SOME APPLICATIONS OF SPECTROFLUORIMETRY IN ANALYTICAL CHEMISTRY.

by

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ABSTRACT

The technique of solution spectrofluorimetry as a means of inorganic trace analysis has been investigated and several analytical applications are suggested. Thallium (I) (1 to 8μg.) is determined by measurement of its violet fluorescence in a hydrochloric acid - potassium chloride medium. Extraction as thallium (III) chloride into ether effects its quantitative separation from all major interferences in this method.

Methods have been devised for the determination of submicrogram amounts of aluminium and beryllium using their blue fluorescent complexes with 2-hydroxy-3-naphthoic acid at pH5.8 and 7.5, respectively. Adaptation of standard optical methods indicates the formation of 1:1 complexes between the reagent and these metals. Aluminium and other metal ions are prevented from interfering in the beryllium determination by masking with the complexone, CDTA. Several reagents structurally similar to 2-hydroxy-3-naphthoic acid have also been investigated qualitatively.

An investigation of carminic acid as a spectrofluorimetric reagent for molybdenum and tungsten,

with which it forms red fluorescent complexes, is described. By incorporation of a thiocyanate extraction procedure, this reaction has been applied to the determination of the molybdenum content (0.01 - 0.1%) of some B.C.S. mild steels. No spectrofluorimetric method has hitherto been evolved for molybdenum.

The spectrofluorimetric determination of microgram amounts of scandium with salicylaldehyde semicarbazone has been investigated. Scandium is separated from interfering ions by removal of transition metal cupferrates followed by extraction of scandium from acid medium into tri-n-butyl-orthophosphate and back-extraction into water.

In the final chapter, some suggestions are made of promising methods of extending the present limited scope of solution spectrofluorimetry in inorganic analytical chemistry.

ACKNOWLEDGEMENTS.

The work described in this thesis is entirely original except where due reference is 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 1963 and April 1966.

I wish to express my gratitude to my supervisors, Professor T.S. West and Dr. G.F. Kirkbright, for their invaluable help, guidance and advice during this time. Thanks are also due to several of my colleagues for helpful discussions.

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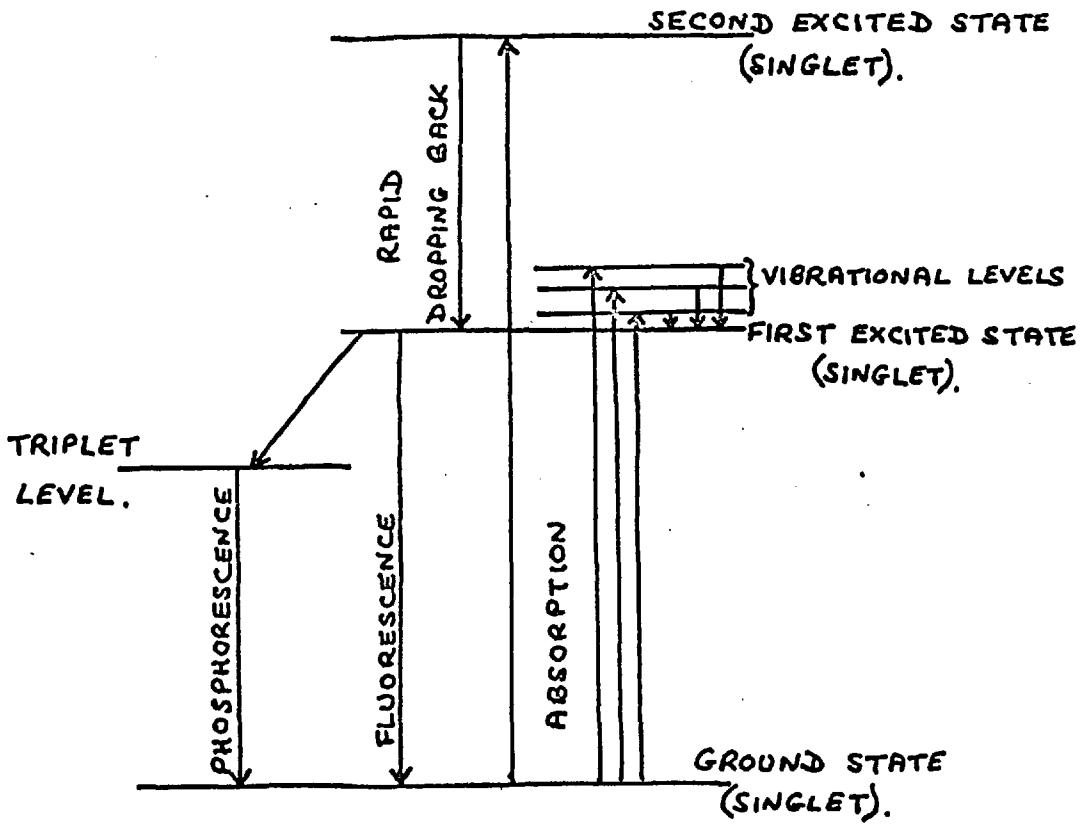
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Introduction.

The theoretical basis of the technique of spectrofluorimetric analysis has been well described by Bowen[1], Pringsheim[2], Bartholomew[3], Parker and Rees[4,5], West[6], and others.

Fluorescence differs from other emission phenomena, *viz.* the Tyndall effect, Raman scattering, etc., in that it involves the absorption and subsequent re-emission of light, at the same or, more generally, at a longer wavelength. When light is absorbed, a molecule is raised to one of the electronically excited levels, see Figure 1. Almost all molecules then drop back very quickly (*ca* 10^{-12} seconds) into the lowest vibrational level of the upper excited state, from which a proportion of the excited molecules may then return to the ground state with the emission of fluorescence. Others lose their excess energy in other ways, e.g. by conversion to the triplet state, by inter-molecular collisions or by photochemical degradation. Although fluorescence may be exhibited by solids, liquids or gases, this discussion will be limited to a consideration of the fluorescence of solutions.

Figure 1



A distinction should be made between fluorescence and phosphorescence. The basic difference is in the lifetime of the emission after excitation has ceased. Fluorescence is an almost instantaneous process, usually occurring within 10^{-8} seconds, while phosphorescence may have a lifetime of up to several seconds. Fluorescence occurs with the singlet-singlet

transition from the first excited state to the ground state. Although it is a spectroscopically forbidden transition, a small proportion of the molecules may fall into the lowest triplet level where their lifetime is increased by the lower probability of inter-molecular collisions. The triplet-singlet transition to the ground state may then occur with the emission of phosphorescence. It should be noted, however, that because of their relatively long lifetime in the triplet state, these molecules are very susceptible to quenching processes and that, for this reason, phosphorescence rarely occurs at normal temperatures. It has been shown, however, that many more substances exhibit phosphorescence at low temperatures, e.g. $77^{o}K$, where quenching by inter-molecular collisions is much reduced. Much work remains to be done on this topic.

If all of the molecules excited by absorption of light return to the ground state with the emission of fluorescence, then one quantum of fluorescent light is produced per quantum of exciting light absorbed and the process has a fluorescence or quantum efficiency of unity. In practice, because of the factors discussed above, this does not occur and a

fraction of the excited molecules returns to the ground state by other processes which do not involve the emission of fluorescence. Thus the fluorescence efficiency is invariably less than one. By definition, the rate of emission of fluorescence equals the rate of light absorption (in quanta per unit time) multiplied by the quantum efficiency of the system, i.e. :-

$$F = [I_0(1 - 10^{-\varepsilon c \ell})].\phi$$

where F = total fluorescence intensity (in quanta per unit time).

I_0 = intensity of exciting light (in quanta per unit time).

ε = molecular extinction coefficient of the solute at the exciting wavelength.

c = molar concentration of the solute.

ℓ = path-length of solution (in cm).

and ϕ = quantum efficiency of fluorescence.

For a very dilute solution in which only a small fraction of the incident light is absorbed, this simplifies to:-

$$F = I_0 . 2.3 \varepsilon . c . \ell . \phi .$$

Thus if all other factors are kept constant, the intensity of fluorescence produced is directly proportional to the concentration of solute present. It can also be seen that the fluorescence intensity varies directly with the intensity of the exciting light. Therefore very low concentrations of fluorescent solutes can be measured simply by using a very high intensity of light to excite their fluorescence. This point shows the basic difference between spectrofluorimetry and absorption spectrophotometry. In the latter technique the detection limit or sensitivity is governed by the minimum measurable difference between two light intensities and the absolute intensity of the radiation has (within limits) no effect on the sensitivity of the measurement. For fluorescence, however, given a high intensity of exciting light and very sensitive amplification and detection system, there should be no limit to the concentrations detectable by solution spectrofluorimetry. In practice, of course, this does not hold. The thermal noise of the photomultiplier and circuit is sometimes significant but the sensitivity of any given method is most often limited by the magnitude of the background (blank) fluorescence.

This may be largely due to the intensity of fluorescence of the organic reagent used under the conditions of the determination, or, if the reagent shows no fluorescence, it may arise from fluorescent impurities in the reagents or solvent, first or second order scattering of the exciting light and Raman scattering by the solvent.

This last possibility has been discussed in detail by Parker[7] who investigated the effect for several solvents. When light falls upon a substance, some of it is scattered in all directions at the same frequency but a small proportion interacts with the vibration and rotational modes of the molecule and is therefore re-emitted with a slightly longer wavelength. The difference in wavelength between the absorbed and emitted radiation corresponds to the energy absorbed during this interaction. Since most of the emission occurs at a longer wavelength than the incident radiation, it may therefore interfere significantly with the measurement of the fluorescence of a solute if this is of very low intensity and of a wavelength near to that of the Raman emission. Only the main Raman band need be considered, the intensity of others being too low to cause interference.

Water, for example, shows its most prominent band at a frequency approximately $0.3\mu^{-1}$ lower than that of the exciting light, e.g. excitation at 365mμ produces a Raman band at 416mμ. While other contributions to the blank fluorescence can be minimised or even eliminated by purification of reagents, etc., Raman emission from the solvent cannot be overcome in this way.

From the above discussion it may appear that any ion or molecule which has absorbed a quantum of light should be capable of yielding fluorescence, but, in practice, only molecules with relatively stable excited states can stabilize the absorbed radiation sufficiently to permit re-radiation of the excess energy as fluorescence. These include:-

a) Ions of transition elements or of rare earths (Sm, Eu, Dy, etc.) and inorganic complexes, e.g. $Pt(CN)_4^{2-}$, UO_2^{2+}, where the excited electron is a 'd' or 'f' electron in the atomic shell and not an outer valency electron.

b) Organic molecules with conjugated π electron systems, where the excitation of a π electron to an upper level has only a small influence on the structure and stability of the molecule.

The author's work has been concerned with the applications of spectrofluorimetry only as applied to inorganic analytical chemistry. The field of organic analysis has not been investigated.

Possibly the greatest limitation to the use of fluorescence as an analytical technique is the phenomenon of "quenching". This general term has been used above for convenience to describe any factor which decreases the observed intensity of fluorescence, although, more correctly, one should clearly distinguish between true "quenching" and "inner-filter" effects. True quenching may be defined as any process which reduces the quantum efficiency of a system. This may be caused by inter-molecular collisions between excited molecules (this is normally termed "self-quenching") or between an excited molecule and other molecules present, e.g. the solvent molecules, which remove the excess electronic energy of some of the excited molecules and thus decrease or eliminate the emission of fluorescence. Similarly, quenching may be caused by the addition of another substance to the system which may either increase the collision frequency of the fluorescent molecules, e.g. by decreasing

the viscosity of the medium, or which may take part in complex formation with them. This subject has recently been treated by West[6] and his paper should be consulted for a discussion of the theoretical aspects of this problem.

It has also been reported[5] that dissolved oxygen is a common quenching agent, particularly in organic fluorimetric analysis, presumably owing to inter-molecular collisions with the fluorescent solute, but this effect was not found to be significant in the systems investigated by the author and is therefore not considered in more detail here. It is advisable, however, to check the effect of de-aerating solutions when a new fluorimetric method is being developed.

Of much greater importance in the author's work have been "inner-filter" effects, which arise when a substance is present which absorbs at the wavelength of either the excitation or the fluorescence emission of the system under investigation. For example; if a small amount of Fe(III) - EDTA solution is added to a solution of salicylic acid which has a fluorescence emission at about 430mμ, although no reaction will occur, a proportion of the fluorescence emitted by the salicylic acid will be absorbed by

the yellow solution added and hence the observed fluorescence intensity will decrease. As most of the fluorescence systems commonly used have excitation and emission maxima in the region 300 - 650mμ, anything which absorbs in the near ultra-violet or visible region will tend to produce inner-filter quenching. Thus in the molybdenum-carminic acid system described later, the reagent, which is highly coloured, has an absorption maximum near the fluorescence emission peak of the molybdenum complex so that, beyond a certain point, any further increase in reagent concentration produces a significant decrease in the measured fluorescence intensity.

A further factor which may reduce fluorescence and which may be regarded as a form of inner-filter quenching, is so-called "concentration quenching". This occurs when the concentration of the fluorescent solute is such that the solution may no longer be regarded as "very dilute" (above about 1g/ℓ for most fluorescent dyes). In such systems a further increase in solute concentration will produce a decrease in observed fluorescence intensity. This has been attributed to the formation of non-fluorescent polymeric species, a process which may also be

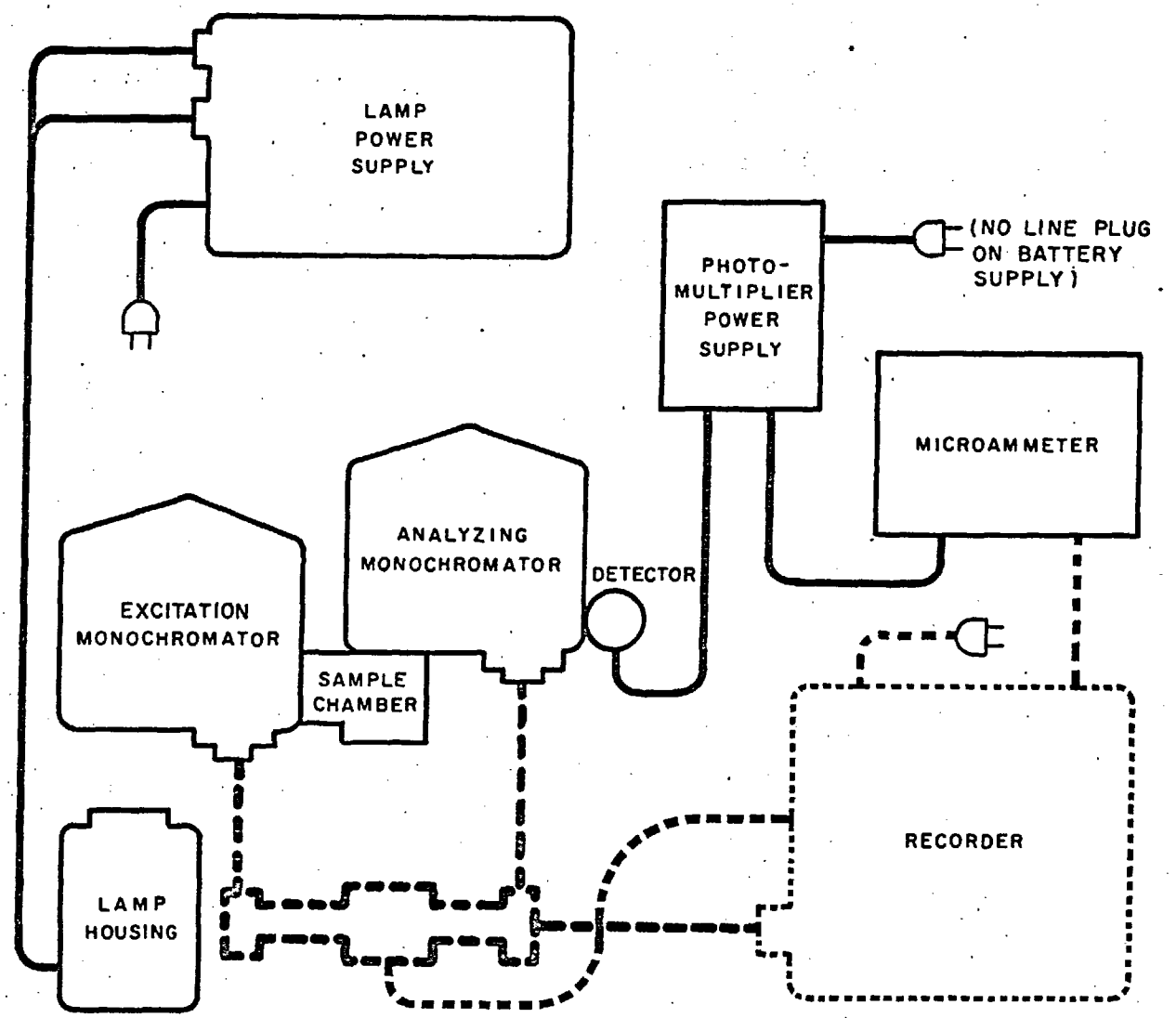
favoured by increase in the electrolyte concentration of the medium.

One further cause of quenching of fluorescence is increase of temperature which increases the probability of inter-molecular collisions. This has been extensively reported in the literature and all of the systems investigated by the author have shown a decrease of fluorescence of the order of 1 - 2% per ^{o}C temperature rise. This is not a great drawback where the variation in working temperature is small ($< \pm 5^{o}C$) but it indicates that if fluorescence measurements could be made at lower temperatures, e.g. $77^{o}K$, quite spectacular improvements in sensitivity would result.

At this stage it is desirable to consider the instrumentation necessary for spectrofluorimetry and this is probably best done by describing the instrument used by the author, which is typical of the instruments commercially available at present.

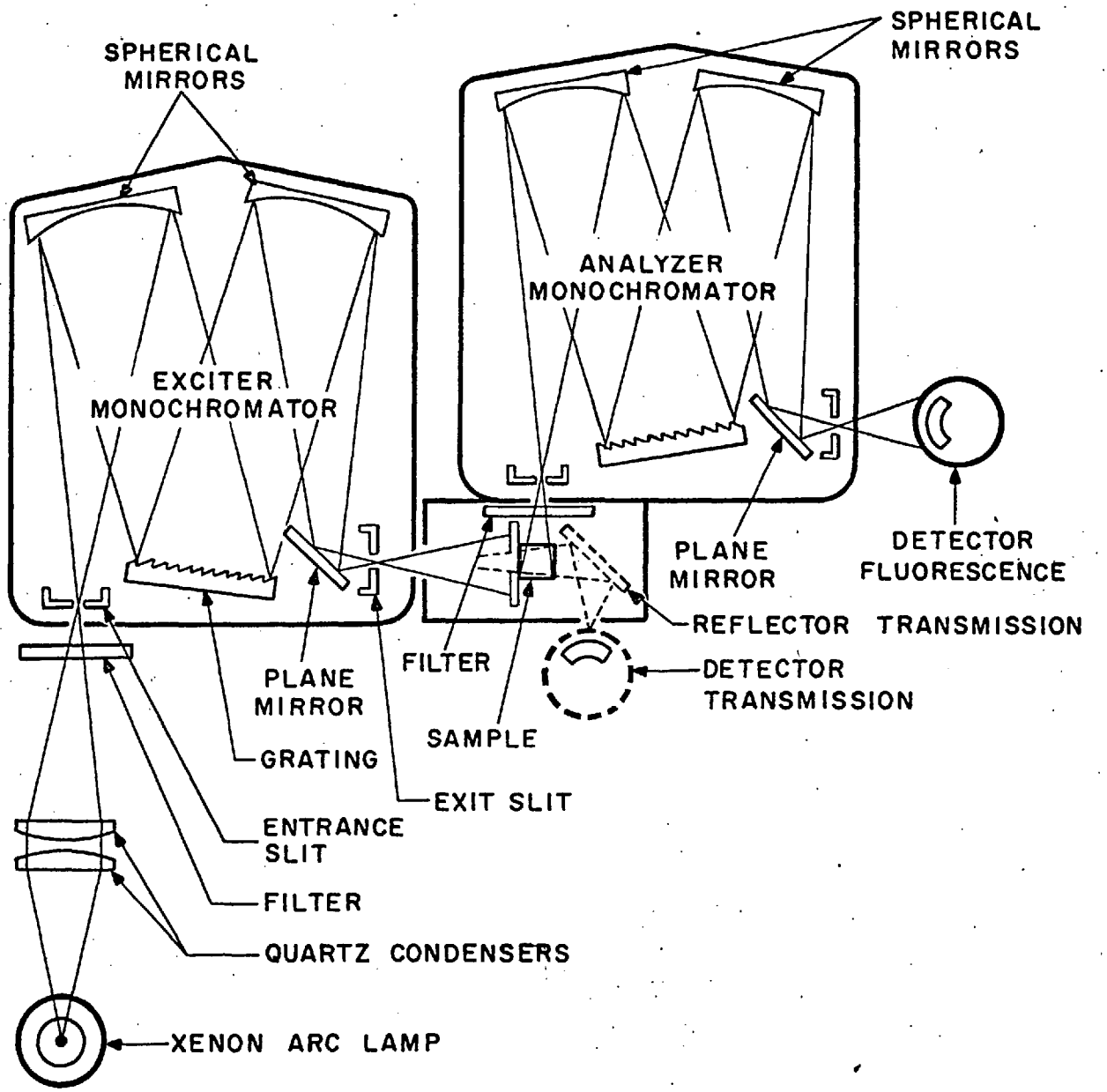
The basic lay-out of the Farrand spectrofluorimeter is shown in Figure 2, the optical diagram in Figure 3 and the actual instrument used in Figure 4. Ultraviolet and visible energy from a xenon arc lamp is focussed on the entrance slit of the excitation

Figure 2



monochromator. Light of the selected wavelength is directed from this monochromator onto the sample and the resultant fluorescent radiation enters the "analysing" monochromator at right angles to the

Figure 3



exciting light. To this monochromator is attached a photomultiplier which produces a signal which is amplified and displayed on a microammeter or transferred

FIGURE 4.


LINE ON
LAMP ON
FUSE 5 AMP.
ON OFF
FARRAND OPTICAL CO. Inc.
10 20 30 40 50 60 70 80 90 100
14.

to a chart recorder.

The light source is a high-pressure 150 watt D.C. xenon arc lamp which gives a continuous spectrum from about 220mμ, through the visible region and well into the infra-red. The lamp power supply uses A.C. mains input and rectifies this to D.C. When the circuit is first switched on, a momentary potential of 25 to 40K volts strikes an arc across the electrodes of the lamp and, after a fraction of a second, the operating voltage of 18-20 volts takes over. During operation the lamp is cooled by air blown by a fan situated in the top of the lamp-housing. The lamp output is focussed onto the entrance slit of the excitation monochromator by means of an off-axis ellipsoidal reflector (not by the quartz condensers shown in Figure 3). A quartz window is fitted in the aperture tube of the lamp-housing to prevent the entry of cold air currents which could adversely affect the stability of the lamp. Light entering the monochromator is collimated by the first spherical concave mirror, then dispersed by the diffraction grating and focussed by the second spherical mirror. It passes via a diagonal mirror through the exit slit to the sample compartment. The monochromators used

each have a wavelength range of 220-650mμ and use replicate diffraction gratings with 14,400 lines per inch. Metal slits with equivalent bandwidths of 5, 10 and 20mμ are provided. It is normally preferable to use the narrowest slits which will provide enough energy for efficient operation of the photomultiplier.

The sample compartment is located between the two monochromators. In all of the author's work, fused quartz cells of size 10 x 20 x 50mm have been used, although smaller cells (10 x 10 x 48mm and 3 x 3 x 40mm), which require less volume of solution, are available. The cell-holder may also be adapted to take a solid sample. The fluorescent radiation, taken at rightangles to the incident light to minimise interference from reflected light and fluorescence from the cell walls, passes through the "analysing" monochromator, which is identical with the excitation monochromator, and falls upon the photocathode of an R.C.A. IP21 or IP28 photomultiplier tube. These tubes vary in their spectral response, current amplification and signal-to-noise ratio. 9-stage IP28 tubes were used throughout the work described in this thesis. The photomultiplier power supply uses

A.C. mains input and rectifies this to a stabilised 900 volts D.C. output.

The R.C.A. micro-ammeter used (Type WV-84C) has six sensitivity ranges which permit the direct measurement of currents from 0.0002 to 1000 μ amps. This has been used in connection with a Minneapolis-Honeywell-Brown chart recorder, the motor of which is geared to drive either of the monochromators; alternatively at fixed wavelengths the effect of time may be studied automatically. The chart width is 10 inches, the response time is one second and paper passes at a rate of 10 inches for a complete spectrum scan, i.e. 220-650mμ. A choice of chart speeds (ranging from $1\frac{2}{3}$ to 40 inches per minute) is available; it is normally preferable to use the slowest possible speed in order to obtain optimum resolution of spectra.

Figure 3 shows that there are two filter positions in the spectrofluorimeter. The first, at the entrance to the excitation monochromator is intended for a filter which transmits ultra-violet but not visible radiation from the source, thus preventing the pick-up of stray light of longer wavelengths in the second monochromator. In some experiments a Corning 7-54 filter (transmittance > 75% over the

range 275 to 375mμ) was used in this position. In the second position, at the entrance to the analysing monochromator, a filter may be placed to eliminate all stray ultra-violet light if the fluorescence being produced by the sample is in the visible region. Corning filters 3-72 and 3-73 (transmittance > 75% above 490mμ and above 455mμ respectively) are suitable for this purpose.

This spectrofluorimeter is a single-beam instrument which measures the fluorescence intensity of any sample solution under given conditions. This measured signal is clearly dependent not only upon the sample being irradiated, but also upon the intensity of the lamp emission at the wavelength used to excite fluorescence, the transmission characteristics of the two monochromators and the sensitivity of the photomultiplier-amplifier response at the wavelength of the fluorescence produced. Thus the fluorescence intensity measured under any given set of instrumental conditions is purely relative and has little absolute significance. Parker and Rees[8] have described methods for the correction of excitation and fluorescence spectra for these factors in order to obtain the "true" spectra for the system. The true excitation

spectrum is obtained by correcting the observed spectrum for the spectral distribution of the source and for the transmission characteristics of the excitation monochromator. Similarly, correction of the observed fluorescence spectrum for the characteristics of the analysing monochromator and for the variation in the detector sensitivity gives the true fluorescence spectrum. Such procedures involve tedious point-by-point corrections and yield spectra of doubtful value to the analytical chemist. Although it is obviously valuable to know the true positions of excitation and fluorescence peaks of a system, the "apparent" or observed maxima are of more practical significance, i.e. the monochromator wavelength settings which will produce maximum response on the operator's own instrument. For practical purposes, therefore, it is preferable to use the uncorrected spectra as obtained directly from the instrument's chart recorder.

As discussed previously, in solution spectrophotometry the measurement made is of a ratio between the absorbances of two solutions. This should, therefore, be largely independent of instrumental variables such as lamp intensity and detector

sensitivity. In spectrofluorimetry, however, the signal measured is a micro-ammeter reading which depends not only upon the sample solution but also upon the lamp intensity, type of photomultiplier used, etc. In the author's experience, using identical conditions at different times, the signal received may vary by about a factor of three. This has been attributed to the xenon arc lamps used which appear to strike different arcs from time to time, thus producing different intensities of light for excitation of fluorescence. Consequently, the intensity of the fluorescence produced by a given solution varies, leading to differences in the signal measured. This demonstrates the need for a fluorescent standard to which observed fluorescence intensities may be related. A dilute solution of quinine in 0.1M sulphuric acid has been recommended for this purpose[6]. This solution has an excitation maximum at 350mμ and its fluorescence emission maximum at 450mμ. By measuring the response due to a standard solution (say 10^{-6}M in quinine bisulphate) at these wavelengths, the sensitivity of the instrument may be checked before and after each experiment. Before it is possible to compare the sensitivities of two

analytical methods, account must be taken of differences in instrumental parameters under the conditions used. Thus if two methods for the determination of, say, 10μg. of aluminium give identical fluorescence intensities when measured at a wavelength of 500mμ with excitation at 350mμ for method A and at 400mμ for method B, if the intensity of the lamp emission is twice as great at 400mμ as it is at 350mμ, it is obvious that the true sensitivity of method A is twice that of method B. This may be allowed for by correcting the observed fluorescence intensity for this difference in lamp intensity (and also, if appropriate, for variations in photomultiplier response at different emission wavelengths), then relating the result to the fluorescence intensity of a standard quinine bisulphate solution similarly corrected. This has been done[9] for some spectrofluorimetric procedures for the determination of aluminium, beryllium and gallium. It should be noted, however, that this type of correction may be misleading because it expresses the true sensitivity of a method and not the relative size of the response which will be observed with a given instrument used under its optimum conditions, which is more important

to an analytical chemist.

The biennial reviews of White[10] give a comprehensive survey of the field of fluorescence analysis and a useful summary has recently been published by the same author[11]. Examples of purely inorganic fluorescent systems are limited and to date there has been only a restricted number of applications in analytical chemistry. Of greater importance are the numerous systems in which an inorganic species reacts with an organic compound to produce a compound which exhibits a different fluorescence. These inorganic-organic systems may be broadly divided into four classes, viz:

(1) those in which the fluorescence of the organic reagent or of a complex is "quenched" by reaction with a cation, e.g. the aluminium-Pontachrome Blue Black R complex with Fe(III),[12] etc:

(2) reaction of a cation with a non-fluorescent organic reagent to produce a complex which exhibits fluorescence, e.g. Aℓ with 8-hydroxyquinoline[13]:

(3) reaction of a metal ion with a fluorescent reagent to produce a complex which fluoresces at a different wavelength, e.g. Aℓ or Be with 2-hydroxy-3-naphthoic acid:

(4) intramolecular energy transfer reactions in which the fluorescence is excited by frequencies absorbed by the organic part of the molecule and is emitted at wavelengths characteristic of the inorganic ion present, e.g. Eu or Sm with thenoyltrifluoroacetone[14].

It is not proposed to give a complete survey of the fluorescence reactions which have analytical applications. To date these appear to have been limited to inorganic ions which have either full or empty 'd' electron shells. Mention should perhaps be made, however, of some of those which have become the classic fluorescence reactions, e.g. Morin (2',4',3,5,7 - pentahydroxyflavone)[15] which gives fluorescent complexes with aluminium, beryllium, gallium, indium, scandium, etc.; 8-hydroxyquinoline (oxine)[13] which has been used to determine a range of metal ions; Pontachrome Blue Black R (4-sulpho-2-hydroxynaphthaleneazonaphthol) which forms a fluorescent complex specifically with aluminium[16].

Because spectrofluorimeters, as opposed to filter-fluorimeters, have become available only within the past decade, there is still considerable scope for expansion in the applications of this method of analysis.

The objectives of the work described in this thesis were, firstly, to investigate the potentialities of solution spectrofluorimetry as an inorganic analytical technique and then to use this technique to extend the rather limited range of metals which may be determined by fluorescence reactions. This has been carried out by developing procedures in a manner similar to that used for spectrophotometry[17]. Methods have been developed for the quantitative determination of several metals, viz. aluminium, beryllium, thallium, scandium, molybdenum and tungsten, based on previously reported qualitative fluorescence tests for these elements.

The method used for the determination of thallium[18] is a purely inorganic system, the fluorescence being attributed to the $TlCl_3^{2-}$ an ion formed in a potassium chloride-hydrochloric acid medium. No other ion investigated reacted similarly, but several interfered with the determination of thallium in other ways. A solvent extraction system was, therefore, devised to separate microgram amounts of thallium quantitatively from the more important interfering cations.

All of the other reactions investigated by the author depended upon the formation of a fluorescent

complex between the metal ion and an organic molecule containing a conjugated π electron system. Aluminium and beryllium were determined down to the 2 and 0.2 parts in 10^9 levels, respectively, by measurement of their blue fluorescent complexes with 2-hydroxy-3-naphthoic acid[19]. Aluminium and other metal ions were prevented from interfering in the beryllium determination by masking with the calcium complex of CDTA (cyclohexanediaminetetra-acetic acid).

The reagent used for the determination of molybdenum and tungsten[20], carminic acid, was selected for two reasons; firstly, because it contains adjacent hydroxyl and carboxylic acid groups on one of the aromatic rings (cf. 2-hydroxy-3-naphthoic acid), and secondly, because, although its reaction with molybdenum had been noted, no fluorimetric method for the determination of this element had previously been described. Both metals form red-fluorescent complexes, but their determinations were subject to several cationic interferences. A simple thiocyanate extraction procedure was, therefore, evolved which was then successfully applied to the determination of 0.01 - 0.1% of molybdenum in some B.C.S. mild steels[21]. The simultaneous extraction of tungsten was prevented by complexation with citric

acid.

Salicylaldehyde semicarbazone, a Schiff's base which forms blue fluorescent complexes with several metals, has been used for the spectrofluorimetric determination of microgram amounts of scandium[22]. Again an extraction procedure was devised to separate scandium from relatively large amounts of other elements including yttrium and aluminium which behave similarly with this reagent[23].

CHAPTER I.

The Spectrofluorimetric Determination of Thallium.

Several colorimetric methods are available for the determination of small amounts of thallium in solution; these utilise the reagents methyl violet[24], Rhodamine B[25], iodine[26], dithizone[27], thionalide[28] and 8-hydroxyquinoline[29]. Onishi[30] has proposed a fluorimetric method for the determination of 2-20µg. of thallium in which the orange-yellow fluorescence of Rhodamine B chlorothallate in benzene solution was measured at about 580mµ. In this method the thallium (III) was separated from other elements by extraction into chloroform as thallium dithizonate and then into benzene as Rhodamine B chlorothallate.

This chapter describes an investigation of the fluorescence reaction between thallium (I) and chloride ion and the development of a method for the determination of microgram amounts of thallium using the violet fluorescence produced. This fluorescence was first reported by Pringsheim and Vogels[31] and subsequently applied to the detection of thallium in ores and other minerals by Sill and Peterson[32]. After completion of this investigation by the writer,

a more recent paper by Bock and Zimmer was noted which described the spectrofluorimetry of thallium (I) in strong sodium chloride solution in the absence of hydrochloric acid. Their note gave the excitation and fluorescence spectra of the system but contained no interference data and did not describe an analytical procedure.

The violet fluorescence was attributed by Pringsheim and Vogels[31] to the existence of the complex anionic $TlCl_3^{2-}$ species, with neither H_2TlCl_3 (formed in the presence of considerable acid) nor thallium (I) ion showing any fluorescence. Their experiments on the absorption spectra of thallium (I) solutions in various chloride media were not repeated here. This fluorescence could only be obtained in a medium containing a vast excess of both alkali chloride and hydrochloric acid. It was, therefore, not possible to employ the mole-ratio, slope-ratio and continuous variations procedures to elucidate the nature of the fluorescent species. The fluorescence emission was at 430mμ with an excitation wavelength of 250mμ in a concentrated hydrochloric acid-potassium chloride medium. Optimum conditions were established for the determination and the effects of numerous ions

were investigated.

Several possible extraction systems were examined for the separation of thallium from the more important interfering ions. At pH5.8, in the presence of EDTA to mask possible interferences, Tℓ(I)-oxinate was found to be extractable into chloroform, which afforded a separation from Cu(II), Fe(III), etc.; excess EDTA, which would otherwise prevent formation of the Tℓ(I)-oxinate, was masked with calcium. The oxinate showed a yellow colour and a weak blue-green fluorescence. Shaking the chloroform extract with potassium chloride-hydrochloric acid solution returned the thallium to the aqueous layer but any resultant $TlCl_3^{2-}$ fluorescence was obscured by the light absorption at 430mμ of the deep yellow coloured solution caused by the presence of oxine.

Attempted removal of interfering ions by extraction of their cupferrates into chloroform resulted in a green organic phase probably caused by an oxidation product of cupferron. The absence of fluorescence in the remaining aqueous phase was attributed to either, (a) extraction of the thallium into the organic phase, or (b) absorption of the

excitation light by non-extracted cupferron or its decomposition products. In either case the method was unsatisfactory. Acetylacetone extraction of interferences was also considered, but rejected because acetylacetone dissolved in hydrochloric acid to give a yellow solution, which reduced the thallium (I)-fluorescence by an inner-filter effect.

The extraction system finally adopted gave an efficient and selective separation of thallium and depended upon oxidation of thallium (I) to thallium (III) with hydrogen peroxide followed by extraction of thallium (III)-chloride into diethyl ether from the optimum concentration of hydrochloric acid. Thallium (I) could not be extracted quantitatively from hydrochloric acid into diethyl ether, but extraction from 1.5M hydrochloric acid separated thallium (III) almost quantitatively from all foreign ions investigated except Au, Bi, Pt (IV) and Sb (V) which also extracted to varying extents. The efficiency of the separation was further improved by washing the ether extract with 1.5M hydrochloric acid to back extract small amounts of Fe (III) which extract with the Tl (III) to an extent of $< .1\%$. The ether phase was then evaporated to dryness, redissolved in water and the

thallium (III) reduced to the univalent state by gaseous sulphur dioxide before the determination. Although the extraction of thallium (III) chloride into diethyl ether had previously been applied on a macro scale[34], this investigation showed that it could also be used successfully for microgram amounts of thallium, with an extraction efficiency of ca. 98% in the range of 1 to 8µg. of thallium.

Experimental.

Reagents.

Standard Thallium (I) solution.

A 10^{-3}M thallium (I) solution was prepared by dissolving 0.2525g. of AnalaR thallous sulphate (Tl_2SO_4) in water and diluting to 1 litre, using water from an all-glass distillation apparatus. This stock solution was diluted to 10^{-5}M as required.

Hydrochloric acid.

AnalaR grade was used. A 2M solution was prepared by dilution of 45ml. of concentrated acid to 250ml. with water.

Saturated potassium chloride solution.

This was prepared by dissolving ca. 150g. of reagent grade potassium chloride in 500ml. of boiling water, filtering the solution while warm and allowing

it to crystallise on cooling. The saturated solution was approximately 4.1M in potassium chloride and was standardised by determining the chloride content by Volhard's method.

Hydrogen peroxide.

6% (20-volume) general purpose reagent grade solution was used.

Diethyl ether.

Solvent grade.

Sulphur dioxide.

Liquefied.

Foreign ions.

0.1M, 0.02M or 0.01M solutions of AnalaR salts were used.

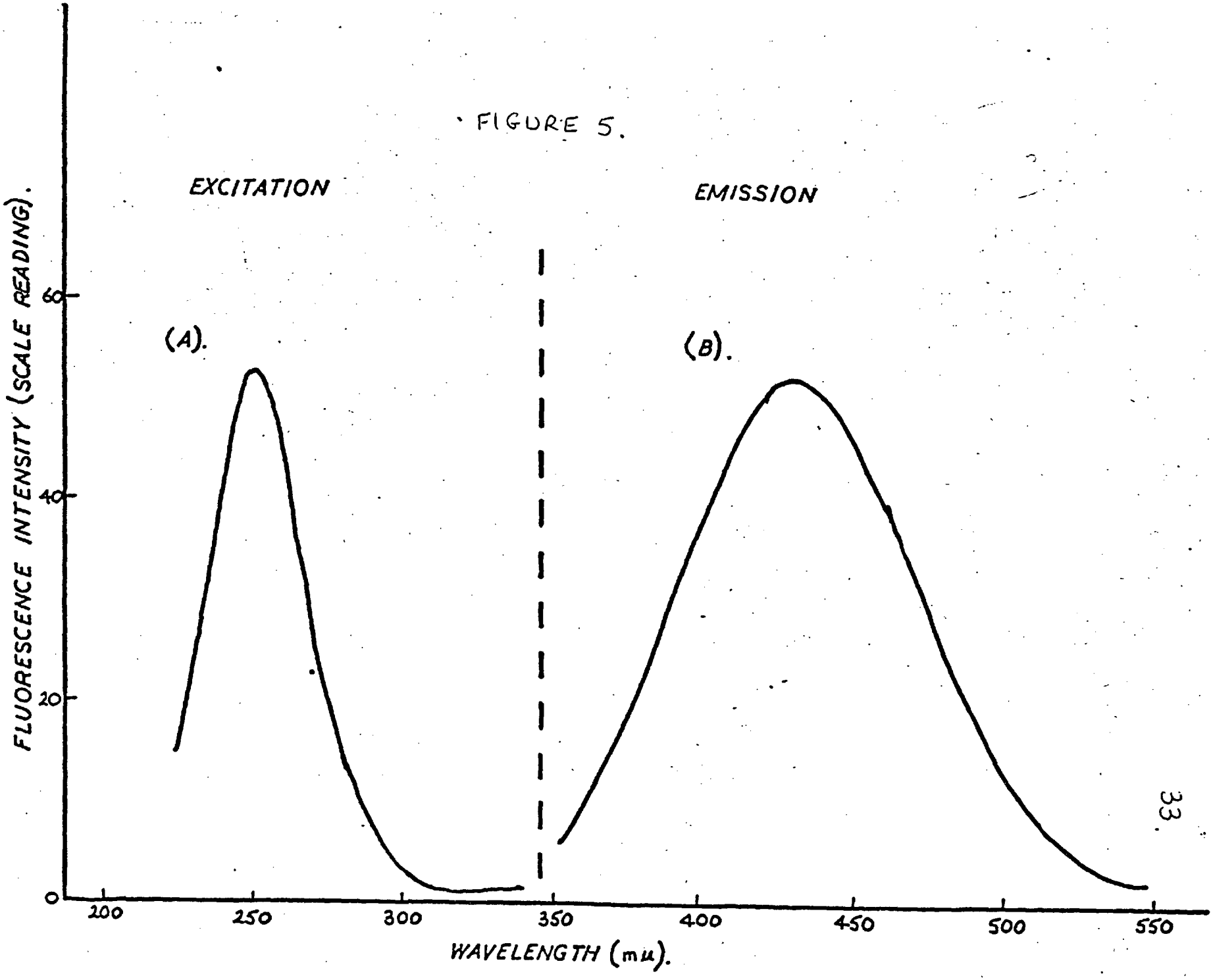
Apparatus.

Fluorescence measurements were made with the Farrand spectrofluorimeter (Farrand Optical Co. Cat. No. 104244) described in "Introduction". 20mμ bandwidth slits were used in both the excitation and analysing monochromators and no filters were used during these experiments.

Spectral Characteristics.

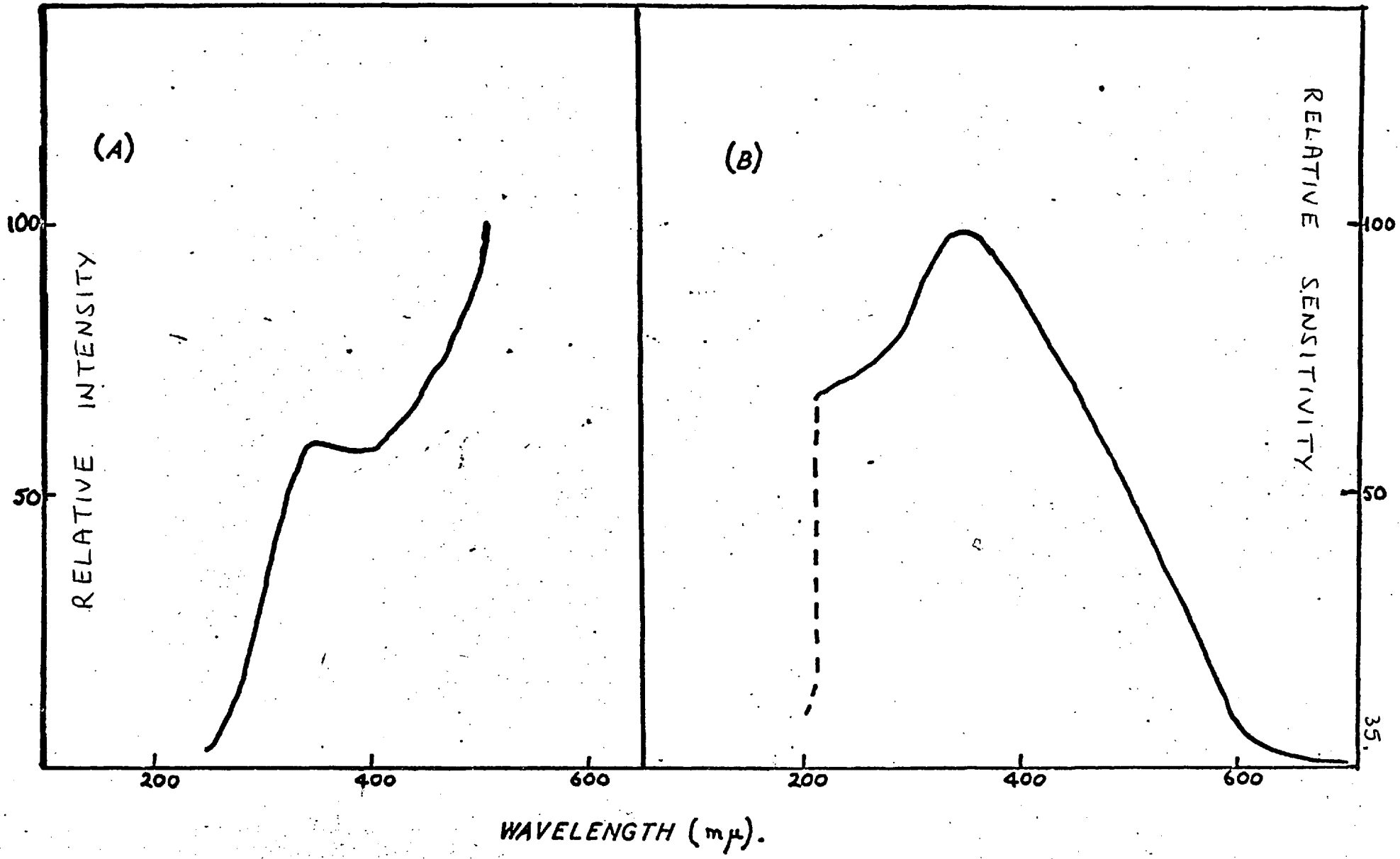
Figure 5 shows the excitation and emission

FIGURE 5.


EXCITATION
EMISSION
(A).
(B).
FLUORESCENCE INTENSITY (SCALE READING).
0
20
40
60
200
250
300
350
400
450
500
550
WAVELENGTH (mμ).

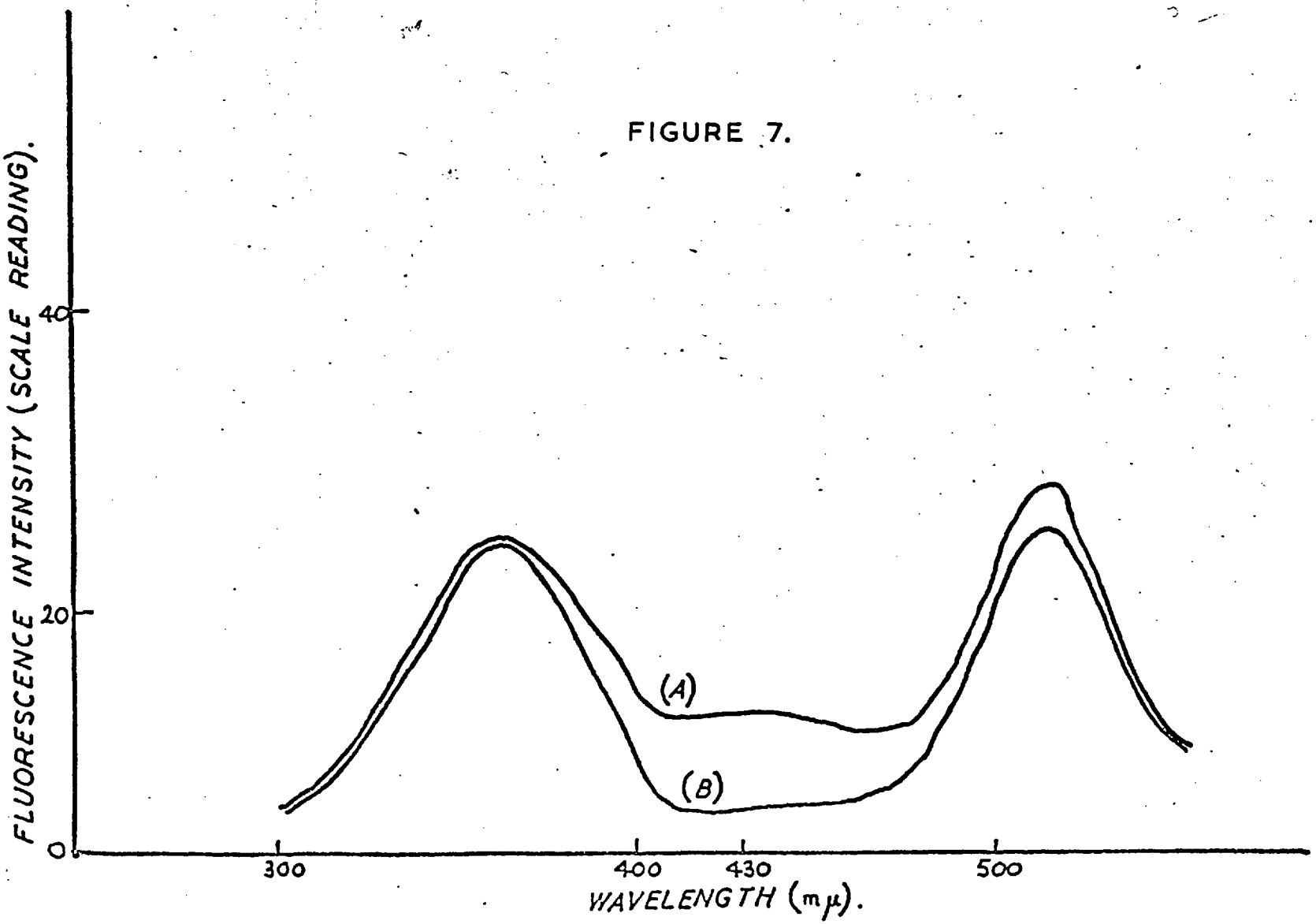
spectra for the thallium (I) ion in hydrochloric acid-potassium chloride solution. The thallium (I) concentration used was 2.5×10^{-4}M and the spectra were plotted on the x 10 sensitivity scale. For curve (A) the emission was measured at 430mμ and for curve (B) the excitation wavelength was set at 250mμ. 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 IP28 photomultiplier. The relevant correction curves are given in Figure 6. Curve (B) shows the relative sensitivity of the photomultiplier against wavelength and is the curve supplied by the manufacturers. Curve (A), the spectral characteristics of the lamp plus the excitation monochromator, was obtained by arranging the instrument for transmission measurements (see Figure 3) and scanning the exitation monochromator over the range 220-650mμ. The "total" spectrum thus measured was then corrected point-by-point for the photomultiplier response to obtain curve (A). Interchanging the monochromators showed that their transmission characteristics were virtually identical.

FIGURE 6.


(A)
(B)
100
50
RELATIVE INTENSITY
RELATIVE SENSITIVITY
100
50
35
200
400
600
200
400
600
WAVELENGTH (mμ).

The excitation maximum occurs at 250mμ and the fluorescence emission maximum at 430mμ. Figure 7 shows the emission spectra of more dilute solutions (uncorrected) at much increased sensitivity with wide (bandwidth 20mμ) slits and no protective filters. For these spectra an excitation wavelength of 250mμ was used and measurements made on the x.1 sensitivity scale. Curve (A) was obtained with a thallium (I) concentration of 4×10^{-7}M and curve (B) with no thallium present. The peak at approximately 510mμ is attributed to second-order diffraction from the analysing (grating) monochromator. The peak at about 360mμ may have been caused by fluorescent impurities in the solvents used, analogous to the effects noted by Parker[35]. Some tests with optical-black paper inside the excitation monochromator designed to minimise internal reflections, however, suggested that the cause was stray light of wavelengths other than 250mμ being reflected by the monochromator walls, reaching the sample cuvette and being scattered by the solution. Using an excitation wavelength of 250mμ, the main Raman band of water appears in the region of 270mμ and, therefore, does not interfere with the fluorescence spectrum.

FIGURE 7.


FLUORESCENCE INTENSITY (SCALE READING).
40
20
0
(A)
(B)
300
400
430
500
WAVELENGTH (mμ).

The "tails" of the two peaks shown in Figure 7 made up the blank "fluorescence" at 430mμ. The increase in signal at this wavelength was then directly proportional to the thallium concentration. For the reasons discussed above, calibration curves prepared at this level of concentration passed above the origin.

Effect of hydrochloric acid and potassium chloride concentrations.

The violet fluorescence of the thallium complex anion is only obtained in the presence of large amounts of hydrochloric acid and an alkali chloride. The optimum amounts of each component for the determination were established by varying their concentrations dependently. In a solution 3.3M in hydrochloric acid, the fluorescence intensity increased with increase of potassium chloride concentration up to 0.8M. Above this level, the fluorescence intensity remained constant and potassium chloride tended to precipitate by the common ion effect. It was also shown that in 0.8M potassium chloride, a 3.3M hydrochloric acid concentration was required to produce maximum fluorescence. Further increase in acid concentration had no effect on the

observed fluorescence intensity. Thus, for optimal sensitivity, the solution should be ca. 3.3M in hydrochloric acid and 0.8M in potassium chloride, i.e. 100ml. of solution should contain 30ml. of concentrated hydrochloric acid and 20ml. of saturated potassium chloride solution.

A slight reduction in the fluorescence intensity (< 10%) was observed when ammonium chloride or sodium chloride was substituted for potassium chloride in the recommended procedure. Replacement of the potassium chloride by the bromide or iodide yielded no measurable fluorescence; in the latter instance the yellow solution absorbed strongly at 430mμ.

Effect of time.

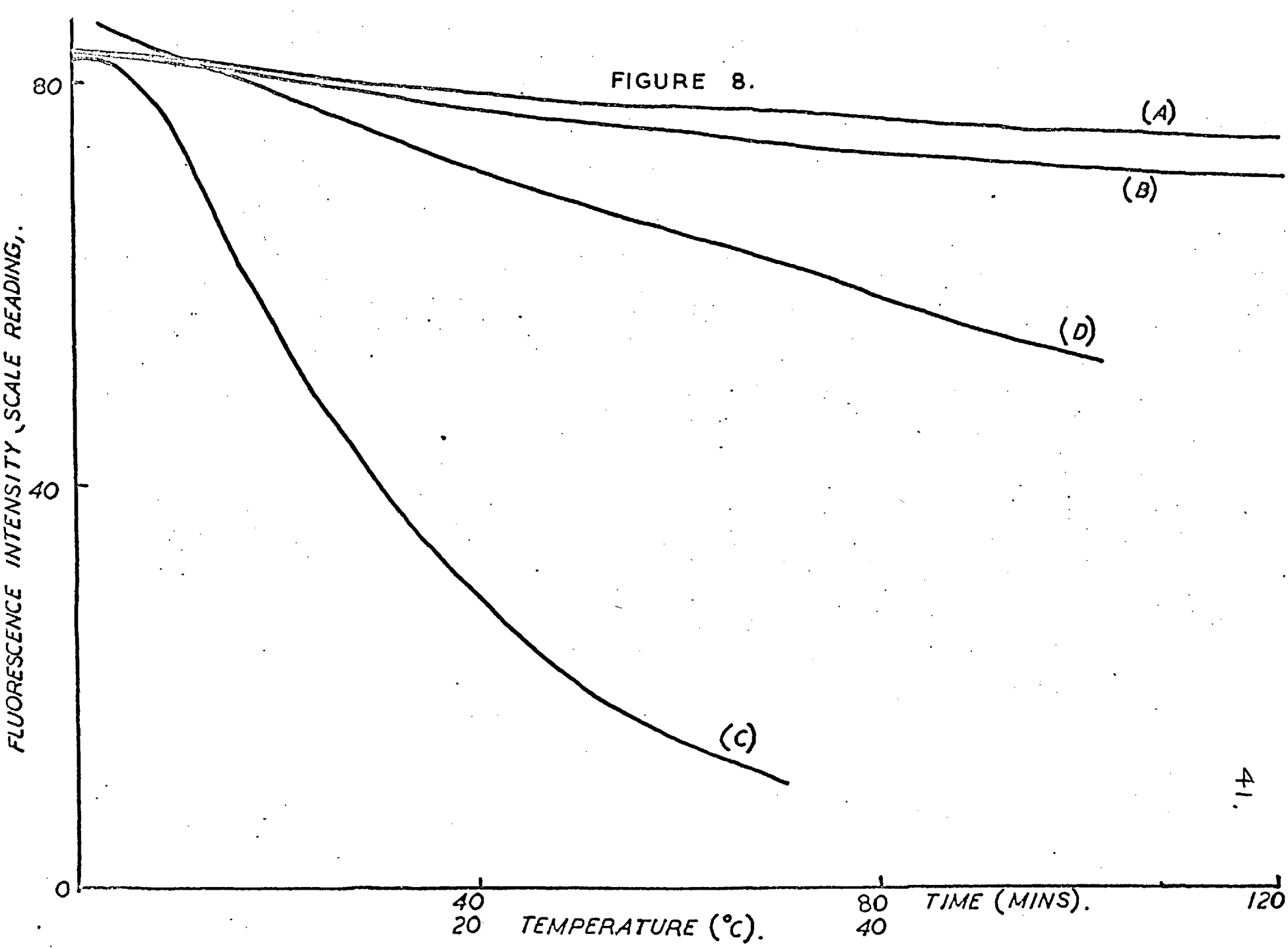
Thallium (I) in a strongly acid medium is oxidised by air to thallium (III) and this process is accelerated by ultraviolet radiation. Since thallium (III) chloride is non-fluorescent, an 8×10^{-7}M solution of thallium (I) prepared by the recommended procedure showed an average reduction in fluorescence intensity of 10% after standing for two hours in darkness (cf. Figure 8A) and a reduction of 14% after standing for two hours under normal laboratory conditions, i.e. under fluorescent-tube

lighting (cf. Figure 8B). However, continuous irradiation of the solution at 250mμ in the spectrofluorimeter completely destroyed the fluorescence within one hour (cf. Figure 8C). In practice this is not a serious limitation because the solution is only irradiated for a short time during analytical measurements.

Effect of temperature.

The effect of temperature on the intensity of fluorescence was investigated by preparing three sets of identical solutions and allowing their fluorescence to develop while immersed in an ice-bath, at room temperature and while immersed in water at ca. 50°C, respectively. Between the limits of the temperature range studied, i.e. 3-43°, a decrease of fluorescence intensity with increase in temperature of development was found which corresponded to a temperature coefficient of ca. 1% per °C (cf. Figure 8D). Under normal laboratory conditions, all measurements of the thallium fluorescence were made at 23±3°C and the observed fluorescent intensities were compared with standards prepared simultaneously, so that the existence of this temperature coefficient did not alter the validity of the results obtained. It was necessary, however,

FIGURE 8.


80
40
0
FLUORESCENCE INTENSITY (SCALE READING).
(A)
(B)
(D)
(C)
40
80
120
TIME (MINS).
20
TEMPERATURE (°C).
40

to minimise the generation of heat of mixing between concentrated hydrochloric acid and water during the preparation of samples for measurement, by adding the acid to as large a volume of water as possible and mixing thoroughly immediately.

Precision.

The precision of the method at its maximum sensitivity was found to be compounded of (a) the chemical precision, i.e. the reproducibility in fluorescence intensity of a series of identical solutions and (b) the instrumental precision, i.e. the reproducibility obtainable in the scale reading using the instrument on its most sensitive scale. These two factors were estimated separately by multiple determinations, (a) using sufficient thallium (80μg) to give readings on a less sensitive scale (x 0.1) where instrumental "noise" was negligible (See Table 1); and (b) using 8μg. of thallium and measuring directly on the most sensitive scale (x 0.01) where instrumental "noise" was appreciable, without treating the solution by the extraction procedure (see Table 2).

Table 1.

Sample no.	Scale reading (x.1)	Net thallium response	Deviation	Variance
1	79.0	72.7	+0.4	0.16
2	79.2	72.9	+0.6	0.36
3	77.5	71.2	-1.1	1.21
4	78.3	72.0	-0.3	0.09
5	79.5	73.2	+0.9	0.81
6	77.3	71.0	-1.3	1.69
7	79.5	73.2	+0.9	0.81
Blank	6.3	-		
		Mean = 72.3		

Standard deviation $= \sqrt{\frac{\Sigma\Delta^2}{n-1}} = \sqrt{\frac{5.13}{6}} = \sqrt{0.85} = 0.92$ units

Table 2.

Sample no.	Scale reading (x.01)	Net thallium response	Deviation	Variance
1	71.5	56.5	-0.6	0.36
2	74.0	59.0	+1.9	3.61
3	72.0	57.0	-0.1	0.01
4	71.5	56.5	-0.6	0.36
5	71.5	56.5	-0.6	0.36
6	72.5	57.5	+0.4	0.16
7	72.0	57.0	-0.1	0.01
Blank	15.0	-		
		Mean = 57.1		

Standard deviation $= \sqrt{\frac{\Sigma\Delta^2}{n-1}} = \sqrt{\frac{4.87}{6}} = \sqrt{0.81} = 0.90$ units

The results shown in Table 1 gave a thallium response of 72.3±0.9 units which indicated a chemical precision of ±1.3%, whilst Table 2 gave a response of 57.1±0.9 units, corresponding to an instrumental precision of ±1.6%. It should be pointed out, however, that, strictly speaking, this was a compromise method of estimating the chemical precision, because, in order to eliminate the instrumental "noise", each sample contained ten times more thallium than the maximum used for the actual determinations.

Accuracy.

The data given in Table 3 were obtained from the results of the analysis of unknown thallium solutions by the recommended procedure (see later). Two standards were prepared and measured with each group of samples, using 0 and 8.16µg. of thallium, respectively. In each case the result quoted in Table 3 was the average of the results of two simultaneous determinations.

Effects of foreign ions.

The effects of 54 foreign ions on the thallium fluorescence in pure solution were studied. None of the cations investigated produced a fluorescence similar to that of thallium, but several, when present

Table 3.

Analysis of Thallium (I) Solutions Treated as Unknown Samples

Sample	µg. Thallium		Error	Error	Foreign ions present Foreign ions present
	Present	Found	(µg.)	(%)	(µg.)
1	4.90	4.57	-0.33	-6.6	Fe(III) (5600)
2	6.92	6.72	-0.20	-2.9	Fe(III) (5600)
3	3.87	3.75	-0.12	-3.1	Fe(III) (5600)
4	3.26	3.26	-	-	Fe(III) (5600)
5	5.91	6.13	+0.22	+3.7	Cu(II)(630), Hg(II)(2000)
6	3.67	3.77	+0.10	+2.8	Cu(II)(630), Hg(II)(2000)
7	3.87	4.04	+0.17	+4.4	Cu(II)(630), Hg(II)(2000)
8	4.08	3.89	-0.19	-4.7	Al(2700), Zn(6500)
9	7.14	6.72	-0.42	-5.9	Al(2700), Zn(6500)
10	5.91	5.87	-0.04	-0.6	Be(900), Mg(2400)
11	7.95	7.78	-0.17	-2.1	Be(900), Mg(2400)
12	3.87	3.75	-0.12	-3.1	Al(2700), Ba(13700)
13	6.31	6.11	-0.20	-3.2	Al(2700), Ba(13700)
14	2.24	2.24	-	-	Be(900), Mg(2400)
15	4.28	4.50	+0.22	+5.2	Be(900), Mg(2400)
16	5.10	4.87	-0.23	-4.5	Co(5900), Fe(III)(5600)
17	7.14	6.68	-0.46	-6.5	Co(5900), Fe(III)(5600)

in relatively large concentrations, showed fluorescence at different wavelengths, e.g. lead (excitation maximum 290mµ / fluorescence maximum 480mµ), copper (I) (390/460mµ), tin (II) (270/480mµ) and cerium (III) 250/350mµ). After consideration of the reproducibility of the determination of 8µg. of thallium in pure solution, ions were considered to interfere at

this level when they caused a variation of fluorescence intensity of $\geq \pm 5\%$. The investigation revealed that the following ions did not interfere under the above conditions: aluminium, ammonium, arsenic (III), arsenic (V), barium, beryllium, cadmium, calcium, chromium (III), cobalt, gallium, indium, lead, lithium, magnesium, manganese (II), nickel, scandium, selenium (IV), silver, sodium, strontium, thorium, tin (II), titanium (IV), tungsten (VI), vanadium (IV), zinc, zirconium; bromide, fluoride, thiocyanate, nitrate, sulphate and tartrate. A 100-fold excess of the other ions investigated caused the error in the fluorescence intensity given in Table 4.

The interfering cations listed in Table 4 were then taken, normally in large excesses (250-2500 fold) over 8µg. of thallium, through the recommended extraction procedure. The results were as shown in Table 5.

Because this method is likely to find application in the determination of thallium in biological and other materials which require dissolution or destruction by wet digestion with nitric or sulphuric acids before determination, the effects of the presence of a large excess (*ca.* 10^6-fold) of sulphate and nitrate on the extraction and determination were investigated.

Table 4.

Ion	Variation in fluorescence intensity	Observations.
antimony (V)	-49%	solution absorbed at 250mμ.
bismuth	-69%	solution absorbed at 250mμ.
cerium (III)	+900%	"tail" of 250/350mμ fluorescence peak.
cerium (IV)	+900%	white cloudiness visible in solution.
copper (II)	-21%	solution absorbed at 250mμ.
gold	-100%	yellow solution absorbing at 430mμ.
iron (II)	-25%	solution absorbed at 250mμ.
iron (III)	-92%	solution absorbed at 250mμ and 430mμ.
mercury (II)	-28%	no visible effect.
molybdenum (VI)	-100%	fluorescence totally quenched.
platinum (IV)	-92%	yellow solution absorbing at 430mμ.
tellurium (IV)	-32%	no visible effect.
tin (IV)	-59%	no visible effect.
uranium (VI)	-18%	yellow solution absorbing at 430mμ.
vanadium (V)	-20%	no visible effect.
iodide	+13%	no visible effect.
bromate	-100%	caused oxidation Tℓ(I)→ Tℓ(III).
iodate	-100%	caused oxidation Tℓ(I)→ Tℓ(III).
sulphite	-100%	fluorescence totally quenched.

The presence of sulphate ions had no effect on the method, but nitrate ions interfered at this level by extraction into diethyl ether with the thallium (III). Nitrate ions absorbed strongly at and below about 250mμ and, therefore, absorbed energy at the wavelength

Table 5.

Cation	Molar excess	Variation in fluorescence intensity.
Antimony (V)	2500	-100%
Bismuth	500	-20%
Gold	10	-68%
Platinum	200	-14%
Cerium (III)	500	$\leq \pm$ 5%
Cerium (IV)	500	$\leq \pm$ 5%
Copper (II)	2500	$\leq \pm$ 5%
Iron (II)	2500	$\leq \pm$ 5%
Iron (III)	2500	$\leq \pm$ 5%
Mercury (II)	250	$\leq \pm$ 5%
Tellurium (IV)	2500	$\leq \pm$ 5%
Tin (IV)	250	$\leq \pm$ 5%
Uranium (VI)	500	$\leq \pm$ 5%
Vanadium (V)	2500	$\leq \pm$ 5%

used for excitation. This had the effect of preventing the thallium (I) fluorescence. Large excesses of nitrate (> 100-fold molar excess over thallium) should, therefore, be removed by fuming the solution with hydrochloric acid before the determination.

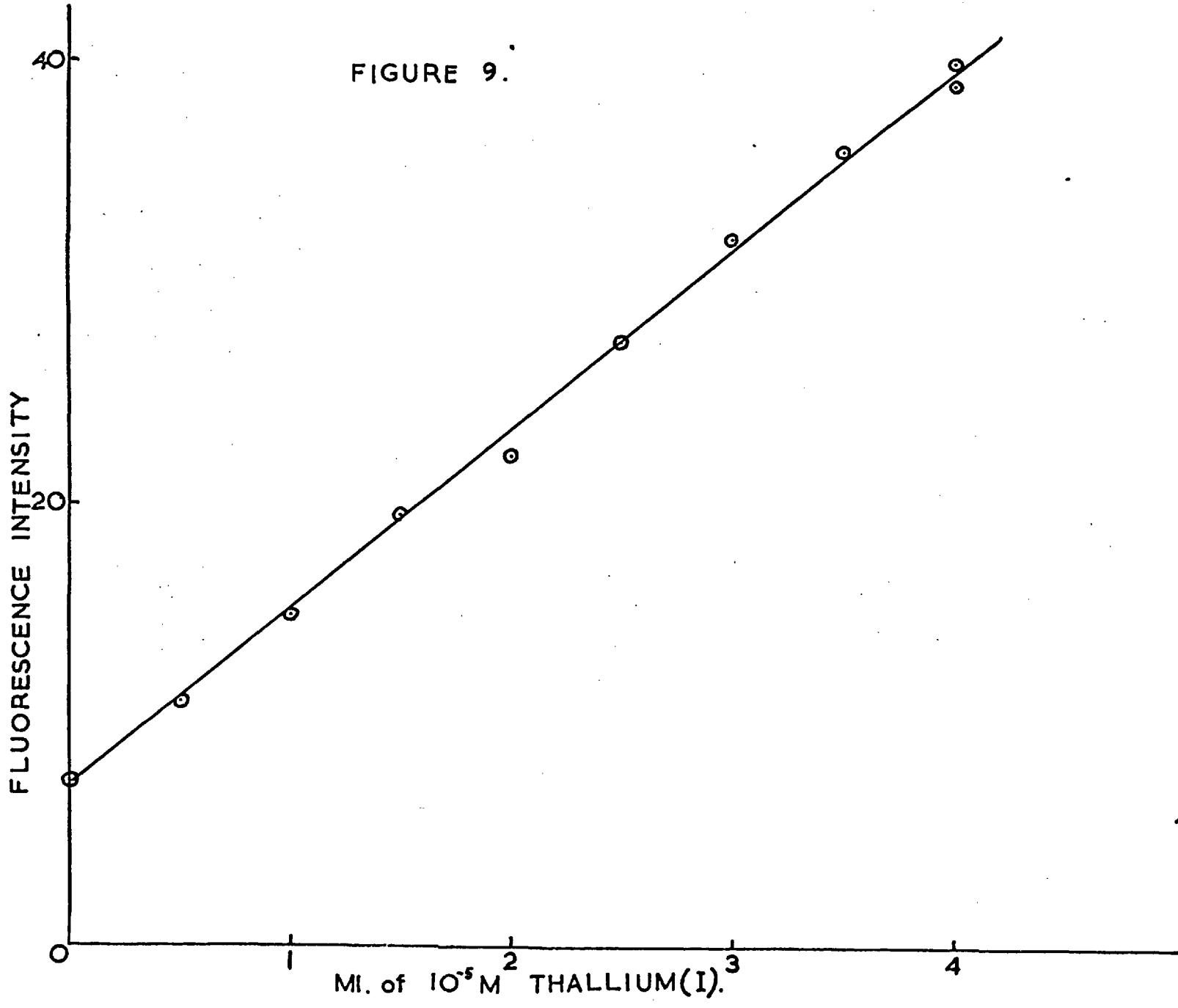
Calibration curve.

To 20ml. of saturated potassium chloride solution, 30ml. of water and 30ml. of concentrated hydrochloric acid in a 100-ml. volumetric flask, were added 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0ml. of standard thallium (I) solution (10^{-5}M ≡ 2.04µg./ml.) respectively. These solutions were diluted to volume with water. After allowing the solutions to stand for 15 minutes, the intensity of the fluorescence was measured at 430mµ with an excitation wavelength of 250mµ. The plot of fluorescence intensity against thallium concentration (0.01-0.08ppm) was a straight line and passed slightly above the origin, see Figure 9. Linearity was shown to extend beyond this upper limit to a final concentration of 2ppm of thallium, i.e. 200µg. in 100ml. of aqueous solution. Two standards were prepared and measured with each group of samples, using 0 and 4ml. of the thallium (I) solution (2.04µg./ml.), respectively.

Extraction procedure.

An aliquot of standard thallium (I) solution (0.5-4.0ml. of 10^{-5}M solution) was transferred to a separating funnel containing 15ml. of 2M hydrochloric

FIGURE 9.



acid and 1ml. of 20-volume hydrogen peroxide. This solution was extracted once into 25ml. of diethyl ether and the aqueous phase was discarded. The organic phase was washed with 10ml. of 1.5M hydrochloric acid and the aqueous phase again discarded. The ether phase was transferred to a 100-ml. beaker, the separating funnel rinsed with a further 10ml. of ether, the ether extracts combined and evaporated to dryness on a water-bath. The residue was dissolved in 50ml. of water and gaseous sulphur dioxide was passed into the solution for five seconds. After the addition of 1ml. of concentrated hydrochloric acid to assist the removal of sulphur dioxide, the solution was evaporated to a volume of 10ml. (It was essential to ensure complete removal of sulphur dioxide becuase it had a strong quenching action on the $TlCl_3^{2-}$ ion fluorescence). The solution was then cooled, transferred to a 100-ml. standard flask containing 20ml. of saturated potassium chloride solution and 30ml. of concentrated hydrochloric acid was added. This was diluted to volume with distilled water and the fluorescence measured after 15 minutes at 250/430mμ. The complete separation and determination of a set of eight samples occupied about three hours.

Discussion.

As a result of these investigations it became apparent that this spectrofluorimeter is an extremely sensitive instrument and, therefore, very suitable for use in the determination of microgram and sub-microgram amounts of metals by fluorescence reactions. In the system studied, the background "fluorescence" was low and constant and, by use of the simple solvent extraction procedure described, the method was freed from the more important interferences without loss of sensitivity.

Bearing in mind that this work was done using a xenon arc lamp for excitation of the fluorescence, it is possible that even greater sensitivity could be obtained by using a mercury discharge or mercury-xenon arc lamp wiich would provide a much greater intensity of radiation at 250mμ, one of the principal mercury lines being at 253.7mμ. This would, at least, have the effect of improving the precision of measurements limited by instrumental "noise" by allowing measurements to be made on a less sensitive micro-ammeter scale. It does not necessarily follow, however, that the solvent extraction procedure could be applied to lower amounts of thallium; a ten-fold increase in sensitivity would make the range of determination 0.1-0.8μg. of thallium (0.001-0.008ppm in the final solution).

Chapter 2.

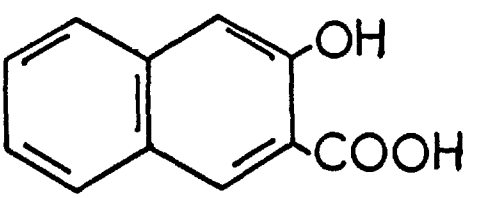
The Spectrofluorimetric Determination of Submicrogram Amounts of Aluminium and Beryllium with 2-Hydroxy-3-naphthoic acid.

Most spectrophotometric methods for the determination of aluminium depend upon its reaction with 8-hydroxyquinoline and its derivatives[36] or the formation of lakes with reagents such as "Aluminon" (aurintricarboxylic acid) or Eriochrome Cyanine R[37]. White[10] has recently reviewed available fluorescence methods. The reaction with Pontachrome Blue Black R (4-sulpho-2-hydroxynaphthaleneazo naphthol)[16] to form a red-fluorescent complex has the advantage of conditional specificity but the disadvantage that most readily available photomultiplier tubes are relatively insensitive in the red region. Other sensitive fluorimetric methods utilise Morin (3, 5, 7, 2', 4'-pentahydroxyflavone)[38], 8-hydroxyquinoline[39] and various salicylaldehyde condensation products[40]. Colorimetric and fluorimetric methods for the determination of trace amounts of beryllium have been reviewed by Kolthoff and Elving[41]. The fluorimetric determination of beryllium with Morin has been described by several workers, including Sill *et al*[42,43]. In this procedure DTPA

(diethylenetriaminepentaacetic acid) was used to complex copper and other metallic ions capable of catalysing the aerial oxidation of Morin in alkaline solution and also to prevent the formation of fluorescent complexes with scandium, yttrium and lanthanum.

Cherkesov[44 - 46] has described the use of 2-hydroxy-3-naphthoic acid (Figure 10) for the detection of aluminium and beryllium.

Figure 10.



2-HYDROXY-3-NAPHTHOIC ACID.

He reported that 0.01µg. of aluminium or beryllium could be detected by formation of blue fluorescent complexes at pH3 and above; the reagent itself exhibits a green fluorescence under these conditions. This chapter reports the spectrofluorimetric investigation of these reactions for the determination of traces of aluminium and beryllium. The optimum

conditions of pH, reagent concentration and development time were established and the effects of a large number of cations and anions were studied. Aluminium and other metal ions were prevented from interfering in the beryllium determination by masking with the calcium complex of CDTA (cyclohexanediaminetetraacetic acid). Continuous variations, mole-ratio and slope-ratio studies indicated the formation of a 1:1 complex between the reagent and aluminium or beryllium and the conditional stability constants for the complexes were evaluated.

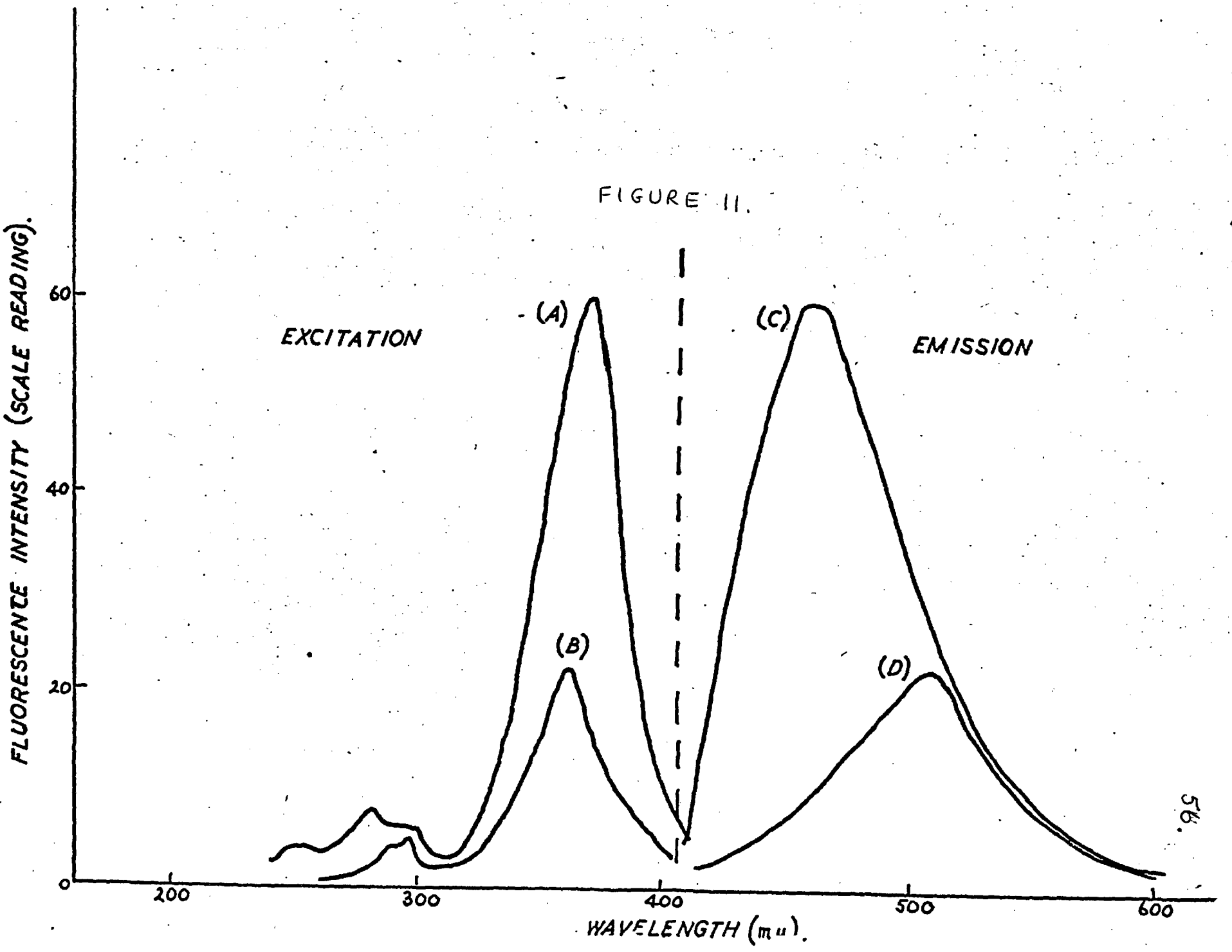
Several reagents structurally similar to 2-hydroxy-3-naphthoic acid were selected and a qualitative examination of their fluorescence reactions with metal ions was undertaken.

Experimental.

Spectral Characteristics.

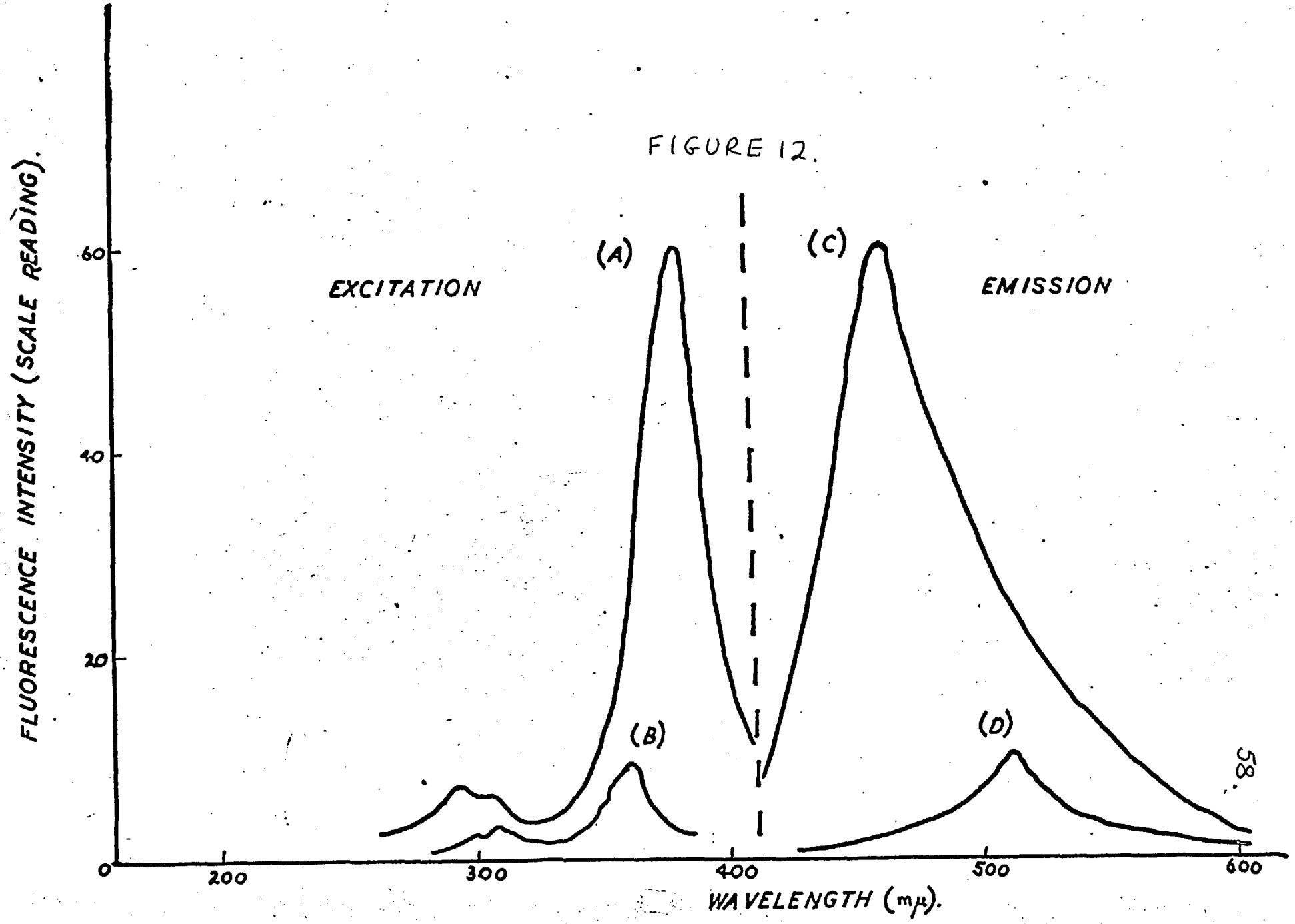
Figure 11 shows the excitation and emission spectra for the reagent and its aluminium complex in aqueous solution buffered at pH5.8. These spectra are uncorrected for variations in the emission characteristics of the lamp, transmission of the monochromators and the response characteristics of the photomultiplier. The relevant correction curves

FIGURE 11.


FLUORESCENCE INTENSITY (SCALE READING).
60
40
20
0
EXCITATION
EMISSION
(A)
(B)
(C)
(D)
200
300
400
500
600
WAVELENGTH (mu).

are given in Figure 6. Curves A and C show the excitation and emission spectra, respectively, of solutions containing equimolar amounts of aluminium and reagent. For curve A the emission was measured at 460mμ and for curve C the excitation wavelength was set at 370mμ. Curves B andD show the excitation and emission spectra, respectively, of the reagent alone. For curve B the emission was measured at 510mμ and for curve D the excitation wavelength was set at 360mμ. Figure 11 shows that 2-hydroxy-3-naphthoic acid, which has a green fluorescence, exhibited its excitation maximum at 360mμ and its fluorescent emission maximum at 510mμ. Its blue fluorescent aluminium complex showed its excitation maximum at 370mμ and exhibited maximum fluorescence at 460mμ.

The spectra of the beryllium complex at pH7.5 were almost identical with those of the aluminium complex. The uncorrected spectra (obtained with the 7-54 filter omitted) are shown in Figure 12. Curves A and C, respectively, show the excitation and emission spectra for solutions containing equimolar amounts of beryllium and reagent. For curve A the emission was measured at 460mμ and for curve C the excitation wavelength was set at 380mμ. Curves B and D

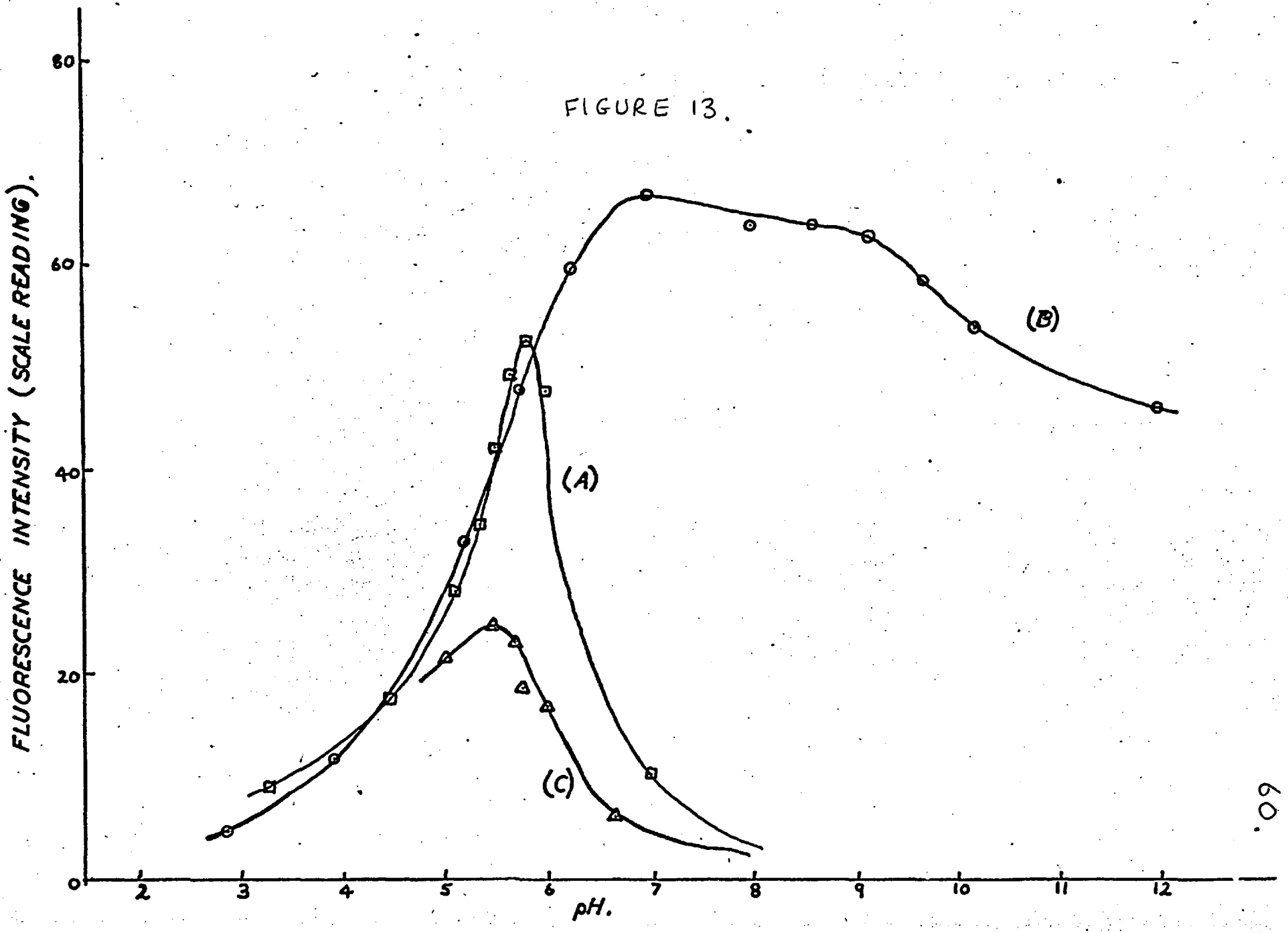

FIGURE 12.
FLUORESCENCE INTENSITY (SCALE READING).
60
40
20
0
EXCITATION
EMISSION
(A)
(B)
(C)
(D)
200
300
400
500
600
WAVELENGTH (mμ).

show the excitation and emission spectra, respeetively, of metal-free reagent at pH7.5. For curve B the emission was measured at 510mμ and for curve D the excitation wavelength was set at 360mμ. As Figure 12 shows, the excitation maximum of the beryllium complex occurred at 380mμ and it exhibited maximum fluorescence at 460mμ.

Influence of pH.

The intensity of the fluorescence of the reagent and of its aluminium and beryllium complex was measured over a range of pH values obtained by adjusting the pH with ammonia or acetic acid. The results are shown in Figure 13. Curve A shows the variation of fluorescence intensity with pH of solutions containing aluminium and a ten-fold excess of reagent; the excitation wavelength used was 370mμ and the emission was measured at 460mμ. The aluminium complex showed maximum fluorescence at pH5.8. The equivalent curve for solutions containing beryllium and a twenty-fold excess of reagent is given in curve B; excitation at 380mμ, emission measured at 460mμ. The pH curve for the beryllium complex showed a broad maximum from ca. pH6.5 to 9. Curve C shows the variation for 2-hydroxy-3-naphthoic acid (360/510mμ).

FIGURE 13.

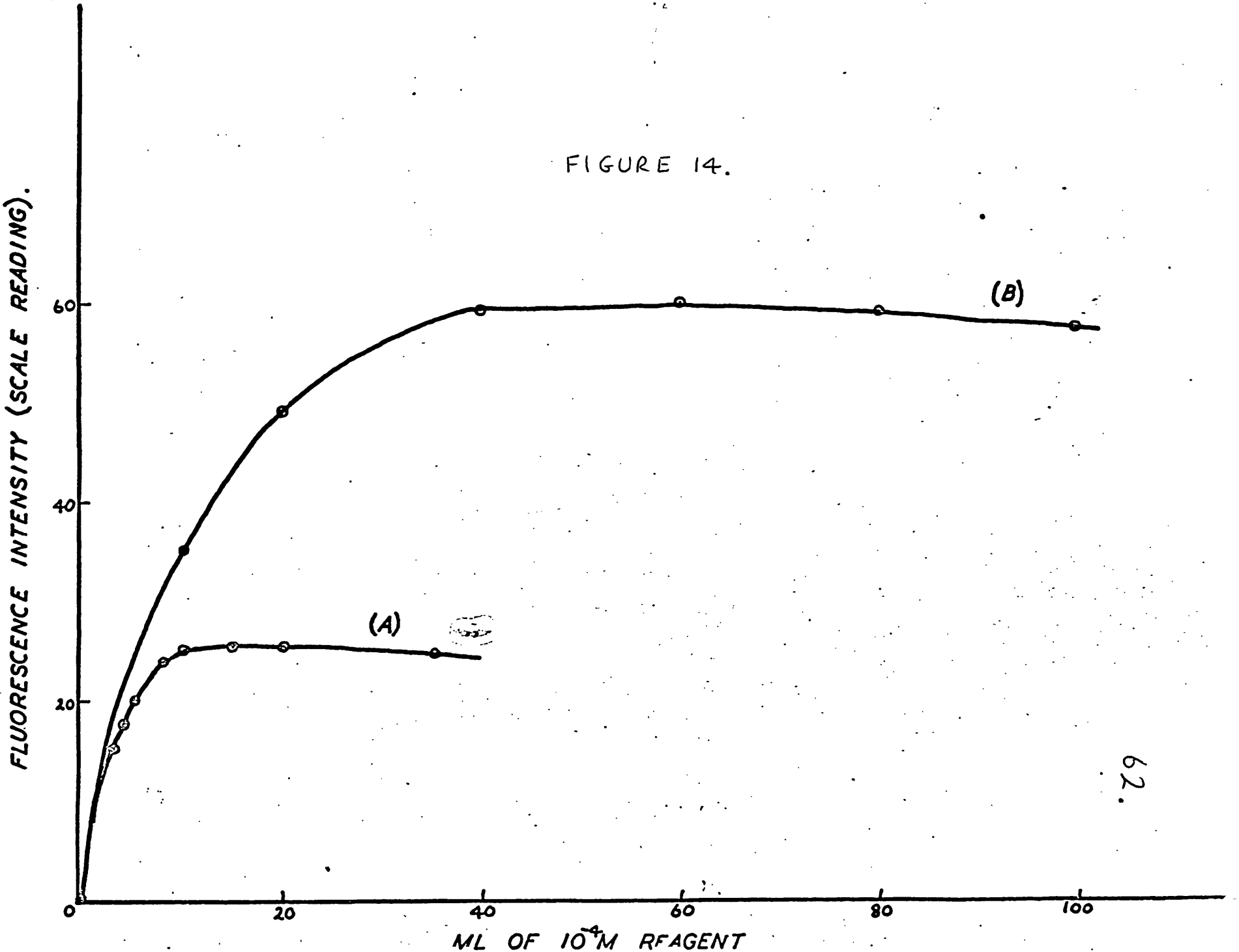

FLUORESCENCE INTENSITY (SCALE READING).
80
60
40
20
0
2
3
4
5
6
7
8
9
10
11
12
pH.
(A)
(B)
(C)

Reagent concentration.

A varying excess of 10^{-4}M sodium 2-hydroxy-3-naphthoate solution was allowed to react for 60 minutes with 1 ml. of 10^{-4}M aluminium solution, buffered at pH5.8. The intensity of the blue fluorescence of the aluminium complex was then measured as usual (excitation wavelength 370mμ, emission measured at 460mμ). As shown in Figure 14A, a minimum ten-fold molar excess of reagent was required to develop maximum fluorescence. This graph was corrected for the contributions to the fluorescence of the varying amounts of reagent present. For the determination of 0.2 to 2.5μg. amounts of aluminium, 10ml. of 10^{-4}M sodium 2-hydroxy-3-naphthoate solution were used (an 11-fold molar excess over the maximum aluminium concentration).

As shown in Figure 14B, which was also corrected for the reagent concentration, a 50-fold molar excess of reagent was required to develop the maximum fluorescence of the beryllium complex, buffered at pH7.5 and fluorescence measured at 380/460mμ. The contribution of the reagent fluorescence measured at 460mμ was considerably less at pH7.5 than at pH5.8 and it was, therefore, possible to use a greater excess of

FIGURE 14.


FLUORESCENCE INTENSITY (SCALE READING).
60
40
20
0
20
40
60
80
100
(A)
(B)
ML OF 10^{-4}M REAGENT

reagent in the determination of beryllium than in the determination of aluminium. For the determination of 0.018-0.18μg. amounts of beryllium, 10ml. of 10^{-4}M sodium 2-hydroxy-3-naphthoate solution were used (a 50-fold molar excess over the maximum beryllium concentration).

Influence of Time.

A 10^{-3}M aqueous solution of the reagent was stable in darkness for a period of weeks, while a similar solution of the aluminium complex showed a decrease in fluorescence intensity of ca. 5% in 18 hours. On exposure to radiation from a mercury discharge lamp for 18 hours, the fluorescence intensity of the reagent decreased by 20%, compared with ca. 60% for the aluminium complex. The intensity of the fluorescence of the complex was reproducible when measured 60 minutes after mixing the solutions and this was the standing time used in all experiments.

During the initial investigations it was noticed that test solutions prepared from the 0.1μg./ml. aluminium standard solution showed progressively lower fluorescence intensities after the standard solution had been allowed to stand for long periods of time. This was not observed when solutions were prepared

immediately from more concentrated (10^{-3}M) stock aluminium solution. This apparent gradual loss of aluminium, which resulted in a decrease of the slope of the calibration curve when older standard solutions were used, was attributed to the adsorption of aluminium ions onto the walls of the volumetric glassware in a manner analogous to the absorption of beryllium observed by Sill[42,43]. The opposite effect of desorption was also noted, i.e. flasks which had previously contained, say, 10^{-4}M aluminium solutions gave erroneously high fluorescence readings when next used for 10^{-7}M solutions. In an attempt to eliminate these effects, before the beryllium studies were begun, all flasks were treated with silicone Repelcote. This produced a considerable improvement without completely eliminating the problem. 2×10^{-7}M solutions of the beryllium-complex then showed a decrease of fluorescence intensity of less than 10% when left in darkness for 24 hours.

Temperature of solutions.

In all experiments the temperature of the solution was $20 \pm 5^{\circ}$C. It was shown that, over the range 2-25°C, the fluorescence intensity increased with decrease of temperature by ca. 1% per °C, but the existence of

this temperature coefficient did not alter the validity of results because, in all cases, measured fluorescence intensities were compared with those of standards prepared simultaneously and under the same conditions.

Effects of foreign ions on the determination of aluminium.

The effects of 39 cations and of 22 anions on the aluminium determination were investigated. 10µg. of aluminium were determined in the presence of a 100-fold molar excess of the foreign ion under investigation. Where the capacity of the ammonia-acetic acid buffer was insufficient (e.g. when the foreign ion was dissolved in strong acid to prevent hydrolysis), the pH was adjusted to 5.8 using dilute ammonia and dilute acetic acid. When an ion was found to interfere, it was further investigated at a lower concentration, viz. 1µg. of aluminium with 0.1 to 1.0mg. of foreign ion. The acceptable variation of fluorescence intensity from that of standard solutions containing 1µg. of aluminium was taken as $\leq \pm 5\%$.

The only positive interferences observed (i.e. ions which also gave a blue fluorescence with the reagent) were with beryllium, scandium and tetraborate, while zirconium and gallium formed precipitates which

themselves exhibited a blue fluorescence. The following ions did not interfere when present in a 1000-fold molar excess over 10µg. of aluminium in 100ml. of aqueous solution: ammonium, arsenic (III), barium, cadmium, calcium, cerium (III), cobalt (II), iron (II), lead, lithium, magnesium, manganese (II), mercury (II), nickel, potassium, silver, sodium, strontium, vanadium (V), zinc, chloride, bromide, iodide, cyanide, thiocyanate, nitrate, carbonate, perchlorate, sulphate and thiosulphate. The effects of other ions are shown in Table 6.

No iron (III) could be tolerated, but its interference at the 1000-fold excess level could be reduced to *ca.* -10%, although not eliminated, by reduction to iron (II) with the required amount of ascorbic acid in the absence of chloride ion. When chloride was present, the solution absorbed at the wavelength of excitation and thus reduced the intensity of the fluorescence observed by an inner-filter quenching effect. A 125-fold molar excess of copper (II) was tolerable and the interference of greater amounts could be removed by complexation with cyanide, the large excess of cyanide (> 1000-fold) being prevented from interfering by the addition of zinc ions.

Table 6.

Effects of 1000-fold molar excesses of ions on aluminium determination.

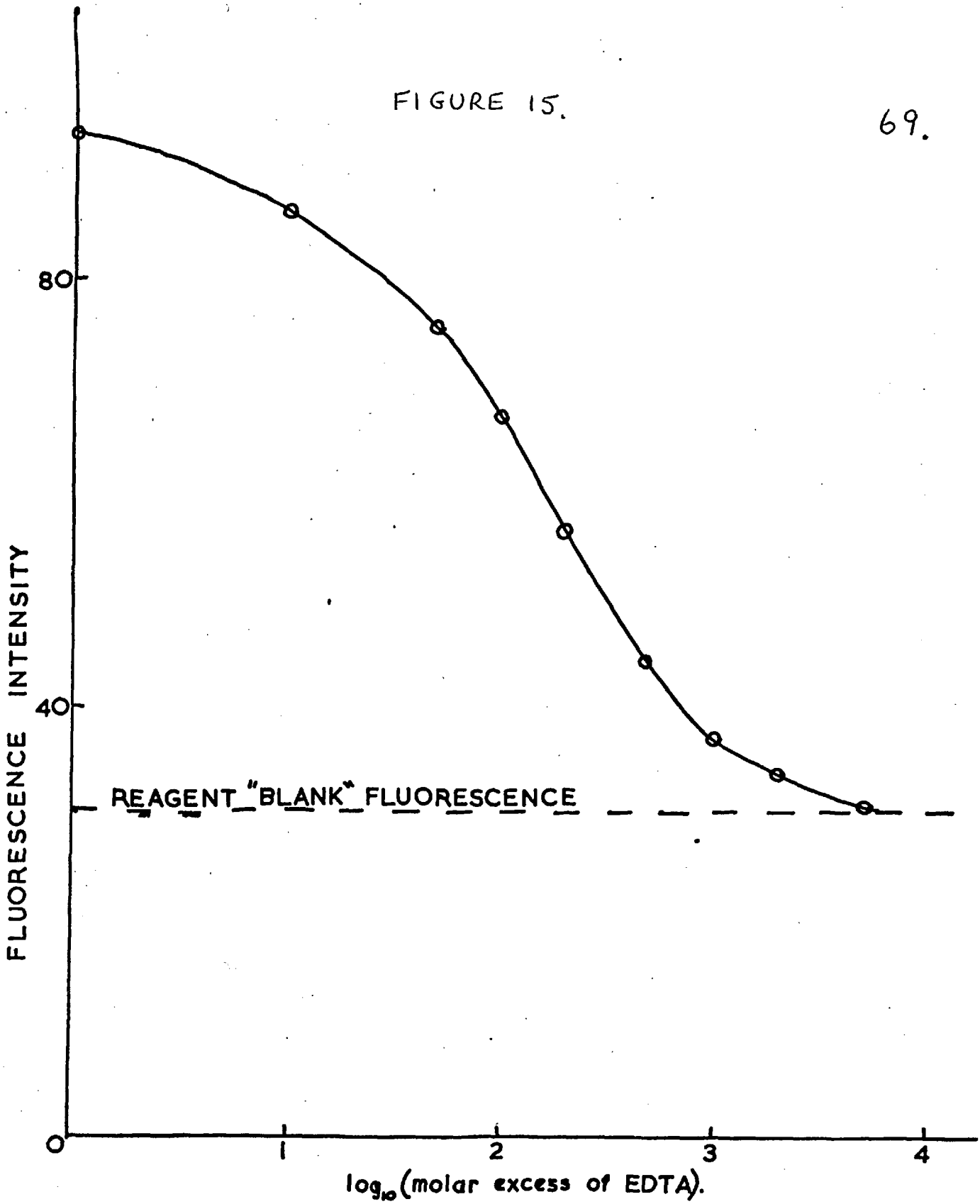
Ion.	Variation in fluorescence intensity	Observations
antimony (III)	-55%	white precipitate
arsenic (V)	-60%	fluorescence greener and weaker
bismuth	-35%	white precipitate
cerium (IV)	-100%	brown precipitate
chromium (III)	-100%	only green reagent fluorescence remained
copper (II)	-24%	solution slightly yellow
indium	-30%	no visible effect
iron (III)	-100%	brown precipitate
thallium (I)	-16%	no visible effect
thorium	-50%	solution cloudy
tin (IV)	-100%	white cloudiness, no blue fluorescence
titanium (IV)	-100%	yellow-green precipitate, no blue fluorescence
uranium (VI)	-50%	solution yellow
chromate	-100%	solution yellow, no fluorescence
fluoride	-100%	only green reagent fluorescence remained
molybdate	-100%	"
nitrite	-10%	no visible effect
phosphate	-100%	only green reagent fluorescence remained
silicate	-100%	"
sulphite	-8%	no visible effect
tungstate	-100%	only green reagent fluorescence remained
citrate	-100%	"
oxalate	-100%	"
tartrate	-100%	"

Among complexing agents investigated, thiourea did not interfere, but 1, 10-phenanthroline (12%), thio-glycollic acid (15%), nitrilotriacetic acid (60%), iminodiacetic acid (75%), acetylacetone (100%), sulphosalicylic acid (100%) and tiron (100%) all reduced the aluminium complex fluorescence when present in a 1000-fold molar excess.

Effects of foreign ions on the determination of beryllium.

It was anticipated that EDTA would mask a number of interfering cations without affecting the beryllium-2-hydroxy-3-napthoic acid complex. At low concentrations of beryllium, however, EDTA prevented the formation of the beryllium-reagent complex, indicating the possible formation of a weak beryllium-EDTA chelate at pH7.5, see Figure 15. The addition of an excess of a non-interfering cation which formed a relatively weak EDTA chelate (calcium) to mask the excess EDTA proved effective. The relatively large amounts of EDTA needed to prevent the formation of the metal-reagent complex indicated that any beryllium-EDTA chelate formed was somewhat less stable than the beryllium-2-hydroxy-3-naphthoic acid complex under these conditions.

FIGURE 15.


80
40
0
FLUORESCENCE INTENSITY
REAGENT "BLANK" FLUORESCENCE
1
2
3
4
log10 (molar excess of EDTA).

The interferences of 37 cations and 21 anions were investigated using 0.18 μg. of beryllium in the presence of 1000-fold molar excesses of the foreign ion under investigation, with a 1250-fold excess of EDTA and a 6000-fold excess of calcium ions to complex the excess EDTA. The pH of each solution was adjusted to ca. 8 before buffering to 7.5. The acceptable variation of fluorescence intensity from that of standard solutions containing 0.18μg. of beryllium was taken as $\leq \pm$ 10%. The investigation revealed that, under the conditions described above, the following ions did not interfere: ammonium, antimony (III), arsenic (III), arsenic (V), barium, cadmium, calcium, cerium (III), cobalt (II), copper (II), gallium, indium, lead, lithium, magnesium, manganese (II), mercury (II), nickel, potassium, silver, sodium, strontium, thallium (I), uranium (VI), vanadium (IV), zinc, zirconium, choride, bromide, iodide, cyanide, thiocyanate, nitrite, nitrate, sulphite, sulphate, thiosulphate, carbonate, perchlorate, molybdate and tungstate. Cationic interferences are given in Table 7. Citrate, tartrate, fluoride, phosphate and silicate all prevented the formation of the beryllium-2-hydroxy-3-naphthoic acid complex.

Table 7

Effects of cations interfering with beryllium method.

Ion	Variation in fluorescence intensity	Observations
bismuth (III)	+155%	solution cloudy
cerium (IV)	+75%	" "
chromium (III)	-80%	solution cloudy, fluorescence greener and weaker.
iron (II)	-20%	solution slightly yellow.
iron (III)	-21%	" " "
scandium (III)	+650%	much more intense blue fluorescence.
thorium (IV)	+37%	no visible effect.
tin (IV)	-100%	only green fluorescence remaining.
titanium (IV)	-33%	solution cloudy, fluorescence bluer but weaker.

The interference of aluminium was reduced but not eliminated by the above method. However, CDTA (cyclohexanediamine-tetra-acetic acid) forms more stable complexes than EDTA with most metals and reacts more rapidly with aluminium. When its calcium salt was used in place of calcium-EDTA, aluminium no longer interfered.

Accuracy data.

The data in Table 8 were obtained from the results of the analysis of solutions for aluminium by the recommended procedure. Similarly, the data for solutions containing beryllium are shown in Table 9. Each of these results represents the average of three simultaneous determinations obtained by comparison with standards prepared simultaneously.

Precision data.

These were obtained by multiple analyses of a series of solutions containing 2.47µg. of aluminium (see Table 10) and another series containing 0.18µg. of beryllium (see Table 11). The precision of the fluorescence intensity measurements for aluminium was ±2.0%, corresponding to ±0.05µg. of aluminium; for beryllium it was ±1.6%, corresponding to 0.003µg. of beryllium.

Possible extraction systems.

2-hydroxy-3-naphthoic acid dissolved in benzene and chloroform to produce a violet and lilac fluorescence, respectively. In ethanol and after extraction into higher-n-alcohols (butanol, amyl alcohol and hexanol), it showed the same green fluorescence as in aqueous medium. The blue fluorescent

Table 8.

Analysis of Aluminium solutions treated as unknown samples.

Sample	µg. Aluminium		Error	Error	Foreign Ions present
	Present	Found	(µg.)	(%)	(µg.)
1	2.00	1.90	-0.10	- 5.0	
2	1.20	1.12	-0.08	- 6.6	
3	0.20	0.15	-0.05	-25.0	
4	0.70	0.72	+0.02	+ 2.9	
5	0.50	0.54	+0.04	+ 8.0	
6	1.10	1.12	+0.02	+ 1.8	
7	2.15	2.08	-0.07	- 3.3	
8	1.14	1.16	+0.02	+ 1.8	Ca(400) + Mg(243)
9	0.34	0.37	+0.03	+ 8.8	Pb(2070) + Zn(654)
10	1.20	1.12	-0.08	- 6.7	Ba(1370) + Mn(II)(550)
11	1.20	1.18	-0.02	- 1.8	Cu(318)
12	1.00	0.92	-0.08	- 8.0	Cu(318)
13	2.00	1.94	-0.06	- 3.0	Cu(635) + CN^-
14	2.00	1.90	-0.10	- 5.0	Cu(635) + CN^-
15	2.00	1.93	-0.07	- 3.5	Cu(635) + CN^-

Table 9.

Analysis of Beryllium solutions treated as unkown samples

Sample	µg. Beryllium		Error	Error	Foreign ions present
	Present	Found	(µg.)	(%)	(µg.)
1	0.078	0.066	-0.012	-15.4	Fe(III)(280), Aℓ(135)
2	0.099	0.097	-0.002	- 2.1	Cu(318), Co(295)
3	0.040	0.040	-	-	Aℓ(135), Cu(318)
4	0.059	0.040	-0.019	-32.2	Fe(III)(280), Co(295)
5	0.146	0.158	+0.012	+ 8.2	Aℓ(135), Cu(317)
6	0.032	0.030	-0.002	- 6.2	Fe(III)(280), Co(295)
7	0.085	0.072	-0.013	-15.3	Fe(III)(280), Cu(317)
8	0.102	0.083	-0.019	-18.6	Fe(III)(280), Cu(317)
9	0.018	0.022	+0.004	+22.2	Mg(122), Zn(325)
10	0.144	0.132	-0.012	- 8.2	Mn(II)(275), Pb(1035)
11	0.136	0.138	+0.002	+ 1.5	Mg(122), Mn(II)(275)
12	0.097	0.097	-	-	Zn(325), Pb(1035)
13	0.072	0.068	-0.004	- 5.5	Mg(122), Pb(1035)
14	0.072	0.070	-0.002	- 2.8	Mn(II)(275), Zn(325)

aluminium complex was not extracted from aqueous solution at pH5.8 into benzene, chloroform, amyl acetate nor di-n-octylmethylamine. Attempted extraction with the higher alcohols resulted in some green-blue fluorescence in the organic phase, probably indicating that the reagent was preferentially extracted.

The beryllium complex, in contrast, was extracted

Table 10.

Sample no.	$\frac{\text{Scale reading}}{\text{*Q.S. reading}} \times 10^4$.	Deviation	Variance
1	856	0	0
2	856	0	0
3	849	-7	49
4	875	+19	361
5	842	-14	196
6	846	-10	100
7	838	-18	324
8	867	+11	121
9	890	+34	1156
10	838	-18	324
	Mean = 856		
Standard deviation = $\sqrt{\frac{\Sigma\Delta^2}{n-1}} = \sqrt{\frac{2631}{9}} = 16.8 = 2.0\%$			

* Q.S. denotes Quinine Sulphate

from aqueous solution at pH7.5 into n-butanol, amyl alcohol and hexanol, but not into benzene, chloroform nor amyl acetate. After extraction into these alcohols, the complex showed a blue fluorescence similar to that in aqueous solution.

Construction of Calibration Curves.

Aluminium.

2.0 to 25.0ml. aliquots of standard aluminium

Table 11.

Sample no.	$\frac{\text{Scale reading}}{\text{*Q.S. reading}} \times 10^4$.	Deviation	Variance
1	643	0	0
2	628	-15	225
3	628	-15	225
4	652	+9	81
5	643	0	0
6	660	+17	289
7	640	-3	9
8	640	-3	9
9	648	+5	25
10	652	+9	81
	Mean = 643		

Standard deviation = $\sqrt{\frac{\Sigma \Delta^2}{n-1}} = \sqrt{\frac{944}{9}} = 10.2 = 1.6\%$

* Q.S. denotes Quinine Sulphate.

solution (3.7×10^{-6}M = 0.1µg. of aluminium per ml.) were transferred accurately into 100ml. volumetric flasks containing 5ml. of pH5.8 buffer solution. Ten ml. of 10^{-4}M sodium 2-hydroxy-3-naphthoate reagent solution were added and the solution was diluted with water from an all-glass distillation apparatus. The intensity of the fluorescence of the solutions was measured after 60 minutes at 460mµ with an excitation

wavelength of 370mμ. The plot of fluorescence intensity against amount of aluminium (0.20 to 2.50μg.) was a straight line and passed slightly above the origin, see Figure 16. The upper limit could, no doubt, be extended beyond 2.5μg. of aluminium, but no special study was made of this.

Beryllium.

1.0 to 10.0ml. aliquots of standard beryllium solution (2×10^{-6}M ≡ 0.018μg. of beryllium per ml.) were transferred by pipette into 100ml. volumetric flasks. Five ml. of calcium-CDTA solution were added and the pH was adjusted to ca. 8 with dilute ammonia solution. Five ml. of pH7.5 buffer solution and 10ml. of 10^{-4}M reagent solution were added and the solution was diluted to 100ml. with water. The intensity of the fluorescence of the solutions was measured after 30 minutes at 460mμ with an excitation wavelength of 380mμ. The plot of fluorescence intensity against amount of beryllium (0.018 to 0.18μg.) was a straight line and passed slightly above the origin, see Figure 17. Again, linearity extended beyond the upper limit examined here. Two standards were prepared and measured with each group of samples, using 1 and 10ml. of the standard beryllium solution,

FIGURE 16.

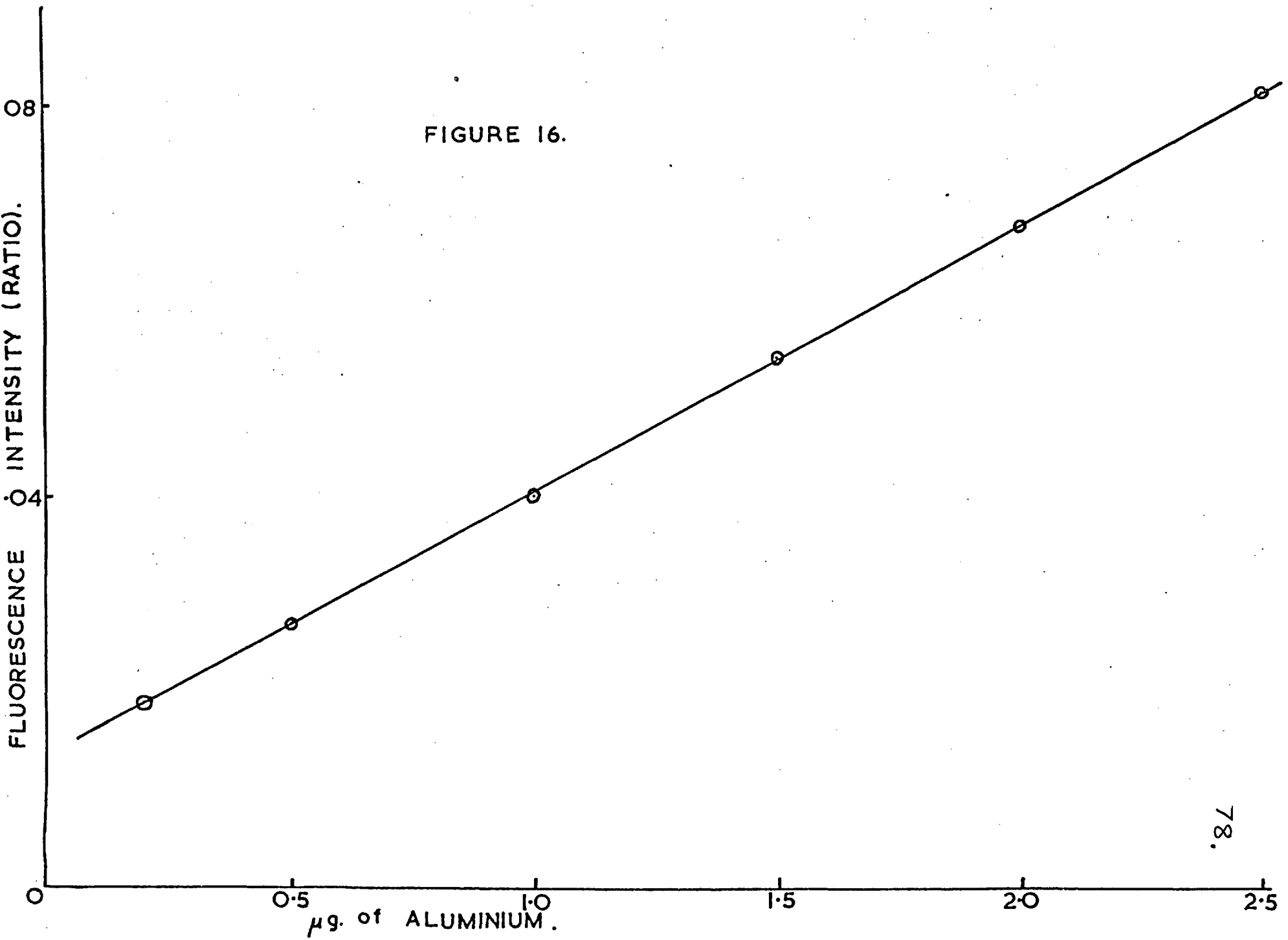
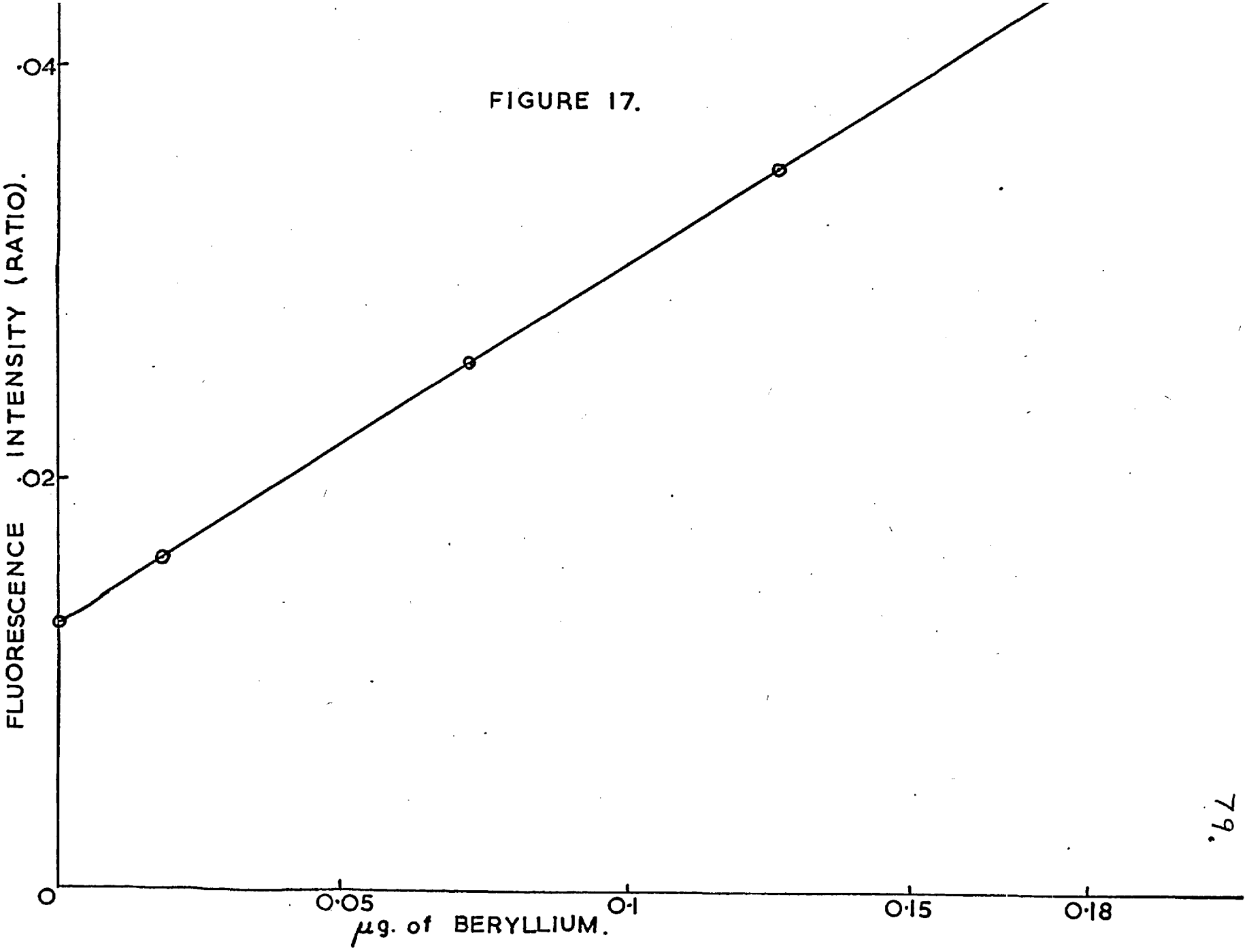

FLUORESCENCE INTENSITY (RATIO).
0·8
0·4
0
0·5
1·0
1·5
2·0
2·5
μg. of ALUMINIUM.

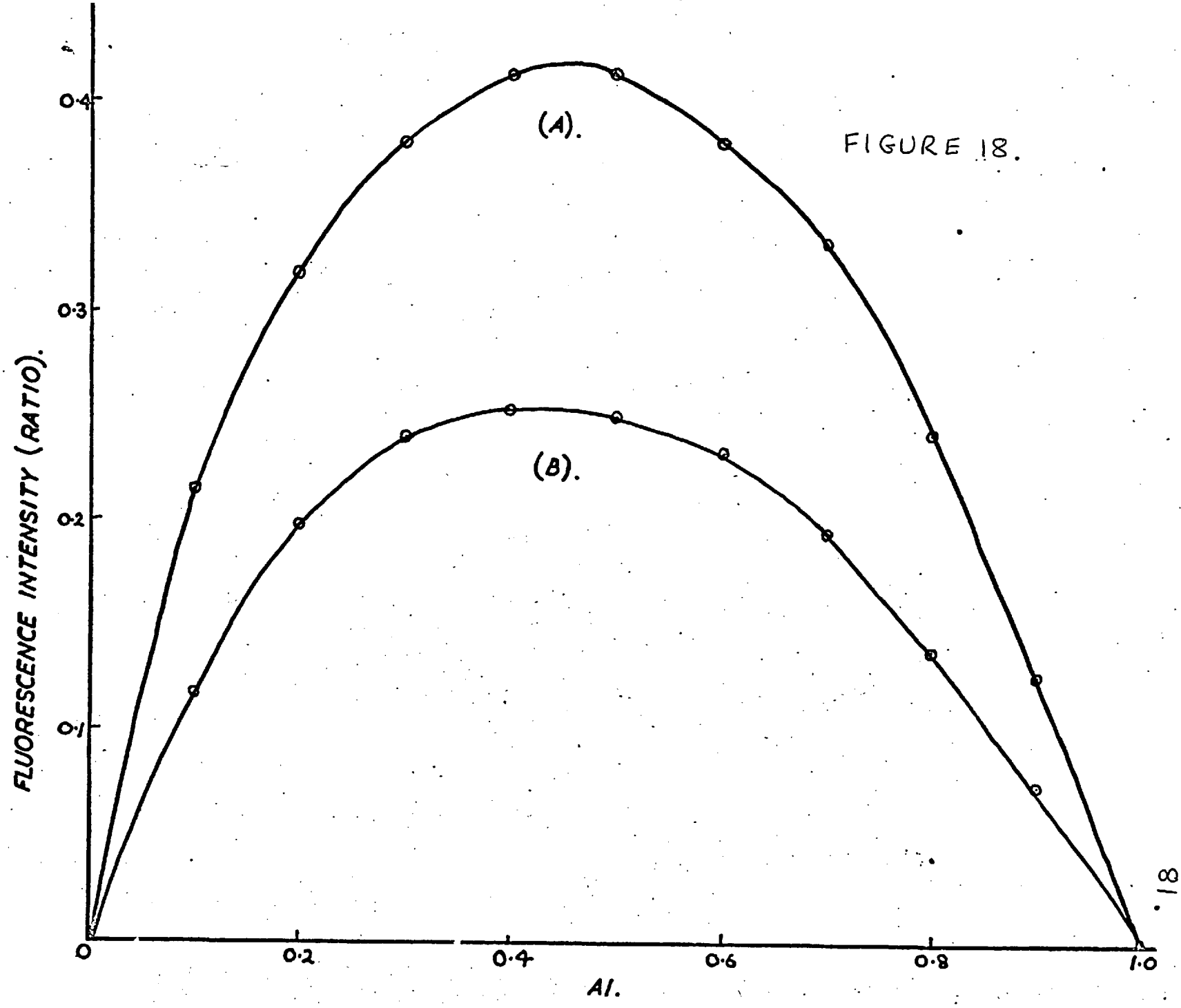
FIGURE 17.

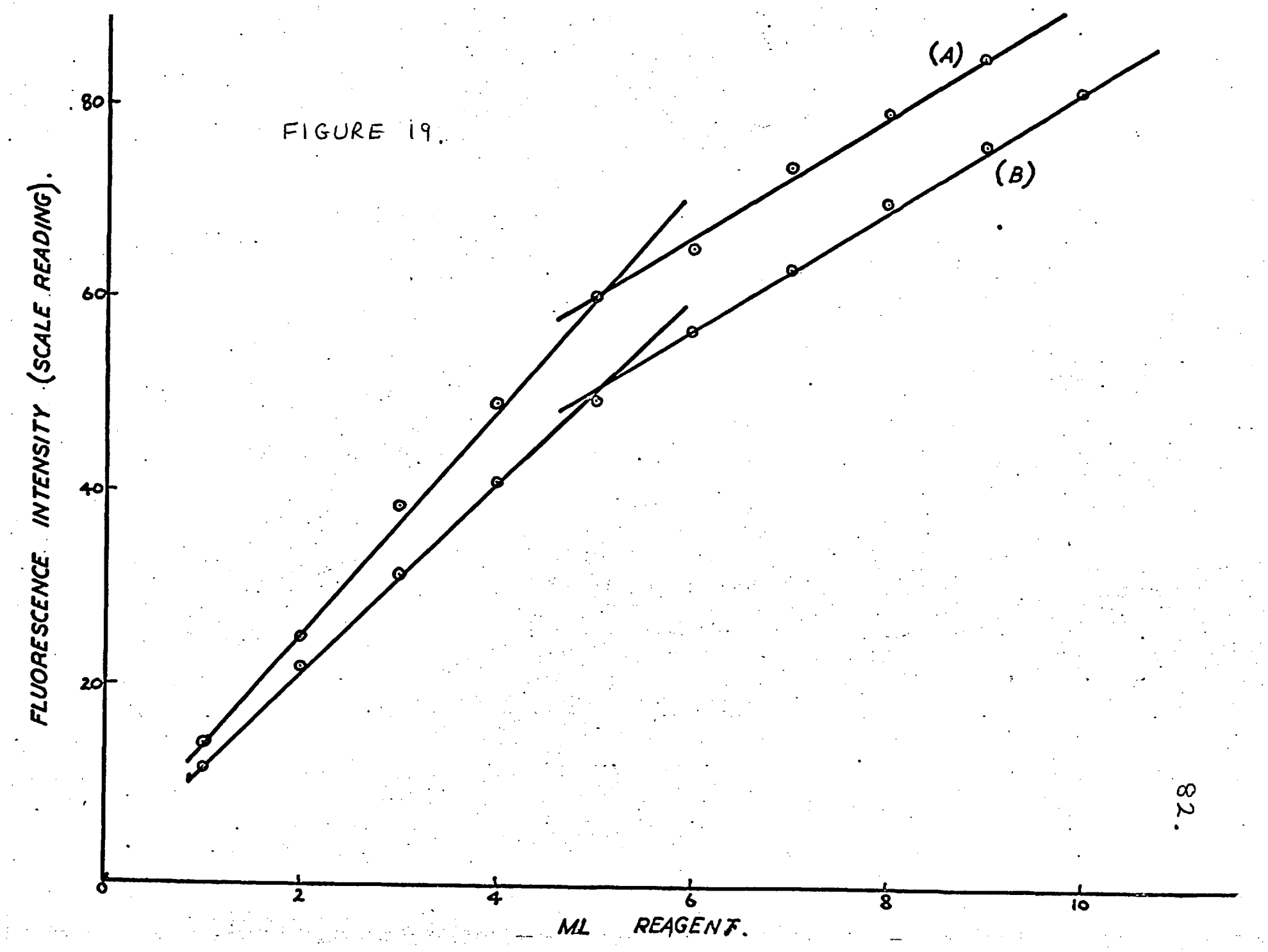

·04
·02
0
FLUORESCENCE INTENSITY (RATIO).
0·05
0·1
0·15
0·18
μg. of BERYLLIUM.

respectively.

Structure of complexes.

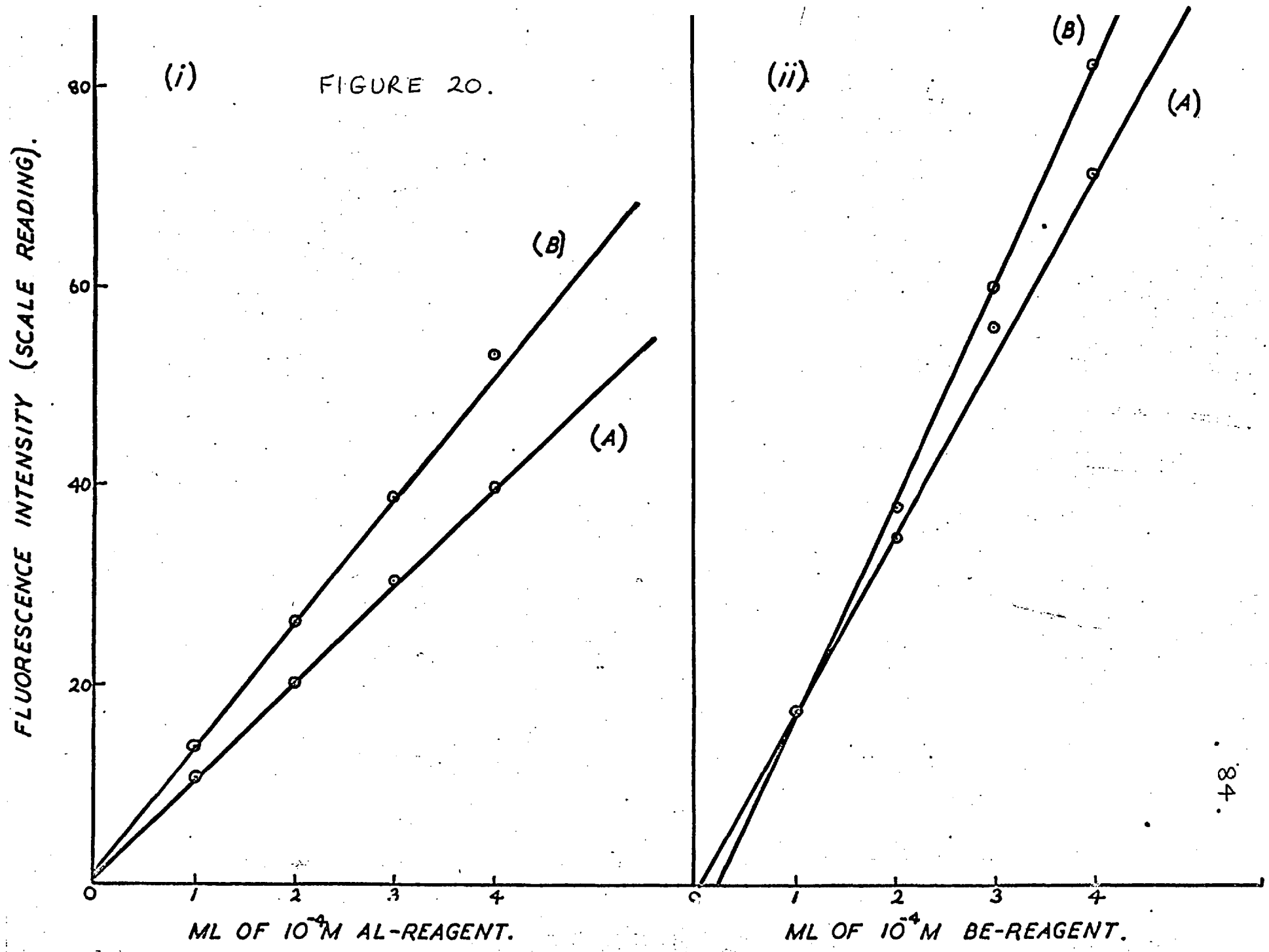
Cherkesov[44] proposed a 1:3 inner-complex structure for the aluminium-2-hydroxy-3-naphthoic acid complex, but the present author's evidence showed only a complex of 1:1 molar proportions. Figure 18 shows the results of an investigation by Job's method of continuous variations[47], which indicate the formation of a 1:1 complex. These curves were corrected for the contribution of the reagent fluorescence. Solutions were buffered at pH5.8, the fluorescence intensity measured at 370/460mμ and then expressed as a ratio compared with the intensity of a standard quinine sulphate solution measured at 350/450mμ. Measurement of the fluorescence at different wavelengths between 450 and 470mμ gave identically shaped curves and, therefore, no indication of the formation of complexes other than 1:1 between aluminium and the reagent. The total amount of aluminium and reagent solutions was 20ml. of 10^{-4}M per 100ml. for curve A and 15ml. of 10^{-4}M per 100ml. for curve B. Yoe and Jones'[48] mole-ratio studies (Figure 19A) also showed evidence of a 1:1 complex. x ml. of 10^{-4}M reagent were added


FIGURE 18.
(A).
(B).
FLUORESCENCE INTENSITY (RATIO).
0·4
0·3
0·2
0·1
0
0·2
0·4
0·6
0·8
1·0
Al.


FIGURE 19.
(A)
(B)
80
60
40
20
0
2
4
6
8
10
FLUORESCENCE INTENSITY (SCALE READING).
ML REAGENT.

to 5ml. of 10^{-4}M aluminium solution buffered at pH5.8 and the fluorescence was measured at 370/460mμ. Ideally the plot of fluorescence intensity should lie parallel to the abscissa, following the break in the curve. The continued slope of the curve, however, could be attributed to increasing association of the relatively weak complex. A Harvey-Manning[49] slope-ratio study, Figure 20 (i), gave evidence of a 1.25:1 reagent to aluminium complex. Curve A contained 10ml. of 10^{-3}M aluminium solution with varying amounts of 10^{-4}M reagent solution; curve B contained 10ml. of 10^{-3}M reagent solution and varying amounts of 10^{-4}M aluminium solution. Both were buffered at pH5.8.

There were also indications that the complex formed was of the adsorption type. The aluminium complex was apparently not formed below pH4. Now aluminium begins to hydrolyse at pH4.1 and would, therefore, be present in a suitable form for adsorption complex formation at pH5.8. Adsorption could be demonstrated by agitating solid aluminium oxide with a 2-hydroxy-3-naphthoic acid solution buffered at pH5.8. The white solid then showed the blue fluorescence characteristic of the complex, compared with the green fluorescence of the excess reagent in

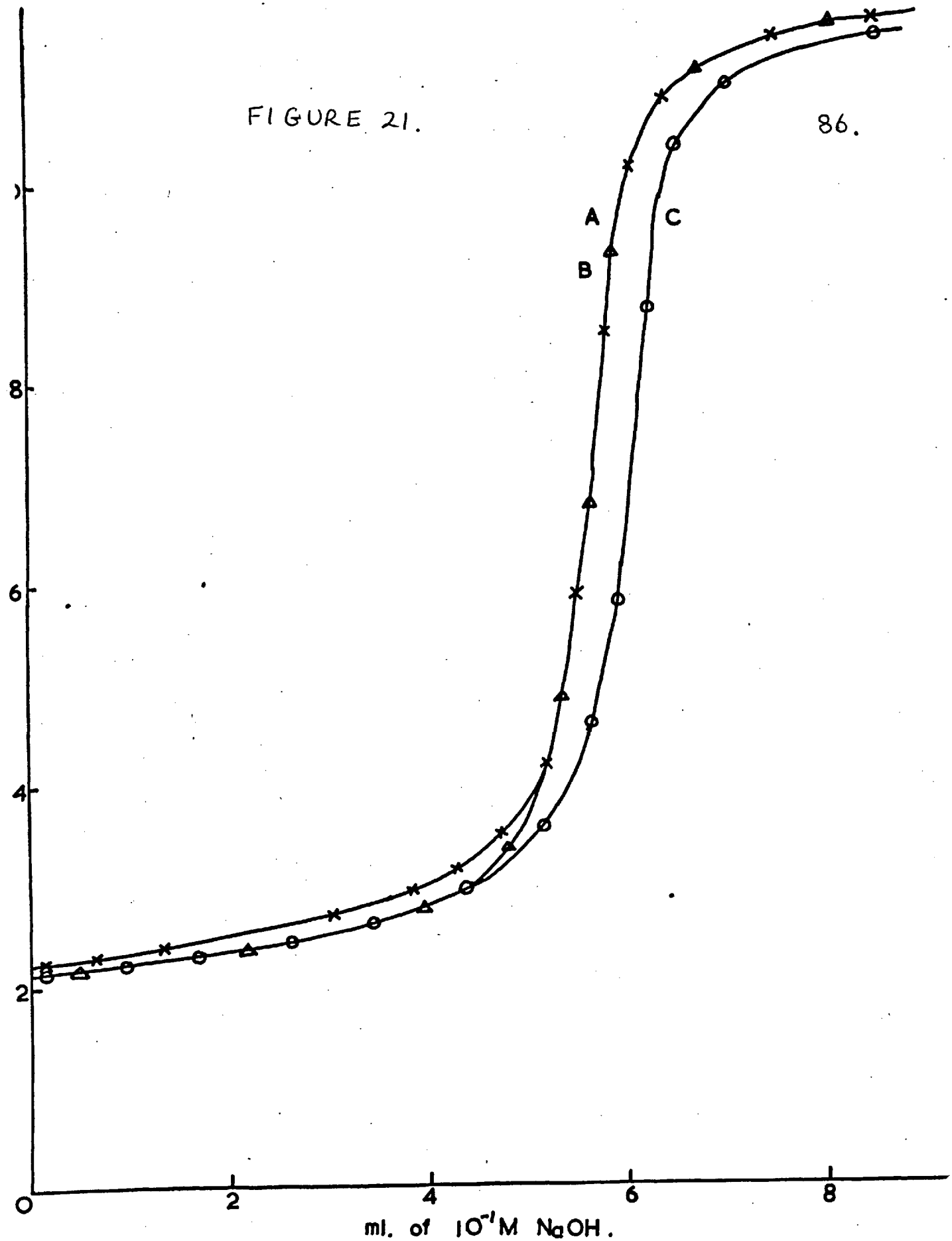

FIGURE 20.
(i)
(ii)
FLUORESCENCE INTENSITY (SCALE READING).
80
60
40
20
0
1
2
3
4
(B)
(A)
ML OF 10^{-3}M AL-REAGENT.
ML OF 10^{-4}M BE-REAGENT.

solution. This showed that some adsorption had occurred.

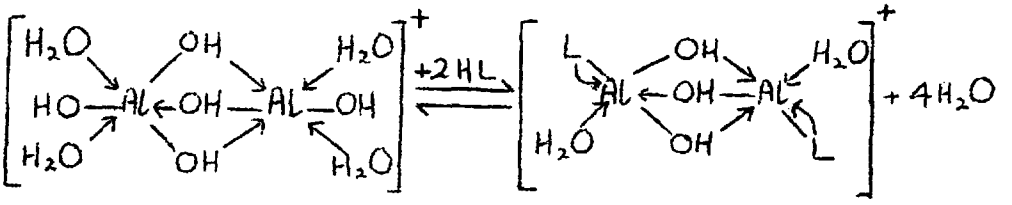
An attempt to obtain a clear break in the mole-ratio plot by making the solutions 20% with respect to acetone resulted in a quenching of all blue fluorescence, i.e. prevention of the reaction. Considered together with the observation that the presence of 20% acetone doubled the intensity of the green fluorescence of the reagent solution (presumably owing to increased solubility, etc.), this was strong evidence that aluminium formed an adsorption complex with sodium 2-hydroxy-3-naphthoate. Feigl[50] has discussed the formation of similar adsorption complexes between aluminium and various dyestuffs, e.g alizarin (1, 2-dihydroxyanthraquinone).

Potentiometric titrations of solutions of aluminium and aluminium plus the sodium salt of the reagent with sodium hydroxide solution showed the presence of no titratable H^+ ions. In Figure 21, curves A and B, which coincide, were the results of the titration of aluminium alone and aluminium plus sodium 2-hydroxy-3-naphthoate, respectively. Curve C, the titration of aluminium plus the free reagent acid, shows a displacement equivalent to

FIGURE 21.



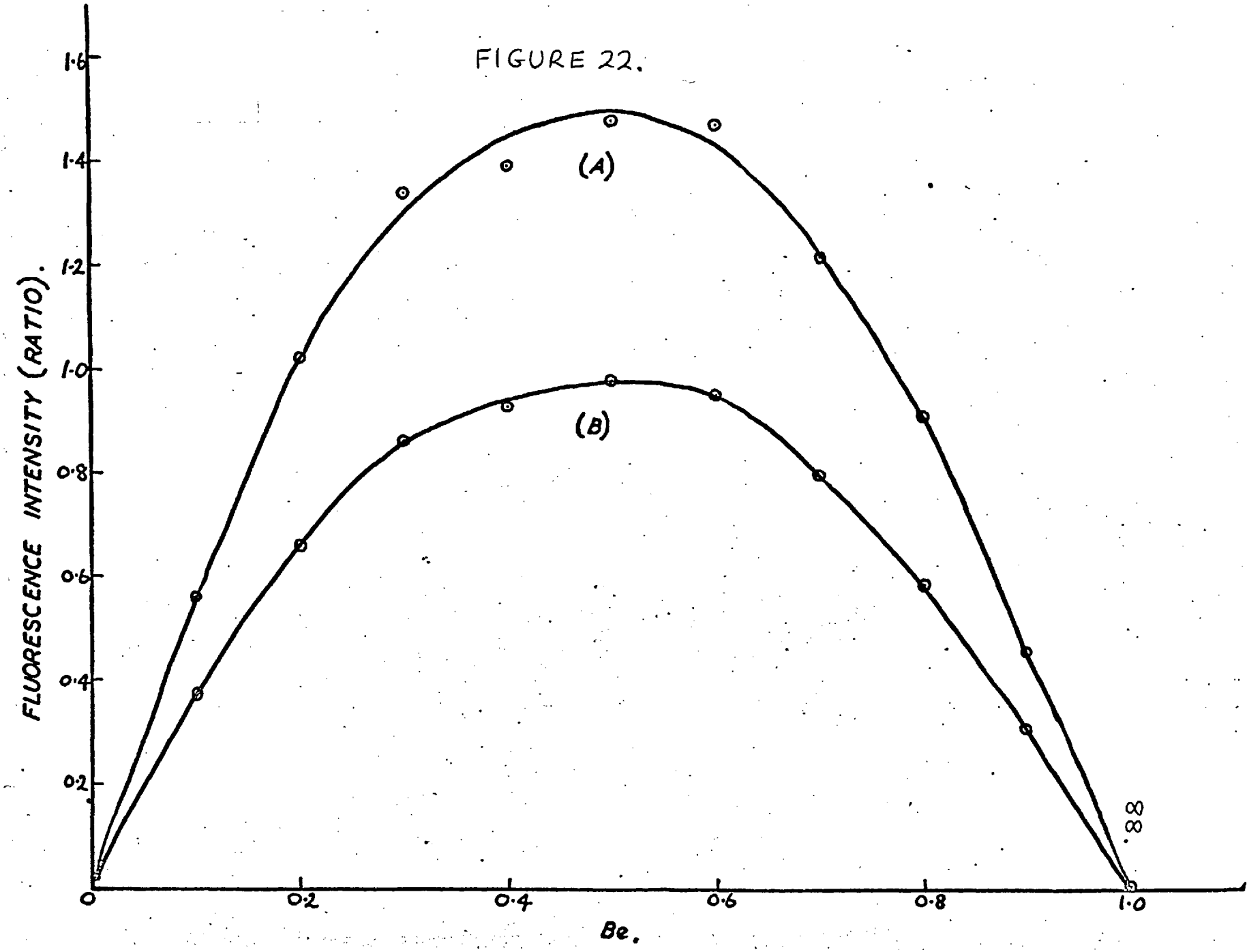
the one titratable H^+ ion released from the carboxylic acid group in the 2-hydroxy-3-naphthoic acid itself. These observations could be explained if the aluminium were in the form of a macromolecule above pH4.1. In this instance, any H^+ ions liberated by complexation would be taken up as water and would not be available for neutralization, as in the reaction:-



Attempts were made to confirm the proposed adsorption complex formation by using the spectrofluorimeter as a sensitive nephelometer to measure the relative amounts of light (at 465mμ) scattered by solutions of (a) aluminium and (b) aluminium with an equal amount of reagent. If an adsorption species were formed, the scattering observed would be expected to increase. The results of these experiments, however, were not reproducible and, therefore, inconclusive.

Cherkesov[45] proposed the formation of a beryllium-reagent complex of 1:2 molar proportions. Figure 22 (Job's method of continuous variations), however,

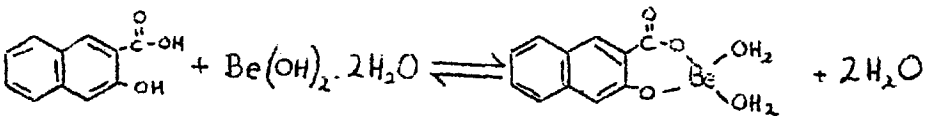
FIGURE 22.


FLUORESCENCE INTENSITY (RATIO).
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
0·2
0·4
0·6
0·8
1·0
Be.
(A)
(B)
88

indicates the formation of a 1:1 complex. These curves were obtained using 10^{-4}M solutions of beryllium and reagent, buffered at pH7.5 and measuring the fluorescence intensity at 380/460mμ. It was unnecessary to correct for the blank reagent fluorescence because this was very slight under these conditions. The total amount of beryllium plus reagent solutions was 20ml. of 10^{-4}M per 100ml. for curve A and 15ml. of 10^{-4}M per 100ml. for curve B. This result was confirmed by a Yoe and Jones' mole-ratio plot (Figure 19B) obtained using 5ml. of 10^{-5}M beryllium solution and varying amounts of 10^{-5}M reagent solution, buffered at pH7.5. A Harvey-Manning slope-ratio study, shown in Figure 20(ii), gave evidence of a 1.2:1, reagent-beryllium complex. Curve A contained 10ml. of 10^{-3}M beryllium solution and varying amounts of 10^{-4}M reagent solution and curve B 10ml. of 10^{-3}M reagent solution with varying amounts of 10^{-4}M beryllium solution. All solutions were buffered at pH7.5 and the fluorescence was measured at 380/460mμ.

The addition of acetone did not prevent the beryllium-2-hydroxy-3-naphthoic acid reaction. Furthermore, unlike the aluminium complex, the beryllium

complex could be extracted into higher alcohols. This was taken as evidence for the formation of a true complex, possibly of the form



since in beryllium solution at pH7.5 the predominant species is $Be(OH)_2$ [51].

Potentiometric titrations of beryllium solution plus reagent with sodium hydroxide were similar to those of aluminium previously described, in that they showed the presence of no titratable H^+ ions. In acid solution, the Be^{2+} ion is extensively hydrolysed and very strongly solvated by water molecules[52]. Thus it is possible that the H^+ ions liberated by complex formation are released as water molecules and are, therefore, not available for neutralisation by the sodium hydroxide.

Stability of complexes.

The apparent or conditional stability constants of the aluminium-reagent and beryllium-reagent 1:1 complexes formed under the experimental conditions described above were evaluated. On the basis of the previous experiments, if it was assumed that a

mononuclear complex, MR, was formed, it was possible to write the conditional stability constant at a stated pH as:-

$$K = \frac{[MR]}{[M][HR]}$$

and, hence the conditional constant as

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

where C represented the concentration of the complex and α represented the degree of dissociation of the complex.

A continuous variations curve could then be used to obtain a measure of the conditional stability constant. This was done using Figure 18 for the aluminium complex and Figure 22 for the beryllium complex. Using the same symbols for K as above, it could be said that at some point, x, on the curve, which corresponded to a certain concentration of the complex,

$$K = \frac{x}{(M-x)(R-x)}$$

where M and R would represent the total molar concentration of M (metal) and R (reagent) if no reaction had taken place between them. Now choosing another point, x' (on the opposite side of the maximum or,

as was done here, on a curve of different concentrations, such that the fluorescence intensities were the same), then

$$K = \frac{x}{(M-x)(R-x)} = \frac{x'}{(M'-x')(R'-x')}$$

and, of course, $x = x'$,

from which,

$$x = \frac{MR - M'R'}{(M+R) - (M'+R')} .$$

Substitution of x in the expression for K, the conditional stability constant, provided the numerical values shown in Table 12 for aluminium and in Table 13 for beryllium. Two values of K were calculated, one for each curve - i.e. x and x', as shown in the following example:

From Figure 22, at fluorescence intensity = 0.4,

$$x = \frac{MR - M'R'}{(M+R) - (M'+R')}$$

$$= \frac{\left(\frac{18.25\times10^{-4}}{100} \times \frac{1.75\times10^{-4}}{100}\right) - \left(\frac{13.0\times10^{-4}}{100} \times \frac{2.0\times10^{-4}}{100}\right)}{(20-15) \times 10^{-6}} .$$

$$= 1.20 \times 10^{-6} .$$

Table 12.

Values of Conditional Constant for Aluminium Complex from Continuous Variations Curve.

Fluorescence Intensity (ratio).	Mℓ. of 10^{-4}M solution				K calculated from	
	M	D	M'	D'	x	x'
0.05	0.4	19.6	0.6	14.4	3.56×10^4	2.63×10^4
0.1	0.85	19.15	1.28	13.72	2.33×10^4	1.46×10^4
0.15	1.3	18.7	2.02	12.98	2.09×10^4	1.72×10^4
0.2	1.85	18.15	3.07	11.93	2.88×10^4	2.24×10^4

Table 13.

Values of Conditional Constant for Beryllium Complex from Continuous Variations Curve.

Fluorescence Intensity (ratio).	Mℓ. of 10^{-4}M solution.				K
	M	D	M'	D'	
0.4	18.25	1.75	13.0	2.0	1.28×10^5
0.5	17.8	2.2	12.4	2.6	0.98×10^5
0.6	17.3	2.7	11.9	3.1	1.72×10^5
0.7	16.9	3.1	11.2	3.8	1.18×10^5
0.8	16.5	3.5	10.5	4.5	1.04×10^5

$$\therefore \text{ from } x,\ K = \frac{x}{(M-x)(R-x)}$$

$$= \frac{1.20 \times 10^{-6}}{(18.25-1.20)\times10^{-6} \times (1.75-1.20)\times10^{-6}} = \underline{1.28\times10^{5}}$$

and from x',

$$K = \frac{1.20 \times 10^{-6}}{(13.00-1.20)\times10^{-6} \times (2.00-1.20)\times10^{-6}} = \underline{1.27\times10^{5}}$$

The average values for K were: for the aluminium complex 2.37×10^{4} and for the beryllium complex 1.44×10^{5}. The accuracy of these calculations was severly limited by the relative imprecision in reading the values of M and R from the Job plot curves. Small errors in these would lead to a large error in the value of x for each value of the fluorescence intensity used.

Discussion.

It is fair to say that the reagent 2-hydroxy-3-naphthoic acid provides a better method for the determination of beryllium than of aluminium on the grounds of both sensitivity and relative freedom from interference. A comparison was made between this method and the other spectrofluorimetric method for beryllium

which uses Morin as reagent[42,43]. The present worker was unable to reproduce the sensitivity reported by Sill, probably owing to unfamiliarity with the method which is experimentally much more complicated than that described above. In both methods the limiting factor in the sensitivity is the contribution of the reagent "blank" fluorescence and every effort must be made to minimise this. The minimum concentration range for the determination of beryllium by either method, as published, is ca. 0.2 to 2 parts per 10^9.

It was noted that one of the few positive interferences in the reaction of aluminium with 2-hydroxy-3-naphthoic acid was that of the tetraborate ion. This reaction was considered to be worth further investigation because probably the best fluorimetric method at present available for the determination of traces of boron is by reaction with benzoin[53], which is a delicate procedure requiring de-oxygenation of solutions before measurement. It was found, however, that the blue fluorescence of the borate-2-hydroxy-3-naphthoic acid complex, in solutions buffered at pH5.8, only began to predominate over the green reagent blank fluorescence when a considerable excess of borate was present. This reaction did not, therefore, seem suitable for analytical application.

Apparatus.

Farrand spectrofluorimeter. To obtain the maximum sensitivity compatible with good definition of maxima, 5- and 20-mμ bandwidth slits were used in the excitation and analysing monochromators, respectively. A Corning 7-54 filter (transmittance > 75% over the range 275 to 375mμ) was used for the excitation radiation and a 3-73 filter (transmittance > 75% above 455mμ) for the emission during the aluminium determination. To increase the sensitivity for beryllium, the 7-54 filter was omitted.

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

Reagents.

Standard aluminium solution: 0.4744g. of AnalaR potassium aluminium sulphate, $Al_2(SO_4)_3.K_2SO_4.24H_2O$, was dissolved in distilled water and diluted to one litre. This stock solution was 10^{-3}M in aluminium and was diluted as required to yield a solution containing 0.1μg. of aluminium per ml. (3.7×10^{-6}M).

Standard beryllium solution: 0.1772g. of AnalaR beryllium sulphate, $BeSO_4.4H_2O$, was dissolved in water, 10ml. of AnalaR perchloric acid, $HClO_4$ (S.G. 1.54), were added and the solution was diluted to one litre.

This stock solution was 10^{-3}M in beryllium and was diluted as required.

All volumetric flasks used were treated with Repel cote, 2% dimethyldichlorosilane in carbon tetrachloride, to minimise adsorption of aluminium and beryllium onto the glass. Water from an all-glass distillation apparatus was used throughout to minimise contamination by traces of heavy metals.

Reagent solution: a 10^{-3}M solution of the sodium salt of 2-hydroxy-3-naphthoic acid (used as supplied by Hopkin and Williams, Ltd., without further purification) was prepared by dissolving 0.2102g. in one litre of water.

Buffer solutions. pH5.8: 40ml. of AnalaR glacial acetic acid were diluted to ca. 800ml. with water. Sufficient AnalaR ammonia solution (S.G. 0.88) was added to adjust the pH to 5.8 (ca. 40ml. required) and the solution was diluted to one litre with water.

pH 7.5: 50ml. of ammonia solution were diluted to ca. 800ml. with water. Sufficient glacial acetic acid was added to adjust the pH to 7.5 (ca. 42ml. needed) and the solution was diluted to one litre with water.

A buffer, pH 5.8, prepared from hydrated sodium acetate, $CH_3COOONa.3H_2O$, and acetic acid was also satisfactory for use in the determination of aluminium.

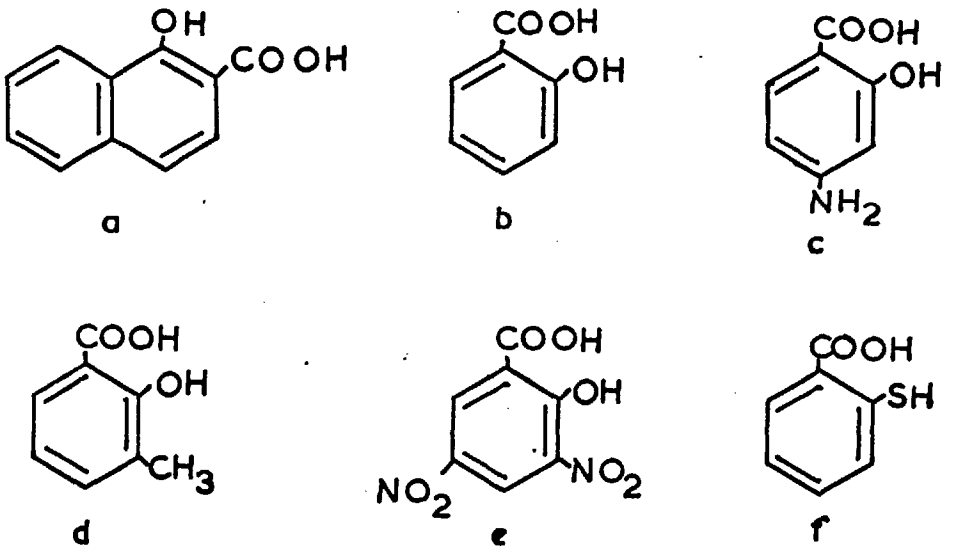
Calcium - CDTA masking solution: 25ml. of 10^{-1}M calcium solution and 6ml. of 10^{-1}M CDTA (34.6g. per litre aqueous solution dissolved in the minimum amount of sodium hydroxide) were measured into a 100ml. flask and diluted to volume with water. This solution contained enough calcium (a four-fold molar excess) to prohibit the existence of free CDTA in solution.

Diverse ions: 0.1M or 0.01M solutions of AnalaR salts were used.

Qualitative studies of reagents related to 2-hydroxy-3-naphthoic acid.

After the completion of the work described above, the fluorescence properties and reactions of a number of organic molecules related to 2-hydroxy-3-naphthoic acid were investigated. These were (see Figure 23) (a) 1-hydroxy-2-naphthoic acid, (b) salicyclic acid,

Figure 23.



(c) 4-amino-salicylic acid, (d) 3-methyl-salicylic acid, (e) 3,5-dinitro-salicylic acid and (f) 2-mercapto-benzoic acid.

(a) 1-hydroxy-2-naphthoic acid.

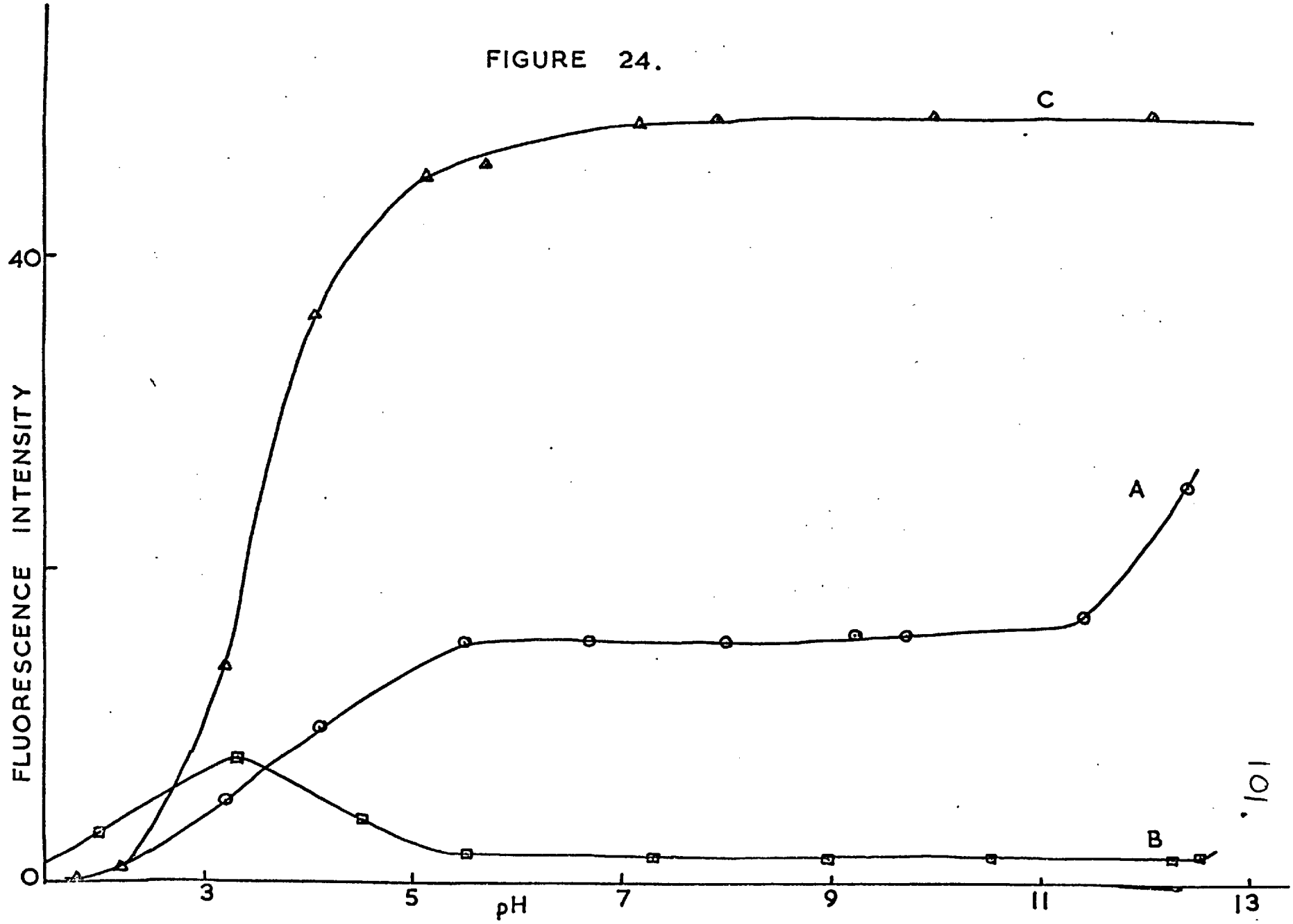
A 10^{-2} M solution was prepared by dissolving 0.188g. of the free acid in a minimum of sodium hydroxide and neutralising with dilute acetic acid. This gave a yellow solution showing a purple fluorescence. The reagent fluorescence was pH dependent, becoming blue at high pH, presumably owing to ionisation of the hydroxyl group. This change was from excitation maximum

360mμ/fluorescence maximum 410mμ (purple) at low pH to 370/460mμ (blue) at high pH. This contrasted with 2-hydroxy-3-naphthoic acid which showed a fluorescence change from 360/510mμ (green) to 370/480mμ (blue-green) at high pH. 1-hydroxy-2-naphthoic acid was tested at pH2, 5.8, 9.2 and 13 with 41 cations, but no potentially useful fluorescence reactions were found.

(b) Salicylic acid.

An aqueous solution of salicylic acid showed a purple fluorescence (excitation maximum 340mμ/fluorescence maximum 410mμ) over the pH range 2 to 13. The variation of the intensity of fluorescence with pH is shown in Figure 24A. The reactions of salicylic acid with 40 cations were tested at pH2, 5.8, 9.2 and 13. The addition of equimolar amounts of aluminium, beryllium and zirconium produced marked increases in the intensity of the purple fluorescence at pH2. The reaction with zirconium was investigated further, but proved to be inapplicable to the determination of traces of the metal because at the optimum pH for zirconium fluorescence development (2.2), the overall intensity was very low. At higher pH's (ca. 5), the contribution of the reagent blank fluorescence was

FIGURE 24.



too large in proportion to the fluorescence due to zirconium and; furthermore; the zirconium underwent slow hydrolysis.

(c) 4-amino-salicylic acid.

An aqueous solution of 4-amino-salicylic acid showed a purple fluorescence (340/400mμ) over the pH range 2 to 13. The variation of the intensity of the fluorescence with pH is shown in Figure 24B. Investigations of this reagent's behaviour with 40 cations at the same pH's as above failed to reveal any analytically interesting reactions.

(d) 3-methyl-salicylic acid.

The variation of the intensity of the purple (330/420mμ) fluorescence of this reagent with pH is shown in Figure 24C. A brief qualitative examination of its reactions revealed the formation of complexes showing increased purple fluorescence with aluminium, beryllium and zirconium. The reaction with zirconium, in particular, warranted further study, but this proved to be difficult because zirconium tended to precipitate as the hydroxide above pH 1.5 and the free reagent precipitated below this pH.

(e) 3,5-dinitrosalicylic acid.

An aqueous solution of this reagent was yellow

and non-fluorescent. No fluorescence reactions were observed.

(f) 2-mercapto-benzoic acid.

2-mercapto-benzoic acid was dissolved in ethanol to give a purple fluorescent solution (excitation maximum 375mμ/fluorescence maximum 425mμ). No fluorescence reactions were observed with aluminium, beryllium, zirconium nor several other cations.

CHAPTER 3.

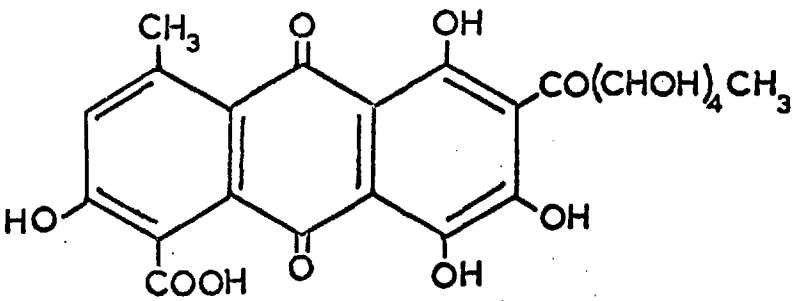
Carminic Acid as a reagent for the spectrofluorimetric determination of molybdenum and tungsten.

The two most common methods for the colorimetric determination of microgram amounts of molybdenum depend upon measuring the absorbances of organic extracts of (a) the yellow molybdenum (V)-thiocyanate complex[54] and (b) the green complex formed with 4-methyl-1,2-dimercaptobenzene (dithiol)[55].

Many of the most satisfactory methods for the determination of traces of metal ions by solution spectrofluorimetry depend on the formation of fluorescent metal complexes with reagents which are either non-fluorescent or which excite or emit at a different wavelength under the same conditions. However, the range of elements which may be determined in this way is still rather limited and one of the objectives of this study of the utility of spectrofluorimetry in trace analysis was to extend the range of elements which could be determined in this way. To the best of the present author's knowledge, no spectrofluorimetric procedure has yet been reported for the determination of molybdenum, although the reaction of

molybdenum salts with tincture of cochineal to produce a "fiery-red" fluorescence has been noted[56]. This chapter describes the use of carminic acid (Figure 25), which is a constituent of cochineal,

Figure 25



CARMINIC ACID

as a reagent for the spectrofluorimetric determination of microgram amounts of molybdenum. The molybdenum-carminic acid complex exhibits a red fluorescence in aqueous solution at pH5. Optimum conditions were established for the determination and the effects of many other ions were investigated.

During the development of the procedure for molybdenum (VI), tungsten (VI) was found to produce a similar red fluorescence with the reagent. A systematic investigation of this reaction was, therefore, also undertaken. Carminic acid formed 1:1

complexes with both molybdenum and tungsten and the conditional stability constants were evaluated.

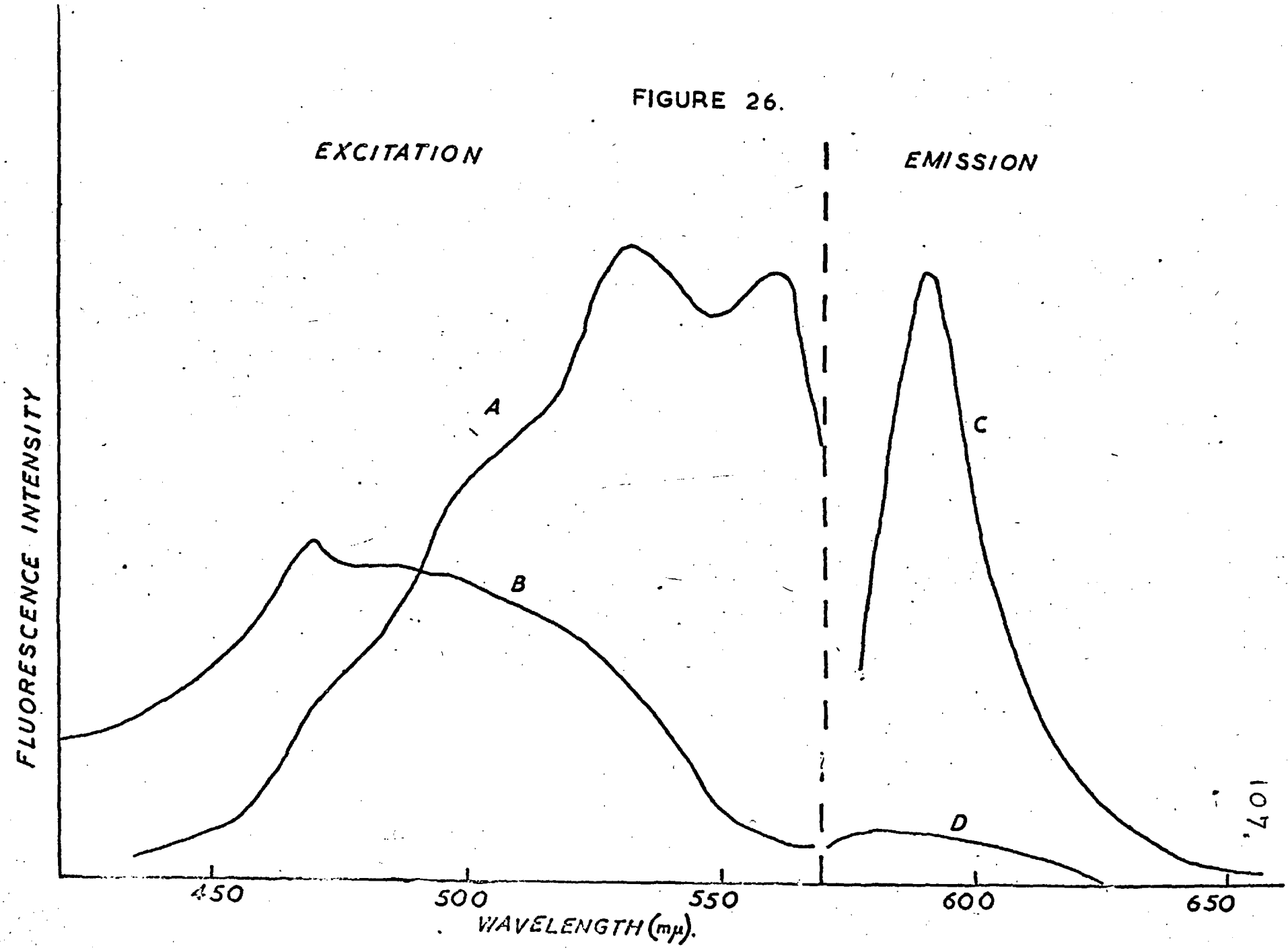
In order to demonstrate the applicability of solution spectrofluorimetry to the analysis of technical materials, the molybdenum-carminic acid system was used to determine molybdenum in certain mild steel down to 0.01%. The reaction of molybdenum (VI) with carminic acid was subject to numerous interferences from other cations, so that a separation of molybdenum from other constituents was necessary before this reagent could be applied. This was effected by separation of molybdenum from the bulk of iron by iso-amyl acetate extraction of molybdenum (V)-thiocyanate.

Part 1. Development of Procedures.

Spectral Characteristics.

Figure 26 shows the excitation and emission spectra of the reagent and its molybdenum complex in aqueous solution at pH5.2. These spectra are uncorrected for the characteristics of lamp emission and photomultiplier response, see Chapter 1. Curve A shows the excitation spectrum of a solution containing 1:1 molar proportions of molybdenum and carminic acid, measuring the fluorescence emission at 590mμ.

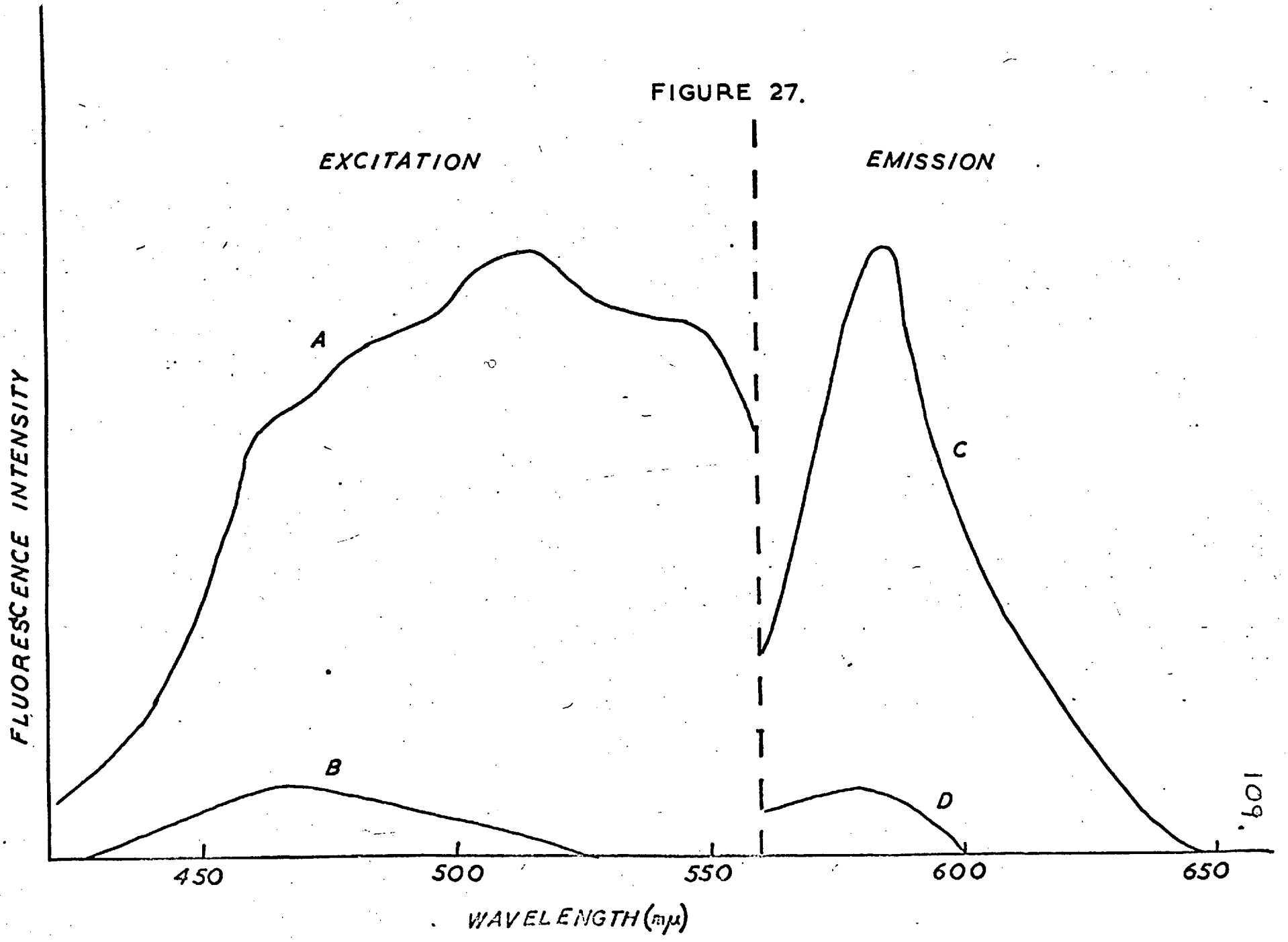
FIGURE 26.


EXCITATION
EMISSION
A
B
C
D
FLUORESCENCE INTENSITY
450
500
550
600
650
WAVELENGTH (mμ).
107
401

The excitation spectrum of the reagent alone is shown in curve B, emission measured at 580mμ. Curves C and D show the fluorescence emission of the molybdenum complex and the reagent alone, respectively; in each instance the excitation wavelength was set at 560mμ. Carminic acid had an excitation maximum at 470mμ and a fluorescence emission maximum at 580mμ with other excitation and emission maxima (not shown in Figure 26) at 355mμ and 440mμ respectively. The molybdenum complex showed two excitation maxima, at 530mμ and 560mμ, and exhibited maximum fluorescence at 590mμ. For quantitative measurements of the fluorescence of the complex, the excitation and emission wavelengths were set at 560mμ and 590mμ, respectively, to minimise the contribution of the "blank" reagent fluorescence.

The excitation and emission spectra of the reagent and its tungsten complex in aqueous solution at pH4.6 are shown in Figure 27. Curve A shows the excitation spectrum of a solution containing equimolar amounts of tungsten and carminic acid with the fluorescence emission measured at 585mμ. The excitation spectrum of the reagent alone is shown in

FIGURE 27.

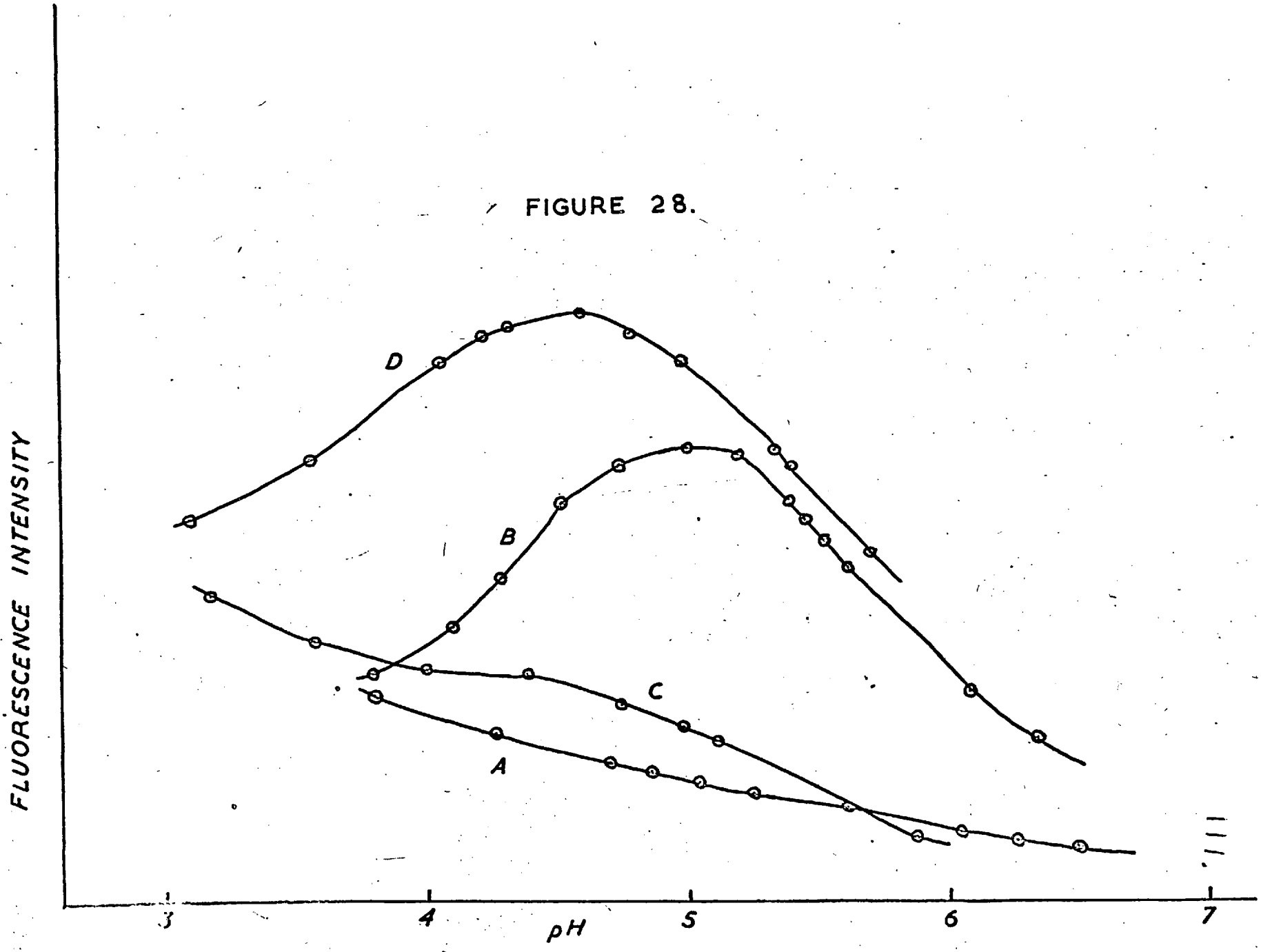

EXCITATION
EMISSION
A
B
C
D
FLUORESCENCE INTENSITY
450
500
550
600
650
WAVELENGTH (mμ)

curve B which was obtained with the emission measured at 580mμ. Curves C and D show the emission spectra of the tungsten complex and the reagent alone, respectively, with the excitation wavelength set at 515mμ. The excitation and emission maxima for carminic acid at pH4.6 were the same as those at pH5.2, i.e. 470/580mμ. The tungsten complex had its excitation maximum at 515mμ and exhibited maximum fluorescence at 585mμ. At these wavelengths the "blank" reagent fluorescence was minimal.

Influence of pH.

Figure 28 shows the results of studies of the variation of fluorescence intensity with pH for carminic acid alone and also for its molybdenum and tungsten complexes. The intensity of the reagent fluorescence, measured at 560/590mμ, gradually fell over the range pH4 to 6.5, see curve A. The fluorescence of the molybdenum complex was measured at 560/590mμ with a ten-fold molar excess of reagent present. Curve B shows the broad peak at pH5 which was obtained. The ratio of complex fluorescence to reagent fluorescence was most favourable at pH5.2 and measurements were carried out at this pH using an ammonia-acetic acid buffer.

FIGURE 28.

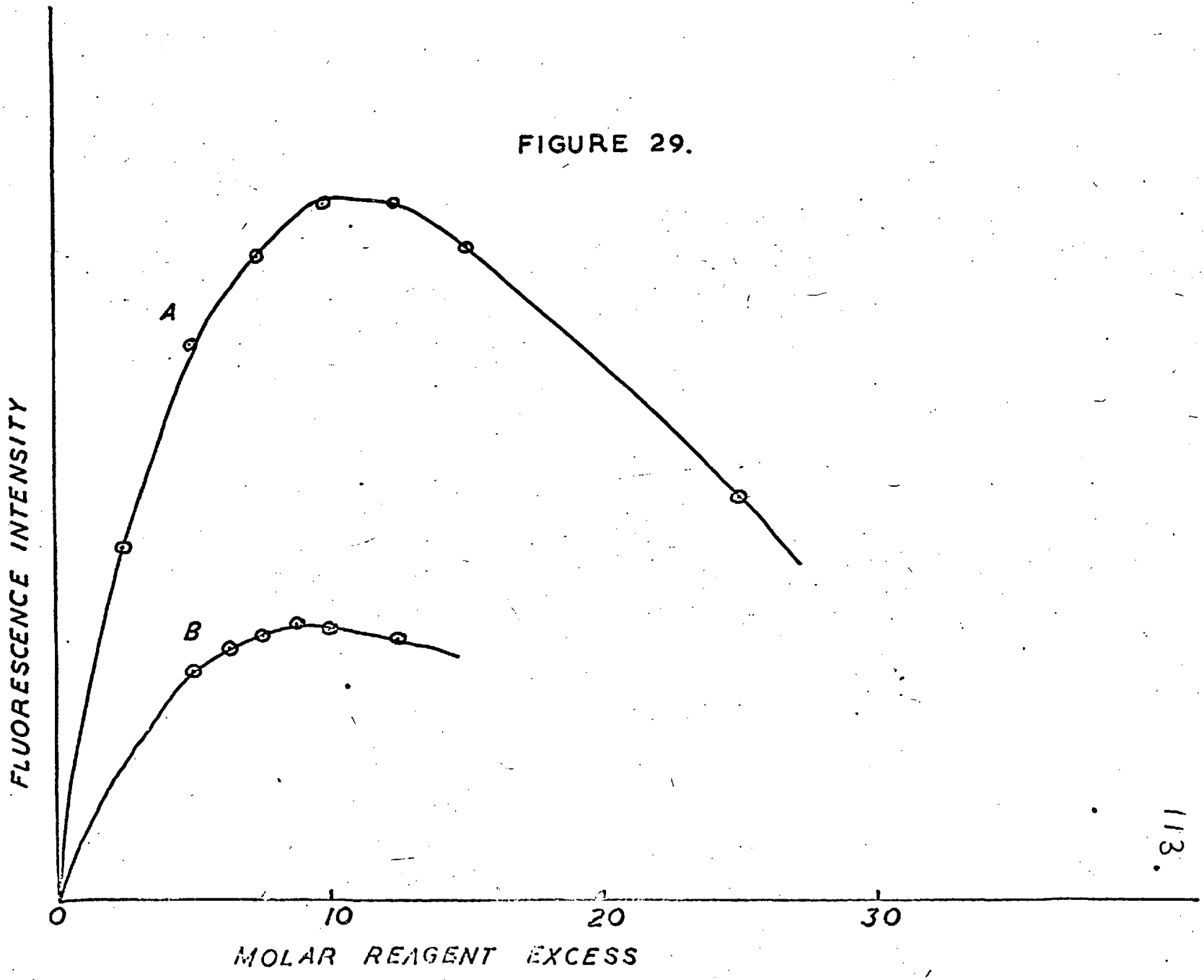


Curves C and D, respectively, show the variation in the intensity of the fluorescence of the reagent and of its tungsten complex, both measured at 515/585mμ. The tungsten complex showed a broad maximum at pH4.6. When measured under these conditions, the contribution of the reagent "blank" fluorescence, curve C, was greater than in the determination of molybdenum.

Reagent Concentration.

Figure 29A shows the effect on the fluorescence intensity of varying the reagent concentration for the molybdenum system. These experiments were carried out at pH5.2 using 1ml. of 10^{-3}M molybdenum (VI) solution with a varying excess of reagent, measuring the fluorescence at 560/590mμ and correcting the curve for the contribution of the reagent fluorescence. A ten-fold molar excess of reagent was required for maximum fluorescence of the molybdenum complex. When a larger excess of reagent was used, the intensity of the fluorescence decreased owing to an inner-filter quenching effect from the reagent which was highly coloured under these conditions. Changes in the concentration of the buffer produced little variation

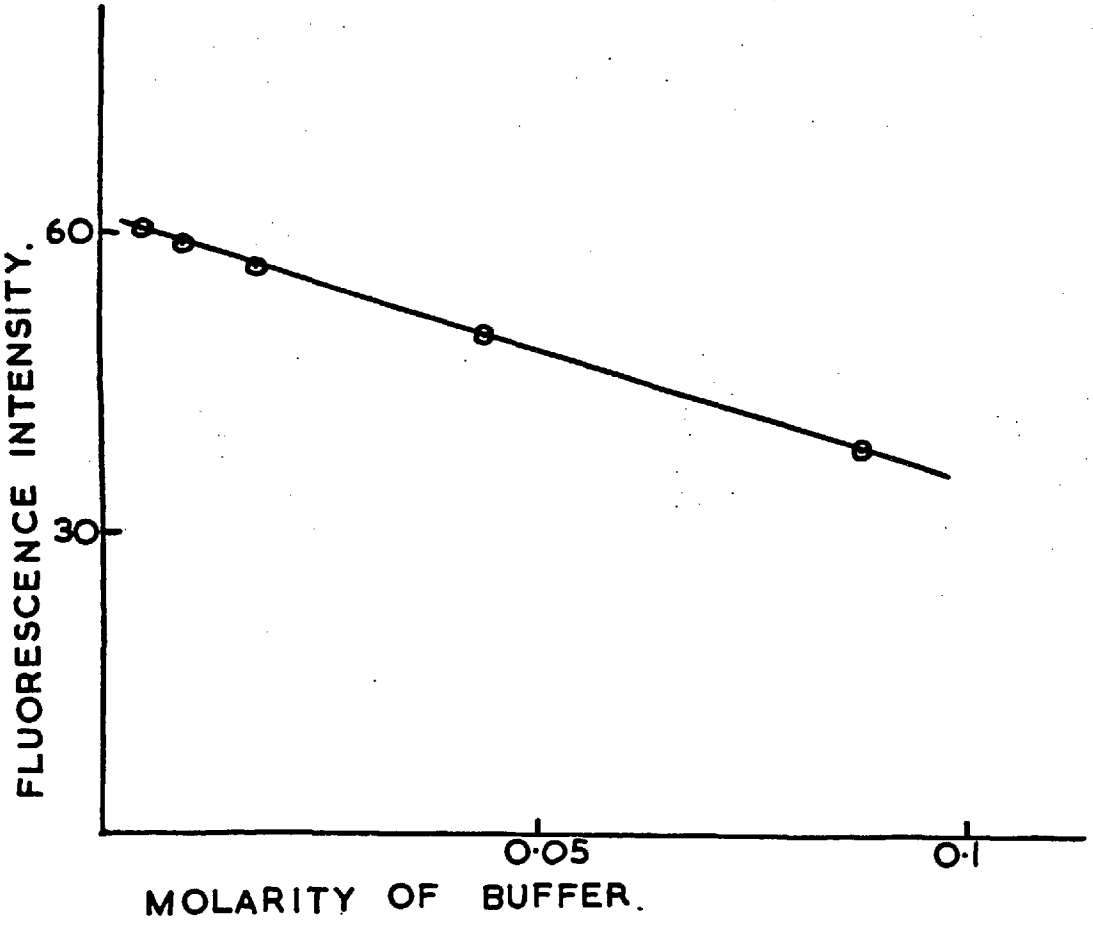
FIGURE 29.



in the observed fluorescence intensity and variation in the order of addition of the reagents was shown to have no effect on the intensity of the fluorescence.

The effect of variation in reagent concentration on the tungsten complex fluorescence is shown in Figure 29B, which was obtained at pH4.6 using 2ml. of 10^{-4}M tungsten (VI) solution with a varying excess of reagent and measuring the fluorescence at 515/585mμ. At the tungsten concentration used in this experiment (0.36ppm.), the optimal reagent concentration was a nine-fold molar excess. At a higher tungsten (VI) concentration (1.83ppm.), the effect of inner-filter quenching by the reagent was such that maximum complex fluorescence was developed by a five-fold reagent excess. Variation in the order of addition of the reagents had no effect on the fluorescence intensity, but the signal due to tungsten decreased with increase in buffer concentration while the reagent "blank" fluorescence was unaffected. In Figure 30 the fluorescence intensity at 515/585mμ is plotted against the molar buffer concentration in the final solution, at pH4.6 using a solution 2×10^{-6}M in tungsten with a suitable reagent excess. When the overall electrolyte concentration was 0.1M,

FIGURE 30.



the observed fluorescence intensity was 40% less than that produced by the same amount of tungsten in the absence of buffer.

Influence of Time.

A 10^{-3}M aqueous solution of carminic acid at pH4.6 or 5.2 was stable for a period of weeks. Maximum fluorescence for both the molybdenum and tungsten complexes was developed immediately and was then constant for several hours. Neither system showed any measurable decay when subjected to normal irradiation in the spectrofluorimeter during analytical examination.

Effect of Temperature.

This was investigated by developing the fluorescence of otherwise identical solutions at a range of different temperatures. Between 2 and 42°C, a decrease of fluorescence intensity with increase in temperature of development was found for the molybdenum complex, corresponding to a temperature coefficient of ca. 2% per °C. For the tungsten complex, over the range 2 to 53°C, this coefficient was ca. 1.2% per °C. All other fluorescence measurements were made at 22±3°C and the observed intensities were compared with those of standards prepared simultaneously, so that these temperature coefficients did not significantly alter the validity of the results

obtained.

Precision Data.

The results of measuring the fluorescence intensities of ten separate solutions each containing 0.96ppm of molybdenum are given in Table 14.

Table 14

Precision data for determination of 96µg. of molybdenum

Sample no.	Scale reading (x.01)	Net Mo. response	Deviation	Variance
1	84.0	64.0	-2.2	4.84
2	83.0	63.0	-3.2	10.24
3	86.0	66.0	-0.2	0.04
4	86.0	66.0	-0.2	0.04
5	87.0	67.0	+0.8	0.64
6	86.5	66.5	+0.3	0.09
7	87.0	67.0	+0.8	0.64
8	88.5	68.5	+2.3	5.29
9	88.0	68.0	+1.8	3.24
10	86.0	66.0	-0.2	0.04
Blank	20.0	-		
		Mean=66.2		

Standard deviation $= \sqrt{\frac{\Sigma\Delta^2}{n-1}} = \sqrt{\frac{25.10}{9}} = 1.66$ units = 2.5%.

The results gave a standard deviation equivalent to 2.5% of the molybdenum response.

The results of similar experiments on solutions containing 0.36ppm of tungsten are shown in Table 15.

Table 15

Precision data for determination of 36µg. of tungsten.

Sample no.	Scale reading (x.01)	Net W response	Deviation	Variance
1	76.2	46.2	+1.3	1.69
2	75.0	45.0	+0.1	0.01
3	75.7	45.7	+0.8	0.64
4	74.5	44.5	-0.4	0.16
5	75.8	45.8	+0.9	0.81
6	74.2	44.2	-0.7	0.49
7	76.0	46.0	+1.1	1.21
8	74.2	44.2	-0.7	0.49
9	73.5	43.5	-1.4	1.96
10	74.0	44.0	-0.9	0.81
Blank	30.0	-		
		Mean=44.9		

Standard deviation $= \sqrt{\frac{\Sigma\Delta^2}{n-1}} = \sqrt{\frac{8.27}{9}} = 0.96$ units $= 2.1\%$

These experiments gave a standard deviation of 2.1% of the tungsten response.

Effect of Foreign Ions.

The effects of 41 cations and 17 anions on the molybdenum determination were investigated at the one

hundred-fold molar excess level over 96µg. of molybdenum. An acceptable variation of fluorescence intensity from that of standard solutions was taken as $\leq \pm 5\%$. Under these conditions, ammonium, arsenic V, cadmium, lithium, manganese II, potassium, selenium IV, sodium, thallium I, fluoride, chloride, bromide, iodide, nitrate, sulphate, phosphate, silicate, cyanide, thiocyanate, perchlorate, sulphite, acetate, hydroxylamine or ascorbic acid did not interfere. Citrate, oxalate, tartrate, EDTA and o-phenanthroline totally extinguished the fluorescence of the complex by reaction with the molybdenum. The other interferences observed are detailed in Table 16.

Table 16.

Ion	Variation in fluorescence intensity	Observations.
aluminium	+100%	purple solution, increased red fluorescence
antimony (III)	-100%	white precipitate
barium	+100%	increased red fluorescence
beryllium	-100%	purple solution
bismuth	-100%	white precipitate
borate	+400%	orange fluorescence
calcium	+220%	increased red fluorescence
cerium (IV)	-100%	purple precipitate

Table 16 continued.

Ion	Variation in fluorescence intensity	Observations.
chromium (VI)	-56%	no visible effect
cobalt (II)	-37%	no visible effect
copper (II)	-100%	purple solution
gallium	-55%	red solution
gold (III)	-100%	yellow solution
indium	-12%	purple solution
iron (II)	-50%	green solution
iron (III)	-100%	green solution
lead (II)	-100%	purple solution
magnesium	+70%	increased red fluorescence
mercury (II)	-16%	yellow solution
nickel	-70%	red solution
scandium	-60%	purple solution
silver (I)	+50%	no visible effect
strontium	+130%	increased red fluorescence
tellurium (IV)	-100%	white precipitate
thorium	-100%	purple solution
tin (IV)	-100%	red precipitate
titanium (IV)	-100%	purple precipitate
tungsten (VI)	+900%	similar red fluorescence
uranium	-100%	green solution
vanadium (V)	-100%	red solution
yttrium	-55%	purple solution
zinc	+280%	increased red fluorescence
zirconium	+16%	purple solution

It can be seen that most of these interferences were caused by the formation of coloured complexes with carminic acid. Many of the interferences could be reduced by the addition of a large excess of ascorbic acid (e.g. iron III), cyanide (e.g. copper II, cobalt II, nickel), or fluoride (aluminium). Even after addition of these masking agents, however, several ions still interfered seriously. This indicated that a separation from other constituents would be necessary before this reagent could be applied to the determination of molybdenum in technical materials. The study of relevant separation procedures and the evolution of a successful method is described later.

No systematic study of possible interferences in the tungsten system was made because this would have been virtually a repetition of the previous investigation undertaken for the molybdenum-carminic acid system. It was assumed, however, that a separation from other constituents would be necessary before application of this reagent to the determination of tungsten in practical samples. It was previously found, however, that the fluorescence of the tungsten complex was reduced by increasing electrolyte concentration and in view of the fact that a more sensitive spectrofluorimetric method for the determination of

tungsten has been reported[57], it was not considered worthwhile to investigate this reaction further.

Structure of Complexes.

Application of the standard optical methods indicated the formation of a relatively weak 1:1 complex between molybdenum and carminic acid. Job's method of continuous variations[47] (see Figure 31) showed a maximum fluorescence intensity for solutions containing equal amounts of molybdenum and reagent. These curves, which were corrected for the contribution of the reagent fluorescence, were obtained at pH5.2, measuring the fluorescence intensity at 560/590mμ. The total concentration of molybdenum and reagent solutions was 10^{-4}M for curve A and 8 x 10^{-5}M for curve B. The results of an investigation by Yoe and Jones' mole-ratio method[48] are shown in Figure 32(i), for which 10ml. of 10^{-4}M molybdenum solution were allowed to react with varying amounts of 10^{-4}M reagent solution. A Harvey-Manning[49] slope-ratio study gave a ratio of 1.2:1 for reagent: molybdenum, see Figure 32(ii). Curve A contained 10ml. of 10^{-3}M molybdenum solution with a varying amount of 10^{-4}M reagent solution and curve B, 10ml. of 10^{-3}M reagent solution with a varying amount of 10^{-4}M molybdenum

FIGURE 31.

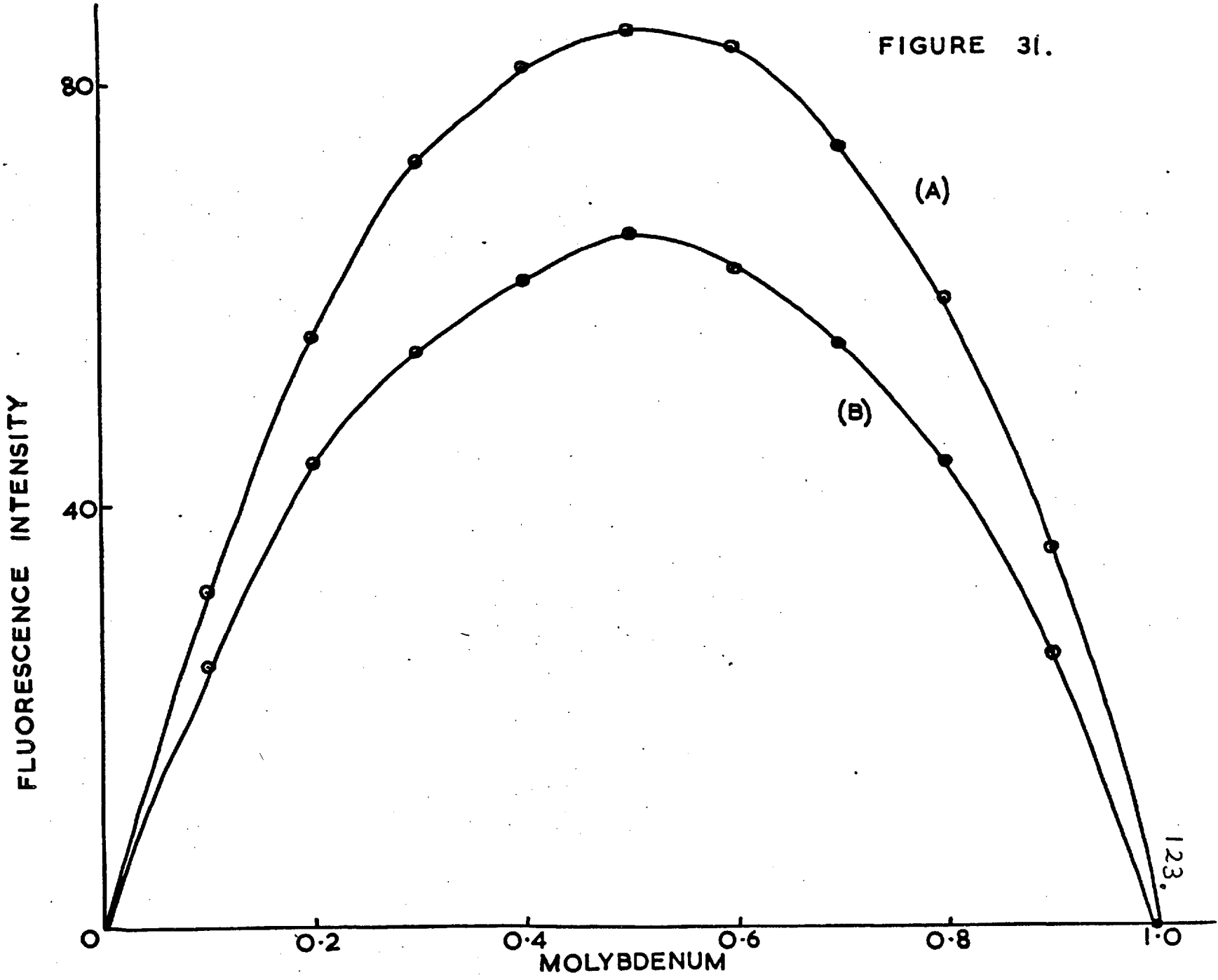
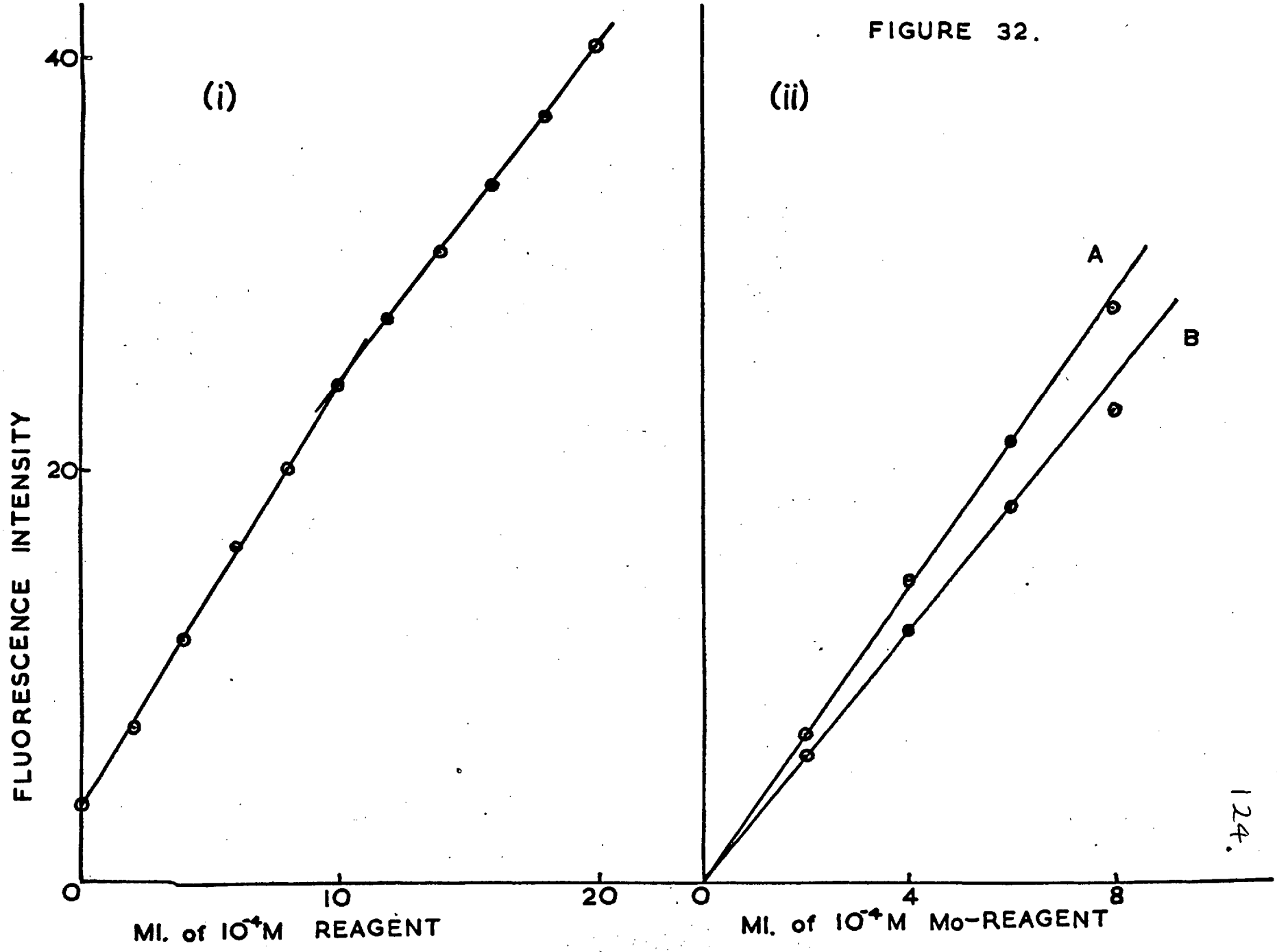


FIGURE 32.


(i)
(ii)
FLUORESCENCE INTENSITY
40
20
0
10
20
0
4
8
Ml. of 10^{-4}M REAGENT
Ml. of 10^{-4}M Mo-REAGENT
A
B

solution. All solutions were buffered to pH5.2 and the fluorescence intensity measured at 560/590mμ.

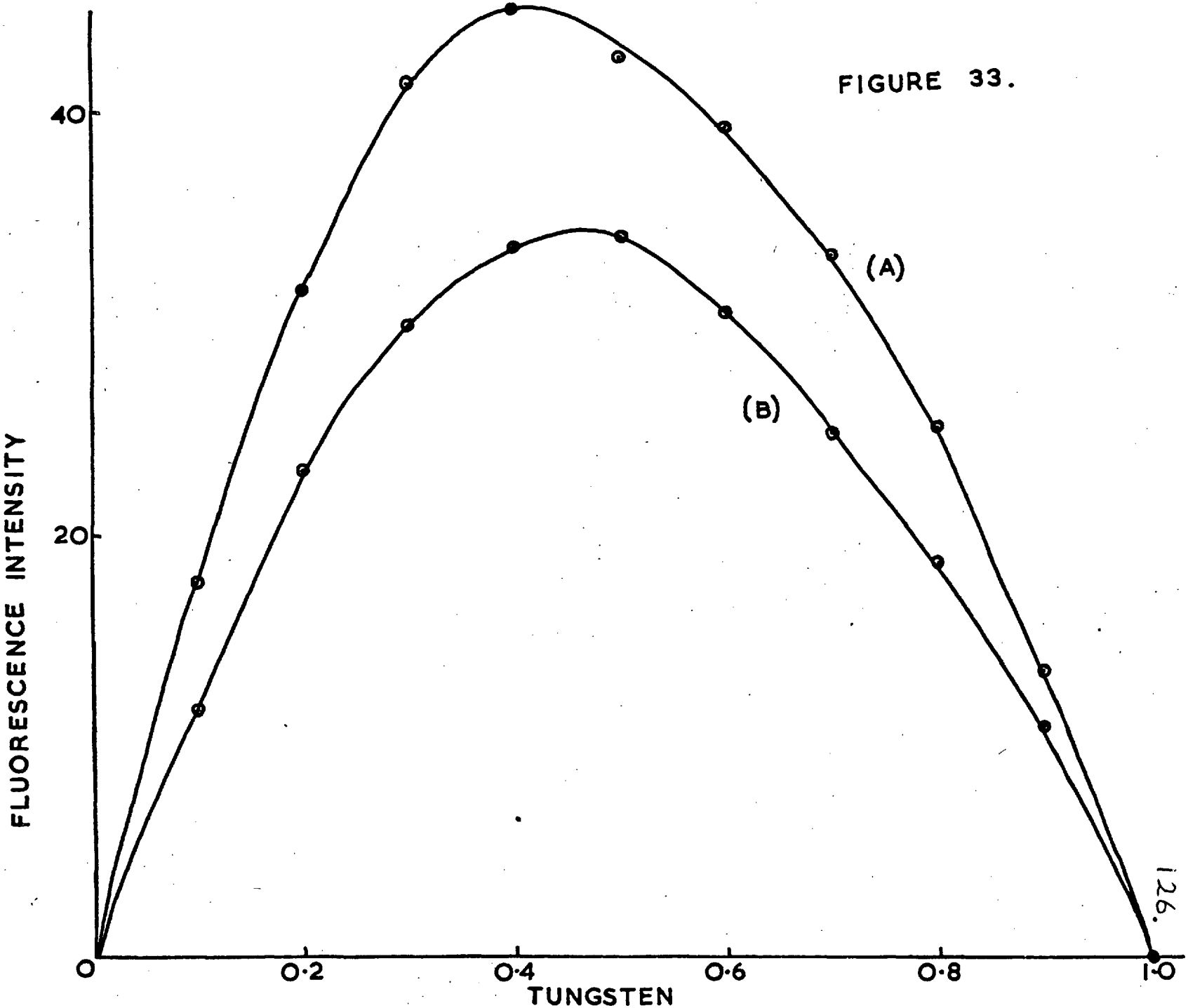
Organic micro-analytical results for the reagent as supplied and used were:-

	% calculated	Lab. No.1	Lab. No.2.	Dried Sample
C	53.6	44.38	44.28	45.28
H	4.07	4.79	5.39	4.43

Overnight drying of the reagent at 100°C produced ca. 7% weight loss and gave the analytical results shown in the last column, indicating that at least a part of the impurity present must have been water. The methods described above were almost certainly affected by self-absorption by the reagent which was orange-coloured at this pH and this effect would distort the shape of the curves obtained. However, it was possible to draw the conclusions from the above analytical results that the reagent had ca. 85% purity and that the molybdenum: carminic acid was of 1:1 molar proportions.

Figure 33 shows the Job plots obtained for the tungsten complex at pH4.6, measuring the fluorescence

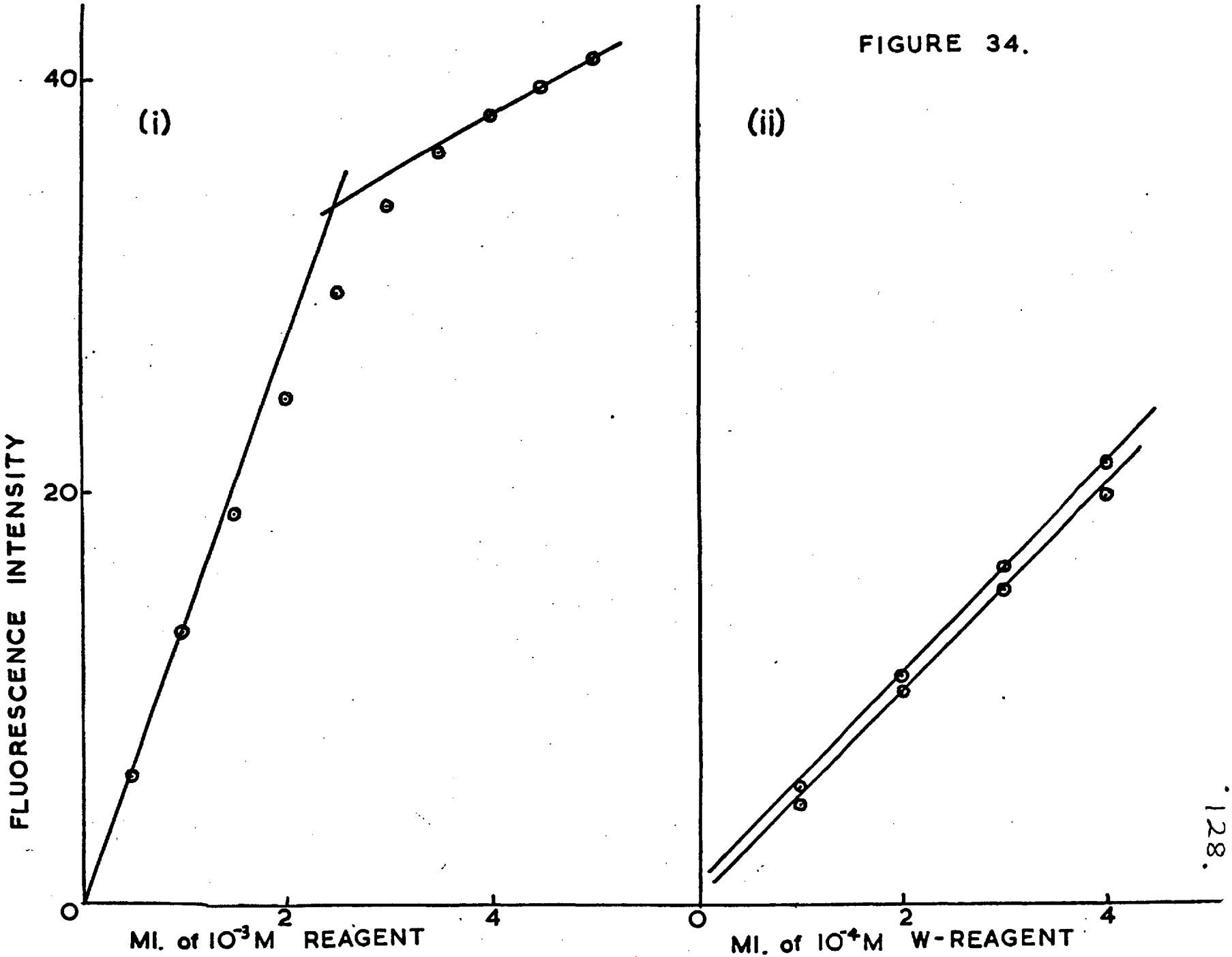
FIGURE 33.



intensity at 515/585mμ. For curve A, the total concentration of tungsten and reagent was 8×10^{-5}M and for curve B, 5×10^{-5}M. Figure 34 shows the results of (i) mole-ratio and (ii) slope-ratio studies which both indicated the formation of a 1:1 complex.

When the solutions were made 20% with respect to acetone, the fluorescence of the tungsten complex was increased by ca. 50% while the reagent fluorescence was little affected. When this experiment was repeated for the molybdenum system, the complex fluorescence increased as before but, at this higher pH, the reagent fluorescence also increased so that there was no increase in the complex signal to blank ratio. While no conclusions could be drawn as far as the molybdenum complex was concerned, these results suggested that the complex formed between tungsten and carminic acid was a true chelate rather than some form of adsorption complex. Both the tungsten-carminic acid complex and the reagent alone were extracted from aqueous solution at pH4.6 into mono- or di-nonylamine dissolved in either benzene or iso-amyl acetate. This indicated that they existed as anionic species which associated with the protonated amine to form

FIGURE 34.


(i)
(ii)
40
20
0
FLUORESCENCE INTENSITY
2
4
Ml. of 10^{-3}M REAGENT
0
2
4
Ml. of 10^{-4}M W-REAGENT

a neutral extractable species. The molybdenum-carminic acid complex at pH5.2 extracted similarly.

Potentiometric titrations of carminic acid with standard alkali showed several inflexions which could be attributed to dissociation of the carboxylic acid and hydroxyl groups, but gave no information as to the nature of the complexes formed with molybdenum and tungsten.

Stability of Complex.

The conditional equilibrium constant of the 1:1 molybdenum complex formed in these experiments was evaluated from the Job plots by the method described in Chapter 2. The results are given in Table 17.

Table 17.

Fluorescence Intensity	Ml. of 10^{-3}M solutions				K
	M	D	M'	D'	
20	9.53	0.47	7.41	0.59	1.5×10^3
25	9.39	0.61	7.24	0.76	2.4×10^3
30	9.23	0.77	7.07	0.93	6.0×10^3
35	9.05	0.95	6.84	1.16	6.1×10^3
40	8.88	1.12	6.60	1.40	5.3×10^3
50	8.45	1.55	6.00	2.00	7.0×10^3

Calculation from dissociation data obtained by

measurement of the intensity of fluorescence of solutions containing (a) 1:1 molar proportions of molybdenum to reagent and (b) a large excess of reagent, gave a value of $K = 6 \times 10^4$ but this was probably higher than the true value owing to the inner-filter effect of the excess reagent similar to that mentioned above.

The asymmetry of the Job plot (Figure 33) did not permit a calculation to be made for tungsten by the first method, but the second method gave a value of $K = 5 \times 10^5$, which was also probably higher than the true value.

Experimental.

Apparatus.

Fluorescence measurements were made with the Farrand spectrofluorimeter using 10mμ slits in both monochromators and the usual fused quartz cells (10mm x 20mm x 50mm). All the reagents were of AnalaR grade except where noted and water from an all-glass distillation apparatus was used throughout.

Reagents.

Standard molybdenum (VI) solution.

0.1766g. of AnalaR ammonium molybdate, $(NH_4)_6\ Mo_7\ O_{24}.4H_2O$, were dissolved in one litre of water. This solution was 10^{-3}M in molybdenum (VI) and was diluted to 10^{-4}M as required.

Standard tungsten (VI) solution.

3.2993g. of AnalaR sodium tungstate, $Na_2WO_4.2H_2O$, were dissolved in one litre of water. This solution was 10^{-2}M in tungsten (VI) and was diluted as required.

Reagent solution.

0.4924g. of carminic acid ("purified", B.D.H. Ltd., used without further purification) were dissolved in 600ml. of water containing a minimum of sodium hydroxide.

Acetic acid was added to pH4.6 or 5.2 as required and the solution was diluted to one litre with water. Assuming 100% purity, this solution was 10^{-3}M in carminic acid. The actual constitution of the reagent used is given on page 125.

Buffer solution, pH5.2.

50ml. of glacial acetic acid were diluted to ca. 800ml. with water. Sufficient ammonia solution was added to adjust the pH to 5.2 (approx. 42ml. needed) and the solution was diluted to one litre with water.

Buffer solution, pH4.6. prepared as above.

Diverse ions. 10^{-1}M and 10^{-2}M solutions.

Hydrochloric acid (sp.gr. 1.18).
Nitric acid (sp.gr. 1.42).
Ammonia solution (sp.gr. 0.88).
Ammonium thiocyanate solution. Aqueous saturated solution.
Citric acid. 1M solution. Dissolved 21.0g. in 100ml. of water.
Ascorbic acid (G.P.R. grade).
Acetic acid, glacial.
Iso-amyl acetate (G.P.R. grade).
Ferric chloride. 1M solution. Dissolved 27.0g. of the hexahydrate in 100ml. of water containing a few drops of hydrochloric acid.

Construction of Calibration Curves.

(a) Molybdenum.

Between 1 and 9ml. of 10^{-4}M molybdate solution

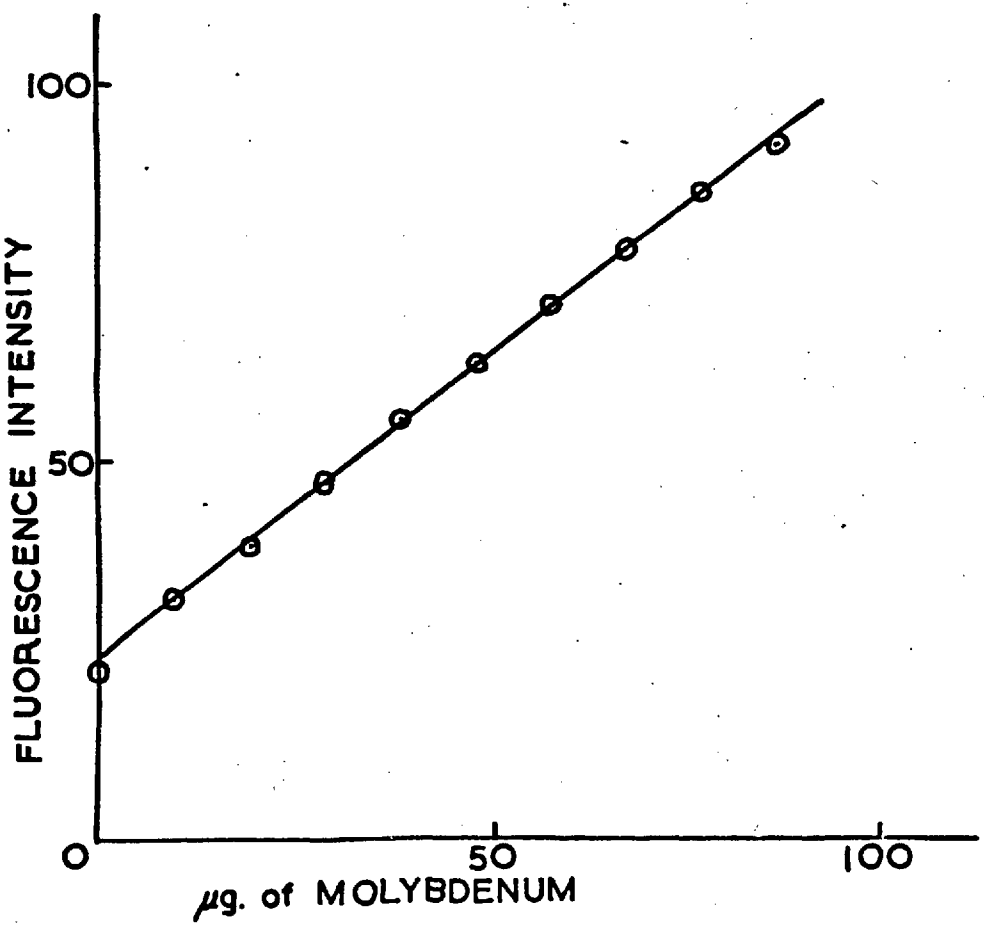
were transferred accurately to 100ml. calibrated flasks containing 5ml. of pH5.2 buffer solution. Ten ml. of 10^{-3}M carminic acid solution were added and the solution was diluted to volume with water. The intensity of the fluorescence of the solutions was measured after 15 minutes at 590mμ at an excitation wavelength of 560mμ. The plot of fluorescence intensity against the amount of molybdenum present (9.6 to 86.4μg.) was a straight line and, after subtraction of the "blank" reagent fluorescence, passed through the origin, see Figure 35.

By suitable reductions in the volumes of reagents added and dilution to a 25ml. volume, molybdenum could be determined similarly in the range 2.4 to 21.6μg.

(b) Tungsten.

Between 0.2 and 2ml. of 10^{-4}M tungstate solution were transferred accurately to 100ml. calibrated flasks containing 1ml. of pH4.6 buffer solution. 1.6ml. of 10^{-3}M carminic acid solution were added and the solution was diluted to volume with water. The intensity of the fluorescence of the solutions was measured after 15 minutes at 585mμ with an excitation wavelength of 515mμ. The plot of fluorescence intensity against the amount of tungsten present

FIGURE 35.



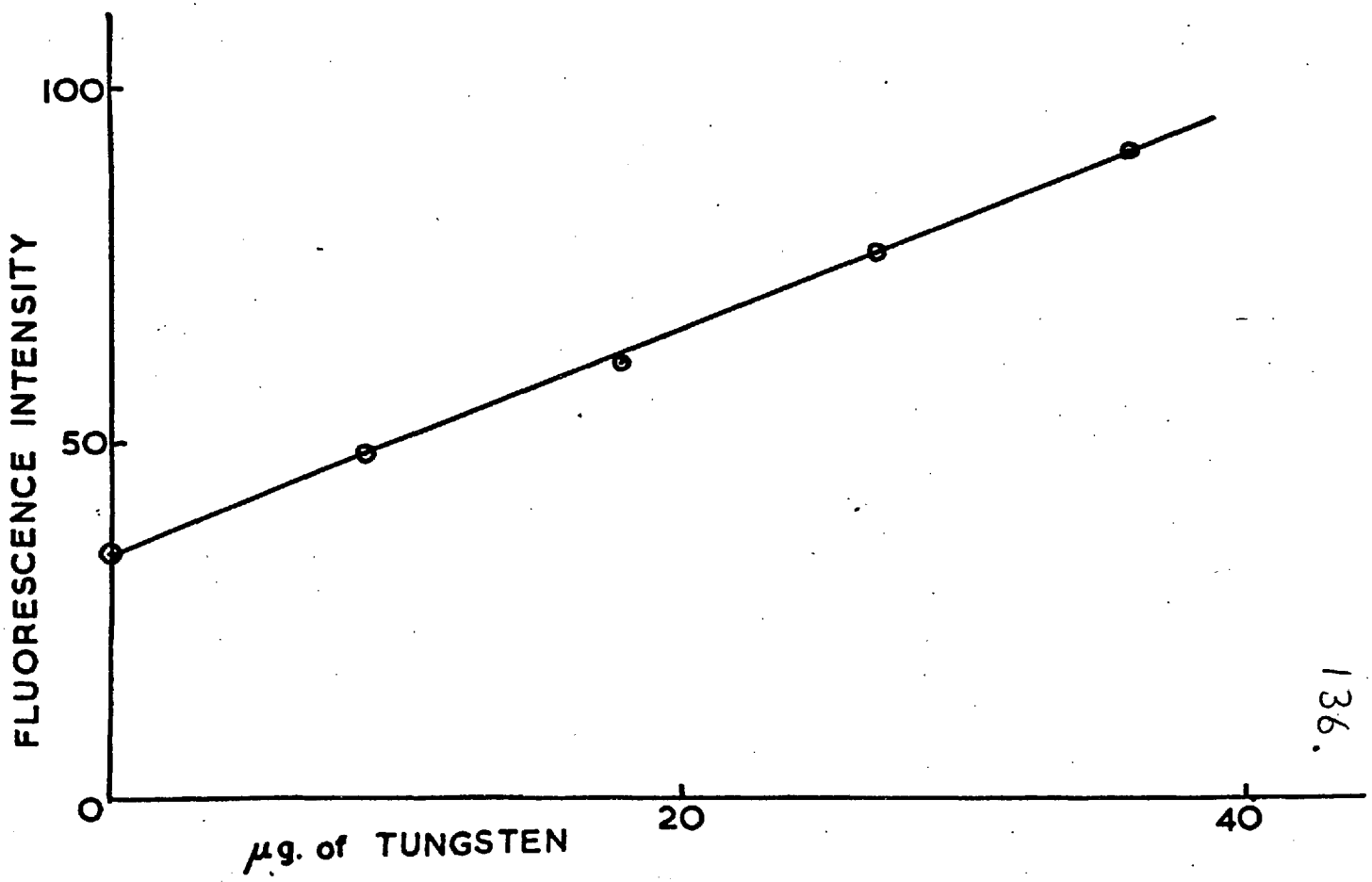
(3.6 to 36µg.)) was a straight line and, after subtraction of the "blank" reagent fluorescence, passed through the origin, see Figure 36.

Discussion.

There were several factors which limited the sensitivity of these determinations, the first being the relative insensitivity of the IP 28 photomultiplier to radiation at 590mµ. The intensity of the reagent "blank" fluorescence obviously also controlled the determination limit. Furthermore, complex organic molecules such as carminic acid tend to show a much lower fluorescence efficiency themselves and in their complexes than simpler molecules, e.g. 2-hydroxy-3-naphthoic acid. In an attempt to reduce this disadvantage by removing the sugar side-chain from the molecule, an alkaline hydrolysis was carried out by refluxing carminic acid for several hours in a strong ammonia solution. A purple solid was obtained which was shown by thin-layer chromatography, using a methanol-water solvent, to differ from the starting material. This product, however, gave no fluorescence reaction with molybdenum and was, therefore, not investigated further.

Given a more sensitive photomultiplier and a

FIGURE 36.



complex with a higher fluorescence efficiency, with a similar reagent it would be possible to use lower concentrations of both reagent and molybdenum. Under these conditions, the problem of inner-filter quenching caused by the intensely coloured reagent would be much reduced, if not eliminated.

Part 2. Determination of Molybdenum in Mild Steel.

Preliminary investigations were made of several extraction systems, e.g. chloride extraction into diethyl ether and 8-hydroxyquinolate extraction into chloroform, in order to find the most convenient method of separation of molybdenum from large amounts of iron. It was found that an efficient separation could be effected by reduction to Mo(V) and Fe(II) respectively with ascorbic acid, followed by solvent extraction of the molybdenum (V)-thiocyanate complex. Iso-amyl acetate was preferred to diethyl ether as the organic solvent because of its lower miscibility with hydrochloric acid and its lower volatility. For a 3M solution of ammonium thiocyanate, molybdenum was found to extract equally well over the hydrochloric acid concentration range 0.1 - 0.8M, giving molybdenum recoveries of > 90%. Ascorbic acid reduction of Fe(III) to Fe(II) was more efficient at the lower

concentration and, as this also produced a lower electrolyte concentration in the final solution, extraction from 0.1M hydrochloric acid was used. After extraction, the amyl acetate phase was back-washed with a solution containing hydrochloric acid, ammonium thiocyanate and ascorbic acid to remove traces of unreduced Fe(III) which extracted as the red ferric thiocyanate complex. The organic phase then contained the molybdenum and showed a yellow colour which has been attributed[58] to the presence of $Fe^{II}[Mo^{V}O(SCN)_5]$ with $Mo^{V}O(SCN)_5^{2-}$ acting as the chromophore. The molybdenum was back-extracted into ammonia solution, to neutralise hydrochloric acid dissolved in the organic solvent, buffered at pH5.2 and determined with carminic acid as previously described.

Tungsten co-extracted with molybdenum in this procedure and, therefore, produced a positive interference in the determination of molybdenum. This, however, could be prevented[59] by the addition of citric acid as a complexing agent for tungsten before the thiocyanate extraction. Tin, which also interfered positively, was removed by previous volatilization as $SnCl_4$.

By this method, amounts of molybdenum in the range 10-70µg. (which corresponds to 0.01 - 0.07% in 0.1g.

of steel) could be separated and determined spectrofluorimetrically as the carminic acid complex.

Experimental Procedure.

A suitable weight of sample (containing 10 to 70µg. of molybdenum) was dissolved in 15ml. of concentrated hydrochloric acid in a 50ml. beaker, heating on a hot-plate to assist solution. After the steel had dissolved completely, heating was continued to evaporate the solution to dryness. The beaker was removed from the hot-plate while the residue was still yellow (Note 1), allowed to cool slightly and the residue was then redissolved in 5ml. of water containing 4-5 drops of concentrated hydrochloric acid. The solution was transferred quantitatively to a 250ml. separatory funnel. Approximately 1g. of solid ascorbic acid and 0.5ml. of M citric acid were added and the solution was made 3M in thiocyanate and 0.1M in hydrochloric acid (Note 2). After adding 25ml. of iso-amyl acetate, the funnel was shaken for two minutes and the aqueous phase was discarded. A further 25ml. of ammonium thiocyanate-hydrochloric acid solution and 1g. of ascorbic acid were added and any extracted Fe(III) was backwashed by shaking for two minutes before discarding the aqueous phase. The molybdenum

was finally back-extracted by shaking the organic phase for $1\frac{1}{2}$ minutes with 25ml. of water containing 0.4ml. of concentrated ammonia solution. The aqueous phase was run into a 100ml. volumetric flask, containing 5ml. of pH5.2 buffer. Eight ml. of 10^{-3}M carminic acid solution were added (Note 3) and the solution was diluted to volume with water. The intensity of the fluorescence was measured after 15 minutes at 590mμ with an excitation wavelength of 560mμ.

All determinations were checked against standards prepared simultaneously by taking 0 and 76.8μg. of molybdenum (8ml. of 10^{-4}M solution) through the extraction procedure described above. The solution, separation and analysis of two steel samples, each in duplicate, could be completed in less than three hours.

Notes on Procedure.

Note 1. If heating was further prolonged, the residue became brown and redissolution was difficult. This tended to cause low recoveries of molybdenum.

Note 2. This was conveniently done by diluting 100ml. of saturated ammonium thiocyanate solution and 4ml. of concentrated hydrochloric acid to 500ml. with water and adding 25ml. of this solution to each separatory flask. The solution was prepared immediately before

use because the thiocyanate tended to decompose on standing, forming sulphide and giving low results for molybdenum.

Note 3. Although the buffering system used was almost always effective, it was a worthwhile precaution before dilution to check that the pH of the final solution was in the range 5.2±0.1. If necessary the pH was adjusted by dropwise addition of acetic acid or ammonia.

Results and Discussion.

The results obtained by application of the proposed procedure to a selection of certificated British Chemical Standard (B.C.S.) mild steels are shown in Table 18. Each result quoted therein was the average value for two analyses run simultaneously.

During the evolution of this procedure, the original method used was to dissolve the steel in concentrated hydrochloric acid containing a few drops of concentrated nitric acid to oxidise the iron present to Fe(III). After cooling, the solution was neutralised by the addition of ammonia solution until $Fe(OH)_3$ began to precipitate and the precipitate was redissolved with 1-2 drops of concentrated hydrochloric acid before proceeding to the solvent

Table 18.

Analysis of molybdenum in B.C.S. certificated steels.

Sample No.	Molybdenum found* %	Average certificate value %	Range in certificate %
B.C.S. 273	$0.04_2, 0.04_9, 0.04_3$.	0.04_5	0.041-0.046
275	$0.09_2, 0.10_3, 0.10_4, 0.08_8$.	0.09_5	0.092-0.100
277	$0.01_1, 0.01_4, 0.01_2$	0.01_5	0.013-0.016
321	$0.06_7, 0.07_5, 0.06_7, 0.06_6, 0.06_1$.	0.068	0.064-0.072
323	$0.09_5, 0.11_0, 0.10_8, 0.10_9, 0.10_2, 0.09_8$.	0.100	0.095-0.105
325	$0.16_3, 0.17_1, 0.17_5, 0.16_4, 0.15_3$.	0.16_	0.15-0.16

* Average of two values.

extraction stage. When applied to B.C.S. mild steels, this method gave satisfactory results for nos. 277, 321, 323 and 325, but nos. 273 and 275 gave consistently high results (ca. 0.075% and 0.13% respectively). This discrepancy was attributed to the presence of a relatively large amount of tin (0.065% and 0.04% respectively in these samples) which was not separated from molybdenum by the extraction procedure used and which interfered by

forming a fluorescent complex with carminic acid. Attempts were, therefore, made to remove tin, firstly by precipitation as metastannic acid and then, when this proved unsuccessful, by volatilization of either $SnCl_4$ or $SnBr_4$. The practice finally adopted, i.e. of evaporation to the point of dryness without further baking, removed tin satisfactorily as $SnCl_4$ without significant loss of molybdenum.

It should be pointed out that, using this separation procedure, the molybdenum content of the steels could have been determined conveniently and more rapidly by measuring the yellow colour of the amyl acetate extracts of molybdenum (V)-thiocyanate. This was not done because this work was undertaken to demonstrate that molybdenum could be separated and determined satisfactorily spectrofluorimetrically.

Although, in the absence of citrate, tungsten gave a considerable positive interference in the determination of molybdenum, it was not possible to devise a similar procedure for tungsten because, as already noted, an increase in the ammonium acetate buffer concentration resulted in a decrease in the fluorescence response due to tungsten. As the final solution after extraction would contain large amounts

of ammonium chloride and thiocyanate as well as buffer, it seemed that this, rather then incomplete extraction of tungsten, was the reason for the reduction in fluorescence. It was found that up to 100µg. of molybdenum produced no interference in the determination of 36µg. of tungsten so that, if this difficulty could be overcome, it should be possible to determine the tungsten content of steel samples without prior removal of molybdenum.

CHAPTER 4.

The Spectrofluorimetric Determination of Scandium using Salicylaldehyde Semicarbazone.

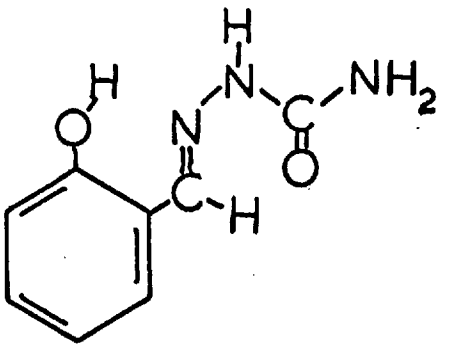
Several colorimetric reagents have been described for the determination of small amounts of scandium in solution, e.g. alizarin red S,[60] 8-hydroxyquinoline[61] and anthrarufin-2,6-disulphonic acid.[62] Morin (3,5,7,2',4',-pentahydroxyflavone)[63] has been used as a fluorimetric reagent for scandium. Concentrations down to 0.1µg./ml. could be determined by measuring the intensity of fluorescence of an ethyl acetate extract from a neutral solution.

Several Schiff's base reagents have been shown to form fluorescent complexes with scandium and other metals of group III[64 - 67]. A brief examination was made of some of these reagents, including salicylaldehyde o-aminophenol, salicylaldehyde phenylhydrazone and salicylaldazine. Several attempts to synthesise salicylaldehyde acetylhydrazone by condensation of salicylaldehyde with hydrazine hydrochloride, followed by acetylation, all yielded the symmetrical salicylaldazine.

This chapter describes the investigation of the

fluorescence reaction of salicylaldehyde semicarbazone (Figure 37) with scandium. It was originally hoped to apply this reagent to the spectrofluorimetric

Figure 37.



SALICYLALDEHYDE SEMICARBAZONE.

determination of zinc, but the sensitivity possible (lower limit 0.1-0.6 ppm. of zinc) was insufficient to merit a detailed investigation. The reagent was prepared by condensation of salicylaldehyde with semicarbazide hydrochloride, followed by recystallization from ethanol. Its scandium complex showed an intense blue fluorescence (excitation maximum 370mμ, fluorescence maximum 455mμ), whereas the reagent itself showed a less intense blue-green fluorescence (325/470mμ) under similar conditions. Continuous variations, mole-ratio and slope-ratio studies indicated

the formation of a 1:1 complex between scandium and the reagent, with a conditional stability constant of 10^5. The probable nature of this complex has been considered.

The effects of time, continuous irradiation at 370mμ and variatioh of temperature on the fluorescence of the complex were examined and optimum conditions were established for an analytical procedure. At pH 6.0, salicylaldehyde semicarbazone could be applied to the spectrofluorimetric determination of scandium in pure solutions in the range 0.2 to 1.8μg. (0.002-0.02 ppm.). The effects of 42 cations and 21 anions on the system were examined. This method was subject to several interferences which could not be eliminated by the use of common masking agents. It was, therefore, necessary to devise a procedure to separate scandium from the more important of these interfering ions and supply it in a state suitable for determination by the proposed method. Separation procedures based on co-precipitation of scandium hydroxide on cadmium hydroxide or extraction of scandium thiocyanate into ether were investigated, but found to be unsatisfactory. The double extraction procedure finally adopted was based on the work of Eberle and Lerner[60] and could

be used for microgram amounts with a recovery of *ca.* 90% in the range 4.5 to 22.5μg. of scandium. Removal of iron (III), vanadium (V), etc. by extraction of their cupferrates from 10% hydrochloric acid into chloroform was followed by extraction of scandium from concentrated hydrochloric acid into tri-*n*-butyl orthophosphate and back-extraction into water. This procedure provided an efficient separation from aluminium and yttrium which, like scandium formed blue fluorescent complexes with salicylaldehyde semicarbazone. Chromium (III), titanium (IV) and zirconium produced low results while tellurium (IV) and tin (IV) completely suppressed the fluorescence of the scandium complex. The reagent was used in ethanolic solution and determinations were consequently carried out in 75% aqueous medium.

Although the sensitivity of this method could not be compared directly with that of other methods unless similar instrumentation were used, the determination of scandium using this reagent appeared to be as sensitive as with morin[63] and considerably more sensitive than available colorimetric methods.[60 - 62]

Experimental.

Reagents.

Standard scandium solution.

0.0690g. of high purity grade scandium oxide, Sc_2O_3, was boiled with 30ml. of concentrated AnalaR hydrochloric acid until fully dissolved. The solution was cooled and diluted to one litre with water. This stock solution was 10^{-3}M in scandium and was diluted to 10^{-4}M when required. Solutions could be standardised by EDTA titration at pH3-5 with Xylenol Orange as indicator[68].

Reagent solution.

Salicylaldehyde semicarbazone was prepared by dissolving 15g. of semicarbazide hydrochloride in 40ml. of water and slowly stirring in 12g. (ca. 10.5ml.) of salicylaldehyde. Stirring was continued for 15 minutes and the solid product filtered off and washed with water followed by ethanol. The white product was dried at 100°C and recrystallised twice from AnalaR ethanol. Its melting-point was 222°C (uncorrected). Final yield was 25%.

Analysis of salicylaldehyde semicarbazone prepared by the above method gave the results shown below:-

	C	H	N	O
Theoretical, %.	53.70	5.06	23.46	17.86
Found, %.	53.47	4.96	23.50	17.77

A 10^{-3}M solution was prepared by dissolving 0.1788g. in one litre of AnalaR ethanol.

Buffer solution, pH6.0.

100g. of G.P.R. grade hexamine (hexamethylene-tetramine) were dissolved in 800ml. of water and hydrochloric acid was added until the pH reacehd 6.0. The solution was diluted to one litre with water.

Diverse ions.

10^{-2}M solutions of AnalaR salts.

Cupferron (AnalaR).

Freshly prepared 1% w/v aqueous solution.

Hydrochloric acid. (AnalaR). S.G. 1.18.

Chloroform (AnalaR)

Tri-n-butyl orthophosphate. (G.P.R. grade).

Ammonia solution. (AnalaR). S.G. 0.88.

Apparatus.

Farrand spectrofluorimeter. 10mμ and 20mμ band-width slits were used in the excitation and analysing monochromators, respectively. The 7-54 filter was used for the excitation radiation.

Vibron pH meter, model 39A.

All calibrated flasks wiich were to contain solutions $\leq 10^{-3}$M in scandium were treated with Repelcote to minimise possible losses by surface adsorption.

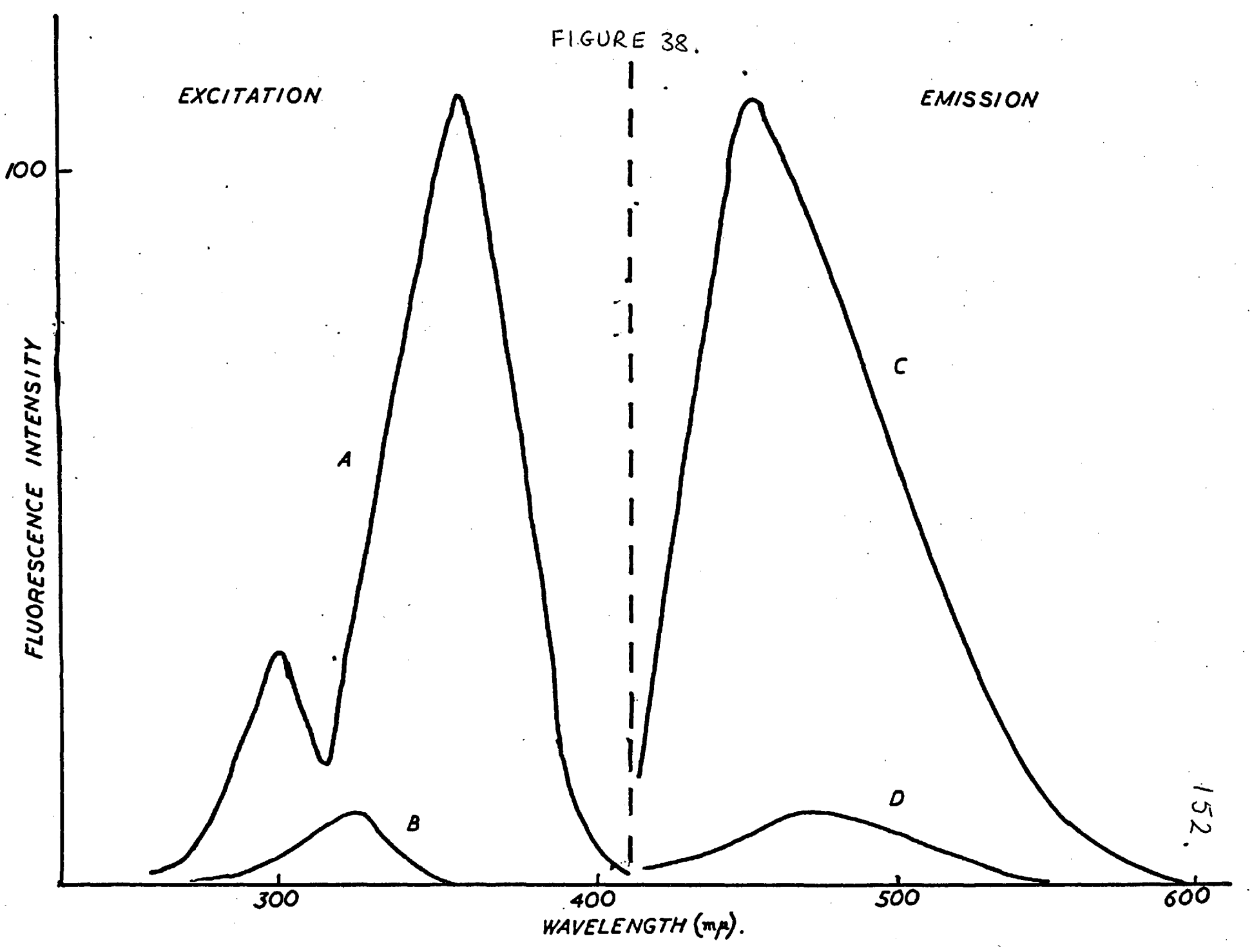
Spectral Characteristics.

Figure 38 shows the excitation and fluorescence emission spectra for the reagent and its scandium complex in aqueous solution at pH6.0. These spectra are uncorrected for the characteristics of lamp emission, monochromator transmission and photomultiplier response. The relevant correction curves are given in Figure 6. Curve A shows the excitation spectrum of the complex (obtained with equimolar amounts of scandium and reagent present), measuring the emission at 455mμ and curve C shows its fluorescence emission spectrum measured with the excitation wavelength set at 370mμ. Curve B shows the excitation spectrum of the reagent alone (emission measured at 470mμ) and curve D, its fluorescence emission spectrum (excitation at 325mμ). As can be seen, salicylaldehyde semicarbazone had its excitation maximum at 325mμ and its fluorescence emission maximum at 470mμ. Its scandium complex showed its excitation maximum at 370mμ and exhibited maximum fluorescence at 455mμ.

Influence of pH.

The intensities of the fluorescence of the reagent and of its scandium complex were measured at 455mμ with an excitation wavelength of 370mμ over a range

FIGURE 38.


EXCITATION
EMISSION
100
FLUORESCENCE INTENSITY
A
B
C
D
300
400
500
600
WAVELENGTH (mμ).

of pH values obtained by adjustment with acetic acid and ammonia. Figure 39 shows the results of these investigations. Curve A was obtained with salicylaldehyde semicarbazone alone and curve B with scandium + reagent, using a 50-fold molar excess of reagent. The fluorescence of salicylaldehyde semicarbazone increased rapidly above pH5, whereas in the presence of scandium the increase occurred at ca. one pH unit lower. Thus, by buffering the solution to pH6, it was possible to obtain a favourable ratio for complex fluorescence to reagent fluorescence (this ratio is plotted in curve C). This pH was readily obtained with hexamine and hydrochloric acid. Owing to the very large rate of change of complex fluorescence with pH, it was necessary to buffer to 6.00 ± 0.05.

Reagent Concentration.

It was shown that a fifty-fold molar excess of reagent was needed to develop fully the complex fluorescence, cf. Figure 40(a) in which the fluorescence intensity (corrected for the fluorescence of the reagent) measured at 370/455mμ is plotted against molar excess of reagent. The solutions were buffered at pH6.0 and contained 1ml. of 10^{-4}M scandium solution with a varying excess of 10^{-4}M ethanolic salicylaldehyde

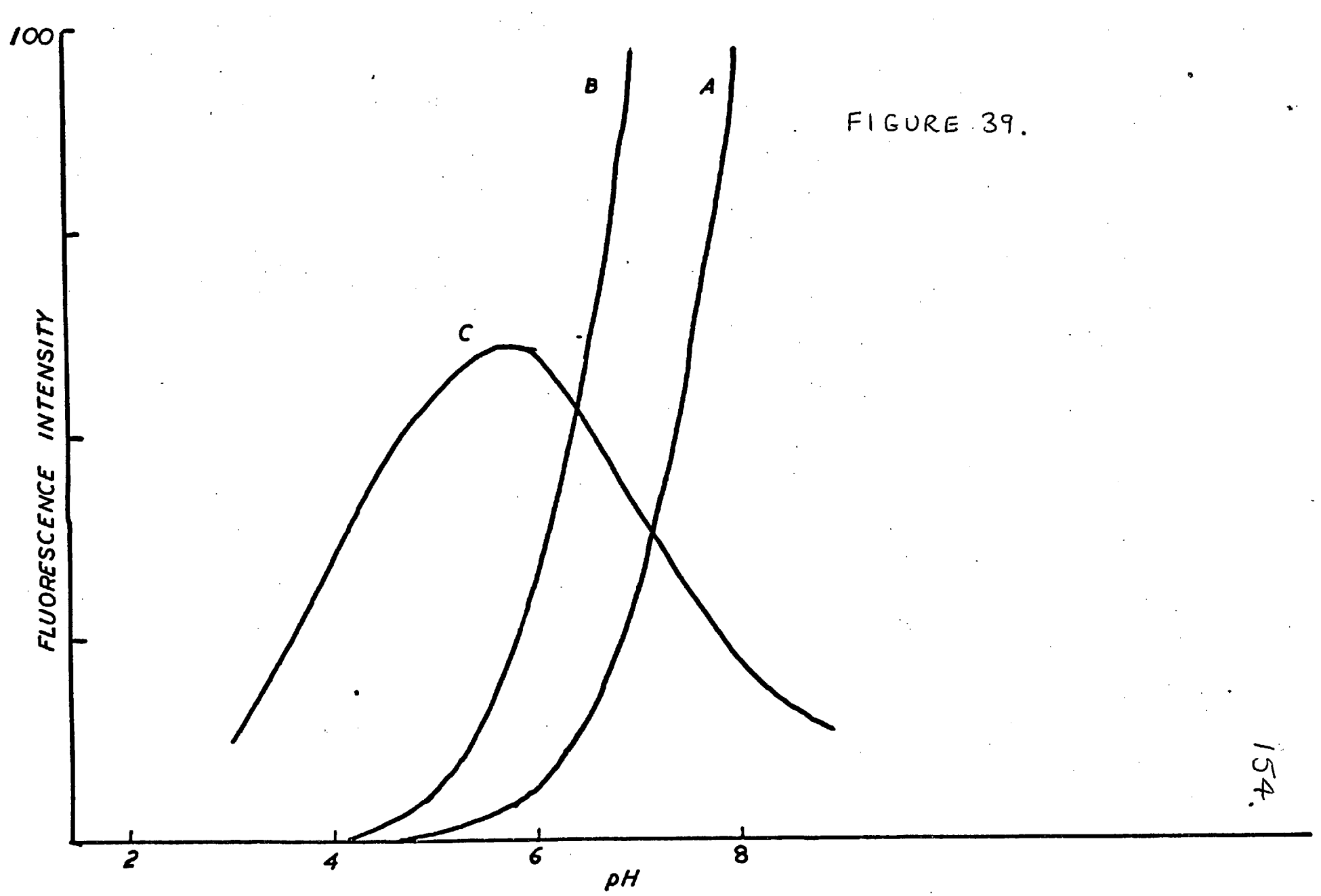
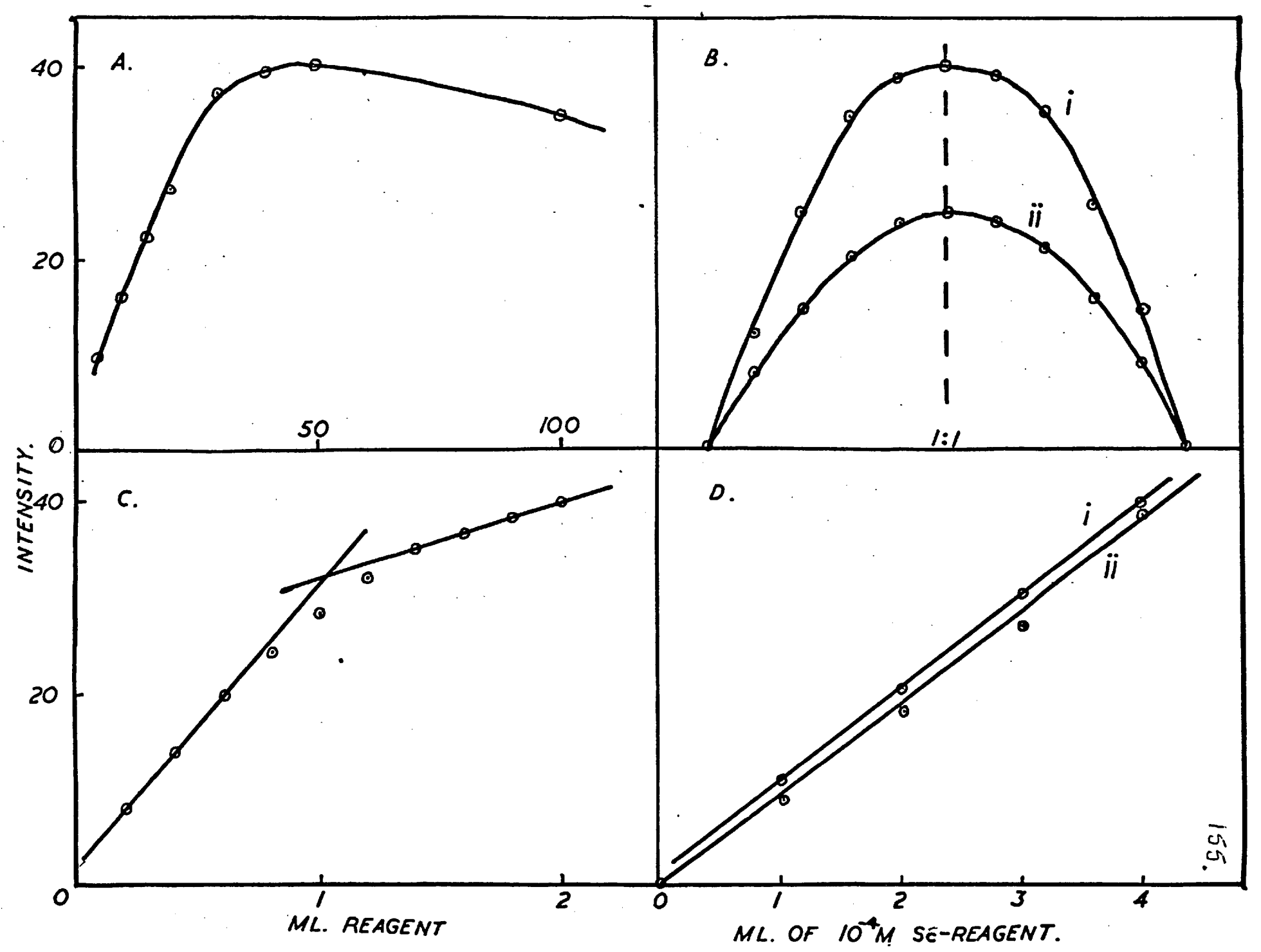


FIGURE 39.


A.
B.
C.
D.
40
20
0
50
100
i
ii
1:1
INTENSITY.
ML. REAGENT
ML. OF 10^{-4} M Se-REAGENT.
1
2
3
4
155.

semicarbazone solution. Increase in ethanol concentration in the final solutions had no marked effect on the intensity of the fluorescence. When measured at the wavelengths of maximum complex fluorescence (370/455mμ), the contribution of the "blank" reagent fluorescence was relatively small, even when the reagent was present in a large excess.

Influence of Time.

A 10^{-3}M ethanolic solution of salicylaldehyde semicarbazone was stable for a period of days in darkness and under normal laboratory lighting. Maximum complex fluorescence was developed within a few minutes and was then constant for more than 24 hours. A period of thirty minutes was selected as a convenient "standing time" before measurement. It was found that deliberate continuous irradiation of the solution at 370mμ in the spectrofluorimeter produced a 50% decrease in complex fluorescence intensity within one hour, but the system showed no measurable decay when subject to normal analytical examinations over a period of several hours.

Effect of Temperature.

Between the limits of the temperature range investigated, viz. 2^{o}-42^{o}C, a decrease of fluorescence

intensity with increase in temperature of development was found, which corresponded to a temperature coefficient of ca. 0.4% per °C. Under normal laboratory conditions, however, all measurements were made at 23±3°C and the observed intensities were compared with those of standards prepared simultaneously, so that the existence of this temperature coefficient did not significantly alter the validity of the results obtained.

Precision data.

These were obtained by measuring the fluorescence intensities of ten separate solutions each containing 1.80µg. of scandium per 100ml. The results are shown in Table 19. These experiments showed the standard deviation to be equivalent to 1.7% of the response due to the scandium complex, corresponding to ± 0.03µg. of scandium.

Effects of foreign ions.

The effects of 42 cations and 21 anions on the scandium determination were investigated at the hundred-fold molar excess level over 1.8µg. of scandium. Under these conditions, ammonium, barium, beryllium, cadmium, calcium, cerium (III), lead, lithium, magnesium, manganese (II), mercury (II), platinum (IV), potassium,

Table 19.

Precision data for determination of 1.80µg. of scandium.

Sample no.	Fluorescence Intensity (x.1)	Net scandium response	Deviation	Variance
1	92.0	76.0	0	0
2	91.0	75.0	-1.0	1.0
3	92.5	76.5	+0.5	0.25
4	94.0	78.0	+2.0	4.0
5	91.0	75.0	-1.0	1.0
6	91.0	75.0	-1.0	1.0
7	93.0	77.0	+1.0	1.0
8	89.5	73.5	-2.5	6.25
9	93.0	77.0	+1.0	1.0
10	92.5	76.5	+0.5	0.25
Blank	16.0	—		
		Mean=76.0		

Standard deviation = $\sqrt{\frac{\Sigma \Delta^2}{n-1}} = \sqrt{\frac{15.75}{9}} = 1.3$ units = 1.7%

sodium, strontium, thallium (I), chloride, bromide, iodide, nitrate, sulphate, cyanide, thiocyanate, nitrite, perchlorate, thiosulphate, acetate and tetraborate did not interfere. The interferences observed are detailed in Table 20.

Table 20.

Effect of foreign ions interfering with scandium determination.

Ion	Variation in fluorescence intensity	Observations
aluminium	+280%	similar blue fluorescence.
antimony (III)	+ 25%	solution cloudy.
arsenic (V)	-100%	fluorescence totally quenched.
bismuth	+124%	white precipitate.
cerium (IV)	-100%	fluorescence totally quenched.
chromium (III)	-100%	fluorescence totally quenched.
cobalt (II)	- 14%	no visible effect.
copper (II)	-100%	fluorescence totally quenched.
gallium	- 16%	probably a pH effect.
gold (III)	- 41%	solution slightly yellow.
indium	- 22%	probably a pH effect.
iron (II)	- 30%	solution slightly yellow.
iron (III)	-100%	solution yellow.
lanthanum	+ 54%	similar blue fluorescence.
nickel	- 23%	no visible effect.
selenium (IV)	- 27%	probably a pH effect.
silver (I)	+ 59%	white precipitate

Table 20. continued.

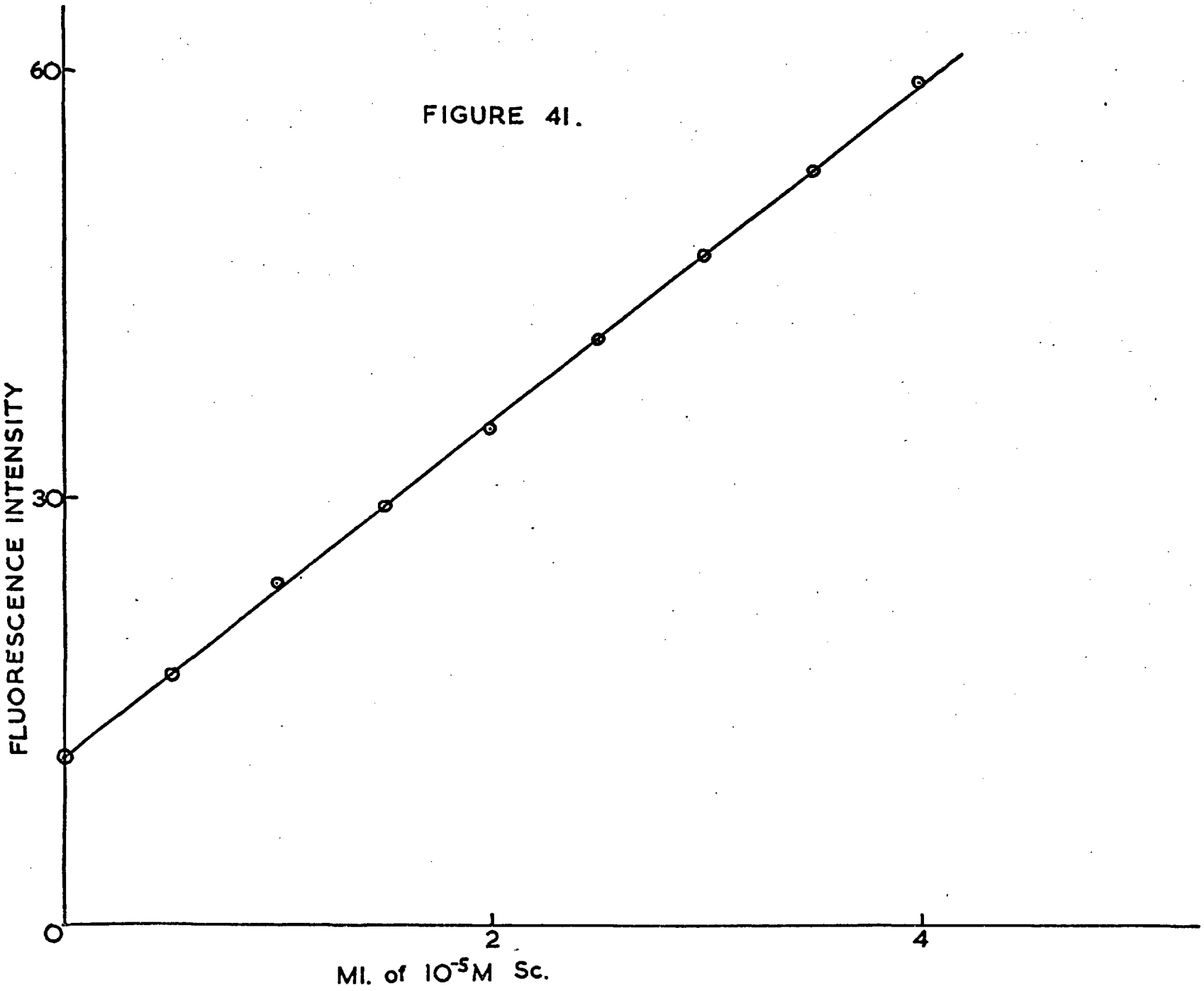
Ion	Variation in fluorescence intensity	Observations
tellurium (IV)	-100%	fluorescence totally quenched.
thorium (IV)	- 45%	solution cloudy.
tin (IV)	-100%	fluorescence totally quenched.
uranium (VI)	-100%	solution yellow.
vanadium (IV)	-100%	fluorescence totally quenched.
vanadium (V)	- 31%	solution yellow.
yttrium	+1000%	similar blue fluorescence.
zinc	+120%	similar blue fluorescence.
zirconium	- 58%	probably a pH effect.
fluoride	-100%	fluorescence totally quenched.
sulphite	- 15%	no visible effect.
silicate	-100%	fluorescence totally quenched.
phosphate	-100%	fluorescence totally quenched.
molybdate	- 50%	no visible effect.
tungstate	-100%	fluorescence totally quenched.
oxalate	-100%	fluorescence totally quenched.
citrate	-100%	fluorescence totally quenched.
tartrate	-100%	fluorescence totally quenched.

The interferences of copper (II), nickel and silver could be eliminated by masking with cyanide and that of zinc with o-phenanthroline. The ions which interfered could be summarised as those which (a) produced a fluorescence similar to that of scandium, e.g. aluminium, yttrium and zinc, (b) precipitated and therefore increased the signal by scattering, e.g. bismuth and antimony, (c) decreased the signal by filter effects, e.g. iron (III) and vanadium (V) and (d) decreased the signal by complexation, e.g. fluoride and tartrate.

Calibration curve.

0.5 to 4.0ml. aliquots of standard 10^{-5}M scandium solution were transferred to 100-ml. calibrated flasks containing 2ml. of pH6.0 buffer solution. Two ml. of 10^{-3}M ethanolic salicylaldehyde semicarbazone were added and the solution was diluted to 100ml. with water from an all-glass distillation apparatus. The intensity of the fluorescence of the solutions was measured after 30 minutes at 455mμ with an excitation wavelength of 370mμ. The plot of fluorescence intensity against the amount of scandium present (0.22 to 1.8μg.) was a straight line and passed slightly above the origin, see Figure 41.

FIGURE 41.



The fluorescence of the reagent blank was equivalent to a scandium content of about 0.2µg. per 100ml. Linearity extended beyond the upper limit examined here. The volume of reagents added was only 4ml., so logically one may deduce that the above procedure could be applied without modification to test solutions with scandium concentrations as low as 0.002ppm.

Structure of Complex.

Figure 40(b) shows the results of two investigations by Job's method of continuous variations[47] at pH6.0, measured at 370/455mµ and corrected for the reagent fluorescence. The total concentration of scandium + reagent solutions was 10^{-5}M for curve A and 7.5×10^{-6}M for curve B. Both clearly indicate the formation of a 1:1 complex. This was confirmed by a Yoe and Jones' mole-ratio study[48], Figure 40(c), in which x ml. of 10^{-3}M reagent solution were added to 1ml. of 10^{-3}M scandium solution at pH6.0 and measured at 370/455mµ. The results of a Harvey-Manning slope-ratio study[49] are shown in Figure 40(d). Curve A was obtained with 10ml. of 10^{-3}M scandium solution plus x ml. of 10^{-4}M reagent solution and curve B with 10ml. of 10^{-3}M reagent solution plus x ml. of 10^{-4}M scandium solution.

Measurements of fluorescence intensity against pH indicated that salicylaldehyde semicarbazone was unionised at pH6.0 and this was confirmed by studying the variations in the absorption and fluorescence excitation spectra which showed different peaks above and below ca. pH7. At pH5 the principal absorption maxima occurred at 255 and 310mμ, whereas at pH10 the peaks were at 275 and 355mμ.

Scandium (co-ordination number, 6) tends to hydrolyse to polymeric species so that the complex formation may be postulated as:-

$$n\ RH + [Sc(OH_3]_n \longrightarrow [RSc(OH)_2]_n + n\ H_2O$$

where RH represents the salicylaldehyde semicarbazone.

This agreed with the results of potentiometric titrations with standard alkali which did not show the liberation of titratable hydrogen ions on complex formation. This could be explained if the hydrogen ions liberated by complexation were converted to water as indicated above.

Stability of Complex.

The apparent or conditional stability constant of the 1:1 scandium-reagent complex formed under the conditions

of these experiments was evaluated by the method described in Chapter 2. The results are given in Table 21.

Table 21.

Values of Conditional Constant for Scandium Complex from Continuous Variations Curves.

Fluorescence Intensity	Ml. of 10^{-4}M solutions M	D	M'	D'	K
12.5	9.20	0.80	6.40	1.10	2.10×10^4
15	9.00	1.00	6.15	1.35	4.45×10^4
17.5	8.80	1.20	5.85	1.65	5.13×10^4
20	8.58	1.42	5.55	1.95	7.70×10^4
22.5	8.35	1.65	5.20	2.30	10.42×10^4

Because of the difficulty of reading M and D accurately from the Job plots, the highest value, $K = 1.04 \times 10^5$, is probably the most reliable of these results.

Another calculation of the stability constant was made from dissociation data obtained by measurement of the intensity of fluorescence of solutions containing (a) 1:1 molar proportions of scandium and reagent and (b) a large excess of reagent. Experiments using 5ml. of 10^{-5}M solutions of scandium and reagent

diluted to 100ml. gave a fluorescence intensity (after subtraction of the reagent blank) of 24.4 x .1, while the same amount of scandium with a hundred-fold excess of reagent gave an intensity of 47.1 x 1. Assuming that the scandium was fully complexed under these conditions, the degree of dissociation in the solutions containing 1:1 molar proportions was, therefore,

$$\frac{47.1 - 2.4}{47.1} = 0.95.$$

Now in these solutions the concentration of the complex (assuming total association) would be 5×10^{-7}M and the stability constant could be written as:-

$$K = \frac{1 - \alpha}{\alpha^2 C} = \frac{1 - 0.95}{(0.95)^2 \times 5 \times 10^{-7}}$$

$$= \underline{1.1 \times 10^5}$$

It will be seen that this value is in close agreement with that obtained by calculation from the continuous variations curves.

Possible extraction systems.

The blue fluorescent scandium complex was not extracted from aqueous solution at pH6.0 into diethyl

ether, iso-butyl methyl ketone, iso-amyl acetate, benzene, chloroform, carbon tetrachloride nor higher n-alcohols.

The number of cationic interferences which could not be eliminated by the use of masking agents necessitated the development of a method for the isolation of microgram amounts of scandium. The first system investigated used a diethyl ether extraction of scandium thiocyanate from dilute hydrochloric acid[69]. This method gave a good signal: blank ratio for relatively large amounts of scandium (50µg.), but the determination of 2µg. was rendered impossible by high and non-reproducible blank values. These were attributed to a positive interference from metallic impurities (e.g. aluminium, zinc, yttrium, etc.) in one or more of the reagents used during the separation procedure, viz. ammonium thiocyanate, diethyl ether, hydrochloric acid and nitric acid. After evaporation to dryness, all three of the above liquids showed a positive fluorescence with salicylaldehyde semicarbazone, that of the nitric acid being most pronounced. The hydrochloric acid interference could be minimised by using only 1ml. of solution and that of diethyl ether was removed by distillation. Redistillation did not remove

the impurity in the nitric acid, however, and this was indispensable in the extraction procedure, being used to destroy the thiocyanate present in the organic extracts. The fluorescence of the impurity was not removed by o-phenanthroline but was prevented by the addition of fluoride which suggested that the probable cause was the presence of aluminium. Owing to this difficulty and the need to destroy thiocyanate, this method was abandoned in favour of a separation of scandium by a co-precipitation procedure.

2.5µg. of scandium could be co-precipitated with cadmium by addition of a sodium tetraborate solution, pH9.2. This procedure gave a quantitative recovery of scandium but was valueless as a means of separation because aluminium also co-precipitated under these conditions.

Eberle and Lerner[60] have described a comprehensive, but extremely lengthy, separation scheme for scandium using both solvent extraction and precipitation processes. An abbreviated, two-stage form of this method was investigated which it was hoped would separate scandium from the more important interferences and supply it in a suitable state for determination by the proposed method. The first stage employed a chloroform

extraction to remove the cupferrates of a number of interferences, viz. antimony (III), iron (III), vanadium (V), etc. Difficulties were encountered in this owing to inner-filter effects caused by decomposition products of cupferron which were not removed by washing with chloroform and/or iso-amyl acetate. These could be destroyed by boiling with a perchloric acid-sulphuric acid mixture and then, after making allowances for the high blank values probably caused by impurities in the reagents, it was shown that scandium was retained quantitatively through this extraction procedure.

In the second stage, scandium was separated from aluminium, chromium, yttrium, etc. by extraction from concentrated hydrochloric acid into tri-n-butyl orthophosphate and back extraction into water. This step also gave rise to a relatively large blank value, which is discussed in more detail later. The final aqueous solution was also shown, by a phosphomolybdate amplification procedure using 2-amino-4-chlorobenzenethiol hydrochloride[70], to contain a small amount of phosphate ion, probably from hydrolysis of the solvent during the extraction procedure. The amount present, however, was not enough to produce significant inter-

ference in the determination of scandium.

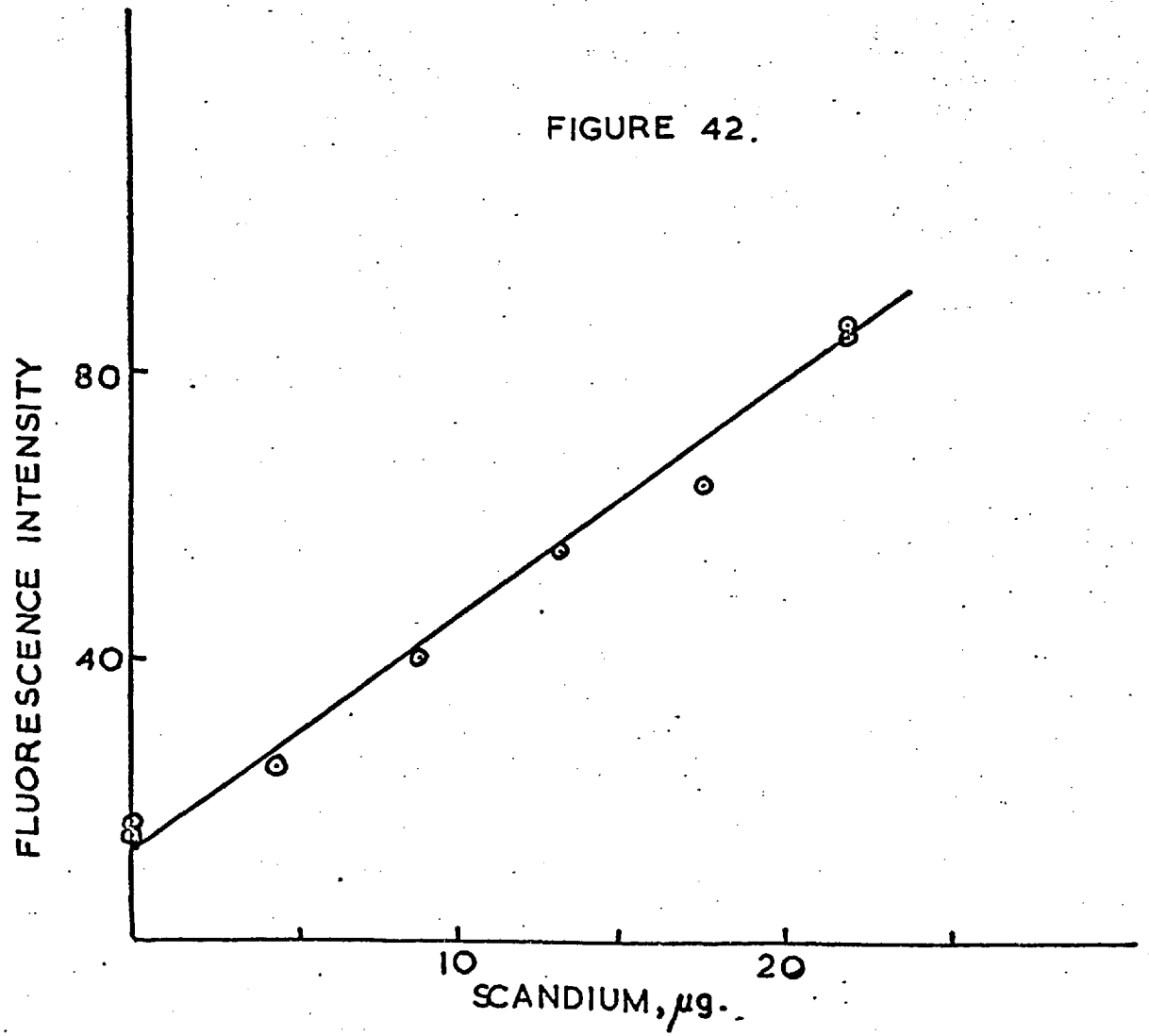
Procedure.

To an aliquot of solution containing 4.5 to 22.5µg. of scandium in a 250ml. separating funnel were added 2.5ml. of concentrated hydrochloric acid and 5ml. of 1% aqueous cupferron solution. After dilution to 25ml. with water, the solution was extracted twice by shaking for one minute with 25ml. of chloroform and the organic (lower phase) extracts were discarded. The aqueous phase was transferred to a 100ml. beaker, the funnel rinsed with water, the washings added to the beaker and the solution then evaporated to a volume of *ca.* 5 ml. The solution was cooled and added to a 250ml. separating funnel containing 25ml. of tri-*n*-butyl orthophosphate. The beaker was rinsed with 25ml. of concentrated hydrochloric acid and the washings were added to the separating funnel. The funnel was shaken for one minute, the phases allowed to separate and the lower, aqeeous layer was discarded. The organic phase was washed twice with 25ml. of concentrated hydrochloric acid and these washings were discarded. The scandium was back-extracted into 25ml. of water containing 1ml. of ammonia solution (the aqueous extract was still acid to litmus at this stage). The

phases were separated and the aqueous phase was shaken twice with 25ml. of chloroform to remove any tri-n-butyl orthophosphate which remained; the chloroform extracts were discarded. The aqueous layer was transferred to a 100-ml. beaker and the washings from the funnel and 10ml. of ammonia were added to the beaker which was covered with a watchglass and evaporated to ca. 20ml. After cooling and redissolving the crystalline ammonium chloride in water, the solution was added to a 100ml. calibrated flask containing 2ml. of 10% hexamine buffer. (pH adjustment was extremely critical in this method, the tolerance as established previously being ± 0.05 units. This combination of ammonium chloride and hexamine consistently produced pH 6.00 ± 0.02). 25ml. of 10^{-3}M reagent solution were added, the solution diluted to the mark with water and the fluorescence intensity measured after 30 minutes at 455mμ, with an excitation wavelength of 370mμ.

The graph of fluorescence intensity against the amount of scandium was linear from 4.5 to 22.5μg. of scandium and passed slightly above the origin (see Figure 42). Two standards were prepared and measured with each group of samples, by taking 0 and 22.5μg.

FIGURE 42.



(5ml. of 10^{-4}M solution) of scandium through the above procedure.

The complete separation and determination of a set of eight samples required 6-7 hours.

Interferences.

The more important interfering cations found previously were taken, normally in 100-fold molar excess over 22.5µg. of scandium, through the recommended extraction procedure. The results are given in Table 22.

This investigation showed that the following ions did not interfere appreciably under the above conditions: aluminium, bismuth, cerium (IV), copper (II), gallium, iron (III), uranium (VI), vanadium (V) and yttrium. The only ions investigated which still interfered were chromium (III), tellurium (IV), tin (IV), titanium (IV) and zirconium. It had already been shown that a number of cations did not interfere while the interference of others could be eliminated by masking with cyanide or o-phenanthroline.

Precision data.

The standard deviation of the combined separation and determination procedures was determined by taking eight solutions, each containing 22.5µg. of scandium

Table 22.

Determination of 22.5µg. of scandium after separation from various ions.

Foreign ions present µg.	Scandium found µg.	Error %
Iron (III)(2,790)	21.4	-4.8
Iron (III)(2,790)+Ce(IV)(7,000)	23.1	+2.7
Yttrium (890)	23.1	+2.7
Yttrium (890)	22.8	+1.3
Aluminium (270)	21.4	-5.0
Aluminium (270)	22.7	+1.1
Bismuth (10,400)	21.7	-3.5
Copper (II)(3,170)	22.1	-1.8
Uranium (VI)(11,900)	21.7	-3.5
Vanadium (V)(2,550)	21.8	-3.0
Gallium (3,500)	22.2	-1.3
Chromium (III)(2,600)	18.2	-19
Titanium (IV)(2,400)	19.1	-15
Zirconium (4,500)	12.6	-44
Tellurium (IV)(6,400)	0	-100
Tin (IV)(5,900)	0	-100

without any foreign ions, through the proposed method. The results are given in Table 23. These experiments showed the standard deviation to be equivalent to 6.2% of the response due to the scandium complex, corresponding to ± 1.4µg. of scandium.

Table 23.

Precision data for determination of 22.5µg. of scandium after extraction procedure.

Sample no.	Fluorescence Intensity (x 1)	Net scandium response	Deviation	Variance
1	22.1	19.4	+0.6	0.36
2	23.5	20.8	+2.0	4.00
3	21.1	18.4	-0.4	0.16
4	21.2	18.5	-0.3	0.09
5	22.0	19.3	+0.5	0.25
6	19.5	16.8	-2.0	4.00
7	22.0	19.3	+0.5	0.25
8	21.0	18.3	-0.5	0.25
Blank	2.7	—		
		Mean = 18.8		

Standard deviation $= \sqrt{\frac{\Sigma\Delta^2}{n-1}} = \sqrt{\frac{9.36}{7}} = 1.16$ units $= 6.2\%$

The standard deviation calculated from the first eleven results in Table 22 was 3.1%. However, all of these determinations were carried out in duplicate and compared with standards prepared simultaneously and in some instances an obviously discrepant result was rejected. The value of 6.2% was, therefore, probably a more realistic estimate of the precision

of this method.

Magnitude and Origin of Blank Fluorescence.

It may be seen from Figure 42 that the fluorescence of the blank was approximately equivalent to that of 4µg. of scandium. This value was caused by the pick-up of some unidentified material during the extraction procedure, which produced a purple fluorescence on addition of the reagent (excitation maximum 350mµ, fluorescence maximum 440mµ) which was unlike that produced by any metal ion investigated. The magnitude of this blank fluorescence governed the range of concentrations over which scandium could be determined with a satisfactory signal to blank fluorescence ratio. The most likely source of this impurity was the tri-n-butyl orthophosphate used, which could not be obtained in analytical grade purity. A prior washing of the TBP with water or concentrated hydrochloric acid effected no improvement. Attempts were made to purify the TBP by redistillation at reduced pressure, but, without a very high vacuum, TBP decomposed at its boiling point to a yellow liquid. More satisfactory results might have been obtained using steam distillation in the presence of sodium hydroxide as a means of purification[71].

CHAPTER 5

Discussion and Suggestions for Future Work.

In the author's opinion, solution spectrofluorimetry as an inorganic analytical technique has now reached a crossroads. It has the basic advantage of considerably greater sensitivity than many other readily available instrumental methods, e.g. solution spectrophotometry and atomic absorption spectroscopy and this, to some extent, compensates for the relatively large number of interferences usually encountered in the spectrofluorimetric methods at present available. Because of its generally superior sensitivity, if the technique can now be applied to a much wider range of elements than hitherto, there is no reason why it should not soon rival spectrophotometry in scope and selectivity. If the technique continues to rest chiefly on the usually unselective reactions of organic reagents with inorganic ions, however, it is likely that its status will decline so that it will gradually be discarded as an interesting but, in general, inapplicable method of analysis. Its future course will obviously be decided by the ingenuity of efforts made (1) to improve the selectivity of the

technique and (2) to extend its scope.

(1) At least two paths may be taken in attempts to improve the selectivity of spectrofluorimetric methods:

(a) a search for "selective" reagents which form fluorescenct complexes with a particular inorganic species and which eliminate the need for prior separation of the element to be determined,

(b) a reappraisal of the technique and improvement of its selectivity by the use of different instrumentation.

Of these two approaches, it seems likely that (b) will be the more rewarding. To follow (a) would be to pursue, from the viewpoint of spectrofluorimetry, the search for functional groupings which react selectively with cations or anions. The results of this approach in spectrophotometry may be taken as a guide for forecasting what may occur in spectrofluorimetry. The search will be made even more complex by the fundamental need for the organic molecule to absorb radiation (as in spectrophotometry) before re-emission. Thus, all the factors which contribute to lack of selectivity of light absorption will be present, as well as factors which affect the emission process.

A rewarding approach under (b) may be to attempt to employ discrete radiation sources and to ensure that the sample irradiated absorbs only at discrete wavelengths. Any methods by which absorption bands in solution can be made narrower will contribute to this end. It is possible that low temperature fluorescence and phosphorescence will be inherently more selective because of considerations of this type. Furthermore, the increase in fluorescence intensity of a system with decrease in temperature is well-known. Recent work in the U.S.S.R.[72] has shown that, at 77°K, many inorganic systems, non-fluorescent under normal conditions, emit a characteristic fluorescence. Although, at its present stage of development, this phenomenon is probably of more interest to a physical chemist, if suitable modifications can be made to existing apparatus relatively easily, there is no reason why this work should not have analytical applications.

(2) The determination of metals such as aluminium, beryllium and zinc which readily form fluorescent complexes with organic reagents has already been investigated fairly thoroughly and there is limited room for further improvements in

this field. The present need is for straightforward methods of determination of those elements which do not fall into this category, the transition metals being an obvious example. Since these cannot be done directly by existing methods, it would seem sensible to look for some simple indirect methods of overcoming this difficulty. The present author has undertaken some preliminary work on two such methods and these are described in the following pages.

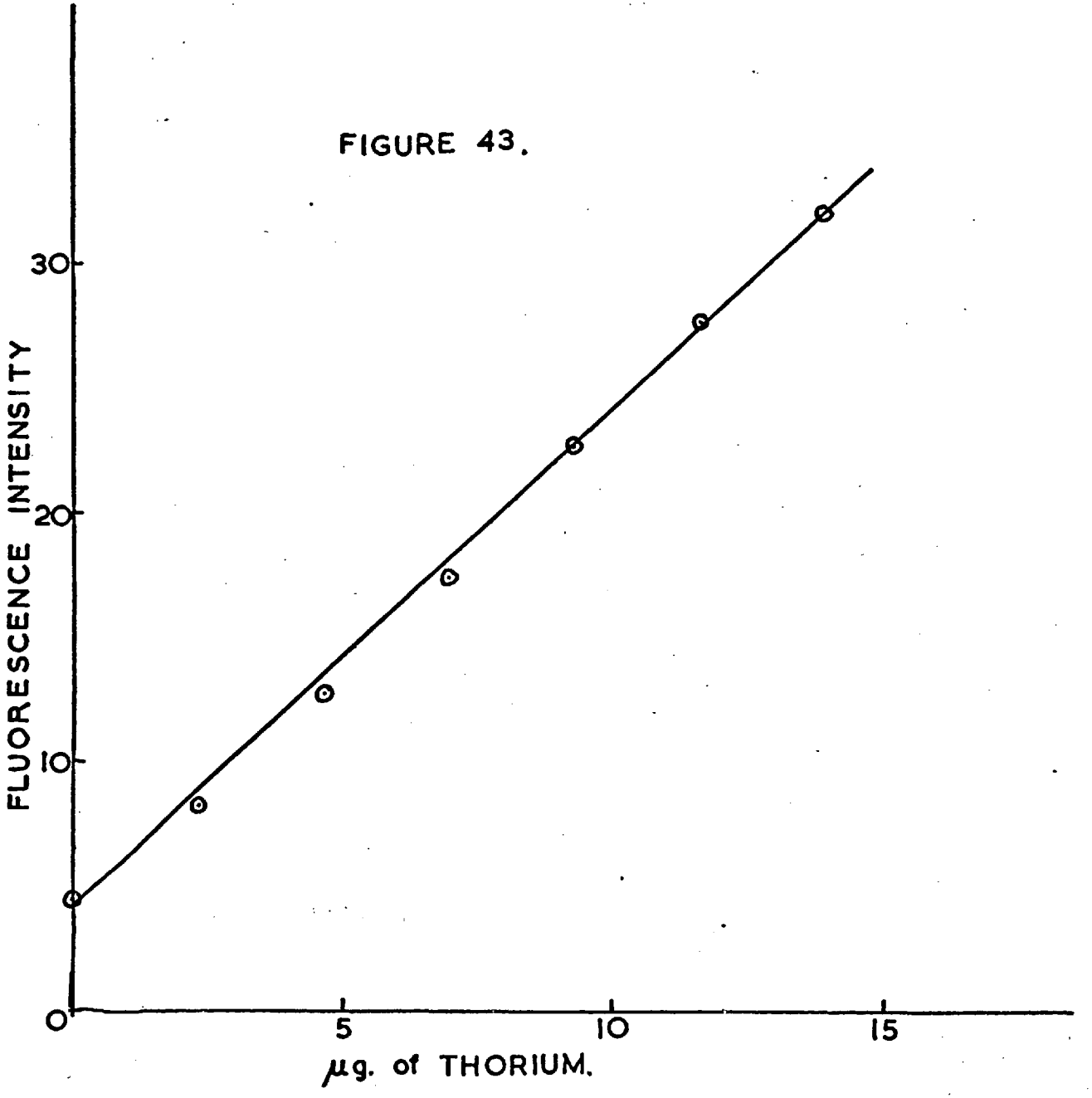
(1) An indirect spectrofluorimetric determination of thorium.

Previous workers[73] have described the indirect fluorimetric determination of microgram amounts of cyanide using a selective replacement reaction. Addition of cyanide removed palladium (II) from its 8-hydroxy-5-quinoline sulphonic acid chelate and the reagent thereby released combined with magnesium ions to form a fluorescent complex. The intensity of fluorescence then measured was directly proportional to the amount of cyanide added. This type of indirect method seemed likely to have applications for the determination of other ions not normally possible by spectrofluorimetry. As a trial, the technique was, therefore, applied to the determination of thorium,

although this has previously been done spectrofluorimetrically using Morin as reagent[74].

The thorium-EDTA complex has a stability constant of 23.2 compared with 15.5 for the aluminium-EDTA chelate. Therefore, if thorium is added to a solution containing equimolar amounts of aluminium and EDTA, aluminium will be released quantitatively and thus will be available to combine with a suitable reagent to form a fluorescent complex. This was carried out by allowing up to 6ml. of 10^{-5}M thorium solution (1-14µg. of thorium) to stand for 60 minutes with 1ml. of 10^{-3}M aluminium-EDTA solution and 50ml. of water. The solution was then buffered to pH5.8 and the aluminium liberated was determined by measurement of its blue fluorescent complex formed with 2-hydroxy-3-naphthoic acid, see Chapter 2. These experiments gave a straight line calibration curve, see Figure 43. Optimum conditions for the method were established, viz. a 20-fold molar excess of aluminium-EDTA solution over the maximum amount of thorium with 60 minutes allowed for the replacement reaction, then addition of 1ml. of pH5.8 acetate buffer, a 20-fold molar excess of 2-hydroxy-3-naphthoic acid over the maximum amount of thorium and dilution to 100ml. with water.

FIGURE 43.



The fluorescence intensity was measured after 30 minutes at 380/460mμ.

In the experiments described above, the replacement reaction took place at an unbuffered pH of ca. 4.7, the exact pH depending upon the amount of thorium present. If the solution was previously buffered to pH5.8, however, using either ammonium acetate or hexamine, no replacement occurred. Furthermore, when the pH was lowered (to 1.0, 3.6 or 4.1) in attempts to accelerate the replacement reaction, little replacement occurred. Therefore, the replacement of aluminium in its EDTA chelate by thorium was much retarded if not prevented by an increase in the ionic strength of the medium.

Although the system described above did not appear to be readily applicable, this method of indirect determination by replacement and subsequent formation of a fluorescent complex has considerable promise if the replacement reactions can be carried out in the presence of foreign ions. Thus the determination of thorium would be improved by cyanide masking of interferences. These investigations were made in detail using thorium, but it was also shown that the above procedure could be applied similarly to the

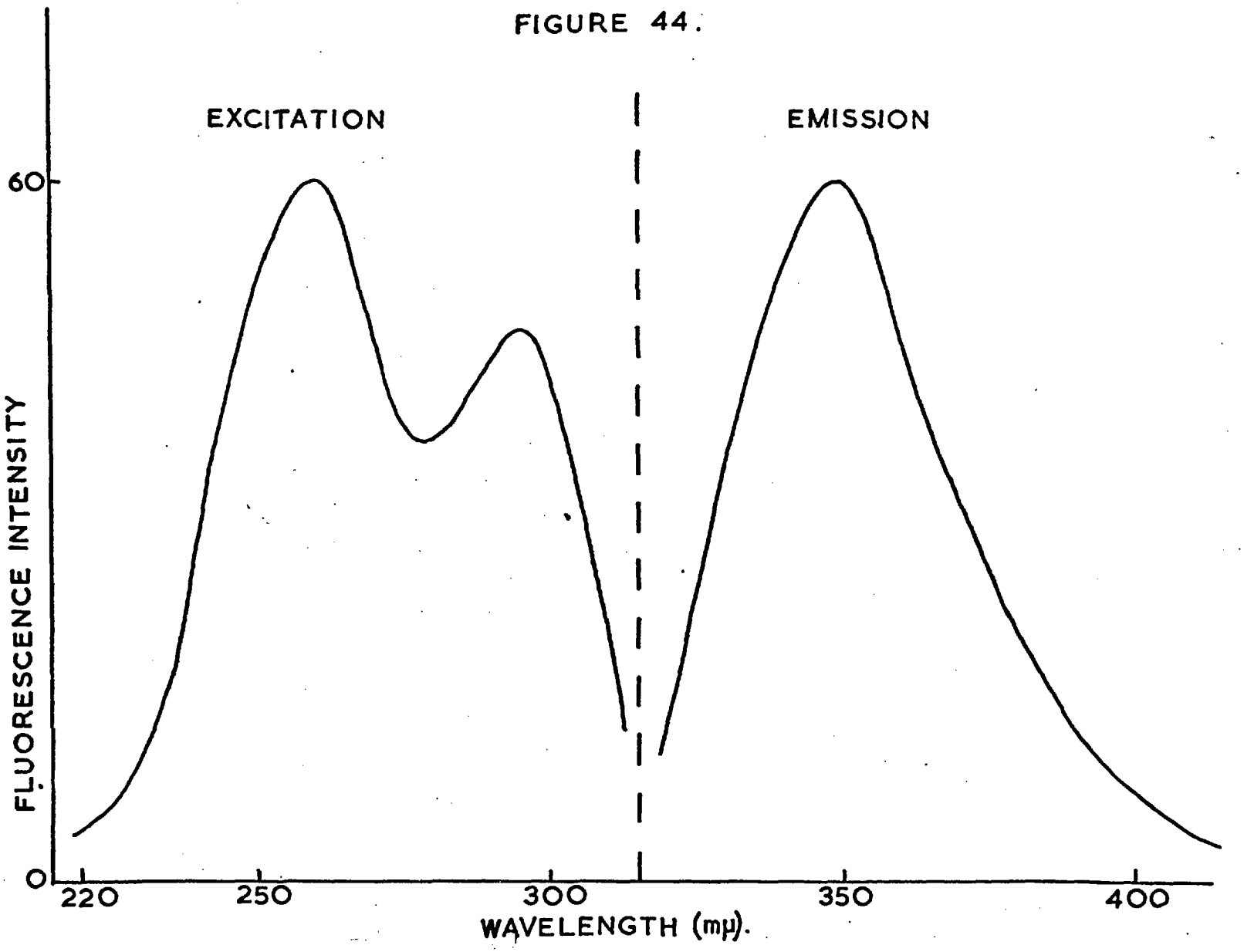
determination of iron (III), which has an EDTA stability constant of 25.1 and for which no direct spectrofluorimetric methods exist.

Cations which form stable EDTA chelates could also be determined by the release of zinc from its EDTA complex, followed by formation of a fluorescent complex with, for example, salicylaldehyde acetylhydrazone[75]. Substitution of another complexing agent, e.g. cyanide, in place of EDTA should lead to further extensions of the scope of this technique to the determination of other ions which do not themselves form fluorescent complexes.

(2) An indirect spectrofluorimetric determination of arsenic (III).

Cerium (III) ions in dilute sulphuric acid exhibit a characteristic fluorescence[76], which, using the Farrand spectrofluorimeter, shows its excitation maximum at 260mμ (with a secondary peak at 295mμ) and fluorescence emission maximum at 350mμ, see Figure 44. This provides a method of determination of cerium (III) at concentrations down to ca. 0.1ppm. The system was shown to be unaffected by change in acid concentration between 0.02 and 1M and by a high concentration of electrolyte.

FIGURE 44.



185.

By using the redox reaction:-

$$2\ Ce(IV) + As(III) \longrightarrow 2\ Ce(III) + As(V)$$

which is catalysed by osmium tetroxide[77], arsenic (III) can be determined indirectly by measurement of the cerium (III) fluorescence produced. It will be noted that there is a two-fold molar amplification of arsenic (III) in this reaction. The variation in the fluorescence intensity produced by a varying amount of cerium (IV) with a constant amount of arsenic (III) is shown in Figure 45.

In 0.05M sulphuric acid and using a two-fold equivalent excess of cerium (IV), the measured fluorescence intensity at 260/350mμ is directly proportional to the amount of arsenic (III) present (0-0.38ppm.), see Figure 46. One drop of 0.05% osmium tetroxide solution is sufficient to promote a rapid reaction and fluorescence measurements are conveniently made 15 minutes after mixing the solutions. Many cations, including the other rare earths, do not interfere with this system at room temperature, but interference is caused by ions which have oxidising or reducing properties under these conditions and by ions which

FIGURE 45

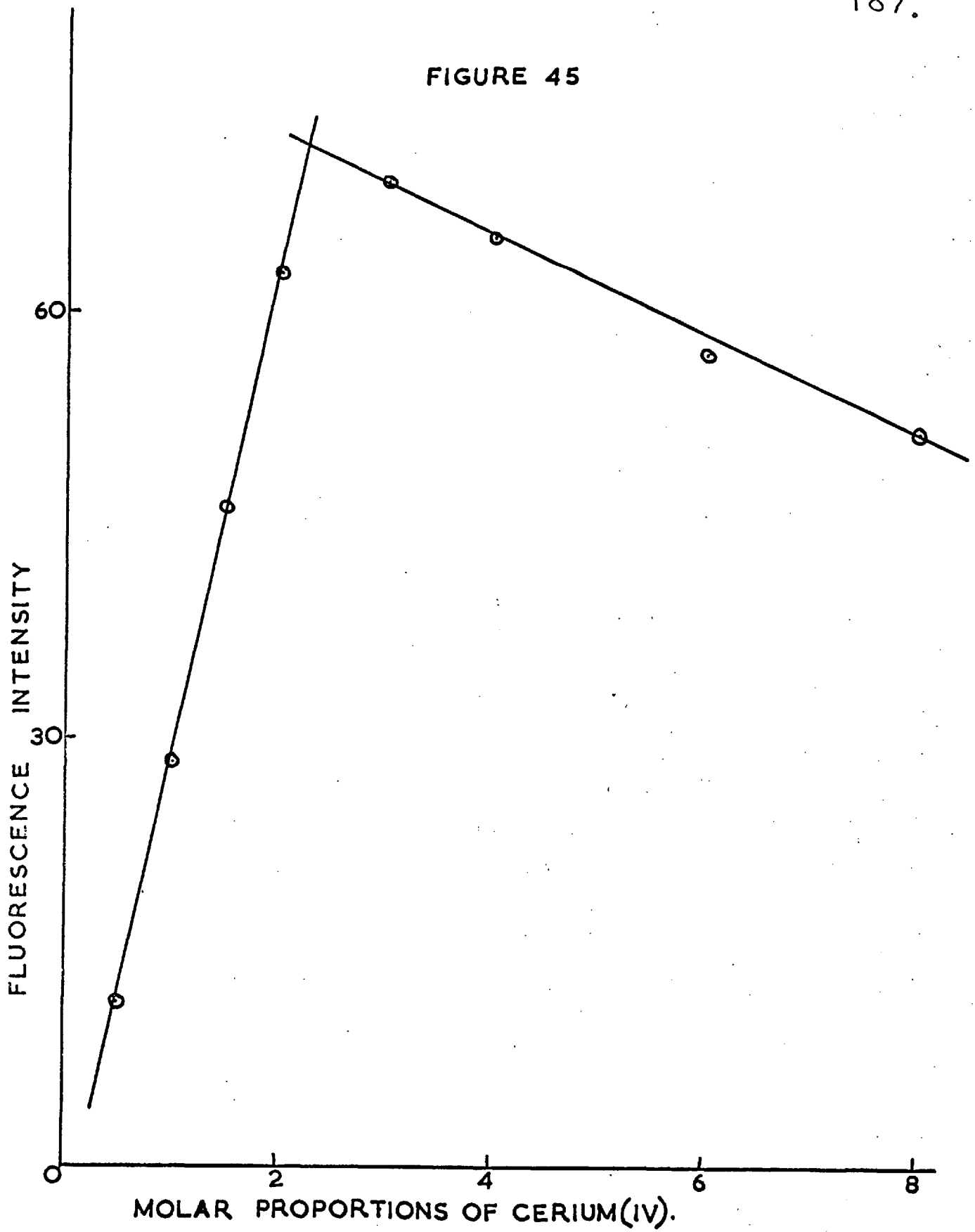
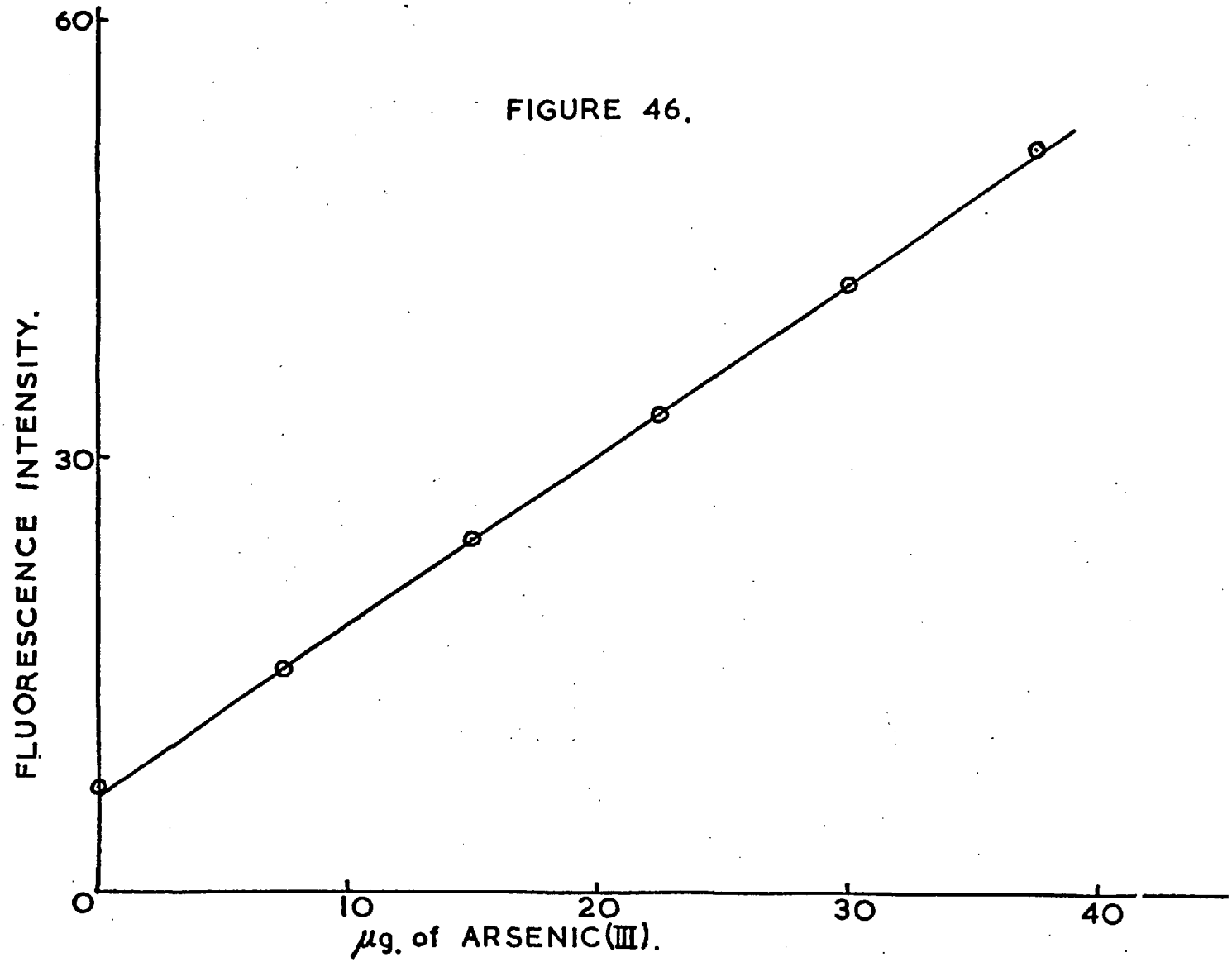

60
30
0
FLUORESCENCE INTENSITY
2
4
6
8
MOLAR PROPORTIONS OF CERIUM(IV).

FIGURE 46.



absorb at either 260mμ or 350mμ, e.g. nitrate, iron (III), etc. The presence of complexing agents (citrate, tartrate, EDTA, etc.) causes high results by favouring the reduction of cerium (IV).

If this system were to be applied to the determination of arsenic in technical materials, a prior separation would probably be necessary. However, the system has considerable interest as an example of a spectrofluorimetric determination of an element which shows no known fluorescence reactions. Furthermore, it may be possible to apply this particular reaction to the determination of trace amounts of osmium in a similar way to the kinetic spectrophotometric method already available[78].

The system should also be applicable to the determination of cerium, either directly as cerium (III) or indirectly as cerium (IV). If an excess of arsenic (III) solution were added to an unknown cerium (IV] solution, the intensity of the cerium (III) fluorescence generated would be proportional to the cerium content.

It is possible to make several suggestions for future investigations in the field of organic spectrofluorimetric reagents. Holzbecher[75] has described investigations of several salicylaldehyde condensation

products as reagents for zinc. Salicylaldehyde acetylhydrazone was particularly recommended and a thorough investigation of this system would be worthwhile.

Hydroxyanthraquinone derivatives should be investigated as possible fluorimetric reagents. Some preliminary studies were made by the author with 1,5-dihydroxy-, 1,8-dihydroxy-, 1,2,4-trihydroxy-, 1,2,5-trihydroxy-, 1,2,6-trihydroxy and 1,2,5,8-tetrahydroxyanthraquinone, but all of this work was handicapped by the lack of a red-sensitive photomultiplier. The metal-complexes formed by these reagents are usually yellow or red so that any fluorescence emitted would be expected to be of wavelength >600mμ.

8-aminoquinoline can be prepared by autoclaving 8-hydroxyquinoline with ammonia and sulphur dioxide at high temperature and pressure[79]. The preparation and investigation of a range of Schiff's base derivatives of this reagent might be a fruitful field of study.

Many examples have been described of titrations which use a fluorescent indication of the end-point. For example, Stolyarov[80] has described micro-titrations of aluminium with salicylate or oxalate, following the course of the titration by measurement of the fluorescence

of the aluminium-Morin complex. Now, photometric titration methods are inherently more accurate than corresponding direct methods, an accuracy and precision of <1% being easily attainable. Therefore, by employing titrations of a metal ion against a suitable fluorimetric reagent, or vice versa, and following the change in fluorescence intensity with titre, it should be possible to combine the advantages of spectrofluorimetry (sensitivity) and titrimetry (precision and accuracy). The design of suitable apparatus for this work should present little difficulty and the technique could find wide application in the determination of metals which form fluorescent complexes instantaneously with organic reagents.

An application of spectrofluorimetry in organic chemistry may be found in the study of the kinetics of reactions which either have fluorescent reactants or a fluorescent product. For example, if salicylaldehyde is allowed to react with semicarbazide hydrochloride in an aqueous-ethanol medium, the formation of the condensation product, salicylaldehyde semicarbazone, may be followed by measuring the intensity of the blue fluorescence of the product. The range of reactions to which this technique could be applied

is obviously limited, but some valuable results could probably be obtained.

The need to extend the scope of spectrofluorimetry has already been discussed. In the course of the author's work on thallium, described in Chapter 1, the fluorescence of lead (II) in chloride medium was observed. A short further examination was made of this and it was shown to be possible to construct a calibration curve for lead in the range 10^{-6} to 10^{-5}M, measuring the fluorescence at 270/480mμ. The fluorescence of tin (II) in chloride medium (260/470mμ) has also been observed, but this has much lower sensitivity then those of thallium (I), lead and cerium (III).

Several workers have studied examples of energy transfer fluorescence reactions in which an organic molecule is caused to react with an inorganic ion which has a characteristic fluorescence. Incident radiation is then absorbed by the organic part of the complex molecule, transferred to the inorganic part and fluorescence is emitted at the wavelength characteristic of the inorganic ion. This method affords a considerable increase in sensitivity, but is obviously restricted to inorganic ions which emit

a characteristic fluorescence in solution. Work to date has largely been restricted to the determination of the rare earths.

A more rewarding field may be found in the study of ternary complexes as a means of determination of metals. Some of the author's colleagues[81] have studied complexes of the type; transition metal: nitrogenous base: anionic dyestuff, e.g. copper (II): o-phenanthroline: Rose Bengal [tetrachloro(P)tetraiodo-(R)fluorescein]. Another well-known example of a ternary system uses the extraction into benzene of the complex between a metal which forms a singly-charged chloroanion (gallium, gold, etc.,) and an organic reagent such as Rhodamine B (Colour Index 45170). This type of system was used, for example, by Onishi[30] for the determination of thallium (III) and is probably capable of wider applications.

The determination of small amounts of phosphorus has been the subject of intensive study. The basis of a sensitive spectrofluorimetric method may be found in the formation of the quininephosphomolybdate complex, which can be precipitated from acid medium[82,83]. If this complex can be separated from the excess quinine present, preferably by solvent extraction,

it will be possible to break the complex and subsequently measure the fluorescence of the quinine released, which should be directly proportional to the amount of phosphate in the original solution.

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