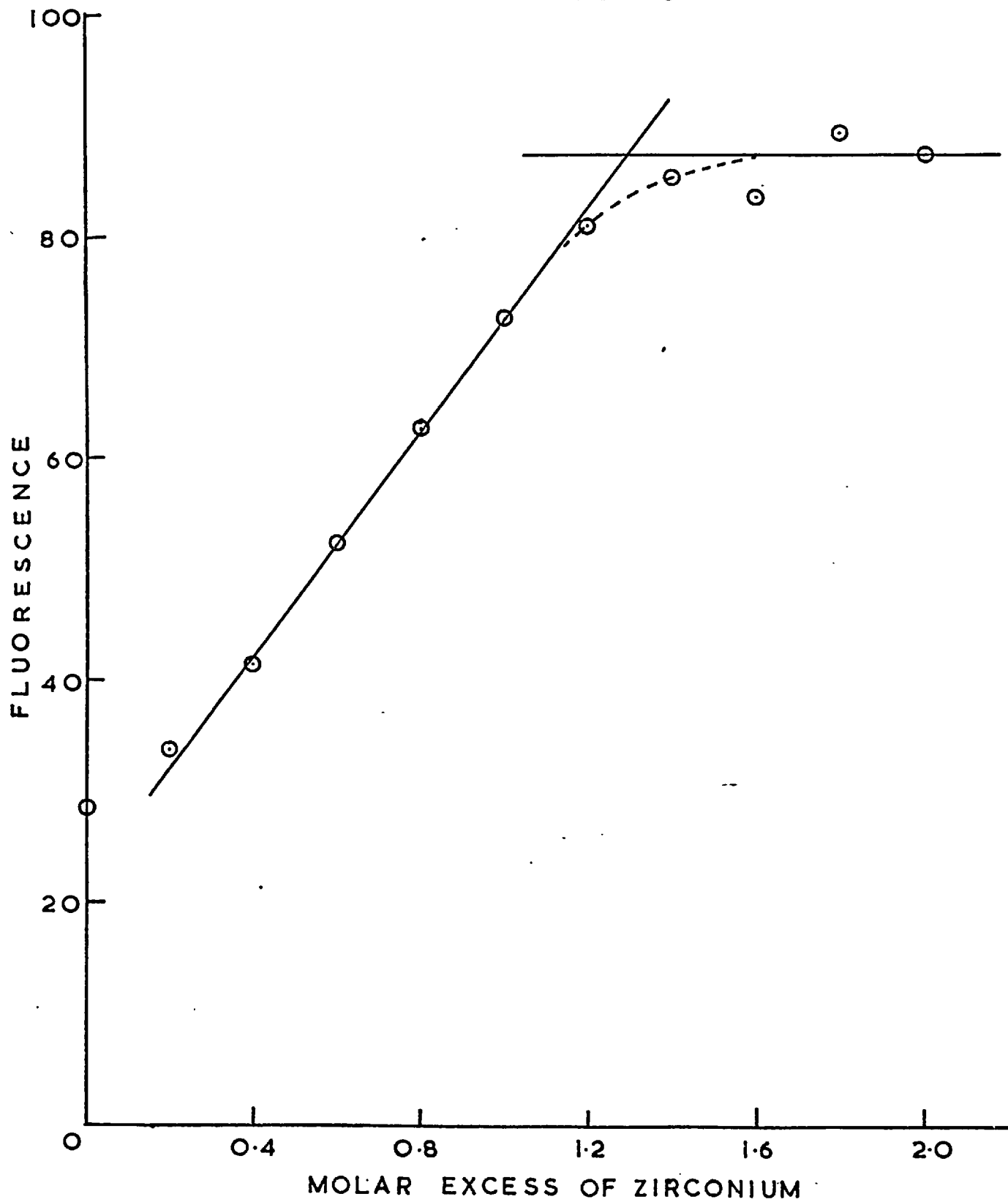
With Calcein Blue prepared pure, more reliable data concerning

the structure of the Zirconium-Calcein Blue complex should be

obtained.

7.3 Experimental

Apparatus

The instrument used was a double-monochromator spectrofluorimeter

(Aminco-Bowman, American Instrument Co., Inc., catalogue No. 4-8202)

fitted with a high intensity xenon arc lamp and an R.C.A. I.P.28

photomultiplier, and used in conjunction with a Bryans X-Y recorder

(Model 21001). Fused quartz cells 12.5 x 12.5 mm square outside,

10 x 10 mm square inside and 48 mm high were used. In order to

obtain maximum sensitivity, 5 mm. slits corresponding to ca. 50 nm

band-pass were used (Aminco-Bowman slit arrangement No. 5), in both

the excitation and analysing monochromators.

pH measurements were made with a Vibron pH meter, Model 39A

(E.I.L. Ltd.)

Reagents

Zirconium solution, 10^{-3}M. Prepared by dissolving 0.1611 g. of

zirconyl chloride ($ZrOCl_2.8H_2O$; purified for use in fluoride

determinations; British Drug Houses Ltd.) in 3M hydrochloric acid,

and diluting to 500 ml. using the same acid. This stock solution

was diluted daily to give a working solution containing 0.1 µg/ml.

of zirconium (10^{-6}M). The dilute zirconium solution was discarded

each day to avoid errors due to polymerisation of the zirconium.

Calcein Blue 5.10^{-4}M. Prepared by dissolving 0.0117 g. of indicator (Hopkin and Williams) in several drops of 0.1M potassium hydroxide and diluting to 50 ml. with freshly distilled water. Suitable aliquots were diluted to 10^{-6}M as required. After 2-3 days, all reagent solutions were discarded and fresh 5.10^{-4}M stock, and 10^{-6}M dilute solutions of Calcein Blue prepared.

Buffer solution, pH 5.5   30 ml. of glacial acetic acid (AnalaR) were diluted to approximately 800 ml. with distilled water and concentrated ammonia solution (AnalaR) added until the pH was 5.5 (approximately 30 ml.)   The solution was then diluted to 1 litre with distilled water.

All volumetric flasks used were treated with silicone Repelcote, 2% dimethyldichlorosilane in CCl_4 (Hopkin and Williams), to minimise adsorption of zirconium onto the glass.

Preparation of Calibration Curve

Transfer accurately 0-1.0 ml. of 10^{-6}M zirconium solution into a series of 100 ml. volumetric flasks containing 1.0 ml. 10^{-6}M Calcein Blue. Wash down the sides of the flasks with distilled water and allow the solutions to mix for at least 5 minutes before adding 5 ml. of pH 5.5 buffer solution to each flask. Dilute to volume with distilled water from an all-glass distillation apparatus.

Measure the intensity of fluorescence at 405 nm with an excitation wavelength of 350 nm. The calibration curve (Fig. 7.7, uncorrected for the fluorescence of the blank) is linear from 20-100 ng. of zirconium (0.0002-0.001 ppm). The deviation from linearity for zirconium concentrations less than 0.02 µg/ml is due to the presence of too large an excess of Calcein Blue reagent for this concentration of zirconium.

The range of calibration can easily be extended by the use of larger quantities of Calcein Blue reagent. Thus, for example, linear calibration extends over the range 0.05-0.2 µg of zirconium (0.0005-0.002 ppm) if 2 ml. of 10^{-6}M Calcein Blue is used as reagent.
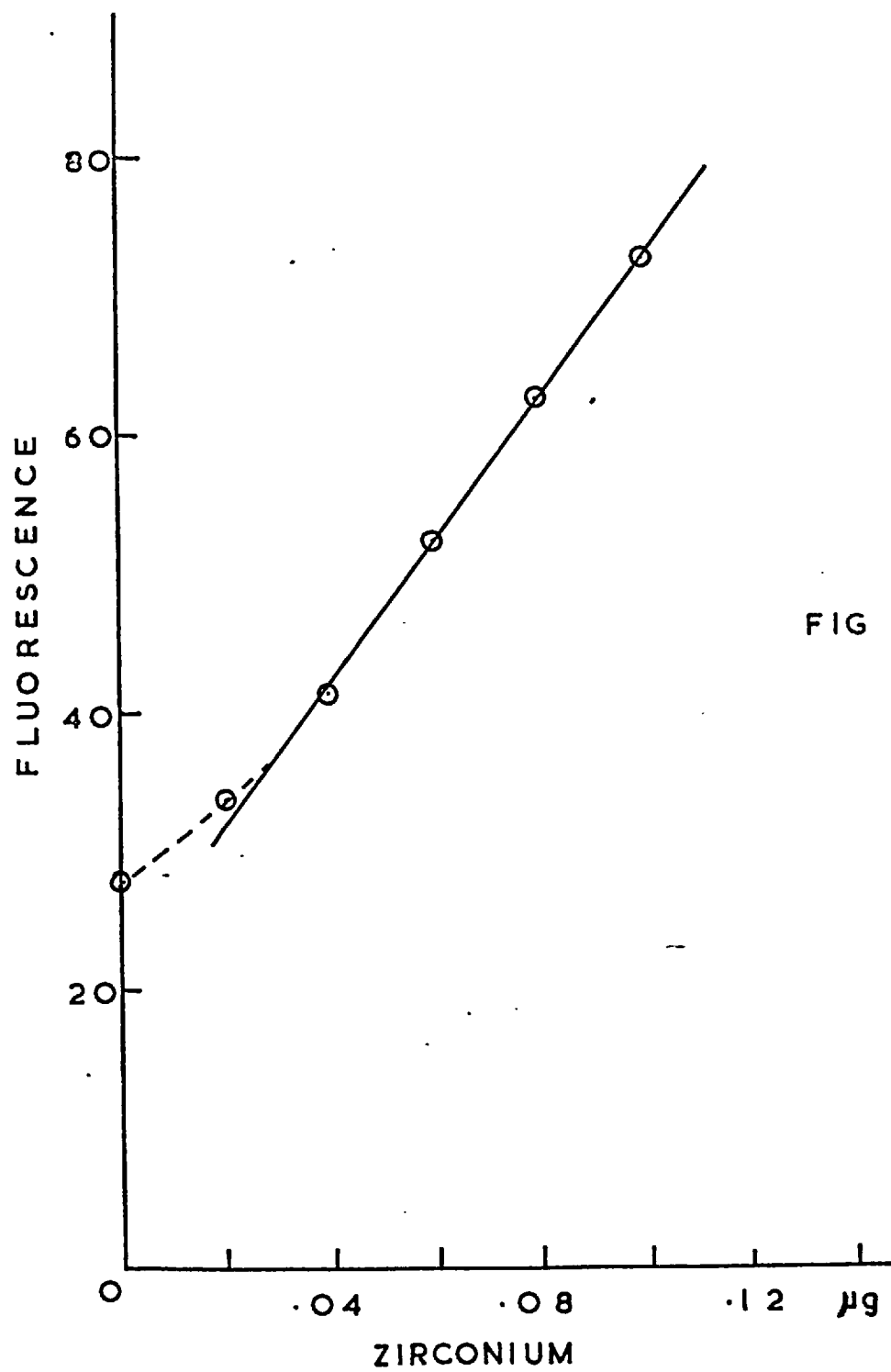
FIG 7.7

CHAPTER VIII

Conclusions and Suggestions for Future Work

The determination of fluoride and sulphate by kinetochromic spectrophotometry has been shown to be sensitive, particularly for fluoride ion. The sensitivity of the procedure for sulphate [124] is conventional at $\varepsilon \sim 2 \times 10^4$, but it is, to the best of the author's knowledge, the only "positive" colour method so far proposed for the sulphate ion. The determination of fluoride by its effect on the zirconium–Methylthymol Blue reaction [138] is considerably more sensitive ($\varepsilon \sim 3 \times 10^5$) than the previous kinetochromic procedures described for this ion with Xylenol Orange as reagent [109], while both kinetochromic procedures are an appreciable advance on the Alizarin Complexan method [102], and are subject to considerably less cationic interference.

The use of solution spectrofluorimetry for kinetochromic analysis with Morin as the reagent, did not prove to be as sensitive as had been hoped, but this is not entirely unexpected, for the reasons already given. The discovery of Calcein Blue as a new spectrofluorimetric reagent for zirconium may, however, prove to be of importance. This reaction has been shown to be of considerably better sensitivity than the spectrofluorimetric reactions for zirconium generally recommended at the present time. Investigations into the use of Calcein Blue in kinetochromic analysis may well

yield even better sensitivities for the determination of fluoride and sulphate than those obtained at present.

The determination of phosphate and arsenate by kinetochromic procedures has not yet been extensively investigated. Quantitative studies of the effect of these anions on the reaction between zirconium and such reagents as Methylthymol Blue, Morin and Calcein Blue could profitably be made, whence very sensitive procedures for the determination of these anions should be obtained.

The kinetochromic methods developed so far are not dauntingly time-consuming, requiring a development time of 60 minutes. The nature of the reaction is such that the procedures are readily available for automation, and traces of fluoride, sulphate, phosphate and arsenate should be determinable within very short development times, ca. two minutes.

The discovery of Calcein Blue as a fluorimetric reagent for zirconium arose from the desire to develop kinetochromic spectro-fluorimetric procedures. This reaction may well prove to be of considerable interest in more conventional solution spectrofluori-metry however. Examination of the interfering effects of foreign ions on the Calcein Blue-zirconium reaction shows that Calcein Blue should prove to be a very sensitive fluorimetric reagent for the determination of thorium, aluminium, titanium, and thallium. Traces of other ions, such as iron, cerium, and phosphate could also be

determined _via_ their quenching effect on the Calcein Blue-zirconium reaction. Of particular interest is the effect of fluoride ion on the determination of zirconium with Calcein Blue, since a _positive_ interfering effect is encountered. This phenomena could, perhaps, be profitably investigated.

REFERENCES

1. R. Belcher and A.J. Nutten. "Quantitative Inorganic Analysis", 2nd Ed., Butterworths, London, 1960.

2. H.B. Weiser and J.L. Sherrick. J.Phys.Chem., 1919, 23, 205.

3. Z. Karaoglanow, Z.Anal.Chem., 1917, 56, 417.

4. R. Pribil and D. Maricova. Chem.Listy., 1952, 46, 542.

5. M. Nishimura, M. Saito and Y. Uzumasa. Japan Analyst, 1964, 13, 544.

6. J.L. Osborne, J. Phys.Chem., 1913, 17, 629.

7. A. Mutschin and R. Pollack, Z.Anal.Chem., 1937, 108, 315.

8. C. Liebermann, ibid., 1875, 14, 359.

9. E. Murmann, Öst.Chem.Stg., 1910, 13, 227.

10. L.W. Winkler, Die Chemische Analyse, XXIX, Stuttgart, 1931.

11. H.A. Fales and W.S. Thompson, Ind.Eng.Chem., Anal.Ed., 1939, 11, 206.

12. E. Schulek and I. Boldizsar, Z.Anal.Chem., 1940, 120, 410.

13. V. Majer, ibid., 1941, 122, 258.

14. I.M. Kolthoff and E.B. Sandell, "Textbook of Quantitative Inorganic Analysis," 3rd Edn., Macmillan Co., New York, 1952.

15. M. Nishimura, Anal.Chim.Acta., 1966, 34, 246.

16. M. Azeem, Analyst, 1967, 92, 115.

17. C. Mahr and K. Krauss, Z.Anal.Chem., 1948, 128, 477.

18. R. Belcher and D. Gibbons, J.Chem.Soc., 1952, 4216.

19. W. Denis, and L. Reed, J.Biol.Chem., 1906, 131; J.Biochem., 1926, 71, 191.

20. G. Toennies and B. Bakay, Anal.Chem., 1953, 25, 160.

21. K. Takiyama and E. Suito, Japan Analyst, 1954, 3, 291.

22. S. Omiti, ibid., 1963, 12, 1032.

23. W.Vólmer and F. Frohlich, Z.Anal.Chem., 1944, 126, 401.

24. J. de la Rubia Pacheco and F. Blasco Lopez-Rubio. Inform.quim. anal., (Madrid), 1951, 5, 1.

25. J. F. Thomas and J.E. Cotton, Water and Sewage Works, 1954, 101, 462.

26. J. Haslam, J.B. Hamilton and D.C.M. Squirrell, Analyst, 1961, 86, 239.

27. J.W. Wimberley, Anal.Chim.Acta, 1968, 42, 327.

28. R. Belcher, A.J. Nutten and W.I. Stephen, J.Chem.Soc., 1953, 1334

29. J.M. Martin and W.I. Stephen, Anal.Chim.Acta., 1967, 39, 175.

30. Idem., ibid., 1967, 39, 525.

31. J.F. Alicino, Anal.Chem., 1948, 20, 85.

32. L.T. Hallet and J.W. Knipers, Ind.Eng.Chem., Anal.Ed., 1940, 12, 360.

33. J.F. Mahoney, and J.H. Mitchell, ibid., 1942, 14, 97.

34. R.T. Sheen and H.L. Kahler, ibid., 1936, 8, 127.

35. R.K. Siegfriedt, J.S. Wiberley and R.W. Moore, Anal.Chem., 1951, 23, 1008.

36. J.O. Sullivan and P. Warneck, Microchem.J., 1964, 8, 241.

37. Y. Suzuki and Y. Murakami, J.Chem.Soc.Japan, Pure Chem.Sect., 1963, 84, 596.

38. C.L. Ogg, C.O. Willitts, and F.J. Cooper, Anal.Chem., 1948, 20, 83.

39. A. Steyermark, E. Bass and E. Littman, ibid., 1948, 20, 587.

40. B.K. Handa, Indian J.Chem., 1965, 3, 368.

41. J.S. Fritz and M.Q. Freeland, Anal.Chem., 1954, 26, 1593.

42. J.S. Fritz and S.S. Yamamura, ibid., 1955, 27, 1461.

43. J.S. Fritz, S.S. Yamamura and M.J. Richard ibid., 1957, 29, 158.

44. B.H. Priscott, T.G. Hand and E.J. Young, _Analyst_, 1966, _91_, 48.

45. H. W. Wharton and L.R. Chapman, _Anal.Chem._, 1964, _36_, 1679.

46. R. Belcher, A.D. Campbell, P. Gouverneur and A.M.G. Macdonald J. Chem. Soc., 1962, 3033.

47. E. Hakoila and P. Noponeu, _Acta.Chem.Scand._, 1965, _19_, 947.

48. B. Budesinsky, _Anal.Chem._, 1965, _37_, 1159.

49. B. Budesinsky and L. Krumlova, _Anal.Chem.Acta._, 1967, _39_, 375.

50. R. Belcher, D. Gibbons and T.S. West, _Chem.and Ind._ 1954, 127.

51. Idem, _Analyst_, 1955, _80_, 751.

52. R. Belcher, R.L. Bhasin, R.A. Shah, and T.S. West, _J.Chem.Soc._, 1958, 4054.

53. D.A. Lewis, _Analyst_, 1962, _87_, 566.

54. R. Belcher, D. Gibbons and T.S. West, _Chem. and Ind._, 1954, 850

55. J.O. Page and W.W. Spurlock, _Anal.Chim.Acta._, 1965, _32_, 593.

56. A.S. Jones and D.S. Letham, _Analyst_, 1956, _81_, 15.

57. R.J. Bertolacini and J.E. Barney, _Anal.Chem._, 1957, _29_, 281

58. Idem., _ibid._, 1958, _30_, 202.

59. R.M. Carbon, R.A. Rosell and W. Vallejos, _ibid._, 1967, _39_, 688.

60. H.W.S. Schafer, _Ibid._, 1967, _39_, 1719.

61. H. Bode, W. Eggeling, and V. Steinbrecht, _Z.Analyt. Chem._, 1966, _216_, 30.

62. T. Braun and J. Tolgyessy, _Talanta_, 1964, _11_, 1543.

63. M.E. Gales, W.H. Kaylor, and J.E. Longbottom, _Analyst_, 1968, _93_, 97.

64. V. Palaty, _Talanta_, 1963, _10_, 307.

65. J.L. Lambert, S.K. Yasuda and M.P. Grotheer, _Anal.Chem._, 1955, _27_, 800.

66. J.C. Guyon and E.J. Lorah, Anal.Chem., 1966, 38, 155.

67. W. Mecklenburg, and F. Rosenkranzer, Z.Anorg.Chem., 1914, 86, 143

68. A.E. Sands, M.A. Grafius, H.W. Wainwright and M.W. Wilson,
     U.S. Bur.Mines., Rept.Invest., No 4547, 1949.

69. C.M. Johnson and H. Nishita, Anal.Chem., 1952, 74, 736.

70. M.L. Cabello-Tomas, Ph.D.Thesis, 1966, University of London.

71. G. Forchhammer, Edinburgh Phil.J. 1850, 48, 345.

72. C. Deladrier, Chem.Weekblad, 1903, 1, 324.

73. A. Gautier and P. Claussman, Compt.rend., 1912, 154, 1469.

74. G. Starck, Z.Anorg.Chem., 1911, 70, 173.

75. R. Belcher and J.C. Tatlow, Analyst, 1951, 76, 593.

76. H.H. Willard and O.B. Winter, Ind.Eng.Chem., Anal.Ed., 1933, 5, 7

77. W.D. Armstrong, ibid., 1936, 8, 384.

78. T.S. Ma and J. Gwirtsman, Anal.Chem., 1957, 29, 141.

79. A.C.D. Newman, Anal.Chim.Acta., 1958, 19, 471.

80. T.S. Light and R.F.Mannion, Anal.Chem., 1969, 41, 107.

81. R.F. Milton, H.F. Liddell and J.E. Chivers, Analyst, 1947, 72, 43

82. W. Selig, ibid., 1968, 93, 118.

83. H.H. Willard and C.A. Horton, Anal.Chem., 1950, 22, 1190.

84. S.E.W. Scott and A.L. Henne, Ind.Eng.Chem.,Anal.Ed., 1935, 7, 299

85. J.H. Saylor and M.E. Larkin, Anal.Chem., 1948, 20, 194.

86. S. Fleury, Anal.Chim.Acta., 1967, 37, 232.

87. J.L. Lambert, Anal.Chem., 1954, 26, 558

88. M.J. Price and O.J. Walker, ibid., 1952, 24, 1593.

89. H.W. Wharton, ibid., 1962, 34, 1296.

90.   J.A. Ruzicka, H. Jakschova and L. Mrklas, _Talanta_, 1966, _13_, 1341

91.   H.E. Bumstead and J.C. Wells, _Anal.Chem._, 1952, _24_, 1595.

92.   A.L. Hensky and J.E. Barney, _ibid._, 1960, _32_, 828.

93.   L. Fine and E.A. Wynne, _Microchem.J._, 1959, _3_, 515.

94.   W.A. Powell and J.H. Saylor, _Anal.Chem._, 1953, _25_, 960.

95.   S. Megregian, _ibid._, 1954, _26_, 1161.

96.   H.M. Nielsen, _ibid._, 1958, _30_, 1009.

97.   O.S. Glaso, _Anal.Chim.Acta._, 1963, _28_, 543.

98.   J. Tusl, _Anal.Chem._, 1969, _41_, 352.

99.   J.G. Sen Gupta, _Anal.Chim.Acta._, 1968, _42_, 119.

100. R. Belcher, M.A. Leonard and T.S. West, _J.Chem.Soc._, 1958, 2390

101. Idem, _Talanta_, 1959, _2_, 92.

102. Idem, _J.Chem.Soc._, 1959, 3577.

103. M.A. Leonard and T.S. West, _ibid._, 1960, 4477.

104. P. Job, _Ann.Chim.(France)_, 1928, _9_, 113.

105. R. Belcher and T.S. West, _Talanta_, 1961, _8_, 853.

106. S.S. Yamamura, M.A. Wade, and J.H. Sikes, _Anal.Chem._, 1962, _34_,
      1308

107. R. Belcher and T.S. West, _Talanta_, 1961, _8_, 863.

108. C.A. Johnson and M.A. Leonard, _J.Pharm.Pharmacol._, 1961, _13_, 164T

109. M.L. Cabello-Tomas and T.S. West, _Talanta_, 1969, _16_, 781.

110. A.K. Babko and G.I. Gridchina, _Russ. J.Inorg.Chem._, 1962, _7_, 458

111. E.S. Pilkington and W. Wilson, _Anal.Chim.Acta._, 1965, _33_, 577.

112. B.C. Sinha and S. Das Gupta, _Analyst_, 1967, _92_, 558.

113. P. Pakalns, _Anal.Chim.Acta._, 1969, _44_, 73.

114. W.B. Blumenthal, "_The Chemical Behaviour of Zirconium_", Van Nostrand, Princeton, N.J., 1958.

115. A.D. Wilson and J.R. Cooke, _Analyst_, 1966, _91_, 135.

116. T.S. West, _Chem.Ind.London_, 1966, 1005.

117. R.B. Hahn, "_Treatise on Analytical Chemistry_" edited by I.M. Kolthoff and P.J. Elving, Part II, volume 5, Interscience, N.Y., 1961,

118. M. Otomo, _Bull.Chem.Soc.Japan_, 1963, _36_, 1577.

119. Idem., _ibid._, 1963, _36_, 809.

120. R. McGillivray and S.C. Woodger, _Analyst_, 1966, _91_, 611.

121. K.L. Cheng, _Anal.Chim.Acta._, 1963, _28_, 41.

122. G.F. Kirkbright, A.M. Smith and T.S. West, _Analyst_, 1967, _92_, 411.

123. M. Honda and M. Tadano, _Japan.Analyst_, 1953, _2_, 451; _Anal. Abstr._, 1955, _2_, 2402.

124. R.V. Hems, G.F. Kirkbright and T.S. West, _Talanta_, 1969, _16_, 789

125. T.R.F.W. Fennell, _Chem. and Ind._, 1955, 1404.

126. A.F. Colson, _Analyst_, 1963, _88_, 26.

127. F.H. Oliver, _ibid._, 1966, _91_, 771.

128. R.J. Hall, _ibid._, 1963, _88_, 76.

129. M.A. Wade and S.S. Yamamura, _Anal.Chem._, 1965, _37_, 1276.

130. W.C.Alford, L. Shapiro and C.E. White _ibid_, 1951, _23_, 1149

131. D.M. Hercules, _Talanta_, 1961, _8_, 485.

132. R.A. Geiger and E.B. Sandell, _Anal.Chim.Acta._, 1957, _16_, 346.

133. C. Woodward Ph.D. Thesis, University of London, 1966.

134. D.H. Wilkins, _Talanta_, 1960, _4_, 182.

135. R.F. Chen Anal.Biochem., 1967, 20, 339.

136. F.L. Moore, Anal.Chem., 1956, 28, 997.

137. H. Freund and F.J. Miner, ibid. 1953, 25, 564.

138. R.V. Hems, G.F. Kirkbright, and T.S. West, Talanta, to be published.