Azo dyes and their metal complexes

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DYES AND THEIR METAL COMPLEXES

by

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Various types of ortho-substituted azo 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 adsorption and kinetic processes were observed in some cases.

The o,o'-dihydrazo azo dyes studied all gave complex steps of displaced half-wave potential with some metal ions, although the displacement was greatest with Co(III) and Cu(II). The hydroxyazo dyes all behaved differently from azo dyes in a number of cases, as the complex step did not replicate the dye step completely, even when excess of metal ion is present.

o,o'-Dihydrazo azo dyes were found to behave similarly to the o,o'-dihydrazo azo dyes.

Complex steps of displaced half-wave potential with other azo dyes were observed only in the instances of iron(II) and with benzidine - electrolytes (4 M KCl).

Polarographic studies of o,o'-dihydrazo and the chlorine complex showed that complex formation involved only one of the three azo groups and one of the three hydroxyl groups. Cottametric results confirmed that two azo on iron were liberated per dihydroxam.

An important new method for the photometric determination of anions 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 Tschudin who examined oxobenzene in buffered alcoholic solutions and suggested that a two-electron reduction to hydrobenzene occurred. Noltlöffel and Liang9 doubted the validity of Tschudin's equation relating half-wave potential to pH:

$$E = -0.05 - 0.069 \log_{10} [H^+]$$

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, Prüll6 separated the cis form of oxobenzene 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 oxobenzene and several of its derivatives7. Robertson8 examined the trans and cis isomers by X-ray methods and showed the structure of the former to be planar as illustrated:

![Diagram of trans isomer]

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

![Diagram of cis isomer]
Nickel and Siebert\textsuperscript{9} studied the isomers polarographically and obtained values of \(-0.3\) and \(-0.27\) volts respectively for their half-wave potentials. Milson and Tinkham\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:

\[ E = -0.35 - 0.05 \text{pH} \]

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 isomers were different. These facts were attributed to the unstable 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 effect the reduction. The overpotential required depended on the isomer reduced, and hence, as the first stage in the reaction was closer at high pH, two steps were formed.

Crater and Taylor\textsuperscript{12} showed that if buffer solutions of sufficient capacity were used, the isomers 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:

\[ E = 0.069 - 0.063 \text{pH} \]

up to pH 12.5.
3.

Kirmmse and Hironi\textsuperscript{17} had previously shown that mixtures of

cis-substituted azobenzene compounds and the corresponding

diazo-azobenzene compounds formed reversible redox systems at

the platinum electrode in acidic solutions. Their results
were confirmed by Benson and Irrett\textsuperscript{18} who showed that even the

mildest reducing agents caused complete reduction to the azine.

The rate controlling reaction was shown to be unimolecular.

Streuli and Zahn\textsuperscript{19} found the azobenzene-diazoazobenzene redox

system to be reversible at the mercury pool electrode.

Nones and Frederichsen\textsuperscript{19} reported that for trans-azobenzene

in 50\% neutral 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 deeter and Haring\textsuperscript{12}

they found this relationship to hold even in acetate buffered

solutions but the molar proportion of buffer to azobenzene had
to be at least 500 : 1. Logarithmic analysis of the diffusion
controlled step was compatible with the reaction being reversible
and involving two 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 0.09 and 0.15 volts and in some

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 experimental error.

The work of Helliker and White\textsuperscript{17}, who determined elongated

diazoazobenzene fluorometrically with m-cresol violet - N.

\[
\begin{align*}
\text{OH} & \quad \text{HO} \\
\text{S}_3\text{N}_2 & \quad \text{N} = \text{N} \\
\end{align*}
\]
and potassium blue black R:

\[ \text{NaSO}_3 \]

led to the first use of dichrothiazine dye complexes as reagents in polarographic analysis. Willard and Bean\(^1\) found that the addition of aluminium to solutions of pentachrome violet \(2\) 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 pentachrome blue black \(4\). 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 andiebert\(^9\), Willard and Bean attributed this displacement, of about 0.2 volts, to the stabilization of the cation form of the dye.

Graham and Turner\(^19\) found the half-wave potentials of twenty-one water solubleazo dyes to be similar and of little use for identification purposes. Nevertheless polaroagraphy can be used for the quantitative determination of these dyes. Graham 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 nzo and the nitro groups in
Both occurred at a more positive potential than nickel was present. The nickel step was completely suppressed.

Harrick and Bryan reported that the equation for half-wave potential versus pH for Pontachrome Violet 53 was:

\[ E_s = -0.029 - 0.06 \text{ pH} \]

between pH 0 and 12 until:

\[ E_s = 0.100 - 0.082 \text{ pH} \]

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 acetate, buffer and three in piprididine buffer.

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

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

They stated that these values correspond to the expected values based on the usual co-ordination number of the cation with a bidentate ligand.
6.

Benn and Iryan\textsuperscript{21} did not consider that the appearance of the second step was due to the stabilizing of a cis form of the ezo group on complex formation; further, they considered that the ezo 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 ezo 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{22}, in a further paper, described an spectrophotometric method for the determination of magnesium using Dextrochrome Violet B. N. Serious interference was experienced from calcium.

Perkins and Reynolds\textsuperscript{23 - 26} developed the Hillard and Benn method for aluminium and made a more detailed study of the reaction between dextrochrome Violet H (Dextrochrome Violet B. N) and aluminium. They examined the interference of beryllium, calcium, manganese (II) and zinc and developed a method for the estimation of aluminium in the presence of beryllium.

Polarographically active complexes with cobalt (II), thorium (IV), titanium (II), vanadium (V), zirconium, iron (III) and nickel (II) were observed\textsuperscript{23}. The copper (II) complex was relatively insoluble. A decrease in the size of the complex step was observed in the presence of fluorine, citrate and oxalate ions.
in proportion to their concentration. Allard and Leen\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 microgram amounts of fluoride by Reynolds et al\textsuperscript{27}, \textsuperscript{28}.

Perkins and Reynolds\textsuperscript{25} found the number of electrons involved in the reduction of Solechrom Violet R to be two, using the microcoulometry technique of Reynolds and Chalgon\textsuperscript{29}. This was in agreement with Leen and Bryan\textsuperscript{26} 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 Solechrom Violet R: 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{36} 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 Leen 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:

\[
\text{Na}_3\text{SO}_4 - \begin{array}{c}
N=N \\
\end{array} - \begin{array}{c}
\text{HO} \\
\end{array}
\]
No modification of the dye wave was observed when aluminium was added to solutions of the first two dyes. Acid Alizarin Black 00 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.

Helochrome Violet 30 has been used to determine a number of other metals polarographically. Intreuk and Azki\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 using the thorium step with acetate ion. Cooney\textsuperscript{23} determined gallium (III) and indium (III), and Iabiloichi et al.\textsuperscript{24} determined uranium. Cooney and Taylor\textsuperscript{25} determined a mixture of aluminium and gallium (III) and Turhlem\textsuperscript{26} determined thorium at high pH. Floresco and Rayke\textsuperscript{27, 28} determined the lanthanides and found the combining ratio to be two dye.
molecules to each metal ion. The lanthanide complexes of
Eriochrome Black T:-

\[
\text{H}_2\text{SO}_4
\]
and Eriochrome Blue G:-

\[
\text{Na}_2\text{SO}_4
\]
showed similar polarographic behaviour to those of Alcoholic
Violet II.

Kecskeméty and Sölver\textsuperscript{35} used Alcoholic Violet II for the
polarographic determination of iron (III). Lither\textsuperscript{35}
determined manganous (III) polarographically using Alcoholic
Violet II.

Florence\textsuperscript{31} used Alcoholic Garnet V:-

\[
\text{H}_2\text{SO}_4
\]
in preference to Alcoholic Violet II, Eriochrome Black T, and
Alcoholic Garnet Blue II:-

\[
\text{Na}_2\text{SO}_4
\]
for the polarographic determination of aluminium in thorium.
Solenochrome Violet has been found to be subject to more interference than the other dyes, 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.

Flournoy and Sylvestre found the reduction of Solochrome Violet 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:

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

followed by:

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

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 m value of four. Reduction of the hydrazo compound was indicated by the presence ofblue in the micro-cell after prolonged reduction.
Ylouard obtained a value of four electrons per dye molecule reduced by comparing the diffusion coefficient obtained from the Illievic equation with that reported for ethylenone by Caster and Sayler \(^1\) (6.41 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}) and that for stilbene reported by Leitmer and Raynor \(^2\) (6.73 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}). Florence and Ylouard suggest that Rehins and Leitner \(^3\) used an incorrect value of Solscrome Violet 70 and that Leitner and Raynor \(^4\) used an incorrect value for the diffusion coefficient in their calculations. Solscromes Black 28 and Chrome Blue Al-

Also gave displaced reduction waves with the leithanines. These two dyes and several anthraquinone dyes were investigated in more detail by Florence \(^5\) but were found to be inferior to Solscromes Violet 70 for analytical purposes.

The disproportionation of hydroxylamine was first suggested by Caster and Frey \(^6\) to explain the rate-controlling step in the potentiometric reduction of ethylenone and a similar explanation was given by Leitmer and Raynor \(^7\) to account for the results of their polarographic and coulometric reduction of p-dimethylamino ethylenone. 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 1.0, 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 1.5 and pH 2.5 but at pH 3.0 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. Hackisch and Hauy had
previously observed a four electron reduction with a number
of the dyes.

Florence and Ayland used controlled potential coulometry
at a stirred mercury cathode to show that four electrons were
involved in the reduction of Deroxime Violet 5B. The
reduction did not follow first order kinetics. The rate could
be related quantitatively to a disproportionation of the hydrated
compound. It should be noted, however, that the use of
coulometry with a micro-electrode to interpret polarographic
results obtained with a macro-electrode, is dangerous as both
the cell and electrode conditions are entirely different.

Florence and Ferrar studied the polarographic behaviour
of trans-dioximes and its sulphonic acid by several techniques
including D.C., A.C. single drop and polarographic
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 hydrate 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 electrode-capillary interface. The
presence of aminomethane was found to improve the reversibility
at pH 9.
Florencet 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. Jihyi et al. found that structural differences in azo compounds gave rise to inductive and mesomorphic effects which caused differences in the half-wave potentials. Florence studied the kinetics of the disproportionation of 4-aminoazobenzene-4-sulphonic acid spectrophotometrically and polarographically. The reaction was found to be first order with respect to both the azo compound and hydrogen ion. The reaction mechanism postulated was based on a rate determining step involving a quinonediimine intermediate, previously suggested by Jonart and Frutt.

Florence et al. used solechrome violet for the volumetric determination of aluminium at a rotating pyrolytic graphite electrode. The processes involved give an increased sensitivity over that obtained polarographically.

Salzer studied the reaction of soleochrome violet with a number of other azo dyes having either diortho-hydroxy or an-ortho-hydroxy group with several metals and found that a polarographically active complex was formed only with the former type of dye.

Salzer and Reynolds and later Wytun and Reynolds studied the reaction of magnesium with soleochrome violet.
at high pH and suggested a technique for overcoming interference from calcium. Richardson has also used this technique as a basis for determining oxalates in calcium carbonate.

Silver and Reynolds found that iron (III) forms a 1 : 2 and a 1 : 4 complex with malachite green. They found that nickel behaved somewhat unusually in that a complex was obtained at pH 3.5 in borate buffer but not in acetate buffer. This was attributed to the nickel ion being protected by its hydration sheath and not being able to interact with the dye cation at pH 3.5 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 methylene derivatives.

Low and Landquist prepared copper complexes of a number of azo substituted triazo dyes. With the dyes containing a single azo group they obtained 1 : 2 (copper : dye) complexes and indicated three possible structures for them:
(5-membered rings -
cis one group)

Brew and Landquist favour structure I by analogy with the copper complexes of salicylidenealines:

\[
\begin{array}{c}
\text{CH}_2 \text{CH} = \text{N} - \text{C} \\
\text{C} \text{H}_2
\end{array}
\]

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

\[
\begin{array}{c}
\text{CH}_2 \text{CH} = \text{N} - \text{C} \\
\text{C} \text{H}_2
\end{array}
\]

prepared by Parrow and Saffey\(^{59}\) and point out that they must have a structure similar to II or III. In contrast Brew and Landquist consider that in the less stable copper complexes of nono-ortho-carboxy-azo dyes the adjacent nitrogen atoms are involved in complex formation (structure IV) giving a six-membered ring rather than the seven-membered ring obtained if the further nitrogen atom were involved (structure V).
With copper (II) and o, o'-disubstituted o-phenylazo compounds only
1 : 1 complexes were obtained (structures VII to IX):

VII

VIII

IX

The o, o'-dihydroxy o-phenylazo compound was considered to be
planar but the o, o'-dicyano o-phenylazo and the o-hydroxy
o'-cyanoxy o-phenylazo compounds were not. Homopiperidine and neo-
quinoline derivatives of VIII were obtained in which pyridine
and quinoline replace a water molecule in the fourth co-
ordination position.

Frey and Haisma found that a single hydroxyl group
was insufficient to co-ordinate an azo 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 o-phenylazo both
1 : 1 and 1 : 2 complexes were obtained (structures IX and
X):

XI

These structures were believed to involve an octahedrally
co-ordinated chromium atom with planar ligand molecules
perpendicular to each other (structure IX), or to three water
molecules (structure IX). These authors also obtained a 1 : 1
o-hydroxy o'-hydroxy azo complex of chromium (III) which was
στην τεχνητή οικονομία και στην ερευνητική επιστήμη. Δεν είναι δυνατόν να επιθυμήσουμε να εμπεδώσουμε τα γεγονότα σε μια μορφή τέτοια ώστε να εμπλουτίσουμε την έννοια και να επιπλέουμε την ιδέα του συμπράξαντος συναρμολογήσεως και κανόνων συμμετοχής. Πρέπει να έχουμε υπόψη, ότι, μόνο με την αποτελεσματική και ισορροπημένη κατανόηση των σημαντικών αίτησης και της διαδικασίας, μπορούμε να επιτύχουμε το έπαθλο της συνεργασίας και της επιτυχίας στο μέλλον.
Later authors expressed doubt as to the existence of these compounds. Batch and Drew also studied aluminium complexes and showed them to be similar to those of chromium. The aquocitrate (structure XIV) was isolated:

\[
\text{H}_2\text{O} \quad \text{XIV}
\]

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

Drew and Banton showed that vanadium (IV) behaved in a similar manner to trivalent actinides. Both 1:1 and 1:2 complexes were obtained with \(\text{a}_{1}^{1}\)-nitrylacyloxy compounds (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-oxide, o'-carboxyazo dyes 1 : 1 and 1 : 2 complexes were obtained with ease comparable to the o.o'-dihydroxyazo compounds.

Heifier and Dacre have indicated possible isomeric forms of o.o'-dihydroxyazo dye complexes of octahedral Cr (III) - structures a - e, to which Martell and Calvin have put forward a further isomer (f):

\[
\begin{align*}
& \text{a} \quad \text{b} \quad \text{c} \quad \text{d} \quad \text{e} \quad \text{f} \\
& \text{O+N+O} \quad \equiv \quad \text{(Diagram)}
\end{align*}
\]

All of the forms above are optically active except structure (d). Heifier and Dacre obtained a leave form of an ozo
sethine dye (XVII) and a dextro and leave form of an ozo
dye (XVIII):

\[
\begin{align*}
& \text{XVII} \quad \text{XVIII} \\
& \text{In the case of the ozo dye, XVII, the centro form had} \\
& \text{almost twice the rotation of the leave form and these authors} \\
& \text{considered them to be optical enantiomorphs of different degrees} \\
& \text{of purity. Martell and Calvin consider that, in view of the} \\
& \text{large number of possible optically active forms, the isolated} \\
& \text{forms may not have been mirror images, but that if they are} \\
& \text{enantiomorphs then form (f) would seem the most likely form.}
\end{align*}
\]
Callis, Nelson and Seiler have preparedazo and amionine dye complexes of nickel (II), cobalt (II and III), chromium (III) and zinc (II). Magnetic susceptibility studies indicate the 1 : 2 nickel complexes of the mono-o-hydroxy substituted azo dyes are planar but that the 1 : 1 complexes formed with o,o'-substitutedazo and amionine (XX - XXX) 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 XXI and XXII a 1 : 1 cobalt (II) complex. Cobalt (III) complexes of o,o'-disubstituted azo dyes could be prepared from the [Co (OH)]^{2+} ion.

Callis and Seiler 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.

Anwely determined the stability constants of a number of azo dye complexes by pH titration in 75% dioxam 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. Thorium (IV) appeared to attain a co-ordination number of nine forming 1 : 3 complexes with o-,o'-dihydroxyazo dyes. Bravely found the order of stability of theazo dye complexes to be as follows:

\[ \text{Fe}^{3+} \text{Cl} \text{Fe}^{3+} \text{Cu}^{2+} \text{Cu}^{2+} \text{Cu}^{2+} \text{Cu}^{2+} \]

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

\[ \text{Cu}^{2+} \text{Co}^{2+} \text{Zn}^{2+} \text{Fe}^{3+} \text{Fe}^{3+} \text{Fe}^{3+} \text{Fe}^{3+} \text{Fe}^{3+} \text{Fe}^{3+} \]

for divalent metals and 

\[ \text{Cu}^{2+} \text{Al} \]

for trivalent species.

Cotens and Sigg\(^{72-73}\) studied Helochrome Violet 22 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 aluminum, 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. T continuous variations plot\(^{74}\) clearly indicated a 1 : 1 complex with copper and no evidence was obtained for the \(\text{Cu}_2\text{Dye}_2\) complex reported by Drew and Fairbairn. No evidence was obtained for the 1 : 3 complexes of calcium and magnesium with Helochrome Black T:--

\[
\begin{align*}
\text{OH} & \quad \text{NH}_2 \\
\text{-N=N-} & \quad \text{-SO}_3\text{N} \text{H} \\
\text{-NO}_2 & \quad \text{-NO}_2
\end{align*}
\]

reported by Young and West\(^{24}\). Using carefully purified

Helochrome Black T, Michel and Issidor\(^{75}\) found only 1 : 1
complexes with magnesium and calcium. This is in agreement with Horrocks and Henderson\textsuperscript{46} who found 1 : 1 complexes of calcium and magnesium with this dye with Eriochrome Blue Black AR:

![Eriochrome Blue Black AR](image)

Eriochrome Blue Black AR:

![Eriochrome Blue Black AR](image)

and with Eriochrome Blue Black AR:

![Eriochrome Blue Black AR](image)

Geary, Hicks and Israël\textsuperscript{71} studied the complexes of radi-

![Eriochrome Blue Black AR](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 co-

ordering without loss of the proton of the o-hydroxyl group.
The same authors have compared the stabilities of the copper (II) complexes of RN with the corresponding cyanins having the structures shown above (XXI and XXIV). The values of log K, the stability constant, in solution were found to be 10.45, 13.68 and 7.30 respectively. This was taken to indicate that the copper RN complex had structure XXIV with its five-membered ring:

![XXI](image1)  ![XXIV](image2)  ![XXV](image3)

Patelidzess et al.\(^2\) observed an unusual aspect in the complexation of p-phenyl-

![p-phenyl](image4)

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

![XXVI](image5)

Servin\(^2\) developed a number of o-hydroxylic, \(\Delta^1\)-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 organic acid protons (XXVII), but the 2:1 thionin complex with pyridine (III) has been shown to involve the loss of both protons$^{30}$ (XXVIII):

![Diagram](attachment:image)

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 as far been observed only with a 2-hydroxyazo dye complexes and it was thought that a polarographic study of the azo dye complexes in which other azo substituents were present in the azo dye molecule would be useful. It was hoped from this study, that existing analytical methods which utilise 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$^{12}$ have determined aluminium by reduction of its eleochrome violet B2 complex at the rotating pyrolytic graphite electrode, and Mittel and Florence$^{14}$ have also determined aluminium by reduction of its aluminium cyanide 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 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 cm.

The current from the cell is chopped by a vibrator relay and then passed through a high stability d.c. amplifier which has a low input resistance; the voltage loss 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 synchro units. The minimum sensitivity corresponds to 0.01 cm.
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 volts in 5 millivolt intervals. This range may be doubled or halved; 0.4 volts may thus be equivalent to 5, 10 or 20 mm. 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 adding accurate potential measurements externally. The degree of damping is varied in six steps and is thus accurately reproducible. However, even at 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 45 mm. per minute and 3.75 in. 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 zero 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 ion recording 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 periodic portion is 0 - 45° 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 true time curvature of such magnitude that distortion of the peaks results. The Univector overcomes this difficulty by the incorporation of a phase sensitive detector to separate the two components, with the result that the curvature of the base line is null, 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 this
makes 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, for
correcting small changes in sensitivity and for 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
case solution is difficult. Attempts to damp these oscillations
usually result in a distorted wave. One of the most successful
smoothing systems is that used in the Cambridge Polarograph.

at 10^{-6} = 10^{-7} M concentrations of the electro reduced
substance, the charging current of the double layer capacitance is much larger than the current due to electrode reaction. It is not easy to compensate for the charging 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 range.

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 substances. 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 0.67 volts 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 Radelkis polarograph by use of a three electrode system.

2. The Radelkis Type UU-102 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., carbon 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 12A polarograph incorporates a three electrode system to remove 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 meter driven potentiometer of the polarograph, 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 is 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 20 cm. full scale deflection, compared with 7.5 and 10 cm. for the other polarographs respectively.

The Hadwiss polarograph is provided with a set of controls to enable the starting voltage to be selected in 6.5 volt steps from +3.0 volts to -2.0 volts. Scales of 0.5, 1.0 or 2.0 volt to 60 cm. of chart paper may be selected, providing scale expansion. These voltage scales may be carried out in 40 seconds, 80 seconds, 8 minutes, 10 minutes, 60 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 circuit is also incorporated, which works off a battery common 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 $10^{-7}$ mV per division to $10^{-10}$ mV 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 thermostatted 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 mechanical faults including the failure of microswitches and the gear box.

3. The Cathode Ray Polargraphic Technique

The cathode ray polarographic technique was first suggested by Daniels. The polarograph described here was designed by Davies and Chestern 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 potentiometric 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 \(^{65}\) 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 wave of conventional shape with a cathode ray polarograph. Smith \(^{66}\) obtained peaked (cathode ray type) polarograms with a conventional d.c. polarograph, by means of a dropping mercury electrode with a drop time of about 2 minutes.

By comparison of the Fender \(^{67}\) and Thoevic \(^{68}\) equations it can be shown that with identical solutions and similar electrodes the ratio of peak current to diffusion current should be about \(3.5 \times 10^{4}\) where \(n\) is the number of electrons involved. The peak current is directly proportional to concentration so 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 \(\alpha^2\) whereas the latter is directly proportional to \(\alpha\) (\(\alpha =\) 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 size of the mercury drop does not alter significantly during the time of sweep - not two seconds of drop life - little variation
of current due to change in drop surface area occurs and implications up to 50 or 100 times that used in conventional d.c. 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 2000:1. The detection limit by this technique is about $10^{-7}$ M to $10^{-5}$ 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 - Coleochrome Violet RS 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 \( \text{H}^+ + e \rightarrow \text{H} \) 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 \( \text{H} \rightarrow e \rightarrow \text{H}^+ \) 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 synchronised 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 synchronisation. Dowell and Reynolds 92 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. 91 The three main branches are:

- Subtractive polarography 92,93 In this technique one cell
contains a blank solution and the other the solution to be
analyzed. The same potential is applied to synchronised
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 obscure
apparent cell before their peak potentials are also eliminated.

"Derivative polarography" is used to denote the technique whereby one cell contains the solution to be analysed 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 (2 x 10^{-4} - 5 x 10^{-5}M). The technique may, however, be used down to 10^{-7}M - 10^{-5}M 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 35.

"Derivative polarography" introduces a small difference (10 or 20mV 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 10mV 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 modulator 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 Unit 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 reference electrode over a range of 100V.

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. Its normal cathode ray polarography is forty to a hundred times as sensitive as a conventional d.c. polarograph. Its 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-Polarograph II also 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 polaro- graph. 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 normal 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 15 mV 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 polaro-gram of 'normal' shape. Automatic compensation for any current, due to previous reductions or to sloping base line, can be made. Satisfactory polaro-grams may be obtained with solutions, whose specific conductivity lies between that of 1 N hydrochloric acid and 0.01 N 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 is a major disadvantage of the square wave polaro- graph.

The importance of impurities in the base electrolyte
καθότι στην πραγματικότητα οι δύο αυτοί οι δρόμοι οικογενειάτου
ησυχίας έχουν την γνώμονα της ίδιας ελπίδας και της ίδιας 
αντιπώτητας.

• Στην άλλη πλευρά, οι προβλέψεις και οι προσπαθίες 
νεκροψίας και αναφοράς φαίνονται να κατεβαίνουν σε 
ευκαιρίες νέους ισχυοφορών. Οι σφραγίδες της 
πάροδου έχουν την ευκαιρία να διατηρήσουν την 
νήματα της ζωής και της ημιοπέδωσης.

• Αυτό είναι αυθεντικό και επίσης σωστό. Ο ισχυρός 
των ενέργειών είναι η ίδια η δυναμική προσπάθεια 
εθνικής ανατροπής και εθνικής διαδραστικότητας.

• Οι καλλωπιστικές προσπάθειες και οι καλλωπιστικές 
προσπάθειες, που είναι το πλεονέκτημα της 
νεκροψίας, δεν μπορούν να διασώσουν την 
νήματα της ζωής και της ημιοπέδωσης.

• Αυτό είναι αυθεντικό και επίσης σωστό. Οι καλλωπιστικές 
προσπάθειες και οι καλλωπιστικές 
προσπάθειες, που είναι το πλεονέκτημα της 
νεκροψίας, δεν μπορούν να διασώσουν την 
νήματα της ζωής και της ημιοπέδωσης.
milli-seconds. 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 base of the capillary tip with solution. This current is eliminated as described at the beginning of this section. As 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 7mV or 20mV are used depending on whether the sensitivity or the resolution is the most important factor. A pulse height of 7mV 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 change in cell impedance as 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 recorder 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 selective. This gives a degree of smoothing to the output. The integration may be made over 1, 3 or 9 drops. The maximum smoothing should be used with the maximum time span 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 previuous reduced substance present in 10^7 times excess over the substance to be determined provided that the assumed reduction occurs at least 0.2 volts earlier than that of the substance to be determined.
Distinction 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 pool 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 characterizing 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:  

\[ \text{I}_d = \frac{60\pi n^2 D t}{n} \]

where \( I_d \) = the limiting diffusion current in micro-amps  
\( n \) = the number of electrons involved in the reaction  
\( D \) = the Perrot, 96,000 cm^2/sec
The diffusional coefficient of the electroactive substance in cm.² sec⁻¹

\( m \) - the mass of mercury flowing (g/sec).

\( t \) - the drop time (seconds).

\( c \) - the concentration of the electroactive substance (millimoles/litre).

It can be shown that the limiting current is proportional to the square root of the height of the mercury column after correction for the interfacial tension at the surface of the drop.

\[ \frac{1}{t} = h_{exp} - h_{basal}, \] where \( h_{basal} \) = 2.4/n²/cm².

\( h_{exp} \) - corrected mercury head, \( h_{basal} \) - measured mercury head.

Heyrovsky has pointed out that in the great majority of investigations it is sufficient to plot limiting current against square root of the actual height of the mercury head.

Further confirmation of diffusion control of a wave can be obtained from the fact that the temperature coefficient of diffusion currents is \( 1 - 2 \) per degree centigrade.

3. **Molten Mercury**

In general, reducible ions reach the surface of the dropping 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 there is no electrolyte in the solution, the current obtained for the reduction of cathodes 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 polaro graphically active substance is strongly adsorbed on 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:

\[ \text{in} = \text{0.2} \times \frac{nF}{nRT} \]

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 in that anions are sometimes produced. The shape of the anions vary from neat peaks to rounded bumps. Anions 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 Hayter and Cottrell and the electrokinetic theory of Nernst and Van Wazer. It is beyond the scope of this thesis to discuss these theories.

4. Kinetic Currents

In some systems the electrode reaction is not the controlling step 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 electrode 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:

\[ i_C = 403.5n^2F^2c(\Delta F/\Delta n) \]

where \( \Delta F \) and \( \Delta n \) 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:

\[ \text{e.m.f.} = E_1 - \frac{2.303} n \log \frac{i}{i_0} \] at 25°C

where

- e.m.f. = 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
- \( i_0 \) = limiting diffusion current
A plot of $\log y$ versus $\log x$ formed a log plot,

$$\log y = \log 10 - \log x$$

is a straight line of slope $n = 0.0591$ with an intercept at the

axis equal to the half-wave potential. This has been verified

experimentally by Jones $164 = 166$ and Boltzmann $167$.

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 $n$ 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 $0.0591$. The above applies to cases where

the solution is reduced to the metal and where the metal ion

is reduced to a lower oxidation state.

Potentiometric titrations

Two cyanides considered in the present study contain at

least one ortho hydroxyl group on one of the aromatic rings.

and are weak bases. On forming a complex the proton of the

ortho hydroxyl group is liberated and this occurs at a lower

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 lose a proton.

Examples of apparent differences in the stoechiometry

determined by this method and by spectrophotometry have been

attributed to this cause.$168$
\[
\frac{\beta}{\theta^{\alpha}} \cdot \left(1 - \frac{\beta}{\theta^{\alpha}}\right) = E
\]

\[
E = \frac{1}{\omega - \alpha} \cdot \sum_{n=0}^{\infty} \frac{(-1)^n}{n!} \left(\frac{\alpha}{\theta^{\alpha}}\right)^n
\]

\[
E = \frac{1}{\sum_{n=0}^{\infty} \frac{(-1)^n}{n!} \left(\frac{\alpha}{\theta^{\alpha}}\right)^n}
\]

These equations and expressions have been derived in the document. Further analysis and discussion are needed to fully understand the implications of these results. The calculations and derivations presented in this study have been used to support the conclusions drawn in the research.
\( t \) = total concentration of ligand
\( v^0 \) = initial concentration of acid
\( v^0' \) = 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
\( \pi \) = degree of formation of the ligand-proton complex

\[ \pi_n = \frac{v^0 \pi y}{v^0 (v' + v'') (v'' - v''')} \]

and if:

\[ v' >> v'' \text{ and } v'' >> v''' \]

then:

\[ \pi_n = \frac{v^0 \pi y}{v'' + v'''} \]

If the volume of alkali added is not negligible, the fraction of \( v^0 \) 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 \( y \); then 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 \( y \) curves over which the complexes are formed and are stable.
General Conditions for Polarography

The dyes studied were each examined in a similar manner throughout. An aliquot of the dye was added to a cell saturated solution, usually containing a total of 10 ml of 24 components, and made up to 50 ml in a graduated flask. Then 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 full plots were carried out on these solutions using the Unicam SP600 spectrophotometer with 2 cm or 5 cm silica cells.

Determination of the Purity of the Dye

The purity of the dyes was determined by potentiometric pH titration or by titration with titaconic chloride solution. In the case where the dye loses a proton at fairly low pH, the titration 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 titaconic chloride was that described by Stagg.
the dye was then calculated from the formula below.

\[
\text{mol. } \text{Fe(III)} \text{. mole } = \text{ mol. } \text{Fe(III) . mole } \times \text{ dil. } \text{ dye } \times \text{ vol. (lit. ) } \times 10^3
\]

where \( n \) = number of hydroxyl ions consumed per dye molecule

- \( n = 4 \) for \( \text{H}_{2} \text{SO}_{4} \)
- \( n = 6 \) for \( \text{H}_{2} \text{SO}_{3} \)

Hiemalometry

The method used was essentially that described by

Reynolds and Shalgany. A micro-cell capable of being

used 0.2 to 0.6 ml. of solution was constructed. The

anode was usually a silver foil and the cathode was a

dropping mercury electrode. In some cases a silver gauze

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 trichloro-

ethylene to prevent any reoxidation 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 calomel reference

was used as the potentialstat.

The dropping mercury electrode was lowered into the

decomposed dye solution and a stop clock was started.

Potentials were 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 Shulzecky.

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:

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

where \( M \) = the molecular weight of substance
\( F \) = the Faraday
\( X \) = the number of coulombs
\( M \) = the weight of sample reduced (calculated from the first and last polarograms)

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

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 arithmetic mean method requires only the limiting currents of the two polarographs. The number of electrons transferred is calculated from the equation:

\[ n = \frac{I_2 - I_1}{I_2 - I_0} \times \frac{1}{i} \times \frac{1}{t} \times \frac{1}{v} \times \frac{1}{u} \]

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 color 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>pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid sodium hydroxide</td>
<td>3.2 - 6.5</td>
</tr>
<tr>
<td>Citric acid sodium hydroxide phosphate</td>
<td>3.2 - 8.5</td>
</tr>
<tr>
<td>Formic acid sodium hydroxide</td>
<td>2.6 - 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>5.8 - 8.3</td>
</tr>
<tr>
<td>Iodic acid - sodium hydroxide</td>
<td>6.0 - 10.0</td>
</tr>
<tr>
<td>Ammonium chloride - ammonium hydroxide</td>
<td>8.5 - 9.2</td>
</tr>
<tr>
<td>Hydrochloric acid - ethanamines</td>
<td>8.6 - 10.4</td>
</tr>
<tr>
<td>Sodium bicarbonate - sodium carbonate</td>
<td>9.3 - 11.0</td>
</tr>
<tr>
<td>Sodium hydrogen phosphate - sodium hydroxide</td>
<td>11.0 - 12.0</td>
</tr>
<tr>
<td>Piperidine - sodium hydroxide</td>
<td>13.5 - 15.0</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:

- $\text{Al}_2\text{(SO}_4\text{)}_3\cdot\text{3H}_2\text{O}$
- $\text{CuSO}_4\cdot\text{5H}_2\text{O}$
- $\text{CoCl}_2\cdot\text{6H}_2\text{O}$ (dissolved in dilute hydrochloric acid)
- $\text{ZnO}\cdot\text{2H}_2\text{O}$
- $\text{FeCl}_3\cdot\text{6H}_2\text{O}$
- $\text{NiCl}_2\cdot\text{6H}_2\text{O}$
Chapter IV
2,4-di-hydroxy azo dyes

Section (1). Coelechrose Violet 3B

Coelechrose Violet 3B

\[
\begin{align*}
\text{C. I. 15670} \\
\text{\includegraphics[width=0.3\textwidth]{image.png}}
\end{align*}
\]

Coelechrose Violet 3B (under the more convenient Violet 3B) was the dye originally used by Millard and Penn to determine aluminium polarographically, and its polarography has been studied more extensively than any other azo dye. The present work follows directly from that of Miller.

The first objective was to develop a method for the polarographic determination of magnesium in the presence of calcium on the lines indicated by Miller and Reynolds and then to study the polarography of a number of other complexes formed by coelechrose violet 3B.

6. Determination of Magnesium in the Presence of Calcium

10-ml. aliquots of standard magnesium solution were placed in 50-ml. graduated flasks to give a final concentration of 2 - 10 g./ml. of magnesium. The solutions were then treated with 50 ml. of 0.05 per cent coelechrose violet 3B solution and 5 ml. of 2N piperidine solution. The solutions were neutralised with 12 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, deoxygenated by passage of hydrogen for about five minutes and polarographed.

These preliminary experiments confirmed that a polarographically active magnesium-coblechrose violet 3B 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 mg/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

- Solochrome Violet RS.
- Dye + Aluminium.

Applied Potential.

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 g ml. and 60 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 d.c. polarography and by cathode ray polarography. The results are tabulated in Table 1.

### Table 1

<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>9020</td>
<td>77</td>
</tr>
<tr>
<td>11.9</td>
<td>800</td>
<td>7</td>
</tr>
<tr>
<td>11.9'</td>
<td>2200</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>5700</td>
<td>25</td>
</tr>
<tr>
<td>11.0</td>
<td>6300</td>
<td>25</td>
</tr>
<tr>
<td>10.5</td>
<td>5200</td>
<td>15.5</td>
</tr>
<tr>
<td>10.0</td>
<td>2220</td>
<td>12.5</td>
</tr>
<tr>
<td>9.5</td>
<td>800</td>
<td>5</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 slope 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 20 μg/ml calcium. In this case all the solutions were heated to 70°C before polarography.

Table 2
3 μg/ml Fe in the presence of 30 μg/ml Ca

<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>5400</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 Calochromes Violet 56 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 mg./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>Peak 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+</td>
<td>6700</td>
<td>35.7</td>
</tr>
<tr>
<td>12+</td>
<td>3400</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>9960</td>
<td>35.0</td>
</tr>
<tr>
<td>10.5</td>
<td>7200</td>
<td>30.0</td>
</tr>
<tr>
<td>10.0</td>
<td>4000</td>
<td>25.0</td>
</tr>
<tr>
<td>9.5</td>
<td>2000</td>
<td>20.0</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 pyochromes Violet B was about 11.5. The peak height was nearly twice that obtained when calcium was present. At the optimum pH the shape of the magnesium dye step was greatly improved (see fig. 5c). 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 10 as previously reported. 44

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 analysis.

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

<table>
<thead>
<tr>
<th>pH</th>
<th>Step Height for Cathode ray polarography (arbitrary units)</th>
<th>Step Height for Cartridge polarography (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5</td>
<td>420</td>
<td>35</td>
</tr>
<tr>
<td>11.5'</td>
<td>3950</td>
<td>35</td>
</tr>
<tr>
<td>11.5''</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>11.0</td>
<td>6240</td>
<td>29</td>
</tr>
<tr>
<td>10.5</td>
<td>3930</td>
<td>17.5</td>
</tr>
</tbody>
</table>

'Solution heated to 55 - 75°C for 5 minutes

'Solution heated to boiling.
Table 5

<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>9300</td>
<td>36</td>
</tr>
<tr>
<td>11.5*</td>
<td>9300</td>
<td>36</td>
</tr>
<tr>
<td>11.0</td>
<td>7500</td>
<td>29.5</td>
</tr>
<tr>
<td>10.5</td>
<td>3600</td>
<td>15</td>
</tr>
</tbody>
</table>

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>Calcium Concentration g./dl.</th>
<th>Ca - Mg Ratio</th>
<th>Peak Height (arbitrary units)</th>
<th>Ammonium Oxalate Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>9300</td>
<td>5 ml. 0.25%</td>
</tr>
<tr>
<td>3</td>
<td>1 : 1</td>
<td>9340</td>
<td>&quot;</td>
</tr>
<tr>
<td>30</td>
<td>1 : 10</td>
<td>8400</td>
<td>&quot;</td>
</tr>
<tr>
<td>30</td>
<td>1 : 10</td>
<td>9300</td>
<td>5 ml. 0.25%</td>
</tr>
<tr>
<td>60</td>
<td>1 : 20</td>
<td>8400</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
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 + Ca**

<table>
<thead>
<tr>
<th>Calcium Concentration g./ml.</th>
<th>Kg./Ca Ratio</th>
<th>Peak Height (arbitrary units)</th>
<th>Ammonium Oxalate Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>1 : 50</td>
<td>9500</td>
<td>5 ml. 0.25%</td>
</tr>
<tr>
<td>300</td>
<td>1 : 100</td>
<td>7000</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>1 : 100</td>
<td>9000</td>
<td>10 ml. 0.25%</td>
</tr>
<tr>
<td>600</td>
<td>1 : 200</td>
<td>10000</td>
<td>5 ml. 0.25%</td>
</tr>
<tr>
<td>600</td>
<td>1 : 200</td>
<td>9000</td>
<td>10 ml. 0.25%</td>
</tr>
<tr>
<td>900</td>
<td>1 : 300</td>
<td>12000</td>
<td>5 ml. 0.25%</td>
</tr>
<tr>
<td>900</td>
<td>1 : 300</td>
<td>12000</td>
<td>5 ml. 0.25%</td>
</tr>
<tr>
<td>1200</td>
<td>1 : 400</td>
<td>12000</td>
<td>10 ml. 0.25%</td>
</tr>
<tr>
<td>1200</td>
<td>1 : 400</td>
<td>1000</td>
<td>12 ml. 0.25%</td>
</tr>
<tr>
<td>1200</td>
<td>1 : 400</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>1 : 600</td>
<td>5700</td>
<td>15 ml. 0.25%</td>
</tr>
<tr>
<td>2000</td>
<td>1 : 667</td>
<td>11700</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1 : 667</td>
<td>8120</td>
<td>0.5% + 5 ml. 0.25%</td>
</tr>
<tr>
<td>3000</td>
<td>1 : 1000</td>
<td>10200</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td>1 : 1000</td>
<td>8400</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td>1 : 1333</td>
<td>7200</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td>1 : 1333</td>
<td>6600</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>1 : 1000</td>
<td>10390</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>1 : 7000</td>
<td>11400</td>
<td>As dissolved in dye solution</td>
</tr>
</tbody>
</table>
### Table 6

**pH 11.5: Magnesium Concentration 3 μg./ml.**

<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 : 2565</td>
<td>15200</td>
<td>25 dissolved in dye solution</td>
</tr>
<tr>
<td>8000</td>
<td>1 : 2067</td>
<td>13900</td>
<td>&quot;</td>
</tr>
<tr>
<td>9000</td>
<td>1 : 2000</td>
<td>15300</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 : 5667</td>
<td>10000</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

1. This solution was allowed to stand two days before polarography.
2. Solution heated to 90°C for five minutes before polarography.
3. Ammonium oxalate was dissolved in the dye solution before addition of calcium and magnesium.
4. Solution containing 1000 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 25 mg 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 in present as Al(OH)$_7^+$ 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 2g 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$^55$ who developed a method, based on the work of Valler and Reynolds,$^54$ 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.

6. **Sulphurea Violet 5G with Aluminium**

Although this reaction had been studied by a number of
workers\(^{18, 20, 21, 23-26}\) 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 350eV, decreased
again to 100 - 150eV when only the complex step was present
(see fig. 7). This occurred at both pH 4.6 and 6.6 where
Peake and Reynolds\(^{24}\) 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 5.7 upwards when 1 \times 10^{-5}M
dye was used. Using a dye concentration comparable to that
used in the polarographic solutions (1 \times 10^{-4}M) a 1:1
complex was observed at pH 4.6 and a 1:2 complex at pH
6.6.

Because of these results it was decided to carry out
continuous variation 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. 8

(1) Curve for HNO$_3$
(2) " " " " + 4x10$^4$ M SVRS
(3) " " " " " " " " + 1x10$^4$ M Al
(see fig. 9). A plot of change of absorption at 10000 cm$^{-1}$ versus mole ratio gave a ratio of 1 : 1 (see fig. 10). A plot of absorption at 25000 cm$^{-1}$ versus mole ratio showed a change in slope at a ratio of 1 : 1. This ratio was confirmed by a mole 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 mole 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$^{25}$. This is possibly due to the pH being close to the pK value for safranine violet. Spectrophotometric studies indicated a ratio of 1 : 2.5, but from the graph of change in absorption versus mole 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 10000 cm$^{-1}$, versus
Concentration of Al ($M \times 10^4$) with $2.56 \times 10^{-4}$ SVRS

**Fig. 11**

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

**pH 4.6**

**Fig. 12**

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

**pH 6.6**
Fig. 13.

Step height in cms. at sens. $1/15$

pH 6.6

mls. $(1 \times 10^{-3} \text{ M AI.})$

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

Fig 14.

$\Delta$ Absorbance at 19000 cm$^{-1}$

pH 5.1

mls. $(1 \times 10^{-3} \text{ M AI.})$

mls. $(1 \times 10^{-3} \text{ M SVRS.})$
The ratio of dye : aluminium indicated that the two species were present, a 1 : 1 and a 1 : 2 complex (see Fig. 14). A similar plot at pH 3.00 cm$^{-1}$ indicated a ratio of 1 : 1. All the spectrophotometry was carried out on a Unicam SP 800 using either 2 cm or 5 cm infra-red 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 1 : 2 complex at pH 6.5 in contrast to the 1 : 2 and 1 : 3 complexes reported by Perkins and Reynolds$^{25}$ 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 Costes and Hing$^{13}$ spectrophotometrically. Florence and Pyke$^{12}$ have suggested that Perkins and Reynolds inadvertently used impure dye in the commercial samples obtained are about 50% pure. The concentration of dye used by Perkins and Reynolds was 1.5 x 10$^{-4}$, assuming 50% purity of the dye, which is of the same order as used in this investigation (4 x 10$^{-4}$) so the discrepancy in ratios cannot be explained as a concentration effect.

It has been reported many times$^{12, 23-25, 54}$ that for the aluminium - melamine violet no complex$^{1}$ 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 $2.5 \times 10^{-4}$ with respect to disoleochrome violet B and $1 \times 10^{-4}$ with respect to aluminium in $2 \times 10^{-3}$ nitric acid was titrated with $0.01N$ sodium hydroxide at 0°C until the 1 : 1 complex was just completely formed (pH 4.75). An aliquot of the solution was removed, deaerated, and polarographed at 0°C. The same aliquot was then heated to 70°C for 10 minutes and then was re-polarographed at 0°C, 40°C, 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 $1.5 \text{ per } ^\circ C$. 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 10 and 40°C was more than that expected from the temperature coefficient for a diffusion controlled step. The increase in step height between 50 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 0.25 volts, in all these determinations.

c. Electrolysis Violation with Iron (III)

As the combining ratios reported by Perkin and Reynolds for aluminium are in error, it was decided to check the ratios for iron (III) reported by Focák and Reynolds\(^5\) who reported ratios of 1:2 and 1:4 at 35°C and 5°C, respectively.

Potentiometric titrations of solutions of iron (III) and Alizarine Violet B, indicated only a 1:2 complex. i.e., four hydroxide ions were released per iron (III) ion. A continuous variations plot at pH 4.0 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 6.5, the complex step
never completely displaced the free dye reduction step.

This phenomenon has not previously been reported. A possible
explanation of this phenomenon is that the two complexes exist;
as one complex and a hydroxo complex, in which the rather
unstable hydroxo compound has been stabilized by complex
formation. The one complex is reduced at the same potential
as the free dye, and the hydroxo complex is then reduced to
the oxine 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. Collochrome Violet K with Nickel

This system was reinvestigated for the same reason as
was the iron-Collochrome Violet K system. Fisher and
Reynolds reported that the behaviour of nickel was unusual
in some respects compared with other metals studied; a
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. Fisher and Reynolds explain this
Fig. 15

Step height in cms. at sens. $1/15$

pH 4.8

mls. of $1 \times 10^{-3}$ Fe

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

Fig. 16.

Step height in cms. at sens. $1/15$

pH 6.6

mls. of $1 \times 10^{-3}$ M Fe

mls. of $1 \times 10^{-3}$ M SVRS
by suggesting that in acetate buffer, the water 'sheath' around the nickel ion is replaced by acetate ions in preference to soleochrome violet RS. 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., -0.26 volts (versus 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 Palmer 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 soleochrome violet RS to pH 4.5 and polarographing, a well-formed complex step was obtained, and as reported by Palmer 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. $1/15$

Fig. 18

Diffusion current

Applied potential (Volts)
acetate buffer. This indicated that the complex formed in
borate 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. Polarochemistry of Solochrome Violet R.

The polarography of Solochrome Violet R at various
pH values within the range 2.4 to 12.0 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:

\[ \eta = -0.0125 - 0.0556 \text{ pH} \]

up to pH 11.7. 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 Steward\textsuperscript{42}
between pH 9.5 and 10.5 (\( \eta = 0.100 - 0.055 \text{ pH} \)), whose
results obtained in piperidine buffer do not fit the
equation. Dean and Iryan\textsuperscript{43} obtained the following
equations:

\[ \eta = -0.355 - 0.35 \text{ pH} \text{ (pH 3 - 12)} \]

and:

\[ \eta = -0.400 - 0.25 \text{ pH} \text{ (pH 3 - 5)} \].
All these values are quoted versus the saturated calomel electrode.

As no other report of the splitting of the monoethene violet Ke wave has been found, it was decided to investigate this phenomenon further. The pH values at which splitting occurs correspond to the pH values for the dye-

\[
\begin{align*}
\text{Na}_2\text{SO}_3 & \quad \text{N} = \text{N} \quad \text{N} = \text{N} \quad \text{N} = \text{N} \\
\text{OH} & \quad \text{OH} \quad \text{OH} \\
\text{H}_3\text{O}^+ & \quad \text{H}_3\text{O}^+ \quad \text{H}_3\text{O}^+ \\
\text{pH} 3 & \quad \text{pH} 7.5
\end{align*}
\]

Lee observed that this type of splitting occurred in the case of Detsill Yellow-

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

but he did not connote 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 3.42 yielded two straight lines, corresponding to 1.5 and 2.76 electrons for the first and second parts, respectively. One 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 6.6 in phosphate/citrate buffer, yielded a curve which
could be divided into two straight line portions, the first
corresponding to 1.18 electrons, and the second 1.6 electrons.

Logarithmic analysis of the split wave at pK 5.3 gave
the straight line portions corresponding to 1.27 and 2.09
electrons, respectively.

The split wave at low pK was investigated by variation
of mercury head; the first part of the step appeared to be
kinetically controlled, and the second part of the curve was
diffusion controlled. Holthoff and Liberti reported the
occurrence of a kinetically controlled step in the reduction
of phenyl nitrate in hydroxylamine at pK values between 7 and 9.
When the first step was small it was completely independent
of mercury head, the overall step for cellosolve violet II
was diffusion controlled. Increasing the temperature from
25 to 36°C almost doubled the step height which also indicates
a kinonic process.

A similar investigation of the wave at pK 4.6 indicated
that it was diffusion controlled. Investigation of the waves
at pK 7.6 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 indicated adsorption. The
splitting at low pK values was unaffected by the drop rate.
At pH 7.6 a "hump" appeared after the main step. This disappeared on the addition of gelatine solution which suggests that this is an adsorption phenomenon.

The above results indicate that the reduction of metachrome violet over a wide pH range is not as 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 anode electrode was used but this gave few results and therefore a silver foil was used. Irreproducible results were obtained with concentrations of cadmium down to 50 μg/l.

Using 0.16 g/l of dye in acetate, ammonium 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 metachrome violet as
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 volumes of solution.
Section III. Prilocaine Black T.

Prilocaine Black T

C.I. 14645

\[
\text{Na}_2\text{SO}_4 - \text{N} = \text{N} \quad \text{H}_2\text{O}
\]

\[
\text{NO}_2
\]

Influence of Prilocaine Black T

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 colchicine violet FS, 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 \(4 \times 10^{-4}\) 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 5.6 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 9 - 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 10.5 - 7 the dye changes from claret to blue.)

At high pH values a small step shaped peak like a
peak, was observed (see fig. 13). 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 lumped
maxima, however, were not completely removed. The drop
time in the dye solution, at zero applied volts, was 4.5
seconds compared with 3.8 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 M 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 11.0 corresponding to the equation:

\[ E_{1/2} = 0.035 - 0.0035 \text{ pH} \]

As before a sharp increase in step height was observed
after pH 7.5 (see fig. 14).

Step height was linearly related to concentration
up to a concentration of 1.6 x 10^-4 M at pH 5 in phosphate
Fig. 19.

Step height in cms. at sens. $^1/1$

Fig. 20

Peak height in cms. at sens. $^1/16$

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 test the metal dye solutions to ensure complete complex formation when studying the polarography 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 Ichinoe \(^{53}\) 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 trimeric species. The tendency for some dyes to exist in aggregates is well known \(^{70},^{71}\) 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 \(4 \times 10^{-4}\) with added buffer, gave rather erratic polarograms, as aqueous solutions having a final dye concentration of \(1 \times 10^{-4}\) 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 scan half-wave potential on the ester group and therefore contributes to the overall step height. The step height increased by up to approximately three times its original value. This could be accounted for by a four
electron one 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. Polarographic 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-
graphy. 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
fillings, nor addition of other reducing agents.

Further fresh solutions were prepared. These were
sealed in capsules under nitrogen or air and were heated to
70°C for 15 minutes. The samples under nitrogen were
unaffected by heating, but those under air rapidly faded and
coloured colour. This indicates that the decomposition is due to oxidation
by atmospheric oxygen. The colour changes were from blue
through violet, grey and green-yellow to yellow.
Modification of the last 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 decolour</td>
<td>Rapidly went 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 Verstergen \(^{118}\) who showed that the fading of ano dyes in light and air is usually due to oxidation and is very slow in an atmosphere of nitrogen. The fading of the colour was followed spectrophotometrically and the colour f and t to decrease with time.

b. *Aristophora flash* with Aluminiun

As reported by Iker \(^{53}\) heating the dye with aluminium solution in acetate, phosphate and succinylsul 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 succinylsul 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 dropt. 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:

\[
E_1 = 0.03 - 0.052 pH
\]

and:

\[
E_2 = 0.012 - 0.017 pH
\]

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

\[
E_3 = 0.155 - 0.028 pH
\]
for pH values within the range pH 5 to 8.

In order to obtain greater resolution of the steps it was decided to use the Southern Harwell Pulse Polarograph using a 0.02 cm gap and counting 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 Centrigrade and pulse polarographs gave smooth curves for up to 0.002 mg/ml. of aluminium. The curve flattened off at 0.01 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. Anisodraza Blue 7 with Iron (III).

Solutions were prepared by adding 0.2 ml. 0.005% iron (III) solution to the dye solution in acetate buffer at pH 4.7 and in ammonical buffer at pH 8.8. 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 6.0 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 (0.25 : 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
Fig. 21

Step height in cm, at sens. 1/7

pH 4.9

μ g/ml. Al with 1x10^{-4} M Eriochrome Black T

Fig. 22

Step height in cm, at sens. 1/7

pH
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 'dough out' above pH 5.

The step height concentration relationship is shown in fig. 29. The step height increased rapidly at first and then flattened off after the addition of 5 mls. 0.001M 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. Driestane Black 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 -0.422 volt to -0.105 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 theazo dye complex being more reversible than that of the hydrated copper ion. In acetic acid buffer the copper step was displaced from -0.217 volt to -0.49 volt. When 5 mls. of 0.001M 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 18.4 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 5.5 and that hydrolysis did not occur below pH 9. Above pH 9 there was evidence of hydrolysis.

6. Principles of Black with Magnesium and Calcium

The addition of 0.1 cm. 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 polarographically active complex. Potentiometric titrations showed that complex formation was almost entirely absent below pH 9.5.
Section (iii). Polychrome Blue Black

Ludens Black 3

C.I. 14640

\[
\text{Na}_2\text{SO}_4 \quad \text{N} = \text{N} \quad \text{H}_2\text{O}
\]

This dye was purified by Soxhlet extraction using acetone as a solvent. The purity of the impure dye was determined by titrimetric chloride 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 - 94.4.

Polychromatography of Coca, rose blue black

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

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

\[ E_1 = + 0.052 - 0.0825 \text{ pH (Cambridge)} \]

\[ E_2 = + 0.045 - 0.0625 \text{ (Medelhia using 0.9 volt scan)} \]

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 minimum at pH 6 - 7 (see fig. 28). This may be attributed to a lack of available hydrogen ions at this pH value.

Logarithmic analysis of the polychromatics obtained at pH 5.9 in formate buffer gave a good straight line.
corresponding to 1.27 electrons, whereas a similar analysis at pH 9.2 gave 1.04 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 1.27 and 1.10 electrons.

The pH dependence was repeated using 20% alcohol and the following equations were found to hold between pH 2.5 and 12.5:

\[ E = +0.057 - 0.0603 \text{ pH (Cambridge)} \]

\[ E = -0.045 - 0.051 \text{ pH (Sadalkin)} \]

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 70 ml. polar buffer, 7 ml. was used. The polarograms obtained with these solutions were better shaped. The equation obtained was:

\[ E = -0.052 - 0.060 \text{ pH (Sadalkin)} \]

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 5 \times 10^{-4} concentration of dye. Both the graph of step height versus mercury bond and that of step height versus square root of mercury bond could be drawn on straight lines. This indicates predominantly diffusion
control with possibly some contribution from adsorption.

At pH 3.5 in alcoholic ammonical buffer the dependence of step height on concentration of dye was linear up to 0.05m dye. Dependence of step height on mercury height indicated some adsorption, but there was some doubt as to the shapes of these curves.

At pH 12.65 in sodium hydroxide/piperidine buffer the step height was again found to vary linearly with concentration and the dependence of step height on mercury height indicated predominantly diffusion control with some influence from adsorption.

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 Calceinone Violet no unsatisfactory results were obtained.

After the electrolysis period two new steps were observed in the polarogram. There was 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.

Then 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 indicates that the dye which is adsorbed onto the mercury drop which may be the cause of our micro-colonomatry results. Some adsorption of the buffer occurred as can be seen from the slightly unsymmetrical form of the curve in the absence of dye (see fig. 23).

b. Polychrome Blue Black with Alumina

9.3 ml of 0.1N alumina solution was added to a mixture of 20 ml of 0.02N polychrome blue black, 10 ml of ethanol, 0.5 ml of 3 acetic acid and 6.25 ml of 0.2N 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 -0.95 volts) and a second step, due to the reduction of the complex (at -0.85 volts versus SCE). Prior heating and cooling of the solution increased the size of the complex step obtained and improved its shape.

At pH 4.5 using acetate buffer, a more continuous variations curve indicated a combining ratio of 1:4.5, while a concentration dependence study for alumina with 0.35M polychrome blue black gave a straight line up to 0.14M alumina, after which the graph flattened off and the points became rather erratic. This concentration of alumina corresponds to a mole ratio of 1:2.

The above procedure was repeated in acetic acid-sodium acetate buffer at pH 4.5 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

--- Base electrolyte
--- Dye

Applied potential in volts

Fig. 24

(1) $8 \times 10^4$ M SBB
(2) " + $2 \times 10^4$ 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 paranin 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 celechrome Blue Black. This curve as 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 broad but 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 aza group. This is the first time that the complexation of an η,η'-dihydroxy aza 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. malachite blue black with Copper

The addition of 5 ml. 10% 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 aza dyes studied and copper is formed. There was no modification of the aza 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 15500 cm⁻¹ for the dye alone.

Similar polarographic and spectrophotometric results were obtained at pH 8.5.

The addition of copper to the dye in piperies/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 10 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 : 1.6 then increased slightly and finally decreased again after a ratio of 1 : 1 had been reached.

A spectrophotometric molar ratio plot using the absorbance at 17500 cm⁻¹ indicated a ratio of 1 : 1.44 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 -0.60 volts (versus S.C.E.) and the absorbance at 2530 nm.

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 7 the titration curves for dye with and without copper
almost merged, which signifies that either one hydrogen ion
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. toluidine 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 had shown that di oath oly xed ool 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 8.6, using the absorbance at 16700 cm⁻¹ and 23800 cm⁻¹ both gave combining ratios of 1 : 2.26. The absorbance at 15500 cm⁻¹ and 14000 cm⁻¹ decreased in size, but changes in the ratios of decrease were noted at mole ratios of 1 : 2.54 and 1 : 2.8. Continuous variations studies, using both the polarographic complex step and the absorbance at 18700 cm⁻¹ indicated a ratio of 1 : 2.5. Using the absorption band at 24000 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 sodium hydroxide solutions in a total of 35 ml. solution. Previously 9.5 ml. of each component were used.

A mole ratio graph using the complex step height for iron (III) in 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 -0.6 volts (versus SCE) was used...
Fig. 25

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

<table>
<thead>
<tr>
<th>$M \times 10^4$ Fe with $4 \times 10^{-4}$ M SBB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
</tr>
<tr>
<td>0.8</td>
</tr>
</tbody>
</table>

Fig. 26

Diffusion current

<table>
<thead>
<tr>
<th>Applied potential in volts</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.1</td>
</tr>
</tbody>
</table>

-0.1  -0.3  -0.5  -0.7  -0.9  -1.1  -1.3
1:2. One of the absorption bands was 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 -0.56 volts (versus Ag) indicated a combining 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 variations plot using the absorbance at 13500 cm⁻¹ also indicated a 1:2 complex.

The addition of iron (II) to a dye solution in alcoholic acetic acid buffer at pH 5.65 caused a change in colour from blue to red and a complex step was formed -2.75 volts (versus Ag). 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 that the solutions were allowed to stand. A dye ratio plot using the absorbance at 15500 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 0.2% dye concentration in 1 cm irradial cells. Similarly, using the absorbance at 17500 and 21500 cm⁻¹ combining ratios of
1 : 5.18 and 1 : 2.02 were obtained.

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

Polarographic continuous variations plots also indicated a 1 : 3 combining ratio, although it was difficult to obtain accurate polarographic measurements, as the complex step became very small when the dye concentration was decreased. Many of the polarograms had a small step (at -0.4 volts [SCE]) 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 : 0.17) was obtained by plotting the height of the additional step at -1.3 volts. Using the absorbance at 10700 cm⁻¹ a ratio of 1 : 1.6 was obtained and using the absorbance at 31000 cm⁻¹ a ratio of 1 : 0.755 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 : 2. Well defined spectrophotometric Job plots were not obtained.

The addition of iron (II) to Colchicine Blue Black, 1 ml. 2N piperidine and 2 ml. 5 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 2

<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. 1x1 CBB</td>
<td>3.94</td>
<td>3.2</td>
<td>3.9 : 1</td>
</tr>
<tr>
<td>10 ml. 1x1 Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1x1 CBB</td>
<td>7.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1x1 Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 ml. 1x1 CBB</td>
<td>11.26</td>
<td>3.29</td>
<td>3.9 : 1</td>
</tr>
<tr>
<td>5 ml. 1x1 Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1x1 CBB</td>
<td>6.5</td>
<td>0.92</td>
<td>7.7 : 1</td>
</tr>
<tr>
<td>2.5 ml. 1x1 Fe(II)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 ml. 1x1 CBB</td>
<td>11.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 ml. 1x1 CBB</td>
<td>6.25</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
c. Molecule Blue Black with magnesium

The same procedure was repeated with the dye and magnesium and no complex step was found at pH 4.6 and 7.1. 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 -1.20 volts observed with the dye alone. The height of this step however, was approximately the same, whether 2 or 30 ml. of 1M 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 Ag). At pH 12.70 a third small step was noticed at -1.1 volts.

A mole ratio plot was obtained using 0.005M dye and the results tabulated below.
Table 10

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1st. Step (cm^3)</th>
<th>2nd. Step (cm^3)</th>
<th>3rd. Step (cm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.24</td>
<td>3.24</td>
<td>0.30</td>
<td>2.23</td>
</tr>
<tr>
<td>0.06</td>
<td>3.64</td>
<td>1.0</td>
<td>3.99</td>
</tr>
<tr>
<td>0.46</td>
<td>3.22</td>
<td>1.94</td>
<td>3.76</td>
</tr>
<tr>
<td>0.26</td>
<td>3.12</td>
<td>2.2</td>
<td>4.56</td>
</tr>
<tr>
<td>0.29</td>
<td>3.2</td>
<td>1.13</td>
<td>3.63</td>
</tr>
<tr>
<td>0.24</td>
<td>5.24</td>
<td>1.3</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 -1.1 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 : 0.69 (mg : dye) was obtained. Examining 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 (v = -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 11.5.
Here the steps were less well separated (-0.8 and -1.1 volts), but the polarograms were better defined.

A monovariant plot using 0.28ml 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 cms$^{-1}$ 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 cms$^{-1}$ a ratio of 1 : 2 was obtained.

f. Colochrome 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 8.8) 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$^{53}$ 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.
106.

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 6 ml. 1 N calcium solution was added to the dye solution at pH 13.5, buffered in sodium hydroxide/propandine 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 20 ml. of 1 N 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 solcotrace blue black.
section (iv). lacticum uine solid us.

constant std 67

\( \text{CH}_3 \text{NO} \)

\( \text{N=N-} \)

\( \text{OH} \)

\( 50 \text{ Na} \)

The constancy of this dye were studied to determine whether the dye and nitro group 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 was hoped that more useful information might be gained.

5. current std 67 with aluminum

When the pH of the free dye gave two steps, the half-wave potentials of which were pH-dependent. At pH 4.10, in acetate buffer the polarogram, of \( 4 \times 10^{-4} \text{m} \) dye gave the following results:

\[ \text{step I} \]

\[ \text{current} = 0.5 \mu \text{A} \text{cc} \cdot \text{sec} \]

\[ \text{current} = 0.6 \mu \text{A} \text{cc} \cdot \text{sec} \]

The addition of 0.5 ml. of 0.05% aluminum solution gave a third step at 3.60 volts (versus mercury cell), which was not affected by prior heating. Further additions of aluminum increased the height of this step.

A Job's constant run plot was obtained using the third step and a combining ratio was found to be 1:3. The first step is almost certainly the first reduction step.

It increased in size on addition of aluminum and disappeared entirely at a stoichiometric ratio of 1:2. The second step, due to the reduction of the nitro group, only decreased slightly (from 2.66 cc to 2.50 cc) on the addition of up to 20 ml. of 1.0% 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 1:4 complex above pH 6. At pH values greater than 6.5, however, appreciable turbidity in the fluid was found to take place. This is probably the cause of the decrease in size of the complex step at pH 9.05.

4. Curdant and 5/ with iron (III)

Addition of progressively larger amounts of iron (III) solution to the dye at pH 4.5 caused a third step to appear as expected. However, a fourth step also appeared. A typical polarogram (6 ml. of iron (III) with 25 ml. of dye in 25 ml. total volume) had the following steps:

Step I  Step II  Step III

j0.194v 0.082 ccm.  j0.20v 0.04 ccm.  j0.24v 0.02 ccm.

Step IV

j0.17v 0.35 ccm.

The third step was rather drawn-out while the fourth step had a maximum superimposed upon it. Due to the rather complicated nature of the polarogram, accurate measurement of the steps was difficult and a Job covariant plot did not give useful results. The combined heights of the dye steps were 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 dye step was little altered. With 50 ml. of 1N iron solution the polarogram gave the following step heights:

<table>
<thead>
<tr>
<th>Step</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.44</td>
</tr>
<tr>
<td>II</td>
<td>0.52</td>
</tr>
<tr>
<td>III</td>
<td>0.59</td>
</tr>
<tr>
<td>IV</td>
<td>0.60</td>
</tr>
</tbody>
</table>

In ammoniacal buffer at pH 9.05 no complex step was observed. Potentiometric titrations showed that two dye molecules were attached to each iron (III) ion above pH 7 and that hydrolysis commenced at about pH 8.4. 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. **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 seems 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 steps was slightly larger in the presence of copper.

Potentiometric titrations indicated that about one were 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. Dissociation End 67 with Iron(III) and Calcium

An potentiometric titrations showed that no complex formation took place at pH values below 10 the polarographic investigations commenced at a pH value higher than this.

In dimethylglycine hydrochloride/cadmium hydroxide 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 -0.65 volts. This step increased linearly with concentration of calcium up to 1 x 10^{-4}M calcium with 4 x 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 salicylaldehyde Violet 5B step by calcium.

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

This is the first indication of a polarographically active complex between anazo dye and calcium.
Eyes of the o,o'-dihydronic and type have been shown by previous workers to give a displacement of the one reduction step in the presence of certain metal ions. The dyes studied in the present work also gave this displacement of the move and the complexes formed had the expected stoichiometry in most cases.

Cobaltroso Violet 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. Viscidin 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 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 cobaltroso blue BL hardly occurred. The shape of the step height versus pH curve (see Fig. 32) 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 one group. An alternative explanation is
Based on the evidence of absorption. 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

\[ \text{\textit{a,p}-Diphenyl pyrazolene dye} \]

Section (4). Colochrome Red dH 125

Per cent red 7

C.I. 19763

\[
\text{NaSO}_3 
\begin{array}{c}
\text{H}_2\text{O} \rightarrow \text{N} = \text{N} - \text{C} \text{N} \\
\text{C}_1
\end{array}
\]

a. Polarography of Colochrome Red dH 125

The dye was examined first for its pH dependence. A

volume of 50 ml. of 0.25 M colochrome Red was pipetted 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 decoloured on standing. The half-wave

potential varied with pH according to the equation:

\[
\frac{E}{2} = -0.440 - 0.0592 \ln \text{pH}
\]

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 doubtful as the first

step had a small residual 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. 36). This

in possibly a double layer effect. At high pH values the
reduction occurred at -0.05 to -1.0 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 solen 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 -0.14, -0.06, -0.02 and -1.07 volts (versus Ag), presumably due to decomposition products. At pH 11 (carbonate buffer) the dye step was reduced in height from 1.6 cm. to 0.2 cm. and additional steps were observed at -0.03 and -0.40 volts, together with a rather drawn out step at -1.3 volts.

At pH 11.2 (phosphate buffer) the dye step was reduced from 0.65 cm. to 0.3 cm. and steps appeared at -0.405 and -1.14 volts.

At pH 12.7 (dimethylglycine hydrochloride/iodine-potassium buffer) the step at -0.17 volts was reduced from 0.12 cm. to 0.3 cm. and was displaced to -0.155 volts. A small step appeared at -0.13 volts, the dye step at -1.4 volts either disappeared, or moved to -2.65 volts and reduced in height from 1.15 cm. to 0.5 cm. The height of the step at -1.1 volts decreased from 0.65 cm. to 0.35 cm. and was displaced slightly to -1.17 volts.
From the data it seems that either a complicated
adsorption is taking place giving rise to several polaro-
graphically 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 13.7. At pH 4.7, the step
height was proportional to the square root of the mercury
height. The dependence of step height on concentration was
not studied successfully as precipitation occurred at dye
concentrations greater than $1 \times 10^{-4}$. Further additions
of alcohol did not improve the results.

At pH 7.0 the nearly constant step was again proportional
to the square root of mercury height. A rather drawn-out step
was also observed at more negative potentials. The step
height of this step was larger than expected for diffusion
control at such mercury height values. This is indicative of
a maximum of the second kind, but unexpectedly the addition
of reactive 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 13.7 the graph of the height of the first step
versus mercury height indicated diffusion control, whereas
the second step appeared to be at least partially kinetically
controlled. Adding reactive 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 gelatine to the alcohol-free solution. The resulting polarogram was identical to that obtained for the gelatine-free alcohol solution. The dye solution in the absence of both gelatin and gelatine gave a curve 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 hydroxide solution was added to the buffer system the second step disappeared. The first step gave a linear plot up to a pH of 12. A concentration of dye.

Investigation with freshly prepared sodium hydroxide solution yielded the well-defined step with a second rather broad 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 portion, the corresponding to 1.15 electrons and the other rather long a portion including the region about the half-wave potential corresponding to 2.37 electrons.

b. Alcoholic solutions with Alumina

The addition of 0.05 g. of alumina to a pH 4.5 solution of the dye in 25% alcohol with added acetate buffer (pH 4.7) yielded two steps, one due to the free dye and the other to the complex. The solution was then heated to 70° for 10 minutes and re-polarographed. The results are shown below.
Before Heating  |  After Heating

<table>
<thead>
<tr>
<th>Sens 1/7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.85 cm.</td>
<td>3.31 cm.</td>
</tr>
<tr>
<td>2.12 cm.</td>
<td>1.78 cm.</td>
</tr>
</tbody>
</table>

Addition of 0.5 g. 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-ethyl-dihydroxyazo dye with respect to aluminium, this has 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 \(5 \times 10^{-4}\) aluminium with \(1 \times 10^{-4}\) dye. The two-fold excess of dye was necessary for linearity.

In 9/34, the addition of aluminium to an unheated solution did not result in the step becoming rounded. The addition of 10 ml. ethyl 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. No other small steps, probably due to impurities, were present in the polarogram of the free dye.
The polarogram of the dye step alone, 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 electrolytes 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 \(2 \times 10^{-5}\) to \(4 \times 10^{-5}\) did not increase the step at \(-0.25\) volts appreciably and resulted in poorly defined polarograms. Without alcohol being present, no step was observed at \(-0.25\) volts.

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

2. Dye step and \(-0.25\) to \(-0.40\) Iron (III)

Liquid at 10 and 10 ml. of \(0.05\%\) iron (III) solution were added to an acetate buffer \(1 \times 10^{-4}\) solution of the dye at \(pH 4.0\) and polarograms were run before and after heating. The colour of the dye changed from orange to green in 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 complex step was observed and the dye step height was unchanged. The effect of heating the solutions was to reduce further the free iron step.
Addition of 0.05M iron (III) to an aqueous solution of the dye caused precipitation but no complex step. The addition of 25 ml. 0.05M iron (III) did not give rise to a second step. Similar results were obtained in glycylglycine/calcium hydroxide buffer pH 12.5.

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

1. Electropolished in 12% 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 -0.365 to -0.375 v.s.m. (versus mercury pool). Heating did not affect the complex: copper reduction step. The addition of 25 ml. of 0.05M copper (the stoichiometric amount for a 1 : 1 complex) caused in three steps being obtained in the polarograms. One step corresponded to the $Cu^{+} \rightarrow Cu^{0}$ reduction, one corresponded to the reduction of copper in the complex and the third step corresponded to the reduction of the dye. The step height of the complex step varied linearly with copper concentration up to $1.5 \times 10^{-3}$.

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

A rather unsatisfactory continuous variations curve was obtained, giving a complexing ratio of 1 : 1. This was confirmed by continuous variations plots at pH 7.1 in a sodium citrate 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 rules and obtained values. The two continuous variations plots confirming the 2:1 stability ratio. The half-wave potential of the copper step was moved from -0.35 volts to -0.369 volts in this solution. As the displacement half-wave potential depends on the dissociation constant of the complex metal ion this small displacement indicated a large dissociation constant. Quantitative determination of the dissociation constant was not made, as the system is not fully reversible.

The continuous variations plot was repeated using the solution twice the concentration of the copper ions. The position of the maximum was only slightly altered confirming the presence of a singly dissociated complex. It can be argued that for an infinitely unstable complex, the position of the maximum on the continuous variations plot is independent of the ratio of concentrations of ligands to catalyst.

The addition of copper to dye at pH 4.5 produced a

olive green from emerald green to red. In less than
the equivalent amount of copper to form a 2:1 complex the complex step and the dye-step were present, with greater
amounts of copper a further step appeared at -0.3 volts (versus
saturated calomel). After heating a further step was found at
-0.40 volts. It is not clear just how difficult to measure owing to their close proximity to the system was
rather unstable due to oxidation non-reproducible results
difficult to obtain, no further work was carried out. It was
noted however, that the addition of copper tended to stabilize
the dye and prevent fading.
Potentiometric titrations indicated that a 1:1 complex was formed from pH 3.5 upwards.

b. A solution containing potassium and calcium

Solutions of potassium and calcium were added to ethylene glycol solutions at pH 4.7, 7.0, and 10.0. No indications of polarographically active complexes were obtained.

Potentiometric titrations indicated that complexes were formed to a slight extent at pH 6.5 but were not fully formed even at pH 7.
section (ii). bromocresol red

Dye: Red 17

This dye is more soluble than metochrom red CR 125 and it was thought that this would be a definite advantage in studying this type of dye.

Laboratory study of bromocresol red 3

This dye gave two well defined steps over the pH range studied. These were separated by about 0.53 volts. In general, the polarograms were not very well shaped, although some reasonable curves were obtained in ammonium and carbonate buffers.

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

\[ E_1 = 0.455 - 0.059 \text{ pH} \]

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:

\[ E_2 = -0.377 - 0.07 \text{ pH} \]

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 3.15 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, as the first step varied linearly with concentration up to a concentration of \(1.25 \times 10^{-4}\) M. 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. i.e., 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 4.5 in acetate similar dependences on mercury bead were obtained. However, at pH 5.0 both steps were completely diffusion controlled. Step heights at various concentrations were erratic as shown in Table 11 below.

<table>
<thead>
<tr>
<th>Concentration (Moles x 10^{-5})</th>
<th>Step Height (cm.)</th>
<th>Step Height (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st. Step</td>
<td>2nd. Step</td>
</tr>
<tr>
<td>2.5</td>
<td>3.64</td>
<td>2.73</td>
</tr>
<tr>
<td>5.0</td>
<td>1.14</td>
<td>3.53</td>
</tr>
<tr>
<td>7.5</td>
<td>5.5</td>
<td>4.91</td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>4.44</td>
</tr>
<tr>
<td>12.5</td>
<td>13.3</td>
<td>3.72</td>
</tr>
<tr>
<td>15.0</td>
<td>7.0</td>
<td>4.76</td>
</tr>
<tr>
<td>17.5</td>
<td>7.3</td>
<td>4.62</td>
</tr>
<tr>
<td>21.0</td>
<td>25.2</td>
<td>19.0</td>
</tr>
</tbody>
</table>
At pH 12.25 in sodium hydrosulfide at high mercury lead, a lump appeared behind the second step. This lump did not appear at mercury lead heights less than 50 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.

Owing to the rather erratic nature of the step heights it was decided to repeat this work using 50% alcoholic solutions. The initial work was carried out on the Cambridge polarograph and later the Haake-Carlson polarograph, using the three electrode system to eliminate the H drop as employed. Graphs of half-wave potential versus pH yielded the following results:

- $i_1 = 1.12 - 0.001 pH$ (first step) Cambridge polarograph
- $i_2 = 0.12 - 0.043 pH$ (second step) polarograph
- $i_3 = -0.05 - 0.075 pH$ (first step) Haake-Carlson polarograph
- $i_4 = 0.25 - 0.42 pH$ (second step) polarograph

With alcohol present the second step gave a graph which was a straight line up to pH 12, using both polarographs. 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...
لأنه لم تكن معروفة وتعرف. • أظهرت النتائج أن هذه المشكلة الرياضية لا يمكن حلها حتى في حالة أن يكون عدد الأعداد في كل مجموعة يساوي 10. • قد يكون له تأثير كبير على فهم الطلاب للرياضيات فيgradable phát triển năng lực giải quyết vấn đề.
127.

i) \( R-N=N-R + 4H^+ + 4e \rightarrow 2NH_2 \)

or \( R-N=N-R + 2H^+ + 2e \rightarrow R-NH-NH-R \)

ii) \[
\begin{align*}
\text{HO-} & \text{C-} \text{N-} \text{R'} \\
\text{R-N}= & \text{N-C} \\
& \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{HO-} & \text{C-} \text{N-} \text{R'} \\
\text{R-N}= & \text{N-C} \\
& \text{CH}_3
\end{align*}
\]

or

\[
\begin{align*}
\text{HO-} & \text{C-} \text{N-} \text{R'} \\
\text{R-N}= & \text{N-C} \\
& \text{CH}_3
\end{align*}
\]

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

iii)

\[
\begin{align*}
\text{HO-} & \text{C-} \text{N-} \text{R'} \\
\text{R-N}= & \text{N-C} \\
& \text{CH}_3
\end{align*}
\]

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

\[
\begin{align*}
\text{HO-} & \text{C-} \text{N-} \text{R'} \\
\text{R-N}= & \text{N-C} \\
& \text{CH}_3
\end{align*}
\]

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).

b. Eriochrome Red G with Aluminium

Aliquots of 5 ml. of 0.25M aluminium solution were added to alcoholic acetate buffer solutions containing 5 ml. 1.0M Eriochrome Red G. The resulting solutions were made up to 50 ml. in graduated flasks. Polarography of these solutions showed that a small step was apparent between the two dye
b. Alizarin Red S with aluminium

Aliquots of 5 ml. of 0.25M aluminium solution were added to alclorile acetate buffer solutions containing 5 ml. 1.0M Alizarin Red S. The resulting solutions were made up to 50 ml. in graduated flasks. Polarography of these solutions showed that a small step was apparent between the two dye steps at -0.11 and -0.34 volts (versus sre). on heating to 70°C for 15 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 Unicorder, did not yield a very useful polarogram probably due to the irreversible nature of the reductions. Increasing the amount of aluminium 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 Hellig polarograph to obtain the continuous variations curve. The steps were well separated when a 75- pulse and a 15 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 aluminium was next added to a 50- alcoholic solution containing 50 ml. 0.3M Alizarin Red S and 5 and an ammoniacal 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 chloride ion.

The addition of aluminium solution to an alcoholic solution of the dye in sodium hydroxide, at pH 7.5, yielded a polargraph having a hump after the first step. This first step was slightly greater than that obtained when aluminium was absent. The hump could be regarded as a change in slope of the plateau of the polargraphic wave.

Potentiometric titrations showed that the dye was slightly acidic in nature even at pH 7.

c. Trycine [Red 8 with iron (III)]

Aliquots of a 0.001N solution of iron (III) were added to an alcoholic solution at pH 4.7 (acetate buffer) and polargraphy was carried out. The procedure was repeated in the presence of 10 ml. 1.0N triethanol amine. The results are shown in Table 12 below using polargraph sensitivity setting:

<table>
<thead>
<tr>
<th>Conc.</th>
<th>0.05</th>
<th>0.15</th>
<th>0.35</th>
<th>0.65</th>
<th>0.95</th>
<th>1.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step height (cm.)</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</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 alone (Dye absent)</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 12

This reaction is observed.
From the above it can be seen that the free iron steps disappear after heating and one well-formed dye step and the very low dye step are left, together with steps due to the iron dye complex.

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

<table>
<thead>
<tr>
<th>Step Height (c.m.)</th>
<th>1.9</th>
<th>0.48</th>
<th>0.35</th>
<th>0.32</th>
<th>4.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>After heating</td>
<td>precipitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron dye</td>
<td>2.19</td>
<td></td>
<td></td>
<td></td>
<td>3.5</td>
</tr>
</tbody>
</table>

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

A concentration dependence using 3 - 15 ml. of 0.001M iron (III) solution with 10 ml of dye solution showed that the complex step heights were approximately constant in this range (see Table 14).

<table>
<thead>
<tr>
<th>Concentration of Fe^3+</th>
<th>10.44</th>
<th>-0.99</th>
<th>-3.29</th>
<th>-2.21</th>
<th>-0.95</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00 x 10^{-7} M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00 x 10^{-7} M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00 x 10^{-7} M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00 x 10^{-8} M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00 x 10^{-8} M</td>
<td></td>
<td></td>
<td></td>
<td></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 variations 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 9. A concentration dependence study gave very erratic results and a continuous variations plot had three peaks corresponding to dye to iron ratios of 2.5 : 1, 1 : 1.7, 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 0.7 : 1 (0.7 : iron) are above 1 : 1, then extrapolated 1 : 1 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 maximum absorption in the visible region was shifted from 520 \( \mu \text{m} \) for the free dye to 534 \( \mu \text{m} \) with excess of iron present. The two absorption bands in the ultra-violet moved only by a few \( \mu \text{m} \). Plotting absorbance at various wavelengths gave bands rather than peaks corresponding to ratios of dye to iron of 3 : 1, 1.07 : 1 : 1.02 and 1 : 1.07 and possibly 1 : 1 at 430, 475, 515, 525 and 550 \( \mu \text{m} \) respectively.

During the polarization investigations it was noted that the complex did not replace the dye step completely, even when a surplus of iron (10 : 1) was present; in fact, when 10 % of 0.001 M iron was added to 10 ml of 0.001 M dye both free dye and complex steps were smaller than those 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 8.5.

At pH 12.4 (electrode cells; sodium hydroxide/methylglycolic hydrochloric acid 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 led to an adverse effect on the already poor step. It was concluded that complex formation took place at this pH, but that the complex was unstable under the conditions studied.

2. Dichloroarsenic with Copper (II)

Suitable dilutions of 0.25 M copper (II) solution were added to 25 ml. of 0.5 M dichloroarsenic solution in acetate buffer at pH 4.5. A step due to the reduction of $Cu^{2+}$ to $Cu^{+}$ at -0.845 volts (versus SCE) disappeared and an increase in step height was noticed for the first of the two dye steps. With the addition of 25 ml. of 0.25 M copper solution the step at -0.845 volts disappeared but was reduced in size from 0.15 cm. to 1.2 cm. at sensitivity $1/4$. The increase in dye step height was found to be O/7 cm. under the same conditions. No step due to the reduction of complexed dye was found. From the above it could seem likely that the reduction of the complex copper reduction with that of the dye dye. This step had a half-wave potential of -0.845 volts (versus SCE).

The addition of copper solution to an anemochoric buffered solution (pH 3) caused an inflection at the base of the dye step which, with the addition of further quantities of copper, developed into a second step having a half-wave potential at -1.053 volts (versus SCE). This corresponds to the half-wave potential of copper in this medium. A close examination of the values was another step was found between the copper step and the dye step which occurred at
-0.44 volts (versus SHE). This additional step was probably due to the reduction of complexed copper. This postulate 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 13 in 0.1M sodium hydroxide solution, with a solution containing 5mls 0.2M copper solution four steps were obtained at -0.17, -0.6, -0.7 and -1.10 volts respectively. The 1st two steps were due to the dye. The height of the more negative dye step was found to vary from run to run. Ammonia copper (50mls of a 0.25M solution) gave an additional step at about -0.35 volts which was difficult to measure owing to the proximity of the other steps. This step was due to copper itself which in this medium is reduced at about -0.5 volts (versus SHE). Heating the dye solution in the
These data give a displaced or reduction step with some metal ions, showing that a chelate group is not a criterion for complex steps of this type. Colchicine (ed) in 126 formed a 1:1 complex with aluminum giving this displacement of the step, but the other metals studied (iron(III), copper, cadmium and magnesium) did not. The first two metals were however shown to form complexes.

Colchicine (ed) and ed gave the displaced step with aluminum and iron(III) at 20°C. Complex formation took place with copper, as slow by potentiometric titrations, but did not result in a displaced step.
Chapter II

Poly- and Multiple Anion Dyes

Section (1): coloured resins and dyes

Vertical from 35

0.1 M HCl

![Chemical Structure]

- Interference of Polyelectrolytes [unspecified page]

A previous investigation of this dye was abandoned because of precipitation of the dye from solution at pH 4.5 and 6.5. The dye concentration used in the present study was 0.001 M in the final solution. Heating the mixture to 50°C was found to dissolve the precipitate, but reprecipitation occurred on cooling. The filtered solution was found to give two steps at -0.21 and 0.62 volts at pH 1.5 and -0.46 and -3.36 volts (versus SCE) at pH 4.5.

Increasing the dye concentration to 1.5 x 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 13.72. In 0.1 m hydrochloric acid precipitation occurred almost immediately. In all cases, polarographic added two steps. In ammoniacal buffer at pH 8.0, however, the steps were not well resolved and in 0.1 M sodium hydroxide buffer at pH 12.72 the second step was poorly defined. The ratios of the two steps varied between 1:1.27 and 1:1.55 except at high pH.
Table 15

<table>
<thead>
<tr>
<th>X</th>
<th>1st. step</th>
<th>2nd. step</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H (V)</td>
<td>(meas.)</td>
<td>H (V)</td>
</tr>
<tr>
<td>6.01</td>
<td>0.45</td>
<td>1.5</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>1.4</td>
<td>0.26</td>
</tr>
<tr>
<td>3.9</td>
<td>0.42</td>
<td>1.5</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>0.62</td>
<td>1.5</td>
<td>0.64</td>
</tr>
<tr>
<td>10.06</td>
<td>0.58</td>
<td>1.7</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>1.27</td>
<td>0.79</td>
</tr>
<tr>
<td>12.72</td>
<td>0.22</td>
<td>0.15</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td>0.15</td>
<td>0.53</td>
</tr>
</tbody>
</table>

at pK 12.72 two more steps were observed at -1.16 and -1.12 volts (versus SSE), respectively.

The effect of heating the solutions to 75°C, cooling and polarographic is shown in Table 15 below:

Table 16

<table>
<thead>
<tr>
<th>X</th>
<th>before Heating</th>
<th>Ratio</th>
<th>after Heating</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H (V) (meas.)</td>
<td>-</td>
<td>H (meas.)</td>
<td>-</td>
</tr>
<tr>
<td>4.16</td>
<td>0.45</td>
<td>1.5</td>
<td>5.75</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.39</td>
<td>1.21</td>
<td>5.23</td>
<td>2.3</td>
</tr>
<tr>
<td>6.9</td>
<td>0.42</td>
<td>1.5</td>
<td>2.42</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>0.68</td>
<td>1.655</td>
<td>0.83</td>
<td>3.25</td>
</tr>
<tr>
<td>10.44</td>
<td>0.45</td>
<td>1.25</td>
<td>0.53</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>0.79</td>
<td>1.15</td>
<td>0.6</td>
<td>2.2</td>
</tr>
</tbody>
</table>
It will be observed that the effect of heating was to increase the dye step—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 2.72 to 12.9 and the first step was found to vary with pH according to the equation:

\[ E = 0.416 - 0.0317 \text{ pH} \]

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.

Some 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 and reduction followed by a six electron nitro reduction, or a four electron nitro reduction followed by a four electron and 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

<table>
<thead>
<tr>
<th>Value</th>
<th>0.36</th>
<th>0.3</th>
<th>0.12</th>
<th>0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.6</td>
<td>0.6</td>
<td>0.78</td>
<td>0.6</td>
</tr>
<tr>
<td>Value</td>
<td>1.1</td>
<td>1.1</td>
<td>1.16</td>
<td>1.3</td>
</tr>
<tr>
<td>Value</td>
<td>1.5</td>
<td>1.5</td>
<td>1.43</td>
<td>0.5</td>
</tr>
</tbody>
</table>
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.\(^{139}\)

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 \(3 \times 10^{-3}\) 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.

5. Polychromism from \(\text{AlCl}_3\) with aluminium

Dilutions of aluminium solution were added to the dye
solution at pH 4.5, 12.0 and 12.9 without any indication of
a third step. Heating to \(70^\circ\)C did not give a third step.
In an attempt to prepare a polarographically active aluminium
complex by refluxing the solution containing the metal ion
and the dye at pH 4.6 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 1.7. After the pH
value of the dye, at pH 7.5, was reached some hydrolysis of the complex was noted, a slight few extra hydrogen ions were liberated. These could not come from the dye as it has only one ionisable hydrogen ion.

c. Addition of 0.25 M iron (III) solution and 0.02 M
iron(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) ions was preceded 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 maximum was found to have been moved from 465 mμ to 450 mμ.

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 ionisable hydrogen atom of the dye 1.45 hydrogen ions per iron(III) ion were released.

d. 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. 0.001 M copper(II) to 20 ml. 0.25 M dye yielded four steps at -0.64, -0.975, -1.47 and -2.52 volts (versus SHE) respectively. Copper under these conditions was reduced at -0.41 volts and the dye at -0.47 and -0.42
volts and hence the step at -0.275 V is attributed to the
reduction of complex as per. The possibility of it being
the reduction of copper(I) was excluded by observing the
effect of addition of copper(I) 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 stage, which indicates that the complex was partially
broken down during heating.

A continuous variations plot indicated a combining ratio
of 1 : 1 : 1. This ratio was confirmed by potentiometric
titration 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 0.9 hydrogen ions released per
copper ion.

Similar polarograms were obtained at pH values of 4.9
and 7.45. An ammonium and dimethylglyoxime/sodium hydroxide
buffer solutions respectively. At pH 11.45 some splitting
of the second dye step was noted but this was difficult to
measure accurately and the work was not continued.

e. Polarography of solutions from 12.135 with Barium and Calcium

A series of barium and calcium solutions were added
to the dye solutions at pH 3.5, 4.9 and 12.4. No new steps
appeared but, in the case of barium at pH 12.4 some
precipitate was formed which was a darker red than the
solution. This might be an insoluble complex.

Potentiometric titration showed that no complex formation
took place at pH values below 10.
section (ii). Auraminotetramine G

Harder from 145

\[
\text{Na}_{2}C_{6}\text{H}_{4}\text{N} = \text{N} \quad \text{NH}_{2} \quad \text{NH}_{2}
\]

This dye was studied to provide confirmatory evidence of the results obtained in section (1) of this chapter.

**Electrochemistry of Auraminotetramine G**

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:

\[ \theta = -0.022 - 0.068 \text{ pH} \]

between pH 3 and 10.5. Step height hardly varied with pH, but increased slightly between pH 5.0 and 10.5. Above this value the step heights decreased sharply. At pH values above 12 two steps were present. Investigation of these steps at pH 12.5 in phosphate/citric acid buffer showed that the second step height did not depend on mercury height 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>Int. Step</th>
<th>2nd. Step</th>
<th>Int. Step</th>
<th>2nd. Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1.72</td>
<td>1.75</td>
<td>33 - 34</td>
<td>2.12</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 biologically controlled and the second diffusion controlled, as the increase in height for this step is within the range expected for a diffusion step (1 – 2. 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^-5 M. 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 M, indicating that the overall reduction process was diffusion controlled.

At pH 4.0 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 99 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. More adsorption was noted from the effect of applied potential on the surface tension of the drop (drop rate).

In an acetic acid buffer at pH 3.9 results were obtained similar to those at pH 4.0.

Experiments with Aluminium

Solutions of aluminium solution were added to buffered solutions of the dye at pH values of 4.0, 3.9 and 2.4.
At pH 4.6 no additional step appeared, even after heating at 70°C for 15 minutes.

At pH 5.5 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.

Immanence 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 range 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 dimethylglycolic dimethylglycolic acid sodium hydrosulphate buffer at pH 5.5 did not yield a coupled step, but merely made the polarogram more erratic, probably due to precipitation of aluminium hydrosulphate.

Potentiometric titrations showed that a 1 : 3 complex was formed at pH values greater than 7.5 and that hydrolysis of the dye occurred at about pH 7.5. The number of hydronium ions liberated above pH 7.5 reached a value of 1.5 per aluminium ion (approximately) on the species formed tended towards $\text{Al}(OH)_3^+$ at high pH.

c. Electron transfer conditions

In acetate buffer at pH 4.6, the iron(II) to iron(III) 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 dimethylglycolic acid sodium hydrosulphate 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 polarisation at pH values below this.

4. 

Potentiometric titrations were obtained when copper was added to the dye at pH 4.5, 3.2 and 2.3. That is to say, the copper step was either suppressed or entirely removed depending on the metal present. A colour change from brown to greenish brown was also noted on the addition of copper.

Potentiometric titrations indicated that a 2:1 complex was formed, but the degree of formation did not approach unity until about pH 6 although some complex formation was apparent from pH 3.5 onwards.

5. 

The addition of magnesium and calcium gave similar results to those obtained for aluminium with this dye. There was an apparent change at pH 4.6. There was a 'false' step at pH 3.8, although in some cases this 'false' step was found in the polarogram of the dye without any metal ions being present. A third minimum appeared after this 'step' which was removed by the addition of potash 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 potash 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>(seconds)</td>
<td>5.9</td>
<td>3.76</td>
<td>4.78</td>
<td>5.81</td>
<td>5.92</td>
<td>5.95</td>
<td>6.2</td>
<td>6.6</td>
<td>7.0</td>
<td>7.3</td>
</tr>
<tr>
<td>(volts)</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>(seconds)</td>
<td>4.57</td>
<td>4.72</td>
<td>4.86</td>
<td>5.0</td>
<td>5.23</td>
<td>5.43</td>
<td>5.7</td>
<td>6.1</td>
<td>6.5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

From the above it seems that the additional step is 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 19.

At pH 12.3 in dimethylglycoluril hydrochloric acid-dimethylglycoluril buffer there was no indication of complex formation.

Summary of primary chloride ion data

This investigation has shown conclusively that the formation of a complex is not necessarily sufficient for the modification of the gas reduction step. Both donors studied were shown to form 1 : 2 complexes with aluminium and iron(III) and 1 : 1 complexes with copper(II) and yet in no instance was part of the reduction step of the gas procedure attributed to a pure negative potential.

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

Calcinose from pH 125 showed the reduction steps, as
was expected from its structure. But Argemittelbraun D,
which has a very similar structure, showed only one reduction
step at pH values below 12. From the comparative shn 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 D, except at pH values above 12. In this
case (below pH 12) it would be expected the ratio of the
height of the combined steps for Calcinose shown to the
height of the Argemittelbraun step to be 6 : 13 (see Table 12)
with a slight correction for the difference in molecular
weights which are 375 and 403 respectively. This correction
however, would be small as the diffusion coefficient is
proportional to the square root of a molecular weight and the
diffusion current is proportional to the square root of
diffusion coefficient.

It can be seen that, within experimental error, this
ratio exists (see Table 12).

Table 12

<table>
<thead>
<tr>
<th></th>
<th>4.5</th>
<th>6.7</th>
<th>12.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>dye</td>
<td>pH</td>
<td>Step Height</td>
<td>R(hv.)</td>
</tr>
<tr>
<td>Calcinose from</td>
<td>.17</td>
<td>2/1</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>.24</td>
<td>27.17</td>
<td>24</td>
</tr>
<tr>
<td>combined</td>
<td>.63</td>
<td>16.35</td>
<td>-</td>
</tr>
<tr>
<td>step height</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 12

<table>
<thead>
<tr>
<th>expmittellbregm</th>
<th>.32</th>
<th>26.5</th>
<th>.43</th>
<th>35.8</th>
<th>.63</th>
<th>45.75</th>
<th>.65</th>
<th>42.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>contined step height</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</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 so well with the experimental evidence.
Chapter VII
p-hydroxy, p'-sulpha re dyes

Section (1). Polychrome No 3 125

Result No 9

\[
\text{C}_{4}H_{8}N_{2} \cdot \text{COONa} \cdot \text{H}_{2} \cdot \text{SO}_{3} \cdot \text{Na}
\]

This type of dye was chosen for study as Bravley \footnote{1} has shown that they can form metal complexes of comparable stability to those with \( \text{o}, \text{o}' \)-dihydroxy re dyes.

\[ \text{pHometric analysis of polychrome No 3 125 with aluminium} \]

Polarography carried out under similar conditions to those described in previous chapters failed to give any displacement of the single red reduction step. The reduction step was found to be pH dependent, as with all the \text{o}, \text{o}'-dye s studied in this investigation. At pH 4.0 the half-wave potential of the step was found to be 0.45 volts (versus mercurypool).

Potentiometric titrations showed that at pH values below 4 and 3.5, one hydroxyl ion is liberated per aluminium ion added. At pH values above 4 the number of hydroxyl ions liberated rose to a value of 3.9 indicating hydrolysis of the complex. Hydrolysis of the complex was suspected during the actual titrations, as the pH values tended to fall slowly. Indications of a 1 : 1 complex between pH 6 and 3.5 were also apparent, but in dilute solutions hydrolysis was evident, even at these low pH values.
b. Isoschizo Red H.155 with Iron(III)

Elutriography 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.

No evidence of the dye step was observed but the free iron(III) to iron(II) step was suppressed.

Potentiometric titrations showed that two hydrogen ions were liberated per iron(II) ion added, at pH values from 3 to 4.5. Above pH 6 hydrolysis of the complex occurred, corresponding to the formation of Fe(OH)₃ at high pH. Iron(III) above it seems that a 1 : 2 complex is formed.

c. Isoschizo Red H.155 with Copper and Nickel.

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⁺ was moved to slightly more negative potentials, indicating a copper-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 5 but with nickel the degree of formation did not reach unity until pH 3.5.

d. Liparosol of isoschizo Red H.155 with fluorescein and oxaline.

Potentiometric titrations showed that no complex formation occurred below pH 12 and polargraphy was carried out only at pH 12.5. No modification of the dye step was observed.

e. Isoschizo Red H.155 with Hemoglobin

MacIntyre[^5] found that hemoglobin formed a complex with this type of dye and not with any of the other types of dye he
studied. An investigation of the system was therefore made. The addition of aliquots of 0.01 M beryllium solution to 5 ml. 0.01 M olecrocene 84 109 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 beryllium. The third portion usually retained the maximum observed for the dye peak. The part of the step proportional to beryllium increased initially but did not reach a value greater than 1.95 c.c. even when 20 ml. 0.01 M beryllium was added.

Potentiometric titrations 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 c.c. with 1.0 ml. 0.01 M olecrocene and 2.125 (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 c.c. of 0.01 M beryllium solution.

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

Potentiometric titrations showed that a 1:1 complex was formed between pH 5 and 7.5 after which some hydrolysis of the complex occurred. This is considered to be an important aspect of the work as polarographically active beryllium compounds are almost unknown and most work is for the determination of beryllium in beryllium-free solutions.
Section 14.2, Solochrome Flavine R 125

Pyroant Funnell 8

2.1. 10821

\[
\begin{align*}
\text{CH}_3
\end{align*}
\]

a. Solochrome Flavine R 125 with Aluminium

Polarography of Solochrome Flavine and aluminium solution under various conditions did not give any modification 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 hydrolys of the complex occurred, which eventually corresponded to an average of 3.5 hydrogen ions liberated per aluminium ion.

b. Solochrome Flavine R 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 8 although at pH values greater than 7 hydrolys of the complex occurred corresponding to the formation of Fe(II).

c. Solochrome Flavine R 125 with Copper

Polarography of Solochrome Flavine R 125 with copper did not yield a displaced step although the reduction step for copper(II) was shifted from -0.25 to -0.29 volts (versus mercury pool).
Potentiometric titrations confirmed complex formation above pH 3. 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.

Solochrome Flavine II 123 with Magnesium and Calcium
titration of these metal ions with Solochrome Flavine
II 123 did not yield a displaced dye step. Potentiometric
titrations, however, indicated that magnesium formed a complex
with the dye corresponding to a combining ratio of 1:1 at
pH 6.0. 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
Potentiometric titrations have confirmed the presence of
complexes of aluminium, iron(III) and copper with both the
dyes studied and with nickel and beryllium for the azo dye
studied. The pyrethrine 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 displaced
by the magnitude of the ionic radius allows it to fit into the
'hole' between the co-ordinating atoms of the complex. None
of the other ions will fit into this 'hole,' without
considerable distortion of the dye molecule.
Chapter VIII

Pyridyl-azo-dyes

Section (1). 1-(pyridylazo)-2-naphthol (IIC)

![Chemical Structure]

c. Polarography of IIC

$1 \times 10^{-3}$ IIC solutions were prepared using various percentage mixtures of ethyl alcohol and water. To avoid precipitation of IIC it was found necessary to use at least 80% v/v alcohol. The addition of potassium chloride base electrolyte, however, caused settling out of the dye and it was found necessary to use 50% alcohol for the polarographic solutions.

Step height varied linearly with concentration up to $8 \times 10^{-4}$ in 0.1M potassium chloride base electrolyte. With increasing concentration of IIC a maximum appeared and increased in size. Logarithmic analysis of the polarographic step indicated a value of 0.99.

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:

$$E_0 = 0.835 - 0.0035 \text{ pH}$$

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, ethylenediamine/sodium hydroxide and ammonia buffer. In all cases the half-wave potentials were more positive than predicted from the equation and in all systems except the ammonia buffer the points fell on the same straight line. In the ammonia system the half-wave potentials were even more positive.

step height varied with pH as shown in fig. 29. 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. Half-wave potential varied slightly with concentration. The ratio of the maximum to the diffusion controlled portion increased at first and then tended to a constant value. Iso-electric 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 1.45 and 1.19 electrons transferred respectively.

The second pH region studied was that of pH 6.48 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
Fig. 29

Step height at sens $10^9$ x 8

\[
\begin{align*}
\text{pH} & \quad 4 & 6 & 8 & 10 & 12 \\
\text{Step height} & \quad 10 & 12 & 14 & 16
\end{align*}
\]

Fig. 30

Step height in cms at sens. $1/10$

\[
\begin{align*}
\text{M} \times 10^5 \text{ Fe with } 2 \times 10^4 \text{ M PAR} & \quad 4 & 8 & 12 & 16 \\
\text{pH 5.3} & \quad 0.4 & 0.8 & 1.2 & 1.6
\end{align*}
\]
could also be drawn as a straight line. The effect of applied potential on drop rate in \( 1 \times 10^{-3} \) 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 11.3 graphs of step height versus mercury head were not very reproducible, the carbonate tended to precipitate and the drop rate became very rapid. The investigation of this pH region was therefore repeated in dimethylglycol:hydrochloric acid:potassium hydroxide buffer at pH 12.55. 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 chloride

Polarography of solutions of Al and aluminium chloride carried out at pH 6.25, 7.0 and 12.03 without any indication of a polarographically active complex being present.

Potentiometric titration of AlCl showed that no hydrogen ions were liberated up to pH value of 12.3. This is in agreement with Collard et al. who showed that the α-hydroxyl
group in 1:1 did not lessen until pH 10.5. The degree of
formulation (\(\beta\)) for the complex was found to be 1:1 i.e.,
two dye molecules per cationic ion. This complex was
fully formed by pH 5. Core precipitation occurred during
these titrations and this could explain why a whole number
value for \(\beta\) was not obtained. No indication of hydrolysis
was apparent.

3. Dye with Iron(II)

Colorimetry of 1:1 with Iron(II) 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 8.7 and 1:1 complex
was formed. In the case of the first complex the remaining
three positions for co-ordination were probably occupied by
water molecules. Ridders\(^{123}\) and co-workers showed that with
the very similar compound \(\text{FeCl}_3\), co-ordination was through the
oxygen of the \(\sigma\)-peripheral group, the pyridyl nitrogen and the
azo group nitro im function from the heterocyclic ring.

4. Copper(I) Compounds

The addition of solutions of Cu to copper solutions
caused the half-wave potential of the ferrocyanide to be moved to
a more negative potential. At pH 6.4 in acetate buffer this
shift was from \(-0.562\) volts (versus Hg) to \(-0.464\) volts, when excess of \(\text{FeCl}_3\) was present. There was also a marked
colour change from yellow to red on the addition of copper
solution to the dye. Owing 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 Lange and Pillement, who claimed a 1:1 complex was formed and stated that the formation constant was $10^{16}$. Using 0.0002% copper and 0.008% Na the stability constant from the polarographic data was found using the following equation:

$$\Delta (E) = -(a \cdot c) + \frac{0.059}{n} \log p - \frac{0.059}{n} \log \log \gamma,$$

where $(a \cdot c) = \text{half-wave potential of the single ion}$

$(a \cdot c) = \text{half-wave potential of the complexed ion}$

$a = \text{number of electrons transferred}$

$p = \text{number of ligands attached to metal ion}$

$c = \text{concentration of ligand}$

$\gamma = \text{stability constant of the complex}$

Therefore:

$$(-0.435) = (-0.1375) = \frac{0.059}{2} \log 1 + \frac{0.059}{2} \log 3 \times 10^{-2}$$

$$0.327 = 0.059 \log 3 + 0.059 \log 2 \times 10^{-2}$$

Therefore $\gamma = 5.0$.

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 $5 \times 10^{-2}$ with respect to acid and $3 \times 10^{-2}$ with respect to alkali, 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 Hanotaux 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 Pb/copper system using potentiometric techniques, but they observed a stable 1 : 1 complex by spectrophotometry.

c. Polarography of Pb with Magnesium and Calcium

Polarography was carried out in diethyglycol
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 3.5. At pH 7.0, a horizontal portion occurred which then fell slightly, probably due to hydrolysis. Eventually the graph rose, the rising portion corresponding to the release of 3.06 hydrogen ions per magnesium ion at pH 11, and to 3.54 hydrogen ions at pH 11.5. 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 Pb-calcium system showed that no hydrogen ions were released until pH 5.7. 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 1.3 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. with nickel.

Colorimetry gave no indication of a polarographically active complex, on the addition of nickel to the solutions, but a colour change from yellow to red and precipitation was noted. Potentiometric titrations showed that the ligands were attached to each nickel ion at pH values above 3.5.
Section (11). 4-(2-pyridylazo) resorcinol red

\[
\begin{align*}
\text{N} & \equiv \text{N} - \text{N} - \text{C}_6\text{H}_4 - \text{ONa} \\
\end{align*}
\]

a. Polarography of 4AR

PAM gave one well defined step, the half-wave potential of which is pH dependent. \(E_1\) corresponded to the equation:

\[
E_1 = 0.145 - 0.002 \text{pH}, \text{(versus Ag)}
\]

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 22, Claxton, Folland et al.\(^{66}\) found the following pH values:

\[
\begin{align*}
\text{pH}_1 &= 2.66; \quad \text{pK}_{\text{OH}^1} = 5.48; \quad \text{pK}_{\text{OH}^2} = 12.51
\end{align*}
\]

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

\[
\begin{align*}
\text{N} & \equiv \text{N} - \text{N} - \text{C}_6\text{H}_4 - \text{ONa} \\
\text{N} & \equiv \text{N} - \text{N} - \text{C}_6\text{H}_4 - \text{O}^-
\end{align*}
\]

The step height was constant up to pH 3.5 above which it increased to twice its height in a similar manner to PAM (see fig. 29).

The logarithmic analysis of the polarographic step at pH 7.02 in phosphate buffer, and at pH 11.21 in phosphate buffer gave a value of 6.8 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
$5 \times 10^{-5}$ with respect to PAH.

In unbuffered solution at pH 7.2, using 0.1M potassium
chloride basic electrolyte, the n value was calculated as
0.7 electrons with $5 \times 10^{-4}$ PAH and 0.73 electrons at $2 \times 10^{-5}$
PAH, which suggests that the reaction becomes more reversible
in more dilute solution.

The variation of step height with concentration and
mercury head indicated that the process is diffusion controlled
under these conditions. In the concentration dependence
investigation it was noted that with concentrations of PAH
between $2 \times 10^{-4}$ and $3.2 \times 10^{-5}$ part of the step was
dispersed and was followed by a rounded maximum. The
remaining 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 changed due to the
uptake of hydrogen ions for the reduction of PAH, 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 head, to be diffusion
controlled.

Similarly the step was shown to be diffusion controlled
in buffered systems. In a tris 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 7th and could be avoided by thoroughly degasifying the solutions before heating. The results from a typical polarogram are given below in Table 20:

<table>
<thead>
<tr>
<th>Before heating</th>
<th>After heating to 70° for 10 mins.</th>
<th>After heating for half an hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>h~.~</td>
<td>h~.~</td>
<td>h~.~</td>
</tr>
<tr>
<td>2.94 cc.</td>
<td>2.94 cc.</td>
<td>2.94 cc.</td>
</tr>
<tr>
<td>-0.49v</td>
<td>-0.49v</td>
<td>-0.49v</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:

<table>
<thead>
<tr>
<th>After heating for half an hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>h~.~</td>
</tr>
<tr>
<td>2.42 cc.</td>
</tr>
<tr>
<td>-0.47v</td>
</tr>
</tbody>
</table>

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 compound which are reduced to hydrous compounds in the region of this potential, involving a four electron reaction.

b. With Barium

Polarography of this system was carried out at pH 4.5, 7.6 and 12.27, 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 PAN and aluminium.

c. With Cadmium

The addition of copper solution to $2 \times 10^{-4}$ PAN 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 \(-0.14\), \(-0.31\) and \(-0.45\) 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 \(2 \times 10^{-3}\) 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 mm, compared with about 2 mm 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 12.3 in dimethylglycine hydrochloride/sodium hydroxide buffer the free copper step was shifted from \(-0.19\) to \(-0.33\) volts (versus mercury pool), while the free dye step
was not affected.

Potentiometric titrations with $2 \times 10^{-3}$ M Cu 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 hydrogens released was found to be 1 : 3 per copper ion. Pollard et al.\textsuperscript{123} using a similar technique, found that both 1 : 1 and 1 : 2 complexes were present and reported that several titrations of each system were necessary before satisfactory results could be obtained.

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

c. \textit{Igpa} with \textit{Irga}(III)

The addition of aliquots of iron(II) solution to $2 \times 10^{-3}$ M Cu 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 2,6'-dihydroxyazo dye, although beryllium did give
a less well defined displacement with a o-carboxy a'-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 $3 \times 10^{-5}$ 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 last 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 6.5. This had the effect of stabilising the solutions and a concentration dependence plot was drawn (see fig. 50). As can be seen from fig. 50, the combining ratio is 1 : 3 although above $3 \times 10^{-5}$ 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 6.9 in acid methanol solution the complex step was very small; even with excess iron it did not attain a height
greater than 6-6 cm. Heating the solution to 70° 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 de-aerated 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
2 : 1 was reached. The points from a ratio of 1 : 2 tended
towards the 1 : 1 ratio, however, became more scattered, even when
excess iron(III) solution was added and the dye step was not
completely eliminated. For example, with $2 \times 10^{-4}$ M iron
and $5 \times 10^{-2}$ M the following results were obtained using
the Cambridge polarograph.

\[
\begin{array}{c|c|c|c}
\text{Iron} & \text{Phosphate} & \text{Iron} & \text{Phosphate} \\
0.00 & 0.00 & 1.65 cm. & 0.00, 0.65 & 1.55 cm. (versus \\ & & & & \text{recessed pool}) \\
\end{array}
\]

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. 41).

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. \(1/10\)

\[ \text{pH 8.9} \]

\[ \text{Mx} \times 10^5 \text{ Fe with } 2 \times 10^{-4} \text{ PAR} \]
From the polarographic data at pH 6.6 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. They found a 1 : 3 ratio for cobalt(III) with the dye by spectrophotometric techniques, and a 1 : 2 ratio by potentiometric methods.

c. Polarography of DII with Iron(III) and Manganese

Polarography was carried out at the three pH values previously mentioned without any indication of a second step, even on heating.
It 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.

This 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 3.0. 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 : (1 : 2)H or two pH values with the addition of further molecules of the 3 : 1. This could occur without the loss of a hydrogen ion (or possibly if a hydrogen is lost 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). Calciachrom

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

The solution 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 actua metals colorimetrically.

2. Calciachrom 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 9 hydrolysis of the complex occurred, as shown by a decrease in pH with time, at pH 7 to 8 of the hydrogen atoms of the calciachrome molecule were ionised in the absence of aluminium.

Electrolysis was carried out in acetate buffer at pH 5.02 using 4 x 10^{-4} m calciachrom solution.

At this pH calciachrom gave one well defined step at -0.42 volts (versus hydrogen pool). The addition of aluminium caused a slight displacement in part of the step so that two steps were now present at -0.42 and -0.63 volts (versus...
curry pool), respectively. With $5 \times 10^{-3}$ aluminium solution a well shaped polarogram was obtained, but on increasing the aluminium concentration precipitation occurred. On reducing the ionic strength by lowering the buffer capacity ten fold to $1.5 \times 10^{-3}$ sodium acetate and $0.1 \times 10^{-3}$ acetic acid the problem of precipitation was overcome, but the two steps were not as well defined. An increase in the aluminium concentration in the range $1 \times 10^{-5}$ to $5 \times 10^{-5}$ did not affect step height of the complex, which remained about 1.7 mV, although variations in the dye step between 1.5 and 2.1 mV at sensitivity of the Cambridge polarograph were noted.

It was then decided to vary the aluminium concentration within the range $3 - 1 \times 10^{-3}$. The polarograms however, now showed a large bump similar to a maximum of the second kind, occurring between -0.7 and -1.3 volts. Still appeared whether aluminium was present or not. Peaks 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 bump to disappear.

A half wave plot using the complex step height indicated a containing ratio of about 1:2, although the complex step height still rose slightly (see Fig. 32).

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. 1/20

Mx10^4 Al with 2x10^4 M Calcichrome

Fig. 33

Diffusion current

Applied potential in volts
hydrogen ions are released per aluminium ion, indicates that a complex of 1 type which involves the coordination of only one of the three end groups to the aluminium, together with bonds to 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. Calciinum with Calcium

Electrophoresis was carried out at pH 12.5 in dimethylglycol
hydrochloride/sodium hydroxide buffer and the polariogram of the free calciinum is shown in fig 22. The addition of calcium did not modify the polariogram even on heating.

Electrophoresis at the same pH in piperidine/sodium hydroxide buffer gave the usual type of wave which calcium did not modify. Glass and factor 624 showed spectrophotometrically at
pH 12, that the ratio of calciin to 2-mold residues in the calciin correspond to 1 : 3 i.e., a 1 : 1 complex.

c. Aluminion with other metal ions

In further electrophoresis was carried out, but potentiometric dilutions were performed with iron(III) and copper(II). In both cases two hydrogen ions per metal ion were found to be liberated above pH 5. Below pH 7 two extra hydrogen ions were liberated and after the pH value of the dye, one extra hydrogen ion was liberated.
Section 3.1. Thorin (1.5 monochloro- and chloroethyl 1.5-oxazolidine-bis-carboxylic acid sodium salt)

This 3.1 is a specific spectrophotometric reagent for tonium; hence, as thorium is not easily determined polarographically, and the simple form of the murea is more stable than that with a diazourea, and with the diazourea and other, it was decided to examine the reaction polarographically.

3. Thorin with Thorin

In the conditions for spectrophotometry usually specify acidic solution, polarography was carried out initially at pH 5.2 in acetate buffer.

The addition of thorin to 2 × 10⁻⁴M 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. A smaller step was also found.

The addition of 5 and 10 ml aliquots of 0.001M thorin solution caused a decrease in step height from 2.5 cm to 1.45 cm, and 0.75 cm, respectively.

The investigation was repeated in cacodylic acid buffer at pH 9 where a single well-defined step was obtained for thorin. The half-wave potential occurred at -0.54 volts (versus mercury pool). Once again the addition of 5 and 10 ml aliquots of 0.001M thorin solution caused a decrease in step height.
The reduction in this case was from 2.62 cm. in the absence of thorin, to 2.47 and 1.78 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:

\[ 2 \times 10^{-4} \text{ thorin, } 1 \times 10^{-5} \text{ thorin,} \]

ammoniacal buffer at \( pH \text{ 9.} \)

<table>
<thead>
<tr>
<th>Temperature ( ^\circ C )</th>
<th>Step Height at Sensitivity ( \frac{1}{2} ) Cambridge Polarograph, cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>20</td>
<td>3.35</td>
</tr>
<tr>
<td>45</td>
<td>3.8</td>
</tr>
<tr>
<td>60</td>
<td>4.9</td>
</tr>
</tbody>
</table>

No indication of splitting was observed in any of these.

5. Thorin with aluminium.

More study of this system was carried out at the above pH and also at \( pH \text{ 4.1.} \)

At \( pH \text{ 4.3} \) the addition of aluminium improved the shape of the polarograph; instead of the ill-defined steps only one now occurred although this was of comparable height to the defined heights of the original steps. In fact, some reduction in step size was noted. This reduction was more
marked on heating, i.e., with a loss of 7. Merin. The addition of 1 ml. 0.05% aluminium caused a decrease in step height from 2.5 to 2.3 cm, with a further reduction to 2.1 cm. on heating.

The polarogram of Merin at pH 4.4 was rather complicated, having five steps of various magnitude. The effect of adding 1 ml. 0.05% aluminium is shown in Table 22 below.

<table>
<thead>
<tr>
<th>Temp</th>
<th>b. 0.05</th>
<th>b. 0.1</th>
<th>b. 0.2</th>
<th>b. 0.3</th>
<th>b. 0.4</th>
<th>b. 0.5</th>
<th>b. 0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3</td>
<td>0.24</td>
<td>0.56</td>
<td>0.75</td>
<td>0.5</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>pH</td>
<td>4.1</td>
<td>0.94</td>
<td>0.36</td>
<td>0.5</td>
<td>1.2</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

This polarogram was difficult to record and even if the increase in the step height at -0.5 volts was due to the complex formation it seems to offer no analytical advantages over the polarelectrode method. - Alumin system. Lying in the complicated nature of the polarograms 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 rate step. Complexes of calcium with calcichrome and thorium with thorin are well established but neither of these give any indication of being polarographically active.

The complex formed by aluminium and calcichrome was shown to involve one of the three one group and one of the three hydroxy groups. This is the first time that this type of complication has been reported.

The unusual shape of the calcichrome polarogram at high potential is probably due to a double layer effect.

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

4. The Displacement of the Half-Plane

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 complex 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 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
in produced. Indium exhibits the same phenomenon, due to the formation of a reversible \( \text{InCl}_4^- \) species\( {136, 137} \). 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:

\[
E = \frac{\Delta E}{2} \ln \frac{K_{ox}}{K_{red}}
\]

where \( K_{ox} \) and \( K_{red} \) 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:

\[
E = \frac{\Delta E}{2} \ln \frac{K_1}{K_{\text{red,1}}}
\]

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 polarographed, 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 anazo dye to the corresponding
hydrate compound, the expected displacement of the half-wave potential on forming a 1 : 1 complex is obtained from the following equations as shown:

\[ \text{H}_2\text{O} + \text{e}^- \rightarrow \text{H}_2\text{O}^+ \quad \Delta \bar{G}_0^o = -221^\circ \]

\[ \text{H}_2\text{O} + \text{e}^- \rightarrow \text{H}_2\text{O}^+ \quad \Delta \bar{G}_0^o = -731^\circ \]

\[ \text{H}_2\text{O} + \text{e}^- \rightarrow \text{H}_2\text{O}^+ \quad \Delta \bar{G}_0^o = -950^\circ \]

\[ \Delta \bar{G}_0^o = \Delta \bar{G}_0^o + \Delta \bar{G}_0^o - \Delta \bar{G}_0^o \]

\[ \Delta \bar{G}_0^o = -731^\circ - 731^\circ \]

\[ \Delta \bar{G}_0^o = -1462^\circ \]

\[ \Delta \bar{G}_0^o = -1462^\circ + \frac{1}{2} \ln \frac{K_{\text{H}_2\text{O}^+}}{K_{\text{H}_2\text{O}^+}} \]

\[ \Delta \bar{G}_0^o = -1462^\circ - \frac{1}{2} \ln \frac{K_{\text{H}_2\text{O}^+}}{K_{\text{H}_2\text{O}^+}} \]

Similarly, for the four electron reduction to the amines:

\[ \Delta \bar{G}_0^o = -\frac{1}{2} \ln \frac{K_{\text{H}_2\text{O}^+}}{K_{\text{H}_2\text{O}^+}} \]

Thus, a displacement of the half-wave potential would not be expected in cases where the hydrate or amine complex were of similar stability to one complex. In fact, the one complexes are much more stable \( \text{H}_2\text{O}^+ \)

In practice, however, it appears from these and other studies that a displacement is only obtained for one dyce containing at least one hydroxyl group and another complexing group in the o and o positions. Only rarely would such displacements be noted in the absence of
α,β-dicarbonyl one groupings. These were with Saldahroo-
and aluminium, 34% with iron(III) and an ε-hydroxy ε'-carboxy
dye, with beryllium. Recently however, Mittel and Florence 31
have observed the displacement to a more positive potential
of the anodic wave of alizarin red S with mircenium (IV)
electrode at the pyrolytic graphite. Florence, Miller and Mittel 32
have also determined aluminium using the anodic wave for the
oxidation of its Solochrome Violet BS complex at a rotated
pyrolytic graphite electrode. Mittel and Florence 31 found
that with alizarin red S and mircenium the displacement
of the irreversible 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 32 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 most systems then 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 displaced step observed. In fact, if the polarographic solutions were not heated and cooled prior to polarography the displaced step was not quantitatively formed. The difference in half-wave potential was constant throughout the investigation. Examination of thorium and lanthanum, 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) - malachite green, nickel(II) - malachite green, and iron(III) - malachite green complexes, are of particular interest from the mechanistic point of view. These reductions are similar and we shall take the first case mentioned as typical. The malachite green is completely complexed with iron(III) and 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 as far as the hydrazo 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 Giardello this type of dye is reduced to the hydrazo compound which then dissociates to theazo dye (which is further reduced) and the azo dye (see page 10). In this case, the hydrazo compound must be stabilized by complex formation with the iron(III). The reduction scheme would be
In summary:

\[ \text{N and reduced} \]

At the potential of the second reduction step it is probable that the jacobinene violet $10^{-}$ - iron (III) complex is reduced directly to the unline as seems to be the case usually with $\alpha$,$\beta$-dimethoxy one dye complex.

An alternative explanation of the first step is that the jacobinene violet $10^{-}$ - iron(III) complex is reduced directly to the corresponding hydroxy - 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 to the unline.

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 so 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) - dye complex at $pH$ 4.6, as in this case the complete dye step is displaced. At $pH$ 5.2 the two steps are obtained as stated.
It is known that at pH 7.5, a polarographic analysis indicated the presence of a 1:2 complex whereas at pH 4.0, a more usual 1:1 complex was obtained. Potentiometric titrations showed that the hydrogen ions are released for iron(III) ions. From these observations the complex at pH 0.5 is probably of the type Fe(II)CO. In acidic solution 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 first 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 FeII 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 an 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 Cu(II) ions and zinc 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 as only one third of the dye step is displaced with excess.
considerable excess of aluminium slightly above 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 groups are liberated on complex formation.

In the case of a dye with an azo and a nitro group (Cordenat 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, and with other dyes incorporating a nitro group was not conclusive.

b. 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\textsuperscript{55} and Poem and Bryan\textsuperscript{53} reported that aluminium formed a 1 : 3 complex with Caledonchrome Violet 25 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 Iglisch\textsuperscript{52} that Jenkins and Reynolds\textsuperscript{55} did not allow for the purity 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, there 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 Costes and King.

Similar investigations of the nickel(II) and iron(III) systems with salicyluronic violet 3B 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 gave a 1:1 complex at pH 4.5. Palmer and Reynolds reported 1:2 and 1:4 complexes for iron(III) at these pH values and a 1:2 complex with nickel(II) at pH 6.5. Rees 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 ammoniacal buffer and a 1:3 complex in pyridine 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 Rees and Bryan work may be due to the fact that nickel, like iron, never fully displaces 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, Hollard et al.

186.
Other o-carboxy o'-hydroxy dyes 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 could have considerable analytical importance, as no good polarographic method exists for the determination of beryllium. Alumina has 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 alumina. Alumina may, of course, be determined in the presence of beryllium by means of Sulphonon Violet 54.

Further work on the calcium-beryllous on Chrome Salicyl 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 2,6-dihydroxy 3,5,5'-tetrachloro diphenyl sulphide, in which the carboxy group is replaced by a sulphide group. No displacement of the step was observed, although the sulphur atom is undoubtedly capable of co-ordination.
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<th>Page(s)</th>
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