ANALYTICAL APPLICATIONS OF TERNARY COMPLEXES

by

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ABSTRACT

The use of two types of ternary complexes in spectrophotometry has been investigated.

The first type involves the association of the complexes formed by certain cations with neutral donor ligands such as 1,10-phenanthroline, with anionic dyestuffs. The use of these complexes has been shown in the development of spectrophotometric procedures for copper and lead utilizing the complexes they form with 1,10-phenanthroline and R.B.E. The procedure devised for copper has been extended to produce a spectrofluorimetric method for that element.

The procedures devised are sensitive and reasonably selective and may, with slight modification be extended to other cations which form similar complexes with 1,10-phenanthroline.

The second type of complex is formed by the association of cationic surfactants with certain binary chelate complexes.

With this type of complex spectrophotometric procedures have been developed for molybdate and tungstate as their Catechol Violet/Cetavalon complexes. Again the methods are sensitive and reasonably selective and the procedures devised are of a general nature for this type of complex.

The general mode of formation of both types of complex have been investigated and some insight has been gained into the mechanisms involved. Also postulations have been made as to the cause of the colour changes occuring when the complexes are formed.

A C K N O W L E D G E M E N T S

The work recorded in this thesis was carried out in the Chemistry Department of Imperial College of Science and Technology between October 1964 and June 1967. It is entirely original except where due reference is made and no part has previously been submitted for any other degree.

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Brian W. Bailey.

C O N T E N T S

INTRODUCTION

Ternary complexes are as the name implies, complexes comprising of three different species. Whilst such complexes are commonplace in inorganic chemistry, their usage in analytical chemistry has, until recently been rather limited. This is possibly because the behaviour of systems containing three constituents is inherently more complex than that of those containing two. It is therefore more difficult to define the various experimental parameters, such as pH, reagent concentration etc., so that the reproducible behaviour, which is essential as a prerequisite for analytical procedures, may be obtained.

There are two main reasons for forming ternary complexes, the first is this, the formation of a ternary complex may enable a cation to be extracted into organic media; after extraction the ternary complex may be determined by conventional techniques. An example is the cadmium/pyridine/iodide complex which can be extracted from excess iodide into benzene. When an I131 tracer is used a very sensitive analytical method for cadmium is obtained.[1] Similarly, the pyridine isothiocyanate complexes of the cations copper, cobalt, nickel, zinc, and manganese can be extracted into organic solvents and then determined polarographically,[2] or spectrophotometrically.[3]

The second reason for forming ternary complexes is that the formation of such a complex can cause an alteration of the spectral characteristics of one of the components invoved, and it is this change which can be used as the basis for a spectrophotometric or a spectrofluorimetric method. Such changes invove alteration of the absorption and/or emission characteristics of one of the constituents. Examples are the terbium/E.D.T.A./sulphosalicylic acid system in which the sulphosalicylic acid absorbs energy at 320mu and then transfers it to the terbium ion which emits its normal line spectrum at 545mu.[4] The presence of the E.D.T.A. is necessary for this process to occur but the exact role it plays is uncertain. It also operates as a mass masking agent for other cations and so gives a highly selective spectrofluorimetric method for terbium.

Another example is the bisphenanthrolinium silver(I)/Bromopyrogallol Red complex. The addition of 1,10-phenanthroline causes a change in the absorbance spectra of the simple silver Bromopyrogallol Red system.[5]

In some cases the formation of the ternary complex, apart from producing a spectral change, also renders previously non-extractable binary complexes extractable, e,g. the ferrous pyridine thiocyanate complex.[6]

In this thesis the author is primarily concerned with the application of ternary complexes to spectrophotometry. The ternary complexes which have been used in this field can generally be said to fall into five main categories.

The first type involves the reaction of various cations with certain electronegative ligands such as chloride, fluoride, and thiocyanate to form complex anions such as $SbCl_6^-$, $SnCl_3^-$, and $FeCl_4^-$. The complex anions then associate with various cationic entities to form coloured complexes. This type of complex can be further subdivided into three subgroups depending on the nature of the cationic entity.

(a) The first and probably the most important sub-group is characterised by the formation of a complex with a cationic chromophore. These chromophores are generally of the triphenyl methane class such as Rhodamine B, Crystal Violet, Methylene Blue etc., as these have very high molar absorptivities. In these types of complex the chromophore does not undergo any spectral change on complexation but the complex may be extracted away from excess dye into a variety of solvents, thus enabling the complex to be determined spectrophotmetrically. Among the elements which have been determined in this way are tin, as the chlorostannate crystal violet complex in heptan-4-one,[7] gallium, as the chlorogallate

brilliant green complex in benzene,[8] and antimony(V), and gold(III), as their chloro-crystal violet complexes in chloroform.[9]

The chromophores used are not limited to dyestuffs, e.g. mercury has been determined as the anionic mercuric iodide complex with the ferrous $\alpha\alpha'$ bipyridyl complex as the counter cation in chloroform.[10]

(b) The second sub-group is characterised by the formation of a coloured complex by the anion with some cation, generally with a view to determining the cation. In the majority of cases, the complex anion is the trichlorostannate species and it has been suggested that this acts as a donor ligand having a similar strength to the chloride ion.[11]

With this type of complex the following cations may be determined spectrophotometrically, palladium,[12] platinum,[13] ruthenium, and rhenium, and indium.[11] The structures of the latter three have been postulated as :- $(Ru\ Cl_2\ (SnCl_3)_2)^{2-}$, $(Rh_2Cl_2(SnCl_3)_4)^{4-}$ and $(Ir_2Cl_6(SnCl_3)_4)^{4-}$ respectively.

(c) The third sub-group is comprised of those complex anions which form extractable coloured complexes with oxygen-containing organic solvents such as ethers and alcohols. In this way iron(III) may be determined by extracting a ferric chloride species into diethyl ether.[14] The structure postulated is:-

$$(C_2H_5)_2O : H^+ \; FeCl_4 \; ((C_2H_5)_2O)_2^-$$

Similarly, the elements iron(II), uranium(VI), bismuth, niobium, cobalt(II), ruthenium(III) and, in the presence of reducing agents, molybdenum and tungsten may be determined as their thiocyanate complexes in diethyl ether.[15]

Whilst these ternary complexes afford very sensitive spectrophotometric procedures, especially those utilising the triphenyl methane dyestuffs, there is one major disadvantage. This is the conditions under which the method must be carried out. In all the procedures the formation of the complex anion requires acidic conditions of pH 1 and less thus making the use of masking agents impossible. A certain degree of selectivity can be obtained however, by strictly controlling the acidity.

The other disadvantage is that the methods are obviously limited to those cations which readily form complex anions.

The second type of ternary complex is characterised by the formation of a complex with a mixed ligand such as phosphomolybdic acid. In this way niobium,[16] titatium,[17] and zirconium[18] have been determined spectrophotometrically as their phosphomolybdates. The general method by which this is accomplished is to reduce the

phosphomolybdate with stannous chloride to form the well known molybdenum blue.[16] However although sensitive, this method is not reproducible and the coloured product is unstable.

The third type of complex is a mixed ligand complex in which a cation forms a complex with two different anionic ligands. The spectral properties of the resulting complex are different from those of the simple binary metal complex with either of the two ligands. Examples of this type of complex are the metal chelate complexes whose absorbance spectra are altered in the presence of fluoride ions.[19] Amongst such systems are the Alizarin complexone complexes of cerium(III) and lanthanum(III)[20] and the Xylenol Orange complex of zirconium.[21] These complexes are used mainly in spectrophotometric procedures for the determination of fluoride.

The fourth type of complex is also a mixed ligand complex but differs from the previous category in that one of the ligands is uncharged. Into this category come the various metal-phenanthroline and metal-pyridine complexes. A number of these complexes are coloured as a result of charge transfer phenomena and may thus be used spectrophotometrically. e.g. determination of copper as the cuprous neocuproin (nitrate) complex[22] or iron as the ferrous phenanthroline (chlorate) complex.[23] Another method by which the spectro-

photometric analysis of the phenanthroline complexes may be accomplished is to measure the absorbance of the phenanthroline in the complex after removing the excess by extraction into chloroform.[24] A third possibility is to associate the positively charged metal phenanthroline with an anionic chromophore as in the silver(1) 1,10-phenanthroline Bromopyrogallol Red complex.[5] In this type of complex the chromophore does under-go a colour change. This change is unique to the ternary system and hence gives rise to a possible spectrophotometric method. However in many instances there is a relatively large overlap in the spectra of the free reagent and the complex and it is preferable to extract the complex away from excess reagent.

This latter type of ternary complex has a number of advantages when used in spectrophotometry. Firstly the methods arising are generally very sensitive with molar absorptivities ranging from 40,000 to 100,000.[25] Secondly the complexes are formed in near neutral solution thus allowing masking agents to be used when necessary. It is to this type of complex that this thesis is mainly devoted.

Some research has also been conducted on the fifth and last type of ternary complex. These complexes are a result of the interactions of various metal chelate complexes with cationic surfactants.

The metal chelate complexes are adsorbed onto the surface of micelle aggregates and in doing so undergo a colour change. In addition their molar absorptivities are frequently increased. This type of system has been used to determine tin as the tin(IV) Catechol Violet complex on a gelatin substrate.[26] Also titanium and indium have been determined in a similar way.[27,28] The main disadvantage of these systems is the non-reproducibility of the dispersing agents such as gelatin. However, this has been overcome by the use of more well-defined surfactants e.g.cetyl trimethyl ammonium bromide (cetavalon). This type of system is also very sensitive, the molar absorptivity of the Tin(IV) Catechol Violet cetavalon complex being 90,000.[29] The other main advantage of this type of system in spectrophotometric analysis is the wide range of cations which may be determined. The formation of the complex is only limited to the formation of a suitable metal chelate complex.

In order to determine the potentialities of ternary complexes in spectrophotometry it is necessary to make a more detailed examination of some of the theoretical aspects on which spectrophotometry is based.

The most important aspect of spectrophotometry, when applied

to trace analysis is the sensitivity which may be obtained and there are two limits which govern this. These limits may be called the 'instrumental limit' and the 'reagent limit'. To consider first the instrumental limit.

The intensity of the absorbance of a coloured solution is measured in 'absorbance units' (A) which may be related to the concentration (C) etc. by the Lambert-Beer Law:-

$$A = \log_{10} I_o/I_t = l.C.E$$

where E is the molecular extinction co-efficient or molar absorptivity of the compound and is a measure of the light capture cross-section of the molecule. 'l' is the path length of the beam in cm. and I_o and I_t are the intensities of the incident and transmitted light beams. It can be seen from the Lambert-Beer law that as I_t tends to I_o, 'A' approaches zero logarithmically. Now in the best spectrophotometers the maximum discrimination is ca. 0.002 absorbance unit, thus the difference in the absorbance of two coloured solutions of different concentration must be at least 0.002 of an absorbance unit before meaningful measurements can be made. This is the instrumental limit.

As a result of this first limit sensitivity is now dependant

on the molar absorptivity of the substance being determined. The greater the molar absorptivity the greater the difference between Io and It for a particular concentration, and so smaller concentrations may be determined. Consider coloured complexes, these may be divided into three groups depending on the mechanism causing the colour.

Firstly there are complexes whose colour is due to a charge transfer between cation and ligand viz. Copper(I) - neocuproin and iron(II) - 1,10-phenanthroline where the colour is dependant an the process:-

$$Cu(I) \overset{h\nu}{\rightleftharpoons} Cu(II)$$

and

$$Fe(II) \overset{h\nu}{\rightleftharpoons} Fe(III)$$

The colour of these charge transfer complexes is quite intense and is also characteristic of the particular cation and ligand involved. However, such complexes are of somewhat limited occurence.

In the second type of complex the colour is a result of d-d transitions in the cation itself e.g. manganese salts. However, such transitions are spin forbidden and the colours are not intense (E ca 10^0) and in consequence are of no use in spectrophotometry.

The last type of coloured complex is that in which a cation (or anion) complexes with a chromophoric ligand. The absorbance of the resulting complex is dependant on the absorbance of the ligand which is usually an organic molecule. The intensity of the absorption bands is a property of the organic molecules and is a measure of the interaction between the molecule and radiation of the appropriate wavelength. The actual wavelength of absorption being dependant on the degree of conjugation in the system.

The molar absorptivity of this last type of complex is dependant on the molar absorptivity of the ligand, which has a maximum theoretical value of the order of 100,000. This maximum value has been calculated by E.A.Braude[30] in the following way:-
Consider a beam of light of intensity I falling on a slice of thickness dl of the absorbant solution in a cell of unit area. Let the concentration of the absorbant be 'c' gm-mol. per litre, also let the average effective absorbing area - 'chromophore area' - of the absorbing molecules in the plane perpendicular to the incident light be 'a', Now we have the

$$\text{Illumination falling on absorbing molecules} = \frac{\text{Area of absorbing molecules}}{\text{Total Area}} \times \text{Total illumination}$$

$$= \frac{c\ Na\ dl}{1000} \times I$$

and the illumination absorbed:-

$$= -dI = \frac{F\ c\ N\ a\ dl}{1000} \times I$$

Where F is the 'light extinction factor' and represents the fraction of light falling on the absorbing molecules which is absorbed, and N is Avogardros number.

Intergrating between the limits I= o and l=l

$$E = \frac{1}{Cl} \log_{10} \frac{Io}{I} = \frac{F\ N\ a}{2.3 \times 1000} = 2.64 \times 10^{20} F\ a$$

Consider a simple molecule when 'a' is taken as the largest cross sectional area (a) of the molecule and ca $10A^2$ and putting F equal to unity we get

$$E \simeq 10^5$$

This figure fits in well with that determined on the basis of classical electromagnetic theory and quantum mechanics, and imposes the second ('reagent') limit on the sensitivity of spectrophotometric procedures.

It is this second point which is of particular interest because, as has been said, most spectrophotometric methods depend on reactions with organic ligands and where these constitute the chromophore E will have a maximum value of the order 100,000.

Consider further the complexes involving organic ligands. In spectrophotometry the technique is to add to the trace of inorganic ion in solution an organic molecule which will react with it to produce an absorbing compound which has different spectral characteristics from either the ion or the ligand. These differences arise because the presence of the ion produces differences in the resonance structure of the molecule which in turn causes differences in the electronic spacings of the molecular orbitals so that different light quanta are necessary for excitation.

Most metallochromic organic reagents are chelating agents processing ionizable protons so that, when these dissociate different absorbing species are formed:-

$$H_2 L \overset{k1}{\rightleftharpoons} H L \overset{k2}{\rightleftharpoons} L^{2-}$$

in binary complexes the cation reacts with the chelating agent viz.

$$M^{2+} + H L^{-} \rightleftharpoons M L \rightleftharpoons M L^{2-}$$

the absorption spectra of which , are different from that of the ligand itself, and generally resemble that of the next higher ionization species viz. L^{2-} in this example.

With ternary complexes the mechanism is more complicated. The first type to be dealt with where a cation complexes with an

uncharged donor ligand, the complex formation may be represented:-

$$M^{2+} + 2L + HR^{-} = M L_2 R$$

This reaction takes place in two stages, the reaction

$$M^{2+} + 2L = (M L_2)^{2+}$$

preceding the step

$$(M L_2)^{2+} + HR^{-} = M L_2 R$$

The second type when all species are ionic, can be represented by:-

$$M^{+} + LOH + HR^{-} = MLR$$

or

$$M^{2+} + HL + HR = MLR$$

In this case the sequence of reaction may not be important and one can have either:-

$$LOH + HR^{-} = LR^{-}$$

then

$$M^{+} + LR^{-} = MLR$$

or

$$M^{+} + HR^{-} = MR^{-}$$

and then

$$MR^{-} + LOH = MLR$$

The absorption spectra of these complexes are also different from those of the reagent though again they are comparable with absorbances of the ligand at higher pH.

The main advantage of the types of ternary complexes investigated is their very high sensitivity, In fact the majority of the complexes have molecular extinction co-efficients in excess of 50,000. Whilst the ultimate sensitivity is dependant on the molar absorptivity of the chromophore, it is interesting to note that the molar absorptivity of binary complexes is usually less than that of the ligand, whereas that of the ternary complex is, in general, of the same order or higher than that of the ligand. The causation of these high molar absorptivities is not really readily determinable, but it has been postulated that introducing the third constituent into a binary complex increases the effective area of light absorption of the molecule. Whilst this is unden-

iably true whether the additional absorption will increase the absorbances at the particular wave-length of maximum absorption of the complex is a moot point.

However, apart from increasing the actual molar absorptivity of the system the formation of a ternary complex tends to raise the sensitivity of an existing spectrophotometric reaction because the difference in the wave-lengths of maximum absorption of the ternary complex and reagent is greater than that of the binary complex and reagent, thus the 'blank' is reduced.

The second advantage is that of increased selectivity. Since most ligands constitute the chromophoric part of a complex it is found that practically any metal of suitable co-ordination geometry will react with a ligand to give the same colour change. Small variations may occur from slight differences in the covalency of the bonding. It can be seen then that there is a very real problem of selectivity as most colour reactions tend to be unselective. One approach to this problem is to make the ligands of such a shape that they will only be able to form complexes with a minimal number of cations because the majority of interfering ions will be prevented sterically from complexing with the ligand, as with calcichrome[31] and neo-cuproin.[22]

The second method of making colour reactions selective is to use ternary complexes. The prospects of other metals duplicating a ternary system are less than those of duplication of a binary system. An example is the bis-1,10-phenanthrolinium/cation/ Rose Bengal Extra system, here complexes are only formed by those cations which will first react with 1,10-phenanthroline.[25]

Yet another advantage of ternary complexes is that they enable a new type of chromophoric reagent to be used. As many ternary complexes are of the ion association type, chromophoric reagents which do not contain complexing centres, such as Rhodamine B, Bromophenol Blue and Rose Bengal Extra, may be used. Apart from the academic interest, this is of particular interest analytically as these reagents have very high molar absorptivities.

It is now readily obvious that there are numerous advantages to be gained by using ternary complexes in spectrophotometry. It is to work in this field and especially to the two particular types of ternary complex mentioned previously that this thesis is devoted.

The basis for investigation was provided by the original studies of Dagnall and West who developed an extremely sensitive spectrophotometric procedure for silver using the ternary silver(I)/ 1,10-phenanthroline/Bromopyrogallol Red complex. Bromopyrogallol

Red (B.P.R.) contains vic hydroxyl groups and has been used as a complexing ligand for various cations.[3] This dyestuff was initially used as a colorimetric reagent for silver(I), but it was found that in the presence of 1,10-phenanthroline the wave -length of the absorption maximum of the resulting complex underwent a bathochromic shift. In addition the molar absorptivity was increased from 10,000 in the absence of phenanthroline to 51,000 with 1,10-phenanthroline. It was postulated at that time that this was due to the formation of the

$$\left\{(\text{phen} - \text{Ag} - \text{phen})_2^{2+}\,\text{B.P.R.}^{2-}\right\}$$

complex and the colour change resulted from either the physical absorption of the dyestuff on to the colloidal bis 1,10-phenanthrolinium silver complex, or a charge transfer effect involving siver(I) and B.P.R.

In view of the sensitivity and selectivity of this reaction and because of the fact that B.P.R. is subject to oxidation, particularly in alkaline media, and the presence of the vic hydroxyl groups render the general reaction unselective - in so far as these groups act as complexing centres for various cations - other reagents were investigated as a possible replacement for B.P.R. Because the

'absorption theory' was generally favoured the dyes investigated were mainly of the so called 'adsorption' indicators. Various dyes were found to give a colour change and a dye similar to that of B.P.R. namely Bromophenol Blue (B.P.B.) was selected as a replacement for B.P.R.

At this point it was decided to investigate the possibilty of forming similar types of ternary complexes with other cations such as copper(II), manganese(II), cobalt(II), nickel(II), iron(II) etc. which were known to form phenanthrolinium complexes.[33] The anionic dye used in this work was another triphenyl methane derivative, namely Rose Bengal Extra. This latter dyestuff was used in preference to B.P.B.and B.P.R., because the acidic group was carboxylic as apposed to sulphonic, a factor which makes extraction into organic solvents easier. Also, the reagent is fluorescent thus giving rise to the possible spectrofluorimetric methods of analysis.

It was found, as expected, that most of the cations which normally form phenanthroline complexes also formed ternary complexes which were extractable into chloroform and ethyl acetate. To investigate these complexes in more detail copper(II) was selected as a'typical' cation and a more detailed investigation of the bis phenanthroline copper(II) R.B.E. complex was made. This resulted in both spectrophotometric and spectrofluorimetric methods for the

determination of copper(II). A similar method was also devised for the spectrophotometric analysis of lead.

A brief investigation was made into the use of other ligands apart from 1,10-phenanthroline, but although similar complexes could be obtained with pyridine and $\alpha\alpha'$ bipyridyl, 1,10-phenanthroline was the best.

At this point interest was focussed on the effect which surfactants had on the ternary systems and on other metal chelate complexes. A spectrophotometric method was devised for the analysis of anionic surfactants based on the formation of a ternary complex between copper(II) 1,10-phenanthroline and the surfactant. This complex was extracted into chloroform and the surfactant was then replaced by R.B.E. with subsequent measurement of the absorbance of this complex.

The ternary complexes formed with cationic surfactants and various metal chelate complexes were next investigated. The reasons for moving away from the 1,10-phenanthroline based ternary complexes was that firstly the 'virtue' of their relative selectivity limits their application to the various phenanthroline complex forming elements and interest was in widening the possible field of application of ternary complexes. The metal chelate-surfactant systems offer this possibility and further, the general

effects occuring in surfactant based ternary complexes are very similar to those in the phenanthroline based systems and it was thought that investigations might help to explain some of the unaccounted phenomena of the phenanthrolinium complexes.

The general reaction mechanism of the ternary systems involving surfactants was determined and a spectrophotometric method was developed for molybdenum as the molybdate/Catechol Violet/cetavalon complex.

An investigation was also made into developing a spectrophotometric method for antimony using a similar type of system.

Finally an attempt has been made to explain the causes of the colour changes which occur in the ternary systems investigated.

C H A P T E R I

INVESTIGATION AND EVALUATION OF REAGENTS

The ternary system silver(I)/1,10-phenanthroline/Bromopyrogallol Red developed by Dagnall and West[5] provides the starting point for the work carried out for this thesis.

Whilst investigating the use of Bromopyrogallol Red (B.P.R.) as a spectrophotometric reagent, it was observed that on the addition of 1,10-phenanthroline to an aqueous solution of silver(I) and B.P.R., instead of the slow removal of the red colour of the reagent and formation of a yellow colour of much lower intensity, there was instantaneous formation of a blue colour of very high intensity. This latter colour change was subsequently found to be a result of the formation of the ternary complex $\{(\text{phen - Ag - phen})_2^{+}\ \text{B.P.R.}^{2-}\}$ and was later used as the basis for spectrophotometric methods of analysis for silver in aqueous and organic media.

In view of the high sensitivity and relative specificity of the reaction it was decided to investigate the formation of similar ternary complexes.

In a ternary complex of this type there are three variables, the cation, the donor ligand, and the counter anion. In the system described the counter anion has two serious disadvantages; firstly

it possesses vic hydroxyl groups which act as complexing centres for various cations. This makes the system liable to interference from cations other than those forming a phenanthrolinium complex. Secondly B.P.R. is prone to oxidation, especially in alkaline solution. It was decided therefore to find initially a substitute for B.P.R.

It was at first thought that the colour change was dependant on the formation of a precipitate, with physical adsorption playing a major part. In view of this the reagents on which particular interest was focused were the class of dyes known as 'adsorption indicators'. This name is derived from their use in precipitation titrations,[34] The mode of reaction of the indicators in such titrations is as follows: an ion such as silver(I) is titrated with an ion of opposite charge such as halide ion. At the equivalence point the system consists of an uncharged precipitate; on further addition of either ion the precipitate becomes charged by adsorption of the excess ion. This charged precipitate then adsorbs the indicator dyestuff which, on adsorption, undergoes a colour change.[34] The indicator dyestuff must of course have an opposite charge to that of the precipitate to which it is to be adsorbed.

A number of adsorption indicators were selected and together with a few other reagents were examined for a colour change in the prescence of silver/1,10-phenanthroline which was different to the colour change,if any, which occured with either silver or 1,10-phenanthroline. The results of the investigation are shown in Table 1.

Of the reagents which underwent a colour change with the silver/1,10-phenanthroline, three which appeared to give the most sensitive colour change were selected for further investigation, These were:-

1) Congo Red

2) Bromophenol Blue

3) Rose Bengal Extra

TABLE I

Reagent	Colour Change	Approx pH
Diphenyl Carbazone	positive	10
Congo Red	positive	4&7
Solochrome Red B	negative	
Phenosafranine	negative	
Acid Alizarin S	positive	7
Chrome Azurol S	negative	
Fluorescein	positive	7
Dichloro (R) fluorescein	positive	7
Diiodo dimethyl (R) fluorescein	positive	7
Tetrabromo (R) fluorescein	positive	7
Tetraiodo (R) fluorescein	positive	7
Tetraiodo (R) tetrachloro (P) fluorescein	positive	7
Mercurochrome	positive	7
Morin	negative	
Beibrich Scarlet	negative	
Calcichrome	positive	7
Bromophenol Blue	positive	4&7
Bromocresol Purple	positive	7
Brilliant Yellow	positive	7
Bromothymol Blue	positive	7
Chromotrope F4B	positive	9

1) Congo Red (C.R.)

Congo red is an acid-base indicator over the range pH7 and has also been used in dyeing.[35] It undergoes a slight colour change in the presence of the bis 1,10-phenanthrolinium silver(I) complex.

Preliminary Spectra

Fig I shows the absorption spectra for Congo Red (curve a) and its silver-1,10-phenanthroline complex (curve b). It can be seen that the shift in the wave-length of the absorption maxima is small at 20mu and the maximum difference in the absorbance between complex and reagent occurs at about 560mu and not at the absorbance maxima of the complex (520mu).

The reagent does not give any visible reaction with silver alone.

Curve (b) was prepared by diluting 5ml 10^{-3}M. C.R., 5ml 10^{-2} M. 1,10-phenanthroline, 5ml of 20% ammonium acetate buffer pH7, and 20ml 10^{-3}M. silver to 100ml distilled water in 1-cm cuvettes. Curve (a) was prepared in a similar manner, but contained no silver. The absorbance was again measured against distilled water in 1-cm cuvettes.

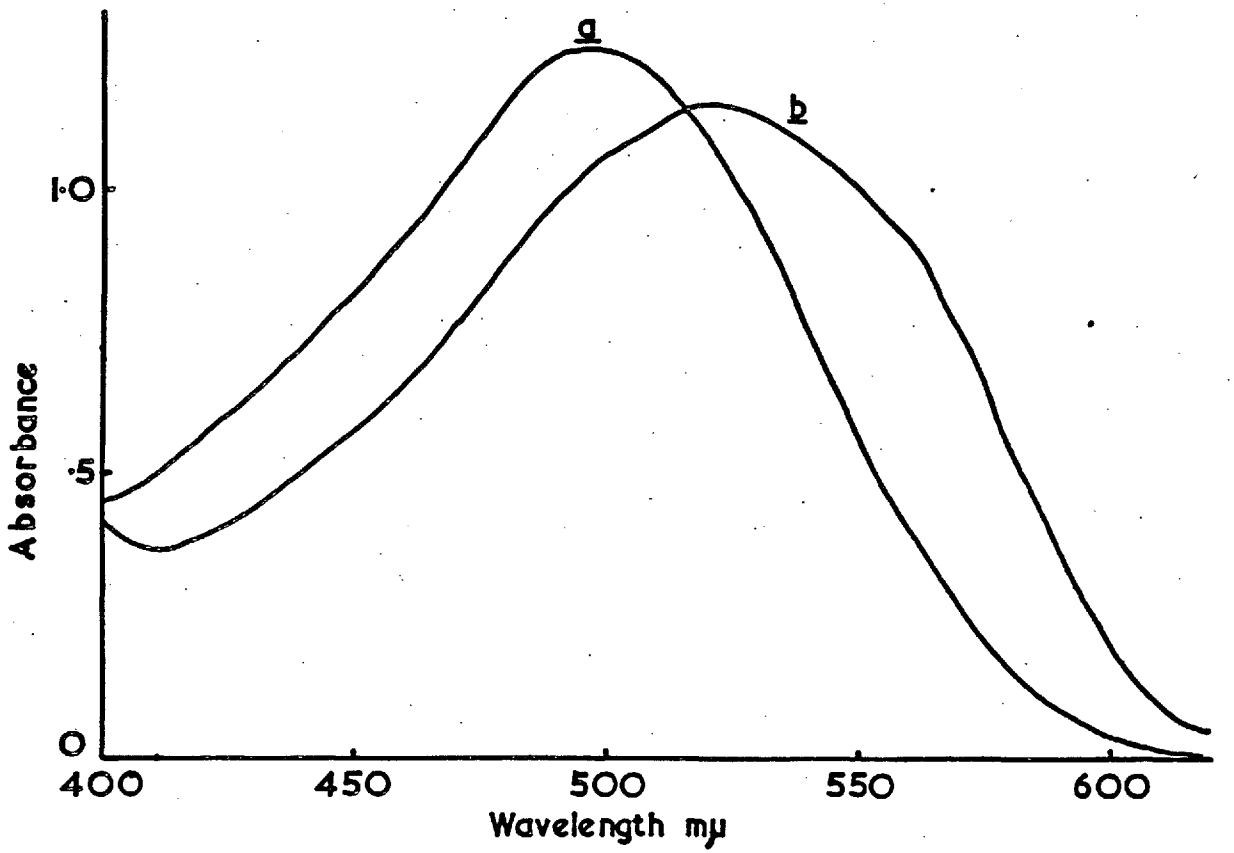


FIG 1

2) Bromophenol Blue

Bromophenol Blue is a similar dyestuff to B.P.R., the main difference being the absence of vic hydroxyl groups. B.P.B. is also similar to Congo Red in that it is an acid base indicator, the colour change being from yellow to purple at pH4. The complex with silver/1,10-phenanthroline at pH7 causes a further bathochromic shift to the blue region.

Preliminary Spectra

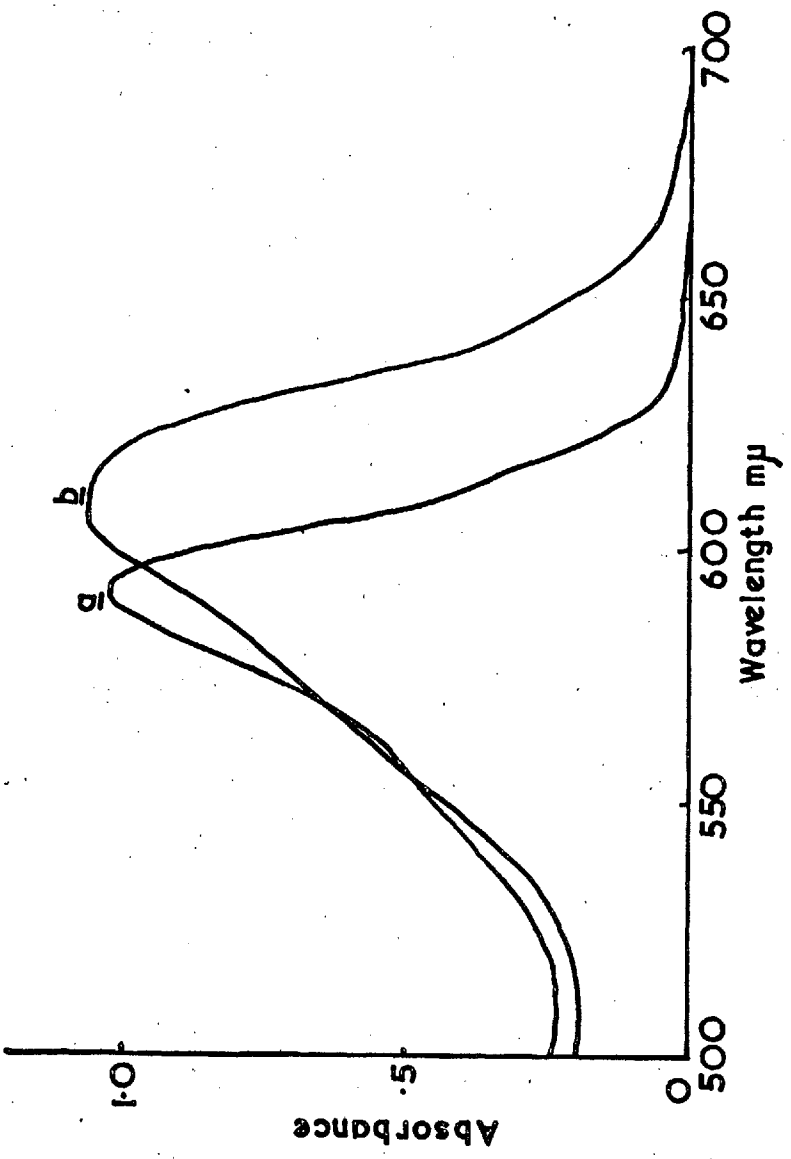
Fig II shows the absorption spectra of the reagent (curve a) and its silver phenanthroline complex (curve b). As with Congo Red the bathochromic shift on complex formation is a small one at 20mu and again the maximum difference in the absorbance between complex and reagent occurs at a higher wave-length than that of the absorbance maxima of the complex.

Similarly the reagent does not give any reaction with silver alone.

Curve (b) was prepared by diluting 4ml 10^{-3}M. B.P.B., 5ml 10^{-2}M. 1,10-phenanthroline, 5ml of 20% ammonium acetate buffer pH7,

and 10ml 10^{-3}M. silver to 100ml of distilled water, in 1-cm cuvetts. Curve (a) was prepared in a similar manner, but contained no silver. The absorbance was again measured against distilled water in 1-cm cuvettes.

FIG II



3) Rose Bengal Extra (R.B.E.)

Rose Bengal Extra or tetrachloro(P)tetraiodo(R)fluorescein is a halogenated fluorescein. All the lesser halogenated fluoresceins and fluorescein itself gave a colour reaction with silver/1,10-phenanthroline. The dyestuff R.B.E. was selected in preference to any of the others because the colour change appeared to be the most sensitive with this reagent.

Apart from its use as an adsorption indicator in precipitation titrations,[36] R.B.E. has also been used in protein staining.[37] In both cases the colour change is from pink to purple and a similar colour change occurs when the reagent complexes with the silver/1,10-phenanthroline entity.

Preliminary Spectra

The absorption spectra of R.B.E. (curve a) and its silver/1,10-phenanthroline complex (curve b) can be seen in Fig III.

The colour reaction is slightly more pronounced than with the previous two reagents, and results in a bathochromic shift of about

30mu occuring. Also, the maximum difference in absorbance between complex and reagent occurs at a wave-length which is only 2mu higher than than the wave-length of maximum absorption of the complex.

As with C.R. and B.P.B. the reagent does not undergo any colour reaction with silver in the absence of 1,10-phenanthroline at these concentration levels.

Curve (b) shows the silver/1,10-phenanthroline/R.B.E. complex and was prepared by diluting 3ml 10^{-3}M. R.B.E., 5ml 10^{-2}M. 1,10-phenanthroline, 5ml of 20% ammonium acetate pH7 buffer and 8ml 10^{-3} M. silver to 100ml with distilled water, and measuring the absorbance against distilled water in 1-cm cuvettes. Curve (a) was prepared in a similar way but contained no silver. The absorbance was again measured against distilled water in 1-cm cuvettes.

A further point of interest with Rose bengal Extra is that like other fluoresceins it is itself fluorescent, and thus has possible application in spectrofluorimetry.

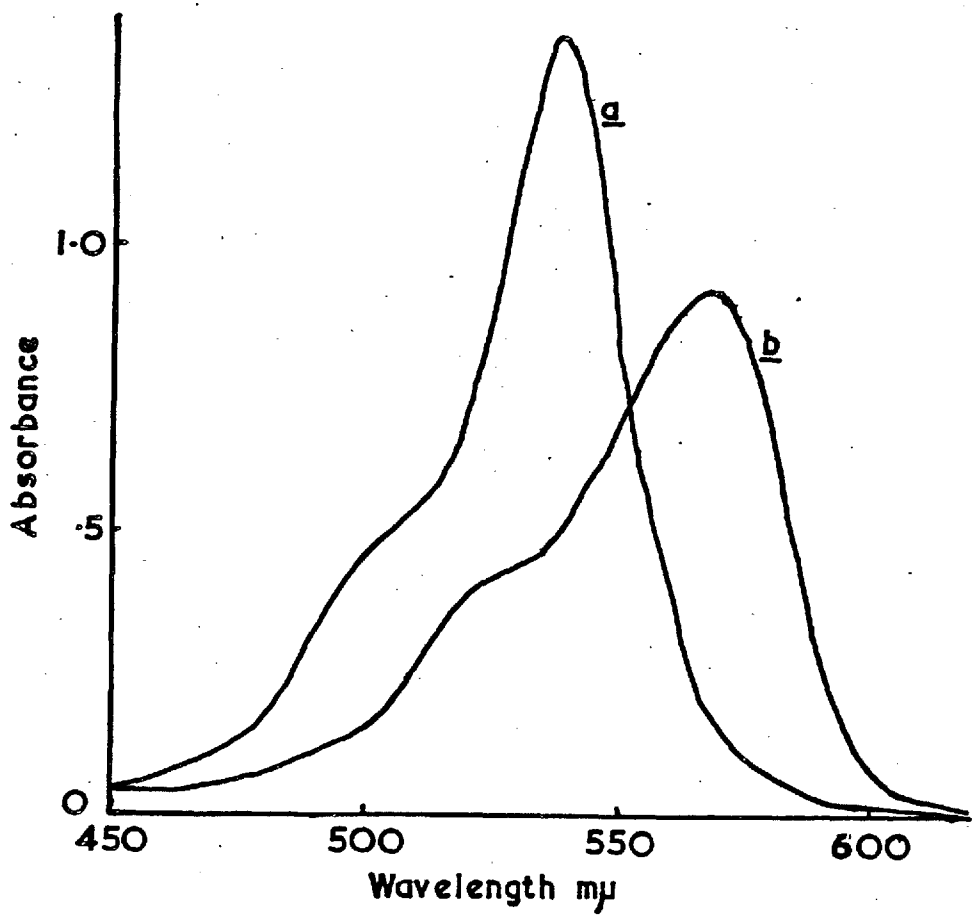


FIG III

NATURE OF COMPLEX

All the complexes examined behaved in a similar way to the silver/1,10-phenanthroline/B.P.R. complex, i.e. they precipitated from aqueous solution on standing, and the colour of the complex was either suppressed or completely destroyed on the addition of large amounts of indifferent electrolyte (such as potassium nitrate) or by heating the solutions.

The structure of the silver/1,10-phenanthroline/B.P.R. complex has been investigated by means of Job and Mole ratio plots,[5] and was found to be

$$2Ag \;:\; 4phen \;:\; 1B.P.R.$$

and it was postulated that this structure corresponded to:-

$$\left\{(phen - Ag - phen)_2^{+}\ B.P.R.^{2-}\right\}$$

Job plots and photometric titrations carried out with the three above reagents gave, for the various respective complexes, results which are in agreement with these viz. silver:reagent ratios such that overall electroneutrality is obtained in the complex. The exception being the results obtained with R.B.E. which indicate ratios of silver 1:R.B.E.1. Because R.B.E. has two removable protons

this seemed anomalous, but it was subseqently found that R.B.E. was ca 50% impure and similar studies using purified R.B.E. gave the expected results i.e. 2 silver:1 R.B.E.*

The curve in Fig IV shows a continuous variation plot for silver and Congo Red, from which the silver to C.R. ratio can be determined as 18:11 or approximately 2:1. The curve was prepared by diluting 0-10 ml 10^{-4} M silver, 5-0 ml 10^{-4}M C.R., 2 ml 10^{-2}M 1,10-phenanthroline and 5 ml 20% ammonium acetate (pH 7) buffer to 50 ml with distilled water. The absorbance was measured in 1-cm cuvettes against blanks containing the corresponding amount of reagent but no silver.

The curve in Fig V shows the results obtained from the photometric titration of silver with C.R. in the presence of excess 1,10-phenanthroline. The ratio of silver:C.R. is 2.5:1 which again approximates to 2:1. The curve was prepared by titrating photometrically 10 ml of 10^{-4}M silver, 2 ml of 20% ammonium acetate (pH 7) buffer with 10^{-3}M C.R.

The curves in Figs VI and VII show the results obtained from continuous variation plots and photometric titrations with silver and B.P.B. (the experimental procedure being the same except B.P.B. is substituted for C.R.). The Mole ratios obtained for

* See Appendix II

silver:B.P.B. are 2:1 and 10:4.5 respectively, i.e. overall ratio of 2:1 for silver:B.P.B.

As previously mentioned anomalous results were obtained with R.B.E. because of the impurity of the reagent. The various systems obeyed Beers Law over the range 2-10ug of silver, but the molar absorptivities were less than that of the B.P.R. complex.

DISCUSSION

It is possible to form the silver/1,10-phenanthroline/dyestuff ternary complexes with most of the anionic adsorption indicators and related reagents. The various complexes have similar physical and chemical properties except that B.P.R. is the only reagent which gives a colour reaction with silver alone. Also the colour change on complexation with the bis 1,10-phenanthrolinium silver complex is much more marked with B.P.R. (shift of ca 70mu) than with other reagents (shifts of 20-30mu). This overlapping of the absorption spectra of the complexes and reagents lowers the effective sensitivity of the colour reaction if used as the basis of spectrophotometric procedures in aqueous solution. To overcome this and to increase the sensitivity the possibility of using solvent

FIG IV

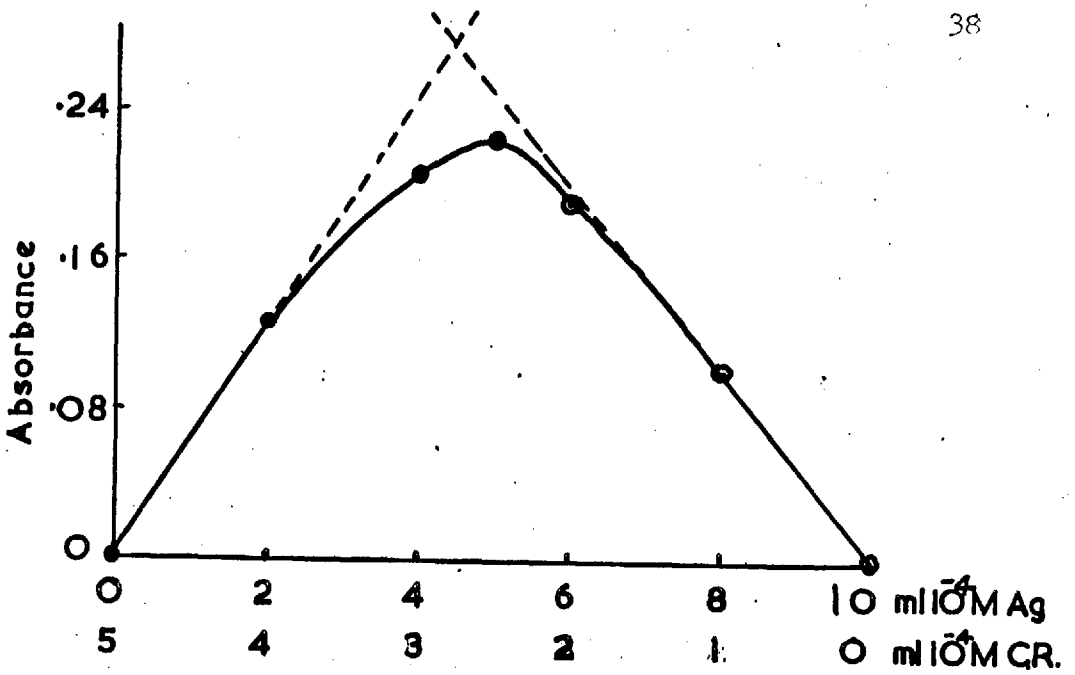

·24
·16
·08
0
Absorbance
0
2
4
6
8
10 ml 10^{-4}M Ag
5
4
3
2
1
0 ml 10^{-4}M C.R.

FIG V

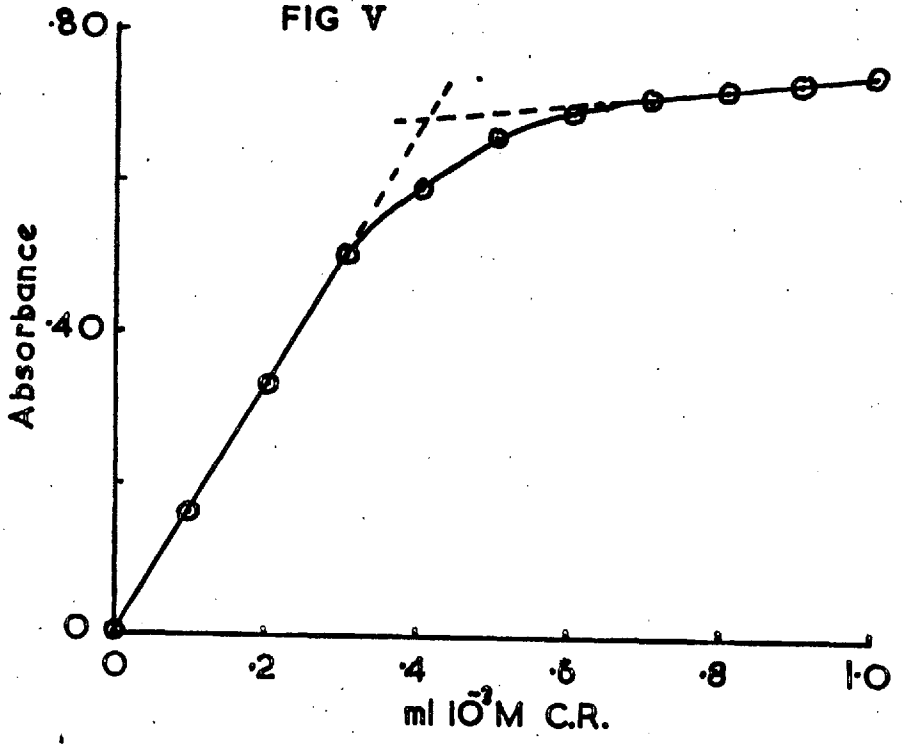

·80
·40
0
Absorbance
0
·2
·4
·6
·8
1·0
ml 10^{-2}M C.R.

FIG VI

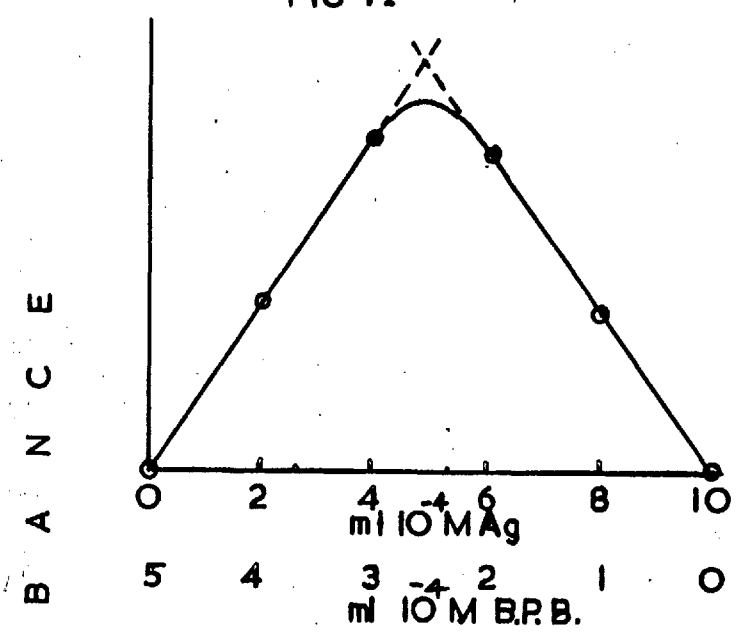
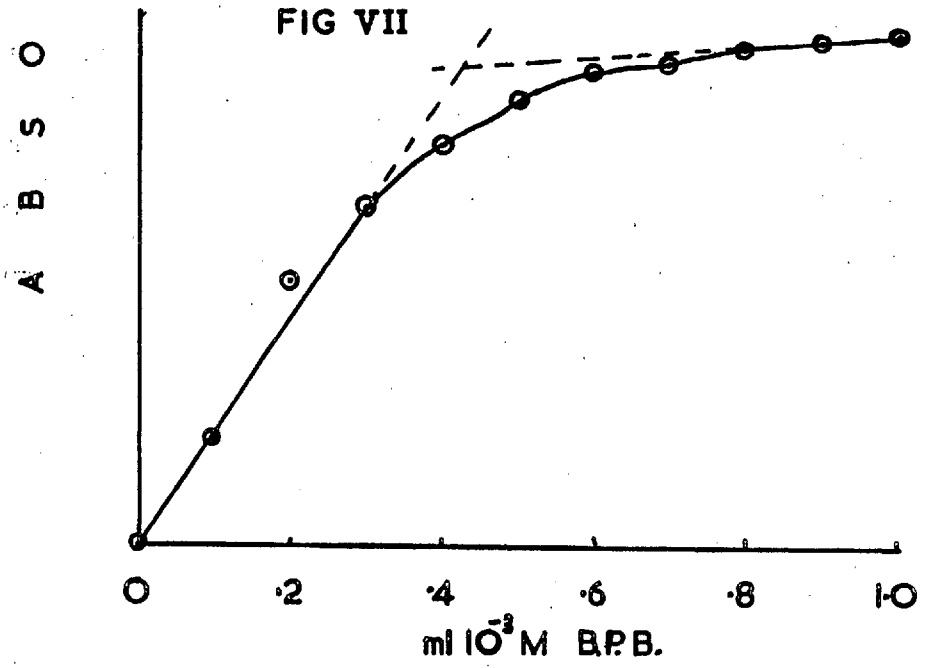


FIG VII



extraction as a means of seperating the complex from excess reagent, was examined.

Solvent Extraction

A selective extraction procedure was devised by Dagnall and West [39] by extracting the silver/1,10-phenanthroline/B.P.R. complex into nitrobenzene from an E.D.T.A. containing medium. Although nitrobenzene is not a particularly pleasant solvent to work with because of its toxicity it was found that other water immiscible solvents such as ether, benzene, carbon tetrachloride, chloroform, methyl isobutyl ketone and higher alcohols all caused the blue coloured complex to collect at the interface of the two phases. Infact only nitrobenzene was capable of extracting the complex.

A number of solvents was again investigated in an attempt to extract the silver/1,10-phenanthroline complexes of C.R., B.P.B., and R.B.E., but it was also found that no extraction occured except into nitrobenzene. The C.R. complex did not even extract into nitrobenzene and this was attributed to the presence of the hydrophillic amino groups. In contrast to this the R.B.E. complex was found to be unsuitable as the reagent itself was extracted to a high degree, especially in the presence of ammonium acetate.

The difference in solubility between R.B.E. and B.P.R. or B.P.B. is because the latter two possess a sulphonic acid group on the phenyl ring as opposed to the less hydrophillic carboxylic acid group in R.B.E.

The B.P.B. complex appeared to behave similarly to the B.P.R. complex in that it was extracted from near neutral aqueous solution and only a little reagent was also extracted.

Structure of B.P.R. Complex in Organic Media

The structure of the B.P.R. complex was only determined in aqueous media by Dagnall and West. It was thought that the structure of the extracted complex differed from that of the aqueous complex, because the wave-length of maximum absorbance of the complex was shifted from 635mu in aqueous solution to 590mu in nitrobenzene. Job continuous variation plots were carried out at the wave-length of maximum absorbance of the complex and at wave-lengths on either side of this to establish the nature of the B.P.R. complex. However, there was no indication that more than one type of complex was formed. The molecular ratio of this complex was the same as in the aqueous solution, viz. silver:B.P.R. of 2:1.

The curve in Fig VIII shows the results obtained from a Job plot prepared by extracting a series of solutions containing 0-10ml

10^{-4}M. Ag, 0-5ml $4x10^{-4}$M. 1,10-phenanthroline, 10-0ml 10^{-4}M. B.P.R., 5-0mldistilled water, 2ml 20% ammonium acetate (pH7)buffer into 25ml nitrobenzene, The absorbances of the nitrobenzene layers were measured at 590mu in 1-cm cuvettes against a similar series of solutions which contained no silver.

Having established the molecular ratios of the silver 1,10-phenanthroline B.P.R. complex in nitrobenzene the complex with B.P.B. in nitrobenzene was subjected to investigation.

Solvent Extraction of B.P.B. Complex

The absorbance spectra of the B.P.B. complex is, in contrast to that of the B.P.R. complex, unaltered on extraction into nitrobenzene. In all other respects the complexes behave in a similar manner.

FIG VIII

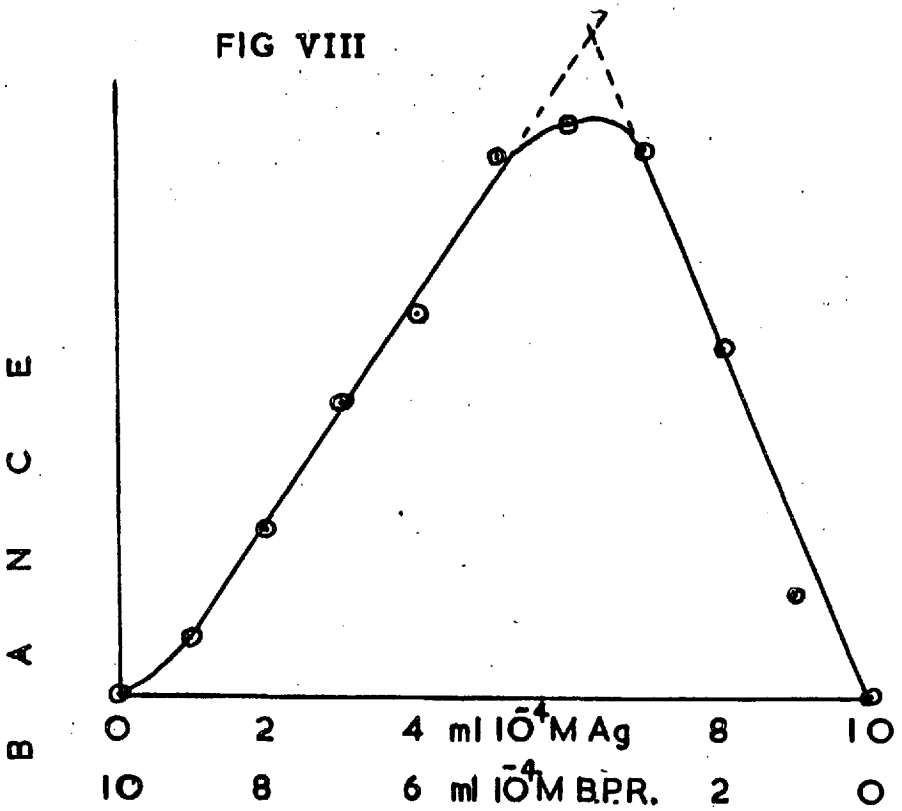
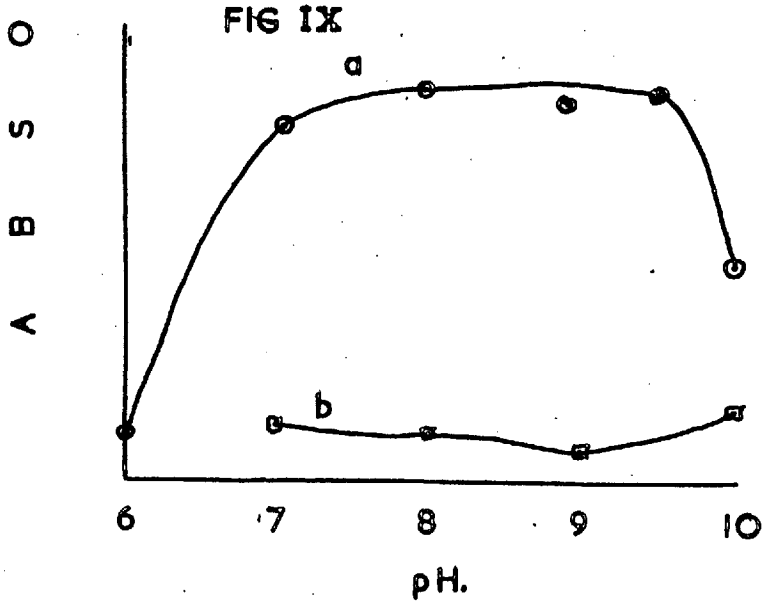


FIG IX



Effect of pH

The curves in Fig IX show the effect of pH on the extraction of the complex and reagent. The maximum extraction occurs over the range pH7-9 , and subsequent measurements were made at pH8.

The curve (a) was prepared by extracting standard amounts of silver, 1,10-phenanthroline and B.P.B. adjusted to various pH's with ammonium acetate into fixed amounts of nitrobenzene and measuring the absorbance at 610mu in 1-cm cuvettes against nitrobenzene after about half an hour.

Curve (b) was prepared in the same manner but contained no silver

Development Time

The absorbance of the complex increases with time, as can be seen from Fig X, which was prepared by measuring the absorbance of a standard amount of the B.P.B. complex into nitrobenzene against a blank containing no silver at various time intervals. The absorbance of the complex increases with time, but after an hour any further increase is slight and measurements can be conveniently made after this time.

Stability of Complex

The complex is stable for 24hrs if it is left in contact with the aqueous phase and not exposed to strong irradiation. However if the nitrobenzene layer is separated from the aqueous layer the colour fades rapidly and for this reason measurements should be made as quickly as possible. Preparation of the aliquot of sample for measurements involves running a small quantity of the nitrobenzene layer into a small beaker and swirling for a few seconds. This clears the turbidity of the nitrobenzene which does not otherwise clear unless left to stand for a period of hours.

Lambert -Beer Law Check

A calibration curve was prepared and Beer's Law was obeyed between 10 and 50ug of silver. The molar absorptivity of the complex in nitrobenzene is 40,000 at 610mu.

Fig XI shows the calibration curve which was prepared by extracting solutions containing 1-5ml 10^{-4}M. Ag, 2ml 2×10^{-3}M. 1,10-phenanthroline, 10ml 2×10^{-4}M. B.P.B. and 2ml of 20% ammonium acetate (pH 8) buffer into 25ml of nitrobenzene. The absorbances were measured in 1-cm cuvettes against a reagent blank at 610mu.

FIG X

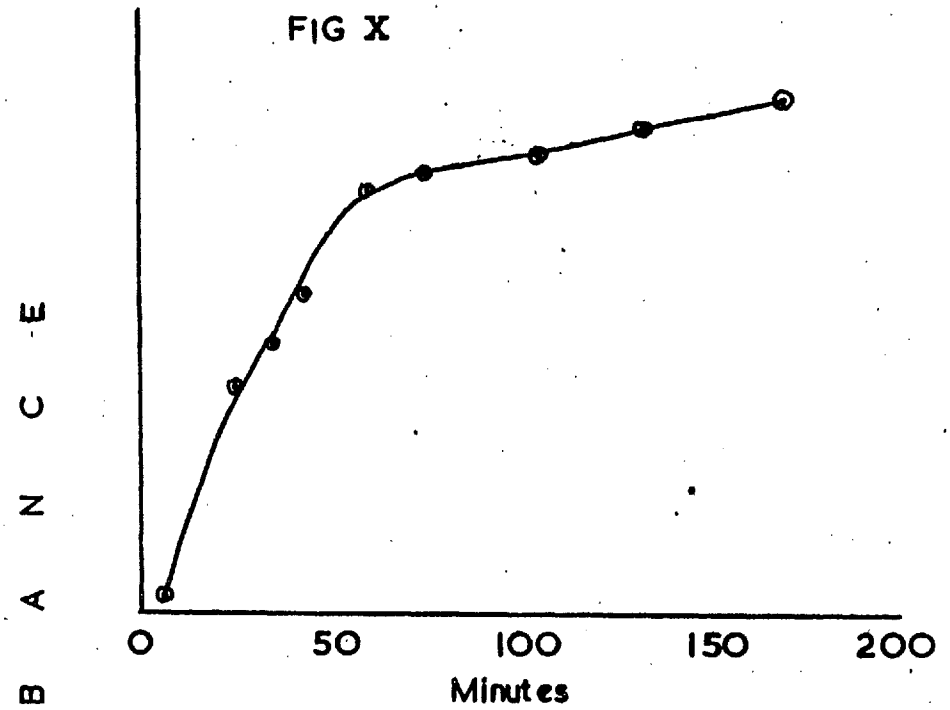
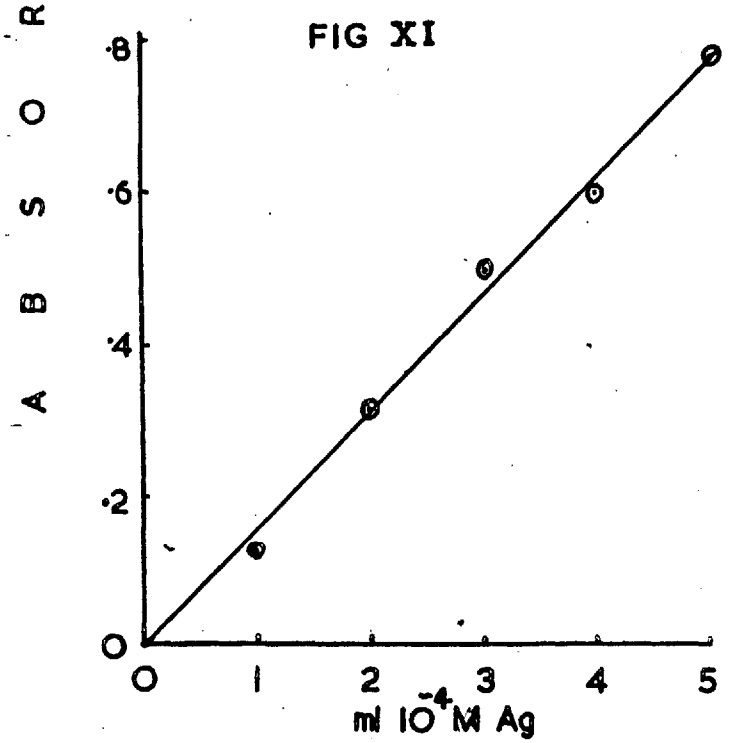

ABSORBANCE
0
50
100
150
200
Minutes

FIG XI


ABSORBANCE
·8
·6
·4
·2
0
0
1
2
3
4
5
ml 10^{-4} M Ag

Nature of Complex

A Job continuous variation plot and a Mole ratio plot gave clear indication of a 2:1 silver:B.P.B. complex.

Fig XII shows a continuous variation plot. The curve was prepared by extracting a series of solutions containing 0-5ml of 10^{-4} M. silver, 5-0ml 10^{-4}M. B.P.B., 1ml 10^{-2}M. 1,10-phenanthroline, 5ml of 20% ammonium acetate (pH8) buffer, into 25ml of nitrobenzene. Absorbance measurements were made after 2 hours at 610mu in 1-cm cuvettes against reagent blanks containing similar amounts of B.P.B.

Fig XIII shows a Mole ratio plot, prepared by extracting a series of solutions containing 4ml 10^{-4}M. silver, 1ml 10^{-2}M.1,10-phenanthroline, 0-5ml 10^{-4}M. B.P.B. and 5ml of ammonium acetate (pH8) buffer into 25ml of nitrobenzene. Absorbance measurements were made as before. The results obtained were as expected because B.P.B. has two replaceable protons.

Interferences

The procedure developed previously, utilising the B.P.R. complex with extraction from an E.D.T.A. medium to acheive selectivity; similarly the B.P.B. complex may also be extracted in this way. However it is interesting to note that those species which

FIG XII

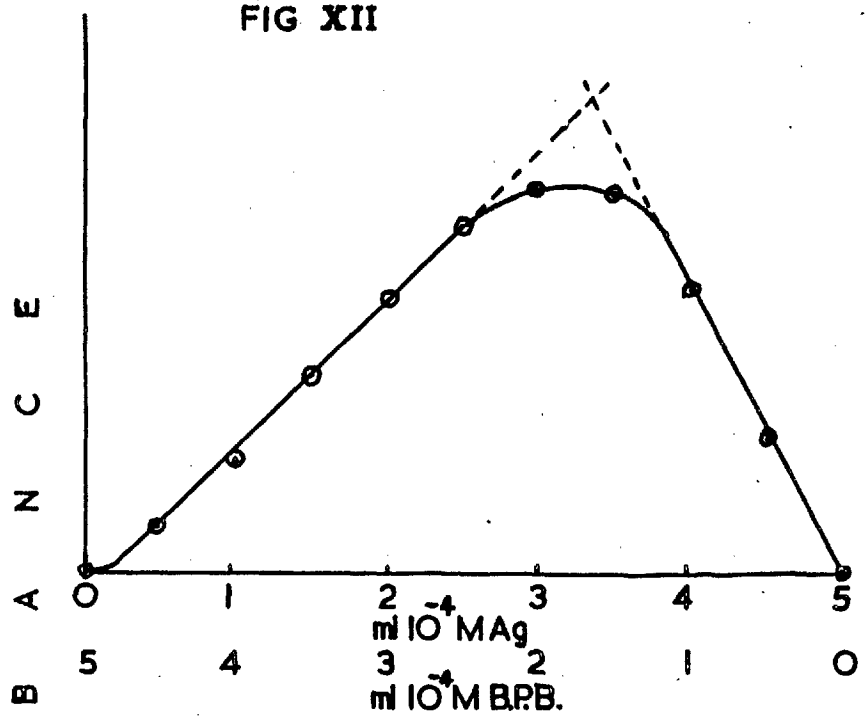
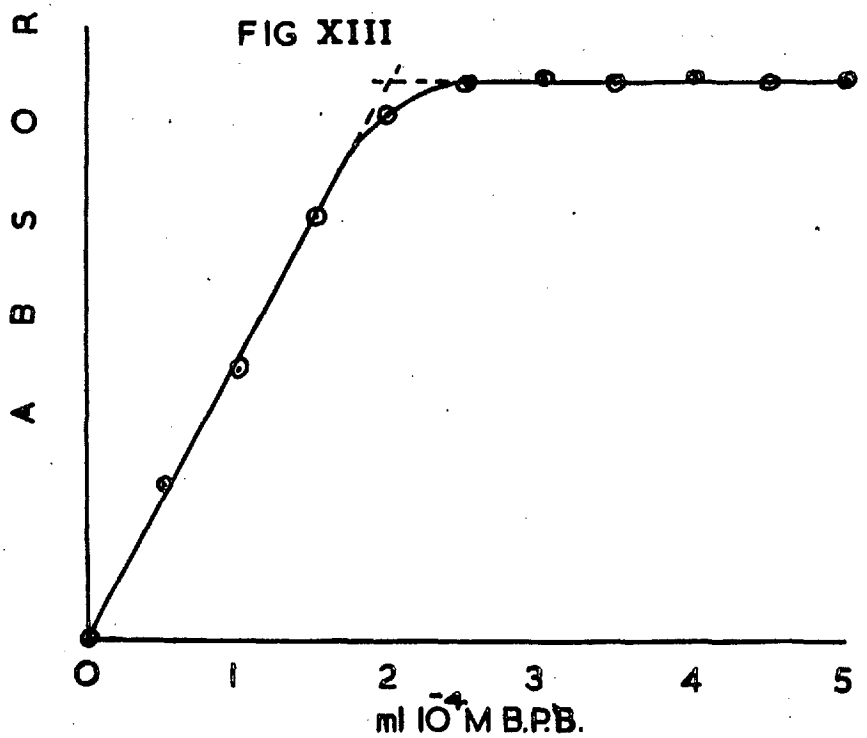


FIG XIII



chelate with B.P.R., such as molybdate, tungstate, niobium(V), and antimony thus give rise to interference effects, do not give any reaction with B.P.B. because it has no complexing centres.

It was found however that large amounts of indifferent electrolyte, such as potassium nitrate seriously depress the extraction, e.g. a 1,000 fold excess depresses the extraction by over 40%. This effect can be obviated by extracting silver as the silver/1,10-phenanthroline/nitrate complex into nitrobenzene. This, on equilibration with an aqueous solution of B.P.B., gives the silver/1,10-phenanthroline/B.P.B. complex and measurements can then be made in the normal manner.

C H A P T E R II

A SPECTROPHOTOMETRIC PROCEDURE FOR THE DETERMINATION OF TRACE AMOUNTS OF COPPER

Introduction

In the previous chapter investigations were centred on the types of dyestuff which would form ternary complexes. The next step was to investigate the possibility of forming similar complexes with cations other than silver. Preliminary investigations showed that most of the phenanthroline forming cations viz. nickel, manganese, copper(II), cobalt, cadmium, would indeed form ternary complexes similar to that with silver, the only exception being iron(II). Moreover the complexes formed with R.B.E. were extractable into such solvents as chloroform and ethyl acetate, whilst those with B.P.B. were not. Thus the previous disadvantage of R.B.E. in possessing a carboxylic rather than a sulphonic group becomes an advantage. When a less polar organic solvent than nitrobenzene is used, the R.B.E. complex is extracted and the B.P.B. complex is not. Further, R.B.E. itself is not appreciably extracted into chloroform.

At this juncture it was decided to select one particular cation

and use this as a 'typical' ion in the subsequent investigations. To this end copper(II) was chosen, mainly because a selective extraction procedure for copper as the copper(I) neocuproin complex could be adapted for use in the development of any subsequent spectrophotometric procedure for the determination of traces of copper.

Preliminary Investigation

Previously, with silver it was found that the results obtained from various Job plots and photometric titrations indicated that the R.B.E. was impure. Initial work with copper tended to confirm this, where Job plots gave a ratio of copper:R.B.E. of 1:2 instead of the expected 1:1. Ultimate analysis results confirmed that it was impure, but attempts to purify the R.B.E. to theoretical composition were unsuccesful.* In view of this, the closely related substance Erythrosin (tetraiodo (R) fluorescein) was used to study the nature of the complex.

Because Erythrosin is widely used as a food additive it was possible to obtain a sample of known purity from I.C.I. Dyestuffs Division. The sample was certified as follows:-

See Appendix II*

Pure dye content (calculated as disoduim salt)	85% min
Molecular Wt.	879.9
Loss on drying at 135°C	12% max
NaCl and Na_2SO_4	2% max
NaI	1% max
Insoluble matter in water	2% max
Matter extractable by ether	1% max
Coloured matter other than Erythrosin	3% max
Cu	10 p.p.m. max
As	1 p.p.m. max
Pb	10 p.p.m. max

The Erythrosin content was taken as 85% i.e. solutions were made up on the basis that 1035g of dye contained 1gm molecule of Erythrosin.

Although Erythrosin was used in determining the structure of the complex it was found preferrable to use R.B.E. for the actual development of procedure as the results with the latter were found to be more reproducible and slightly more sensitive. However, the optimum conditions of reaction were the same for Erythrosin and R.B.E.

Preliminary Spectra

Fig XIV shows the absorption spectra of the copper/1,10-phenanthroline/Erythrosin complex in aqueous solution and following extraction into chloroform. Curve (a) was prepared by diluting 3ml of 10^{-4}M. Erythrosin, plus 3ml of 10^{-3}M. 1,10-phenanthroline and 1ml of phosphate buffer to 25ml with distilled water. Curve (b) was prepared in a similar way but also contained 5ml of 10^{-4}M. copper. Curve (c) was prepared as (b) but after extraction into 25ml of chloroform. The spectra were measured against a distilled water blank in 1-cm cuvettes.

Chloroform was selected in preference to ethyl acetate because it is more dense than water, and may also be used to extract the copper(I) neocuproin complex.

It can be seen that, whereas a mixture of 1,10-phenanthroline and Erythrosin in the absence of copper(II) shows maximum absorption at 525mu, the ternary complex absorbs at 545mu in aqueous solution and chloroform. In the latter medium however, the molar absorptivity of the complex is several times greater, probably as a result of the complex existing as a precipitate in aqueous solution.

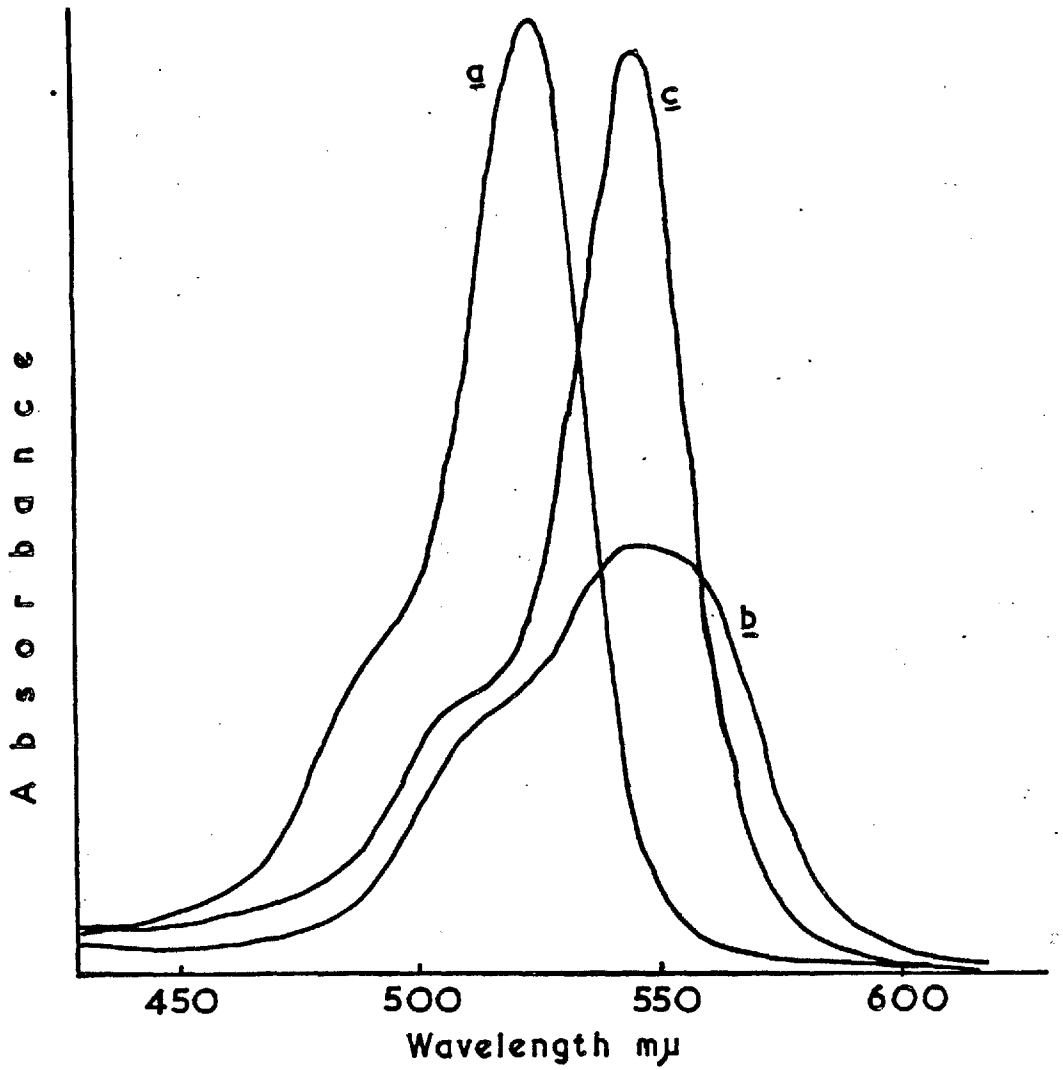


FIG XIV

Effect of pH

Fig XV shows the response to pH of (i) the reagent system (curve b), in the absence of copper(II) and (ii) the ternary complex (curve a) with copper(II) following extraction into chloroform. Curve (c) represents the resultant of curve (a) compared with curve (b). Curve (a) was prepared by adjusting to the required pH with ammonia and acetic acid a solution containing 1ml 10^{-4}M. copper, 1ml of 10^{-2}M. 1,10-phenanthroline and 20ml of 10^{-4}M. Erythrosin. The whole was then extracted into 25ml of chloroform. Curve (b) was prepared in a similar manner, but contained no copper. The absorbance was measured at 545mu in 1-cm cuvettes against a chloroform blank. From curve (c) it can be seen that maximum difference between the absorbance of the complex and the blank occurs in the region pH 7-9, the higher value was selected for further studies as a matter of convenience. A disodium hydrogen phosphate buffer was found to be most suitable as ammonium acetate tends to produce variable results in the extraction of the reagent; there was no apparent deleterious effect of the phosphate ion on the colour response of the copper.

FIG XV

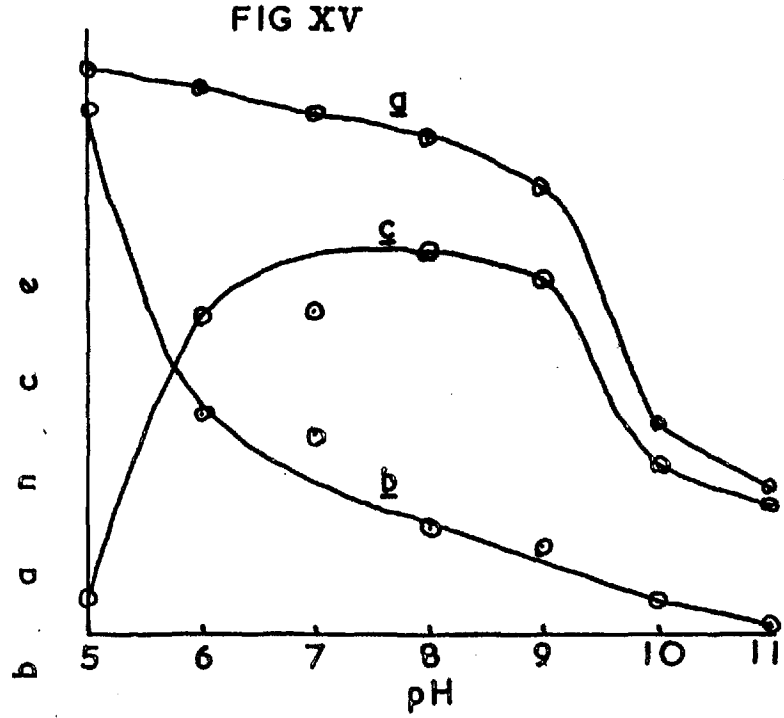
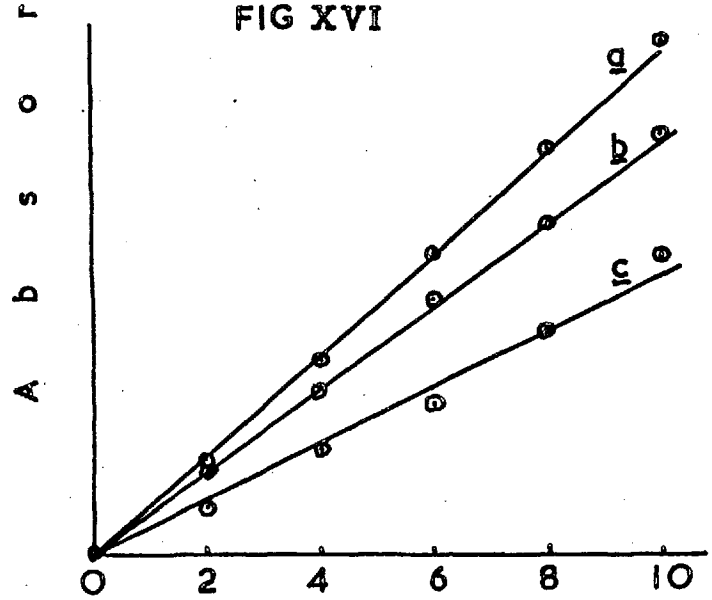


FIG XVI



(a) Cu
ml 10^{-5} M (b) Erythrosin
(c) 1,10-phen.

Nature of Complex

As with the previously described silver complexes, it was found that the present complex also precipitated from aqueous solution after about one hour, particularly when its concentration was greater than 10^{-4}F. Chloroform extracts were quite stable over a period of four days when standing exposed to normal laboratory conditions of illumination. Irradiation by bright sunlight caused a slow fading, however. The time necessary for maximum colour to develop after extraction was found to be 30 minutes, however further standing does result in a further slight increase in absorbance as with the $Ag(phen)_2$B.P.B. system. The order of addition of reagent solutions did not appear to be critical in any way.

Reagent Excess

To obtain the maximum colour response it was found necessary to maintain a minimum five-fold molar excess of 1,10-phenanthroline and a two-fold molar excess of Erythrosin over copper(II). In subsequent work leading to the development of an analytical procedure a minimum ten-fold excess of Erythrosin or R.B.E. and a hundred-

fold minimum excess of 1,10-phenanthroline were employed.

Composition of the Complex

The ternary complex between silver, 1,10-phenanthroline and B.P.R. or B.P.B. has been shown by the usual optical procedures to have the empirical formula $\left\{ \left[Ag\ (phen)_2^{+} \right]_2\ Dye^{2-} \right\}$. Because Erythrosin also carries two negative charges and copper has a co-ordination number of four or six, a similar but somewhat simpler structure was expected for the present system. Job and Mole ratio plots were carried out and results (Fig XVII and XVIII) revealed the structure to be as expected - $\left\{ Cu\ (phen)_2^{2+}\ \ Ery^{2-} \right\}$. Figure XVI shows the results of a Harvey-Manning slope ratio plot obtained by varying one constituent at a time whilst maintaining a constant large excess of the other two. The reacting ratios of copper(II) to Erythrosin and of copper(II) to 1,10-phenanthroline balance well at 1:1 and 1:2, but the value of the 1,10-phenanthroline to Erythrosin ratio is more indeterminate and is closer to 2:3. Nevertheless the situation is complicated by the extraction procedure and taken in conjunction with the more definite evidence of the Job and Mole ratio plots the complex appears to be as formulated.

The curve in Fig XVII (a) shows the variation of copper and

FIG XVII a

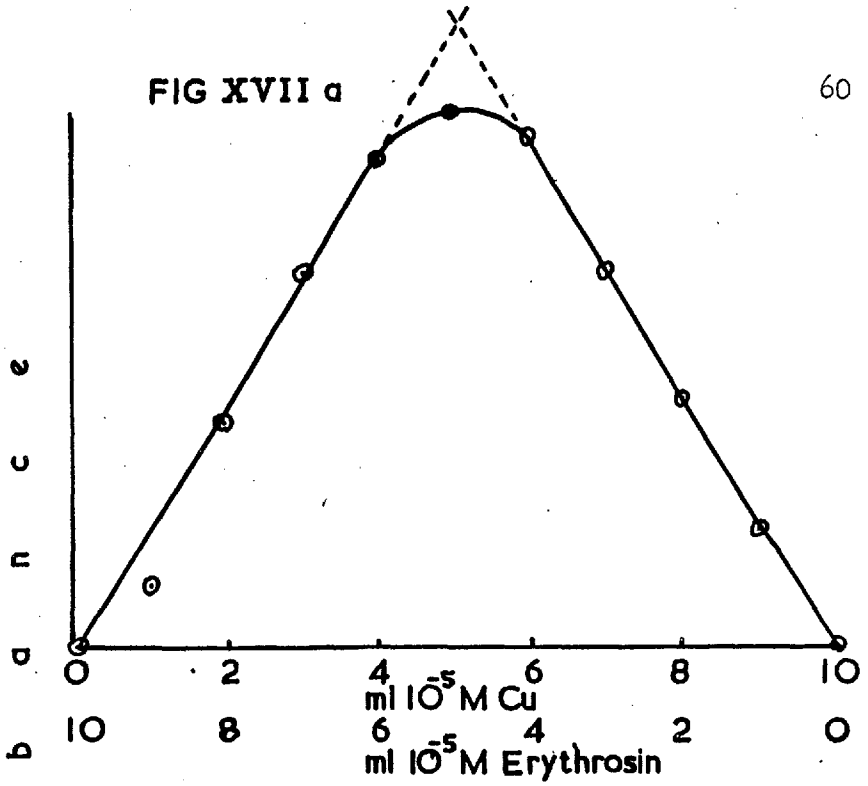


FIG XVII b

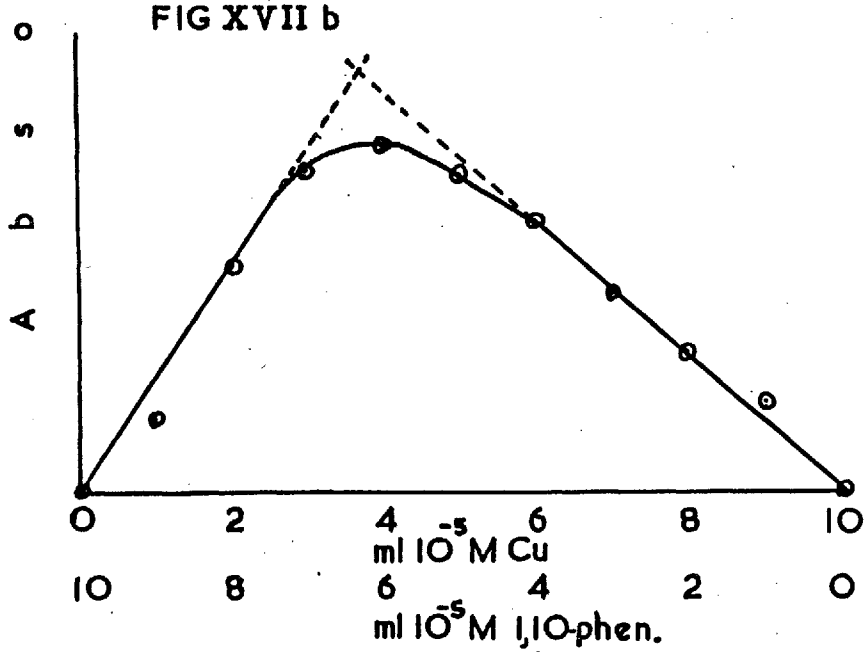


FIG XVIII a

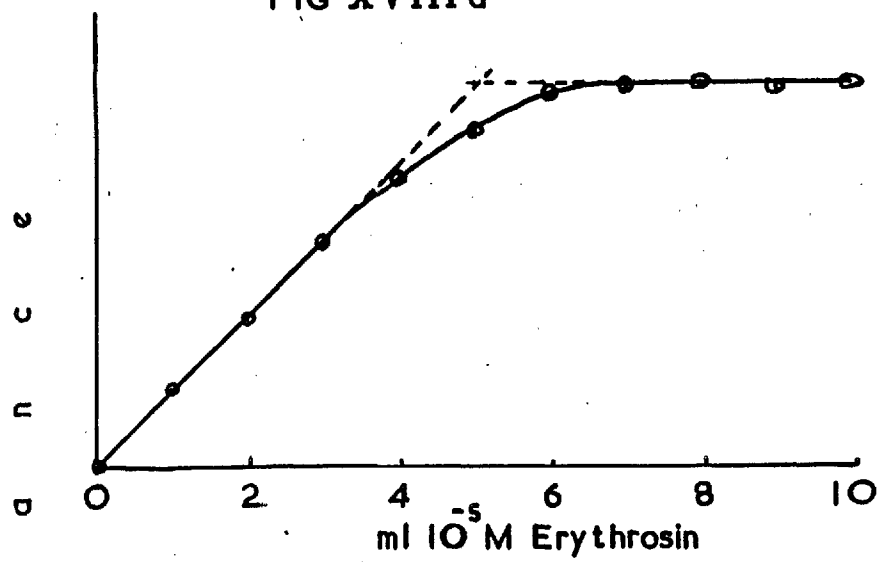
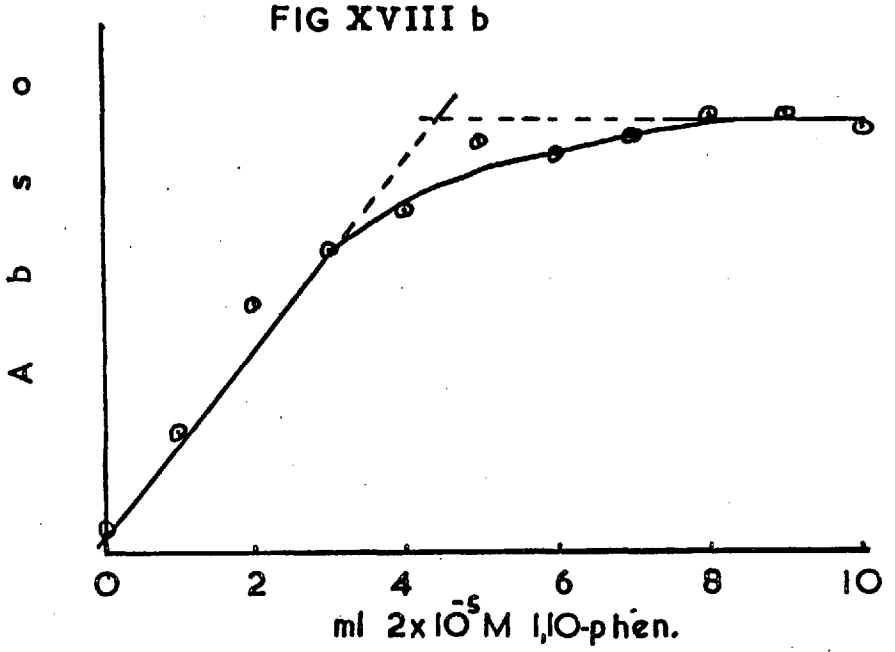


FIG XVIII b



Erythrosin and was prepared by extracting into 25 ml of chloroform a series of solutions containing 0-10 ml of 10^{-5} M copper, 10-0 ml 10^{-5} M Erythrosin, 1 ml 10^{-3} M 1,10-phenanthroline and 1 ml of phosphate pH9 buffer. The absorbances were measured at 545 mu in 1-cm cuvettes against a blank prepared in a similar way but containing no copper or phenanthroline.

The curve in Fig XVII (b) shows the variation of copper and 1,10-phenanthroline and was prepared in a similar way, but with 10-0 ml of 10^{-5} M 1,10-phenanthroline and 5 ml of 10^{-4} M Erythrosin. The absorbances were measured at 545 mu in 1-cm cuvettes against blanks prepared in a similar manner but containing no copper.

The curve in Fig XVIII (a) represents the variation of Erythrosin against constant copper and was prepared by extracting into 25 ml of chloroform a series of solutions containing 0-10 ml of 10^{-5} M Erythrosin, 5 ml of 10^{-4} M copper, 1 ml of phosphate pH9 buffer, and sufficient distilled water to give a constant volume. Absorbances were measured in 1-cm cuvettes at 545 mu against chloroform.

The curve in Fig XVIII (b) represents the variation of 1,10 phenanthroline against constant copper and was prepared in a similar manner to the previous curve but with 0-10 ml of 2×10^{-5} M 1,10 phenanthroline and 5 ml of 10^{-4} M Erythrosin. Absorbances were measured in the same manner as before.

The results obtained from these slope ratio plots are shown in Fig XVI. Curve (a) was prepared by extracting into 25ml of chloroform a series of solutions containing 0-10ml 10^{-5}M. copper, 5ml 10^{-4}M. Erythrosin, 1ml of 10^{-3}M. 1,10-phenanthroline, 1ml of phosphate pH9 buffer and sufficient distilled water to make a constant volume. The absorbances were measured at 545mu in 1-cm cuvettes against a blank prepared in a similar manner, but containing no copper.

Curve (b) was prepared in a similar manner but with 0-10ml of 10^{-5}M. Erythrosin and 5ml of 10^{-4}M. copper, and 1ml of 10^{-3}M. 1,10-phenanthroline. The absorbances were measured as before.

Curve (c) was also prepared in a similar way but with 0-10ml of 10^{-5}M. 1,10-phenanthroline 5ml of 10^{-4}M. copper and 5ml of 10^{-4}M. Erythrosin. The absorbances were measured as before.

Development of Analytical Method

An attempt was made to use the extraction of copper(I)as its Neo-cuproine (2,9-dimethyl-1,10-phenanthroline) complex to separate copper from the other ions likely to interfere. The extraction of copper(I) Neo-cuproine is reported in the literature to give a specific colour reaction for copper(I) in the prescence of some 56 metals and numerous anions except cyanide.[39-41] Morrison and Frieser suggest that it may be used as a specific extractant for copper(I).[15] However in the experiments that were carried out, it was found that when Neo-cuproine was used to seperate copper from the other ions likely to interfere in the proposed method(i.e. those cations which were mentioned previously as forming the phenanthroline/R.B.E. complexes) it was found that cadmium, cobalt, nickel and zinc still interfered.

This disagreement with Morrison and Freiser results from the fact that the colour reaction with Neo-cuproine which is specific for copper(I) and is as a result of a charge transfer phenomena has been taken to be a specific reaction for copper(I). Work by Irving and Mellor has shown that the cations cobalt, nickel, zinc and cadmium all form at least the bis Neo-cuproine complexes.[33]

However, when E.D.T.A. was used as a masking agent in conjunction

with the Neo-cuproine only copper(I) was extracted. Infact silver(I) was also found to form a Neo-cuproine complex, but this only will interfere if present in such large amounts as to consume sufficient Neo-cuproine.

In the recommended analytical procedure copper(II) is first reduced and extracted into chloroform as its copper(I)/Neo-cuproine complex from aqueous solution containing E.D.T.A. and citrate at pH4-6. This gives a quantitive and specific extraction from all other cations examined. The copper extract is then equilibrated with a pH9 phosphate buffer containing 1,10-phenanthroline and R.B.E. and the absorbance of the copper/phenanthroline/R.B.E. complex in the chloroform layer is measured after 30 minutes at 570mu. Oxidation by air proved as efficient as sodium persulphate and more satisfactory because the R.B.E. was not attacked.

The copper(I)/Neo-cuproine complex can also form an ion-association complex with Erythrosin in the chloroform layer at pH9 but the absorbance of this complex is only half that of the copper(II)/1,10-phenanthroline system. This presumably is because of the difference in charge on the central ion of the complex whereby two copper(I) Neo-cuproine complexes are required for association with one Erythrosin molecule. Fortunately the copper(II)/phenanthroline complex

is more stable at pH 9 and is formed quantitatively when the Neocuproine complex is treated with excess of 1,10-phenanthroline.

Because the order of addition of reagents is not critical, a composite solution containing E.D.T.A., sodium nitrate, sodium citrate and hydroxylamine hydrochloride pre-adjusted to pH 4-6 and a separate Neo-cuproine solution for the initial extraction of the copper(I) complex was used. Similarly a composite solution containing 1,10-phenanthroline and R.B.E. and a separate pH 9 phosphate buffer solution were used to convert the copper(I) Neoccuproine complex to the $Cu(phen)_2$ R.B.E. system.

Lambert-Beer Law Check

The calibration curve with R.B.E. was found to be linear for 1-6 ug of copper and probably beyond by the procedure described below; also its extension passes through the origin. Under these conditions in a 1-cm cuvette, at 570 mu, 1.27 ug of copper gave an absorbance of 0.050, while 6.35 ug gave 0.254 absorbance units following extraction into 25 ml of chloroform. Extraction of 1.27 ug of copper from 500 ml of solution into 25 ml of chloroform gave exactly similar results, i.e., with test solution at the 0.0024-p.p.m. level. Smaller amounts are best determined using 4-cm cuvettes.

EXPERIMENTAL

Reagents

All reagents are of analytical grade unless otherwise stated.

Copper Sulphate Solution, 10^{-5}M

Composite Solution A, 0.1 M sodium nitrate, 0.01 M E.D.T.A., 30% sodium citrate, and 1% hydroxylamine hydrochloride. Dissolve 8.5 g of sodium nitrate, 3.7 g of E.D.T.A., 300 g of sodium citrate, and 10 g of hydroxylamine hydrochloride in distilled water, adjust the pH to 4-6 with aqueous ammonia and dilute to 1l.

Neo-cuproine (2,9-dimethyl-1,10-phenathroline) solution in ethanol, 0.1% w/v

Composite Solution B, 10^{-3}M 1,10-phenanthroline and ca 10^{-4}M Rose Bengal Extra solutions. Dissolve 0.20 g of 1,10-phenanthroline and ca 0.20 g of Rose Bengal Extra distilled water and dilute to 1l.

Phosphate buffer, pH 9 Dissolve 20 g of disodium hydrogen phosphate in distilled water and dilute to 100 ml (A 2 ml aliquot of this solution diluted to 20 ml gives pH 9-9.2)

Apparatus

Spectrophotometer Unicam SP 600 spectrophotometer with 1-cm glass cuvettes

Procedure

Calibration Curve Pipette 2-10 ml of 10^{-5}M copper sulphate solution A, and 1 ml of a 0.1% Neo-cuproine solution into 100 ml separating

funnels. Add distilled water to make the volume to 25ml. Finally add 25ml of chloroform and shake for 1 min. Allow the phases to separate and run off the chloroform extracts into another series of 100ml separating funnels. Pipette into these 10ml of the composite solution B and 2ml of the phosphate buffer, add about 10ml of distilled water and shake for 1min. Allow the phases to separate and after 30 min run off the chloroform extracts into 1-cm cuvettes. Measure the absorbance at 570mu against a blank containing no copper.

Determinations Proceed as above, taking an aliquot containing 1-6ug of copper(II) in 500ml of nearly neutral solution.

C H A P T E R III

A SPECTROFLUORIMETRIC PROCEDURE FOR THE DETERMINATION OF SUBMICROGRAM AMOUNTS OF COPPER

It is well known that the halogenated fluoresceins and fluorescein itself are fluorescent. However as they do not contain complexing centres their fluorescence properties have not been subjected to much examination. It is known that when used in protein staining the fluorescence of the reagent (R.B.E.) is quenched on adsorption onto the protein. It was found that similar quenching occurs when the R.B.E. is adsorbed onto the various cation-phenanthrolines and it is possible to devise a method of fluorimetric analysis which is based on this effect.

To illustrate this a calibration curve was constructed which shows (Fig. XIX) the decrease of the fluorescence intensity of R.B.E. on complexation with silver/1,10-phenanthroline. The curve is linear for 2-8ug of silver. It was prepared by diluting 0-8ml 10^{-5}M. silver, 10ml 10^{-4}M. 1,10-phenanthroline, 5ml 4x10^{-5}M. R.B.E., and 10ml 20% ammonium acetate pH7 buffer to 100ml with distilled water. The fluorescence intensity was measured in 10x20x50 mm. cuvettes with excitation at 343mu and emission at 570mu.

The frequency used for excitation does not correspond to the

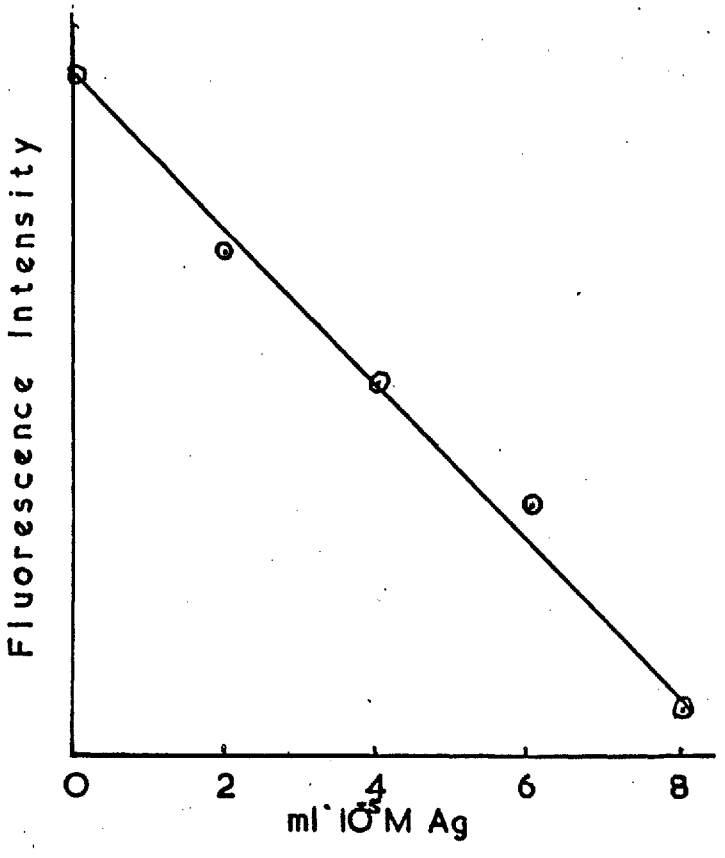
most sensitive peak which occurs at 550 mu. The reason for using the less sensitive peak will be explained later.

The use of fluorescence quenching as a basis for a spectrofluorimetric method is not as acceptable as one in which an increase of fluorescence with ion concentration is used. In view of this attempts were made to modify the system to this end. In aqueous solution the complexes exist as precipitates and as such, do not fluoresce. On extraction, when the various complexes are in solution the case is somewhat altered.

An examination of the bisphenanthroline/R.B.E. complexes of cadmium, cobalt(II), copper(II), manganese(II), nickel, lead, and zinc, showed that the complexes with the transition metal ions were, with the exception of manganese(II) nonfluorescent, whilst the others were fluorescent. In each instance the fluorescence emission occured at 570 mu and the major excitation at 560 mu. These wavelengths coincide exactly with the emission and excitation wavelengths for R.B.E. in aqueous solution.

The fluorescence reactions observed appear to be in accord with the generally accepted but little understood 'd^{10}' theory whereby complexes which use vacant 'd' orbitals from the cation for bonding are nonfluorescent. Manganese is thought to be an exception because

FIG XIX



it has the relatively stable d^5 configuration. As a consequence it was considered that whilst the ternary complex involving (d^9) copper(II)is non-fluorescent the corresponding (d^{10}) complex of copper(I) should be fluorescent.

A fluorimetric method for the determination of copper was of considerable interest because, at present, there are no direct spectrofluorimetric methods for any of the transition metals. Most methods involve fluorescence-quenching of an organic reagent or complex. The catalytic addition of copper(II) on the decomposition of lumocupferron has however recently been used as the basis of an indirect method by Russian workers.[42]

Development of Method

Preliminary investigation of the copper(I) complex showed it to be fluorescent as expected. The fluorescence spectrum was, in addition, identical to that of R.B.E. in aqueous solution and in chloroform. Fortunately, the R.B.E. itself is only very slightly extractable (ca 1%) into chloroform. However development of such a procedure would limit the general applicability to the non-transition ions and for the previously mentioned reasons there is a particular interest in developing a method for the transition metals.

It was observed in earlier work on the development of the spectrophotometric procedure for copper (Chapter II) that the complex was not extracted at pH values greater than 10. This was presumably a result of dissociation of the complex at this pH. It is this fact which gives rise to the following possible spectrofluorimetric procedure. If the chloroform extract of the transition ion/ 1,10-phenanthroline/ R.B.E. complex is equilibrated with an aqueous medium containing a few ml of .880 ammonia the complex is back-extracted into the aqueous phase as the dissociated complex. The fluorescence intensity of the free (ionised) R.B.E. may then be measured. This procedure, however, is rather lengthy and a more direct method was eventually evolved.

As was mentioned previously, the copper(I) complex of R.B.E. was fluorescent.

It was also observed that the fluorescence intensities of both the complex and R.B.E. in chloroform were increased by the addition of acetone, presumably because of the increase of the dissociation of the ternary complex with increasing dielectric constant of the solvent mixture. A 4-fold dilution with ammoniacal acetone has an even more marked effect; the fluorescence is enhanced by a factor of ten. It is probable that the dissociation of the complex is complete in the latter

case, and that the resulting fluorescence is entirely due to free (ionised) R.B.E. Provided that the reagent blank can be kept low the addition of ammoniacal acetone would, therefore, seem to be beneficial.

These experiments showed that whilst the copper(I) complex is itself fluorescent it can be completely broken down to yield free, fluorescent R.B.E. Now, whilst the copper(II) complex is of itself non-fluorescent, it was also found possible to break it down by means of addition of ammoniacal acetone, again liberating the free fluorescent R.B.E. In this instance, however, the R.B.E./ copper(II) ratio is twice that for the corresponding copper(I) complex, thus offering a method of determination which is twice as sensitive.

In order to establish this point, the solution of the copper(I) complex was compared with a similar solution of the copper(II) complex at half the dilution of the former. In this way, according to the formulae of the complexes, equal amounts of R.B.E. should be produced from each complex upon treatment with ammoniacal acetone. It will be seen from the results obtained (TableII) that this has been largely substantiated. A slight difference in distribution ratio could account for the small difference between the two results.

TABLE II

Comparative Sensitivity for Cu(I) and Cu(II) complexes in Ammoniacal Acetone Medium.

Chloroform extract from	Fluorescence Intensity (scale units)
5×10^{-7} M Cu(II)-$phen_2$R.B.E.	67.5
1×10^{-6} M $(Cu(I)\text{-}phen_2)_2$R.B.E.	62.5
R.B.E. in absence of Cu(I) or Cu(II) i.e., blank value	5.0

The method subsequently developed for copper(II) has the advantage that it can also be applied to the other transition cations as well as the non-transition cations, provided suitable separation procedures are devised.

SELECTION OF SPECTROFLUORIMETRIC REAGENT

It is well known that the quantum efficiency of fluorescence of the halogenated fluoresceins decreases markedly with increase in the degree of halogenation.[43] In addition, the order of the fluorescence efficiency for substituted fluoresceins is Cl > Br > I because of the relative strengths of their nuclear fields which promotes increased triplet state degenerations of the singlet excited-state molecule. Thus, substitution of a less fully halogenated fluorescein

dyestuff for R.B.E. would be expected to increase the overall sensitivity. It was found, however, that although ternary complexes were formed with all the derivatives of fluorescein in aqueous solution, their degree of extraction into chloroform decreased with decrease in halogenation and with decrease in the atomic number of the halogen substituent. The complex with fluorescein itself is, for all practical purposes, non-extractable for concentrations of the order of 10^{-6} M. Thus despite their favourable fluorescence these lower halogen homologues are not as suitable as R.B.E. in the proposed method. To illustrate this, a series of solutions containing a fixed amount of copper(II), 1,10-phenanthroline, and a phosphate buffer pH 9 together with similar amounts of R.B.E., dibromo (R) fluorescein, and fluorescein, respectively were extracted into a fixed amount of chloroform. A similar series containing no copper was also prepared. The absorbances were measured at the wavelengths of the absorption maxima of the particular complex, and then aliquots of the various solutions were diluted to 100 ml with ammoniacal acetone and their fluorescent intensities were measured at the appropriate wavelengths of excitation and emission. The results are listed in Table III.

TABLE III

	Absorbance	Fluorescence Intensity
Fluorescein "blank"	0.000	1
" complex	0.000	1.5
Dibromo R. Fluorescein "blank"	0.010	8
" complex	0.227	89.5
Rose Bengal Extra "blank"	0.026	6
" complex	0.389	91

The most sensitive fluorescent reagent is R.B.E. because a greater degree of extraction is obtained with this reagent. For this reason it was finally selected.

Fluorescence Spectra

The excitation and emission spectra of the copper/ 1,10-phenanthroline/ R.B.E. complex after dilution of the chloroform extract with ammoniacal acetone are shown in Figures XX and XXI. Curve (a) in Figures XX and XXI show the excitation spectrum analysed at 570 mu. Curve (b) shows the emission spectrum excited at 560 mu and 343 mu respectively.

These spectra are uncorrected for variations of the Xenon lamp and the response characteristics of the photomultiplier. Correction

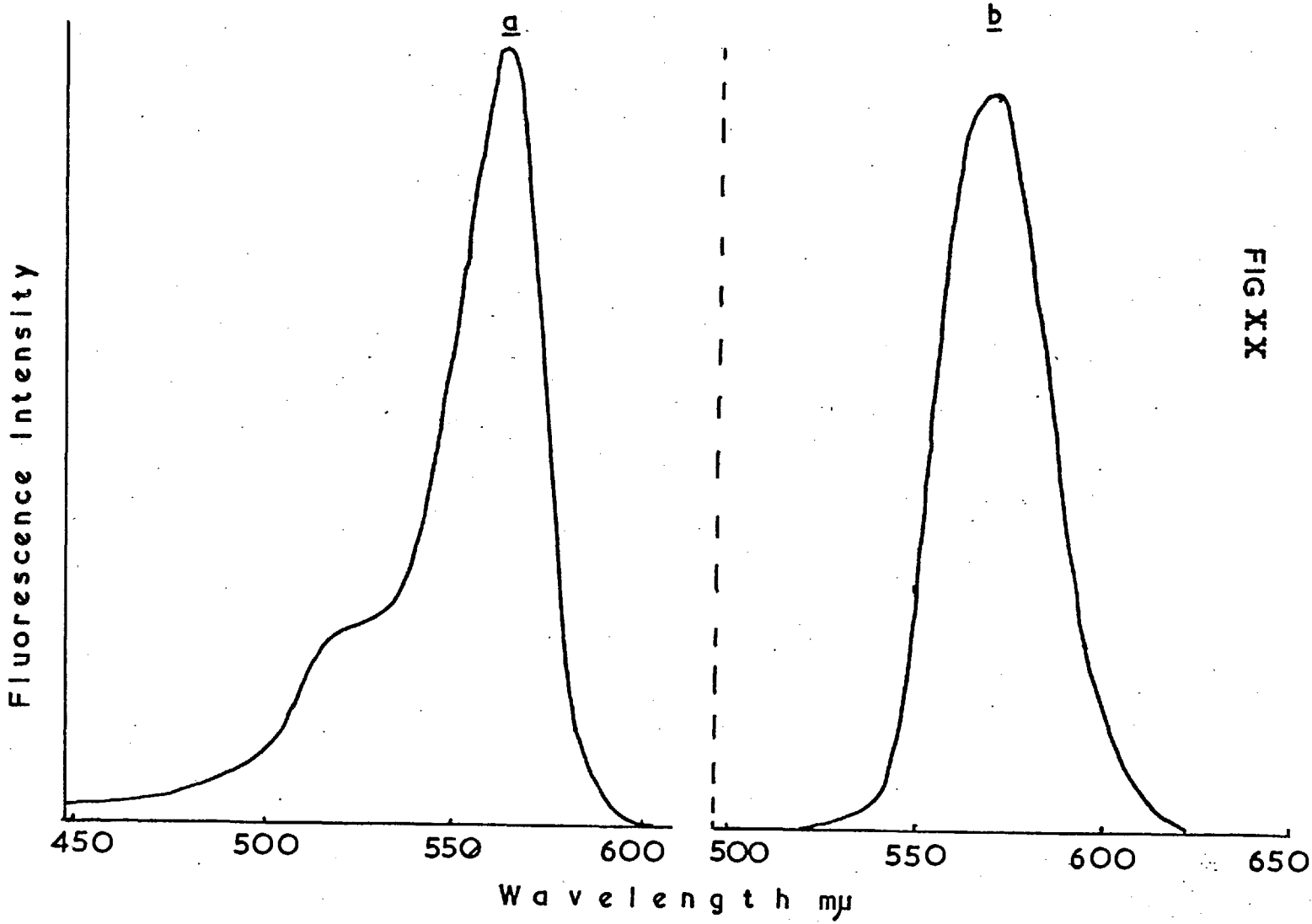

a
b
Fluorescence Intensity
450
500
550
600
500
550
600
650
Wavelength mμ

FIG XX

Fluorescence Intensity
300
350
400
450
Wavelength mμ
to peak at 560 mμ
550
600

FIG XXI

data for the instrument have, however, been reported elsewhere.[44] The excitation spectrum is continuous to about 300 mu with an absorption maximum at 343 mu. (Figure XXI) The fluorescence intensity obtained by excitation at this wavelength is about 100 times less than that obtained at 560 mu.

It was considered initially that the 10-mu bandwidth slits used for both the exciting and analysing monochromators would lead to a large overlap of excitation and emission spectra and that the inner-filter effect would be serious. It was found, however, that scattering in the 560-570 mu range was negligible, and that the inner-filter effect was imperceptible with the concentrations of copper and R.B.E. used in these experiments.

Interferences

As in Chapter II, the reaction is rendered conditionally specific for copper by a preliminary chloroform extraction of the copper(I)-neocuproine complex from a medium containing E.D.T.A. and citrate. The copper(I) complex of neocuproine is then readily converted into the $Cu(II)phen_2$ R.B.E. complex by shaking with 1,10-phenanthroline and R.B.E. in a phosphate buffer at pH 9. The method, as a result, is free from the interference of 56 cations and numerous anions. Only large amounts of cyanide interfere.

The temperature coefficient of the fluorescence reaction was not measured since little variation was observed under the normal fluctuations of laboratory temperatures. Dissolved oxygen did not quench the fluorescence. Indeed, the stability is such that the fluorescence was observed to be unchanged after several days.

EXPERIMENTAL

Reagents

All reagents are analytical grade unless otherwise stated. It was found that there was insufficient copper in the reagent to warrant further purification; the blank is largely due to the extracted R.B.E.

Copper Sulphate Solution 10^{-6} M. A stock 10^{-1} M solution was prepared, standardised by E.D.T.A. titration, and diluted as required immediately before use.

Composite Solution "A" This solution is 0.1 M in sodium nitrate and 0.01 M in E.D.T.A. and contains 30% of sodium citrate and 1% of hydroxylamine hydrochloride. Dissolve 8.5 g of sodium nitrate, 3.7225 g of E.D.T.A. disodium salt dihydrate, 300 g of trisodium citrate dihydrate and 10 g of hydroxylamine hydrochloride in distilled water. Adjust the pH to 4-6 with ammonia and dilute the mixture to one litre.

Neocuproin (2,9-dimethyl-1,10-phenanthroline) solution in ethanol 0.1% w/v.

Composite Solution "B" This solution is 10^{-3}M in 1,10-phenanthroline and ca. 10^{-5}M in Rose Bengal Extra. Prepare by a 10-fold dilution of a stock solution containing 1.982 g of 1,10-phenanthroline and ca 0.2 g of Rose Bengal Extra, C.I. 45440, (B.D.H. Ltd.) dissolved in distilled water and diluted to 1 l.

Phosphate Buffer pH 9. Dissolve 20g of disodium hydrogen phosphate (duodeca hydrate) in distilled water and dilute to 100ml. (A 2-ml aliquot of this solution diluted to 20ml gives pH 9-9.2).

Ammonia (sp. gr. 0.880) in reagent-grade acetone, 2% v/v.

Apparatus

Spectrofluorimeter A double monochromating spectrofluorimeter (Farrand Optical Co., catalogue No. 104244) fitted with a 150-watt xenon arc lamp (Hanovia Division, catalogue No. 901 C-1) and a R.C.A. I.P.28 photomultiplier, and equipped with a Honeywell-Brown recorder. Fused quartz cells (10x20x50 mm) were used throughout. Slits giving a 10-mu bandwidth were used for both the exciting and analysing monochromators.

Procedure

Calibration Curve Transfer by pipette into 100-ml separating funnels

0-10 ml of 10^{-6}M copper sulphate, 10ml of the composite solution "A" and 1 ml of the 0.1% neocuproine solution. Dilute to 25 ml with distilled water add 25 ml of chloroform. Shake the funnels for 1 min, let the phases separate, and run off the chloroform extracts into another series of 100-ml separating funnels. Transfer by pipette into these 2 ml of the phosphate buffer and 10 ml of the composite solution "B". Then add about 10 ml of distilled water and shake for 1 min. After 30 mins, transfer the chloroform extracts to a series of 100-ml graduated flasks and dilute to the mark with a 2% solution of concentrated aqueous ammonia in acetone. Measure at 570 mu the fluorescence intensity excited at 560 mu.

The calibration curve is linear for amounts of copper in the range 0.1-0.6 ug. Calibration curves ranging up to 6 ug can be prepared by the use of more concentrated reagents.

Determination of the Sensitivity of the Fluorimetric Procedure

At present there is a lack of a suitable method whereby the sensitivities of a fluorescent reaction may be measured. Some approaches have been discussed by Bartholomew[45] but many of these are dependant on the type of apparatus used.

The method used by the author is one developed by Smith.[46] This involves measuring the fluorescence intensity of a solution of the substance to be investigated at its absorption and emission maxima, and comparing this with the fluorescence intensity of a solution of quinine in M/10 sulphuric acid measured at its own excitation and emission maxima (350/450 mu). Allowance must be made for the spectral variations of the excitation and detector systems.

Using this technique it is possible to make a comparison of the true sensitivities of different fluorimetric procedures.

For the sensitivity measurement a calibration curve was prepared for the copper/phenanthroline/R.B.E. using the afore mentioned procedure. Two quinine solutions of different concentration and a distilled water blank for quinine were used as the reference. The equation used to evaluate the sensitivity was:-

$$\text{Sensitivity} = \frac{\text{C quinine}}{\text{C' metal}} \times \frac{\text{S metal}}{\text{H quinine}} \times \frac{(\text{IxP}) \text{ quinine}}{(\text{IxP}) \text{ metal}}$$

where

C = Molar concentration quinine

C' = Molar concentration metal (copper)

H = Maximum fluorescence intensity quinine

S = Fluorescence intensity of metal

I = Lamp correction factor for excitation wavelength

P = Photomultiplier correction factor for fluorescent wavelength

Procedure

The calibration curve was prepared in the recommended manner. The spectrofluorimeter was fitted throughout with 5 mu half bandwidth slits and was adjusted to give 95% scale deflection for the most concentrated solution. The intensity of the fluorescence of the solutions prepared were measured at 560/570 mu. A stock solution of quinine sulphate was then diluted with M/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 R.B.E. measurements. The fluorescence of the quinine was measured at the excitation and emission maximum

viz. 350 and 450 mu. A distilled water blank value was subtracted from the quinine fluorescence intensity. The correction factors for lamp output and detector response for the instrument were supplied by R.Smith. The results are shown below.

$C = 1.62 \times 10^{-5} M$

$C' = 8 \times 10^{-8} M$

$H = 49$

$S = 76$

$I\ 350 = .74$

$I\ 560 = 4.91$

$P\ 450 = 2.51$

$P\ 570 = .641$

$$\therefore \quad \text{Sensitivity} = \frac{76}{49} \times \frac{1.62 \times 10^{-5}}{8 \times 10^{-5}} \times \frac{0.74 \times 2.51}{4.91 \times 0.641}$$

$$\text{Sensitivity} = 185$$

The over all sensitivity of the procedure is compared with the sensitivities of other procedures in Table IV.[47]

It can be seen that the sensitivity is very high and is only

approached by that of Rhodamine B. It is thought that the very high sensitivity is a result of the close proximity of the excitation and emission wavelengths, as with Rhodamine B.

TABLE IV

Comparative Sensitivity of the method

Cation	Reagent	Maxima Absorption/ Fluorescence mμ	Sensitivity Index
-	Quinine	350/450	1
Al^{3+}	8-Hydroxyquinoline in chloroform	395/515	0.25
Be^{2+}	Morin	440/530	0.35
Ga^{3+}	Rhodamine B	560/580	38
Cu^{2+}	R.B.E.	560/570	185

Reproducibility

The precision of the method was obtained at the level of concentration used in the calibration curve from measurements made on a series of eight solutions. The results are shown in Table (V) from which it will be seen that for a final concentration of copper of 10^{-7}M there is a standard deviation of 6.3%.

TABLE V

No.	Scale Reading	Deviance D	D^2
1	40	-2.8	7.84
2	45	2.2	4.84
3	45.5	2.7	7.29
4	46	3.2	10.24
5	40	-2.8	7.84
6	40.5	-2.3	5.29
7	43.5	0.7	0.49
8	41.5	-1.3	1.69

Total = 342 = 45.52

Average = 42.8

$$\text{Standard Deviation} = \sqrt{\frac{D^2}{n-1}}$$

$$\text{Standard Deviation} = \sqrt{\frac{45.52}{7}}$$

$$\text{Standard Deviation} = 2.55$$

$$\text{Per cent Standard Deviation} = \frac{2.74}{42.8} \times 100$$

$$6.3\%$$

Results and Discussion

The spectrofluorimetric procedure developed for copper can, in conjunction with appropriate separation procedures, be used for all cations which form ternary complexes with R.B.E. and 1,10-phenanthroline.

If the complex solutions in ammoniacal acetone were left to stand for a period of days, a yellow colour was observed to develop. Furthermore, the intensity of the colour was proportional to the concentration of the complex and increased with time; if the

solutions were left for a period of two weeks an apparent molar absorptivity of the order of 10^6 was obtained. This phenomenon is dealt with more fully in Appendix III.

C H A P T E R IV

SOME ASPECTS OF TERNARY PHENANTHROLINE COMPLEXES

In the previous two chapters the development of the copper(II)/1,10-phenanthroline/R.B.E. system for use in both spectrophotometric and spectrofluorimetric procedures has been described. It was also mentioned that a number of other cations also form similar complexes.

Table VI shows the results of a preliminary study of the sensitivities of some of these complexes in various solvents and also those for the corresponding dithizone complexes.

TABLE VI

Tentative sensitivities of ternary ion-association systems

Ion determined	Molar absorptivity	
	Ternary Complex	Dithizone
Cd	92,000(E.A.)	85,000
Co	92,000(E.A.)	59,000
Cu(II)	78,000(E.A.	45,000
Mn	65,000(E.A.)	32,000
Ni	50,000($CHCl_3$)	34,000
Pb	70,000(N.B.)	72,000
Zn	95,000(E.A.)	94,000

E.A. Ethyl Acetate N.B. Nitrobenzene

The Table shows that the ternary complexes are, on the whole, capable of yeilding greater sensitivity than dithizone which is one of the most sensitive of all spectrophotometric reagents. Furthermore, the stability of the colour system is very much superior to that of the dithizone systems.

The use of such complexes, coupled with selective extraction and/or masking procedures allows the development of very sensitive spectrophotometric and spectrofluorimetric procedures for the various cations mentioned.

However there are two anomalous points; the first is that the well known ferrous phenanthroline complex does not form a ternary complex with R.B.E. and the second concerns the various molar absorptivities of the complexes. If, as has been postulated, the complexes are of the ion-association type then it would seem reasonable to expect a greater degree of similarity in the molar absorptivities of the various complexes than was obtained.

Formation of Ternary Complexes

It has been shown that with both silver(I) and copper(II) it is the bisphenanthrolinium entity which associates with the R.B.E. It is well known for copper (and the other transition

metals) that the formation of the phenanthroline complexes occurs step wise viz.

$$Cu^{2+} + \text{phen} \overset{K1}{\rightleftharpoons} \left[Cu\ (\text{phen})\right]^{2+}$$

$$\text{phen} + \left[Cu\ (\text{phen})\right]^{2+} \overset{K2}{\rightleftharpoons} \left[Cu\ (\text{phen})_2\right]^{2+}$$

$$\text{phen} + \left[Cu\ (\text{phen})_2\right]^{2+} \overset{K3}{\rightleftharpoons} \left[Cu\ (\text{phen})_3\right]^{2+}$$

and that the values of the formation constants fall with each succeeding step viz. $K1 > K2 > K3$.[33] The situation with the ferrous phenanthroline system is very different because, due to spin orbital stabilization, the formation constant for the third step K3 is greater than that for the second step K2. This results in a trisphenanthroline complex of very high stablity. It was postulated that the ternary cation/phenanthroline/R.B.E. complexes could only be formed by the bis (or mono) phenanthroline complexes, because an ion-association system between the trisphenanthrolines and a bulky anion such as R.B.E. is sterically impossible. If this were so, in a solution containing cation, phenanthroline and R.B.E., there would be two competing reactions, the first:-

$$\left[M(phen)_2\right]^{2+} + phen \underset{}{\overset{k_3}{\rightleftharpoons}} \left[M(phen)_3\right]^{2+}$$

and the second:-

$$\left[M(phen)_2\right]^{2+} + R.B.E.^{2-} \underset{}{\overset{k_r}{\rightleftharpoons}} \left\{\left[M(phen)_2\right]^{2+} R.B.E.^{2-}\right\}$$

and the ternary R.B.E. complex would only be formed if $k_r > k_3$. The values for log k_3 for the cations previously mentioned as forming chloroform extractable complexes are of the order of seven or less, whilst the value of log k_3 for iron(II) is exceptionally high at ten. It was postulated then that as a result of this high k_3 value, iron would not form a ternary complex system with R.B.E.

Further evidence in support of this theory was obtained from the ternary system between copper(II), 1,10-phenanthroline and another anionic dyestuff Chrome Azurol S. The use of Chrome Azurol S. (C.A.S.) has been suggested as a spectrophotometric reagent for both copper(II) and iron(II).[50] Investigations showed that addition of 1,10-phenanthroline to the copper(II)/C.A.S. complex resulted in a colour change due to the formation of the copper(II)/phenanthroline/C.A.S. complex. With the iron(II)/C.A.S. complex addition of 1,10-phenanthroline resulted in

destruction of the complex and subsequent formation of the red tris.phenanthroline/iron (II) complex as was expected. However the most interesting point is that formation of the copper/phenanthroline/C.A.S. was very dependant on the copper(II):1,10-phenanthroline ratio. A series of photometric titrations were carried out and the results are shown in Fig XXII and XXIII. Fig XXIII shows the effect of addition of copper to a solution of 1,10-phenanthroline and C.A.S. As can be seen there is no evidence of formation of the ternary complex until the ratio of 1,10-phenanthroline to copper(II) is less than 3:1. Between the ratios 3:1 and 2:1 there is a rapid increase corresponding to increased complex formation. There is a further slight increase as the ratio falls from 2:1 to 1:1.

The effect of addition of 1,10-phenanthroline to a solution of the copper(II)/C.A.S. complex is shown in Fig XXIII. Complex formation appears to reach a maximum as the 1,10-phenanthroline concentration increases until the ratio of copper(II): 1,10-phenanthroline is 1:1; between this ratio and 1:2 the degree of complex formation remains the same, but once the ratio 1:2 is exceeded, the complex is destroyed and the colour of the solution reverts to that of the free C.A.S.

These results show that the ternary complex copper(II)/

1,10-phenanthroline/C.A.S. is only formed by the mono- and bis-phenanthroline /copper(II) complexes. It would seem than that for the system K3 > KR. It is interesting to note that for the system

$$Cu^{2+} + \text{C.A.S.} \underset{\rightleftharpoons}{Ks} \text{Cu C.A.S.}$$

log $K_s \simeq 4$ [50] This is lower than log K_3 (7) and because it is unlikely that log KR will be greater than log k_s, the previous postulation for the failure of the iron(II) to form the ternary complexes seems to be correct.

The curve in Fig XXII was prepared by titrating photometrically (using the E.E.L. titrator) a solution containing 2 ml 10^{-3}M copper, and 10 ml 2×10^{-4}M C.A.S., with 10^{-3}M 1,10-phenanthroline at a pH of 7.

The curve in Fig XXIII was prepared in a similar manner by titrating a solution containing 10 ml 2×10^{-4}M C.A.S., and 4 ml of 10^{-3}1,10-phenanthroline with 10^{-3}M copper(II) at pH 7.

Variations in Molar Absorptivity

The second, apparently, anomalous point was the variation in the absorbances of the R.B.E./phenanthroline complexes of the

FIG XXII

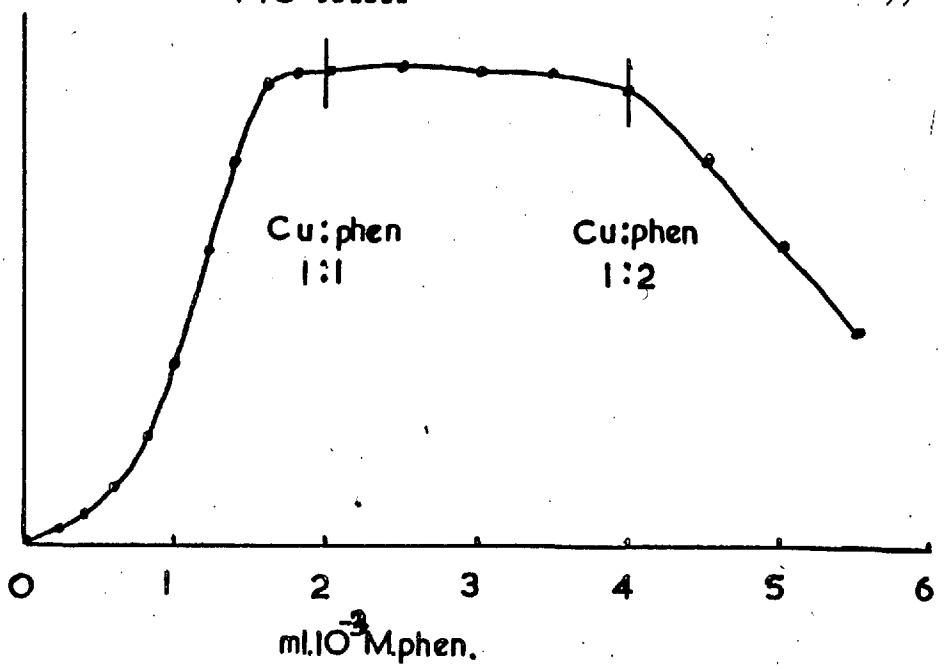
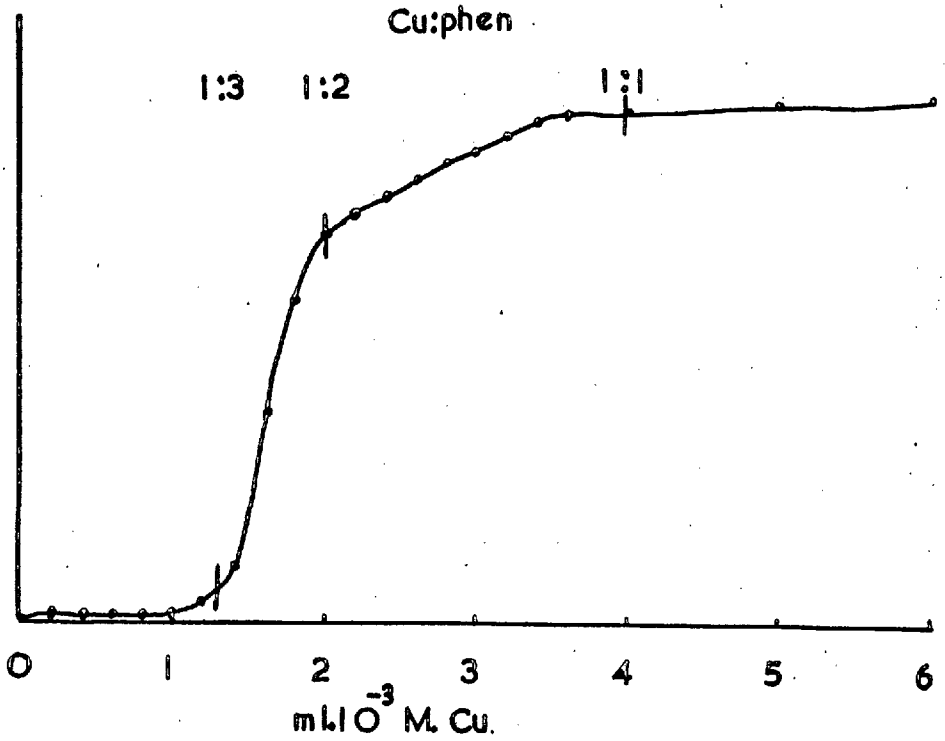


FIG XXIII



various divalent cations both in aqueous and organic media. Since the system is thought to be an ion-association system between the metal/phenanthroline and R.B.E. differences in the molar absorptivities would not be expected to occur. The charge/size ratio for the bisphenanthroline complexes is the same because the size of the phenanthroline molecules is large enough to nullify any small differences in the sizes of the cations and the charge is 2 for all cations. However in the light of the previously mentioned work with the copper/1,10-phenanthroline /C.A.S. system it was thought that molar absorptivities of the complex may depend on the ratios of K_3:K_s. The results in Table VII compare the molar absorptivities with values of log K_3 (determined by Irving and Mellor).[33]

The molar absorptivities were determined by measuring the absorbances of a series of solutions containing 2×10^{-4} M M^{2+}, 10 ml 10^{-2}M 1,10-phenanthroline, 25 ml 2×10^{-4} R.B.E., and 5 ml 20% ammonium acetate (pH8) buffer and diluted to 100 ml with distilled water. The absorbances were measured at 570 mu against a blank containing no cation.

TABLE VII

Cation	Molar absorptivity 570 mu	Log K_3
Mn	87,000	3
Fe(II)	-	10
Co	67,000	6.2
Ni	59,000	7.6
Cu(II)	55,000	5.0
Cd	65,000	4.2

There is a reasonable correlation between the molar absorptivity and the value for K_3, the major exception being copper. However it must be remembered that the measurements are not made on true solutions but on finely dispersed precipitates and not necessarily under the optimum conditions. The situation is very different in an organic phase, because the relative stabilities of the bis-phenanthroline metal complexes are now involved.

C H A P T E R V

EXAMINATION OF NITROGENOUS LIGANDS

The one variable which so far has not been altered is the donor ligand, 1,10-phenanthroline being used in all the complexes discussed.

Subsequent results showed that the best results are indeed obtained with this particular ligand.

In the search for a substitute for 1,10-phenanthroline, it soon became apparent that a ligand which on complexation with a cation, produced a positively charged entity, is necessary to enable the subsequent association with the anionic dyestuffs used. The cations forming the ternary complexes of the type investigated have been monovalent or divalent, and a neutral donor ligand such as 1,10-phenanthroline is necessary. Other similar nitrogen donor ligands have been examined to determine their potentialities.

Pyridine

Preliminary investigations using pyridine produced a silver/pyridine/R.B.E. complex which in contrast to the corresponding phenanthroline complex was chloroform extractable. This is

probably due to the increased basicity of the ligand. However, the use of pyridine is limited by two factors; firstly the concentration of R.B.E. has to be kept low,otherwise it is extracted in large amounts,and secondly the pH range over which the pyridine complex may be extracted is very small, about 0.2 of a pH unit(Fig XXIV).

Curve (a) shows the effect of pH on the extraction of the complex prepared by extracting standard amounts of silver, pyridine, and R.B,E. buffered at various pH's with ammonium acetate into fixed amounts of chloroform. The absorbance was measured in 1-cm cuvettes at 560 mu against a distilled water blank.

CurveN(c) was prepared in the same way but contained no silver.

Beers law was obeyed over the range 20-100 ug of silver but the molar absorptivity was low (ca 16,00). The curve in Fig XXV shows a calibration curve prepared by extracting into 25 ml of chloroform a series of solutions containing 2-10 ml 10^{-4}M silver, 10 ml 10^{-4}M R.B.E., 1 ml pyridine and adjusted to pH 7.3-7.4 with ammonium acetate. The absorbance was measured in 1-cm cuvettes at 560 mu against a blank carried through the procedure but containing no silver.

FIG XXIV

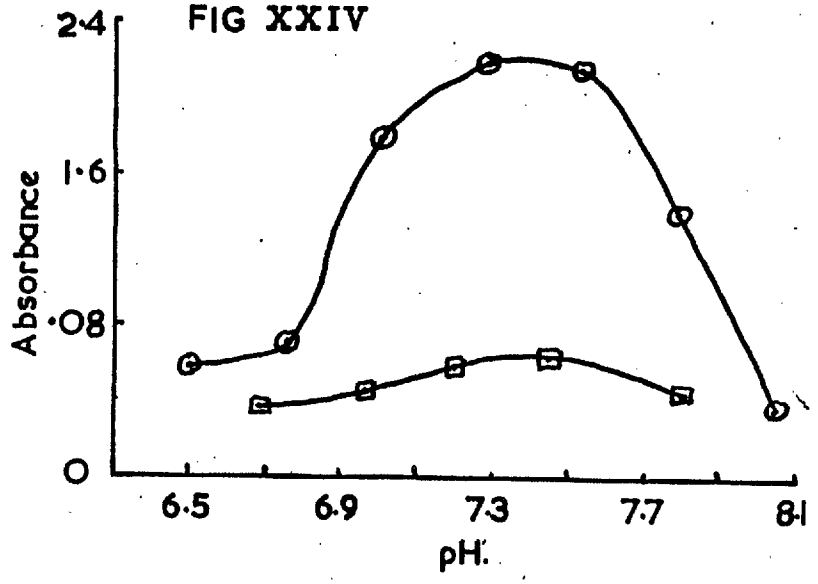
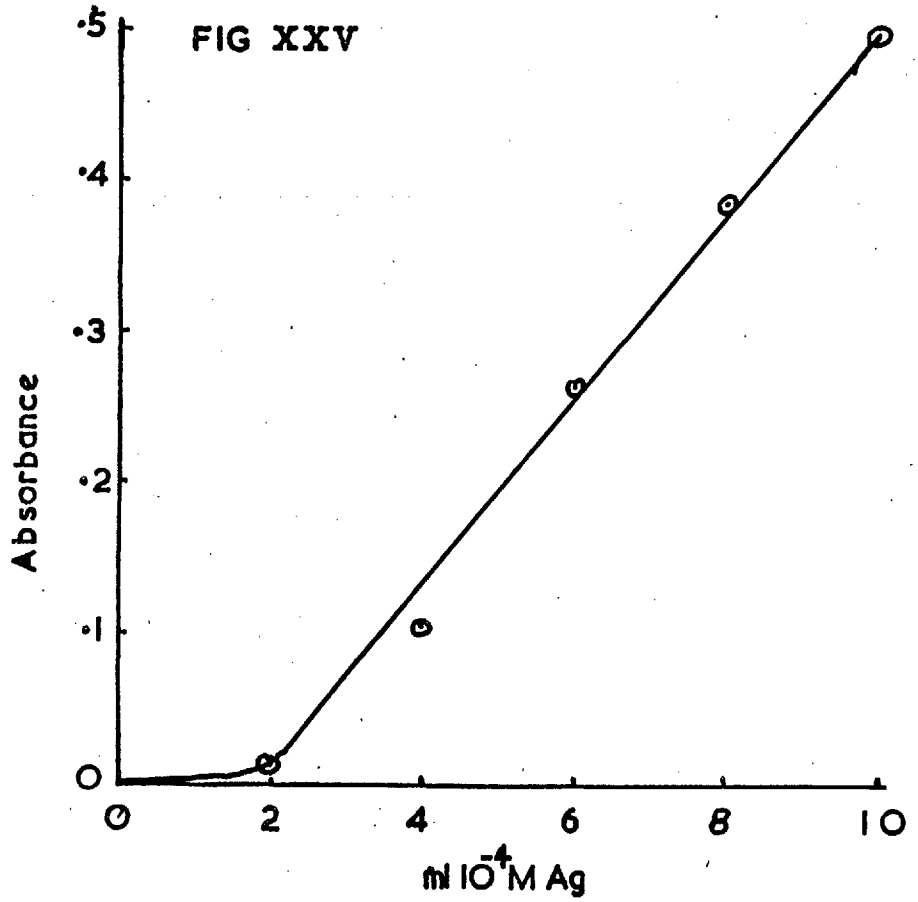


FIG XXV



Other investigations in this field have been carried out by El-Ghamry [48] and he has reported the formation of a pyridine R.B.E. complex with palladium. This complex is also extractable into chloroform though again the pH is critical, appreciable extraction only occurring in the range pH 8-8.5. The molar absorptivity however is very high at ca 125,000.

α α' Bipyridyl

The next ligand to be investigated was α α'bipyridyl, a ligand very similar to 1,10-phenanthroline. Preliminary investigations showed that this behaved in a very similar way to 1,10-phenanthroline. The complexes with R.B.E. and manganese, cobalt, nickel, copper(II), zinc, and cadmium are all capable of being extracted into chloroform. The molar absorptivities are shown in Table VIII.

The molar absorptivities are, in general, lower with α α'bipyridyl than with 1,10-phenanthroline, probably because the bis-α α'bipyridyl complexes of the various cations are less readily formed than the corresponding bis-1,10-phenanthroline complexes.

TABLE VIII

Cation	Molar absorptivity (in chloroform)
Mn	6,000
Cu	63,000
Ni	50,000
Cu	65,000
Zn	15,000
Cd	4,000

Tetraphenylphorphyrin

The final ligand to be investigated was tetraphenylphorphyrin (T.P.P.) which is known to form 1:1 complexes with a number of cations including tin(IV) and has been proposed as a spectrophotometric reagent,[49] although no procedure for its analytical application have been reported.

T.P.P. is different to the previous ligands examined in that it has a cage structure and two replaceable protons. The complexes

formed with divalent metal ions are thus uncharged but if the valency is greater than two the resulting complex will be charged. An attempt was made to form the tin(IV)/T.P.P./R.B.E. complex but it was found that the tin/T.P.P. complex would not form an additional complex with R.B.E.

The T.P.P. complex of tin(IV) was prepared by boiling about 10 mg of T.P.P. with excess cation in glacial acetic acid. The complex was extracted into chloroform and then equilibrated with an aqueous solution of R.B.E. at pH 7. There was no resulting extraction of an R.B.E. complex into the chloroform.

Discussion

The formation of the ternary complexes of the type investigated can be accomplished with most nitrogen ligands which have donor properties. However, 1,10-phenanthroline was found to be the most satisfactory.

C H A P T E R VI

A SPECTROPHOTOMETRIC PROCEDURE FOR LEAD AS THE BISPHENANTHROLINIUM LEAD R.B.E.COMPLEX

Introduction

Undoubtedly the most widely used spectrophotometric procedures for the determination of lead are those based upon reaction with dithizone.[51-58] Although the formation of lead dithizonate provides one of the most sensitive methods for determining lead- E 520 mu = 65,000[58] - the method suffers many disadvantages arising from non-selectivity, photodecomposition of the dithizone and insolubility of the complex in aqueous media.

More recently 4-(2-pyridylazo)-resorcinol has been developed for the spectrophotometric determination of lead in alloys and steels (E520=40,000).[59] However it is also unselective and preliminary extraction procedures to seperate lead from most metal ions are necessary.

In view of the relative dearth of good spectrophotometric procedures for lead it was decided to examine the possibility of utilising the bis 1,10-phenanthrolinium/Rose Bengal Extra complex of lead.

As has been mentioned previously, this reaction is reasonably specific and it was thought that by the use of selective seperations and masking agents a specific procedure could be obtained.

Preliminary Investigation

The behaviour of the lead/ 1,10-phenanthroline/R.B.E. complex is very similar to the corresponding copper(II) complex. The complex may be extracted at pH 8 into chloroform, ethyl acetate, and nitrobenzene, although the molar absorptivity is greatest in nitrobenzene. However, the high degree of extraction of the 'blank' into nitrobenzene tends to complicate the issue (Chapter I). This can be overcome by using a mixed solvent of chloroform and nitrobenzene, or a buffer of limited amounts of ammonium acetate.

Lambert - Beer Law Check

Beers law is obeyed over the range 4-20 ug of lead and probably higher. Fig XVII shows a calibration curve prepared by extracting a series of solutions containing 2-10 ml 10^{-5}M lead, 10 ml 10^{-4}M Erythrosin, 2 ml 10^{-3}M 1,10-phenanthroline, and 2 ml 20% ammonium acetate pH 8 buffer into 50 ml of a 50/50 mixture (v/v) of nitrobenzene and chloroform. The absorbances were

measured in 1-cm cuvettes against a blank prepared in a similar manner but containing no lead. The molar absorptivity calculated from the slope of the graph is 75,000.

Structure of Complex

The structure of the complex was assumed to be very similar to that of the corresponding copper complex and so a detailed investigation was not carried out. A Harvey Manning slope ratio plot (Fig XVIII) showed that the ratio of lead to Erythrosin was, as expected, 1:1. However the phenanthroline to lead ratio obtained of 4:1 is obviously wrong. This result was probably caused by incomplete formation of the lead/phenanthroline complex at low concentrations.

Curve (a) was prepared by extracting into 50 ml of a 50/50 v/v nitrobenzene/chloroform mixture a series of solutions containing 2-10 ml 10^{-5}M lead, 10 ml 10^{-4}M Erythrosin 2 ml 10^{-3}M 1,10-phenanthroline and 2 ml of ammonium acetate pH 8. The absorbances were measured in 1-cm cuvettes at 570 mu against blanks prepared in a similar manner but containing no lead.

Curve (b) was prepared in a similar manner but with 2-10 ml of 10^{-5}M Erythrosin, and 10 ml of 10^{-4}lead.

FIG XXVI

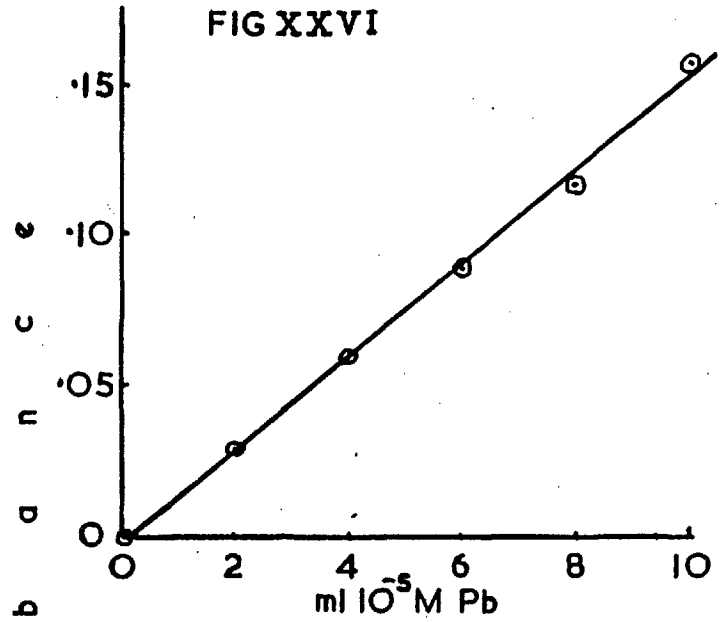
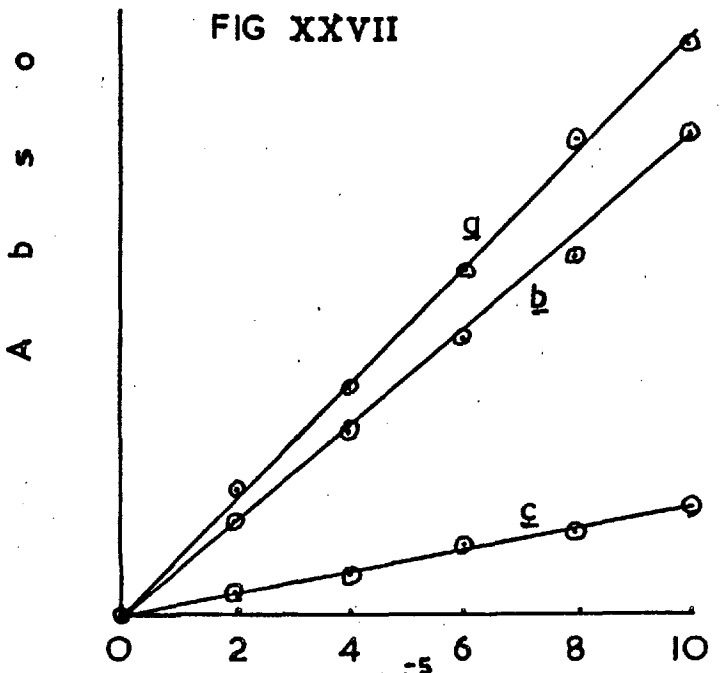


FIG XXVII



Curve (c) was also prepared in a similar way but with 2-10 ml 10^{-5}M 1,10-phenanthroline and 10 ml 10^{-4}M lead and Erythrosin.

Spectra

The absorbance spectra of the lead of the lead complex was identical to that of the corresponding copper complex.

Development of Method

The major problem was to acheive by either selective masking and/or extraction, separation from the other elements which also form phenanthroline/R.B.E. complexes.

In masking procedures the resulting solution contains a fairly high concentration of indifferent electrolyte which tends to interfere with the extraction of the ternary systems. It is necessary then first to devise a method of seperation of the lead from such solutions. With silver it was found to be relatively easy to extract the silver/1,10-phenanthroline/nitrate or acetate into nitrobenzene and this was subsequently equilibrated with an aqueous solution of the appropriate dye, which then replaced the other anion. A similar process was investigated for lead, but it was found that anions such as nitrate and acetate did not give

appreciable extraction of the lead/phenanthroline. Further investigations showed that the use of iodide and thiocyanate as counter anions afforded a certain degree of extraction and perchlorate gave very good extraction and was selected as the counter ion.

Effect of pH

Fig XXVIII shows the effect of pH on the extraction of the lead/phenanthroline/perchlorate complex (curve a). As can be seen maximum extraction occurs between pH 7 and 8.5 and subsequent work was carried out within this range.

The curve in Fig XXVIII (a) was prepared by extracting into 25 ml of nitrobenzene a series of solutions containing 2 ml of 10^{-4}M lead, 2 ml 10^{-2}M 1,10-phenanthroline, 5 ml 10% ammonium perchlorate, and adjusted to the various pH's with ammonia and acetic acid. The nitrobenzene layer was run off and equilibrated with an aqueous solution containing 5 ml of 10^{-4}M R.B.E., and 1 ml of 20% ammonium acetate buffer. The absorbance of the resulting R.B.E. complex in the nitrobenzene was measured in 1-cm cuvettes at 570 mu against distilled water.

Curve (b) shows a series of blanks containing no lead which were carried through the same procedure.

Effect of Perchlorate Concentration

The curve in Fig XXIX shows the effect of excess perchlorate on the extraction of the lead/1,10-phenanthroline. The minimum concentration which insures optimum extraction is 2 ml of 10% perchlorate, subsequently 10 ml of 10% perchlorate was used. The curve was prepared by extracting into 25ml of nitrobenzene a series of solutions containing 1 ml of 10^{-4}M lead, 2 ml 10^{-2}M 1,10-phenanthroline, 5 ml of 20% ammonium acetate pH8 buffer and amounts of ammonium perchlorate corresponding to .2, 1, 10, and 100, ml of 10% perchlorate. The nitrobenzene phase was run off and equilibrated with 25 ml of an aqueous solution containing 5 ml of 10^{-4}M R.B.E. and 1 ml of 20% ammonium acetate pH8 buffer. The absorbance of the nitrobenzene phase was measured in 1-cm cuvettes at 570 mu against distilled water.

Lambert- Beer Law Check

Having established that lead can be extracted as the lead/phenanthroline/perchlorate complex, a calibration curve was prepared to see if Beer's law was obeyed for the whole procedure The calibration curve in Fig XXX shows that Beer's law is obeyed over the range 10-40 ug of lead. The final molar absorp-

FIG XXVIII

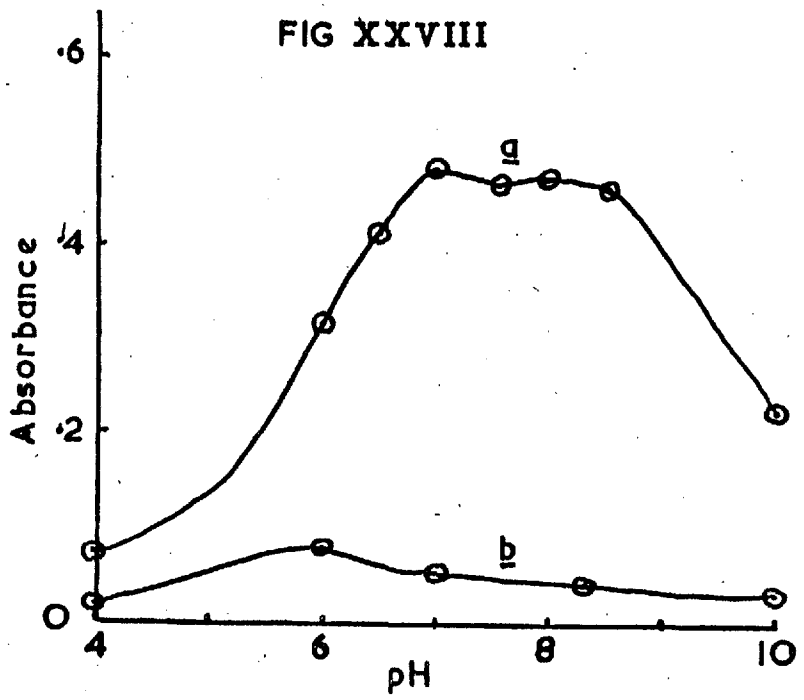
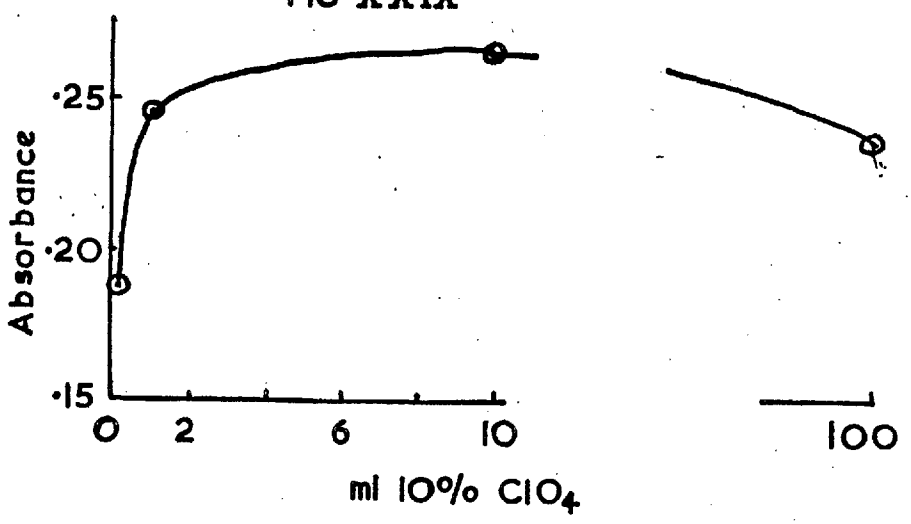


FIG XXIX



tivity calculated from the curve is 52,000.

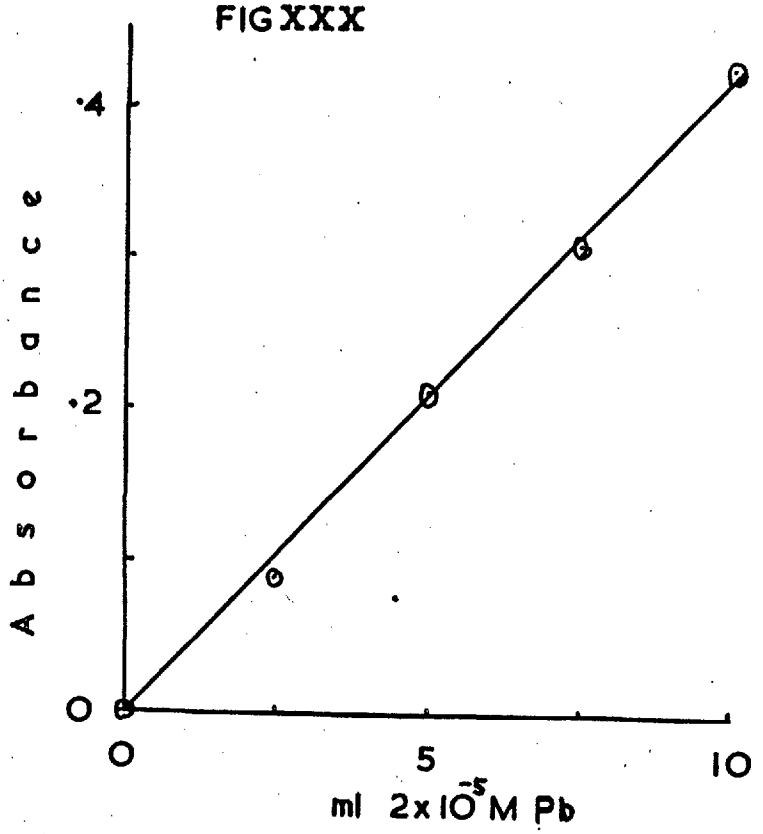
The calibration curve was prepared by extracting into 25 ml of nitrobenzene a series of solutions containing 2.5-10 ml of 2×10^{-5} M lead, 2 ml 10^{-2} M 1,10-phenanthroline, 5 ml 10% ammonium perchlorate, and 5 ml 20% ammonium acetate pH 8 buffer. The nitrobenzene phase was run off and equilibrated with a solution containing 5 ml 20% ammonium acetate (pH 8) buffer and 5 ml 10^{-4} M R.B.E. The absorbance of the R.B.E. complex in the nitrobenzene phase was measured at 570 mu in 1-cm cuvettes against a blank carried through the procedure, but containing no lead.

The effect of masking agents on the system was next examined.

The one examined first was cyanide, as it is known to form only a very weak complex with lead , but very stable complexes with copper(II), cobalt, zinc, and cadmium.

It was found that cyanide does not interfere with the procedure if added to the aqueous solution after the phenanthroline (i.e. the lead phenanthroline complex is not destroyed by cyanide.) However, the cyanide, if added before the phenanthroline, prevented extraction of the lead. Also, although cyanide is said to mask the previously mentioned cations, it was found that they could not be masked completely by cyanide when present in large excesses,

FIG XXX



because the strength of the phenanthroline complexes and cyanide complexes are very similar (Table IX).

TABLE IX

Comparison of the Stability Constants of 1,10-phenanthroline and Cyanide with Various Cations [60]

Cation	Log Bn of ligand 1,10-phenanthroline		Cyanide	
	n		n	
Cd	3	15.79	4	16.85
Co	3	20.10	6	19.09
Cu(II)	3	20.41	4	25.00
Fe(II)	3	21.00	6	24.00
Fe(III)	3	15.00	6	31.00
Mn	3	12.70	-	-
Ni	3	23.70	6	15.46
Zn	3	17.05	4	16.90
Pb(II)	-	-	4	10.30

Another possible masking procedure examined was that of using phosphate, with subsequent extraction of the interfering cations as their phenanthroline perchlorates. However, it was found impossible to demask the lead effectively.

An attempt to separate the lead by a preliminary extraction as the dithizone complex with subsequent destruction of this complex in acid media was abandoned because of the unreproducible results obtained.

The final separation procedure investigated was one based on the extraction of the interfering cations as their pyridine thiocyanate complexes. It has been reported[61] that the pyridine thiocyanates of copper(II), manganese, zinc, cadmium, nickel, cobalt, and iron(II), could be extracted completely into chloroform whilst the lead is not effected. The lead could subsequently be extracted into nitrobenzene as the phenanthroline/perchlorate complex.

EXPERIMENTAL

Reagents

All reagents are analytical grade unless otherwise stated.

Lead Nitrate Solution 2×10^{-5}M A stock 10^{-1}M solution was prepared, standardised by E.D.T.A. titration, and diluted as required immediately before use.

Ammonium Thiocyanate Solution in distilled water 10% w/v.

Ammonium Perchlorate Solution in distilled water 10% w/v.

Ammonium Acetate Buffer pH 6 Dissolve 20 gm of ammonium acetate in 100 ml of distilled water and adjust to pH 6 with acetic acid.

Ammonium Acetate Buffer pH 8 Dissolve 20 gm of ammonium acetate in 100 ml of distilled water and adjust to pH 8 with ammonia.

1,10-phenanthroline 10^{-2}M Dissolve 2.0 g of 1,10-phenanthroline in distilled water and dilute to 1 litre.

Rose Bengal Extra ca 10^{-4}M Dissolve ca 0,20 g of Rose Bengal Extra in distilled water and dilute to 1 litre.

Apparatus

Spectrophotometer Unicam S.P. 600 with 1-cm glass cuvettes.

Recomended Procedure

Pipette 2-10 ml 2×10^{-5} lead nitrate, 1 ml pyridine, 10 ml 10% ammonium thiocyanate, 5 ml of ammonium acetate pH 6 buffer into a series of 250 ml separating funnels and add sufficient distilled

water to give a constant volume. Add 25 ml of chloroform and shake for 1 minute, allow phases to seperate and discard the chloroform phase. To the aqueous phase add a further 25 ml of chloroform and .2 ml of pyridine and repeat the procedure. To the remaining aqueous phase pipette 2 ml 10^{-2}M 1,10-phenanthroline, 5 ml 10% ammonium perchlorate and 5 ml 20% ammonium acetate pH 8 buffer (pH at this point 7.5). Add 25 ml nitrobenzene and extract by shaking for 1 minute. Run off the nitrobenzene layer into a series of 100 ml separating funnels. Into these pipette 1 ml 20% ammonium acetate pH 8 buffer, 5 ml 10^{-4}M R.B.E. and about 20 ml of distilled water. Equilibrate the two phases by shaking for 1 minute, and allow the phases to seperate. After 30 minutes, run off a portion of the nitrobenzene into a 100 ml beaker and swirl the solution until the nitrobenzene phase clears and then measure the absorbance in 1-cm cuvettes at 570 mu against a blank carried through the procedure, but containing no lead.

A linear calibration curve which passes through the origin is obtained similar to that shown in Fig XXX..

Interferences

The results obtained when other ions are present are shown in table X. If iron is present it must be reduced to iron(II) first, by hydroxylamine hydrochloride as iron (III) interferes. Manganese also interferes when present in 100 fold excess but not appreciably at 10 fold excess.

TABLE X

Interferences:- 100 fold molar excess unless otherwise stated.

Standard	Interference	Absorbance	% Interference
10 ml 10^{-5}M Pb	-	.210	-
"	Ni	.201	-4.3
"	Co	.198	-5.7
"	Cd	.206	-1.9
"	Zn	.199	-5.2
"	Cu(II)	.218	3.8
"	Fe(II)	.208	-
"	Mn	.600	300

TABLE X (continued)

Standard	Interference	Absorbance	% Interference
10 ml 10^{-5}M Pb	Mn 10 fold xs	.222	5.7
"	Mn equimolar	.200	-5.1
"	I^-	.200	-5.1
"	PO_4^{2-}	.195	-7.1
"	CN^-	.203	-3.3
"	SCN^-	All	0
"	ClO_4^{2-}	present	0
"	NO_3^-	in normal	0
"	CH_3COO^-	procedure	00

Discussion

The method developed shows the general applicability of the cation/1,10-phenanthroline/R.B.E. complexes to spectrophotometry. The procedure is sensitive and has been made reasonably specific by the use of a selective extraction procedure to remove interferences.

The method could also be modified to give a spectrofluorimetric procedure in a similar way to that devised for copper previously.

C H A P T E R VII

SPECTROPHOTOMETRIC PROCEDURE FOR THE ESTIMATION OF ANIONIC SURFACTANTS

In previous chapters it has been reported that the extraction of ternary complexes of the type:-

$$\left\{\left[M(phen)_2\right]^{n+}Dye^{2-}\right\} \qquad (n = 1 \text{ or } 2)$$

into various organic solvents is suppressed by the presence of large amounts of indifferent electrolytes. In such instances the anion from the electrolyte replaces the anionic dye molecule. Furthermore, the complexes resulting from the association of the metal phenanthrolines with anions such as nitrate, perchlorate, and thiocyanate may be extracted into organic solvents. If the organic extracts of the complex are subsequently equilibrated with an aqueous solution of the dyestuff the exchange process is reversed, i.e. the dye replaces the anion and the metal phenanthroline dyestuff is formed and extracted into the organic solvent. At this point there was speculation on the feasibility of using such a procedure for the quantitive determination of anionic entities and in particular anionic surfactants.

Present methods for the spectrophotometric estimation of surfactants depend on the reaction of the dye-antagonist- a term coined by Tschoegl[62] - with subsequent extraction of the dye-surfactant

salt into some organic solvent. The absorbance of the dye in the organic phase is then measured and is proportional to the amount of surfactant present.[63] The dyestuff used carries an opposite charge to the surfactant. In the proposed procedure the preliminary extraction is accomplished using copper(II)/1,10-phenanthroline as the antagonist. The organic extract of the copper(II)/1,10-phenanthroline surfactant complex is then equilibrated with an excess of R.B.E. which displaces the surfactant. The absorbance of the copper/phenanthroline/R.B.E. complex is measured and this is directly proportional to the concentration of surfactant.

Preliminary Investigations

The anionic surfactant used in the investigations was di (2-ethyl hexyl) sulphosuccinate or Manoxol O.T. (Aerosol O.T.) as this has been used previously as a'standard' anionic surfactant in the developement of other spectrophotometric procedures.[64] It was decided that the bis phenanthroline/copper(II) complex should be used as it has already been utilised together with R.B.E. for the development of a spectrophotometric method for copper(II).

It was found that, as postulated, in the presence of Manoxol

the copper/phenanthroline could be extracted into chloroform at a pH of 7 or thereabouts.

Optimum pH of Extraction

The curve in Fig XXXI shows the effect of pH on the extraction of the surfactant complex (curve a) and the copper/phenanthroline (curve b). As can be seen the complex may be extracted over the range pH 4-9, but the optimum pH range is 5-6.

Curve (a) was prepared by extraction into 25 ml a series of solutions containing 2 ml 10^{-4}M copper, 1 ml 10^{-2}M 1,10-phenanthroline, and 10 ml 0.001% Manoxol, ammonia and acetic acid were used to obtain the required pH. The chloroform extract was run off and equilibrated with a solution containing 5 ml 2×10^{-4} M R.B.E., and 2 ml of phosphate buffer pH 9. The absorbance of the R.B.E. in the organic phase was measured after 30 minutes in 1-cm cuvettes at 570 mu against distilled water.

Curve (b) was prepared in a similar manner but contained no Manoxol.

Optimum Copper-Phenanthroline Concentration

Fig XXXII shows the effect of copper-phenanthroline concentration on the extraction of the complex (curve a) and 'blank' (curve b). It can be seen that between 5 and 10 ml of 10^{-4}M copper is sufficient to ensure complete extraction.

Curve (a) was prepared by extracting into 25 ml of chloroform a series of solutions containing 5 ml .001% Manoxol O.T.,1 ml 10^{-2} M 1,10-phenanthroline, 2 ml phosphate pH9 buffer and 1-10 ml 10^{-4} M copper. The chloroform phase was run off and equilibrated with an aqueous solution containing 5 ml $2x10^{-4}$M R.B.E. and 2 ml of phosphate buffer pH 9. The absorbance of the R.B.E. in the organic phase was measured after 30 minutes in 1-cm cuvettes at 570 mu against distilled water.

Curve (b) was prepared in a similar way but containing no Manoxol.

Lambert-Beer Law Check

Beers law is obeyed over the range 20-100 p.p.m. as can be seen from the calibration curve in Fig XXXIII. The molar absorptivity for the method, calculated from the calibration curve, is high at 73,000.

FIG XXXI

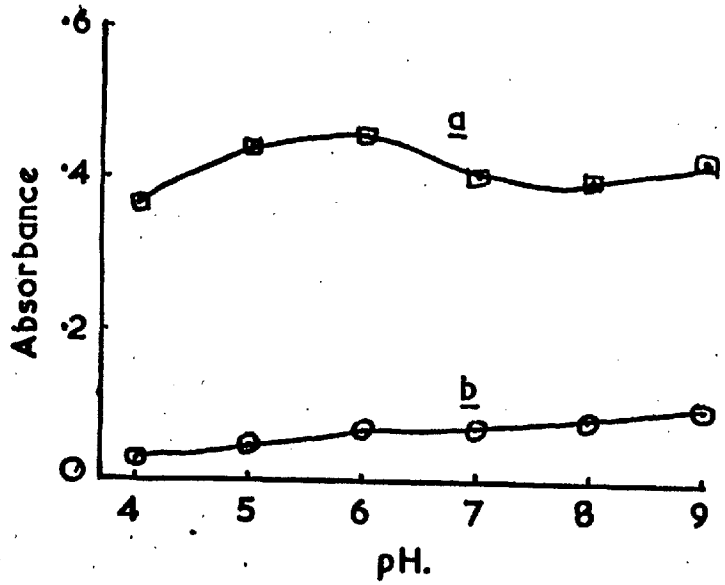


FIG XXXII

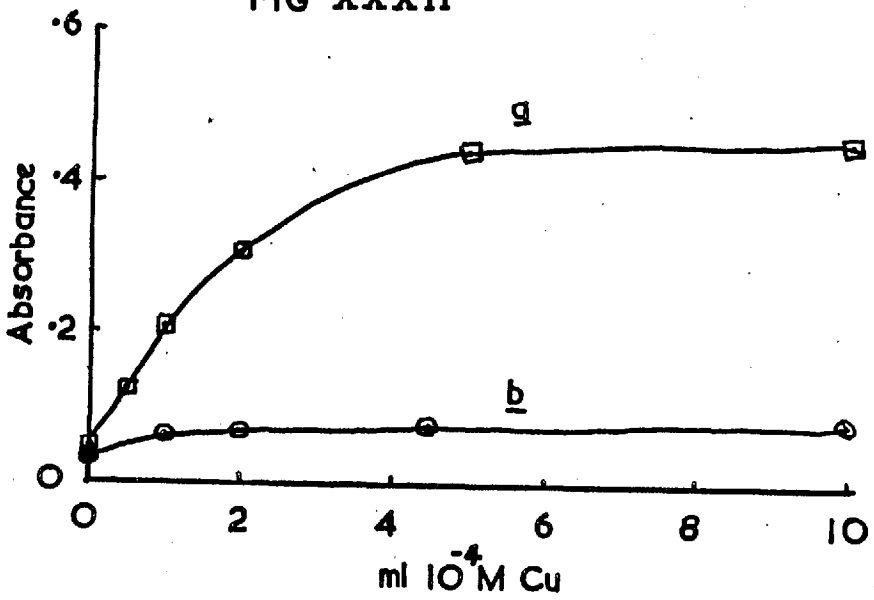
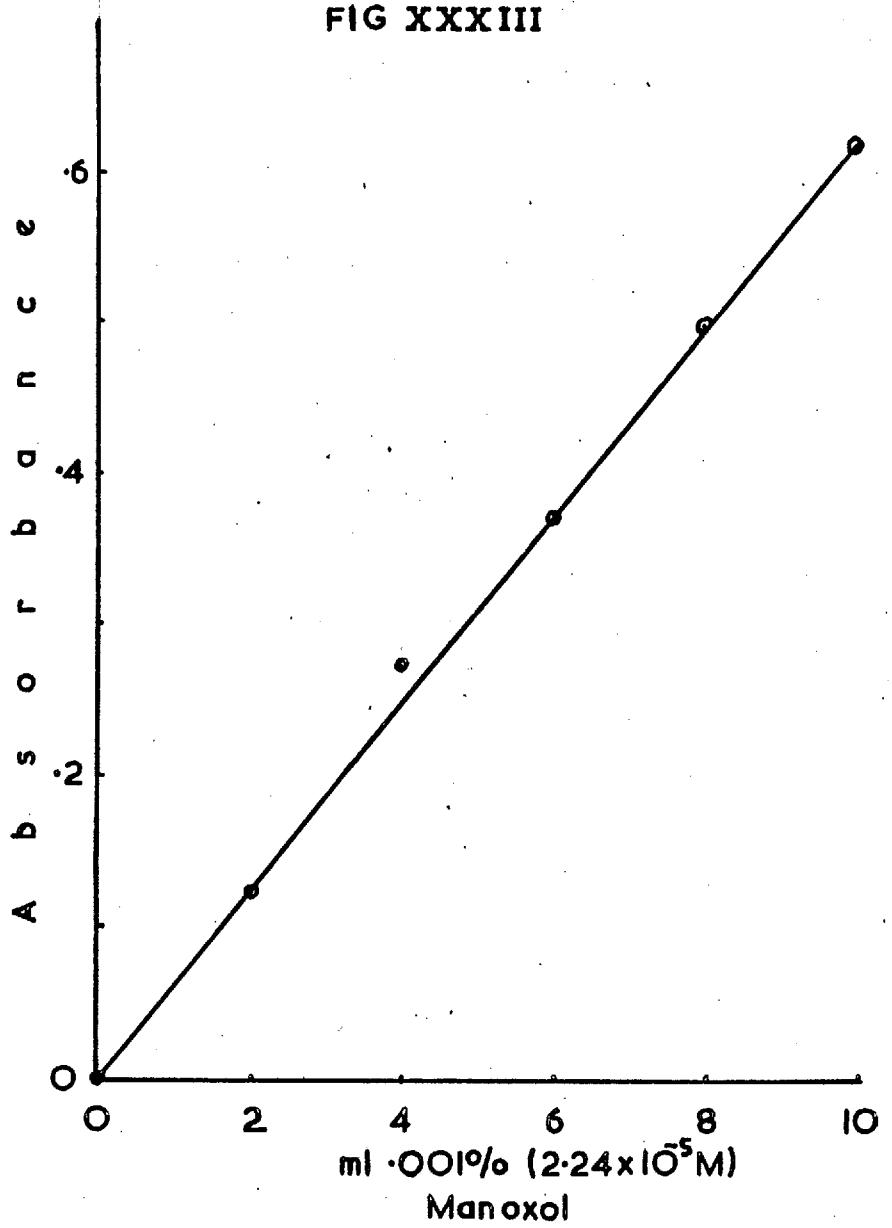


FIG XXXIII



The curve in Fig XXXIII was prepared by extracting into 25 ml of chloroform a series of solutions containing 2-10 ml 0.001% Manoxol, 10 ml 10^{-4}M copper, 2 ml phospate pH 9 buffer. The chloroform phase was run off and equilibrated with an aqueous solution containing 5 ml $2x10^{-4}$M R.B.E. and 2 ml phosphate pH 9 buffer. The absorbance of the R.B.E. complex was measured after 30 minutes in 1-cm cuvettes at 570 mu against a blank containing no Manoxol which was carried through the same procedure.

Interferences

The main interferences in the previously developed spectrophotometric procedures come from those anions which form extractable complexes with the various dyes used. The most serious interferences arise from thiocyanate and perchlorate.[64]

It was found that the ions such as nitrate, bromide, chloride, sulphate, sulphite, persulphate and carbonate did not interfere when present in 1000 fold excess. However, thiocyanate and perchlorate could only be tolerated up to 100 fold excess and thereafter caused serious interference.

Discussion

The proposed method for the estimation of anionic surfactants compares well with the other recognised spectrophotometric procedures. Samples of 20-100 ug of surfactant can be readily determined as compared with 20-150 ug in the Jones method as modified by Longwell and Maniece[64] and 5-60ug in the Jones method as modified by Moore and Kolbeson.[65] Furthermore, the sensitivity of the spectrophotometric procedure could be increased by using the extraction procedure as a method of concentration. Also, if the procedure was further modified, in the way previously described for copper, a spectrofluorimetric method for samples containing as little as 0.1 ug of surfactant may be obtained.

C H A P T E R VIII

TERNARY COMPLEXES INVOLVING SURFACTANTS

In the previous chapters the development and use of various ternary complexes in spectrophotometric analysis has been described. These complexes which are characterised by their very high molar absorptivities have all involved phenanthrolinium metal complexes with anionic dyestuffs. The colour changes produced on complexation is similar to that which the dyestuffs undergo when they form salts with cationic surfactants. It was this latter point, coupled with the desire to extend the use of ternary complexes to cations other than those which formed phenanthroline complexes, that led to the investigation of complexes formed between anionic ligands, cations, and cationic surfactants.

It has long been known that certain metal chelate complexes in the presence of disperse agents such as gelatin have their absorptivities increased, and also may have their wavelength of maximum absorbance shifted (usually bathochromically). This phenomenon has been termed 'sensitization' and several spectrophotometric procedures involving bathochromic shift or the production of a new colour body have been produced.[26-29]

Malat and co-workers have used the Catechol Violet complexes of the cations tin, titanium, and indium adsorbed onto a

gelatin substrate as the basis for spectrophotometric procedures for these elements.[26-28] More recent work[29] has shown that results obtained using gelatin are rather variable and the surfactant Cetyl trimethyl ammonium bromide has been used instead.

Cetyl trimethyl ammonium bromide (C.T.A.B.) has the added advantage that its exact composition and structure is known, wheras with gelatin they are undefined, also use of C.T.A.B. 'sensitizes' the tin(IV)/Catechol Violet system more than the gelatin.

Other workers in this field have also used C.T.A.B. in conjunction with the Xylenol Orange complexes of a number of cations.[67] The action of the surfactant here is quite different to that of the previous case in that its presence does not give rise to a bathochromic shift or the production of a new colour body. The purple-red alkaline (pH 10.5) solutions of Xylenol Orange are decolorized by addition of cationic long chain quarternary salts - such as C.T.A.B. - and the addition of metal ions - such as calcium, zinc, and manganese - restores the original reagent colour.

In this latter case the formation of micelle aggregates

was believed to be resonsible for the changes, although no conclusive experimental evidence was presented in support. The mechanism involving the colour change was considered only in terms of a supposed adsorption phenomena. The authors admit the theory is incomplete and they present the system as a means of determining complexometrically such ions as calcium and magnesium with an indicator not normally applicable under the alkaline conditions used.

This latter type of surfactant complex is of less interest, with regard to spectrophotometry than the former, because it is formed in alkaline media, and the precipitation of metal ions occurs, thus giving rise to serious interference as adsorption occurs onto these as well as the surfactant. Investigations were therefore concentrated on the former type of complex.

The complexes involving surfactants have not been regarded as ternary complexes but more as binary complexes adsorbed onto substrates. The preliminary investigations were, firstly to determine whether the complexes formed are ternary complexes, these being defined as - an association of three species combined in a definite ratio - and secondly, to examine the mechanism involved in the formation of these complexes.

Preliminary Investigations

The tin(IV)/C.V./C.T.A.B. complex was selected for use in the preliminary investigations because this is the only complex of this type developed to date for use in spectrophotometry which utilized C.T.A.B.[29]

The fist point to be examined was the composition of the complex.

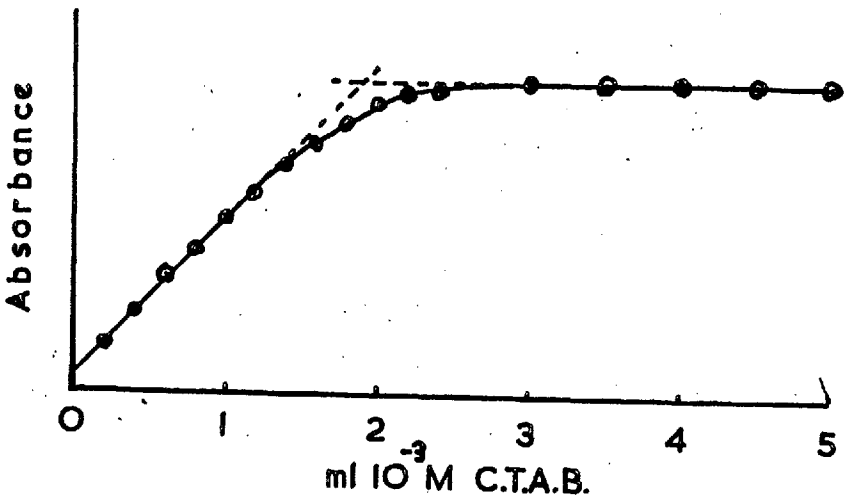
Composition of Complex

Previous work has shown that the tin(IV)/Catechol Violet complex has a molecular ratio of 1:2.[29] Photometric titrations confirmed this and showed that this entity forms one to two complex with C.T.A.B. i.e. a ratio of tin(IV):C.V.:C.T.A.B. of 1:2:2: - Fig XXXIV

The curve in Fig XXXIV was prepared by titrating with an E.E.L. photometric titrator a solution containing 10 ml 2×10^{-4}M C.V., 1 ml 10^{-3}M tin (adjusted to pH 2 with sulphuric acid) with a 10^{-3}M solution of C.T.A.B.

Investigations with other complexes involving C.V. and C.T.A.B. gave similar results. The complex with molybdate has a molecular ratio of 1:1:2, molybdate:C.V.:C.T.A.B. (Chapter IX).

FIG XXXIV



Antimony forms two different coloured complexes with C.V. and C.T.A.B. depending on the relative ratios of the reagents (Chapter X).

These results showed that the ternary systems involving surfactants are not micelle aggregates of variable composition as has been supposed, but complexes of fixed composition - and thus ternary complexes.

Effect of Micelle Formation

The importance of micelle formation in the production of ternary complexes was next investigated.

It has been postulated that the prescence of micelles is necessary for the formation of the Xylenol Orange complexes.[67] Preliminary investigations indicated that this is also true of the type of complex being investigated, as they are not formed with quarternary ammonium salts such as tetra ethyl and tetra butyl ammonium bromide - which are not surfactants (and so do not produce micelles).

To examine the effect of micelle concentration it was necesary to determine the critical micelle concentration (C.M.C.) of the surfactant used (C.T.A.B.). The C.M.C. may be defined as,

the minimum concentration of detergent at which micelles are formed. The simplest and most widely used method for determining the C.M.C. of a surfactant is by means of the marked colour change which occurs in solutions containing both surfactant and a suitable dyestuff of the opposite charge, when the concentration of surfactant reaches the C.M.C.[68] This colour change can be observed either visually during the course of a titration or instrumentally.[69]

Eosin and fluorescein have both been usee for determining the C.M.C. of cationic surfactants[68] and the author used the closely related R.B.E. in view of its previous use in ternary systems.

The C.M.C. was determined by this method in the following manner; an aliquot of R.B.E. was pipetted into 100 ml flask and diluted to about 90 ml; various amounts af C.T.A.B. were added until the range over which the colour change occured was found. This was determined - visually - to be when the concentration (of C.T.A.B.) was about 4×10^{-6}M overall. A series of solutions were made up containing 1 ml 10^{-4}M R.B.E., and 1-5 ml 10^{-4}M C.T.A.B. in 100 ml flasks. The absorbances of these solutions were measured at 570 mu (the absorbance maximum of the surfactant-dye salt) in 1-cm cuvettes against a blank containing no C.T.A.B. The

results were plotted as the rate of change in absorbance with concentration and these are shown in Fig XXXV(a). The curves (b) and (c) were prepared in a similar way but contained 2 ml and 4 ml 10^{-4}M R.B.E. respectively. From the results it can be seen that the C.M.C. (the point where d abs/dv is a maximum) is indeed when the concentration of C.T.A.B. is $4x10^{-6}$M.

The procedure was repeated using 2.5 ml 10^{-4}M tin and 5 ml 10^{-4}M C.V. (and sulphuric acid to pH 2) instead of R.B.E. The absorbances were measured in a similar manner but at 660 mu (the absorbance maxima of the tin/C.V./C.T.A.B. complex). The results show that an inflection is again obtained at the point where the C.T.A.B. concentration is the C.M.C. viz. $4x10^{-6}$M. From these results it can be seen that the formation of micelles does have an effect on complex formation.

Further work was carried out in an aqueous/acetone media. The use of aqueous/acetone has the effect of raising the C.M.C. This enables the use of a concentration of C.T.A.B. such that the colour change through the C.M.C. is more easily observed. The C.M.C. of C.T.A.B. was determined in a similar manner to the previous determination for various concentrations of acetone in

water. A 30% v/v acetone/water media gave a C.M.C. for C,T,A,B. of 10^{-4}M and was used in further investigations with the tin(IV)/ C.V./C.T.A.B. system. As before the ternary complex first began to form appreciably when the concentration of C.T.A.B. reached the C.M.C.

Fig XXXVII shows the results obtained from an investigation carried out in a similar manner to that previously described for aqueous media. The inflection again occuring at the C.M.C.

The curve in Fig XXXVII was prepared by pipetting 1 ml 10^{-3}M C.V.,and 5 ml 10^{-4}M tin(IV) into a series of 100 ml graduated flasks, 30 ml of acetone was then added followed by 2-7 ml of $2x10^{-3}$M C.T.A.B. The solutions were made up to 100 ml with distilled water and the absorbances were measured at 660 mu in 4-cm cuvettes against a blank containing no C.T.A.B.

It was noticed that with the aqueous/acetone media the order of addition of the reagents was very important. If the C.T.A.B. was added to the tin(IV)/C.V. complex before the acetone (i.e. if the ternary complex is formed before the micelles are destroyed by the addition of acetone) the ternary complex is not destroyed on subsequent addition of acetone.

A comparison of the absorbances of solutions of the ternary

FIG XXXV

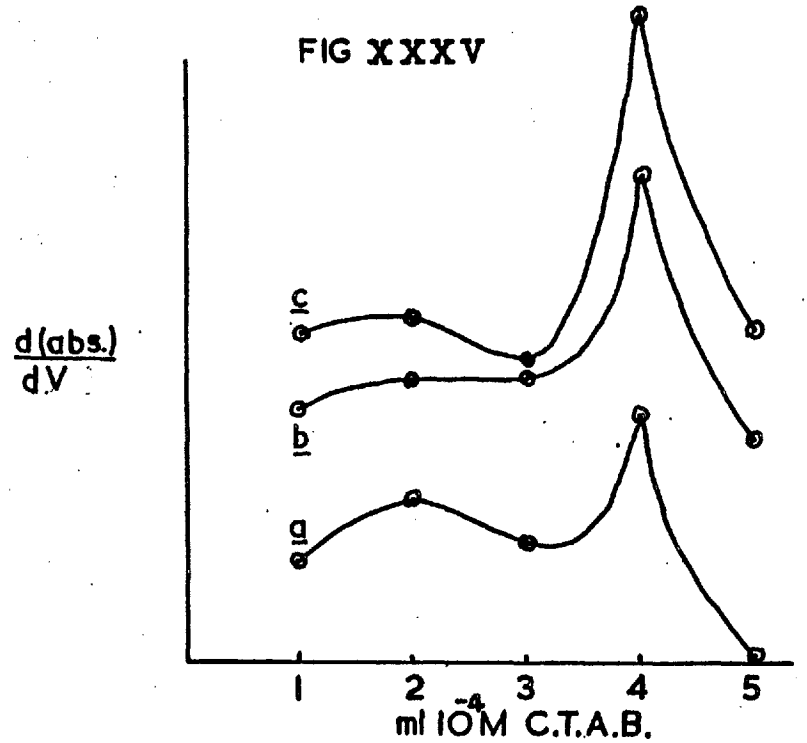


FIG XXXVI

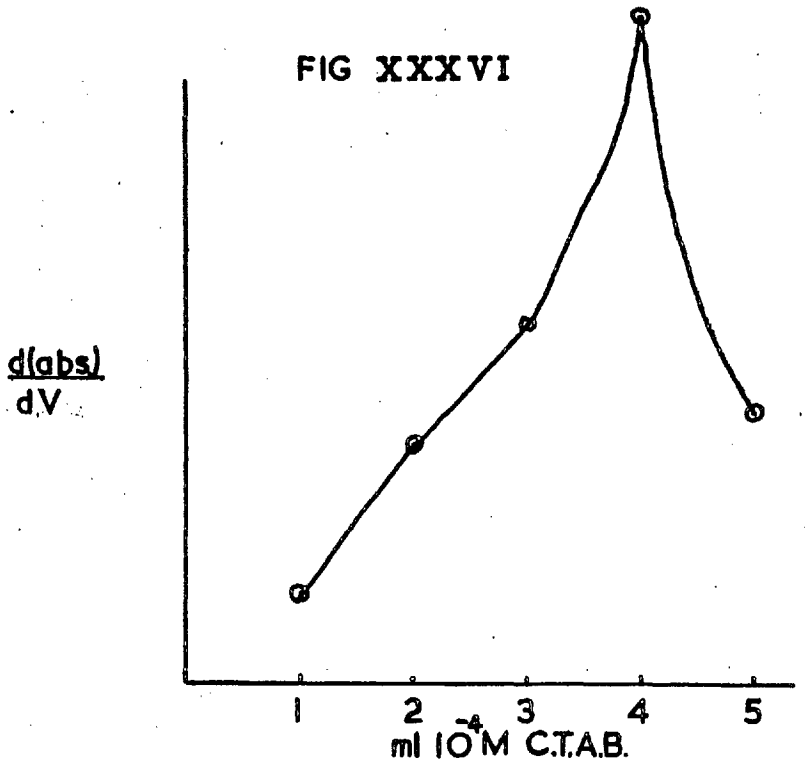
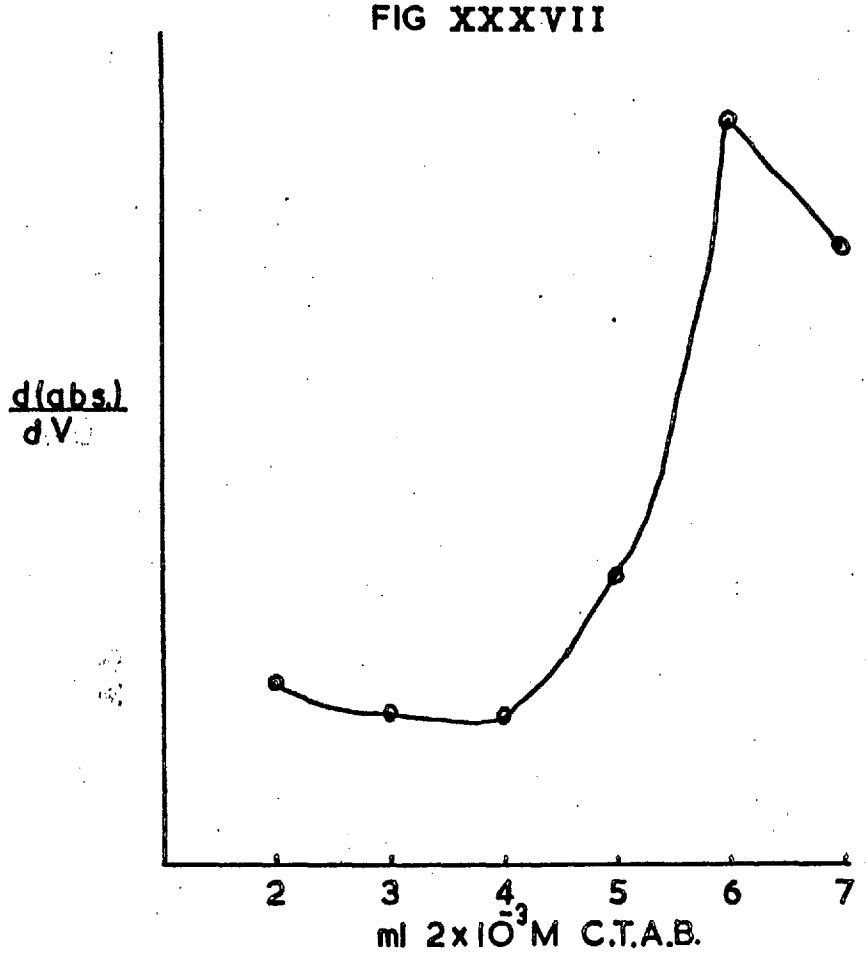


FIG XXXVII



complex of the same concentration is shown in Table XI. In the first case the C.T.A.B. is added after the acetone, in the second case before, and in the third case the media is aqueous.

TABLE XI

Final Molar Conc. of C.T.A.B.	C.T.A.B. Added (a) after acetone	(b) before acetone	Aqueous media
4×10^{-5}	.018	.288	.490
14×10^{-5}	.095	.341	.533

(C.M.C. 10×10^{-5}M)

These results may be contrasted with the results obtained with R.B.E. in which the order of reagent addition has no effect on the degree of salt formation in the final solution.

The conclusion drawn from this work was that it is necessary to form micelles to obtain the complex, but once the complex is formed the C.T.A.B. concentration can fall below the C.M.C. without unduly affecting the complex. Thus the C.M.C. is also a critical reaction concentration below which little or no complexation occurs.

Further Applications of the Surfactant Complexes

At the beginning of this chapter it was stated that one of the reasons for investigating the surfactant type of complexes was to extend the range of applicability of the ternary systems by using these complexes.

Further investigations have shown that a number of other elements, namely antimony, bismuth, titanium, zirconium, gallium, indium, and also molybdate and tungsgate give similar colour reactions to tin with C.V. and C.T.A.B. A more detailed study of the complexes with molybdate and antimony is given later.

Some of the complexes with the above mentioned ions are chloroform extractable in contrast to the tin complex. Furthermore, the extraction of the complexes is not dependent on the quarternary ammonium salt being a surfactant. With both molybdenum and antimony the complexes can be extracted when the quarternary ammonium group is either a tetra-ethyl or a tetra-butyl ammonium halide.

Investigations with Other Reagents

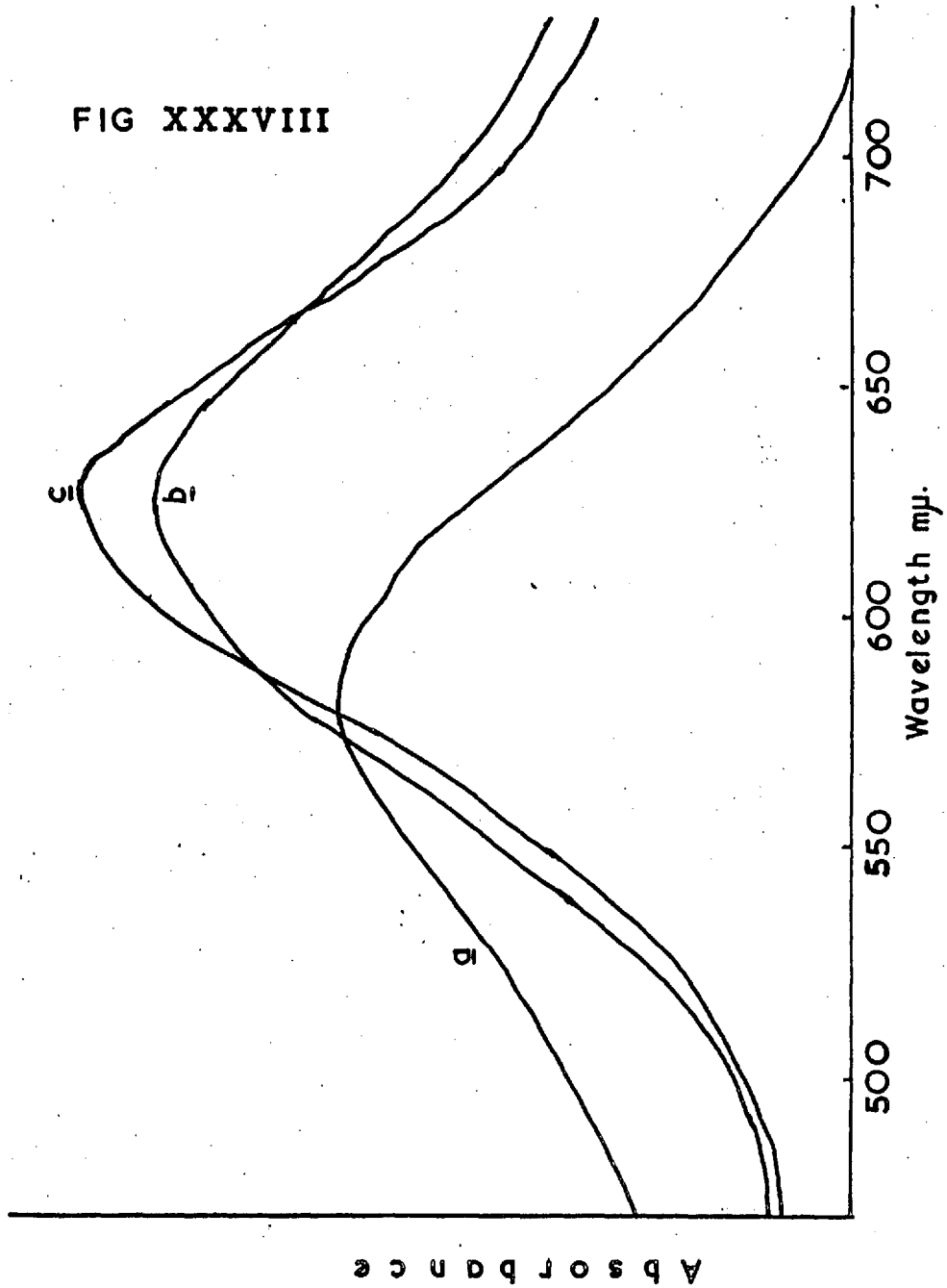
Complexes with ligands similar to C.V. (triphenyl methanes)

containing more hydroxyl or carboxylic, groupings than are used in complexation have been shown to form ternary complexes with surfactants e.g. the Bromopyragallol Red complex of antimony and the Chrome Azurol S complex of copper. This latter complex is especially interesting as the two ternary complexes of copper/C.A.S. one with 1,10-phenanthroline and the other with C.T.A.B. have identical spectra. The curves in Fig XXXVIII show the absorption spectra (a) of the copper(II)/C.A.S. complex, (b) the copper(II)/1,10-phenanthroline/C.A.S. complex and (c) the copper(II)/C.A.S./C.T.A.B. complex. The molar concentrations of the various complexes are the same.

This similarity between the two types of complexes prompted an investigation into whether the copper/1,10-phenanthroline, and other phenanthrolines, acted as a micelle in the formation of the ternary complexes (in aqueous solution) in a similar manner to C.T.A.B.. However , the results obtained from a study carried out in a similar way to that with tin(IV) gave inconclusive results.

If the copper/1,10-phenanthroline forms micelles, the C.M.C. is too low for it to be determined in aqueous solution by the previously described method.

FIG XXXVIII



Experiments using an aqueous acetone media showed that increasing the percentage of acetone decreased the amount of complex formation. This would appear to indicate that micelles are formed, however it was not possible to obtain any results which showed a definite increase in complex formation at any particular concentration of copper/1,10-phenanthroline for a given aqueous acetone media.

Discussion

It has been shown that the formation of new colour bodies which occur when surfactants are added to certain binary complexes are a result of a definite association with a fixed number of the quarternary ammonium molecules with the binary complex.

However this association is not purely ionic, as the association is also dependent (in aqueous media) on the formation of micelles by the surfactant.

The ternary complexes may, in some cases, be extracted into organic media, but here the situation is slightly changed. The quarternary nitrogen compounds need not be surfactants thus indicating that in organic media the association is purely ionic.

A similarity in the mechanism of complex formation between the ternary phenanthroline and ternary surfactant complexes has been observed.

This information is of use in selecting replacements for 1,10-phenanthroline. These should be strongly hydrophobic in order to produce micelle forming entities on complexation with cations.

C H A P T E R IX

A SPECTROPHOTMETRIC PROCEDURE FOR MOLYBDENUM

The number of spectrophotometric procedures for the determination of traces of molybdenum are rather limited. Probably the best known and most sensitve colour reaction for molybdenum is the one involving the reduction of the heteròpoly phosphomolybdic acid to form molybdenum blue.[51] However, this gives unreproducible results and is not generally regarded as satisfactory for use in spectrophotometry.

The sensitivities of the few recognised spectrophotometric procedures for molybdenum are listed in Table XII[58]

As can be seen,the molar absorptivities are not particularly high and furthermore, determinations must be made in organic solvents

TABLE XII

Reagent	Solvent	Molar absorptivity
Thiocyanate	diethyl ether	20,000
Dithiol	isoamyl acetate	18,000
Oxine	chloroform	8,000
(Catechol Violet) (C.T.A.B.)	(aqueous)	(46,000)

In view of this lack of suitable sensitive methods for molybdenum it was decided to develop the ternary complex which molybdenum forms with Catechol Violet and C.T.A.B.

Preliminary Investigation

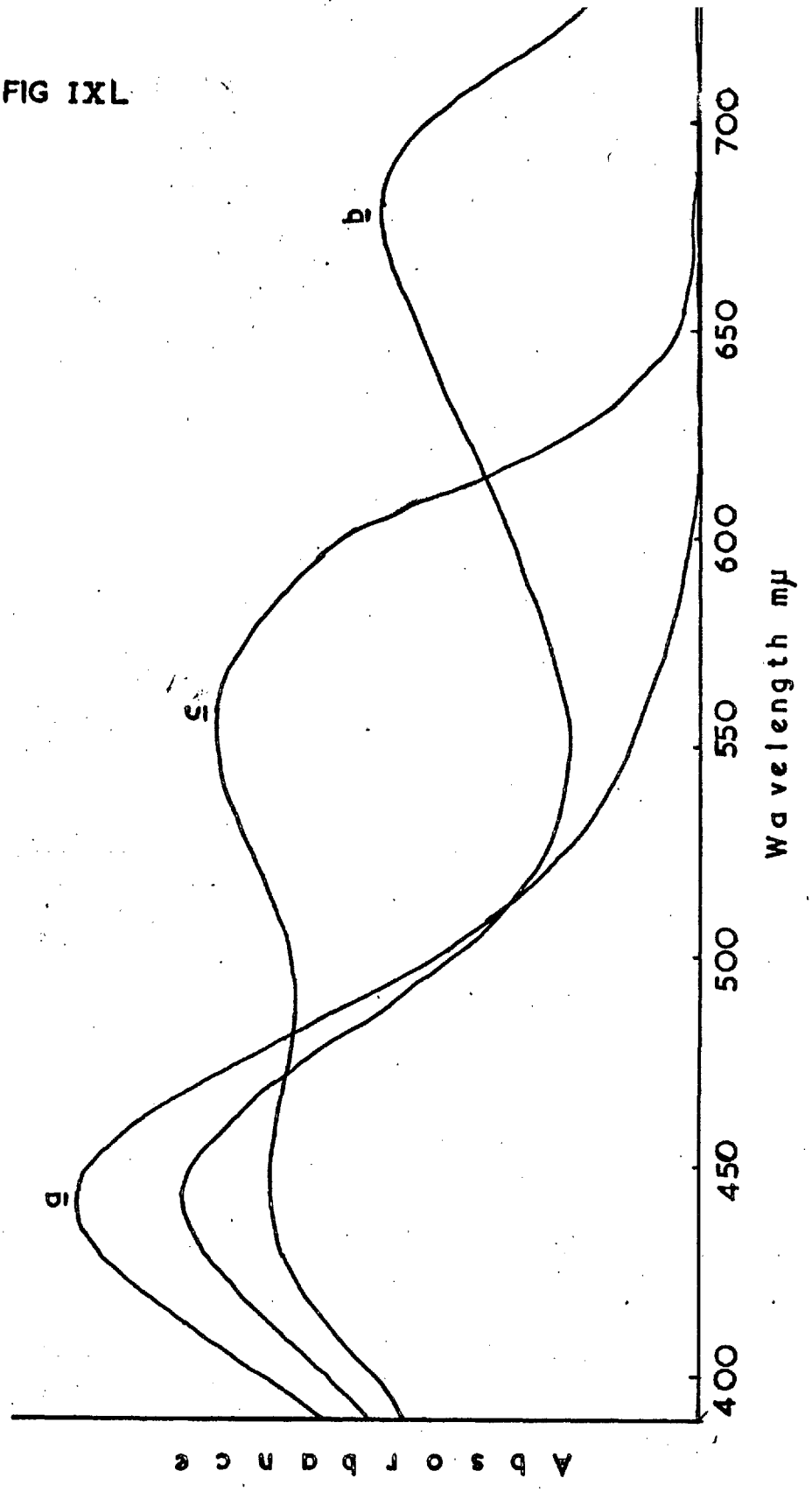
It was reported in the previous chapter (page 147) that molybdenum as molybdate formed a ternary complex with C.V. and C.T.A.B. It has long been known that molybdate undergoes a colour reaction with organic ligands such as catechol which contain vic hydroxyl groups, but these have been used exclusively in spot tests for the organic ligands.[70]

It was found that molybdate forms a coloured complex with Catechol Violet but the molar absorptivity is low ($E_{540} = 8000$). On addition of C.T.A.B. the wavelength of maximum absorbance is shifted from 540mu to 670mu and the molar absorptivity is increased to about 46,000.

Preliminary Spectra

The curves in Fig. IXL show the absorbance spectra of Catechol Violet (curve a), the molybdate/Catechol Violet complex (curve c) and the molybdate/Catechol Violet/C.T.A.B. complex (curve b).

FIG IXL



Curve (b) was prepared by measuring the absorbance of a solution 10^{-5} M in the ternary molybdate/C.V./C.T.A.B. complex.

Curve(c) was prepared by measuring the absorbance of a solution 10^{-4}M in molybdate/C.V. complex.

Effect of pH

The curves in Fig XL show the effect of pH on the complex (curve a) and the reagent (curve b). Maximum colour development occurs in the range pH 3-5. Above pH 6 the reagent blank increases rapidly.

Curve (a) was prepared by measuring the absorbance at 675 mu in 1-cm cuvettes of solutions 10^{-5}M in molybdate and $5x10^{-5}$M in C.V. and C.T.A.B..

Curve (b) was prepared in a similar manner, but contained no molybdate.

Reagent Excess

A series of photometric titrations were carried out to determine the amounts of C.T.A.B. and C.V. neccessary for maximum colour development. The results (Fig XLII and XLIII) show that a minimum 2.5 fold molar excess of C.T.A.B. and a

1.5 fold molar excess of C.V. over the molybdate insures maximum colour development. A greater excess of C.T.A.B. causes a slight increase in absorbance, but the reverse occurs with C.V.

The curve in Fig XLII was prepared by titrating photometrically a series of solutions containing 25 ml 10^{-5}M molybdenum and 1.25 ml 10^{-3}M C.V. with 10^{-3}M C.T.A.B.

The curve in Fig XLIII was prepared in a similar way, but the concentrations of C.V. and C.T.A.B. were reversed.

Lambert-Beer Law Check

Beers law is obeyed over the range 10-100 ug of molybdate. The curve in Fig XLI shows a calibration curve prepared by diluting to 100 ml a series of solutions containing 1-10 ml 10^{-4}M molybdate, 5 ml 10^{-3}M C.V., 15 ml 10^{-3}M C.T.A.B., and 5 ml acetate pH 3.85 buffer. The absorbance was measured in 1-cm cuvettes at 675 mu against a blank prepared in a similar manner, but containing no molybdate. The molar absorptivity calculated from the curve is 46,000.

FIG XL

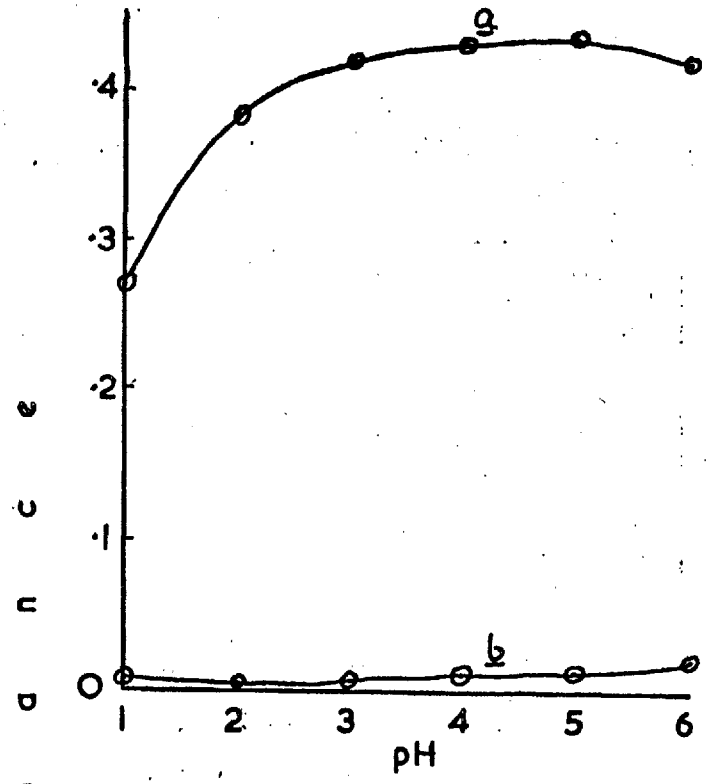


FIG XLI

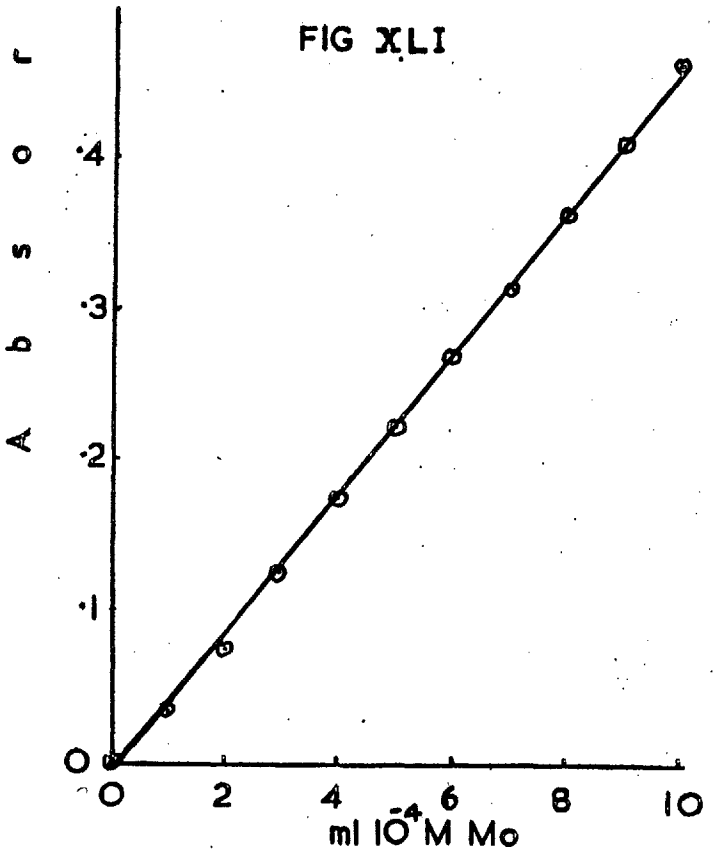


FIG XLII

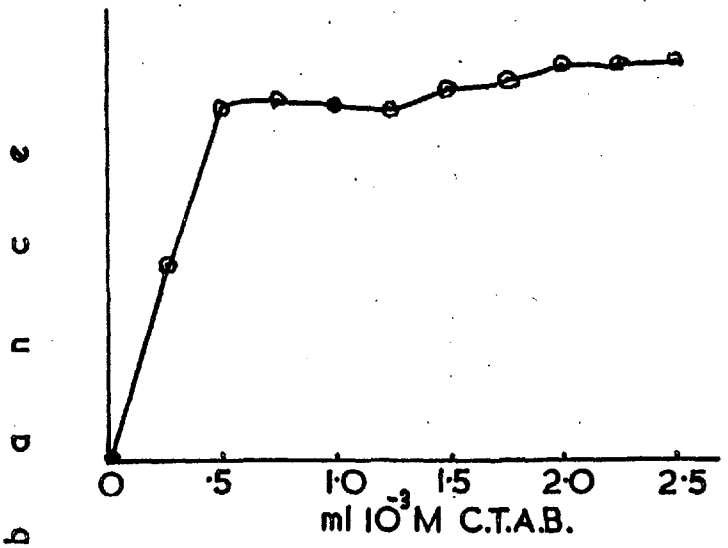
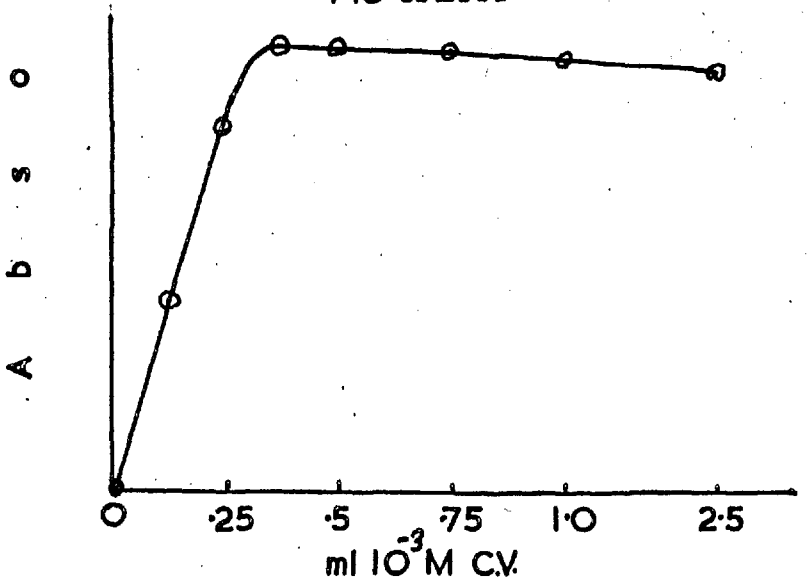


FIG XLIII



Nature of Complex

The colour of the complex, like the corresponding tin(IV) complex is destroyed by the addition of excess acetone. The structure of the complex was investigated by means of Job continuous variation and slope ratio plots Fig XLIV,XLV and XLVI. The results obtained show unequivocally that the ratio of molybdate:C.V.:C.T.A.B. is 1:1:2. The molybdate to C.V. ratio in the binary complex was determined by means of a Job contiuous variation plot as 1:1. Fig XLVII

The curve in Fig XLIV was prepared by diluting to 100 ml a series of solutions containing 0-10 ml of 2×10^{-4}M molybdate, 10-0 ml 2×10^{-4}M C.V. and 5 ml of 10^{-3}M C.T.A.B. adjusted to pH 3.5 withacetic acid. The absorbances were measured in 1-cm cuvettes at 675 mu against a blank prepared in a similar way, but containing no molybdate.

The curve in Fig XLV was prepared in a similar way but with the concentrations of C.T.A.B. and molybdate reversed.

The curve in Fig XLVI was prepared by diluting to 100 ml a series of solutions containing 1-5 ml 2×10^{-4}M molybdate, 5 ml 10^{-3}M C.V. and C.T.A.B. adjusted to pH 3.5 with acetic acid.

The absorbances were measured in 1-cm cuvettes at 675 mu against a blank prepared in a similar way, but containing no molybdate.

Curves (b) and (c) were prepared in a similar manner, but with 1-5 ml of 2×10^{-4}M C.V. and C.T.A.B. respectively. The same excess of other reagents was maintained.

Interferences

The effect on the colour system of some 18 cations and various masking agents was examined and the results are listed in Table XIII.

The major interferences come from those elements already known to form similar ternary complexes. Lead interferes by precipitating the molybdate. Some of the interferences may be removed by judicious use of masking agents e.g. zirconium may be masked by fluoride. However, it is proposed that if used in a spectrophotometric procedure the method should be coupled with the selective seperation procedure for molybdenum developed by Woodward et al.[71]

FIG XLIV

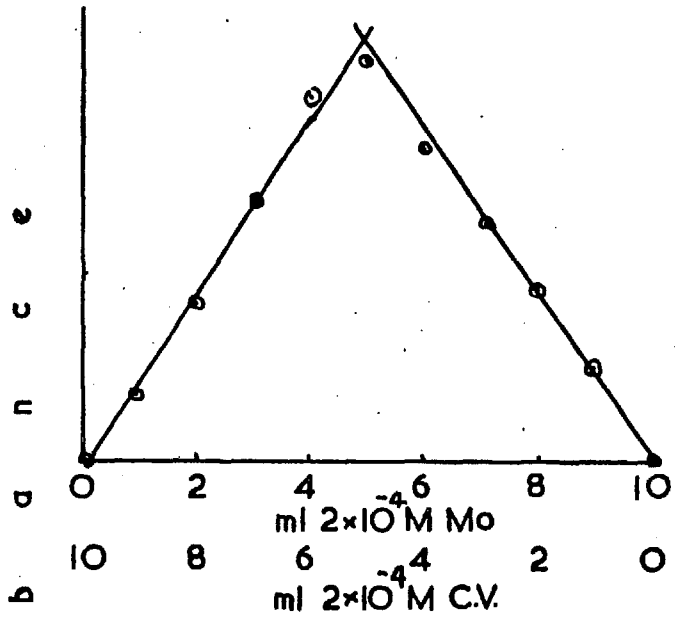


FIG XLV

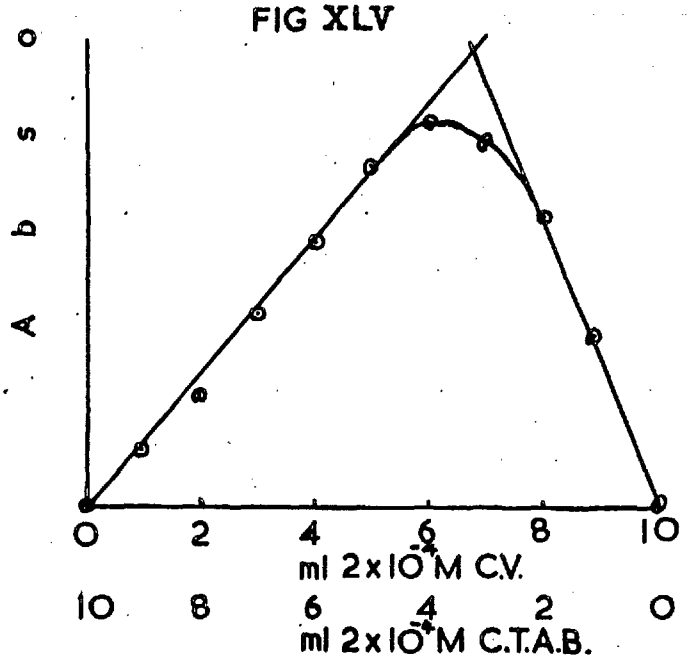


FIG XLVI

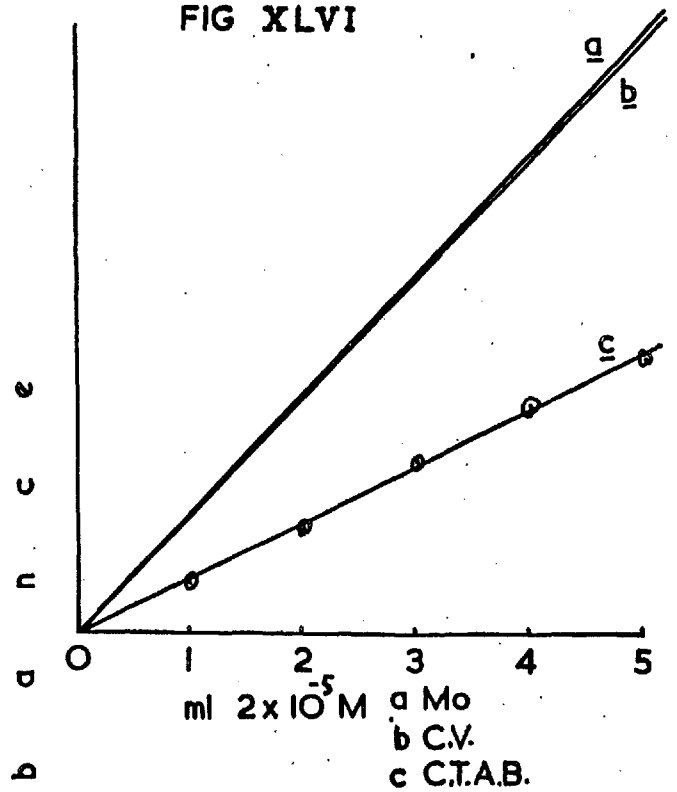


FIG XLVII

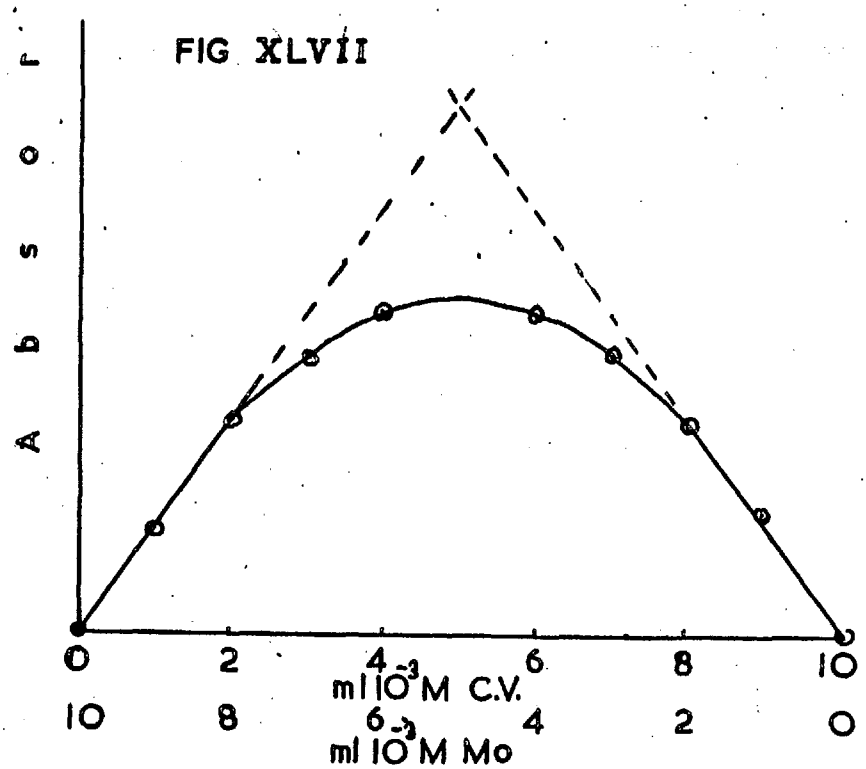


TABLE XIII

Standard	Interference	Molar Excess	Absorbance 675 mu	% Interference
10 ml 10^{-4} M Mo	none	-	.455	-
"	Mn	100	.455	0
"	Cu(II)	100	.460	1.1
"	Co	100	.452	0
"	Ni	100	.438	-3.7
"	Zn	100	.438	-3.7
"	Pb	100	.040	-91
"	Mg(II)	100	.438	-3.7
"	Fe(III)	100	.355	-22
"	Al	200	.460	1.1
"	In	100	.490	7.7
"	Ga	10	.650	43
"	Bi	100	.370	-18.5
"	Cr	100	.380	-16.5
"	Sb	100	.542	19.1
"	Zr	100	.280	-38.5

TABLE XIII (continued)

Standard	Interference	Molar Excess	Absorbance 675 mu	% Interference
10 ml 10^{-4}M Mo	none	-	.455	-
"	Th	100	.480	5.5
"	Ti	100	.390	-14.3
"	Chromate	100	.275	-39.5
"	Tungsgate	100	.024	-95
"	Vanadate	100	.380	-16.5
"	Thiocyanate	1000	.455	-
"	Tartrate	100	.458	-
		1000	bleaches solution	
"	Oxalate	100	.420	-7.7
"	Fluoride	1000	.460	-
"	Cyanide	75	.463	1.75
"	Ascorbate	100	.455	-
"	Citrate	100	.381	-16.25
"	Sulphosalyalic Acid	1000	.468	3.5
"	E.D.T.A.	1000	.124	-72.5

Solvent Extraction

The complex may be extracted into chloroform but the reagent excess is critical. The ratio of C.V. to C.T.A.B. is also critical and must be ca 1:1 for maximum extraction.

The wavelength of maximum absorbance of the complex is shifted from 675 mu in aqueous to 635 mu in chloroform.

EXPERIMENTAL

Reagents

All reagents are of analytical grade unless otherwise stated.

Ammonium Molybdate Stock Solution 10^{-2}M Dissolve 1.766 g of the heptamolybdate hexahydrate in 1 litre of distilled water, 10^{-4}M Solution prepared by appropriate dilution of stock solution.

Catechol Violet Solution 10^{-3}M Dissolve .387 g of Catechol Violet in (Hopkin and Williams) 1 litre of distilled water.

Cetyl Trimethyl Ammonium Bromide Solution 10^{-3}M Dissolve .364 g of C.T.A.B. (G.P.R.) in 1 litre of distilled water.

Acetate Buffer pH 3.85 Dissolve 50 ml of glacial acetic acid

in distilled water and adjust to pH 3.85 with ammonia, make up solution to 250 ml.

Apparatus

Unicam S.P.600 Spectrophotometer, fitted with 1-cm glass cuvettes.

CHAPTER X

A COLOUR REACTION FOR ANTIMONY

The development of a sensitive colour reaction for antimony is of particular interest because there are very few sensitive spectrophotometric methods available for this element. Perhaps the best known is that involving the formation of a ternary complex with Rhodamine B and antimony chloride ($SbCl_6^-$).[52] Although this reaction is reasonably sensitive (E545 = 34,000), it involves initial oxidation of the antimony(III) to the unstable antimony(V) and it has been reported that it has been found difficult to obtain reproducible results.[72]

More recently a method has been developed utilizing the antimony(III) /Bromopyrogallol Red complex[72] which, although sensitive (E560 = 39,000) involves measuring a decrease in the absorbance of the reagent on complex formation which is not particularly satisfactory.

It was thus decided to investigate the possibility of developing a spectrophotmetric procedure utilizing the antimony (III)/C.V./C.T.A.B. complex

Preliminary Investigation

Choice of Reagent

It was mentioned previously (page 147) that antimony was observed to form a complex with Catechol Violet and C.T.A.B. However, because the antimony-B.P.R. system has already been used spectrophotometrically and because B.P.R. is a similar dyestuff to Catechol Violet, an attempt was initially made to 'sensitize' the existing procedure involving B.P.R. by the addition of C.T.A.B. Unfortunately C.T.A.B. itself causes a colour change with B.P.R. and although the prescence of antimony causes an additional colour change, the sensitivity was too low ($E = 10,000$) to warrant further investigations.

The formation of the antimony/C.V. complex is not accompanied by a distinct colour change as with molybdate and tin. The colour reaction consists only of a slight broadening of the absorption spectra of the C.V. The resulting complex has the same wavelength of maximum absorbance as C.V., but the absorbance of the reagent is decreased on complexation (similar to B.P.R.) When C.T.A.B. is present a distinct colour change occurs. Further investigations showed that not one, but two, different

complexes are formed depending on the relative ratios of the reagents.

When the C.V. is in two fold excess with regard to the C.T.A.B., a blue coloured complex (similar to the corresponding molybdate and tin(IV) complex) is predominantly formed. However, if the two reagents are present in equimolar proportions or the C.T.A.B. is in excess, a red complex is predominantly formed.

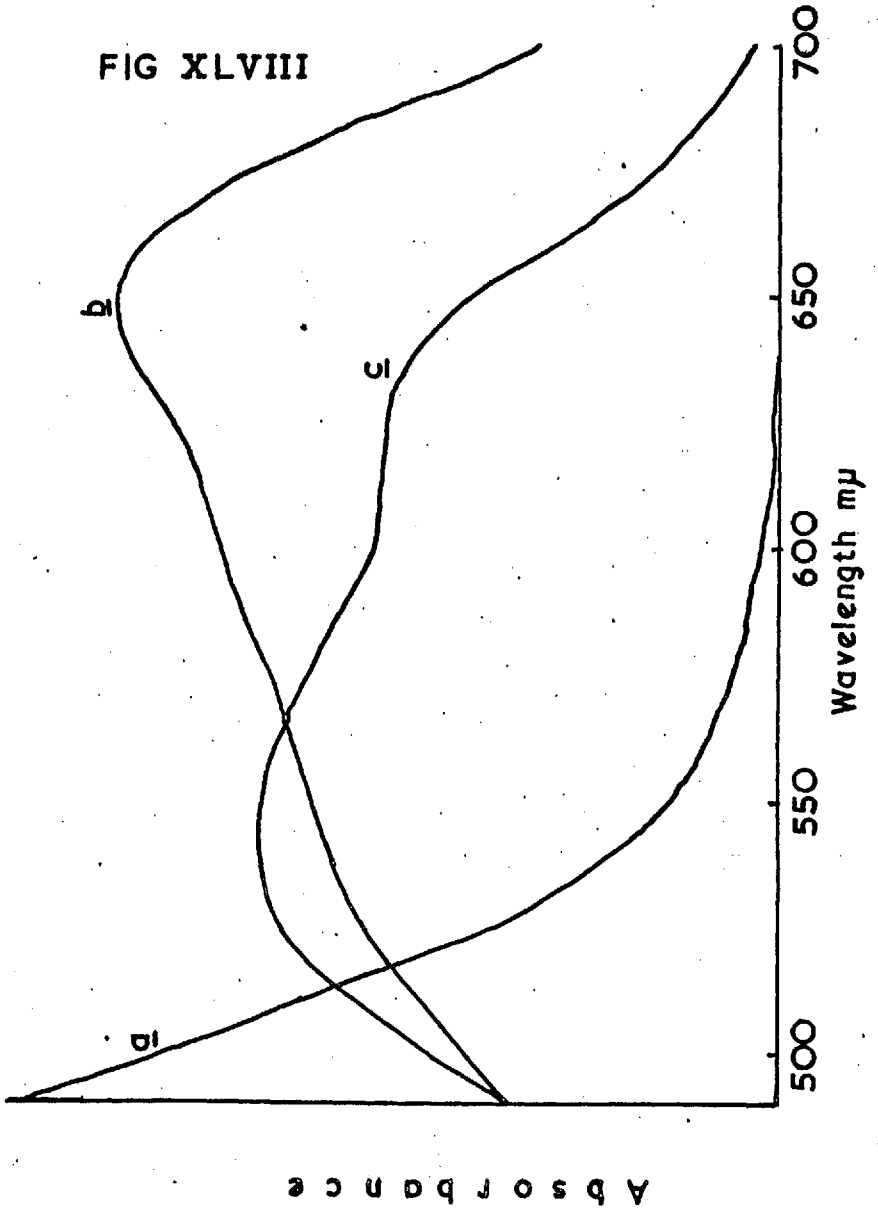
Preliminary Spectra

The absorption spectra of the two complexes is shown in Fig XLVIII. Curve (b) shows the absorption spectra of the complex with a spectra of the complex with a ratio of C.T.A.B. to C.V. of 1:2.

Curve (c) shows the absorption spectra of the complex when the ratio C.T.A.B.:C.V. is 1:1. The concentration of antimony was the same in both cases and the absorbances were measured against a reagent blank (curve a).

The molar absorptivity of the 'blue' complex is slightly higher (E 660 ca 20,000) than the 'red' complex (E540 ca 17,000) however the molar absorptivity of the latter is increased when

FIG XLVIII



a five fold excess of C.T.A.B. over C.V. is used.(E530 ca 30,000) In consequence it was decided to continue investigations using the 'red' complex.

Effect of pH

The curve in Fig XLIX shows the effect of pH on the complex (curve a) and the reagent system. The optimum pH is 4-5 however, the effect of pH is not critical over the range pH 2-6. Above pH 6.5 the reagent blank increases considerably.

Curve (a) was prepared by adjusting a series of solutions containing 1 ml 10^{-3}M antimony, 2 ml 10^{-3}M C.V., and 10 ml 10^{-3}M C.T.A.B. to the required pH with ammonia and acetic acid (for pH 2.3 nitric acid was used).

Curve (b) was prepared in a similar way but contained no antimony.

Nature of Complex

It was found impossible to determine the composition of the complex by the normal spectrophotometric techniques due to the competitive formation of the two different complexes with the different reagent concentrations. An attempt was made to find

the ratio of antimony to C.V. when C.T.A.B. was in excess using a Mole ratio plot. It was found that when antimony is added to a series of solutions containing fixed amounts of C.V. and antimony, the absorbance of the blue complex increases with respect to that of the red complex, as the antimony:C.V. ratio tends to unity. A meaningful interpretation of the results was impossible.

Development of Analytical Procedure

Previous methods for antimony e.g. that using B.P.R. have utilised E.D.T.A. as a mass masking agent to achieve selectivity. The effect of E.D.T.A. on the present colour reaction was examined and it was found that the colour of the complex in its presence was very pH dependant Fig L (a). The complex was not formed at pH 3 and the optimum pH was 6.

The curves in Fig L were prepared in a similar manner to those in Fig XLIX except they also contained 1 ml of a 10^{-1}M E.D.T.A. solution.

Reagent Excess

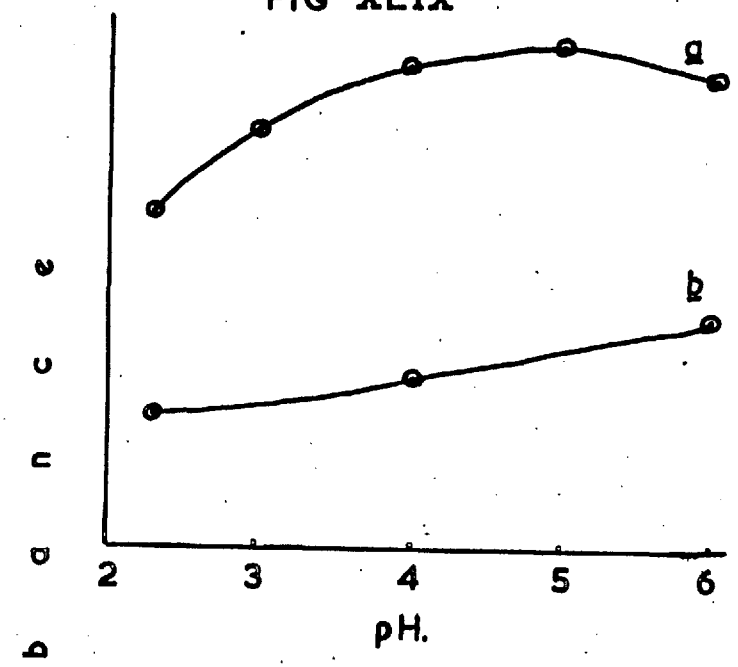
A minimum 5 fold molar excess of C.T.A.B. over C.V. must

be used to obtain maximum sensitivity. The excess of C.V. over antimony is also important; as the excess of C.V. is increased the wavelength of maximum absorbance of complex, measured against a reagent blank, undergoes a bathochromic shift and sensitivity falls. A minimum 2.5 fold molar excess of C.V. over antimony was found to be necessary for Beer's law to be obeyed over the range 0.3-3.0 ug/ml. of antimony - Fig LI. When the excess falls below 2.5 M the absorbance of the 'blue' complex increases at the expense of the 'red' and as a result Beer's law is no longer obeyed.

Lambert-Beer Law Check

Beers law is obeyed over the range 0.3-3.0 ug/ml of antimony - Fig LI. The calibration curve in Fig LI was prepared by diluting to 100 ml with distilled water a series of solutions containing .5-5 ml 5×10^{-4}M antimony, 5 ml 10^{-3}M. C.V., and 25 ml 10^{-3}M C.T.A.B. , and 2 ml 20% ammonium acetate (pH 5.8) buffer. The absorbances were measured at 530 mu in 1-cm cuvettes against a reagent blank prepared in a similar manner, but containing no antimony.

FIG XLIX



Absorbance

FIG L

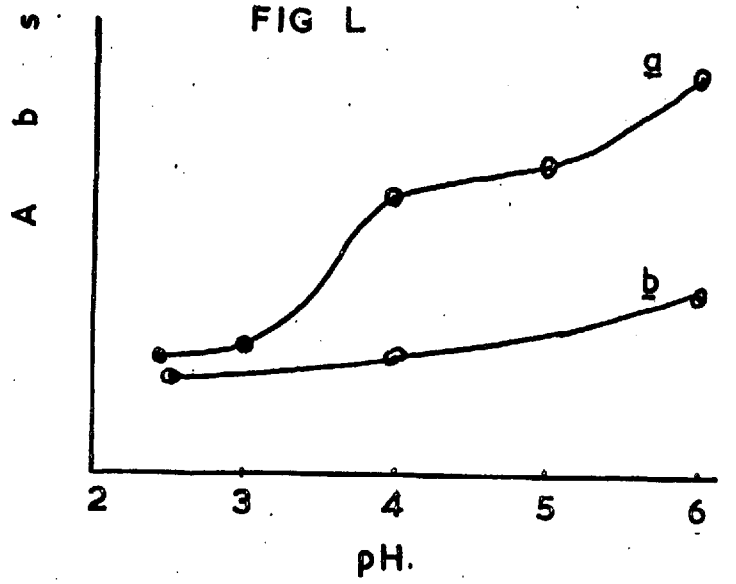
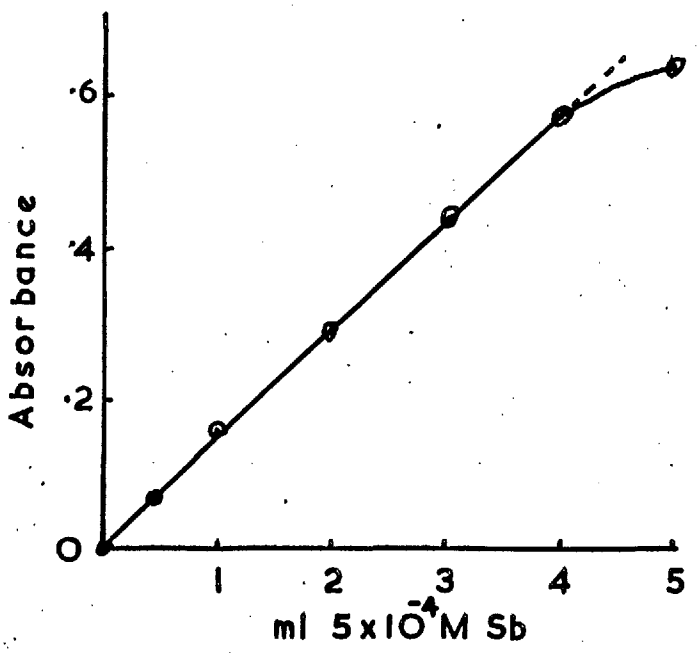


FIG LI



Sensitivity

The molar absorptivity of the system calculated from the calibration curve is 30,000, but the prescence of E.D.T.A. lowers this to 28,000 for a 100 fold molar excess and 24,000 for a 1,000 fold molar excess.

Interferences

The major interferences are similar to those in the B.P.R. procedure namely niobium(V), zirconium, uranium, molybdate and tungstate.

Solvent Extraction

Preliminary Investigation

The antimony/C.V./C.T.A.B. complex may be extracted into chloroform. However, reagent excesses are not as critical as was found with molybdate. The reagent ratios are still important, because the two different coloured species (red and blue) are still evident in chloroform, but the 'red' complex is now the preferred form. The curve (c) in Fig LII shows the absorption spectra of the antimony complex after extraction into chloroform

when the ratio of C.T.A.B.:C.V. is 1:1. There is little evidence of the 'blue' complex in this instance.

Curve (b) in Fig.LII shows the absorption spectra of the complex in chloroform when the ratio of C.T.A.B.:C.V. is 1:2.

In both cases the antimony and C.V. concentrations are the same and absorbance measurements were made under the same conditions against a reagent blank (curve a).

The sensitivity of the procedure (using the red complex) is increased by extraction to give a molar absorptivity of 40,000. The presence of 100 fold and 1,000 fold excess of E.D.T.A. depress the molar absorptivity to 34,000 and 33,000 respectively at pH 6. Beers law is obeyed over the same range as in aqueous solution.

The increase in sensitivity caused by extraction does not seem worth the additional step and it is proposed that determinations be made in aqueous solution where possible.

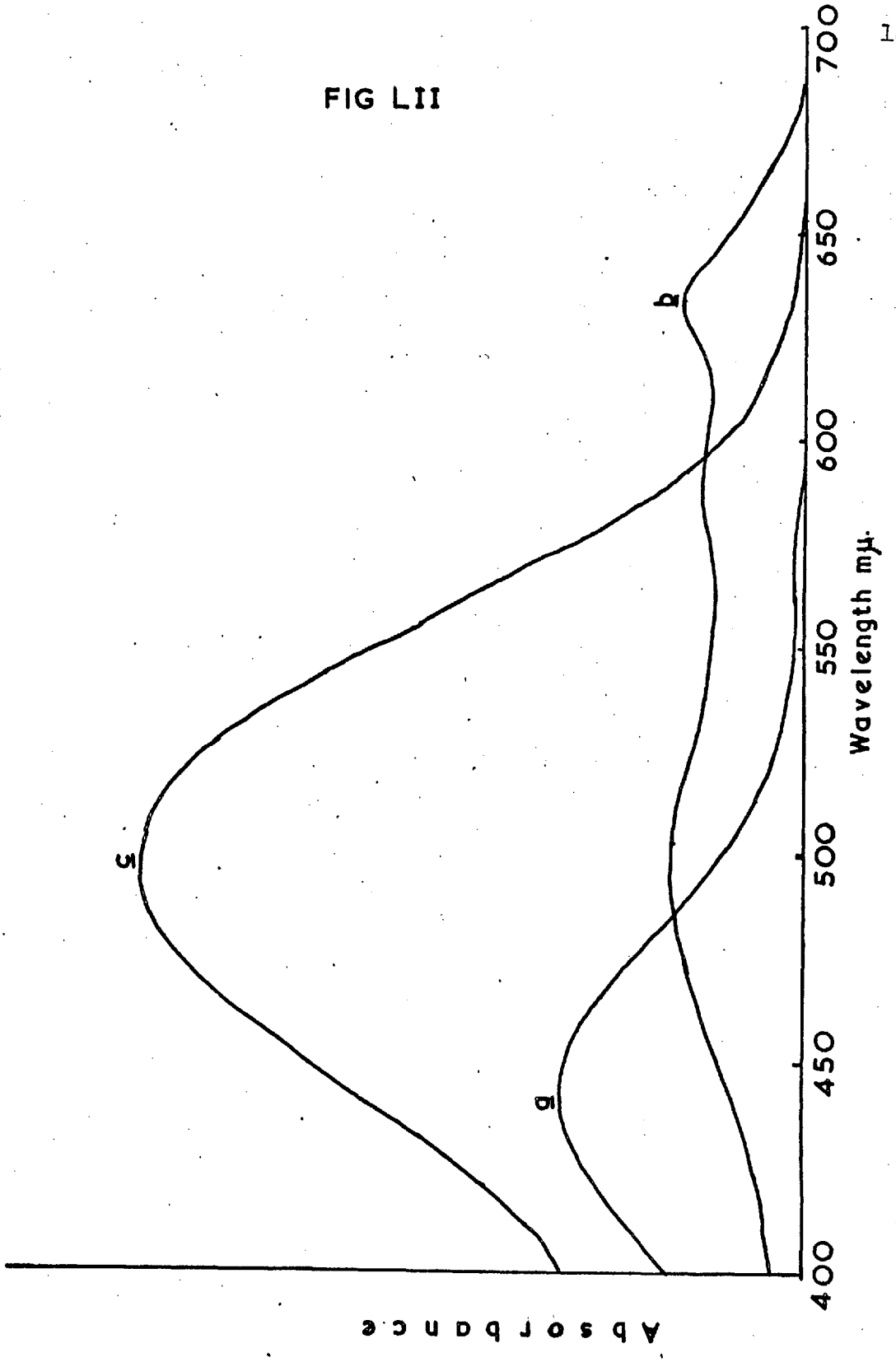
Experimental

Reagents.

All reagents are of analytical grade unless otherwise stated.

Potassium Antimonyl Tartrate solution. Prepared by appropiate dilution of standardised (bromate method) 10^{-2}M potassium antimonyl tartrate.

FIG LII



Catechol Violet 10^{-3}M solution prepared by dissolving 0.387 g. of Catechol Violet in 1 litre of distilled water.

Cetyl Trimethyl Ammonium Bromide 10^{-3}M solution prepared by dissolving 0.364 g. of C.T.A.B. (G.P.R.) in 1litre of distilled water.

Ammonium Acetate pH 5.8 buffer. Prepared by dissolving 20g. of ammonium acetate in distilled water, adjusting to pH 5.8 with acetic acid and diluting to 100ml.

Apparatus

Unicam S.P.600 spectrophotometer fitted with 1-cm. glass cuvettes.

C H A P T E R XI

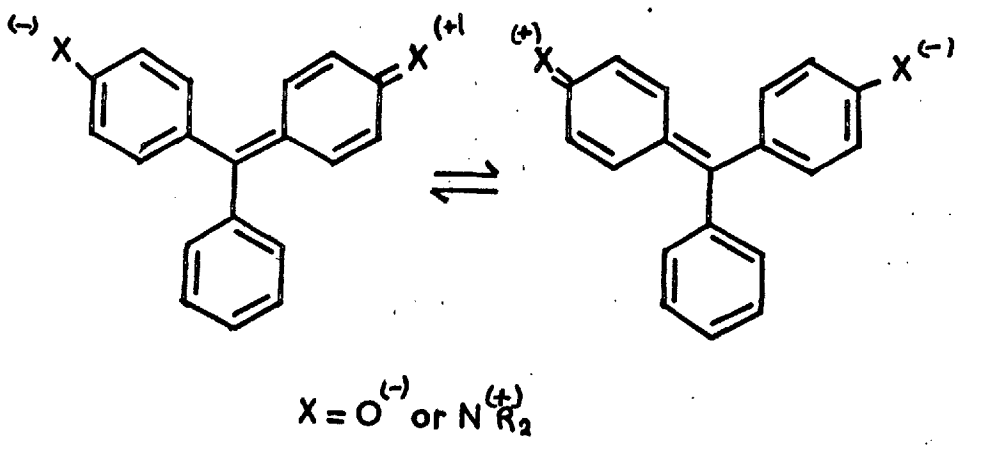
SOME ASPECTS OF COLOUR REACTIONS IN TERNARY COMPLEXES

The causation of the colour changes which occur on formation of ternary complexes will be dealt with in this chapter. The situation is rather complex and it is therefore not intended to give a complete explanation of the particular colour changes in each system, but rather to provide an overall explanation of why these colour changes occur.

The two types of ternary complexes investigated (i.e. the phenanthroline and surfactant complexes) have been shown to have distinct similarities and the situation is examined for both simiItaneously.

In determining the cause of the colour changes in these two systems it is best to distinguish between the two types of dyestuffs used. The first type do not contain complexing centres and do not react with metal ions, e.g. Rose Bengal Extra and Bromophenol Blue; whilst the second type do e.g. Bromopyrogallol Red, Catechol Violet, Chrome Azurol S.

The colour of triphenyl methane dyestuffs is due to resonance of the type:-



In general the phenyl group takes little part in the transition.[73] Initially it was thought that the colour reaction was a result of a charge transfer phenomenum or electrostatic interactions with charged micelles, both of these involving (in the dyes mentioned) with the hydroxyl groups in the rescorcinol part of the molecule.

The first type of dye to be considered only formed ternary complexes with the metal phenanthrolines. Since the colour change on complexation with the various metal phenanthroline complexes were identical, it seems unlikely that the change is caused by charge transfer. Furthermore, because the colour of the complexes are the same in aqueous and organic media, and because in the latter instance the complexes are in solution (hence no micelles exist), it seems that the adsorption (to micelles) theory must also be discounted.

It has already been stated that the colour of the dyestuff is primarily due to resonance in the resorcinol molecule. In the

phenanthroline complexes it has been established from the molecular ratios obtained for various complexes that both the resorcinol and and phenyl groups are involved in complexation, Interaction with the carboxylic acid group may, for steric reasons, cause the phenyl group to twist out of the plane of the resorcinol molecule. This would cause a change in the inductive effect of the phenyl group on the resorcinol part of the molecule and may thus cause a slight colour change.

However, it has been reported[74] that the esterified tetrabromo (D)fluorescein undergoes similar colour changes on adsorption to proteins as do the non-esterified fluoresceins such as R.B.E. This indicates that it is interactions with the hydroxylic group in the resorcinol part of the molecule that causes the colour change.

The theory postulated is that the formation of the complex, i.e. the association of the dyestuff with the charged phenanthroline, cause a slight change in the resonance structure of the dye as a result of an inductive and/or resonance effect via the hydroxyl group. This would account for the slight colour changes observed.

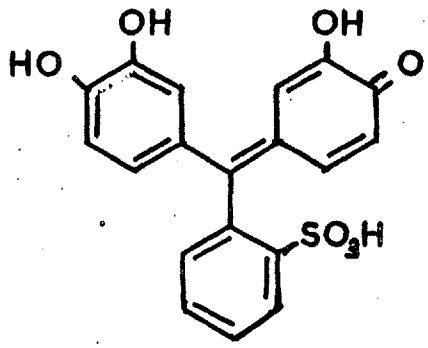
If this is so it would be theoretically possible to increase the molarabsorptivities of the complexes by a factor of 2 if dyes with blocked carboxylic acid groups are used, as the molecular ratio of

dyestuff to complex will be increased by a factor of 2.

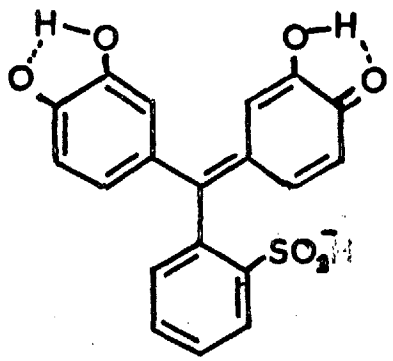
With the second type of dyestuff the situation is more complicated. Cations, and in some cases complex anions such as molybdate and tungstate, chelate directly with the dyestuff and effect a colour change. The addition of a surfactant or 1,10-phenanthroline causes a further colour change. The process can also occur in reverse in some instances; the dye can undergo a colour reaction with the surfactant and then further change when a cation is added.

The initial colour change in the system is a result of the manner in which the particular cation or anion affects the resonance system of the dye and may or may not be unique. The addition of a surfactant or 1,10-phenathroline will affect the system in one of two ways; the first is by complexation with the cation (as with 1,10-phenathroline), thereby changing the effect the cation has on the resonance system. In the second case (as with the quarternary nitrogen systems) the surfactant becomes directly attached to the resonance system, displacing a hydrogen ion thereby affecting the resonance system and causing a colour change. This latter point is illustrated by Catechol Violet and its ternary complexes. The colour changes with the pH and on complex formation are very similar and the mechanism postulated is shown below.

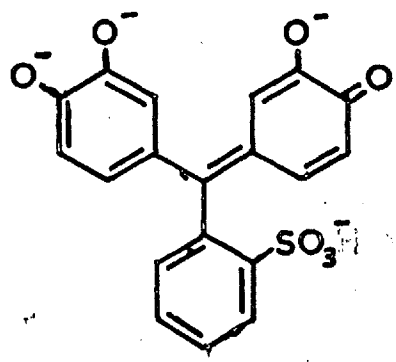
pH 2-6 yellow



pH 6-12
red-purple

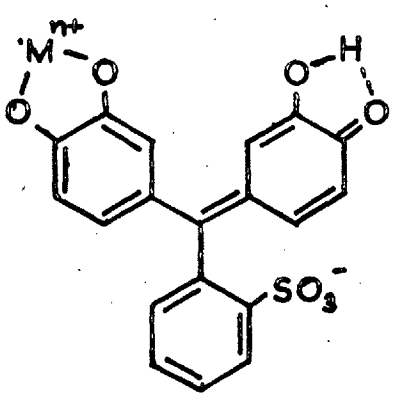


pH 12
blue

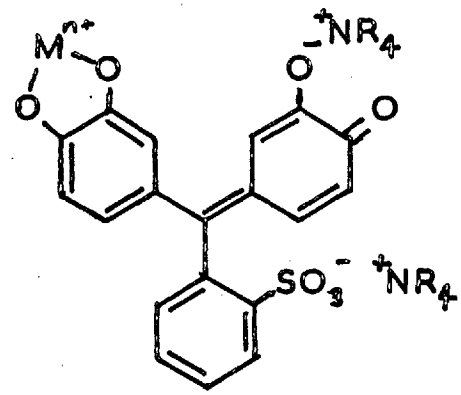


pH 2-6

red-purple



blue



In some instances a mixture of the two mechanisms occurs, as with C.A.S., where the copper/1,10-phenanthroline complexes at one end of the ligand and acts as a surfactant at the other.

Although the colour change explanations that have been postulated are obviously not the 'whole' truth, they do give some insight to the reaction mechanism involved in these ternary complex systems. Thus it becomes possible to predict the formation etc. of a particular ternary system.

C H A P T E R XII

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

A number of spectrophotmetric procedures using ternary complexes have been developed. The sensitivity and relative specificity of the reactions involved illustrates the potentialities of such ternary complexes in spectrophotometry.

In the various investigations carried out in the search for suitable reagents the full circle has been completed. Initially B.P.R. was abandoned for the more specific reagents which do not contain complexing centres. Finally a return to reagents containing complexing centres was made in order to increase the scope of ternary complexes to include ions which do not form phenanthroline complexes.

Some insight has been gained into the mode of complex formation in both types of complex and postulations have been made as to the causation of the colour changes.

The scope for future work in this field is very large, To consider first the ternary phenanthroline complexes. The development of spectrophotometric (and spectrofluorimetric) procedures for the determination of the various ions forming complexes with 1,10-phenanthroline may now be accomplished .

Increased sensitivity with this type of complex may be obtained using reagents similar to R.B.E. but with the carboxylic acid group blocked. In this way it may be possible to obtain complexes with molar absorptivities of the order of 200,000.

The substitution of sulphur for nitrogen in the donor ligands affords another line for further development. Use of such ligands will enable other cations to be determined with this type of complex and yet a reasonable degree of selectivity will be retained.

The design of ligands to replace 1,10-phenanthroline provides another interesting field for future work. The ideal ligands should be strongly hydrophobic so that on complexation with a cation a surfactant type molecule would be produced.

The compound envisaged would contain a long chain alkyl group attached to the chelating part of the molecule.

With the second type of ternary complex there is much work to be done in investigating the formation of other similar complexes. There are a number of existing spectrophotometric procedures utili-

zing the chelate complexes of ligands similar to Catechol Violet.

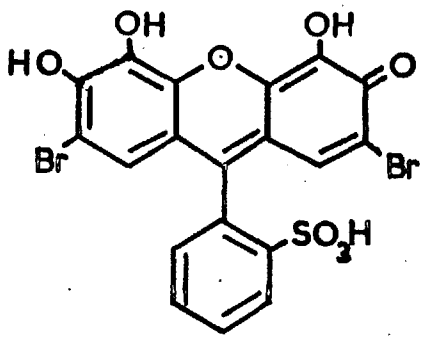
Many of these complexes may also form ternary complexes with surfactants with a resulting increase to their sensitivity.

Finally the substitution of quarternary phosphonium salts for the quarternary ammonium salts may provide interesting results.

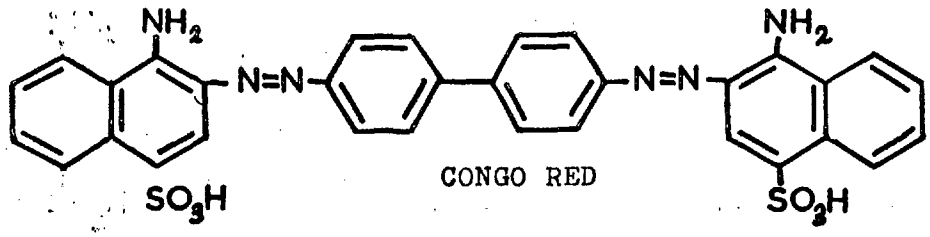
A P P E N D I X

APPENDIX I

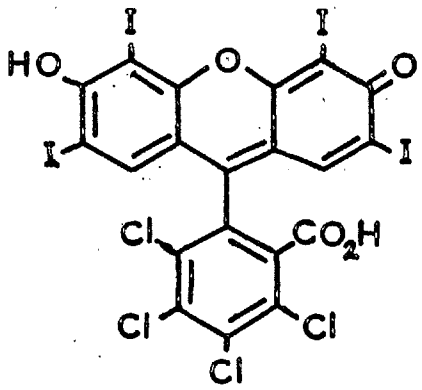
STRUCTURAL FORMULAE OF DYESTUFFS



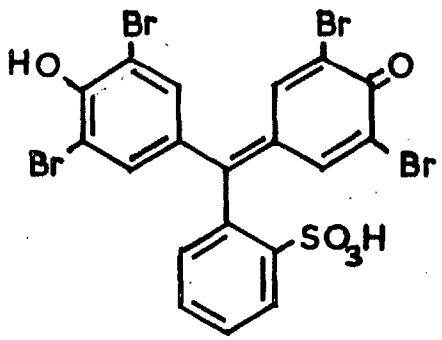
BROMOPYRAGALLOL RED



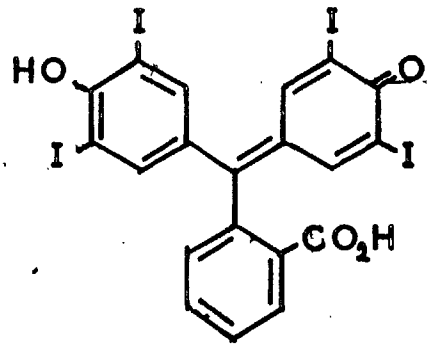
CONGO RED



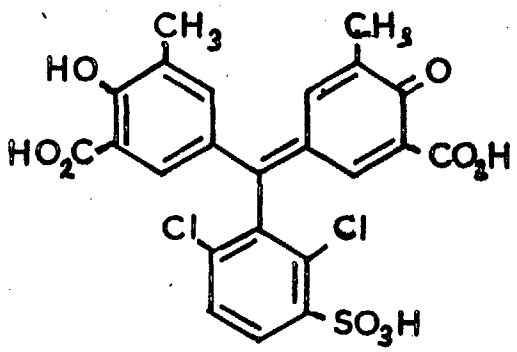
ROSE BENGAL EXTRA



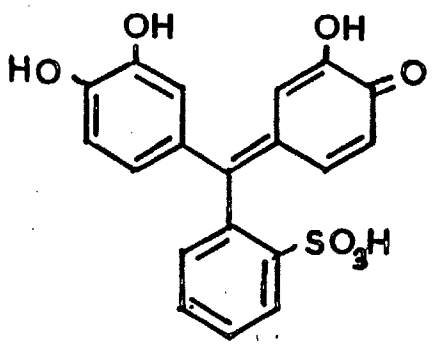
BROMOPHENOL BLUE



ERYTHROSIN



CHROME AZUROL S



CATECHOL VIOLET

APPENDIX II

PURIFICATION OF ROSE BENGAL EXTRA

Anomalous results were obtained in attempts to determine the structures of the various metal/phenanthroline/R.B.E. complexes .

R.B.E. contains 2 replaceable protons, yet the molecular ratios of M^{n+}:R.B.E. obtained for the various ternary complexes were n:1 rather than the expected n:n/2.

The microanalysis results of a sample of R.B.E. showed that the compound was indeed impure (the results are shown in Table XIV)

A further sample was subjected to a thin layer chromatographic examination run on a silica gel substrate using a solvent of .880 ammonia ethanol and water in the ratio 4:80:16. The crude R.B.E. dissolved in the solvent, but left a whitish crystalline residue. The chromatogram showed that R.B.E. was the major coloured constituent and examination of the chromatogram under U.V. light confirmed that lesser halogenated fluoresceins were only present in trace amounts.

It was thought that the major impurity must be that portion which is insoluble in the ammonia/ethanol/water solvent.

The following attempted method was used to purify the R.B.E. The crude dye was dissolved in ammonaical ethanol, and filtered.

The dye was precipitated (in the acid form) by the addition of concentrated acid to the filtrate. The precipitate was washed and dried in an oven at 60°C.

The results obtained from microanalysis of the sample (Table XIV) showed that the compound was still impure. However a potentiometric titration of a sample (Fig LIII) showed that two replaceable hydrogens per molecule were present. Furthermore Mole ratio plots etc. with various metal phenanthrolines gave results which indicated that the dye was reasonably pure.

TABLE XIV

Microanalysis Results

	%Composition	
	C	H
Calculated	23	.2
Crude R.B.E.	20	2.2
Purified (1) R.B.E.	25	.78
Purified (2) R.B.E.	29	.4

Another attempted purification was as follows. The R.B.E. was extracted from 4N sulphuric acid media into chloroform. The chloroform

phase was washed with a slightly acidic aqueous solution. The chloroform extract of the R.B.E. was then carefully evaporated to dryness and the dye was dried at 60°C.

The analysis results (TableXIV) showed that the dye was still impure; but as before a potentiometric titration (Fig LIV) showed two replaceable protons per molecule and Mole ratio studies again gave results which indicated that the dye was pure.

Further evidence indicating a reasonably pure dye was obtained by comparing molar absorptivities in aqueous solution of the crude R.B.E., purified R.B.E. and Erythrosin (of known purity). The results (Table XV) show that the purified R.B.E. is at least double the purity of the crude and in comparison with Erythrosin is reasonably pure.

TABLE XV

	Molar Absorptivity (540 mu R.B.E.;525 mu Erythrosin)
Crude R.B.E.	46,000
Purified R.B.E.	91,000
Erythrosin	92,000

It was concluded therefore that the purified R:B.E. is inspite of the analysis results better than 90% pure.

EXPERIMENTAL

The curve (a) in Fig LIII was prepared by titrating potentiometrically 0.0973 g of R.B.E. (purified by the first method mentioned) in 50 ml of distilled water against N/10 sodium hydroxide.

Curve (b) shows the blank containing no R.B.E.

The curves in Fig LIV were prepared in a similar manner using 0.0428 g of R.B.E. purified by the second method.

Results

Purification I

Wt. of R.B.E. sample = .0973 g

ml N/10 NaOH consumed = 2.2-.3

= 1.9

Assuming R.B.E. to be dibasic

21 N NaOH ≡ 974 g R.B.E.

i.e. 2 ml N/10 NaOH ≡ .0974 g R.B.E.

The result obtained deviates 5% from the theoretical.

Purification 2

Wt. of R.B.E. sample = .0428

ml N/10 NaOH consumed = 1.1-.25

= .85

As before assuming R.B.E. to be dibasic

21 N NaOH ≡ 974 g R.B.E.

i.e. .85 ml N/10 NaOH ≡ $\frac{.0974}{2}$ x .85 g R.B.E.

≡ .0414 g R.B.E.

i.e. the result obtained deviates 3% from the theoretical.

FIG LIII

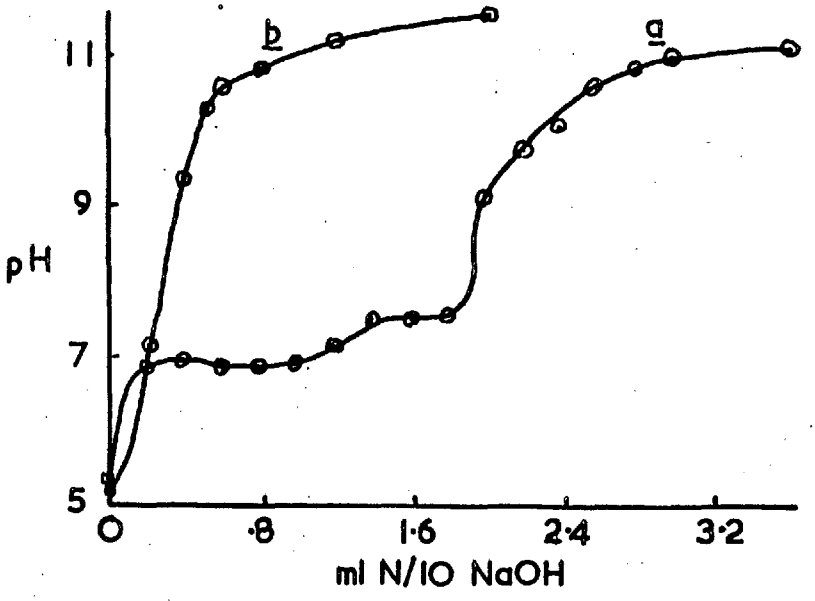
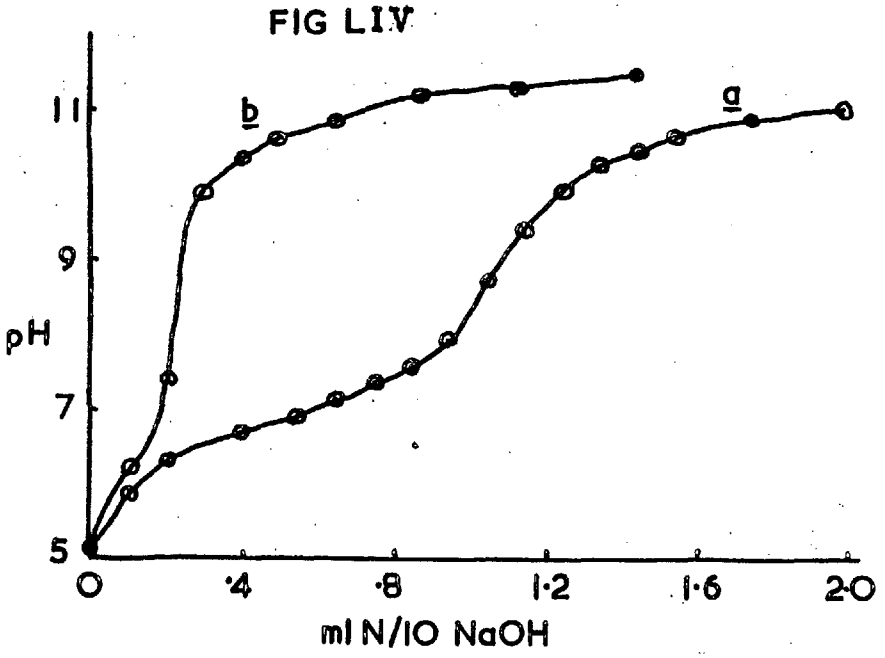


FIG LIV



APPENDIX III

In chapter III it was mentioned that if the solutions of copper/1,10-phenanthroline/R.B.E. in the ammoniacal/acetone/chloroform media were left for a few days , a yellow colour (maximum absorbance 445 mu) developed and furthermore the intensity of the colour was proportional to the concentration of the complex.

The intensity of the colour increased with time, and if the solutions were left for a period of four weeks an apparent molar absorptivity of 10^6 was obtained. In view of this very high sensitivity it was decided to investigate the phenomena further and attempt to accelerate the process.

The very high molar absorptivity indicated that the phenomena was caused by some sort of catalytic action. The only reagent, apart from the solvent, liable to be present in excess was the 1,10-phenanthroline. At first it was thought that the colour was due to the decomposition of the 1,10-phenanthroline. However, subsequent work carried out showed that this was not true , and that the yellow colour was developed in ammoniacal/acetone/chloroform solutions of R.B.E. alone. However, the R.B.E. itself is not affected as the absorbance of the R.B.E. is the same before and after the for-

mation of the yellow colour. Fig LV shows the absorbance spectra of a solution 10^{-5}M in R.B.E. in an ammonia/acetone/chloroform media before (curve(a)) and after (curve (b)) the formation of the yellow product.

The yellow product is also produced if Erythrosin is used instead of R.B.E.

In both cases the prescence of ammonia is necessary for the colour formation to occur. It seems then that the colour is a result of catalytic action of the fluorescein based dyes on the solvent, probably the acetone as it is known to undergo polymerization in basic media.

Isolation of Product

An attempt was made to isolate the yellow product. An ammonia/acetone/ chloroform solution containing the yellow product was diluted with distilled water until the chloroform phase became immisible with the rest. The yellow product remained in the chloroform phase whilst the R.B.E. remained in the aqueous phase. Attempts to crystallise the yellow product from a number of solvents including chloroform, benzene, acetone, ethanol, and petether were unsuccessful. In all cases a yellow-brown oil was the only

product obtained.

A sample of the oil was examined by thin-layer chromatography and found to be a complex mixture rather than a pure compound.

Acceleration of Reaction

A solution of R.B.E. in ammonia/acetone/chloroform was subjected to strong irradiation (both U.V. and visible) and heating , but the rate of formation of the yellow product was not noticably increased.

Discussion

Whilst the colour reaction is of interest in view of the high sensitivity , its application to spectrophotometry is limited because of the length of time taken for the colour development.

A calibration curve (Fig LVI) was obtained following the procedure devised for the spectrofluorimetric determination of copper. The solutions were then left for 5 days under normal laboratory conditions and the absorbances were then measured in 1-cm cuvettes at 445 mu against a blank carried through the same procedure, but containing no copper. The final concentrations of copper(and R.B.E.) were in the range $2\text{-}10\times10^{-7}$M.

The molar absorptivity after 5 days was calculated from the calibration curve as ca 154×10^{3}.

FIG LV.

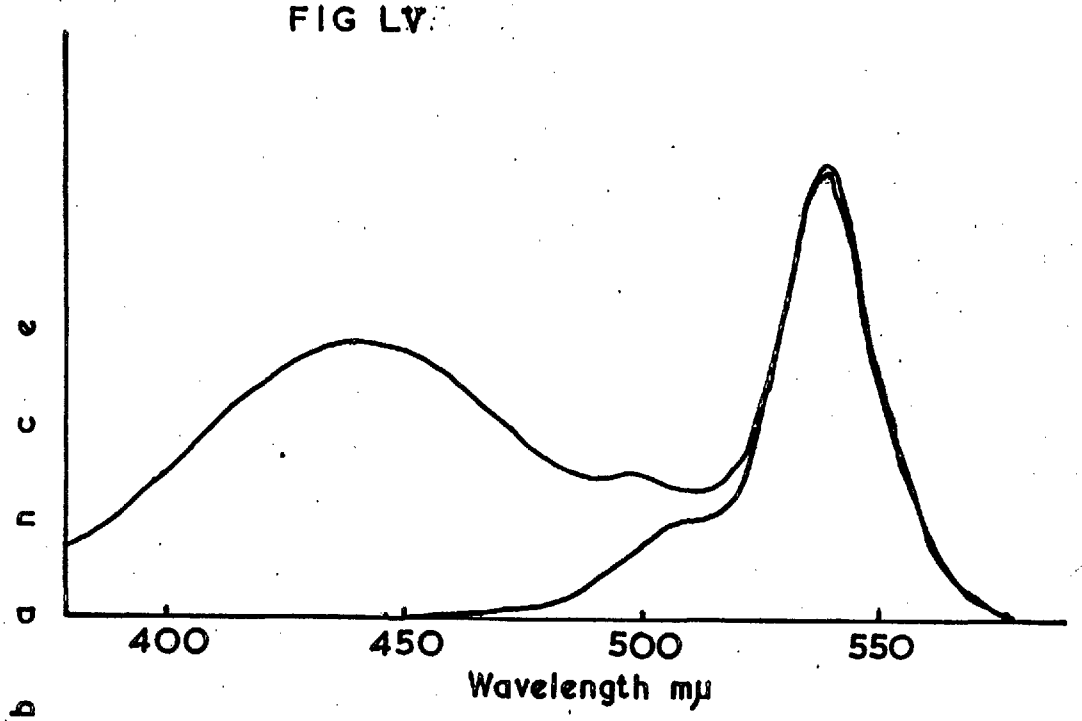
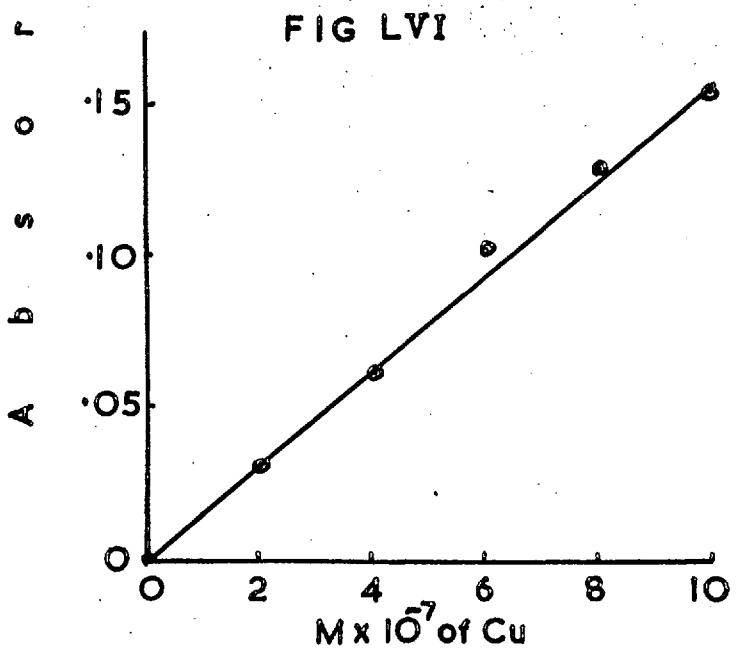


FIG LVI



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