Azo dyes and their metal complexes

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AND DYES AND THEIR METAL COMPLEXES

by

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Index

Acknowledgement (2)

Summary of Thesis (11)

Chapter I Introduction

Chapter II Polarographic Instrumentation

Chapter III Theoretical basis of Measurements, General Techniques and Reagents Used

Chapter IV α,α'-dihydroxy azo dyes

  Section (1) Cellochrome Violet 50
  Section (11) Cellochrome Black 7
  Section (111) Cellochrome Blue Black
  Section (iv) Bordeaux on Chroma Solido II

Summary

Chapter V α,α'-dihydroxy pyrazolone dyes

  Section (1) Cellochrome Red 7X 125
  Section (11) Cellochrome Red G

Summary

Chapter VI α-hydroxy α'-amine azo dyes

  Section (1) Cellochrome Brown AS 125
  Section (11) Organmittelbraun C

Summary

Chapter VII α-hydroxy o-carboxy azo dyes

  Section (1) Cellochrome Red B 125
  Section (11) Cellochrome Yellow II 125

Summary

Chapter VIII Cylindroazo dyes

  Section (1) PMG
  Section (11) PMK

Summary

109
Chapter IX: Other dyes investigated
  Section (i) Calcein brom
  Section (ii) Thiorin
  Summary
Chapter X: Discussion and Conclusions
References
Acknowledgement

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Various types of ortho-substituted benzene dyes and their complexes have been studied by polarography. The polarography of the dyes themselves has been shown to involve a more complicated mechanism than a pure diffusion step. The influence of reduction and kinetic processes was observed in some cases.

The o,o'-dihydronaphtho-oxazone dyes studied all gave complex steps of displaced half-wave potential with one metal ion, although the displacement was greatest with naphthol blue-black and o,o'-dihydronaphtho-oxazone. The other dyes behaved differently; the dyes which form a number of dyes, so the complex step did not remove the dye step completely, even than excess of metal ion is present.

o,o'-Dihydronaphtho-oxazone dyes were found to behave similarly to the o,o'-dihydronaphtho-oxazine dyes.

Complex steps of displaced half-wave potential with other oxazine dyes were observed only in the instance of o,o'-dihydronaphtho-oxazine and with terpillar - naphtho-oxazine.

Polarographic studies of o,o'-dihydronaphtho-oxazine and its chromium complex showed that complex formation involved only one of the three ring groups and two of the three hydrazyl groups.

Catalytic reduction results confirmed this; two hydrazyl on iron were liberated per diimine dianion.

An important step in the polarographic determination of hydrazines has been developed and a method
for the removal of calcium has been discussed. It has been shown that complex formation does not necessarily give a displacement of the dye step, and that such cases are the exception rather than the rule. Theories accounting for the occurrence of the displaced step have been suggested.
Introduction

The first polarographic study of the compounds was made in the period 1931-1933 by Teodoro and Ballesteros who examined acenaphthene in buffered alcoholic solutions and suggested that a two-electron reduction to hydroacenaphthene occurred. Woltoff and Duggan doubted the validity of Teodoro's equation relating half-wave potential to pH:

$$E_1 = E_0 - 0.059 \log_{10} pH$$

because he had used varying amounts of alcohol and, in addition, did not take into account the effect of alcohol on the pH of the solutions.

In 1937, Arlton separated the cis form of acenaphthene from the trans form by extraction procedures and in 1938 he published further data on the equilibrium mixtures in various solvents and the rates of thermal conversion for acenaphthene and several of its derivatives. Abbott's examination of the trans and cis isomers by X-ray methods and showed the structure of the former to be planar as illustrated:

\[
\text{cis form}
\]

whereas for the cis isomer steric hindrance caused the rings to be rotated from the $C=C=C=C$ plane by $40^\circ$ as can be seen below.

\[
\text{cis form}
\]
Hinkel and Elbert\textsuperscript{9} studied the isocitrate polarographically and obtained values of \(-0.8\) and \(-0.97\) volts respectively for their half-wave potentials. Williams and Birnbaum\textsuperscript{10}, on the other hand, found that the half-wave potentials were identical below pH 6.5 and varied with pH according to the equation:

\[ E = 0.09 - 0.06 \text{pH} \]

In contrast Volpi\textsuperscript{11} reported the half-wave potential to be independent of pH. At higher pH values the half-wave potentials of the isocitrate were different. These facts were attributed to the reversible cis form being converted rapidly to the trans form which was then reduced. The speed of conversion was faster than the electrode processes; hence, only one step was produced. As the pH was increased the reduction became more irreversible and a larger overpotential was required to affect the reduction. The overpotential required depended on the isocitrate reduced, and hence, on the first stage in the reaction was closer at high pH, two steps were formed.

Cantor and Taylor\textsuperscript{12} showed that if buffer solutions of sufficient capacity were used, the isocitrate had identical half-wave potentials up to pH 12.5. When citrate-phosphate buffer was used the reduction was reversible and involved two electrons; in acetate buffer the reaction was not fully reversible. In the former buffer system the half-wave potentials were found to vary linearly with pH according to the equation:

\[ E = 0.060 - 0.062 \text{pH} \]

up to pH 12.5.
Widman and Alm\textsuperscript{15} had previously shown that mixtures of
alkyl substituted azobenzene compounds and the corresponding
hydroazobenzene compounds formed reversible redox systems at
the platinum electrode in acidic solution. Their results
were confirmed by Currell and Ibbett\textsuperscript{16} who showed that even the
mildest reducing agents caused complete reduction to the azine.
The rate controlling reaction was shown to be unimolecular.
Staal and Wate\textsuperscript{15} found the azobenzene-hydroazobenzene redox
system to be reversible at the mercury pool electrode.

Widman and Fredericksen\textsuperscript{15} reported that for trans-azobenzene
in 50% ethanol solutions they obtained a linear relationship
between pH and half-wave potential; the half-wave potential
varied by \(0.059\) volts per pH unit. Unlike Currell and Ibbett\textsuperscript{16},
they found this relationship to hold even in acetic buffered
solutions but the molar proportion of buffer to azobenzene had
to be at least 500 : 1. Lepworth's analysis of the diffusion
controlled step was compatible with the reaction being reversible
and involving the electrons. The cis isomer was found to be
reduced at a slightly more positive potential than the trans
isomer at all pH values in the range studied. The difference
in potential varied between 0.04 and 0.45 volts and in one or
two cases the reduction of the cis isomer occurred at a more
negative potential than the reduction of the trans isomer,
although this was probably due to experimetal error.

The work of Currell and Wate\textsuperscript{16}, who determined azobenzene
fluorenespectically with potassium iodate \((\text{KIO}_3\text{)}).

\[
\begin{align*}
\text{OH} & \quad \text{N=N} \\
\text{SO}_3\text{Na} & \quad \text{HO}
\end{align*}
\]
and fastonatrine blue black R.

\[
\begin{align*}
\text{NaSO}_3 & \quad \text{OH} \\
\text{O} & \quad \text{N=N} \\
\end{align*}
\]

led to the first use of diesthioharmine dye complexes as reagents in polarographic analysis. Willard and Dean found that the addition of aluminium to solutions of fastonatrine violet caused part of the diffusion step to be displaced to more negative potentials. The height of the second part of the step was proportional to the aluminium concentration and was also dependent on the pH of the solution. Similar results were obtained with the less soluble fastonatrine blue black A. Interference was observed in the presence of iron (III), copper (II), nickel (II), cobalt (II), titanium (IV) and vanadium (V). Beryllium (II), magnesium (II), calcium (II), zinc (II) and manganese (II) did not interfere, unless present in great excess. In view of the work of Nickel and Dickert, Willard and Dean attributed this displacement, of about 0.2 volts, to the stabilization of the cis form of the dye.

Gabriel and Turner found the half-wave potentials of twenty-one water solubleazo dyes to be similar and of little use for identification purposes. Nevertheless, polaro graphic can be used for the quantitative determination of these dyes. Gabriel and Turner suggested that both two and four electron reductions were possible, depending on the electron withdrawing or donating properties of the other groups in the molecule. They also noted that the reduction of the azo and the nitro groups in
stand occurred at a more positive potential when nickel was present. The nickel step was completely suppressed.

Bean and Bryan\textsuperscript{15} reported that the equation for half-wave potential versus pH for Pontochrome Violet G, \( E_0 \), was:

\[
E_0 = -0.035 - 0.06 \text{ pH}
\]

between pH 0 and 12 and:

\[
E_0 = 0.100 - 0.052 \text{ pH}
\]

between pH 3 and 5. They reported that in the presence of aluminium a maximum of three dye molecules were co-ordinated to each aluminium ion; at higher pH values hydroxyl ions probably compete with the dye anion for co-ordination to the aluminium. They reported the maximum number of dye molecules per metal ion to be two for zinc, one for cadmium, one for iron (III), three for cobalt (II), one for magnesium and three for manganese (II). With nickel the complex formed depended entirely on the nature of the buffer. Two dye anions were attached in oxalic acid buffer and three in pyridine buffer.

The dye to metal ratios, \( n \), was calculated using the equation:

\[
n = \frac{\text{weight of second dye}}{\text{combined step height}} \times \frac{\text{total dye concentration}}{\text{total aluminium concentration}}
\]

They stated that these values correspond to the expected values based on the usual co-ordination number of the cation with a tridentate ligand.
Benn and Iryan\textsuperscript{31} did not consider that the appearance of
the second step was due to the stabilising of a dio form of
the ene group on complex formation; further, they considered
that the ene group was not involved in co-ordination with the
metal ion and that the new step therefore could not appear
because of any increased difficulty of reducing a co-ordinated
ene group. They suggested that the most probable explanation
was that the rigidity of the complex imposed a strain on the
molecule and made it more difficult to reduce polarographically.
The increase in separation of the two waves with increase in
ionic radius of the complexing cation seemed to support this
hypothesis.

Benn and Iryan\textsuperscript{32}, in a further paper, described an
empirical method for the determination of magnesium using
centroschre Violet 82. Serious interference was experienced
from calcium.

Perkins and Reynolds\textsuperscript{33, 34} developed the Billard and Benn
method for aluminium and made a more detailed study of the
reaction between centroschre Violet 82 (centroschre Violet 3 82 )
and aluminium. They examined the interference of beryllium,
calcium, manganese (II) and zinc and developed a method for the
determination of aluminium in the presence of beryllium.

Polarographically active complexes with cobalt (II), thorium (IV)
titanium (IV) vanadium (V), zirconium, iron (III) and nickel (II)
were observed\textsuperscript{35}. The copper (II) complex was relatively
insoluble. A decrease in the size of the complex step was
observed in the presence of fluoride, citrate and oxalate ions.
in proportion to their concentration. Allard and Bean\textsuperscript{18} first reported that fluoride formed a more stable complex than the dye with aluminium and this was developed as a basis for the determination of micromolar amounts of fluoride by Reynolds et al.\textsuperscript{27, 28}. Perkins and Reynolds\textsuperscript{25} found the number of electrons involved in the reduction of Solechno Violet 3G to be two, using the microcalorimetry technique of Reynolds and Chalgesky\textsuperscript{29}. This was in agreement with Bean and Bryan but in conflict with Gilbert and Mills\textsuperscript{30} who obtained a value of four electrons by a similar technique to Reynolds. Perkins and Reynolds showed that four electrons were involved in the reduction of the Solechno Violet 3G aluminium complex at pH 4.5, indicating that two dye molecules were attached to the metal. This was confirmed by Job's method of continuous variations\textsuperscript{24} by plotting the height of the second step against the composition of the solution. At pH 6.5 a dye to metal ratio of three to one was obtained in agreement with Bean and Bryan\textsuperscript{27}. The slope of the first step was consistent with the reduction of the free dye being irreversible.

Perkins and Reynolds\textsuperscript{26} examined the polarography of aluminium with Orange II:

\[
\text{Na}_2\text{SO}_4 \quad \text{N=NN} \quad \text{HO}
\]
Pernigrinel Yellow:

\[
\text{Na}_{\text{SO}}_{\text{3}} \quad \text{N} = \text{N} \quad \text{N} = \text{N} \quad \text{OH}
\]

and Acid Mizarin Black 43:

\[
\text{OH} \quad \text{N} = \text{N} \quad \text{OH} \quad \text{H}_{\text{O}} \quad \text{N} = \text{N} \quad \text{SO}_{\text{3}} \quad \text{Na} \quad \text{SO}_{\text{3}} \quad \text{Na}
\]

No modification of the dye wave was observed when aluminium was added to solutions of the first two dyes. Acid Mizarin Black 43 has two reduction waves presumably due to the two azo groups being reduced at slightly different potentials. In addition of aluminium a third wave appeared and the first dye step became smaller. The second dye step increased in height but it was not clear whether this was due to a displacement of part of the first step or not.

Selenochrome Violet B has been used to determine a number of other metals polarographically. It took and Okul\textsuperscript{22} determined mixtures of zirconium and thorium by measuring the combined complex step due to both metals and then that due to zirconium alone, by making the thorium step with acetate ion. Cooney\textsuperscript{23} determined gallium (III) and indium (III), and Ishibashi et al\textsuperscript{24} determined uranium. Cooney and Sealy\textsuperscript{25} determined a mixture of aluminium and gallium (III) and Turnham\textsuperscript{26} determined thorium at high pH. Floresco and Whitt\textsuperscript{27, 29} determined the lanthanides and found the combining ratio to be two dye.
molecules to each metal ion. The lanthanide complexes of

Eriochrome Black T:-

and methyl blue 2:-

showed similar polarographic behaviour to those of eolechrome

Violet B.

Dawson and Volier 29 used eolechrome Violet B for the
polarographic determination of iron (III). Lither 35
determined lithium (III) polarographically using eolechrome

Violet B.

Hincenb 31 used euperchrome Garnet 3:-

in preference to eolechrome Violet B, eireochrome Black 4:-

and eolechrome Bath Blue:-

for the polarographic determination of aluminium in thorium.
Selenochrome Violet II was found to be subject to more interference than the super dyes I and the other two dyes were less sensitive. Interference was experienced from cobalt (II), iron (III), nickel (II), lead (II), uranium, vanadium (V) and zirconium.

Thorne and Aylward found the reduction of selenochrome Violet II to involve four electrons and reconciled this to the two electron process indicated by logarithmic analysis of the diffusion step by postulating a mechanism involving the disproportionation of the unstable hydrazo intermediate involved in the reduction:

\[
\text{Selenochrome Violet} + 2H^+ + 2e^- \rightarrow \text{Hydrazo Intermediate} + 2H_2O
\]

followed by:

\[
2\text{Hydrazo Intermediate} \rightarrow \text{Selenochrome Violet} + \text{Hydrazo Compound}
\]

They state that the diffusion current data and reversibility studies may be reconciled if the hydrazo intermediate disproportionation within the life time of the drop. The regenerated dye is then available for further reduction, finally resulting in an n value of four. Reduction of the hydrazo compound are indicated by the presence of azine in the micro-cell after prolonged reduction. Thorne and
Julian obtained a value of four electrons per dye molecule reduced by comparing the diffusion coefficient obtained from the Fick's equation with that reported for anthracene by Gaster and Taylor (4.69 x 10^{-5} cm^2 sec^{-1}) and that for stilbene reported by Leite and Hugenholtz (4.35 x 10^{-5} cm^2 sec^{-1}).

Florence and Julian suggest that Rehbein and Lengsfield used an impure sample of Solochrome Violet B and that Leite and Fryer used an incorrect value for the diffusion coefficient in their calculations. Solochrome Black B and Chrome Blue Al-

![Chemical structure](attachment:chemical_structure.png)

also gave displaced reduction waves with the leucoforms. These two dyes and several anthraquinone dyes were investigated in more detail by Florence but were found to be inferior to Solochrome Violet B5 for analytical purposes.

The disproportionation of hydrosolubilene was first suggested by Giese and Furst to explain the rate controlling step in the potentiometric reduction of solubilene and a similar explanation was given by Leite and Hugenholtz to account for the results of their polarographic and coulometric reduction of p-diisobutylamino anthracene. The latter authors reported that the disproportionation was so slow at pH 4.5 that the polarographic step corresponded to a two electron reduction. At pH 1.9, on the other hand, the disproportionation was more rapid and a four electron reduction was observed. At the stirred mercury pool a four electron reduction was observed at pH 1.9 and pH 2.5 but at pH 3.5 at the dropping mercury
electrode an intermediate number of electrons were apparently involved owing to the rate of disproportionation being comparable with the drop time. Bateck and Lacey had previously observed a four electron reduction with a number of the dyes.

Florence and Aylward used controlled potential coulometry at a stirred mercury cathode to show that four electrons were involved in the reduction of Echolochrome Violet 2B. The reduction did not follow first order kinetics. The rate could be related quantitatively to a disproportionation of the hydrazo compound. It should be noted, however, that the use of coulometry with a micro-electrode to interpret polarographic results obtained with a micro-electrode, is dangerous as both the cell and electrode conditions are entirely different.

Florence and Barker studied the polarographic behaviour of trans-resorcinol and its sulphonic acid by several techniques including d.c., i.e., single drop and micro-polarography. Current-potential curves were recorded also at the stirring mercury electrode and these provided information on the reversibility of the electrode processes. Current-time curves were used to study the effects of adsorption. The rate of the two to hydrazo reduction was dependent on pH and was a minimum at pH 9 where maximum adsorption occurred. Reversibility was approached at very low and very high pH values. The reversibility changes were attributed to strong adsorption of both components near the electro-capillary maximum. The presence of ammonium ions was found to improve the reversibility at pH 9.
Florence\textsuperscript{[9]} investigated the effect of substituents in the aromatic rings on the polarographic reduction of azo compounds and found that the electron donating or attracting ability of the substituent affected the half-wave potential and also the number of electrons involved. The presence of electron donating groups such as the hydroxyl, amino, and dimethylamino groups increased the number of electrons involved in the reduction from two to four. Tahiya et al.\textsuperscript{[10]} found that structural differences in azo compounds gave rise to inductive and resonance effects which caused differences in the half-wave potentials. Florence\textsuperscript{[1]} studied the kinetics of the disproportionation of 4-aminohydroxazobenzene-4-sulphonic acid spectrophotometrically and polarographically. The reaction was found to be first order with respect to both the hydroazo compound and hydrogen ion. The reaction mechanism postulated was based on a rate determining step involving a quinonediimine intermediate previously suggested by Jonass and Fritz.\textsuperscript{[11]}

Florence et al.\textsuperscript{[12]} used solochrome violet II for the voltammetric determination of aluminium at a rotating pyrlytic graphite electrode. The processes involved gave an increased sensitivity over that obtained polarographically.

Palmer\textsuperscript{[13]} studied the reaction of solochrome violet II and a number of other azo dyes having either diortho-hydroxy or one ortho-hydroxy group with several metals and found that a polarographically active complex was formed only with the former type of dye.

Palmer and Reynolds\textsuperscript{[14]} and later Lyburn and Reynolds\textsuperscript{[15]} studied the reaction of magnesium with solochrome violet II.
at high pH and suggested a technique for overcoming interference from calcium. Richardson\textsuperscript{26} has also used this technique as a basis for determining magnesium in calcium carbonate.

Almer and Reynolds\textsuperscript{27} found that iron (III) forms a 1:2 and a 1:4 complex with toluidine blue O. They found that nickel behaved somewhat unusually in that a complex was obtained at pH 4.5 in borate buffer but not in acetate buffer. This was attributed to the nickel ion being protected by its hydration sheath and not reacting with the dye when at pH 4.5 in acetate buffer.

The stoichiometry and structure of the dye complexes have been studied even more extensively by other methods. An attempt has been made here to list the numerous uses of the dyes in spectrophotometry and as indicators. Nevertheless, the structures of the dye complexes are important in the present studies and it is convenient to review here a number of papers which are relevant to later discussion. The following structures show the important features of the complexes but do not represent the actual compounds which may be nephelometric derivatives.

Kowal and Vanquart\textsuperscript{18} prepared copper complexes of a number of extra-substitutedazo dyes. With one dye containing a single ethyl hydroxyl group they obtained 1:2 (copper: dye) complexes and indicated three possible structures for them: 

\[
\text{(6 saturated rings - two nzo group)}
\]
(5 nembered rings -
cis one group)

Kreuz and Landquist favour structure II by analogy with the copper complexes of acetylidinoline:-

\[
\begin{align*}
\text{CH}_2 & \text{N} \quad \text{N} \\
\text{O} & \text{H}
\end{align*}
\]

which must have a structure similar to I. These authors drew attention to the copper complexes of:-

\[
\begin{align*}
\text{CH}_2 & \text{N} \quad \text{N} \\
\text{O} & \text{H}
\end{align*}
\]

prepared by Kemps and Saffy\(^{59}\) and point out that they must have a structure similar to II or III. In contrast Kreuz and Landquist consider that in the less stable copper complexes of mono-ortho-carboxylazo dyes the adjacent nitrogen atom are involved in complex formation (structure IV) giving a six nembered ring rather than the seven nembered ring obtained if the further nitrogen atom were involved (structure V).
With copper (II) and o,o'-disubstituted one does only 1:1 complexes were obtained (structures VII to XIII):—

![Chemical structures]

The o,o'-dihydroxy naphthalene compound was considered to be planar but the o,o'-dioxybenzene compound and the o-hydroxy o'-carboxy naphthalene compound were not. Isoxazolines and mesoquinoline derivatives of VIII were obtained in which pyridine and quinoline replace a water molecule in the fourth co-ordination position.

Lew and Fairairs found that a single hydroxyl group was insufficient to co-ordinate an one group with a chromium (III) and hence were unable to prepare any complexes of this type. In this respect chromium (III) was similar to iron (III), copper (III) and zinc (II) but complexes were obtained with copper (II), nickel (II) and cobalt (II). With o,o'-dihydroxy naphthalene both 1:1 and 1:2 complexes were obtained (structures IX and XI):—

![Chemical structures]

These structures were believed to involve an octahedrally co-ordinated chromium atom with planar ligand molecules perpendicular to each other (structure X), or to three water molecules (structure IX). These authors also obtained a 1:1 o-hydroxy o'-sulphonyl naphtho complex of chromium (III) which was
Later authors expressed doubt as to the existence of these compounds. Smith and Drew also studied aluminium complexes and showed them to be similar to those of chromium. The aluminium (structure XIV) was isolated:

Evidence for a 1 : 2 complex was found in solution but the complex was not isolated.

Drew and Pusten showed that vanadium (IV) behaved in a similar manner to trivalent actinides. Both 1 : 1 and 1 : 2 complexes were obtained with 2,6-dinitroxy complexes (structures XV and XVI).
The co-ordinated water in structure XV could be replaced by pyridine. With o-hydroxy, o'-carboxyazo compounds an impure (probably) 1 : 1 complex was obtained; however, with o-amino, o'-carboxyazo dyes 1 : 1 and 1 : 2 complexes were obtained with ease comparable to the o,o'-dihydroxyazo compounds.

Heffier and Cour55 have indicated possible isomeric forms of o,o'-dihydroxyazo dye complexes of octahedral Cr (III) — structures a — e, to which Martell and Calvin65 have put forward a further isomer (f):—

\[
O + N + O = \text{structure } d
\]

All of the forms above are optically active except structure (d). Heffier and Cour55 obtained a laevor form of an azo retazine dye (XVII) and a dextro and laevor form of an azo dye (XVIII):—

In the case of the azo dye, XVIII, the dextro form had almost twice the rotation of the laevo form and these authors considered them to be optical enantiomorphs of different degrees of purity. Martell and Calvin consider that, in view of the large number of possible optically active forms, the isolated forms may not have been mirror images, but that if they are enantiomorphs then form (f) would seem the most likely form.
Callis, Nelson and Boller \(^6\) have preparedazo and amethine dye complexes of nickel (II), cobalt (II and III), chromium (III) and zinc (II). Magnetic susceptibility studies indicate that the 1 : 1 nickel complexes of the mono o-hydroxy substitutedazo dyes are planar but that the 1 : 1 complexes formed with o,a'-substituted azo and amidine dyes (XX - XXXI) are tetrahedral.

When a cobalt (II) salt was used to prepare the cobalt complexes of dyes, XIX gave a 1 : 3 cobalt (III), XX a 1 : 2 cobalt (II) complex, and XIXI and XXII a 1 : 1 cobalt (II) complex. Cobalt (III) complexes of o,a'-disubstituted azo dyes could be prepared from the \([\text{Co}(\text{III})]_{\text{6}}^{2+}\) ion.

Callis and Boller \(^6\) made a further study of similar dyes with cobalt, chromium and zinc and suggested that mono ortho-substituted azo dyes could satisfy one primary and two secondary valences of the metal and that diortho-substituted azo dyes could satisfy two primary valences and three secondary valences.

Smith \(^9\) determined the stability constants of a number of azo dye complexes by pH titration in 75% dioxane in water.

The stoichiometry of the complexes observed was in good agreement with the previous preparative results. Copper (II) was restricted to a maximum 1 : 2 complex with bidentate dyes and to 1 : 1 complexes with tridentate dyes. Other bi- and
trivalent metals appeared to be able to attain a co-ordination number of six, and formed 1 : 3 and 1 : 2 complexes with di- and tridentate dyes respectively. Iodine (IV) appeared to attain a co-ordination number of nine forming 1 : 3 complexes with o,o'-dihydroxyazo dyes. Brønsted found the order of stability of the cobalt complexes to be as follows:

\[ \text{Co}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+} \]

and the order of stability of metal ions with these dyes to be

\[ \text{Cu}^{2+} > \text{Co}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+} \]

for divalent metals and

\[ \text{Co}^{3+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+} \]

for trivalent species.

Contes and Vigilino\(^{72}\) studied Colsochrome Violet 3B potentiometrically and spectrophotometrically and obtained thermodynamic data on the dye and its self-association.\(^ {73} - 74\)

Spectrophotometric evidence was obtained for 1 : 1 and 1 : 2 complexes with aluminium, but attempts to detect a 1 : 3 complex failed. These authors\(^ {73}\) determined formation constants of a number of metal-dye complexes and found that most metals formed 1 : 1 and 1 : 2 complexes with the ligand acting as a tridentate system. A continuous variations plot\(^ {74}\) clearly indicated a 1 : 1 complex with copper and no evidence was obtained for the \(\text{Cu}^{2+} \text{NH}_2\) complex reported by Brown and Fairbanks. No evidence was obtained for the 1 : 3 complexes of calcium and magnesium with Colsochrome Black T-

![Diagram of Colsochrome Black T structure](image)

reported by Young and West\(^ {73}\). Using carefully purified Colsochrome Black T, Michel and Lindstrom\(^ {75}\) found only 1 : 1
complexes with magnesium and calcium. This is in accordance with Horrocks and Henderson, who found 1:1 complexes of calcium and magnesium with this dye with Eriochrome Blue Black Br-

![Eriochrome Blue Black Br-](image)

and with Eriochrome Blue Black Ar-

![Eriochrome Blue Black Ar-](image)

Geary, Richless, and Jollard studied the complexes of rhod-

![Rhodamine](image)

both spectrophotometrically and by pH titration. They found that in a number of cases, higher complexes were indicated by spectrophotometry than were indicated by pH titration. They suggested that this effect was due to one molecule of dye coordinating without loss of the proton of the α-hydroxyl group.
The same authors have compared the stabilities of the copper (II) complexes of RR with the corresponding dyes having the structures shown above (XXII and XXIV). The values of \( \log K \), the stability constant, in solution were found to be 16.45, 13.65 and 7.29 respectively. This was taken to indicate that the copper RR complex had structure XX with the five-membered ring:-

![Structure XX](image1)

![Structure XXI](image2)

Patterson et al.\(^7\) observed an unusual aspect in the complexation of \( \text{p-ANS} \):-

![Structure XX](image3)

Complex formation occurs only after the proton of the para hydroxyl group is ionised, although this group cannot be involved directly in coordination. It was thought that the ligand in this case chelates as shown (XXVI):-

![Structure XXV](image4)

Mavro\(^7\) developed a number of \( \alpha \)-hydroxyl, \( \beta \)-naphthoic acidazo dyes as colorimetric reagents. The complexes formed
were extremely stable and formed even in strongly acidic solution. Complex formation usually involved only the loss of one of the aromatic acid protons (XXVII), but the 1:1 thorium complex with oxine (III) has been shown to involve the loss of both protons\(^\text{50}\) (XXVIII):  

\[ \text{XXVII} \]

\[ \begin{array}{c}
\text{O} \\
\text{N} \\
\text{O} \\
\text{H} \\
\text{A}_5 \\
\text{O} \\
\text{O} \\
\text{X} \\
\text{M} \\
\end{array} \]

\[ \text{XXVIII} \]

\[ \begin{array}{c}
\text{O} \\
\text{N} \\
\text{O} \\
\text{H} \\
\text{A}_5 \\
\text{O} \\
\text{O} \\
\text{T} \\
\text{X} \\
\text{M} \\
\end{array} \]

The present work was undertaken to gain further information about the polarography of azo dyes and their metal complexes with a view to understanding the requirements of a polarographically active metal dye complex. This activity had so far been observed only with \(\alpha,\alpha\)-dihydroxy azo dye complexes and it was thought that a polarographic study of the azo dye complexes in which other ortho substituents were present in the azo dye molecule would be useful. It was hoped from this study, that existing analytical methods which utilize the displaced azo dye complex step would be improved and that new methods would be developed.

It is of interest to note that since this work was commenced two other examples of the displacement of part of the ligand step have appeared in the literature. Florence, Miller and Mittel\(^\text{12}\) have determined aluminium by oxidation of its oleochrome violet 53 complex at the rotating perspeticene graphite electrode, and Mittel and Florence\(^\text{14}\) have also determined aluminium by reduction of its alizarin red 5 complex.
Chapter II

Polarographic Instrumentation

Introduction

In the following pages the polarographs used in this work have been described. The purpose of the description is to facilitate an appreciation of the properties and advantages which influenced the choice of a particular polarograph in the various sections of the work.

1. The Centrifugal-Recording Polarograph

This is a conventional d.c. polarograph consisting of a cell, a dropping mercury electrode with stand, a recorder unit and a thermostatic bath. The polarographic cell is convenient to use and the solutions are easily deoxygenated and are afterwards kept free of oxygen by a water seal. The recorder unit consists of an ink-on-paper recording galvanometer operated by the cell through an amplifier. The chart movement is coupled to the potentiometer drive so that a record of current against applied voltage is obtained. A disadvantage of this instrument is the relatively small chart size of 7.5 x 17 cms.

The current from the cell is chopped by a vibrator relay and then passes through a high stability a.c. amplifier which has a low input resistance; the voltage drop is about 0.1 millivolts for full scale deflection. The output goes through a galvanometer carrying the recorder pen. The amplification is variable in accurately adjusted steps using a series of syphon dividers. The maximum sensitivity corresponds to 400 mV.
of chart per microamp and the minimum 0.4 in. of chart per microamp.

The potentiometer is graduated from 0.4 volt to 1.0 volt in 5 millivolt intervals. This range may be doubled or halved; 0.1 volt may thus be equivalent to 5, 10 or 20 cm. on the chart. The current through the potentiometer is usually adjusted by a milliammeter and rheostat but for greater precision adjustment can be made against a built-in standard cell, the voltage of which is balanced against the potentiometer, thus reducing errors of calibration.

The damping introduces a slight potential displacement of a few millivolts so that the current is virtually unaltered; usually with resistance capacity damping the wave is flattened and distorted. The system in this instrument allows the recording of substances with half-wave potentials which lie close together and allows the true shape of the curves to be determined. The damping has no appreciable effect on the input resistance of the amplifier. This displacement in potential can be readily allowed for by making accurate potential measurements externally. The degree of damping is varied in six steps and is thus accurately reproducible.

However, even on damping setting zero there is some damping provided, which may be a disadvantage in some fundamental studies.

The chart and potentiometer are driven by a synchronous motor with a two speed gear; the speeds are 15 cm. per minute and 3.75 cm. per minute when the supply is 50 cycles per second.
A compensating current is provided which is used to produce large changes in the galvanometer zero to compensate for the diffusion current for any large steps which occur before the one that is to be studied. This compensating current is independent of the sensitivity setting of the recorder and is obtained from a potentiometer in series with a resistance. This resistance is variable in steps, each value being appropriate to a small range of recorder sensitivities. Within this range the compensating current can be adjusted from a small positive value to a negative value equivalent to approximately ten times the full scale deflection of the recorder. The normal zero adjustment of the recorder is made by a separate control which covers about half of the full scale. The current supply for these two controls is obtained from a separate dry cell within the recorder case.

A counter current adjustment is provided which gives a negative current increasing in magnitude linearly with applied voltage. The slope of the counter current is adjustable by a potentiometer. The counter current, which compensates for baseline slope often due to capacity current, varies linearly over small potential range. It is arranged so that the slope for any setting varies directly with recorder sensitivity, so that once set the counter current will be correct for all sensitivities. The counter current circuit causes slight errors in applied potential and is therefore not used when this can be avoided.

If necessary an external recorder may be used with this
instrument, merely by connecting to the 'recorder' terminals. The potentiometer circuit can also be isolated from the amplifier and the recorder circuit so that both may be used independently.

Cambridge Univector polarograph

This instrument is always used in conjunction with the Cambridge galvanometer polarograph.

A small alternating voltage is superimposed on the D.C. voltage and the resulting a.c. component of the cell current is recorded as a function of applied d.c. voltage. A series of peaks corresponding to the normal polarographic waves is usually obtained. The basic principle is not the same as that of derivative polarography but the waves obtained have approximately the same form. For reversible processes the magnitude of the peaks is much greater than that of a true derivative peak.

The alternating current is composed of two parts. The derivative portion is 90° out of phase with the applied voltage whilst the other portion is 90° out of these, and is due to capacitive effects in the region of the electrode. If the total current is measured the peaks are superimposed on a standing current which is relatively large at high sensitivities and this results in base line curvature of such magnitude that distortion of the peaks results. The Univector overcomes this difficulty by the incorporation of a photo-sensitive detector to separate the two components, with the result that the curvature of base line is small, then working at high sensitivities there is a secondary disturbance of the base line.
due to the resistance in series with the electrode; this is partly the resistance of the solution around the electrode and partly the resistance of the instrument itself. This effect may be minimized by means of a compensating circuit, but then the potential reading more slightly negative in the absence of reducible species. The instrument is provided with a means of adjusting the sensitivity on the voltmeter, of correcting small changes in sensitivity, and of checking the sensitivity. A disadvantage of the a.c. superposition method employed by this unit is that it is not sensitive to irreversible processes. It was found to be unsuitable for the present system.

Disadvantages of Conventional D.C. Polarography

Conventional d.c. recording polarographs suffer from a number of disadvantages. These are discussed below:

1. Limitations are imposed on the sensitivity by the dropping of the mercury which gives rise to a growth and fall in current, and to the double layer capacitance associated with the drop surface. The large fluctuations in current are proportional to the current flowing and are thus largest at the top of a polarographic step. Therefore the accurate determination of a minor constituent, which is reduced at a more negative potential than another major constituent in the same solution is difficult. Attempts to damp these oscillations usually result in a distorted wave. One of the most successful damping systems is that used in the Cambridge polarograph:

at $10^{-9} - 10^{-5}$ concentrations of the electro reduced
substance, the changing current of the double layer capacitance is much larger than the current due to electrode reaction. It is not easy to compensate for the changing current as it has a complex relationship to the voltage. The type of circuit which must be used if the cost is to remain reasonable involves rectilinear compensation which is effective only over a short voltage span.

ii. The resolution between adjacent steps is not good. The resolution is determined by the two half-wave potentials, the number of electrons involved in the reduction and the relative concentration of the reducible substance. For reasonably good separation the steps need to be about 0.15 to 0.2 volts apart. Some instruments are provided with one or two derivative circuits which will resolve reduction steps of species of equal concentrations at about 0.07 volt apart. Resolution can be improved by the use of scale expansion as provided on the Cambridge polarograph.

iii. Another disadvantage of the conventional polarograph is that the waves are distorted when the resistance of the cell is greater than about 500 ohms. This difficulty has been overcome in the Model B polarograph by use of a three electrode system.

2. The Model B Type III - 132 Polarograph

In d.c. and a.c. polarography, electrolysis is usually carried out with a polarisable dropping mercury electrode and a non-polarisable large area electrode (e.g. calomel electrode or mercury pool). The current flowing through the cell is determined by the processes taking place at the dropping mercury.
electrode, which acts as an indicator electrode. The potential of the reference electrode remains effectively constant. In such a system, the polarogram obtained corresponds very closely to actual current-potential curve, provided that the total resistance of the cell is less than about 100 ohms. If the resistance is much greater than this, the wave is distorted and displaced.

The Model 111 polarograph incorporates a three electrode system to solve this problem. The polarographic cell is fitted with three electrodes - a dropping mercury electrode, a reference electrode (usually a calomel electrode) and a working electrode (usually the mercury pool). An automatic electronic compensating circuit controls the current flowing between the dropping mercury electrode and the working electrode. By this means, the potential difference between the dropping electrode and the reference electrode is always kept equal to the voltage appearing on the motor driven potentiometer of the polaroscope, regardless of the resistance of the system. Hence the in drop due to a high cell resistance is continuously made good and does not cause a voltage drop as in the case with the conventional d.c. circuit. The use of this technique has an obvious advantage in the polarography of aqueous or partially aqueous systems where resistances are always high.

A further advantage of this system is that a calomel electrode of the type normally used for pH determinations may be used. This is more readily kept uncontaminated than with low resistance calomel electrodes normally used in polarography.
With this type of in drop compensating circuit, two further errors which are present when the potentiometer is used as the current supply are eliminated. The first error arises from the nonlinearity of voltage with respect to the position of the sliding contact, and the other is the voltage loss due to the contact resistance of the sliding contact. In this compensation system no current is supplied by the potentiometer, and consequently, the above errors do not occur.

A further advantage of this polarograph over the Cambridge polarograph and the cathode ray polarograph (see later) is that the recorder chart size is 50 cm. full scale deflection, compared with 7.5 and 10 cm. for the other polarographs respectively.

The Haldane polarograph is provided with a set of controls to enable the starting voltage to be selected in 0.5 volt steps from +2.0 volts to -2.0 volts. Sets of 0.5, 1.0 or 2.0 volt to 50 cm. of chart paper may be selected, providing scale expansion. These voltage sets may be carried out in 45 seconds, 25 seconds, 8 minutes, 16 minutes, 40 minutes or 120 minutes, making the polarograph suitable for kinetic studies. The diffusion current compensation is provided by an eleven-step control, with a potentiometer fine-adjustment. A condenser current compensating current is also incorporated, which works off a battery supply to the diffusion current compensating circuit. The diffusion compensation is obtained by means of resistors and may be varied continuously by a ten-turn potentiometer up to 1.5 micro-amp per volt. Filtering is provided by parallel T type filters, the attenuation of which
is very high within the narrow frequency band of the usual drop time. This avoids the polarographic curve being distorted by the filter and hence accurate half-wave potentials may be obtained. A reverse drive mechanism which will show up any delay in the pen recording system is useful in ensuring accurate half-wave potentials. An R.C. derivative circuit is provided. This is brought into operation by short-circuiting the normal circuit with a pair of contacts by means of a switch. The amplifier sensitivity is adjustable in each of five ranges from 10^-7 mpa per division to 10^-5 mpa per division.

In principle, this instrument has many advantages, giving accurately measurable half-wave potentials and allowing the step heights to be measured with a high degree of precision. Unfortunately, however, no thermostated bath is provided and the large polarographic cell supplied allows air to diffuse in to it unless nitrogen is passed continuously over the surface of the solution.

The instrument used in the present work has been susceptible to frequent technical faults including the failure of microswitches and the gear box.

3. The Southern Analytical Device Differential Cathode Ray Polarograph

The cathode ray polarographic technique was first suggested by Daniels. The polarograph described here was designed by Davies and Jaenisch and was developed from a Daniels type polarograph in which many of the possible variables were fixed. In cathode ray polarography drop wave effects are avoided by...
plotting the polarogram in the life of one drop. The drop is allowed to grow undisturbed for five seconds to a stable and reasonable size where the rate of change of the surface area of the drop is small. A 0.5 volt sweep is then applied over two seconds. The drop and the sweep are synchronized, the drop time (seven seconds) being controlled mechanically.

At the fall of the drop the decrease in cell current is utilized to trigger the instrument back to its rest condition so that the process may be repeated. A long persistence cathode ray tube is used to display the current change with voltage. Permanent records may be obtained by photographing the screen; a camera attachment is provided. If a permanent record is not required the peak height is read off from the oscillograph screen.

The rapid voltage sweep gives rise to a polarographic wave with a peak superimposed on the curve. This is not due to a polarographic maximum, but arises because, at the reaction potential of a species, all of that species is stripped out of solution by a process that is not diffusion controlled, and which gives rise to a cell current which is larger than the limiting diffusion current normally obtained. Once this has taken place, however, the depleted layer around the drop surface must be replenished if the reaction is to continue. This can only take place by the normal processes of diffusion, so that the initial current falls exponentially to the limiting diffusion value, or to some value below this if the ions cannot fully make up the initial concentration in the time available.
Lowell and Reynolds\textsuperscript{55} have shown that the wave form in cathode ray and conventional polarography are simply a function of the ratio of drop time to rate of change of potential. By using a very fast drop time they obtained a step of conventional shape with a cathode ray polarograph. Smith\textsuperscript{54} obtained peaked (cathode ray type) polarogram with a conventional d.c. polarograph, by means of a dropping mercury electrode with a drop time of about 3 minutes.

By comparison of the Randles\textsuperscript{57} and Ikevic\textsuperscript{58} equations, it can be shown that with identical solutions and similar electrodes the ratio of peak current to diffusion current should be about \(3.5 n^2\) where \(n\) is the number of electrons involved. The peak current is directly proportional to concentration as is the diffusion current; the use of the peak current leads to increased sensitivity.

A further advantage of the technique is that the ratio of faradaic current to double layer charging current is increased by the fact that the former is only a function of \(\mathcal{A}^2\) whereas the latter is directly proportional to \(\mathcal{A}(\mathcal{A} =\) sweep rate in volts/second). The peaked wave obtained is easier to measure than the usual polarographic wave and the resolution between adjacent waves is better. It is possible to resolve separate peaks which are about 0.1 volt apart; when the derivative circuit is used peaks only 30 - 50 mV apart can be separated, although some loss in sensitivity is unavoidable. As the time of sweep - that is seconds of drop life - little variation
of current due to change in drop surface area occur and
amplifications up to 10 or 100 times that used in conventional
dc. polarography can be used. The selectivity can be improved
over that of conventional polarography by setting the start
potential to a more negative value than the half-wave
potential of a previously reduced substance such that the
first reduction is diffusion controlled. The diffusion current
may then be compensated for by a static current in opposition
to it; this is greatly enhanced by the effective concentration
ratio now being 5.5 to 1.

The above procedure usually leaves an increasing base line
which may be corrected by a linear slope compensator similar
to 'counter current,' but this becomes imperfect at high
sensitivities and limits preceding reductions to 3000 - 5000 : 1.
The detection limit by this technique is about \(10^{-7}\) \(\text{M}\) - \(10^{-5}\) \(\text{M}\)
of reducible substance.

Because of the rapid sweep each component may be determined
in about twenty seconds. Another effect of the rapid scan is
that reducible substances which are polar are often adsorbed
on to the surface of the drop which results in an increased
current and therefore an increased sensitivity. The aluminium -
Malachite Green complex is adsorbed in this way allowing
aluminium to be determined down to 0.001 p.p.m. The use of
maximum suppressors are rarely necessary using this type of
instrument. If maxima occur they do so at high concentrations
and are easily recognised.

Normally peak potentials and not half-wave potentials are
quoted, as the accurate measurement of half-wave potential with this instrument is not easy. The peak potentials are usually more negative than the half-wave potentials by about 0.05 volts. The applied potential is measured relative to the working electrode.

This instrument may be used to differentiate between reversible and irreversible reactions. If the sweep is applied so that the cathode becomes more negative during the scan then the reaction will be \( \text{H}^+ + e \rightarrow \text{H}(\text{g}) \) whether the reaction is reversible or not. If the starting potential is now set at a more negative potential than the reduction potential then the reduction will take place continuously during the rest period and will cease during the sweep to more positive potentials. If the reaction is reversible, then the reaction \( \text{H}^+ + e \rightarrow \text{H}(\text{g}) \) occurs and an inverted polarogram results. If, however, the reaction is irreversible then no peak will be obtained on reverse sweep and there will only be a gradual decrease in current from diffusion level to zero current. This can be of great advantage when a reversible reaction is to be studied in the presence of an irreversible one.

Because the cathode ray polarograph has a high sweep rate the polarogram is much more dependent on the kinetics of the reaction. It is therefore important, for analytical purposes, to have a system in which the electrode reaction is fast.

The instrument can be operated on twin polarographic cells and is thus much more versatile in its mode of operation than other polarographs. The signal from a reference cell may be subtracted from the signal from the sample cell. For this the
electrodes must be synchronized and their characteristics must be closely matched. It is usual to cut a capillary in half and use one half for each cell. Slight differences in mercury flow rate and drop size may be adjusted by moving the mercury reservoirs independently. Electrochemical drop separation ensures synchronization. Dewell and Reynolds have explained some of the difficulties involved in matching systems.

There is some confusion over the nomenclature associated with this technique. The system given here is that applied by the designer and other important workers in this field.

'Differential polarography' is a general term used to denote the measurement of the difference between currents in two cells under identical conditions of drop growth and rate of potential change. The three main branches are:

1. Subtractive polarography. In this technique one cell contains a blank solution and the other the solution to be analyzed. The same potential is applied to synchronized dropping mercury electrodes in the two solutions and the difference in cell currents is measured. This eliminates unwanted cell currents such as those arising from impurities or from previous reductions or from the slight increase in the surface area of the drop during the sweep period. This latter may become appreciable when high concentrations of ions are being reduced at the start potential. This also eliminates the cell current due to the charging of the Helmholtz double layer and hence full use may be made of the high sensitivity of the instrument. Currents due to the onset of reductions later than the one of interest but large enough to become
apparent cell before their peak potentials are also eliminated.

Electrochemical polarography \( \text{ECP} \) is used to denote the technique whereby one cell contains the solution to be analyzed and the other a similar solution of accurately known composition. The same potential is applied to the dropping mercury cathode and the difference in current is amplified and measured. This technique is best used to measure small differences in fairly concentrated solutions \( \left( 2 \times 10^{-5} - 5 \times 10^{-2} \right) \). The technique may, however, be used down to \( 10^{-7} - 10^{-3} \) concentrations but here there may be insufficient instrumental sensitivity to take full advantage of the method. Coefficients of variation better than 0.1% have been reported using this technique.

Derivative polarography introduces a small difference (13 or 30 mV in the potentials of the two electrodes during the voltage sweep. Under these conditions the display approximates to the first derivative of the current waveform of a single cell. R.C. circuits are also provided for single cell derivative operation. The resolution of the 10 mV twin circuit is at least as good as the ten millisecond R.C. circuit and is about four times as sensitive. Similarly the resolution of the first twin cell circuit is as good as the 50 millisecond circuit and is three times as sensitive.

It is possible to use R.C. circuits in conjunction with the twin cell circuits to give a second derivative curve with even better resolution but lower sensitivity than that obtained with the first derivative circuit.
The current zero control is provided in each circuit by means of a ten-turn potentiometer, which introduces current into the input circuit of the rectifier in the opposite sense to the diffusion current. A sensitivity balance is present for adjustment of the relative sensitivities of the input circuits which may vary slightly as the circuit warms up. There are two means of adjusting the sensitivity. An amplifier scale factor varies the amplifier in decade steps with an accuracy of better than 2% and a current scale factor, which has sixteen positions, attenuates the cell current before input to the amplifier. The latter has an accuracy of better than 0.1%.

A starting potential control is provided to govern where the 0.5 volt sweep commences. The potential balance adjusts the relative starting potential applied to cells I and II and is used to cancel any inequalities in the potentials of the two reference electrodes over a range of 10 mV.

In normal single cell operation this polarograph is about seven times as sensitive as the usual cathode ray polarograph and its derivative operation is up to fifty times as sensitive. The normal cathode ray polarography is forty to a hundred times as sensitive as a conventional d.c. polarograph. The resolving power is superior and previous reductions are tolerated better than with other cathode ray polarographs. Using comparative and subtractive techniques the superiority to conventional polarography is further emphasized.

4. The Southern Analytical Southern-Hammond Type J Polarograph

This polarograph was designed specifically to provide a polarographic technique of the highest sensitivity. It is
a development of the principle employed in the square wave polarograph. The limitations to the sensitivity previously fixed by 'capillary noise' have been overcome by providing a compensating current which is proportional to the pulse height at any instant and is fed back to the input circuit, and the noise level of the electronic circuitry which had been kept at an exceptionally low level. The problem of the capacity current has been removed by measuring the forensic current in the latter half of the pulse life, when the exponentially decaying current is virtually zero.

In one of the two possible modes of operation, a single pulse of 7 or 150V amplitude is applied once in the life of each drop. The 40 millisecond pulse is superimposed on a voltage ramp. This mode of operation gives peaks of 'derivative' shape. In the other mode of operation a pulse of increasing size (between 0 and 1 volt) is superimposed upon a preselected 'start' potential. This gives rise to a polarogram of 'normal' shape. Automatic compensation for any current, due to previous reductions or a sloping base line, can be made. Satisfactory polarograms may be obtained with solutions, whose specific conductivity lies between that of 1N hydrochloric acid and 0.01N potassium chloride. With the use of the more dilute base electrolytes, the problem of introducing impurities is diminished. The necessity for using concentrated base electrolytes in a major disadvantage of the square wave polarograph.

The importance of impurities in the base electrolyte
milliseconds. As the measurement of current is only made during the second half of the pulse life, the effect of double layer capacity is eliminated.

At higher sensitivities a current due to the capillary response becomes appreciable. This is due to wetting of the cone of the capillary tip with solution. This current is eliminated as described at the beginning of this section. If the surface tension of the drop changes at the start of a polarising pulse it causes the drop to oscillate slightly and modulate the background current. Although the background current is rejected by the filter circuits, the oscillating current may pass through when the current is high. This effect is only present when the drop is large, and for this reason, the pulse is applied at the half life of the drop. Compounds which are strongly adsorbed onto the mercury drop may influence the shape of the derivative polarogram and may cause a sloping base line on 'normal' presentation. A capillary compensation control is used to counter this but is not completely successful. Another pulse heights of 7nV or 25nV are used depending on whether the sensitivity or the resolution is the most important factor. A pulse height of 7nV gives the greater resolution, but there is a five-fold decrease in sensitivity.

The instrument may be used with a free fall mercury drop or with an electrochemical drop separator. In the first mode of operation, the large drop in cell incidence and the mercury drop is detached, starts a two second time delay. At the end of the two seconds the 40 millisecond voltage pulse in
applied to the cell. Correct setting is ensured by adjustment on the front panel and a drop indicator bulb. When a mechanical drop indicator is used, a pulse is generated at the end of the 40 millisecond period. This energizes the area synchronizer and starts a one second time delay. After one second the 40 millisecond voltage pulse is applied to the cell and the cycle is repeated.

The curve plotted by the recorder is a series of pulses because of the change in current as the drop is charged. The distance between each pulse represents the drop period. The amplitude of each pulse is proportional to the integrated value of the current in the cell during the final 20 millisecond period of the polarizing pulse.

An integrator selector switch is provided which allows the response of the output stage to be made more sluggish. This gives a degree of smoothing to the system. The integration may be made over 1, 3 or 9 drops. The maximum smoothing should be used with the maximum time seen setting to avoid loss of resolution or sensitivity.

This instrument is very sensitive and has high resolving power. It can overcome the problem of a previously reduced substance present in 100 times excess over the substance to be determined provided that the assumed reduction occurs at least 1.2 volts earlier than that of the substance to be determined.
Distinctions between the main types of limiting currents

Five main types of limiting current may be distinguished under polarographic conditions, namely: diffusion, migration, kinetic, adsorption and catalytic currents.

These may be distinguished by the effect of systematic variation mercury drop on the step height and by studying the concentration dependence of the step. In some cases, study of the effect of temperature may help in characterising the type of current.

1. Diffusion Currents

These are the usual and the most important type of polarographic current. The magnitude of the diffusion current depends on the rate of diffusion of the electroactive species from the bulk of the solution to the depleted layer near the surface of the electrode. The rate of diffusion is proportional to the difference in concentration in the bulk of solution and at the electrode. If sufficient supporting electrolyte is present to eliminate migration currents (see 2 below), then the limiting diffusion current is directly proportional to the concentration of electroactive material. The magnitude of this limiting current is given by the Ilkovic equation. 96

\[ i_d = \frac{60\pi D n^2 c_0}{R T} \]

where \( i_d \) = the limiting diffusion current in micro-amps

\( n \) = the number of electrons involved in the reaction

\( D \) = the Perkamp, 96,000 c-amps
2. Electrolysis

In general, reducible ions reach the surface of the growing mercury electrode under the influence of two forces, a diffusive force, proportional to the concentration gradient, and an electrical force proportional to the potential gradient at the electrode.

If the electrolyte is not present then the current obtained for the reduction of cations is generally about twice that obtained when excess base electrolyte is added to the solution. The addition of small amounts of base electrolyte rapidly reduced
this current until finally when the base electrolyte concentration is about 50 times that of the reducible species the current remains constant. For anions the current is smaller in the absence of base electrolyte. This is because the transference number of the reducible ion decreases rapidly as base electrolyte is added and approaches zero when base electrolyte is present in large excess. For electrically neutral species the limiting current is entirely diffusion controlled and the limiting current should be constant and independent of the presence of base electrolyte.

3. Adsorption Currents

If a polarographically active substance is strongly adsorbed on to the mercury drop then an adsorption wave may result. The height of an adsorption wave at first increases with concentration, but becomes independent of concentration when the surface of the drop is completely covered with the electroactive species. The height of the step is given by the equation

$$\text{in} = 0.087\text{Fnt}^{-1}$$

where $n$ is the number of moles of electroactive species adsorbed per square centimeter of surface, the other symbols have their usual significance.

It can be shown that the step height is proportional to the height of the mercury column.

An important result of adsorption is that anima are sometimes produced. The shape of the anima very from neat peaks to rounded bumps. Anima are reproducible but very
sensitive to conditions. After the maximum the current falls exponentially until it is diffusion controlled. Then the maximum is very acute the current-voltage curve is a straight line, the slope of which is equal to the reciprocal of the cell resistance.

Maxima are usually eliminated by the addition of a surface-active agent. In this study the concentration of dyes used did not usually give a maximum.

The theory of maxima is not fully understood but what occurs is probably somewhere between the adsorption theories of Hayworth and Kinotic and the electrokinetic theory of Martin and Van Steenbergh. It is beyond the scope of this thesis to discuss these theories.

4. Kinetic Currents

In some systems the electrode reaction is not the slowest one and the step height may be partly or wholly controlled by a chemical reaction which occurs prior to the electrode reaction. The resulting step is known as a kinetic step.

This type of step is usually proportional to the concentration of the electroactive species but is independent of mercury bond height. Another characteristic of this type of step is the very large temperature coefficient; normally, the step height is doubled for about each ten degree rise in temperature whereas a diffusion step usually increases by only 1 - 1.5 per degree centigrade. The kinetic current is given by the equation

\[ i_k = \frac{4030 nF^2 c (k_f / k_r)}{n} \]

where \( k_f \) and \( k_r \) are the forward and reverse rate constants for the chemical reaction.
5. Catalytic Currents

There are two kinds of catalytic steps. The first type is characterized by the step height of an electroactive substance being increased by the presence of a catalyst and the second type is characterized by a polarographically inactive substance causing a shift in half-wave potential of an electroactive species to more positive potentials. A common example of the latter type of reaction involves the lowering of the overpotential of hydrogen allowing its reduction to take place at a more positive potential.

As the catalyst is regenerated continuously the step height usually many times that of a diffusion step. With increasing concentration of catalytic species the step height usually reaches a limiting value. Buffer concentration and pH also affect the step height considerably. The mechanism of catalytic steps is not well understood.

Logarithmic Analysis of the Polarographic Wave

The equation of the polarographic wave for a reversible process may be expressed as:

\[ E_{\text{d.m.e.}} = E_1 - \frac{0.0591}{n} \log \frac{i}{i_d - 1} \text{ at } 25^\circ C \]

where

- \( E_{\text{d.m.e.}} \) = the potential at the dropping mercury electrode
- \( E_1 \) = the half-wave potential
- \( n \) = the number of electrons involved in the reduction
- \( i \) = the current at any given point on the wave
- \( i_d \) = limiting diffusion current
A plot of \( \log \frac{a}{m} \) versus \( \log \frac{a}{m} \) formed a log plot, \( \log a \frac{a}{m} \), in a straight line of slope \(-2.303 \) with an intercept at the x-axis equal to the half-wave potential. This has been verified experimentally by Jones, 106 and Holton and Lingane, 107.

Thus the slope of the log plot is dependent only on the number of electrons involved in the reduction and is independent of the concentration of reducible species. A limitation on the use of the method of determining \( a \) is that the reduction has to be reversible. For irreversible systems a curve may result, or the slope of the expected straight line will be more negative than \(-2.303 \). The above applies to cases where the action is reduced to the metal and where the metal ion is reduced to a lower oxidation state.

Potentiometric Titration

The cyan dyes considered in the present study contain at least one ortho hydroxyl group on one of the aromatic rings and are weak acids. On forming a complex the proton of the ortho hydroxyl group is liberated and this occurs at a lower \( \mathrm{pH} \) than when the metal ion is absent.

From these curves it is possible to obtain an indication of the stoechiometry of the complexes being formed in solution. A limitation of this method is that it will not detect the co-ordination of a ligand which does not have a proton.

Examples of apparent differences in the stoechiometry determined by this method and by spectrophotometry have been attributed to this cause. 102
\[
\frac{A + B}{(C - D)} = E
\]

The terms on the left of the equation are:

\[A < 0 \quad \text{and} \quad B < 0\]

and the right:

\[(C - D) \cdot (E - F) = G
\]

The terms on the right of the equation are:

\[H \quad \text{and} \quad I\]

The left-hand side of the equation is:

\[(J + K) \cdot L = M
\]

The terms on the right of the equality are:

\[N \quad \text{and} \quad O\]

The left-hand side of the equation is:

\[P \quad \text{and} \quad Q\]

The terms on the right of the equality are:

\[R \quad \text{and} \quad S\]

The left-hand side of the equation is:

\[T \quad \text{and} \quad U\]

The terms on the right of the equality are:

\[V \quad \text{and} \quad W\]

The left-hand side of the equation is:

\[X \quad \text{and} \quad Y\]

The terms on the right of the equality are:

\[Z\]
\( T \) = total concentration of ligand
\( \theta \) = initial concentration of acid
\( V' \) = initial volume of solution
\( V', V, V'' \) = volumes of alkali added to reach a particular pH meter reading (see Fig. 1)
\( n \) = normality of standard alkali solution
\( y \) = number of ionizable hydrogen atoms on the ligand
\( \eta \) = degree of formation of the ligand-proton complex
\[
\eta_a = \frac{2^{1/2} \theta + (V'' - V') \theta^{1/2} (2^{1/2} - \theta^{1/2})}{\theta^{1/2} + \theta}^{-1/2}
\]
and if:
\[ n >> 1/2 \theta \text{ and } V'' >> V' \]
then:
\[
\eta_a = \frac{2^{1/2} \theta + (V'' - V') \theta^{1/2}}{\theta^{1/2} + \theta}^{-1/2}
\]
If the volumes of alkali added is not negligible fraction of \( V' \) then the approximate equations may be used without any appreciable loss of accuracy.

For these results obtained to be accurate the solution must be allowed to equilibrate; complications may also arise from hydrolysis of the metal ions at high \( \theta \); when this occurs, a considerable time is needed to reach equilibrium and a drawn-out curve is obtained. However, in cases such as this the titrations are useful for obtaining the \( \eta \) curves over which the complexes are formed and are stable.
General Conditions for Polarographic Study

The dyes studied were each examined in a similar manner throughout. A aliquot of the dye was added to a cell buffered solution, usually containing a total of 10 ml. of 3 components, and made up to 50 ml. in a graduated flask. When the complexes were being studied an aliquot of the metal ion solution was added and in some cases (e.g., in the case of aluminium), the solutions were heated to 70°C in a water bath to ensure complete formation of the polarographic step. The solutions were then added to a polarographic cell, degassed with nitrogen and polarographed. In certain cases spectrophotometric Job plots were carried out on these solutions using the Unicam G300 spectrophotometer with 2 cm. or 5 cm. silica cells.

Determination of the Purity of the Dyes

The purity of the dyes was determined by potentiometric pH titration or by titration with titriplex chloride solution. In the case where the dye loses a proton at fairly low pH the estimation was straightforward but where the proton was not lost until pH values greater than about 7, aliquots of metal ion solution were added progressively until all the dye present lost a proton or protons and the purity was estimated from the amount of metal ion that was required. For this, some knowledge of the stoichiometry of the complex is needed. The procedure used for the determination with titriplex chloride was that described by Viggia.10
the dye was then calculated from the formula below.

\[ \text{mol. Co(III) chloride} = \text{mol. Co(III)pyrocatechol} \times \text{mol. Co(III) chloride} \times 100 \]

where \( n \) = number of hydrogen atoms consumed per dye molecule

- \( n = 4 \) for \( \text{HCl} \)
- \( n = 6 \) for \( \text{HC} \)

Electrochemistry

The method used was essentially that described by Reynolds and Shelton. A micro-cell capable of being used 0.2 to 0.6 mC of solution was constructed. The anode was usually a silver foil and the cathode was a dropping mercury electrode. In some cases a silver gauge was used in place of the silver foil in order to give a greater surface area to avoid polarization of the anode. The mercury pool was covered with a layer of trifluoroethylene to prevent any recrystallization of the reduction products.

The reduction potential was adjusted to be more negative - usually by 0.2 volts - than the half-wave potential of the ion to be reduced. A constant current source was used as the potentiostat.

The dropping mercury electrode was lowered into the deaerated dye solution and a stop clock was started. A polarogram was recorded to give the initial limiting current and then with the potentiometer drive disconnected from the chart drive, the potential was kept constant whilst
the limiting current was recorded against time (the slower chart speed was used.) It can be shown that the current should fall exponentially with time. When the end of the chart was reached the electrolysis was stopped; the solution was stirred by passing oxygen free nitrogen through it and a second polarogram was obtained. The number of electrons involved in the reduction was calculated using either the integration method or the arithmetic mean method, described by Reynolds and Shalgozky.

In the former method, the current versus time chart is cut out and weighed. From this the total number of coulombs passed (X) is calculated. The number of electrons transferred is then calculated, using the equation:

\[ n = \frac{M}{F} \times \frac{X}{W} \]

where \( M \) = the molecular weight of substance

\( F \) = the Faraday

\( X \) = the number of coulombs

\( W \) = the weight of sample reduced (calculated from the first and last polarograms)

\[ W = \frac{i - i_t \times V \times C}{i_0} \]

where \( i_0 \) = the initial current

\( i_t \) = the current after time \( t \)

\( V \) = the volume of sample

\( C \) = the initial concentration of the electroactive species
The anodic form method requires only the limiting currents of the two polarograms. The number of electrons transferred is calculated from the equation:

\[ n = \frac{I_i \times t \times \frac{1}{I_0} + \frac{1}{I_0}}{\frac{1}{I_0} - \frac{1}{I_i}} \times 1 \]

where \( t \) = the total electrolytic time

The other symbols have the same significance as in the previous equation.

**Reagents Used**

In all cases the reagents used were A.R. quality chemicals.

The buffer solutions were made up one molar except where stated. The ratio of the two constituents was varied to give the required pH values within the range shown below.

<table>
<thead>
<tr>
<th>Buffer System</th>
<th>( \text{pH Range} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid sodium hydroxide</td>
<td>3.3 - 6.9</td>
</tr>
<tr>
<td>Citric acid sodium hydroxide phosphate</td>
<td>2.8 - 5.5</td>
</tr>
<tr>
<td>Formic acid sodium hydroxide</td>
<td>2.3 - 4.6</td>
</tr>
<tr>
<td>Succinic acid borax</td>
<td>3.9 - 5.9</td>
</tr>
<tr>
<td>Acetic acid - sodium acetate</td>
<td>4.7 - 6.6</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate - sodium hydroxide</td>
<td>5.6 - 8.3</td>
</tr>
<tr>
<td>Iodic acid - sodium hydroxide</td>
<td>8.9 - 10.9</td>
</tr>
<tr>
<td>Ammonium chloride - ammonium hydroxide</td>
<td>8.3 - 9.2</td>
</tr>
<tr>
<td>Hydrochloric acid - ethanolic sodium</td>
<td>2.6 - 10.4</td>
</tr>
<tr>
<td>Sodium bicarbonate - sodium carbonate</td>
<td>9.2 - 11.0</td>
</tr>
<tr>
<td>Sodium hydrogen phosphate - sodium hydroxide</td>
<td>11.0 - 12.9</td>
</tr>
<tr>
<td>Piperidine - sodium hydroxide</td>
<td>10.5 - 13.0</td>
</tr>
<tr>
<td>Dimethylglycine - sodium hydroxide</td>
<td>11 - 13</td>
</tr>
</tbody>
</table>
The solutions of the metal ions were made up from the following reagents at concentrations given in the text:

\[ \text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O} \]

\[ \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \]

\[ \text{CaCO}_3 \text{ (dissolved in dilute hydrochloric acid)} \]

\[ \text{Ca}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O} \]

\[ \text{CaCO}_3 \cdot 2\text{H}_2\text{O} \]

\[ \text{BaCO}_3 \cdot 2\text{H}_2\text{O} \]

\[ \text{LaCO}_3 \cdot 7\text{H}_2\text{O} \]
Chapter IV
2,4-Dihydroxyazo dyes

Section (1). Coelochrome Violet 52

Coelochrome Violet 52 (under the name Coelochrome Violet 40) was the dye originally used by Hillard and Macfarlane to determine aluminium polarographically, and its polarography has been studied more extensively than any other azo dye. The present work followed directly from that of Hillard and Reynolds and then to study the polarography of a number of other complexes formed by Coelochrome Violet 52.

6. Determination of Magnesium in the Presence of Calcium

A series of standard magnesium solution were placed in 50 ml. graduated flasks to give a final concentration of 0 - 10 g./ml. of magnesium. The solutions were then treated with 50 ml. of 0.05 per cent Coelochrome Violet 52 solution and 5 ml. of 20 per cent piperidine solution. The solutions were neutralised with 0.05 hydrochloric acid and then their pH values were adjusted to 13 with 10 per cent sodium hydroxide solution. Finally the solutions were diluted to 50 ml. with distilled water and were heated for five minutes at 70°C in a water bath. After cooling, aliquots were placed in a polarographic cell, degassed by passage of hydrogen for about five minutes and polarographed.

These preliminary experiments confirmed that a polarographically active magnesium-Coelochrome Violet 52 complex
was formed, part of the azo reduction step being displaced to a more negative potential. The height of the second step was proportional to the magnesium concentration up to about 7\(\mu\)g/ml. of magnesium. At higher concentrations the increase in step height was less than that expected. Although the step was measurable it was not of a very satisfactory shape. The step for aluminium is compared with that for magnesium in figures 2 and 3.

A further series of aliquots of the standard magnesium solutions were treated as above except that an aliquot of standard calcium solution was added to give a comparable concentration to that of magnesium. A good calibration curve was difficult to obtain due to the deterioration in the shape of the magnesium step on addition of even a small amount of calcium. The deterioration in the shape of the step was accompanied by a change in colour of the solution from deep violet to orange as previously reported. With amounts of calcium present which were previously found to be tolerated the magnesium step could only be measured with the greatest difficulty.

Although the dye step was not affected polarographically by the presence of calcium, the colour change in the solution indicated that a complex was formed.

In view of the fact that the deterioration of the magnesium - dye wave was caused by the calcium complex, efforts were made to discover conditions under which the calcium complex was not formed.
Fig. 2

Diffusion Current

Applied Potential.

- Solochrome Violet RS.
- Dye + Aluminium.

Fig. 3

3 microg. Mg per ml. in presence of Solochrome Violet RS. pH 13

Diffusion Current

Applied potential (Volts versus Hg pool)
A series of solutions was prepared in 50 ml graduated flasks such that the final concentration of magnesium and calcium in each solution was 3 \( \mu \text{g} \) ml. and 60 \( \mu \text{g} \) ml. respectively. Each solution was treated as previously described but this time the final pH was adjusted within the range pH 9.5 - 12.5. Some of the solutions were examined after heating to 70°C or 100°C and cooling. Other solutions were examined without this preliminary heating and cooling.

A calcium-free magnesium solution was also polarographed for comparative purposes. The solutions were examined by conventional a.c. polarography and by cathode ray polarography. The results are tabulated in Table 1.

**Table 1**

3 \( \mu \text{g} / \text{ml.} \) Mg in the presence of 60 \( \mu \text{g} / \text{ml.} \) Ca

<table>
<thead>
<tr>
<th>pH</th>
<th>Peak Height for Cathode Ray Polarography (arbitrary units)</th>
<th>Step Height for Cathode Ray Polarography (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0***</td>
<td>9620</td>
<td>27</td>
</tr>
<tr>
<td>11.9</td>
<td>900</td>
<td>7</td>
</tr>
<tr>
<td>11.9*</td>
<td>2700</td>
<td>16</td>
</tr>
<tr>
<td>11.9**</td>
<td>4200</td>
<td>22</td>
</tr>
<tr>
<td>11.5</td>
<td>4200</td>
<td>15</td>
</tr>
<tr>
<td>11.5†</td>
<td>2700</td>
<td>25</td>
</tr>
<tr>
<td>11.0</td>
<td>6300</td>
<td>25</td>
</tr>
<tr>
<td>10.5</td>
<td>9200</td>
<td>15.5</td>
</tr>
<tr>
<td>10.0</td>
<td>2420</td>
<td>12.5</td>
</tr>
<tr>
<td>9.5</td>
<td>300</td>
<td>3</td>
</tr>
</tbody>
</table>
Solution heated to 55 - 70°C before polarography.

Solution boiled before polarography.

Calcium free solution.

Examination of the above results shows that the optimum pH is 11 - 11.5. This corresponds to the solution which had the deepest purple-red colour. Heating to 55 - 70°C or boiling the solutions produced an increase in the step height and an improvement in the shape of the step at non-optimum pH values.

Table 2 shows the results for a similar set of experiments with 3 μg/ml magnesium and 50 μg/ml calcium. In this case all the solutions were heated to 70°C before polarography.

Table 2

<table>
<thead>
<tr>
<th>pH</th>
<th>Peak Height for Cathode Ray Polarography (arbitrary units)</th>
<th>Step Height for Cambridge Polarography (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0</td>
<td>1</td>
<td>change of slope only</td>
</tr>
<tr>
<td>11.5</td>
<td>4500</td>
<td>17</td>
</tr>
<tr>
<td>11.0</td>
<td>5100</td>
<td>21</td>
</tr>
<tr>
<td>10.5</td>
<td>5400</td>
<td>20</td>
</tr>
<tr>
<td>10.0</td>
<td>4200</td>
<td>20.4</td>
</tr>
<tr>
<td>9.5</td>
<td>very small</td>
<td>5</td>
</tr>
</tbody>
</table>
From the above results it can be seen that for these magnesium and calcium concentrations the optimum pH is between 10.5 and 11.0.

In the above case, the increase in sensitivity at the optimum pH range was accompanied by a considerable improvement in step shape. A typical wave at pH 11 is shown in fig. 4. This may be compared with the wave for magnesium at pH 13 (fig. 3).

Ten-fold dilution of both Calochrome Violet R and the magnesium did not lead to any improvement in the polarographic waves.

During the above experiments one, or sometimes two, small steps occurred in addition to the two large steps normally obtained. One of these was due to failure to remove the last traces of oxygen and the other may be due to adsorption at the mercury drop.

A further series of solutions were prepared in which the magnesium-calcium ratio was varied. It was confirmed that the optimum pH is dependent on the concentration of calcium. This had already been indicated by the results shown in Tables 1 and 2. Solutions containing 3/4 ml. magnesium without any calcium were examined and the results given in Table 3.
Fig. 4

3 microg. per ml. Mg and 60 microg. per ml. Ca in presence of Solochrome Violet RS
pH 11

Fig. 5

3 microg. per ml. Mg in presence of Solochrome Violet RS.
pH 11.5 (Piperidine buffer.)
Table 2

<table>
<thead>
<tr>
<th>pH</th>
<th>Peak Height for Cathode Ray Polarography (arbitrary units)</th>
<th>Stop Height for Cambridge Polarography (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>7320</td>
<td>32.0</td>
</tr>
<tr>
<td>12.5</td>
<td>9700</td>
<td>35.7</td>
</tr>
<tr>
<td>12.5</td>
<td>9600</td>
<td>40.0</td>
</tr>
<tr>
<td>11.5</td>
<td>9720</td>
<td>40.0</td>
</tr>
<tr>
<td>11.0</td>
<td>9900</td>
<td>36.0</td>
</tr>
<tr>
<td>10.5</td>
<td>9900</td>
<td>38.0</td>
</tr>
<tr>
<td>10.0</td>
<td>4500</td>
<td>33.0</td>
</tr>
<tr>
<td>9.5</td>
<td>2500</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Solution heated to 55 - 70°C for 5 minutes

Solution boiled

The above results showed that the optimum pH for polarography of magnesium in the absence of calcium with malachite green and pH was about 11.5. The stop height was nearly twice that obtained when calcium was present. At the optimum pH the shape of the magnesium dye stop was greatly improved (see fig. 5). The results obtained show that the method can be made more sensitive and precise by carrying out the polarography at the optimum pH instead of at pH 13 as previously reported.

In view of the above results and the fact that the presence of calcium caused a considerable decrease in the
height of the magnesium - dye step, it was desirable to separate the magnesium and calcium before polarographic
a number of methods were considered, including chromatography
and ion exchange. A number of complexing and precipitating
agents were also tried. It was found that an effective
method was to precipitate the calcium with ammonium oxalate;
very little magnesium was lost in this process. The
precipitate was decanted off before the solution was
polarographed, and in some cases, centrifuged to aid the
settling of the precipitate. The excess oxalate had a
negligible effect on the shape of the magnesium step, which
had a sensitivity approaching that obtained in the absence
of calcium, as can be seen from Tables 4 and 5.

Table 4

| pH  | Step Height for Cathode ray | Step Height for | Graphy (arbitrary units) | Graphy (arbitrary units) |
|-----|----------------------------|----------------|--------------------------|
|     |                            |                |                          |
| 11.5 |                           | 520            |                         |
| 11.0 |                           | 3350           | 35                       |
| 10.5 |                           | 2400           | 53                       |
| 10.0 |                           | 2390           | 17.5                     |

*Solution heated to 55 - 70°C for 5 minutes

**Solution heated to boiling.
In order to find the calcium : magnesium ratio which could be tolerated the cathode ray polarograph was used because of its rapidity and sensitivity. The results are given in Table 6, and a typical cathode ray polarogram is shown in Fig. 6.

**Table 6**

<table>
<thead>
<tr>
<th>pH</th>
<th>Peak Height for Cathode Ray Polarography (arbitrary units)</th>
<th>Step Height for Cambridge Polarography (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5</td>
<td>9000</td>
<td>35</td>
</tr>
<tr>
<td>11.5*</td>
<td>9500</td>
<td>35</td>
</tr>
<tr>
<td>11.0</td>
<td>7500</td>
<td>22.5</td>
</tr>
<tr>
<td>10.5</td>
<td>3600</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calcium Concentration g./dl.</th>
<th>Ca : Mg Ratio</th>
<th>Peak Height (arbitrary units)</th>
<th>Ammonium Exalate Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 : 1</td>
<td>9000</td>
<td>5 ml., 0.25%</td>
</tr>
<tr>
<td>3</td>
<td>1 : 1</td>
<td>8940</td>
<td>&quot;</td>
</tr>
<tr>
<td>50</td>
<td>1 : 10</td>
<td>8400</td>
<td>&quot;</td>
</tr>
<tr>
<td>10</td>
<td>1 : 10</td>
<td>9300</td>
<td>5 ml., 0.25%</td>
</tr>
<tr>
<td>60</td>
<td>1 : 20</td>
<td>5400</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
Fig. 6

3 microg. magnesium per ml. in presence of Solochrome Violet RS. 1000 microg. calcium per ml. precipitated by addition of 2g. ammonium oxalate. pH 11.5 (Piperidine buffer.)
Table 6

PH 11.5 Magnesium Concentration mg/mL

<table>
<thead>
<tr>
<th>Calcium Concentration mg/mL</th>
<th>Mg:Ca Ratio</th>
<th>Peak Height (arbitrary units)</th>
<th>Arsenious Oxalate Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>1:50</td>
<td>9000</td>
<td>5 ml, 0.253</td>
</tr>
<tr>
<td>300</td>
<td>1:100</td>
<td>6780</td>
<td>&quot;</td>
</tr>
<tr>
<td>300</td>
<td>1:100</td>
<td>9000</td>
<td>10 ml, 0.253</td>
</tr>
<tr>
<td>600</td>
<td>1:200</td>
<td>10600</td>
<td>5 ml, 0.253</td>
</tr>
<tr>
<td>900</td>
<td>1:300</td>
<td>5000</td>
<td>10 ml, 0.253</td>
</tr>
<tr>
<td>1200</td>
<td>1:300</td>
<td>13200</td>
<td>5 ml, 0.253</td>
</tr>
<tr>
<td>1200</td>
<td>1:300</td>
<td>12500</td>
<td>5 ml, 0.253</td>
</tr>
<tr>
<td>1200</td>
<td>1:400</td>
<td>2400</td>
<td>10 ml, 0.253</td>
</tr>
<tr>
<td>1500</td>
<td>1:400</td>
<td>8700</td>
<td>12 ml, 0.253</td>
</tr>
<tr>
<td>1500</td>
<td>1:480</td>
<td>6720</td>
<td>&quot;</td>
</tr>
<tr>
<td>2000</td>
<td>1:423</td>
<td>5700</td>
<td>15 ml, 0.253</td>
</tr>
<tr>
<td>2000</td>
<td>1:667</td>
<td>11700</td>
<td>&quot;</td>
</tr>
<tr>
<td>2500</td>
<td>1:720</td>
<td>2120</td>
<td>0.5g + 5 ml, 0.253</td>
</tr>
<tr>
<td>3000</td>
<td>1:1000</td>
<td>10200</td>
<td>As dissolved in dye solution</td>
</tr>
<tr>
<td>3000</td>
<td>1:1000</td>
<td>8400</td>
<td>&quot;</td>
</tr>
<tr>
<td>4000</td>
<td>1:1233</td>
<td>7500</td>
<td>&quot;</td>
</tr>
<tr>
<td>4000</td>
<td>1:1233</td>
<td>1000</td>
<td>&quot;</td>
</tr>
<tr>
<td>5000</td>
<td>1:1697</td>
<td>10500</td>
<td>&quot;</td>
</tr>
<tr>
<td>5000</td>
<td>1:7100</td>
<td>11400</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
Table 6

<table>
<thead>
<tr>
<th>Calcium Concentration (μg/ml)</th>
<th>Mg : Ca Ratio</th>
<th>Peak Height (Arbitrary units)</th>
<th>Ammonium oxalate added</th>
</tr>
</thead>
<tbody>
<tr>
<td>7000</td>
<td>1 : 2333</td>
<td>15200</td>
<td>2g dissolved in dye solution</td>
</tr>
<tr>
<td>6000</td>
<td>1 : 2067</td>
<td>13900</td>
<td>&quot;</td>
</tr>
<tr>
<td>9000</td>
<td>1 : 3000</td>
<td>15500</td>
<td>&quot;</td>
</tr>
<tr>
<td>10000</td>
<td>1 : 3333</td>
<td>16500</td>
<td>&quot;</td>
</tr>
<tr>
<td>11000</td>
<td>1 : 3667</td>
<td>10800</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

1. This solution was allowed to stand two days before polarography.
2. Solution heated to 75°C for five minutes before polarography.
3. Ammonium oxalate was dissolved in the dye solution before addition of calcium and magnesium.
4. Solution containing 10000 μg/ml of calcium and above had a distinct orange tint at pH 11.5.
5. The wave obtained from the solution was poorly defined.

A series of solutions were prepared as above but containing 1000 μg/ml calcium and 2g ammonium oxalate, added as the solid. The magnesium concentration in the polarographic solution was varied within the range 1 - 5 μg/ml. The results showed a good linear relationship between the step height and the magnesium concentration in the range 1 - 5 μg/ml.
The effect of interference by aluminium was also studied. It was shown that, provided the pH of the final solution was greater than 11, no aluminium - dye step was obtained and no interference with the magnesium determination was encountered. It is probable that the aluminium is present as Al (OH)₄⁻ at high hydroxyl ion concentrations.

From Table 6 it can be seen that calcium interference can be eliminated by addition of ammonium oxalate, provided that at least twice the stoichiometric amount of oxalate ion is added. There was some evidence that, in solutions of low calcium content with a high residual concentration of oxalate, some depression of the magnesium complex step occurred. It was found to be essential to ensure that all the crystals of ammonium oxalate had dissolved before adding the magnesium and calcium solutions. If this was not done some loss of magnesium occurred by co-precipitation in the regions of high oxalate concentration surrounding the undissolved crystals. The excess of calcium which could be tolerated was limited by the solubility of ammonium oxalate. At room temperature approximately 3g of ammonium oxalate may be dissolved in 50 ml of dye solution.

This procedure for removing calcium interference was simultaneously and independently used by Richardson, who developed a method, based on the work of Balmer and Reynolds, for the determination of magnesium in luminous and pharmaceutical quality calcium carbonate. Richardson also studied the interference of a number of ions including iron, but did not carry out extensive studies of
the interaction of magnesium and calcium, such as reported in this work.

b. **Solechrome Violet AS with Aluminium**

Although this reaction had been studied by a number of workers, it was re-investigated here, initially, to see if the separation between the free dye step and the complex step varied with concentration of aluminium. It was found that the separation between steps increased linearly with aluminium concentration at first but, after reaching a maximum separation of 300 eV, decreased again to 100 - 150 eV when only the complex step was present (see fig. 7). This occurred at both pH 4.5 and 6.5 where Jenkins and Reynolds reported a 1:2 and 1:3 complex respectively.

To check these containing ratios potentiometric titrations were carried out. The results indicated that a 1:2 complex was formed from pH 3.7 upwards when $1 \times 10^{-3}$ dye was used. Using a dye concentration comparable to that used in the polarographic solutions ($1 \times 10^{-4}$), a 1:1 complex was observed at pH 4.5 and a 1:2 complex at pH 6.6.

Because of these results it was decided to carry out continuous variations plots using the height of the complex step as ordinate. At pH 4.6, where potentiometric titrations indicated only the 1:1 complex to be present (see fig. 6), a ratio of 1:1.4 (metal : dye) was obtained, although the largest step was obtained with a ratio of 1:1.
Fig. 7.

\[
\Delta E_{\text{L}} (\text{mV})
\]

\[
\begin{align*}
\text{pH} & 4.6 \\
\text{pH} & 6.6
\end{align*}
\]

\[
\text{M} \times 10^4 \text{ AI with } 1 \times 10^{-4} \text{ M SVRS}
\]
(1) Curve for HNO₃
(2) " " " + 4x10⁴ M SVRS
(3) " " " " " " " " + 1x10⁴ M Al
(see fig. 9). A plot of change of absorption at 13000 cms\(^{-1}\) versus molar ratio gave a ratio of 1 : 1 (see fig. 10). A plot of absorption at 24000cms\(^{-1}\) versus molar ratio showed a change in slope at a ratio of 1 : 1. This ratio was confirmed by a molar ratio plot (see fig. 11).

The investigation was repeated at pH 6.6, the region where potentiometric titrations indicated only a 1 : 2 complex to be present. Both molar ratio and continuous variations plots indicated a 1 : 2 complex (see fig. 12 and 13 respectively) although some of the step heights were somewhat erratic as previously noted by Perkins and Reynolds.\textsuperscript{25} This is possibly due to the pH being close to the pH value for Dyes/cm Violet \(D_c\). Spectrophotometric studies indicated a ratio of 1 : 2.5, but from the graph of change in absorption versus molar ratio, it was difficult to obtain an accurate ratio.

The investigation was repeated at pH 5.1 and a ratio of 1 : 1.74 was obtained. The reason for this non-stoichiometric ratio is that more than one species is present in the solution at this pH. From the potentiometric titration curves a definite contribution from the 1 : 2 complex can be observed (see fig. 1). Spectrophotometry again showed that no obvious changes in absorption due to complexed formation were apparent. Therefore the absorption due to the free dye was recorded at each dye concentration and subtracted from that of the dye plus aluminium solution, plotting the change in absorption, at 13000 cms\(^{-1}\), versus
Fig. 9.

Step height in cms. at sens 1/5

pH 4.6

mls. (1 x 10^{-3} M Al.)

mls. (1 x 10^{-3} M SVRS)

Fig. 10.

Δ Absorbance at 18000 cm^{-1}

pH 4.6

mls. (1 x 10^{-3} M Al.)

mls. (1 x 10^{-3} M SVRS)
**Fig. 11**

Step height in cms. at sens. $\frac{1}{20}$

Concentration of Al ($M \times 10^4$) with $2.56 \times 10^{-4}$ SVRS

**pH 4.6**

**Fig. 12**

Step height in cms. at sens. $\frac{1}{20}$

Concentration of Al ($M \times 10^4$) with $2.56 \times 10^{-4}$ SVRS

**pH 6.6**
Fig. 13.

Step height in cms. at sens. 1/15

pH 6.6

mls. (1x10^-3 M Al.)

9 7 5 3 1

mls. (1x10^-3 M SVRS.)

Fig. 14.

Δ Absorbance at 19000 cm^-1.

pH 5.1

mls. (1x10^-3 M Al.)

9 7 5 3 1

mls. (1x10^-3 M SVRS.)
The ratio of dye : aluminium indicated that two species were present, a 1 : 1 and a 1 : 2 complex (see fig. 14). A similar plot at pH 5 and at pH 6 indicated a ratio of 1 : 1. All the spectrophotometry was carried out on a Unicam SP850 using either 2mm or 5mm Infracel cells. In all the three investigations there was no apparent change in the absorption in the ultra-violet region, showing that the resonance of the dye was little affected by complexation.

From the above evidence it is seen that a 1 : 1 complex is present at pH 4.6 and a 1 : 2 complex at pH 6.5 in contrast to the 1 : 2 and 1 : 3 complexes reported by Perkins and Reynolds at these pH values. Examination of the diagrams in the paper by these authors reveals that, for the same concentration of aluminium, the complex step at pH 6.5 is twice that at pH 4.6. Assuming similar diffusion coefficients, this indicates that twice as much dye is present in the complex formed at pH 6.5 as for that formed at pH 4.6. These ratios agree with those obtained by Coster and King spectrophotometrically. Florence and Ayward have suggested that Perkins and Reynolds inadvertently used impure dye in the commercial sample obtained and the pure. The concentration of dye used by Perkins and Reynolds was $4.5 \times 10^{-4}$, assuming 100% purity of the dye, which is of the same order as used in this investigation ($4 \times 10^{-4}$). So the discrepancy in ratios cannot be explained as a concentration effect.

It has been reported many times (19, 23-25, 54) that for the aluminium - toluidine blue complex step to be
fully developed it is necessary either to heat the solution at 60 - 70°C for about 5 - 10 minutes or to allow the mixture to stand at room temperature for a day or more. Potentiometric titrations, on the other hand, indicate that the aluminium complexes are completely and rapidly formed even at 0°C. It was therefore decided to investigate this aspect more closely.

A solution 2.5 x 10^{-3} M with respect to electric blue Violet 80 and 1 x 10^{-4} M with respect to aluminium in 2 x 10^{-3} M nitric acid was titrated with 0.01 M sodium hydroxide at 0°C until the 1 : 1 complex was just completely formed (pH 4.75). An aliquot of the solution was removed, decolorized and polarographed at 0°C. The same aliquot was then heated to 70°C for 10 minutes and then was re-polarographed at 0 ; 40 and 60°C. The results showed clearly that the complex step was enhanced by the heating process although the polarogram obtained at 0°C was not well shaped due to precipitation. At 40°C the complex step was somewhat less than half the total step height as would be expected for a 1 : 1 complex with the constituents in the above ratio. The separation between the steps remained about the same at all temperatures. The polarogram at 40°C was larger than would be expected from the usual temperature coefficient of 1.5 \text{ per } °C. This was because the precipitate was fully dissolved at this temperature.

The titration of the bulk of the solution was continued and a second aliquot was removed at pH 7.2 when the
The 1 : 2 complex was completely formed. This liquid was polarographed under the same conditions as described above.

On heating to 70°C and then cooling again to 0°C, the second step was increased considerably, leaving the dye step about one third of the total height. Theoretically, it should be about one fifth of the total height. As the total step height was somewhat smaller in this case, however, this effect could have been due to the precipitation of the complex.

In the polarograms at 40°C and 60°C, both steps were increased, leaving the free dye step one fifth of the total step height. The increase in step height between 0°C and 40°C was more than that expected from the temperature coefficient for a diffusion-controlled step. The increase in step height between 0°C and 60°C, however, did approximate closely to this value. The difference in the half-wave potentials of the two waves remained essentially constant, at 0.05 volts, in all these determinations.

c. Potentiometric titrations with iron (III)

As the contining ratios reported by Verhins and Reynolds for aluminium are in error, it was decided to check the ratios for iron (III) reported by Huber and Reynolds, who reported ratios of 1 : 2 and 1 : 4 at pH 4.5 and 5.5, respectively.

Potentiometric titrations of solutions of iron (III) and alcocrome violet R, indicated only a 1 : 2 complex i.e., four hydrogens ions were released per iron (III) ion.

A continuous variations plot at pH 4.5 using the complex
step height yielded a rather indefinite graph, which probably indicated a combining ratio of 1 : 1 (see fig. 15).

At pH 6.6 a continuous variations plot (see fig. 16) yielded a ratio of 1 : 2.

An interesting point about these polarograms is that, although potentiometric titrations indicated that a 1 : 2 complex is completely formed by pH 5.5, the complex step never completely displaced the free dye reduction step.

This phenomenon had not previously been reported. A possible explanation of this phenomenon is that two complexes exist: an one complex and a hydrazo complex, in which the rather unstable hydrazo compound has been stabilised by complex formation. The one complex is reduced at the same potential as the free dye, and the hydrazo complex is then reduced to the azine at a more negative potential. This accounts for the two steps being equal, even when a considerable excess of iron (III) is present. Both complexes are reduced, involving two electrons in each reaction.

4. Colloferrite Violet II with Nickel

This system was reinvestigated for the same reason as was the iron-Coloferite Violet II system. Isler and Reynolds reported that the behaviour of nickel was unusual in some respects compared with other metals studied; no second step was formed at pH 4.5 in acetate buffer whilst at pH 6.5, a second step, corresponding to a 1 : 2 complex, was obtained. In borate buffer, the second step was obtained at both pH 4.5 and pH 6.5. Isler and Reynolds explain this...
Fig. 15

Step height in cms. at sens. \( \frac{1}{15} \),

\( \text{pH} 4.8 \)

\(|\text{mls. of } 1 \times 10^{-3} \text{ Fe}| \)

\(|\text{mls. of } 1 \times 10^{-3} \text{ M SVRS}| \)

Fig. 16.

Step height in cms. at sens. \( \frac{1}{15} \),

\( \text{pH} 6.6 \)

\(|\text{mls. of } 1 \times 10^{-3} \text{ Fe}| \)

\(|\text{mls. of } 1 \times 10^{-3} \text{ M SVRS}| \)
by suggesting that in acetate buffer, the water 'sheath' around the nickel ion is replaced by acetate ions in preference to Cellosolve Violet 3R. Potentiometric titrations showed that two hydrogen ions are released per nickel ion, indicating a 1 : 1 complex at pH 5 and above.

A continuous variations plot at pH 6.55 using the complex step height, indicated a 1 : 1 complex (see fig. 17) during these polarograms, a third step was noted between the dye step and the complex step i.e., -0.66 volts (voltage mercury pool) which did not correspond to the reduction of free nickel but may have been due to the reduction of complexed nickel as opposed to a complexed dye.

Polarography in acetate buffer at pH 4.5 yielded a small complex step. This is in accord with the potentiometric titrations, which indicated only slight complex formation at this pH.

The validity of Neal and Reynolds' results using borate buffer at pH 4.5 are doubtful, as this pH is outside the buffer region for borate buffer; the present author found that one drop of dilute alkali or acid changed the pH considerably. By adjusting the pH of a solution of nickel and Cellosolve Violet 3R to pH 4.5 and polarographing, a well-formed complex step was obtained, and as reported by Neal and Reynolds, polarographing a similar mixture i.e., containing the same amount of borate but this time, buffered with succinic acid and sodium hydroxide, yielded only a small complex step similar to that obtained in
Fig. 17.

Step height in cms. at sens. 1/5

mls. of $1 \times 10^{-6}$ M Ni
mls. of $1 \times 10^{-3}$ M SVRS

Fig. 18

Diffusion current

Applied potential (Volts)
acetate buffer. This indicates that the complex formed in
bicarbonate buffer at pH 4.5 is due to insufficient buffer capacity
allowing the pH to be increased in the vicinity of the
mercury drop when hydrogen ions are used up in the reduction
of the azo group.

e. Polarography of Solochrome Violet R

The polarography of Solochrome Violet R at various
pH values within the range 2.4 to 13.0 was studied using
various buffers, to ensure that the solutions were adequately
buffered at all pH values. At low pH values (i.e., ca pH 2)
and also at pH 7.5 to 8.2 the dye step was split into two
parts. Using the second part of the step at low pH values
and the first part at high pH values, a straight line was
obtained for half-wave potential versus pH, corresponding
to the equation:

$$E_1 = -0.0125 - 0.0556 \text{ pH}$$

up to pH 7.7. Above this pH there was a fairly large
shift of half-wave potential to more negative values,
probably due to the increased irreversibility of the re-
duction in piperidine buffer. This equation agrees
reasonably well with that of Florence and Wyllie42
between pH 3.5 and 7.5 ($E_1 = -0.100 - 0.065 \text{ pH}$), whose
results obtained in piperidine buffer do not fit the
equation. Bean and Iryna43 obtained the following
equations:

$$E_1 = -0.055 - 0.05 \text{ pH} (\text{pH 3 - 15})$$

and:

$$E_2 = -0.100 - 0.025 \text{ pH (pH 3 - 5).}$$
All these values are quoted versus the saturated calomel electrode.

As no other report of the splitting of the valence bond was found, it was decided to investigate this phenomenon further. The pH values at which splitting occurs correspond to the pH values for the dyes:

\[
\text{Na}_2\text{SO}_3\text{N=N=N} \xrightarrow{\text{pH 3}} \text{SO}_3\text{N=N=N} \quad \text{and} \quad \text{pH 7.5}
\]

Hao observed that this type of splitting occurred in the case of Patent Yellow:

\[
\text{Na}_2\text{SO}_3\text{N=N-NH-}
\]

but he did not coment upon it.

The various steps were investigated by logarithmic analysis and the effect of the height of the mercury head was also studied.

Logarithmic analysis of the steps at pH 2.42 yielded two straight lines, corresponding to 1.5 and 2.76 electrons for the first and second parts, respectively. No difficulty was experienced in estimating where one step ended and the other began. Logarithmic analysis of the combined steps was a curve, indicating an irreversible process.

Logarithmic analysis of the polarographic wave at pH 4.6 in phosphate/citrate buffer, yielded a curve which
could be divided into two straight line portions, the first corresponding to 1.16 electrons, and the second 1.6 electrons.

Logarithmic analysis of the split wave at pH 5.3 gave the straight line portions corresponding to 1.27 and 2.00 electrons, respectively.

The split wave at low pH was investigated by variation of mercury height; the first part of the step appeared to be kinetically controlled, and the second part of the curve was diffusion controlled. Belthoff and Liberti reported the occurrence of a kinetically controlled step in the reduction of phenylnitroso-hydroxylamine at pH values between 7 and 9; when the first step was small it was completely independent of mercury height. The overall step for Calechres Violet 12 was diffusion controlled. Increasing the temperature from 25 to 35°C almost doubled the step height which also indicates a kinetic process.

A similar investigation of the wave at pH 4.6 indicated that it was diffusion controlled. Investigation of the waves at pH 7.0 indicated that the first part of the wave was diffusion controlled and that the second part of the wave was at least partially adsorption controlled. The total wave was diffusion controlled. The dependence of the step height on the concentration of dye confirmed this. It was also noted that the splitting of the waves was most evident at slow drop times. This also indicates adsorption. The splitting at low pH values was unaffected by the drop rate.
At pH 7.6 a "knee" appeared after the main step. This disappeared on the addition of gelatin solution which suggests that this is an adsorption phenomenon.

The above results indicate that the reduction of methylene blue over a wide pH range is not so straightforward as has previously been indicated. Differently protonated forms of the dye appear to be reduced at slightly different potentials, resulting in splitting of the reduction wave. It would also appear that the reduction step of one of the species depends on the rate at which one form can change into another. At pH values around 7.6 one of the species is quite strongly adsorbed giving rise to a step that is at least partially adsorption controlled.

In view of the fact that logarithmic analysis of the polarographic wave did not give whole numbers it was decided to carry out micro-coulometry. To check the experimental details cadmium solution was used as a control. At first a silver gauze anode was used but this gave low results and therefore a silver foil was used. Irreproducible results were obtained with concentrations of cadmium down to 50 μg/ml.

Using 0.16 g/l of dye in acetate, ammoniacal and piperidine buffers at pH values 4.6, 9.0 and 12.5 respectively gave the inclusive results. The good results obtained with cadmium solutions showed that the method worked. The results obtained with methylene blue.
suggested that some reaction was interfering with the electrode processes to different extents in the series of experiments. This may have been adsorption or dissociation of the dye.

Using the electromechanical drop separator at high speeds gave inconsistent results which may have been due to stirring of the small volume of solution.
Section II. Triphenylamine Black II

Ferronate Black II

C.I. 14645

\[
\begin{align*}
\text{OH} & \\
\text{Na}_2\text{SO}_3 & \\
\text{NO}_2 & \\
\text{N} & = \text{N} & \\
\text{Ph} & \\
\end{align*}
\]

Electronic Study of Triphenylamine Black II

An investigation of the pH dependence of the half-wave potential of the dye was carried out. The first series of experiments gave rather erratic results, probably due to insufficient buffering, as the system was that used for magnesium and solochrome violet II, adjusted to the appropriate pH values. The work was therefore repeated using some of the buffer systems listed in Chapter III. The dye concentration was \(4 \times 10^{-3}\)M throughout.

It was found that solutions of the dye decomposed fairly readily and leaving overnight caused the step height recorded for a solution buffered at pH 5.0 to be reduced by 15-20%. It was, therefore, necessary to make up the stock solution each day and when this was done the step heights could be reproduced within 5%.

In studying the pH dependence of the wave it was found that there was a sharp increase in step height at pH 7, but after pH 12, the step height was reduced slightly. Evidence of splitting of the step at pH 5-7 was found, but this could not be reproduced consistently. It is
probably due to a pH value for the dye being in this region. (above 6.5 - 7 the dye changes from clear to blue.)

At high pH values a small step shaped plateau like a peak, was observed (see fig. 4). This appears to be a maximum of the second kind. The addition of gelatine solution caused this step to become flatter and smaller, but it also caused splitting of the main step in some cases.

The optimum amount of gelatine was found to be 0.15 ml. of 0.1 solution which removed the peak formed at high pH values and did not distort the step seriously. The humped maxima, however, were not completely removed. The drop time in the dye solution, at zero applied voltage, was 1.5 seconds compared with 3.3 seconds in distilled water, at this applied potential, showing a strong adsorption.

In some precipitation was noted in the polarographic cell the pH dependence was repeated using a final dye concentration of 1 x 10^{-4} and adding 0.15 ml. of 0.1 gelatine solution. At low pH some coagulation of the dye was observed when the potential was applied. The plot of half-wave potential versus pH was a straight line between pH 2.4 and 11.6 corresponding to the equation:

\[ E_1 = 0.092 - 0.0635 \text{pH} \]

As before a sharp increase in step height was observed after pH 7.6 (see fig. 10).

Step height was linearly related to concentration up to a concentration of 1.6 x 10^{-4} at pH 5 in phosphate
Fig. 19. Step height in cms. at sens $\frac{1}{5}$.

Fig. 20. Peak height in cms. at sens $\frac{1}{16}$.

mls. of $1.8 \times 10^3$ M Fe added to $1 \times 10^{-4}$ M dye.
buffer, after which precipitation of the dye occurred.

As it is necessary to heat the metal dye solutions to ensure complete complex formation when studying the
photometry of the complexes, the effect of heating the
dye solutions alone was studied. The step height of the
dye was found to increase on heating. This explains the
results of Balasch53 who found that an increase in dye step
height occurred, on the addition of metal ion and heating.

This increase is probably caused by an increase in the amount
of monomeric dye species present after heating, as opposed
to the dimeric and trimmeric species. The tendency for one
dye to exist in aggregates is well known79, 71 and has
considerable significance. Alcohol was added to the dye
solutions, but this caused a decrease in step height and
the effect of heating became more pronounced. Alcoholic
solutions of dye (4 x 10^-4) with added buffer, gave rather
attractive polarograms, as aqueous solutions having a final
dye concentration of 1 x 10^-4 were once more employed.

Heating again caused an increase in step height but at
high pH values there was a decrease in some cases. These
changes in step height were not very marked.

The large increase in step height above pH 7 is
almost certainly due to the reduction of the nitro group
which occurs at the open half-wave potential on the one
hand and therefore contributes to the overall step height.
The step height increased by up to approximately three times
its original value. This would be accounted for by a four
electron and reduction plus a six electron nitro reduction.

It was noted that, at high pH in pyridine buffer, the
colour of the dye solution changed from red to yellow on
standing. Colorimetric examination of these aged solutions
showed that the height of the dye step had been considerably
decreased. The same result was noticed using pulse polaro-
geraphy. This indicates that the decomposition involves the
azo group. Additions of hydrogen peroxide did not restore
the red colour, neither did heating in the presence of iron
filings, nor addition of other reducing agents.

Further fresh solutions were prepared. These were
sealed in ampoules under nitrogen or air and were heated to
70°C for 12 minutes. The samples under nitrogen were
unaffected by heating, but those under air rapidly faded and
changed colour. Thus the decomposition is due to oxidation
by atmospheric oxygen. The colour changes were from blue
through violet, grey and greenish-yellow to yellow.

Identification of the lost solutions gave a colourless
solution. The results are presented in Table 8.

Table 8

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample under N₂</th>
<th>Sample under Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating to 70°C</td>
<td>Did not decolourise</td>
<td>Rapidly gone green</td>
</tr>
<tr>
<td>Storage in the light</td>
<td>Stable for several days</td>
<td>Gone green within hours and faded further</td>
</tr>
<tr>
<td>Storage in the dark</td>
<td>Stable for several months</td>
<td>Gone grey in a few days</td>
</tr>
</tbody>
</table>
This is in agreement with the findings of Kemetergan\textsuperscript{118} who showed that the fading of an dye in light and air is usually due to oxidation and in very slow in an atmosphere of nitrogen. The fading of the colour was followed spectrophotometrically and the colour faded to decrease with time.

b. Rhodamine black 7 with aluminium

As reported by \textsuperscript{63} Yelling the dye with aluminium solution in acetate, phosphate and borax buffer increased the dye step height, but the present author has shown that this is due to heating the dye alone and not to complex formation as previously claimed. In borate and borax buffer, however, a new step was apparent on close examination of the polarograms. This step was difficult to measure because it is so close to the dye step and because the addition of aluminium caused the plateau of the dye step to slope more steeply, probably by modification of the double layer around the mercury drop. In phosphate buffer there was some indication of a complex step but it was not as well formed as in the other buffers, probably due to interference by complex formation between the phosphate and aluminium. The effect of pH on the half wave potential of the dye step with and without the addition of aluminium is given in the following equations:

\[ \alpha_1 = 0.03 - 0.05 \text{ pH} \]

and:

\[ \alpha_2 = 0.042 - 0.01 \text{ pH} \]

respectively. Within experimental error these equations are identical. The equation for the complex step is:

\[ \alpha_1 = 0.125 - 0.063 \text{ pH} \]
for pH values within the range pH 5 to 6.

In order to obtain greater resolution of the steps it was decided to use the Cottrell Faraday Pulse Polarograph using a 70.8 pulse and scanning 1 volt per 15 minutes for maximum resolution. Two well separated peaks were obtained.

A study of the concentration dependence drawn from results obtained on the Cottrell and pulse polarographs gave smooth curves for up to 5.0 mg/ml of aluminium. The curve flattened off at 6.0 mg/ml of aluminium (see Fig. 21).

A combining ratio determination using the method of Job, employing the pulse polarograph results, indicated the presence of a 1:1 complex at pH 4.9 in acetate buffer. This agrees with the potentiometric titrations which showed that two hydrogen ions are released per aluminium ion at pH 5.5.

c. Methylene Blue with Iron (III).

Solutions were prepared by adding 0.5 ml of 0.001M iron (III) solution to the dye solution in acetate buffer at pH 4.7 and in ammonium buffer at pH 6.5. These gave a second reduction peak when examined on the pulse polarograph. In a similar solution buffered with pipericine solution at pH 12.5, no second peak was observed. The peak at pH 6.5 was somewhat larger than the one at pH 4.7. Heating the solutions to 70°C decreased the height of the second peak.

A continuous variations plot at pH 4.7 indicated the presence of a 1:1 complex (0.05:1.05) although after the equivalence point (i.e., with excess iron (III)) the step heights were rather more erratic. In the presence of iron
Step height in cms. at s.e.n.s 1/4

Fig. 21

Step height in cms. at s.e.n.s 1/4

Fig. 22
the purple colour of the dye deepened significantly.

The results were in good agreement with the potentiometric titration curve which indicated a 1 : 1 complex at pH 4.5.

As the pH was increased hydrolysis of the iron (III) appeared to take place, since the curve was rather 'drawn out' above pH 5.

The step height concentration relationship is shown in fig. 20. The step height increased rapidly at first and then flattened off after the addition of 5 ml. of 0.0015 M iron (III), which is equivalent to almost twice the amount of dye present. The step due to the iron complex was only about half the size of that due to the aluminium complex at comparable concentration. This phenomenon is discussed later.

d. **Trichrome Block with Copper**

The addition of copper to buffered solutions of the dye caused displacement of the copper reduction step. In acetate buffer the shift was from -0.122 volt to -0.165 volt (versus mercury pool) i.e., the reduction was moved to a more positive potential. This can only be due to the reduction of copper in the azo dye complex being more reversible than that of the hydrated copper ion. In ammoniacal buffer the copper step was displaced from -0.197 volt to -0.12 volt. When 5 ml. of 0.0015 M copper solution was added, a reduction in the dye step height of about 60% was noted, which is in accord with a 1 : 1 complex being formed. In piperidine buffer, the copper step was moved to a more negative potential (-0.06 to -0.316 volt). In this case, the height of the
copper step was greatly reduced from 110.5 to 13.4 arbitrary units due to precipitation of the copper. Further evidence of complex formation was the change in colour from red to blue between pH 4.7 and 9.1 on addition of copper.

Potentiometric titrations indicated that a 1 : 1 complex was formed completely, above pH 3.5 and that hydrolysis did not occur below pH 9. Above pH 9 there was evidence of hydrolysis.

b. Prussian black with magnesium and calcium

The addition of 0.1 mg. of magnesium ion to the dye solution at pH 10.5 (carbonate buffer) gave a slight indication of a new peak but, as the dye was not very stable at high pH values, the polarograms were difficult to interpret. Using the Cambridge polarograph, it was shown that the height of the dye step was reduced on adding magnesium. Optimum pH conditions for complex formation seem to lie between pH 10 and 11.

Calcium gave no indication of forming a polaro graphically active complex. Potentiometric titrations showed that complex formation was almost entirely absent below pH 9.5.
Section (iii). Colochrome Blue Black

Lavender Black 3
C.I. 14640

This dye was purified by solvent extraction using acetone as solvent. The purity of the impure dye was determined by titration with chloric titration. That of the purified dye was estimated from the increase in absorption of the dye. The results showed that the purity of the dye used was 92 – 94%

Electrochemistry of Colochrome Blue Black

20 ml. of 0.35% dye solution was added to solutions buffered at various pH values in 50 ml. volumetric flasks and the resulting solutions were diluted to volume.

Electrochemistry was carried out on the Cambridge and on the Rodelkin polarographs and the half-wave potential of the well-defined step was plotted against pH. The following equations were obtained:

\[ E_2 = + 0.002 - 0.0025 \text{ pH (Cambridge)} \]

\[ E_1 = + 0.015 - 0.0025 \text{ pH (Rodelkin using 0.9 volt scale)} \]

Colour changes were noted at pH 7.4 and at about the pH corresponding to the pH values of the dye.

A plot of step height versus pH showed a maximum at pH 6 – 7 (see fig. 23). This may be attributed to a lack of available hydrogen ions at this pH value.

Logarithmic analysis of the polarographs obtained at pH 5.9 in formate buffer gave a good straight line.
corresponding to 1.67 electrons, whereas a similar analysis at pH 9.0 gave 1.64 electrons. In the aqueous solutions tended to precipitate on standing 20% alcoholic solutions were used. Logarithmic analysis of the polarograms obtained for a solution in formate buffer in this solvent yielded two straight line portions corresponding to 1.13 and 1.56 electrons.

The pH dependence was repeated using 20% alcohol and the following equations were found to hold between pH 2.8 and 12.3:

\[ E_y = +0.057 - 0.0603 \text{ pH} \text{ (Cambridge)} \]
\[ E_y = -0.045 - 0.061 \text{ pH} \text{ (Mandelka)} \]

As some precipitation was still evident, the strength of the buffer solutions was reduced to a tenth of the original concentration i.e., instead of using a total of 20 ml of polar buffer, 2 ml were used. The polarograms obtained with these solutions were better shaped. The equation obtained was:

\[ E_y = -0.062 - 0.060 \text{ pH} \text{ (Mandelka)} \]

The polarographic step height versus pH was of the same shape as that obtained without the addition of alcohol.

The concentration dependence in 20% alcoholic acetate buffer at pH 4.0 proved to be a straight line up to at least \( 2 \times 10^{-4} \) concentration of dye, both the graph of step height versus mercury head and that of step height versus square root of mercury head could be drawn as straight lines. This indicates predominantly diffusion
control with possibly some contribution from adsorption.

At pH 3.5 in alcoholic ammoniacal buffer the dependence of step height on concentration of dye was linear up to 0.001 M dye. Dependence of step height on mercury head indicated some adsorption, but there was some doubt as to the shapes of these curves.

At pH 12.69 in sodium hydroxide/piperidine buffer the step height was again found to vary linearly with concentration and the dependence of step height on mercury head indicated predominately diffusion control with some influence from adsorption.

So the logarithmic analysis results indicated that irreversible reduction processes were taking place it was decided to try to determine the number of electrons involved in the reduction by micro-coulometry, as with Sulphhemate Violet no unsatisfactory results were obtained.

After the electrolysis period two new steps were observed in the polarogram. These had the appearance of maxima of the second kind being rather broad. To test this hypothesis polarograms were obtained for solutions in which the concentration of base electrolyte was reduced. Maxima of the second kind usually occur in fairly concentrated solutions of base electrolyte. The slope of the plateau region was different but the two waves remained. The colour of the dye changed from blue to purple on electrolysis.

When the electro-capillary curve was plotted (drop time versus applied potential) it was noted that the position
of the maximum was moved to a more negative potential in the presence of the dye. This indicated that the dye which had adsorbed onto the mercury drop which may be the cause of poor micro-coulometery results. Some adsorption of the buffer occurred as can be seen from the slightly unsmoothed form of the curve in the absence of dye (see fig. 23).

b. Pecokhrene blue black with aluminium

1 ml of 10% aluminium solution was added to a mixture of 20 ml of 0.1M Pecokhrene Blue Black, 10 ml of ethanol, 0.5 ml of 3 acetic acid and 0.25 ml of 5N sodium acetate solution in a 50 ml volumetric flask. The resulting solution was made up to volume with water. Polarography yielded two steps, one due to the reduction of the dye (at -0.25 volts) and a second step, due to the reduction of the complex (at -0.785 volts versus SCE). Prior heating and cooling of the solution increased the size of the complex step obtained and improved its shape.

At pH 4.9 using acetate buffer, a Job continuous variations curve indicated a combining ratio of 1 : 1.45, while a concentration dependence study for aluminium with 0.25M Pecokhrene Blue Black gave a straight line up to 0.14M aluminium, after which the graph flattened off and the points became rather erratic. This concentration of aluminium corresponds to a mole ratio of 1 : 2.

The above procedure was repeated in alcoholic
acetic acid buffer at pH 4.9 using 0.5 ml of each buffer component instead of the usual 5 ml. Two steps were observed, which were somewhat less well resolved than
Fig. 23

Drop rate in seconds

Applied potential in volts

- Base electrolyte
- Dye

Fig. 24

(1) 8 \times 10^4 \text{ M} \text{ SBB}
(2) " + 2 \times 10^4 \text{ Fe}

pH

mls. 0.0097 M NaOH
those obtained at pH 4.9. The half-wave potentials were
-0.575 and -0.741 volts (versus SCE), respectively. The
complex step was relatively larger than that obtained at
pH 4.9. This might be due to the stoichiometry of the
complex being changed, the number of electrons involved
in the reduction being different, the diffusion constant
having changed or the complex being more completely formed.
Prior heating and cooling of the solutions enhanced the
second step at the expense of the first and shifted the
half-wave potentials by 0.04 volt in the positive direction.
This was found to be due to the loss of paranitro on heating.
A Job continuous variations plot indicated a combining
ratio of 1:2, although a dip in the graph also occurred
at a ratio corresponding to 1:1.3.

A concentration dependence study gave a smooth curve
up to 0.125m aluminium concentration with 0.25m concentration
of celestatone Blue Black. This curve on a mole ratio plot
confirms the 1:2 ratio previously obtained.

At pH 12.6 in piperidine-sodium hydroxide buffer only
one well shaped step, corresponding to the dye reduction,
was obtained. A second, churned out step associated with the
dye was also observed. This second step however, varied
with aluminium concentration in an irreproducible manner.

Potentiometric titrations showed that up to pH 5 one
hydrogen ion was liberated per aluminium ion added. Above
this pH a second hydrogen ion was released. Taken in
conjunction with the polarographic work these results
indicate the formation of a 1:1 and a 1:2 complex in which the dye is attached to the metal ion by only one of its hydroxyl groups and by the azo group. This is the first time that the complexation of an o,o'-dihydroxy
azo dye has been observed in this manner. The fractional ratio at pH 4.9 was probably obtained because the 1:2 complex is not completely formed at this pH. On the other hand, the potentiometric titrations indicate that it is completely formed at this pH. The conditions for the two investigations were not identical; however, in that the dye concentration for the titrations was twice that used in the polarographic studies and that the polarographic solutions were buffered.

c. polychrome blue black with copper

The addition of 5 ml. 1% copper solution to the dye solution at pH 4.9, under similar conditions to those previously described caused an increase in the height of the dye step; at the same time a point of inflection appeared in the wave. The purple-red colour of the dye solution changed to a deeper purple colour. The step due to the reduction of copper in the absence of the dye did not appear under these conditions. It would appear that a similar complex to those formed between the previous dyes studied and copper is formed. There was no modification of the dye step. Prior heating and cooling of the solution caused the step height to increase. The dye step and the complex step were almost coincidental and could not be
resolved satisfactorily.

Spectrophotometry showed an absorption band with a maximum at 16300 cm⁻¹ for the complex compared with 15900 cm⁻¹ for the dye alone.

Similar polarographic and spectrophotometric results were obtained at pH 8.3.

The addition of copper to the dye in pipericine/sodium hydroxide buffer at pH 11.9 resulted in a step being obtained before the dye step. Further the drop-out step previously referred to was better defined. Heating the solutions to 70°C for 30 minutes and cooling before polarography resulted in polarograms of improved shape.

A graph of the height of the complex step versus the concentration of copper indicated a combining ratio of 1 : 1.7. The dye step decreased to a minimum at a ratio of 1 : 1.4 then increased slightly and finally decreased again after a ratio of 1 : 1 had been reached.

A spectrophotometric mole ratio plot using the absorbance at 17500 cm⁻¹ indicated a ratio of 1 : 1.49 although neither this, nor the polarographic investigation gave a straight line suitable for analytical purposes.

Continuous variations plots from polarographic and spectrophotometric results yield ratios of 1 : 1.42 and 1.52 respectively, using the step at -0.64 volts (versus S.C.E.) and the absorbance at 2530Å.

Potentiometric titrations indicated that 1.65 hydrogen ions were released per copper ion added. The titration
curves were indicative of a fully formed complex. Above
pH 4.7 the titration curves for dye with and without copper
almost merged, which signifies that either the hydrogen was
liberated per copper ion, or that the complex dissociated
at this pH. This latter possibility, however, is not in
accord with the polarographic evidence.

The results obtained for this system are not very
consistent but seem to indicate the formation of a complex,
Cu(Dye).

d. Alizarin red S blue black with Iron (III)

Unusual and in some ways uncertain results were
obtained with this system. Potentiometric titrations seemed
to indicate that a 1 : 1 complex was formed i.e., two
hydrogen ions were released per iron (III) ion. Previous
work has shown that diethyldihydroxyazo dyes normally form
1 : 2 complexes. On the other hand, the polarographic and
spectrophotometric results tend to indicate that a 1 : 2
complex is formed. These present results can be reconciled
only if one phenolic proton is liberated per dye molecule
on complex formation. The potentiometric titrations
(see fig. 24) of the dye, with and without iron (III)
present, tend to merge at pH values above the pH value
of the dye. The slight displacement in the iron (III)
curve at these higher pH values is probably due to hydrolysis.
The fact that the curves tend to merge supports the theory
that only one hydrogen ion is liberated per iron (III) ion
added.
Due to the uncertainty of the results and for completeness, the results are recorded without further comment. Further work is required on this system before firm conclusions can be drawn.

**Results**

Spectrophotometric mole ratio graphs, at pH 8.0, using the absorbance at 16700 cm\(^{-1}\) and 21000 cm\(^{-1}\) both gave combining ratios of 1 : 2.26. The absorbance at 15500 cm\(^{-1}\) and 16000 cm\(^{-1}\) decreased in size, but changes in the ratios of decrease were noted at mole ratios of 1 : 2.64 and 1 : 2.8. Continuous variations studies, using both the polarographic complex step and the absorbance at 16700 cm\(^{-1}\) indicated a ratio of 1 : 2.5. Using the absorption band at 24900 cm\(^{-1}\), a ratio of 1 : 2.7 was indicated. Some indications of complexes containing more than one iron (III) ion were obtained.

These results are suspect as the buffer capacity of the solutions subsequently was found not to be large enough to cope with the acid used to stabilize the stock iron (III) solution. The buffer capacity was therefore increased by using 2 ml. of ammonium chloride and 2 ml. of ammonium hydroxide solutions in a total of 35 ml. solution. Previously 0.5 ml. of each component was used.

A mole ratio graph using the complex step height for iron (III) is shown in Fig 25. The combined eye and complex step height was somewhat erratic, well-defined continuous variations plots were not obtained at any wavelength but when the complex step at -0.8 volts (versus SCE) was used
Fig. 25

Step height in cms, at sens. $10^8 \times 3$

$M \times 10^4$ Fe with $4 \times 10^{-4}$ M SBB

Fig. 26

Diffusion current

Applied potential in volts
the cellophane...
1 : 2. Some of the absorption bands were increased in
size. A plot of dye step height versus concentration of
iron (II) showed a minimum at a ratio of 1 : 2 and then
increased to a maximum at a ratio of 1 : 1. The combined
complex and dye step height was fairly constant up to a
1 : 1 ratio and then decreased almost to zero. The small
complex step at -0.26 volts (versus Fe) indicated a
combinable ratio of 1 : 2. It was difficult to measure
this step accurately as it never attained a height more
than a quarter that of the dye step. A continuous
variation plot using the absorbance at 12500 cm⁻¹ also
indicated a 1 : 2 complex.

The addition of iron (II) to a dye solution in alcoholic
acetic acid buffer at pH 3.65 caused a change in colour from
blue to red and a complex step was formed -0.70 volts
(versus Fe). Prior heating and cooling did not increase
the complex step height. The polarographic results were
erratic, probably due to precipitation; although no
precipitate was observed during polarography. It was,
however, noticed when the solutions were allowed to stand.
A dye ratio plot using the absorbance at 12500 cm⁻¹ and
subtracting the contribution from the free dye, indicated
a ratio of 1 : 2. The major contribution to the absorbance
was from the dye itself, however, that due to the complex
being small. These results were obtained using a 2% dye
concentration in the alkaline cells. Similarly, using the
absorbance at 12500 and 21000 cm⁻¹ combining ratios of
1 : 3.18 and 1 : 2.02 were obtained.

Spectrophotometric continuous variations plots at 10600 cm⁻¹ and 15400 cm⁻¹ indicated combining ratios of 1 : 3 and 1 : 2 respectively although the latter was indicated by a change in slope and not a maximum.

Potentiographic continuous variations plots also indicated a 1 : 3 combining ratio, although it was difficult to obtain accurate potentiographic measurements, as the complex step became very small when the dye concentration was decreased. Many of the potentiograms had a small step (at -0.4 volts) preceding the dye step.

At this stage the buffer capacity of the system was increased as reported in the previous section. A continuous variations plot for iron (II), using the complex step at -0.8 volts (versus SCE) gave a combining ratio of 1 : 1 and a similar result (1 : 1.17) was obtained by plotting the height of the additional step at -1.5 volts. Using the absorbance at 16700 cm⁻¹ a ratio of 1 : 1.6 was obtained and using the absorbance at 61000 cm⁻¹ a ratio of 1 : 0.725 was obtained.

A mole ratio plot using the complex step height was a smooth curve for which it was difficult to determine the equivalent point. The ratio was probably 1 : 3. Well defined spectrophotometric Job plots were not obtained.

The addition of iron (II) to Calchroom Blue Black, 1 ml. 20 piperidine and 2 ml. 3 sodium hydroxide buffer in a total of 25 ml. gave a complex step after heating to 70°C for 10 minutes and cooling, but rather erratic
results were again obtained, due to precipitation. A mole ratio plot indicated a ratio of about 1 : 2, but when a continuous variations plot was carried out, good complex steps were not obtained. At this pH the dye was rather unstable and the colour tended to fade. This deterioration resulted in the appearance of two additional polarographic steps.

It seemed that not only the ratio of dye : metal but also the concentration of dye present (at constant dye : metal ratio), affected the ratio of dye : complex step heights. Table 2 shows these results.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Dye Step (cm.)</th>
<th>Complex Step (cm.)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ml. 1% CBB</td>
<td>8.94</td>
<td>3.2</td>
<td>2.79 : 1</td>
</tr>
<tr>
<td>10 ml. 1% Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1% CBB</td>
<td>7.55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1% Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 ml. 1% CBB</td>
<td>11.29</td>
<td>3.59</td>
<td>3.3 : 1</td>
</tr>
<tr>
<td>5 ml. 1% Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1% CBB</td>
<td>6.9</td>
<td>0.92</td>
<td>7.7 : 1</td>
</tr>
<tr>
<td>2.5 ml. 1% Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 ml. 1% CBB</td>
<td>11.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1% CBB</td>
<td>6.25</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
c. Molechrome Blue Black with Magnesium

The same procedure was repeated with the dye and magnesium and no complex step was found at pH 4.0 and 3.1. The lack of complex formation was confirmed by potentiometric titration. A slight colour change was observed, but was not sufficiently pronounced to show up in the spectrophotometric examination which followed. Addition of magnesium to an alcoholic solution of the dye in sodium hydroxide/piperidine buffer at pH 12.65 increased the small step at -1.26 volts observed with the dye alone. The height of this step however, was approximately the same, whether 2 or 20 ml. of 1N magnesium solution was added. Prior heating and cooling increased the size of the dye step and decreased the second step. This could be caused by the dissociation of complex. On heating a colour change was noted from blue to red-purple, similar to that noted on the addition of excess magnesium.

A series of experiments designed to find the optimum pH for separation of the steps was carried out. This was found to be 12.70. At this pH the half-wave potentials were -0.3 and -1.435 volts (versus S.C.). At pH 12.70 a third small step was noticed at -1.4 volts.

A mole ratio plot was obtained using 0.025M dye and the results tabulated below.
Table 10

<table>
<thead>
<tr>
<th>Concentration (1st. Step) (mm.)</th>
<th>2nd. Step (mm.)</th>
<th>3rd. Step (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.34</td>
<td>3.24</td>
<td>0.35</td>
</tr>
<tr>
<td>0.38</td>
<td>3.24</td>
<td>1.9</td>
</tr>
<tr>
<td>0.42</td>
<td>3.22</td>
<td>1.94</td>
</tr>
<tr>
<td>0.46</td>
<td>3.12</td>
<td>2.2</td>
</tr>
<tr>
<td>0.50</td>
<td>3.8</td>
<td>1.13</td>
</tr>
<tr>
<td>0.54</td>
<td>5.24</td>
<td>1.9</td>
</tr>
<tr>
<td>0.58</td>
<td>5.24</td>
<td>4.16</td>
</tr>
</tbody>
</table>

From these figures curves can be drawn representing 1 : 2 and 1 : 3 complexes for the steps at -1.4 and -1.4 volts respectively but owing to the scatter of the experimental points, these results should be treated with reserve especially as the step at -1.4 volts occurs to some degree in polarograms of the free dye at this pH.

Polarographic and spectrophotometric continuous variations curves were obtained. Using the step at -1.4 volts a ratio of 1 : 0.59 (mg : dye) was obtained. Examine the dye step closely showed that this step could be divided into two parts, both of which were decreased in size when the concentration of the dye was lowered. The second step could also be divided. The first part of this step (-1.4 volts) gave the ratio indicated above, whereas the height of the second part of the step remained constant. Spectrophotometrically the absorbance fell linearly with decrease in dye concentration.

In view of the rather unsatisfactory results obtained it was decided to carry out the investigation at pH 4.9.
Here the steps were less well separated (−0.3 and −1.1 volts), but the polarograms were better defined.

A monovariant plot using 0.28mM dye gave a combining ratio of 1 : 2 using the step at −1.1. The dye step fell exponentially. Only one step was observed with the free dye although the slope of the plateau region after the step rose fairly sharply. Using the absorbance at 18700 cm⁻¹ a 1 : 2 ratio was again observed.

A polarographic continuous variations plot had very scattered points and no conclusions could be made. Using the absorbance at 18700 cm⁻¹ a ratio of 1 : 2 was obtained.

**Dolochrome Blue Black with Calcium**

The addition of calcium solution to the dye at pH 4.9 gave no indication of complex formation even on heating. Potentiometric titration indicated that no complex formation took place below pH 9, which was the maximum pH studied in this series of experiments.

In ammoniacal buffer (pH 3.0) addition of calcium caused a bump to appear on the steeply rising region; in addition an increase in step height was noted. This effect was noted by Palmer who attributed it to the usual type of complex formation. The present author considers this explanation to be in error as the sum of the heights of the dye plus complex steps is usually constant or slightly lower than the height of the free dye step because the diffusion coefficient is slightly lowered by complexation.
A probable explanation is that the dye becomes less aggregated on the addition of calcium. Freshly prepared dye solution gave a larger step than a solution which had aged. The results obtained were not reproducible.

Then 0.1 ml. 4% calcium solution was added to the dye solution at pH 12.5, buffered in sodium hydroxide/ speridine buffer; a colour change from blue to red was observed. This colour change could be induced by heating the dye solution in the absence of calcium. The dye step height increased slightly but further additions of up to 30 ml. of 4% calcium solution did not cause a further increase. The addition of calcium caused the absorption band at 15400 cm\(^{-1}\) to diminish in size and caused a shoulder to appear on the absorption band at 17750 cm\(^{-1}\). This effect could be obtained on heating the dye solution in the absence of calcium. From the above, it seems unlikely that a stable complex is formed between calcium and solochrome blue black.
Section (iv). Luminous na-mercury Halides.

constant 857

\[
\begin{align*}
&\text{C}_{12}\text{CH}_8\text{NO} \\
&\text{H} = \text{O} \\
&\text{SO}_3\text{Na}
\end{align*}
\]

The analyses of this dye were studied to determine
whether both am and nitro stear were modified by complexation.
Results reported in this thesis for similar dyes are not
conclusive. In the structure of this dye is somewhat similar
it is hoped that some useful information might be gained.

4. REACTING NO3 WITH ALUMINUM

When only 0.005 mol of the free dye gave two steps, the half-
wave potentials of which were pH dependent. At pH 4.12,
in acetate buffer the polarogram of 4 x 10^{-4} M dye gave the
following results:

<table>
<thead>
<tr>
<th>Step I</th>
<th>Step II</th>
</tr>
</thead>
<tbody>
<tr>
<td>complex</td>
<td>( h = 0.25 \pm 0.03 \text{ cm} )</td>
</tr>
</tbody>
</table>

The addition of 0.1 ml. of 0.025 M aluminum solution
gave a third step at -3.63 volts (vitreous mercury pool),
which was not affected by prior heating. Further additions
of aluminum increased the height of this step.

A Job oscillator plot was obtained using the third
step and a combining ratio was found to be 1 : 8. The
first step is almost certainly the nitro reduction step.
It is depressed in acid on addition of aluminum and disappeared
entirely at -stoechiometric ratio at 1 : 2. The second
step, due to the reduction of the nitro group, only
appeared slightly (from 2.35 cm. to 2.50 cm.) on the
addition of up to 20 ml. of 1.25 aluminum solution.
Increasing the pH of the solution to 9.05 resulted in a decrease in the height of the complex step and a converging of the two dye steps.

Potentiometric titrations indicated the formation of a 1:2 complex above pH 6. At pH values greater than 7.5 however, appreciable hydrolysis was found to take place. This is probably the cause of the decrease in size of the complex step at pH 9.05.

b. Potentiometric titrations with iron (III)

Addition of progressively larger amounts of iron (III) solution to the dye at pH 4.3 caused a third step to appear as expected, however, a fourth step also appeared. A typical polarogram (6 ml. of iron (III) with 55 ml. of dye in 50 ml. total volume) had the following steps:

Step I


Step II


Step III


The third step was rather drawn-out while the fourth step had a maximum superimposed upon it. Owing to the rather complicated nature of the polarogram, accurate measurement of the steps was difficult and a Job nonvariant plot did not give useful results. The combined heights of the dye steps was fairly constant, but the combined 'complex' steps reached a maximum at 14 ml. of iron (III) solution. Individual ratios could not be obtained.
A difference between this and the polarography with aluminium was that here the height of the one step was little altered. With 20 ml. of iron solution the polarogram gave the following step heights:

<table>
<thead>
<tr>
<th>Step</th>
<th>Step II</th>
<th>Step III</th>
</tr>
</thead>
<tbody>
<tr>
<td>h_0 = 1.25 cm.</td>
<td>h = 3.44 cm.</td>
<td>h = 0.25 cm.</td>
</tr>
</tbody>
</table>

In ammoniacal buffer at pH 6.85 no complex step was observed. Potentiometric titrations showed that two dye molecules were attached to each iron (III) ion above pH 5 and that hydrolysis commenced at about pH 4.5. The onset of hydrolysis was indicated by the fact that the colour of the dye plus iron (III) solution returned to the red colour, which is characteristic of the free dye above pH 6.5.

c. Borscht red 67 with Copper

The addition of copper to the dye solution did not result in the displacement of either dye step, but the copper step was removed, indicating complex formation. Addition of copper above a 1:1 ratio resulted in the formation of a free copper step. Copper also seemed to cause the dye steps to merge and it was impossible to measure the individual steps with any degree of accuracy. The combined height of the dye step was slightly larger in the presence of copper.

Potentiometric titrations indicated that about one more hydrogen ion is liberated from the dye in the presence of copper as expected for a 1:1 complex. The number of extra
hydrogen ions liberated however, does not reach unity which suggests that the complex is not fully formed.

d. Maintain pH 6.7 with Potassium and Calcium

An indicator titrations showed that no complex formation took place at pH values below 6.5 the polarographic investigations centred at a pH value higher than this.

In diethyldiamine hydrochloride/calcium hydrate buffer at pH 12.2 the dye gave two well separated steps of similar size at −0.54 and −1.11 volts (versus mercury pool) respectively.

The addition of magnesium solution did not modify the polarogram noticeably but the addition of calcium caused the appearance of a new step at −3.65 volts. This step increased linearly with concentration of calcium up to 1 × 10^{-2}M calcium with 4 × 10^{-3}M dye. The effect of heating the solutions for 10 minutes at 70°C was to reduce the complex step and to increase the dye steps. The effect of magnesium was to suppress the calcium step. This is analogous to the suppression of the magnesium sulochrome violet B step by calcium.

The calcium step was found to be very dependent on the conditions. Solutions at the same pH but without diethyldiamine hydrochloride gave results which were very much inferior to those obtained with diethyldiamine hydrochloride.

This is the first indication of a polarographically active complex between an ace dye and calcium.
Dyes of the o-chloro-phenylazo type have been shown by previous workers to give a displacement of the end reduction step in the presence of certain metal ions. The dyes studied in the present work also gave this displacement of the wave and the complexes formed had the expected stoichiometry in most cases.

Sulphonazon Violet 13 was found to give the best formed complex step, and the most consistent and reproducible results of all the dyes studied, when a nitro group was present in the dye the polarographic reduction step of this group was not affected even when the one step was displaced. Beraut and 67, a dye containing a nitro group, was found to form a complex step with calcium, which is the first to be reported. Magnesium, although not giving a complex step itself, was found to suppress the calcium step.

An important part of the work was the development of a polarographic method for the determination of magnesium.

The reduction of the dyes themselves were not as straightforward as reported by previous workers and in certain cases, were not fully diffusion controlled. In neutral solution the reduction of solochrome Blue S1 hardly occurred. The shape of the step height versus pH curve (see fig. 82) is similar to those for acid base catalysed reactions but in this case may be due to lack of hydrogen ions needed for the reduction of the azo group. An alternative explanation is
Based on the evidence of adsorption, if the reduction products are adsorbed at around pH 7 then the mercury drop will be covered after the reduction of a small amount of dye preventing further reduction. The reduction products will be more soluble in both acid and alkaline medium and can therefore be more easily desorbed allowing the reduction step to be fully developed.
Chapter V

c.01-Dimethyl pyrazolene dye

Section 1. Collochrome Red 425

The dye was examined first for its pH-dependence. A
volume of 50 ml. of 0.25M Collochrome Red was injected into
a 50 ml. volumetric flask and 10 ml. of normal sodium-
buffer solution was added and the flasks were made up to
50 ml. with distilled water. In some cases, with some
buffers, precipitation occurred and in the more alkaline
solutions the dye decolourised on standing. The half-wave
potential varied with pH according to the equation:

\[ E_1/2 = -0.408 - 0.0454 \text{pH} \]

At pH values between 3 and 5 the polarographic wave
exhibited a small, very drawn-out portion which may have
been another step. In phosphate buffer two other steps
appeared, but these were not very reproducible. A polarogram
of the base electrolyte showed a slight rise in this region.

At pH 11.2, two steps seemed to be present, but whether
or not the second step was genuine in doubt, as the first
step had a small anodal and the second step only rose to a
level which the diffusion current would have attained if the
dip after the maximum had been absent (see Fig. 35). This
is possibly a double layer effect. At high pH values the
reduction occurred at -0.5 to -0.8 volts, at which applied potential the mercury drop would be negatively charged and the outer part of the double layer would be positively charged. If these charges were reversed, possibly by adsorption, then the negatively charged dye step would be repelled causing a dip such as that observed.

At pH 12.7 a small third step appeared at -0.15 volts. On allowing the carbonate-buffered solution (pH 12.7) to stand for 60 hours, the height of the dye step decreased from 1.62 cm. to 0.22 cm. and additional steps appeared at -0.4, -0.6, -0.82 and -1.07 volts (versus SCE), presumably due to decomposition products. At pH 11 (carbonate buffer) the one step was reduced in height from 1.6 cm. to 0.2 cm. and additional steps were observed at -0.3, and -0.49 volts, together with a rather drawn out step at -1.5 volts.

At pH 11.2 (phosphate buffer) the one step was reduced from 0.26 cm. to 0.2 cm. and steps appeared at -0.435 and -1.11 volts.

At pH 12.7 (dimethylglycine hydrochloric sodium pyridine buffer) the step at -0.17 volts was reduced from 0.12 cm. to 0.3 cm. and was displaced to -0.43 volts. A small step appeared at -0.53 volts, the one step at -1.14 volts either disappeared, or moved to -2.66 volts and reduced in height from 1.52 cm. to 0.5 cm. The height of the step at -1.1 volts decreased from 0.35 cm. to 0.35 cm. and was displaced slightly to -1.17 volts.
From the wave it seems that either a complicated
variation is taking place giving rise to several polarographically active compounds, or to a single compound
having several polarographic steps.

The nature of the wave was investigated at three pH
values - pH 4.7, pH 7.0 and pH 12.7. At pH 4.7 the step
height was proportional to the square root of the mercury
head. The dependence of step height on concentration was
not studied successfully as precipitation occurred at dys
concentrations greater than 1 x 10^{-4}. Further additions
of alcohol did not improve the results.

At pH 7.0 the well shaped step was again proportional
to the square root of mercury head. A rather drawn-out step
was also observed at a more negative potential. The step
height of this step was larger than expected for diffusion
control at high mercury head values. This is indicative of
a maximum of the second kind, but unexpectedly the addition
of solution solution caused this step to increase slightly.
This may be due to the step becoming better defined in which
case it is not due to a maximum.

At pH 12.7 the graph of the height of the first step
versus mercury head indicated diffusion control, whereas
the second step appeared to be at least partially kinetically
controlled. Adding solution to the solution had the effect
of increasing the size of the second step whereas the first
step was reduced in size. The experiment was repeated,
adding gelatin to the alcohol free solution. The resulting
polargraph was identical to that obtained for the gelatin
free alcohol solution. The dye solution in the absence of
both gelatin and gelatin gave a wave of somewhat different
shape. Alcohol appears, in this instance, to be acting as a
maximum suppressor. Concentration dependence studies yielded
surprising results. When freshly prepared sodium hydrosulfite
solution was added to the buffer system the second step
disappeared. The first step gave a linear plot up to
5 x 10^{-4} concentration of dye.

Investigation with freshly prepared sodium hydrosulfite
solution yielded one well-defined step with a second rather
dreadful ill-defined step. The well-defined step proved to
be diffusion controlled. Heating this polarographic solution
resulted in three well-defined steps being obtained.

Empirical analysis of the polarograms obtained from the
solution containing carbonate buffer gave the straight line
portions, the corresponding to 1.45 electrons and the other
rather long a portion including the region about the half-
wave potential corresponding to 3.47 electrons.

2. Electrochromic Behavior with Anthraquinone

The addition of 0.05 g of Alizarin to a 7 x 10^{-4}
solution of the dye in 20% alcohol with added acetate buffer
(pH 4.72) yielded two stages, one due to the free dye and the
other to the complex. The solution was then heated to 70°C
for 10 minutes and repolarographed. The results are shown
below.
addition of 0.5 mg. of aluminium and heating yielded two
steps, 0.26 cm. and 2.12 cm. respectively. Since it is
evident that this type of dye behaves in a similar manner to
the di-oxy-dihydroxyazo dyes with respect to aluminium,
is not been previously reported.

A continuous variations curve was obtained under the
conditions stated above and a combining ratio of 1:1 resulted.
A colour change from pink to orange was noted on heating aluminium
with the dye solution.

A concentration dependence study showed that a straight
line could be obtained up to 5 x 10^{-4} aluminium with
1 x 10^{-4} dye. The two-fold excess of dye was necessary for
linearity.

In 0.2 ml. the addition of aluminium to an isomolecular of
the dye resulted in the step becoming rounded. The addition
of 10 ml. methyl alcohol resolved this into two steps, the
combined height being less than that of the rounded step
without the addition of alcohol. This effect was perfectly
reproducible. Heating increased the height of the second
step at the expense of the first. Two other small steps,
probably due to impurities, were present in the polarogram
of the free dye.
The polarograms of the dye step shown in this investigation, yielded three steps probably due to the dye solution having deteriorated—a freshly prepared dye solution again yielded only one step. The polarograms of the base electrolyte did not show these steps. The addition of alcohol to aluminium free solution resulted in the dye step being reduced in height and a poorer polarogram being obtained. Increasing the amount of aluminium concentration from $2.5 \times 10^{-5}$ to $1 \times 10^{-3}$ did not increase the step at -0.67 volts appreciably and resulted in poorly defined polarograms. Without alcohol being present, no step was observed at -0.67 volts.

Potentiometric titrations indicated that the hydronium ions were released per aluminium ion. This corresponds to the formation of a 1:1 complex. A precipitate was formed at pH 3 to 5 but dissolves more about pH 7 after which there are evidence of hydrolysis of the aluminium (III).

c. Solutions of 0.00001 M iron (III)

Digital of 0.1 and 10 ml. of 0.0001 M iron (III) solution were added to an acetate buffered 1 x $10^{-5}$ solution of the dye at pH 6.7/6 and polarograms were run before and after heating. The colour of the dye changed from orange to green on addition of the iron (III) indicating complex formation. Although the step due to the reduction of the iron (III) to iron (II) was reduced considerably, no similar step was observed and the dye step height was unaltered. The effect of heating the solutions was to reduce further the free iron step.
Adding 1 ml. of 0.060M Fe(III) to an optical solution of the dye caused precipitation but no complex step. The addition of 10 ml. of 0.025M Fe(III) did not give rise to a second step. Similar results were obtained in glycine/carbonic acid buffer, pH 4.25.

Potentiometric titrations indicated a 1 : 1 combining ratio with some hydrolysis taking place.

1. Electrolyzed in 0.02M with copper.

Adjusts of copper were added to solutions of the dye at pH 4.75 as described previously. The half-wave potential of the copper step was moved from -0.255 to -0.175 V (versus mercury pool). Seeding did not affect the complex-copper reduction step. The addition of 25 ml. of 0.25M copper (the stoichiometric amount for a 1 : 1 complex) caused in three steps being obtained in the polarogram, the step corresponding to the Cu⁺ → Cu²⁺ reduction, the second corresponding to the reduction of copper in the complex and the third step corresponding to the reduction of the dye. The step height of the complex step varied linearly with copper concentration up to 4.3 x 10⁻⁴.

Thus a three-fold excess of the dye is needed for linearity.

A rather unsatisfactory continuous variations curve was obtained, giving a combining ratio of 1 : 1. This was confirmed by continuous variations plots at pH 4.75 in a normal buffer solution. Although, at higher copper concentration, it became difficult to measure the height of the complex step accurately since a step due to the reduction of free copper was obtained with increasing copper concentration,
both indicate the existence of a non-continous variation plot confirming the 1:1 stoichiometry ratio. The half-wave potential of the copper step was moved from -0.435 volts to -0.403 volts in this solution. As the displacement half-wave potential depends on the dissociation constant of the complex until ion this small displacement indicated a lower dissociation constant. Quantitative determination of the dissociation constant was not made, so the system is not fully reversible.

The continuous variations plot was repeated using the solution twice the concentration of the copper ion. The position of the maximum was only slightly altered confirming the presence of a singly dissociated complex. It can be shown that for an indefinitely unstable complex, the position of the maximum on the continuous variations plot is independent of the ratio of concentrations of ligand to metal ion.

The addition of copper to dye at pH 1.7 produced a color change from green to yellow to red. Less than the equivalent amount of copper to form a 1:1 complex for complex step and the dye step were present, with greater amounts of copper a further step appeared at -0.88 volts (versus Saturated Calomel). After adding a further step was found at -0.43 volts. The heights of the volt anions were difficult to measure owing to their close proximity. As the system was rather unstable due to oxidation and reproducible results difficult to obtain, no further work was carried out. It was noted however, that the addition of copper tended to stabilise the dye and prevent fading.
Potentialometric titrations indicated that a 1:1 complex was formed from pH 3.5 upwards.

a. Potassium, sodium, calcium, and cobalt

Solutions of potassium and calcium were added to amionic dye solutions at pH 4.7, 7.0 and 11.3. No indications of polarographically active complexes were obtained.

Potentialometric titrations indicated that complexes were formed to a slight extent at pH 6.5 but were not fully formed even at pH 7.
This dye is more soluble than alcian blue BR 125 and it was thought that this would be a definite advantage in studying this type of dye.

b. Polarography of alcian blue BR 2

This dye gave too well defined steps over the pH range studied. These were separated by about 0.33 volts. In general the polarograms were not very well shaped, although some reasonable curves were obtained in aminocel and carbonate buffers.

The plot of half-wave potential versus pH, for the first step is a straight line corresponding to the equation:

\[ E_1 = 0.433 - 0.059 \text{pH} \]

between pH 3 and 10.5, after which the increase in half-wave potential with pH was greater than that indicated by this equation. The second step gave a straight line corresponding to the equation:

\[ E_2 = -0.077 - 0.07 \text{pH} \]

up to pH 7.4. At pH values higher than 7.4 the half-wave potential was slightly more positive. Precipitation in several solutions precluded the preparation of a useful graph of step height versus pH. In acid solutions the yellow dye tended to precipitate readily, whereas the magenta coloured alkaline solutions did not.
At pH 7.10 in formate buffer the first step was diffusion controlled, whereas the height of the second step was proportional to the mercury head and therefore seemed to be at least partially adsorption controlled. The dependence of step height on concentration tended to confirm these conclusions. On the first step varied linearly with concentration up to a concentration of \(1.25 \times 10^{-4}\%\). At higher concentrations the step heights were lower, probably due to precipitation. At low dye concentrations (up to \(5 \times 10^{-5}\%\)) the second step was absent. The height versus concentration for the second step rose then fell then rose again. This pattern was reproducible. At concentrations greater than \(1.25 \times 10^{-4}\%\) the first step followed a similar pattern.

At pH 4.0 in acetate similar dependences on mercury head were obtained. However, at pH 9.0 both steps were completely diffusion controlled. Step heights at various concentrations were erratic as shown in Table 11 below.

<table>
<thead>
<tr>
<th>Concentration (mol/L)</th>
<th>Step Height (cm.)</th>
<th>Step Height (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>1st. Step</td>
<td>2nd. Step</td>
</tr>
<tr>
<td>0.002</td>
<td>0.04</td>
<td>2.75</td>
</tr>
<tr>
<td>0.003</td>
<td>0.11</td>
<td>3.53</td>
</tr>
<tr>
<td>0.004</td>
<td>1.6</td>
<td>4.11</td>
</tr>
<tr>
<td>0.005</td>
<td>1.6</td>
<td>4.01</td>
</tr>
<tr>
<td>0.006</td>
<td>13.3</td>
<td>3.75</td>
</tr>
<tr>
<td>0.007</td>
<td>7.9</td>
<td>3.76</td>
</tr>
<tr>
<td>0.008</td>
<td>7.23</td>
<td>3.62</td>
</tr>
<tr>
<td>0.009</td>
<td>25.2</td>
<td>19.0</td>
</tr>
</tbody>
</table>
At pH 12.65 in sodium hydroxide at high mercury lead, a lump appeared behind the second step. This lump did not appear at mercury lead heights less than 56 cm, and did not seem to be a polarographic minimum. The first step height was found to be proportional to the square root of mercury lead, whereas the second step height was proportional, neither to the mercury lead, nor to the square root of mercury lead.

Being to the rather erratic nature of the step heights it was decided to repeat this work using 5% alcoholic solutions. The initial work was carried out on the Cambridge polarograph and later the Incolic polarograph, using the three-electrode system to eliminate the H drop us employed.

Graphs of half-wave potential versus pH yielded the following results:

- \( \Delta E = 0.042 \pm 0.001 \text{ mV} \) (first step) Cambridge polarograph
- \( \Delta E = 0.162 \pm 0.041 \text{ mV} \) (second step) polarograph
- \( \Delta E = 0.332 \pm 0.025 \text{ mV} \) (first step) Incolic polarograph
- \( \Delta E = 0.33 \pm 0.45 \text{ mV} \) (second step) polarograph

With alcohol present the second step gave a graph which was a straight line up to pH 12, using both polarographs. Whereas in the absence of alcohol the linear portion extended only as far as pH 7.4. The points for both these plots were less scattered than those obtained without alcohol. Step height remained constant with variation of pH, except in phosphate buffer where low results were obtained and at pH values greater than 11, where the height of the first step
i) \[ R-\text{N}=\text{N}-R' + 4H^+ + 4e \rightarrow 2\text{NH}_2 \]
or \[ R-\text{N}=\text{N}-R + 2H^+ + 2e \rightarrow R-\text{NH}-\text{NH}-R \]

ii) \[
\begin{align*}
R-\text{N}=\text{N}-C & - + 4H^+ + 4e \rightarrow R-\text{N}=\text{N}-C - & \text{NH}_2 \\
& - \text{CH}_3 & \text{CH}_3
\end{align*}
\]

OR

\[
\begin{align*}
R-\text{N}=\text{N}-C & - + 2H^+ + 2e \rightarrow R-\text{N}=\text{N}-C - & \text{NH} \\
& - \text{CH}_3 & \text{CH}_3
\end{align*}
\]

The scission of the C-N bond is unlikely as it would require too much energy.

iii) \[
\begin{align*}
R-\text{N}=\text{N}-C - & + 2H^+ + 2e \rightarrow R-\text{N}=\text{N}-C - & \text{ZH}_2 \\
& - \text{CH}_3 & \text{CH}_3
\end{align*}
\]

iv) As the C-C is in a conjugated system this may also be reduced.

\[
\begin{align*}
\text{HO-C-N-R'} & + 2H^+ + 2e \rightarrow \text{HO-C-NH}_2 \\
& - \text{CH}_3 & \text{CH}_3
\end{align*}
\]

From the ratio of the step heights it seems likely that the first step is due to a 4 electron reduction of the azo group followed by one of the two electron reductions probably the one proposed in reaction (ii).

5. **Iriochrom Red G with Aluminium**

Aliquots of 5 ml. of 0.25m aluminium solution were added to alcoholic acetic buffer solutions containing 5 ml. 1.Om Iriochrom Red G. The resulting solutions were made up to 50 ml. in graduated flasks. Potentiography of these solutions showed that a small step was apparent between the two dye
b. Tris(hydroxyamino)methane-aluminum

A aliquots of 5 ml. of 0.25 M aluminum solution were added to alcoholic acetate buffer solutions containing 5 ml. of 0.25M tris(hydroxyamino)methane and 3. The resulting solutions were made up to 10 ml. in graduated flasks. Polarography of these solutions showed that a small step was apparent between the two dye steps at -0.11 and -0.54 volts (versus SBE), on heating to 70°C for 30 minutes this third step increased in size, although it was difficult to measure owing to the proximity of the three steps. i.e., polarography, using the Cambridge Universal, did not yield a very useful polarogram probably due to the irreversible nature of the reductions. Increasing the amount of aluminum increased the size of the complex step at the expense of the first step.

As the resolution of the three steps was not good it was decided to use the Rals polarograph to obtain the continuous variations curve. The steps were well separated when a 70°C pulse and a 10 minute scan time were used. The continuous variations plot indicated a ratio of 1:1. Potentiometric titration confirmed this ratio and also provided evidence of hydrolysis from pH 5 upwards.

A volume of 5 ml. of 0.35M aluminum was next added to a 20 ml. alcoholic solution containing 25 ml. of 0.3M tris(hydroxyamino)methane and 3 and an acetic-acetic buffer. The solution was made up to 50 ml in a graduated flask. Polarography of this solution, before and after heating to 70°C, did not yield a complex step.
Due to hydrolysis of the aluminous ion,

the addition of aluminium solution to an alcoholic solution of the dye in sodium hydroxide, at pH 9, yielded a polarogram having a bump after the first step. This first step was slightly greater than that obtained when aluminium was absent. The bump could be regarded as a change in slope of the plateau of the polarographic wave.

Potentiometric titrations showed that the dye was slightly acidic in nature even at pH 3.

c. Triacetoxybenzene 8 with iron (III)

Aliquots of a 0.001 M solution of iron (III) were added to an alcoholic solution at pH 4.7 (acetate buffer) and polarography was carried out. The procedure was repeated in the presence of 10 ml. 0.03 M triacetoxybenzene 8. The results are shown in Table 12 below using polarograph sensitivity setting:

<table>
<thead>
<tr>
<th>Iron salts</th>
<th>0.05</th>
<th>0.10</th>
<th>-0.35</th>
<th>-0.55</th>
<th>-0.75</th>
<th>-1.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step height (cm.)</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>After heating</td>
<td>-</td>
<td>0.02</td>
<td>0.02</td>
<td>0.06</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>Iron salts (dye absent)</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 12

Fe³⁺ Fe⁺ Fe⁺ | - | - | - | - | - | Fe⁺
From the above it can be seen that the free iron steps disappear after heating and one well defined dye step and the very small dye step are left, together with steps due to the iron dye complex.

For a solution containing 20 ml of 0.001 M iron (II), the following steps were obtained at polarograph sensitivity setting (Table 13).

<table>
<thead>
<tr>
<th>Step Height (cm)</th>
<th>1.9</th>
<th>0.16</th>
<th>0.35</th>
<th>0.2</th>
<th>0.32</th>
<th>0.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>After heating</td>
<td>precipitation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Free slope (cm)</td>
<td>2.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In the absence of iron the height of the dye steps were 1.5 and 1.0 cm, respectively. Hence it can be seen that both steps are greatly reduced on complexation.

A concentration dependence using 5 - 15 ml of 0.001 M iron (II) solution with 20 ml of the dye solution showed that the complex step heights were approximately constant in this range (see Table 14).

<table>
<thead>
<tr>
<th>Concentration to Eq E&lt;sub&gt;p&lt;/sub&gt;</th>
<th>10.25</th>
<th>-0.25</th>
<th>-0.27</th>
<th>-0.42</th>
<th>-0.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mol x 10&lt;sup&gt;-7&lt;/sup&gt; M</td>
<td>-0.2</td>
<td>-0.3</td>
<td>0.33</td>
<td>0.33</td>
<td>0.34</td>
</tr>
<tr>
<td>Increased to 50%</td>
<td>-0.2</td>
<td>-0.3</td>
<td>0.33</td>
<td>0.33</td>
<td>0.34</td>
</tr>
<tr>
<td>2 x 10&lt;sup&gt;-4&lt;/sup&gt; M</td>
<td>-0.2</td>
<td>-0.3</td>
<td>0.33</td>
<td>0.33</td>
<td>0.34</td>
</tr>
<tr>
<td>Reaction</td>
<td>$R_{2}$</td>
<td>$R_{3}$</td>
<td>$R_{4}$</td>
<td>$R_{5}$</td>
<td>$R_{6}$</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Heated to $70^\circ$C</td>
<td>0.02</td>
<td>0.7</td>
<td>0.5</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>$2.25 \times 10^{-4}$</td>
<td>0.43</td>
<td>0.63</td>
<td>1.23</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Heated to $70^\circ$C</td>
<td>0.16</td>
<td>0.53</td>
<td>0.73</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>$5.25 \times 10^{-4}$</td>
<td>0.36</td>
<td>0.63</td>
<td>1.13</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Heated to $70^\circ$C</td>
<td>0.15</td>
<td>0.55</td>
<td>0.75</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>$5.35 \times 10^{-4}$</td>
<td>0.45</td>
<td>0.66</td>
<td>1.21</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Heated to $70^\circ$C</td>
<td>0.16</td>
<td>0.53</td>
<td>0.77</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

A continuous variations plot indicated a ratio of 1 : 1.

Potentiometric titrations indicated that two hydroxyl ions were liberated for each iron (II) ion added, thus confirming the 1 : 1 ratio. The continuous variation plot was carried out using the step at -0.3 volts, since the second step was too small to provide any useful information.

The complex was investigated next at pH 3. A concentration dependence study gave very erratic results and a continuous variations plot had three peaks corresponding to iron to iron ratio of 2.5 : 1, 1 : 1/4, and 1 : 1. It is probable that these latter results are due to the hydrolysis of the iron producing erratic polarograms, especially at high iron concentrations, if the points up to a ratio of 0.5 : 1 (i.e., iron) are above 1 : 1.5 and extrapolated to lowest iron ratio is obtained. During these investigations a change in colour of the solutions from a deep purplish red through orange to yellow was noted, it is possible that a 1 : 1 complex is formed, which undergoes...
Hydrolysis to form ferric hydroxide which co-precipitates some of the dye.

To ascertain whether the peaks on the Job plot were 'real' or due to other effects, the determination was repeated using spectrophotometric determinations. The maximal absorption in the visible region was shifted from 530 μ for the free dye to 500 μ with excess of iron present. The two absorption bands in the ultra-violet moved only by 1 μ to 40 μ. Plotting absorbance at various wavelengths gave bands rather than peaks corresponding to ratios of dye to iron of 3:1, 1.67:1:1, 1.33:1 and 1:1.67 and maxima 5:1 at 405, 475, 615, 725 and 570 μ respectively.

During the spectrophotometric investigations it was noted that the copper did not replace the dye step completely, even when we consider the excess of iron (10:1) was present; in fact, when 10 ml of 1.67 μM iron was added to 10 ml of solution the dye was still present, though some complex was formed. The complex of dye and copper was smaller than that previously due to precipitation at this higher iron concentration.

Potentiometric titrations confirmed the presence of a 1:1 complex and showed that one of the ionizable hydrogen atoms of the dye is indeed at low pH and the second at about pH 5.5.

At pH 12.5 (Dialysis cell a glycine/glutaraldehyde hydrochloride buffer) no indications of a complex step were observed, although the step was poorly defined, amounting to no more than a change in slope, and not accurately
measurable and was not very reproducible. Heating had an
adverse effect on the already poor step. It was concluded
that complex formation took place at this pH, but that the
compound was unstable under the conditions studied.

5. Indications of Copper (II) solution were added to
20 ml. 0.05M trichloroacetic acid buffered solution
containing 10% of alcohol. The step due to the reduction of
$Cu^{2+}$ to $Cu^{0}$ at -0.375 volts (versus SCE) disappeared and an
increase in step height was noticed for the first of the
two step steps. With the addition of 30 ml. 0.05M copper
solution the step at -0.375 volts reappeared but was reduced
in size from 0.55 cm. to 1.3 cm. at sensitivity 1/7. The
increase in step height was found to be 0.7 cm. under
the same conditions. No step due to the reduction of
complexed cuprous ions was found. From the above it would seem
likely that the reduction of the complex copper solution
with that of the cuprous step had a half-wave
potential of -0.375 volts (versus SCE). The
addition of copper solution to an unbuffered
buffered solution (pH 3) caused an inflection at the base
of the cuprous step which, with the addition of further quantities
of copper, developed into a second step having a half-wave
potential at -0.375 volts (versus SCE). This corresponds
to the half-wave potential of copper in this medium. A
close examination of the inflection saw another step was found
between the copper step and the cuprous step which occurred at
-9.44 volts (versus SCE). This additional step was probably due to the reduction of complexed copper. This possibility is supported by the fact that the free copper step is about half the expected size in the presence of the dye. Examination of the polarographic solution using the false polarograph confirmed that there were four steps present. The ratio of peak heights using the false polarograph was found to be different to that of the step heights obtained using the Cambridge polarograph. Using the Cambridge polarograph the complex step seemed quite small, although accurate measurement was difficult, owing to the proximity of the steps. With the false polarograph the complex peak was much larger in comparison to the other peaks. The percentage of complex product was found to increase slightly on heating, indicating that true equilibrium was not attained in the cold. A continuous variations plot indicated a ratio of 1 : 1.

The above investigation was repeated at pH 15 in 0.1 M sodium hydroxide solution. With a solution containing 5 ml. 0.02N copper solution four steps were obtained at -9.15, -9.67, -9.7, and -10.0 volts respectively. The last two steps were due to the dye. The height of the more negative dye step was found to vary from run to run. Almost copper (50 ml. of a 0.25M solution) gave an additional step at about -6.85 volts which was difficult to ascribe owing to the proximity of the other steps. This step was due to copper itself which in this solution is reduced at about -1.5 volts (versus SCE). Heating the dye solution in the
not translated
Some data gave a displaced one reduction step with some metal ions, showing that a phenyl group is not a criterion for complex steps of this type. Selenides and S formed a 1:1 complex with aluminum, giving this displacement of the step, but the other metals studied (iron(III), copper, cadmium, and mercury) did not. The first two metals were known shown to form complexes.

Selenides and Se also gave the displaced step with aluminum and iron(III) at pH 0.6. Complex formation took place with copper, as shown by potentiometric titrations, but did not result in a displaced step.
Chapter II

polyarylaminoazo dyes

Section (1). Sulfonurea type II

Synthesis from N-3

G.I. 1929

\[
\begin{align*}
\text{OH} & \quad \text{N} \quad \text{NH}_2 \\
\text{N} & \quad \text{N} \quad \text{NH}_2 \\
\text{NO}_2 & \quad \text{S}_2\text{O}_3\text{Na}
\end{align*}
\]

a. Interatomic bond distances [mm in Table II]

A previous investigation of this dye was abandoned\(^5\) because of precipitation of the dye from solution at pH 4.5
and 6.5. The dye concentration used in the present study
was 0.00125 M in the final solution. Heating the mixture to
80°C was found to dissolve the precipitate, but reprecipitation
occurred on cooling. The filtered solution was found to give
the steps at -0.21 and 0.02 volts at pH 4.5 and -0.25 and -0.55
volts (versus NHE) at pH 4.5.

Reducing the dye concentration to 1.5 × 10\(^{-4}\) M gave solutions
which gave no precipitate for at least five days at pH
values of 4.9, 5.9, 10.44 and 12.73. In 0.2N hydrochloric
acid precipitation occurred almost immediately. In all cases,
polarography yielded two steps. In ammonium buffer at pH
5.9, however, the steps were not well resolved and in
phosphate/acetate hydroxide buffer at pH 12.73 the second step
was poorly defined. The ratios of the two steps varied between
1 : 1.27 and 1 : 1.54 except at high pH.
Table 15

<table>
<thead>
<tr>
<th>pH</th>
<th>1st. step</th>
<th>2nd. step</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mV)</td>
<td>(mV)</td>
<td></td>
</tr>
<tr>
<td>6.06</td>
<td>0.15</td>
<td>1.5</td>
<td>1.25</td>
</tr>
<tr>
<td>6.38</td>
<td>0.146</td>
<td>1.5</td>
<td>1.19</td>
</tr>
<tr>
<td>7.09</td>
<td>0.43</td>
<td>-</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>1.9</td>
<td>0.64</td>
</tr>
<tr>
<td>10.90</td>
<td>0.56</td>
<td>1.7</td>
<td>0.76</td>
</tr>
<tr>
<td>10.48</td>
<td>0.68</td>
<td>1.27</td>
<td>0.79</td>
</tr>
<tr>
<td>12.72</td>
<td>0.38</td>
<td>0.15</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td>0.15</td>
<td>0.52</td>
</tr>
</tbody>
</table>

At pH 12.72 two more steps were observed at -1.16 and -1.12 volts, (versus AgCl), respectively.

The effect of heating the solutions to 75°C, cooling and polarography is shown in Table 16 below.

Table 16

<table>
<thead>
<tr>
<th>pH</th>
<th>before heating</th>
<th>ratio</th>
<th>after heating</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mV) (mV)</td>
<td></td>
<td>(mV) (mV)</td>
<td></td>
</tr>
<tr>
<td>4.56</td>
<td>0.145 1.6</td>
<td>1 : 1.07</td>
<td>0.55 1.04</td>
<td>1 : 1.5</td>
</tr>
<tr>
<td>6.33</td>
<td>0.27 1.7</td>
<td>1 : 1.21</td>
<td>0.39 1.4</td>
<td>1 : 1.5</td>
</tr>
<tr>
<td>8.9</td>
<td>0.82 1.5</td>
<td>1 : 1.1</td>
<td>0.42 1.4</td>
<td>1 : 1.2</td>
</tr>
<tr>
<td>10.46</td>
<td>0.43 1.25</td>
<td>1 : 1.59</td>
<td>0.53 1.5</td>
<td>1 : 1.64</td>
</tr>
<tr>
<td>12.74</td>
<td>0.15</td>
<td></td>
<td>0.5 2.2</td>
<td>1 : 1.4</td>
</tr>
</tbody>
</table>
It will be observed that the effect of heating was to increase the dye steps — especially the second step — except at high pH (12.72) where the first step decreased appreciably.

A pH dependence study was carried out within the range pH 3.72 to 12.9 and the first step was found to vary with pH according to the equation:

\[ E_s = 0.116 - 0.0017 \text{pH} \]

The second step did not give a good straight line and in fact, the steps converged to within 0.13 volts at pH 7. Step heights remained fairly constant up to pH 11 after which the polarograms were difficult to measure.

Dye precipitation occurred at pH values between 5 and 6.5 in citrate/phosphate buffer.

In the majority of the polarographic waves the ratio of step heights was about 2:3, which could correspond, either to a four electron one reduction followed by a six electron nitro reduction, or a four electron nitro reduction followed by a four electron one reduction together with the second part of the nitro reduction involving a further two electrons. The former explanation is the more likely. Nitro compounds containing hydroxyl and amino give six electron reductions at
all pH values whereas most other aromatic nitro compounds give two steps corresponding to 4 and two electron reductions respectively in acid medium and only a four electron reduction in alkaline conditions.

The first step height was found to be proportional to the square root of mercury head indicating diffusion control although the plot of step height versus mercury head also appeared to be a straight line at pH 2.5. The response of the second step to variation of mercury head showed it to be at least partially adsorption controlled. This also applied to the total step height. Both step heights varied linearly with concentration up to $8 \times 10^{-5}$ dye showing overall diffusion control, with the higher concentrations a maximum developed on the second step.

At pH 4.6 both steps were diffusion controlled. In all the well-shaped polarograms, the ratio was either exactly or close to 2 : 3.

b. Polychromate from li 125 with aluminium

Aliquots of aluminium solution were added to the dye solution at pH 4.5, 6.5 and 12.0 without any indication of a third step. Heating to 70°C did not give a third step.

In an attempt to prepare a polaroagraphically active aluminium complex by refluxing the solution containing the metal ion and the dye at pH 4.5 for four hours also failed.

Potentiometric titrations showed that very nearly two hydrogen ions were liberated per aluminium ion added. The formation of the complex was not complete as shown by the fact that the degree of formation was 1/2 after the pH
value of the dye, at pH 7.5, was reached. Some hydrolysis of the complex was noted, a small four extra hydrogen ions were liberated. These could not come from the dye as it has only one ionizable hydrogen ion.

3. Selection from [II] with iron (III)

Dilutions of 0.25% iron (III) solution and 0.05 M

(III) were added to suitable volumes of the dye solution. A change in colour of the solution from a light yellow colour to red was noted. The reduction of iron(III) to iron(II)

was suppressed unless excess iron(III) was present, and the dye step was slightly reduced in height.

The absorption spectra of the solution at pH 6.6 was examined and the absorption maxima were found to have been moved from 445 nm to 465 nm.

Potentiometric titrations showed that a 1:2 complex was formed at pH 5 upwards. At about pH 6 hydrolysis of the complex commenced and after the release of the only ionizable hydrogen atom of the dye, 1.47 hydrogen ions per iron(III) ion were released.

4. Inhibition of selection from [II] with copper.

Addition of copper solution to the dye at pH values 5.5 and 6.5 showed that the reduction step of copper(II) was only present if excess copper was in the solution. The addition of 5 mL, 0.05 M copper(II) to 2 mL, 0.3 M dye yielded four steps at -0.74, -0.775, -0.47 and -2.62 volts (versus SHE) respectively. Copper under these conditions was reduced at -0.74 volts and the dye at -0.47 and -0.62
volts and hence the step at -0.75 V was attributed to the
reaction of complex 1 as per. The possibility of it being
the reduction of copper(1) was excluded by observing the
effect of addition of copper(1) to the system.

Heating the solution to 70°C before polarography caused
a decrease in the complex step and an increase in the free
copper step, which indicates that the complex was partially
broken down on heating.

A continuous variations plot indicated a combining ratio
of 1:1:1. This ratio was confirmed by potentiometric
titrations which showed the formation of a 1:1 complex
from pH 3 upwards. Again some hydrolysis of the complex
was noted at pH values above 7.5. This time the hydrolysis
corresponded to an average of 0.3 hydrogen ions released per
copper ion.

Similar polarograms were obtained at pH values of 7.9
and 11.3 in oxalic acid and dimethylglyoxime/sodium hydroxide
buffer solutions respectively. At pH 11.3 some splitting
of the second dye step was noted but this was difficult to
assess accurately and the work was not continued.

6. Colorimetry of Dyes from Zn, Cu, Fe with Barium and Calcium

A 2% of magnesium and calcium salt was used added
to the dye solutions at pH 7.4, 9.9 and 12.4. No new steps
appeared but, in the case of barium at pH 12.4 some
precipitate was formed which was a lighter red than the
solution. This might be an insoluble complex.

Potentiometric titrations showed that no complex formation
took place at pH values below 10.
section (ii). Arcamittellipum 0

Serdent from 148

\[
\text{NaO}_3 \quad \text{N} = \text{N} \quad \text{NH}_4 \quad \text{NH}_4
\]

This dye was studied to provide confirmatory evidence of the results obtained in section (i) of this chapter.

**Electrochemistry of Arcamittellipum 0**

The dye was examined in a manner similar to that of other dyes studied. The half-wave potential varied with pH according to the equation:

\[
\text{E} = -0.02 - 0.052 \text{pH}
\]

between pH 3 and 12.5. Step height hardly varied with pH, but increased slightly between pH 5.0 and 13.5. Above this value the step heights decreased sharply. At pH values above 12 the steps were present. Investigation of these steps at pH 12.5 in phosphate/sodium hydroxide buffer showed that the second step height did not depend on mercury bead and was, therefore, probably kinetically controlled, whereas the first step and the combined steps were diffusion controlled. Raising the temperature from 25°C to 23 - 34°C however, gave the following results (Table 17).

**Table 17**

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>1st Step</th>
<th>2nd Step</th>
<th>3rd - 4th Step</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1.75</td>
<td>1.55</td>
<td>33 - 44</td>
<td>2.55</td>
<td>1.6</td>
</tr>
</tbody>
</table>

---
a 63. increase was thus obtained for the first step and only a 16. increase for the second step. From these results it seems that the first step was kinetically controlled and the second diffusion controlled, as the increase in height for this step is within the range expected for a diffusion step (1 - 25 per degree centigrade). A concentration dependence study at this pH gave curved graphs for both the reduction steps - over the concentration range 0 to 2 x 10^{-4}. This was taken as indicative of adsorption of the dye. As the concentration of the dye increased, however, the combined step heights gave a straight line up to a concentration of 1 x 10^{-4}, indicating that the overall reduction process was diffusion controlled.

At pH 4.5 the single step was completely diffusion controlled, as shown by the variation of the step height with mercury head and with concentration. At high mercury heads (i.e., above 20 cm) the step heights were larger than required for purely diffusion control probably due to a stirring effect caused by the higher drop rate at these pressures of mercury. Were adsorption was noted from the effect of applied potential on the surface tension of the drop (drop rate).

In an anionic buffer at pH 3.9 results were obtained similar to those at pH 4.5.

**Apparatus and Method**

Solutions of aluminium solution were added to buffered solutions of the dye at pH values of 4.5, 5.9 and 12.4.
At pH 4.6 no additional step appeared, even after heating at 90°C for 10 minutes.

At pH 5.3 two small steps due to impurities occurred after the dye step; the addition of aluminium caused the appearance of another step which was greatly reduced on heating. Further additions of aluminium gave smaller steps at this potential.

Irradiation of the electro-capillary curve showed that strong adsorption was taking place at this potential in the presence of aluminium. This was the probable cause of the change of step angle encountered in successive determinations under the same conditions. Heating caused the dye step to increase both in the presence, and absence of aluminium.

The addition of aluminium solution to the dye in dimethylglycoluril hydrochloride/sodium hydroxide buffer at pH 12.6 did not yield a coupled step, but merely made the polarography more erratic, probably due to precipitation of aluminium hydroxide.

Potentiometric titrations showed that a 1 : 3 complex was formed at pH values greater than 7.5 and that hydroydrol of the dye remained at about pH 7.0. The number of hydroydrol ions liberated above pH 7.0 reached a value of 1.6 per aluminium ion (approximately) as the species formed tended towards Al(OH)₄⁻ at high pH.

c. Manganese Determination

In acetate buffer at pH 6.6, the iron(III) to iron(II) reduction step was decreased in height in the presence of the dye. Further heating caused a further reduction in this step.

In ammonium and in dimethylglycoluril/sodium hydroxide buffer
precipitation occurred and therefore polarography was not carried out.

Potentiometric titrations showed that a 1:1 complex was formed from pH 4 onwards with only slight polarization at pH values below 7.5.

f. Interaction with Copper

Similar results were obtained when copper was added to the dye at pH 4.6, 9.2 and 12.5. That is to say, the copper step was either suppressed or entirely removed depending on the pH and presence, colour change from brown to greenish brown also noted on the addition of copper.

Potentiometric titrations indicated that a 1:1 complex was formed, but the degree of formation did not approach unity until about pH 6 although some complex formation was apparent from pH 3.7 onwards.

g. Interaction by Aluminium and Calcium

The addition of magnesium and calcium gave similar results to those obtained for aluminium with this dye. There was no apparent change at pH 4.6. There was a 'false' step at pH 3.5, with step in the case this 'false' step was found in the polarogram of the dye without any metal ions being present. The first minimum appeared after this 'step' which was removed by the addition of palatine solution. The other additional step was enhanced slightly by the addition of magnesium or calcium ions and seemed very susceptible to slight changes in conditions. The addition of palatines reduced it in size.
The effect of adsorption on to the mercury drop can be seen
to be appreciable from the effect of applied potential on drop
time as shown in Table 18 below:

<table>
<thead>
<tr>
<th>volts</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>2.5</td>
<td>3.05</td>
<td>4.75</td>
<td>4.9</td>
<td>5.22</td>
<td>5.0</td>
<td>5.95</td>
<td>5.2</td>
<td>4.55</td>
<td>5.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>volts</th>
<th>1.0</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
<th>1.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>4.67</td>
<td>4.78</td>
<td>4.68</td>
<td>4.95</td>
<td>4.88</td>
<td>5.5</td>
<td>2.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

From the above it seems that the additional step in due to
adsorption and not to complex formation. This is borne out by
potentiometric titrations, which show that no complex formation
takes place up to pH 9.

At pH 92.5 in dimethylglycol hydrochloride/hydroxylamine buffer there was no indication of complex formation.

Summary of Primary Examination

This investigation has been conclusively that the formation
of a complex is not necessarily sufficient for the modification
of the rate reduction step. Both dyes studied were shown to form
1 : 2 complexes with aluminium and iron(III) and 1 : 1 complexes
with copper(II). Yet in no instance was a part of the reduction
step of the dye group dissociated to some negative potential.

In the case of iron(III) and of copper(II), further evidence
for the presence of complexes was obtained from the removal of
suppression of the metal ion reduction step and in the case of
cadmium from the 1 : 2 reduction step attributed to complexed
copper considered.
The polarography of both the dyes studied indicated that absorption processes were appreciable, and under certain conditions this affected the reduction of the dye.

Calcium green A355 showed two reduction steps, as was expected from its structure. But Argemittelbraun G, which has a very similar structure, showed only one reduction step at pH values below 12. From the comparative size of the steps of the two dyes, it is probable that the nitro and azo reductions are taking place at the same potential for Argemittelbraun G, except at pH values above 12. In this case (below pH 12) it could be expected the ratio of the height of the combined steps for Calcium green to the height of the Argemittelbraun step to be 4 : 1 (see Table 13) with a slight correction for the difference in molecular weights which are 378 and 402 respectively. This correction however, would be small as the diffusion coefficient is proportional to the square root of the molecular weight and the diffusion current is proportional to the square root of the diffusion coefficient.

It can be seen that, within experimental error, this ratio exists (see Table 13).

<table>
<thead>
<tr>
<th>pH</th>
<th>A355</th>
<th>A7</th>
<th>A10</th>
<th>A12</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>17</td>
<td>21</td>
<td>17.9</td>
<td>17</td>
</tr>
<tr>
<td>6.7</td>
<td>17</td>
<td>21</td>
<td>17.9</td>
<td>17</td>
</tr>
<tr>
<td>12.5</td>
<td>17</td>
<td>21</td>
<td>17.9</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 13

Calcium green | A355 | A7 | A10 | A12 |
---|------|----|-----|-----|
| 17 | 21 | 17.9 | 17 |
| 17 | 21 | 17.9 | 17 |
| 17 | 21 | 17.9 | 17 |

Combined step height | 17 | 21 | 17.9 | 17 |
Table 12

<table>
<thead>
<tr>
<th>diameter (mm)</th>
<th>2.5</th>
<th>4.5</th>
<th>6.5</th>
<th>8.5</th>
<th>10.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>step height</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

An alternative explanation of the results obtained involves the suggestion that only one of the two possible solutions is, in fact, taking place. It is considered, however, that this does not accord as well with the experimental evidence.
Chapter VII

azo-dyes, o-thermo and diazo

Section III. Bolschwein and B. 475

The mechanism and reaction of this dye, 8.475, is shown below:

This type of dye was chosen for study because it has been shown that they can form metal complexes of comparable stability to those with o-diazopyrrole and azo dyes.

A. Electrochemistry of Bolschwein and B. 475 with Aluminium

Voltammetry carried out under similar conditions to those described in previous chapters failed to give any indication of the single or reduction step. The reduction step was found to be pH dependent, as with all the other dyes studied in this investigation. At pH 4.7, the half-wave potential of the step was found to be -0.70 volts (versus normal salt).

Potentiometric titrations showed that at pH values between 4 and 5.5, one hydrogen ion is liberated per aluminium ion added. At pH values above this the number of hydrogen ions liberated rose to a value of 3.9 indicating hydrolysis of the complex. Hydrolysis of the complex was confirmed during the actual titrations, as the pH values tended to fall slowly.

Indications of a 1 : 2 complex between pH 6 and 8.7 were also apparent, but in dilute solutions hydrolysis was evident, even at those low pH values.
b. 

**Polyethyleneglycol 1000 with Iron(III)**

Colorimetry of the dye and iron(III) was carried out at pH 4.0 and pH 6.0; where complex formation had been shown, by potentiometric titration, to be complete.

In modification of the dye step was observed but the free iron(III) to iron(II) step was suppressed.

Potentiometric titrations showed that two hydroxyl ions were liberated per iron(III) ion added, at pH values from 3 to 3.5. Above pH 6 hydrolysis of the complex occurred, corresponding to the formation of Fe(OH)$_2$ at high pH. Iron above it areas that a 1:2 complex is formed.

c. 

**Polyethyleneglycol 1000 with Copper and Iodine**

The addition of either of these metal ions to the dye solution at pH 5.5 did not modify the dye step. With copper, however, the reduction step for the Cu$^{2+}$ ion was moved to slightly more negative potentials ($\Delta E = 0.65$ volts), indicating some complex formation.

Potentiometric titrations showed that in both 6 and 7 complexes were formed. In the case of copper the degree of formation was fairly complete above pH 3 but with iodine the degree of formation did not reach unity until pH 5.5.

d. 

**Polyethyleneglycol 1000 with Iodine**

Potentiometric titrations showed that no complex formation occurred below pH 10 and spectrophotometry was carried out only at pH 12.5. On modification of the dye step was observed.

e. 

**Polyethyleneglycol 1000 with Hemin**

Hemin$^{55}$ found that hemin formed a complex with this type of dye and not with any of the other types of dye in
studied. An investigation of the system was therefore made.

The addition of aliquots of 0.01% beryllicium solution to 5 ml. 0.01% elecrochrome and 0.125 in acetate buffer at pH 4 caused a modification of the dye step as shown in fig. 27. The

polarogram can be divided into three regions; the foot, then a steeply rising middle portion and finally a third portion which was proportional to the concentration of beryllicium. The

third portion usually retained the maximum observed for the dye - one. The part of the step proportional to beryllicium increased initially but did not reach a value greater than

1.05 e.v. even when 10 ml. 0.01% beryllicium was added.

titration showed that the complex was not completely formed until pH 7.5 and hence the polarograms were run at various pH values all greater than 4. The best polarograms were obtained at pH 7.5. A concentration dependence study gave a smooth curve up to 10 ml. with 1.0 ml. 0.01% elecrochrome and 0.125 (see fig. 28).

Above this concentration it became difficult to resolve the three portions. The step height-concentration relationship was almost linear up to 5 ml. of 0.01% beryllicium solution.

The combined heights of the steps, in all cases, remained equal to the height of the dye step in the absence of beryllicium.

Potentiometric titrations showed that a 1 : 1 complex

was formed between pH 5 and 7.5 after which basic hydrolysis of the complex occurred. This is considered to be an important aspect of the work as polarographically active beryllicium compounds are almost insoluble and met with do for the determination of beryllicium buffer interference free elecrochrome.
Fig. 27

Diffusion current

Applied potential in volts

Fig. 28

Step height in cms. at sens. 1/50

$M \times 10^4$ Be with $1 \times 10^{-3}$ M Solochrome Red
Section 4.12, Selenochrome Flavine 3 125

a. Polarography of Selenochrome Flavine 3 125 with Aluminium

Polarography of Selenochrome Flavine and aluminium solution under various conditions did not give any indication of the single dye step. Potentiometric titrations, however, indicated that a 1:2 complex was fully formed at pH 6.0. At pH values above 7 hydrolysis of the complex occurred, which eventually corresponded to an average of 3.5 hydrogen ions liberated per aluminium ion.

b. Selenochrome Flavine 3 125 with Iron(III)

Polarography of the dye with iron(III) at pH 4.9 did not modify the dye step. The iron(III) reduction step was suppressed unless excess iron(III) was present, in which case the step was reduced in size. A colour change was noted on heating the dye - iron solution prior to polarography. This colour change was from yellowish-brown to brown.

Potentiometric titrations showed that a 1:2 complex was formed over the pH range 3 to 10 although at pH values greater than 7 hydrolysis of the complex occurred corresponding to the formation of Fe(OH)

c. Selenochrome Flavine 3 125 with Copper

Polarography of Selenochrome Flavine 3 125 with copper did not yield a displaced step although the reduction step for copper(II) was shifted from -0.25 to -0.39 volts (versus mercury pool).
Potentialometric titrations confirmed complex formation above pH 5. The combining ratio was found to be 1:1 but above pH 6.5 some hydrolysis occurred giving finally about 1.5 hydrogen ions liberated per copper(II) ion.

B. Salochrome Flavine II 125 with Iron(III) and Calcium.

Telegraphy of these metal ions with Salochrome Flavine II 125 did not yield a displaced dye step. Potentialometric titrations, however, indicated that magnesium formed a complex with the dye corresponding to a combining ratio of 1:1 at pH 8.0. At pH values above this, accurate results could not be obtained due to the shape of the graphs.

With calcium there was some indication of complex formation but from the graphs it was apparent that the degree of formation was very low.

Summary

Potentialometric titrations have confirmed the presence of complexes of aluminium, iron(III) and copper with both the dyes studied and with nickel and beryllium for theeryl dye studied. The pyrocatechol dye was found to form a complex with magnesium in addition to the usual complexes. None of the complexes formed, with the exception of those of beryllium, caused any modification of the dye step.

A possible explanation of the specific formation of a beryllium complex which gives a half-wave potential displacement is that its small ionic radius allows it to fit into the 'hole' between the co-ordinating atoms of the molecule. None of the other ions will fit into this 'hole,' without considerable distortion of the dye molecule.
Chapter VIII

Pyridyl-azo-dyes

Section (II). \(1\text{-(pyridylazo)}\text{-}2\text{-naphthal} (I)

\[
\text{\begin{align*}
\text{I} & = \begin{array}{cc}
\text{N} & \text{N} \\
\text{H} & \text{H}
\end{array}
\end{align*}}
\]

c. Polarography of I

A solution was prepared using various percentage mixtures of ethyl alcohol and water. To avoid precipitation of I, it was found necessary to use at least 75% v/v alcohol. The addition of potassium chloride base electrolyte, however, caused settling out of the base and it was found necessary to use 15% alcohol for the polarographic solutions.

Step height varied linearly with concentration up to \(5 \times 10^{-4}\) M in 0.1M potassium chloride base electrolyte. With increasing concentration of I, a maximum appeared and increased in size. Logarithmic analysis of the polarographic step indicated a value of 0.90.

The step height was proportional to the square root of the mercury head, but a straight line could be drawn through the points on the step height versus mercury head graph. From the above the overall process would seem to be diffusion controlled with some contribution from adsorption.

The half-wave potential of the well-shaped step varied with \(pH\) according to the equation:

\[
E' = 0.675 - 0.0665 \text{ pH}
\]

over the range \(pH\) 2.5 to 12.7. In the \(pH\) range 7.1 to 9.8,
however, the points were slightly more positive than predicted from this equation. This latter range was studied using a number of buffer systems, including borate, phosphate, glycine/ sodium hydroxide, ethanolic acid/sodium hydroxide and ammoniacal buffer. In all cases the half-wave potentials were more positive than predicted from the equation and in all systems except the ammoniacal buffer the points fell on the same straight line. In the ammoniacal system the half-wave potentials were even more positive.

Step height varied with pH as shown in fig. 27. In view of the shape of graph it was decided to investigate certain regions (marked )

At pH 4.75 in formic acid/sodium hydroxide buffer the step height was proportional to the square root of the mercury head and the step height varied linearly with concentration. In fact, the process was diffusion controlled. The ratio of the maximum to the diffusion controlled portion increased at first and then tended to a constant value.

In-cathodic analysis of the polarographic step gave a slight curve indicating some degree of irreversibility in the reaction, but two straight line portions could be drawn corresponding to 1.15 and 1.99 electrons transferred respectively.

The second pH region studied was that of pH 6.42 in acetic acid/sodium acetate buffer. The step height once more varied linearly with both concentration and square root of mercury head, but again step height versus mercury head
could also be drawn as a straight line. The effect of applied potential on drop rate in $1 \times 10^{-4}$ and $1 \times 10^{-5}$ KCl was studied. The considerable adsorption on the positive side of the electro-capillary maximum and the moving of the electro-capillary maximum to more negative potentials indicated the adsorption of an anion. From the above it seems likely that the overall process is diffusion controlled with some contribution from adsorption.

In carbonate buffer at pH 11.5 graphs of step height versus mercury head were not very reproducible, as carbonate tended to precipitate and the drop rate became very rapid. The investigation of this pH region was therefore repeated in dimethylglycine hydrochloride/sodium hydroxide buffer at pH 12.5. The results indicated a diffusion controlled process. Logarithmic analysis again gave a curve which could have two straight line portions drawn giving values of 1.5 and 2.17 electrons.

At high pH values it was necessary to record the polarograms as soon after mixing up the solutions as possible, in order to avoid irreproducibility.

a. Al with aluminium

Polarography of solutions of Fe(III) and aluminium were carried out at pH 6, 7, 9, 10 and 12.5 without any indication of a polarographically active complex being present.

pHimetric titration of Fe(III) showed that no hydrogen ions were liberated up to a pH value of 12.5. This is in agreement with Iolland et al. who showed that the o-hydroxyl
group in 2.2 did not interact with Fe III. The degree of interaction \( \mathrm{Fe}^{2+} \) for the complex was found to be 2.42 i.e., two Fe ions per uric acid ion. This complex was fully formed by pH 5. Free precipitation occurred during these titrations and this could explain why a whole number value for (5) was not obtained. No indication of hydrolysis was apparent.

d. **Complex with \( \text{Fe(II)} \)**

Colorimetry of \( \text{Fe(II)} \) with \( \text{Fe(III)} \) did not give a displaced dye step, when carried out at pH 6.4 in acetate buffer, even though potentiometric titration indicated that a 1 : 1 complex was formed at this pH. At pH values above 6.5 a 1 : 2 complex was formed. In the case of the first complex the remaining three positions for co-ordination were probably occupied by water molecules. Results**123** and elsewhere showed that with the very similar compound Fe(III) co-ordination was through the oxygen of the \( \text{CO}_2 \)-group, the pyridyl nitrogen and the naphthyl nitrogen furthest from the heterocyclic ring.

e. **Solutions of Fe(II)**

The addition of solutions of Fe(II) to copper solutions caused the half-wave potential of the former to be moved to a more negative potential. At pH 6.4 in acetate buffer this shift was from -0.612 volts (versus \( \text{Fe(II)} \)) to -1.272 volts, thus excess of \( \text{Fe(II)} \) was present. There was also a marked colour change from yellow to red on the addition of copper solution to the dye. Going to the proximity of the two steps it was difficult to measure the heights of the free
copper step and the complexed copper step accurately. In plotting the free copper step versus concentration of copper, however, a marked change in slope of the graph was noted at a ratio of 1:1. This ratio was in agreement with Hoge and Millings who claimed a 1:1 complex was formed and stated that the formation constant was $10^{16}$. Using 0.005M copper and 0.01M ligand the stability constant from the polarographic data was found using the following equation:

$$\left(\frac{b}{c}\right) - \left(\frac{b}{2}\right) = \frac{2b}{a} \log \frac{2b}{a}$$

where $\left(\frac{b}{c}\right)_a$ = half-wave potential of the simple ion.

$\left(\frac{b}{c}\right)_c$ = half-wave potential of the complexed ion.

$n$ = number of electrons transferred.

$p$ = number of ligands attached to metal ion.

$s$ = concentration of ligand.

$\beta$ = stability constant of the complex. Therefore

$\left(\frac{b}{c}\right)_a - \left(\frac{b}{2}\right) = \frac{2b}{2} \log 2 + \frac{2b}{2} \log 2 = 2 \times 10^{-2}$

$\left(\frac{b}{c}\right)_c = \frac{2b}{2} \log 2 = 2 \times 10^{-2}$

therefore $\beta = 5 \times 10^{-2}$

Potentiometric titrations indicated that two hydrogen ions were released per copper ion added above pH 5.9 indicating the formation of a 1:2 complex. These results are difficult to reconcile with the polarographic evidence, but it should be noted that the potentiometric titrations were carried out in solutions 5 \times 10^{-4} M with respect to metal and 3\times 10^{-5} M with respect to Cu, whereas the polarography was carried out in solutions about ten times more dilute. Attempts to reduce the strength of the reagents ten fold for potentiometry gave
irreproducible results, partly due to unstable pH readings. This difficulty was also experienced by Irving and Canadell who had to repeat each titration several times before they obtained reasonable results.

During the potentiometric titrations precipitation again occurred. Corsini et al. have reported that they did not obtain reliable information for the TMA/copper system using potentiometric techniques, but they observed a stable 1:1 complex by spectrophotometry.

e. Polarography of TMA with Magnesium and Calcium

Polarography was carried out in diethylyglycine hydrochloride/sodium hydroxide buffer at pH 12.26. No evidence of polarographically active complexes was obtained with either metal, even after heating to 70°C.

Potentiometric titration of the dye and magnesium indicated that no hydrogen ions were released up to pH 9.5. At pH 9.5 a horizontal portion occurred which then fell slightly, probably due to hydrolysis. Eventually the graph rose, the rising portion corresponding to the release of 3.67 hydrogen ions per magnesium ion at pH 11, and to 3.54 hydrogen ions at pH 10.5. This indicates that hydrolysis is taking place at the high pH value required for complex formation. The glass electrode required a considerable time to attain equilibrium and the readings were rather erratic.

Potentiometric titration of the TMA-calcium system showed that no hydrogen ions were released until pH 9.5. In this case the pH did not increase rapidly, but if the displacement in the curves at pH 11.75 was used, an average
of 1.2 hydrogen ions released per calcium ion, was obtained. From the above it seems that a 1:2 complex is formed but this is only fully established at pH values above 12. Again precipitation occurred during the titrations.

f. \( \text{Ca}^2+ \) with Nickel

Colorimetry gave no indication of a polarographically active complex, on the addition of nickel to \( \text{Ca}^2+ \) solutions, but a colour change from yellow to red and precipitation was noted. Potentiometric titration showed that the ligands were attached to each nickel ion at pH values above 3.5.
Section (11), 4-(3-triphenylazo) resorcinol Red

![Chemical Structure](image)

### a. Polargraphy of HII

HII gave one well-defined step, the half-wave potential of which is pH-dependent. It corresponded to the equation:

$$E_1/2 = 0.145 - 0.002 \text{ pH} \text{ (versus Ag)}$$

But at pH values below 4.5 close examination of the step showed it was split, probably due to the presence of two ionic forms. In 59, Sivanesan, Sollad et al. found the following pK values:

$$\text{pK}_{1} = 2.66; \text{pK}_{\text{OH}^{-}} = 5.48; \text{pK}_{\text{OH}^{2-}} = 12.51$$

The splitting was probably caused by an equilibrium mixture having constituents with slightly different half-wave potentials:

$$\begin{align*}
\text{N=N} & \rightarrow \text{N=N} \\
\text{H} & \rightarrow \text{H}
\end{align*}$$

The step height was constant up to pH 5.5 above which it increased to twice its height in a similar manner to HX (see fig. 29).

Analysing the polargraphic step at pH 5.62 in phosphate buffer, and at pH 11.21 in phosphate buffer gave a value of 0.8 and 1.21 electrons respectively. From these figures it would seem that the mechanism of the reduction in these cases is different. Both steps, however,
seen to be irreversible and probably correspond to the transfer of one and two electrons respectively. These plots were carried out using the polarograms of solutions $3 \times 10^{-5}$ M with respect to PAR.

In unbuffered solution at pH 7.2, using 0.1M potassium chloride base electrolyte, the a value was calculated as 0.7 electrons with $3 \times 10^{-5}$ M PAR and 0.73 electrons at $2 \times 10^{-5}$ M PAR, which suggests that the reaction becomes more reversible in more dilute solution.

The variation of step height with concentration and mercury bond indicated that the process is diffusion controlled under these conditions. In the concentration dependence investigation it was noted that with concentrations of PAR between $2 \times 10^{-5}$ and $3.2 \times 10^{-4}$ part of the step was displaced and was followed by a rounded minimum. The combined sections of the step fit the linear concentration dependence. An explanation of this may be that the pH in the vicinity of the drop may have been altered due to the uptake of hydrogen ions for the reduction of PAR, allowing the species of the dye to be present in this region, and giving rise to this split step. Both portions of the step appear, from variation of mercury bond, to be diffusion controlled.

Similarly, the step was shown to be diffusion controlled in buffered systems. In a monochloric buffer it was noted that heating caused the step to be slightly reduced in size and a second step to form. This was found to be due to oxidation
of the pH, and could be avoided by thoroughly deoxygenating the solutions before heating. The results from a typical polarogram are given below in Table 20:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Current</th>
<th>Time</th>
<th>pH</th>
<th>Current</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before heating</td>
<td>1.34 cc.</td>
<td>9.37v</td>
<td>6.0%</td>
<td>9.36 cc.</td>
<td>9.59v</td>
</tr>
<tr>
<td>After heating to 70°C for 10 mins.</td>
<td>3.38 cc.</td>
<td>9.59v</td>
<td>2.3%</td>
<td>3.38 cc.</td>
<td>9.59v</td>
</tr>
</tbody>
</table>

This second step was rather drawn out and reached its maximum value after the solution had been heated for about half an hour. After this period the results were:

After heating for half an hour | 2.42 cc. | 9.59v | 6.6% | 2.47v |

After this period the first step was diminished in size further but the second step did not increase any more. It is considered that oxidation would most likely be to the anhydrous compounds which are reduced to hydrate complexes in the region of this potential, involving a four electron reaction.

2. Alkali with Aluminium

Polarography of this system was carried out at pH 4.5, 6.0 and 12.0, as before, without any indication of a second step.

Complications, (as mentioned above,) arise at pH 9 if the solutions were heated, but it was shown that no polarographically active complex existed between pH 2 and aluminium.

3. Alkali with Copper

The addition of copper solution to 2 x 10⁻⁴ M Cu initially
caused the free copper step to be removed until an equivalent concentration of metal ion had been added. A small free copper step then appeared followed first by a dye step, approximately two-thirds the size of the free dye step, and then by a third step due to complexed copper. The half-wave potentials of these steps were -0.74, -0.51 and -0.43 volts (versus mercury pool), respectively, in acetate buffer at pH 4.6.

Below this concentration of copper only one step was observed (i.e., that of the dye itself) which was somewhat smaller than expected. Further additions of copper solution (above 2 x 10⁻⁶ M copper) did not increase the complex step although there was a tendency for this step to split. The free copper step, however, did increase. The dye step was difficult to measure as the copper step became larger but it appeared to be fairly constant at about 1.6 cm, compared with about 2 cm, for the complex step.

The complex step would not be expected to increase in size if all the ligand was reacted by an equivalent amount of copper. If this is the case the ratio of the complex is probably 1:1.

The addition of copper to the dye solution at pH 9, in acetic acid buffer, did not give a complex step and the free copper step was not shifted significantly from -0.54 volts (versus mercury pool). The dye step was reduced slightly in size when copper was present.

At pH 12.5 in dimethylglycine hydrochloride/sodium hydroxide buffer the free copper step was shifted from -0.40 to -0.53 volts (versus mercury pool), while the free dye step
potentialometric titrations with $2 \times 10^{-3} \text{M}$ dye indicated that two hydrogen ions were released per copper ion added, thus suggesting the formation of a 1:2 complex. Using dye solution an order of magnitude more dilute, the average number of hydrogen released, was found to be 1:1 per copper ion. Pollard et al.\textsuperscript{128} using a similar technique, found that both 1:1 and 1:2 complexes were present and reported that several titrations of such system were necessary before satisfactory results could be obtained at this solution. Kulicheva and Demch\textsuperscript{129} have also shown that in acidic solution complexes of the type $[(\text{Cu})_2]$ are formed and in alkaline solution complexes of the type $\text{Cu}[(\text{Cu})_2]$ are obtained.

The results quoted above are in general agreement with those obtained in this work, except that no indication of complex formation was found potentiographically in mannitol buffer. This was probably due to competition between the buffer and the dye for the complexing species.

c. react with $\text{Fe}^{3+}$

The addition of aliquots of iron(III) solution to $2 \times 10^{-4} \text{M}$ dye in acetate buffer at pH 4.5 resulted in a decrease in step height of the free iron(III) step and in a displacement of part of the dye reduction step. The displacement was well developed. This is the first time that such a displacement has been observed with any dye other than an o,o'-dihydroxyazo dye, although beryllium did give
a less well-defined displacement with a co-ordinating o-hydroxy
azo dye. The colour of the solution changed from yellow
to red on addition of iron(III). On heating, the solution
became brown in colour.

The height of the displaced portion of the dye step
increased linearly with concentration of iron(III) up to
a concentration of $2 \times 10^{-5}$ iron(III) i.e., a ten-fold
excess of dye was required for linearity. Above this
concentration the increase in step height was less than
that required for linearity and at concentrations above
$5 \times 10^{-5}$ iron the results became very erratic, due to
precipitation. In standing, the solutions changed colour
from red to brown and a precipitate was observed at the
higher concentrations. In this investigation the dye step
gradually got smaller and was not observed when $3 \times 10^{-5}$
iron was present. The least solution to show a residual dye
step was that containing $7 \times 10^{-5}$ iron(III). This
corresponds, approximately, to a $1 : 5$ iron to dye ratio.

10 ml alcohol was added to solutions similar to
those above and the pH was found to be 5.5. This had the
effect of stabilising the solutions and a concentration
dependence plot was drawn (see fig. 30). As can be seen
from fig. 30, the combining ratio is 1 : 3 although above
$6 \times 10^{-5}$ iron the scatter of the points increases. The
total step height remains constant up to $6 \times 10^{-5}$ iron
after which the value falls.

At pH 8.0 in aminosalicylic solution the complex step was
very small; even with excess iron it did not attain a height
greater than 0.4 cm. Heating the solution to 75° for 10 minutes increased the height of the complex step considerably, but it was difficult to measure accurately, owing to the step caused by oxidation of the dye during the heating process.

The investigation at this pH was repeated using boiled out demineralized distilled water to make up all the solutions and with passage of nitrogen through the solutions before and during the heating.

Results obtained under these conditions showed that the complex step continued to increase at least until a ratio of 1 : 1 was reached. The points from a ratio of 1 : 2 towards the 1 : 1 ratio however, became more scattered, even when excess iron(III) solution was added all the dye step was not completely eliminated. For example, with $1 \times 10^{-3}$ M iron and $2 \times 10^{-5}$ M for the following results were obtained using the Clarkidge polarograph:

<table>
<thead>
<tr>
<th>m</th>
<th>0.65 cm.</th>
<th>0.65 cm. (versus hydrogen pool)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.65 cm.</td>
<td>2.06 cm.</td>
<td>1.66 cm.</td>
</tr>
</tbody>
</table>

The above investigation was repeated using freshly prepared solutions and immediate polarography. In this case the complex step stabilised at a ratio of 1 : 1 (see fig. 31).

Potentiometric titrations indicated that two hydrogen ions were liberated per iron(III) ion added which indicated a 1 : 2 combining ratio. This is not in accord with the polarographic data. At low pH values precipitation occurred during the titration, a possible explanation of these results is that a 1 : 1 complex is formed of the type
Fig. 31

Step height in cms. at sens. $\frac{1}{10}$

$pH 8.9$

$10^5 Fe$ with $2 \times 10^{-4}$ PAR
From the polarographic data at pH 3.6 it appears that three dye molecules are co-ordinated with the loss of two hydrogen ions. This kind of behaviour has been reported previously by Holland et al. who found a 1:1 ratio for cobalt(III) with HPA by spectrophotometric techniques, and a 1:2 ratio by potentiometric methods.

c. Polarography of HPA with iron(III) and malachite

Polarography was carried out at three pH values previously mentioned without any indication of a second step, even on heating.
As has been found with other compounds, although this is known to form complexes with the metals studied, no indication of a polarographically active complex was obtained.

It only gave a displaced site with iron(III).

Apparently a 1:3 complex is formed at pH 4.0, but only a 1:1 at pH 2.0. Potentiometric titrations indicated that two hydrogen ions were liberated for each iron(III) ion added. This indicates the formation of a complex of the type I-(2 H)_2.

At low pH values with the addition of further molecules of the dye. This could occur without the loss of a hydrogen ion (or possibly if a hydrogen ion last then another is added to one of the dye molecules probably to the pyridyl nitrogen). However, this explanation is not fully satisfactory.
Chapter IX
Other Dyes Investigated

Section (1). Calcischiron

This dye was investigated because it has been shown to be a highly sensitive colorimetric reagent for calcium.

The reaction is free from interference by barium, strontium, and magnesium and it was hoped that this reagent might provide a method for determining calcium and possibly other metals colorimetrically.

3. Calcischiron with Aluminium

Colorimetric titrations were carried out and it was found that a complex was fairly completely formed from pH 4.5 upwards. The equivalent of two hydrogen ions were released per aluminium ion added but above about pH 8 hydrolysis of the complex occurred, as shown by a decrease in pH with time,

at pH 7.5 of the hydrogen atoms of the calcischiron molecule

and released in the absence of aluminium.

Colorimetry was carried out in acetate buffer at pH

5.62 using 4 x 10^{-4}M calcischiron solution,

at this pH calcischiron gave one well defined step at

-0.12 volts (versus mercury pool). The addition of aluminium caused a slight displacement in part of the step so that two steps were now present at -0.12 and -0.65 volts (versus
mercury pool), respectively. With $5 \times 10^{-4}$ aluminum solution a well defined polarogram was obtained, but on increasing the aluminum concentration, precipitation occurred. On reducing the ionic strength by lowering the buffer capacity ten fold to $1.5 \times 10^{-3}$ sodium acetate and $0.4 \times 10^{-3}$ acetic acid the problem of precipitation was overcome, but the two steps were not as well defined. An increase in the aluminum concentration in the range $1 \times 10^{-3}$ to $5 \times 10^{-3}$ did not affect step height of the complex, which remained about 1.7 cm, although variations in the step between 1.5 to 2.4 cm at sensitivity a (cathode polarograph) were noted.

In the then decided to vary the aluminum concentration within the range $3 - 1 \times 10^{-3}$. The polarograms, however, did show a large step similar to a minima of the second kind, occurring between -0.7 and -1.3 volts. Thus appeared whether aluminum was present or not. Tailing of the second kind do not usually occur in solutions with base electrolyte concentrations as dilute as that used in this study. Increasing the base electrolyte concentration to its original strength caused the hump to disappear.

A clear cut plot using the complex step height indicated a combining ratio of about 1 : 2, although the complex step height still rose slightly (see Fig. 2).

At the ratio of 1 : 1 the complex step was one third of the combined dye and complex steps. This, together with the fact that potentiometric titrations indicated that two
Fig. 32

Step height in cms. at sens. $\frac{1}{20}$

$10^4$ Al with $2 \times 10^4$ M Calcichrome

Fig. 33

Diffusion current

Applied potential in volts
hydrogen ions are released per aluminium ion, indicated that a complex of this type which involves the coordination of only one of the three carboxyl groups to the aluminium, together with one or two of the three available oxygen atoms. The fact that the step height still increases slightly even after the 1 : 1 ratio is reached can be explained by the entry of another aluminium ion into the space available. This is difficult, due to the small space available, so that nothing in the nature of a quantitative reaction is achieved.

b. Calcium with Aluminium

Polarography was carried out at pH 7.5 in diethylglycol
hydrochloride/calcium hydroxide buffer and the polarogram of
the free calcium ion is shown in fig 27. The addition of
calcium did not modify the polarograms even on heating.

Polarography at the same pH in diethylglycol/calcium hydroxide
buffer gave the usual type of wave which calcium did not
modify. Glass and fast (124) showed spectrophotometrically at
pH 12, that the ratio of calcium to &-oid residues in the
complex corresponds to 1 : 3 i.e., a 1 : 1 complex.

c. Aluminium with other Metal Ions

A further polarography was carried out, but potentiometric
titrimetric titrations were performed with iron(III) and copper(II). In
both cases two hydrogen ions per metal ion were found to be
liberated above pH 3. Below pH 7 two extra hydroxyls were
liberated and after the pH value of the dye, one extra
hydrogen ion was liberated.
Section (41). Thorin (1.3 chloroethyl-3-ethyl-1,1,1-
trichloroethy1 chloride salt, sodium salt)

This 3,3 is a specific spectrophotometric reagent for iron.

Once, as thorium is not easily determined polarographically,
and the signals formed is more stable than that with 3,3-
dihydroxy napthol, it was decided to examine the reaction
polarographically.

a. Thorin with thorin

In the conditions for spectrophotometry usually specify
acidic medium, polarography was carried out initially at a
pH 3 in acetate buffer.

The addition of thorin to 2 x 10^{-4}M thorin solution
caused the combined step height of the two, rather ill-
defined steps, occurring at about -0.45 volts, to be reduced
in size. In addition two steps were found.

The addition of 5 and 10 mL aliquots of 0.02% thorin
caused a decrease in step height from 2.5 cm to 1.45 cm,
and 0.75 cm, respectively.

The investigation was repeated in acetate buffer at
pH 9 where a single well defined step was obtained for thorin.

The half-wave potential occurred at -0.45 volts (reference
mercury pool). Once again the addition of 5 and 10 mL aliquots
of 0.02% thorin solution caused a decrease in step height.
The reduction in this case was from 2.62 cm. in the absence of thorium, to 2.47 and 1.70 cm. respectively.

The effect of temperature on the single wave was studied to see if cooling, or heating gave an indication of the wave splitting. The results are shown below in Table 21. The conditions were as follows:

\[ 2 \times 10^{-5} \text{ mol} \text{ HCl, } 1 \times 10^{-5} \text{ mol thorium, } \]

ammonical buffer at pH 9.

**Table 21**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Step Height at Sensitivity (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td>20</td>
<td>3.05</td>
</tr>
<tr>
<td>40</td>
<td>3.0</td>
</tr>
<tr>
<td>60</td>
<td>4.9</td>
</tr>
</tbody>
</table>

No indication of splitting was observed in any of these.

3. Thorin with Aluminium.

More reply of this system was carried out at the above.

At pH 4.4 the addition of aluminium improved the slope of the polarogram; instead of the ill-defined steps only one was occurred although this was of comparable height to the confined heights of the original steps. In fact, some reduction in step sizes was noted. This reduction was more
marked on heating, i.e., with \( h = 10^{-4} \) MeV. The
addition of 1 ml. 0.0037M aluminium caused a decrease in
step height from 2.7 to 2.3 cm., with a further reduction
to 2.1 cm., on heating.

The polaron of Chernin at pI 4.4 was rather
complicated, having five steps of various magnitude. The
effect of adding 1 ml. 0.0037M aluminium is shown in Table
23 below.

<table>
<thead>
<tr>
<th>( h ) (cm.)</th>
<th>( 0.26 )</th>
<th>( 0.24 )</th>
<th>( 1.7 )</th>
<th>( 0.5 )</th>
<th>( 0.3 )</th>
<th>( 0.22 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( h ) (cm.)</td>
<td>( 0.26 )</td>
<td>( 0.24 )</td>
<td>( 1.7 )</td>
<td>( 0.5 )</td>
<td>( 0.3 )</td>
<td>( 0.22 )</td>
</tr>
</tbody>
</table>

This polaron was more difficult to measure and even if the
increase in the step height at \( h = 0.5 \) cm. was due to complex
formation it seems to offer no analytical advantage over the
polaron at \( h = 1 \). - Aluminia system. Owing to the
complicated nature of the polaron as obtained it would not be
wise to draw any conclusions without carrying out further work
on the system.
Once again, it has been confirmed that complex formation is not the sole criterion for displacement of part of the cate
step. Complexes of calcium with salicylhydrazine and thorium with thorin are well established but neither of these give any
indication of being polarographically active.

The complex formed by aluminum and salicylhydrazine was
shown to involve one of the three ace groups and two of the
three hydroxyl groups. This is the first time that this type
of complexation has been reported.

The unusual shape of the salicylhydrazine polarogram at high
ph values is probably due to a double layer effect.

Further work in the thorium-thorin system is necessary if the
decrease in step height with addition of thorium is useful for
an analytical procedure, may prove fruitful.
Chapter 4
Discussion and Conclusions

4. The Displacement of the Step

The displacement of the half-wave potential of metal ions to more negative values by complex formation is well known and has frequently been used to determine the stability constants of complex species. A metal ion in a complex species is usually more difficult to reduce to a lower oxidation state than the corresponding hydrated ion but there are many cases where complex formation causes a shift of half-wave potential in the positive direction. This is due to the greater reversibility of the combined ion compared to that of the simple hydrated ion. An example of this phenomenon is provided by nickel. In dilute solution, nickel is reduced at a half-wave potential which shows a considerable overvoltage upon the value calculated from thermodynamic data. However, in pyridine solution the magnitude of this overvoltage is sharply reduced and the half-wave potential approaches the reversible value predicted by standard energy data. Similarly, it has been shown \(^{125}\) that in high concentrations of chloride ion (1M) the half-wave potential is close to the thermodynamic reversible value. Evidently, under these conditions of high chloride concentration and low water free energy the 'irreversible' water sheath around the nickel ion is ruptured and a reversible chloride complex
is produced. Indium exhibits the same phenomenon, due to the formation of a reversible $\text{InCl}_4^-$ species\textsuperscript{126}, \textsuperscript{127}. When the metal ion is not reduced to the zero oxidation state the stability of the metal ion in its reduced oxidation state must be considered. The change in half-wave potential in this case is given by the equation:

$$E = \frac{\Delta G}{2F} \ln \frac{K}{K_0}$$

where $K$ and $K_0$ are the stability constants of the metal ion in its oxidised and reduced oxidation states, respectively.

From similar considerations of stability when the ligand is itself reduced a displacement of its reduction wave should be observed on complexation. Further, if the reduced form of the ligand is also capable of complexation the change in half-wave potential will be dependent on the relative stabilities of the complexes formed by the ligand and the reduced form of the ligand. The displacement in half-wave potential is given by the equation:

$$E = \frac{\Delta G}{2F} \ln \frac{K_1}{K_{\text{red,1}}}$$

where $K_1$ and $K_{\text{red,1}}$ are the stability constants of the ligand complex and the reduced ligand complex respectively. If a complex species of an electropositive metal, such as aluminium, is polarographically reduced, the reduction of the ligand may be observed in the absence of reduction of the metal ion at normal working potentials.

For the reduction of an one dye to the corresponding
hydrazo compound, the expected displacement of the half-wave potential on forming a 1:1 complex is obtained from the following equations as shown:

\[\ce{H_2O + H_2O2 -> hydrazo}; \quad \Delta \mu_1^0 = -nF\]
\[\ce{H_2O + H_2O2 -> 2 HO2}; \quad \Delta \mu_2^0 = -2nF\]
\[\ce{H + hydrazo -> N + hydrazo}; \quad \Delta \mu_3^0 = -nF\]

\[\ce{H_2O + H_2O2 -> hydrazo}; \quad \Delta \mu_4^0 = -nF\]

\[\Delta \mu_2^0 = \Delta \mu_4^0 - \Delta \mu_3^0 - \Delta \mu_1^0\]

\[\Delta \mu_2^0 = -3nF\]

\[\Delta \mu_2^0 = -3nF\ln K_{mess} \text{ hydrazo}\]

\[\Delta \mu_2^0 = -2nF\ln K_{mess} \text{ hydrazo}\]

Similarly, for the four electron reduction to the amine:

\[\Delta \mu_2^0 = -4nF\ln K_{mess} \text{ amine}\]

Thus, a displacement of the half-wave potential would not be expected in cases where the hydrazo or amine complex were of similar stability to the complex. In fact, the amine complexes are much more stable.

In practice, however, it appears from these and other studies that a displacement is only obtained forazo dye containing at least one hydroxyl group and another complexing group in the o or o' position, only rarely were such displacements been noted in the absence of...


σ, π-diarylxyone grouping. These were with Solochrome and aluminium, and with iron(III) and an o-hydroxy π-carboxy dye, with beryllium. Recently however, Mittel and Florence \(^1\) have observed the displacement to a more positive potential of the anodic wave of alizarin red S with mircenium (IV) at the pyrolytic graphite. Florence, Miller and Mittel \(^2\) have also determined aluminium using the anodic wave for the oxidation of its Solochrome violet R3 complex at a rotated pyrolytic graphite electrode. Mittel and Florence \(^3\) found that with alizarin red S and mircenium the displacement of the irreversibly anodic step was greater and better defined than the displacement of the reduction step which was more irreversible and erratic in nature. Mittel and Florence \(^3\) developed an analytical procedure for mircenium based on this method.

From thermodynamic considerations such as those mentioned above, one would expect this displacement of the ligand step to be exhibited by more systems than has proved the case.

The thermodynamic treatment above is strictly true only for reversible systems, but even when applied to irreversible systems useful information should be obtained without the introduction of serious errors although the change in half-wave potential is not predicted accurately by the equation. It has been shown that the reduction of the dye and that of its metal complex are not equally reversible and hence it would be expected that this alone would cause some difference in the half-wave potentials. The difference between half-wave
60°C, but in no case was a decrease in the height of the
dissolved step observed. In fact, if the polarographic
solutions were not heated and cooled prior to polarography
the dissolved step was not quantitatively formed. The
difference in half-wave potential was constant throughout
the investigation. Examination of thorium and thorium, a
system in which complex formation is well known, failed to
give any displacement of the dye step at 0°C. It must be
assumed therefore that the temperature variation was not
sufficient to make an appreciable difference in the rate of
the dissociation reaction, or that the displacement is due to
some other mechanism.

The reductions of the iron(III) - toluidine violet A,
nickel(II) - toluidine violet A and iron(III) - toluidine (at
pH 3.3) complexes, are of particular interest from the
mechanistic point of view. These reductions are similar and
will take the first case mentioned as typical. Then
toluidine violet A is completely complexed with iron(III)
only half of the dye reduction wave is displaced. The two
steps, therefore, appear to involve two electrons each, and
the first reduction appears to proceed only so far as the hydroxide
stage. Since the first step occurs at the same half-wave
potential as the free dye it seems probable that the
reduction proceeds via the free dye. According to Florence
and Giese[2] this type of dye is reduced to the hydrazo
compound which then dissociates to the free dye (which is
further reduced) and the azine (see page 10). In this
case, the hydrazo compound must be stabilized by complex
formation with the iron(III). The reduction scheme would be
as follows:

\[
\text{Fe}^{3+} + \text{e}^{-} \rightarrow \text{Fe}^{2+} \\
\text{red} \\
\text{Fe(III) complex} \leftrightarrow \text{Hydrazo} \quad (\rightarrow \text{no emission not favoured})
\]

At the potential of the second reduction step it is probable that the selenium violet \(3.3 - \text{iron(III)}\) complex is reduced directly to the azine as seems to be the case usually with \(o,p'\)-dihydrazo azo dye complexes.

An alternative explanation of the first step is that the selenium violet \(3.3 - \text{iron(III)}\) complex is reduced directly to the corresponding hydrazo - iron(III) complex, the stability constants of these two complexes being similar and hence not causing a displacement of the step. The second step would again be due to the reduction of the complex at the azine.

This latter mechanism has the advantage that free iron(III) - which can be reduced to iron(II) is not liberated. Until concentrations of iron(III) greater than that required to complex all the dye present were used as iron(III) to iron(II) reduction step was observed. To account for the non-appearance of an iron(III) to iron(II) reduction step the final product would have to be sufficiently stable to resist reduction of iron(III) in the range of applied potential studied.

The above explanation does not apply to the iron(III) - FeN complex at \(pH 4.6\), as in this case the complete dye step is displaced. At \(pH 5.3\) the two steps are obtained as stated.
It pH 4.6 a polarographic coupling ratio determination by the
method of Job indicated the presence of a 1 : 2 complex whereas
at pH 7.9 a more usual 1 : 1 complex was obtained. Potenti-
ometric titrations show that the hydrogen ions are released for
iron(III) ions. From these observations the complex at pH 4.6
is probably of the type H(OM)3. In acidic medium the other
two dye molecules are associated with the metal without
liberating extra hydrogen ions. As the 1 : 2 ratio was obtained
using the height of the complex step which is part of the one
step displaced it is likely that the one grouping is involved
in the complexation of the other two dye molecules. For
the structure of the complex to fit in with the evidence it
is necessary for co-ordination to involve the hydroxyl group
and the one group for two of the H2 molecules and the
heterocyclic nitrogen and the one group for the other. It
appears that two very different complexes are formed at the
two pH values studied, although there is no indication of a
transition from one form to the other in the potentiometric
curves.

The results described in earlier chapters indicate
that the one grouping must be co-ordinated if a displaced
step in to be obtained. The results with Cr dichloride and
aluminum support this view. In this case the polarographic-
results indicate that only one of the three one groups in
the molecule is co-ordinated to the metal so only one third
of the dye step is displaced with excess aluminum. With
considerable excess of aluminium slightly more of the step is displaced probably due to another aluminium ion entering the limited space available with considerable difficulty. This is confirmed by the fact that only two of the three hydroxyl protons are liberated on complex formation.

In the case of a dye with an azo and a nitro group (Sordent Red 57) only one of the two steps was displaced on the addition of aluminium. This indicates that the nitro group is not affected by complex formation. Work with other dyes incorporating a nitro group was not conclusive.

5. Comparison with previous results

Several aspects of the present work are not in agreement with previous results. These differences are discussed in this section.

Several authors have quoted combining ratios for the metal - dye complexes which have been shown to be in error. Jenkins and Reynolds reported that aluminium formed a 1 : 3 complex with Dolechrome Violet 35 at pH values above 6.5 but the present work using polarography, spectrophotometry and potentiometric titrations gave no indication of this complex. The results showed conclusively that a 1 : 1 complex was formed at pH 4.6 and a 1 : 2 complex at pH 6.5. The explanation of Florence and 'glander that Jenkins and Reynolds did not allow for the polarity of the dye seems to be supported. Dean and Bryan did not carry out job combining ratio determinations but relied on the
comparative heights of the two steps to assign ratios. In the opinion of the present author this is not valid, since examples have been shown, in this thesis, where not all the dye step is eliminated, even in the presence of excess metal ion. The present work is supported by the spectrophotometric evidence obtained by Halden and Sigg.\textsuperscript{12}.

Similar investigations of the nickel(II) and iron(III) systems with dyeleum violet B5 have yielded 1:1 complexes at pH 4.5 and a 1:2 complex at pH 6.5 for iron(III). The nickel(II) system gives a 1:1 complex at pH 4.5. Palmer and Reynolds\textsuperscript{7} reported 1:2 and 1:4 complexes for iron(III) at these pH values and 1:2 complex with nickel(II) at pH 6.5. Peck and Bryan also report that the nature of the nickel complex depends entirely on the nature of the buffer and that a 1:2 complex was formed in ammonium buffer and a 1:3 complex in piperidine buffer. The ratios obtained by the present author are again consistent with Palmer and Reynolds using dye of about 95% purity. The error in the Peck and Bryan work may be due to the fact that nickel, like iron, never fully displaced the dye step and so, their method of calculation is not valid. Another important point shown in the present work is that the displacement of the dye step in borate buffer, but not acetate buffer at pH 4.5, is due to insufficient buffer capacity and not to the reasons given by Palmer and Reynolds.

Care must be taken in interpreting results obtained by one technique only, for example, Halden et al.\textsuperscript{10}. 
Other o-carboxy o'-hydroxy diaryl compounds should be studied with the objective of finding a dye which gives a better displacement of the wave than that studied in this work. In view of the specific complex reaction for beryllium reported in this thesis, it would have considerable analytical importance, as no good polarographic method exists for the determination of beryllium. Aluminum was found not to give a polarographically active complex with this type of dye, which is extremely important in view of the difficulty of determining beryllium in the presence of aluminum. Aluminum may, of course, be determined in the presence of beryllium by means of Chromeazurol Violet.

Further work on the calcium-borole on Chrome B system might prove fruitful in providing a useful and convenient method for the determination of calcium.

Another investigation of probable value would be the study of other o,o'-dihydroxy compounds in which the carboxy group was replaced by other groups. Displacement of the step in complex formation could be of particular interest.

The present author was able to make only a cursory examination of one compound of this type. This was 2,3' dihydroxy 5,5' tetrachloro diphenyl sulfide, in which the carboxy group is replaced by a sulfide group. No displacement of the step was observed, although the sulfide atom is undoubtedly capable of co-ordination.
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