

THE SOLUTION SPECTROFLUORIMETRY OF INORGANIC

CHELATE COMPLEXES.

by

RICHARD SMITH B.Sc. (London), A.R.C.S.

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Department of Chemistry,
Imperial College of Science and Technology.

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ABSTRACT

Some aspects of the use of solution spectrofluorimetry, as applied to the determination of trace quantities of metals in solution, have been examined. The investigation of quinine as a spectrofluorimetric primary standard was followed by using quinine as the basis of a scale of reaction sensitivities. The use of quinine in the determination of quantum yields of fluorescence for some standard substances was also developed; at the time of writing, this procedure is being employed on a routine basis in the research group.

Several azomethine (Schiff base) compounds were prepared with some metal ions investigated. From this, an analytical method was developed for the spectrofluorimetric determination of aluminium using salicylidene 2, aminophenol. In view of the lack of selectivity of the reagent, a simple clean-up procedure was devised employing a diethyldithiocarbamate extraction of the interfering ions into ethylacetate. The reaction of salicylidene 2, aminophenol with gallium was also investigated but was found to be of limited analytical value.

The preparation, structural determination and chelation reactions of the cage molecule bis salicylidene 2,3, diaminobenzofuran (SABF) have been described. A spectrofluorimetric method and extraction procedure for magnesium have been developed and have been applied to the determination of magnesium in blood plasma and municipal water samples. The fluorogenic reaction of gallium with this reagent has also been investigated.

A specific determination of terbium using sulphosalicylic acid and ethylenediamine tetraacetic acid has been

described together with an examination of similar rare earth energy transfer systems.

In the final chapter, some suggestions for future work have listed, and at the time of writing, some of these suggestions are being examined in detail.

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 1964 and April 1967.

I wish to express my gratitude to my supervisors, Professor T. S. West and Doctor R. M. Dagnall, for their invaluable help and guidance. I am grateful to the Science Research Council for the award of a Research Studentship and also for the award of a grant (to Professor West) for the purchase of the spectrofluorimeter used in this work.

Thanks are due to Dr. J. E. Baldwin and Mr. D. J. Elythin of the Organic Chemistry Department for their help in the determination of the structure of bis salicylidene 2,3 diaminobenzofuran, and finally I wish to thank several of my colleagues for helpful discussions and my wife Mrs. F. B. Smith for typing the manuscript.

A handwritten signature in cursive script, appearing to read 'R. Smith'.

Richard Smith.

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INTRODUCTION.

The increasing need for quantitative analysis of metallic ions in the submicrogram range has led to a re-examination of phenomena which have been regarded for many years as interesting, but of limited practical value. The recent analytical use of atomic absorption spectroscopy is a classic example. Solution spectrofluorimetry is gradually becoming recognised as a trace analysis technique as the number of metals capable of determination becomes greater. The lack of suitable reagents and the need for somewhat sophisticated apparatus has perhaps retarded the development of fluorimetry in the past, but the expanding research and instrumental development of recent years has done much to remedy the situation.

The phenomenon of light emission from solutions has been noted for many centuries. Chemiluminescence emission from marshes, seawater and phosphorus being perhaps the most celebrated examples. The chemiluminescence of phosphorus was noted at its discovery in 1674 or 1675 by Brand of Hamburg¹ and Boyle¹ in 1681 published the first method of isolating this element. Boyle² also made the first recorded observation of true fluorescence when he noted the emission of blue light from the yellow solution of a vegetable extract. Stokes³ in 1852 coined the word "fluorescence" as a result of light scattering experiments in which he showed that the emitted light was of a different wavelength to that of the incident light. The first recorded observation of fluorescence from metal chelates was made in 1868 by Goppelschröder⁴ when he showed that the non-fluorescent dye Morin gave a fluorescence reaction with aluminium.

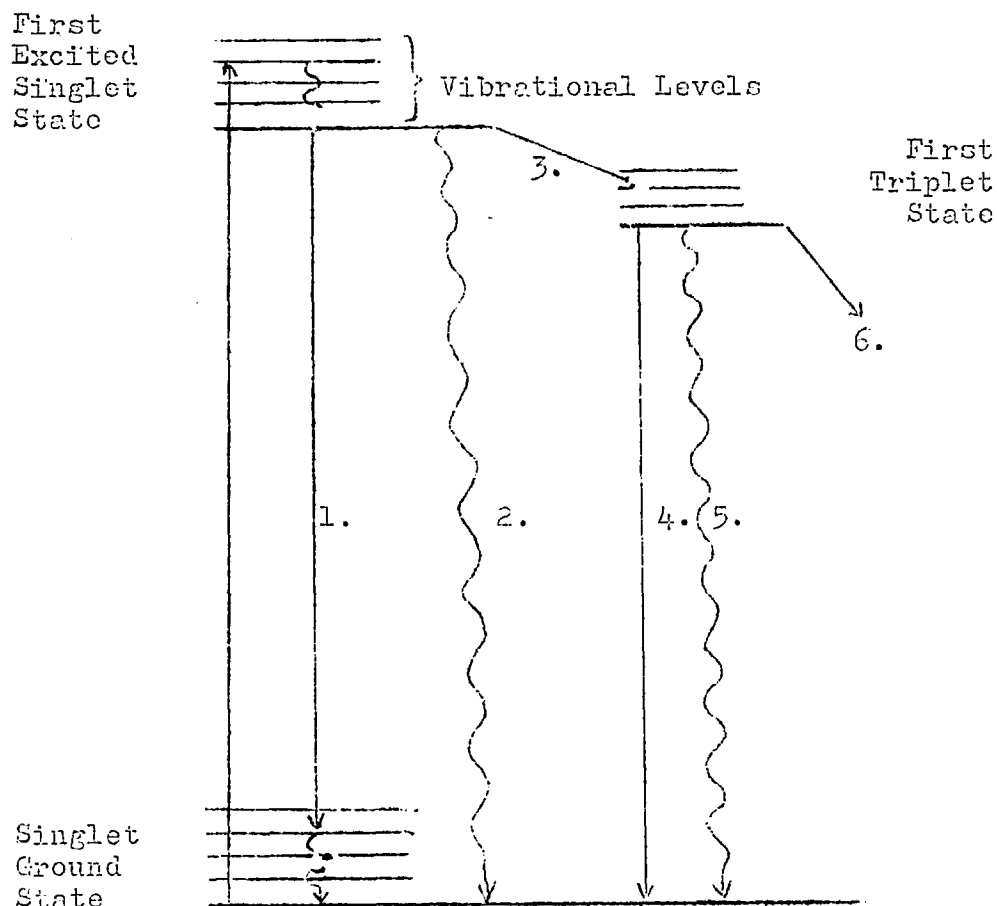
Since then many semi-quantitative and qualitative analytical uses of fluorimetry have been developed and have been well summarised by Radley and Grant⁵. Several reactions listed in this book have provided the basis of subsequent quantitative determinations, for example the determination of molybdenum using carminic acid⁶.

The application of solution spectrofluorimetry to the determination of trace metals has been reviewed in the books by Radley and Grant⁵, Schlezinger-Konstantinova⁷ and White⁸ in addition to the exhaustive coverage given by White⁹ in the Analytical Chemistry biennial reviews. The theoretical and instrumental aspects of the technique have been the subjects of books by Pringsheim¹⁰, Bowen¹¹, and Hercules¹², and review articles by Bartholomew¹³, Parker and Rees¹⁴, and West¹⁵. A very useful theoretical account of fluorescence and related phenomena is included in the book of Calvert and Pitts¹⁶.

THEORY.

Figure 1 shows an energy level diagram representing the electronic states of a light absorbing polyatomic molecule. Each of the electronic states has a number of vibrational sublevels associated with it, and because of this, the energy of excitation does not correspond to a discrete wavelength, but to a range of wavelengths. Hence the absorption (and fluorescence) spectrum occurs as a band and not a sharp line.

On absorption of a photon of the appropriate energy, an electron in the electronic and vibrational ground state of the molecule undergoes excitation to any vibrational level of a higher electronic energy state. Within 10^{-12} sec, the excited molecule loses energy by radiationless processes, and finally occupies the lowest vibrational level of the first excited singlet state. The lifetime of

Figure 1.

state is 10^{-9} to 10^{-7} sec, and the energy of the state may be dissipated in several ways:-

- 1) Fluorescence - The molecule emits a photon corresponding to the energy difference between the lowest vibrational level of the first excited singlet state and any vibrational level of the electronic ground state. The fluorescence decay time is effectively equal to the lifetime of the first excited singlet state.

- 2) Internal Conversion - Many large molecules which do not possess rigid structures can dissipate the excess energy of the excited state by vibrational processes which take approximately 10^{-13} sec.
- 3) Intersystem Crossing - Although electronic transitions involving a change in spin multiplicity are forbidden, singlet triplet transitions from the first excited state are very efficient processes. A singlet triplet transition is approximately 10^5 times less likely than a singlet singlet transition. However, a vibrational process can occur in 10^{-13} sec so that a vibrational coupling of the excited singlet and triplet states can result in a process requiring $10^{-13} \times 10^5$ i.e. 10^{-8} sec. Therefore the singlet triplet transition occurs in approximately the same time as does the fluorescence process. The triplet state has a long lifetime in non-chelated molecules (ca. 10^{-4} to 10 sec) because it can only be deactivated by spin forbidden or diffusion processes controlled as described below:
- 4) Phosphorescence - Triplet-singlet transition to the electronic ground state accompanied by emission of a photon.
- 5) Energy Transfer - Because of the long lifetime of the triplet state, collisional probability is high and transfer of energy to a suitable acceptor molecule may occur. Such molecules include chelated or non-chelated rare earth ions, molecules of the same compound or other molecules having suitable absorption characteristics.
- 6) Photolysis - Because of the long lifetime of the triplet state, chemical processes such as decomposition, isomerisation, or phototropism can occur.

CLASSIFICATION OF FLUORESCENCE REACTIONS.

Classifications of the fluorescence reactions of metal ions with organic molecules have already been proposed^{12,15,17} and are based on the fluorescence characteristics of the free and chelated reagent. The following classification is based on the type of complex formed, rather than on the outward appearance of the reaction.

- 1) Formation of a Fluorescent Ion - Many reagents are non-fluorescent at the pH at which chelation occurs, but they do in fact form fluorescent anions on raising the pH. In this instance the process of chelation serves only to remove a proton(s) at a lower pH value. Generally the fluorescence characteristics of the anion and chelated ligand are very similar. Reactions of this type include Sc(III)/Salicylaldehydesemicarbazone¹⁸, Al/Salicylaldehyde¹⁹ and the Ga Tetrachloride/Rhodamine B ternary complex extraction system²⁰.
- 2) Formation of a Fluorescent Complex - Because of deactivating processes such as internal conversion or intersystem crossing, many reagents fluoresce only weakly or not at all, irrespective of the pH of the solution. On chelation, the metal ion prevents the competitive process and a fluorescent complex is formed. With 8-hydroxyquinoline the presence of a long wavelength absorption band, corresponding to an $n \rightarrow \pi^*$ transition from the non-bonding electrons on the nitrogen to the excited singlet state, is a decisive factor encouraging the rate of intersystem crossing²¹. On chelation the lone pair on nitrogen is displaced towards the metal ion, as a result the $n \rightarrow \pi^*$ transition shifts to a wavelength lower than that of the $\pi \rightarrow \pi^*$ transition and a fluorescence chelate can be

formed with suitable metal ions. This behaviour is analogous to the solvent dependent fluorescence of quinoline²², which is non-fluorescent in hydrocarbon solvents, but fluorescent in hydrogen bonding solvents. In azo and azomethine reagents, this effect is also coupled with the prevention of free rotation on complexing with a metal thus reducing internal conversion processes²³.

3) Energy Transfer Fluorescence - Radiation absorbed by the donor, which need not be chelated, is transferred intermolecularly to an acceptor and fluorescence characteristic of the acceptor is observed. This type of fluorescence is made use of in the analysis of the rare earth elements and because the metal 4f electrons are surrounded by other electrons, the fluorescence signal is not broadened by solvation effects. However, in ruthenium phenanthroline complexes²⁴, the 4d shells are susceptible to solvation effects and these chelates exhibit a band fluorescence sharper than that of a typical organic molecule, but broader than that of the rare earth ions.

4) Quenching Methods - These include the formation of a non-fluorescent species from a fluorescent reagent. Examples are the reaction of transition metals with calcein²⁵, and salicylic acid. This would also include indirect quenching methods, for example the determination of fluoride by its depression of the Al/Pontachrome BER fluorescence²⁶.

5) Chemiluminescence - The emission of a photon of light as the product of a reaction involving a metal ion. Other than the reaction of luminol, lucigenin siloxene⁷ with transition metals in the presence of hydrogen peroxide, the scope of this type of reaction is virtually

unexplored. Because of the difficulty of predicting the occurrence of this type of reaction and because of the few systems known, it is unlikely to find immediate widespread use.

6) Catalytic Methods - The metal ion to be determined catalyses the destruction or formation of a fluorescent compound. For example Lumocupferron²⁷ provides a sensitive selective reagent for copper. In this reaction it is believed that copper catalyses the formation of a fluorescent dimer. Because of the greater sensitivity obtainable using catalytic methods and because of their potential selectivity this type of reaction would be well worth future investigation. Such methods could be used for the analysis of transition metal ions, and anions. The use of catalytic methods in conjunction with enzymes could provide a very high degree of selectivity.

7) Metal Ion Fluorescence - Solutions of certain metallic ions present as aquo- oxo- or chloro- etc., complexes give fluorescence, characteristic of the metal ion. Examples are the thallium(I)^{17,28} and lead fluorescence in the presence of chloride ions, the fluorescence of platinocyanide complexes and the fluorescence of some rare earth ions in the absence of energy donors. The analytical methods based on this type of fluorescence can be made highly selective^{17,28} but the low absorption of such complexes often results in weak fluorescence. However, the low blanks encountered with such methods often compensate for this.

8) Other Methods - Several other methods are now known in which a fluorescence may be generated. These bear resemblances to techniques already used in spectrophotometry

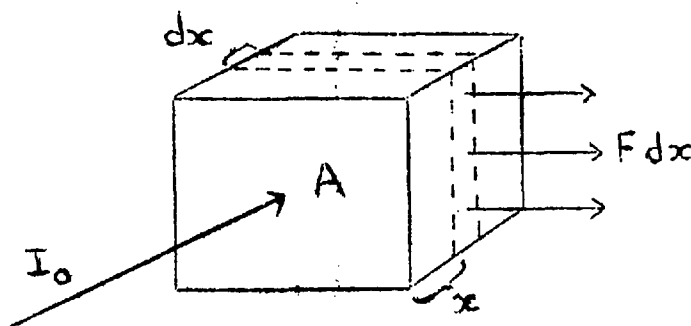
and therefore do not merit separate headings. They include:

- (a) Redox systems in which a fluorescence is destroyed or generated on changing the valence state of the ion being determined, e.g. the Ce/As Redox system^{17,29}.
- (b) Indirect systems in which a non-fluorescent complex is isolated and a fluorescent species generated on destruction of the complex e.g. Cu/o-phen/Rose Bengal B.O.³⁰ Extra system.
- (c) Displacement systems in which one metal is displaced from a complex by the metal being determined and the displaced metal is then determined by an already developed procedure, for example, the proposed system for determination of thorium¹⁷, in which aluminium is displaced from its EDTA complex and then determined by 2, hydroxy 3, naphthoic acid³¹.

SOME PRACTICAL ASPECTS OF SPECTROFLUORIMETRY.

Consider the light of intensity I_0 falling on an area A of sample cuvette and giving a total emission of fluorescence F .

Figure 2.



Then according to the Lambert/Beer Law of Absorbance

$$I_t = AI_0 e^{-klc}$$

I_t = intensity of transmitted light

l = cell path length

c = molar concentration of absorbing species.

Therefore the light intensity on the element of width dx at a distance x is $AI_0 e^{-kxc}$ and the light intensity transmitted by the layer $x + dx$ is $AI_0 e^{-kc(x + dx)}$. Therefore the energy absorbed by the element dx is:

$$\begin{aligned} dE &= AI_0 [e^{-kxc} - e^{-kc(x + dx)}] \\ &= AI_0 e^{-kxc} [1 - e^{-dx.k.c.}] \end{aligned}$$

Now since dx and c are very small

$$dE = AI_0 kce^{-kxc} dx$$

And
$$dF = KAI_0 . kce^{-kxc} . dx$$

Integrating over the whole path length of the cuvette

$$\begin{aligned} F &= AKI_0 kc \int_0^l e^{-kxc} dx \\ &= AKI_0 [1 - e^{-klc}] \end{aligned}$$

On expanding by series

$$F = AKI_0 \left[klc - \frac{(klc)^2}{2!} + \frac{(klc)^3}{3!} - \dots \right]$$

For c small

$$F = K'I_0 klc$$

Or
$$F = K'' \frac{I_0 \epsilon lc}{\epsilon}$$

Where ϕ is the quantum efficiency of fluorescence

ϵ is the molar extinction coefficient.

This relationship is particularly important since it serves to illustrate the basic differences between spectrofluorimetry and spectrophotometry.

In the latter the dependence of analytical signal, I/I_0 , on concentration is logarithmic
i.e. $\log_{10} \frac{I}{I_0} = -\epsilon cl$ (Lambert Beer Law)

whereas in fluorimetry it is linear. Also in spectrophotometry, we measure a ratio

$$\log_{10} \frac{I_0 - I_a}{I_0}$$

and when I_a is very small, there is a practical limit to

the measurement of absorbance, ca 0.001. In fluorimetry, the sensitivity is limited by the sensitivity of the detector. An increase in the detector sensitivity or incident light intensity will increase the analytical signal whereas in spectrophotometry this would have no advantageous effect.

In practice there are two factors which limit the sensitivity of the fluorimetric method:

- (a) With instruments which are not fitted with a blank "backing-off" device, the limit is set by the value of the blank. This can arise in many ways, e.g. light scattering, Raman scattering, residual fluorescence, stray light or second order diffraction.
- (b) With instruments which are fitted with a blank "backing-off" device the limit of determination is set by the instrumental noise level and the reproducibility of the fluorescence reaction at low metal ion concentrations.

The various processes which compete with fluorescence have already been discussed. However, in practice there are several other factors which distort the analytical signal.

- (1) Inner filter effects - If any substance in the solution absorbs light at the wavelength of excitation or emission of the fluorescent species, a reduction in intensity of fluorescence will result. When the absorbing species itself is very concentrated a "saturation inner filter effect" may occur where the fluorescence emission intensity differs with distance from the front face of the cuvette.
- (2) Oxygen Quenching - The oxygen molecule is paramagnetic and the pairing energy for the two unpaired electrons is relatively low. When the difference between the first excited state of the fluorescent species and the first

excited triplet state is of the same order, intersystem crossing to the triplet state can be increased by the presence of dissolved oxygen. This results in a decrease in fluorescence.

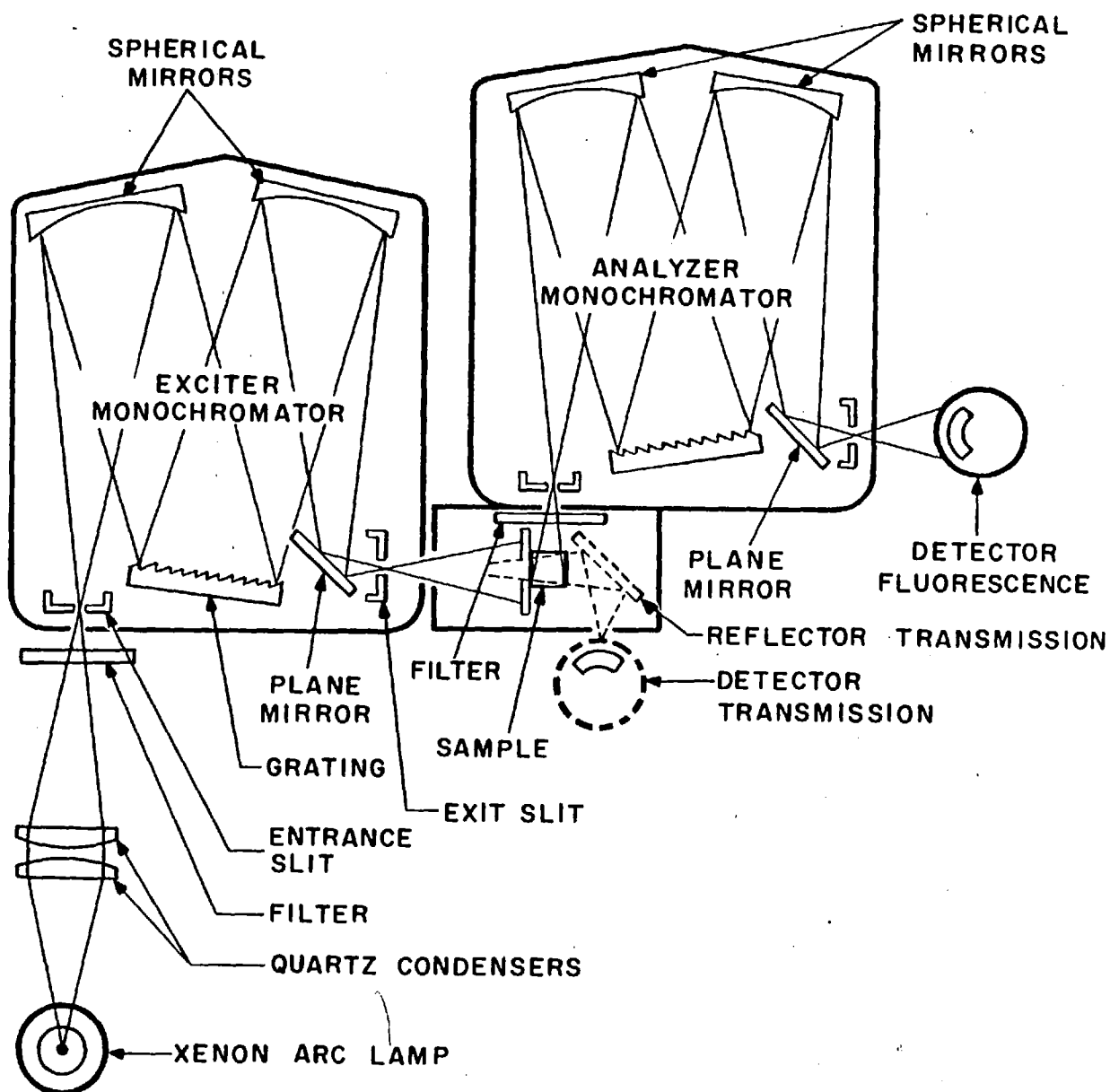
(3) Collisional Quenching - Because of the long lifetime of the triplet state, triplet molecules can undergo collision with other molecules in solution. Molecules in the first excited singlet state will not undergo significant collisional quenching because of the short lifetime of this state. However, increased collisional deactivation of the triplet state will tend to increase triplet formation at the expense of fluorescence processes. Use of viscous or rigid solvents and low temperatures will tend to reduce this effect. The use of rigid solvents and low temperatures will also reduce vibrational quenching of the first excited state.

(4) Raman Scattering³² - At the high instrumental amplifications encountered in analytical fluorimetry, Raman scattering becomes observable. Radiation of any wavelength is absorbed and radiation of a slightly longer wavelength re-emitted (ca. 30 mμ or 50 mμ difference). The position of the Raman emission peak is always dependent on the wavelength of the incident light and because of this it can be distinguished easily from fluorescence. Raman scatter can increase the value of the blank when the instrument is used at high sensitivity.

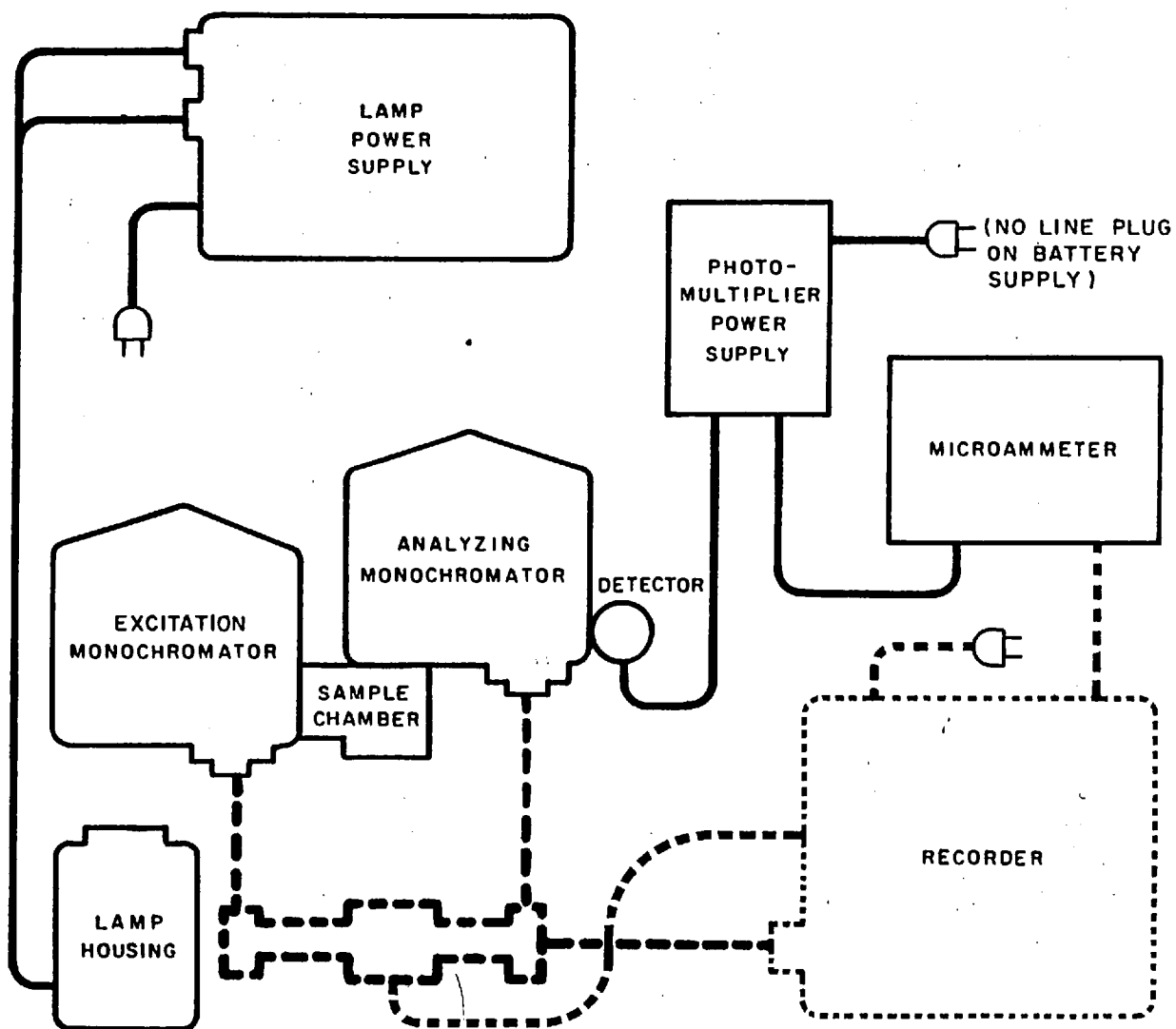
INSTRUMENTATION.

The instrument used in this work was a Farrand Optical Company spectrofluorimeter, for block diagrams of the optical and electrical systems refer to figures 3 and 4. The light source is a high pressure 150 watt D.C. xenon arc lamp which gives a usable light intensity from 250 mμ to beyond 600 mμ. During operation the lamp is cooled by a fan situated at the top of the lamp housing.

Figure 3.



The monochromators each have a wavelength range of 220-650m μ and use replica gratings with 14,400 lines/inch. Interchangeable metal slits with spectral halfbandwidths of 1, 5, 10 and 20m μ are provided for the entrance and exit ports of each monochromator.

Figure 4.

The sample compartment, situated between the two monochromators, is suitable for the use of 10x20x50 mm quartz cells (used in all this work) or 10x10x50 mm quartz cells. Alternatively, a micro cell holder with focussing lens can

be inserted into the cell compartment, thereby accomodating 3x3x40 mm cells or cylindrical cells, 2 mmx40 mm. The cell compartment can also be fitted with a solid sample holder or can be replaced by a cell compartment fitted with a water circulating block for temperature control. A dull surface aluminium mirror is provided for direct measurement of transmission using the photomultiplier in the alternative position next to the cell compartment (see diagram 3). These accessories are all standard Farrand fitments.

The analysing monochromator is identical to the excitation monochromator and is used in conjunction with an R.C.A. 1P 21 or 1P 28 photomultiplier tube. These tubes vary only slightly in their spectral response, and were operated from a mains input/900 volts DC output stabilised power supply.

The RCA microammeter has six decade sensitivity ranges which permit measurements of current from 0.0002 to 1,000 μ a. This is used in conjunction with a Honeywell chart recorder, the motor of which is geared to drive either of the monochromators. The chart width is 10 inches and a complete spectrum scan (220-650 m μ wavelength linear) occupies 10 inches. A chart speed of $1\frac{2}{3}$ inches/min. was used in all the work, although other speeds up to 40 inches/min. can be used.

There are two positions (figure 3) for filters: at the entrance to the excitation and analysing monochromators. Filters are used to eliminate unwanted radiation which may come through the monochromators by internal scattering or second order diffraction. Alternatively the slits may be removed completely and the instrument used as a simple filter fluorimeter. The filters supplied with the instrument were: A Corning 7-54 (75% transmittance between 275 and 375 m μ) which was used in conjunction with the excitation monochromator, a Corning 3-72 (75% transmittance above 490 m μ)

and a Corning 3-73 (75% transmittance above 455 mμ) both of which were used with the analysing monochromator.

OBJECTIVES

The work described in this thesis was undertaken in order to extend the present understanding of solution spectrofluorimetry as a trace analysis technique for metal ions. This has necessitated the examination of several different types of reaction system e.g. energy transfer, fluorogenic chelation reactions, charge transfer systems, etc. Where an analytically useful reaction has presented itself, this has been exploited and a method of analysis developed. Some attempt has also been made to investigate the critical factors involved in the production of a fluorescent chelate compound.

CHAPTER I.

CALIBRATION OF THE SPECTROFLUORIMETER.

The instrument used in this work is not fitted with any device which might compensate for the spectral variations of lamp intensity, monochromator transmission or photomultiplier response. It is sufficient, however, to calibrate the lamp and excitation monochromator as one unit and the photomultiplier and analysing monochromator as another. The task of calibration is made considerably more easy because of this.

The methods available for calibrating lamps and detectors have been discussed in many publications dealing with spectrofluorimetry, but the papers of Parker and Rees³³, Parker³⁴, and White, Ho and Weimer³⁵ are perhaps the most comprehensive. In general, however, there are four main methods of calibration summarised as follows:

- (1) The use of a detector of known spectral response.

This can be a calibrated photomultiplier, a thermopile, a photographic plate or a chemical actinometer. In this way the lamp of the instrument can be calibrated.

- (2) The use of an illumination source of known spectral distribution to calibrate the detector. Generally, calibrated tungsten or mercury lamps are used for this purpose.

- (3) The use of quantum counters. A very concentrated solution of a fluorescent dyestuff such as fluorescein or Rhodamine B is placed in the sample cuvette arranged at an angle to the incident light beam, in such a way that the fluorescence of the front face of the cuvette can be measured. Because the solution is very concentrated, all the incident light is absorbed at the surface and the

fluorescence emission, the wavelength of which is independent of the wavelength of the exciting light, is proportional to the number of quanta of light incident upon the cuvette. The method relies on the constancy of the quantum yield of the screen with wavelength, and can be used from 200 mμ to 400 mμ. (4) The use of fluorescent substances whose spectral characteristics have already been published. By adjusting the observed excitation spectrum to the true excitation spectrum, the lamp characteristics can be determined. In a similar way the photomultiplier can be calibrated from the emission spectrum.

(A) Calibration of Wavelength Scale.

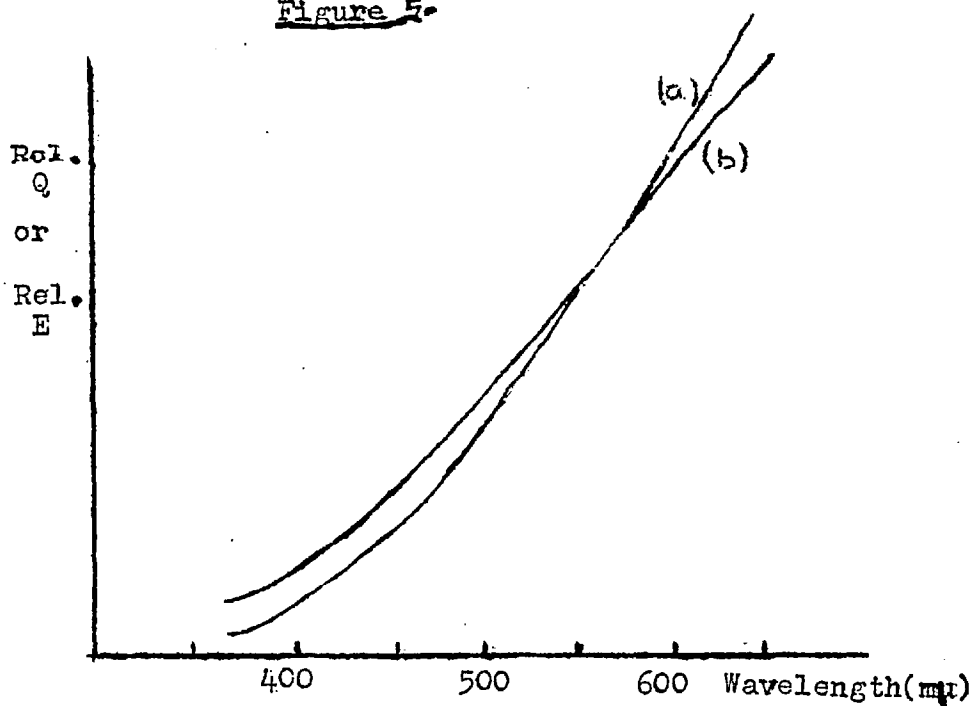
The cell compartment of the spectrofluorimeter was first removed and a mercury vapour lamp placed so as to illuminate the entrance slit of the analysing monochromator. The monochromator was fitted with 1 mμ slits, the entrance slit having been almost completely masked with adhesive tape so as to reduce the incident light intensity. The wavelength scale of the analysing monochromator was then set to read the 546 mμ mercury line. There was no alteration in the wavelength reading on adjusting the position of the mercury lamp laterally.

The cell compartment was replaced and the wavelength dial of the excitation monochromator was then set by turning on the xenon arc lamp of the apparatus and measuring the scatter peak at 450 mμ of a quartz cell filled with distilled water. This was repeated at other wavelengths. The "backlash" error of each monochromator on mechanically scanning the spectrum was then measured by scanning the mercury lamp spectrum and noting the wavelength dial readings simultaneously. The error was between 1 and 2 mμ and has been neglected.

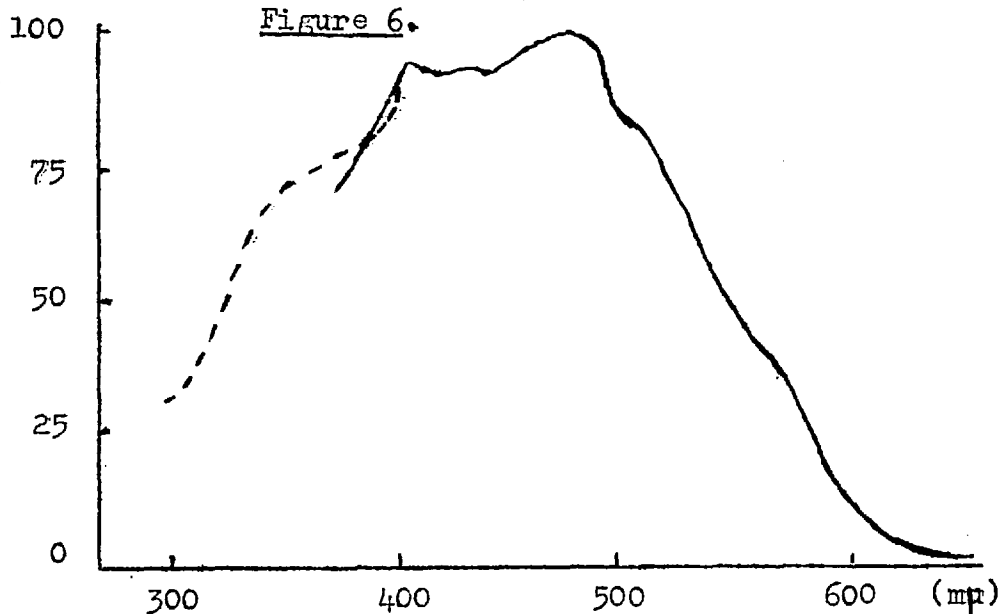
(B) Calibration Using Standard Lamp.

The cell compartment was replaced with a 1 Kw glass envelope standardised tungsten lamp run at a colour temperature of 2854°K . The analysing monochromator was scanned mechanically and a plot characteristic of the tungsten lamp, photomultiplier and monochromator was obtained. The lamp was calibrated in energy units (watts) and the calibration data points were multiplied by wavelength to convert to quanta per unit wavelength interval (figure 5). On dividing the lamp characteristic curve point by point into the total curve, the spectral response of the detector system as a whole was obtained for the wavelength range 380 mμ - 650 mμ (figure 6). The emission of the tungsten lamp is small at wavelengths below 380 mμ because of the absorption of the glass envelope.

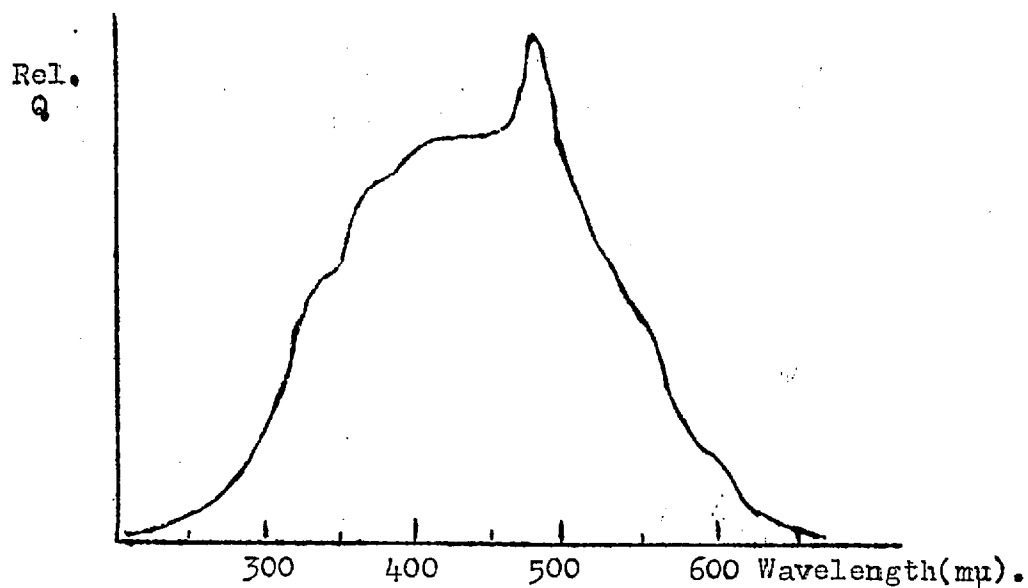
The cell compartment was replaced and 5 mμ slits fitted throughout. The entrance slit of the excitation monochromator was masked with adhesive tape at top and bottom so as to maintain the original bandwidth and an aluminium mirror, freshly coated with a thick layer of freshly deposited magnesium oxide, was placed in the cell compartment to reflect the light from the excitation monochromator to the analysing monochromator. Magnesium oxide acts as a diffuse reflector whose reflectivity is practically independent of wavelength. The intensity of the reflected light was then measured from 220 mμ to 650 mμ at 10 mμ intervals and a spectral curve was obtained (figure 7) which was the product of the characteristics of the excitation and detector units of the apparatus. By dividing the spectral response curve of the detector system into this "total" curve, the spectral variation of lamp and excitation monochromator could be obtained for the range 380 mμ to 650 mμ (figure 8).

Figure 5.

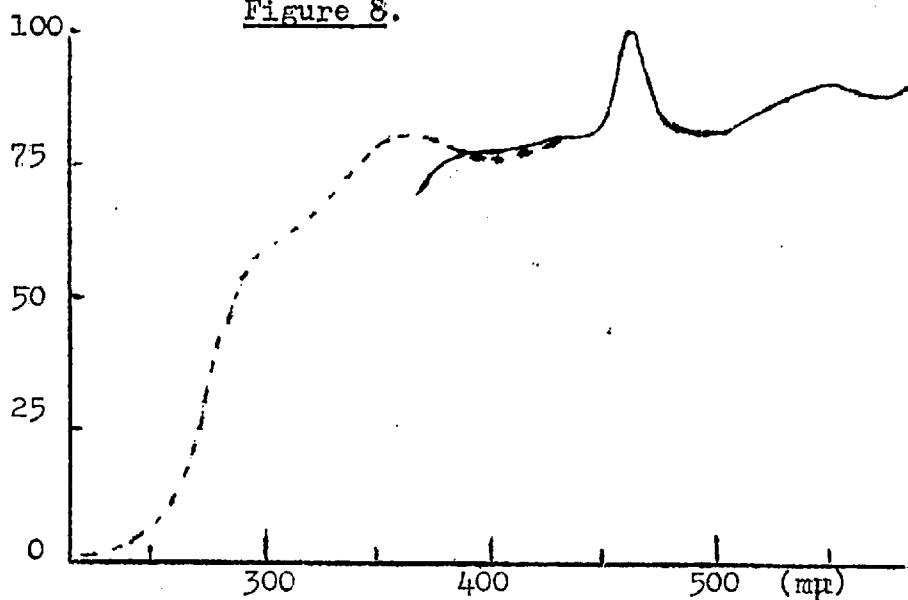
Spectral response of standard lamp in units of (a) quanta (b) energy.

Figure 6.

Correction factors for 1P28 photomultiplier and analysing monochromator as determined by quantum counter (dotted line) and standard lamp (solid line).

Figure 7.

Product of correction factors for excitation and detector units of spectrofluorimeter.

Figure 8.

Correction factors for Xenon lamp and excitation monochromator as determined by the tungsten lamp (solid line) and quantum counter (dotted line).

(C) Calibration using Quantum Counters.

For wavelengths below 400 mμ, the apparatus was calibrated using the quantum counter or fluorescent screen technique. Three screens consisting of 4 g/l Rhodamine B in ethylene glycol, 4 g/l Rhodamine B + 1 g/l acriflavine in glycerol and 4.4×10^{-3} fluorescein in aqueous 0.1M sodium carbonate, 0.1M sodium bicarbonate were used separately. The solutions were placed in a quartz cell set at an angle of about 30° to the incident light beam. On mechanically scanning the analysing monochromator three plots of the excitation system of the instrument were obtained for 220 mμ - 400 mμ (figure 8). By division of the average of the plots into the total response curve obtained by reflection, the detector characteristics below 400 mμ could be plotted (figure 6). Finally, the two sets of correction factors obtained by the standard lamp and fluorescent screen methods were normalised to give the same value at 400 mμ. The validity of the factors was tested by correcting the spectra of anthracene and quinine sulphate solution and comparing with published data^{36,37}.

Apparatus - Tungsten Lamp.

The tungsten lamp used in the calibration was calibrated by G.E.C. Ltd., Central Research Laboratories and was a H.V. 1,000 watt class A.1. Osram lamp operating at a colour temperature of 2854°K at 186 volts and 3.725 amps. A Variac variable transformer attached to the mains was used as a power supply and the voltage rating was checked before and after use. Identical results were obtained when the lamp was operated at 180 and 188 volts and it would appear that the voltage does not critically effect the spectral distribution of the lamp.

We thank Dr. K. Ruddock of the Department of Physics, Imperial College for the loan of the lamp on several occasions.

EXAMINATION OF QUININE AS A FLUORESCENCE STANDARD.

In solution spectrophotometry the limits of any measurement are set by 0% and 100% transmission. All readings must, therefore, fall between these limits. In spectrofluorimetry, only the lower limit of zero fluorescence or blank value has any meaning and as a result, comparison of fluorescence intensities between laboratories is impossible without reference to a standard substance. In addition, the sensitivities of the instrumental components may vary from day to day and the use of a standard substance is again imperative if fluorescence intensity comparisons are to be made. If a standard substance of known quantum fluorescence yield is chosen, other quantum yields may be calculated by relative methods. Because of their simplicity relative methods are becoming increasingly used and are well described in the literature^{16,33,36}.

Quinine bisulphate solution or quinine in M/10 sulphuric acid has become widely accepted as a fluorescence standard³⁸. Melhuish³⁷ and Eisenbrand³⁹ have carried out some investigations as to its suitability as a standard. Parker and Rees³³ have compared quinine with other substances e.g. fluorescein, anthracene, etc., and have shown it to be the most suitable as a standard, provided the quinine content of the sample was specified.

Because of the importance of quinine standards in some of the later work, it was decided to make a thorough investigation of the fluorescence characteristics of the quinine/sulphuric acid system. Unless stated otherwise, fluorescence measurements were carried out at 450 mμ using excitation light of 350 mμ and using solutions of quinine in M/10 sulphuric acid.

Purity of Quinine.

Quinine samples were examined for the presence of other cinchona alkaloids by thin layer chromatography using a

system described by Randerath⁴⁰. Chromatograms were run on Shandon apparatus using silica gel as the stationary phase and a solvent consisting of petroleum ether (BP100° - 120°)/diethylamine/acetone (23/9/9 by volume). Chromatograms were developed for 40 mins, were examined under ultraviolet light and were then sprayed with Dragendorff Reagent, the alkaloids appearing as brown spots. Dragendorff Reagent was prepared by dissolving 1.7 g bismuth subnitrate and 20 g tartaric acid in 80 ml of water and adding this to 16 g of potassium iodide in 40 ml of water. To each 50 ml of the mixture was added 100 g of tartaric acid and 500 ml of water.

The mean result of 6 runs is given in Table 1.

Table 1.

Thin-Layer Chromatography of Quinine Samples.

Sample	Rf	Source	Fluorescence
(a) Quinine (B.P.C.)	0.37	1	Blue green
(b) Quinine (purified by Cohen's method)	0.51	1	Blue green
(c) Quinine (G.P.R.)	0.36	2	Blue green
(d) Quinine (G.P.R. recrystallised)	0.36	3	Blue green
(e) Quinidine (G.P.R.)	0.56	2	Blue green
(f) Cinchonine(G.P.R.)	0.63	2	Non fluorescent
(g) Cinchonidine(G.PR)	0.55	2	Non fluorescent

1. Lake & Cruickshank Ltd.
2. B.D.H. Ltd.
3. B.D.H.(G.P.R.) 10 g quinine was recrystallised from 250 ml of boiling water containing sufficient ethanol to dissolve the sample. The residue was washed well with cold water and dried to constant weight at 100°C (about 12 hours).

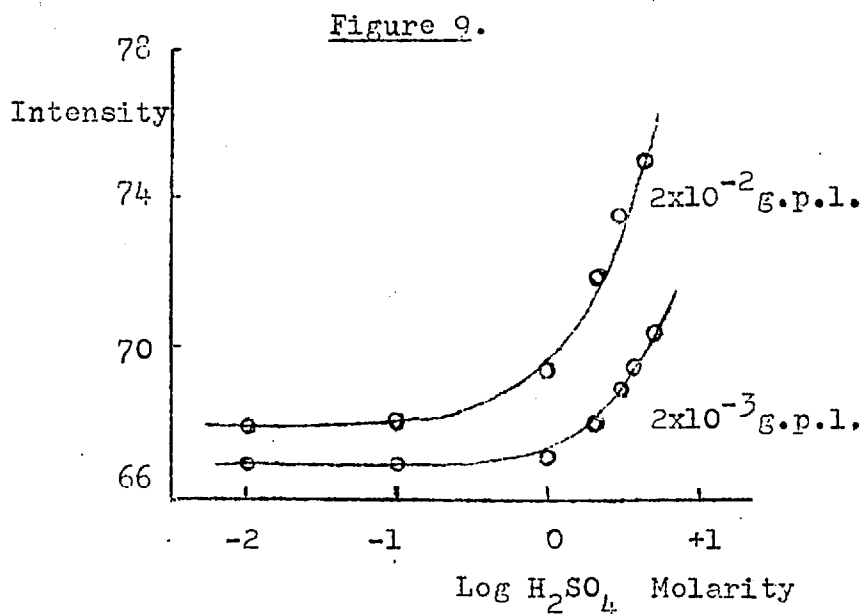
Analysis Results - for Recrystallised (G.P.R.) quinine
 Calculated for anhydrous quinine $C_{20}H_{24}N_2O_2$ (MW 324.48)
 Found C 73.7%; H 7.3%; N 8.5%
 Calculated C 73.9%; H 7.4%; N 8.6%

Some of the Rf values were obtained from mixtures of two or more alkaloids; the method is easily capable of separating all four alkaloids. There appeared to be no major impurities in the quinine samples taken, however the "quinine" purified by Cohen's method⁴¹ was shown to have the same chromatographic characteristics as quinidine and to be quite different from quinine. As this particular sample was a gift, we have no knowledge of its history and can offer no explanation for this curious change.

Effect of Acid Molarity.

Solutions containing 5×10^{-4} g/l and 5×10^{-3} g/l of quinine were examined for the effect of sulphuric acid molarity on the fluorescence emission.

The results are summarised in figure 9.



Quinine is normally used in M/10 sulphuric acid and this would appear to be a suitable acid concentration, since there is little fluorescence variation with acidity at this level. There was no change in the position of the excitation or emission spectrum over the acidity range examined.

Effect of Ultraviolet Radiation.

There was no change in the fluorescence intensity of a 5×10^{-4} g/l solution of quinine after irradiation with 350 mμ light for 60 mins in the cell compartment of the spectrofluorimeter. However, on exposing the same solution to light direct from the window of the lamp housing for 30 secs, the fluorescence intensity was reduced by 20%. Irradiation for 2 mins reduced the intensity by 57%.

These results show that although quinine is not subject to photolysis under the normal conditions of use, care must be taken not to expose the solution to strong ultraviolet light and to store solutions in the dark.

Stability of Quinine Solutions.

A solution of 5×10^{-2} g/l quinine was kept in a dark cupboard and diluted immediately before measurement to give a 5×10^{-3} g/l solution. The absorption spectrum of this solution was plotted to determine the extent of deterioration of the fairly concentrated quinine solution. After three months there was no apparent decrease in the quinine absorbance at 246 mμ and all the absorption spectra were superposable.

The 5×10^{-3} g/l solution was diluted tenfold and used as a standard to measure the deterioration of a 5×10^{-4} g/l quinine solution stored in a volumetric flask and exposed to the normal laboratory illumination. The two solutions

were compared by measuring their fluorescence intensities, but after three months no apparent deterioration of the dilute quinine solution was noted.

When more dilute solutions of quinine are required (i.e. less than 5×10^{-4} g/l) it is probably better to obtain these by dilution rather than rely on the stability of very dilute solutions as there may be adsorption losses on the walls of the vessel. This source of error has been reduced by coating the storage vessels with Silicone Repelcote.

Effect of Oxygen Quenching.

A solution of 5×10^{-4} g/l quinine was deoxygenated by bubbling nitrogen for 15 mins, and measuring the fluorescence. Oxygen and nitrogen were then bubbled in turn and the fluorescence intensity was measured after the passage of each gas.

Table 2.

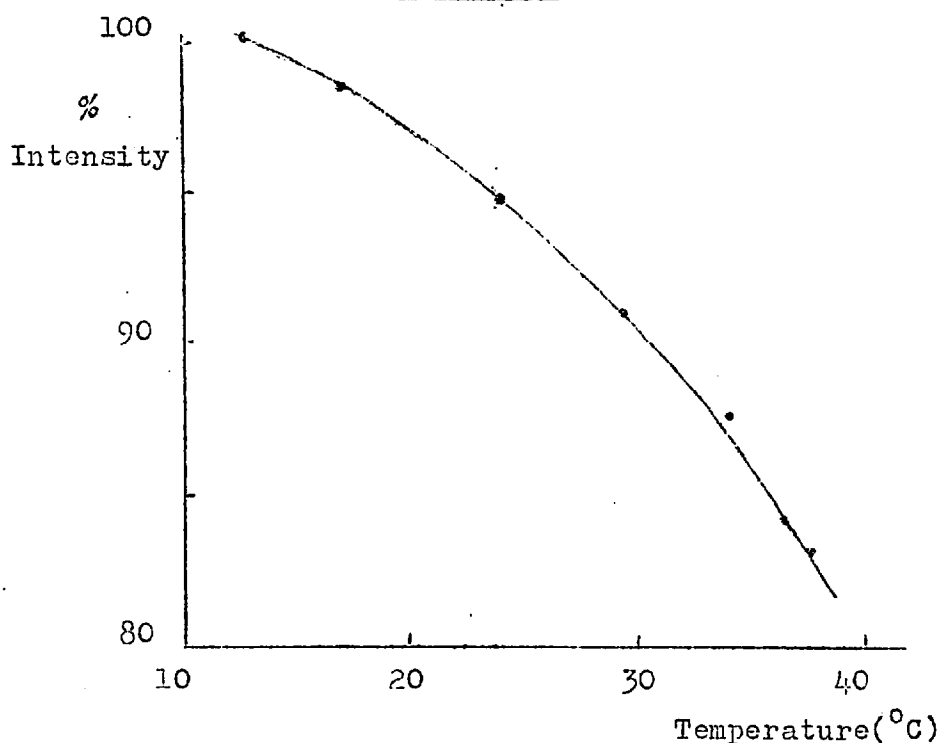
	Fluorescence Intensity
Original Solution	85
Standard	85
N ₂ bubbled for 15 mins	92
Standard	85
O ₂ bubbled for 10 mins	91
Standard	84
N ₂ bubbled for 15 mins	92
Standard	85

The increase in fluorescence intensity after the initial deoxygenation can be attributed to the cooling effect of the expanding gas and the negative temperature coefficient of fluorescence of quinine.

Effect of Temperature.

The cell compartment incorporating a water circulation block was fitted to the spectrofluorimeter. Water from a large, stirred thermostated water-bath was circulated through the cell compartment and fluorescence measurements made when the cell compartment had attained a steady temperature. The temperature was measured directly in the cell using a 0-50°C thermometer graduated in half degrees. The temperature dependence of fluorescence was measured on a 5×10^{-3} g/l quinine solution over a range 14°C - 38°C (figure 10). In the range 14°C - 24°C, the temperature coefficient was found to be -0.54% per °C.

Figure 10.



Effect of Quinine Concentration.

Linear concentration dependence of fluorescence intensity was found for quinine concentrations in the range from 10^{-8} to 10^{-5} M. This covers the concentration range over which quinine is likely to be used as a standard. Because of the negligible overlap of excitation and emission spectra, the area under the emission peak should also bear a linear relationship to the concentration.

Summary.

Quinine has been shown to be a suitable primary fluorescence standard. General purpose reagent and B.P.C. quinine were both adequately pure, although the water content is high and variable. The fluorescence of quinine in M/10 sulphuric acid has been shown to be influenced only slightly by acid molarity, moderate u.v. radiation, and dissolved oxygen. The solutions are stable over a period of months and a linear dependence of fluorescence intensity on concentration exists over a wide range. The temperature coefficient of fluorescence is high, but most fluorescent substances have appreciable temperature coefficients. The simplicity of the excitation spectrum tends to minimise errors due to non-monochromatic light.

DETERMINATION OF FLUORESCENCE REACTION SENSITIVITIES.

At present there exists no satisfactory method by which the sensitivities of a fluorescence reaction can be measured. Some approaches have been discussed by Bartholomew¹³, but many of these appear to be dependent on the type of apparatus used. In the proposed method for comparing fluorescence sensitivities¹⁵, the fluorescence intensity of a solution is measured at its absorption and emission maxima and compared with the fluorescence intensity of an equimolar amount of quinine in M/10 sulphuric acid measured at its own excitation and emission maxima (350/450 mμ). Allowance must be made for the spectral variations of the excitation and detector systems. In this way a comparison of the true maximum intensities of the absorption bands of fluorescent chelates can be made. As the measurement is being made from the point of view of the analytical chemist only the peak height need be considered (i.e. not the area). The measurements are made under the optimum conditions as supplied in the literature references in Table 3.

For each sensitivity measurement six concentrations of metal ion covering the linear concentration/fluorescence range were chosen together with a blank. Two quinine solutions of different concentration and a distilled water blank for quinine were chosen as the reference. In this way a graph may be plotted for the metal ion, and the slope used. The equation used to evaluate the sensitivities was:

$$\text{Sensitivity} = C \text{ quinine} \times \frac{S \text{ metal}}{H \text{ quinine}} \times \frac{(I.P.) \text{ quinine}}{(I.P.) \text{ metal}}$$

where

C = Molar concentration

H = Maximum fluorescence intensity

I = Lamp correction factor for excitation wavelength

P = Photomultiplier correction factor for fluorescence wavelength

S = Slope of metal ion calibration plot.

Table 3.

Sensitivities of Fluorescent Systems.

System	Sensitivity	pH	Ref.
Al/Morin	7.46x10 ⁻² 6.81x10 ⁻² 7.14x10 ⁻² 7.60x10 ⁻²	3.0	42
Al/Oxine	2.24x10 ⁻¹ 2.22x10 ⁻¹ 2.10x10 ⁻¹	5.0	43
Al/Pontachrome BBR	5.9 x10 ⁻³ 3.8 x10 ⁻³ 5.8 x10 ⁻³ 3.5 x10 ⁻³	5.0	44
Al/2,Hydroxy 3, Naphthoic Acid	7.73x10 ⁻³ 7.97x10 ⁻³ 7.65x10 ⁻³	5.8	17,31
Al/Salicylidene 2,Aminophenol	1.0 x10 ⁻²	5.6	45,46
Ga/Oxine	8.43x10 ⁻² 8.52x10 ⁻² 8.08x10 ⁻²	5.0	47
Ga/8,Hydroxyquinaldine	9.10x10 ⁻² 9.86x10 ⁻²	5.0	48
Ga/Rhodamine B	1.33	6M HCl	49
Ga/Salicylidene 2,Aminophenol	4.0 x10 ⁻³	4.0	45
Be/3,Hydroxy 2,Naphthoic Acid	7.96x10 ⁻² 7.94x10 ⁻²	7.5	17,31

Procedure

The working concentration ranges of each method were taken from the literature and calibration curves made up exactly as recommended in the references. The spectrofluorimeter was fitted throughout with 5mμ halfbandwidth slits and was adjusted to give 95% scale deflection for the

most concentrated solution. The intensity of fluorescence of the metal ion solutions was then measured at the emission and excitation peaks. Aliquots of a stock quinine solution were diluted with 11/10 sulphuric acid until a scale reading between 25% and 75% of the maximum was obtained on the same instrumental sensitivity scale as used for the metal chelate measurements. The quinine fluorescence was measured at 450 mμ using an excitation maximum of 350 mμ and a distilled water blank value was subtracted from the quinine fluorescence intensity.

The temperature of the measurement was checked and was about $20^{\circ}\text{C} \pm 2$. Fortunately this does not involve as large an error as would be expected from the quinine temperature coefficient of -0.54% per $^{\circ}\text{C}$ since the metal chelate also has a negative temperature coefficient.

The results of Table 3 indicate the sensitivity of the methods themselves independent of instrumental factors. Although a large survey of all existing fluorimetric methods would perhaps be of value, it is felt that this type of comparison is not worth the effort in view of the possible errors involved. Errors in each of the correction factors will be of the order of 3% giving a 12% total error due to the correction factors alone. Temperature effects, measurement errors, and irreproducibility of the chemical system could increase the total possible error to 20%. In addition the analyst must then change the "true" sensitivity factors to give "apparent" sensitivities for his own instrument thereby increasing each total possible error to 26%. Results of this type have no theoretical value and it is most likely that analysts will compare sensitivities of methods by examination of the limits of determination.

DETERMINATION OF QUANTUM YIELDS OF FLUORESCENCE.

The quantum yield of fluorescence is the ratio of the number of quanta emitted per second to the number of quanta absorbed per second by a solution of a fluorescing species. Absolute measurements of the quantum yield of fluorescence involve a determination of the incident light intensity falling on the sample and a determination of the fluorescence intensity emitted in all directions. The determination of absolute quantum yields of fluorescence was beyond the capabilities of the apparatus at our disposal. Recently, however, relative fluorescence intensity measurements have become widely used because of the comparative ease with which they may be made^{16,33}. Generally, the area under the fluorescence emission spectrum from a sample solution is compared with the emission from a substance of known quantum efficiency. The wavelength of irradiation is usually the same in both cases (it need not be at the excitation peak of either compound) and the ratio of the areas under the fluorescence emission spectra enables a determination of the quantum yield of the unknown to be made. In most of these relative measurements, quinine in M/10 sulphuric acid has been used as the standard, because of the small overlap of its excitation and fluorescence emission spectra.

The total fluorescence emission from a solution is given by the equation (page 9).

$$F = k\phi I_0(\xi.c.l) \quad (i)$$

For a standard and an unknown irradiated at the same wavelength the equation becomes:

$$\begin{aligned} \frac{F}{F_s} &= \frac{\phi(\xi.c.l.)}{\phi_s(\xi.c.l.)_s} \\ \text{or } \phi &= \frac{F \cdot l_s}{F_s \cdot l} \cdot \phi_s \quad (ii) \end{aligned}$$

Where A = The absorbance of the solution at the wavelength of excitation.

F = The total area under the fluorescence emission spectrum expressed in quanta per unit frequency interval against a linear frequency abscissa.

ϕ = Quantum yield of fluorescence.

s = Subscript referring to standard.

The method relies on the uniformity of ϕ and ϕ_s irrespective of the wavelength of irradiation. However, to avoid errors arising from the solution having an appreciable absorbance, the optical density must not be allowed to exceed 0.02 absorbance units. If the optical density exceeds this value, the intensity of the exciting light will vary appreciably with the distance from the front face of the sample cuvette. Alternatively these errors can be overcome, if the optical densities of the standard and unknown solutions are exactly equal at the wavelength of excitation.

In practice, distorted fluorescence emission spectra are obtained due to spectral variations in the photo-multiplier/monochromator unit. The apparent spectra are corrected point by point to the true fluorescence emission spectra by dividing by the detector unit correction factor for the particular wavelength. This gives the true emission spectrum with abscissa linear in wavelength and ordinate linear in quanta per unit wavelength. In order to compare the area under the curve with that of the standard, the abscissa must be converted to a linear frequency scale, and the ordinate values must be converted to quanta per unit frequency interval. This is done by dividing the ordinate values by the square of the frequency, using the equation (iii) on page 34.

$$R = \frac{H}{S\nu^2} \quad (\text{iii})$$

Where R = the corrected response in quanta per unit frequency.

H = the measured response in quanta per unit wavelength.

S = the correction factor for the particular frequency.

ν = the frequency

The above equation is used for the correction of every data point on the emission curve. In our determinations of quantum yield the calculations were carried out by computer.

Procedure.

The accuracy of the method was assessed by calculating the quantum yield of fluorescence for substances whose quantum yields had already been determined. Ten milligrams of each substance were dissolved in 100 ml of the appropriate solvent and the absorbance measured at 350 m μ using a Beckmann DB spectrophotometer. If the absorbance was small or coincided with a sharp rise in the absorption spectrum, an absorbance minimum or peak, near 350 m μ , was chosen instead.

Absorbance measurements for many of the compounds were not made at the absorption peak, and there was a great variation of absorbance with slit width of the spectrophotometer. To overcome this difficulty, the spectrofluorimeter photomultiplier was placed in the alternative position (figure 3) adjacent to the cell compartment. The magnesium oxide reflector was fitted behind the cell, so as to reflect the transmitted excitation light on to the photomultiplier. The excitation light was attenuated by masking

the entrance slit of the excitation monochromator with tape in such a way that the spectral bandwidth of the slit was not affected. The 100% transmission was set with a cuvette of the appropriate solvent and 0% transmission adjusted by closing the photomultiplier shutter. In this way the percentage transmission of a concentrated solution could be measured under exactly the same conditions as the fluorescence was measured on the corresponding diluted solution. This arrangement reduces errors due to non-monochromatic radiation and variation of absorbance with spectral bandwidth.

An aliquot of the solution was then diluted one hundred-fold and a solution of quinine was diluted with M/10 sulphuric acid so as to give an approximately equal absorbance at the wavelength of excitation. The solution of the unknown was then deaerated by bubbling nitrogen for 20 mins, and the spectrofluorimeter cell compartment purged with nitrogen before use. The fluorescence emission spectra were scanned for the unknown and the quinine standard using the same instrumental amplification and excitation wavelength. The fluorescence intensity was read from the spectra at frequency intervals of $0.02\mu^{-1}$ using a brass scale, specially constructed for this purpose, and the results were put on IBM punch cards. A value of 0.55 was assumed for the quantum yield of quinine in M/10 sulphuric acid.

Computer Program.

The computer program devised by the author is included in Appendix I and is considerably different to that of Drushel et al.⁵⁷. Data is presented on IBM punch cards using columns 1-4 of each card for the frequency in μ^{-1} , columns 6-10 for the corresponding correction factor for the frequency, and columns 12-72 for fluorescence intensity data.

In this way, the fluorescence intensities from one to ten separate emission spectra can be put on one card; each card representing one frequency value. A total of 51 cards are punched for each complete spectrum. The program is designed to read the cards, correct each point using equation (iii) on page 34, and sum the area under each corrected spectrum using Simpson's Rule. The corrected and uncorrected spectra are then normalised to give a maximum intensity of 80 units and the corrected fluorescence emission spectrum is then printed out using asterisks and the uncorrected spectrum using full stops. When an asterisk and a full stop coincide, only a full stop is printed. In this way, data for up to ten substances may be treated simultaneously.

Programs were run on the Imperial College IBM 7090/1401 unit using the PUFFT system. Total computing time, including compilation, was about 0.2 min. for four substances and the results could be obtained from the computing centre within one-and-a-half hours. Punching a set of 51 cards took about 10 to 15 minutes for four substances, and about 20 minutes for 10 substances.

Quantum efficiencies of fluorescence were then calculated using equation (ii) (page 32).

Results and Discussion.

The results, literature comparisons and relevant experimental data are summarised in Table 4. An error of about $\pm 2\%$ is to be expected in each absorbance reading, giving a total possible absorbance error of $\pm 4\%$. Fluorescence intensity measurements (including correction factor errors) will each have an error of $\pm 4\%$, but these are likely to cancel each other as 51 intensities are treated in the calculation. It is estimated that an overall accuracy of $\pm 8\%$ should be possible, assuming the chemicals are pure. Because

Table 4.

Determination of Quantum Yield of Fluorescence using Quinine in M/10 Sulphuric Acid as a Standard ($\phi = 0.55$).

Substance	Solvent	Conc. ($\mu\text{g/ml}$)	Freq. (μ^{-1})	ϕ	Literature
Acriflavine	water	4.0	2.86	0.57	0.56 ⁵¹ , 0.54 ⁵²
Anthracene	ethanol	0.5	2.82	0.28, 0.26	0.30 ⁵² , 0.27 ³⁷ , 0.28 ⁵³
Anthranilic Acid	ethanol	0.5	2.86	0.34	0.58 ³⁷
Coronene	chloroform	10.0	2.65	0.30	0.3 ⁵⁴
Eosin	ethanol	5.0	2.86	0.36	
Eosin	M/10 NaOH	2.0	2.86	0.12	0.15 ⁵⁵ , 0.19 ⁵² , 0.12 ⁵⁶
Fluorescein	M/10 Na ₂ CO ₃ ⁺	1.0	2.86	0.82,	0.79 ⁵⁶ , 0.77 ⁵⁶ ,
	M/10 NaHCO ₃			0.81	0.80 ⁵⁵ , 0.85 ³³
Rhodamine B	ethanol	1.0	2.86	0.66	0.97 ⁵² , 0.69 ³³ , 0.76 ⁵⁷
Rubrene	benzene	10.0	2.86	1.02	1.02 ⁵⁶ , 1 ¹⁶

dilute solutions were used, no allowance has been made for self-absorption or self-quenching.

The results for the determination of quantum yields of fluorescence, described in the literature, vary considerably. In general, the results for aromatic hydrocarbons such as anthracene, rubrene, etc., are consistent, but the results for the quantum yields of dyestuffs show great variation. This is probably due to the difficulties encountered in

obtaining the pure dyestuff, and also to the effect of ionisation of the dyestuff.

Eosin, in particular is difficult to obtain in a pure form because of the presence of other partially brominated fluorescein derivatives. The presence of these derivatives will tend to give a spuriously high value for the quantum yield of eosin. In addition, Parker and Rees³³, report the instability of dilute solutions of eosin on standing. Thin-layer chromatography of the eosin (B.D.H. Indicator)⁵⁸ showed the presence of a small amount of other dyestuffs (ca. 7%).

The quantum yield of fluorescein varies from 0.66 at pH 7⁵², 0.84 at pH 9.6³³, to 0.93 in M/10 NaOH^{51,52}. A value of 0.78 to 0.85 for the quantum yield at pH 9.6, however, appears to be highly probable in view of the agreement in the results of recent workers. Thin-layer chromatography⁵⁸ showed the fluorescein (B.D.H. Indicator) to be free from other dyestuffs.

Perhaps the greatest variations in report quantum yield, however, are those for rhodamine B, (0.69 to 0.93) and may arise from variations in the degree of ionisation of the dye. All the reported measurements have been carried out on ethanolic solutions and, therefore, the quantum yield would be expected to be dependent on the mode of preparation of the dye. This could be overcome by using aqueous solutions of known acidity.

The result obtained for anthranilic acid of 0.34 (0.58³⁷) is the most divergent from literature values. This could be a concentration effect: our measurements were carried out on 0.5 µg/ml ethanolic solutions whilst Melhuish's were carried out on 1.7×10^3 µg/ml ethanolic solutions. Unfortunately this was the only literature comparison we were able to obtain.

CHAPTER II.

PHENOLIC AZOMETHINES AS FLUORIMETRIC REAGENTS.

The fluorescent chelating reactions of several O,O' dihydroxyazo- and azomethine- compounds with metals have been investigated by Freeman and White⁵⁹. Holzbecher⁶⁰ lists a table of fluorescence intensities for the reactions of some azomethines with aluminium. Workers in the U.S.S.R.⁶¹ have also investigated the fluorescence reactions of these chelating agents with metal ions and a cursory study of the fluorescence characteristics of some of the metal chelate compounds was reported by Umland et al.⁶².

Azomethines (Schiff Bases) were selected as reagents for study for the reasons listed below:-

- (1) Easy preparations from readily available chemicals.
- (2) Similar in structure to azo-dyes, which have been studied extensively in this department. Unlike azo-dyes, however, the corresponding azomethine compounds generally absorb at much lower wavelengths. Consequently fluorescence is emitted at a lower, more convenient wavelength for measurement, usually between 350 mμ and 550 mμ. Because of the fewer number of nitrogen atoms in azomethines, the possibility of long wavelength $\pi \rightarrow \pi^*$ transitions does not arise, although with azo- chelates this could be a principal cause of non-fluorescence. Long wavelength $\pi \rightarrow \pi^*$ absorption bands give rise to triplet formation in the excited state and hence reduction of fluorescence emission.
- (3) The work of Holzbecher^{60,63,64,65} and White^{59,66} indicated the suitability of these reagents for fluorimetry.

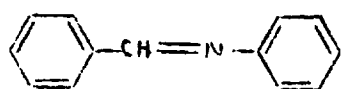
Preparation of Schiff Bases.

All the compounds with the exception of bis salicylidene 2,3, diamino benzofuran prepared were made by direct interaction of an amine dissolved in ethanol with an aromatic

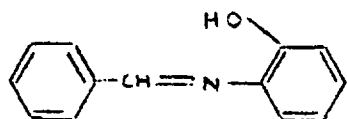
aldehyde. Amines which contained ionising groups e.g. $-\text{SO}_3\text{H}$ or $-\text{HN}^+\text{Cl}^-$ were dissolved in ethanol and a little concentrated ammonia solution. The preparation of all the azomethine compounds are given in the literature and are summarised in table 5. The reactions and properties of bis salicylidene 2,3, diamino benzofuran are treated separately (Chapter III).

Table 5.

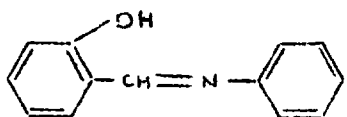
Synopsis of Synthesis of Schiff. Base Reagents.



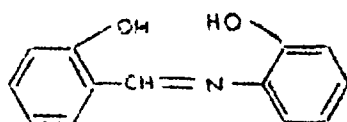
(I). White solid, obtained in 40% yield. Recrystallised from pet. ether, M.Pt. $52^\circ(54^\circ)$.



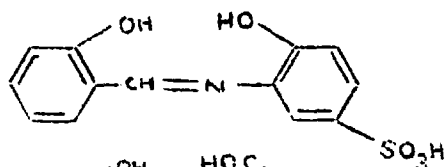
(II). White solid, obtained in 45% yield. Recrystallised from pet. ether, M.Pt. $88^\circ(89^\circ)$.



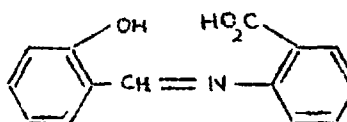
(III). Yellow solid, obtained in 64% yield. Recrystallised from ethanol, M.Pt. $52^\circ(52^\circ)$.



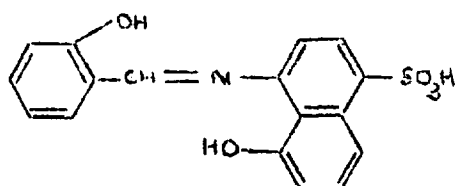
(IV). Red/brown solid, obtained in 82% yield. Recrystallised from ethanol, M.Pt. $185^\circ(184^\circ)$.



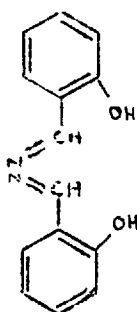
(V). Yellow solid, obtained in 72% yield. Not recrystallised. M.Pt. 155° .



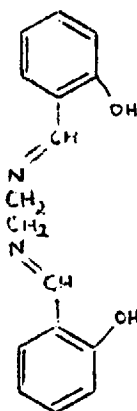
(VI). Red solid, obtained in 37% yield. Recrystallised from ethanol, M.Pt. $206^\circ(205^\circ)$.



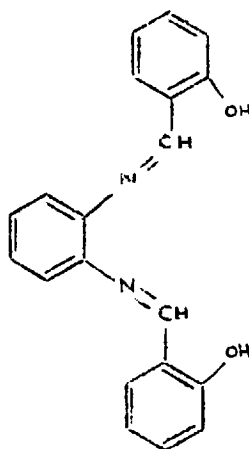
(VII). Brown solid, obtained in 35% yield. Not recrystallised. M.Pt. 196° .

Table 5. (cont'd)

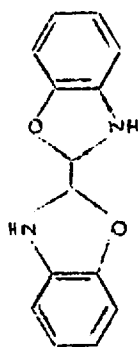
(VIII). Pale yellow solid, obtained in 78% yield. Recrystallised from xylene, M.Pt. 213° (213°).



(IX). Yellow solid, obtained in 90% yield. Recrystallised from ethanol, M.Pt. 125° (126°).



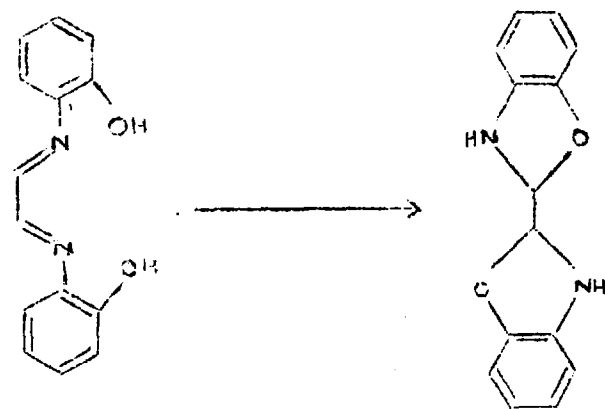
(X). Yellow solid, obtained in 75% yield. Recrystallised from benzene, M.Pt. 163° (164°).



(XI). Sample belonging to Prof. West

Schiff Base Structures.

Freiser⁶⁷ has reported the rearrangement of some o-hydroxy Schiff bases to benzoxazolines. The case in point was that of glyoxal bis 2, aminophenol which spontaneously formed a bis benzoxazoline:



The Schiff Base form was, in fact, never isolated. Thiophenols were reported⁶⁷ as behaving in a similar way.

In view of the possibility of oxazoline formation, infrared spectra were run on all the products as nujol or hexachlorobutadiene mulls. Because of very strong intramolecular hydrogen bond formation, the phenolic -OH stretching absorption is broadened and is not detectable in many cases. A very strong absorption in this region ($2800-3300\text{cm}^{-1}$) is, therefore, more indicative of an -NH stretching in an oxazoline. In Schiff Base compounds a $\text{C}=\text{N}$ stretching ($1640-1600\text{cm}^{-1}$) is present, but may be complicated by the presence of phenyl ring vibrations around 1600cm^{-1} . The positions of the $\text{C}=\text{N}$ stretching frequencies are summarised in Table 6.

In addition to infrared spectra, n.m.r. spectra were obtained for some of the compounds prepared. Schiff base formation is indicated by the existence of a singlet proton resonance at 1.4-2.0 τ . Oxazoline formation is indicated

by the presence of a more complicated spectrum in which the -NH resonances are split by the presence of the protons on the adjacent carbon atoms. The n.m.r. spectra of selected compounds are also summarised in Table 6.

From these physical measurements it is evident that glyoxal bis 2, aminophenol existed as the oxazoline, in accordance with published data. All other compounds gave the spectra of typical Schiff bases.

Table 6.

Physical Measurements Carried out on Schiff Bases.

	Compound	Azomethine Proton Shift (τ)	Infrared (cm^{-1}) -CN Stretch
I	Benzylidene aniline	1.72(CCl_4)	1627
II	Benzylidene 2,aminophenol	1.53(CCl_4)	1626
III	Salicylidene aniline	1.46(CCl_4)	1618
IV	Salicylidene 2,aminophenol	1.25(dioxan)	1632
V	Salicylidene 2,aminophenol 4, sulphonic acid	insoluble	1630
VI	Salicylidene 2,amino- benzoic acid	1.45(dioxan)	1628
VII	Salicylidene 1,amino 8, hydroxynaphthalene 4, sulphonic acid	insoluble	1630
VIII	Bis salicylidene azine	insoluble	1612
IX	Bis salicylidene ethylenediamine	1.53(dioxan)	1638
X	Bis salicylidene 1,2 diaminobenzene	1.05(acetone)	1630
XI	Glyoxal bis 2, aminophenol	2.20(DMSO^*) 2.28 4.70 4.80	Absent (3380 strong NH stretch)
	*Dimethylsulphoxide		

Fluorescence Reactions of Schiff Bases with Metals.

The fluorescence characteristics of the reagents themselves were first examined in neutral, alkaline (ca. pH9-10) and strongly alkaline dimethylformamide solutions. Under all these conditions, salicylidene-2-amino benzoic acid(VI) and salicylidene-1-amino-8-hydroxynaphthalene-4-sulphonic acid(VII) gave an intense blue fluorescence which masked any fluorescence reaction on chelation. The reagents were then tested under similar conditions with solutions of the following elements:

Al, Ag, As(V), Au, B, Ba, Be, Bi(III), Ca, Cd, Ce(IV), Co(II), Cr(III), Cr(VI), Cu(II), Fe(II), Fe(III), Ga, Hg(I), Hg(II), In, Mn(II), Mo(VI), Nb(V), Ni, Pb, Pt(IV), Sb(III), Se(IV), Sn(II), Sn(IV), Sr, Te(IV), Th(IV), Ti(IV), Tl(I), U(VI), Zn and Zr(IV). Positive fluorescence reactions are summarised in Table 7.

All the reagents were tested for the so-called "indicator-reversal" effect⁶⁸. Some fluorescent indicators such as calcein exhibit a fluorescence in alkaline solutions (ca. pH10) which disappears on increasing the alkalinity (pH 12) of the medium. Addition of certain metal ions (e.g. Ca, Sr) to the strongly alkaline solution regenerates the fluorescence⁶⁸. None of the reagents prepared showed this effect on testing with Al, Mg, Ca, Sr, Ba and Zn.

In view of the sensitivity of the reactions of aluminium and gallium with salicylidene 2,aminophenol it was decided to investigate these reactions more thoroughly with a view to development of analytical methods.

Table 7.

Positive Fluorescence Reactions of Schiff Bases
in Dimethylformamide.

Substance	Metal	Fluorescence	Acidity
I	-	None	
II	-	None	
III, IV, V, X	Al	Strong Green	Neutral
	Ga	Strong Green	"
	Zn	Weak Blue-Green	Sl. Alkaline
	Be	Blue	"
	Mg	Green	Alkaline
	Ca	Green	Alkaline
	Ba & Sr	(Weak Green)	Alkaline
VIII	Zn	Green	Neutral
	Al	Blue	"
	Ga	Strong Blue	"
	Be	Strong Blue	"
	Zn	Strong Blue	Sl. Alkaline
	Cd	Weak Blue	"
	In	Weak Blue-Green	"
	Mg	V. strong blue	Alkaline
	Ca	V. weak green	"
XI	-	None	
Residual intense blue fluorescence of Reagents (VI), (VII), masks any effect produced on chelation.			

CHAPTER III

SALICYLIDENE 2, AMINOPHENOL

THE DETERMINATION OF ALUMINIUM USING SALICYLIDENE 2, AMINOPHENOL^{45,46}.

The standard fluorimetric methods for the determination of aluminium utilise morin⁴² (3,5,7,2',4', pentahydroxy-flavone); Pontachrome Blue Black R⁴⁴ (4, sulpho 2, hydroxy 1, naphthalene-azo 2', naphthol); 8, hydroxyquinoline⁴³; or more recently 2, hydroxy 3, naphthoic acid^{17,31}. 8, Hydroxyquinoline is used in an extraction procedure, but many other metal ions form co-extractable fluorescent complexes while morin gives a sensitive, but unselective fluorescence reaction. Pontachrome BBR requires a development time of nearly one hour, but gives a fairly selective fluorescence reaction with aluminium. Salicylidene 2, aminophenol was chosen as a reagent for study because of its widespread use as a fluorescence indicator in Eastern Europe^{60,63,64,65}, and because of its sensitivity in comparison with other azomethine reagents.

The reagent is easily prepared from readily available materials and can be easily purified. Many cations interfere with the reaction by forming coloured, non-fluorescent complexes, but the majority can be removed by extraction as their diethyldithiocarbamates into ethyl acetate at the pH of the determination without adverse effect on the subsequent spectrofluorimetric determination of aluminium. Out of 46 metal ions examined, the only interferences which could not be removed readily were those due to chromium(III), scandium and thorium.

Procedure

Add a 10 ml aliquot of aluminium solution containing 2.7×10^{-2} μ g to 2.7 μ g of aluminium, to a 250 ml separatory-funnel containing 10 ml pH 5.6 buffer and 30-35 ml of ethyl acetate. Add 10 ml of 0.2% sodium diethyldithiocarbamate solution and shake for 30 secs. Reject the organic phase, which contains the undesired foreign ions and transfer the aqueous phase to a 100 ml standard flask.

Add 5 ml of 0.1% salicylidene-o-aminophenol reagent and dilute to 100 ml with water. Measure the fluorescence after 20 mins at 520 m μ using an excitation wavelength of 410 m μ .

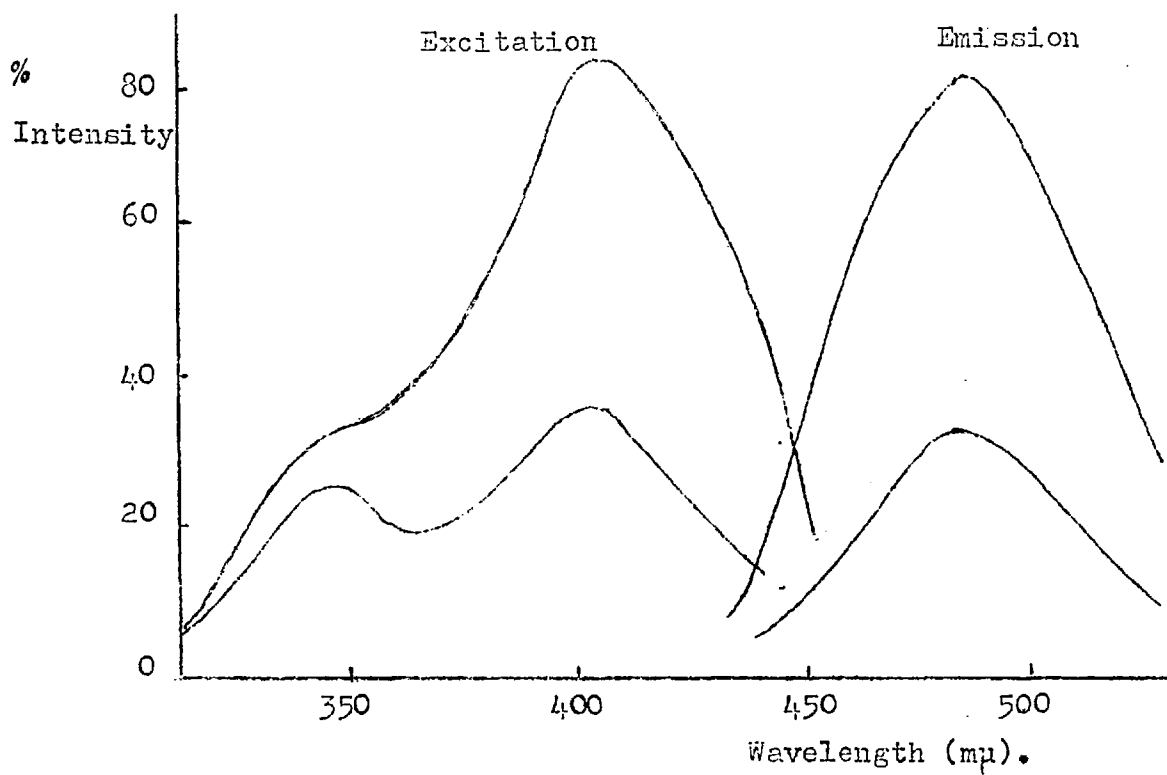
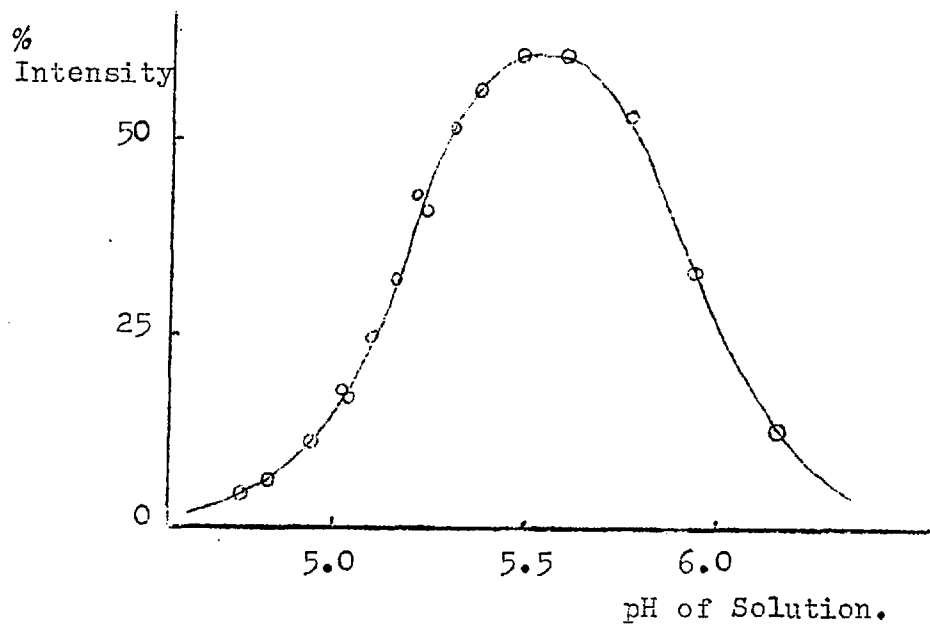
The calibration curves should be prepared as above, using aliquots of 10^{-4} M to 10^{-6} M aluminium solutions freshly prepared by dilution of the 10^{-2} M or 10^{-3} M stock solution. The curves pass above the origin because of the blank value.

Spectral Characteristics

The uncorrected emission and excitation spectra are given in figure 11. The excitation spectrum has maxima at 410 and 340 m μ in agreement with the absorption spectrum of the reagent while the fluorescence emission maximum occurs at 520 m μ .

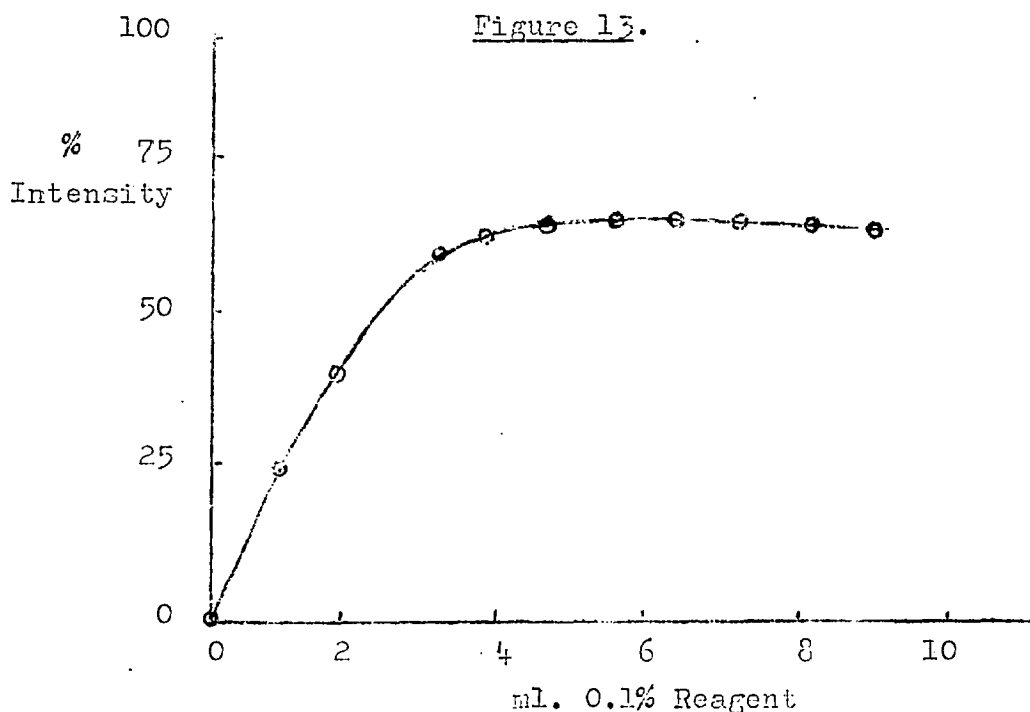
Influence of pH

The intensity of fluorescence was measured over a range of pH values obtained by adjusting the pH of the solution with ammonia or acetic acid (figure 12). The aluminium complex formation shows a sharp pH dependence with a maximum at pH 5.6 in agreement with other work.⁸ It is therefore advisable to check the pH before measurement. The tolerance is ± 0.1 pH unit but in this respect the method does not demand closer limits than other spectro-fluorimetric procedures.

Figure 11.Figure 12.

Reagent Concentration

The effect of reagent concentration on the fluorescence intensity of the aluminium complex is shown in figure 13. In the recommended procedure, 5 ml of 0.1% reagent are used, corresponding to a 1,000-fold excess over the upper limit of aluminium used in this investigation.



Influence of Time

The fluorescence intensity of a $10^{-6}M$ (0.027ppm) aluminium test solution was measured and found to be steady 15 mins after mixing. A constant ratio was obtained thereafter when measured against a quinine sulphate standard throughout the next 4 hours; on standing overnight the ratio had dropped by only 3%.

On the addition of the reagent, a yellow colour is first observed, both in the absence and presence of

aluminium, but gradually fades after dilution. The fading process takes 10-15 mins, after which measurements can be made. The fading is independent of lighting conditions, because a reagent blank, freshly diluted and kept in a dark cupboard, fades at the same rate as one exposed to normal laboratory illumination. Because the fading is rapid in solutions of low pH, it is thought that the reagent, but not the aluminium complex, is hydrolysed in the acid solution. Although aluminium reacts with salicylaldehyde, one of the hydrolysis products, to give a green fluorescence, this latter reaction is not as sensitive as with the Schiff Base reagent.

Limits of Determination.

A linear relationship between fluorescence intensity and concentration of the final solution exists for aluminium in the range 10^{-8} M (2.7×10^{-4} ppm) up to 10^{-6} M, i.e. for amounts ranging from 27 ng to 2.7 μ g in 100 ml. The linearity probably extends above this range. If due allowance is made for the volume of reagents to be added to this procedure, this corresponds to a working concentration range of 4×10^{-4} to 4×10^{-2} ppm for the original aluminium test solution. The blank value corresponds to 0.1 μ g of aluminium. The limit of determination of aluminium using the 2, hydroxy-3-naphthoic acid^{17,31}, method, previously developed in this laboratory, was ca. 270 ng so that the present procedure is approximately 10 times sensitive.

Attempts were made to reduce the value of the blank of the determination by preparing the reagent in the presence of EDTA in slightly ammoniacal ethanol to remove trace metals from the starting materials, and by repeated recrystallisation of the reagent from various solvents to remove fluorescent impurities. The use of appropriate

filters on emission and excitation beams to minimise random scatter within the monochromator and the variation of slit width was also examined. None of these approaches caused any significant reduction of the blank value. The blank is thought to be due to a residual fluorescence caused by the reagent; reduction of the blank accompanies a reduction of the reagent concentration, although there is no improvement in the signal to blank ratio.

Interferences

The effects of 100-fold molar excesses of foreign ions were examined for $2 \times 10^{-7} \text{M}$ aluminium ($0.135 \mu\text{g}$) - see Table 8. The limiting error was taken as $\pm 5\%$ of the fluorescence intensity value obtained in the absence of foreign ions. Marginal interferences were examined in more detail. The following 34 cations did not interfere beyond the diethyldithiocarbamate extraction:

Ag, As(III), Ba, Be, Ca, Cd, Ce(III), Co(II), Cu(II), Fe(II), Fe(III), Ga, Hg(II), In, K, La, Li, Mg, Mn(II), Mo(VI), NH_4 , Ni, Pb, Se(IV), Sn(IV), Sr, Te(IV), Tl(I), Tl(III), V(IV), Y, Zn.

Antimony(III), bismuth and uranium(VI) were quantitatively removed after two or more extractions and did not subsequently interfere. It was more convenient to use carbon tetrachloride for the initial extractions and ethyl acetate for the final extraction in view of their densities relative to water.

Tungsten formed a yellow product with the reagent which gave rise to low results, but only when present in greater than 10-fold molar excess. Quantitative extraction of tungsten using diethyldithiocarbamate occurs at lower pH values. A similar interference caused by vanadium(V) and cerium(IV) could be eliminated completely by reduction with ascorbic acid before extraction. Reduction with ascorbic

Table 8.

Interferences in the determination of aluminium using salicylidene 2, aminophenol. Foreign ions are present in 100-fold molar excess.

Ion	% Deviation		% Deviation Further Treatment
	Without Extraction	With Extraction	
Sb(V)	+23	+9	+1 Repeat Extraction
Bi(III)	+32	+10	+5 Repeat Extraction
Ce(IV)	-6	-6	0 Reduce to Ce(III) and Extract
Cu(II)	-81	+2	
Cr(III)	-88	-82	-82 No Elimination Procedure
Fe(II)	-95	-1	
Fe(III)	-95	-2	
Hg(II)	+63	+1	
Mo(VI)	+91	+3	
Pt(II)	+20	+20	-1 Mask with CN^-
Sc(III)	+22	+22	+22 No Elimination Procedure
Se(IV)	+11	0	
Te(IV)	+18	+1	
Th(IV)	+16	-6	-6 No Elimination Procedure
Sn(II)	-50	-50	0 Oxidise to Sn(IV) and Extract
W(VI)	-31	-6	-1 Repeat Extraction
U(VI)	+15	-3	0 Repeat Extraction
V(V)	-6	-1.2	0 Reduce to V(IV) And Extract
Zr(IV)	-50	-50	-3 Add mandelic acid

acid also improved the extraction of large quantities of iron and the stability of the reagent to oxidation at pH5.6. Tin(II) was best removed by extraction of tin(IV) diethyldithiocarbamate following oxidation with hydrogen peroxide and removal of the peroxide by heating. Zirconium gave low results because of precipitation, but this interference could be entirely removed by the addition of large excesses of mandelic acid after the extraction. A 100-fold excess of platinum gave a turbidity which yielded high results; the addition of cyanide masked this interference.

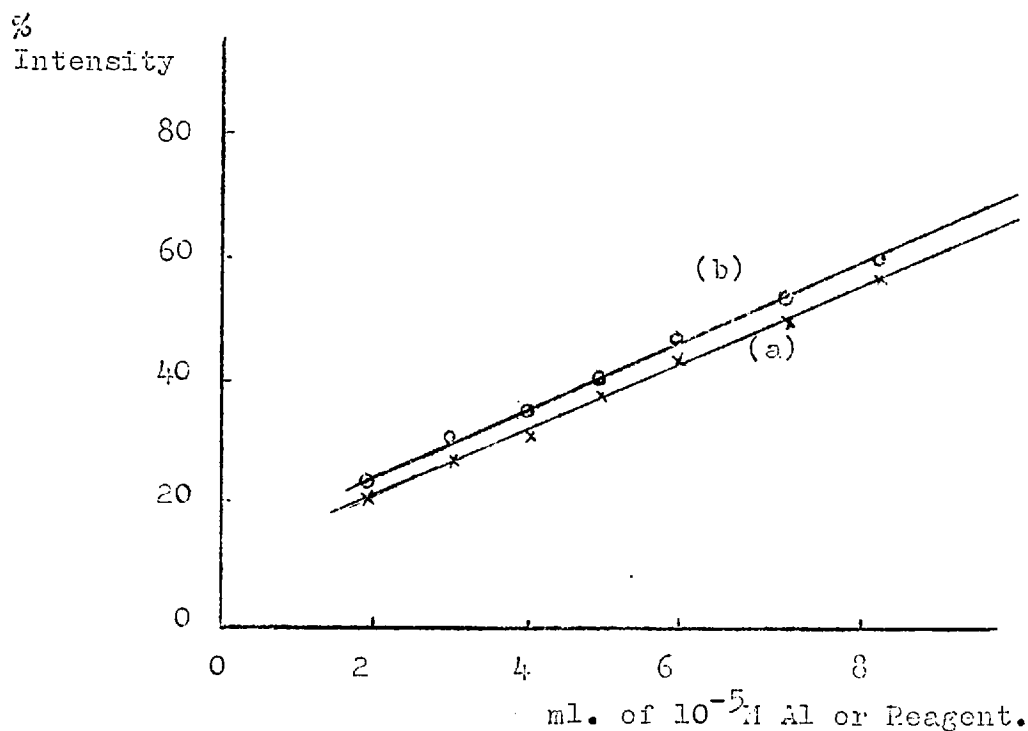
The remaining cations were thorium, scandium and chromium(III). Thorium gave slightly low results (ca.10% with 100-fold excess) with no visible signs of precipitation or complex formation. Scandium appeared to form a fluorescent complex and chromium(III) gave very low results. The effects of niobium and tantalum were not examined because these cations could only be obtained in fluoride or tartrate solutions which are mentioned below as anionic interferences.

Anionic interferences were examined in 100-fold molar excess and no interference was noted from acetate, chloride, bromide, iodide, sulphate, nitrate, perchlorate, cyanide and carbonate. Small quantities of fluoride, tartrate, citrate and oxalate interfered seriously by diminishing the fluorescence due to aluminium, similar behaviour was encountered from members of the complexan series: EDTA, NTA, IDA and CDTA. The metal complexes zinc/EDTA, manganese(II)/EDTA and cadmium/EDTA did not affect the fluorescence but proved ineffective as masking agents.

Beryllium, even in 1,000-fold excesses, did not interfere with the determination as it does with most other methods for aluminium. It is, however, noteworthy that at higher pH values (ca.9-10), beryllium gives a blue fluorescence with salicylidene 2, aminophenol. Because beryllium is not extracted as a diethyldithiocarbamate, this could form the basis of a sequential determination of beryllium and aluminium. The reagent is, however, not very sensitive for beryllium.

Slope ratio plots (figure 14) indicate a metal:ligand ratio of 1:1 but no solid complex could be isolated for further examination. It is probable that the complex

Figure 14



- (a) Aliquots of $10^{-5}M$ aluminium solution with 5ml of 0.1% reagent solution at pH 5.6 in 100 ml.
- (b) Aliquots of $10^{-5}M$ reagent solution with 5 ml of $10^{-5}M$ aluminium solution in 100 ml.

is polynuclear and similar to those proposed for the aluminium complexes of Pontachrome BBR and 2, hydroxy 3, naphthoic acid. This is supported by the following observations:

1. Although no aluminium complex could be isolated a green fluorescence was produced when a 0.1% solution of salicylidene 2, aminophenol was dropped on to either freshly precipitated, washed alumina or strongly heated, inert aluminium hydroxide. The surface of the alumina was stained yellow, the colour of the reagent, and the colour was not removed by washing with water, acetone or ethanol.
2. The pH of formation of the complex is pH5.6, which is considerably higher than the pH at which aluminium hydroxide precipitates (approximately pH4.1).
3. The fluorescent aluminium complex of salicylidene 2, aminophenol has several properties similar to the complexes of aluminium with 2, hydroxy 3, naphthoic acid^{17,31} and pontachrome BBR⁴⁴. The pH range for formation is narrow and occurs over a similar range in all three instances: Pontachrome BBR, pH5; 2, hydroxy 3, naphthoic acid, pH5.8; salicylidene 2, aminophenol pH5.6. (With 8, hydroxyquinoline complete complex formation occurs above pH3.5 and with morin at pH3.0). The pH of formation of these aluminium complexes, would, therefore seem to be dependent more on the degree of hydrolysis of the aluminium aquo complex than on the state of ionisation of the ligand.

The above evidence suggests that the aluminium/reagent adduct may exist as an entity in which the reagent molecules are adsorbed chemically on to the surface of colloidal aluminium hydroxide by replacement of surface hydroxyl groups.

There is other evidence, however, which favours the formation of a more definite chemical compound. For example, it is possible to extract the aluminium/salicylidene 2, aminophenol complex into water-immiscible solvents such as n-butanol, isopentanol, n-pentanol and n-hexanol. This behaviour is more characteristic of a true aluminium complex than a simple adsorption compound. It is relevant to note that only alcohols were effective extracting solvents, and it is possible that these molecules substituted for solvated water molecules to form the extractable species. The differences in the partial extraction of the aluminium complex into methyl isopropyl ketone (ca.50%) and into methyl iso-butyl ketone (ca.5%) is an interesting anomaly and may be due to steric effects of the two ketones in the solvated complex; the donor properties of the carbonyl group are unlikely to be substantially different for the two ketones.

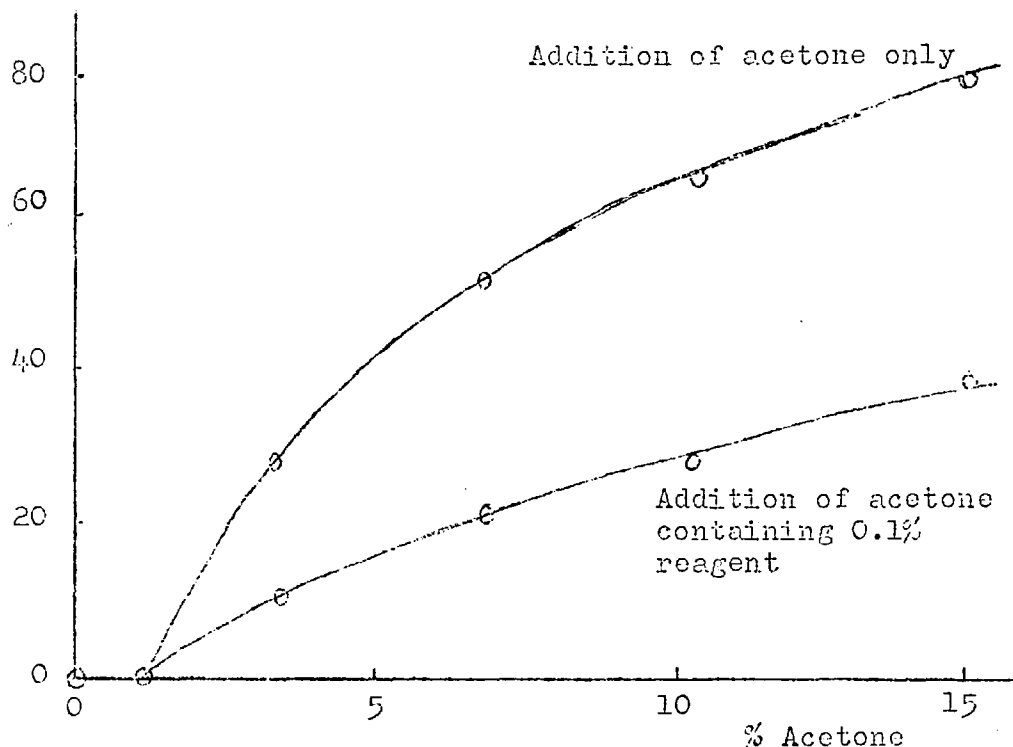
The 1:1 stoichiometry found for the compound also favours the existence of a true complex rather than an adsorption compound.

To investigate the solubilising effect of the reagent on the aluminium solution at pH5.6, light scattering experiments were carried out using the spectrofluorimeter. Inner filter and fluorescence effects were minimised by setting both monochromators at 560 mμ. It was initially found that the addition of reagent produced a higher degree of scatter than was obtained from the aluminium solution. Subsequently it was observed that the acetone in which the reagent was made up was responsible for at least some of the increased light scattering. This can be attributed to aggregation of aluminium hydroxide on reducing the dielectric constant of the medium. When allowance was made for the addition of acetone with reagent it was found that correspondingly less scatter was obtained in the presence of larger excesses

Figure 15.

%

Intensity

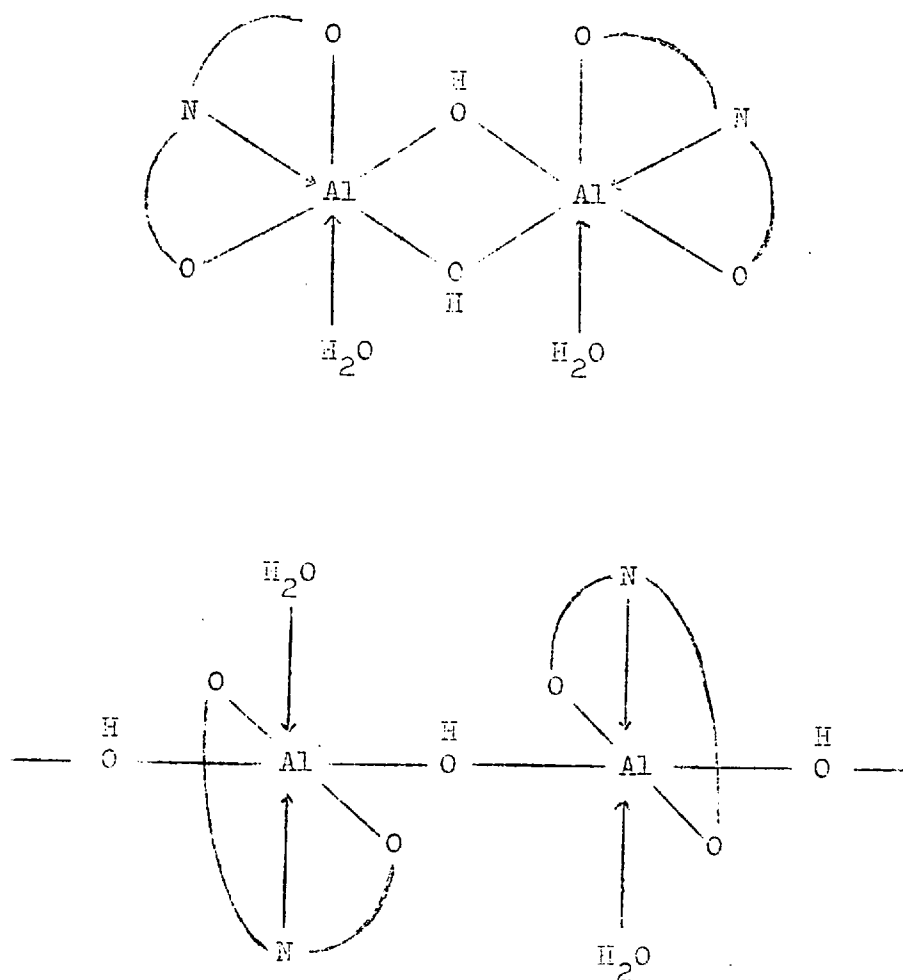


of reagent (figure 15). Although it is not possible to interpret these results unequivocally, it would appear that the reagent definitely opposes the formation of insoluble aluminium hydroxide species. It probably does so by the suggested mechanism, whereby chemical combination of the ligand through replacement of a hydroxyl group in the hydrated alumina unit terminates the possibility of further of these units through cross-linking.

With this evidence in mind it appears that the complex is neither a simple mononuclear one, nor an adsorption of reagent on to polymeric alumina, but most probably resembles a low order polynuclear species such as the ones suggested

under (figure 16). The properties of such complexes should be consistent with the observed resistance to precipitation; 1:1 stoichiometry; degree of light scattering; narrow pH zone of stability; extractability into water-immiscible, oxygen donor solvents.

Figure 16.



Reagents.0.1% Salicylidene 2, Aminophenol Reagent.

2, Aminophenol (5g) was dissolved in hot ethanol (20 ml) and salicylaldehyde (10ml) added dropwise with stirring to the hot solution. On cooling slowly to room temperature and subsequently in an ice bath, large, red-brown, needle shaped crystals separated. The product was filtered, washed with ethanol (5ml), recrystallised from ethanol and dried in a vacuum over CaCl_2 .

Yield (before recrystallisation) 32%.

Melting Point $185 - 6^\circ\text{C}$ (184°C).

The micro analysis results for two different preparations are given:

Found C:73.2%,72.9%; H:5.63%,5.08%; N:6.47%,6.77%.

Calculated C:73.3%; H:5.63; N:6.6%

Dissolve 0.5g of the solid salicylidene 2, aminophenol in 500 ml acetone. This reagent solution is stable for several weeks.

Buffer Solution (pH5.6).

Add sufficient glacial acetic acid (ca.10ml) to 50g of ammonium acetate in 400ml of water to give $\text{pH}5.60 \pm 0.05$.

 10^{-2}M Aluminium Solution.

Dissolve 0.4744 g of potassium alum, $[\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$, in 100 ml of water. Dilute this stock solution as required before use for the preparation of calibration curves. Solutions more dilute than 10^{-3}M should not be stored for any length of time, even in siliconed flasks because of the tendency for aluminium to be adsorbed strongly on glassware. 1 ml of 10^{-6}M solution = $0.027\mu\text{g}$ of aluminium.

0.2% Sodium Diethyldithiocarbamate.

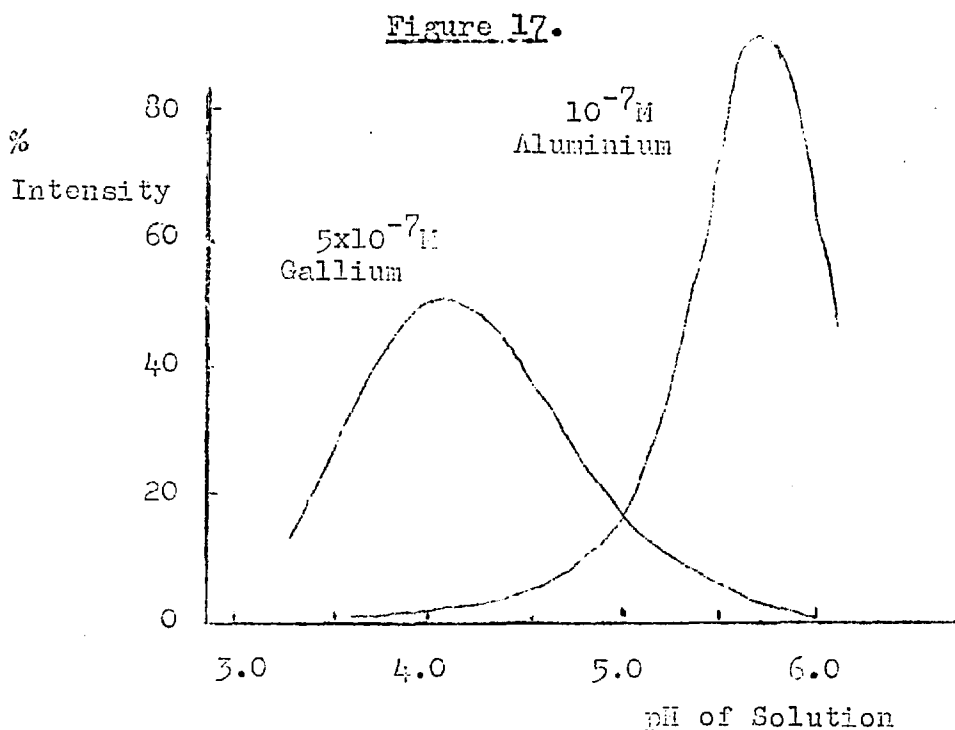
Dissolve 1g sodium diethyldithiocarbamate (Hopkin & Williams Ltd.) in 500 ml of water. This solution is subject to oxidation and should be made up daily.

INVESTIGATION OF THE GALLIUM/SALICYLIDENE 2, AMINOPHENOL SYSTEM^{4,5}.

Salicylidene-2, aminophenol has been described as giving a fluorescence reaction with gallium. In view of the potential use of this reagent with aluminium, it was thought that an investigation of the gallium fluorescence reaction would be useful. However, the reactions proved relatively insensitive and hence the following investigation was not a rigorous one.

Influence of pH.

The combined curves for the formation of the gallium and aluminium salicylidene-2, aminophenol complexes are



given in figure 17. Gallium shows a fluorescence intensity maximum at pH4.0 and aluminium at pH5.6. Because of the pH of formation of the complexes, gallium does not interfere with the determination of aluminium provided the molar gallium concentration does not exceed that of aluminium. However, only one tenth molar amounts of aluminium can be tolerated in the gallium determination.

Spectral Characteristics.

The excitation and emission characteristics for the two complexes are: gallium (410/520 mμ) and aluminium (420/510 mμ). The spectra for the gallium complex are practically superposable over those of the aluminium complex (figure 11) and are not repeated here.

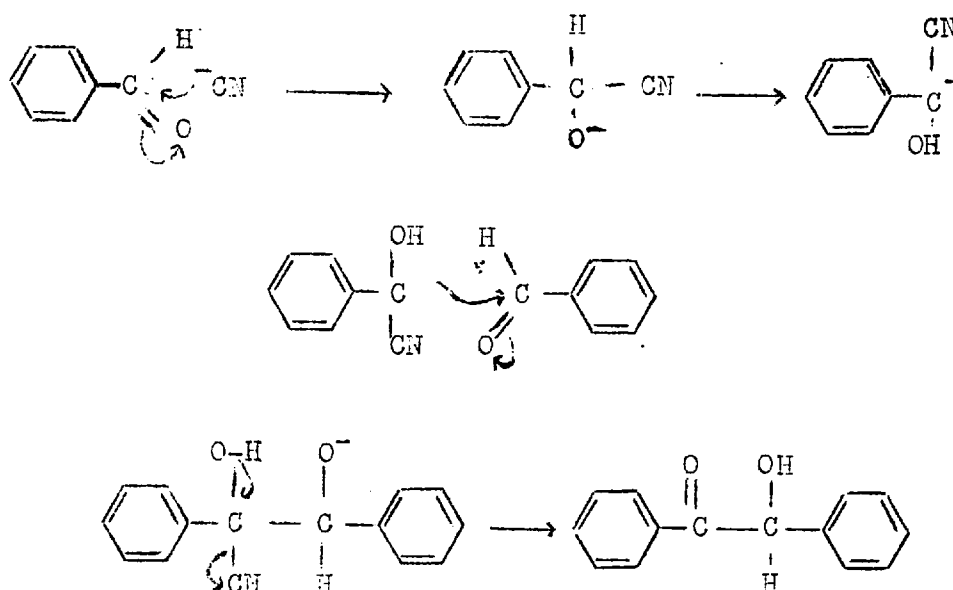
Limits of Determination.

A linear relationship between fluorescence intensity and concentration exists for gallium in the range $10^{-7}M$ (7×10^{-3} ppm) up to $10^{-5}M$ and probably beyond. (For aluminium the range was $10^{-8}M$ to $10^{-6}M$ and probably beyond). The blank in the gallium determination corresponds to $9 \times 10^{-8}M$ (6.3×10^{-3} ppm).

CHAPTER IV

BIS SALICYLIDENE 2,3, DIAMINOBENZOPURAN.

The "benzoin condensation" is a well-known, but little understood reaction in organic chemistry. On treating benzaldehyde with potassium cyanide in ethanol the reaction below occurs:



The author used this reaction to attempt to prepare 2,2', dihydroxybenzoin by an analogous procedure starting with salicylaldehyde. No product could be isolated from the reaction mixture, but on the addition of concentrated ammonia, a red precipitate separated in ca.25% yield. Assuming this to be an ammonium salt of 2,2', dihydroxybenzoin, the author tested the reagent with solutions of metal ions and found that only magnesium and gallium gave appreciable fluorescence reactions. A literature survey indicated the preparation of the compound as long ago as

1865^{69,70}, but there was no further mention of this reaction until Phadke⁷¹ reported the preparation of apparently the same compound, and assumed it to be 2,2' dihydroxybenzoin.

On obtaining infrared spectra from a nujol mull of the compound it was found that the strong C = O stretching absorption expected around 1660 cm^{-1} , and present in benzoin itself, was absent. Moreover, the n.m.r. spectrum of the compound in dioxan could not possibly have been explained on the basis that the compound was 2,2', dihydroxybenzoin. In view of the promising fluorescence reactions with magnesium and gallium it was decided to undertake a structural determination of the unknown compound. This work was carried out in co-operation with J.E. Baldwin and D.J. Blythin⁷² of the Organic Chemistry Department and the work carried out by these two has been included in Appendix II. The author's own work is described below.

PREPARATION OF BIS SALICYLIDENE 2,3, DIAMINO BENZOFURAN.

The preparation is an improved version of the one outlined before. Instead of precipitating the product with concentrated ammonia, this reagent is added at the beginning of the reaction.

A mixture of salicylaldehyde (50 ml) and ethanol (80 ml) was deaerated by bubbling nitrogen for 20 mins and concentrated ammonia (15 ml) was then added. A white or pale lemon precipitate was formed immediately. The mixture was placed in an ice-bath and potassium cyanide (6g) in water (9ml) was added with stirring. During the course of the reaction the precipitate changed from lemon to orange-yellow and, after ca. two hours, set to a solid mass. The solid was broken up, ethanol (50 ml) added and after stirring for a further 90 mins concentrated ammonia was added (15 ml). The reaction was stirred for 30 mins, filtered and washed well with:

(i) 50% (V/V) water/methanol to remove cyanide, (ii) methanol, until the filtrate was a clear yellow. The residue was removed from the filter, triturated with methanol (ca. 300 ml), filtered and dried in a vacuum dessicator over calcium chloride.

Yield 29.5 g (90% based on KCN).

MPt. 181 - 3°C.

Samples were recrystallised from benzene/pet. ether (red crystals), dioxan/water (orange powder) or acetone (yellow crystals). Despite the variation of the colour of the recrystallised products, they were all shown to have identical infrared spectra, visible spectra and n.m.r. spectra.

The product was shown to be a single substance by thin layer chromatography on silica gel using benzene and chloroform as solvents.

MPt. 182 - 3°C (recrystallised from benzene/pet. ether).

STRUCTURAL DETERMINATION OF BIS SALICYLIDENE 2,3-DIAMINO BENZOFURAN.

Microanalysis Results.

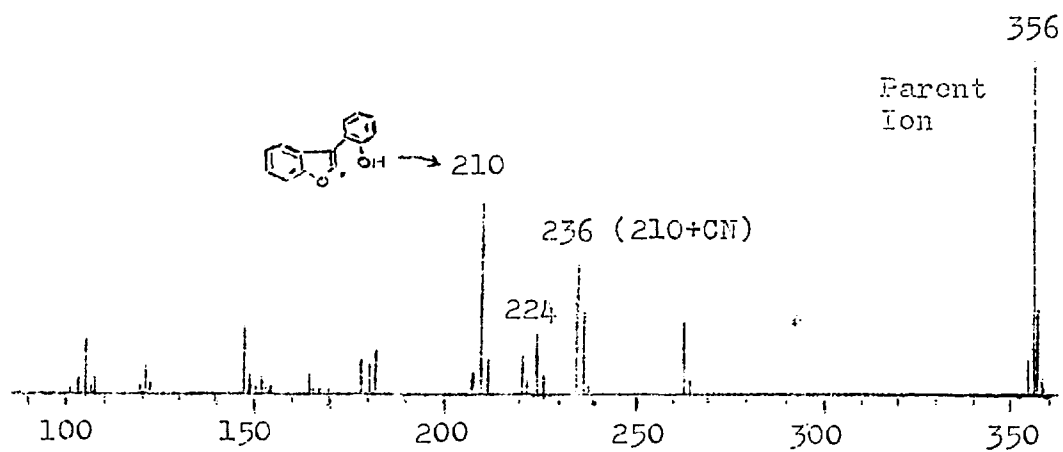
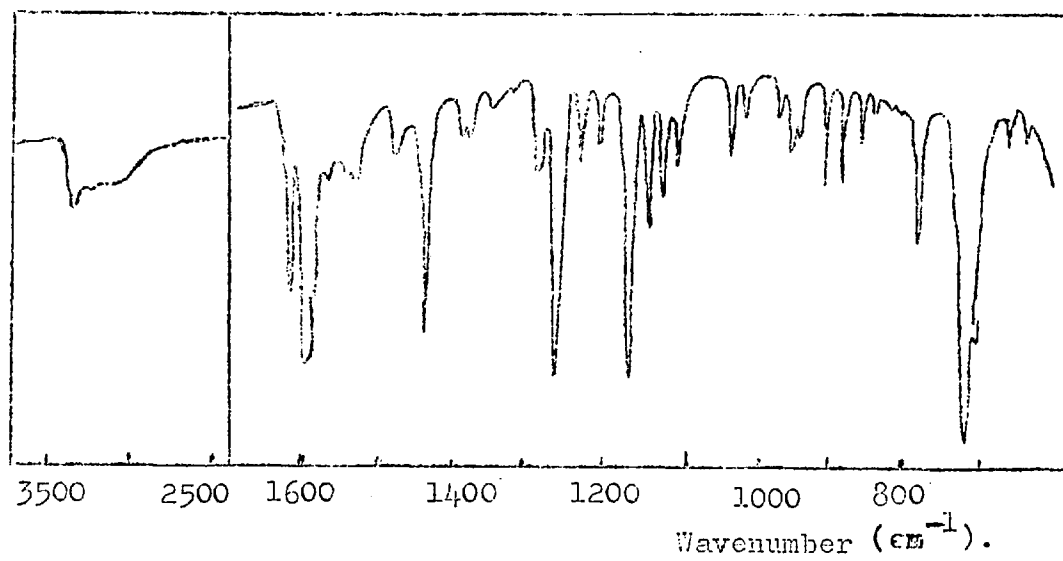
Calculated for $C_{22}H_{16}N_2O_3$ (Mr 356).

Found : C 74.0%; H 4.4%; N 7.8%.

Calculated: C 74.2%; H 4.5%; N 7.8%.

Mass Spectrum.

The mass spectrum gave a parent ion peak at mass 356 consistent with the molecular weight obtained by microanalysis. The mass spectrum is shown in figure 18, the identity of the peaks being assigned after the structure had been determined.

Figure 18.Figure 19.

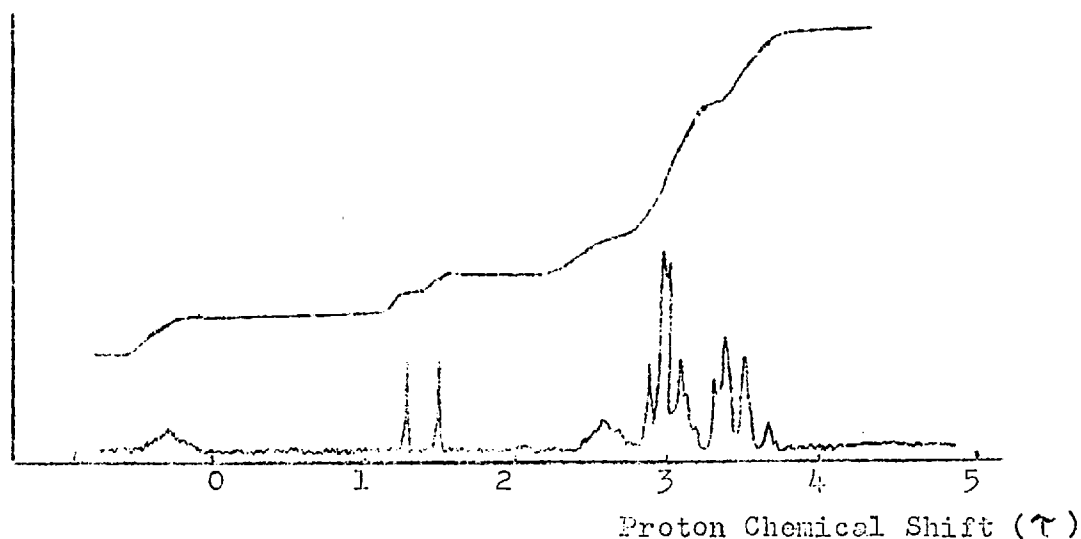
Infrared Spectrum.

The infrared spectrum is shown in figure 19. Characteristic functional group absorptions are at 1630cm^{-1} (C = N stretching); 1610cm^{-1} , 1600cm^{-1} , 1490cm^{-1} and 1450cm^{-1} (aromatic ring vibrations); 1275cm^{-1} (phenolic OH out of plane bending, confirmed by deuteration); 1185cm^{-1} (possibly phenolic CO stretching); and 740cm^{-1} (1,2 disubstituted benzene ring). In addition there was a little, inconclusive absorption around 3200cm^{-1} typical of a very strongly hydrogen-bonded -OH group. The infrared spectrum showed the complete absence of any -C = O, -NH₂ or -NH groups.

N.M.R. Spectrum.

The n.m.r. spectrum of the compound in CDCl₃ is shown in figure 20. The spectrum shows a broad resonance which

Figure 20.



disappears on deuteration, characteristic of two -OH protons at 0.7τ , together with two singlets at 1.26τ and 1.38τ characteristic of two azomethine protons. (Aldehyde protons would appear in this region, but $C = O$ has been shown to be absent. Protons on the α -carbon atom in an unsaturated nitrogen heterocyclic ring would also appear in this region). In addition there is resonance in the aromatic proton region around 2.7τ characteristic of twelve aromatic protons.

The number of protons indicated by the n.m.r. confirms the analysis result.

Chemical Evidence.

(a) Acid Hydrolysis - The physical evidence obtained indicates the possible presence of one or two azomethine $-CH = N$ -groups. Such groups are subject to rapid hydrolysis under mild conditions and the effect of acid hydrolysis was therefore examined.

The compound (0.12 g) was dissolved in hot dioxan (5 ml) and concentrated hydrochloric acid (10 ml) and hot water (30 ml) added. The solution was kept hot, without boiling for 30 mins and precautions taken to avoid accidental steam distillation. The 2:4 DNP reagent⁷³ (80 ml) was added dropwise to the warm solution which was then allowed to cool to room temperature. The precipitate was filtered on a sintered glass crucible, washed with 2N hydrochloric acid (20 ml) and water (20 ml), and dried to constant weight over phosphorous pentoxide in a vacuum. (The reagent solution was made by saturating 2N hydrochloric acid (120 ml) with 2:4 dinitrophenylhydrazine, filtering and adding 2N hydrochloric acid (20 ml).

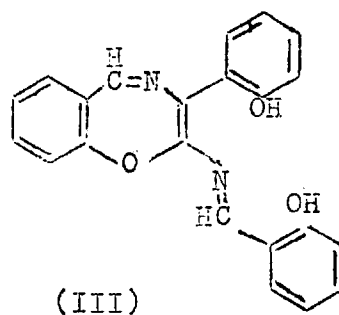
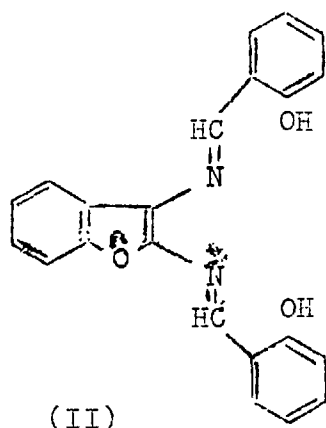
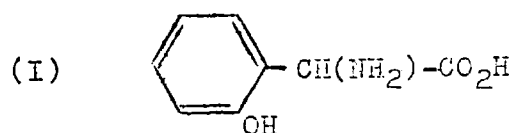
The product gave a single spot $R_f = 0.6$ when run as a thin layer chromatogram in silica gel with chloroform as solvent, or $R_f = 0.4$ with benzene as solvent.

After two recrystallisations from ethanol/dimethylformamide (ca. 5/1 V/V) the melting point of a small sample became constant at 245°C the specimen decomposed on melting. (Salicylaldehyde 2:4 DNP melts at 250°C with decomposition).

A specimen of the product gave an infrared spectrum identical with that of the salicylaldehyde 2:4 DNP derivative. Weight of original compound = 0.120 g 3.37×10^{-4} mole. Final weight of derivative = 0.2143 g 7.12×10^{-4} mole. Therefore from 1 mole of the original compound, 2.11 mole of 2:4 DNP derivative was obtained.

These results indicate that the unknown compound contains two salicylidene residues.

In a separate experiment (Appendix II) the other hydrolysis product was identified as the amino-acid (I):



Two structures can be written for the unknown, (II) and (III), on the basis of the evidence described. These are the only structures which would explain the experimental findings satisfactorily. The chemical evidence used to distinguish

between the two structures (II) and (III) is given in Appendix (II). However, the author was able to make a less rigorous distinction on the basis of their expected chelation properties.

(b) Glass Electrode Titration - Stuart Molecular models indicated that (II) could form square-planar complexes with metal ions involving both phenolic OH groups. Compound (III) could react with a metal ion through both OH groups to give a chelate that was not square-planar, and which contained a seven-membered chelate ring.

A glass-electrode titration was carried out for the reagent and nickel in 80% dioxan/water, the results are shown in figure 21. On chelation with one mole of nickel it is evident that two protons are released and from figure 22 it is evident that the release of protons occurs on a single step. Therefore, both phenolic OH groups are involved in chelation and the single step process would indicate a fairly symmetrical complex.

Spectrophotometric mole-ratio studies were carried out in 50% dioxan with the addition of 1ml 1:4 diluted ammonia.

The absorbance was measured after 10 mins at 500 m μ using 4cm glass cells and a distilled water blank. The results (figure 23) indicated the formation of a 1:1 nickel/reagent complex.

(c) Magnetic Studies on the Nickel Complex - Nickel chloride $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (2.4 g) was dissolved in hot dimethylformamide and a solution of the unknown (3.6 g) in hot dimethylformamide (20 ml) was added. The solution was heated almost to boiling, allowed to cool slowly to room temperature, and finally cooled in ice for 30 mins. The deep red crystals obtained were filtered, washed well with ether and dried in a vacuum over silica gel. Yield 1.7 g (ca. 40%).

Figure 21.

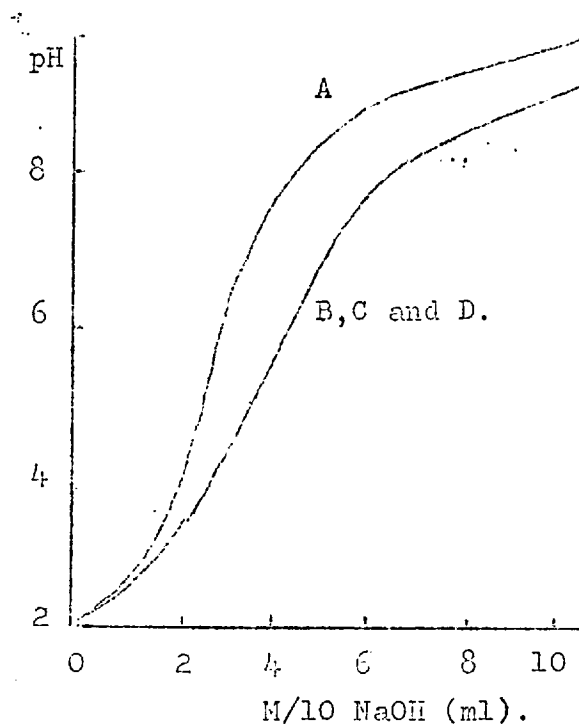
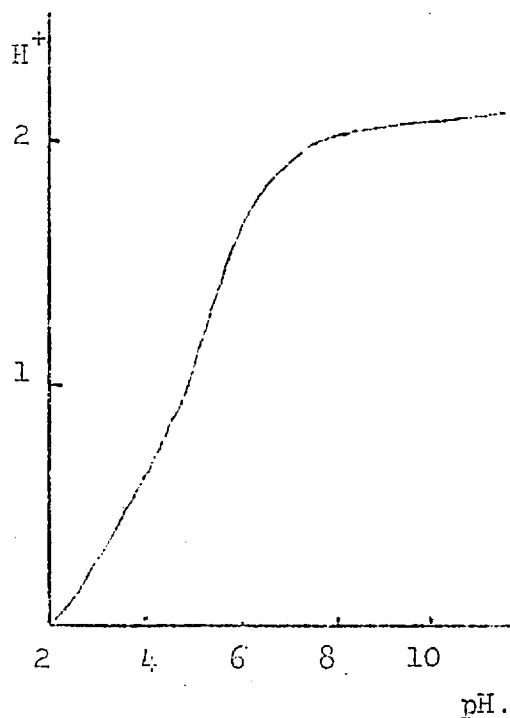


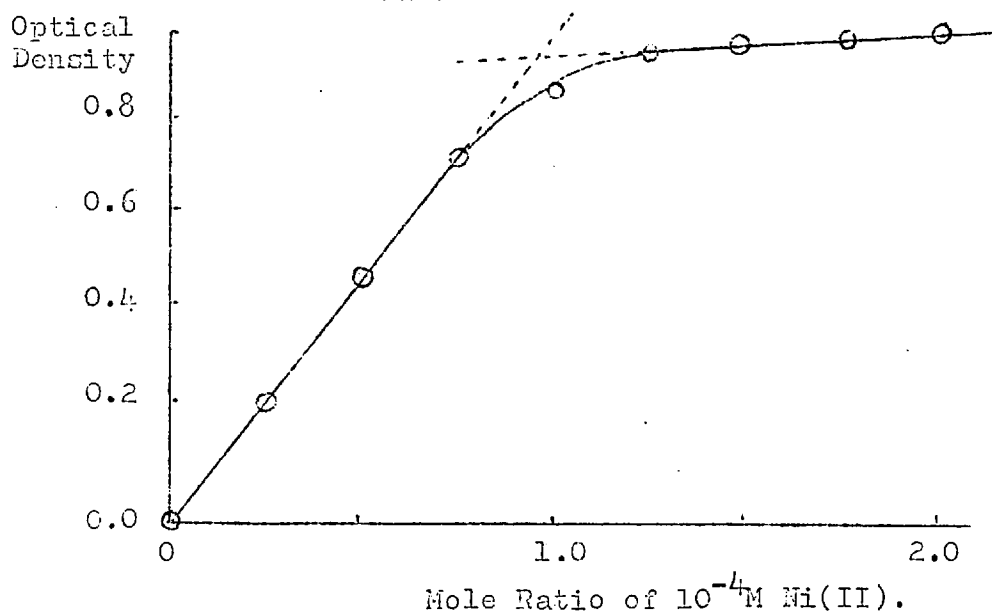
Figure 22.



- A, 0.001M SABF.
 B, 0.001M SABF+0.001M Ni(II).
 C, 0.001M SABF+0.0005M Ni(II).
 D, 0.001M SABF+0.002M Ni(II).

Number of protons released
 (H^+) in titration.v.pH.

Figure 23.



Analysis: Nickel analysis by the pyridine thiocyanate gravimetric procedure⁷⁶ Found: Ni = 14.05%.

Calculated ($\text{NiC}_{22}\text{H}_{14}\text{N}_2\text{O}_3$)Ni = 14.3%.

Measurements of paramagnetic susceptibility on 0.1343 g of the nickel complex at 21°C gave a magnetic susceptibility of 0.790 B.M. This is consistent with the formation of a planar nickel complex^{74,75}. An octahedral nickel complex would have an expected susceptibility ca. 2.83 B.M. and a tetrahedral complex ca. 3.5 B.M.^{74,75}.

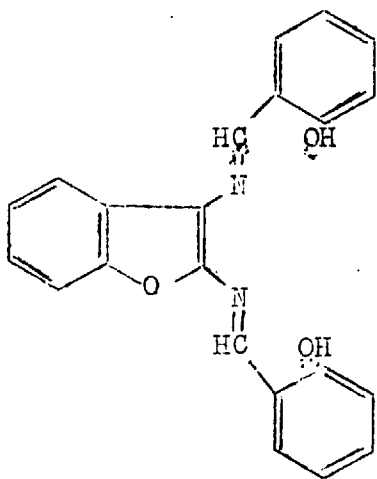
In infrared spectrum of the nickel complex indicated the absence of H_2O , dimethylformamide or free reagent. The 1630 cm^{-1} C = N stretching frequency of the free liquid was shifted into the region due to the strong aromatic ring absorptions and the 1275 cm^{-1} phenolic OH bending of the ligand was absent in the complex.

On the basis of the chelating tendencies of the ligand, the structure (II) was thought to be more probable than (III). Later evidence (Appendix II) confirmed this.

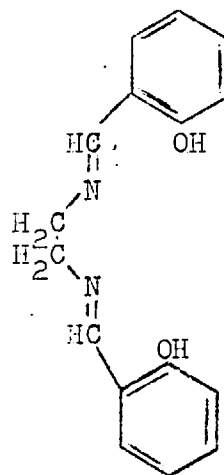
THE STEREOCHEMISTRY OF BIS SALICYLIDENE 2,3, DIAMINOBENZOFURAN (SABF)⁷⁷.

Four geometric isomers of bis salicylidene 2,3, diaminobenzofuran (SABF) are possible due to cis/trans isomerism about the azomethine bonds. Apart from the "all trans" configuration, these isomers have one, or both, salicylidene groups aligned at right angles to the benzofuran ring. The consequent loss of conjugation would be expected to render these isomers thermodynamically less stable than the "all trans" configuration, and they would be expected to show different behaviour on thin-layer chromatography. Thin-layer chromatography (on silica gel using benzene or chloroform) indicated the presence of only one compound.

SABF has the same reactive grouping as bis salicylidene ethylenediamine (SALen), the reagent proposed by Cuttitta and White⁷⁸ for the determination of magnesium. A comparison of the two reagents is interesting in view of the differences in their properties.



Bis salicylidene 2,3,
diamino benzofuran

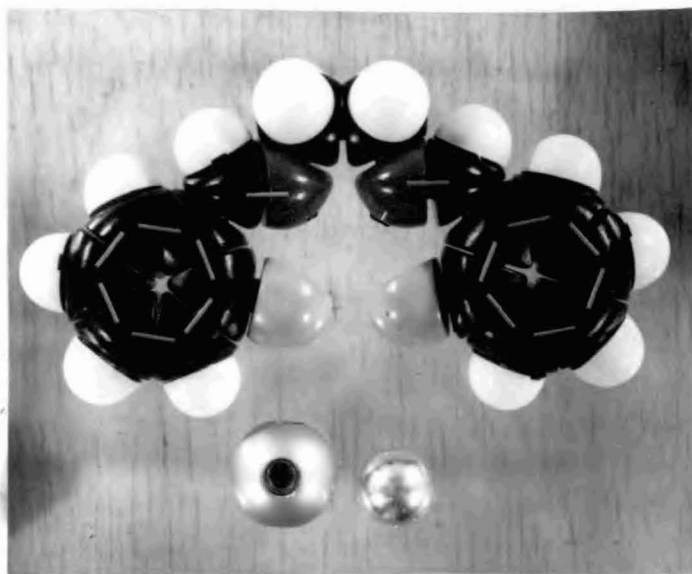


Bis salicylidene
ethylenediamine

In SALen, free rotation about the central carbon-carbon bond is possible. Introduction of a double bond prevents rotation and shortens the bond distance. Thus the amount of tolerable strain on chelation with large ions is decreased and the effective size of the cavity available for chelation is reduced. The distance between the two azomethine nitrogen atoms in SALen can widen to accomodate large cations, but steric interactions in SABF prevent this. An increase in the distance between the azomethine nitrogen atoms of SABF would result in an interaction between the hydrogen atom in the 4-position of the benzofuran ring with

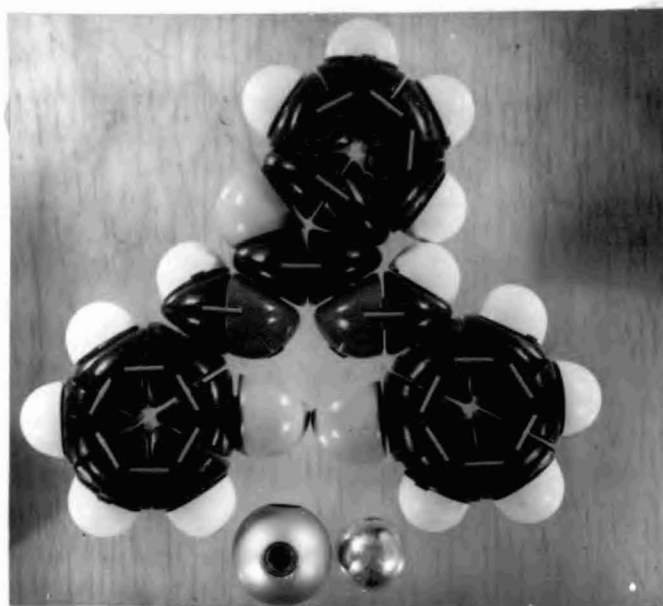
Figure 24.

73.



NN' bis Salicylidene Ethylenediamine

Figure 25.



NN' bis Salicylidene 2,3 Diamino
Benzofuran

the hydrogen atom on the neighbouring azomethine group. (figures 24 and 25). Also, closure of the two nitrogen atoms would result in an interaction of the two ionised phenolic oxygen atoms. Consequently the dimensions of the cavity available for chelation in the planar ligand will be strictly limited by these steric effects. The largest ion which could be accommodated should have an ionic diameter equal in size to that of the largest square cavity which can be made. Stuart and Briegleb scale models of SABF were constructed and measurements made of the size of the square planar cavity. From these approximate measurements an upper limit in ionic radius of $1.00\text{--}1.04\text{\AA}$ was deduced. There is excellent agreement between this and the experimental results shown in table 9.

Although too much emphasis must not be placed on the nature of the ions which do not react with SABF, it is worth noting that all the ions examined with radii between 0.5 and $0.8\text{\AA}$ reacted, whilst none with radii greater than $1\text{\AA}$ did. The range was limited to those cations existing in aqueous solution as simple aquo complexes and whose ionic radii were known. The alkali metals were not considered because of their low powers of co-ordination.

FLUORESCENT CHELATION REACTIONS OF BIS SALICYLIDENE 2, 3, DIAMINO BENZOFURAN⁷⁷.

The reactions of SALen with various metal ions in dimethylformamide⁷⁸ were checked and are compared here with the results reported in table 9. All the cations which reacted with SABF also reacted with SALen. In addition, lead (ionic radius $1.27\text{\AA}$) gave a yellow complex with SALen but not with SABF. The most important differences, however, are shown in the fluorescence reactions of the two ligands. SALen gives a strong blue fluorescence in neutral

Table 9.

Reactions of Metal Ions with SADF.

Ion	* Ionic Radius Å	Reaction
Be ²⁺	0.38	No reaction.
Al ³⁺	0.52	Slight orange fluorescence.
Ga ³⁺	0.60	Bright orange fluorescence
Cr ³⁺	0.62	No visible reaction due to kinetic inertness.
Co ³⁺	0.63	Green colour obtained on oxidation of Co ²⁺ complex.
Fe ³⁺	0.64	Red brown soluble complex.
Mg ²⁺	0.66	Bright orange fluorescence.
Cu ²⁺	0.72	Red precipitate, not masked by CN ⁻ .
Zn ²⁺	0.72	Red ppt. weakly red fluorescent.
Ni ²⁺	0.73	Red colouration.
Co ²⁺	0.74	Very deep red colouration.
Fe ²⁺	0.76	Reddish precipitate.
Mn ²⁺	0.78	Pink colour.
In ³⁺	0.81	Weak pink colour, large In ³⁺ excess.
Sb ³⁺	0.92	No reaction.
Cd ²⁺	0.96	Pink, non-fluorescent precipitate
Tl ³⁺	0.96	No reaction
Ca ²⁺	0.99	Red complex with large Ca ²⁺ excess
Bi ³⁺	1.08	No reaction
Sn ²⁺	1.10	" "
Hg ²⁺	1.10	" "
Ce ³⁺	1.14	" "
Sr ²⁺	1.15	" "
La ³⁺	1.16	" "
Ag ⁺	1.21	" "
Pb ²⁺	1.27	" "
Ba ²⁺	1.37	" "
Tl ⁺	1.59	" "

* Octahedral crystal radius

or basic dimethylformamide with magnesium, beryllium, zinc, aluminium, gallium and indium. All have fluorescence excitation maxima at 350 - 360 m μ and emission maxima at 440 - 450 m μ . However, only gallium and magnesium were found to give strongly fluorescent chelates with SABF. The excitation spectra are similar in both cases; the maximum of the longest wavelength absorption band being at 475 m μ in 50% methanol/water with an emission maximum at 545 m μ . Aluminium and zinc give weakly fluorescent complexes with SABF, the indium complex is non-fluorescent and beryllium does not appear to react. These differences can be related directly to the radii of the ions involved.

It is interesting to consider the effect of ionic radius for any particular periodic group. For example, beryllium (group IIa) does not react because its small radius prevents formation of octahedral or planar complexes. The formation of a beryllium/SABF complex, of metal:ligand ratio 2:1, is possible but no indication of such a complex has been observed. Magnesium reacts to give a fluorescent complex, calcium reacts to give a non-fluorescent complex and strontium and barium are too large to react. Similar comparisons can be made for the aluminium and zinc group metals.

Allison and Becker⁷⁹, in their examination of the fluorescence and phosphorescence of metallo-porphyrins, have noted the dependence of fluorescence intensity on ionic radius for the zinc, cadmium, barium and magnesium complexes. The decrease in fluorescence with increase in the radius of the metal was found to be much greater than could be accounted for solely by an atomic weight effect. These considerations could be expected to apply in part to SABF which has a rigid structure comparable with the porphyrins. The non-fluorescence of transition metal

complexes has been attributed⁷⁹ to the introduction of energy levels which, as a result of ligand field splitting, are intermediate between the excited and ground states of the ligand d -electron system. Intramolecular energy transfer can, therefore, occur through these levels in preference to a direct transition back to the ground state. A similar behaviour is observed with transition metal complexes of 8-hydroxyquinoline⁸⁰.

THE SPECTROFLUORIMETRIC DETERMINATION OF MAGNESIUM USING BIS SALICYLIDENE 2,3, DIAMINOBENZOFURAN⁸¹.

There are few reagents currently available for the spectrofluorimetric determination of submicrogram amounts of magnesium in the presence of calcium. Lumomagneson IREA⁸² (2, hydroxy 3, sulpho 5, chlorobenzene azo-barbituric acid) and 22' dihydroxyazobenzene⁸³ give fluorescence reactions with magnesium, but both reagents form coloured calcium complexes which give rise to inner-filter effects when calcium is present in greater than ten-fold molar excess over magnesium. The magnesium complexes of these two reagents can only be extracted into organic solvents with difficulty. The most sensitive fluorimetric reagent for magnesium proposed to date is bis salicylidene ethylenediamine (SALen)⁷⁸ which will detect $\text{ca. } 10^{-5}$ ppm magnesium. However, the use of this reagent is restricted to anhydrous dimethylformamide solutions. This presents severe difficulties to its practical application and, in addition, calcium forms a coloured, non-fluorescent complex.

Other reagents which have been proposed for the spectrofluorimetric determination of magnesium include 8, hydroxyquinoline⁸⁴, methyl salicylate⁸⁵ and calcein.^{68,25} Although these reagents are sensitive, they also give a strong fluorescence reaction with calcium and the other alkaline earths.

Using the reagent SABF with the recommended procedure, the detection limit for magnesium (10^{-3} ppm) is at least as good as that using lumomagneson⁸² and probably better than 22' dihydroxyazobenzene⁸³, although Diehl et al.⁸³ have not quoted sensitivity figures for the latter. Similar excesses of calcium may be tolerated with both these reagents and SABF. In contrast, however, SABF is easily prepared and purified. Furthermore, traces of magnesium can be quantitatively extracted from aqueous solution into methyl iso butylketone. This extraction procedure is unique in that only a single extraction is necessary, cf. dihydroxyazobenzene, and that a non-fluorescent calcium complex is extracted cf. 8, hydroxyquinoline. The extraction of the coloured Ca/SABF complex may be suppressed by the addition of Sr/EDTA.

Procedure.

1. Development of Fluorescence in 50% Methanol.

To each 50 ml volumetric flask add 25 ml of methanol, 1ml of pH10.5 buffer and a solution containing between 0.1 μ g and 0.6 μ g of magnesium. Add masking agents if necessary (see "Interferences") at this point and allow to stand for 5 mins. Add 5 ml 0.02% SABF reagent in dioxan, dilute to the mark with deionised water and measure the fluorescence intensity at 545 m μ , using an excitation wavelength of 475 m μ , between 15 and 45 mins after the addition of reagent.

2. Analysis of Blood Plasma Samples.

The procedure above was followed using 50 μ l of untreated blood plasma and standard calibration solutions containing 0.1 and 2 μ g magnesium. No masking agent was used, and it was not found necessary to deproteinate the samples.

3. Analysis of Water Samples.

The above procedure was followed using 0.5, 0.25 or 0.05 ml of water and calibration solutions containing 0, 2, 4, 6 μg of magnesium. Use 2 ml of Sr/EDTA masking agent to overcome the interference due to calcium.

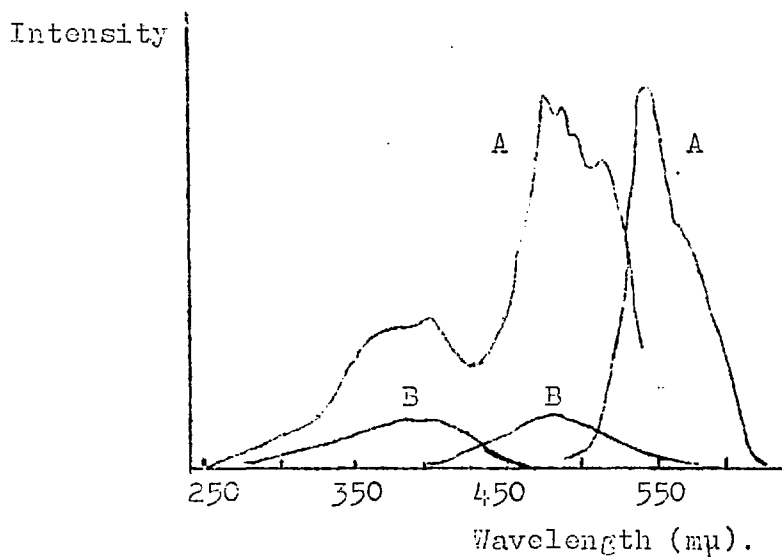
4. Extraction Procedure.

To ca. 25 ml of aqueous solution containing 0.1 μg to 6 μg of magnesium in a 250 ml separating funnel add masking agent if required (see "Interferences") and then add 2 ml pyridine and 1 ml diethylamine. Add 25 ml of methyl iso butylketone and 2 ml of 0.1% SABF reagent in dioxan. Shake thoroughly for 1 min, separate and discard the aqueous layer. Dilute the organic layer with the ketone solvent to 50 ml in a graduated flask and measure the fluorescence intensity at 555 m μ using an excitation wavelength of 525 m μ .

Results and Discussion.

Spectral Characteristics.

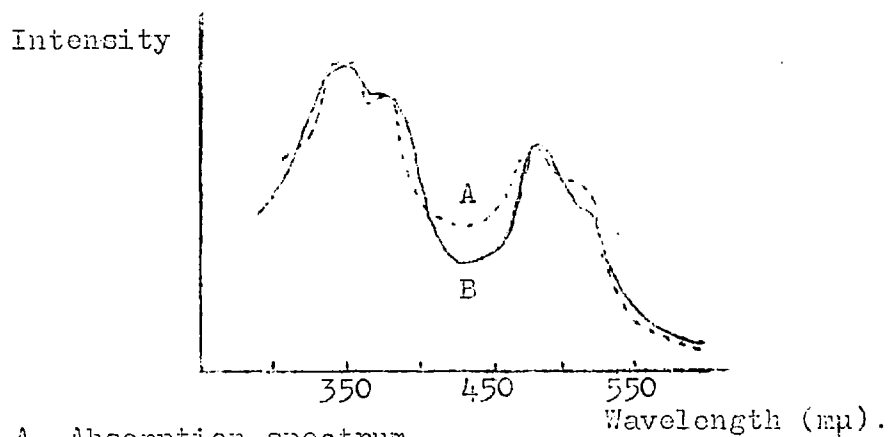
All Schiff bases hydrolyse to some extent in aqueous solution and the effects of hydrolysis have been carefully evaluated for SABF. The uncorrected fluorescence excitation and emission spectra for the complex and blank are shown in figure 26. The fluorescence of this blank was measured in the presence of EDTA and is, therefore, due entirely to the hydrolysis products rather than to the presence of foreign cations. The emission maximum of the blank fortuitously coincides with the excitation maximum of the magnesium complex and, as a result, the blue fluorescence of the hydrolysis products does not interfere with the measurement of the orange fluorescence of the magnesium complex. The corrected fluorescence excitation for the Mg/SABF complex is compared with the absorption spectrum for the complex in figure 27.

Figure 26.

Fluorescence emission and excitation spectra for:
 A, 1ml of $10^{-4}M$ magnesium solution with 5ml of
 0.02% reagent in 50% aqueous methanol.

B, Reagent blank

Spectra are uncorrected for spectral variations
 in lamp and photomultiplier.

Figure 27.

A, Absorption spectrum.

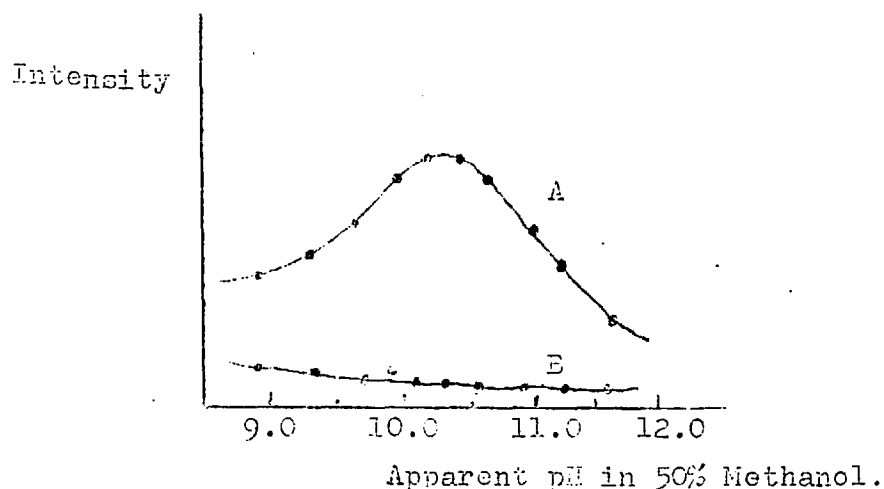
B, Corrected fluorescence excitation spectrum.

The fluorescence excitation maximum for the methyl iso butylketone extract is 525 m μ . This peak is not the same as that used for excitation in 50% aqueous/methanol. The fluorescence emission maximum is shifted from 545 m μ in 50% aqueous methanol to 555 m μ in the ketone extract. These may be genuine solvent effects or may be due to inner-filter quenching caused by the larger excess of reagent.

Influence of pH.

The fluorescence intensity was measured over a range of apparent pH values for 50% aqueous methanol. The pH was adjusted by the addition of diethylamine and hydrochloric acid (figure 28). The corresponding blanks were obtained by adding one drop of 10^{-1} M EDTA to each of the solutions

Figure 28.



Variation of fluorescence intensity with apparent pH in a 50% aqueous methanol medium for:

A, SABF/magnesium complex.

B, Reagent blank.

once the fluorescence intensity of the magnesium complex had been measured. Maximum fluorescence intensity was obtained at $\text{pH} 10.5 \pm 0.2$. This pH value can be adequately maintained using the recommended buffer and the fluorescence intensity is independent of the volume of buffer used.

Reagent Concentration.

Maximum fluorescence intensity was obtained using 4 to 10 ml of a 0.02% solution of the reagent in dioxan. The recommended volume of reagent (5 ml) corresponds to a concentration of approximately $5.6 \times 10^{-5} \text{M}$ in the final solution. This represents a ten-fold molar excess over the highest point on the calibration curve, i.e. 6 μg magnesium in 50 ml of solution. There is a tendency for precipitation to occur when larger excesses of reagent are used or if the pH falls below ca. pH 10.

Effect of Methanol.

Methanol has been used with other reagents to improve their sensitivity towards magnesium. These reagents were found to give results which were extremely dependent on the methanol concentration. With SABF, however, there is no difference in fluorescence intensity for all methanol concentrations between 30% and 40%. Below 40% methanol precipitation of the reagent occurs. The use of methanol concentrations higher than 50% reduces hydrolysis of the excess reagent.

Development Time.

The fluorescence intensity increases during the first 10 mins after addition of the reagent. The heat generated on mixing methanol and water is sufficient to raise the temperature of the solution to above 30°C . As the solution cools, the fluorescence intensity increases as a result of

the negative temperature coefficient which is common to most fluorescence systems. A temperature effect also applied to the hydrolysis of the excess reagent. In this instance the speed of hydrolysis increases with increase of temperature. Since the reagent becomes hydrolysed, its inner-filter effect is reduced and the fluorescence intensity rises. If the solutions are allowed to reach room temperature before the addition of reagent, the rate of hydrolysis of excess reagent is slow. As a result of these two opposing temperature effects, no meaningful temperature coefficient could be evaluated for the system. If no cooling time is allowed, the fluorescence intensity, relative to a quinine bisulphate standard, is constant between 15 and 45 mins after the addition of the reagent.

With the extraction procedure, on the other hand, the fluorescence intensity may be measured immediately after dilution and the fluorescence is stable for at least two hours.

Extraction Procedure.

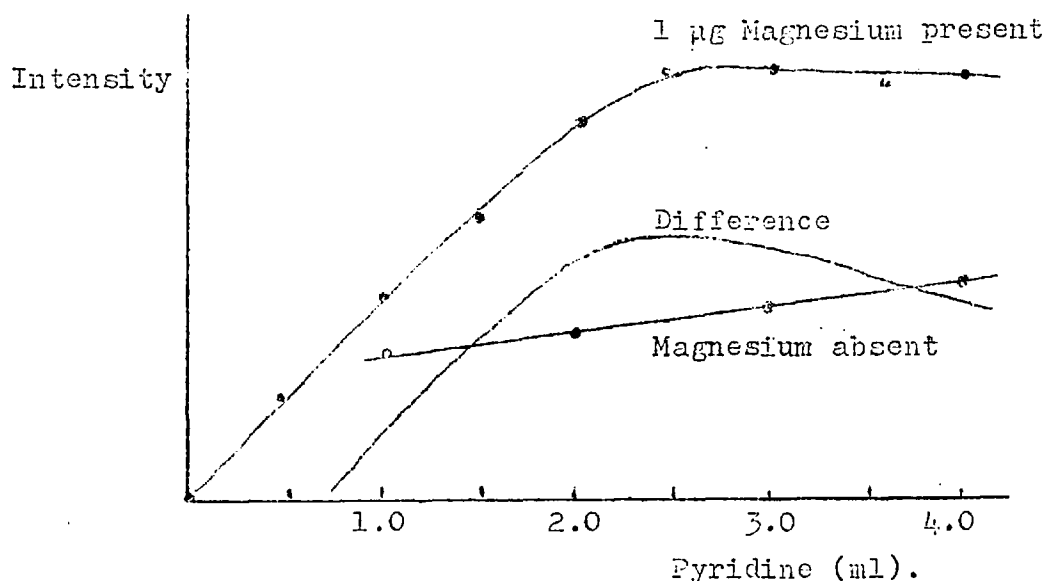
(a) Influence of Diethylamine Concentration: Tests were carried out using pyridine (2 ml), standard magnesium (1 μ g) and 0.1% SADF reagent (2 ml). There was no extraction in the absence of diethylamine but quantitative extraction with $\frac{1}{2}$ -5 ml of diethylamine.

(b) Influence of Pyridine Concentration: Tests were carried out using diethylamine (1 ml), standard magnesium (1 μ g) and 0.1% SADF reagent (2 ml). The results are summarised in figure 29, and consequently 2 ml pyridine has been recommended for the extraction.

(c) Influence of 0.1% SADF reagent solution: Tests were carried out using diethylamine (1 ml), standard magnesium

(1 μ g) and pyridine (2 ml). Maximum extraction was obtained using 3 ml of 0.1% SABF reagent in dioxan. This corresponds to molar reagent excess of approximately two hundred-fold.

Figure 29.



(d) Influence of pH: The pH of the aqueous phase was varied using concentrated hydrochloric acid or 10% sodium hydroxide solution. The optimum pH for extraction (12.4) could be obtained using diethylamine and pyridine only.

(e) Percentage Extraction: Solutions were made up according to the recommended procedure, extracted and the aqueous and organic layers separated. The aqueous layer was then shaken with more methyl iso butylketone, diethylamine etc., and the fluorescence intensities of the first and second organic extracts compared. For 0.2, 0.6, 1.0, 3.0 and 5.0 μ g of magnesium, extraction was found to be at least 98% complete.

(f) Calibration Curves: The limits of determination were the same as for the 50% methanol procedure (i.e. 0.01 μg of magnesium). However, no advantage was taken of the increase in sensitivity obtainable by extraction from a large volume of the aqueous phase into a small volume of the organic phase.

Interferences.

The effects of one hundred-fold molar excesses of foreign ions on the fluorescence intensity produced by 5 ml of 10^{-5}M magnesium solution (ca. 1.2 μg) were examined (table 10). The limiting error was taken empirically as $\pm 5\%$ of the magnesium standard containing no foreign ion. Marginal interferences were examined in more detail.

The following ions caused no interference in the 50% aqueous methanol procedure: As(III), As(V), Ba, Be, BO_3^{3-} , Cr(VI), Ga, Ge(IV), Hg(II), K, Mo(VI), Na, Sr, Tl(I), Ti(IV) and V(V).

In the absence of masking agents, one hundred-fold amounts of Ca, Cd, Cr(III), Co(II), Cu(II), Fe(II), Fe(III), Mn(II), Ni, Pd(II) and Zn interfered by formation of coloured, non-fluorescent complexes which gave rise to low results.

Aluminium formed a fluorescent complex at this pH; the complex was only weakly fluorescent, but gave rise to high results. The even more weakly fluorescent zinc complex gave rise to low results because the large excess of zinc over reagent prevents the formation of the magnesium complex.

The following interfered by precipitation as chlorides or hydroxides: Ag, Hg(I), Pb, Sn(II), Sn(IV), Th and Zr.

In addition, Bi, In, LaSb(III), Se(IV), Te(IV) and Y gave results which were low by ca. 10% although they did not show any visible signs of precipitation or colour formation.

The effects of various masking agents on selected interferences were, therefore, examined. Calcium could be tolerated in ten-fold molar excess without the use of masking

Table 10.

Ions interfering in the determination of magnesium with S/BF. Foreign ions are present in 50 fold molar excess.

Ion	Deviation %		Observation for 50% MeOH
	50% MeOH	Extraction + Sr/EDTA	
Al(III)	+60	0	Fluorescent complex
Sb(III)	-12	+1	Oxychloride precipitate
Bi(III)	-11	-12	Oxychloride precipitate
Cd(II)	-88	-31	Pink precipitate
Ca(II)	-46	0	Pink precipitate
Cr(III)	-83	0	No visible interference
Co(II)	-97	-100	Black precipitate
Cu(II)	-78	-96	Strong pink colouration
Fe(II)	-32	-62	Brown colouration
Fe(III)	-43	-53	Brown colouration
La(III)	-13	0	Hydroxide precipitate
Pb(II)	-61	0	Chloride precipitate
Mn(II)	-96	0	Pink precipitate
Hg(I)	-41	0	Chloride precipitate
Ni(II)	-96	-67	Pink precipitate
Pd(II)	-98	0	Red colouration
Se(IV)	-15	-9	Bleaching of reagent
Ag(I)	-96	0	Chloride precipitate
Te(IV)	-6	-6	No visible interference
Th(IV)	-41	-35	Hydroxide precipitate
Sn(II)	-72	0	Hydroxide precipitate
Sn(IV)	-73	0	Hydroxide precipitate
Y(III)	-8	0	No visible interference
Zn(II)	-86	-79	Red precipitate
Zr(IV)	-78	-80	Hydroxide precipitate

agents and the interference due to larger amounts of calcium was completely suppressed using Sr/EDTA solution as a masking agent. Two hundred-fold excesses of calcium could be tolerated when 2 ml of the above mixture was used as a masking agent. The interference of one hundred-fold excesses of La and Y was also prevented in this way. Some metals which form very stable EDTA complexes in slightly acid solution, e.g. Al, transition metals, etc., were not masked using Sr/EDTA because of the high pH of the determination.

The interferences due to Ag, Cd, Co(II), Cu(II), Ni and Pd(II) were completely suppressed by the addition of 1 ml of 3% potassiumcyanide, but Fe(II), Fe(III) and Zn were not masked in this way.

The addition of 5 ml of a 10^{-1} M sodium fluoride solution completely suppressed the interference of one hundred-fold excesses of Se(IV) or Te(IV). However, if this masking agent was used in the presence of large amounts of calcium (ca. 100 ug), coprecipitation of CaF_2 and MgF_2 occurs and low readings are obtained.

The interference due to one hundred-fold excesses of Fe(III), Al or In could be reduced by about 50% using 0.5 ml of triethanolamine. This reagent may, however, be useful for use with lower amounts of these metal ions. Manganese, however, could not be prevented from interfering.

The effects of other masking agents were considered.

One hundred-fold molar excesses of oxalate, citrate, tartrate, NTA, CDTA and EDTA reduced the fluorescence intensity of the magnesium complex to below the blank value. A one hundred-fold molar excess of phosphate gave a reduction in signal of about 40%. No interference, however, was noted from acetate, bromide, chloride, cyanide, fluoride, iodide, nitrate, perchlorate, sulphate or sulphide when present in one hundred-fold molar excess over magnesium. Similar

concentrations of oxidising agents such as chromate or vanadate caused no interference.

Of those metals interfering in the 50% aqueous methanol procedure, no interference was noted in the extraction procedure from Al, Hg(I), Mn(II), Pb and Sb(III) in one hundred-fold molar excesses over magnesium (table 10). All the other interfering metal ions resulted in low recoveries. Various masking agents, e.g. thiourea, tiron, sodium sulphide, o-phenanthroline, sodium fluoride and potassium cyanide were used in an attempt to improve the selectivity of the extraction. Cyanide prevented the interference of copper only, and the other masking agents proved ineffective. However, using Sr/EDTA, calcium could be tolerated in two thousand-fold molar excess over magnesium. This is a considerable improvement on the 50% aqueous methanol procedure.

Large amounts of the above interfering ions could be probably removed quantitatively by one or two pre-extractions from aqueous solution using either cupferron or sodium diethyldithiocarbamate.

Practical Application of the Method.

The method was applied to the analysis of magnesium in blood plasma and municipal water samples (see "Recommended Procedures"). These materials were chosen because there was no extensive pre-treatment necessary. Blood plasma, in particular, was chosen as there is an urgent need for a simple, rapid fluorimetric procedure for the analysis of small amounts of magnesium. Water samples were chosen in order to demonstrate the validity of the method in the presence of large concentrations of calcium. The results are summarised in table 11 and 12.

Table 11.

Determination of Magnesium in Blood Plasma.

Sample	Found ($\mu\text{g/ml}$)			Flame Photometry ($\mu\text{g/ml}$)
1	28	28	26	28
2	30	28	23	30
3	23	21	22	22
4	27	26	27	
5	26	26	27	
6	25	23	25	
7	21	26	24	
8	29	25	27	
9	29	29		
10	24	26		
11	14	15		
12	19	20		
13	25	26		
14	27	27		

Table 12.

Analysis of Water Samples.

Origin	Magnesium ($\mu\text{g/ml}$)	Magnesium ($\mu\text{g/ml}$)		Calcium ($\mu\text{g/ml}$)
	Flame Photometry	Fluorimetry		EDTA Titration
Witney	5.8	5.4	6.2	170
Gloucester	14.2	14.4	14.8	115
Pontypool	6.3	5.6	6.0	36
Marlbrook	1.8	1.4	1.2 1.6	32
Clacton	46	48.4	44.0	210
London	6.8	7.2	6.7	260
Bagshot	7.5	7.8	7.6	135
Marlow	2	2.8	1.7 2.3	110
Exeter	6.0	5.6	6.4	64

Structure of the Magnesium Complex.

The magnesium complex of SABF has been assigned a 1:1 metal ligand ratio on the basis of fluorimetric mole ratio, slope ratio and Job plots carried out in 50% aqueous methanol (Figures 30, 31 and 32.).

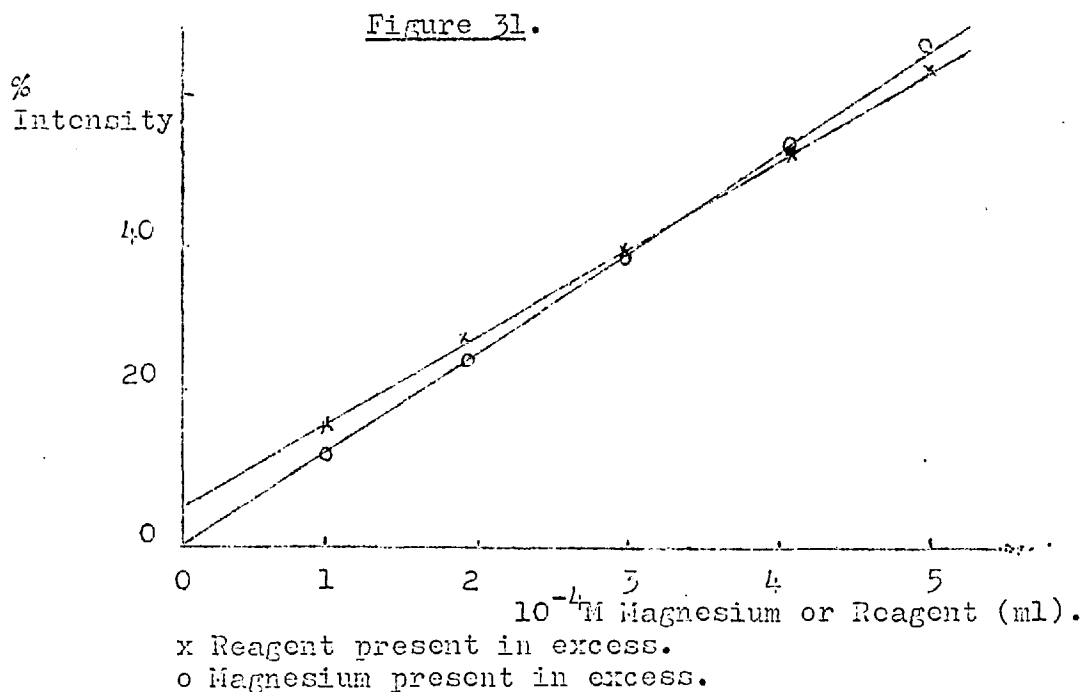
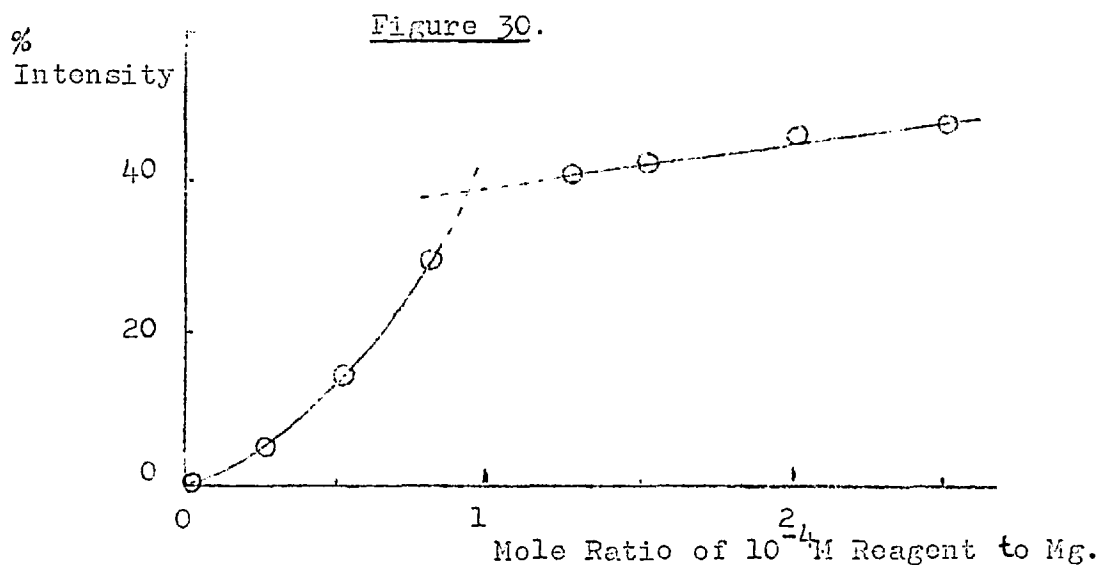
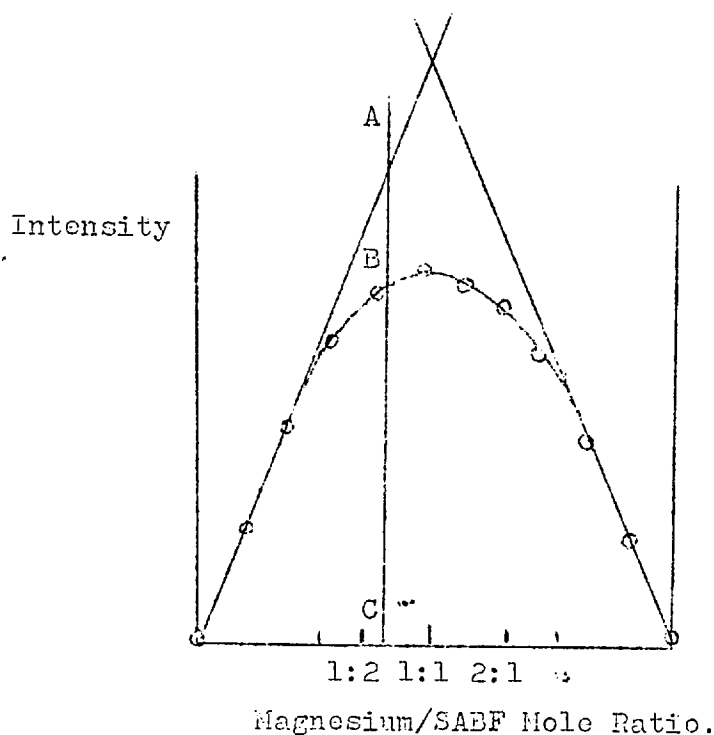


Figure 32.

A conditional stability constant for the complex has been evaluated from the Job plot; the effects of hydrolysis being minimised by taking fluorescence measurements as soon as possible after the addition of reagent.

It is normal procedure to derive the degree of dissociation of a metal complex from a Job plot at the point corresponding to the stoichiometric ratio. A simple modification of this procedure using equation (i) enables the conditional stability constant to be determined for any point on the Job plot.

Table 13.

Values of Conditional Stability Constant obtained from Job Plot.

Magnesium conc. $\times 10^{-6}M$	Reagent conc. $\times 10^{-6}M$	x $\times 10^{-6}M$	α	$K \times 10^6$
2.0	5.0	3	0.161	1.74
2.5	4.5	2	0.223	1.36
3.0	4.0	1	0.305	1.01
3.5	3.5	0	0.362	1.62
4.0	3.0	1	0.278	1.39
4.5	2.5	2	0.210	1.49
5.0	2.0	3	0.143	1.79

Average $K = 1.4 \times 10^6$

$$K = \frac{\text{Complex}}{\text{Metal} \cdot \text{Ligand} \cdot (1-\alpha)}$$

$$K = \frac{\text{Complex}}{\alpha^2 c + \alpha x} \quad (i)$$

Where α = degree of dissociation of the complex given by

$$\frac{AB}{AC} = \alpha \quad (\text{figure 32}).$$

c = Concentration of complex assuming $\alpha = 0$.

x = Excess magnesium or ligand concentration over 1:1 stoichiometry.

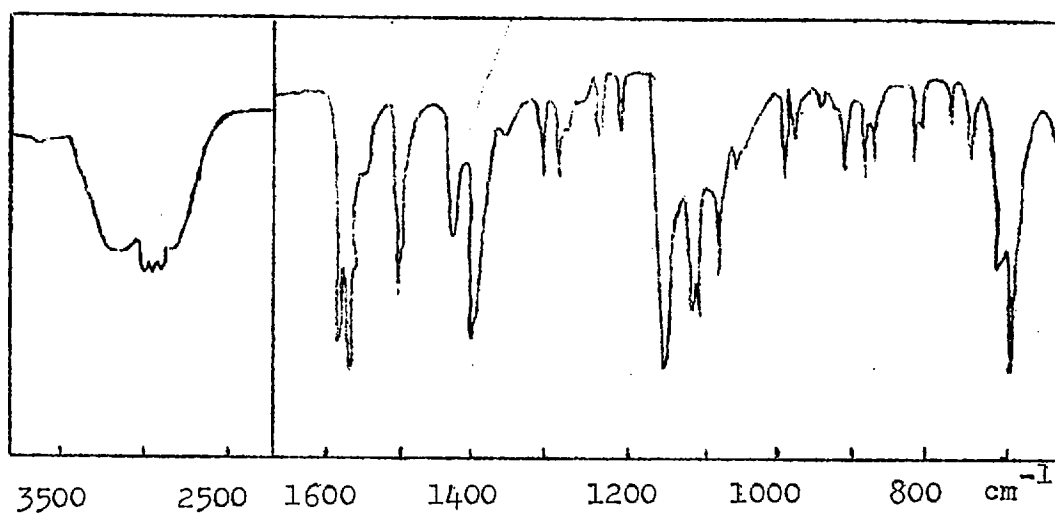
Conditional stability constant values derived for seven different metal:ligand ratios are given in table 13.

An orange-red solid was obtained by precipitation from aqueous dimethylformamide and was shown to be the magnesium/SABF complex. Anhydrous magnesium perchlorate (0.2 g) in dimethylformamide (30 ml) was added to a solution of SABF (1g) in dimethylformamide (70 ml) and diethylamine (1ml).

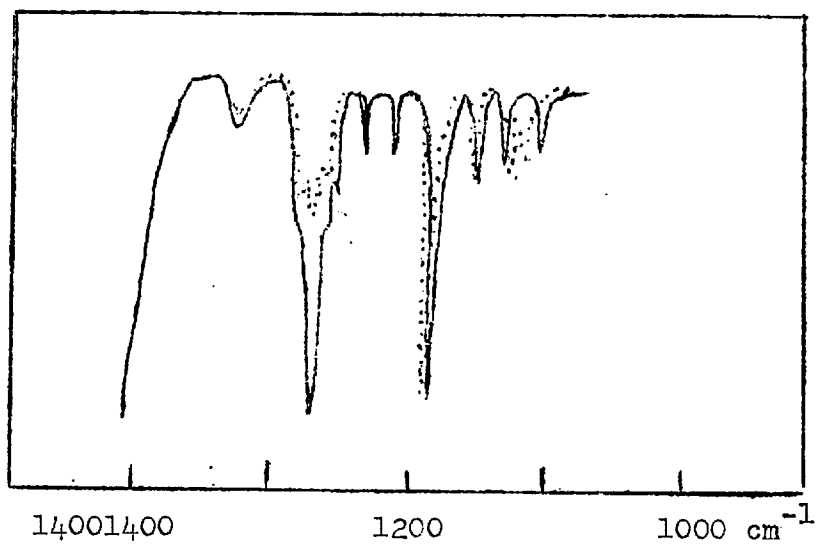
Water (30ml) was added, and the precipitate triturated with acetone, filtered, washed with petroleum spirit (BP.40-6C) and dried under vacuum. Micro analytical results are not quoted because they indicated contamination of the sample by coprecipitated magnesium to the extent of ca.3%. Solutions of the solid in dioxan or dimethylformamide showed an intense red fluorescence. However, the complex could not be recrystallised, nor was it sufficiently soluble in solvents suitable for n.m.r. studies. Consequently, physical measurements were limited to infrared spectra and thermogravimetry.

The assignable, characteristic infrared absorption frequencies are summarised in table 14 for the magnesium/SABF complex and the free ligand. The infrared spectrum is given in figure 33, and that for the free ligand is in figure 19 (page 65).

The -OH bending frequency was assigned by shaking a solution of the reagent in carbon disulphide with D₂O. The carbon disulphide layer was separated, dried overnight with anhydrous sodium sulphate and an infrared spectrum was run on the clear deuterated solution. The peak at 1275 cm⁻¹ disappeared on this treatment, but reappeared when the remainder of the carbon disulphide solution had been equilibrated with water, separated and dried as before. The disappearance of this -OH bending frequency on reacting with magnesium indicates the formation of a true complex involving both of the phenolic -OH groups. The presence of solvated water is also indicated by the infrared spectrum.

Figure 33.

Infrared spectrum of magnesium complex of SABF.

Figure 34.

Dotted line Spectrum of deuterated SABF
 Continuous line Spectrum of SABF

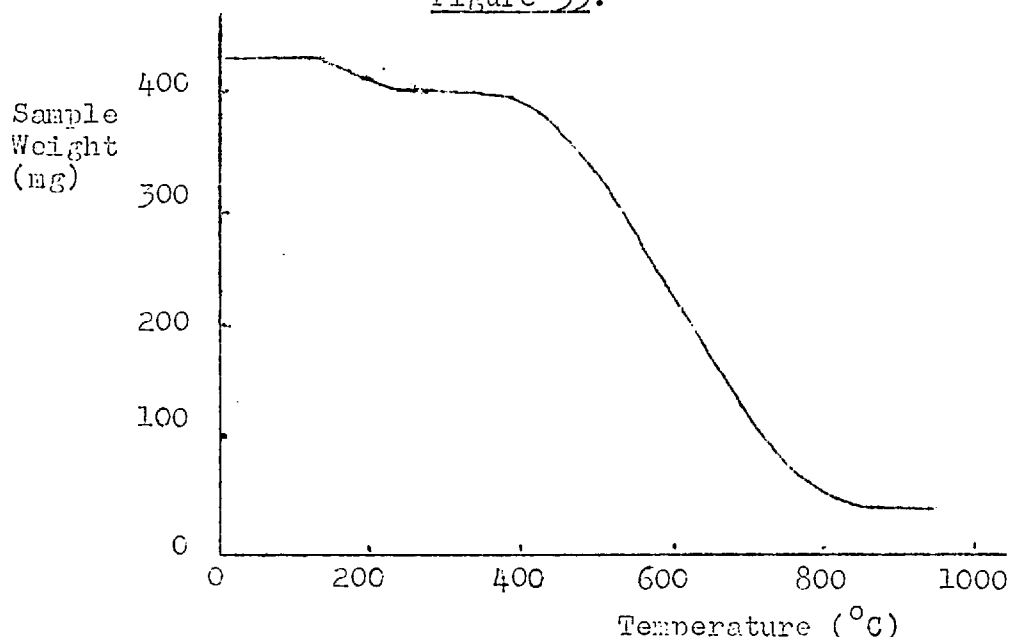
Table 14.

Characteristic infrared frequencies for Mg/SABF complex and SABF.

Frequency (cm^{-1})		Assignment
SABF	Mg/SABF	
3300(w)	3100(s)	O-H stretch of H_2O ligand Phenolic OH weak because of hydrogen bonding.
1630	1580(?)	C=N stretch of azomethine.
1610	1610	Phenyl ring absorption.
1600	1600	" "
1490	1465	" "
1450	1445	" "
1275	-	Phenolic OH out of plane bending.
1185	1180	Phenolic CO stretching.
740	740	Out of plane bending of -H on, 1,2 disubstituted benzene rings.

Thermogravimetric measurements were carried out between room temperature and 950°C using a temperature rise of 4°C per minute. The first weight loss occurs between 120°C and 250°C and corresponds to the loss of two molecules of H_2O from the complex. Oxidation begins at 350°C (see figure 35). Specimens of the complex have been heated on a melting-point apparatus to 300°C without carbonisation, although a slight darkening of the specimen did occur above 110°C .

Figure 35.



Thermogravimetry of Mg/SABF complex, using temperature rise of 2° per minute on Stanton recording thermobalance

Reagents.

SABF Reagent: 0.01% SABF solid in dioxan was used in the 50% aqueous methanol procedure.

0.1% SABF solid in dioxan was used in the extraction procedure.

Standard Magnesium Solution: Analytical reagent grade magnesium oxide was dried at 120°C for 3 hours. 1.663g were dissolved in the minimum amount of 2N hydrochloric acid and the solution was diluted to 1 litre with de-ionised water. This gave a solution containing 10^3 ppm of magnesium from which a stock solution containing 10 ppm of magnesium was obtained by dilution. Solutions containing 1 ppm or less of magnesium were not stored for longer than two weeks because of possible adsorption losses.

Methanol, analytical-reagent grade.

Methyl isobutyl ketone, general purpose reagent grade.

Pyridine, general purpose reagent grade.

Diethylamine, general purpose reagent grade.

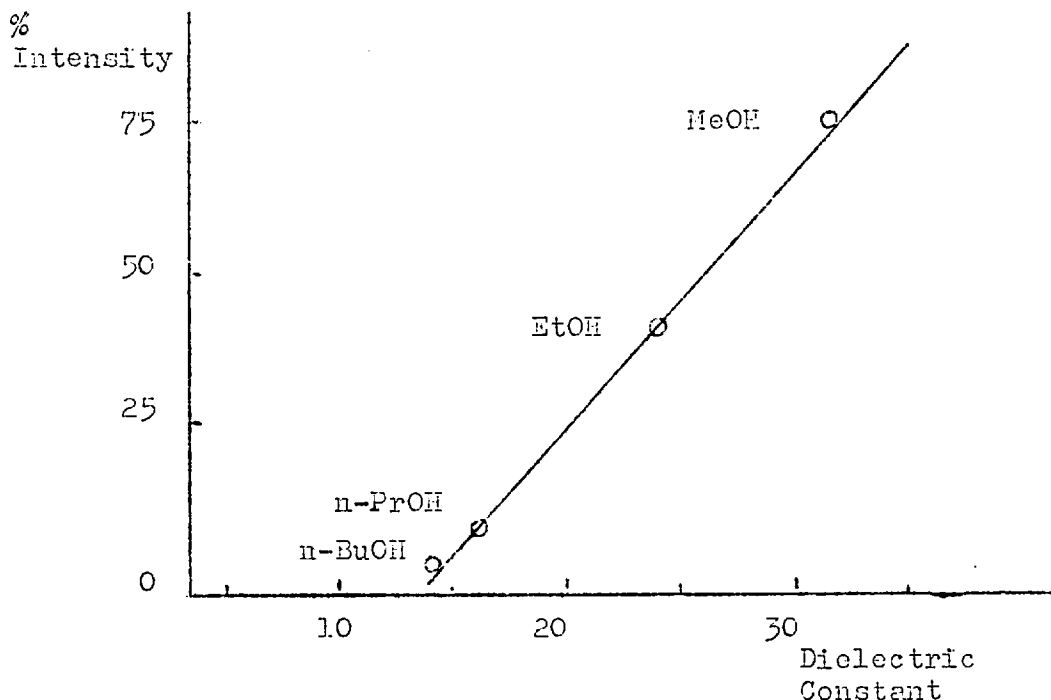
Distilled water from an all-glass distillation apparatus was de-ionised by passing it through a 15 x 5cm column of Zeo-Karb 225 (Permutit Co., Ltd., London) cation-exchange resin in the hydrogen ion form.

INVESTIGATION OF THE GALLIUM/BIS SALICYLIDENE 2,3 DIAMINOBENZOFURAN SYSTEM.

Gallium reacts with SABF to give a complex exhibiting similar fluorescence characteristics to the magnesium/SABF complex⁷⁷. The reaction is of potential analytical value but no thorough investigation has been carried out in view of the many reagents available for the determination of gallium.

Choice of Solvent.

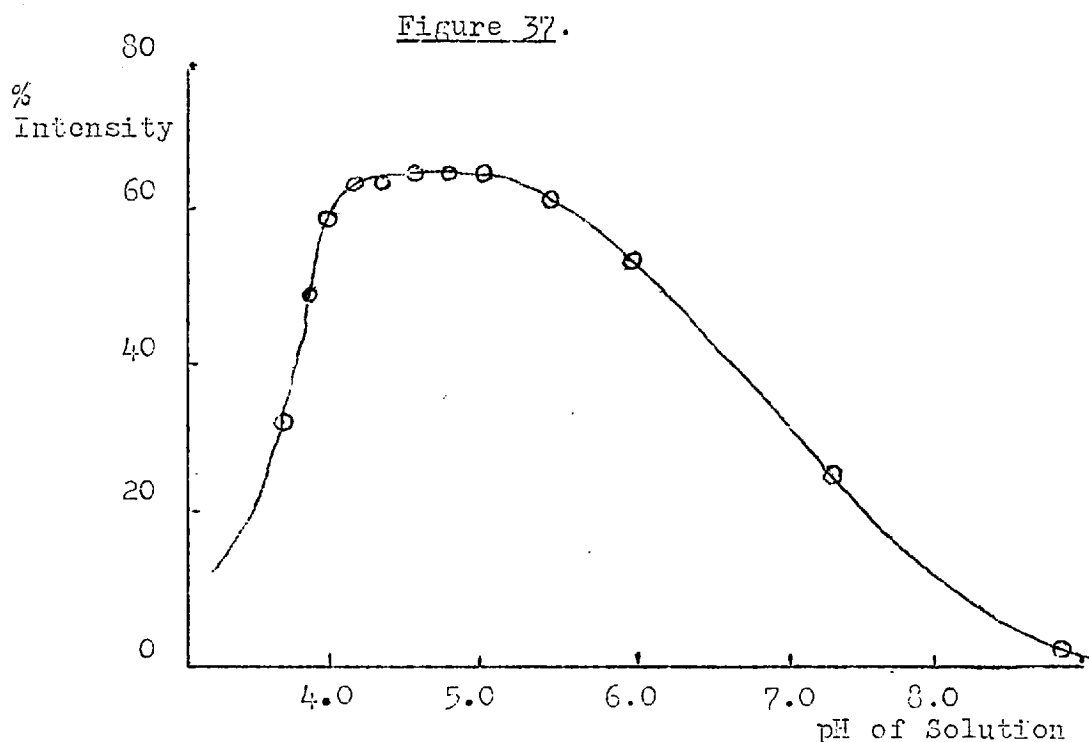
Because of the insolubility of SABF and its complexes in water, semi-aqueous media were examined to find a suitable solvent. Using 2ml of a pH4 buffer, 2ml of 10^{-3} M gallium solution and 5ml of 0.01% reagent in ethanol the following solvents were examined as development media. Dimethyl-formamide (90%), ethanol (95%), acetone (90%), n-amyl alcohol (90%), methanol (90%), n-butanol (90%), dioxan (90%). The most intense fluorescence was obtained in methanol. An interesting variation of fluorescence intensity with solvent was noted for the range of straight chain alcohols examined (figure 36). A linear dependence of fluorescence intensity with the dielectric constant of the solvent was observed. However, the reasons for this can only be determined by a more extensive investigation, which was not justified at the time.

Figure 36.Spectral Characteristics.

The fluorescence excitation, fluorescence emission and the absorption spectra of the gallium complex are identical with those of the magnesium complex (figure 26), and are not reproduced here. The usual analytical parameters were determined using an excitation wavelength of 475m μ or 365m μ . The signal to blank ratio was better using the 475m μ excitation and the intensity measured was about 50% greater than using 365m μ excitation. Variation of spectral bandwidth from 5 - 20m μ on both excitation and emission monochromators did not affect the signal to blank ratio.

Influence of pH.

The apparent pH of the 50% aqueous methanol solution, containing 5ml of 10^{-4} M gallium and 5ml 0.02% SABF reagent,



was adjusted with dilute ammonia or dilute acetic acid. The fluorescence intensity of the solutions was measured (figure 37), and a constant maximum fluorescence intensity was observed between the apparent pH 4.0 - 5.5.

Reagent Excess.

0.1% SAMF reagent in dioxan was added to 5ml $10^{-4}M$ gallium in 50% aqueous methanol at an apparent pH 4.5. Maximum fluorescence intensity was obtained using $\frac{1}{2}$ to 2ml of reagent but there was visible precipitation with 5 to 8ml of reagent solution.

Limits of Determination.

The limit of determination using the optimum parameters already evaluated was 3.5 μ g of gallium in 100ml solution (i.e. $5 \times 10^{-8}M$). This is of the same order of sensitivity as the fluorescence of salicylidene 2, amino-phenol with gallium.

Interferences.

The effects of one hundred-fold molar excesses of foreign ions were examined on the determination of 10^{-7} m gallium (ca. 70µg in 100ml).

The following ions caused no interference:

Beryllium, cobalt(II), manganese(II) and nickel.

The following ions gave rise to the interference indicated in parentheses:

Aluminium (+180%), chromium(III) (-96%), chromium(VI) (-96%), copper(II) (-50%), indium (+30%), iron(II) (-95%) and iron(III) (-95%).

In view of the interference of aluminium and indium, studies on this reaction were discontinued.

CHAPTER V.RARE EARTH, ENERGY TRANSFER SYSTEMS.

Intramolecular energy transfer in rare earth chelates has been the subject of several investigations. The phenomenon was reported by Weissman⁸⁶ who found that excitation of a metal complex in the region of light absorption associated primarily with the ligand resulted in a high yield of sharp line fluorescence characteristic of the metal ion. Crosby et al.⁸⁷ have reviewed the theoretical aspects of the phenomenon, and carried out work on a variety of rare earth complexes^{88,89}.

Analytical chemists have been attracted by the possibility of a simultaneous determination of several rare earth ions in solution and have investigated the fluorescence of rare earth chelates fairly thoroughly. There appears to be a distinction between sensitised fluorescence, in which the absorbing molecule is not directly chelated to the metal, and ligand energy-transfer where the absorbing molecule forms part of a complex with the rare earth ion. In practice, however, the distinction may not be very clearly defined and transient complexes may be formed in several cases of sensitised fluorescence.

Alberti and Massucci⁹⁰ have studied the simultaneous determination of Dy, Eu, Sm and Tb in 0.6M sodium tungstate solutions, while Sevchenko and Kuznetsova⁹¹ have employed 1,10-phenanthroline in a simultaneous determination of the same elements. Both methods are subject to interference from other rare earth ions.

Kononenko et al.⁹² have used a 1,10-phenanthroline/salicylic acid system for the extraction of rare earth ions into benzene in a fluorimetric determination of Eu and Tb.

Both determinations are subject to interference from some of the other rare earth ions. A similar extraction system using 1,10 phenanthroline and thenoyltrifluoroacetone has been used for the fluorimetric determination of Eu and Sm⁹³. Ballard and Edwards have examined the thenoyltrifluoroacetone/trioctylphosphate extraction system in the determination of Eu⁹⁴. Determinations of Eu using 2,phenyl 4, quinoline carboxylic acid⁹⁵ and of Dy and Tb using 4, sulphophenyl 5, methyl 5, pyrazolone⁹⁶ also suffer from the interelement effects of other rare earth ions.

McCarthy and Winefordner⁹⁷ have examined the possibilities of simultaneous analyses of Dy, Eu, Sm and Tb in non-aqueous solutions containing aromatic carbonyl compounds. Although interelement effects are present, the choice of carbonyl compound has been shown to increase the selectivity of the method considerably.

THE DETERMINATION OF TERBIUM USING SULPHOSALICYLIC ACID AND EDTA⁹⁸.

In the course of investigations into the laser properties of rare earth chelates, Charles and Riedel⁹⁹ reported a fairly strong fluorescence from the Tb/EDTA/Sulphosalicylic acid complex in aqueous solution. The reaction appeared to be well worth further study since EDTA could be expected to act as a general masking agent and might also protect the chelated Tb ion from the interelement effects of other rare earth ions.

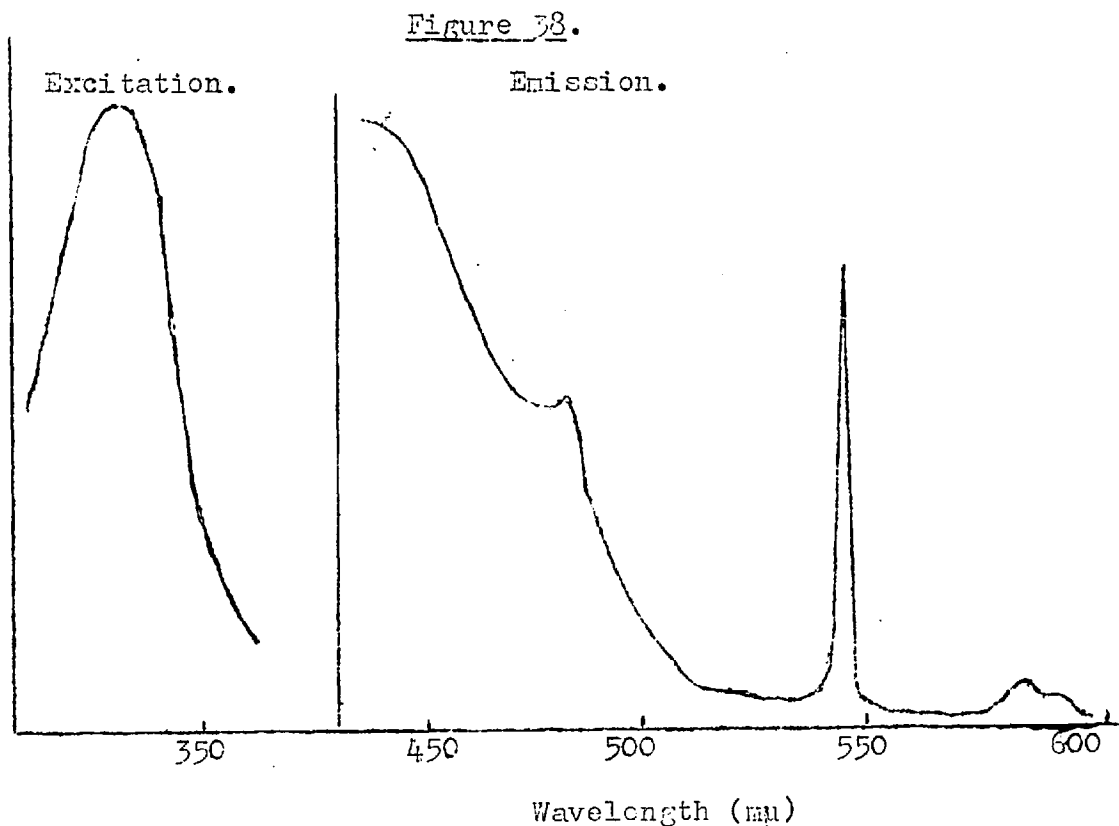
Procedure.

An aliquot of solution containing between 0.2µg and 400µg of terbium was added to each volumetric flask. 1ml of 10^{-1} M EDTA, 0.5ml of 10^{-1} M sulphosalicylic acid (SSA) and 1ml of pH11.8 buffer was added and the solution diluted to volume with distilled water.

The fluorescence was measured after 10 mins at 545m μ using 20m μ halfbandwidth slits on the analysing monochromator. An excitation wavelength of 320m μ can be used in conjunction with 20m μ slits on the excitation monochromator, but it was thought preferable to remove the slits from the excitation monochromator and to use a Corning 7-54 (9863) filter instead. This filter transmits radiation between 230 and 400m μ (bandwidth at 10% transmission) giving maximum transmission at 350m μ .

Spectral Characteristics.

Figure 38 shows the uncorrected excitation and emission spectra of the Tb/EDTA/SSA system as used analytically.



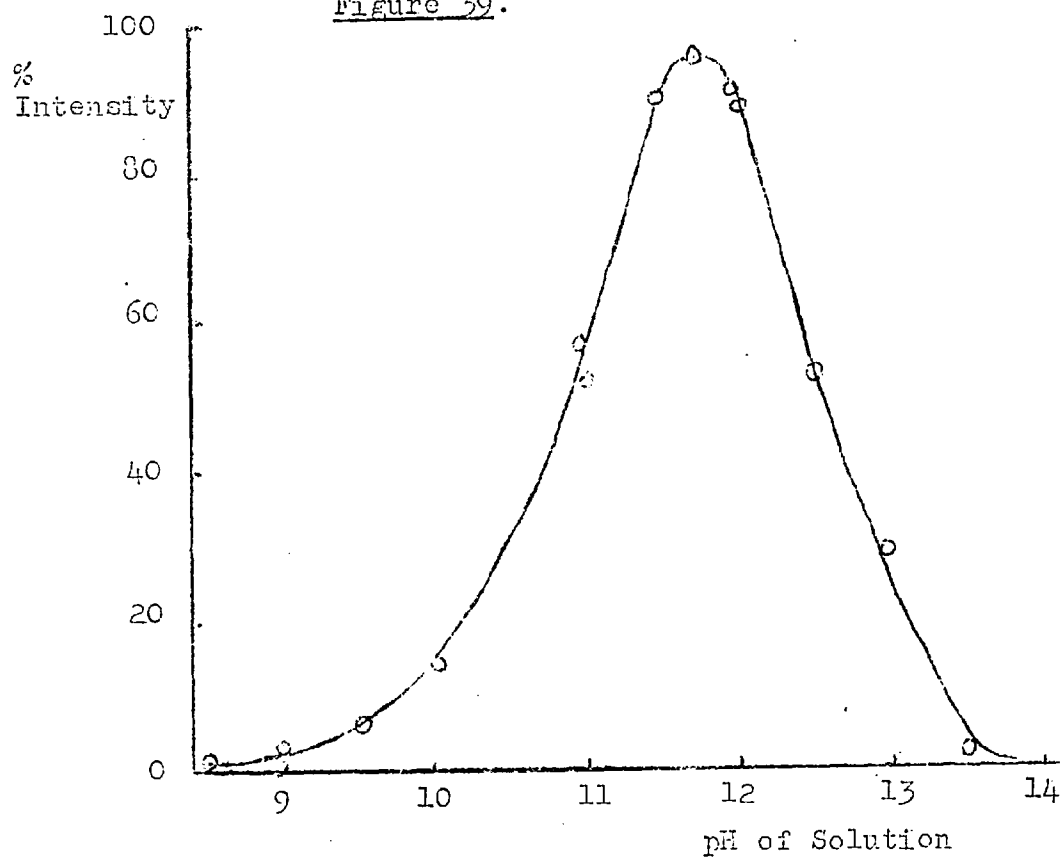
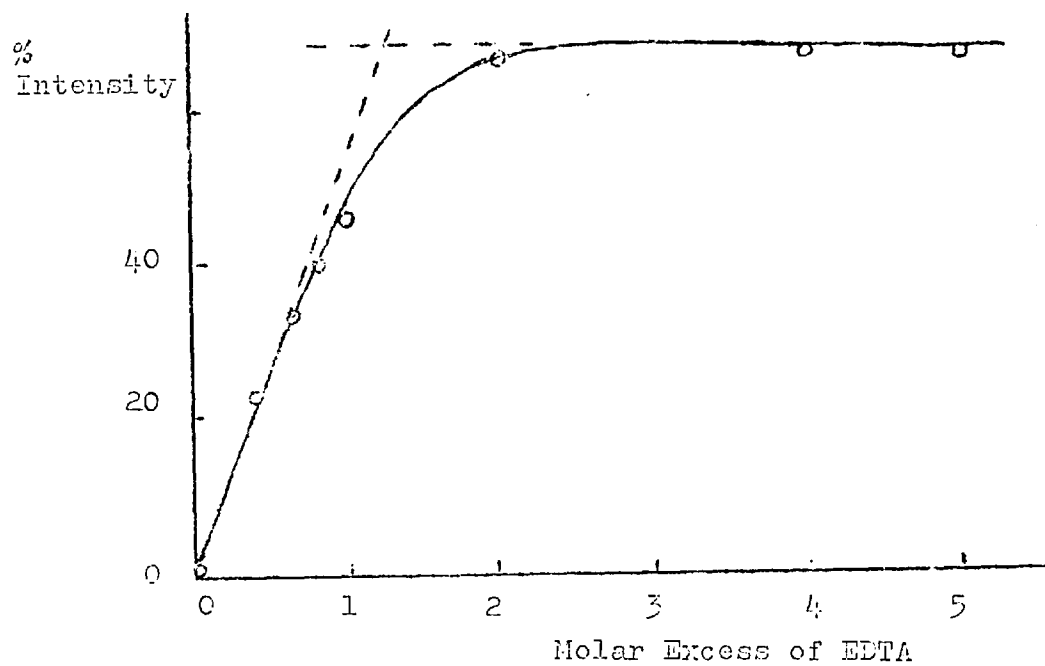
The excitation wavelength maximum (320m μ) coincides with the absorbance maximum for the SSA^{3-} ion, while the emission consists of a broad band (not shown completely) characteristic of the SSA^{3-} ion together with line emission characteristic of Tb^{3+} . The line emission of Tb^{3+} has been assigned⁹⁹ to transitions from the $^5\text{D}_4$ level to the $^7\text{F}_6$, $^7\text{F}_5$ and $^7\text{F}_4$ levels at 485m μ , 545m μ and 575m μ respectively. A fourth emission peak, due to the $^5\text{D}_4 - ^7\text{F}_3$ transition, occurring at longer wavelengths (ca. 630m μ) was not observed because of insensitivity of the photomultiplier in this region. With the apparatus described, only the peak at 545m μ is analytically useful.

Influence of pH.

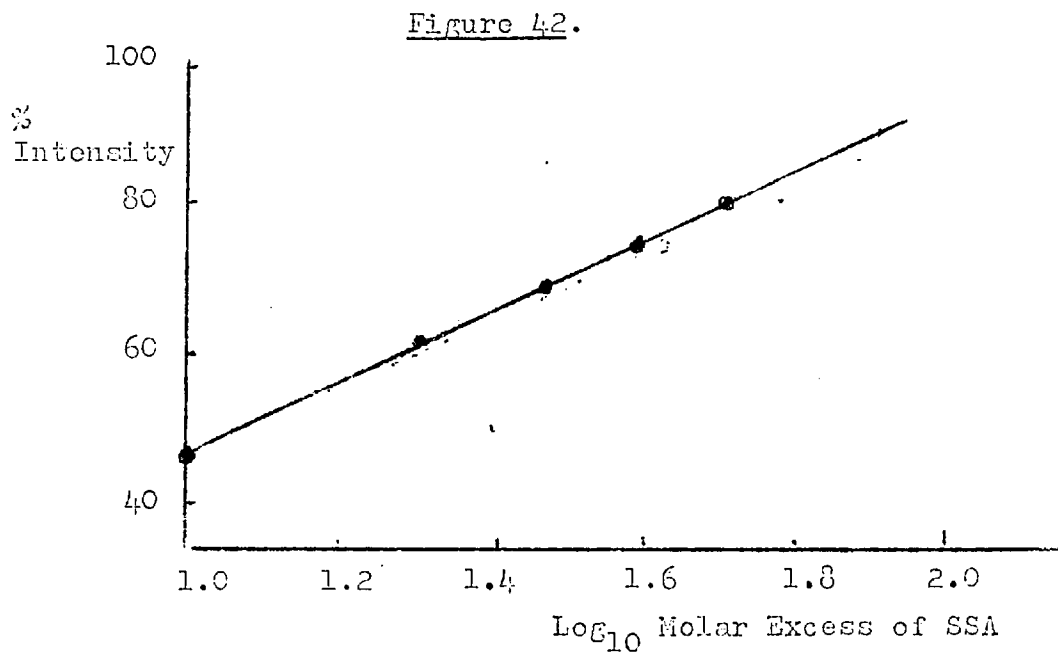
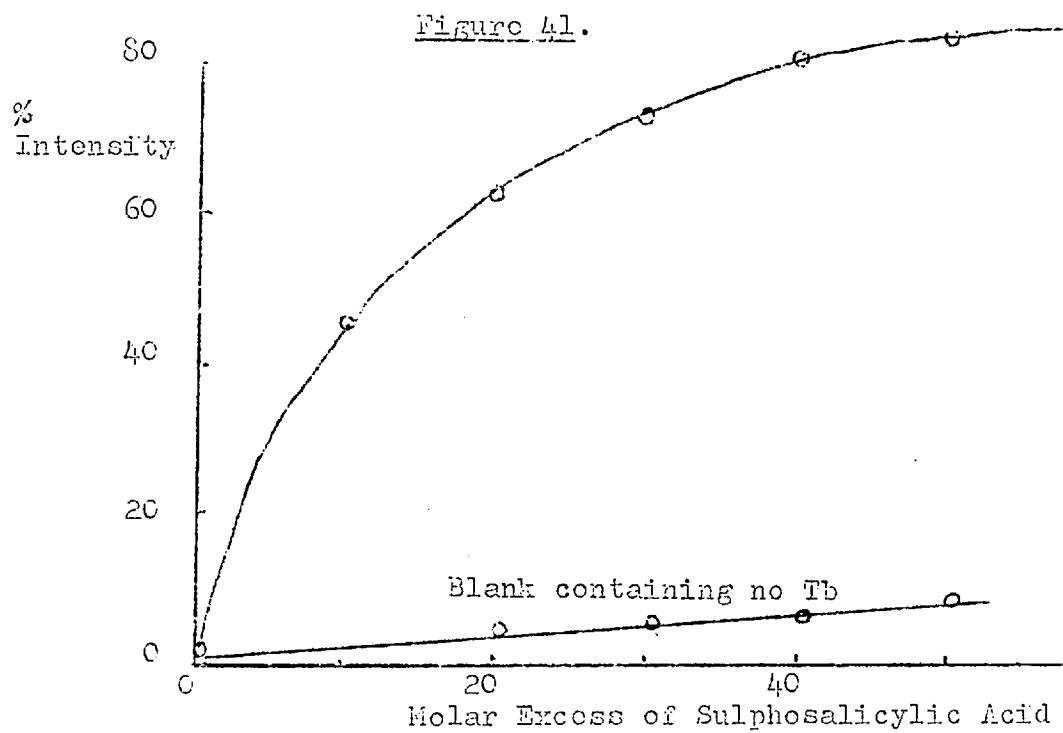
The fluorescence intensity of a 10^{-5}M terbium solution with tenfold molar excesses of EDTA and SSA was measured over a range of pH values from pH8 - 13, using ammonium hydroxide and sodium tetraborate as buffer and adjusting the pH with hydrochloric acid or sodium hydroxide solution. Blank solutions containing no terbium were prepared separately. Maximum fluorescence intensity was obtained at pH11.6 - 11.9 (figure 39) and a diethylamine/hydrochloric acid buffer was chosen as most suitable for this range. The fluorescence intensity was independent of buffer concentration at this pH and there was adequate buffering capacity if neutral or alkaline metal ion solutions were used.

Reagent Excess.

The effect of reagent excess was extensively investigated for both EDTA and SSA. Investigations were carried out on 10^{-5}M terbium solutions and indicated that the fluorescence intensity increased linearly with EDTA concentration up to a 1:1 stoichiometry and that excesses of EDTA (e.g. one thousand-fold) did not affect the fluorescence further (figure 40).

Figure 39.Figure 40.

Because of the masking action of EDTA on possible interferences, a 10^4 molar excess of EDTA over terbium is recommended.

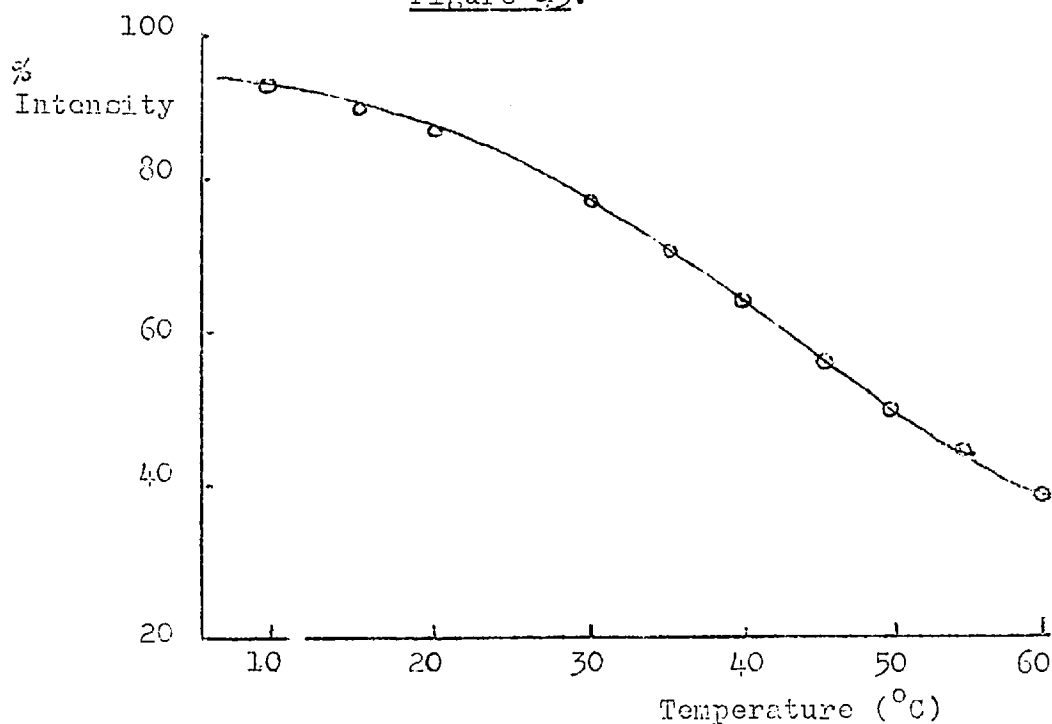


A logarithmic relationship was obtained between SSA concentration and fluorescence intensity (figures 41,42). With lower concentrations of terbium (ca. $10^{-7}M$) no improvement in signal to blank ratio was obtained when the SSA concentration was decreased from the recommended 10^{-4} molar excess to a tenfold molar excess.

Other Analytical Parameters.

The fluorescence intensity of solutions containing $10^{-7}M$ and $10^{-6}M$ terbium were measured and related to that of a quinine sulphate standard using the same excitation filter, but measuring the quinine sulphate fluorescence at 450mp. Constant fluorescence intensity was noted from ten minutes after dilution to one hundred hours later when observations were discontinued. During the first ten minutes after dilution, the fluorescence decreased by about 5% to 10%.

Figure 43.

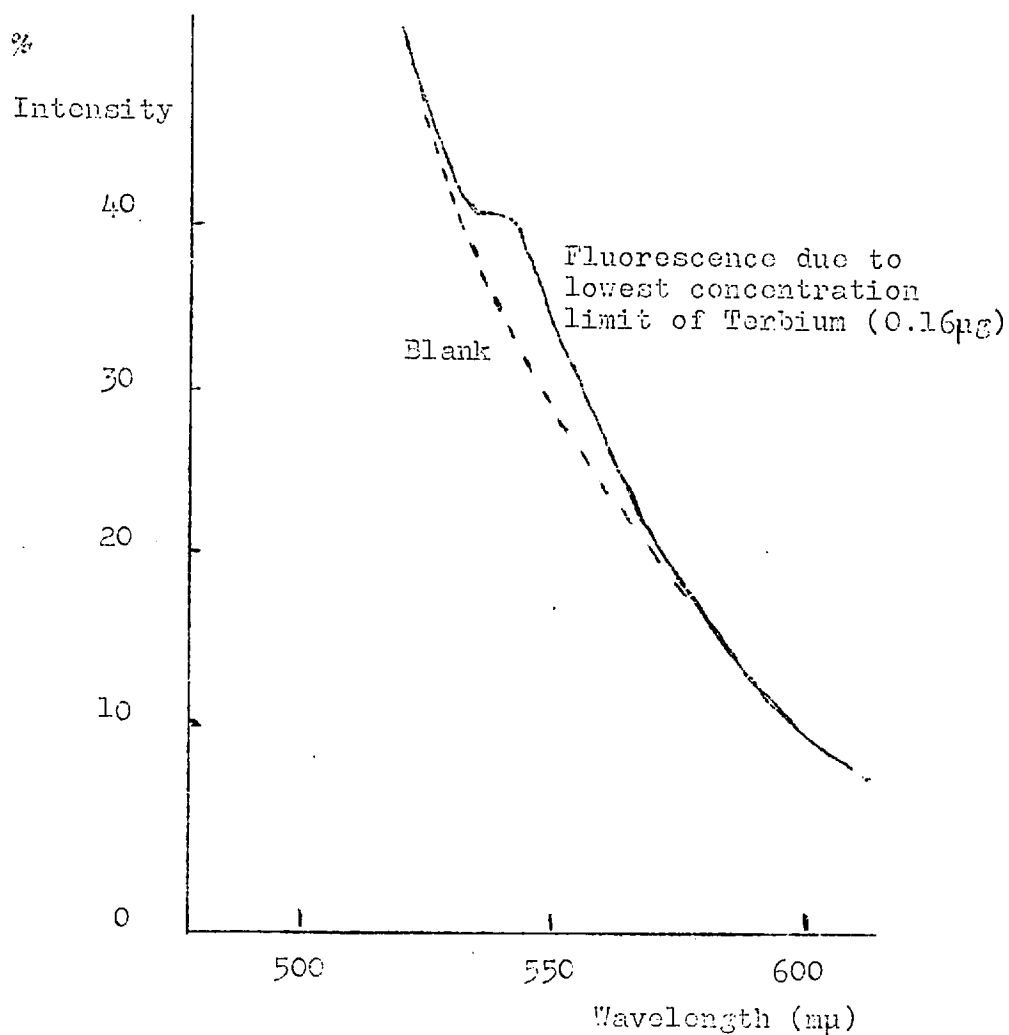


The effect of temperature on the fluorescence of a solution containing 4×10^{-6} M terbium was observed over the range $10 - 60^{\circ}\text{C}$, (figure 43). A high terbium concentration was chosen so that blank variations were negligible. For the range $15 - 30^{\circ}\text{C}$ the temperature coefficient was constant and had the value $-0.37\%^{\circ}\text{C}^{-1}$. Charles and Riedel⁹⁹ have found the quantum efficiency of the Tb/EDTA/SSA system in D_2O was constant over the temperature range $5 - 25^{\circ}\text{C}$.

The effect of dissolved oxygen was investigated by measuring the intensity of fluorescence of a solution containing 10^{-7} M terbium, before and after degassing with nitrogen for 20 mins. Oxygen was then bubbled for 5 mins and the fluorescence intensity measured again. Three identical readings were obtained showing that no quenching of fluorescence due to dissolved oxygen occurred. Care was taken to make these measurements at the same temperature as bubbling the gases caused some cooling.

Limits of Determination.

A linear relationship between fluorescence intensity and terbium concentration was obtained for solutions containing $6.4\mu\text{g}/\text{ml}$ (4×10^{-8} M) terbium to $320\mu\text{g}/\text{ml}$ (2×10^{-5} M) terbium. The intensity of fluorescence of the blank was equivalent to $6 \times 10^{-3}\mu\text{g}/\text{ml}$ (4×10^{-7} M) terbium and, therefore, the lowest calibration graph was for the range 0.16 to $1.6\mu\text{g}$ terbium in 25ml of solution (i.e. 4×10^{-8} M to 4×10^{-7} M). The blank at this level is 50% of the maximum scale deflection and has, therefore, been set as a limiting value. Suitable amplification of the signal would enable the method to detect ca. $0.05\mu\text{g}$ terbium using a blank value corresponding to 90% of the full scale deflection. A fluorescence emission spectrum for the blank and $0.16\mu\text{g}$ terbium is shown in figure 44.

Figure 44.

Narrowing the slit width in the analysing monochromator also improves the signal to blank ratio but higher amplification must be used to compensate for this. This is a direct result of the sharp line emission of the terbium chelate superimposed on the comparatively uniform spectral distribution of the blank. However, because the narrow slits (5mμ) did not isolate the whole of the terbium emission line, it was found that non-linearity of the

calibration curves resulted for lower concentrations of terbium, (i.e. less than 1.0 μ g). For this reason, 20mp slits are recommended in the analysing monochromator; these isolate all the terbium fluorescence and the analytical signal is proportional to the area under the fluorescence peak rather than to the height. Using 20mp slits, linear calibration curves for 0.1 μ g to 1.6 μ g terbium were obtained.

The use of ultraviolet filters in the analysing monochromator to eliminate stray light did not improve the signal to blank ratio.

Effect of Foreign Ions.

Fifty-fold molar excesses of foreign ions were added together with 4 μ g of terbium as in the recommended procedure. The following trivalent rare earth ions were examined and caused no interference: La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm and Yb.

The following metal ions were also examined in fifty-fold molar excess. None interfered: Ag(I), Al, As(III), Ba, Fe, Ca, Cd, Co(II), Cr(III), Cr(VI), Cu(II), Fe(II), Fe(III), Ga, Hg(I), Hg(II), K, Li, Mg, Mn(II), Mn(VII), Mo(VI), Na, Nb(V), Ni(II), NH₄, Pb, Se(IV), Sr, Ta(V), Te(IV), U(VI) and Zn.

The following anions were examined in one thousand-fold molar excess over 4 μ g of terbium; none caused any interference: acetate, bromide, carbonate, chloride, iodide, nitrate, oxalate, perchlorate, phosphate, sulphate, sulphide, sulphite, tartrate and thiosulphate.

One thousand-fold excesses of lead, however, gave a visible white precipitate, probably lead chloride, which reduced the fluorescence by ca.20%. Similar excesses of mercurous ion gave a fine grey suspension which reduced the fluorescence intensity by ca.2%. One thousand-fold excesses

Table 15.Analysis of Samples Treated as Unknowns.

	Terbium µg	Foreign Ions µg	Found µg	Error µg	Error %
1.	0.16		0.19	+0.03	+18.8
2.	0.16		0.16	0.00	0.0
3.	0.80		0.80	0.00	0.0
4.	0.80		0.80	+0.03	+3.6
5.	1.27		1.29	+0.02	+1.6
6.	1.27		1.26	-0.01	-0.8
7.	0.95	Eu(159), Gd(158), Sm(150).	0.94	-0.01	-1.1
8.	0.95	Nd(144), Pr(141), Yb(346), Er(334).	0.95	0.00	0.0
9.	0.64	Al(270), Be(90).	0.65	+0.01	+1.5
10.	0.64	(NH ₄) ₂ S ₂ O ₈ (210), CrVI(52), MnVII(55).	0.64	0.00	0.0
11.	0.64	Pb(207), Ce(140), La(140).	0.58	-0.06	-10.3
12.	1.19	La, Eu, Gd, Sm, Er, Nd, Dy, Tm, (16).	1.24	+0.05	+4.0
13.	1.19	Th(23), Ho(17), Yb(17), Nb(19).	1.21	+0.02	+1.7
14.	1.19	Cu(63), MnII(55), Fe II(56), CoII(59).	1.18	-0.01	-0.9

of uranium(VI) gave a strongly yellow coloured EDTA complex which gave rise to inner filter effects and reduced the fluorescence by ca.18%.

The analytical results on various prepared mixtures are given in table 15.

Structure of the Complex.

Mole ratio and Job plots have been carried out fluorimetrically for EDTA and SSA; a ratio of 1:1:1 for the Tb/EDTA/SSA was found. This is in complete agreement with the results of Charles and Riedel⁹⁹. Our optimum pH value for the fluorescence of 11.6 - 11.9 compares well with pH titration data which shows that the complex is completely formed at this pH but not below.

A systematic study of the structure would have to determine the co-ordination number of terbium, and this could be greater than or equal to six. The work of Charles and Riedel⁹⁹ indicated the formation of a complex of the formula $\text{Na}_4\text{Tb}(\text{EDTA})(\text{SSA})$, in which the SSA was bonded directly to the terbium atom. The absence of oxygen quenching and the sharpness of the terbium emission line would support this.

Table 16.

Relative Fluorescence of Other Tb/SSA Complexone Systems.

Complexone	Fluorescence	
Ethylenediaminetetraacetic acid	100	
1,2 Diaminopropanetetraacetic acid	100	
Trans 1,2 cyclohexanediaminetetraacetic acid	85	
Diaminodithylene other tetraacetic acid	65	
Hexamethylenediamine tetraacetic acid	37	
Iminodiacetic acid	9	
2,6 Diaminopyridine tetraacetic acid	7	No detectable Tb^{3+} fluorescence. Impure sample.
Ethylenediamine-N,N'-diacetic acid	4	Weak Tb^{3+} fluorescence
Nitrilotriacetic acid	0	No fluorescence due to Tb^{3+} was detected even on high amplification of the instrument.
Diethylenetriamine pentaacetic acid	0	

We have also carried out fluorescence measurements using other complexones (table 16). The solutions were made up as in the recommended procedure except that 10 ml of 10^{-2}M complexone was substituted for 1 ml of 10^{-1}M EDTA solution. None

of the complexones, except 1,2 diaminopropane tetraacetic acid, gave a terbium fluorescence intensity equal to that obtained with EDTA, and some gave no fluorescence even on high amplification. In instances where a measurable fluorescence was observed, no change in wavelength of the line fluorescence was noted, nor was there any appreciable change in the bandwidth of the emitted line. Similar experiments were carried out using europium, dysprosium and samarium solutions in place of terbium, but no characteristic fluorescence for these ions was obtained.

OTHER ENERGY TRANSFER SYSTEMS.

Europium/EDTA/p-Benzoylbenzoate.

In their work on laser materials, Charles and Riedel⁹⁹ investigated the Tb/EDTA/Sulphosalicylic acid chelate described earlier. In a later communication¹⁰⁰, the same authors described the europium complex, Eu/EDTA/p-benzoylbenzoate. In this complex, the p-benzoylbenzoate anion is not bound to europium and acts as an energy donor by collision in solution. As a result, high benzoylbenzoate concentrations (0.2M) are necessary and the system is subject to severe oxygen quenching.

In view of the selectivity of the terbium method, it was decided to investigate the Eu/EDTA/p-benzoylbenzoate system. The reagent p-benzoylbenzoic acid was made as follows:

Melting point p-methylbenzophenone 58°C (59°C)¹⁰¹.

Yield: 54% Recrystallised from petroleum ether.

Melting point p-benzoylbenzoic acid 148°C (148°C)¹⁰².

Yield: 50% Recrystallised from ethanol.

Infrared spectra show characteristic absorption at:

- (I) 1665cm^{-1} (aromatic C = O stretching).
 840cm^{-1} (2-adjacent ring-H bending).
 740cm^{-1} (5-adjacent ring-H bending).
 (II) 1680cm^{-1} - 1720cm^{-1} (aromatic $-\text{CO}_2\text{H}$).
 1656cm^{-1} (aromatic C = O stretching).
 820cm^{-1} (2-adjacent ring-H bending).
 750cm^{-1} (5-adjacent ring-H bending).

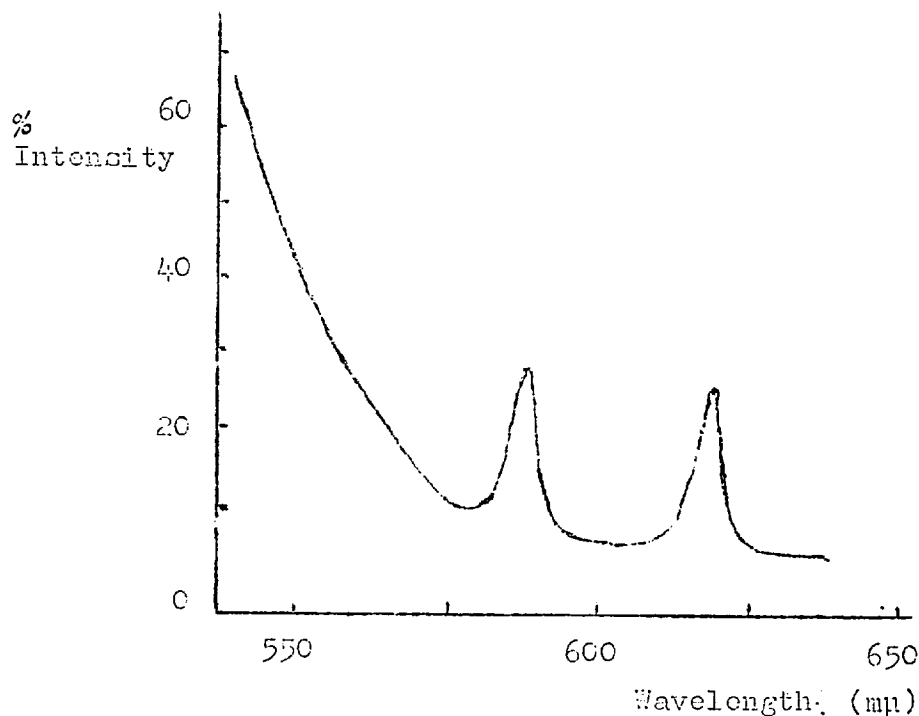
N.M.R.

- (I) CH_3 singlet τ 7.62 τ
 Aromatic multiplet 2-3 τ
 (II) Aromatic multiplet 2-3 τ

The preparations were carried out using modifications of the literature methods for (I)¹⁰¹ and (II)¹⁰².

Measurements on the europium/EDTA/p-benzoylbenzoate system were carried out at pH9 using dilute sodium carbonate solution as a buffer. The instrumental arrangement was similar to that used for the Tb/EDTA/Sulphosalicylic acid system: the excitation monochromator slits were removed, a 350m μ filter inserted to eliminate visible light, but 5m μ slits were used in the analysing monochromator instead of the 20m μ slits used in the terbium determination.

The fluorescence emission spectrum (figure 45) was obtained using 5ml of 10^{-3}M europium solution, 1ml 10^{-1}M EDTA solution and 0.1M p-benzoylbenzoate in 25ml of solution at pH9-10. Unlike the terbium/EDTA/SSA fluorescence, the emission from the europium was fairly weak and extremely

Figure 45.

sensitive to the presence of dissolved oxygen. On bubbling nitrogen for ten minutes the intensity increased by more than three-fold. In comparison with the terbium/EDTA/SSA fluorescence emission spectrum, the emission due to europium appears much broader, suggesting a collisional energy transfer rather than transfer from a chemically bonded *p*-benzoylbenzoate ion. The large excess of *p*-benzoylbenzoate necessary to promote the europium fluorescence and the high degree of oxygen quenching also suggests the absence of a chemical bond between the donor and acceptor species.

The analytical exploitation of this system was discontinued for the reasons below:

- (a) A sensitive, selective method is available for europium using sodium carbonate as the reagent¹⁰³.

- (b) Although no interference studies were carried out, the sensitivity of the system to oxygen quenching would suggest similar interference from paramagnetic metal ions.
- (c) The apparatus when used on full sensitivity would be able to determine an estimated concentration of only $10^{-6}M$ europium.

Systems Employing Other Energy Donors.

Energy transfer from an organic donor molecule to a rare earth acceptor ion is believed to occur through a mechanism involving the triplet state of the organic molecule. The fluorescence emission expected from the rare earth ion can be found in the literature and is fairly independent of the nature of the donor. The triplet state energy of the donor can be guessed at from the wavelength of the fluorescence emission of the donor, hence it is possible to obtain some indication of whether energy transfer is likely or not.

The following organic compounds were used in conjunction with EDTA and deaerated aqueous solutions of rare earth ions:

- (a) Glycine: is known to form ternary complexes of the type $\text{Eu/EDTA/glycine}^{104}$. Fluorescence 450m μ .
- (b) Anthranilic acid: Fluorescence 450m μ , contains $-\text{CO}_2\text{H}$ group and is, therefore, similar to sulphosalicylic acid.
- (c) Salicylaldehyde: Chemically similar to sulphosalicylic acid but fluorescence maximum occurs at 510m μ .
- (d) 8, Hydroxyquinoline: Promotes line fluorescence in rare earth complexes in absence of EDTA in organic solvents. The free reagent is non-fluorescent and probably forms a stable triplet state.
- (e) Anthranilic acid diacetic acid: Fluorescence occurs at 450m μ , used in presence and absence of EDTA as it contains

both donor and complexone groupings.

Only with anthranilic acid diacetic acid in the absence of EDTA was there any rare earth type fluorescence; a fluorescence with terbium was noted but the intensity was only about one tenth of that of the Tb/EDTA/SSA system.

CHAPTER VI.

DISCUSSION AND SUGGESTIONS FOR FUTURE WORK.

Without doubt, the greatest disadvantage inherent in the use of solution spectrofluorimetry is the lack of selectivity of most reagents and the susceptibility of current analytical methods to interference from other elements. Because there are two spectra, i.e. excitation and emission, the probability of elemental interference is twice that encountered in solution spectrophotometry. In view of this, it would appear that some research in inorganic spectrofluorimetric analysis must be directed towards improving selectivity; a few possibilities are listed below:

1. Metal(I)/Pyridine/Halide Complexes.

The fluorescent precipitates formed by the cadmium, cuprous, lead and silver ions with pyridine in the presence of iodide ions are well-known.¹⁰⁵ In a brief investigation of some of these complexes the fluorescence data listed in table 17 was obtained and from this some conclusions may be drawn.

The complexes of silver(I), cadmium and lead with pyridine and halide ions all show a similarity in fluorescence characteristics. Most probably, the fluorescence is due to the $\pi^* - \pi$ transition in the co-ordinated pyridine. Pyridine itself, however, is non-fluorescent and this has been attributed to the presence of low energy $n - \pi^*$ transitions which increase the probability of intersystem crossing from the pyridine $n\pi^*$ singlet state to the triplet.²¹ On complexation with a metal ion, the non-bonding electrons on nitrogen are localised to some extent near the metal ion, and the $n - \pi^*$ transition moves to a wavelength lower than that of the lowest energy $\pi - \pi^*$ transition. As a result,

fluorescence corresponding to the $\pi^* \rightarrow \pi$ transition of pyridine may be observed.

With cuprous pyridine halides, however, a different type of fluorescence is observed (figure 46). The excitation wavelength (ca. 390m μ) is the same, or approximately so, irrespective of the halide ion. The fluorescence emission, on the other hand, is critically dependent on the halide ion. This is explained by the possibility of charge-transfer from the readily oxidisable cuprous ion of the pyridine complex to the ligand π or π^* orbital. The fluorescence corresponds to the return charge transfer from the ligand π^* orbital to the metal d orbital. The energy of the fluorescence emission ($\pi^* \rightarrow d$ transition) is a measure of the electronegativity of the halogen atom.

It was not possible to isolate a stable dry sample of cuprous pyridinium chloride but stable samples of the bromide and iodide were obtained. Additional compounds were prepared using quinoline, acridine and 22' dipyridyl instead of pyridine, and their fluorescence characteristics examined (figure 47). With some of the compounds, e.g. cuprous bipyridyl iodide, the absorption was at a fairly long wavelength ca. 550-600 m μ so that any fluorescence would be difficult to observe visually and impossible to observe instrumentally.

It was impossible to dissolve any of the cuprous pyridine halides in organic solvents so as to retain the fluorescence. However, work is in progress at the time of writing, to attempt to precipitate the cuprous pyridine halide as a fine suspension. If this work is successful it may be possible to determine bromide and iodide (and perhaps chloride), simultaneously in the presence of each other.

Table 17.

Fluorescence characteristics of solid metal halide
Complexes of N-aromatic heterocyclics.

Complex	Excitation (mμ)	Emission (mμ)	Colour
Cd.pyr.Chloride	*	350	White
Cd.pyr.Bromide	*	350	"
Cd.pyr.Iodide	*	350	"
Cd.quin.Iodide	*	390	"
Cd.acr.Iodide	*	475	"
Ag(I).pyr.Nitrate	*	350	"
Ag(II).pyr.Persulphate	+	+	Brown
Cu(I).pyr.Bromide	390	535	White
Cu(I).pyr.Iodide	390	470	"
Cu(I).quin.Iodide	*	565	Yellow
Cu(I).acr.Iodide	+	+	Red
Cu(I).bipy.Iodide	+	+	Red
Cu(I).phen.Iodide	+	+	Blue

* No definite excitation peak can be located.

+ Non-fluorescent over range 300-600mμ.

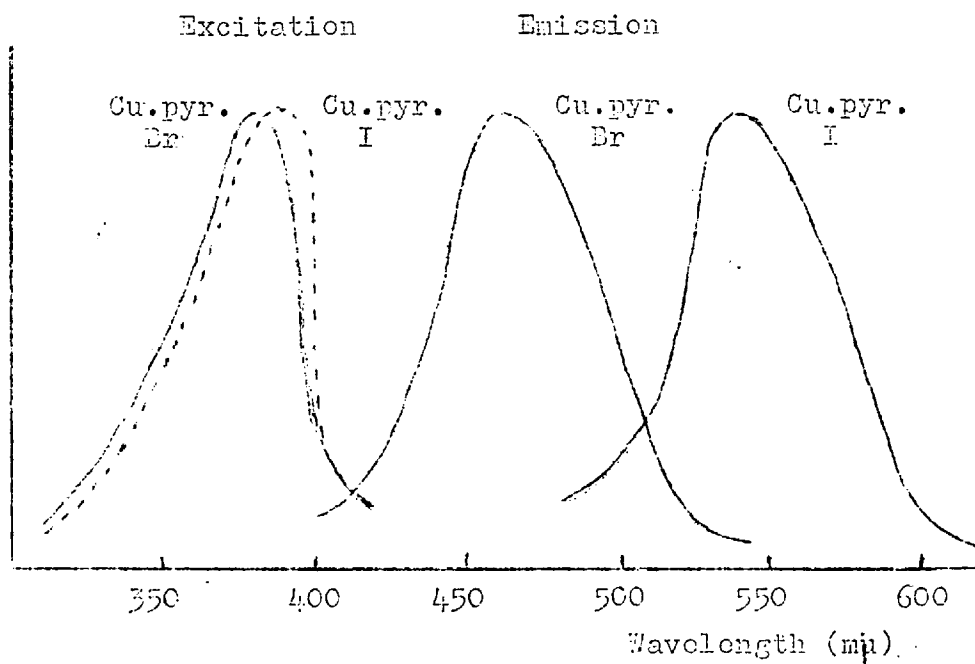
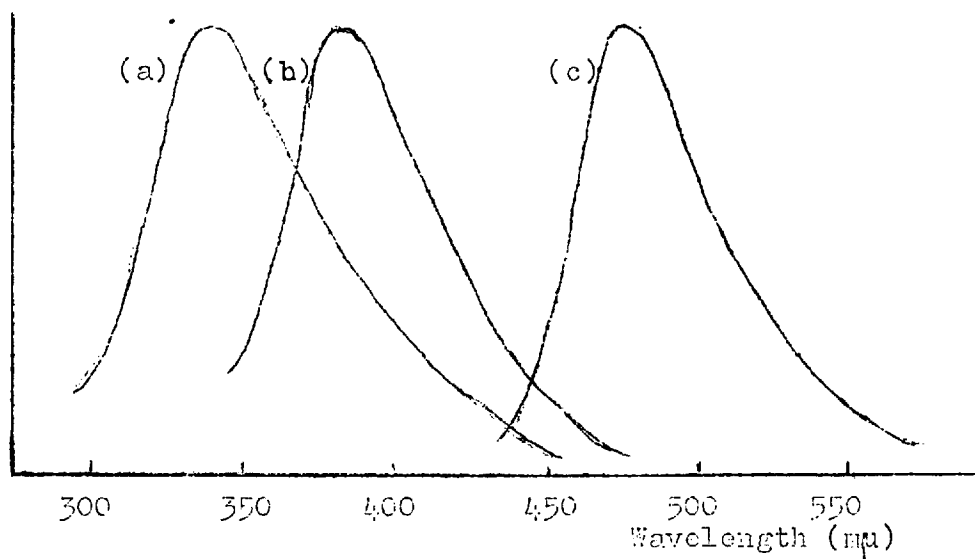
pyr. Pyridine.

quin. Quinoline.

acr. Acridine.

bipy. 2,2' Bipyridyl.

phen. 1,10 Phenanthroline.

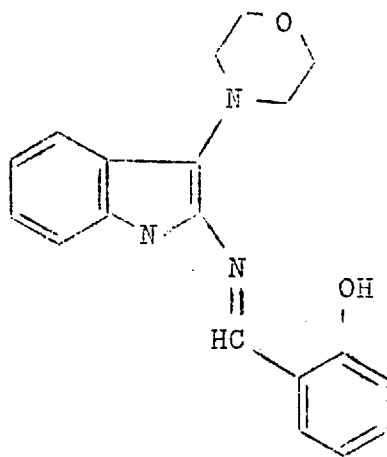
Figure 46.Figure 47.

Fluorescence emission spectra of:

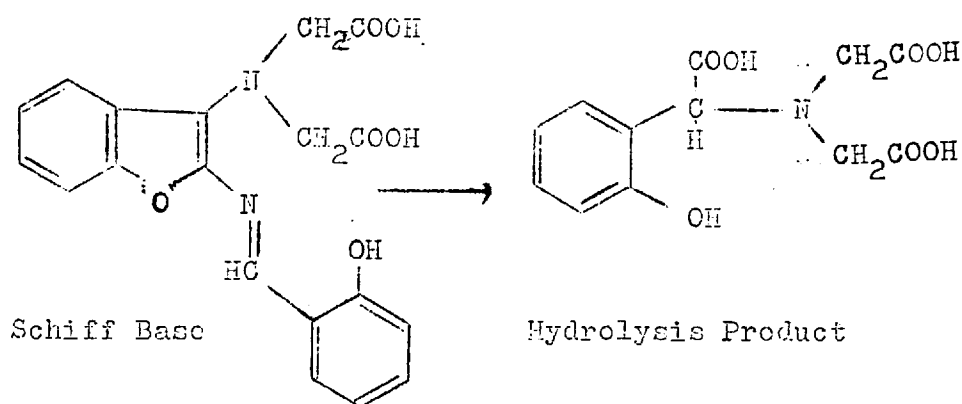
- (a) Cd/pyridinium/iodide
- (b) Cd/quinolinium/iodide
- (c) Cd/acridinium/iodide

2. Synthesis of New Reagents.

Although many new reagent structures could be suggested for possible synthesis, it would be particularly interesting to follow up the preparation of bis salicylidene 2,3, diamino-benzofuran(SABF). If morpholine, a secondary amine, is used instead of ammonia in the SABF synthesis, a compound having the structure below is isolated:



This compound and the piperidine analogue was prepared by the author during an investigation of the SABF preparation. Although the morpholine and piperidine derivatives are of little analytical importance, the reaction does point to a facile preparation of related compounds which may have co-ordinating properties. For example, the condensation of salicylaldehyde, cyanide and iminodiacetic acid would be expected to yield:



The analytical properties of both complexones may prove of interest. From the point of view of the organic chemist, the reaction is useful as it provides an easy preparation of 2,hydroxyphenylglycine, a hitherto unreported amino acid, the preparation of which would otherwise be fairly difficult.

3. The Use of High Intensity Sharp Line Sources.

The rare earth elements have been determined spectrophotometrically in concentrated solutions ($\text{ca. } 10^{-1} \text{ M}$) by means of their sharp absorbance lines¹⁰⁶. Unfortunately, these lines are of low extinction ($\epsilon_{\text{max}} \sim 10$) and, therefore, trace determinations by spectrophotometry have been rendered impossible. The use of spectrofluorimetry for the determination of rare earth ions has been confined to the highly paramagnetic ions dysprosium, europium, gadolinium, samarium and terbium, because they readily accept energy from suitable donors. The direct measurement of these ions

using a high intensity, xenon lamp and low resolution monochromators is impossible because the blank amplification is also high. If high intensity line sources were used, such as hollow cathode lamps or electrodeless microwave excited discharge tubes, the amplification of the blank would be small in comparison with that of the rare earth absorption. The technique would, in fact, be very similar to atomic fluorescence. Alternatively, rare earth lasers could be used as selective excitation sources if very high intensities were found necessary. In this way, the range of rare earth ions capable of being determined by spectrofluorimetry could be extended.

4. Polarised Fluorescence.

The effect and theory of polarised fluorescence has been investigated extensively by Perrin¹⁰⁷ and comprehensive reviews on the subject have been published by Weber.^{108,109} If a number of fluorescent molecules in solution are irradiated with polarised light, then only those molecules which are suitably oriented will absorb the polarised light. If the molecules do not undergo rotation while in the excited state, the fluorescence emission will also be polarised, although not necessarily in the same direction as the incident radiation.

The author has carried out some exploratory work on the effect using two pieces of polaroid plastic as the polarising and analysing polars, If measurements of the fluorescence are made with "crossed" and "uncrossed" polaroids the polarisation (p) can be found from the expression.

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Where $I_{\parallel}$ is the fluorescence intensity
with uncrossed polars
 $I_{\times}$ is the fluorescence with
crossed polars.

Initial experiments, using zinc and aluminium 8, hydroxyquinolates, were carried out to find the differences exhibited by two substances having similar spectral characteristics but very different molecular geometries. The results below (table 18) indicate that the fluorescence from the zinc chelate is polarised to a higher degree than that of aluminium chelate. The results also show that the

Table 18.

Polarisation of the Fluorescence of some Metal Chelate Compounds.

Complex	Solvent	Conc.	Polarisation
Zn(oxinate) ₂	Glycerol	$10^{-7}M$	0.5(0)
"	"	$10^{-5}M$	0.4(5)
"	CTAB	"	0.3(3)
"	$CHCl_3$	"	0.1(7)
Al(oxinate) ₃	Glycerol	$10^{-7}M$	0.3(0)
"	"	$10^{-5}M$	0.2(7)
"	CTAB	"	0.1(9)
"	$CHCl_3$	"	0.1(4)
Al(SAP)	Glycerol	$10^{-6}M$	0.5(0)
Mg(SABF)	Glycerol	$10^{-5}M$	-0.03

CTAB $10^{-3}M$ cetyltrimethyl ammonium bromide in water.

SAP Salicylidene 2, aminophenol.

SABF Bis Salicylidene 2,3 diaminobenzofuran.

fluorescence becomes depolarised as the concentration increases and as the viscosity of the solvent is decreased; in both cases re-orientation of the excited species becomes more probable.

The problem of re-orientation of the excited molecules has been overcome by the use of glycerol-water as a solvent. The very high viscosity of this type of medium hinders molecular rotation, but the solvent is inconvenient and messy. The use of long chain surface active agents or colloidal protein molecules is currently being examined as a means of reducing molecular rotation. Additional selectivity may be obtained because of the different adsorption characteristics of the species involved.

Another possible use of polarised fluorescence may be found in the determination of rare earth ions. With high instrumental amplification, the blank value caused by scattered light is a limiting factor; this would be even more serious with the resonance fluorescence systems postulated in section 5 of this chapter. Our present work has shown that polarised light does not become appreciably depolarised on scattering; in contrast, the symmetrical, fluorescent rare earth ions would be expected to completely depolarise the fluorescence emission, relative to the incident radiation. The use of crossed polaroid filters would, therefore, be expected to improve the fluorescence to blank ratio considerably.

The use of polarised fluorescence techniques in the determination of fluorescent, optically active compounds, e.g. quinine in the presence of quinidine, is another aspect of this phenomenon worthy of inspection.

In conclusion, it might be pointed out that besides being a useful analytical technique for the determination of traces of materials, spectrofluorimetry has been used in the solution of general chemical problems. The technique has been employed in the investigation of organic molecular complexes¹¹⁰; in the examination of potential rare earth laser materials⁹⁹; in investigations of the chemistry of spectroscopically excited states^{111,112}; and polarised fluorescence has been used in the determination of molecular dimensions of large molecules in solution.^{107,108} In view of this, it would appear that this is an area of trace analysis which will undergo considerable development and yield very valuable results in the near future.

APPENDIX I.FORTRAN IV COMPUTER PROGRAM, USED TO CALCULATE
QUANTUM YIELDS OF FLUORESCENCE.

Time for job:

0.13 min. PUFFT Two Spectra.
0.6 min. IBSYS Two Spectra.
0.23 min. PUFFT Four Spectra.
0.7 min. IBSYS Ten Spectra.

The program corrects experimentally determined spectra for variations in quantum response of photomultiplier tube and analysing monochromator, sums the area under the corrected curve using Simpson's Rule, normalises the corrected and uncorrected spectra, and prints out both in the form of a graph. The complete program is given in this appendix together with the output for one substance, anthracene.

\$IBFTC DICK

DICK - EFN SOURCE STATEMENT - IFN(S) -

```

      DIMENSION FREQ(60),FACTOR(60),HEIGHT(60,12),ODDS(12),EVENS(12),
      2TRUEHT(60,12),AREA(12),UPLIM(12),XY(12),I3(12),I1(12),YAXIS(80),
      3HIGH(60,12),UPL(12)
      DATA BLANK,POINT,PLOT/1H,1H*,1H./
C TO OBTAIN CORRECTED INTENSITY IN QUANTA PER UNIT FREQUENCY INTERVAL
      DO 5 I=1,51
      READ(5,1000) FREQ(I),FACTOR(I),(HEIGHT(I,J), J=1,10)
1000  FORMAT(F4.2,1X,F5.2,10(1X,F5.1))
      DO 10 J=1,10
C CORRECTION OF SPECTRA
      HIGH(I,J)=HEIGHT(I,J)/FREQ(I)**2
      TRUEHT(I,J)=(HEIGHT(I,J)/FACTOR(I))/FREQ(I)**2
10  CONTINUE
5  CONTINUE
C TO SUM AREA UNDER CORRECTED CURVE BY SIMPSON'S RULE
      DO 15 J=1,10
      ODDS(J)=0
      EVENS(J)=0
      DO 20 I=2,50,2
      EVENS(J)=EVENS(J)+TRUEHT(I,J)
20  CONTINUE
      DO 25 I=3,49,2
      ODDS(J)=ODDS(J)+TRUEHT(I,J)
25  CONTINUE
      AREA(J)=(0.02/3.0)*(TRUEHT(1,J)+TRUEHT(51,J)+4.0*ODDS(J)
      2 +2.0*EVENS(J))
15  CONTINUE
C TO DETERMINE UPPER LIMIT OF CURVE AND PLOT CORRECTED AND AND UNCORRECTED SPECT
      DO 30 J=1,10
      UPLIM(J)=0
      UPL(J)=0
      WRITE(6,1004) AREA(J)
1004  FORMAT(1H131H CORRECTED EMISSION SPECTRUM OF/6H AREA=F13.4/
      217H FREQ. INTENSITY/6X,11HUNCORR CORR,6X,2H*0,18X,3H*20,17X,
      33H*40,17X,3H*60,17X,3H*80/)
      DO 35 I=1,51
      IF(TRUEHT(I,J).GT.UPLIM(J)) UPLIM(J)=TRUEHT(I,J)
      IF(HIGH(I,J).GT.UPL(J)) UPL(J)=HIGH(I,J)
35  CONTINUE
      DO 45 I=1,51
      TRUEHT(I,J)=TRUEHT(I,J)*80.0/UPLIM(J)
      HIGH(I,J)=HIGH(I,J)*80.0/UPL(J)
      DO 40 K=1,80
40  YAXIS(K)=BLANK
      I1(J)=TRUEHT(I,J)
      I2=I1(J)
      IF(I2.LE.1) I2=1
      IF(I2.GE.80) I2=80

```

YAXIS(I2)=POINT
I3(J)=HIGH(I,J)
I4=I3(J)
IF(I4.LE.1) I4=1
IF(I4.GE.80) I4=80
YAXIS(I4)=PLOT

C TO PRINT OUT RESULTS AS A GRAPH OF CORRECTED SPECTRUM V UNCORRECTED

WRITE(6,1005)FREQ(I),HIGH(I,J),TRUEHT(I,J),YAXIS

108

1005 FORMAT(1X,F4.2,2X,F5.1,2X,F5.1,4X,1H.,80A1)

45 CONTINUE

30 CONTINUE

STOP

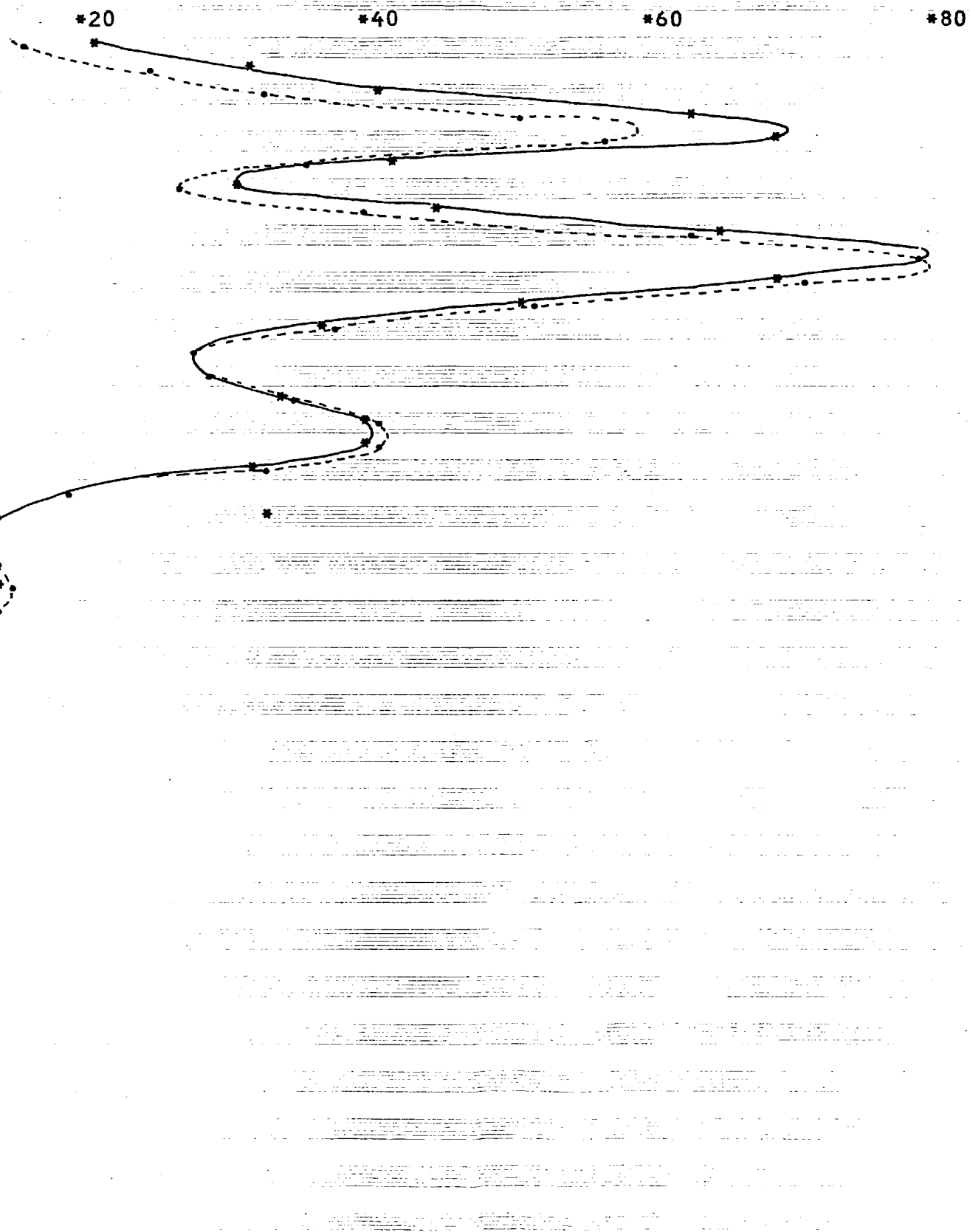
END

CORRECTED EMISSION SPECTRUM OF ANTHRACENE

AREA= 0.1093

FREQ. INTENSITY

	UNCORR	CORR	*0
2.72	16.3	21.4	.
2.70	25.2	32.3	.
2.68	33.3	41.8	.
2.66	51.2	63.3	.
2.64	57.2	69.6	.
2.62	36.3	42.8	.
2.60	27.8	31.4	.
2.58	40.8	45.2	.
2.56	63.8	65.7	.
2.54	80.0	80.0	.
2.52	71.1	69.7	.
2.50	52.5	51.5	.
2.48	38.5	37.8	.
2.46	28.9	28.7	.
2.44	29.5	29.2	.
2.42	35.2	34.6	.
2.40	41.4	40.6	.
2.38	41.4	40.7	.
2.36	33.0	32.4	.
2.34	19.7	19.2	.
2.32	14.8	33.8	.
2.30	13.6	13.0	.
2.28	14.4	13.7	.
2.26	15.4	14.5	.
2.24	14.7	13.8	.
2.22	12.4	11.6	.
2.20	9.7	9.0	.
2.18	6.2	5.7	.
2.16	4.4	4.1	.
2.14	3.6	3.3	.
2.12	2.9	2.7	.
2.10	2.1	2.0	.
2.08	-0.	-0.	..
2.06	-0.	-0.	..
2.04	-0.	-0.	..
2.02	-0.	-0.	..
2.00	-0.	-0.	..
1.98	-0.	-0.	..
1.96	-0.	-0.	..
1.94	-0.	-0.	..
1.92	-0.	-0.	..
1.90	-0.	-0.	..
1.88	-0.	-0.	..
1.86	-0.	-0.	..
1.84	-0.	-0.	..
1.82	-0.	-0.	..
1.80	-0.	-0.	..
1.78	-0.	-0.	..
1.76	-0.	-0.	..
1.74	-0.	-0.	..
1.72	-0.	-0.	..



APPENDIX II.CHEMICAL PROOF OF THE STRUCTURE OF BIS SALICYLIDENE
2,3, DIAMINO BENZOFURAN (SABF).

Work carried out by J. E. Baldwin and D. J. Blythin on the structural determination of SABF is described here.

Acid Hydrolysis of SABF.

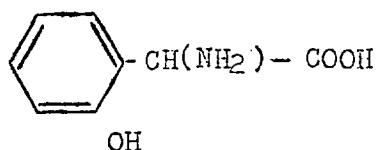
SABF in refluxing ethanol was hydrolysed with the calculated volume of concentrated hydrochloric acid for 30 minutes. The solution was evaporated to dryness, water added and extracted twice with ether. Salicylaldehyde was obtained from the ether extracts as the 2:4 dinitrophenylhydrazone and as the semicarbazone. The aqueous layer was evaporated to a small volume, more hydrochloric acid added and refluxing continued for 3 hours. The mixture was evaporated to dryness to remove hydrochloric acid, a small volume of water added and then silver oxide until neutral. Silver chloride was filtered off, the filtrate evaporated to dryness, the residue recrystallised from ethanol/water, and finally dried over phosphorus pentoxide for 10 days at 60°C to remove water of crystallisation.

Analysis:

Found: C = 57.5%, H = 5.5%, N = 8.1%.

Calculated: C = 57.5%, H = 5.4%, N = 8.4%

The mass spectrum gave a molecular weight of 165 and the infrared and n.m.r. spectra were consistent with the structure below:



The amino acid could have been formed from either (II) or (III) on page 68.

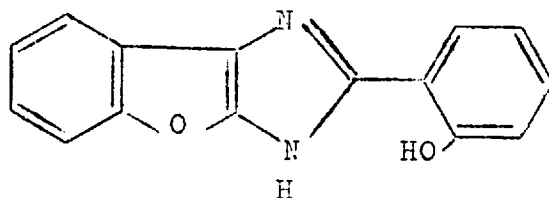
Iodine Reaction.

SABF (5g) in refluxing ethanol was treated with iodine (1.85g) in ethanol. The mixture was refluxed overnight with more iodine (1.85g) and for a further 5 hours. The solvent was removed under vacuum, the residue dissolved in ethyl acetate, washed with water, sodium thiosulphate solution, and water. The dark green solution remaining was dried (with magnesium sulphate), evaporated and extracted with benzene to leave a yellow solid which was filtered and washed with benzene.

Yield 623 mg (18%).

The product was purified by chromatography on an alumina column to yield 484 mg of material Melting Point $271 - 273^{\circ}\text{C}$ (with decomposition). The purified compound was then sublimed at 0.5 mm, 230°C to give a compound of molecular weight 250 (mass spectrum).

The structure below was suggested for the iodine reaction product:-



Analysis:

Found: C = 71.8%, H = 4.2%, N = 10.7%, I = 0%.
Calculated: C = 72.0%, H = 4.3%, N = 11.2%.

The infrared spectrum was in accordance with the structure proposed and the molecular formula was finally confirmed by mass measurement.

Bromine Reaction.

The isolation of the amino acid and possibly the iodine reaction product could be explained from the structures (II) or (III) on page 68. The bromination reaction was designed to distinguish between (II) and (III), according to the reaction scheme overleaf.

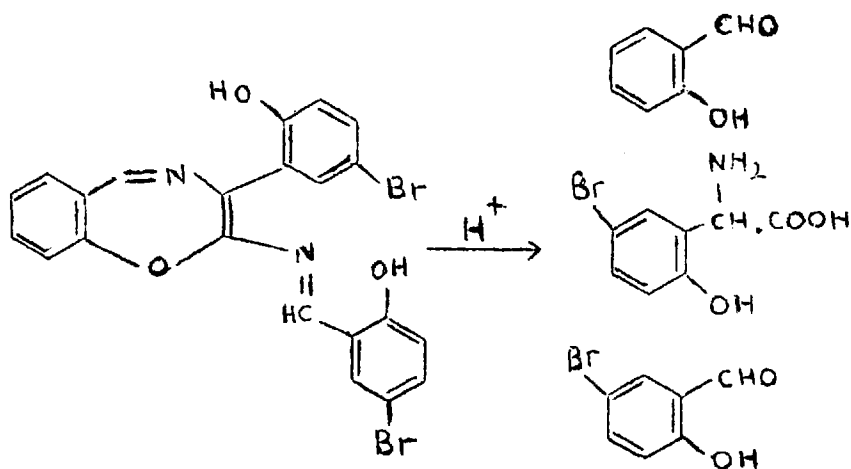
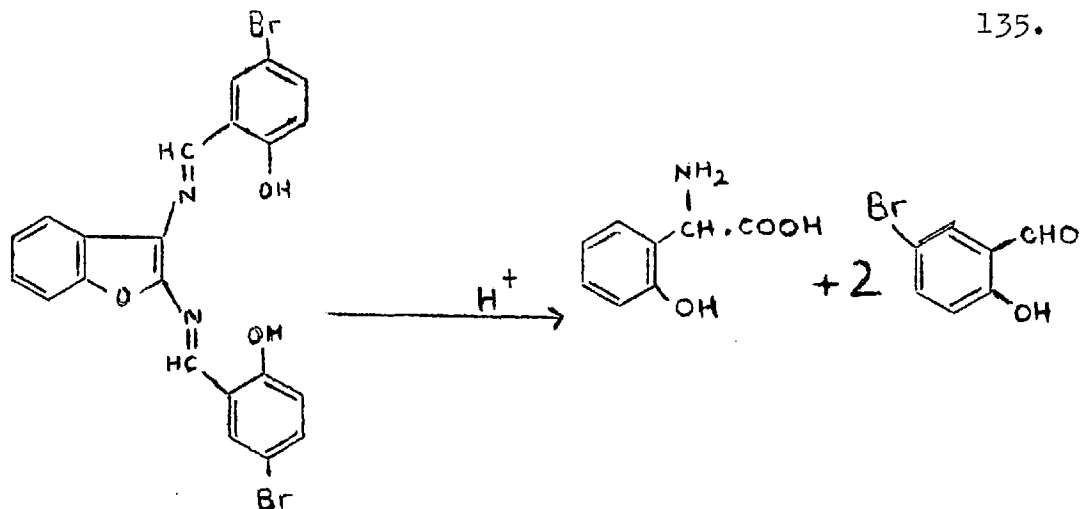
SABF (5g) was dissolved in benzene (150 ml) and was stirred at room temperature. To this was added a benzene solution of pyridine perbromide (21.0g prepared in situ) during several hours. When T.L.C. showed the absence of starting material, the mixture was filtered, the organic layer washed with sodium thiosulphate solution, water and finally dried using sodium sulphate. The solution was evaporated to a dark oil and a solid, treated with a small volume of hot benzene, cooled and filtered to give an orange-red solid which was dried and used for the hydrolysis.

Analysis:

Found: Br = 31.9%.
Calculated: Br = 31.2% (Dibromide).

The brominated adduct was hydrolysed using concentrated hydrochloric acid as before and the ethereal and aqueous extracts examined.

Ether extract - Examined by n.m.r., mass spectroscopy, T.L.C. and G.L.C. No salicylaldehyde could be found. The



product appeared to consist of mono bromosalicylaldehyde with some dibromo salicylaldehyde.

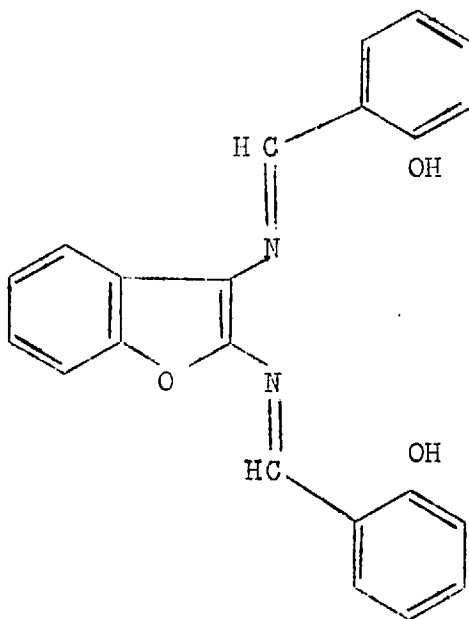
Aqueous extract - The extract was evaporated to dryness (90 mg solid), dissolved in cold water and refluxed for 3 hours with concentrated hydrochloric acid (1ml). The solution was evaporated to dryness (63mg) to give a mixture of the amino acid and ammonium chloride from the hydrolysis.

Total halogen 26.17%

Ionic halogen 26.24%

This demonstrates the absence of halogenated amino acid. The residue was examined further by mass spectroscopy and shown to give a similar mass spectrum to the amino acid obtained by direct hydrolysis of SABF.

This work proves the structure of SABF to be:



Preparation of Analogous Compounds.

Various secondary amines have been substituted for ammonia in the SABF preparation. The author has prepared the morpholine and piperidine analogues by a similar method to that described for SABF. The isolation and purification of other adducts is easy and will not be described because of the similarity to the SABF preparation.

Amine Used	MPt.	Found %			Calculated %		
		C	H	N	C	H	N
Dimethylamine	71°	73.06	5.62	10.04	72.84	5.75	9.99
Diethylamine	-	74.17	6.55	9.00	74.00	6.54	9.08
Pyrrolidine	119°	74.30	6.10	9.13	74.49	5.92	9.14
Piperidine	140°	74.84	6.27	8.57	74.97	6.29	8.74
N-Methyl-o-toluidine	151°	77.44	5.73	7.77	77.51	5.66	7.86

APPENDIX III.LIST OF PUBLISHED WORK.

1. R.M. Dagnall, R. Smith and T.S. West,
"Spectrofluorescence of Aluminium and Gallium with
Salicylidene-o-Aminophenol", Chem. Ind., 1499, 1965.
2. R.M. Dagnall, R. Smith and T.S. West,
"Spectrofluorimetric Determination of Submicrogram
Amounts of Aluminium using Salicylidene-o-Aminophenol",
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3. R. Smith,
"NN' Bis Salicylidene 2,3 Diaminobenzofuran (SABF),
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Magnesium". Proc. Soc. Anal. Chem, 3, 144, 1966.
4. R.M. Dagnall, R. Smith and T.S. West,
"Spectrofluorescence of the Magnesium Complexes
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5. R.M. Dagnall, R. Smith and T.S. West,
"The Spectrofluorimetric Determination of
Magnesium with NN' Bis salicylidene 2,3, diamino-
benzofuran", Analyst, 92, 20, 1967.
6. R.M. Dagnall, R. Smith and T.S. West,
"A Specific Spectrofluorimetric Determination of
Terbium Using Sulphosalicylic Acid and EDTA."
Analyst, 92, 1967. (To be published).
7. J.E. Baldwin, D.J. Blythin, R.D.G. Cooper and R. Smith,
"The Structure of Hydrocyansalide, A New Reagent for
Magnesium".
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ERRATA

Abstract 2nd para, 1st sentence Read "Several azomethine (Schiff Base) compounds were prepared and their reactions with some metal ions investigated".

Last para Read "....some suggestions for future work have been listed....."

Page 33 7th line up for "wavelenght" read "wavelength"

Page 104 2nd line up for "stoiometry" read "stoichiometry"

Page 142 Ref 57 for "Drushell" read "Drushel"