The analytical chemistry of o,o'-dihydroxydiphenyl sulphides and methanes

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THE ANALYTICAL CHEMISTRY OF

o,o'-DIHYDROXYDIPHENYL SULPHIDES

AND METHANES

by

A. GRAY B.Tech.

SUPERVISORS:

Dr. D. Thorburn Barnes
Dr. A.G. Fogg

Submitted for the Degree of Doctor of Philosophy of
Loughborough University of Technology, September 1970.
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4-1</td>
<td>Practical Details of Stability Constant Determinations and Other</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>4-2</td>
<td>Verification of the Composition of Complexes by the Method of Continuous Variations</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>5-1</td>
<td>Results and Discussion of Stability Constant Determinations and Metal Complex Formations of o,o'-Dihydroxydiphenyl Sulphides and Methanes</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>5-2</td>
<td>Ligand-proton Stability Constants</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metal-ligand Stability Constants</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>6-1</td>
<td>Solvent Extraction and Spectrophotometric Determination of Iron(III) with Ethionol</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>6-2</td>
<td>Brief Review of Solvent Extraction and Spectrophotometric Methods of Determining Iron</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>6-3</td>
<td>Spectrophotometric Determination of Iron(III)</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>6-4</td>
<td>Solvent Extraction Procedure for the Spectrophotometric Determination of Iron(III)</td>
<td>63</td>
</tr>
</tbody>
</table>
6-5 Discussion

Chapter 7 Determination of Mercury(II) with Bithionol
    7-1 Attempted Gravimetric Determination
    7-2 Brief Review of Gravimetric Methods
    7-2 Titrmetric Determination

PART TWO
INVESTIGATION OF METHODS OF SEPARATING AND DETERMINING o,o'-DIHYDROXYDI-PHENYL SULPHIDES AND METHANES

Chapter 8 A Review of Existing Methods
    8-1 Spectroscopic Methods
    8-2 Chromatographic and Ion-Exchange Methods
    8-3 Electrical Methods
    8-4 Methods Based on the Determination of Chlorine

Chapter 9 The Use of Acid Dissociation Constants in Selecting Buffers to Optimise the Electrophoretic Separation of Bithionol, Fenticlor, Hexachlorophene, Bromchlo-Phene, Dichlorophene, Tetrachlorophene, and 4-Hexylresorcinol
    9-1 Introduction
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-2</td>
<td>Determination of Acid Dissociation Constants</td>
<td>110</td>
</tr>
<tr>
<td>9-3</td>
<td>Electrophoresis</td>
<td>113</td>
</tr>
<tr>
<td>9-4</td>
<td>Results and Discussion</td>
<td>114</td>
</tr>
<tr>
<td><strong>Chapter 10</strong></td>
<td>Photometric Studies on the Determination of Bithionol with Iron(III)</td>
<td>120</td>
</tr>
<tr>
<td>10-2</td>
<td>Attempted Colorimetric Determination</td>
<td>120</td>
</tr>
<tr>
<td>10-3</td>
<td>Photometric Titration</td>
<td>124</td>
</tr>
<tr>
<td><strong>Chapter 11</strong></td>
<td>Anodic Polarography of Bithionol in which a Mercury(II)-bithionol Precipitate is Formed at the Dropping Mercury Electrode</td>
<td>127</td>
</tr>
<tr>
<td><strong>Chapter 12</strong></td>
<td>General Discussion and Suggestions for the Extension of this Study</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>Appendix - Computer Programs</td>
<td>1</td>
</tr>
</tbody>
</table>
Acknowledgements

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The author wishes to thank Dr. H. Thun for making available a copy of his computer program and Dr. W. J. Eilbeck for much helpful discussion.

Finally, the author wishes to thank his wife for encouragement during this study, and also for typing this thesis.
The formation of a number of metal complexes of the o,o'-dihydroxydiphenyl methane and sulphides, hexachlorophene, bithionol and fenticlor have been investigated, and their stability constants determined in 3+1 v-v ethanol-water solution at ionic strength one. The proton-ligand stability constants of dichlorophene, bromchlorophene and tetrachlorophene have also been determined under these conditions. A solvent extraction procedure has been developed for the colorimetric determination of iron(III) with bithionol as reagent. The use of bithionol as a gravimetric reagent for mercury(II) has been investigated, but the reproducibility of recoveries obtained for similar samples was generally poor.

Methods of separating and determining o,o'-dihydroxydiphenyl sulphides and methanes have been investigated. Acid-base titration data and acid dissociation constants have been used to optimise the selection of buffer solutions in which to effect electrophoretic separation of hexachlorophene, bithionol, bromchlorophene, tetrachlorophene, fenticlor, dichlorophene and 4-hexylresorcinol. The use of iron(III) as a colorimetric reagent for bithionol has been studied, but was found to give low and un reproducible results. The formation of the mercury(II)-bithionol precipitate at the dropping mercury electrode during anodic polarography has been investigated in an attempt to develop a method of determining bithionol based on
on this reaction, but no specific wave which could be attributed to bithionol, was observed.
CHAPTER ONE

GENERAL INTRODUCTION

\(\text{o,o'}-\text{Dihydroxydiphenyl sulphones and methanes possess strong bacteriostatic and fungistatic properties, and for this reason they are important pharmaceutical compounds. Hexachlorophene is particularly active and has found widespread use, for example, in medicated soaps, shampoos and toothpastes. Other compounds including bithionol, fenticlor and dichlorophene are available commercially for use as bacteriostats, fungicides, spermicides and anthelmintics. These compounds are also used as antioxidants and stabilizers in polymers, lubricants, rubbers, motor fuels and other organic materials, and have been used as curing agents in the vulcanization of rubbers.}\)

The structures of some of the more important compounds of this type are shown in Table 1, together with their generic and structural names.

**Table 1**

The Structure and Nomenclature of \(\text{o,o'}-\text{Dihydroxydiphenyl Sulphones and Methanes}\)

-\(\text{R}_1\) OH \(\text{HO}\) \(\text{R}_1\)
-\(\text{Cl} \quad \text{X} \quad \text{Cl}\)
-\(\text{R}_2 \quad \text{R}_2\)
<table>
<thead>
<tr>
<th>Generic name and structure</th>
<th>Name based on structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorophene X=CH₂, R₁, R₂=H</td>
<td>2,2'-Methylenebis(4-chlorophenol)</td>
</tr>
<tr>
<td></td>
<td>Bis(5-chloro-2-hydroxyphenyl)methane</td>
</tr>
<tr>
<td></td>
<td>2,2'-Dihydroxy-5,5'-dichlorodiphenyl methane</td>
</tr>
<tr>
<td>Tetrachlorophene X=CH₂, R₁=Cl</td>
<td>2,2'-Methylenebis(4,6-dichlorophenol)</td>
</tr>
<tr>
<td>R₂=H</td>
<td>Bis(3,5-dichloro-2-hydroxyphenyl)methane</td>
</tr>
<tr>
<td></td>
<td>2,2'-Dihydroxy-3,5,5'-tetrachlorodiphenyl methane</td>
</tr>
<tr>
<td>Bromchlorophene X=CH₂, R₁=Br</td>
<td>2,2'-Methylenebis(6-bromo-4-chlorophenol)</td>
</tr>
<tr>
<td>R₂=H</td>
<td>Bis(3-bromo-5-chloro-2-hydroxyphenyl)methane</td>
</tr>
<tr>
<td></td>
<td>2,2'-Dihydroxy-3,3',5'-dibromo-5,5'-dichlorodiphenyl methane</td>
</tr>
<tr>
<td>Hexachlorophene X=CH₂, R₁,R₂=Cl</td>
<td>2,2'-Methylenebis(3,4,6-trichlorophenol)</td>
</tr>
<tr>
<td></td>
<td>Bis(3,5,6-trichloro-2-hydroxyphenyl)methane</td>
</tr>
<tr>
<td></td>
<td>2,2'-Dihydroxy-3,5,6,3',5',6'-hexachlorodiphenyl methane</td>
</tr>
</tbody>
</table>
Halogenated o,o'-thiobis and methylenebis phenols are colourless, crystalline, non-volatile, and stable, usually melting in the range 100° to 200°c. They are practically odourless when pure. Their aqueous solubility is very low (0.0004% for bithionol at 25°C) but they are soluble in aqueous alkali solution, alcohols, glycols, ketones, esters and ethers; slightly soluble in aromatic hydrocarbons; and practically insoluble in petroleum solvents. Like other phenols they darken on exposure to bright light, especially in alkaline media.

Bechhold and Ehrilich first demonstrated the antibacterial action of bis-phenols in 1906. The linkage of two phenol molecules, either directly or by means of an alkylene-group, greatly increases their antibacterial potency. Halogenated bis-phenols were prepared in
Germany by I.G. Farbenindustrie A.G. (1927), and Weiler, Wark and Stotter (1.29), and were patented as mothproofing agents. Wesenberg and Nuth also patented compounds with sulphur (1932) and oxygen linkages (1936) as disinfecting and preserving agents. In the U.S.A., Dunning et al. (1931) and Nones et al. (1932) studied the antibacterial action of many thiobis-phenols. Harden and co-workers (1932 and 1937) continued this work with alkalenebis-phenols.

Although the bacteriostatic and fungistatic activity of these compounds had been noted previously, they were not used commercially until the early 1940's. Dichlorophene was used primarily as a fungicide and hexachlorophene was used for the preparation of antiseptic soaps. This usage stimulated work on similar compounds and many halogenated phenols, linked directly or with alkylene or sulphur bridges, were recommended as ingredients for anti-septic soaps. These compounds now have wide pharmaceutical application.

In general one or more halogen atoms (preferably chlorine) must be present in each benzene ring for the compounds to be pharmaceutically active, and in addition, one of these halogen atoms must be para to the hydroxyl group. The mechanism of the pharmaceutical activity of certain drugs is known to involve complexation of metal ions; either the metal complex formed in vitro is potent to the infecting organism or the organism is deprived of essential trace metals by complexation. Adams made a
A semi-quantitative study of metal complexes of bithionol and hexachlorophene potentiometrically and colorimetrically as a means of demonstrating that their pharmaceutical action could involve complexation of trace metals.

Adams' work indicated some degree of complex formation between bithionol and iron(II), iron(III), copper(II), cobalt(II) and manganese(II), and between hexachlorophene and iron(II), iron(III) and copper(II). With this exception, very little work had been published on the metal complex formation of o,o'-dihydroxydiphenyl sulphides and methanes. These compounds are possible chelating agents, and as such their chelating properties are of interest analytically and pharmaceutically.

The present author was primarily interested in these compounds from the analytical viewpoint, and investigated the metal complex formation and utilisation of o,o'-dihydroxydiphenyl sulphides and methanes as reagents for metals. The widespread pharmaceutical use of o,o'-dihydroxydiphenyl sulphides and methanes has necessitated the development of methods of determining these compounds. The majority of methods in present use, however, suffer from the disadvantage that related compounds cause interference, and so improved methods of separating and determining these compounds have also been investigated.

As part of this investigation the proton-ligand and ligand-metal formation curves and stability constants of bithionol, fenticlor, hexachlorophene, and several of their metal complexes have been determined potentiometrically. A procedure for the determination of iron(III) with
bithionol has been developed, and the gravimetric
determination of mercury(II) with bithionol has been
investigated. Methods of separating and determining o,o'-
dihydroxydiphenyl sulphides and methanes have been re-
viewed. Acid dissociation constants were used to optimise
the selection of buffers for the electrophoretic separation
of hexachlorophene, bithionol, bromchlorophene, tetrachlor-
ophene, fentilol, dichlorophene and 4-hexylresorcinol.
The anodic polarography of bithionol in which a mercury(II)-
bithionol precipitate is formed at the dropping mercury
electrode was investigated, and the application of the
iron(III) bithionol reaction to the determination of bith-
ionol was examined.
PART ONE

A STUDY OF THE METAL COMPLEXES FORMED BY o,o'-DIHYDROXY-DIPHENYL SULPHIDES AND METHANES, AND THE UTILISATION OF THESE COMPOUNDS AS ANALYTICAL REAGENTS FOR METALS

CHAPTER TWO

INTRODUCTION

As stated previously, Adams observed that bithionol, and hexachlorophene formed complexes with many metals. Patents have been filed on the use of metal complexes of bithionol, hexachlorophene and dichlorophene as pharmaceutical compounds, but very little formation data is given. The present author has investigated the complex formation of the o,o'-dihydroxydiphenyl sulphides and methane, bithionol, fenticlor and hexachlorophene, by determining the formation curves and stability constants of some of their metal complexes. Acid association constants of dichlorophene, tetrachlorophene, bromochlorophene and 4-hexylresorcinol have also been determined. The latter compound is included as a comparison compound for the later electrophoresis study, chapter nine. The utilisation of these compounds as analytical reagents for metals was then investigated, in particular the determination of iron(III) and mercury(II) with bithionol has been studied. The thio compounds are of particular interest being potential trichelating ligands, in contrast to the methylene compounds which are potentially only
dichelating ligands. For this reason the thio compounds have been investigated in much greater detail.

Very little information is available on trichelating ligands similar to o,o'-dihydroxydiphenyl sulphides and dichelating ligands similar to o,o'-dihydroxydiphenyl methanes, from which an indication of their chelating properties could be inferred. Peri-diphenols form strong complexes with many metals. The stability of chelates formed through phenolic hydroxyl groups usually agrees with the schematic order, o- and peri-diphenols > o-phenol carboxylic acids > phenols with isolated hydroxyl groups. Chromotropic acid especially forms very strong complexes with iron(III), having an overall stability constant for the 1:2 complex of about 33-34, depending on solution conditions. These compounds give some idea of the strength of the double phenolic group, but are not directly comparable with the thio and methylene compounds. When peri-diphenols chelate a six membered ring is formed, whereas methylene compounds would form less stable eight membered rings.

O,o'-Dihydroxydiphenyl sulphides are more closely related to o,o'-dihydroxy azo dyes than peri-diphenols. The azo compounds chelate with many metals, with the azo compound reacting as a trichelating ligand as shown.
This structure is stabilized by resonance, with strain free five and six membered rings being formed.

Suzuki and Yamasaki have worked with aliphatic compounds similar to o,o'-dihydroxydiphenyl sulphides and methanes. The stability constants of several metal complexes of glutaric, diglycolic, and thiodiglycolic acids were determined. The general level of stability of the chelates formed with these compounds is low, but this is probably owing to the fact that the compounds are aliphatic. The stability of the chelates formed with the two ligands containing oxygen and sulphur is significantly higher than those of glutaric acid. This indicates an additional chelate ring is formed in the chelates of these two acids, and that coordination of the oxygen or sulphur takes place.

Perrin states that where multidentate chelates are
formed in which ligand atoms serve as links between different portions of the complexing agent, the preferred ligand atoms are nitrogen and phosphorus. Oxygen and sulphur are very weakly basic and have only a weak tendency to coordinate. One way of achieving increased selectivity, however, would be to design ligands in which the coordinating atoms are phosphorus, sulphur, selinium or arsenic, so that stronger complexing and greater differences in stability constants would be expected among transition metal ions in their lower valency states and among metal ions with filled d\textsuperscript{10} shells. Many such compounds are unstable. Jørgensen\textsuperscript{11} states that sulphur containing ligands would be expected to bind strongly to Ahrland, Chatt and Davies' type B central ions, viz. copper(I), silver(I), gold(I) and mercury(II), which are d\textsuperscript{10} systems. Sidgwick\textsuperscript{12} observed that the effect of substitution in aliphatic sulphur compounds is opposite to that for oxygen and nitrogen compounds, R\textsubscript{2}S\textsuperscript{2}\rightarrow RSH\rightarrow H\textsubscript{2}S, that is the greater the substitution, the greater the donor power of the sulphur atom. This view, however, is not supported by Jørgensen\textsuperscript{11}.

Thus while peri-diphenols and o,o'-dihydroxy azo compounds chelate strongly with many metals, o,o'-dihydroxydiphenyl sulphides would be expected to form slightly less stable chelates. The formation of two five membered rings containing a sulphur atom should enhance the stability of the sulphide chelates. The phenolic oxygen-metal bond is stronger than any sulphur-metal bond in these compounds, and as such it dominates the selectivity.
of the ligands, but the sulphur atom should contribute to some extent.

The methylene group in o,o'-dihydroxydiphenyl methanes will not participate in any metal bonding, so that if these compounds act as bidentate ligands an eight membered ring would be formed. The formation of such a ring would be difficult, but considering the high stability of the peri-diphenol chelates, this may be possible, especially in 3+1 v-v ethanol-water solution, which was used as solvent in the present work.

2-1 Previous Determination of Acid Dissociation Constants, $K_A$, and $K_{A_2}$ of o,o'-Dihydroxydiphenyl Sulphides and Methanes for Pharmaceutical Use

The pharmaceutical importance of o,o'-dihydroxydiphenyl sulphides and methanes has necessitated the determination of the $K_A$ values of many of these compounds. The published values are given as $pK_A$ i.e. $-\log K_A$ values in Table 2.

Unfortunately these figures cannot be used in the present work, because the solvents used differ from each other and from the solvent used in the present work. Further, the ionic strengths of the solutions used are not the same, and only practical constants appear to have been determined. The accuracy of some of these constants is difficult to assess. Thus only the general trend and not the actual figures can be compared with those determined in the present work.


### TABLE 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\text{PK}_A^1$</th>
<th>$\text{PK}_A^2$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorophene$^{13}$</td>
<td>7.6</td>
<td>11.5</td>
<td>Water</td>
</tr>
<tr>
<td>Tetrachlorophene$^{13}$</td>
<td>5.6</td>
<td>10.65</td>
<td>Water</td>
</tr>
<tr>
<td>Hexachlorophene$^{13}$</td>
<td>-</td>
<td>10.1</td>
<td>Water</td>
</tr>
<tr>
<td>Hexachlorophene$^{13}$</td>
<td>5.4</td>
<td>10.85</td>
<td>3+7 v-v ethanol-water solution</td>
</tr>
<tr>
<td>Hexachlorophene$^{14}$</td>
<td>5.56</td>
<td>-</td>
<td>4+1 v-v dimethylformamide-water solution</td>
</tr>
<tr>
<td>Bithionol$^1$</td>
<td>4.82</td>
<td>10.50</td>
<td>methanol-water mixtures</td>
</tr>
</tbody>
</table>
CHAPTER THREE

THEORETICAL CONSIDERATIONS OF STABILITY CONSTANT CALCULATION

3.1 Introduction

Most complex systems reach equilibrium rapidly at ordinary temperatures and can be studied by equilibrium methods. These methods include the determination of concentration variables, of colligative properties, and of other physical properties, such as absorption spectra, or conductivity.

o,o'-Dihydroxydiphenyl sulphides and methanes are dibasic weak acids. Consequently, complex formation depends on competition between metal ions and protons for the ligand, and so it was convenient to determine the proton-ligand and metal-ligand stability constants from pH potentiometric titration data using the Irving and Rossotti procedure. This procedure is based on that used by Calvin and Wilson.

The method involves pH potentiometric titration of these solutions with standard sodium hydroxide solution,

1. a solution containing a small amount of acid and the ionic background,
2. a solution containing the same concentrations of constituents with the addition of a certain concentration of ligand, and
3. a solution containing the same concentration of constituents as solution 2, with the addition of a certain
concentration of metal ions, such that the ratio of ligand to metal concentration exceeds five to one. A series of values of $V_1$, $V_2$, and $V_3$, corresponding to the volumes of $N$ molar sodium hydroxide solution giving the same pH meter reading in the above three titrations are noted. The initial volume, $V_0$, the initial molarity of strong acid, $E_0$, the initial total concentration of ligand, $A_0$, and the initial total concentration of metal ions, $M_0$, are also noted. The proton-ligand and metal-ligand stability constants are then calculated from this data using the equations which will be derived later.

The small amount of acid is added to the above solutions in order to lower the pH and enable the low pH region to be examined. Also if only solutions 2 and 3 were titrated, large errors would arise at low pH levels through small errors in the measurement of hydrogen ion concentration. In order to reduce these errors, solution 1 which contains the same concentration of acid in the same matrix as solutions 2 and 3, is also titrated.

3-2 Basic Principles

The stepwise formation of complexes up to a maximum of $MA_n$ in solution, can be expressed as,\textsuperscript{13}

\[
\begin{align*}
M + A & \rightleftharpoons MA \\
MA + A & \rightleftharpoons MA_2 \\
& \quad \vdots \\
MA_{n-1} + A & \rightleftharpoons MA_n
\end{align*}
\]

with charges omitted for ease of representation. At a given temperature, the activity of the species $MA_n$ (repre-
sented as \( \{MA_n\} \) can be related to the activities of \( M \) and \( A \) thus\(^5\),

\[
\{MA_n\} = T_{Bn} \{M\} \{A\}^n = T_{Kn} \{MA_{n-1}\} \{A\}
\]

\( T_{Bn} \) is the overall stability constant, and the step stability constant, \( T_{Kn} \), is the activity or thermodynamic constant of the reaction step \( MA_{n-1} + A \leftrightarrow MA_n \). The overall and step stability constants are related by the expression

\[
T_{Bn} = T_{K1} \cdot T_{K2} \cdot \cdots \cdot T_{Kn}.
\]

In a solution of constant ionic strength, the activity coefficient \( \gamma \) of each species is constant, and the stoichiometric stability constants can be defined in terms of equilibrium concentrations, \( K_n \) or \( B_n \):

\[
i.e. \quad K_n = \frac{[MA_n]}{[MA_{n-1}][A]} = T_{Kn} \left( \frac{\gamma MA_{n-1} \cdot \gamma A}{\gamma MA_n} \right)
\]

\[
and \quad B_n = \frac{[MA_n]}{[M][A]^n} = T_{Bn} \left( \frac{\gamma M \cdot \gamma A^n}{\gamma MA_n} \right)
\]

In all the stability constant determinations described here, the ionic strength of the solutions was kept constant by the addition of a fixed large excess of electrolyte. The ionic background chosen in a particular case should not affect the measured physical property either by complex formation with the species under investigation or by formation of insoluble salts. In the present work the ionic background was one molar (i.e. ionic strength one) in sodium perchlorate.
3-3 Ligand-proton (Acid Association) Stability Constants, $K_{H_1}$ and $K_{H_2}$

$\text{o,o'\text{- Dihydroxydiphenyl sulphones and methanes}}$

associate with protons in the stepwise manner,

\[
\begin{align*}
\text{\text{O-}} & \quad \text{X} & \quad \text{O-} \\
\text{HO} & \quad \text{X} & \quad \text{HO} \\
\text{OH} & \quad \text{X} & \quad \text{OH}
\end{align*}
\]

which may be represented as,

\[
\begin{align*}
A + H & \rightleftharpoons HA \\
& \\
HA + H & \rightleftharpoons H_2A
\end{align*}
\]

\[
\begin{align*}
K_{H_1} & = \frac{1}{K_{A_2}} = \frac{[HA]}{[A][H]} \\
K_{H_2} & = \frac{1}{K_{A_1}} = \frac{[H_2A]}{[HA][H]}
\end{align*}
\]

$K_{A_1}$ and $K_{A_2}$ are the respective dissociation constants.

By definition $B_{H_1} = K_{H_1}$ and $B_{H_2} = K_{H_1} \cdot K_{H_2}$. The formation function of a protonated ligand species, $\bar{n}_H$, is defined as being the average number of hydrogen ions bound per ligand molecule.

\[
\bar{n}_H = \frac{[\text{protons bound to ligand}]}{[\text{total ligand}]}
\]

In this case,

\[
\begin{align*}
\bar{n}_H & = \frac{B_{H_1} \cdot [H] + 2 \cdot B_{H_2} \cdot [H]^2}{1 + B_{H_1} \cdot [H] + B_{H_2} \cdot [H]^2} \\
& = \frac{K_{H_1} \cdot [H] + 2 \cdot K_{H_1} \cdot K_{H_2} \cdot [H]^2}{1 + K_{H_1} \cdot [H] + K_{H_1} \cdot K_{H_2} \cdot [H]^2}
\end{align*}
\]
A plot of $n_H$ against $\log[H]$ is referred to as the proton-ligand formation curve.

For the ligands studied in the present work, the values of $K_{H_1}$ and $K_{H_2}$ are sufficiently different to assume that in solutions with $n_H$ between zero and one only $A$ and $HA$ are present, and in solutions with $n_H$ between one and two only $HA$ and $H_2A$ are present. Thus when $0 < n_H < 1$,

$$n_H = \frac{[HA]}{[H] + [HA]} = \frac{K_{H_1} \cdot [H]}{1 + K_{H_1} \cdot [H]}$$

and, therefore, $\log K_{H_1} = pH + \log \left( \frac{n_H}{1 - n_H} \right)$

When $1 < n_H < 2$,

$$n_H = \frac{[HA] + 2[HA]}{[HA] + [H_2A]} = \frac{K_{H_1} \cdot [H] + 2K_{H_2} \cdot K_{H_1} \cdot [H]^2}{K_{H_1} \cdot [H] + K_{H_1} \cdot K_{H_2} \cdot [H]^2}$$

and, therefore, $\log K_{H_2} = pH + \log \left( \frac{n_H - 1}{2 - n_H} \right)$

These two equations enable $\log K_{H_1}$ and $\log K_{H_2}$ to be calculated from values of $n_H$ and $pH$. The $pH$ is measured during the titrations, and it is possible to calculate $n_H$, at a particular $pH$, directly from an equation derived by Irving and Rossotti, for a dibasic weak acid,

$$n_H = 2 - \frac{(V_o - V_1)(N + E_o)}{(V_o + V_1) \cdot A_o}$$

A computer program based on the equations derived...
above was written in Fortran 4 for use with an I.C.T. 1905 computer, for the calculation of $K_{H_1}$ and $K_{H^2}$. A copy of the program is given in Appendix A. In practice values for $K_{H_1}$ are only determined over $0.2 < \bar{n}_H < 0.8$, and values for $K_{H^2}$ are only determined over $1.2 < \bar{n}_H < 1.3$, because values of $K_{H_1}$ and $K_{H^2}$ calculated from data outside these $\bar{n}_H$ ranges tend to be inaccurate. This program also provides data from which proton-ligand formation curves can be plotted.

3-4 Effect of Non-aqueous Solvents and Ionic Background on Measured pH. (pH meter reading)

In aqueous solution it can be assumed that pH meter reading equals the minus log hydrogen ion activity, once the pH meter and electrodes have been standardised with buffer solutions of known hydrogen ion activities. In partly non-aqueous solutions, such buffer solutions are not defined. Van Uitert and Hass have suggested a general relationship between hydrogen ion activity and pH meter reading, which also applies to partly non-aqueous solutions, 

$$-\log \left( \bar{H} \right) = \text{pH meter reading} + \log(\text{activity coefficient}) + \log(\text{constant}).$$

The ionic strength of the solution also has considerable effect on measured pH, and has to be taken into account.

In the work described here, the pH meter was standardised against two aqueous buffer solutions before each titration and checked again after each titration. Also the pH meter readings were calibrated against stoichiometric pH (minus log hydrogen ion concentration) by acid-
base titration in the solution matrix used for the stability constant determinations. 

A linear relationship resulted which differed slightly from the general relationship given above,

\[ \text{stoichiometric } \text{pH} = (\text{pH meter reading } \times 0.94) + 0.03. \]

The difference is the small constant 0.03. This could be due to inaccuracy of measurement, but as a large number of readings were taken, it is probably caused by a slight electrode deviation. This factor is very small and affects the value of stoichiometric pH to less than 1%.

By converting pH meter readings to stoichiometric pH, all the constants produced are stoichiometric rather than practical constants. Irving and Rossotti have shown that this is only important in the calculation of proton-ligand stability constants. In the calculation of metal-ligand stability constants, values of \( B_{H1} \) and \( B_{H2} \) are multiplied by hydrogen ion concentration, and so the non-stoichiometry cancels out, provided that there is either consistent use of practical units or consistent use of stoichiometric units. For the present work stoichiometric units have been used, unless otherwise stated.

### 3-5 Metal-ligand Stability Constants

As stated previously, the stepwise formation of complexes up to a maximum of \( MA_n \) can be characterized by the overall and step stability constants, \( B_n \) and \( K_n \) respectively. The total concentrations \( M \) and \( A \) of the metal and ligand are given by,
\[ M = [M] + [MA] + [MA_2] + \ldots + [MA_n] = [M] \sum_{i=0}^{n} B_n \cdot [A]^n \]
\[ A = [A] + [MA] + 2[MA_2] + \ldots + n \cdot [MA_n] = [A] + [M] \sum_{i=1}^{n} n \cdot B_n \cdot [A]^n \]

where \([M]\) and \([A]\) are the corresponding free concentrations of metal and ligand.

When both these free concentrations can be determined, the stability constants can be calculated directly. When only one of these terms is determined, as in the case of pH potentiometric titrations, it is convenient to make use of a secondary concentration variable, such as \(\bar{n}\) or \(\phi\) and also the previously used term \(\bar{M}\). \(\bar{n}\) is the formation function of the metal complexes, and \(\phi\) is the degree of complex formation.

\[
\bar{n} = \frac{A - [A]}{[M] + [MA] + [MA_2] + \ldots} = \frac{\sum_{i=1}^{n} n \cdot B_n \cdot [A]^n}{\sum_{i=0}^{n} B_n \cdot [A]^n}
\]

\[
\phi = \frac{M}{[M]} = \frac{[M] + [MA] + [MA_2] + \ldots}{[M]} = 1 + \sum_{i=1}^{n} B_n \cdot [A]^n
\]

Also it is possible to relate \(\phi\) and \(\bar{n}\),

\[
\frac{\text{d}\phi}{\text{d}[A]} = B_1 + 2 \cdot B_2 \cdot [A] + 3 \cdot B_3 \cdot [A]^2 + \ldots
\]

\[
\text{thus } \bar{n} = \frac{\text{d}\phi}{\phi} \cdot [A]
\]

-20-
and \( \ln \phi = \bar{n} \cdot d \ln[A] + \text{constant} \)

Irving and Rossotti also derived equations which can be used with their titration procedure for the calculation of \( \bar{n} \) at any particular pH. When \( B_{H^+} \) and \( B_{H^2} \) have been determined, the relevant \( p[A] \) value can also be calculated.

\[
\bar{n} = \frac{(V_0 - V_\phi) [N + E_0 + A_0 \cdot (2 - \bar{n}_H)]}{(V_0 + V_\phi) \cdot \bar{n}_H \cdot M_0}
\]

and \( p[A] = -\log[A] \)

\[
= \log \frac{(V_0 + V_\phi) \left[B_{H^+} \cdot 10^{-pH} + B_{H^2} \cdot (10^{-pH})^2 \right]}{V_0 \cdot (A_0 - \bar{n}_H \cdot M_0)}
\]

The plot of \( \bar{n} \) against \( \log[A] \) is referred to as the metal-ligand formation curve.

\section*{3-6. Calculation of Stability Constants}

The calculation of the proton-ligand stability constants has already been discussed; the method used is reasonably simple and gives accurate results. A similar procedure cannot be used for the calculation of metal-ligand stability constants, because the difference in these stability constants, \( K_1 \) and \( K_2 \), is too small in many cases, so that the species \( M, MA \) and \( MA_2 \) can occur in solution together.

Graphical and simple approximation methods have been surveyed\textsuperscript{18, 32}. In general these tend to suffer some of the following disadvantages, (a) often full use
is not made of the available experimental data,
(b) subjective smoothing occurs in graphical procedures,
and (c) tedious calculations are necessary in certain cases. In this section, only those methods which have been applied to the systems investigated in this thesis will be discussed.

The basic assumptions made in the present work were that each o,o'-dihydroxydiphenyl sulphide or methane ligand molecule loses two protons per metal ion on complexation, and that only 1:1 and 1:2 metal-ligand complexes are formed. The volumes of sodium hydroxide consumed, after formation of the singly charged ligand ion, was equivalent to the ionisation of one proton per metal ion for a 1:1 complex, and was equivalent to the ionisation of two protons per metal ion for a 1:2 complex. Also the formation curves indicated no metal-ligand complex formation of a higher order than 1:2. Thus the theory associated with the calculation of $K_1$ and $K_2$ will be discussed in this section.

(a) Interpolation at Half $n$ Values

Approximate values for $K_1$ and $K_2$ can be obtained from a simple approximation introduced by Bjerrum. The equation for the degree of formation of the metal complexes, $\bar{n}$, can be written in the form,

$$\bar{n} = \frac{K_1[A] + 2\cdot K_1\cdot K_2\cdot[A]^2}{1 + K_1[A] + K_1\cdot K_2\cdot[A]^2}$$

ie. $$\bar{n} + (\bar{n} - 1)\cdot K_1[A] + (\bar{n} - 2)\cdot K_1\cdot K_2\cdot[A]^2 = 0$$
$K_1 = \frac{1}{[A]} \cdot \frac{n}{(1-n) + (2-n) \cdot K_1 \cdot [A]}$

$K_2 = \frac{1}{[A]} \cdot \frac{n + (n-1) \cdot K_1 \cdot [A]}{(2-n) \cdot K_1 \cdot [A]}$

and $\log K_1 = pA_{\nu_2} + \log[2/ (1 + \sqrt{1 + 12K_2/K_1})]$

$\log K_2 = pA_{\nu_2} - \log[2/ (1 + \sqrt{1 + 12K_2/K_1})]$

where $pA_n$ represents $-\log[A]$ when $n = n$. When $K_{n-1} \gg K_n$, approximately equal amounts of $M_{A_{n-1}}$ and $M_{A_n}$ will be present at $n = n-\nu_2$ and other species can be neglected. Thus,

$\log K_n = pA_{n-\nu_2}$

This method may only be used when $K_1/K_2 \gg 10^{\nu_2}$, otherwise large errors can result, and only a small section of the available data is used in the approximation.22

The constants obtained from this approximation have been used as a preliminary check on the constants computed by the other methods used, and for the selection of buffer solutions for electrophoresis of o,o'-dihydroxydiphenyl sulphides and methanes, chapter nine, where only approximate values were required.

(b) Least-squares treatment

The equation,

$\bar{n} + (\bar{n} - 1) \cdot K_1 \cdot [A] + (\bar{n} - 2) \cdot K_1 \cdot K_2 \cdot [A]^2 = 0$

can be rewritten in the form,

$\frac{\bar{n}}{(n-1) \cdot [A]} = \frac{(2-n) \cdot [A]}{(n-1)} \cdot K_1 \cdot K_2 - K_1$

-23-
This is an equation of a straight line and corresponds to the equation,
\[ y = x \cdot a + b \]
where \( y \) and \( x \) are variables, and \( a \) and \( b \) are constants.
The stability constants are difficult to evaluate by graphical methods because the values of \([A]\) often vary by several powers of ten, and so the method of least-squares approximation is often used with the aid of a computer. A disadvantage of this method is that in the region \( 0.95 < \bar{n} < 1.05 \), the two variables become very large and sensitive to slight errors in \( \bar{n} \). Points in this region are therefore not used.

A computer program based on the least-squares fit of a straight line was written in Fortran 4 for use with an I.C.T. 1905 computer. A copy of this program is given in Appendix B. In use, however, the program was found to be unsuitable, spurious results were obtained when the experimental data showed more than a slight amount of scatter.

On examination, two factors were thought to be relevant. The least-squares approximation was applied over the full range \( 0 \leq \bar{n} \leq 0.95 \) and \( 1.05 \leq \bar{n} \leq 2 \), and some of the data used for the calculations varied by \( 10^{18} \) (i.e. some of the input data varied by \( 10^9 \) and in the calculation the squares of this data are summed). Thus some points are going to have more significance than others, and unless an extremely large number of points is used, the possibility of inaccurate results being obtained is high. The problem can be overcome to some extent by applying the
approximation separately over the two $\tilde{n}$ ranges, but this is not entirely satisfactory and alternative procedures were examined.

It is possible to reduce the errors incurred further with the above least-squares method, by using a weighting procedure. The method of weighted least-squares approximation devised by Eilbeck et al. was examined. This is essentially a modification of that described by Sullivan et al., and gives accurate results from the data presented, because Eilbeck and co-workers checked the calculated constants using a similar procedure to that described in section 3-6(d).

(a) Calculation of Stability Constants Using a Computer Program Devised by Thun, Verbeek and Vanderlaan

A computer program devised by Thun et al., however, was used in the computation of stability constants described in this thesis. The method is a modification of Fronaeus' graphical integration method which is based on extrapolating a series of functions to the limit $[A] \to 0$.

\[
\ln \phi = \int \frac{\tilde{n}}{[A]} \cdot d[A] + \text{constant}
\]

and a function can be defined such that,

\[
F([A]) = \frac{\phi - 1}{[A]} = K_1 + K_1K_2[A] + K_1K_2K_3[A]^2 + \text{etc.}
\]

The function $F([A])$ approaches $K_1$ as the ligand concentration approaches zero,
Extrapolation of \([A]\) to zero in a system with coordinates \(F([A])\) and \([A]\) gives \(K_1\). Another function can be defined such that,

\[
G([A]) = \frac{F([A]) - K_1}{[A]} = K_1K_2 + K_1K_2[A] + \text{etc.}
\]

This function \(G([A])\) approaches \(K_1K_2\) as the ligand approaches zero,

\[
\lim_{[A] \to 0} G([A]) = K_1K_2
\]

Extrapolation of \([A]\) to zero in a system of coordinates \(G([A])\) and \([A]\) gives \(K_1K_2\). In this particular case, where constants higher than \(K_2\) are not being considered, the extrapolation is merely averaging the values of the function \(G([A])\). Combination of the two results enables the constants \(K_1\) and \(K_2\) to be determined.

Thun and co-workers start with the formation function,

\[
\bar{n} = \frac{K_1[A] + 2K_1K_2[A]^2}{1 + K_1[A] + K_1K_2[A]^2}
\]

and differentiate such that,

\[
\frac{\bar{n}}{[A]} = \frac{d \ln(1 + K_1[A] + K_1K_2[A]^2)}{d[A]}
\]

which leads to,

\[
K_1 + K_1K_2[A] = \exp \left( \int_{[A]}^{[A]} \frac{\bar{n}}{[A]} \, d[A] \right) - 1
\]
Fronaeus' \( F([A]) \) is in fact equal to the right hand side of the above equation. It is thus possible to determine \( K_1 \) and \( K_2 \) by integrating the above function and extrapolating to the limit \( [A] \to 0 \).

Thun et al.\(^{25}\) calculate the integral \( \int_0^\infty \frac{n}{[A]} \, d[A] \) by parabolic integration; intervals are marked by the measured values of \( [A] \), and through every three successive points a parabola of the form,

\[
\frac{n}{[A]} = a [A]^2 + b[A] + c
\]

is drawn. After introduction of the co-ordinates a set of three linear equations is obtained from which the coefficients \( a, b \) and \( c \) can be derived.

An iteration procedure is incorporated in the computer program such that values of stability constants are repeatably calculated and compared with previous values, until the difference is less than a predetermined value (in the present work 0.001). If the error has not been reduced to this value after a certain number of iterations (in the present work 5), some of the peripheral \( \bar{n} \) and free ligand concentration input data is removed and the procedure restarted. At each iteration step the extrapolation is carried out only once and an accumulation of errors due to consecutive extrapolations is avoided.

It was only necessary to make minor modifications to the original Fortran 4 program in order to make it suitable for use with an I.C.T. 1905 computer. This program does not calculate values of \( \bar{n} \) and \( [A] \), and so a program was written to compute this data, prior to its use in the
above program. Copies of both computer programs are given in Appendices C and D.

(d) Check on Calculated Constants

Where errors are introduced by computer calculations, they can be very difficult to identify. This is particularly true with the normal least-squares procedure in which standard deviations are calculated. As the stability constant data used in least-squares approximation can vary by $10^{16}$, the standard deviation can be calculated on a very small number of points. Thus although the resultant standard deviation may be small, in fact the computed constants may be erroneous and unrepresentative.

In the present work, values for the proton-ligand and metal-ligand stability constants were determined and the formation curves were then recalculated. For particular values of $[H]$ or $[A]$, values of $n_H$ or $n$ were calculated from the equations,

$$n_H = \frac{K_{H_1} [H] + 2K_{H_1} K_{H_2} [H]^2}{1 + K_{H_1} [H] + K_{H_1} K_{H_2} [H]^2}$$

$$n = \frac{K_1 [A] + 2K_1 K_2 [A]^2}{1 + K_1 [A] + K_1 K_2 [A]^2}$$

The recalculated formation curves were compared with the experimental curves, and if necessary minor modifications were made to the constants in order to obtain a better fit. A more reliable value for the error was then obtained.

It is realised that this procedure may be slightly
subjective. The initial constants, however, are calculated objectively, major errors are prevented, and minor modifications to the constants were normally within the errors quoted.

A computer program to recalculate the formation curves and so check the constants was written in Fortran 4 for use with an I.C.T. 1905 computer. A copy of this program is given in Appendix E.

3.7 Determination of the Presence and Composition of Chelates by the Method of Continuous Variations

A number of methods used to determine the composition and stability constants of complexes \( MA_n \), are based on the measurement of a property, which is dependent on the concentrations of the species \( MA_n \) and \( A \). Optical density is the most common property measured but conductivity measurements are also used. The basic assumption in these methods is that only one complex is formed under the particular solution conditions used.

The method of continuous variations, which was first applied to the study of complex formation by Job, is the most commonly used method. A series of solutions are prepared such that the ligand concentration varies from zero to a maximum, \( A_0 \), as the metal concentration varies inversely from a maximum, \( M_0 \), to zero. Also \( A_0 = b \cdot M_0 \). Their optical densities are then measured, and these values are plotted against concentration.

At any point on the curve, the total concentration of metal is \( M \), and the total concentration of ligand,
\[ A = \frac{A_0 \cdot (M_0 - M)}{M_0} \]

\[ = b \cdot (M_0 - M) \]

Maximum optical density for a stable complex \( MA_n \) occurs when,

\[ A / M = n \]

Therefore, \( n = \frac{b \cdot (M_0 - M)}{M} \)

\[ = b \cdot \left( \frac{M_0}{M} - 1 \right) \]

and, \( \frac{M}{M_0} = \frac{b}{n+b} \).

When equimolar solutions are used, as in the present study, \( b = 1 \) and,

\[ \frac{M}{M_0} = \frac{1}{n+1} \]

Under these conditions maximum optical density for a complex \( MA \) occurs at, \( M / M_0 = 0.50 \),

for a complex \( MA_2 \), at \( M / M_0 = 0.33 \),

and for a complex \( MA_3 \), at \( M / M_0 = 0.25 \).

Most complexes show slight dissociation near the maximum.

This is indicated by the deviation of the curve from the intersecting tangents of the curve drawn at the points \( M / M_0 = 0 \) and \( M / M_0 = 1 \).

Although equations have been derived for the determination of stability constants with this procedure, it is not recommended, except where only the first complex \( MA \)
is formed. This is because it is extremely unlikely that only one complex will be present in solution, except in these circumstances. In the present study, the method of continuous variations has only been used to determine the composition or confirm the presence of complexes formed under specific conditions.

The possibility of other coloured complexes being present in solution in major amounts, as well as the complex under investigation, can be eliminated. Optical density measurements are made at the absorption spectrum maximum of the complex under investigation, and also at two other wavelengths. Provided that at all the wavelengths studied, maxima or minima occur at the same $M/M_0$ ratio, the presence of other coloured complexes can be neglected. This, however, does not eliminate the presence of other complexes which are either colourless or only slightly coloured.

Allowance can also be made for the effect of coloured metal ions or ligand on the optical density measurements of the complex. The optical density of the metal ions or ligand is subtracted from that of the complex.
CHAPTER FOUR

PRACTICAL DETAILS OF STABILITY CONSTANT DETERMINATIONS
AND OTHER COMPLEX FORMATION INVESTIGATIONS

4.1 Stability Constant Determinations

(a) Evaluation of Solvent Systems

o,o'-Dihydroxydiphenyl sulphides and methanes are only sparingly soluble in water. Thus, in order to dissolve these compounds and also the metal ions and background electrolyte, a mixed organic solvent-water solution is necessary. A 3+1 v-v dioxan-water solution was used by Dr. Fogg in a preliminary examination of bithionol. In this solution, it is not possible to dissolve the molar sodium perchlorate used as background electrolyte, and in addition the second proton of bithionol is not neutralised until a pH meter reading in excess of 14, which makes the determination of \( \log K_{H^+} \) extremely difficult.

A number of organic solvent-water solutions of varying compositions were examined in an attempt to find a more suitable solvent for this study. The organic solvents examined were methanol, ethanol, dimethylformamide, dimethylsulphoxide and diglyme. A 3+1 v-v ethanol-water solution was found to be the most suitable solvent for the present work.

On mixing ethanol with water, there is an apparent contraction in the total volume. The initial volume of the titration solution is required for stability constant
calculation, and so the contraction on mixing a 3+1 v-v ethanol-water solution was calculated from tables, and confirmed by measurements using a density bottle. On mixing 37.50 ml of ethanol with 12.50 ml of water the final volume is 48.50 ml.

(b) Reagents

Analytical-reagent grade chemicals were used where available. Water was double distilled in silica apparatus, and was purged with nitrogen before use. Absolute ethanol of British Pharmacopoeia quality was used, and was purged with nitrogen.

Sodium perchlorate solution 6.47 M. This was prepared from analytical-reagent grade sodium perchlorate, and was filtered before use.

Standard carbonate-free sodium hydroxide solution, 0.1 M in 3+1 v-v ethanol-water. This was prepared from an ampoule of B.D.H. concentrated volumetric solution and was made one molar in sodium ion by the addition of sodium perchlorate. The hydrogen ion molarity of the solution was confirmed by titration against potassium hydrogen phthalate, and the solution was kept under nitrogen to protect it from carbon dioxide in the air.

0.00647 M solutions in ethanol

Eithionol (Cyclo Chemicals Ltd; melting point 188-190°, lit. value 186-189°); hexachlorophene (Kock-Light Ltd.; melting point 156-160°, lit. value 161-167°); dichlorophene (May and Baker Ltd.; melting point 170-173°, lit. value 177-178°); tetrachlorophene (Unilever...
research sample; melting point 160-161°, lit. value 166-167°); bromochlorophene (Unilever research sample; melting point 184-185°, lit. value 188°); and 4-hexylresorcinol (Kock-Light Ltd.; melting point 62-63°, lit. value 62-67°) were used as received. Fenticlor (Calmic Ltd.) was recrystallised from toluene (melting point 167-169°, lit. value 175°). In all cases with the exception of 4-hexylresorcinol, the purity was confirmed by potentiometric titration, and was found to be 98.5%, 99.9%, 100.1%, 100.3%, 100.7% and 100.1% for bithionol, fenticlor, dichlorophene, tetrachlorophene, bromchlorophene, and hexachlorophene respectively. 4-Hexylresorcinol is too weak an acid to be titrated with sodium hydroxide solution.

Standard nitric acid solution, 0.04 M (approx.). A stock solution of approximately 0.8 M nitric acid was prepared by diluting 100 ml of concentrated nitric acid (micropanalytical-reagent grade) to 2 litres in a volumetric flask. The 0.04 M standard solution was prepared by diluting 25.0 ml of this solution to 500 ml in a volumetric flask. On subsequent standardisation the dilute solution was found to be 0.0403 M in nitric acid.

Standard metal solutions, ca. 0.01 M in metal ion and 0.0403 M in nitric acid. These were prepared by dissolving the appropriate metal nitrate in 25.0 ml of the 0.8 M nitric acid solution prepared as above, and diluting the resulting solution to 500 ml in a volumetric flask. The solutions of nickel(II), copper(II), cobalt(II), manganese(II), zinc(II), magnesium(II), cadmium(II), lead(II) and
iron(III) were standardised by EDTA titration. Murexide was used as indicator for nickel, copper and cobalt; Erichrome black T for manganese, zinc and magnesium; Xylenol orange for cadmium and lead; and Pyrocatechol violet for iron(III). The mercury(II) solution was made up from triple distilled mercury and the silver(I) solution was standardised by potentiometric titration with potassium chloride.

(c) Apparatus

pH measurements were made with a Pye 290 pH meter, an E.I.L. GHSN33 (E7) screened glass electrode and a saturated calomel reference electrode. This direct reading pH meter has a 135 mm scale and can either be operated in the 0-14.0 pH range or one of 14 ranges of 1.40 pH units each. The latter mode was used for the present work, and although it is possible to discriminate to 0.002 pH unit, measurements were made to 0.005 pH unit only. The instrument has a very low drift - less than 0.002 per 24 hours non-cumulative is quoted by the makers and confirmed in the present work - because solid state circuitry and a photo-chopper amplifier circuit is used. There is also negligible error when changing from one scale to the next. The response of the E.I.L. glass electrode was found to remain linear at high pH values and a sleeve type reference electrode was used in order to obtain good contact between the aqueous electrode solution and the partly non-aqueous titration solutions.

Grade 'A' burettes, pipettes and volumetric flasks were used throughout. The drainage time of the burettes
during titration was governed by the time taken to obtain a steady pH meter reading, but was fairly consistent throughout the titrations. For the pipettes a period of approximately five seconds was allowed after initial drainage.

The 100 ml titration vessel used in the potentiometric investigations was as shown in Fig. 1. It was necessary to construct the main body in glass because partly non-aqueous solutions were used. For ease of construction the top was made of perspex, and glass tubes sweated into conical holes in the perspex were used to hold the electrodes and the other equipment clear of the bottom of the vessel. During the titrations, care was taken to ensure that the titration solution did not come into contact with the perspex. Two smaller vessels of similar design were used to contain the buffer solutions used to standardise the pH meter. All three vessels were immersed in a tank containing water thermostated at 25°±0.01°.

Gentle stirring and exclusion of carbon dioxide and oxygen was achieved by means of B.O.C. oxygen-free nitrogen which was bubbled through the solution. The nitrogen was first scrubbed with 0.1 M sodium hydroxide solution to remove any traces of carbon dioxide, and then was passed through two scrubbers containing 3+1 v-v ethanol-water at 25°, to saturate the gas with solvent and prevent any concentration changes in the sample solution due to evaporation. Although the above titration vessel was not tightly sealed, only small gaps were left round the electr-
Screened Glass Electrode

Calomel Electrode

Burette

FIG. 1.

TITRATION VESSEL
odes and the other equipment, so that the internal pressure of nitrogen was high enough to exclude carbon dioxide and oxygen. A glass rod of suitable shape was incorporated into the apparatus to remove drops of sodium hydroxide from the burette tip.

(d) pH Measurements

The pH meter was standardised before each titration and the standardisation checked after each titration with an 0.05 M aqueous solution of potassium hydrogen phthalate (B.D.H. primary pH standard grade), the meter being initially set at pH 4.005. The reproducibility of the response of the electrodes was checked daily with this buffer and with an 0.01 M borax A.R. solution. On the British Standards' scale this latter buffer solution has a pH of 9.18 at 25°, but a pH meter reading of 9.12 was obtained consistently.

The response of the glass electrode in 3+1 v-v ethanol-water solution containing one molar sodium perchlorate was checked by potentiometric titration of a standard nitric acid solution with standard sodium hydroxide solution. The stoichiometric pH, i.e. the negative logarithm of the hydrogen ion concentration (\(-\log[H^+]\)) was calculated at various points on the titration curve from the known amounts of sodium hydroxide and nitric acid added. A plot of stoichiometric pH against pH meter reading was linear of the form,

\[
\text{stoichiometric pH} = (\text{pH meter reading} \times 0.94) + 0.03
\]

and for the calculation of stability constants all pH meter readings were converted to stoichiometric pH values.
(e) Potentiometric Titrations

In order to determine the proton-ligand and the metal-ligand stability constants of bithionol, fenticlor and hexachlorophene using the Irving and Rossotti procedure, solutions prepared from the following amounts of reagents were titrated with the standard sodium hydroxide solution,

(1) 5 ml of 0.0403 nitric acid solution
   37.5 ml of ethanol
   7.5 ml of 6.47 M sodium perchlorate solution
(2) 5 ml of 0.0403 nitric acid solution
   37.5 ml of 0.00647 M solution of ligand in ethanol
   7.5 ml of 6.47 M sodium perchlorate solution

and
(3) 5 ml of 0.01 M (approx) standard metal solution containing 0.0403 M nitric acid
   37.5 ml of 0.00647 M solution of ligand in ethanol
   7.5 ml of 6.47 M sodium perchlorate solution

For the determination of the proton-ligand stability constants of dichlorophene, bromchlorophene, tetrachlorophene and 4-hexyresorcinol only the first two solutions were titrated. The metal solutions titrated were those detailed in a previous section, viz. nickel, copper, cobalt, manganese, zinc, magnesium, cadmium, lead, iron(III), mercury(II) and silver with bithionol; nickel, copper, cobalt, manganese and zinc with fenticlor; and nickel and copper with hexachlorophene. Each titration was performed at least twice to check reproducibility, and in the case of bithionol-nickel and fenticlor-nickel, the titrations were also carried out at a nickel concentration
one-half of that used previously, in order to confirm the absence of polymeric species. With the exception of this halved nickel concentration, the initial concentrations of sodium perchlorate, nitric acid, ligand and metal were 1.00, 0.00415, 0.005 and (approximately) 0.001 M respectively, owing to the contraction on mixing the above solutions.

The titration curves were drawn on large graph paper, 30 by 22 inches, so that the volumes of sodium hydroxide solution, $V_1$, $V_2$ and $V_3$, giving the same pH meter readings in the three titrations indicated above respectively, could be read off accurately.

4-2 Verification of the Composition of Complexes by the Method of Continuous Variations

In order to check the copper-bithionol and copper-hexachlorophene formation data obtained from the stability constant determinations, the method of continuous variations was used. The stability constant work indicated the formation of light-brown 1:1 and 1:2 copper-bithionol complexes and a darker-brown 1:1 copper-hexachlorophene complex, at values of pH meter reading 4.3, 8.0 and 5.5 respectively. This work also indicated the formation of a 1:2 copper hexachlorophene complex at a pH meter reading of approximately 9.5. At this pH meter reading, however, the solution becomes very much paler in colour and a blue precipitate forms on standing indicating extensive hydrolysis. Hence the formation of the 1:2 copper-hexachlorophene complex was not examined by the method of
A series of solutions were prepared from a 0.01 M aqueous copper nitrate AR solution and a 0.005 M solution of bithionol in ethanol. These solutions were prepared in 3:1 v-v ethanol-water solution such that the concentration of copper varied from 0-0.001 M and the concentration of bithionol varied inversely from 0.001-0 M. Also the total concentration of copper and bithionol in each solution was constant and equal to 0.001 M. The pH meter reading of all these solutions was adjusted to the value of 4.3 with sodium hydroxide solution. The spectrum of the most intensely coloured solution was measured on the Hilger Uvispek ultra violet and visible spectrophotometer and a single broad absorption band was observed in the visible region with a maximum at 430 nm.

Optical density measurements were made on all the solutions using 4 cm cells, again with the Hilger Uvispek, at 430 nm and also at 400 and 500 nm. The values obtained for optical density at each wavelength were plotted against concentration and the result is shown in Fig. 2(a).

At each wavelength studied there was a pronounced maximum in optical density at $\frac{M}{M^o} = 0.5$ and $\frac{M}{A} = 1$, corresponding to the formation of a 1:1 copper-bithionol complex.

(b) 1:2 Copper-bithionol Complex

A series of solutions were prepared with the same concentrations of copper, bithionol, ethanol and water as those above, and the pH meter reading of these solutions
Fig. 2.

(a) 1:1 Copper-bithionol Complex

(b) 1:2 Copper-bithionol Complex

(c) 1:1 Copper-hexachlorophene Complex
was adjusted to the value 8.0 with sodium hydroxide solution. The spectrum of these solutions was similar to that of the above solutions, and so optical density measurements were made at 430 nm and also at 500 nm and 550 nm, using 4 cm cells. A graph of optical density at each wavelength against concentration is given in Fig. 2(b).

At each wavelength studied there was a pronounced maximum in optical density at \( M/M^0 = 0.33 \) and \( M/A = 0.5 \), corresponding to the formation of a 1:2 copper-bithionol complex.

(c) 1:1 Copper-hexachlorophene Complex

A series of solutions were prepared with the same concentration of copper, ethanol and water, and the same concentration of hexachlorophene as bithionol in the above solutions. The pH meter reading of these solutions was adjusted to the value of 5.5 with sodium hydroxide solution. The spectrum of these solutions was measured and a single broad absorption band was observed in the visible region with a maximum at 440 nm. In this case, however, the optical density was approximately four times higher than for the copper bithionol complexes. Optical density measurements were made at 440 nm and also at 470 nm using 4 cm cells. A graph of optical density at each wavelength against concentration is given in Fig. 2(c).

At each wavelength studied, there was a pronounced maximum in optical density at \( M/M^0 = 0.5 \) and \( M/A = 1 \), corresponding to the formation of a 1:1 copper-hexachlorophene complex.
CHAPTER FIVE

RESULTS AND DISCUSSION OF STABILITY CONSTANT DETERMINATIONS AND METAL COMPLEX FORMATION OF o,o'-DIHYDROXYDIPHENYL SULPHIDES AND METHANES

5.1 Ligand-proton Stability Constants of o,o'-Dihydroxydiphenyl Sulphides and Methanes

The logarithms of the proton-ligand (acid association) stability constants of certain o,o'-dihydroxydiphenyl sulphides and methanes, determined in 3+1 v-v ethanol-water solution and in an ionic background of molar sodium perchlorate, are given in Tables 3 and 4. Table 3 details the computed results obtained over a range of $E_H$ values, for $\log K_{H1}$ between 1.2 and 1.8, and for $\log K_{H2}$ between 0.2 and 0.8. Table 4 details the mean values of these results, and also the final values of $\log K_{H1}$ and $\log K_{H2}$ obtained after comparison with recalculated formation curves, together with their estimated error values.

The ease of combination of ligands with metal ions often follows the same order as the ease of combination of ligands with protons. This is because some of the factors which influence the stability of proton-ligand bonds have a similar influence on the bonding of metal ions with the ligand. These factors are ionic and coulombic effects, which include the charge and radius of the cation, and the charge and charge distribution of the anion. The other factors which do not have similar effects on the bonding of metal ions and protons, include such factors as changes in
### TABLE 3

Values of Ligand-proton Stability Constants Calculated
Over a Range of pH Values

<table>
<thead>
<tr>
<th></th>
<th>Bithionol</th>
<th>Fenticlor</th>
<th>Hexachlorophene</th>
<th>Bromochlorophene</th>
</tr>
</thead>
<tbody>
<tr>
<td>log$K_{H_1}$</td>
<td>log$K_{H_2}$</td>
<td>log$K_{H_1}$</td>
<td>log$K_{H_2}$</td>
<td>log$K_{H_1}$</td>
</tr>
<tr>
<td>9.094</td>
<td>5.344</td>
<td>10.47</td>
<td>7.703</td>
<td>11.51</td>
</tr>
<tr>
<td>9.090</td>
<td>5.335</td>
<td>10.46</td>
<td>7.706</td>
<td>11.51</td>
</tr>
<tr>
<td>9.055</td>
<td>5.346</td>
<td>10.40</td>
<td>7.695</td>
<td>11.47</td>
</tr>
<tr>
<td>9.041</td>
<td>5.330</td>
<td>10.38</td>
<td>7.677</td>
<td>11.57</td>
</tr>
<tr>
<td>9.021</td>
<td>5.333</td>
<td>10.36</td>
<td>7.671</td>
<td>11.46</td>
</tr>
<tr>
<td>9.017</td>
<td>5.316</td>
<td>10.35</td>
<td>7.671</td>
<td>5.087</td>
</tr>
<tr>
<td></td>
<td>5.299</td>
<td>10.33</td>
<td>7.666</td>
<td>5.088</td>
</tr>
<tr>
<td></td>
<td>10.29</td>
<td>7.663</td>
<td>5.081</td>
<td>11.00</td>
</tr>
<tr>
<td></td>
<td>10.29</td>
<td>7.651</td>
<td>5.073</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tetrachlorophene</th>
<th>Dichlorophene</th>
<th>4-Hexylresorcinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>log$K_{H_1}$</td>
<td>log$K_{H_2}$</td>
<td>log$K_{H_1}$</td>
</tr>
<tr>
<td>11.02</td>
<td>6.090</td>
<td>11.91</td>
</tr>
<tr>
<td>11.05</td>
<td>6.090</td>
<td>11.94</td>
</tr>
<tr>
<td>11.06</td>
<td>6.090</td>
<td>11.95</td>
</tr>
<tr>
<td>11.05</td>
<td>6.090</td>
<td>12.08</td>
</tr>
<tr>
<td>11.07</td>
<td>6.097</td>
<td>12.13</td>
</tr>
<tr>
<td>11.05</td>
<td>6.093</td>
<td>12.26</td>
</tr>
<tr>
<td>11.02</td>
<td>6.093</td>
<td>12.17</td>
</tr>
<tr>
<td>11.03</td>
<td>6.094</td>
<td>8.271</td>
</tr>
<tr>
<td>11.04</td>
<td>6.094</td>
<td>8.261</td>
</tr>
<tr>
<td>11.01</td>
<td>6.106</td>
<td>8.260</td>
</tr>
<tr>
<td>11.01</td>
<td>6.116</td>
<td>8.261</td>
</tr>
<tr>
<td>11.00</td>
<td>6.127</td>
<td>8.251</td>
</tr>
<tr>
<td></td>
<td>Final values after comparison with recalculated formation curves</td>
<td>Initial mean values</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>log$_{K_H}$</td>
<td>log$_{K_H}$</td>
</tr>
<tr>
<td>Bithionol</td>
<td>9.05±0.03</td>
<td>5.33±0.02</td>
</tr>
<tr>
<td>Fenticlor</td>
<td>10.38±0.03</td>
<td>7.68±0.02</td>
</tr>
<tr>
<td>Hexachlorophene</td>
<td>11.50±0.05</td>
<td>5.10±0.03</td>
</tr>
<tr>
<td>Bromchlorophene</td>
<td>11.1±0.1</td>
<td>5.75±0.02</td>
</tr>
<tr>
<td>Tetrachlorophene</td>
<td>11.03±0.05</td>
<td>6.10±0.03</td>
</tr>
<tr>
<td>Dichlorophene</td>
<td>≈12.1</td>
<td>8.26±0.03</td>
</tr>
<tr>
<td>4-Hexyresorcinol</td>
<td>≈12.6</td>
<td>10.53±0.05</td>
</tr>
</tbody>
</table>

Resonance and steric effects. An appreciation of the stability of the proton-ligand bonds, thus provides an indication of the metal complexing ability of the ligand. As 4-hexyresorcinol is not directly related to the compounds under investigation and has only been included for the later electrophoresis study, this compound will not be discussed in this section.

Examination of both proton-ligand stability constants for bithionol and for fenticlor shows the effect of the extra electron-withdrawing chlorine atom in each benzene ring of bithionol, which results in bithionol being a stronger acid than fenticlor. A similar effect is indicated by the log$_{K_H}$ values of hexachlorophene, tetrachlorophene and dichlorophene; as the number of chlorine atoms per benzene ring decreases from three to one, the log$_{K_H}$...
values increase, indicating a decrease in acidity. The values of $\log K_H$ for these three compounds, however, do not follow this trend. On comparison of the proton-ligand stability constants of all five compounds, it would appear that the value of $\log K_H$ for hexachlorophene is high. The equivalent thio linked compound was not studied and thus cannot be compared.

The value of $\log K_H$ for hexachlorophene also appears high, when the values obtained by previous workers shown in Table 2 are examined, and some allowance is made for the effect on pH meter reading of the different non-aqueous solvent-water mixtures and different ionic backgrounds used by these workers. Constants determined in the region above stoichiometric pH 11 are not as accurate as those determined below this level, because of the potentiometric procedure used. The accuracy of constants determined in this pH region, therefore, should be treated with caution. Approximate titrations carried out for the electrophoresis study, however, confirm the value of $\log K_H$ for hexachlorophene determined in the present study.

If only the inductive effect of the halogen atoms is considered, when the bromine atom in each benzene ring of bromchlorophene is replaced by a chlorine atom, as in tetrachloro9hene, the acidity of the phenol groups should increase slightly. Examination of the proton-ligand stability constants of these two compounds indicates that this is not so. The difference in values, however, is small, and in the case of the two $\log K_H$ values, the difference is less than the quoted error.
In the two thio linked compounds, bithionol and fenticlor, the sulphur atom has two lone pairs of electrons available for conjugation with the two benzene rings. In the methylene linked compounds, however, the carbon atom has no such lone pairs of electrons. Diphenyl sulphide shows a lack of conjugation, because the sulphur atom and both benzene rings are not coplanar, owing to steric hindrance. If bithionol and fenticlor are conjugated, then the electron withdrawing effect of each chlorine atom of these compounds should be transmitted through both benzene rings, and they should be more acidic than their methylene linked equivalents, tetrachlorophene and dichlorophene. Examination of the proton-ligand stability constants of these four compounds shows that the thio linked compounds are more acidic, and this may indicate some degree of conjugation in these compounds, in contrast to diphenyl sulphide.

5-2 Metal-ligand Stability Constants of o,o'-Dihydroxy-diphenyl Sulphides and Methanes

The logarithms of the metal-ligand stability constants of certain metal complexes of bithionol, fenticlor and hexachlorophene, determined in 3:1 v-v ethanol-water solution and in an ionic background of molar sodium perchlorate, are given in Table 5. Both the initial values of log $K_1$ and log $K_2$, calculated by the computer program devised by Thun, Verbeek and Vanderleen, and also the final values of these constants, which were obtained after comparison with recalculated formation curves, are given.
together with their estimated error values.

The colour changes during the titration of solutions of bithionol, fenticlor, and hexachlorophene containing metal ions are given in Table 6. The formation curves of certain metal complexes of bithionol, fenticlor and hexachlorophene are given in Figs. 3, 4 and 5, respectively.

**TABLE 5**

**Metal-ligand Stability Constants of Bithionol, Fenticlor and Hexachlorophene**

<table>
<thead>
<tr>
<th>Bithionol</th>
<th>Final values after comparison with recalculated formation curves</th>
<th>Initial values calculated with Thun et al. program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\log K_1$</td>
<td>$\log K_2$</td>
</tr>
<tr>
<td>Manganese(II)</td>
<td>$5.32 \pm 0.05$</td>
<td>$4.02 \pm 0.04$</td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>$7.80 \pm 0.03$</td>
<td>$6.56 \pm 0.03$</td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>$8.06 \pm 0.02$</td>
<td>$7.75 \pm 0.02$</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>$9.75 \pm 0.03$</td>
<td>$7.05 \pm 0.02$</td>
</tr>
<tr>
<td>Zinc(II)</td>
<td>$8.06 \pm 0.03$</td>
<td>$5.86 \pm 0.02$</td>
</tr>
<tr>
<td>Cadmium(II)</td>
<td>$6.76 \pm 0.02$</td>
<td>$5.21 \pm 0.02$</td>
</tr>
<tr>
<td>Silver(I)</td>
<td>$4.55 \pm 0.05$</td>
<td>$2.60 \pm 0.05$</td>
</tr>
<tr>
<td>Iron(III)</td>
<td>$\simeq 11.1$</td>
<td></td>
</tr>
<tr>
<td>Magnesium(II)</td>
<td>$\simeq 2.6$</td>
<td></td>
</tr>
</tbody>
</table>

It was not possible to obtain stability constants for the lead(II) and mercury(II) complexes of bithionol because of precipitation.

-47-
### TABLE 5 CONTINUED

<table>
<thead>
<tr>
<th>Fenticlor</th>
<th>Final values after comparison with recalculated formation curves</th>
<th>Initial values calculated with Thun et al. program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \log K_1 )</td>
<td>( \log K_2 )</td>
</tr>
<tr>
<td>Manganese(II)</td>
<td>5.95 ± 0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>8.86 ± 0.02</td>
<td>6.29 ± 0.02</td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>8.86 ± 0.02</td>
<td>7.52 ± 0.02</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>10.73 ± 0.05</td>
<td>6.20 ± 0.05</td>
</tr>
<tr>
<td>Zinc(II)</td>
<td>9.07 ± 0.02</td>
<td>4.87 ± 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hexachlorophene</th>
<th>Final values after comparison with recalculated formation curves</th>
<th>Initial values calculated with Thun et al. program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \log K_1 )</td>
<td>( \log K_2 )</td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>9.55 ± 0.05</td>
<td>9.63</td>
</tr>
</tbody>
</table>
Fig. 3  Formation Curves of Certain Metal Complexes of Bithionol
Fig. 4  Formation Curves of Certain Metal Complexes of Fentlclor
Fig. 5  Formation Curves of Certain Metal Complexes of Hexachlorophene
Colour Changes During the Titration of Solutions of Bithionol, Fenticlor and Hexachlorophene containing metal ions. (All pH values included in this table are pH meter readings)

<table>
<thead>
<tr>
<th></th>
<th>Bithionol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese(II)</td>
<td>Colourless at pH 3, turns yellow-brown at pH 4 and at pH 10.5 a precipitate forms</td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>Faint pink colour pH 3 - 10</td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>Light-green colour pH 3 - 10</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>Light-brown colour pH 3 - 10</td>
</tr>
<tr>
<td>Zinc(II)</td>
<td></td>
</tr>
<tr>
<td>Cadmium(II)</td>
<td>Colourless pH 3 - 10</td>
</tr>
<tr>
<td>Magnesium(II)</td>
<td></td>
</tr>
<tr>
<td>Silver(I)</td>
<td>Colourless at pH 3, and turns yellow at pH 8</td>
</tr>
<tr>
<td>Iron(III)</td>
<td>Intense red-brown colour at pH 3, and a brown precipitate forms above pH 8</td>
</tr>
<tr>
<td>Mercury(II)</td>
<td>Yellow precipitate forms above pH 3</td>
</tr>
<tr>
<td>Lead(II)</td>
<td>White precipitate forms at pH 3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Hexachlorophene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel(II)</td>
<td>Colourless at pH 3, turns faint yellow-green at pH 6 and a precipitate forms at pH 7.5</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>Colourless at pH 3, turns faint brown at pH 4 which darkens to a maximum at pH 7, and at pH 10 a precipitate forms</td>
</tr>
</tbody>
</table>
### TABLE 6 CONTINUED

<table>
<thead>
<tr>
<th>Metal</th>
<th>Colour and pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese(II)</td>
<td>Colourless at pH 2.5, turns to light-brown at pH 7 and dark-brown at pH 8. A precipitate forms above pH 11</td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>Colourless at pH 3, turns to faint pink above pH 6</td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>Colourless at pH 3, turns to light-green above pH 7</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>Colourless at pH 3, turns to light-brown at pH 5 which turns to dark-brown at pH 7</td>
</tr>
<tr>
<td>Zinc(II)</td>
<td>Colourless pH 3 - 10</td>
</tr>
</tbody>
</table>

The ease of combination of ligands with metal ions, for the 1:1 metal complexes of bithionol and fenticlor, follow the same order as the ease of combination of ligands with protons. The values of $\log K_1$ for fenticlor are slightly greater than those for bithionol. The values of $\log K_a$ for bithionol, however, are slightly greater than those for fenticlor.

Values of $\log K_1$ for complexes of bithionol and fenticlor with the divalent ions of the first transition series follow the Irving-Williams' order, viz. Mn < Co < Ni < Cu > Zn. ($\log K_1$ values for iron(II) were not determined owing to the ready oxidation of iron(II) - bithionol solutions). Values of $\log K_a$ for nickel(II) were greater than those for copper. This effect has been noted previously for several ligands. 40, 41
Irving and Fernelius observed that the empirical order of stability constants of divalent metal complexes, \( \text{Cu} > \text{Ni} > \text{Pb} > \text{Co} > \text{Zn} > \text{Cd} > \text{Mg} \), holds with minor variations for many oxygen and nitrogen type ligands, but lead and cadmium fall out of line when the ligand contains sulphur, i.e. \( \text{Cu} > \text{Pb} > \text{Cd} > \text{Zn} > \text{Ni} \). The values of \( \log K_1 \) for the divalent metal complexes of bithionol are in the order, \( \text{Cu} > \text{Ni} \approx \text{Zn} > \text{Co} > \text{Cd} > \text{Mn} > \text{Mg} \). This indicates that for bithionol the two metal-oxygen bonds are stronger than the metal-sulphur bond.

Jørgensen has commented on the probability that thio-ether groups in many multidentate ligands such as thenoyltrifluoroacetone, \( \text{C}_4\text{H}_3\text{SCOCHCOOF}_3^- \), and methionine, \( \text{CH}_3\text{SCH}_2\text{CH}_2\text{CH(\text{H}_2\text{S})COO}^- \), do not participate in complexation. These particular compounds, however, cannot be used to predict the bonding characteristics of the sulphur atoms in bithionol and fenticlor, because they are not directly comparable. Also, examination of the structures of thenoyltrifluoroacetone and methionine indicates that metal-sulphur bonding in these compounds would be more difficult than in bithionol and fenticlor.

The formation of the distorted octahedral configuration for the 1:2 copper-bithionol complex, if the bithionol is acting as a tridentate ligand, would be expected to be difficult. Thus, either the formation of six co-ordinate copper(II) with tridentate bithionol or fenticlor, or the formation of a square planar complex with bidentate bithionol or fenticlor (in which the sulphur is non-co-ordinating), could explain why the values of \( \log K_2 \)
for complexes of these ligands are smaller for copper than for nickel. The formation of the square planar complex with bidentate bithionol or fenticlor, however, would involve the formation of an eight-membered ring, and this is unlikely.

The nickel-bithionol and the nickel-fenticlor formation curves drawn from pH potentiometric data, obtained from solutions containing two different concentrations of nickel ions, 0.001 M and 0.0005 M, showed good agreement. This confirms the absence of polymeric species of nickel-bithionol and nickel-fenticlor.

Jørgensen has shown from ultra violet and visible spectra, that 2,2'-diaminodiethyl sulphide gives high-spin octahedral complexes with nickel. This complex has absorption bands at 533 nm (ε = 15) and at 354 nm (ε = 19). The 1:2 complex of nickel with bithionol, in 3+1 v-v ethanol-water solution buffered at pH meter reading 6.0 with sodium acetate-acetic acid buffer, has an absorption band at 650 nm (ε = 25). The spectral region of this complex below 400 nm is difficult to study because of the strong absorption of bithionol, but by comparison of the spectral regions available, the 1:2 nickel-bithionol complex is similarly octahedral. The much lower approximate value of log K, obtained for the nickel-hexachlorophene complex compared with the corresponding values for bithionol and fenticlor, appears to indicate that in the case of the nickel complexes the co-ordination of the sulphur atom has a considerable stabilising effect.

Hexachlorophene might be expected at most to act as
a bidentate ligand, but, in this case, the complex formed would contain an eight-membered ring. The formation of an eight-membered ring is more likely in the 3:1 v-v ethanol-water solution used in the present study than in water, because of the relatively lower concentration of hydroxide ions in competition with the ligand for the metal. Hydrolysis, as well as complex formation, was evident in the copper-and nickel-hexachlorophene systems, but it is uncertain from the present work whether mixed hexachlorophene-hydroxo metal complexes are formed. At a pH meter reading of 5.5 the colour of the copper-hexachlorophene system was dark brown, and the volume of sodium hydroxide solution consumed, over and above that required to form the singly charged hexachlorophene ion, corresponded to the ionisation of one proton per copper ion. The method of continuous variations indicated the formation of a 1:1 copper-hexachlorophene complex. Thus, the complex formed at this pH meter reading could be either \((\text{Cu A})\) or \((\text{Cu(OH)HA})_n\). At higher pH meter readings the solution became much paler in colour, and a blue precipitate formed on standing, indicating extensive hydrolysis.

The method of continuous variations was also applied to the copper-bithionol system. At a pH meter reading of 4.3, where the sodium hydroxide consumed, over and above that required to form \(\text{HA}^-\), corresponds to the ionisation of one proton per copper ion, the formation of a 1:1 copper-bithionol complex was indicated. At a pH meter reading of 8.0 (two extra protons per copper ion beyond
HA⁻) the formation of a 1:2 copper-bithionol complex was indicated.
CHAPTER SIX

SOLVENT EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF IRON(III) WITH BITHIONOL

6-1 Introduction

During the investigations into the metal complex formation of o,o'-dihydroxydiphenyl sulphides and methanes, iron(III) was observed to form an intense red-brown coloured complex with bithionol. The other metal ions studied formed either colourless or weakly coloured complexes. This reaction was investigated, and a solvent extraction and spectrophotometric method of determining iron(III) with bithionol was developed.

6-2 Brief Review of Solvent Extraction and Spectrophotometric Methods of Determining Iron

(a) Inorganic Reagents

There are few inorganic reagents of any value for the colorimetric determination of iron, apart from thiocyanate, ferricyanide and ferrocyanide. Iron may be determined colorimetrically as ferric chloride and ferrous sulphate. It is possible to extract the chloride into many organic solvents including diethyl ether, diisopropyl ether, B,B'-dichlorodiethyl ether, amyl acetate, methyl isobutyl ketone, chloroform and isoamyl alcohol.

Iron(III) reacts with thiocyanate in acid solution to give a complex which may be extracted into many organic
solvents including amyl alcohol-ether, isobutyl alcohol, tri-n-butyl phosphate, ethyl acetate, and methyl isobutyl ketone. A number of ions interfere with spectrophotometric measurements on the extracted complex.

(b) Organic Reagents

Acetylacetone Many metals form complexes with acetylacetone. Iron(III) can be extracted from aqueous solution over a wide pH range with the reagent itself or mixed with most organic solvents including chloroform, xylene and butyl acetate, and determined spectrophotometrically. Titanium, chromium, bismuth, uranium, manganese and copper interfere.

8-Hydroxyquinoline (Oxime) Iron(III) can be extracted with Oxime from aqueous solution over a wide pH range into chloroform, prior to spectrophotometric determination. Interference from titanium, vanadium, nickel and copper can be masked.

7-Iodo-8-hydroxyquinoline-5-sulphonic Acid (Ferron)

Tributyl phosphate and isooamy alcohol have been used to extract iron(III) from aqueous solution at pH 4.7 with this reagent, prior to spectrophotometric determinations on the green complex. Many interfering metal ions can be masked by cyanide and citrate.

N-nitroso-N-phenylhydroxylamine (Cupferron) Iron(III) can be extracted from acidic aqueous solution with the reagent and ether, chloroform, ethyl acetate or isooamy acetate, and determined spectrophotometrically. E.D.T.A. can be used to mask most interfering ions.
Titanium and vanadium interfere.

1-Nitroso-2-naphthol  The iron(II) complex of this reagent may be extracted from aqueous solution at pH 1.5 with chloroform and used for the spectrophotometric determination of iron(II). No interference is caused by nickel, vanadium, molybdenum, copper and citrate.

1-{(2-Pyridylazo)-2-naphthol(PAN)}  Iron(III) may be extracted from aqueous solution at pH 4-8 with the reagent and benzene, prior to spectrophotometric determination. The coloured complexes of manganese, cadmium, mercury gallium and yttrium are also extracted at various pH levels.

2-Thienyltrifluoroacetone  This reagent may be used to extract iron(III) from 10 M nitric acid solution into xylene or benzene. Spectrophotometric measurements are not normally useful because a large number of common ions interfere.

Phenyl-2-pyridylketoxime  Coloured complexes formed by iron(II) and iron(III) with this reagent can be extracted from alkaline solution into chloroform, carbon tetrachloride, or isooctyl alcohol and used to determine iron spectrophotometrically. Extractable coloured complexes are also formed by copper(I), copper(II), cobalt(II) and manganese(II).

Quinoline-8-carboxylic acid  Iron(II) can be determined spectrophotometrically with this reagent. The complex can be extracted from aqueous solution into pyridine or chloroform. Copper, nickel, cobalt, zinc and aluminium interfere.

-57-
2-Mercaptopyridine-K-oxide (Thione). The iron(III) chelate of Thione can be used to determine iron(II) and iron(III) spectrophotometrically, after extraction from aqueous solution into chloroform. Gross interference is caused by copper and nitrite.

N-Phenylnbenzohydroxamic Acid. The complexes of many metals formed with this reagent can be extracted into chloroform. This reaction has been used to determine iron(III) spectrophotometrically. Many interfering ions can be masked by hydrogen peroxide and tartrate, but iron(II) interferes.

1,10-Phenanthrolene. This is a very sensitive spectrophotometric reagent for iron(II), but many other metal ions also form coloured complexes. Many interfering ions can be masked by extracting the iron(II) complex as the perchlorate salt from aqueous solution into nitrobenzene. Copper and cobalt cause interference. Extraction of the complex as the iodide from aqueous solution containing E.D.T.A. into chloroform avoids interference by these metals.

4,7-Diphenyl-1,10-phenanthrolene (Bathophenanthrolene). Bathophenanthrolene is a very sensitive and selective though expensive, reagent for iron(II). Iron(III) can be reduced with hydroxylamine hydrochloride and the iron(II) complex extracted into n-hexanol or isoamyl alcohol, prior to spectrophotometric determination. No common ions interfere. In certain circumstances isoamyl alcohol produces turbidity when perchlorates are present in solution, and nitrobenzene should be used.
2-(2'-pyridyl)-benzimidazole and 2(2'-pyridyl)-imidazoline

Both reagents resemble 1,10-phenanthroline in their reactions, giving intensely coloured complexes with copper(I), copper(II), cobalt(II), iron(II) and iron(III). The ferrous complex of 2(2'-pyridyl)-benzimidazole may be extracted from aqueous solutions over a wide range of pH with isoamyl alcohol, but the 2,(2'-pyridyl)-imidazoline complex may only be extracted from aqueous solutions containing perchlorate. Iron(III), copper (I), copper(II) and cobalt(II) also form coloured complexes with these reagents.

Tris (2'-pyridyl)-1,3,5-triazine An intense violet coloured complex is formed with this reagent and iron(II). The complex may be extracted as the perchlorate from aqueous solution at pH 2.7 - 7.0 with nitrobenzene. This reagent is nearly as sensitive as Bathophenanthroline, cheaper, but many common ions including cobalt, copper and nickel interfere with the spectrophotometric determination of iron(II).

2,6-Bis(4-phenyl-2-pyridyl)-4-phenyl pyridine (Terrosite)

This is a more sensitive spectrophotometric reagent for iron(II) than Bathophenanthroline. Iron(II) can be extracted quantitatively from aqueous solution over the pH range 2 - 8 with Terrosite, ethanol and chloroform. This reagent is difficult to prepare and consequently very expensive.

3-(4-phenyl-2-pyridyl)-5,6-diphenyl-1,2,4-triazine This also is a more sensitive spectrophotometric reagent for iron(II) than Bathophenanthroline and its selectivity is
comparable. The iron(II) chelate forms over pH range 3 - 8 and can be completely extracted from aqueous solution with isoamyl alcohol. Again, however, the synthesis of this reagent is complex and expensive.  

**2,2'-Dipyridyl** This is a sensitive reagent for the spectrophotometric determination of iron(II) over the pH range 2 - 9.5. The 2,2'-dipyridyl-iron(II) complex can be extracted from aqueous solution into chloroform containing teepol. Silver, cobalt, chromium, copper, mercury(II), nickel, molybdenum and tungsten all interfere to a certain extent, and the amounts present must therefore be controlled.

**Disodium-1,2-dihydroxybenzene-3,5-disulphonate (Tiron)**

This reagent is specific for iron(III), no other ion gives a red colour with this reagent. Molybdate, copper(II), uranium, vanadate and titanium form coloured complexes but can be removed by means of hydrogen sulphide. This reagent becomes oxidised by strong oxidising agents, such as perchloric acid but it is possible to determine iron(III) in the presence of large amounts of fluoride, citrate, tartrate, oxalate and phosphate.

**Salicylic Acid** Iron(III) reacts with salicylic acid in acetic acid medium to form an amethyst colour suitable for colorimetric estimation. Many ions interfere including, tartrate, oxalate, citrate, orthophosphate, pyrophosphate, arsenate, cyanide, tungstate, sulphate, fluoride, sulphite, thiosulphate, iodide, copper, nickel, cobalt and manganese.

**Sulphosalicylic Acid** An intense red colour is produced
by the reaction of iron(III) with this reagent which is suitable for the spectrophotometric determination of iron(III). In alkaline solution this reagent reacts with both iron(II) and iron(III) to form a yellow colour, so this reagent may be used to determine both forms of iron. In acid solution, iron(II) causes slight interference, and phosphate, copper, nickel, cobalt, and oxidising agents interfere. Copper, however, can be masked by cyanide.

Salicylaldoxime This is a sensitive spectrophotometric reagent for iron(III) which can be used over a wide pH range. Many ions interfere including tartrate, citrate, oxalate, cyanide, carbonate, borate, phosphate, iodide, sulphite, molybdate, cobalt and uranyl ions.

1-Nitroso-2-hydroxy-3,6-naphthalene Disodium Sulphonate (Nitroso-R-salt) Iron(II) forms a green colour with this reagent which is suitable for colorimetric estimation. Excess reagent must be absent because it is yellow. Aluminium, antimony, bismuth, mercury(II), and silver precipitate from solution, and cobalt, copper, nickel, cerium, chromium, dichromate and uranium interfere by colour formation. Citrate, fluoride and cyanide prevent full colour formation. The sensitivity is comparable with o-phenanthroline and 2,2'-dipyridyl methods.

Mercapto-acetic Acid (Thioglycollic acid) This reagent forms a red-purple colour with both forms of iron in alkaline solution suitable for the spectrophotometric determination of iron. Many metal hydroxides precipitate at the pH used, and cyanide and large amounts of the
alkali metals interfere.

α-Nitrosophenol This is a sensitive spectrophotometric reagent for iron(II). The green complex can be extracted from aqueous solution into benzene and petroleum ether. Other coloured complexes of copper, nickel, mercury and zinc can be screened out, and the use of acid medium avoids the colour of alkali salts of the reagent.

Dimethylglyoxime This is a suitable spectrophotometric reagent for iron(II) in alkaline medium. At the pH used, however, many ions precipitate or form colours with the reagent including aluminium, beryllium, chromium, magnesium, manganese, thorium, uranium, barium, calcium, strontium, lead, copper and nickel.

2-Hydroxymethyl-5-hydroxy-1-pyrone (Kojic Acid) The orange iron(III) complex of this reagent can be used for the spectrophotometric determination of iron. The colour depends partly on the excess of reagent used. Citrate, oxalate, pyrophosphate, aluminium, arsenic, bismuth, chromium, dichromate, copper, tin, and vanadate all interfere seriously.

4-Hydroxydiphenyl-3-carboxylic Acid This reagent is virtually specific for iron(III); uranium (orange-red) and copper (green) are the only other ions to form coloured complexes with this reagent. The violet iron(III) complex can be used to determine iron spectrophotometrically in 2+3 v-v ethanol-water solution at pH 2.3 - 3.2. Fluoride, phosphate, phthalate, oxalate, tartrate interfere, copper can be removed with hydrogen sulphide, and titanium does not interfere at pH 2.5.
Spectrophotometric Determination of Iron(III) with Bithionol

The formation of the iron(III)-bithionol complexes was studied initially by potentiometric titration, as described in the previous chapters. Since bithionol is insoluble in water, the measurements were made in 3+1 v-v ethanol-water solution. The titration data indicated that under the solution conditions used, the 1:2 iron(III)-bithionol complex is formed completely in solutions of pH meter reading above about 5.0. Precipitation of ferric hydroxide was observed in solutions of pH meter reading greater than 7.0.

Spectrophotometric measurements were made with a Hilger Uvispek ultra violet and visible spectrophotometer. Again measurements were made on 3+1 v-v ethanol-water solutions. These solutions were buffered with an equimolar acetic acid-sodium acetate buffer which gave a pH meter reading of 5.9. The spectrum of the iron(III)-bithionol complex was found to have a single broad absorption band in the visible region with a maximum at 484 nm. Bithionol itself is colourless.

The composition of the complex was confirmed by the method of continuous variations. Solutions were prepared containing iron(III) and bithionol in 3+1 v-v ethanol-water solution and buffered with equimolar acetic acid-sodium acetate buffer solution, such that the iron(III) concentration varied from 0 to 0.00035 M and the bithionol concentration varied inversely from 0.00036 to 0 M. Also the total iron(III) and bithionol concentration was con-
stant and equal to 0.00036 M. Absorbance measurements were made on these solutions with 1 cm glass cells at 450, 484 and 550 nm, and the values of optical density were corrected for the colour of iron(III) acetate. The values obtained for optical density at each wavelength were plotted against concentration and the result is shown in Fig. 6. At each wavelength there was a pronounced maximum in optical density at $M/M^0 = 0.33$ and $M/A = 0.5$, corresponding to the formation of a 1:2 iron(III)-bithionol complex.

**Determination of Iron(III)**

In making the stability constant measurements referred to earlier, the solutions used were made 0.005 M with respect to bithionol. To avoid precipitation of bithionol at this concentration it was necessary to make the measurements on 3+1 v-v ethanol-water solutions.

Pretreatment of iron-containing samples, however, will usually yield an aqueous solution, and, thus, if maximum sensitivity is to be attained with the method, it is essential that the amount of ethanol added be minimised. It was found that the 1+1 v-v ethanol-water solutions which were 0.001 M with respect to bithionol remained clear. This concentration of bithionol is a sufficient excess for the determination of iron, and the use of 1+1 v-v ethanol-water solutions was adopted. The spectrum of the iron(III)-bithionol complex and its molar absorptivity at 484 nm were unchanged at this lower ethanol concentration. The molar absorptivity of the complex at 484 nm was found to be 5,600, and Beer's Law was obeyed in the
Fig. 6 Method of Continuous Variations Applied to the Iron(III) - Bithionol Complex

Optical Density

Iron(III) molarity x 100,000

Bithionol molarity x 100,000
useful analytical range using 1 cm cells, 0 - 8 ppm (0 - 0.00014 M) of iron(III).

Reagents

Bithionol solution 0.004 mg per ml (approx. 0.01 M) in ethanol.

Buffer solution One molar with respect to both acetic acid and sodium acetate. This solution buffers aqueous solutions at pH 4.6, but the pH meter reading in 3+1 and 1+1 v-v ethanol-water solution is 5.9 and 5.6 respectively.

Standard iron(III) solution 0.1 mg per ml (approx. 0.002 M) Analytical reagent grade iron(III) ammonium sulphate (0.864 g) was dissolved in water, concentrated hydrochloric acid (10 ml) added, and the solution diluted to one litre.

Procedure

An aliquot of the standard iron(III) solution (or of a weakly acid sample solution) containing up to 0.4 mg of iron(III) was pipetted into a 50-ml volumetric flask. Buffer solution (5 ml), ethanol (20 ml), and bithionol solution in ethanol (5 ml of 0.004 mg per ml) were added, the solution was made up to volume with water and mixed thoroughly. The absorbance of the solution was measured against water at 484 nm in 1 cm glass cells. The absorbance of a blank solution, containing the reagents but no iron(III), was also measured against water, and this was then deducted from the previous value.

The calibration curve obtained with the above procedure was linear, and the mean absorbance value of ten solu-
tions containing 0.23 mg (approx. 5 ppm) of iron(III) was 0.466 with a standard deviation of 0.001.

**Interferences**

When iron(II) salts are treated by the above procedure the iron(III)-bithionol complex is formed; this oxidation reaction was shown to be quantitative up to 0.4 mg of iron(II). When the iron(II) and reagent solutions are deoxygenated by bubbling nitrogen through them prior to mixing, however, no oxidation occurs and a colourless solution is obtained. From this it is apparent that dissolved oxygen is responsible for the oxidation of the iron(II). The addition of hydroxylamine salts does not prevent the oxidation.

No interference (i.e. an effect less than 1 per cent) was observed at the 0.23 mg (approx. 5 ppm) of iron(III) level in the presence of 25 mg of iodide, chloride, sulphate, tetraborate, bromide, nitrate or acetate, 5 mg of zinc, cobalt, nickel, magnesium, manganese(II) or aluminium ions, 1 mg of fluoride, 0.3 mg of copper(II) ion, or 0.15 mg of dichromate ion. Severe interference was observed at this level of iron from an equal amount of mercury(II), chromium(III), tartrate, oxalate or citrate ions. Mercury(II) forms a precipitate with the reagent and the other ions cause the colour intensity of the solution to be reduced. On further study it was observed that lead also forms a precipitate with bithionol.

**Determination of Iron in Aluminium Alloys**

The method was applied to the determination of iron in two high-silicon aluminium alloys supplied by the
British Aluminium Company Limited. Suitable quantities of the samples (from 0.2 - 0.5 g) were dissolved in diluted hydrochloric acid (10 ml of 1+1 v-v). Most of the excess acid was boiled off and the solutions were diluted to 100 ml in volumetric flasks. Aliquots (5 ml) of these solutions were treated, after neutralisation to Congo red (pH 3-5), by the procedure described earlier. Further aliquots (5 ml) of these sample solutions were treated by the o-phenanthroline method described by Furman. The results, together with the spectrographic analysis values supplied by British Aluminium, are given in Table 7.

The results obtained with bithionol agree extremely well with those obtained with o-phenanthroline. From these results, the main source of error would appear to be in the method used to dissolve the samples of aluminium alloys.

| TABLE 7 |
| Estimation of Iron in Aluminium Alloys |

<table>
<thead>
<tr>
<th>Solution number</th>
<th>Iron found (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample number</td>
</tr>
<tr>
<td>Bithionol method</td>
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</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>o-Phenanthroline</td>
<td>1</td>
</tr>
<tr>
<td>method</td>
<td>2</td>
</tr>
<tr>
<td>Spectrographic analysis</td>
<td></td>
</tr>
</tbody>
</table>
6-4 Solvent Extraction Procedure for the Spectrophotometric Determination of Iron(III) with Bithionol

On further investigations, it was found that the iron(III)-bithionol complex could be extracted from 1:1 v-v ethanol-water solution into organic solvent. It was initially thought that from its structure the complex would be ionic and carry a single negative charge. For the complex to extract into organic solvent, it must be neutral. The complex thus appears to have reacted with a single proton per molecule of complex. This assumption is supported by the work described in chapter ten. In attempting to increase the amount of the iron(III)-bithionol complex formed in solution containing excess iron(III), the addition of an amount of a large positive cation was found to be partly successful.

Several solvents, viz. chloroform, carbon tetrachloride, diethyl ether, n-butanol, ethyl acetate, cyclohexane and n-hexane extract the iron(III)-bithionol complex from 1:1 v-v ethanol-water solutions of pH meter reading 5.6. Chloroform seemed to be the most suitable solvent and extractions with chloroform were studied further.

Because the reagent is only sparingly soluble in chloroform and water, ethanol must be included in the aqueous phase to prevent precipitation of bithionol. A proportion of the ethanol enters the chloroform phase. The absorption spectrum of the complex in chloroform-ethanol is identical to that of the 1:2 iron(III)-bithionol complex formed in ethanol-water, and is independent of the chloroform to ethanol ratio. A single broad
absorption band occurs in the visible region with maximum absorption at 484 nm and the molar absorptivity at this wavelength is 5,600.

The method of continuous variations was used to confirm that the iron(III) is extracted as the 1:2 iron(III)-bithionol complex. The reagent concentrations were similar to those used in the previous section, and the solutions were treated as described in the section entitled 'Extractive Determination of Iron(III)'. Absorbance measurements were again made at 450, 484 and 550 nm using 1 cm glass cells. The results obtained were similar to those obtained in the previous section, confirming the formation of a 1:2 iron(III)-bithionol complex in the extracted solution.

Distribution Determinations

The effect of pH on the extraction of iron(III), and on the extraction of several other metals, with bithionol was examined. The following procedure was used.

A 1:1 v-v ethanol-water solution (50 ml), containing bithionol (20 mg) and metal ion (0.25 mg), was adjusted to the required pH meter reading with hydrochloric acid, sodium acetate or sodium hydroxide. It was then extracted successively with 8, 4 and 3 ml of chloroform. In the case of iron(III) the combined chloroform-ethanol extract (about 30 ml) was made up to 50 ml in a volumetric flask with ethanol, and the absorbance of the solution at 484 nm was used to determine the proportion of iron(III) extracted.

In the case of the other metals, the unextracted
metal ion was determined in the aqueous layer remaining after extraction. Copper(II) and nickel(II) were determined colorimetrically with sodium diethyldithiocarbamate, cobalt(II) with nitroso-R-salt and chromium(III) (after oxidation with sodium peroxide) as chromate.

The results of the investigation are shown in Fig. 7. Iron(III) is extracted quantitatively from solutions of pH meter reading 5.1 - 6.0. Complete extraction of copper, nickel and cobalt, on the other hand, is only possible from solutions of pH meter reading 8 - 10. At pH meter reading 5.6, which is suitable for the quantitative extraction of iron(III), only one-half of the copper and much smaller amounts of nickel and cobalt are extracted. Small amounts of chromium(III) are extracted at pH meter reading 1 - 12.

**Extractive Determination of Iron(III)**

The reagents used were of the same concentrations as those used in the previous section.

An aliquot of the standard iron(III) solution (or of a weakly acid sample solution) containing up to 0.4 mg of iron(III) was pipetted into a 100 ml separating funnel. Buffer solution (5 ml), ethanol (20 ml) and bithionol solution (5 ml of 0.004 mg per ml) were added. The solution was diluted to approximately 50 ml with water and extracted successively with 8, 4 and 3 ml quantities of chloroform. The chloroform extracts were combined in a 50-ml volumetric flask, diluted to volume with ethanol, and mixed thoroughly. The absorbance of the solution was measured against ethanol at 484 nm in 1 cm glass cells.
Fig. 7 Effect of pH on the Extraction of Iron(III), Chromium(III), Copper, Nickel, and Cobalt with Dithionol.
The absorbance of a blank solution, containing the reagent but no iron(III), was also measured against ethanol, and this was then deducted from the previous value.

The calibration curve obtained with the above procedure was linear, and the mean deviation of twelve solutions containing 0.23 mg (approx. 5 ppm) of iron(III) was 0.466 with a standard deviation of 0.002.

Interferences

No interference (i.e. an effect less than 1%) was observed at the 0.23 mg (approx. 5 ppm) level of iron(III) in the presence of 80 mg of sulphate, nitrate, chloride, iodide, bromide, acetate, perchlorate or borate; 20 mg of zinc, nickel, magnesium, cobalt(II), manganese(II), cadmium, aluminium, calcium, potassium, sodium or ammonium; 2.3 mg of dichromate; 1.4 mg of fluoride ion; 0.9 mg of copper(II); 0.23 mg of chromium(III) or 0.15 mg of tartrate. Mercury(II) forms a precipitate with the reagent but 2.5 mg could be tolerated.

Oxalate, phosphate and citrate at the 0.08 mg level reduced the absorbance by 3%, 5% and 10% respectively.

6-5 Discussion

Bithionol is a stable, white, crystalline solid, which is produced commercially in a relatively pure form at reasonable cost. Its solutions in ethanol are colourless and are stable if they are stored in the dark.

The initial spectrophotometric method of determining iron(III) with bithionol is fairly selective, and the coloured solutions produced have been shown to be stable
for a week. The sensitivity of the method is similar to that of the o-phenanthroline method, with which it compares favourably as a reagent for iron, and the reagent is considerably cheaper.

Extraction techniques are used in colorimetric analysis to limit interference, to increase sensitivity, to prevent decomposition of the coloured species, or to increase its solubility. Alternatively, extraction techniques may be used to remove a large excess of an interference from the substance to be determined.

With the extraction procedure described there is a significant increase in the amounts of the interfering elements that can be tolerated compared to the previous bithionol method. In particular three times the amount of copper(II), or ten times the amount of dichromate, over iron(III), can now be tolerated. The increase in tolerance to copper(II), nickel(II), cobalt(II) and chromium(III) is predictable from the distribution curves shown in Fig. 7.

The procedure as it stands does not concentrate the complex, but a two fold concentration can be effected by extracting with 7, 2 and 1 ml aliquots of chloroform and diluting to 25 ml. The complex is soluble and stable to decomposition in chloroform-ethanol solution.

Bithionol is a relatively inexpensive reagent, and its use in removing a large excess of iron might be advantageous in some applications. Using 12.5 ml of a 0.04 mg per ml (approx. 0.1 μ) solution of bithionol in ethanol, 7.5 mg of iron(III) can be removed with the
extraction procedure as described. Larger amounts of iron can be removed if the amounts of chloroform are increased.

Many reagents have been suggested for the extraction and spectrophotometric determination of iron. The reagent, bithionol, has several limitations, but its use should be advantageous in several applications, particularly as it is relatively inexpensive, and will soon be readily available, as Hopkin and Williams Ltd. have agreed to market the reagent in small quantities suitable for analytical use.
CHAPTER SEVEN

DETERMINATION OF MERCURY(II) WITH BITHIONOL

7-1 Attempted Gravimetric Determination of Mercury(II) with Bithionol

Introduction

When bithionol is added to a solution of mercury(II) in a partly alcoholic medium, a yellow crystalline precipitate is formed. This precipitate is stable for at least a month, and is formed over a wide pH range (pH meter reading 2-8, approximately). No precipitate is formed when bithionol is added to a solution of mercury(I) under similar conditions. Thus the formation of the insoluble mercury(II)-bithionol complex was investigated in an attempt to develop a simple gravimetric procedure for the determination of mercury(II).

Brief Review of Gravimetric Methods of Determining Mercury

The majority of methods available for the gravimetric determination of mercury involve either reducing the mercury salt to the metal, or the use of an inorganic reagent. Few organic reagents are in common use.

Furman recommends the following as standard gravimetric methods for mercury: (1) precipitation as mercuric sulphide using ammonium sulphide (Volhard's method), (2) dry distillation and collection as an amalgam on gold or silver, and (3) electrolytic deposition on to platinum.

-74-
or gold.

**Determination in the form of mercury(II) sulphide with ammonium sulphide (Volhard's method**). Strong acid solutions containing mercury are partly neutralised by the addition of sodium carbonate, and mercury(II) sulphide is precipitated by the addition of ammonium sulphide. The precipitates are then redissolved by the addition of sodium hydroxide as Na₂[HgS₂] and any elemental sulphur present in solution dissolved as sodium polysulphide. The solutions are boiled, and mercury(II) sulphide reprecipitated by the addition of ammonium nitrate. The precipitates are then dried at 110°C.

Reliable determination of mercury by this procedure is not possible in the presence of metal ions which form insoluble sulphides. Cadmium, in particular, causes large errors due to mixed crystal formation with mercury(II) sulphide.

**Determination as mercury(II) sulphide in acid medium with hydrogen sulphide** Mercury can be determined in hydrochloric acid solution by saturating the solution with hydrogen sulphide, thus precipitating mercury(II) sulphide.

Copper, cadmium, tin, zinc, thallium, silver, arsenic and antimony interfere. Cyanide can be used to mask copper. Oxidising agents such as nitric acid, chlorine and iron(III) must also be absent. The precipitate is liable to be contaminated with sulphur and this can be removed with carbon disulphide.

**Determination as Mercury(I) Chloride** Phosphorus trichloride is hydrolysed with water to form a solution of phosphor-
ous acid and hydrochloric acid. This solution is then used to precipitate mercury as mercury(I) chloride from dilute sulphuric acid solution.

This method is fairly selective because only lead and silver form precipitates under similar conditions. Slightly low results are obtained, however, because of the slight solubility of the precipitate, especially in solutions which contain additional chloride ions. The precipitate also has the tendency to disproportionate to form metallic mercury and mercury(II) chloride, and so the solutions should not be heated above 70°C. Once dried, however, the precipitate is stable below 130°C.

Dry distillation method

Metallic mercury can be distilled from dry material in a reducing medium. Either iron, lime, or in samples containing arsenic, zinc oxide can be used as the reducing agent. Metallic mercury is then collected by amalgamation with gold or silver. The distillation can be carried out in a crucible which has a tight fitting cooled lid into which gold or silver foil is fitted. Gold is preferable to silver.

In the presence of chlorides or sulphates which have low volatility, the distillation must be carried out with lead chromate in a current of carbon dioxide.

Electrolytic deposition

Slightly acid solutions of mercury salts can be electrolysed and metallic mercury collected as an amalgam on gold or gold coated platinum electrodes. This procedure can be lengthy.

Where the mercury is present as mercury(II) - sulphide, it is preferable to carry out the electrolysis in an
alkaline sodium sulphide solution.

Reduction to metallic mercury with ascorbic acid. Both mercury(I) and mercury(II) compounds can be reduced with ascorbic acid to metallic mercury which can then be determined gravimetrically. When gold and silver are present, they precipitate with the mercury. In this case the precipitate is weighed before and after the mercury has been evaporated from the precipitate, and the mercury content is determined by difference.

No interference is caused by lead, cadmium, zinc, large amounts of nitrates or small amounts of nitric acid, but copper and platinum interfere. The solutions cannot be heated above 70° because of the low volatility of metallic mercury.

This method is preferable to the method using calcium hypophosphite, because the solution is not contaminated with calcium and phosphate ions which are difficult to remove.

Determination as mercury(II) iodate $\text{Hg} \left( \text{IO}_3 \right)_{2}$. Mercury can be determined gravimetrically in nitric or sulphuric acid solution with the addition of iodic acid to form sparingly soluble mercury(II) iodate.

Halide ions prevent the precipitation of mercury as the iodate and so must be absent from solution. Silver, lead, bismuth, titanium, zirconium and iron(III) interfere by precipitation. Tin and antimony can be separated in the preparation of the nitric acid sample solution.

Determination as mercury(II) periodate $\text{Hg} \left( \text{IO}_4 \right)_{2}$. Mercury can also be determined gravimetrically as mercury(II)
periodate in nitric acid solution by the addition of sodium periodate.  

Iron interferes by precipitation as iron(III) periodate. Halides must be absent because they prevent the precipitation of mercury as the periodate, and nitric and sulphuric acid concentrations must be limited to 0.15 N and 0.1 N respectively.

Determination of mercury with Thionalide Hg(C_{6}H_{11}CHOC,H_{2}SH). This reagent can be used to determine mercury gravimetrically from dilute acid solutions. The solution can be heated to 80-85° and the precipitate dried at 105°.

If nitric acid solutions of mercury(II) nitrate are used, the mercury(II) nitrate should be converted into mercury(II) chloride by the addition of chloride ions. The chloride concentration, however, should not exceed 0.1 N. Iron(III), copper, silver and bismuth must be absent, the former because it acts as an oxidising agent, and the remainder because they also form precipitates with Thionalide under similar conditions.

Determination as copper ethylenediamine mercuri-iodide 
\[\text{[Cu (NH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{2})\textsubscript{2}I\textsubscript{2}]}.\] A boiling aqueous concentrated solution of copper ethylenediamine sulphate or nitrate is added to a neutral or slightly ammoniacal mercury solution, which previously had been heated nearly to boiling point, and excess potassium iodide is added. Mercury precipitates as copper ethylenediamine mercuri-iodide and can be determined gravimetrically in this form.

Cadmium also forms a precipitate with copper ethyl-
enediamine nitrate or sulphate under similar conditions and should be absent. Many other compounds similarly interfere.

Structure of the Mercury(II)-bithionol Complex

The pH potentiometric titrations, carried out on solutions containing bithionol and mercury(II) in 3:1 v-v ethanol-water solution with sodium hydroxide solution, have already been described in chapter four. The metal-ligand stability constants of the mercury(II)-bithionol complexes were not determined because mercury(II) was observed to form a yellow precipitate with bithionol above a pH meter reading of 3. It was only possible to obtain an approximate formation curve and this is shown in Fig. 8. It indicates that a 1:1 mercury(II)-bithionol complex is formed completely between pH meter reading 2.6 and 7.2.

Mercury(II) readily forms covalent complexes. The majority of these complexes have a coordination number of four and a tetrahedral structure. A few complexes such as \([\text{Hg (ethylene diamine)}_3][(\text{NO}_3)_2]\) attain a coordination number of six. A coordination number of two may possibly exist in \(\text{HgS}_2^{2-}\), and a coordination number of three may be possible in some halide complexes such as \(\text{K(HgX}_3\)). The tertiary phosphine complexes \(\text{HgX}_2\text{PR}_3\) have been shown to be dimers, and the analogous dialkyl sulphide complexes are thought to have a similar structure, viz.

-79-
Fig. 8 Formation Curve of the Mercury(II)-bithionol Complex
where X represents a halide atom

Mercury(II) also forms more highly bridged complexes with halides and tertiary phosphines such as,

Mercury(II) would thus be expected to sustain tetrahedral coordination in a 1:1 mercury(II)-bithionol complex. The following are suggested as possible structures, either (I) a polymer or (II) a hydrated complex,

I  

II
Investigation of Bithionol as a Gravimetric Reagent for Mercury(II)

Reagents

Mercury(II) solution, ca. 0.1 M  This was prepared by dissolving 10.9008 g of triple distilled mercury metal in the minimum concentration of warm nitric acid. Nitrous fumes were boiled off, and the solution made up to 500 ml in 0.02 N nitric acid. 

Bithionol solution 0.06 M in absolute ethanol.

Buffer solution  This was one molar sodium acetate - one molar acetic acid solution, which has a pH meter reading of 5.9 in 3+1 v-v ethanol-water solution.

A disodium hydrogen phosphate-citric acid buffer solution, which was initially used, was found to form a white precipitate with mercury(II).

Initial Procedure

The initial study was carried out in 3+1 v-v ethanol-water solution with a concentration of mercury which gave a precipitate weighing approximately 0.65 g.

Three solutions were prepared containing 0.005 M mercury(II), 0.015 M bithionol and 0.05 M sodium acetate-acetic acid buffer in 3+1 v-v ethanol-water solution. The precipitates were collected in number four porosity sintered glass crucibles which had previously been heated at 110° to constant weight. These were then washed with 3+1 v-v ethanol-water solution, ethanol and ether, and heated in an oven at 110°. After each period of heating, the crucibles were cooled in a desiccator for half an hour before weighing. The precipitates did not reach a constant weight.
weight, and the final weighings were all low with regard to both proposed structures for the mercury(II)-bithionol complex. The results obtained are given in Table 8.

<table>
<thead>
<tr>
<th>Heating time (hours)</th>
<th>Weight of mercury(II) added = 0.2180 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight of mercury(II)-bithionol precipitate recovered and percentage</td>
</tr>
<tr>
<td></td>
<td>recovery calculated from proposed structures I and II</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample A</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.5968 g (I 99.0%) (II 95.9%)</td>
</tr>
<tr>
<td>16</td>
<td>0.5901 g (I 97.9%) (II 94.9%)</td>
</tr>
<tr>
<td>17</td>
<td>0.5887 g (I 97.7%) (II 94.6%)</td>
</tr>
<tr>
<td>18</td>
<td>0.5875 g (I 97.5%) (II 94.4%)</td>
</tr>
<tr>
<td>Sample B</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.5897 g (I 97.8%) (II 94.8%)</td>
</tr>
<tr>
<td>16</td>
<td>0.5831 g (I 96.8%) (II 93.7%)</td>
</tr>
<tr>
<td>17</td>
<td>0.5820 g (I 96.6%) (II 93.5%)</td>
</tr>
<tr>
<td>18</td>
<td>0.5807 g (I 96.4%) (II 93.3%)</td>
</tr>
<tr>
<td>Sample C</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.5697 g (I 94.5%) (II 91.5%)</td>
</tr>
<tr>
<td>16</td>
<td>0.5689 g (I 94.4%) (II 91.4%)</td>
</tr>
<tr>
<td>17</td>
<td>0.5683 g (I 94.2%) (II 91.3%)</td>
</tr>
<tr>
<td>18</td>
<td>0.5674 g (I 94.1%) (II 91.2%)</td>
</tr>
</tbody>
</table>
Modified Procedure

To avoid decomposition of the precipitate during drying, a vacuum oven set at 50° was used. The concentration of mercury(II) was reduced to give a precipitate weighing approximately 0.2 g, and the molar concentration of bithionol was reduced to 120 per cent of the new mercury(II) molar concentration. At this concentration of bithionol it is possible to use a lower concentration of ethanol, and 1:1 v-v ethanol-water solution was used as solvent in the subsequent study.

Three solutions were prepared containing 0.004 M mercury(II), 0.0046 M bithionol and 0.05 M sodium acetate-acetic acid buffer in 1:1 v-v ethanol-water solution. The precipitates were filtered in number four porosity sintered glass crucibles, which had previously been heated to constant weight, washed with water and with ethanol, and heated to constant weight. It was observed that the washings were coloured slightly yellow, indicating that the precipitate is slightly soluble in the solvents used. The recoveries obtained are shown in Table 9. These were again low, and showed poor reproducibility.

TABLE 9

<table>
<thead>
<tr>
<th>Weight of mercury(II) added = 0.03721 g</th>
<th>Weight of mercury(II)-bithionol precipitate recovered and percentage recoveries calculated from proposed structures I and II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A 0.2256 g</td>
<td>Sample B 0.2227 g</td>
</tr>
<tr>
<td>I        94.8%</td>
<td>II      91.7%</td>
</tr>
<tr>
<td>Sample C 0.2337 g</td>
<td></td>
</tr>
<tr>
<td>I        96.9%</td>
<td>II      93.8%</td>
</tr>
</tbody>
</table>
The above procedure was repeated but in this case the precipitates were washed with 1+1 v-v ethanol-water solution only. The results obtained are given in Table 10. The recoveries obtained were slightly higher than those obtained previously, but still had poor reproducibility.

**TABLE 10**

<table>
<thead>
<tr>
<th>Weight of mercury(II) added</th>
<th>Weight of mercury(II)-bithionol precipitate recovered and percentage recoveries calculated from structures I and II.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>Sample B</td>
</tr>
<tr>
<td>0.2458 g</td>
<td>0.2370 g</td>
</tr>
<tr>
<td>I 102.6%</td>
<td>II 99.4%</td>
</tr>
<tr>
<td>II 99.4%</td>
<td>II 95.3%</td>
</tr>
</tbody>
</table>

The effect of salting out the precipitate was then examined by increasing the concentration of buffer solution twenty times. This, however, had an adverse effect, and the weight of the precipitate recovered was greatly reduced. Saturated sodium nitrate was then used in place of the increased buffer concentration. Also the procedure was modified slightly, as indicated below, in an attempt to increase both recoveries and precision.

Solutions were prepared containing 0.004 M mercury(II), 0.05 M sodium acetate-acetic acid buffer and approximately 10% v-v saturated sodium nitrate in 1+1 v-v ethanol-water solution. The solutions were heated to 40° and bithionol solution was added dropwise, whilst the solutions were vigorously stirred, until the bithionol concentration
reached 0.0048 M. The precipitates were filtered through number four porosity sintered glass crucibles, which previously had been heated to constant weight. They were washed with water and 1+1 v-v ethanol-water solution, and were heated to constant weight. The recoveries obtained were approximately quantitative for that calculated from structure I, but the reproducibility was very poor with a range of ±6%. The data obtained is given in Table 11.

<table>
<thead>
<tr>
<th>TABLE 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight of mercury(II) added = 0.08721 g</strong></td>
</tr>
<tr>
<td><strong>Weight of mercury(II)-bithionol precipitate recovered and percentage recoveries calculated from structures I and II</strong></td>
</tr>
<tr>
<td>Sample A</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>0.2548 g</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>105.7%</td>
</tr>
<tr>
<td>Sample D</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>0.2250 g</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>93.3%</td>
</tr>
</tbody>
</table>

The above procedure was repeated on further samples but the precipitates were washed with 1+1 v-v ethanol-water solution only. The results obtained are given in Table 12. The recoveries were higher than those obtained previously, being approximately quantitative for that calculated from structure II, and although the reproducibility was improved, it was still poor with a range of ±11%. 

-85-
TABLE 12

Weight of mercury(II) added = 0.08721 g

Weight of mercury(II)-bithionol precipitate recovered and percentage recoveries calculated from the proposed structures I and II.

<table>
<thead>
<tr>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2481 g</td>
<td>0.2511 g</td>
<td>0.2509 g</td>
</tr>
<tr>
<td>I</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>102.9%</td>
<td>99.6%</td>
<td>104.1%</td>
</tr>
</tbody>
</table>

Sample D

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2458 g</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>101.9%</td>
<td>98.7%</td>
</tr>
</tbody>
</table>

Discussion

The reproducibility of recoveries of the mercury(II)-bithionol precipitate, obtained for similar samples, was poor (the best possible precision being ±1% on four samples). Erdey**, however, obtained similar precision with certain sets of samples using the mercury(II) iodate and mercury(I) chloride methods.

The variation of recoveries depended on the solution conditions and wash solutions used, which indicates that experimental conditions are critical. This could be caused by a number of factors, (1) slight solubility of the precipitate in the solvents used, (2) co-precipitation of sodium nitrate, sodium acetate-acetic acid buffer or bithionol, and (3) possible non-stoichiometric 1:1 mercury (II)-bithionol complex formation. Lead also forms a
precipitate with bithionol above a pH meter reading of 3.5 and would be expected to interfere. This study was thus terminated.

It was also not possible to make any final conclusions concerning the structure of the 1:1 mercury(II) - bithionol complex, because of the poor results obtained.

7-2 Titrimetric Determination of Mercury(II) with Bithionol

A number of methods of complexometric titration, in which a precipitate is formed, are in common use. In many of these methods incomplete complex formation, unknown structure and solubility are no problem. Thus, although it was not possible to develop a precise gravimetric procedure for determining mercury(II) with bithionol, the titrimetric methods of determining mercury(II) with bithionol were investigated as follows: (a) direct titration using iron(III) as indicator, (b) conductometric titration, (c) high frequency titration, and (d) pH potentiometric (alkalimetric) titration.

(a) Direct Titration of Mercury(II) with Bithionol

The investigation of metal-ligand stability constants indicated that the pale yellow 1:1 mercury(II)-bithionol complex was more stable than the intense red-brown 1:2 iron(III)-bithionol complex. On titration of a solution containing mercury(II) ions and iron(III) ions in a sodium acetate-acetic acid buffer with bithionol solution, the bithionol would be expected to react preferentially with
mercury(II), and only after the equivalence point should the iron(III)-bithionol complex be formed. Thus a direct titration of mercury(II) with bithionol, using iron(III) as indicator, was studied.

Solutions containing 0.005 M mercury(II), 0.2 M sodium acetate-acetic acid buffer and 0.0001 M iron(III) in 1:1 v-v ethanol-water solution were titrated with 0.01 M bithionol in ethanol solution. The yellow mercury(II)-bithionol precipitate formed, but it was not possible to distinguish the equivalence point accurately. This was because near the equivalence point the colours of the two complexes were indistinguishable.

The iron(III) complex appeared to form just prior to the equivalence point and was probably co-precipitated.

(b) Conductometric Titration of Mercury(II) with Bithionol

Conductometric methods can be used to follow a number of titrations in which a precipitate is formed. This is because during the formation of the precipitate, one pair of ions is substituted for another, so there is little change of conductance until after the equivalence point, when the conductance should rise as excess ions are added.\(^{113}\)

The precipitation reaction of mercury(II) with bithionol was investigated using conductometric titration. Slight irregularities owing to solubility or non-stoichiometric complex formation of the precipitate at the equivalence point should not affect the accuracy of this procedure greatly, because results other than those near the
equivalence point are used to estimate the equivalence point.

When bithionol forms complexes with metal ions, protons are released. These protons have high mobility and produce a marked increase in conductance. It was thought probable that the titration of mercury(II) with bithionol would be followed by measuring the conductance changes caused by the proton release. Most metal impurities present in solution, however, would also cause protons to be released by complexation, and thus interfere. For the procedure to be specific, only the conductance changes caused by the precipitation reaction should be measured. Only lead is known to form a precipitate with bithionol at low pH levels apart from mercury(II).

To prevent interference by the release of protons, buffering the titration solutions was examined. An equimolar sodium acetate-acetic acid buffer solution was used for this purpose, because it gives a pH of approximately 6 in the 3+1 v-v ethanol-water solution used as solvent, and at this pH bithionol is partly ionised and the mercury(II)-bithionol precipitate is formed.

In order to examine the effect of the release of protons on complexation and the addition of buffer solution, titrations were carried out on both unbuffered and buffered solutions.

Reagents and Apparatus

Mercury(II) solution, 0.01 M This solution was prepared by dissolving 0.8566 g of mercuric nitrate (analytical reagent grade) in 250 ml of distilled water containing
the minimum concentration of nitric acid. Bithionol in ethanol solutions (0.1 and 0.01 M) and one molar sodium acetate-acetic acid buffer solution were prepared as described previously.

A Lock Conductivity Bridge, type NCB1 was used to measure the conductance of the solutions. The bridge current is energized by an internal oscillator at 1500 c/s. Any out of balance voltage is amplified and fed to a phase sensitive detector, and a minimum meter reading indicates when the bridge is balanced. The cell constant was 0.45.

Procedure

Solutions containing bithionol (0.001 M) in 50 ml of 1+1 v-v ethanol-water solution were titrated with mercury(II) nitrate solution (0.1 M). After each addition of titrant, the solution was thoroughly stirred, and the conductance measured. The titration was continued after the equivalence point and all measurements were corrected for dilution. The conductance increased with the addition of mercury(II) solution, and continued to increase at a slightly lower rate after the equivalence point. No sharp end point could be detected.

Mercury(II) nitrate solutions (0.001 M) in 100 ml of 1+1 v-v ethanol-water solution were titrated conductometrically with bithionol solution (0.01 M). The conductance, corrected for volume changes, increased linearly to a maximum at the equivalence point and then decreased linearly. This procedure was then repeated with similar solutions containing 0.01 M sodium acetate-acetic acid buffer, and the conductance remained approximately constant

-90-
Mercury(II) nitrate solutions (0.05 M) in 50 ml of 
1+1 v-v ethanol-water solution were titrated conducto-
metrically with bithionol solution (0.01 M). The corrected 
results obtained were similar to those obtained in the 
procedure above, with well defined maxima at the equiva-
\[...\]

Discussion

Although it is possible to determine mercury(II) with 
bithionol using conductometric titration, the signal is 
produced by the release of protons by bithionol on com-
plexation with mercury(II). A signal corresponding to the 
precipitation of the mercury(II)-bithionol complex was not 
observed.

Thus the procedure is non-specific, and most metal 
ions present in solution would interfere. Also the con-
ductance could not be measured very accurately. The proton 
signal was probably reduced to some extent by the precipi-
tation signal.

(c) High Frequency Titration of Mercury(II) with 
Bithionol

In high frequency titrations, conductance is not 
measured, but rather molecular and orientation polariza-
[...] Also external electrodes are used which do not
Fig. 9 Conductometric Titration of Mercury(II) with Bithionol.

Conductance corrected for volume changes

Theoretical equivalence point

Volume of bithionol (ml of 0.01 M)
make contact with the titration solution, and so cannot be fouled by the deposition of precipitate. The precipitation reaction of mercury(II) with bithionol was examined with this technique in an attempt to observe the signal due to the precipitation alone.

**Reagents and Apparatus**

The reagents used were of similar concentration to those used for the conductometric titrations.

A Pungar high frequency titrimeter was used for the titrations. A glass titration cell is located between two external metal ring electrodes, which are connected to a 130 Mc/s oscillating circuit. Changes in the impedance of a solution placed in the titration cell are determined by measuring the grid current of the valve in the oscillating circuit.

**Procedure**

Mercury(II) nitrate solutions (0.0025 M) in 100 ml of 1+1 v-v ethanol-water solutions were titrated with bithionol in ethanol solution (0.01 M). After each addition of titrant, the solutions were thoroughly stirred with a magnetic stirrer, and the impedance measured. The titration was continued after the equivalence point. The meter reading decreased linearly to a minimum at the equivalence point and then increased but at a slightly lower rate.

This procedure was then repeated but with bithionol solution (0.1 M) as titrant, and improved titration curves were obtained. An example of these titration curves is shown in Fig. 10. In both cases, however, no equivalence
Fig. 10 High Frequency Titration of Mercury(II) with Dithirol

Volume of bithionol (ml of 0.1 M)
point was observed (i.e., the meter reading remained approximately constant) when these titrations were repeated with the addition of sodium acetate-acetic acid buffer (0.02 M) to the titration solution.

Discussion

The results obtained from examination of the precipitation reaction of mercury(II) and bithionol with high frequency titration were similar to those obtained previously with conductometric titration. No significant improvement was observed, and so the same conclusions apply.

(d) Alkalimetric Titration of Mercury(II) with Bithionol

In the conductometric and high frequency titrations, the signal in both cases depended on the release of protons on complexation. Thus the alkalimetric titration of mercury(II) with bithionol was briefly examined.

A Pye 290 pH meter was used to measure pH in conjunction with an E.I.L. GHEN 33 E°7 screened glass electrode and a sleeve type saturated calomel reference electrode. It was found necessary to adjust the pH meter reading of the titration solutions to 5.0 in order to obtain a reasonable response on the pH meter to small changes in hydrogen ion concentration. A pH meter reading higher than this could not be used because bithionol has a log $K_{H^+}$ ($pK_{A1}$) value of approximately 5.5 - 6.0 under the solution conditions used.

Bithionol in ethanol solution (0.0065 M) was added slowly to solutions containing 0.001 M mercury(II) in
50 ml 1+1 v-v ethanol-water solution, and the pH meter reading adjusted back to 5.0 with the addition of sodium hydroxide solution, until further addition of bithionol caused no change.

Approximately theoretical results were obtained but the change of pH meter reading, caused by the addition of bithionol solution, was too small to be measured accurately, and so the equivalence point could not be located accurately (approximately ±5%). The procedure involves a change in ethanol concentration of the solution which affects pH meter reading to some extent, and so complicates the titration curve obtained.

It was calculated that molar concentrations of reagents would be required to obtain a change of pH meter reading which could be measured accurately. At this concentration level, however, the precipitate would probably be too large and would interfere with pH measurements. The procedure would not be selective or sensitive, so this work was discontinued.
PART TWO

INVESTIGATION OF METHODS OF SEPARATING AND DETERMINING
O,O'-DIPYROXIDIPHENYL SULPHIDES AND METHANES

CHAPTER EIGHT

A REVIEW OF EXISTING METHODS

8-1 Spectroscopic Methods

(a) Ultra Violet Spectrophotometric Methods

Lord, McAdam and Jones\textsuperscript{115} used ultra violet spectrophotometry to determine hexachlorophene in aqueous alkali solution (0.1 M). The spectrum of this solution has an absorption band in the ultra violet region with a maximum at 320 nm. Similar solutions of dichlorophene and bithionol have absorption bands with maxima at 304 and 328 nm respectively. The maxima are sufficiently wide for identification of a particular bisphenol, but dichlorophene and bithionol would interfere with the determination of hexachlorophene. Although simple substituted phenols absorb less strongly in this region, they too would interfere to some extent. This procedure has been applied to the analysis of hexachlorophene in a number of soaps.\textsuperscript{115} Allowance for irrelevant absorption by the soap was made using the Morton-Stubbs\textsuperscript{116} three point geometric correction. This method is limited in scope because it depends on complete dissolution of the sample of soap in the solvent.

Clements and Newburger\textsuperscript{117} used a series of solvent
extraction and filtration stages to separate hexachlorophene, dichlorophene or bithionol from soaps and cosmetic preparations, prior to ultra violet spectrophotometric determination on solutions of these compounds in alcohol. The procedure is tedious. Any one of these three compounds can be determined in a particular sample but the other two compounds would interfere if present. Any other phenolic compound present would interfere also. The identity of the extracted compound can be established by examination of its ultra violet spectrum. The ultra violet spectra of hexachlorophene, dichlorophene and bithionol in ethanol solution have absorption bands with maxima at 300, 288 and 308 nm respectively. Account is taken of irrelevant absorption of the sample, by subtracting half the sum of both absorbance readings measured at two wavelengths close to the maximum wavelength from that measured at the maximum wavelength, and comparing these values to those obtained at the same wavelengths on a standard solution.

Childs and Parks modified the procedure of Lord, McdAdam and Jones, and developed a differential ultra violet spectrophotometric method of determining hexachlorophene in liquid soaps. A 9+1 v-v ethanol-water solution of the soap containing the hexachlorophene is adjusted to pH 8, and the absorbance of the solution is measured at 312 nm against a blank solution containing the same concentrations of constituents, including hexachlorophene, but adjusted to pH 3 with 0.3 M acetic acid. This procedure is based on the increase in the extinction coefficient of -96-.
hexachlorophene due to ionization at pH 8. This eliminates irrelevant absorption due to the soap base and permits assay of the sample without removal of the soap. Again other phenols interfere.

A simplified extraction procedure has been developed by Hilton\textsuperscript{119} for the analysis of phenolic fungicides, including dichlorophene and hexachlorophene, in mildew-proof materials. The phenol is extracted from the material by aqueous alkali and is extracted subsequently into 2,2,4-trimethylpentane prior to ultra violet spectrophotometric determination. The phenol is identified from its ultra violet spectrum. Other phenols interfere.

The method of Childs and Parks\textsuperscript{118} was applied to the analysis of hexachlorophene and bithionol in solid and liquid soaps, emulsions and dusting powders by Van der Pol\textsuperscript{120}. In order to overcome turbidity with solutions of solid soap, the use of 99+1 v-v methanol-water solution instead of 9+1 v-v methanol-water solution is suggested. Also 0.3 N sodium acetate was used as alkaline reagent, and 0.3 N acetic acid containing 1.5 ml of 4 N hydrochloric acid per litre was suggested as the acid reagent.

Matuszak, Bope and Harris\textsuperscript{121} however, found that the buffer solutions of the Van der Pol method\textsuperscript{120} are not required in the assay of bithionol in liquid soaps. For highly alkaline soaps, the use of 0.5 N hydrochloric acid in 9+1 v-v methanol-water solution is proposed as acid reagent to ensure suppression of the ionization of bithionol. The rapid deterioration of alkaline solutions of bithionol in bright sunlight was also investigated by
these workers. Elvidge and Peutrell suggest the use of tris-(hydroxymethyl)aminomethane-hydrochloric acid in 9:1 v-v methanol-water solution to buffer the sample solutions at pH 8, in the determination of hexachlorophene and other phenols in a number of soaps and cosmetic preparations.

In order to determine hexachlorophene in ointment preparations containing both salicylic acid and methyl p-hydroxybenzoate, Bryant, Mantle and Yoder removed the salicylic acid selectively into pH 8 buffer and saponified the methyl p-hydroxybenzoate. Hexachlorophene was then determined by the 'difference' method of Childs and Parks with no interference from the other components.

The method of determining germicide mixtures in soaps and detergents developed by Jungermann and Beck enables the determination of hexachlorophene, dichlorophene and bithionol in the presence of halogenated salicylanides and halogenated carbanilides. Interfering substances are eliminated by extraction with dimethylformamide followed by evaporation to dryness. The residue is then taken up either in acidic or in basic alcohol. Ultra violet spectra of both solutions can be used to identify the particular compound present, and the absorbance at the maximum used to determine it. Other phenols interfere.

Maody, Kaik and Agrawal used ultra violet spectrophotometric measurements on both chloroform and aqueous alkali solutions to determine bithionol and chloroxylenol in combination. By examination of both spectra, the interference of chloroxylenol on the determination of
bithionol was estimated, and vice versa. Lee and Putnam determined hexachlorophene and chloroxyleneol by extraction with hydrochloric acid and methanol prior to their ultra violet spectrophotometric determination at 296 and 279 nm respectively.

Hexachlorophene has been determined in the presence of zinc phenolsulphonate in aerosol deodorants by Will and Varsel. The hexachlorophene is extracted with sodium hydroxide from chloroform solution and determined spectrophotometrically at 320 nm.

(b) Colorimetric Methods

Gottlieb and Marsh first applied the red colour reaction of phenols with 4-aminoantipyrine, in the presence of the alkaline oxidising agent potassium ferricyanide-sodium carbonate, to the colorimetric determination of phenolic fungicides, including hexachlorophene and dichlorophene. The phenolic fungicide is extracted from fabric with sodium carbonate solution, prior to the addition of the other reagents. The pH of the medium in which the colour is developed must be between 10.4 and 10.6. Minor deviations from these values cause differences in colour and colour stability. Application of this method to the analysis of hexachlorophene, dichlorophene and bithionol in soaps and cosmetic preparations produces unreliable results, possibly owing to interference from co-extracted pigment, perfume or soap constituents. Most other phenols interfere.

Johnson and Savidge investigated the determination of hexachlorophene and dichlorophene in pharmaceutical
preparations using this procedure. They found it necessary to control the pH carefully and an ammonia buffer solution was recommended.

Johnson and Porcaro\textsuperscript{131} separated hexachlorophene from organic matter prior to colorimetric determination with the above method. Organic matter is dried at 30° in a vacuum oven, and hexachlorophene extracted with ethanol. After evaporating this solution to dryness, hydrochloric acid is added to the residue and hexachlorophene is extracted again with chloroform, prior to colorimetric determination using the 4-aminoantipyrine method. This procedure has been adapted for the autoanalysis of hexachlorophene in soap by Hoover, Ginn and Jungermann.\textsuperscript{132}

The 4-aminoantipyrine method was applied to the colorimetric determination of bithionol in the presence of methiotriazsamine in poultry tissues by McGchesney, Koss and Shekosky.\textsuperscript{133} Sugii, Kabasawa, Nagasawa and Tone\textsuperscript{134} also determined bithionol with this method but used ammonium persulphate as oxidant. Acetone, ethanol and tyrosine were found to interfere.

Ashton\textsuperscript{135} found that the extinction coefficient of the sample solutions depended on the concentration of potassium ferri cyanide used with the 4-aminoantipyrine procedure. The use of this reagent also produces high blanks and the colour is unstable. Lower blanks and more stable colour formation can be obtained with the use of potassium periodate as oxidising agent.

Dichlorophene in fabric was determined colorimetrically by Shiraeff\textsuperscript{136} after extraction with chlorox and
sodium hydroxide. A yellow colour is formed. The reagent also extracts most coloured material from the fabric, and so calibration curves are only applicable to a particular fabric.

Iron(III) chloride has been developed for use as a colorimetric reagent for the determination of hexachlorophene in soaps by Larson. Most phenols form a red colour with this reagent and so it is not specific. Hexachlorophene is separated from the soap by precipitating the soap from alcohol solution with barium bromide. This method requires very careful control of temperature and timing of experimental operations. Also the colour fades and this is a severe disadvantage. Tamura and Totani used iron(III) chloride in 1:1 pyridine-water solution to determine hexachlorophene and bithionol in cosmetic cream.

Singer and Stern used 2,6-dibromoquinone chloroimide as a colorimetric reagent for the determination of hexachlorophene and dichlorophene. These compounds are extracted with aqueous alkali and the solutions are buffered at pH 9.5 prior to the addition of the reagent. The extinction coefficient is very sensitive to pH change. This procedure is not specific, as other phenols interfere.

In order to determine hexachlorophene in the presence of methyl salicylate in cosmetic preparations, Achmeteli used methylene blue as the colorimetric reagent. Anionic detergents are removed by washing the chloroform extract of hexachlorophene with water. Aqueous methylene blue solution is then added to the chloroform solution, and the blue coloured species is extracted into the chloroform.
layer. 4-Aminoantipyrine is added to improve the extraction of the coloured species. As this species is probably an ion association complex and the 4-aminoantipyrine would be expected to make the solution slightly basic, this would increase the ionization of hexachlorophene enabling it to form an ion association complex quantitatively. An alternative buffer may be preferable. Other phenols interfere.

Atzeni and Giannessi used bromophenol blue and silver nitrate to determine fenticlor colorimetrically in wines and foods. Both ether extracts and paper chromatograms were analysed.

Both Walker and Klinge have compared certain of the colorimetric and ultra violet spectrophotometric methods already described.

(c) Infrared Spectrophotometric Methods

Phenolic antioxidants including dichlorophene have been determined by near-infrared spectrophotometry by Goddu. The use of the 2.7 to 3.0 micron range for the qualitative and quantitative analysis, and the effects of substitution and intramolecular bonding on the free hydroxyl stretching band are described. It is possible to determine as little as 25 ppm of dichlorophene with this procedure. Certain other phenols interfere.

The infrared spectra of many compounds of pharmaceutical interest including hexachlorophene have been compiled by Sammul, Brannon and Hayden.

(d) Nuclear Magnetic Resonance Method

A rapid high resolution proton nuclear magnetic
resonance procedure has been developed for the determination of hexachlorophene, halogenated carbanilides and halogenated salicylanilides in soaps and detergents by Dietrich and Keller. This procedure can be used to determine the relative concentrations of these compounds in their two component mixtures.

It may be necessary, however, to separate these compounds from the soap or detergent prior to nuclear magnetic resonance analysis.

The proton nuclear magnetic resonance spectra of many o,o'-methylene bisphenols with arsenic trichloride as solvent have been compiled by Szymanski and Bluemle. The compounds studied include o,o'-methylene bisphenol and alkyl and alkyl alcohol derivatives.

B-2 Chromatographic and Ion-exchange Methods
(a) Paper and Thin-layer Chromatographic Methods

The paper chromatographic separation and identification of many phenol derivatives including dichlorophene have been obtained by Re1o. The solvent systems and methods of identification, which were used, are listed.

Mixtures of hexachlorophene and dichlorophene have been separated by ascending paper chromatography and thin-layer chromatography using n-heptane saturated with glacial acetic acid as eluent by Ordenes and Hernandez. A solution of potassium ferricyanide and iron(III) chloride is used to detect both compounds, which appear as blue spots. For quantitative determination of the compounds, the ultra violet absorption curves of the pure products are determined in n-heptane saturated with acetic acid.
Maximum absorbance is found between 285 and 300 nm.

The Rf values of several bisphenols including hexachlorophene and bithionol have been measured using thin-layer chromatography by Lienert and Jahn. Silica gel G suspended in 0.1 N hydrochloric acid was used as the stationary phase and benzene was used as eluent.

Shimizu separated and identified a number of bis-alkyl phenols used as antioxidants in lubricating oils by partition paper chromatography. These compounds included alkyl derivatives of \(\omega,\omega^1\)-dihydroxydiphenyl sulphides and methanes. \(\alpha\)-cetane, liquid-paraffin or chloronated paraffin was used as the stationary phase and 3+2 or 4+1 v-v ethanol-water solution was used as the mobile phase.

Hexachlorophene has been separated from quaternary ammonium compounds with thin-layer chromatography by Henry, Jacobs and Achmeteli. Various mixtures of ethanol, chloroform and water were used as the eluent and silica gel was used as the stationary phase. Bromthymol blue and methyl red in ethanol were used to detect the components.

Mixtures of hexachlorophene, dichlorophene, bithionol and 4-hexylresorcinol have been separated using two way thin-layer chromatography by Inoue and Juniper. Silica gel was used as the stationary phase and the plates were developed firstly in \(n\)-heptane saturated with formic acid and, then at ninety degrees to the first development with toluene saturated with formic acid. The components were located with aqueous potassium ferricyanide-iron(III) chloride solution.

-104-
(b) Liquid - liquid Chromatography

Derry, Holden and Newburger\textsuperscript{183} developed a liquid-liquid partition chromatographic procedure to separate mixtures of hexachlorophene, dichlorophene and p-hydroxybenzoates, prior to their determination by ultra violet spectrophotometry.

A chromatographic column is prepared, the lower half of which contains silane treated Celite in 1+1 v-v n-heptane-chloroform, and the upper half contains these constituents plus the sample, which is applied as a solution in 1+1 v-v n-heptane-chloroform. The column is eluted with 1+9, 7+15 and 3+2 v-v ethanol-water solutions and the fractions collected and analysed. The hexachlorophene sample may be contaminated with dichlorophene. This can be recognised from the shape of the ultra violet spectrum and the extent of contamination estimated by measuring other absorption bands.

(c) Ion-Exchange Separation

Suffis and Dean\textsuperscript{184} separated hexachlorophene and bithionol from soap or soap based preparations by ion-exchange, prior to their determination by ultra violet spectrophotometry. Chlorinated phenols are stronger acids than non-chlorinated phenols, carboxylic acids and the soap base, and so can be separated from the remainder on a strongly basic anion-exchange resin. The soap base and other less acidic compounds are eluted from the ion-exchange column with methanol saturated with sodium chloride, and the more acidic compounds are then eluted with 9+1 methanol-concentrated hydrochloric acid solution.
attempt appears to have been made to separate specific o,o'-dihydroxydiphenyl sulphides or methanes from mixtures of these compounds.

(a) Gas-liquid Chromatography

Hexachlorophene, dichlorophene, bithionol and fentinol have been separated and determined by Porcaro using gas-liquid chromatography with a flame ionization detector. Quarter inch outside diameter by twelve inch glass or eight inch copper columns were used. These were packed with 10% DC-710 silicone oil on Chromport XXX-mesh solid support, and maintained at 225° or 250°. Helium was used as carrier gas and the sample was injected either as a solid sample or dissolved in a suitable solvent. Rapid separation and determination was obtained. The procedure was applied to cosmetic preparations.

An alternative gas chromatographic procedure was developed for the analysis of hexachlorophene in soap by Wisniewski. Hexachlorophene is extracted and converted to a trimethyl silyl ether derivative, and is then determined by gas-liquid chromatography using a flame ionization detector with dioctyl phthalate as internal standard.

8-3 Electrical Methods

(a) Potentiometric Titration

Pure samples of hexachlorophene or bithionol can be conveniently determined by pH potentiometric titration with standard sodium hydroxide solution. Phenols and other acidic compounds interfere.

-106-
(b) Polarographic Method

A number of phenolic compounds which have antioxidant properties have been investigated with anodic polarography at the wax-impregnated graphite electrode by Nash, Skauen and Purdy.\textsuperscript{158} The polarograms were obtained in 1+1 v-v ethanol-water solution at a pH meter reading of approximately 7.1 and a temperature of 25°. Hexachlorophene, dichlorophene and bithionol have half wave potentials of 0.406, 0.452 and 0.457 volts when measured against a saturated calomel electrode.

8-4 Methods Based on the Determination of Chlorine

Jenkins, Waters and Beal\textsuperscript{159} determined the chlorine content of dichlorophene. The sample was oxidised with potassium permanganate and the excess potassium permanganate was reduced with potassium nitrite. The chlorine content was then determined either by Volhard's method\textsuperscript{38} (by the addition of silver nitrate and titration of the excess), or by turbidimetric titration.

Carrero and Ossorio\textsuperscript{160} determined hexachlorophene or dichlorophene by alkali fusion in the presence of an oxidant followed by the determination of the chloride content with silver nitrate as above. The results obtained for dichlorophene were inaccurate and this was thought to be because of tetrachlorophene impurity. Other chlorine compounds present in solution interfere.
CHAPTER NINE

THE USE OF ACID DISSOCIATION CONSTANTS IN SELECTING BUFFERS TO OPTIMISE THE ELECTROPHORETIC SEPARATION OF BITHIONOL, FENTICLOR, HEXACHLOROPHANE, BROMOCHLOROPHANE, DICHLOROPHENE, TETRACHLOROPHENE AND 4-HEXYLRESORCINOL.

9-1 Introduction

In the majority of procedures developed for the determination of o,o'-dihydroxydiphenyl sulphides and methanes, difficulty is experienced in distinguishing between individual compounds, and in avoiding interference from similar compounds. Chromatographic procedures for the separation of these compounds prior to their determination have been described. Mixtures of bithionol, hexachlorophane, dichlorophene and 4-hexylresorcinol have been separated by two-way thin-layer chromatography, and mixtures of bithionol, fenticlor, hexachlorophane and dichlorophene have been separated and determined by gas-liquid chromatography with a flame ionization detector.

The present chapter describes an investigation into the electrophoretic separation of several o,o'-dihydroxydiphenyl sulphides and methanes and also 4-hexylresorcinol, which is found in certain cosmetic products as an alternative to o,o'-dihydroxydiphenyl sulphides and methanes. As these compounds are dibasic acids, their electrophoretic mobility will increase with their degree of ionization. A knowledge of their acid dissociation constants ($K_a$), therefore, is of value in selecting suitable buffer solu-
tions in order to optimise their electrophoretic separation. Acid dissociation constants rather than association constants are used in this chapter for convenient interpretation of acid-base titration curves. \((\log K_{H^+} = -\log K_N)\)

The effect of size on the electrophoretic separation of these compounds was not investigated in this study because they are all relatively similar in size.

The procedure for determining acid dissociation constants described in chapter four, involved the use of constant ionic background (molar sodium perchlorate) and careful temperature control, titration technique and pH measurement. Dissociation constants of sufficient accuracy for the present purpose may be obtained by a simplified procedure which is described later.

The value of the dissociation constant is affected considerably by the ionic strength of the solution. In the present electrophoretic study, buffer solutions of approximately 0.1 M concentration were used, and therefore the dissociation constants used were determined in an ionic background of 0.1 M sodium perchlorate. 3+1 v-v ethanol-water solution was also used in the present electrophoretic study, owing to the low water-solubility of o,o'-dihydroxydiphenyl sulphides and methanes, and so the dissociation constants were determined in this solvent.

In order to investigate the validity of using acid dissociation constants to select buffers to optimise electrophoretic separation, the separation of bithionol, fenticlor and hexachlorophene was studied initially at a number of pH meter readings. All seven compounds were
then separated at the optimum pH meter readings.

9-2 Determination of Acid Dissociation Constants

Reagents

Sample solution, 0.0065 M solution in ethanol. Halogenated o,o'-dihydroxydiphenyl sulphide or methane, or 4-hexylresorcinol (0.0065 mole) was dissolved in ethanol (B.P. grade) and the solution diluted to 100 ml with ethanol.

Standard sodium hydroxide solution, 0.1 M in 3+1 v-v ethanol-water solution. This was prepared from a BDH concentrated volumetric solution by appropriate dilution.

Sodium perchlorate solution, 0.4 M and 4 M solution in water. This was prepared from analytical reagent grade sodium perchlorate and was filtered before use.

Procedure

Sample solution in ethanol (37.5 ml) and sodium perchlorate solution (12.5 ml) were added to a 100 ml glass titration vessel and titrated with standard sodium hydroxide solution (added from a 10 ml burette). The titration was followed potentiometrically using a Pye 290 pH meter, a pH responsive glass electrode (E.I.L. CHSE 33 E°7) and a saturated calomel reference electrode.

The titration curves for the o,o'-dihydroxydiphenyl sulphides and methanes studied here and 4-hexylresorcinol are shown in Fig. 11.

Estimation of Acid Dissociation Constants

The method of interpolation at half $\bar{n}$ values has been used for the estimation of approximate acid dissociation.
Figure 11: pH Potentiometric Titration Curves

Sodium hydroxide solution (ml of 0.1 M)
constants. It is possible to use this method because the two hydroxyl ions in the compounds studied react with protons in two quite separate pH regions. Thus it may be assumed that in the region where the first proton reacts only \( H_A^+ \) and \( HA^- \) are present, and in the region where the second proton reacts only \( HA^- \) and \( A^- \) are present.

### TABLE 13

**Acid Dissociation Constants in 3:1 v-v Ethanol-water Solution** (All values based on pH meter readings)

<table>
<thead>
<tr>
<th>Compound</th>
<th>( pK_{A1} )</th>
<th>( pK_{A2} )</th>
<th>( pK_{A1} )</th>
<th>( pK_{A2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexachlorophene</td>
<td>5.6</td>
<td>12.4</td>
<td>5.4</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>5.39 ± 0.03</td>
<td>12.20 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bithionol</td>
<td>5.9</td>
<td>10.5</td>
<td>5.65</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>5.64 ± 0.02</td>
<td>9.60 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bromchlorophene</td>
<td>6.4</td>
<td>12.3</td>
<td>6.1</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>6.09 ± 0.02</td>
<td>11.81 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrachlorophene</td>
<td>6.75</td>
<td>12.2</td>
<td>6.5</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>6.46 ± 0.03</td>
<td>11.70 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenticlor</td>
<td>8.4</td>
<td>11.8</td>
<td>8.2</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>8.14 ± 0.02</td>
<td>11.01 ± 0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 13 CONTINUED

<table>
<thead>
<tr>
<th></th>
<th>In 0.1 M a sodium perchlorate</th>
<th>In 1 M a sodium perchlorate</th>
<th>In 1 M b sodium perchlorate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dichlorophene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK_{A1}$</td>
<td>9.1</td>
<td>8.8</td>
<td>$8.78 \pm 0.03$</td>
</tr>
<tr>
<td>$pK_{A2}$</td>
<td>12.5</td>
<td>11.9</td>
<td>$\approx 12.8$</td>
</tr>
<tr>
<td><strong>4-Hexylocrocinol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK_{A1}$</td>
<td>11.6</td>
<td>11.2</td>
<td>$11.17 \pm 0.05$</td>
</tr>
<tr>
<td>$pK_{A2}$</td>
<td>12.6</td>
<td>12.0</td>
<td>$\approx 13.4$</td>
</tr>
</tbody>
</table>

(a) Obtained by the method described in this chapter.

(b) Obtained by the more accurate method described in chapter 4.

The values of $pK_{A1}$ and $pK_{A2}$ obtained for bithionol without the addition of sodium perchlorate were 6.2 and 11.5 respectively.

At the first half neutralised point on the titration curve $[HA^-] = [H_2A]$, $\overline{\text{pH}} = \frac{1}{2}$ and $pK_{A1} = \text{pH} \ (\text{at} \ [HA^-] = [H_2A]).$

Similarly at the half neutralised point of the second proton, $[A^{2-}] = [HA^-]$, $\overline{\text{pH}} = \frac{1}{2}$ and $pK_{A2} = \text{pH} \ (\text{at} \ [A^{2-}] = [HA^-]).$

In order to examine the accuracy of the titration procedure and method of calculation used in the present study, approximate acid dissociation constants were determined in molar sodium perchlorate and compared with the constants determined by the more accurate method described.
in chapter four. An approximate value for the acid dissociation constant of bithionol was also determined with no ionic background added, so that the effect of ionic strength in lowering the value of acid dissociation constants could be illustrated.

The values of \( pK_{A1} \) and \( pK_{A2} \) of the \( o,o' \)-dihydroxydiphenyl sulphones and methanes studied here and 4-hexylresorcinol are given in Table 13. From these results, it can be seen that reasonably accurate \( pK_a \) values can be determined with the procedure described in this chapter.

All pH values quoted in this chapter are pH meter readings, and the values of dissociation constants refer to 3+1 v-v ethanol-water medium at the particular ionic strength indicated.

9-3 Electrophoresis

Cellulose acetate membranes (Sartorius Membranfilter GmbH, 17 x 5 or 2.5 cm) were used in conjunction with a Gelman electrophoresis tank having 10 cm between supports. A Shandon Voken stabilised power supply was operated in the constant current mode at 0.1 - 0.2 mA per cm width and at an initial setting of 400 volts. Buffer solutions (approx. 0.1 M in 3+1 v-v ethanol-water) were prepared from sodium chloride-hydrochloric acid, sodium acetate-acetic acid, ammonium acetate-ammonium hydroxide, and glycine-sodium chloride-sodium hydroxide. The pH of each buffer solution was measured with a Pye 290 pH meter and combined glass-calomel electrode. Minor adjustments to the pH were made where necessary by the addition of small amounts of
strong acid or base. The membranes did not stretch in these buffer solutions.

Aliquots of the compounds studied were applied to the membranes by the microscope cover slip technique 5 cm from the cathode end of the membrane. During the electrophoresis period, of up to a maximum of three and a half hours, the voltage dropped slightly to about 390 volts. When the current was limited to 0.1-0.2 mA per cm width and approximately 400 volts, evaporation of solvent from the membranes did not exceed an acceptable level.

After electrophoresis the compounds were located by immersing the membrane in a freshly prepared aqueous solution of iron(III) nitrate and potassium ferricyanide. The membrane was then washed with water and dried at room temperature. The compounds developed as various shades of blue, which were readily detectable against the white background of the cellulose acetate strip.

2.4 Results and Discussion

(a) Electrophoretic Separation of Bithionol, Fenticlor and Hexachlorophene

From examination of the titration curves shown in Fig. 11, buffer solutions of pH meter reading 5.6 and 6.3 were thought to be most suitable for the electrophoretic separation of bithionol, fenticlor and hexachlorophene. Buffer solutions of pH meter reading 6.8 and 11.0 were thought to be suitable for the separation of at least one of these compounds from the other two, and with buffer solutions at pH meter reading 3.1 and 12.3 no separation
was expected.

The results obtained are given in Table 14.

All three compounds were separated from buffer solutions of pH meter reading 5.6 and 6.3. The latter buffer solution produced a better separation, because the ratio of concentrations of singly charged to uncharged species, and hence the distances moved by bithionol and hexachlorophene are greater. Fenticlor and hexachlorophene were separated from the other two components in buffer solutions of pH meter reading 8.8 and 11.0 respectively. The other buffer solutions examined were not expected to effect electrophoretic separation of bithionol, fenticlor and hexachlorophene, and in fact did not do so.

**TABLE 14**

**Electrophoretic Separation of Bithionol, Fenticlor and Hexachlorophene**

<table>
<thead>
<tr>
<th>pH meter reading</th>
<th>Length of run (minutes)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>150</td>
<td>Bithionol, fenticlor and hexachlorophene all moved 0.4 cm. No separation</td>
</tr>
<tr>
<td>5.6</td>
<td>195</td>
<td>Bithionol, fenticlor and hexachlorophene moved 1.0, 0.5 and 1.55 cm respectively. Good separation.</td>
</tr>
<tr>
<td>6.3</td>
<td>105</td>
<td>Bithionol, fenticlor and hexachlorophene moved 2.2, 1.1 and 2.8 cm respectively. Good separation.</td>
</tr>
</tbody>
</table>
### TABLE 14 CONTINUED

<table>
<thead>
<tr>
<th>pH meter reading</th>
<th>Length of run (minutes)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.8</td>
<td>75</td>
<td>Bithionol, fenticlor and hexachlorophene moved 2.0, 1.0 and 2.1 cm respectively. Separation of fenticlor from bithionol and hexachlorophene.</td>
</tr>
<tr>
<td>11.0</td>
<td>90</td>
<td>Bithionol, fenticlor and hexachlorophene moved 3.3, 3.3 and 2.5 cm respectively. Separation of hexachlorophene from bithionol and fenticlor.</td>
</tr>
<tr>
<td>12.3</td>
<td>75</td>
<td>Bithionol, fenticlor and hexachlorophene moved 3.5, 3.6 and 3.4 cm respectively. No separation.</td>
</tr>
</tbody>
</table>

(b) **Electrophoretic Separation of Hexachlorophene, Bithionol, Bromchlorophene, Tetrachlorophene, Fenticlor, Dichlorophene and 4-Hexylresorcinol.**

From an examination of the titration curves shown in Fig. 11, it was considered that a buffer solution of pH meter reading 6.2 would be most suitable for the electrophoretic separation of hexachlorophene, bithionol, bromchlorophene, and tetrachlorophene from each other and from the other compounds. Good electrophoretic separation was effected at this pH, but from an examination of the developed membrane and the titration curves, it seemed possible that the optimum separation could be effected at
the slightly higher pH meter reading of 6.4. Optimum separation was in fact obtained at this pH.

Similarly, for the electrophoretic separation of fentinol, dichlorophene and 4-hexylresorcinol, a buffer solution of pH meter reading 8.9 was selected. This proved to give optimum separation. The results of the electrophoretic runs at pH meter reading 8.9 and 6.4 are given in Table 15.

In the above separations, the optimum pH meter reading in each case proved to be 0.2 higher than the mean pKₐ values of the particular group of compounds. Thus, for hexachlorophene, bithionol, bromchlorophene and tetra-chlorophene, the mean pKₐ value is 6.2 and the optimum pH meter reading for separation is 6.4. Similarly, the mean pKₐ value of fentinol and dichlorophene is 8.7, and the optimum pH meter reading for separation is 8.9. Electrophoresis at these slightly higher pH meter reading values is necessary in order to obtain slightly greater movement of the less ionized compounds.

At all pH values studied in the present work, the electrophoresis zones were well defined with no evidence of absorption. This can be seen from the illustration of the developed membranes, run at pH meter reading 8.9 and 6.4, shown in Fig. 12.

The limit of detection of the compounds is determined by the weakest colour intensity obtained with a particular staining technique. The volume of solution applied to the membrane from an 18 mm microscope cover slip is approximately 3 - 3.5 µl, and this volume of a 0.002 M solution
Fig. 12 Full Scale Illustration of the Developed Membranes

1. Hexachlorophene
2. Bithionol
3. Bromchlorophene
4. Tetrachlorophene
5. Fenticon
6. Dichlorophene
7. 4-Hexylresorcinol
8. Mixture of all seven components

pH meter reading 6.4

pH meter reading 8.9
of hexachlorophene, the weakest coloured of the stained compounds, may be detected when stained with iron(III) nitrate and potassium ferricyanide solution.

**TABLE 15**

Results of the Electrophoresis of All Seven Compounds

<table>
<thead>
<tr>
<th></th>
<th>pH 6.4</th>
<th>pH 8.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexachlorophene</td>
<td>5.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Bithionol</td>
<td>4.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Bromochlorophene</td>
<td>2.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Tetrachlorophene</td>
<td>1.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Fenticlor</td>
<td>0.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Dichlorophene</td>
<td>0.7</td>
<td>2.5</td>
</tr>
<tr>
<td>4-Hexylresorcinol</td>
<td>0.7</td>
<td>0.5</td>
</tr>
</tbody>
</table>

(c) General Discussion

The use of acid dissociation constants in selecting buffer solutions to optimise electrophoretic separation of particular compounds is illustrated by reference to the compounds separated in the present study. The \( pK_a \) values given in Table 13 were used to select buffer solutions of suitable pH to effect their optimum electrophoretic separation, the results of which are given in Tables 14 and 15. When titration data is available the selection of buffers can be made more conveniently directly from the titration curves (Fig. 11). Alternatively, the formation curves \( \ln[H] / \text{combined proton concentration/total reagent concentration} - \text{versus pH} \) can be calculated from the values of the dissociation constants determined at the
correct ionic strength. Provided that the two protons in each compound are ionized in quite separate pH regions, the following approximate formulae are valid.

\[ \frac{1 + 2 \text{antilog}(pK_{A1} - \text{pH})}{1 + \text{antilog}(pK_{A1} - \text{pH})} \text{ for } \text{pH} = pK_{A1} \pm 1 \]

and

\[ \frac{\text{antilog}(pK_{A2} - \text{pH})}{1 + \text{antilog}(pK_{A2} - \text{pH})} \text{ for } \text{pH} = pK_{A2} \pm 1 \]

For routine use in electrophoretic work, the prior determination of acid dissociation constants (if these are not already known) can in many cases effect a considerable saving of time, and because the pH data can be used in conjunction with the electrophoretic data, identification is simplified and made more certain. It is assumed throughout that specific absorption effects and strong ion-association with ions in the buffer solution are absent, as was the case in the present study.
CHAPTER TEN

PHOTOMETRIC STUDIES ON THE DETERMINATION OF BITHIONOL
WITH IRON(III)

10.1 Attempted Colorimetric Determination of Bithionol
with Iron(III)

In chapter six, the colorimetric determination of iron(III) using bithionol as reagent is described. Excess bithionol and one molar sodium acetate-acetic acid buffer solution were used in 1+1 v-v ethanol-water solution. Under these conditions the intense red-brown coloured 1:2 iron(III)-bithionol complex is formed. The spectrum of the complex contains a single large absorption band in the visible region with a maximum at 484 nm. The colorimetric determination of iron(III) was then improved with the use of solvent extraction.

Larson \(^{139}\) developed a method of determining hexachlorophene using iron(III) chloride as reagent. With this method, the timing of operations is critical, and it is necessary to make measurements after a fixed time interval, to within \(\pm\) 10 seconds. The use of iron(III) chloride to determine both hexachlorophene and bithionol colorimetrically in 1+1 v-v pyridine-water solution \(^{137}\) would be expected to show the same disadvantages for the determination of hexachlorophene as Larson's method. \(^{139}\)

Also the pH of 1+1 v-v pyridine-water solution is too high (approximately pH meter reading 10.5) for the quantitative colorimetric determination of bithionol.
The extinction coefficient of the iron(III)-bithionol complex is of the same order as that of the iron(III)-hexachlorophene complex, and from the earlier studies the 1:2 iron(III)-bithionol complex would appear to have greater stability. Thus the formation of the iron(III)-bithionol complex was studied in an attempt to develop an improved colorimetric method of determining bithionol based on this reaction. Iron(III), being the reagent, would thus be present in excess.

Procedure

Solutions containing 0.000012 M to 0.00024 M (approximately 0.6 to 13 ppm) of bithionol, 0.012 M iron(III) and 0.1 M sodium acetate-acetic acid buffer were made up to 50 ml in 1:1 v-v ethanol-water solution. The bithionol was added in ethanol solution, and the iron(III) was added as a solution of ammonium ferric sulphate (analytical reagent grade) in dilute hydrochloric acid. Slight turbidity appeared in these solutions and was probably caused by iron(III) precipitating out of solution. The ethanol concentration of the solutions was thus reduced and the above solutions prepared in 2:3 v-v ethanol-water solution. These solutions were clear, and so their absorbances were measured, against a water blank, using the Hilger ultraviolet and visible spectrophotometer. For convenience water was used in place of 2:3 v-v ethanol-water solution for the blank. Both solvents are colourless. The absorbance readings on duplicate samples showed poor reproducibility. Also, when these readings were compared with those obtained with the previous studies on the determina-
tion of iron(III), for equivalent concentrations the readings were approximately 74% of that expected.

In an attempt to increase the intensity of the colour formed, similar solutions were prepared, and the iron(III)-bithionol complex extracted with 8, 4 and 3 ml quantities of chloroform. The extracts were then made up to 50 ml with ethanol and their absorbances measured. These were found to be increased but still only equivalent to 79% of that expected from the previous work.

It was noted that the colour of the extracts were orange rather than red. This was probably caused by the partial extraction of the yellow-orange coloured iron(III) acetate or chloride. In an attempt to limit this effect, solutions were prepared containing the same concentrations of constituents as above, with the exception that the iron(III) concentration was reduced to 0.0024 M. The solutions were again extracted with 8, 4 and 3 ml quantities of chloroform and made up to 50 ml. Their absorbances were then measured. The colour of the extracts were less yellow, but the absorbances were lower, being approximately 64% of that expected.

Spectra of some of the above solutions were measured and compared with those obtained in the previous work on the determination of iron(III), in which bithionol was present in excess. The spectra were similar and all had single absorption bands in the visible region with maxima at 484 nm. There were, however, very slight shoulders on the spectra which could be attributable to the yellow colour.
The 1:2 iron(III)-bithionol complex would be expected to have a single net negative charge per molecule in solution. As it was found possible to extract the complex into organic solvent, however, the complex must be neutral. This is possible if each molecule of complex reacts with a single proton. An attempt was thus made to increase the intensity of the colour produced by the addition of large positively charged onium compounds. Small amounts of diphenyliodonium chloride and tetraphenylarsonium chloride were added to solutions containing the original concentrations of constituents in 2+3 v-v ethanol-water solution, prior to extraction.

The absorbance readings were slightly more reproducible and were higher, but they were still only equivalent to 81% and 82% respectively of those expected.

Discussion

The increase in absorbance readings and their reproducibility on the addition of the onium compounds prior to extraction of the iron(III)-bithionol complex into chloroform indicates that the methods of Larson, and of Tamura and Totani can be improved by this procedure and in particular by the addition of tetraphenylarsonium chloride.

The absorbance readings obtained with these solutions are low, however, when compared with those obtained with solutions containing excess bithionol. Also the reproducibility obtained on duplicate samples using the improved method is still low, indicating that the reaction conditions remain critical.
If the reaction conditions, especially time, were very carefully controlled, then acceptable results could probably be obtained. In this form, however, the procedure would not be suitable for routine analytical use. Thus the work was discontinued.

In the presence of excess iron(III), it is probable that the 1:2 iron(III)-bithionol complex is not completely formed, and that the colourless or weakly coloured 1:1 iron(III)-bithionol complex is partly formed. This explanation is supported by the increase in colour intensity on the addition of the large positively charged onium compounds. The 1:1 complex would be expected to have a single net positive charge per molecule, and so only the negatively charged 1:2 complex would be expected to react with the onium compounds.

10-2. Photometric Titration of Bithionol with Iron(III)

The previous section described the attempted colorimetric determination of bithionol with iron(III), which gave low and erratic results. In an attempt to circumvent this difficulty, a photometric titration study was made of the reaction. Solutions containing iron(III) were titrated with bithionol solution, so that an excess of bithionol was present after the equivalence point, in which case the 1:2 iron(III)-bithionol complex should be completely formed.

Procedure

The instrument used for the photometric titrations was the EEL titrator. This employs a series of glass
filters to select the required wavelengths. Light is focused through the sample, and it then passes through the filter before being measured by a barrier layer photocell. The signal is recorded on a galvanometer. The filter selected had a transmission maximum at 490 nm.

Three solutions containing 0.000063 M iron(III) and 0.1 M sodium acetate-acetic acid buffer in 30 ml of 2+1 v-v ethanol-water solution were separately titrated with 0.005 M bithionol in ethanol solution using the EEL titrator. The galvanometer readings were corrected for volume changes, and plotted against the volume of bithionol solution added. An example of the titration curves is shown in Fig. 13. The three curves were similar, but showed equivalence points which were much higher than that expected from the concentrations used (i.e. approximately six times the theoretical volume of bithionol was required).

Similar titrations were repeated using three solutions containing 0.000042 M iron(III) and 0.3 M sodium acetate-acetic acid buffer in 2+1 v-v ethanol-water solution. Similar high results were obtained.

The pH meter readings of the above solutions were checked before and after the titrations. They were found to have changed from 5.7 to 6.0, as would be expected from the change of ethanol concentration. This change of pH meter reading is not large enough to affect the formation of the 1:2 iron(III)-bithionol complex.

Fresh standard solutions were prepared, and the titrations carried out with two solutions containing 0.000067 M iron(III) and 0.2 M sodium acetate-acetic acid.
Fig. 13 Photometric Titration of Iron(III) with Bithionol
buffer in 30 ml of 2+1 v-v ethanol-water solution with 0.001 M bithionol in ethanol solution as titrant. These titrations were then repeated with two solutions containing 0.000033 M iron(III). With both sets of solutions, equivalence points were observed at approximately three times the theoretical amounts of bithionol.

Discussion

This work was discontinued because of the high and erratic equivalence points obtained from the titrations.

The probable explanation for these results is again the incomplete 1:2 iron(III)-bithionol complex formation in the presence of excess iron(III). The absorbance readings during the early part of the titration were low, probably because of iron(III) being present in excess. Complete 1:2 complex formation and subsequent maximum absorbance was not reached until there was a sufficient excess of bithionol.
CHAPTER ELEVEN

ANODIC POLAROGRAPHY OF BITHIOCOL IN WHICH A MERCURY(II)-BITHIOCOL PRECIPITATE IS FORMED AT THE DROPPING MERCURY ELECTRODE

11-1 Introduction

In the presence of inorganic ions which form sparingly soluble salts with mercury (e.g. chloride, hydroxyl, sulphide and hydrogen sulphide ions) anodic polarographic waves are produced. The anodic polarization of the dropping mercury electrode produces mercury(I) and mercury(II) ions, which react with suitable anions and precipitate. This precipitation reaction disturbs the electrode equilibrium and the anodic current increases, for additional mercury ions are produced in an attempt to maintain equilibrium. When a supporting electrolyte is present the reaction becomes diffusion controlled. The current is governed by the diffusion of anions towards the dropping mercury electrode surface, and the limiting current is proportional to the anion concentration. The half wave potential also is a function of the concentration of the anion.

The anodic polarography of certain organic compounds which form sparingly soluble mercury complexes have been investigated, in particular thiol compounds, derivatives of urea and thiourea. In most of these cases the anodic waves are distorted by absorption effects, but these waves are used for analysis.
In chapter seven the attempted gravimetric determination of mercury(II) with bithionol is described. This was unsuccessful owing to the low precision obtained. In this chapter the investigation into the formation of the mercury(II)-bithionol precipitate by anodic polarography, in an attempt to develop a method for determining bithionol, is described. This procedure was expected to be specific for those compounds forming a precipitate with mercury under the particular pH conditions used.

11-2 Apparatus

A Cambridge Pen Writing Polarograph was used in the normal mode in conjunction with a dropping mercury electrode, and either a mercury(I) sulphate or saturated calomel reference electrode.

The mercury(I) sulphate reference electrode was prepared by adding a small amount of solid mercury(I) sulphate (about 10-20 mg) to the surface of a mercury pool electrode. Molar sodium sulphate solution containing approximately 0.1 M sulphuric acid to suppress hydrolysis was then added. Electrical contact was maintained through a capillary side arm of the reference electrode, which also contained some of the sodium sulphate solution. A diagram of the polarographic cell containing this reference electrode is shown in Fig. 14.

The saturated calomel reference electrode was prepared by covering a mercury pool electrode with saturated potassium chloride solution. In this case electrical contact was maintained by a salt bridge filled with a
Fig. 14 Anodic Polarographic Cells
saturated solution of potassium nitrate. A diagram of the polarographic cell containing this reference electrode is also shown in Fig. 14.

11-3 Experimental Procedure

For all the anodic polarographic investigations described in this chapter, the following general conditions were observed. The polarographic cell was immersed in a water bath thermostated at a temperature of 25\(^\circ\) and the height of the mercury reservoir was adjusted to give a drop time of approximately 3 seconds. Before each polarographic run, the dropping mercury electrode and the two sintered glass ends of the salt bridge or capillary side arm were thoroughly washed with water and with ethanol, and the solution to be polarographed was deoxygenated by bubbling B.O.C. oxygen-free nitrogen through the solution for at least twenty minutes. After deoxygenation the cell was closed to prevent air re-entering. The solutions were then polarographed from -0.8 to +0.8 volts, the degree of sensitivity and damping being selected depending on the concentration of reagents. The counter current and compensating current circuits were not used.

An initial examination of the formation of the mercury(II)-bithionol precipitate at the dropping mercury electrode was made using the polarographic cell with the mercury(I) sulphate reference electrode. Erratic and unreproducible results were obtained. This was found to be due to a precipitate forming at the junction of the reference electrode capillary tube with the sample solu-
tion.

A saturated calomel reference electrode, in which electrical contact was maintained through a saturated sodium nitrate salt bridge, was used in further studies. The saturated calomel electrode was preferred to the mercury(I) sulphate electrode for use with the salt bridge owing to the ease of preparation and the ease of cleaning the sintered glass of the salt bridge.

Both aqueous and ethanol-water solutions of bithionol were used in the anodic polarographic investigations. The aqueous solutions were prepared by dissolving the bithionol either in sodium hydroxide solution, or in the minimum of sodium hydroxide solution and adding this solution to borax or sodium acetate-ammonium acetate buffer solutions. When aqueous 0.0005 M bithionol solution containing the minimum sodium hydroxide was added to potassium hydrogen phthalate or equimolar sodium acetate-acetic acid buffer solutions, the bithionol precipitated. Hence aqueous solutions were only used above pH 8.

The following solutions were polarographed using the conditions described. Basically these solutions were either of pH 4 - 7 in ethanol-water solution or aqueous solutions of pH 8 - 12. The mercury(II)-bithionol precipitate was found to form at high pH levels in aqueous solution, and so the aqueous solutions were polarographed in an attempt to reduce the solubility of the precipitate compared to the ethanol-water solutions. In each case a blank solution, containing all the reagents except bithionol, was also polarographed.
1. Bithionol 0.002 to 0.0001 M, 0.1 M sodium nitrate and 0.005% gelatin in 1+1 v-v ethanol-water solution.

2. Bithionol 0.002 to 0.0005 M, 0.03 M sodium acetate-acetic acid buffer and 0.005% gelatin in 1+2 v-v ethanol-water solution.

3. Bithionol 0.0002 M, 0.03 M sodium acetate-acetic acid buffer, 0.1 M sodium nitrate and 0.005% gelatin in 1+2 v-v ethanol-water solution.

4. Bithionol 0.0025 M and 0.05 M sulphuric acid in 1+1 v-v ethanol-water solution.

5. Bithionol 0.002 to 0.0005 M, 0.01 M sodium hydroxide, 0.05 M sodium nitrate and 0.005% gelatin in aqueous solution.

6. Bithionol 0.002 to 0.00001 M, 0.02 M borax, approximately 0.005 M sodium hydroxide and 0.005% gelatin in aqueous solution.

7. Bithionol 0.001 M, 0.02 M borax, 0.03 M sodium nitrate and 0.005% gelatin in 1+5 v-v ethanol-water solution.

8. Bithionol 0.005 M, 0.1 M ammonium acetate-sodium acetate buffer, approximately 0.005 M sodium hydroxide, 0.1 M sodium nitrate and 0.005% gelatin in aqueous solution.

II-3 Discussion

Polarographic waves were obtained with the majority of the above solutions, but when these were compared the results obtained with the blank solutions, no specific wave could be attributed to bithionol, although a yellow precipitate, presumably the mercury(II)-bithionol complex,
formed during polarography.

It is not known why an anodic wave was not observed for the formation of the mercury(II)-bithionol precipitate. There was, however, very strong absorption of either bithionol or the precipitate on the mercury surface. This may have adversely affected the result.

11-4 Final Studies

To conclude the investigation into the polarographic behaviour of the mercury(II)-bithionol complex, solutions containing mercury(II) and bithionol were polarographed in an attempt to obtain a cathodic wave corresponding to the reduction of the complex. Owing to its low solubility, even in 3+1 v-v ethanol-water solution, it was necessary to reduce the concentration level to 0.000025 M before the complex remained in solution. Sodium nitrate 0.1 M was added as supporting electrolyte to bithionol solutions of 0.000005 to 0.000025 M and these solutions polarographed.

No specific wave was observed which could be attributed to a reduction of the mercury(II)-bithionol complex.
CHAPTER TWELVE

GENERAL DISCUSSION AND SUGGESTIONS FOR THE EXTENSION OF THIS STUDY

The formation of a number of metal complexes of the o,o'-dihydroxydiphenyl methane and sulphides, hexachlorophene, bithionol and fenticlor have been investigated, and their stability constants determined in 3+1 v-v ethanol-water solution at ionic strength one.\textsuperscript{144} The values of log $K_1$ for complexes of bithionol and fenticlor follow the Irving-Williams's order, but the values of log $K_2$ for nickel are greater than those for copper. The proton-ligand stability constants of dichlorophene, bromchlorophene and tetrachlorophene have also been determined under these conditions. A solvent extraction procedure has been developed for the colorimetric determination of iron(III) using bithionol as the reagent.\textsuperscript{165, 166} Bithionol is a sensitive, fairly selective, non-toxic, stable, reasonably priced, colourless and readily obtainable reagent for iron(III). The use of bithionol as a gravimetric reagent for mercury(II) has been investigated. It was not possible to determine the structure of the 1:2 mercury(II)-bithionol complex from this study, and the reproducibility of recoveries obtained for similar samples was generally poor, the best precision observed being ±1\% on four samples.\textsuperscript{147} Bithionol was further investigated as a reagent for mercury(II) by direct titration using iron(III) as indicator, and by conductometric, high frequency and alkalimetric
titration. This work was only partly successful.

Methods of separating and determining o,o'-dihydroxy-
diphenyl sulphides and methanes have been investigated.
Acid-base titration data and acid dissociation constants
have been used to optimise the selection of buffer solu-
tions in which to effect electrophoretic separation of
hexachlorophene, bithionol, bromchlorophene, tetrachloro-
phene, fenticlor, dichlorophene and 4-hexylresorcinol. With a buffer solution of pH meter reading 6.4, optimum
separation of hexachlorophene, bithionol, bromchlorophene
and tetrachlorophene is obtained, and the optimum separa-
tion of the other three compounds is obtained with a buffer
solution of pH meter reading 8.9. The use of iron(III) as
a reagent for bithionol was attempted to improve the un-
reliable procedure described by Larson. Addition of
positively charged onium compounds prior to solvent ex-
traction was found to improve the method, but not suffi-
ciently for routine analytical use. Photometric titra-
tion of bithionol with iron(III) also gave inaccurate
results. The formation of the mercury(II)-bithionol
precipitate at the dropping mercury electrode during
anodic polarography has been studied, in an attempt to
develop a method of determining bithionol based on this
reaction. Widely varying conditions were examined but in
each case no specific wave was observed which could be
attributed to bithionol, although a yellow precipitate,
presumably the mercury(II)-bithionol complex, formed
during polarography.

A logical extension of the present study would be
to investigate the complex formation and if possible determine the stability constants of complexes formed between o,o'-dihydroxydiphenyl sulphides and methanes, and certain less common metals, which are of analytical interest. Such metallic ions would include cerium(III), uranium(VI), thorium(IV), and vanadium(IV). The compound o,o'-dihydroxydiphenyl sulphide has been synthesised using published methods. It would be interesting to investigate this compound by pH potentiometric titration and determine its proton-ligand and certain of its metal-ligand stability constants so that these values could be compared with those obtained for the halogenated compounds, bithionol and fenticlor. This compound may also have useful analytical properties; it would, for instance, be expected to fluoresce or phosphoresce on complexation with metal ions, in contrast to the halogenated compounds bithionol and fenticlor, in which case more sensitive analytical methods may be possible.

Suffis and Dean developed an ion-exchange procedure to separate either hexachlorophene or bithionol from a soap base, prior to ultra violet spectrophotometric determination. It may be possible to use acid dissociation constants to select the optimum pH conditions to effect the separation of a number of o,o'-dihydroxydiphenyl sulphides and methanes using ion-exchange resins. This would be analogous to the electrophoretic work described in chapter nine.

o,o'-Dihydroxydiphenyl sulphides have been shown to be trichelating compounds, and bithionol found to be a
useful colorimetric reagent for iron(III). The linkage of metal atom to ligand through one sulphur and two oxygen bonds is not common. It would be interesting to attempt to design and synthesise specific ligand molecules based on this type of linkage. The rigid compound, for instance, formed by bridging the 4 or 5 to 4' or 5' carbon atoms of the phenolic rings in a compound such as bithionol, may have useful analytical implications.
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The computer used in the present work was an I.C.T. 1905 computer, and all the programs are written in Fortran 4. The computer programs in appendices A, B and D are also written for batch monitor input. This is a simplified computer processing procedure which is designed for short programs. The remaining programs are written for normal input.

List of Computer Programs

Appendix A - Computer program for the calculation of acid association constants.

Appendix B - Least-squares approximation program for the calculation of metal-ligand stability constants.

Appendix C - Computer program for the calculation of values of $\tilde{n}$ and free ligand concentration. These values are printed on paper, and punched on cards as input data for the Thun et al. program.

Appendix D - Computer program for the calculation of stability constants devised by Thun, Verbeck, and Vanderleen.

Appendix E - Check on constants computer program. Either proton-ligand or metal-ligand formation curves can be recalculated with appropriate input data.

Key to Symbols Used in the Computer Programs for Input and Output Data

<table>
<thead>
<tr>
<th>Fortran 4 Programs</th>
<th>Description of Symbol</th>
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<td>YC $A, B, C$</td>
<td>number of protons capable of dissociation per ligand</td>
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<td>Fortran 4</td>
<td>Programs</td>
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<td>----------</td>
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<td>A,B,C</td>
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<tr>
<td>VO</td>
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-III-
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<tr>
<td>U</td>
<td>D</td>
<td>free ligand concentration</td>
</tr>
<tr>
<td>Z</td>
<td>D</td>
<td>$\overline{n}$</td>
</tr>
<tr>
<td>Z(1)</td>
<td>D</td>
<td>first calculated value of $K_1$</td>
</tr>
<tr>
<td>HM</td>
<td>D</td>
<td>number of $M$ actually used in the calculation</td>
</tr>
<tr>
<td>H</td>
<td>D</td>
<td>value of constant $K_1$ or $K_2$</td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>error value</td>
</tr>
<tr>
<td>NB</td>
<td>D</td>
<td>number of constants calculated</td>
</tr>
<tr>
<td>I</td>
<td>D</td>
<td>order of the constant, in this case either 1 or 2</td>
</tr>
<tr>
<td>J</td>
<td>E</td>
<td>number of data packs</td>
</tr>
<tr>
<td>DIF</td>
<td>E</td>
<td>value to be added on to PL value at each loop</td>
</tr>
</tbody>
</table>

-III-
<table>
<thead>
<tr>
<th>Fortran 4</th>
<th>Programs</th>
<th>Description of Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>E</td>
<td>initial value of $p[A]$ or pH</td>
</tr>
<tr>
<td>PKA1</td>
<td>E</td>
<td>$K_a$ or $K_{H1}$</td>
</tr>
<tr>
<td>PKA2</td>
<td>E</td>
<td>$K_a$ or $K_{H2}$</td>
</tr>
<tr>
<td>BARN</td>
<td>E</td>
<td>$\bar{n}$ or $\bar{n}_H$</td>
</tr>
</tbody>
</table>
APPENDIX A

*FORTRAN H003. ACID ASSOCIATION CONSTANTS BY HENDERSON EQUNS. FOGG-GRAY

MASTER H003

0J 9 K=1.4

1 FORMULA (1X 9HFOGG-GRAY/1X.4PHACID ASSOCN CONSTS. DETN. WITH HENDEN
1 150N 1/5 N /2X.4HPHR,5Y.4HPST,4X.5HBARNA.9X.4HPKA2.9X.4HPKA1)
2 READ(2,1)TC,TV,V0,V0,XN,XN
3 READ(1,3)PHIP,V1,V2
4 FORMULA (3F6 3)
5 BARNAT=V=-(V2-V0)*(XN+4)/(4*(V0-V1))
6 HST=PHIP+0.04+0.03
7 IF(BARNA GT.1.0) GO TO 8
8 PHA2=PHST+ALOG11((BARNA/(1.1-BARNA)))
9 WRITE(2,4)PHIP,PHST,BARNA,PKA2
10 READ(2,4)PHIP,PHST,BARNA,PKA2
11 FORMULA (2X13,F6.3),3X,E12.5,2Y,E12.4
12 GO TO 6
13 FORMULA (2X13,F6.3),3X,E12.5,2Y,E12.4
14 CONTINUE
15 CONTINUE
16 CONTINUE
17 CONTINUE
18 STOP
19 END

DATA -V-
*FORTRAN HO55 STABILITY CONSTANTS

MASTER HO55

DIMENSION V2(00), V3(99), BAN(99), BAR(99), PH(99), PL(99)

READ(1,11) YC, TI0, TM0, VO, FO, XN, N

11 FORMAT (F9.0, 2F9.6, F9.3, 4F9.6, F7.5/F13)

READ(12,12) (BAN(LA), V2(LA), PH(LA), LA=1,N)

12 FORMAT (F9.0, 3F9.4, E10.4, 4F9.1, E10.4)

READ(14,14) (BAN(L), E10.4, B3, B2)

14 FORMAT (F9.0, 4F9.1, E10.4)

YSUM=0
YSUM=0
YSUM=0
YSUM=0
G=N

N=4 L=1 N
BARN=V3(L) - V2(L) * (XN - 00 + TLO + (YC - BAN(L))) / (BAN(L) + TM0 + (VO + V2(L)))

PH(L) = NH / TLO * O4 + 0.03

Q=10 ** (-NH(1))

CL=(B+H+RET+H'B+H'B*+V) / (V0+V3(L)) / ((TLO+BARN+TM0)*V0)

IF (BARN GT 95.0D AND (BARN LT 105.0 OR (BARN GT 200)) GO TO 32

Y=BARN / (BARN - 1) + (BARN - 1) / C1

YSUM=YSUM + X

YSUM=YSUM + Y

YSUM=YSUM + X

YSUM=YSUM + Y

GO TO 33

32 N=0-1

33 BAR(1) = BARN

4 PL(L) = ANGT0(C1)

B=(Q*XYSUM - XSUM + YSUM) / (Q*X2SUM - XSUM + XSUM)

A=(K*SUM - XYSUM - XYSUM) / (Q*X2SUM - XSUM + XSUM)

BET1 = A

BET2 = A/R

UPM(T, S) = BET2

5 FORMAT (6HBF31=, E10.4, 5X, 6HBF31=, E10.4)

6 FORMAT (1X, TIO + X2SUM, Y2SUM, XSAM, XSBM, YSUM)

7 FORMAT (1X, F7.3, 2F11.4, 2E12.4, F10.4)

8 IF (2.8) YC, TI0, TH0, VO, FO, XN, N
APPENDIX B CONTINUED

8 FORMAT (1HN, 4V, PHYC, 4X, HTLO, 8X, 3HTMD), 13X, 2HV0, 7X, 2HE0, 7X, 2HN, 9X, 1
HNN/1X, F5, 2X, 2(F11.8, 2X), 2(F9.6, 1X), F11.6, 2X, 1I4/1H1)
READ (?, 16)
16 FORMAT (2H truncated, F4.0)
STOP
END
*DATA
APPENDIX D

LIST(LP)
SEND TO (FD,FORTCOMAREA ONE)
PROGRAM(H041)
INPUT=CRD
OUTPUT;Z=1,60
TRACF
END
MASTER H041
DIMENSIONu(4),h(4),z(40),71(40),HM(11)
READ(1,1) IJT
DO 98 IJK=1,1JT
READ(1,4)H,B,IAT,NA
1 FORMAI(I2,F15.4,?12)
WRITE(2,901)H,R,IAI,NA
901 FORMAI(7H M=12,3H B=,E11,4,5H IAT=,12,4H NA=,12)
(1)=0
WRITE(2,902)
902 FORMAI(11X,10HFREEL1GAND.14X,4HBARN)
DO 30 I=1,4
READ(1,5)U(I+1),Z(I+1)
5 FORMAI(F11.4,F12.5)
WRITE(2,903)U(I+1),Z(I+1)
903 FORMAI(10X,E11,4,10X,E12 5)
300 /(I+1)=/(I+1)/x(I+1)
300 =U 8 I=2,7
8 HM(I)=0,0
DO 2 I=1.1,2.5
2 HM(J+1)=HM(J+1)+U(I+1)*J
7(I)=7(I)+U/I(3)+U(I(2))/U(3)-U(2)/)
WRITE(2,6)7(*)
6 FORMAT(E15 8)
GO TO 800
A16 IF(Z(2)-Z(I))802,803,803
803 DO 801 J=2,4
U(J)=U(I+1)
7(J)=7(I)+J+1
800 WRITE(2,700)I(J),Z(J)
700 FORMAI(2F14.7)
M=M-1
GO TO 900
802 HHM=M+1
HM(I)=M
WRITE(2,6)M(1)
DO 9 JJ=2,4,M-2
L=JJ-1
IF(JJ=MH)10,1,10
11 I=L-1
10 DO 12 I=1,3
K=L-1
APPENDIX D CONTINUED

H(I, 4) = \gamma (K + 3)
DO 12 J = 1, 3
IF(U(K+3)) 120, 121, 120
121 H(I, J) = 0
H(I, 3) = 1
GO TO 12
120 H(I, J) = U(K + 3)**(3 - J)
12 CONTINUE
T = 1
NB = 3
NE = 4
NC = 4
GO TO 36
14 DO 9 I = 1, 2
K = L - 1
G1 = U(K - 1)
G2 = U(K)
9 7I(K-1) = (G2 - G1) * (H(1, 4)/3, G*(G1 + G2) + G2 + G1) + 0.5D0 * H(2, 4) * (G1 + G2 + 1) + H(3, 4))
DO 80 I = 3, 11
80 HM(I) = 0
IAT = IAT - 1
I = 0
DO 17 I = 2, M
17 7I(I) = 7I(I) + 7I(I-1)
DO 18 I = 1, M
18 7I(I) = 0.5D0 * (7I(I) - 1.0) / U(I+1)
G1 = G1 + 7I(I) * 2
DO 18 J = 8, 11
18 HM(J) = HM(J) + 7I(I) * U(I+1)**(J-8)
T = 2
DO 21 NB = 2, NA
DO 20 I = 1, NB
DO 20 J = 1, NB
L = I + J
20 H(I, J) = HM(L - 1)
NC = NB + 1
NE = NC + NB
DU 22 I = 1, NB
H(I, NC) = HM(I + 7)
NH = NC + NB
I = NC + 1
DU 43 K = 1, NH
43 H(I, K) = 0
KH = NC + I
22 H(I, NH) = 1
36 I = 1
32 N = I + 1
DO 30 J = N, NE
30 H(I, J) = H(I, J) / H(I, I)
APPENDIX D CONTINUED

33 IF(I-NB)33,34,34
34 DU 31 K=N,NB
35 DU 31 J=N,NE
36 H(K,J)=H(K,J)=H(K,I)*H(I,J)
37 I=I+1
38 GO TO 32
39 N=I-1
40 H(K,J)=H(K,J)=H(K,I)*H(I,J)
41 I=I-1
42 IF(I=i)34,35,34
43 GO TO (14,23),1ST
44 N=01
45 G2=N-1-NB
46 DU 24 I=1,NB
47 H=H(I,NC)*H(I+7)
48 DU 25 I=1,NB
49 K=NC+1
50 H(I,K)=H(I,K)+H(I+62)
51 IF(NB-4)200,201,200
52 H(4,7)=-H(4,7)
53 H(4,7)=DSQRT(H(4,7))
54 IF(I=2,77)H(4,5),H(4,7)
55 GO 26 I=7,NB
56 I=NB-I+2
57 K=NC+1,
58 H(L,NC)=H(I,NC)/(L-1,NC)
59 H(L,K)=H(I,K)*H(L,NC)**2*H(L-1,K-1)/(L-1,NC)**2
60 DU 21 I=1,NB
61 N=NC+1
62 D=H(I,NC)
63 IF(D)220,21,222
64 N=-D
65 DU 21 1=2,77)H(I,NC),D,NB,1
66 FORMAI:(2E16,8,214)
67 G1=Z(I)-H(1,N)+1)
68 IF(G1)23,29,29
69 G1=G1
70 IF(G1)N=7(1))98,97,97
71 IF(1)=H(1,NA+1)
72 IF(IA)316,98,816
73 CONTINUE
74 STOP
75 END
76 FINISH
77 LIST(LP)
*FORTRAN H053, CHECK ON CONSTANTS FOGG+GRAY

MASTER H053
READ(1,7)J
7 FORMAT(12)
READ(1,12)N,DIF
12 FORMAT(13,F4.2)
DO 5 K=1,J
WRITE(2,1)
1 FORMAT(1X,4HFOGG/1X,18HCHECK ON CONSTANTS/5X,4HNBAR,12X,2HPL)
READ(3,7)PL,PKA1,PKA2
2 FORMAT(3F6.3)
DO 4 I=1,N
FL=10 **(-PL)
BLOG1=PKA1
11=10 **BLOG1
BLOG2=PKA1+PKA2
R2=10 **BLOG2
WRITE(2,5)BADN,PL
3 FORMAT(15,6,F5.2)
4 PL=PL+DIF
WRITE(2,6)
6 FORMAT(1X,5X,4HPKA1,6X,4HPKA2)
WRITE(2,8)PKA1,PKA2
8 FORMAT(2/4X,F6.3),/:///)
5 CONTINUE
C/OP
FLD

*DATA