COMPLEX FORMATION BETWEEN METALS 
AND SULPHUR-CONTAINING LIGANDS

A Thesis
submitted for the
Degree of Doctor of Philosophy
in the
Australian National University
by
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December 1966
The work described in this Thesis was carried out by the Candidate at the Australian National University. The work of others has been appropriately credited.

J. H. Sarge
This Thesis reports results of a study of the metal complexes formed by a number of ligands containing the sulphydryl ion. Although most of these systems had been investigated previously, it was apparent that the results reported in the literature were in many cases either unreliable or incorrect, because their determination had involved certain simplifying assumptions which have now been shown to be invalid. The use of a wider range of concentrations, coupled with improvements in computational and experimental techniques has made possible the determination of more satisfactory constants, and enabled the recognition of a number of unusual complex species.

The results of the present work have been based mainly on pH titration data, obtained using a specially developed titration cell. Spectrophotometric studies, and e.m.f. measurements with a zinc amalgam electrode, have also been used where possible to provide confirmatory evidence for the proposed species. The very extensive calculations which were necessary have been carried out using an electronic digital computer, for which a number of programmes were written. The most important of these was a general non-linear least-squares equilibrium constant refinement programme, developed from a simpler one written by Tobias.
The new programme is capable of calculating the constants for any type of complex formation reaction which can occur between a metal and a ligand in water, even though a number of such equilibria may occur simultaneously. The treatment can be extended to deal with a mixture of several metals and several ligands, where any mixed species may be postulated, and its stability constant (in principle) be calculated.

The stability constant studies have demonstrated that (b) class metals* form very stable complexes with thiol-type ligands. Three features are apparent which were unrecognized in most of the previous studies. In several of the systems examined, values of the second stability constant \( (K_2) \) are greater than the first \( (K_1) \), and in several cases where it is sterically possible polynuclear species have been shown to be present in the solution. Also a number of systems contain protonated complexes. In those systems where both protonated species and polynuclear species are formed, the possibility of protonated polynuclear species exists. In such cases, only a tentative and approximate description of the system is possible. These effects may be partly a result of \( \pi \)-bonding between the metal and the ligand.

* see P.3
Publications based on the present work include:


(c) "Computer Calculation of Equilibrium Concentrations in Mixtures of Metal Ions and Complexing Species", D. D. Perrin and I. G. Sayce, Talanta, submitted for publication.
ACKNOWLEDGEMENTS

The Author is much indebted to his supervisor, Dr. D. D. Perrin, for his guidance and encouragement throughout the project, also to his colleagues in the laboratory, and to the Staff of the C.S.I.R.O. Computing Research Section, Canberra, for helpful discussions. Grateful acknowledgement is also made to Professor R. S. Tobias, of the University of Minnesota, for providing details of a computer programme from which the programme used in the present work was developed, and to the Australian National University for the award of a Scholarship.
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CHAPTER 1

INTRODUCTION

The formation of metal complexes in which bonding is through sulphur atoms has received relatively little reliable quantitative study. This is at first sight surprising because many sulphur-containing ligands are known, including numerous substances of biological importance. There are several reasons for the deficiency. Firstly, many of the ligands are unstable and readily undergo decomposition or oxidation.¹ For example, thiols are rapidly converted to disulphides either by traces of oxygen in the presence of metal ions of variable valency,² or by the direct reduction of suitable metal ions (e.g. copper(II)³,⁴,⁵). Again, whereas a nitrogen or an oxygen atom usually coordinates to only one metal ion in solution, sulphur commonly binds to two metal ions, giving rise to polynuclear complexes.⁶,⁷ Finally, complexes of sulphur-containing ligands are generally less soluble in water than are the corresponding species in which bonding is through oxygen, because of the decreased ability of sulphur to form hydrogen bonds.

There is a wide range of ligands in which bonding can occur through a sulphur atom. It includes the sulphydride,
sulphite, thiosulphate, and thiocyanate ions, thiols, thioethers (organic sulphides), disulphides, thioureas, thioamides, thioethers, thioketones, xanthates, dithiocarbamates, thiophosphates, triphenylphosphine sulphide, and (with palladium(II) and platinum(II)) dimethyl sulphoxide. Work on these ligands has recently been reviewed, and the majority of the relevant stability constant studies are available in published Tables.

Of the organic sulphur-containing ligands, the most important biologically are the thiols, thioethers and disulphides, and of these only the thiols form strong complexes with many metals in aqueous solution. Although a number of sulphur-containing ligands have been shown to form polynuclear species, to date the existence of such complexes has been considered in only very few stability constant studies. For this reason their importance has now been investigated in aqueous solutions of some divalent metal ions with some thiol-type chelating agents.

Nature of the Sulphur-Metal Bond

Although sulphur and oxygen occur in the same group of the Periodic Table, they show a number of important differences as donor atoms. Ahrland, Chatt, and Davies have divided metals into two broad classes. Those metals
which form their most stable complexes with ligands of the first row of the Periodic Table (N, O, F) are placed in class (a), and those which form stronger complexes with ligands in the second (P, S, Cl) or a subsequent row, are termed class (b). Their classification is shown in Fig.1.01.

N.B. Ahrland et al. regarded zinc as a class (a) metal. However, as the present work shows zinc to have pronounced (b) character in its thiol complexes, this metal has been placed in the border region in the Figure.

Class (b) metals form a roughly triangular group at the centre of the Table, and the boundary between the classes is diffuse. Metals at the centre of the group show (b) character only in their lower oxidation states, while metals to the right of the group show some (b) character even in their higher oxidation states.
Craig and Nyholm\textsuperscript{10} discussed the formation of metal halide complexes in terms of the ionisation potential of the metal, the electron affinity of the anion, the loss of hydration energy of the two ions, and the energy involved in bringing together the two ions from infinity. They found that the division into class (a) or (b) depended largely on the ionisation potential of the metal, the high ionisation potential of the (b) class metals reflecting the high polarising power of these acceptors, and their ability to form covalent bonds. However this model takes no account of the ability of many metals of pronounced (b) character to donate electrons back to the ligand in the formation of a \(\pi\)-bond (as is found in the coordination of \(\text{C}_2\text{H}_4\), \(\text{CO}\) etc.), the factor regarded by Ahrland et al. as being the most important.

Pearson\textsuperscript{11} has extended the classification of Ahrland et al., pointing out that metals and ligands could be regarded as Lewis acids and bases. Class (a) metals may be regarded as "hard" (i.e. non-polarizable), and prefer to bind to hard bases. Class (b) metals on the other hand are "soft" (i.e. polarizable), and prefer to bind to soft bases. These observations may be explained in terms of the theories of ionic and covalent bonding, \(\pi\)-bonding, electron correlation effects, and the nature of the solvent (if any). These
various factors contribute to different extents, depending on the example considered.

For ligands in the same period, forming complexes with most of the metals of class (a), the order of stability of the complexes is, for example, $N > O > F$, corresponding to the decreasing availability of the lone pair electrons with increasing electronegativity of the ligand. With class (b) metals this order is again observed, the differences being enhanced because the filled $d$ orbitals of these metals cause repulsion with the non-bonding lone pairs of the ligand. The order is repeated with ligands of the next period i.e. $P > S > Cl$, but here the differences are smaller because the electronegativity of the ligand is decreased, and the vacant $3d$ orbitals of the donor give rise to $\pi$-bonding with reduced lone pair repulsion.

This type of $\pi$-bonding appears to be important in thiol-metal complexes. First reported by Syrkin in 1948, it has been discussed in detail by Craig et al. Bond formation by sulphur may be considered to entail first the donation of a pair of electrons from the sulphur $sp^3$ orbital to the metal $d_{x^2-y^2}$ orbital. Formation of this dative $\sigma$-bond depletes the charge on the ligand, and a metal orbital of symmetry $d_{xz}$ now overlaps with one of the sulphur $d_{\pi}$-orbitals to form a dative $\pi$-bond between the two centres.
The original charge transfer is partly neutralised, and each form of bond enhances the other. In planar complexes two, and in octahedral complexes three, dπ orbitals are available on the metal for this type of bonding. These orbitals are orthogonal, and π-bonding may influence the stability of cis and trans isomers in a square-planar complex MX₂Y₂. Thus if Y can form a σ-bond with the metal (M) but X cannot, then in the cis isomer both Y atoms can be π-bonded, but in the trans isomer only one π-bond would be possible, although resonance between the two positions might occur. This is an example of the trans- effect and results in the greater stability of the cis isomer.

Although the position of the group RS⁻ in the spectrochemical series (for the ligand field splitting parameter Δ) has not yet been established, the existence of dπ-dπ bonding for ligands of this type suggests that high field strengths should be expected, so that the formation of low spin complexes would be encouraged.

The separations between the electronic energy levels of transition metal ions (manifested by the electronic spectra) are related to the electronic repulsions between the d electrons. Certain ligands reduce these repulsions, and reduce the energy-level separations relative to the free ion. The effect has been ascribed to the property of the ligand of expanding
the electron cloud on the metal as a result of covalent bond formation. Both metals and ligands may be arranged in order of magnitude of this effect (the nephelauxetic effect).\textsuperscript{15} For metals the order is that of increasing oxidising character of the ions, and the order for ligands is that of increased reducing character. This order for some typical ligands is $\text{F}^- < \text{H}_2\text{O} < \text{NH}_3 < \text{oxalate} < \text{ethylenediamine} < \text{NCS}^- < \text{Cl}^- < \text{CN}^- < \text{Br}^- < \text{I}^-$. Sulphur ligands appear to be very high in this series. The complexes of thiourea with cobalt(II) exhibit an exceptionally large nephelauxetic effect, indicating a large degree of covalent bond formation.\textsuperscript{16}

Other properties which may be partly the result of $\pi$-bonding are the comparatively high values of the second stability constants of certain metal complexes with sulphur-containing ligands, and the ability of sulphur to coordinate with more than one metal atom. The effect of "back-donation" described above means that less negative charge is transferred to the metal ion than would otherwise have been expected. The stability constant $K_2$ is therefore higher than would have been predicted from $K_1$ in the absence of back-donation. In the same way the return of some negative charge depletes the charge on the sulphur atom less than otherwise, leaving sulphur free to form a bond with a second metal ion. It is as a result of this
property that sulphur-containing ligands can form polynuclear metal complexes. Of the other donor atoms in the second row of the periodic table, phosphorus, well known for its $\pi$-bonding ability in the phosphine complexes, cannot bond to a second metal atom because of the absence of a second lone pair of electrons. Chlorine on the other hand is found as a bridging atom in a number of complexes, but only as the ion: alkyl halides are ineffective as electron donors.

**Complexes of Thiol Ligands**

(i) Qualitative Studies

(a) Monodentate Ligands:— As discussed in the previous section, the $RS^-$ ion forms strong bonds with class (b) metal ions, the name "mercaptan" arising from the avidity of thiols for mercury. The complex of nickel(II) with ethane thiol, first reported by Jensen,\(^{17}\) who suggested a polymeric structure, has recently been shown by X-ray crystallography to have the hexameric structure I (Fig.1.02), in which each nickel ion is surrounded by a square planar arrangement of sulphur atoms (Woodward et al.)\(^{18}\) Gould and Taylor have proposed an analogous structure from X-ray data for the nickel(II) complex of 2-mercaptoethanol, where (except possibly at high pH) the hydroxyl groups do not participate in bonding.\(^{19}\)
Fig. 1.02
In addition to another monothio complex, that of hexamethylenetetramine cobalt(III), the species is reported to have a three-dimensional lattice structure. 20

Chatt and Hart have studied a number of dimeric platinum(II) complexes in which two metal atoms are bridged by two thioether bridges, or by one thioether and one thiolether bridge, the latter suggesting the occurrence of a pseudo symmetrical structure in the cis configuration which appears to possess some degree of "amatoxin" character. Of particular interest is their observation that in the complex cis-PSP-cis-symmetric dichlorobis(1-alkylphosphine)-chloroethylthio-diplatnum (structure II, Fig. 1.02), there is greater stability associated with the cis structure, than with the unsymmetrical or trans-bridged structures, where the sulphur atom would be trans to one or both of the phosphine groups. Phosphorus is another ligand atom expected to show a different bonding in metal complexes, and the remarks of Craig warn against the possibility of the electronegative phosphorus substituents would occur with the phosphorus atoms, and sulphur would be expected to occupy "cis" positions using orthogonal orbitals.

In their second paper, 22 Chatt and Hart examined the structure of di-thio-bridged complexes depending in a manner at present not fully understood, on the nature of the groups attached to the bridging sulphur atoms. When both
In studies of another monothiol complex, that of hexanethiol with cobalt(III), the species is reported to have a three-dimensional lattice structure. 20

Chatt and Hart have studied a number of dimeric platinum(II) complexes in which the metal atoms are linked by two thio-bridges, or by one thio- and one chloro-bridge. 21,22 They suggest that delocalisation occurs in the S-Pt-S-Pt ring which appears to possess some degree of "aromatic" character. Of particular interest is the observation that in the complex cis-PSP-cis-symmetrical-dichlorobistri-n-propylphosphine-µ-chloro-µ'-ethylthio-diplatinum (structure II, Fig.1.02), there is greater stability associated with the cis structure, than with the unsymmetrical or trans-bridged structures, where the sulphur atom would be trans to one or both of the phosphine groups. Phosphorus is another ligand atom expected to show dπ-dπ bonding in metal complexes, and the remarks of Craig et al.13 again apply. To avoid the competition for back-donated electrons which would occur with trans-bonding, phosphorus and sulphur would be expected to occupy cis positions using orthogonal orbitals.

In their second paper, 22 Chatt and Hart demonstrate that the structure of di-thio-bridged complexes depends, in a manner at present not fully understood, on the natures of the groups attached to the bridging sulphur atoms. When both
are aliphatic the cis structure III (Fig. 1.02) is more stable, but if one is aromatic the corresponding trans isomer is preferred.

(b) Bidentate and Terdentate Ligands:—Jicha and Busch have demonstrated the formation of polynuclear species by nickel(II), cobalt(II), palladium(II), and cadmium(II) with 2-mercaptoethanolamine. These authors have shown that the trinuclear species IV (Fig. 1.02) is a major component in solutions of nickel(II) containing this ligand. Crystalline specimens of this complex, and of a number of similar species, have been prepared.24,25

From titration data for solutions of mercaptoacetic acid in the presence of various metals, Cabrera and West deduced the formation of certain species.26 With zinc(II) the titration ended with the formation of a species of stoichiometric formula ML₃, whereas cadmium(II) formed the highly soluble species M₂L₃. For mercury(II), the formation of M₂L₂, as postulated by Stricks et al.,27 was proposed. Lead(II) was reported to form the simple complex ML₂, while with silver(I) the reaction stopped with formation of ML.

Shindo and Brown reported the infra-red spectra of solutions and solid specimens of complexes of zinc(II), cadmium(II), mercury(II), and lead(II) with L-cysteine,
S-methyl-L-cysteine, methyl-L-cysteinate, and 3-mercaptopropionic acid. A shift was observed in the carboxylate (or carbonyl) stretching frequency of the series of ML₂ complexes of various metals with cysteine and methyl cysteinate. The similarity of the spectra with these two ligands indicated that the carboxyl group of cysteine was not bound in the ML₂ species, and the shift was interpreted as an inductive effect associated with the strength of bonding of the amino group. Such a series was not observed with 3-mercaptopropionic acid, in which the carboxyl group took part in bonding. With cysteine, all the metals formed ML and ML₂ complexes, binding with -S⁻ and -NH₂ groups only. However at pH values below 2, solutions of zinc(II) and cadmium(II) chloride (or bromide) in cysteine yielded crystals of a different type of complex, of empirical formula MLX. It was suggested that the most likely structure for these compounds was the trinuclear species V, analogous to IV (Fig. 1.02), but with binding through carboxyl and sulphydryl groups; the amino groups would be protonated and not take part in the bonding.

In the last four years a large number of papers have appeared concerning a new class of â-dithiol metal chelates. These complexes have been studied mainly by three groups of workers. All are of the square-planar general
structure VI (Fig.1.02), and compounds with a variety of metals (M), substituents (R), and charges (z), have been reported. It has been suggested that the interesting electronic effects observed may best be explained by postulating an extensive π-orbital extending over the entire complex. Two typical ligands of the class are maleonitrile dithiol and toluene-3,4-dithiol.

The formation of polynuclear species by these ligands has been observed. Evidence has been presented to indicate that bis(toluene-3,4-dithiolato)tin is polynuclear, and X-ray structure determination has shown the dimeric nature of the cobalt species VII (Fig.1.03).

(ii) Quantitative Studies

Stability constants have been measured for a number of chelates in which the ligand contains one or more sulphur atoms. As the present work concerns thiols, we shall limit our discussion to stability constant studies for ligands of this type. Details of other studies may be found in the Stability Constant Tables and in other recent reviews.

Stability constants in aqueous media have been reported for the complexes of some metal ions with the following monothiol chelating agents: mercaptoacetic acid, 3-mercaptopyruvic acid, 2-mercaptoethylamine, cysteine, penicillamine (ββ-dimethylcysteine), methyl-α-amino-β-mercaptopropionate, and mercaptosuccinic acid. In most
cases, these constants have been calculated on the arbitrary assumption that only mononuclear species were formed. Stability constants have also been reported for metal complexes with dithiol chelating agents, \( \alpha \beta \)-dimercapto-
succinic acid, ethane-1,2-dithiol, and 2,3-dimercaptopropionic acid. Evidence was found for polynuclear complex formation by some metals with the latter two ligands, but the possibility was not considered with \( \alpha \beta \)-dimercaptosuccinic acid.

The quantitative results from the above studies are summarised (using the notation and conventions of the Stability Constant Tables\(^8\)) in Tables 1.01 to 1.10 (p.23 et seq.).

Results for the nickel(II) complexes of mercaptoacetic acid (Table 1.01) illustrate why many of these values are likely to be incorrect. Leussing studied the complexes of cobalt(II), manganese(II), nickel(II), and zinc(II) assuming the formation of 1:1 and 2:1 complexes.\(^38\) In the light of subsequent suggestions as to the nature of nickel-sulphur bonding,\(^23\) Leussing, Laramy, and Alberts reconsidered the complex formation of nickel(II) with mercaptoacetic acid,\(^39\) and suggested the formation of \( \text{NiL}_2^{2-} \), \( \text{Ni}_4 \text{L}_6^{4-} \) (structure VIII, Fig.1.03) and small amounts of \( \text{Ni}_3 \text{L}_4^{2-} \) (analogous to structure IV). Further work, with a view to establishing
whether or not polynuclear complexes are formed by the other metal ions, has not been forthcoming, although some otherwise unpublished work by the group has been listed in the Stability Constant Tables. These results do not invoke polynuclear species for cobalt(II), manganese(II), and zinc(II), but irregular trends may be discerned in the apparent constants as the temperature is changed.

Li and Manning did not postulate polynuclear species in their work on lead(II) and zinc(II) with mercaptoacetic acid, and contrary to the present, and certain other, work on sulphur-containing ligands, the value of log $K_2$ for the zinc(II) complex was less than log $K_1$. This conclusion, also reached by Leussing, may have resulted from the calculation of the constants of $K_1$ and $K_2$ from the values of $-\log[L]$ at $\bar{n}=0.5$ and $\bar{n}=1.5$ on the formation curve (of $\bar{n}$ versus $-\log[L]$). This involves an approximation which, though satisfactory in some cases, is invalid when $K_2$ is not considerably smaller than $K_1$.

Of the remaining results in Table 1.01, it should be noted that the values for mercury(II) require correction because the measurements were carried out in acetate buffers in which, as a result of the strong complex formation between mercury(II) and the acetate ion, there would have been only a very small concentration of the free metal ion.

The results of published studies of the complexes of 3-mercaptopropionic acid are shown in Table 1.02. It is
interesting to note that although the values reported by Fernando and Freiser are only approximate, having been calculated from the formation curves, the nickel(II) species are shown to be less stable than those of zinc(II), and also the six-membered chelate ring is very much less stable than the five-membered ring formed by the thioglycollic acid.\(^{43}\)

None of the previously published studies of the stability constants of 2-mercaptoethylamine (Table 1.03) has considered the possibility of polynuclear complex formation, although the work of Jicha and Busch would suggest the formation of such species, at least in the case of nickel(II).\(^{23}\) Nor have any of the studies covered a sufficiently wide range of concentration to have revealed the existence of polynuclear complexes, although from their work with tenfold excess of metal ion, Felder, Rescigno, and Radica\(^{44}\) found evidence for the formation of the protonated species \(\text{MHL}^+\) by cadmium(II), nickel(II), lead(II), and zinc(II). The methods of calculation used by the remaining workers were again approximate and could not be expected to indicate whether polynuclear species were present, or to show any reversal of the order of \(K_1\) and \(K_2\). The results of Knoblock and Purdy\(^ {46}\) for cadmium(II), cobalt(II), copper(II), and zinc(II) give only "apparent" values, because the work was carried out in phosphate and carbonate buffers, and the meaning of their figure for the
copper(II) complex is questionable. With rare exceptions, copper(II) ion oxidises sulphhydryl groups to disulphides. The present work confirms that this reaction, which is well known for cysteine\(^3-5\), occurs also with such ligands as mercaptoethanolamine and penicillinamine. Hence it would appear that any values quoted for stability constants of copper(II)-thiol complexes must be regarded with suspicion.

Table 1.04 lists the stability constant studies which have been reported for cysteine. In the majority of these the constants have been obtained from potentiometric data, again by approximate methods, and most studies have employed only one set of reagent concentrations, so that the presence of polynuclear species, while not postulated, cannot be excluded. Lenz and Martell employed two concentrations, but did not discuss the agreement, or the lack of agreement, between the results for these two sets of conditions.\(^53\)

Of relevance to the present work, is the observation of Kroll who studied the manganese(II)-cysteine system, and pointed out that with this terdentate ligand protonated species might be formed, in which bidentate chelation would occur.\(^56\) Once again the values obtained using polarographic techniques are not absolute, the measurements having been made in a variety of complex-forming buffers, and that reported for copper(II) is of doubtful significance.\(^57\)
Three groups of workers have studied complex formation by penicillamine $^{53,55,57}$ (Table 1.05). The stability constants reported by Kuchinskas and Rosen $^{55}$ were calculated from the formation curves, while those of Lenz and Martell $^{53}$ were obtained using a least-squares method. From an examination of the formation curves, the latter report that whereas in complexes with cadmium(II), mercury(II), and lead(II), cysteine and penicillamine both appear to be terdentate, the nickel(II) and zinc(II) chelates appear to be bidentate. Once again the constants reported for copper(II) $^{55,57}$ are suspect in view of the likelihood of oxidation of the ligand.

The stability constants for complexes of methyl-$\alpha$-amino-$\beta$-mercaptopropionate reported by Li and Manning $^{35}$ (and listed incorrectly in the Stability Constant Tables $^8$), are shown in Table 1.06. That the values for zinc(II) are smaller with this ligand than with cysteine is a reflection of the lower $pK_a$ values of the former ligand, indicating a decreased tendency to share the lone pair electrons of nitrogen. Greater differences between the two ligands appear in the lead(II) complexes, because the former ligand (an ester) can only form a bidentate chelate, whereas cysteine is probably terdentate. Various workers have measured stability constants for mercaptosuccinic acid...
complexes (Table 1.07), but no protonated or polynuclear species have been reported.\textsuperscript{58-60} Nor have any polynuclear complexes been postulated for \( \alpha \beta \)-dimercaptosuccinic acid (Table 1.08) although a number of protonated species have been described.\textsuperscript{58,60} Constants for complexes with these ligands were obtained by selecting buffer regions in the neutralisation curve, and assuming that each corresponded to only one equilibrium. Assuming that the remaining constants were known, or negligible, the constant corresponding to this equilibrium could be calculated. This method can give approximate constants when the species are known, but is less satisfactory than the simultaneous treatment of all regions of a series of titrations, as was possible using the computer programme developed in the present study.

Leussing and coworkers have published several papers on complex formation by dithiol ligands. Table 1.09 shows the stability constants reported for the nickel(II) complexes of ethane-1,2-dithiol.\textsuperscript{62} The experimental data were interpreted in terms of the species \( \text{NiL}_2^{2-} \) and \( \text{Ni}_2\text{L}_3^{2-} \) (structure IX, Fig.1.03). The observed deviations from a Job's plot were explained as arising from dissociation of the binuclear species. An alternative explanation, not considered by these authors, is that the deviations were due to the presence of more than one polynuclear species. Hydroxy
species appeared to be unimportant at the pH used (10.8).

The complexes of 2,3-dimercaptopropan-1-ol (BAL) with several metals have been studied, and the reported constants are summarised in Table 1.10. Manganese(II)-BAL titrations showed some deviations from the expected superimposable formation curves for different total metal concentrations, but the authors do not invoke any species other than ML and ML₂.⁶⁴ Among other possible sources of error, catalytic oxidation of the ligand in the presence of manganese(II) might have caused the observed discrepancies.

Data for the zinc(II)-BAL system⁶⁴ were treated by the method of Sillén.⁶⁷,⁶⁸ It was shown that the data could be fitted by assuming the formation of a "core plus links" series of the general formula L(ML)²⁻. Members of this series (structure X, Fig. 1.03) with chain lengths of up to seven or eight units appeared to be present in significant concentrations, and precipitation of higher members occurred. The link species is neutral, hence the high degree of association in this system.
The complexes formed by nickel(II) with this ligand were studied spectrophotometrically. The major species in alkaline solutions were reported to be $ML^2_2$ and $M_2L_3OH^3_-$, calculations using the alternative polynuclear species $M_2L^2_3$ and $M_2L_3(OH)^4_2$ giving a much less satisfactory fit to the data. It was later suggested that the $OH^-$ group apparently present, actually corresponded to the ionisation of a proton from the hydroxyl group of one of the ligands. Polynuclear complexes of nickel(II) with BAL were more stable than those of zinc(II).

In a final paper in this series it was shown that iron(II) forms a "core plus links" series with BAL, of probably form $L(ML)^2_n$, and chain lengths again up to about eight units. Results for this system were rendered less accurate by the catalytic oxidation of the ligand, by the slow attainment of equilibrium, and by precipitation of insoluble polynuclear species.
<table>
<thead>
<tr>
<th>Metal</th>
<th>Method</th>
<th>Temp. (°C)</th>
<th>Medium</th>
<th>Log of equilibrium constant</th>
</tr>
</thead>
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<tr>
<td>H⁺</td>
<td>gl</td>
<td>25</td>
<td>0.15 KNO₃</td>
<td>$K_1$ 9.78, $K_{12}$ 3.58</td>
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<th>Medium</th>
<th>Log of equilibrium constant</th>
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<th>Medium</th>
<th>Log of equilibrium constant</th>
<th>Ref.</th>
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<td>$K_2^{16.02}$</td>
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<td>0.264</td>
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<td>$K_1^{10.22}, K_2^{8.68}$</td>
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<td>gl</td>
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TABLE I. 04

Stability constants of complexes of cysteine (H₂L).

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<th>Medium</th>
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<th>Ref.</th>
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<td>0.264</td>
<td>K₂ 16.0</td>
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<tr>
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<td>Hg²⁺</td>
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TABLE I. 05

Stability constants of complexes of penicillamine (H₂L).

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<th>Ref.</th>
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<td>gl</td>
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<td>0.1 KNO₃</td>
<td>K₁ 10.88</td>
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TABLE 1.06
Stability constants of complexes of methyl-α-amino-6-mercaptopropionate (ML).

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TABLE 1.07
Stability constants of complexes of mercaptosuccinic acid (H₃L).

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</table>

TABLE 1.08
Stability constants of complexes of a-s-dimercaptosuccinic acid (H₄L).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Method</th>
<th>Temp.(°C)</th>
<th>Medium</th>
<th>Log of equilibrium constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺</td>
<td>gl</td>
<td>20</td>
<td>0.1 KCl</td>
<td>K₁ 11.82, K₁₂ 9.44, K₁₃ 3.46, K₁₄ 2.40</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>gl</td>
<td>25</td>
<td>0.1 KNO₃</td>
<td>K₁ 10.79, K₁₂ 8.89, K₁₃ 3.48, K₁₄ 2.71</td>
</tr>
<tr>
<td>KNi²⁺</td>
<td>-</td>
<td>-</td>
<td>K₂ 10.4</td>
<td></td>
</tr>
<tr>
<td>KNi²⁺ H₂L²⁻ &amp; NH₃L⁺</td>
<td>13.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KNi²⁺, HL²⁻</td>
<td>9.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ni(NH₃)L⁺ H⁺ &amp; NiL₂⁻]</td>
<td>11.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K[NiL₂H²⁺ &amp; H⁺ &amp; NiL²⁻]</td>
<td>10.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 1.08 continued

<table>
<thead>
<tr>
<th>Complex</th>
<th>Method</th>
<th>Temp. (°C)</th>
<th>Medium</th>
<th>Log of equilibrium constant</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Zn}^{2+} + \text{gl} )</td>
<td>20</td>
<td>0.1 KCl</td>
<td></td>
<td>15.87, ( K_1 ) 3.57</td>
<td>61</td>
</tr>
<tr>
<td>(\text{Zn}^{2+} + \text{gl} )</td>
<td>25</td>
<td>0.1 KNO₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Zn}^{2+} + \text{gl} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Ni}^{2+} + \text{gl} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Fe}^{2+} + \text{gl} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Fe}^{3+} + \text{gl} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Mn}^{2+} + \text{gl} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Ni}^{2+} + \text{sp} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Zn}^{2+} + \text{gl} )</td>
<td>30</td>
<td>0.1 KCl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1.09

Stability constants of complexes of ethane-1,2-dithiol.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Method</th>
<th>Temp. (°C)</th>
<th>Medium</th>
<th>Log of equilibrium constant</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{H}^+ )</td>
<td>E</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 10.54, ( K_{12} ) 8.96</td>
<td>62</td>
</tr>
<tr>
<td>(\text{Ni}^{2+} )</td>
<td>sp</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 25.6, ( K_2 ) ( K_3 ) 47.3</td>
<td>62</td>
</tr>
<tr>
<td>(\text{Fe}^{2+} )</td>
<td>gl</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 15.8, ( K_2 ) ( K_3 ) ( K_4 ) ( K_5 ) ( K_6 ) ( K_7 ) ( K_8 ) ( K_9 ) ( K_{10} ) ( K_{11} )</td>
<td>63</td>
</tr>
<tr>
<td>(\text{Mn}^{2+} )</td>
<td>gl</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 5.23, ( K_2 ) 10.43</td>
<td>64</td>
</tr>
<tr>
<td>(\text{Ni}^{2+} )</td>
<td>sp</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 22.78, ( K_2 ) ( K_3 ) ( K_4 ) ( K_5 ) ( K_6 ) ( K_7 ) ( K_8 )</td>
<td>65</td>
</tr>
<tr>
<td>(\text{Zn}^{2+} )</td>
<td>gl</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 13.48, ( K_2 ) 23.3</td>
<td>64</td>
</tr>
</tbody>
</table>

TABLE 1.10

Stability constants of complexes of 2,3-dimercaptopropan-1-ol.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Method</th>
<th>Temp. (°C)</th>
<th>Medium</th>
<th>Log of equilibrium constant</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{H}^+ )</td>
<td>gl</td>
<td>25</td>
<td>0.1</td>
<td>( K_1 ) 10.79, ( K_{12} ) 8.69</td>
<td>63</td>
</tr>
<tr>
<td>(\text{Ni}^{2+} )</td>
<td>sp</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 10.59, ( K_{12} ) 8.62</td>
<td>64</td>
</tr>
<tr>
<td>(\text{Fe}^{2+} )</td>
<td>gl</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 15.8, ( K_2 ) ( K_3 ) ( K_4 ) ( K_5 ) ( K_6 ) ( K_7 ) ( K_8 ) ( K_9 ) ( K_{10} ) ( K_{11} ) ( K_{12} ) ( K_{13} ) ( K_{14} )</td>
<td>63</td>
</tr>
<tr>
<td>(\text{Zn}^{2+} )</td>
<td>gl</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 5.23, ( K_2 ) 10.43</td>
<td>64</td>
</tr>
<tr>
<td>(\text{Ni}^{2+} )</td>
<td>sp</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 22.78, ( K_2 ) ( K_3 ) ( K_4 ) ( K_5 ) ( K_6 ) ( K_7 ) ( K_8 )</td>
<td>65</td>
</tr>
<tr>
<td>(\text{Zn}^{2+} )</td>
<td>gl</td>
<td>30</td>
<td>0.1 KCl</td>
<td>( K_1 ) 13.48, ( K_2 ) 23.3</td>
<td>64</td>
</tr>
</tbody>
</table>
Ligands Chosen For Study

While it was apparent that many sulphur-containing ligands deserved more detailed study than had previously been attempted, the monothiol chelating agents appeared most likely to lead to tractable results because the presence of only one sulphur atom would not be expected to encourage the formation of long chains. Thus the study of monothiol ligands formed the major part of the present work.

One of the simplest of these ligands is di-anion of mercaptoacetic acid, for which stability constants had already been measured for the polynuclear nickel(II) species. This work was repeated and extended, and the complex formation of this ligand with zinc(II) and other metal ions was examined in order to establish the existence, or otherwise, of polynuclear species in these systems.

2-Mercaptoethylamine mono-anion was the next simple ligand studied, polynuclear species being expected with nickel(II), and also possibly for zinc(II). Poor solubility of the complex species presented a problem with this ligand. To overcome this difficulty, in order to study bidentate aminothiol complexes at high concentration, an attempt was made to prepare a new complexing agent, 3-mercapto-2-aminopropane-1-sulphonic acid.

The aminothiol residue is present in two further ligands studied in this work, cysteine and penicillamine.
While the carboxyl group might here be expected to increase the solubility of the species, it may also participate in some bonding, increasing the number of species to be considered, and rendering interpretation of data more difficult.

The final complexing agent studied was 2,3-dimercaptopropane-1-sulphonic acid ("Unithiol"). This compound might be expected to form very stable and highly soluble "core plus links" species, analogous to those of BAL.

The structures of these, and of some other important sulphur-containing ligands, are shown in Figure 1.04.

**Biological Significance of Ligands Selected**

Interest in the ligands chosen for this study stems, not only from the unusual nature of the complexes formed, but also from the considerable importance of such sulphur compounds in biological processes.

The aminothiol residue is a constituent of the important amino acid cysteine, found in glutathione and many other peptides and proteins. The active end-group of coenzyme A is a mercaptoethylamine residue,\(^69\) acylated at nitrogen by pantothenic acid, which is itself linked by a pyrophosphate moiety group to an adenylic acid group. The entire
Some important sulphur-containing ligands.

Mercaptoacetic acid

\( \text{CH}_2-\text{CO}_2\text{H} \)

\( \text{SH} \)

\( \text{I} \)

\( \text{I} \)

Cysteine

\( \text{CH}_2-\text{CH}-\text{CO}_2\text{H} \)

\( \text{SH} \)

\( \text{NH}_2 \)

\( \text{C}-\text{CH}-\text{CO}_2\text{H} \)

\( \text{NH} \)

\( \text{2-Mercaptoethylamine} \)

\( \text{2,3-Dimercaptopropane-1-sulphonic acid} \)

\( \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{SO}_3\text{H} \)

\( \text{SH} \)

\( \text{SH} \)

\( \text{3-Mercapto-2-aminopropane-1-sulphonic acid} \)

\( \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{SO}_3\text{H} \)

\( \text{SH} \)

\( \text{NH}_2 \)

\( \text{2,3-Dimercaptopropan-1-ol} \)

\( \text{CH}_2-\text{CH}-\text{CH}_2-\text{OH} \)

\( \text{SH} \)

\( \text{SH} \)

\( \text{Dihydrolipoic acid} \)

\( \text{CH}_2-\text{CH}_2-\text{CH}-(\text{CH}_2)_4-\text{CO}_2\text{H} \)

\( \text{SH} \)

\( \text{SH} \)

\( \text{6-Mercaptopurine} \)

Fig. 1.04 Some important sulphur-containing ligands.
molecule contains a number of chelating groups and appears to be involved in a wide range of syntheses and degradations proceeding through $C_2$ units.

An important dithiol, found in the body and in plants, is dihydrolipoic acid, whose properties have been summarised by Reed. It is involved in the decarboxylation of $\alpha$-keto acids, and may also act as a photosynthetic intermediate in plants. Although a $\beta$-dithiol, dihydrolipoic acid will form stable chelates. Inhibition of the pyruvate oxidation system of the brain by arsenicals, and reversal of this inhibition by BAL but not by monothiols, is suggested as evidence for the presence of protein-bound dihydrolipoic acid, and is a manifestation of its chelating ability.

Thiols are found to protect living organisms from damage by radiation. This property may arise from their action as radical scavengers, it may be associated with their ability to form mixed disulphides with the essential thiol groups of enzymes, so protecting them from attack by radicals, or it may be connected with their ability to chelate metals. Jones, and Knoblock and Purdy, have described correlations between stability constants and the radioprotective ability of a number of compounds, but some of their conclusions are invalidated by their use of erroneous values for the stability constants of copper(II)-thiol complexes. However although the equilibria
which are important are little understood, the involvement of chelation in radioprotection cannot be ruled out.

Thiols have found several medical applications. 2-Mercaptoethylamine has been used in the treatment of leukaemia, and 6-mercaptopurine is now widely used in the retardation of this disease. In the treatment of various types of poisoning, thiols are again useful. The toxicity of lead and arsenic appears to arise from complex formation with sulphydryl groups in nerve cells.

Treatment of this poisoning with BAL has been useful for some years, but the drug has some unpleasant side-effects and may not be taken orally. "Unithiol" on the other hand is stable, less toxic, and forms highly soluble complexes. It is currently being tested as a metal-poisoning antidote in Russia. Wilson's disease is accompanied by an excess of non-excretable copper in the body. DL-penicillamine and its analogue N-acetyl-DL-penicillamine may both be taken orally, and are very effective in the removal of this metal (as the cuprous complex). Treatment may take some months, as the metal must migrate to sites accessible to the drug. DL-cysteine is much less effective in this work, possibly because it is too rapidly metabolised, or perhaps because of the insolubility of the cuprous complex.
In view of the biological importance of these compounds, and the fact that this importance arises, or may arise, from their ability to form metal complexes, it is desirable to know the nature and stability of these chelates. Only then may their mode of action be understood, and the effect of disturbing the biological equilibrium by the introduction of a foreign chelating agent be predicted.

The above considerations made it seem likely that many of the stability constants reported for metal complexes with ligands of the types selected for study were, in fact, likely to be incorrect because of the use of oversimplified treatments. The possibility of polynuclear complex formation in metal-thiol systems, meant that, if satisfactory results were to be obtained, potentiometric measurement should be of a much higher level of accuracy than had previously been attained, that a sufficiently wide range of metal ion and ligand concentration should be studied, and that much more refined methods of calculation should be used.

In the study reported in this Thesis, the necessary instrumental precision has been obtained by using a recording pH meter which was reproducible to ± 0.002 pH unit, in conjunction with a specially designed titration cell.
The rapid quantitative mathematical analysis of titration data from systems containing polynuclear or protonated species is impractical by the use of desk calculators and other conventional devices. Instead, it was necessary to develop a generalised equilibrium constant refinement programme for use with a large capacity, high-speed, digital computer. With this programme it was possible to examine the quality of fit to the data that could be obtained using various combinations of possible complexes, and thus to determine the nature of the species present with far greater reliability than had hitherto been possible.
CHAPTER 2

CALCULATION METHODS

The quantitative study of chemical equilibria commenced in the latter half of the last century with the derivation of the Law of Mass Action, based mainly on the work of van't Hoff,\textsuperscript{83} and Guldberg and Waage.\textsuperscript{84} Ostwald was the first to calculate acid-base equilibrium constants,\textsuperscript{85} and in the early part of this century various workers began to examine metal complex equilibria. Present day calculation concerning metal complex systems are based largely on the methods of N.\textsuperscript{86} and J. Bjerrum\textsuperscript{87} and others.\textsuperscript{88,89}

Stability Constant Definitions

N. Bjerrum\textsuperscript{86} considered the successive reaction of a metal M with a series of ligand molecules (or ions) to occur in a sequence of equilibria*

\[
\begin{align*}
M + L & \rightleftharpoons ML \\
ML + L & \rightleftharpoons ML_2 \\
& \quad \ldots \quad \\
ML_{N-1} + L & \rightleftharpoons ML_N
\end{align*}
\]

* In the interests of clarity, charges on ionic species will be omitted here, and elsewhere in this Thesis, where they are unimportant to the discussion.
For each of these reactions it is possible to define an equilibrium constant. The stepwise formation constants apply to the process of association and are defined as

\[ K_1 = \frac{[ML]}{[M][L]} \]
\[ K_2 = \frac{[ML_2]}{[ML][L]} \]
\[ \ldots \ldots \ldots \]
\[ K_N = \frac{[ML_N]}{[ML_{N-1}][L]} \].

These constants may be combined to give the stability or complexity constant, \( \beta_N \) for the \((N)\)th species being the product of the individual stepwise formation constants, i.e., for the equilibrium

\[ M + N(L) \rightleftharpoons ML_N, \]

\( \beta_N \) is given by

\[ \beta_N = \frac{[ML_N]}{[M][L]^N} = K_1 K_2 \ldots K_N. \]

N. Bjerrum defined the quantity \( \bar{n} \) as the average number of ligands bound to each metal atom, thus

\[ \bar{n} = \frac{[ML] + 2[ML_2] + \ldots + N[ML_N]}{[M] + [ML] + [ML_2] + \ldots + [ML_N]} = \frac{\sum_{n=1}^{n=N} n \beta_n [L]^n}{\sum_{n=0}^{n=N} \beta_n [L]^n} \]

This equation has been termed the formation function, and the plot of \( \bar{n} \) versus \( \log[L] \) is the formation curve. The quantity \( \bar{n} \) is a function of \([L]\) only, provided all the complexes formed are mononuclear. In this case the formation curve for any given ligand is independent of the total metal ion concentration.
Sillén 67 has discussed the case when polynuclear complexes form, but where all complexes may be described as comprising one central species (the "core") and a number of units of another species (the "link"). Any member of the "core plus links" series can thus be described by one general formula. This type of system is often found in metal hydrolysis, and gives rise to a series of formation curves, for different values of total metal concentration \([M_T]\). The curves all have similar shape, but are shifted parallel to the log\([L]\) axis. The spacing is regular, \(\Delta \log[M_T]/\Delta \log[L]\) being constant. From the magnitude of the spacing it is possible to calculate the compositions of the core and linking species, and by a variety of methods the relevant equilibrium constants may be obtained. 67, 68

The treatment often gives very satisfactory results with simple systems. In metal chelate systems, however, there may be no good reason to assume that a particular "core plus links" series should predominate, and it is often impossible to obtain accurate results over a very wide range of total metal concentrations. This means that the "core plus links" hypothesis if used, should be applied with caution, because an apparent fit to the data, by a given series over a restricted range of concentrations, may at times be purely fortuitous.
In order to calculate \( \bar{n} \) we can use another equation which follows from the definition of \( \bar{n} \), namely that

\[
\bar{n} = \frac{[L_T] - [L_U]}{[M_T]} \quad 2.05
\]

where \([L_T]\) and \([M_T]\) are the total concentrations of ligand and metal respectively and \([L_U]\) is the concentration of ligand not complexed with metal, this quantity including free ligand and any protonated species which are present.

The stability constant, as defined above, may in principle be determined by any method which gives the concentrations in equation 2.03, and numerous methods are available. The essence of all of these is the measurement of the requisite quantities without disturbing the equilibrium. Sometimes one component may be measured potentiometrically or polarographically. If the spectra of the various species are known, and sufficiently different from one another, it may be convenient to measure the concentrations spectrophotometrically. Solubility, ion-exchange, and a variety of other equilibrium processes have also been employed to give the necessary data.

Potentiometry is one of the most accurate methods available for measuring concentrations, and where it is convenient it is generally the method of choice.
Potentiometry using a glass electrode is a particular example of a class of methods in which a competition is studied between the metal and another ion for the ligand. In this case the other ion is the proton, and since the equilibrium constant for the reaction between the ligand and the proton may be separately determined, measurement of the hydrogen ion concentration may, under suitable circumstances, provide all the data necessary to derive the stability constants. This process will now be considered in detail.

Evaluation of $pK_a$ Values of Ligand

Ligands are generally weak acids or weak bases, and the degree of their dissociation in solution varies with pH. If the ligand is monobasic, of the form HL, we may employ a calculation of the following type, which is also suitable for a dibasic ligand if the $pK_a$ values are separated by more than 2.5 to 3 pH units. We have the equilibrium

$$\text{HL} \rightleftharpoons \text{L}^- + \text{H}^+$$

with the "practical" dissociation constant

$$K_a = \frac{[\text{H}^+][\text{L}]}{[\text{HL}]} = 2.06$$

Here and elsewhere we shall employ the convention that square brackets [ ] denote molar concentration and braces { } denote activities.
The total ligand concentration \([L_T]\) is given by

\[
[L_T] = [L^-] + [HL]
\]

\[
= [L^-] \left\{ 1 + \frac{[H^+]}{K_a} \right\}
\]  

(2.07)

and from the principle of electroneutrality we have total positive charge equal to total negative charge, i.e.

\[
[H^+] + [K^+] = [L^-] + [OH^-] + [ClO_4^-]
\]  

(2.08)

where \([K^+]\) = concentration of added inorganic base, and

\([ClO_4^-]\) = concentration of inorganic acid (if any).

Equation 2.08 may be rewritten in the form

\[
Q = [H^+] + [K^+] - [OH^-] - [ClO_4^-] = [L^-]
\]  

(2.09)

Then from equation 2.07, we have

\[
Q = \frac{[L_T]}{1 + \frac{[H^+]}{K_a}}
\]  

(2.10)

and hence

\[
K_a = \frac{[H^+]Q}{([L_T] - Q)}
\]  

(2.11)

or

\[
p_{K_a} = pH + \log \left\{ \frac{[L_T] - Q}{Q} \right\}
\]

(2.12)

The calculation is less simple if the two \(pK_a\) values of a dibasic ligand are less than 2.5 pH units apart, but a method similar to that of Speakman may be employed.
If, for example, we consider a species such as 2-mercaptoethylamine (HL), then we have the following equilibria

\[ \begin{align*}
H_2L^+ & \rightleftharpoons HL + H^+ \\
HL & \rightleftharpoons L^- + H^+ 
\end{align*} \]

with equilibrium constants

\[ K_{a1} = \frac{\{H^+\}\{HL\}}{[H_2L^+]} \quad \text{and} \quad K_{a2} = \frac{\{H^+\}\{L^-\}}{[HL]} \]. \quad 2.12

The total ligand equation now becomes

\[ [L_T] = [L^-] + [HL] + [H_2L^+] \quad 2.13 \]

and electroneutrality gives us

\[ [H_2L^+] + [H^+] + [K^+] = [L^-] + [OH^-] + [ClO_4^-] \quad 2.14 \]

Manipulation of equations 2.12, 2.13 and 2.14 yields the relationship,

\[ \frac{\{H^+\}^2 ([L_T] + Q)}{([L_T] - Q)} = K_{a1} Q\{H^+\} + K_{a1} K_{a2} Q^{-[L_T]} \]. \quad 2.15

By plotting the left hand side of equation \( 2.15 \) versus \( \frac{Q\{H^+\}}{Q^{-[L_T]}} \), \( K_{a1} \) and \( K_{a1} K_{a2} \) may be obtained as the slope and intercept respectively.
Small Fortran computer programmes were written to carry out both of these calculations. The second calculation involves either a graphical plot of the computed figures, or automatic least-squares analysis by the computer itself. After the development of the programme GAUSS (see below), both of these types of calculation were unnecessary, unless it was desired to obtain initial estimates of the constants, for subsequent refinement by the larger programme. In the latter programme the constants must be defined as the cumulative association constants, and it is in this form that they are reported in Chapter 5, i.e.

\[ \beta_1 = \frac{[HL]}{[L][H^+]} \quad \beta_2 = \frac{[H_2L]}{[L][H^+]^2} \]

2.16

**Stability Constant Calculation**

The pK\textsubscript{a} values of the ligand having been determined, we can now calculate values of free ligand concentration and \( \bar{n} \) in a potentiometric titration in the presence of metal. Equation 2.05 becomes, for our particular ligand \( L^- \), with protonated forms HL, H\(_2\)L\(^+\),

\[ \bar{n} = \frac{[L_T^-] - [L^-] (1 + \frac{(H^+)}{K} + \frac{(H^+)^2}{K_2})}{[M_T]} \]

2.17
If we could assume complete formation of the \((n)\)th complex before the \((n+1)\)th species starts to form, then, following Flood and Loras,\(^9^2\) we would expect to find that for 1:1 and 2:1 complexes,

\[
\begin{align*}
K_1 &= \frac{\bar{n}}{(1-\bar{n})[L^-]} \\
K_2 &= \frac{(\bar{n}-1)}{(2-\bar{n})[L^-]}
\end{align*}
\]

and in general we could obtain \(K_n\) from our formation curve as the value of \(1/[L^-]\) at the point where \(\bar{n} = (n-1/2)\).

In fact, although these approximations have been widely used in work with sulphur ligands, they have given rise to many erroneous results. Successive formation constants in some cases increase, and usually, if a decrease is observed, it is not sufficiently great for accurate constants to be obtained in this way.

On the other hand the graphical method of Irving and Rossotti,\(^9^3\) for a system of 1:1 and 1:2 complexes, is rigorous and is analogous to the calculation of overlapping \(pK_a\) values. The equation follows from equation 2.04, i.e.

\[
\frac{\bar{n}}{(1-\bar{n})[L]} = \frac{(2-\bar{n})[L]n_2 + K_1}{(1-\bar{n})}\]
This expression may be solved graphically, in which case the terms to be plotted may be calculated using a small Fortran programme. Alternatively \( K_1 \) and \( \beta_2 \) may be obtained by least-squares analysis, using the computer. This method gives satisfactory results where only the two species \( ML \) and \( ML_2 \) exist, although where \( K_1 \) is small relative to \( \beta_2 \), the value of \( K_1 \) obtained may be uncertain because the intercept is close to the axis. When further species are present, in particular when these are polynuclear, protonated, or hydrolysed complexes, it is necessary to use more advanced methods.

In the course of the present study a number of computer programmes were written, based on the assumption that only one additional species existed. As this had been suggested to be the case for 2-mercaptoethylamine and nickel(II), and was a fair approximation in the case of mercaptoacetic acid and zinc(II), the equations will be discussed briefly.

Let us postulate a system where all the metal complex equilibria can be written in the form

\[
p^M + qL \rightleftharpoons \frac{M}{p} \cdot \frac{L}{q}
\]

where \( p \) and \( q \) are integers, and no protonated or hydrolysed metal complex species exist. The stability constant of each species is then defined by an expression of the form
\[ \beta_{qp} = \frac{[LP]}{[MP][L]^q} \] 2.21

and irrespective of the range of \( p \) and \( q \), or of the number of pairs of values of \( p \) and \( q \) which apply, the free ligand concentration \([L]\) and value of \( \bar{n} \) may still be calculated using the equations derived above.

Now if we have only the species \( ML, ML_2 \), and one additional species \( ML \), from the definition of \( \bar{n} \), we can derive the following relationship;

\[ \bar{n} [M_T] - [ML] - 2[ML_2] = \frac{p q}{q \log [H] + q \log [L]}. \] 2.22

and hence

\[ \log (\bar{n} [M_T] - [ML] - 2[ML_2]) = \log (\beta_{qp}) + p \log [H] + q \log [L]. \] 2.23

Under favourable circumstances it is possible to measure the free metal concentration, and if we can obtain an estimate of \( K_1 \) and \( \beta_2 \) under conditions of negligible complex formation, then the term on the left hand side of equation 2.23 can be calculated and plotted in two different ways. These are (a) versus \( \log [M] \) at constant \([L]\), and (b) versus \( \log [L] \) at constant \([M]\).

If our assumptions prove valid, plot (a) should yield a straight line of slope \( p \), and plot (b) a straight line of slope \( q \). Both plots should give an intercept from which \( \beta_{qp} \) could then be calculated.
This method was employed for the zinc(II)-mercapto-
acetic acid system, but the necessity of determining
separately either $[M]$ or $[L]$ (and of obtaining reasonably
accurate estimates of the values of $(N-1)$ of the constants
of a system of $N$ complex species) greatly restricts the use
of this technique. Several iterative computer programmes
were written for this type of system, for the treatment of
$pH$ titration data when the above conditions were not
satisfied. One such programme will be briefly discussed,
although, with the development of the programme GAUSS, all
of these elementary computer methods have been rendered
obsolete.

The calculation was based on a modification of the
Irving-Rossotti equation (equation 2.19). If we include
a single species $M^pL^q$, the relationship becomes
\[
\frac{\bar{n}}{(1-\bar{n})[L]} + \beta^{qp} \frac{[M]^{p-1}[L]^{q-1}}{(\bar{n}-1)} = \frac{(2-\bar{n})[L]}{(1-\bar{n})} \beta_2 + K_1. \tag{2.24}
\]
When the computer was provided with the experimental data,
with trial values of $p$ and $q$ and with initial approximations
for $K_1$ and $\beta_2$, it calculated and printed, for each
experimental point, an approximate value of $\beta^{qp}$. As shown
in the simplified flow sheet (Fig.2.01), by a suitable choice
of a sense-switch the computer could be made to accept
alternative estimates of $K_1$ and $\beta_2$ (in an attempt to reduce
the spread of values of $\beta^{qp}$), or more usually, to accept a
Fig. 2.01. Simplified flow chart for computer programme to calculate constants when one polynuclear species is present.
"best estimate" of $\beta_{qp}$ from the range provided. This meant that the left hand side of equation 2.24 was now calculable, and for each point in the titration we could obtain quantities which could be plotted to yield $\beta_2$ and $K_1$ as in the simple Irving-Rossotti case. If required these were plotted, otherwise the calculation was carried out automatically by the least-squares method, and the computer then entered an automatic cycle in which $\beta_{qp}$ was adjusted to give the most nearly linear plot for equation 2.24. When the best fit was achieved, values of all the relevant data were printed out. Alternative values of $p$ and $q$ were then tested in order to find those values which gave the best fit to the data.

This method, although simple in approach, would be quite satisfactory if we had only one polynuclear, and no protonated or hydrolysed, species to consider. It soon became apparent that the systems under study were not as simple as this, and so a more general approach became necessary. For this purpose, the programme GAUSS4 was developed.
CHAPTER 3

PROGRAMME GAUSS

Two basic methods for the treatment of data for polynuclear systems have been discussed in the literature. The first and simplest is the least-squares approach. This technique has been used in simple cases by several groups, and was first written in a general form by Tobias. The mathematical approach has been discussed by Wentworth. The second method is due to Sillén and is employed in a series of programmes of the "LETAGROP" or "pit-mapping" type. In these programmes the problem is still to find a set of constants which minimize an appropriate error-square sum, but rather than doing this by the conventional least-squares technique, the error-square sum function for the parameters is defined as a "paraboloid in n-dimensional space", the equation of this surface is calculated, and the minimum or "pit" is searched for.

Both of these approaches can give satisfactory results if used properly. The "pit-mapping" method is more unwieldy to programme, and, at least in its earlier versions, was the less reliable; however, some of these problems appear to have been overcome by employing more sophisticated mathematical techniques.
The conventional least-squares programme was selected for development and use in the present study for various reasons. The non-linear least-squares approach is widely used and has for some years been the standard technique employed by X-ray crystallographers in the reduction of data, and by other workers with similar types of problem. The approach is simpler and possibly somewhat more reliable, and finally, a version of the basic programme (GAUSSG written by Tobias) was available in Fortran,\textsuperscript{102} while the only version of the latest LETAGROP programme was written in Algol,\textsuperscript{101} a computer language not commonly employed in Australia, and for which facilities for compilation (or translation) were not available.

The present programme will be referred to simply as GAUSS, although various versions have been written (all in Fortran). The one to be described is the latest development (GAUSS4). In basic approach it is quite similar to the programme GAUSSG of Tobias,\textsuperscript{96} but the present programme includes some important modifications and extensions, so that its efficiency has been greatly improved, and its usefulness in treating pH titration data for complicated systems much increased. For this reason the programme will be described in full. Discussion of the subroutines employed, which have been only slightly altered, will be found in Appendix II.
Both the present and Tobias's programmes are designed to refine equilibrium constants to fit data for any of the following types of system, or a combination of these: metal-ion hydrolysis, dissociation of weak acids, simple mononuclear complex formation (up to any coordination number), polynuclear complex formation, and the formation of any complexes where the species are protonated or hydrolysed. The use of either programme for these various systems requires only that all of the species present be specified, together with their approximate equilibrium constants. Unlike certain comparable programmes, no further programme-writing is required to deal with different systems.

As in the original programme, refinement, which may be of one or more constants at a time, consists of minimizing the sum of the squares of the weighted residuals in a quantity we may term the "analytical hydrogen ion concentration". This quantity, $C_H$, is defined as

$$C_H = L_T \cdot X + A \cdot V/(V+T) - B \cdot T/(V+T)$$

where $L_T$ is the total ligand concentration, $X$ is the number of displaceable protons on the undissociated complexing species as added to the solution, $A$ is the initial concentration of inorganic acid, $B$ is the concentration of titrant base, $T$ is the titre and $V$ the total initial volume.
The residuals, $R$, are then given by

$$ R = C_H - \frac{(H^+)(OH^-)}{f^\pm} + \sum f_m s_n [L]^1 [M]^m (H^+)^{-n} \beta_{1mn} $$

Where $\{H^+\}$ and $\{OH^-\}$ are the activities of hydrogen and hydroxyl ions, $f^\pm$ is the mean activity coefficient, $[L]$ and $[M]$ are the concentrations of free ligand and free metal, and $\beta_{1mn}$ is the general stability constant given by

$$ \beta_{1mn} = \frac{[M]^n [L]^1 (H^+)^n}{[M]^n [L]^1 (OH)^n} $$

The value of $n$ in these equations is positive for hydrolysed species and negative for protonated species. Equation 3.02 differs from that employed by Tobias, since we are here calculating "practical" constants, as defined by equation 3.03 and this necessitates the inclusion of the activity coefficient.

In the weighted least-squares refinement each value of $R^2$ is divided by its variance. Thus the process of refinement is designed to adjust the various equilibrium constants so as to minimize $S$ in the equation

$$ S = \sum w_i R_i^2 $$

Where $I$ is the total number of data points, $R_i$ is the
residual for the (i)th point, and \( w_i \) is the reciprocal of the variance of \( R_i \), and may be termed the statistical weight of \( R_i^2 \). The present programme calculates the weights by the method of Hugus from the errors in pH (\( \delta \text{pH} \)), and in total metal (\( \delta M_T \)), total ligand (\( \delta L_T \)), and analytical hydrogen ion (\( \delta C_H \)), concentrations. The calculation of these quantities (not provided in Tobias's programme) is carried out as follows. With \( M_T \) equal to the total metal concentration, \( M_0 \) and \( L_0 \) equal to the total initial metal and ligand concentrations, and the other symbols as defined above, we have

\[
M_T = M_0 \cdot V / (V+T)
\]

3.05

\[
L_T = L_0 \cdot V / (V+T)
\]

3.06

and \( C_H \) as defined by equation 3.01. Denoting the errors in \( M_0 \), \( L_0 \), \( V \), \( T \), \( A \) and \( B \) by \( \delta M \), \( \delta L \), \( \delta V \), \( \delta T \), \( \delta A \), \( \delta B \) respectively, we may now calculate the required errors by first partially differentiating the relevant equations. Then, assuming that the input errors are independent, the variances (\( \delta M_T \))^2 etc. are obtained by summation of the squared terms, i.e. by taking the vector sum of the independent component vectors. Hence

\[
(\delta M_T)^2 = \left( V \cdot \delta M \right)^2 \left( V + T \right) + \left( \frac{[(V+T)M_0 - M_0 \cdot V] \delta V}{(V+T)^2} \right)^2 + \left( \frac{-M_0 \cdot V \cdot \delta T}{(V+T)^2} \right)^2
\]

3.07
\[(\delta L_T)^2 = \left(\frac{V \cdot \delta L}{V+T}\right)^2 + \left(\frac{[L_0 - L_0 \cdot V] \cdot \delta V}{(V+T)^2}\right)^2 + \left(\frac{-L_0 \cdot V \cdot \delta T}{(V+T)^2}\right)^2\]

and

\[(\delta C_H)^2 = (\delta L_T)^2 + P + Q\]

where \(P\) and \(Q\) are terms involving acid and base, given by

\[P = \left(\frac{V \cdot \delta A}{V+T}\right)^2 + \left(\frac{[(V+T)A - V \cdot A] \cdot \delta V}{(V+T)^2}\right)^2 + \left(\frac{-A \cdot V \cdot \delta T}{(V+T)^2}\right)^2\]

\[Q = \left(\frac{T \cdot \delta B}{V+T}\right)^2 + \left(\frac{[(V+T)B - T \cdot B] \cdot \delta T}{(V+T)^2}\right)^2 + \left(\frac{-B \cdot T \delta V}{(V+T)^2}\right)^2\]

In this way, the errors of the input quantities having been specified, \(\delta pH, \delta M_T, \delta L_T,\) and \(\delta C_H\) may be calculated for use later in the programme in the calculation of the weight of each reading.

Now, for the \((i)th\) experimental point the residual \(R_i\) is a function of the constants \(\beta_1, \beta_2\) etc.

\[R_i = f_i(\beta_1, \beta_2, \ldots)\]

and we require to minimize the function \(\Sigma w_i R_i^2\), where \(w\) is the weight. If we expand the function in Taylor's series about the trial values of the constants, which are assumed to be close to their actual values, then neglecting terms beyond the first order, we find that for a minimum, i.e.,

\[f(x) = x^2 + x + 1 = 0\]
terms beyond the first order, we find that for a minimum value of $\sum w_i R_i^2$, the so called "normal equations" apply, i.e.

$$\sum w_i \left( \frac{\delta f_i}{\delta \beta_1} X(1) + \frac{\delta f_i}{\delta \beta_2} X(2) + \frac{\delta f_i}{\delta \beta_3} X(3) + \ldots + R_{oi} \right) \left( \frac{\delta f_i}{\delta \beta_1} \right) = 0$$

3.13

$$\sum w_i \left( \frac{\delta f_i}{\delta \beta_1} X(1) + \frac{\delta f_i}{\delta \beta_2} X(2) + \frac{\delta f_i}{\delta \beta_3} X(3) + \ldots + R_{oi} \right) \left( \frac{\delta f_i}{\delta \beta_2} \right) = 0$$

etc.

where $X(1)$, $X(2)$ etc. are the shifts to minimum for $\beta_1$, $\beta_2$, etc., and $R_{oi}$ is the residual for the (i)th point using the initial values of the constants. These equations may be rewritten in the form of the matrix:

$$\begin{pmatrix}
\sum w_i \frac{\delta f_i}{\delta \beta_1} & \sum w_i \frac{\delta f_i}{\delta \beta_2} & \ldots & \sum w_i \frac{\delta f_i}{\delta \beta_j} \\
\sum w_i \frac{\delta f_i}{\delta \beta_1} & \sum w_i \frac{\delta f_i}{\delta \beta_2} & \ldots & \sum w_i \frac{\delta f_i}{\delta \beta_j} \\
\sum w_i \frac{\delta f_i}{\delta \beta_1} & \sum w_i \frac{\delta f_i}{\delta \beta_2} & \ldots & \sum w_i \frac{\delta f_i}{\delta \beta_j} \\
\sum w_i \frac{\delta f_i}{\delta \beta_1} & \sum w_i \frac{\delta f_i}{\delta \beta_2} & \ldots & \sum w_i \frac{\delta f_i}{\delta \beta_j}
\end{pmatrix}
\begin{pmatrix}
X(1) \\
X(2) \\
\vdots \\
X(j)
\end{pmatrix}
= 
\begin{pmatrix}
-\sum w_i R_{oi} \frac{\delta f_i}{\delta \beta_1} \\
-\sum w_i R_{oi} \frac{\delta f_i}{\delta \beta_2} \\
\vdots \\
-\sum w_i R_{oi} \frac{\delta f_i}{\delta \beta_j}
\end{pmatrix}$$

3.14

etc.

The derivatives in this matrix are obtained numerically by applying a small change to each constant and then since the term $C_H$ in equations 3.01 and 3.02 does not change as a result of this increment, we may replace terms in $f_i$ with terms in $F_i$ which from 3.02 is given by
Inversion of this matrix leads to the solution of the normal equations for the shifts $X$, and when these are applied to the initial constants we obtain a set of improved values for the constants. The cycle may then be repeated if further refinement is required.

In the above broad outline the programme is similar to that of Tobias, but for greater convenience and reliability, as well as improved speed of calculation, the following additions and changes have been made.

Firstly the form of the input data has been altered. Since we are only concerned with data from pH titrations, the first part of the new programme is concerned with the reading of the primary data from an individual experiment, or from a set of experiments which deal with a single system whose results are to be refined simultaneously. The data for a set of experiments are correlated, and all the concentrations, pH's and errors are set up ready for the calculation. The main programme then calls the appropriate subroutine in CØNC5, CØNC6, or CØNC7, in which the concentrations of free metal and free ligand are calculated using the input estimates of the constants. CØNC5 incorporates a Newton-Raphson iterative procedure (see Appendix II) for the simultaneous solution of the

\[
F_i = \frac{\{H^+\} - \{OH^-\}}{f^+} + \xi \sum_{m \geq n} \beta_{lmn} [L_i]^{m}[M_i]^{n}[H_i]^{-n} 3.15
\]
equations for total metal and total ligand concentrations, and is used for general metal complex calculations. C\text{ONC6} calculates free ligand, and C\text{ONC7} free metal, concentrations. These subroutines are brought into use for calculations of acid dissociation constant, and metal hydrolysis respectively. At the end of each subroutine the analytical hydrogen ion concentration corresponding to the estimated constants is calculated, as is the variance in this quantity. The latter is obtained by the method of Hugus. Control then returns to the main programme.

The subroutines used in the present programme are very similar to those in the original, but a number of small changes greatly increase their efficiency. The approximations now used to commence the iteration for free metal and free ligand concentrations, assume for the first point (at low pH) that the free metal is equal to the total metal concentration, and that the free ligand concentration equals the value that would be found at the appropriate pH if there was no complex formation. Subsequently, for the (n+1)th point, the initial estimates for free metal and free ligand concentrations are taken as the final values for the (n)th point. In the event of non-convergence of the (n)th point, the starting approximations for the (n+1)th are the same as those for the first point. These changes mean that the calculation in the subroutines is
usually complete in 2 to 4 cycles per point, as opposed to 20 to 30 cycles in the original programme, saving a considerable amount of time. In the unusual event of repeated non-convergence in the concentration subroutines, which is usually the result of faulty data, an indicator has now been included which leads to the abandonment of the faulty experimental set, and the programme moves on to the next set of data.

The present programme is designed to deal with poorer initial estimates than was the original form. Thus while the latter programme could cope with only small overshifts, it was found useful in the present version to include a step which prevented any excessively large shifts, restricting the maximum change per cycle to ±0.5 log units, while retaining the check on small overshifts. This means we have rapid shifts where required without permitting these to be impossibly large. Where the initial estimates of the constants are much too low, adjustment of the constants by the earlier programme did not occur, since the increment employed in the differentiation process was too small to have an appreciable effect. This problem has been overcome by permitting successive increases in the value of the increment (H(I), in the programme), until the differential term is large enough to be significant.
A message is printed out when this occurs and after the differentiation the increment is returned to its former value.

Another change in the programme was necessary at this stage. The original programme carried out the numerical differentiation by successively incrementing each constant $E(I)$ by $H(I)$, subtracting $2*H(I)$, and finally adding $H(I)$. With the IBM 360/50 the quantities are not stored as whole numbers, and this cycle does not return $E(I)$ to exactly its original value. The error is significant if many successive differentiations are required, and if $H(I)$ is large. The problem is avoided by storing the original value of $E(I)$ as $E_{\text{ORIG}}(I)$, and returning to this value after each cycle.

The matrix inversion subroutine is now a standard IBM programme. It is used in preference to the one incorporated in the original programme: the latter, because of failure to initialise two components of the matrix, led to erroneous results when it was used on a C.D.C 3600 computer. More recently an IBM 360/50 computer has been used, and for this machine it was necessary to employ double precision variables for the concentration subroutines and the section of the programme dealing with the differentiation and shifts in the constants. It is this version of the programme which is shown below.
After each cycle to refine the constants the computer prints out their new values together with their estimated standard deviations, and the weighted variance and unweighted sum of the squares of the residuals. The refined values are then used as initial estimates in the next cycle. After a specified number of cycles (five being usually sufficient) the computer uses the final values of the constants to calculate and print out a table which lists for each experimental point, the concentrations of total metal and total ligand, the pH, the analytical hydrogen ion concentration, the residual in this quantity, its statistical weight, the concentrations of free metal, free ligand and of each of the complex species, $n$, the actual titre of base, and the difference between this quantity and that calculated using the refined constants. This latter quantity provides a convenient measure of the closeness of fit of the experimental data. After printing this table, the standard deviation in the titre is printed out, followed by the elements of the matrices used in the last cycle. The computer then advances to the next experiment, or set of experiments, in the data stack. As described below, it is possible, before the exit to a new set of data, to repeat the calculation with a new initial value for one of the constants. This feature is very useful and can save much trouble in the unnecessary duplication of data decks.
We shall now consider some aspects of the use of the programme GAUSS. If an attempt is made to obtain constants for an unimportant species, this fact manifests itself in either of two ways. In successive cycles the values of the constant may fail to converge to a satisfactory limit with only a small standard deviation, and the weighted variance and unweighted sum of the squares of the residuals may even increase as the calculation proceeds. Alternatively, the constant assigned to the species may be steadily decreased in successive cycles, with only slight changes in the values of the other constants. Either of these effects may also be observed if the initial estimates of any constants are very poor.

Initial estimates for the constants may be obtained graphically or from the literature. However if, for a postulated species, an initial value is not available, this may be obtained by carrying out a series of calculations in which the value of the unknown constant is held steady at a series of trial values while the remaining constants are refined as described later; this facility has been incorporated into the programme. In this way is found the value of the constant which gives the lowest variance, and this may be used in a subsequent calculation in which it is refined automatically. In general, when searching for the value of a given constant using the programme, it
is best to vary only the constants for those species which are believed to be major components in a given experiment, values of constants for other species being fixed at the best available estimates.

Once sufficiently good initial estimates have been obtained for all the constants, these may be refined simultaneously in a calculation which employs the entire range of experimental data. It is preferable that this final refinement be carried out only with good initial values of the constants, otherwise refinement, i.e. the minimization of the sum of the squares of the weighted residuals, may proceed to an alternative minimum, which corresponds to a different set of complex species and gives a poorer fit to the experimental data. This effect is also observed with other equilibrium constant refinement programmes.\textsuperscript{98,103}

The input data and formats required for GAUSS are listed below.

Item 1:- The number of different systems (i.e. experiments or sets of experiments for which refinement is to be carried out (I2)).

Item 2:- The number of experiments which together make the first (second, third etc.) set to be refined simultaneously (I2). This feature makes it possible simultaneously to refine
data for more than one experiment applying to a given system. Runs under a range of experimental conditions may thus be combined up to a maximum number of 200 experimental readings in the present programme.

Item 3:- The number of complex species, including protonated forms of the ligand (I2). The number of displaceable protons on the ligand in the form in which it was added to the solution (I2). The convergence parameter for iteration in the mole balance equation for total metal (F8.6). The calculation returns to the main programme when \( \frac{B_{\text{calc}} - B_{\text{expt}}}{B_{\text{expt}}} \) is less than this quantity (e.g. 0.0000001), where \( B_{\text{calc}} \) and \( B_{\text{expt}} \) are the calculated and experimental total metal ion concentrations respectively. A similar convergence parameter (F8.6) for iteration in the total ligand equation (e.g. 0.0000001). An index (II) which is greater than zero if errors are to be supplied by the user, to be employed in weighting the residuals. An index (II) which is zero unless it is desired that the calculation be repeated a number of times with new initial values of a given constant, in which case this index
corresponds to that number. A final index (Il) which is only greater than zero if it is desired that no final back calculation and tabulation be carried out.

Item 4:- The number of ligands, metals and hydroxy groups bound in each of the complexes (6012). As up to 20 complex species are permitted, the card(s) carries these quantities in sets of three up to 20 sets. If the complex is protonated the number of "hydroxy groups" is entered as a negative integer.

Item 5:- The title of the particular experiment whose titration data follows immediately. This title will be printed at the head of the output sheet and may contain any characters in columns 1-80.

Item 6:- A card carrying the experimental conditions (5F10.5). The initial concentrations of total metal, total ligand and inorganic acid (if any), the concentration of base used as titrant, and the initial volume.

Item 7:- If errors are supplied (as indicated in (3)) these are to be inserted on this card (7F10.5). If unit weights are to be assumed this card is omitted. The errors are listed in the following
order; error in initial metal, ligand, and acid concentrations, error in the titrant base concentration, and in the total volume, these to be expressed as percentage errors. Then the estimated actual errors in titres, and pH values, which are assumed not to change significantly in the course of the titration.

Item 8:- There follows a series of cards bearing the readings of titre and pH in the above experiment (2F10.5). The last of these cards must bear an index greater than zero in column 25. If we are refining data for a single experiment as indicated in item (2) we now proceed to (9), but if more than one experiment is to be considered we return to (5), and proceed as above to (8) and repeat this process until all experiments have been included, and then proceed to (9). The dimensions of the present programme permit up to 200 titration readings.

Item 9:- The next card carries the ionic product of water expressed in terms of activity, as the negative logarithm (i.e. -14.167 at 20°) and the activity coefficient (e.g. 0.78 at 20°, I=0.1). These are entered in a format (2F8.4).
Item 10:- On the next card(s) are punched the constants applying to each of the species enumerated in (3) format (20F8.4). They must appear in the corresponding order, and be expressed as the logarithm of the cumulative association constant i.e. $\log \beta_{1mn}$.

Item 11:- The number of cycles desired in the refinement, 5 being usually sufficient, followed by the number of constants to be varied (2I2).

Item 12:- Each of the next cards carries the index, corresponding to (4), of the constant being varied, and the increment to be applied in the numerical differentiation procedure (I2,E10.3). The usual value of the increment used has been $+1.400E-03$. There is one such card for each constant to be varied. We now return to (2) to commence refinement for another set of experiments unless the indicator in (3) specifies a repeated calculation with new initial values, in which case we proceed to (13).

Item 13:- If in (3) we specified e.g. "n" repeated calculations, then the following "n" cards carry the index of the constant and its new value ($\log \beta_{1mn}$) in the format (I2,F8.4).
We now return to (2) if there remain any sets of data to be refined (corresponding to the number of jobs on (1)), otherwise the computer passes on to the next programme.

Set out below (Fig. 3.01) is part of the output of a simple typical calculation, in which two constants are refined to fit data for two potentiometric titrations for the nickel(II)-penicillamine system. The calculation is here over only four (as opposed to the usual five) cycles, and a tabulation of quantities of interest then follows. Only part of this tabulation is shown. This Figure is followed by a detailed flow chart of the programme GAUSS4. A listing of the programme will be found in Appendix III.
Fig. 3.01. Part of output for refinement of \( \beta_{11} \) and \( \beta_{21} \) from data for two pH titrations of penicillamine in the presence of nickel(II) ions.
FLOW CHART FOR PROGRAMME GAUSS4

START

100 READ NPTC

WRITE 900

Sets to zero counter recording number of experimental sets already considered.

Sets to zero counter recording number of experimental sets already considered.

Sets to zero counter recording number of repeat calculations involving changed values of initial constants.

READ (1,1)

READ (1,2)

1:N

AL(I)=ML(I)

AM(I)=MM(I)

AN(I)=MN(I)

READ TITLE

WRITE TITLE

Sets to zero indicator for total number of readings.

Sets to zero indicator for total number of readings.

Reads I, number of complex species; NOP, number of displaceable protons; EX1, EX2 convergence parameters for total metal and total ligand; IWS, indicator telling if weights supplied; IHS, indicator for number of repeat calculations with one input constant changed; INO, indicator for no output listing at end of calculation.

Reads ML, MM, MN, number of ligands, metals and protons in each complex.

End of chart.
Sets to zero counter for number of point in a given experiment.

Reads concentrations: TM, total metal; TL, total ligand; ACID, inorganic acid present at start; BASE, concentration of titrant base; VOL, initial volume of solution
Prints these quantities.

If errors are supplied by user, reads estimated errors:
DM, in metal; DL, in ligand; DACID, in acid; DBASE, in base (these as percentages of initial concentrations). DV, percentage error in initial volume; DET, actual error in titre, assumed constant throughout titration; DPH, actual error in pH.
Prints these quantities.

Advances indicator for point number.

Reads TITRE (I), ZT (I), INDEX; titre, and pH for (I)th point in experiment. INDEX = 0 except for last point of experiment.

Sets indicator for number of readings in experiment.

Prints this quantity.

Performs intermediate calculation of concentration of inorganic acid, base, volume, total metal, total ligand, analytical hydrogen ion concentration.
If weights supplied, converts percentages to actual errors.

Calculates intermediate variables and actual errors in various quantities, for the particular experiment:
- Error in total metal,
- Error in total ligand,
- Intermediate variable,
- Intermediate variable,
- Error in pH,
- Error in analytical hydrogen ion concentration.

Sets KMT, counter for total number of data points so far read.
Sets LMT, counter used with IMT, KMT and MNT to correlate data points in successive experiments of a given set.

Calculates final arrays which include for all data points of all experiments of set:
- total metal,
- total ligand,
- analytical hydrogen ion concentration,
- pH,
- inorganic acid,
- added base,
- initial volume,
- titre.
Correlates for all points of all experiments of set, errors in total metal, total ligand, pH and analytical hydrogen ion concentration.

Returns to read data for next experiment unless last of set has been read.

Requisite quantities have now been calculated for all points of all experiments of set. MT is total number of data points of set.

Reads CKWL, ionic product of water; F, activity coefficient and E(I), the respective values of the equilibrium constants.

Prints number of displaceable protons, ionic product, activity coefficient.

Prints number of constants, and total number of readings in the set of experiments.

Reads NCD, number of cycles desired, and NCV, number of constants to be varied.

Prints number of cycles desired.

Prints whether unit weights, or user's weights are to be applied.

Prints table of species assumed and initial values of respective constants.

Except when calculation is a repeat refinement using a different initial value for one constant, reads IG(I), index, and H(I), increment for each constant to be varied.

Preserves original values of increments.
Sets up 2.303...
Sets to zero counter for number of cycles calculated.
Counter \( > 0 \) if concentration subroutine has already been used once in this refinement cycle for this point.
Sets up ionic product in exponential form.

Sets up indicator for which concentration subroutine to be used later.

Exponentiates pH's.

Sets up errors in pH.

Advances counter for cycle being calculated.
Sets to zero counter for total number of non-convergent iterations in subroutine.
Sets to zero \( \sum w_i R_i^2 \) sum of squares of weighted residuals.
Sets to zero \( \sum w_i R_i^2 \) with input constants.
Sets to zero \( \sum R_i^2 \) unweighted sum of squares.
Sets to zero indicator \( > 0 \) if any increments (H) changed.

Sets to zero indicator that increment changed for (I)th point.

Preserves values of each constant at start of each cycle.
Sets to zero indicator for change in increment for (I)th constant.
Sets to zero constant vector.
Sets to zero coefficient matrix

Counter used after shifts applied and sum of squares with new constants is to be calculated.

Counter for data point number.
Sets up total metal for (K)th point.
Sets up total ligand for (K)th point.
Counter for number of non-convergent iterations in subroutine for (K)th point.
Index used in application of increments in differentiation.
Index used in control of logic during differentiation.

Sets up errors for (K)th point.

Analytical hydrogen ion concentration for (K)th point. 
1/(H+) for (K)th point.
Convergence parameters for (K)th point
Choice of initial estimates for free ligand (TX) and free metal (VX).

For first passage through concentration subroutine (ICC=0) and for first point, initial approximations are VX=total metal and TX as calculated from pKa's assuming no complex formation at this pH. For subsequent points we assume TX, VX have initial values equal to final value for previous point, unless this was non-convergent, in which case the first approximations above are again applied. After first passage through subroutine for a given point (ICC > 0), initial values of TX, VX are taken as final values for that point in previous cycle.

The adjacent routine involving variables DM and DMY selects each protonated ligand species and uses it in an equation of the form

\[ TX = \frac{L_{total}}{1 + \left(1 + \frac{K_a}{K_a} \right)^2} \]

Calculates exponential value of each constant.

Calls appropriate subroutine which calculates free metal and free ligand concentration CONC5 for metal complexing, CONC6 for pKa's and CONC7 for simple metal hydrolysis.

Exits to next job if non-convergence (after 99 cycles) occurs more than 30 times in subroutine.

If first cycle reserves final VX and TX for use as initial estimates next cycle.

If errors not supplied by user, sets unit weights.
Calculates weight of residual.
Calculates unweighted residual.
Calculates weighted residual.
Causes exit to final backcalculation with refined constants.

Causes exit to calculate residual square sum with refined constants.
Advances counter used in numerical differentiation.

Calculates sum of squares of residuals with input constants.
Reserves value of weight.
Value of analytical \(H^+\) with input constants.
Residual with input constants.
Counter for constant used in differentiation.
Index of constant used in differentiation.
Increases constant.
Advances counter for number of times concentration subroutine used.
Second value of analytical \(H^+\).
Decreases constant.
Third value of analytical \(H^+\).
Restores constant to original value.

Checks to ensure that the range \(2H(J)\) is large enough to give measurable difference \((H\theta_2-H\theta_3)\). If not \(H(J)\) is increased by five times.
Indicator for change in increment.
Indicator for index of changed increment.
Indicator for point for which increment changed.

Derivative \(\frac{\partial R_i}{\partial \theta_j} = W_i(k_{i2}-R_{i3})/\theta_j \).
Restores \(H(J)\) to original value, if changed.
Exits only after calculation of all derivatives
Advances counter for derivatives taken
Restores M to start differentiation again
Sets up index of new constant to be varied
Changes next constant

Sets up components of constant vector.

\[ \sum_{i} R_{o}(\alpha R_{i}/\alpha \beta_{j}) \]

Sets up elements of coefficient matrix

\[ \sum_{i} W_{i}(\alpha R_{i}/\alpha \beta_{j})(\alpha R_{i}/\alpha \beta_{k}) \]

Checks counter for number of non-convergent iterations, and if > 0
writes point number, pH and number of iterations.

Advances to next point
Restores to zero counter for number of times concentration subroutine used.

Returns to 37 until derivatives calculated for all points

The matrix BC(I,J) will be inverted
CC(I,J) is to be retained unchanged.

The adjacent section checks whether any increments were changed, and if so prints out the index of any changed increment.
This section calculates the number (NICH) of points for which increments were changed and prints this quantity.

Calls matrix inversion subroutine. A standard IBM programme is used. \([BC]=\left[BC\right]^{-1}\)

Initialises all shifts.

Calculates shifts
\[X_i = \sum_j \frac{BC_{ij} \cdot CK_j}{C_k}
\]

Sets to zero indicator for extreme overshifts.

Checks whether absolute value of any shift is greater than 1 log unit in which case IE0S=1.

If IE0S=1 any shift greater than 1 log unit is reduced to 0.5 and any less than -1 to -0.5.

Extreme overshift warning, index of constant, and shift are printed.
If no extreme overshifts found checks whether absolute value of any shift greater than 0.5 log unit in which case indicator for overshift (I0S)=1. Prints overshift warning, index of constant and value of shift.

If I0S=1 all shifts are reduced to half

Calculates $\Sigma w_i r_i^2 / (MT-NCV)$

i.e. weighted variance, for input constants of this particular cycle.

Sets index of each constant

Applies shifts to give improved constants.

Resets indicator for overshift

For first cycle writes weighted variance using input constants.

Advances L from zero so that on return to earlier part of programme to calculate the sum of the squares of the residuals with new constants, an exit occurs at statement number 24.

Calculates $\Sigma w_i r_i^2$ using new constants.

Calculates $\Sigma r_i^2$ using new constants

Advances counter for point number

Returns to statement number 37 if further points remain.

Calculates weighted variance with new constants.
Prints values of weighted variance and unweighted sum of squares of deviations.

Sets up index of constant
Calculates errors in constants $\sigma^2 = \frac{\sum (y_i - \bar{y})^2}{MT - NCV}$.
Avoids taking square root of negative number, and prints warning. This never occurs when programme functioning satisfactorily.

Calculates standard deviation
Prints index, constant estimated standard deviation, and shift from former value.

Compares initial weighted variance with that using refined constants.

If fit is worse using new constants shift has been too great. All shifts are now halved and a message to this effect is printed out.

Prints number of cycles calculated.
If further cycles required returns to 11, otherwise continues.
Indicator zero unless repeat calculation with new initial estimate required.

INN is zero except when no final tabulation of concentrations etc. is required.

If further initial estimates are required these are read here, at each cycle until IHS=IEC (indicator for E changed).

Reads new value of index and constant.

Resets indicator and returns to commence refinement.

Prints headings for table.

Sets to zero variable used in calculation of \( \bar{n} \).

Checks whether metal and/or ligand present in this run.

N.B. \( \bar{n} \) calculated in this section is only valid in absence of protonated complexes.

Checks each species in turn to find if this is a metal complex species.

COMPL is the total complexed ligand.

Calculates \( \bar{n} \).

Calculates \( \log_{10}(\text{free ligand concentration}) \).

Prints index of point, total metal, total ligand, pH, \( C_b \) calculated from constants, unweighted residual, weight, free metal, free ligand, \( \bar{n} \) and \( \log_{10}(\text{free ligand concentration}) \).
For clarity in final tabulation concentrations of species less than $10^{-20}$, are set at 0.0 and will be printed out as such.

From calculated analytical hydrogen ion concentration ($H_i$) using new constants, value of titre to be expected at each pH is computed. ERR0R is the difference between this quantity and the experimental titre (TITR(K)).

Prints index of point, concentration of the first eight species, experimental titre and error. Advances to next point.

Returns to consider next point until last reached. Advances counter for number of jobs calculated. Sets to zero variable used to sum (ERR0R)².

Calculation of ESDTIT, the estimated standard deviation in titre.

Sets first 8 concentrations to zero. If this is not done, if the next job contains fewer species erroneous values of C(J) would be printed in table (although the calculation of constants would be correct). Advances to fresh sheet, and starts to print matrices used in last cycle.

Prints COEFFICIENT MATRIX.

Prints values of this matrix.

Prints CONSTANT VECTOR and values.

Prints INVERSE MATRIX.
Programme Development

While the interesting developments of this programme could be extensive, the most important will be mentioned.

The first diagram follows, which the programme considers to form K(ON) a
formed by one metal and one ligand. The subroutine C0NCS5 calculates the concentration of each species C and R.

This calculation is carried out by the solution of the set of simultaneous equations obtained by the Newton-Raphson method.

This code can be generalised for a mixture of several metal complexes, in which we have a series of complexes formed from metal and ligands

\[ \begin{align*}
L_{1} & \rightarrow \left[ M_{1}L_{1} \right]_{1} = M_{1}^{2}C_{1}^{2} = M_{1}^{2}C_{1}^{2}L_{1}^{2} \\
L_{2} & \rightarrow \left[ M_{2}L_{2} \right]_{2} = M_{2}^{2}C_{2}^{2} = M_{2}^{2}C_{2}^{2}L_{2}^{2} \\
& \vdots \\
L_{n} & \rightarrow \left[ M_{n}L_{n} \right]_{n} = M_{n}^{2}C_{n}^{2} = M_{n}^{2}C_{n}^{2}L_{n}^{2}
\end{align*} \]

and the metal may be a positive integer or zero and a may be a positive integer (for a hydrolysed species)

or a negative integer (for a protonated species).

Prints values of inverse matrix.

Prints SOLUTION VECTOR and values, i.e. shifts applied in last cycle.

Prints CORRELATION MATRIX.

Guards against taking square root of negative number. This never occurs if programme running satisfactorily.

Calculates and prints elements of correlation matrix

\[ \text{CC}(I,J) = \frac{BC(I,J)}{\sqrt{BC(I,I) \cdot BC(J,J)}} \]

Advances to fresh sheet.

Returns to read new input data and start new calculation if required, otherwise terminates programme.
Programme Developments

While several interesting developments of this programme could be described only the most important will be mentioned. The first of these follows from the way in which the programme GAUSS considers the general complex \( L_1^M (O H)_n \) formed by one metal and one ligand. The subroutine C\( \text{ONC5} \) (Appendix II) calculates the concentration of each species \( C_j \) from the relation,

\[
C_j = \beta^{1mn} [L]^1 [M]^m [H]^n
\]

This calculation is carried out by the solution of the two simultaneous equations in total metal and total ligand equations by the Newton-Raphson method.

This process can be generalised for a mixture of several metals and several ligands. Thus if we have a series of metals \( M^a, M^b, M^c \) ... and a series of ligands \( L^r, L^s, L^t \) ... any complex which can be formed from these can be represented by

\[
(M^a)^\alpha (M^b)^\beta (M^c)^\gamma ... (L^r)^\rho (L^s)^\sigma (L^t)^\tau ... (OH)^\omega
\]

Where \( \alpha, \beta, \gamma, ..., \rho, \sigma, \tau, ..., \omega \) may be positive integers or zero, and \( \omega \) may be a positive integer (for a hydrolysed species), zero, or a negative integer (for a protonated species).
The concentration of any species $C_j$ (including protonated ligand species, and hydrolysed metal ions) is then given by

$$C_j = \beta_j [M^a]^\alpha [M^b]^\beta [M^c]^\gamma \ldots [L^r]^\rho [L^s]^\sigma [L^t]^\tau \ldots [OH]^\omega$$

3.18

where $\beta_j$ is the overall formation constant. The total concentration of metal $M^i$ is thus

$$[M^i] = [M^i] + \sum_{j=1}^{n} P_{ij} C_j$$

3.19

Where $P_{ij}$ is the number of ions of $M^i$ in the species $j$, and $n$ is the total number of species. A similar set of equations may be written for total ligand concentrations.

This approach makes possible two different types of calculation. Firstly, given the stability constant for each complex present in a mixture the concentration of all components may be calculated. Secondly, given the potentiometric titration data, the stability constants of unknown mixed species may be calculated.

The first of these calculations has successfully been incorporated into a computer programme. The computer is supplied with a set of values of $\alpha \beta \gamma \ldots \omega$ for each complex, together with its equilibrium constant,
and also total concentrations of each metal, \([M_T^i]\), and ligand, \([L_T^X]\), and the pH values for which the calculation is to be carried out. As a first approximation \([M^i]\) is assumed to equal \([M_T^i]\) and \([L^X]\) is calculated from \([L_T^X]\) using the appropriate \(pK_a\) values and assuming negligible complex formation. The quantity on the right hand side of equation 3.18 is calculated for each metal ion and similarly for each ligand species, to give quantities to be designated \([M_T^i]\)\(^{\text{calc}}\) and \([L_T^X]\)\(^{\text{calc}}\) respectively. The initial estimates of \([M^i]\) and \([L^X]\) are then replaced with quantities \([M^i]\).\([M_T^i]\)\(^{\text{calc}}\)^{1/2} and \([L^X]\).\([L_T^X]\)\(^{\text{calc}}\)^{1/2}. These new estimates are then used in a repeat calculation of \([M^i]\) and \([L^X]\). This process of successive approximations is continued until all values of \([M_T^i]\)\(^{\text{calc}}\) and of \([L_T^X]\)\(^{\text{calc}}\) differ from the corresponding values of \([M_T^i]\) and of \([L_T^X]\) by less than a specified amount (e.g. 0.001%). The final values of \([M^i]\) and \([L^X]\) now satisfy all equations to this accuracy, and are used to calculate the concentrations of each species, which are then printed out in tabular form.

This programme successfully calculated the concentrations of each species in a test calculation, in which ten metals and ten ligands were used. Including all types of complex, equilibrium constants were available for 195 species. Using an IBM 360/50 computer 7.8 minutes
were necessary for the calculation at pH 10 of all species concentrations. This was simply a test of the method. Of greater importance was a second calculation in which the method was applied to data for mixed complex formation by nickel(II) and zinc(II) with pyruvate and glyoxalate ions and glycine.\textsuperscript{106} Equilibrium concentrations of 28 different species were calculated in 16 seconds for a solution at pH 9 and 0.001 M in each total metal ion and each total ligand concentration. The results are shown in Table 3.01.

The above calculation method proved quite satisfactory for very complicated systems, but for simpler systems containing mixed species a much more efficient method is available. For the mixture of \( m \) metals and \( l \) ligands we may write \((m + l)\) non-linear equations for the total concentrations. It was found possible to generalise the Newton-Raphson iterative procedure (normally used for one or two unknowns) for \((m + n)\) unknown quantities. The method is complicated and details would be out of place in this Thesis, but the calculation is considerably faster than the alternative method, when used with simple mixtures. However, where more than three metals and three ligands are present, even the "Double Precision" facility of the IBM 360/50 does not give sufficient accuracy for the Newton-Raphson method to converge reliably, and so for the more complicated systems the simpler programme described earlier is the method of choice. Details of both procedures are to be published elsewhere.
At present very few values of stability constants are available, despite their importance for an understanding of metalloenzyme-substrate interaction and no generally acceptable method exists for the calculation of stability constants of these and other mixed species. However the above approach makes possible their determination from potentiometric titration data. For this purpose the Gauss-Raphson method appears the more suitable, and this programme can be incorporated into a refinement program of the form of GAUSS. The resulting general programme will make possible the calculation of the stability constant of any species which can be expressed in the form of 3.17, and will thus fill one of the few gaps which remain in different stability constant calculation methods.

<table>
<thead>
<tr>
<th>Species</th>
<th>Glycine</th>
<th>Pyruvate</th>
<th>Glyoxalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>2.48</td>
<td>818</td>
<td>879</td>
</tr>
<tr>
<td>HL</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H₂L</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NiL</td>
<td>382</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>NiL₂</td>
<td>67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NiL₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZnL</td>
<td>143</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>ZnL₂</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZnL₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Ni(gly)(pyruv) 81 Zn(gly)(pyruv) 62
Ni(gly)₂(pyruv) 15 Zn(gly)₂(pyruv) 3
Ni(gly)₂(pyruv)₂ 3 Zn(gly)₂(pyruv)₂ 1
Ni(gly)(glyox) 90 Zn(gly)(glyox) 9
Ni(gly)₂(glyox) 15 Zn(gly)₂(glyox) -
Ni(gly)₂(glyox)₂ 1 Zn(gly)₂(glyox)₂ -

At pH 9. All concentrations in µM. Total concentrations of each metal ion and complexing species, 1000µM. Free nickel ion, 341µM; free zinc ion, 764µM.
At present very few values of stability constants are available for complexes of one metal with two different ligands, despite their importance for an understanding of metalloenzyme-substrate interactions, and no general method exists for the calculation of stability constants of these and other mixed species. However the above approach makes possible their determination from potentiometric titration data. For this purpose the Newton-Raphson method appears the more suitable, and this programme can be incorporated into a refinement programme of the form of GAUSS. The resulting general programme will make possible the calculation of the stability constant of any species which can be expressed in the form of 3.17, and will thus fill one of the few gaps which remain in present stability constant calculation methods.
EXPERIMENTAL

Apparatus and Techniques

(i) pH Determination

Measurements of pH values during the titration of a ligand solution in the presence of metal ions have been widely used in the calculation of the stability constants of metal complexes. Where systems are believed to contain polynuclear or protonated species, in addition to simple complexes, it becomes important to obtain pH values of the highest possible accuracy. A number of instruments are commercially available for this purpose, but the accuracy of the determination depends also on the form of the pH cell used. Hitherto, potentiometric titrations have generally been carried out in a vessel comprising a simple water-jacketed beaker, closed at the top by a rubber or plastic stopper, through which pass the electrodes and an inert gas supply. The latter has sometimes been used to stir the solution, but in most cells stirring has been achieved mechanically.

Such a cell was tested at the start of this work. The pH of buffer solutions of 0.05 M potassium hydrogen phthalate was measured using an E.I.L. GHS 33 internally shielded glass electrode, a saturated calomel electrode, and "Vibron"
electrometer C33 B (E.I.L.) which was connected to a "Rectifier" recording milliammeter (Texas Instruments Inc.). The apparent pH of the buffer solution maintained at 20.0 ± 0.05°C was found to vary with the temperature of the room in which the apparatus was housed, changes of up to ±0.05 pH unit being observed over several hours. If the room temperature was controlled to ±0.5°C the effect was diminished, and a sinusoidal variation of ±0.01 pH unit was observed. These variations may have been due to an electrothermal effect in the head of the glass electrode. They were eliminated when the upper end was maintained at constant temperature.

Another unexpected source of error, which has been avoided in the present apparatus, is the photosensitivity of the glass electrode assembly. The magnitude of the effect varies with the electrode, but differences of up to 0.05 pH unit were observed between readings in bright diffuse daylight and near-darkness. The effect was reversible and presumably resulted from the sensitivity to light of the silver-silver chloride electrode, commonly used as an internal reference in glass electrodes.

These difficulties have been overcome by using the cell shown in Figure 4.01, in a windowless air-conditioned room, maintained at 20 ± 2°C under constant artificial illumination.
Fig. 4.01. pH titration cell for precise measurements.
The cell consisted of two water-jacketed parts, joined by a ground glass joint. The lower portion was the usual type of water-jacketed beaker, while the upper portion, serving to enclose the upper ends of the electrodes, was sealed at the top with a perspex stopper and rubber O-ring, through which passed the electrode leads, inert gas supply, thermometer, and reagent addition tube. At the bottom of this upper section, the electrodes etc. passed through a rubber stopper into the cell. The gas supply tube passed through a teflon bush, so that its height could be adjusted to permit flushing of the solution, or of the space above, with inert gas. The reagent addition tube served as an airlock for addition of easily oxidised materials to the solution and also for the addition of titrant solution.

The titrant was added from an "Agla" micrometer syringe and passed into the solution through a polythene cannula which was supported by a stainless steel tube.

The reference electrode was a miniature saturated calomel electrode closed by a sintered glass plug, and supported inside another tube sealed with a glass sinter, which contained a bridge solution of agar (3%), sodium nitrate (0.20 M) and ammonium nitrate (1.40 M). This solution gives equal transference of charge by positive and negative ions, and should minimise the junction potentials due to the bridge. The use of the gel resulted in negligible flow of potassium hydrogen phthalate, pH 4.001 at 20°C, or 0.05 M.
chloride, permitted the use of perchlorate solutions for stability constant studies, and gave an electrode which seldom required attention. The electrode could not be used for long periods above pH 10 because dissociation of the ammonium ion affected the reproducibility of the results. The solution was stirred magnetically.

All trouble from electrostatic effects was eliminated by placing an earthed brass disc on the underside of the perspex stopper, and by using a dilute electrolyte solution as thermostat liquid. Since the thermostat bath was itself earthed, complete screening was thus achieved.

The cell has proved to be most convenient in use, and to give very accurate and reproducible results. When the water circulating in the jacket was kept at 20 ± 0.02°C the recorded pH values of, for example, phthalate buffer remained constant to within ±0.002 pH for one week. This figure is the stated accuracy of the "Vibron" instrument, itself, and thus represents the present limit of experimental accuracy. After standardisation of the apparatus with phthalate buffer, the measured pH for a 0.05 M solution of sodium tetraborate generally lay within ±0.004 pH units of the literature value for this buffer.

(ii) Titration Procedure

Prior to a stability constant or pKₐ titration, the cell was standardised against a buffer solution (either 0.05 M potassium hydrogen phthalate, pH 4.001 at 20°C, or 0.05 M
sodium tetraborate, pH 9.228, or both). The upper section of the cell was then removed, and the electrodes were rinsed and immersed in water at the temperature of the experiment. The lower section was washed, rinsed with acetone, and dried, and the various reagent solutions (excluding the oxidisable ligand) were then added. The electrodes in the upper portion were rinsed with distilled water, dried by gently draining with absorbent tissue, and the cell was then closed. Nitrogen, purified and presaturated as described below, was passed through the cell for at least 30 minutes to remove oxygen, and the appropriate quantity of ligand solution was added through the reagent addition tube, which was simultaneously flushed with nitrogen to prevent entry of air. The titrant supply tube having been inserted, the cell was sealed and the titration, with carbonate-free potassium hydroxide, was commenced.

Each titration mixture contained 10 ml. of 0.5 M sodium perchlorate, and as the total volume of the solution at the start was 50 ml., the ionic strength was 0.1 (NaClO₄). This ionic strength, and the temperature of 20.00 ± 0.05° were employed throughout this study.

Readings of pH were taken only after the attainment of equilibrium as observed on the pen-recorder. After the first two or three readings of a titration this generally required about 5 minutes, but in some titrations (e.g. nickel(II) with 2,3-dimercaptopropane-1-sulphonic acid)
equilibrium in certain regions was reached only after some hours. Stirring of the solution was not stopped and the flow of nitrogen was maintained while measurements were made, as neither of these operations affected the pH readings. After the titrations the cell was checked with standard buffer solution. The titration results were rejected if buffer balance was not within 0.01 pH; agreement was usually better than this. The "scale length" of the glass electrode was regularly checked against the two buffer standards, and the electrode was changed if agreement was not within 0.01 pH.

(iii) E.M.F.Titrations

In some experiments the titration procedure was modified, and the cell was fitted with a saturated zinc amalgam electrode. It was then possible to determine the concentration of free zinc ions in the solution. The electrode was prepared by immersing the tip of a moulded zinc rod into an acidified solution of mercuric chloride, the remainder of the rod being coated with picein wax. As reference electrode the calomel electrode was again used, the cell being calibrated under nitrogen at the appropriate pH, using 0.1 M sodium perchlorate solutions containing known concentrations of zinc perchlorate. The e.m.f. of the cell was measured with a Tinsley type 4046B potentiometer connected to a Pye "Scalamp" mirror galvanometer. Provided that zinc concentrations exceeded $10^{-5}$ M, readings were
reproducible, and a plot of e.m.f. (in volts) versus $\log_{10} [Zn^{2+}]$ was linear with the theoretical slope (1/0.02906). The concentration of zinc ions in the solution could then be calculated from the equation

$$\log_{10} [Zn^{2+}] = (E_0 - E)/0.02906$$

where $E$ is the measured e.m.f. and $E_0$ is the standard e.m.f. of the cell, obtained from the calibration plot. At concentrations below $10^{-5} \, M$ the reproducibility was poor.

Pure zinc electrodes, moulded from A.R. zinc, annealed just below the melting point, and etched as described by Clayton and Vosburgh\textsuperscript{111} were unsatisfactory, possibly because the degassing procedure recommended by these authors could not be attempted in the potentiometric cell used in these studies.

When $[Zn^{2+}]$ was to be measured using this apparatus, a different titration procedure was adopted. The requisite volumes of water and sodium perchlorate solution were placed in the cell, and flushed with nitrogen. The cell was then opened briefly and any oxide layer on the zinc electrode was removed by rinsing with perchloric acid (0.1 M) and distilled water. After draining the excess water from the electrode the cell was closed, and again flushed with nitrogen. The ligand solution was then added anaerobically and after addition of an aliquot of zinc perchlorate from a burette the pH was adjusted to 5.50 (±0.01) by the addition of carbonate-free potassium hydroxide from a micrometer syringe.
After the attainment of steady readings of the e.m.f. of the zinc electrode, and of pH, both were measured accurately. The potential of the zinc amalgam electrode was measured against the calomel electrode, which for this purpose was disconnected from the pH meter by a suitable switch. Over the useful region of the titration, bubbling of nitrogen, and magnetic stirring, had negligible effect on e.m.f. measurements. Further aliquots of zinc perchlorate were then added and the procedure was repeated, so that, for a given system, titration pH data could be recorded as a function of the alkali added, and in terms of free and total zinc ion concentrations.

(iv) Spectrophotometric Titrations

A number of experiments were carried out in which the formation of a polynuclear species was followed spectrophotometrically at a given pH. The pH was initially maintained using buffers of tris(hydroxymethyl)methylamine ("Tris"), but it became apparent that complex formation with the buffer might complicate the interpretation of results, and, since it was further evident that complete exclusion of air was desirable, a special flow-apparatus was developed to overcome these difficulties. The apparatus consisted of a mixing chamber (capacity 100 ml.) sealed by a rubber stopper through which passed glass and calomel electrodes, burette tip, inert gas supply and micrometer syringe delivery tube.
From the mixing chamber the solution was led through a flow-cell in a Hilger "Uvispek" spectrophotometer, into a small teflon and perspex centrifugal pump and back to the mixing chamber. With this apparatus aliquots of reagent (metal or ligand) could be added to the solution, brought to the required pH by addition of acid or alkali, and the spectrum of the resulting solution measured. The spectrophotometric readings taken after equilibration were treated by Job's method.\textsuperscript{112} With this technique an equilibrium of the form

$$A + nB \rightleftharpoons AB_n$$

may be studied by observing some property (e.g. optical density) of the solution, in which $AB_n$ differs markedly from the components A and B. The enhancement of this property, i.e. the difference between its experimental value, and that calculated assuming no reaction, is plotted against composition, and from the position of the maximum $n$ may be calculated.

Reagents, Preparation, Purification, and Analysis

(i) Metal Solutions

In order to minimize complex formation of the metal ions with all components of the solutions except the ligands under study, the only inorganic anion permitted was perchlorate, and all metals were added as their perchlorates. In some preliminary work using 2-mercaptoethylamine, the metal nitrates were used, and the inert electrolyte was potassium...
nitrate. In these cases, oxidation of the ligand may have occurred because the pH was observed to fall and not to come to equilibrium. This reaction did not occur when the perchlorates were used.

Cadmium(II), copper(II), manganese(II), nickel(II), and zinc(II) perchlorates were prepared by the method of Serjeant. A solution of the A.R. salt (0.1M chloride, nitrate or sulphate) was passed through a cation exchange column (B.D.H. I.R. 120-H) in the protonated form, until the pH of the ingoing and effluent solutions were identical. Then, after rinsing the column with glass-distilled water, the required salt was eluted with a solution of barium perchlorate (0.025M, Fluka Puriss. P.A.), until a test portion showed an opalescence (barium sulphate) with sulphuric acid. The metal content of these solutions was determined by complexometric titration against standard solutions of ethylenediaminetetra-acetic acid by standard methods. The solution was then diluted with water and sufficient standard perchloric acid was added to make the final solutions 0.05M in metal and 0.001M in acid, the acid serving to repress hydrolysis on storage. For most metals, strongly bound to the resin and little hydrolysed, the eluted solution was assumed to be neutral before addition of acid. The acid strength of the cadmium and mercury perchlorates were checked
after elution, and prior to the addition of acid by careful adsorption of an aliquot of the metal solution on a column of cation exchange resin in the acid form and titration of the eluant and washings against standard alkali. The titre was equivalent to twice the concentration of the (divalent) metal ion plus the concentration of acid originally present.

Many mercury(II) compounds (e.g. the chloride and sulphate) are appreciably covalent in character, and hence are only weakly held by ion exchange resins. However, mercuric perchlorate appears to be ionic in character. For this reason it was prepared by dissolving mercuric oxide (B.D.H., L.R. grade, 99%) in standard perchloric acid, and purified by adsorption on an ion-exchange column, washing with 0.001M perchloric acid, followed by elution with a solution of barium perchlorate (0.052M) and perchloric acid (0.001M). The acid and metal contents were then determined as above.

(ii) Ligands

Mercaptoacetic acid (Fluka Purum, 98%) was twice distilled under nitrogen (74-76°/0.06mm.). The pure compound was kept at 0° under vacuum, and stock solutions were made using air-free water, and stored under nitrogen. As with the other ligands used in this work, air-free solutions were stored in sealed flasks with specially designed stoppers (Fig.4.02) which permitted withdrawal of solution under pressure of nitrogen. Access of air was thus prevented.
Solutions of mercaptoacetic acid were analysed by the method of Leussing and Kolthoff, using standard potassium iodate solutions. The ligand was found in this way to be 99.9% pure. Jacobsen and Lund have reported the decomposition of 0.1M solutions of mercaptoacetic acid stored under nitrogen. They describe the formation of hydrogen sulphide as one product, and suggest that the decomposition is catalysed by this or another product of the reaction. Under nitrogen, the rate of decomposition was reported to be 1% per week falling to 0.1% per week if the solution was flushed daily with nitrogen.

Fig. 4.02 Flask used to store ligand solutions under nitrogen.
Slight decomposition was detected in the present work, but (although the vessel was not flushed daily with nitrogen), the rate did not exceed 0.1% per week. Where solutions were old enough for the decomposition to be significant, allowance for the reaction was made in the calculations. If the products were assumed to be gaseous, or unimportant in the potentiometric titration (e.g. $\text{H}_2\text{S}, \text{CO}_2, \text{CH}_3\text{OH}$) a slight improvement in the fit to the data was noted. However the slight extent of the reaction (not exceeding about 0.5%) had no significant effect on the conclusions of the study.

2-Mercaptoethylamine (Fluka Purum >99%) was twice resublimed under nitrogen (60-70°/0.5mm.). The hygroscopic product was handled in a dry-box under nitrogen. A stock solution, made using air-free water, was stored under nitrogen. Standardisation by oxidation with standard iodine solution followed by back titration with sodium thiosulphate, gave a purity of 100.2±0.2%. Potentiometric titrations for $pK_a$ values confirmed this purity.

L-Cysteine (Fluka Puriss.Chr.) was dried under vacuum over phosphorus pentoxide at room temperature. Stock solutions were prepared using air-free water and stored under nitrogen. Although iodimetric titration can be a convenient method of estimating thiols, addition of iodine to the disulphide formed may result in the reaction of a non-stoichiometric quantity of the oxidising agent. However,
the following method was found to be satisfactory. The
cysteine solution (0.02M, 5ml.) was added to glacial acetic
acid (25ml.) and the mixture was cooled to 0°. The cold
solution was titrated with standard iodine solution in the
presence of starch, until a pink colour became evident.
On addition of water (30ml.) the colour changed to purple.
The excess iodine was then back-titrated with sodium
thiosulphate. This method gave reproducible results
indicating a purity of 99.9±0.2%.

**D,L-Penicillamine** (Fluka Puriss.) was dried under
vacuum over phosphorus pentoxide at room temperature. In
some titrations the solid was added to the solution, but in
most cases a stock solution in air-free water, stored under
nitrogen, was used. All of the iodimetric titration
methods which were attempted resulted in the reaction of
non-stoichiometric amounts of iodine, but potentiometric
titrations confirmed a purity of >99.5%.

**Sodium 2,3-dimercaptopropane-1-sulphonate** ("Unithiol")
was prepared following the method of Petrunkin.\(^{119}\) Allyl
bromide (155g.) was added during 1 hour to a solution of
anhydrous sodium sulphite (162g.) in water (500ml.) at 60°.
After heating at 60° for a further hour, the mixture was
heated on a steam bath under reflux until homogeneous.
The allyl sulphonic acid was not separated but brominated
directly. To the above solution, bromine (ca. 230g.) was
added dropwise with stirring at $10^\circ$ until a permanent light yellow colour was produced. The solution was neutralised with sodium carbonate (48g. in 140ml. of water) and left overnight. The precipitate of sodium 2,3-dibromopropane-1-sulphonate was filtered off, washed briefly with a little cold water, and dried at room temperature. Half of this product was treated with potassium hydrogen sulphide to yield the dithiol. A solution of potassium hydroxide (120g.) in water (600ml.) was saturated with hydrogen sulphide (until a test portion did not colour phenolphthalein). Sodium 2,3-dibromopropane-1-sulphonate (250g.) was added to the solution and the mixture was heated at $50^\circ$ (1 hr.). The solution was made just acid with glacial acetic acid, flushed with nitrogen to remove hydrogen sulphide, and to the warm mixture was added a solution of basic lead acetate (225g.) and glacial acetic acid (50ml.) in water (450ml.). The yellow precipitate of $\text{Pb}_2(\text{CH}_2\text{S.CH}_2\text{SO}_3)_2$ was collected and repeatedly washed with boiling water until the washings were free of bromide ion (no precipitate with silver nitrate). Yield 129g. (51% overall).

The finely ground lead salt (40g.) suspended in ethanol (200ml.) was treated with hydrogen sulphide for 5 hours at room temperature. The lead sulphide was filtered off and excess hydrogen sulphide was removed from the filtrate by flushing with nitrogen. The solution was then neutralised
with excess sodium carbonate at 60-70° and filtered hot. On cooling, crystals of sodium 2,3-dimercaptopropane-1-sulphonic acid were deposited. Yield 10.5g. (49%). The product was recrystallised twice from boiling ethanol and dried under vacuum over phosphorus pentoxide.

Petrunkin analysed his product iodometrically. However with this method the sample obtained above consistently yielded an apparent purity of over 100% with respect to thiol. Satisfactory results were not obtained with attempted complexometric titrations with zinc, or redox titrations with copper, but a sensitive potentiometric method was developed. Silver was found to form a very stable complex with the ligand, so that if a slight excess (5%) of silver ion was added to the ligand solution, the liberated acid could be titrated potentiometrically under nitrogen with carbonate-free potassium hydroxide. A plot was then drawn of the change in pH per aliquot of alkali added (dpH/dV) and the maximum of this plot corresponded to the equivalence point. (A large excess of silver was found to obscure the end-point as a result of hydrolysis of the metal.) By this method the ligand was shown to be at least 99.4% pure. Stock solutions of the ligand were again stored in sealed glass vessels under nitrogen.
3-Mercapto-2-aminopropane-1-sulphonic acid.—An attempt was made to prepare this ligand (which is at present unknown) because of its potential usefulness in the study of the formation of amino-thiol complexes, because insolubility of the complexes and ambiguity as to the chelating groups would present no problem with this complexing agent. Unfortunately the compound proved somewhat intractable, and lack of time prevented the preparation of any quantity of the pure ligand.

Of various routes attempted, the most successful synthetic approach followed the work of Crawhall, Elliott, and Hooper. The method is outlined below.
Reactions 1, 2, 4, and 5 were described by Crawhall et al., and reaction 3 was described by Elliott. Stages 6, 7, and 8 have not previously been reported. Steps 1 to 5 consist of the reduction of cystine to cysteine, and the formation of cysteine ethyl ester. Ethyl benzimidate was prepared from benzonitrile and then treated with the cysteine ester to yield 4-ethoxycarbonyl-2-phenylthiazoline, which was reduced with lithium aluminium hydride to give 4-hydroxyethyl-2-phenylthiazoline.

4-Chloromethyl-2-phenylthiazoline.- 4-Hydroxyethyl-2-phenylthiazoline (4.85g.) was dissolved in a mixture of pyridine (2ml.) and chloroform (25ml.). The mixture was cooled in ice and thionyl chloride (3.3g.) was added slowly with stirring. The mixture was heated under reflux (20 min.) and the solution was then evaporated to dryness. The resulting oil was extracted with boiling benzene (6 x 50ml.) and the combined extracts were evaporated to dryness.

Recrystallization of the product from light petroleum yielded pale yellow crystals, m.p. 49.5⁰ (5.07g., 95%). Found: C, 57.05; H, 5.1; Cl, 16.6; N, 6.6. Calc. for C_{10}H_{10}ClNS: C, 56.7; H, 4.7; Cl, 16.8; N, 6.6%.

Sodium 2-phenylthiazoline-4-methylsulphonate.- 4-Chloromethyl-2-phenylthiazoline (2.1g.) was added to a suspension of sodium sulphite (5.0g.) in aqueous alcohol (150ml., 40% EtOH by volume). Potassium iodide (0.1g.) was
added as catalyst, and the mixture was heated in a sealed tube (150°, 20 hr.). The tube was opened, the solvent was removed, and the solid residue was extracted with boiling ethanol (3 x 150ml.). Evaporation of the solvent left a deliquescent white powder (2.3g., 84%). A good analysis was not obtained for this solid, which did not melt, but charred above 200°. It is possible that the material could have been purified via the barium salt which can be precipitated from aqueous solution.

3-Mercapto-2-aminopropane-1-sulphonic acid.—This was only obtained in the form of a nickel complex of indeterminate structure. The crude sodium 2-phenylthiazoline-4-methylsulphonate (0.2g.) was added to a mixture of fuming hydrochloric acid (5ml.) and butane thiol (0.5ml.) which was placed in a Carius tube and sealed under nitrogen. After heating at 140° (6 hr.) the tube was opened and the solution was evaporated to dryness under nitrogen. A little water was added and the solution was extracted with ether to remove benzoic acid. The ether extract on evaporation yielded benzoic acid crystals corresponding to ca. 75% reaction. Excess nickel chloride solution was added to the aqueous extract, and sufficient sodium hydroxide to bring the pH to 6.5. The dark purple solution was evaporated to dryness, the solid taken up in a little water and the solution was filtered. Ethanol was
added to precipitate the complex which was washed with ether and dried under vacuum. Yield 0.05g., 34% if trimeric complex. The purple complex was recrystallized from aqueous ethanol, but the analytical figures fitted no structure which was proposed, and it must be assumed that the material was contaminated. Found: C, 17.0; H, 3.7; N, 6.0; Ni, 8.9. Calc. for trinuclear complex $\text{C}_{12}\text{H}_{28}\text{N}_4\text{Ni}_3\text{O}_{12}\text{S}_8$: C, 16.0; H, 3.1; N, 6.2; Ni, 19.6%. 

While the constitution of the products of the last two reactions remains unproven, several conclusions may be drawn from this investigation. Firstly, it will be desirable to obtain a pure specimen of the thiazoline sulphonic acid, possibly via its barium salt, before proceeding with the synthesis. Secondly, more suitable conditions are required for the final stage. The above reaction employed vigorous conditions and used butane thiol as a scavenger for any oxygen present. While butane thiol itself is easily removed by evaporation from the reaction product, and the disulphide is unlikely to form complexes, the mixed disulphide, which may well form under these conditions, would be hard to separate from the desired product. This suggests that if an oxygen scavenger is required, Cleland's Reagent (2,3-dihydroxy-1,4-butane dithiol), or possibly 1,4-dimercaptobutane itself, might be more suitable, as the stability of the six-membered disulphide ring would reduce
the tendency to form mixed disulphides. Alternatively, by refluxing the thiazoline in concentrated hydrochloric acid under nitrogen the cleavage of the ring might be effected at lower temperature, and the reaction enhanced by the removal, in the gas stream, of the benzoic acid produced. Finally, a satisfactory extraction and purification procedure for the ligand is required. Precipitation of the lead salt followed by the regeneration of the ligand with hydrogen sulphide, as with 2,3-dimercaptopropane-1-sulphonic acid, might be suitable. While the ligand offers a number of interesting applications, its unusual properties render its isolation somewhat difficult.

(iii) Other Reagents

**Buffers.**—The primary pH standard used in this work was a solution of potassium hydrogen phthalate (B.D.H., A.R.). The solid, dried over calcium chloride, was dissolved in glass-distilled water to make a 0.05M solution, for which the pH was assumed to be 4.001. The secondary pH standard employed was sodium tetraborate (A.R.), for which the pH of a 0.05M solution in boiled-out glass distilled water was assumed to be 9.228. The pH values of solutions of this salt vary negligibly for minor differences in concentration, caused by slight loss of water from the decahydrate, so that no precautions were taken to maintain this degree of hydration, apart from storing the solid in an airtight container.
Ethylenediaminetetra-acetic acid obtained as the disodium salt (B.D.H., L.R.) was recrystallized as described by Vogel, and dried at 80° for 24 hr. This yielded the dihydrated salt. For some purposes the salt was dried at 120-140° for 12 hr. when the anhydrous salt was produced. Standard solutions of 0.05 and 0.01M were prepared using glass-distilled water and stored in polythene containers.

"Carbonate-free" potassium hydroxide was prepared by washing potassium hydroxide (A.R.) sticks with boiled-out glass-distilled water until approximately half had dissolved. The remaining solid was dissolved in boiled-out glass-distilled water. The stock solution so obtained was diluted to 1.0N and standardised against standard hydrochloric acid using either methyl orange or phenolphthalein indicators. The differences in the results corresponded to dissolved carbonate, and was never more than 0.2% of the total alkali. The stock solution of base was stored (under a soda-lime guard tube) in a polythene container from which solution could be removed without access of carbon dioxide.

Hydrochloric acid solutions, used as primary standards for acid-base titrations were made using A.R. acid, and standardised in the usual way against anhydrous sodium carbonate.
Nitrogen was "oxygen-free" grade. As used in the pH titrations it was washed with water, passed through two columns containing acidified chromous chloride solution in contact with zinc amalgam, and then through a bottle containing alkaline sodium dithionite solution with added sodium anthraquinone-2-sulphonate catalyst, before finally washing with water at 20.0°C and passing into the cell. Presaturation with water, and removal of traces of oxygen and carbon dioxide were achieved by this method.

Other reagents used in quantitative studies were of A.R. grade.

Mercaptoacetic Acid

(i) Acid Association Constants

Potentiometric titrations of mercaptoacetic acid afforded acid association constants which after refinement by the programme GAUSS had the values (and estimated standard deviations) shown in Table 5.01. These fit the experimental data (24 points) to within an estimated standard deviation in titre of 0.0021 ml. (the complete titration requiring 0.5 ml.). The values are also in reasonable agreement with recent published values (Table 1.01).
CHAPTER 5

RESULTS

All constants reported in this Chapter were determined at 20.00 ± 0.05°, and in the presence of 0.1 M sodium perchlorate which served to maintain the ionic strength at an almost constant value. Also, for convenience, and because this is the form in which the constants must appear in the general refinement programme, the acid strengths reported in this Thesis are given, not as pKₐ values, but as the logarithm to base 10 of the overall acid association constants, as defined by equations 2.16, p.42.

Mercaptoacetic Acid

(i) Acid Association Constants

Potentiometric titrations of mercaptoacetic acid afforded acid association constants which after refinement by the programme GAUSS had the values (and estimated standard deviations) shown in Table 5.01. These fit the experimental data (24 points) to within an estimated standard deviation in titre of 0.0021 ml. (the complete titration requiring 0.5 ml.). The values are also in reasonable agreement with recent published values (Table 1.01).
Table 5.01

Acid association constants (with estimated standard deviations) of mercaptoacetic acid at 20.0°C, I=0.1 (NaClO₄)

\[ \log \beta_1 = 10.204 \pm 0.006 \]
\[ \log \beta_2 = 13.721 \pm 0.009 \]

(ii) Zinc(II) Complexes

Data from potentiometric titrations of various mixtures of zinc(II) perchlorate and mercaptoacetic acid are shown in Table A.01 (Appendix I) and plots of \( \bar{n} \) versus log [\( L^{2-} \)] are shown in Fig. 5.01. The formation curves for different ligand or metal concentrations are not identical, indicating the probable presence of polynuclear complexes. On the other hand the curves are not parallel with a spacing \( \Delta \log [L^{2-}] \) proportional to \( \Delta \log [M_T] \) so the system does not contain predominantly a "core plus links" series of complexes. Instead, the spacing between the curves decreases as the concentration of total metal is reduced, suggesting that the curves are converging towards the "mononuclear wall" of Biedermann and Sillén. An Irving-Rossotti graphical plot using data for the most dilute solution gave the approximate values of \( K_1=5.4 \times 10^7 \) and \( \beta_2=1.1 \times 10^5 \) for the stability constants of the 1:1 and 2:1 complexes.
Fig. 5.01. Formation curves for pH titrations of zinc(II) ions and mercaptoacetic acid. The points are experimental; the curves are calculated from the refined constants. Experiment: (i) ■; (ii) ▲; (iii) ●; (iv) ▼.
These experiment numbers refer to experiments listed in Table A.01.
In further experiments the titration method was modified and, using a zinc amalgam electrode, $[Zn^{2+}]$ was measured, together with the reagent concentrations and $pH$. Assuming that protonated metal complexes were formed, it was possible to calculate $\Delta p$, and thus to estimate the concentrations of $ML$ and $M_2L_2$ from $K_1[Zn^{2+}][L^-]$ and $K_2[Zn^{2+}][L_2^-]$, respectively. Then, assuming the formation of only one polynuclear species ($M_nL_q$), equation 2.23 was used, and, as described in Chapter 2, the plots were drawn to obtain $p$ and $q$. For $p$, log($M^{2+}$) was plotted against $\log(n[ML]_{2}[ML]_{2})$ at constant $[L^{2-}]$, and for $q$, log($L^{2-}$) was plotted against $\log(n[ML]_{2}[ML]_{2})$ at constant $[M]$. These plots were found to be roughly linear, with the slope $p = 2$ (at $\log(L^{2-}) = -7.50$) and $q = 3$ (at $\log(M^{2+}) = -4.00$). Thus it appeared that, in this region $M_nL_q$ is the major polynuclear species, although the increased presence of $M_2L_2$ at higher concentrations indicated the increased stability of the polynuclear species under these conditions.

Extrapolation of the data from the two plots yielded similar intercepts for which an approximate $K_{2}$ was calculated to be $25$.

This value and the approximate values of $K_{1}$ obtained above were used as initial estimates in a polynomial refinement of the data from the plot of normal fit assuming the existence of only these species was quite accurate.
In further experiments the titration method was modified and, using a zinc amalgam electrode, \([Zn^{2+}]\) was measured, together with the reagent concentrations and pH. Assuming that no protonated metal complexes were formed it was possible to calculate \([L^{2-}]\), and thus to estimate the concentrations of ML and ML\(_2\) from \(K_1[Zn^{2+}][L^{2-}]\) and \(\beta_2[Zn^{2+}][L^{2-}]^2\) respectively. Then, assuming the formation of only one polynuclear species \((M_pL_q)\), equation 2.23 was used, and, as described in Chapter 2, the plots were drawn to obtain \(p\) and \(q\) (Fig. 5.02). To find \(p\), \(\log[M^{2+}]\) was plotted against \(\log(n[M]-[ML]-2[ML_2])\) at constant \([L^{2-}]\), and for \(q\), \(\log[L^{2-}]\) was plotted against \(\log(n[M]-[ML]-2[ML_2])\) at constant \([M^{2+}]\). These plots were found to be roughly linear, with slope \(p=2\) (at \(\log[L^{2-}]=-7.50\)) and \(q=3\) (at \(\log[M^{2+}]=-4.00\)). Thus it appeared that in this region \(M_2L_3\) was the major polynuclear species, although the increased curvature at higher concentrations indicated the presence of further polynuclear species under these conditions. Extrapolation of the two plots yielded similar intercepts from which an approximate value of \(\log \beta_{32}\) was calculated to be 26.

This value and the approximate values of \(\beta_{11}\) and \(\beta_{21}\) obtained above were used as initial estimates in a preliminary refinement (by the programme GAUSS) of the data from the set of normal stability constant titrations. The closeness of fit assuming the existence of only these species was quite
good, but was considerably improved by the inclusion of a further species \( M_3L_4 \). This gave a much better fit than either of two other possible species, i.e. \( M_2L_2 \) and \( M_4L_6 \).

In addition to these species the possibility was considered of the formation of a simple carboxylic acid complex \( MHL \), in which the thiol group is not ionised.

A tentative value of \( \log \beta_{MHL} = 2.3 \) \( \beta_{MHL} = \frac{[MHL]}{[M][HL]} \) was chosen (to be roughly comparable with the stability constant of the zinc acetate complex \(^8\)), but attempted refinement by the computer, using data for the low pH region, led to a progressive decrease in \( \log \beta_{MHL} \), so that the species \( MHL \) could safely be neglected.

The above calculations employed data for experiments (i) to (iv) in Table A.01, in which \( \bar{n} \) was less than 2. However, values of \( \bar{n} \) greater than 2 were obtained in the titration of a solution of zinc(II) ions and mercaptoacetic acid in a ratio of 1:4 (experiment (v), Table A.01). The species \( ML_3 \), previously postulated by Cabrera and West,\(^{26}\) appeared most likely. Its stability constant was calculated for readings of \( \bar{n} > 2 \) using the refined values of \( \log \beta_{11} \), \( \log \beta_{21} \), \( \log \beta_{32} \), and \( \log \beta_{43} \) obtained from the remainder of the data. A final refinement of all these constants over the entire range of experimental data yielded the constants, listed with their estimated standard deviations, in Table 5.02.
Table 5.02

Stability constants (with estimated standard deviations) of complexes of zinc(II) with mercaptoacetic acid at 20.0°, I=0.1 (NaClO₄)

\[
\begin{align*}
\log \beta_{11} &= 7.796 \pm 0.008 \\
\log \beta_{21} &= 14.958 \pm 0.008 \\
\log \beta_{31} &= 17.799 \pm 0.016 \\
\log \beta_{32} &= 25.20 \pm 0.10 \\
\log \beta_{43} &= 36.465 \pm 0.029 \\
\end{align*}
\]

Using these constants the theoretical formation curves were calculated and these are shown in Fig. 5.01. The agreement between experimental points and the calculated curves is good except, in some cases, at the extreme ends, where reduced accuracy is to be expected.

As a further measure of closeness of fit, the difference between the experimental titre and that computed from the refined constants was calculated for each experimental point. Over the whole range of experimental data the standard deviation so found was 0.0015 ml.
(iii) Nickel(II) Complexes

Titration data for solutions of nickel(II) perchlorate and mercaptoacetic acid are given in Table A.02. Formation curves derived from them are shown in Fig.5.03. They indicate that this system, also, contains polynuclear species. In this case the curves do not converge rapidly towards a "mononuclear wall" so that polynuclear complex formation in dilute solution is more extensive than with zinc(II). However, the lack of a suitable electrode reversible to nickel(II) ions prevents the direct determination of free metal ion concentrations, and hence of the composition of the polynuclear complexes present. Instead it is necessary to find, by successive trials, the set of complexes which most nearly reproduces the experimental results.

The assumption that nickel(II) formed the same types of complexes as zinc(II) led to a good fit of the experimental data, but complexes of the type ML (and also $M_2L_2$) appeared to be unimportant. The fit was improved significantly by the inclusion of the additional species $M_4L_6$ first postulated by Leussing et al.\textsuperscript{39} A titration in which the metal:ligand ratio was 2.5:1 yielded a value of $\log \beta_{11} = 6.2 \pm 0.2$ when data were refined with the remaining constants maintained at the values obtained above. However, insertion of this value into calculations for experiments
Fig. 5.03. Formation curves for pH titrations of nickel(II) ions and mercaptoacetic acid. The points are experimental; the curves are calculated from the refined constants. Experiment: (i) □; (ii) ○; (iii) ●; (iv) ▼.
(i) to (v) showed that concentrations of ML in these experiments were negligible, and this species was therefore ignored in the final computation.

Table 5.03
Stability constants (with estimated standard deviations) of complexes of nickel(II) with mercaptoacetic acid at 20.0°C, I=0.1 (NaClO₄)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Log Stability Constant ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁₁</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>S₂₁</td>
<td>13.009 ± 0.005</td>
</tr>
<tr>
<td>S₃₁</td>
<td>14.987 ± 0.028</td>
</tr>
<tr>
<td>S₃₂</td>
<td>22.68 ± 0.07</td>
</tr>
<tr>
<td>S₄₃</td>
<td>33.267 ± 0.015</td>
</tr>
<tr>
<td>S₆₄</td>
<td>49.849 ± 0.019</td>
</tr>
</tbody>
</table>

The refined constants for complexes of nickel(II) with mercaptoacetic acid are shown in Table 5.03. These values were used in a calculation to reproduce the experimental data. Calculated and experimental titres agreed to within an estimated standard deviation of 0.0016 ml. The calculated constants were also used to compute the formation curves shown in Fig.5.03. Again experimental points agree well with the calculated curves except at the extreme ends of the titrations. In the experiments using highest concentrations further polynuclear species may also be present.
(iv) Manganese(II) Complexes

The results of four titrations of manganese(II) perchlorate in the presence of mercaptoacetic acid are shown in Table A.03 and plotted as the formation curves in Fig. 5.04. Interpretation of these results is rendered somewhat more difficult than with the above metals because, as a metal of variable valency, manganese catalyses the oxidation of the thiol group. As noted in Chapter 1, Leussing and Tischer observed a similar effect in their work on the manganese(II)-BAL system, in which slight oxidation caused their results for manganese to be less accurate than those with zinc. 64

The curves in Fig. 5.04 show some spread as the total metal concentration is varied, but this is less than that reported for the manganese(II)-BAL system. The order of the curves is the reverse of that shown, from the results for nickel(II) and zinc(II), to be caused by polynuclear complex formation. Three of the solutions exhibit marked deviations towards the end of the titrations, where pH values of over 10 were reached. The only titration not to show such a deviation ended at pH 9.5 (n = 1.58). These deviations from the coincident plots expected for a mononuclear system are such as might result from the oxidation of a small amount of ligand by traces of oxygen, and from hydrolysis in the latter part of the titration. There does not appear to be any evidence for polynuclear complex formation in this system.
The refinement by CAUSE was thus confined to considering the species $ML$ and $ML_2^-$, for which approximate constants were obtained from an Irving- Rossotti plot, and $MOH^+$, for which a constant

$$\log \beta_{11} = -11.10 \left( \frac{[MOH^+][H^+]}{[H^+]^2} \right)$$

was obtained from the literature.

With this value for the hydrolysis constant, refinement of the two complex stability constants indicated that only very small quantities of hydrolysed metal were present. Alternatively, a much better fit of the data was obtained if the hydrolysis constant was also treated as a variable. This constant, after refinement, was found to have the value $-9.66 \pm 0.04$, which differs considerably from the literature values. Instead, it was probable that a small proportion of oxidation of the metal and was responsible for the deviations observed. The data were therefore interpreted in terms of $ML$ and $ML_2^-$ only, employing readings at $\bar{n}$ values below 1.0. Deviations from the expected pure curves occurred.

These values show in Table 5.04.

These constants fit the experimental data (117 points) with an error of $\pm 0.01$.

Fig. 5.04. Experimental formation curves for pH titrations of manganese(II) ions and mercaptoacetic acid. Experiment: (i) ■; (ii) ▲; (iii) ●; (iv) ▼.
The refinement by GAUSS was thus confined to considering the species ML and ML₂, for which approximate constants were obtained from an Irving-Rossotti plot, and MOH⁺, for which a constant

\[ \log \beta_{011} = -11.10 \left( \frac{[\text{MOH}^+][\text{H}^+]}{[\text{M}^{2+}]} \right) \]

was obtained from the literature.¹²⁴

With this value for the hydrolysis constant, refinement of the two complex stability constants indicated that only very small quantities of hydrolysed metal were present. Alternatively, a much better fit of the data was obtained if the hydrolysis constant was also treated as a variable, but this constant, after refinement, would have had the value \(-9.66 \pm 0.04\), which differs considerably from the literature values. Instead, it is probable that a small amount of oxidation of the ligand was responsible for the deviations observed. The data were therefore interpreted in terms of ML and ML₂ only, employing readings at \(\bar{n}\) values below where deviations from the expected curve occur. These give the stability constants shown in Table 5.04.

These constants fit the experimental data (117 points) with an estimated standard deviation of 0.0037 ml. in titre.
Table 5.04
Stability constants (with estimated standard deviations)
of complexes of manganese(II) with mercaptoacetic acid
at 20.0°C, I=0.1 (NaClO₄)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Log β₁¹</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.329</td>
<td>± 0.007</td>
</tr>
<tr>
<td>2</td>
<td>7.473</td>
<td>± 0.009</td>
</tr>
</tbody>
</table>

(v) Cobalt(II) Complexes

Several side reactions complicated the study of the complex formation of cobalt(II) with mercaptoacetic acid. The metal catalysed the oxidation of the ligand by traces of air, so that an inert atmosphere was essential. As with most of the metals studied, little complex formation occurred before the addition of one equivalent of alkali, to deprotonate the carboxylic acid group. With cobalt(II) however, equilibrium was never reached at this point. The pH decreased steadily, and the rate of this reaction was unaffected by the use of potassium chloride or nitrate as alternative swamping electrolyte. Oxidation by air seemed unlikely because a steady stream of thoroughly purified nitrogen was passing through the solution. However an increase in the flow of gas did somewhat decrease the rate of the reaction, so a possible explanation is that cobalt(II) catalyses the decomposition of thioglycollic acid, as does one of the gaseous reaction products, which is removed in the gas stream. A similar but more rapid reaction was observed in a titration of 2-mercaptoethylamine in the
presence of cobalt(II).

While the presence of such a side-reaction renders the exact interpretation of the data impossible, a number of rapid titrations (2-4 minutes equilibration time per aliquot of titrant) were carried out, in which decomposition was minimal. Another side-reaction was then observed. It was found that, at $n$ values of about 1, equilibration was taking steadily longer until after a time the pH rose steadily, and equilibrium was not reached after some hours. An interpretation of this behaviour is inevitably speculative. Cobaltous cyanide in an oxygen-free aqueous solution can reduce water to hydrogen, being itself oxidised to the cobaltic complex. The cyanide and sulphhydryl complexes of cobalt(II) have some features in common, e.g. both cobaltous cyanide and cysteinate complexes react with carbon monoxide to give the carbonyl hydride Co(CO)$_4$H in aqueous solution. It thus appears that cobalt(II) sulphhydryl complexes might also reduce water, and hence cause the observed rise in pH.

The results of a number of rapidly executed titrations are listed in Table A.04 and plotted as the formation curves in Fig.5.05. The separation of the formation curves is indicative of polynuclear complex formation, as is the dark olive-yellow to black colour of the solutions. The separation of the curves cannot, in the circumstances, lead to a reliable definition of the nature of the species.
In order to obtain approximate values of the constants, it was assumed that, in a rapid titration, decomposition and other side-reactions were negligible, and that any polynuclear species present were either of the form $\text{ML}_2$ or $\text{ML}_4$.

The closeness of fit of three sets of species in the results is evidenced by the relative values of the second moments of the residuals in analytical hydrate concentration fits obtained from the refinement of the mononuclear species, as seen in Table 5.01. A fourth set of species was not included because of the uncertainty of the experimental data; however, in view of the above reports on the likely presence of polynuclear species, those polynuclear species are also considered.

The set $\text{ML}_2$, $\text{ML}_4$, $\text{ML}_6$, given the best fit and, for these data tested, this provides the best description of the system. The values of the dissociation constants are listed with the estimated standard deviations in Table 5.05.

**Fig. 5.05.** Experimental formation curves for pH titrations of cobalt(II) ions and mercaptacetate acid. Experiment: (i) ■; (ii) ▲; (iii) ●.
The ratio $\Delta \log[M_T]/\Delta \log[L]$ is not constant over the range studied, indicating that a "core plus links" series is not predominant.

In order to obtain approximate values of the constants it was assumed that, in a rapid titration, decomposition and other side-reactions were negligible, and that any polynuclear species present were either of the form $M_2L_3$ or $M_3L_4$. The closeness of fit of three sets of species is indicated by the relative values of the weighted variance of the residuals in analytical hydrogen ion concentration ($W.V.$), obtained from the refinement programme GAUSS (Table 5.05).

Further sets of species were not tested because of the uncertainty of the experimental data, however, in view of the obvious importance of the polynuclear species $M_2L_3$, it is not unlikely that further polynuclear species are also present.

Table 5.05

Weighted variance of residuals for possible cobalt(II)-mercaptoacetic acid complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ML$ $M_2L_3$</td>
<td>0.152</td>
</tr>
<tr>
<td>$ML$ $M_2L_4$</td>
<td>0.316</td>
</tr>
<tr>
<td>$ML$ $ML_2$</td>
<td>3.55</td>
</tr>
</tbody>
</table>

The set $ML$, $ML_2$, $M_2L_3$ gives the best fit and, of the sets tested, this provides the best description of the system. The values of the relevant constants are listed, with their estimated standard deviations in Table 5.06.
Table 5.06

Stability constants (with estimated standard deviations) of complexes of cobalt(II) with mercaptoacetic acid at 20.0°, I=0.1 (NaClO₄)

<table>
<thead>
<tr>
<th>Log β₁₁</th>
<th>β₁₂</th>
<th>Log β₂₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.614 ± 0.057</td>
<td>11.801 ± 0.035</td>
<td>21.195 ± 0.026</td>
</tr>
</tbody>
</table>

This set reproduces the experimental data to within an estimated standard deviation in titre of 0.0030 ml. for two experiments (29 readings) where the above side-reactions were not important.

(vi) Cadmium(II) Complexes

Titrations of cadmium(II) perchlorate with mercaptoacetic acid were marred by the precipitation of a white complex at an early stage in the titration, even in the most dilute solution used, e.g. precipitation occurred at \( n = 0.24 \) in a solution of \( \text{Cd}^{2+} \) (0.000519M) and mercaptoacetic acid (0.0009964M). The precipitate did not redissolve until \( n > 1.5 \) when equilibration was very slow, suggesting the formation of polynuclear complexes in this region. The data that were obtained for the system are shown in Table A.05. In order to obtain an approximate figure for the stability of the 1:1 complex it was assumed that in the most dilute solution, before precipitation occurred, the only complex...
species present was $ML$, and a calculation indicated that
$\log \beta_{11}$ was about $9.26 \pm 0.04$ at $20.0^\circ$, $I=0.1$ ($\text{NaClO}_4$).

It seems likely that the insoluble species in these
experiments is the neutral complex $ML$. Cabrera and West
proposed the formation of the species $\text{Cd}_2L_3^{2-}$, but reported
that this was highly soluble, thus it is not likely to
have precipitated under the present conditions. The
formation curves are not reproduced here because only a
small portion was obtained for each titration. Over this
range however they were not coincident, and thus confirmed
the existence of some polymeric species. No further work
was carried out on this system.

(vii) Mercury(II) Complexes

A number of titrations of mercury(II) perchlorate in
the presence of mercaptoacetic acid were carried out, and
data and formation curves for some of these are shown in
Table A.06 and Fig.5.06 respectively. An unusual feature
of this system was the formation of a very stable species
at the start of the titration which prevented the attainment
of $\bar{n} < 1.1$. When acid was added in order to decrease $\bar{n}$,
a white precipitate resulted.

From polarographic data Stricks et al., suggested
the formation of the species $ML_2$, $MHL_2$, $MH_2L_2$, $M_2L_2$.
From the pH ranges at which these various species might
exist, it appears that of the complexes proposed, only
$M_2L_2$ and $ML_2$ should be important in the present work.
Fig. 5.06. Experimental formation curves for pH titrations of mercury(II) ions and mercaptoacetic acid. Experiment: (i) ■; (ii) ▲; (iii) ●.
The "core plus links" hypothesis could not be applied, because the spacing between the curves was not constant. The assumption that the only complex present was $ML_2$, led to convergence in the refinement programme. When any other species was included convergence was not rapid and automatic, and values of these constants could only be found using the search procedure incorporated in the programme, in which one constant is held constant at a series of values while a further one or more are varied. The necessity of resorting to this procedure is itself evidence that the system is unusual in some way, and the process requires prolonged calculations. The inclusion of $M_2L_2$ led to an improvement in fit, as did $M_3L_3$. The species $M_4L_6$ and similar highly associated complexes might well be present but could not be tested because, for these complexes to be present in significant concentrations, their stability constants would have to have very high values. This would lead to a calculation in which certain quantities would exceed the limits of the computer ($10^{75}$ to $10^{-75}$). The refinement programme will have to be modified to treat these numbers in the logarithmic form before such species can be considered.
Table 5.07

Weighted variance of residuals for possible mercury(II)-mercaptoacetic acid complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML₂ M₂L₂</td>
<td>5.64</td>
</tr>
<tr>
<td>ML₂ M₃L₃</td>
<td>5.64</td>
</tr>
<tr>
<td>ML₂ MH₂L₂</td>
<td>13.3</td>
</tr>
<tr>
<td>ML₂ M₂H₂L₂</td>
<td>47.8</td>
</tr>
<tr>
<td>ML₂ M₄L₆</td>
<td>49.1</td>
</tr>
</tbody>
</table>

* No convergence.
† Quantities in calculation too large for computer.

Some of the species tested are shown with their respective weighted variances (W.V.) in Table 5.07. It appears that ML₂ is the major species at the end of the titration, but earlier there are undoubtedly considerable quantities of polynuclear species. It is quite possible that some of these species may be protonated. In which case the calculation once more involves very large numbers, and it would be necessary to modify the refinement programme to deal with such complexes. The very extensive calculations which would be necessary to define the nature of the system more closely render this impossible at present.
Values of the constants for three sets of species are shown, with the estimated standard deviations in the titre, in Table 5.08. However none of these sets can be said to provide a satisfactory description of the system.

Table 5.08
Stability constants (with estimated standard deviations) of complexes of mercury(II) with mercaptoacetic acid, assuming various sets of species. 20.0°, I=0.1 (NaClO₄)

<table>
<thead>
<tr>
<th>Log β₁₁</th>
<th>Log β₂₂</th>
<th>Log β₂₁--₂⁺</th>
<th>E.S.D. in Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.04 ± 0.03</td>
<td>32.0*</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>23.61 ± 0.04</td>
<td>30.9*</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>20.43 ± 0.09</td>
<td></td>
<td>0.064</td>
<td></td>
</tr>
</tbody>
</table>

* Constant held at this value.
† For the definition of the constant for this protonated species, see p.148.

2-Mercaptoethylamine
(i) Acid Association Constants

In studying complex formation by 2-mercaptoethylamine, two acid dissociations must be considered, the loss of the proton from the (protonated) amino group, and the dissociation of the sulphydryl group. Which dissociation occurs first is a matter of some controversy, and it is quite probable that the two monoprotonated species exist...
simultaneously. The dissociation constants were measured
gaptentiometrically in the usual way. Treating the constants
as an overlapping pair, an initial calculation gave
preliminary values for the constants. These were then
refined using GAUSS. The resulting constants are shown,
with their estimated standard deviations, in Table 5.09.
These constants fit the experimental data (2 experiments,
81 readings) within an estimated standard deviation in
titre of 0.0013 ml.

Table 5.09

Acid association constants (with estimated
standard deviations) of 2-mercaptoethylamine
at 20.0°, I=0.1 (NaClO₄)

\[
\log \beta_1 = 10.811 \pm 0.002 \\
\log \beta_2 = 19.204 \pm 0.003
\]

(ii) Nickel(II) Complexes

The data for a series of titrations of solutions of
2-mercaptoethylamine containing nickel(II) perchlorate are
shown in Table A.07, and the corresponding formation curves
are shown in Fig.5.07. These curves suggest the presence
of polynuclear species, but in the conditions studied such
species evidently do not predominate. It was not possible
to study the system at concentrations as high as those
employed with other ligands because of precipitation of the
sparingly soluble green ML₂ complex.²³
However, included in Table A.07 are the data for one experiment at higher concentration, in which the metal to ligand ratio was 3:4, where a considerable amount of polynuclear complex formation occurs before precipitation (at $n=0.46$, curve (i)).

This is another system where the "core plus links" hypothesis is not applicable, and it is necessary to test sets of species by fitting each set to the data using GAUSS. Some of the combinations tried are listed in Table 5.10.

Table 5.10. Weighted variance of residuals for possible nickel(II)-2-mercaptoethylamine complexes.

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ML_2$</td>
<td>0.116</td>
</tr>
<tr>
<td>$M_2L_3$</td>
<td>0.118</td>
</tr>
<tr>
<td>$M_4L_6$</td>
<td>0.267</td>
</tr>
<tr>
<td>$ML_4$</td>
<td>0.0031</td>
</tr>
<tr>
<td>$M_2L_3$</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

* Species negligible.

Fig. 5.07. Experimental formation curves for pH titrations of nickel(II) ions and 2-mercaptoethylamine. Experiment: (i) $\circ$; (ii) $\bullet$; (iii) $\triangle$; (iv) $\bigcirc$; (v) $\triangledown$.

Expt. (i) (Table A.07) alone.
However, included in Table A.07 are the data for one experiment at higher concentration, in which the metal to ligand ratio was 3:4, where a considerable amount of polynuclear complex formation occurs before precipitation (at \( \bar{n}=0.46 \), curve (i)).

This is another system where the "core plus links" hypothesis is not applicable, and it is necessary to test sets of species by fitting each set to the data using GAUSS. Some of the combinations tried are listed in Table 5.10.

Table 5.10

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML ML2 M(_2)L(_3)</td>
<td>0.116</td>
</tr>
<tr>
<td>ML (_2) M(_2)L(_3)</td>
<td>0.118</td>
</tr>
<tr>
<td>ML ML2 M(_4)L(_6)</td>
<td>0.267</td>
</tr>
<tr>
<td>ML ML2 M(_3)L(_4)</td>
<td>0.291</td>
</tr>
<tr>
<td>ML ML2</td>
<td>0.405</td>
</tr>
<tr>
<td>ML ML2 M(_3)L(_4) MHL*</td>
<td>-</td>
</tr>
<tr>
<td>ML ML2 M(_2)L(_3) M(_3)L(_4)*</td>
<td>-</td>
</tr>
</tbody>
</table>

Expt. (i) (Table A.07) alone

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML ML2 M(_2)L(_3)</td>
<td>0.0031</td>
</tr>
<tr>
<td>ML ML2 M(_3)L(_4)</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

* Species negligible.
If only one polynuclear species is assumed to be present, the complex $M_2L_3$ offers the greatest improvement in fit. The inclusion of a further species $M_3L_4$ or $MHL$ only resulted in the steady reduction of the constants for these species, indicating that these complexes were negligible under these conditions. This is a little surprising because both of these species have been reported to exist. $MHL$ was reported for solutions containing a great excess of metal,\textsuperscript{44} but appears to be unimportant under the present conditions. The trinuclear complex $M_3L_4$ was obtained as a crystalline sample from concentrated solution of 2-mercaptoethylamine containing nickel(II) ions.\textsuperscript{23} Spectrophotometric evidence was cited as indicating the existence of polynuclear species in more dilute solution, and it was suggested that the only polynuclear species present was $M_3L_4$. The experiment that was described employed Job's method of continuous variations.\textsuperscript{112} Successive portions of a solution of nickel(II) ions were added to a solution of the $ML_2$ complex in water, and the corresponding change in optical density was observed. However, in this study no attempt appears to have been made to exclude air, to measure the pH of the solution, or to maintain this pH at a constant value. Fluctuations in pH must have occurred in this experiment and the results...
so obtained must be considered unreliable. The linear plot which was observed may well have been fortuitous.

In the present work these precautions were taken in the study of the nickel(II)-cysteine system, and the evidence suggested a mixture of polynuclear species. When the method was applied to the nickel(II)-2-mercaptoethylamine system, difficulties arose due to the insolubility of the \( \text{ML}_2 \) complex. When carried out at the concentrations employed by Jicha and Busch\(^ {23} \) (0.001M), at a pH high enough to ensure little dissociation of the complex (pH 7 to 9), precipitation of the \( \text{ML}_2 \) complex could not be prevented. That the above workers were able at the same reagent concentrations to obtain a solution, suggests that the pH employed was considerably lower, probably as a result of absorption of carbon dioxide. It was also observed that at a pH above 7, little of the intensely coloured polynuclear species was formed. This suggests that either this coloured complex is a protonated species which deprotonates to give a less strongly absorbing solution, or that, at this pH, hydrolysis occurs leading to breakdown of the polynuclear species. Whatever the reason, it appeared that spectrophotometry was not an ideal method for determining unambiguously the species present, and this work was discontinued.
so obtained must be considered unreliable. The linear
plot which was observed may well have been fortuitous.

In the present work these precautions were taken in
the study of the nickel(II)-cysteine system, and the
evidence suggested a mixture of polynuclear species.
When the method was applied to the nickel(II)-
2-mercaptoethylamine system, difficulties arose due to
the insolubility of the ML₂ complex. When carried out
at the concentrations employed by Jicha and Busch
(0.001M), at a pH high enough to ensure little
dissociation of the complex (pH 7 to 9), precipitation
of the ML₂ complex could not be prevented. That the
above workers were able at the same reagent concentrations
to obtain a solution, suggests that the pH employed was
considerably lower, probably as a result of absorption
of carbon dioxide. It was also observed that at a pH
above 7, little of the intensely coloured polynuclear
species was formed. This suggests that either this
coloured complex is a protonated species which deprotonates
to give a less strongly absorbing solution, or that, at this
pH, hydrolysis occurs leading to breakdown of the
polynuclear species. Whatever the reason, it appeared
that spectrophotometry was not an ideal method for
determining unambiguously the species present, and this
work was discontinued.
It therefore seems likely that, under the conditions employed in the stability constant titrations, the predominant polynuclear species is \( M_2L_3 \). In view of the comparatively small degree of polynuclear complex formation at the concentrations which may be studied by this method, the inclusion of further species does not appear to be justified, but under suitable conditions further polynuclear species (e.g. \( M_3L_4 \), \( M_4L_6 \)) will probably be present. In fact it was found that in the most concentrated solutions studied, \( M_2L_3 \) and \( M_3L_4 \) were of comparable importance (experiment (i) in Table A.07, and Table 5.10).

Stability constants corresponding to some of the sets of complexes tested are shown in Table 5.11. The set giving the best fit consists of \( ML \), \( ML_2 \), and \( M_2L_3 \), but it is apparent that \( ML \) is of little importance (and therefore has a high estimated standard deviation). The pair of species \( ML_2 \), \( M_2L_3 \), provides a satisfactory description of this system, and \( ML \) may be neglected under these conditions, with little decrease in accuracy.
Table 5.11

Stability constants (with estimated standard deviations) of complexes of nickel(II) with 2-mercaptoethylamine assuming various sets of species.

$20.0^\circ$, $I=0.1$ (NaClO$_4$)

<table>
<thead>
<tr>
<th>$\log \beta_{11}$</th>
<th>$\log \beta_{21}$</th>
<th>$\log \beta_{32}$</th>
<th>$\log \beta_{43}$</th>
<th>$\log \beta_{64}$</th>
<th>E.S.D. in Titre (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.447±0.005</td>
<td>32.291±0.012</td>
<td></td>
<td></td>
<td></td>
<td>0.0037</td>
</tr>
<tr>
<td>19.458±0.008</td>
<td>32.262±0.020</td>
<td></td>
<td></td>
<td></td>
<td>0.0037</td>
</tr>
<tr>
<td>19.532±0.009</td>
<td></td>
<td>45.010±0.057</td>
<td></td>
<td></td>
<td>0.0053</td>
</tr>
<tr>
<td>19.528±0.008</td>
<td></td>
<td></td>
<td>67.725±0.052</td>
<td></td>
<td>0.0056</td>
</tr>
<tr>
<td>19.590±0.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0081</td>
</tr>
</tbody>
</table>
(iii) Zinc(II) Complexes

Data for a number of titrations of solutions of 2-mercaptoethylamine containing zinc(II) perchlorate are listed in Table A.08 and the formation curves are plotted in Fig. 5.08. Values of \( \bar{n} \) shown in these curves are calculated in the usual way, assuming the absence of protonated complexes. The curves have a form similar to those of the zinc(II)-penicillamine system (below). These are not of the type expected for a series of polynuclear complexes, but are likely to be a result of the formation of protonated species. The calculation of \( \bar{n} \), as required for these plots, may only be carried out if it is assumed that protonated metal complexes are absent. If this is not the case, \( \bar{n} \) does not have its usual significance.

A number of possible sets of complexes were tested using the refinement programme. These are listed in order of size of the weighted variance (W.V.) after refinement (Table 5.12). As some polynuclear complex formation had been shown to occur with nickel(II), the effect of inclusion of polynuclear species was also tested with the zinc(II) system.
Fig. 5.08. Experimental formation curves for pH titrations of zinc(II) ions and 2-mercaptoethylamine. Experiment: (1) ■ ; (II) △ ; (III) ● ; (IV) ▽.

Table 5.12

Weighted variance of residuals for possible zinc(II)-2-mercaptoethylamine complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ML^2)</td>
<td>0.190</td>
</tr>
<tr>
<td>(M_2L)</td>
<td>0.193</td>
</tr>
<tr>
<td>(MHL)</td>
<td>0.193</td>
</tr>
<tr>
<td>(MHL_2)</td>
<td>0.190</td>
</tr>
<tr>
<td>(ML^2)</td>
<td>0.252</td>
</tr>
<tr>
<td>(M_2L_2)</td>
<td>0.451</td>
</tr>
<tr>
<td>(MHL_2)</td>
<td>0.563</td>
</tr>
<tr>
<td>(ML)</td>
<td>0.567</td>
</tr>
<tr>
<td>(M_2L_2)</td>
<td>0.607</td>
</tr>
<tr>
<td>(MHL_2)</td>
<td>0.652</td>
</tr>
<tr>
<td>(ML_2)</td>
<td>0.656</td>
</tr>
<tr>
<td>(MHL)</td>
<td>0.657</td>
</tr>
<tr>
<td>(MHL_2)</td>
<td>0.877</td>
</tr>
</tbody>
</table>

*Species negligible
Table 5.12
Weighted variance of residuals for possible zinc(II)-2-mercaptoethylamine complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML₂ M₂L₃ MHL₂</td>
<td>0.190</td>
</tr>
<tr>
<td>ML₂ MHL₂</td>
<td>0.193</td>
</tr>
<tr>
<td>ML₂ M₃L₄ MHL₂</td>
<td>0.193</td>
</tr>
<tr>
<td>ML M₂L₃ MHL₂</td>
<td>0.198</td>
</tr>
<tr>
<td>ML* M₂L₃* MHL₂</td>
<td>0.208</td>
</tr>
<tr>
<td>ML* M₂L₃ MHL₂</td>
<td>0.252</td>
</tr>
<tr>
<td>ML M₂L₃ MH₂L₂</td>
<td>0.451</td>
</tr>
<tr>
<td>ML M₂L₃</td>
<td>0.565</td>
</tr>
<tr>
<td>ML M₂L₃ MHL</td>
<td>0.567</td>
</tr>
<tr>
<td>ML₂ M₂L₃ MH₂L₂</td>
<td>0.568</td>
</tr>
<tr>
<td>ML M₂L₃ MH₂L₂</td>
<td>0.607</td>
</tr>
<tr>
<td>ML M₂L₃</td>
<td>0.652</td>
</tr>
<tr>
<td>ML M₃L₄ MH₂L₂</td>
<td>0.656</td>
</tr>
<tr>
<td>ML M₃L₄ MHL</td>
<td>0.657</td>
</tr>
<tr>
<td>ML M₄L₆</td>
<td>0.684</td>
</tr>
<tr>
<td>ML₂ M₃L₄ MHL</td>
<td>0.877</td>
</tr>
<tr>
<td>ML M₂L₃</td>
<td>1.07</td>
</tr>
<tr>
<td>ML₂ M₂L₃ MHL</td>
<td>1.09</td>
</tr>
<tr>
<td>ML M₂L₃ MH₂L₂*</td>
<td>1.18</td>
</tr>
<tr>
<td>ML M₂L₃ MHL*</td>
<td>1.74</td>
</tr>
<tr>
<td>ML₂ MH₂L₂</td>
<td>5.29</td>
</tr>
<tr>
<td>ML₂ MHL</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*Species negligible
The best fit to the data is provided by either of the sets $\text{ML}_2$, $\text{M}_2\text{L}_3$, $\text{MHL}_2$, and $\text{ML}_2$, $\text{MHL}_2$. The species $\text{M}_2\text{L}_3$ is not very important as the fit is only very slightly worse if this species is omitted. While $\text{M}_2\text{L}_3$ and other polynuclear species may be significant at higher concentrations, they can be neglected in more dilute solutions. The inclusion of hydrolysed MOH (with a value held constant at $\log \beta_{011} = -9.43$) was tested in order to ascertain its importance in these solutions. The improvement in fit, and the concentration of this species in the mixture, were negligible.

The values of the stability constants corresponding to the set giving the best fit, and some other sets of interest are shown in Table 5.13.
Table 5.13

Stability constants (with estimated standard deviations) of complexes of zinc(II) with 2-mercaptoethylamine, assuming various sets of species. 20.0°C, I=0.1 (NaClO₄)

<table>
<thead>
<tr>
<th>log β₁₁</th>
<th>log β₂₁</th>
<th>log β₃₂</th>
<th>log β₄₃</th>
<th>log β₂₁₋₁</th>
<th>E.S.D. in Titre (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.515±0.006</td>
<td>24.725±0.008</td>
<td></td>
<td></td>
<td></td>
<td>0.0032</td>
</tr>
<tr>
<td>17.510±0.007</td>
<td>28.389±0.214</td>
<td></td>
<td></td>
<td></td>
<td>0.0032</td>
</tr>
<tr>
<td>17.514±0.006</td>
<td>40.591±0.218</td>
<td>24.717±0.010</td>
<td></td>
<td></td>
<td>0.0032</td>
</tr>
<tr>
<td>9.205±0.023</td>
<td>17.608±0.016</td>
<td>29.832±0.041</td>
<td></td>
<td></td>
<td>0.0084</td>
</tr>
<tr>
<td>9.364±0.022</td>
<td>17.710±0.018</td>
<td></td>
<td></td>
<td></td>
<td>0.0110</td>
</tr>
</tbody>
</table>

The constants for the protonated species in the Table above, and those elsewhere in this Thesis, are denoted by a subscript whose form is similar to that used in entering the species in the input data for GAUSS.

Thus

$$\beta_{11-1} = \frac{[\text{MHL}]}{[\text{M}][\text{L}]}$$, \hspace{1cm} $$\beta_{21-1} = \frac{[\text{MHL}_2]}{[\text{M}][\text{L}][\text{H}^+]}$$ \hspace{1cm} and \hspace{1cm} $$\beta_{21-2} = \frac{[\text{M(HL)}_2]}{[\text{M}][\text{L}][\text{H}^+]^2}$$
Penicillamine

(i) Acid Association Constants

Penicillamine may have up to three ionisable protons. However, in the pH regions studied in the present work only two protonated forms are important, the protons being bound to the amino group (in the zwitterion), and to the sulphydryl group. The relevant equilibrium constants were determined potentiometrically. Initial values calculated by the modified Speakman method were subsequently refined by GAUSS. The final values, from two experiments (78 readings) considered together, are shown with their estimated standard deviations in Table 5.14. These values fitted the experimental titres to within an estimated standard deviation of 0.0020 ml.

<table>
<thead>
<tr>
<th>Table 5.14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid association constants (with estimated standard deviations) of penicillamine</td>
</tr>
<tr>
<td>at 20.0°, I=0.1 (NaClO₄)</td>
</tr>
</tbody>
</table>

Both values are in agreement with those reported in the literature. Also, previous research has shown that log $\beta_1$ is greater than log $\beta_2$ (12.14). It is seen that $\log \beta_1$ (10.679) is less than log $\beta_2$ (18.714). It is because the species ML can only be present in low concentrations, whereas the more stable in the determination of $\log \beta_1$ is somewhat lower than estimated.

(ii) Nickel(II) Complexes

Titration data for four sets of experimental conditions for the nickel(II)-penicillamine system are given in Table A.09 and are plotted, as the formation curves, in Fig.5.09.
The curves for the four experiments are coincident. It was not possible, in solutions of metal:ligand ratio of 1:4, to obtain \( \bar{n} \) values of greater than 2. These findings indicate that under these conditions only the mononuclear species ML and ML\(_2\) are present. An Irving-Rossotti plot yielded approximate values of log \( K_1 \) (11.0) and log \( K_2 \) (23.0), and these estimates were refined over the entire range of experimental data. The final values (Table 5.15) fitted the data (167 points) to within an estimated standard deviation in titre of 0.0022 ml. As expected, the inclusion of various polynuclear species did not significantly improve the fit.

Table 5.15

<table>
<thead>
<tr>
<th>Stability constants (with estimated standard deviations) of complexes of nickel(II) with penicillamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 20.0(^\circ), ( I=0.1 ) (NaClO(_4))</td>
</tr>
<tr>
<td>( \log \beta_{11} = 10.749 \pm 0.018 )</td>
</tr>
<tr>
<td>( \log \beta_{21} = 22.886 \pm 0.002 )</td>
</tr>
</tbody>
</table>

Both values are somewhat higher than those in the literature. Also, previous workers\(^{53,55}\) have reported that \( \log K_1 \) was greater than \( \log K_2 \). But the present study, using both Irving-Rossotti and GAUSS refinement methods, shows that \( \log K_1 \) (10.75) is less than \( \log K_2 \) (12.14). It is because the species ML can only be present in low concentrations that the accuracy in the determination of \( \log K_1 \) is somewhat lower than usual.
Job's spectrophotometric method was used to confirm the absence of polynuclear species in this system. Polynuclear species of 2-mercaptoethylamine and cysteine with nickel(II) give rise to absorption at 380, and 376 nm, respectively. No enhancement of absorption was detected in this region of the spectrum of nickel(II)-penicillamine solutions, under conditions where penicillamine formed polynuclear species (see below).

(iii) Nickel(II) Complexes

Titration data for solutions of zinc(II) perchlorate and penicillamine (Table 5.10) yielded the formation curves shown in Fig.5.10. These are clearly more complicated than those for nickel(II) ions with this ligand, and furthermore are not of the form expected for a "core plus links" series of complexes. The nickel(II) fails to form polynuclear species with penicillamine suggests that the same may be true for zinc(II). Finally, zinc(II) has been shown to form protonated complexes with this ligand, while simple and mononuclear complexes with nickel(II) cannot account for the unusual shape of the formation curves. Thus, we have thus expect to find up to five mononuclear species: $ML_3$, $ML_2$, $MHL$, $MHL_2$, and $MHL_3$.

There was no evidence for any $ML_3$, since values of $n$ greater than 2 were never obtained. These various species were tested using the refinement programme, and yielded the weighted variances shown in Table 5.16.
Job's spectrophotometric method$^{112}$ was used to confirm the absence of polynuclear species in this system. Polynuclear species of 2-mercaptoethylamine and cysteine with nickel(II) give rise to absorption at 380, and 376 $\mu$m respectively. No enhancement of absorption was detected in this region of the spectrum of nickel(II)-penicillamine solutions, under conditions where cysteine formed polynuclear species (see below).

(iii) Zinc(II) Complexes

The titration data for solutions of zinc(II) perchlorate and penicillamine (Table A.10) yielded the formation curves shown in Fig.5.10. These are clearly more complicated than those of nickel(II) ions with this ligand, and furthermore are not of the form expected for a "core plus links" series of complexes. That nickel(II) fails to form polynuclear species with penicillamine suggests that the same may be true for zinc(II). Finally zinc(II) has been shown to form protonated complexes with some ligands which form simple complexes with nickel(II),$^{126}$ and such behaviour could account for the unusual shape of the formation curves.

With this terdentate ligand we might thus expect to find up to five mononuclear species; $\text{ML, ML}_2, \text{MHL, MHL}_2, \text{MH}_2\text{L}_2$. There was no evidence for any $\text{ML}_3$, since values of $\bar{n}$ greater than 2 were never obtained. These various species were tested using the refinement programme, and yielded the weighted variances shown in Table 5.16.
Fig. 5.10. Experimental formation curves for pH titrations of zinc(II) ions and penicillamine. Experiment: (i) ■; (ii) ▲; (iii) ●; (iv) ◀.

The inclusion of the postulated species greatly improved the fit to the data. It is evident that ML₂ is not an important species, because the refinement reduces the concentration of this species to a negligible quantity in which it was tried. And according to it, the other combinations of species are the major components under the conditions employed. The constants relating to this set of species are listed in Table 5.17. Those constants fit the experimental titrations (200 points) to within an estimated standard deviation of 0.005 ml.
Table 5.16
Weighted variance of residuals for possible zinc(II)-penicillamine complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML</td>
<td>0.193</td>
</tr>
<tr>
<td>ML ML₂</td>
<td>0.348</td>
</tr>
<tr>
<td>ML MHL</td>
<td>0.691</td>
</tr>
<tr>
<td>ML ML₂</td>
<td>1.01</td>
</tr>
<tr>
<td>ML MHL</td>
<td>2.14</td>
</tr>
<tr>
<td>ML ML₂</td>
<td>2.16</td>
</tr>
<tr>
<td>ML MHL</td>
<td>3.80</td>
</tr>
<tr>
<td>ML ML₂</td>
<td>7.30</td>
</tr>
</tbody>
</table>

* Species negligible

The inclusion of protonated species greatly improves the fit to the data, but it is evident that MHL is not an important species, because the refinement reduces the apparent concentration of this species to a negligible quantity in three of the four possible sets of complexes in which it was tried. Of the other combinations the set ML, ML₂, MHL₂, MH₂L₂ provides the best fit to the data, and accordingly it is suggested that these species are the major components under the conditions employed. The constants relating to this set of species are listed in Table 5.17. These constants fit the experimental titres (200 points) to within an estimated standard deviation of 0.0039 ml.
Table 5.17
Stability constants (with estimated standard deviations) of complexes of zinc(II) with penicillamine

at 20.0°C, I=0.1 (NaClO₄)

log $\beta_{11}$ = 9.589 ± 0.008
log $\beta_{21}$ = 19.559 ± 0.007
log $\beta_{21-1}$ = 25.547 ± 0.011
log $\beta_{21-2}$ = 31.171 ± 0.021

It was shown for the mercaptacetate complexes of zinc(II) and nickel(II) ions that the small values of the estimated standard deviation in titre corresponded to a close fit between the calculated and experimental formation curves. Such a comparison is not possible for a system containing protonated species, because values of $\bar{n}$ calculated in the usual way have no meaning. However in order to provide a graphical description of the quality of fit to the data, the calculated and experimental titration curves (of pH plotted against titre) may be compared. This has been done for two experiments of the set of five used to obtain stability constants for the zinc(II)-penicillamine system. The plots are shown in Fig.5.11, where it is evident that at each point in the titration there is close correspondence between experimental values and those calculated using the refined values of the constants.
Cysteine, like penicillamine, has three sites for protons addition or removal. However, in the pH regions of interest, only protons bound to the amine and sulphydryl group are important. The results of three titrations (readings) were calculated by the modified Speakman method and refined together by the programme CHANS to give the calculated and estimated standard deviations shown in Table 5.16.

Fig. 5.11. Calculated curves for pH titrations of zinc(II) and penicillamine, and experimental points (a) for experiment (11), and (b) for experiment (14).
Cysteine

(i) Acid Association Constants

Cysteine, like penicillamine, has three sites for proton addition or removal. However, in the pH regions of interest, only protons bound to the amino and sulphydryl groups are important. The results of three titrations (56 readings) were calculated by the modified Speakman method, and refined together by the programme GAUSS to give the constants and estimated standard deviations shown in Table 5.18. These fitted the titration data to within an estimated standard deviation in titre of 0.0027 ml.

Table 5.18

Acid association constants (with estimated standard deviations) of cysteine

\[
\begin{align*}
\log \beta_1 &= 10.498 \pm 0.003 \\
\log \beta_2 &= 19.330 \pm 0.004
\end{align*}
\]

at 20.0°C, I=0.1 (NaClO₄)

(ii) Nickel(II) Complexes

(a) Spectrophotometric studies.—Jicha and Busch²³ used Job's method,¹¹² to obtain spectrophotometric evidence for the existence of the trimeric nickel(II)-2-mercaptoethylamine complex (structure IV, Fig.1.02). Successive portions of a nickel(II) solution were added to a solution of the ML₂ species, and the enhancement in spectroscopic absorption was
followed at 380 mµ (the "enhancement" being defined as the
difference between the observed optical density and that
calculated assuming no reaction of the component solutions).
This led to a plot for which the two parts approximated
closely to linearity with a maximum a mole fraction of
ML₂ of 0.66, corresponding to the presence of a
predominant species M₃L₄.

For reliable results it appeared that this method
required the maintenance of a constant pH, and complete
exclusion of air. Buffers were avoided, as these
interfered by complex formation, so pH adjustment by
addition of acid or alkali was adopted. The method and
apparatus have been described in Chapter 4. Successive
portions of a nickel(II) perchlorate solution were added
to a solution of bis(cysteinato)nickel(II) at pH
8.00 ± 0.01, while the absorption at 376 mµ was measured.
This wavelength corresponded to the maximum in the peak
which develops under these conditions. The results were
plotted as optical enhancement versus mole fraction of
ML₂ (Fig.5.12). In the experiment shown, the concentrations
of the complex at the start, and of nickel(II) perchlorate
added were both 0.01M. The plot shows a maximum enhancement
at the mole fraction 0.75, corresponding to the species
M₂L₃. Unlike the plot reported for 2-mercaptopoethylamine,
the legs of the plot deviate somewhat from linearity, and
under some conditions the peak lay nearer 0.56, which would correspond to the formation of $M_2L_4$. Thus while $M_2L_3$ appears to be the predominant species, the presence of other polynuclear species, possibly $M_3L_4$, cannot be excluded.

(b) Potentiometric studies.— The pH titration data and formation curves are shown in Table A.1 and Fig. 5.13 respectively. The curves follow the pattern expected for polymer complex formation (in keeping with the spectrophotometric evidence) and support the "mononuclear" at low concentrations. Polynuclear complex formation does not appear to be predominant under the conditions employed, and it is not possible to use the "core plus link" hypothesis on the data. A trial and error approach was therefore adopted. A variety of sets of possible complexes were tested in calculations with GAMS, and the respective fit to the entire range of experimental data is shown by the mode of the weighted variance of the residuals (Table A). A little improvement was possible on the species of the form $M_2L_3$ (as found in nickel(II)-mercaptacacetate complexation) which may improve the fit in a small way but are not likely to be important for a tartardate ligand.

Fig. 5.12. Job's plot for solutions of bis(cysteinato)-nickel(II) ions containing various proportions of nickel(II) perchlorate. Maximum concentration of either species 0.01 M, cell width 0.1 cm.; wavelength 376 m.µ.
under some conditions the peak lay nearer 0.66, which would correspond to the formation of $M_3L_4$. Thus while $M_2L_3$ appears to be the predominant species, the presence of other polynuclear species, possibly $M_3L_4$, cannot be excluded.

(b) Potentiometric studies.— The pH titration data and formation curves are shown in Table A.11 and Fig.5.13 respectively. The curves follow the pattern expected for polynuclear complex formation (in keeping with the spectrophotometric evidence) and approach the "mononuclear wall" at low concentrations. Polynuclear complex formation does not appear to be predominant under the conditions employed, and it is not possible to use the "core plus links" hypothesis on the data. A trial and error approach was therefore adopted. A variety of sets of possible complexes were tested in calculations with GAUSS, and the respective fit to the entire range of experimental data is shown by the magnitude of the weighted variance of the residuals (Table 5.19). Little emphasis was placed on the species of the form $M_4L_6$ (as found in nickel(II)-mercaptoacetate complexes) because the species did not greatly improve the fit in simple cases, and species of this type are not likely to be important for a terdentate ligand.
Fig 5.13. Experimental formation curves for pH titrations of nickel(II) ions and cysteine. Experiment: (i) • ; (ii) • ; (iii) • ; (iv) • .
### Table 5.19  Weighted variance of residuals for possible nickel(II)-cysteine complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML ML₂ M₂L₃ M₃L₄ MHL</td>
<td>0.016</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ M₃L₄ MHL</td>
<td>0.018</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ M₂L₂ L₂</td>
<td>0.033</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ MHL</td>
<td>0.038</td>
</tr>
<tr>
<td>ML* ML₂ M₂L₃ MHL</td>
<td>0.038</td>
</tr>
<tr>
<td>ML ML₂ M₂L₄ M₂L₂ L₂</td>
<td>0.060</td>
</tr>
<tr>
<td>ML ML₂ M₂L₄ MHL</td>
<td>0.061</td>
</tr>
<tr>
<td>ML ML₂ M₂L₄ MHL</td>
<td>0.067</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₂L₃ M₃L₄</td>
<td>0.068</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₄L₆</td>
<td>0.078</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₃L₄</td>
<td>0.090</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₃L₄ MHL₂</td>
<td>0.094</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₃L₄</td>
<td>0.098</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₃L₄ M₃L₄</td>
<td>0.099</td>
</tr>
<tr>
<td>ML M₃L₄ MHL</td>
<td>0.133</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₂L₃ MHL₂</td>
<td>0.147</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ MHL</td>
<td>0.191</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ M₄L₆</td>
<td>0.197</td>
</tr>
<tr>
<td>ML ML₂ M₄L₆</td>
<td>0.229</td>
</tr>
<tr>
<td>ML₂ M₂L₃ M₃L₄</td>
<td>0.314</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₂L₂</td>
<td>0.378</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₃L₄ M₄L₆</td>
<td>0.401</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ MHL*</td>
<td>0.748</td>
</tr>
<tr>
<td>ML ML₂</td>
<td>0.896</td>
</tr>
</tbody>
</table>

* Species negligible.
No unambiguous set of complexes may be assigned on the basis of the data of Table 5.19. However the closeness of fit of several combinations of species is evident, as is the poor fit of the pair of species ML, ML$_2$ usually assumed to predominate. Of the three-member sets, ML, ML$_2$, and M$_3$L$_4$ form the best set neglecting protonated species, but if we include one protonated species (MHL) the fit improves greatly and the best three-member set becomes ML$_2$, M$_2$L$_3$ and MHL. Of the four variable sets, ML$_2$, M$_2$L$_3$, M$_3$L$_4$, MHL is the best of those tested. Inclusion of ML improves the fit slightly, but no more than expected from the inclusion of a further variable. The species ML$_3$ does not appear to be significant and no values of $\bar{n}$ greater than 2 were obtained. Thus the system may be described by the species ML$_2$, M$_2$L$_3$, M$_3$L$_4$ and MHL, and these fit the data to within an estimated standard deviation in titre of 0.0036 ml. This fit is not as good as has been obtained with some other systems, and further species must be present. The best set contains both polynuclear and protonated species, and it is very likely that there are present further complexes of these types, and also protonated polynuclear species. The number of such possible complexes makes it unreasonable to attempt to fit any further species to the data at present.

Values of the constants, and estimated standard deviations in both the constants and the experimental titres are shown for several sets of species in Table 5.20.
Table 5.20

Stability constants (with estimated standard deviations) of complexes of nickel(II) with cysteine, assuming various sets of species.

\[ 20.0^\circ, I=0.1 \text{ (NaClO}_4 \text{)} \]

<table>
<thead>
<tr>
<th>log $\beta_{11}$</th>
<th>log $\beta_{21}$</th>
<th>log $\beta_{32}$</th>
<th>log $\beta_{43}$</th>
<th>log $\beta_{11-1}$</th>
<th>E.S.D.in Titre (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.977±0.094</td>
<td>20.156±0.003</td>
<td>33.005±0.024</td>
<td>45.719±0.029</td>
<td>15.426±0.016</td>
<td>0.0032</td>
</tr>
<tr>
<td>20.158±0.003</td>
<td>33.084±0.014</td>
<td>45.713±0.034</td>
<td>15.485±0.010</td>
<td>0.0036</td>
<td></td>
</tr>
<tr>
<td>9.714±0.021</td>
<td>20.111±0.004</td>
<td>45.714±0.021</td>
<td>0.0061</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.711±0.031</td>
<td>20.072±0.007</td>
<td>32.759±0.026</td>
<td>0.0068</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.097±0.025</td>
<td>20.167±0.010</td>
<td></td>
<td></td>
<td>0.0133</td>
<td></td>
</tr>
</tbody>
</table>
(iii) Zinc(II) Complexes

After some preliminary titrations to obtain approximate values for $\beta_1, \beta_2$ for this system a series of e.m.f. titrations were attempted by a method similar to that used in the zinc(II)-mercaptoacetic acid system. The data, when plotted graphically, failed to yield straight lines, and it was suspected that this might be due to the presence of protonated species. Further titrations confirmed this.

The data for titrations of solutions of cysteine in the presence of zinc(II) perchlorate are shown in Table A.12, and the formation curves are plotted in Fig. 5.14. The formation curves are again derived from calculations which assume negligible protonated complex formation, but they are somewhat more complicated than those obtained for the zinc(II)-penicillamine system (Fig. 5.11). In the latter, the curves for different ligand concentrations crossed, but those for the same ligand concentration but different total metal concentrations did not cross. The curves for the zinc(II)-cysteine system show both features.
The crossing of curves at different total ligand concentrations is in keeping with protonated species, while the crossing of curves at different total metal concentrations implies the presence of polynuclear species. The combination of these two properties implies that cysteine may form both protonated and polynuclear species.

A conclusion might also be reached by comparing the complexes formed by zinc(II) and cysteine (protonated), of nickel(II) and penicillamine (simple), and of nickel(II) with cysteine (polynuclear and probably protonated). It is also borne out by the comparison of the weighted variance of the residuals corresponding to the various sets of complexes tested (Table 5.32).

Finally, at high concentrations, a white precipitate was formed during the titration (corresponding to the breakage of curve (v in Fig. 5.14)). With nickel(II) protonated species, the more important and common form was therefore a mononegative precipitate nickel(II) complex was a protonated species. It dissolved on addition of...
The crossing of curves at different total ligand concentrations is in keeping with protonated species, while the crossing of curves at different total metal concentrations implies the presence of polynuclear species. The combination of these two properties implies that cysteine may form both protonated and polynuclear species.

This conclusion might also be reached by comparing the complexes formed by zinc(II) with penicillamine (protonated), of nickel(II) with penicillamine (simple), and of nickel(II) with cysteine (polynuclear and probably protonated). It is also borne out by the comparison of the weighted variances of the residuals corresponding to the various sets of complexes tested (Table 5.21). Finally, at high concentrations, a white precipitate was formed during the titration (corresponding to the break in curve (i), Fig. 5.14). With nickel(II) protonated species were less important and no precipitate was formed, therefore it is probable that the precipitated zinc(II) complex was a protonated species. It dissolved on addition of further alkali.
Table 5.21  Weighted variance of residuals for possible zinc(II)-cysteine complexes

<table>
<thead>
<tr>
<th>Species</th>
<th>W.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML₂ M₃L₄ MHL₂ MH₂L₂</td>
<td>0.137</td>
</tr>
<tr>
<td>ML₂ M₃L₄ MHL* MHL₂ MH₂L₂</td>
<td>0.138</td>
</tr>
<tr>
<td>ML* ML₂ M₃L₄ MHL MHL₂ MH₂L₂</td>
<td>0.145</td>
</tr>
<tr>
<td>ML* ML₂ M₂L₃* M₃L₄ MHL MHL₂</td>
<td>0.185</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ M₃L₄</td>
<td>0.473</td>
</tr>
<tr>
<td>ML ML₂ M₃L₄</td>
<td>0.538</td>
</tr>
<tr>
<td>ML₂ M₂L₃ MHL₂ MH₂L₂</td>
<td>0.575</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ MHL₂ MH₂L₂</td>
<td>0.771</td>
</tr>
<tr>
<td>ML ML₂ M₃L₄ MHL MHL₂</td>
<td>0.790</td>
</tr>
<tr>
<td>ML ML₂ M₄L₆</td>
<td>0.817</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ M₃L₄ MHL</td>
<td>0.860</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₃L₄</td>
<td>0.921</td>
</tr>
<tr>
<td>ML ML₂ M₃L₄ MHL</td>
<td>0.925</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ MHL</td>
<td>0.973</td>
</tr>
<tr>
<td>ML ML₂ M₃L₄ M₄L₆</td>
<td>1.00</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃</td>
<td>1.08</td>
</tr>
<tr>
<td>ML* ML₂ M₂L₃ MHL* MHL₂ MH₂L₂</td>
<td>1.19</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂</td>
<td>3.60</td>
</tr>
<tr>
<td>ML ML₂</td>
<td>4.25</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₂L₃</td>
<td>- †</td>
</tr>
<tr>
<td>ML ML₂ M₂L₂ M₄L₆</td>
<td>- †</td>
</tr>
<tr>
<td>ML ML₂ M₂L₃ M₄L₆</td>
<td>- †</td>
</tr>
</tbody>
</table>

*Species negligible.  †No convergence.
As may be seen from Table 5.21, with this system the set of complexes giving the best fit is not that which contains the most species, but over the range studied the set $\text{ML}_2$, $\text{M}_3\text{L}_4$, $\text{MHL}_2$, $\text{MH}_2\text{L}_2$ gives a good approximation to the data. The stability constants corresponding to this set, and to several other sets of interest, are shown in Table 5.22 with the estimated standard deviations of the constants and of the titres (as obtained by back-calculation using the refined constants).

Once again, the comparatively large value of the estimated standard deviation in titre indicates that even the best set of species gives only an approximation to the situation, and there is little doubt that further species, in particular protonated polynuclear complexes, are also present. However, present experimental techniques do not justify attempts to fit further species to the data.
Table 5.22

Stability constants (with estimated standard deviations)
of complexes of zinc(II) with cysteine, assuming various sets of species.

20.0\(^{\circ}\), I=0.1 (NaClO\(_4\))

<table>
<thead>
<tr>
<th>log (\beta_{11})</th>
<th>log (\beta_{12})</th>
<th>log (\beta_{32})</th>
<th>log (\beta_{43})</th>
<th>log (\beta_{21-1})</th>
<th>log (\beta_{21-2})</th>
<th>E.S.D. in Titre (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.220 ± 0.007</td>
<td>43.563 ± 0.036</td>
<td>24.771 ± 0.020</td>
<td>30.724 ± 0.027</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.326 ± 0.038</td>
<td>18.241 ± 0.013</td>
<td>43.979 ± 0.038</td>
<td></td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.343 ± 0.034</td>
<td>18.220 ± 0.131</td>
<td>30.575 ± 0.095</td>
<td>43.822 ± 0.054</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.838 ± 0.047</td>
<td>18.161 ± 0.047</td>
<td></td>
<td></td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2,3-Dimercaptopropane-1-sulphonic acid

(i) Acid Association Constants

The dissociation of the two sulphhydryl protons of 2,3-dimercaptopropane-1-sulphonic acid was studied in the usual way. Preliminary constants, obtained by the simple calculation, were refined using GAUSS, and yielded the acid association constants shown (with their estimated standard deviations) in Table 5.23. The refined constants fitted the experimental data (24 points) to within an estimated standard deviation in titre of 0.0012 ml.

Table 5.23

Acid association constants (with their estimated standard deviations) of 2,3-dimercaptopropane-1-sulphonic acid at 20.0°C, I=0.1 (NaClO₄)

<table>
<thead>
<tr>
<th>Log β₁</th>
<th>11.849 ± 0.004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log β₂</td>
<td>20.553 ± 0.005</td>
</tr>
</tbody>
</table>

These may be compared with literature values and estimated standard deviations of 11.88 ± 0.2, 20.52 ± 0.2 at 25.0°C, I=0.1 (KCl),¹²⁷ and 11.17 ± 0.02, 19.9 ± 0.04*¹²⁸

These values may have been obtained using impure samples.

* The experimental conditions for the latter two values were not specified in Chemical Abstracts.
(ii) Nickel(II) Complexes

Several titrations were attempted with nickel(II) perchlorate. However, even in quite dilute solution, the equilibration became very slow at an early stage in the titration. The solution, originally pale green, became dark mauve-brown, and then black. This intense absorption was not the result of decomposition, since addition of acid showed the process to be quite reversible. It was thus evident that highly associated polynuclear complexes were formed, which, unlike those of BAL, were very soluble and did not precipitate. Long chains may thus be expected to form, and such a process is manifested in the long equilibration time observed in the titrations (several hours near $\bar{n} = 1.0$ in solutions of $[\text{Ni}_T]=0.002\text{M}$, $[\text{L}_T]=0.004\text{M}$).

The data for two experiments are presented in Table A.13, and these give the formation curves shown in Fig.5.15. The completely different form of these two curves, for experiments employing the same concentration of total metal, indicates that the calculation of $\bar{n}$ is not valid, and that protonated species are present. In view of the high degree of association expected for this system, the presence of polynuclear species greatly extends the number of possible species.
If it could be assumed that protonated and polynuclear species were absent, and that the value of $\log K_{21}$ could be calculated from the formation curve ($-2 \log [L]$ at $n=1$), then a value of $\log K_{21} = \log 3.5$ would be obtained, but this very high value is all that can at present be provided to estimate the chelating ability of this unusual ligand. Because of the complexity of the mixture, and the long time of the reaction, the system was not studied in greater detail because equilibration was more rapid. The data for experiments are shown in Table A.14 and the corresponding formation curves are shown in Fig. 5.15. The maximum (negative) $\log [M_n]/[L]_n$ is not constant as expected for the "core and links" series of complexes, but appears to change with the concentration of metal. The maximum might represent a trend to $-2$, which would correspond to the existence of mononuclear species at low concentrations and of the metal at $\log [L]/[M_n] = -1$ in the region $n=1$, as we employed the same total metal concentration but different total ligand.
If it could be assumed that protonated and polynuclear species were absent, and that the value of log $\beta_{21}$ could thus be calculated from the formation curve ($-2 \log[L]$ at $\bar{n} = 1.0$), then a value of log $\beta_{21} \approx 33.6$ would be obtained. These assumptions are not valid, but this very high figure is all that can at present be provided to demonstrate the chelating ability of this unusual ligand. Because of the complexity of the mixture, and the long equilibration time of the reaction, the system was not studied further.

(iii) Zinc(II) Complexes

Complex formation with zinc(II) was studied in greater detail because equilibration was more rapid. The data for several experiments are shown in Table A.14 and the corresponding formation curves are shown in Fig.5.15. The spacing ($\Delta \log[M_T]/\Delta \log[L]$) is not constant as expected for a simple "core plus links" series of complexes, but appears to change with the concentration of metal. The maximum (negative) value observed for this ratio is $-1.5$. This might represent a trend from 0 to $-2$, which would correspond to the existence of mononuclear species at low concentrations and of the series $M(ML_2)_n$ at $\Delta \log[M_T]/\Delta \log[L] = -2$ in the region $\bar{n} < 1.67$. However in experiments which employed the same total metal concentration but different total ligand
concentrations, the curves do not coincide. This indicates the presence of protonated species, and implies that as well as forming the dithiol chelate complexes, the system also contains protonated species in which the metal is bound by only one sulphhydryl group, possibly with some participation by the sulphonic acid group. In the latter part of the titration this system also exhibits slow equilibration, presumably the result of the formation of long polynuclear chains. Some calculations were carried out assuming mononuclear species, and a particular "core plus links" series, in which the successive stability constants were related by a constant quantity, but no satisfactory constants were obtained. It appears that for a system of this degree of complexity, with protonated as well as normal polynuclear species possible, it is not at present possible to provide meaningful stability constants for the wide variety of species formed.

As a very approximate indication of the stability of the zinc(II) complexes of this ligand, it was assumed that in the most dilute solutions studied ([Zn\textsubscript{T}] = 0.0002M, [L\textsubscript{T}] = 0.0008M) polynuclear and protonated species were absent, and from \( n = 1.0 \) on the formation curve a value of \( \log \beta_{21} \approx 25 \) was obtained.
Reactions of Copper(II) Ion with Thiols

The reaction of copper(II) ions with three of the above ligands was studied briefly in order to investigate certain discrepancies in studies reported in the literature (see Chapter 1).

With 2-mercaptoethylamine a low pH was observed throughout the titration, and calculations revealed that $n$ apparently had the value of $1.0 \pm 0.1$ throughout the titration. This indicated that the cupric ion had quantitatively oxidised the ligand.

Kuchinskas and Rosen\textsuperscript{55} and Knoblock and Purdy\textsuperscript{57} reported stability constants for the copper(II) complexes of penicillamine. An attempt was made to repeat this work using carefully degassed solutions and adding the copper(II) solution as the last component of the mixture.

A purple colouration was observed on mixing, but this faded in a few seconds. A reaction obviously occurred, and this was presumably the oxidation of ligand as was observed above.

Similarly a titration was attempted using cysteine and copper(II). Even in anaerobic conditions an intense purple/black colouration appeared for an instant (in the acidic solution), before fading with the formation of cystine and cuprous cysteinate as a precipitate. This confirms the observations of previous workers\textsuperscript{2-5}, and indicated that pH titrations would yield little information about these systems.
The original aim of the project was to provide a general study of complex-formation by sulphur-containing ligands, and chelation by mono-thiol ligands was chosen as a starting point because some stability constant studies for these systems had already been reported in the literature. In the course of the work it became clear that many of these literature values were unsatisfactory because their determination involved various simplifying assumptions which the present work has shown to be invalid. It was evident that only by a very detailed study could meaningful results be obtained for these systems, and while a number of the more common metals have been examined, such a detailed study has proved possible in only a few cases.

The majority of the work has concerned the ions of nickel(II) and zinc(II). The reasons for this selection are threefold; firstly, these are not ions of variable valency, and catalytic oxidation of the unstable ligands is therefore not favoured. Secondly, in the study of (b) class metals it became evident that the heavier...
metals of this group, with stronger (b) class character, were likely to form complexes which were highly associated, and would often be of low solubility, while nickel(II) and zinc(II), having weaker (b) character were more likely to lead to tractable results. The final reason for the selection of the ions of these metals was that their complexes with a wide range of other ligands are well known, and these other studies provided a convenient frame of reference with which the more unusual complexes found in the present work could be compared. The range of species which might have been tested was so large that it was necessary to employ, where possible, conclusions derived from studies of related systems, in the selection of possible complexes.

However, with these limitations, it has been possible to extend considerably our knowledge of the way in which these important ligands bind with metals.
Table 6.01

Summary of constants for most probable species, expressed as log of equilibrium constant followed by estimated standard deviations; all measurements at 20.0°C, I=0.1 (NaClO₄).

**Mercaptoacetic acid**

<table>
<thead>
<tr>
<th>Species</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H^+ )</td>
<td>10.204±0.006</td>
<td>13.721±0.009</td>
</tr>
<tr>
<td>( Cd^{2+} )</td>
<td>9.26 ±0.04</td>
<td></td>
</tr>
<tr>
<td>( Co^{2+} )</td>
<td>5.614±0.057</td>
<td>( \beta_2 11.801±0.035 ), ( \beta_3 21.195±0.026 )</td>
</tr>
<tr>
<td>( Hg^{2+} )</td>
<td>20.43 (?)</td>
<td></td>
</tr>
<tr>
<td>( Mn^{2+} )</td>
<td>4.329±0.007</td>
<td>( \beta_2 7.473±0.009 )</td>
</tr>
<tr>
<td>( Ni^{2+} )</td>
<td>&lt;6.2</td>
<td>( \beta_2 13.009±0.005 ), ( \beta_3 14.987±0.028 ), ( \beta_4 33.267±0.015 ), ( \beta_6 49.849±0.019 )</td>
</tr>
<tr>
<td>( Zn^{2+} )</td>
<td>7.796±0.008</td>
<td>( \beta_2 14.958±0.008 ), ( \beta_3 17.799±0.016 ), ( \beta_4 36.465±0.029 )</td>
</tr>
</tbody>
</table>

**2-Mercaptoethylamine**

<table>
<thead>
<tr>
<th>Species</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H^+ )</td>
<td>10.811±0.002</td>
<td>19.204±0.003</td>
</tr>
<tr>
<td>( Ni^{2+} )</td>
<td>19.447±0.005</td>
<td>32.291±0.012</td>
</tr>
<tr>
<td>( Zn^{2+} )</td>
<td>17.515±0.006</td>
<td>( \beta_2 24.725±0.008 )</td>
</tr>
</tbody>
</table>

**Penicillamine**

<table>
<thead>
<tr>
<th>Species</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H^+ )</td>
<td>10.679±0.003</td>
<td>18.714±0.003</td>
</tr>
<tr>
<td>( Ni^{2+} )</td>
<td>10.749±0.018</td>
<td>22.886±0.002</td>
</tr>
<tr>
<td>( Zn^{2+} )</td>
<td>9.589±0.008</td>
<td>( \beta_2 19.559±0.007 ), ( \beta_2 25.547±0.011 ), ( \beta_2−1 31.171±0.021 )</td>
</tr>
</tbody>
</table>
Cysteine

\[ H^+ \begin{array}{l} \beta_1 \ 10.498 \pm 0.003, \\ \beta_2 \ 19.330 \pm 0.004 \end{array} \]

\[ \text{Ni}^{2+} \begin{array}{l} \beta_{21} \ 20.158 \pm 0.003, \\ \beta_{32} \ 33.084 \pm 0.014, \\ \beta_{43} \ 45.713 \pm 0.034 \end{array} \]

\[ \beta_{11-1} \ 15.485 \pm 0.010 \]

\[ \text{Zn}^{2+} \begin{array}{l} \beta_{21} \ 18.220 \pm 0.007, \\ \beta_{43} \ 43.563 \pm 0.036, \\ \beta_{21-1} \ 24.711 \pm 0.020 \end{array} \]

\[ \beta_{21-2} \ 30.724 \pm 0.027 \]

2,3-Dimercaptopropane-1-sulphonic acid

\[ H^+ \begin{array}{l} \beta_1 \ 11.849 \pm 0.004, \\ \beta_2 \ 20.553 \pm 0.005 \end{array} \]

\[ \text{Ni}^{2+} \begin{array}{l} \beta_{21} \sim 33(?) \end{array} \]

\[ \text{Zn}^{2+} \begin{array}{l} \beta_{21} \sim 25(?) \end{array} \]
Mercaptoacetic Acid

Using the stability constants listed in Table 6.01 the concentration of each of the metal complexes has been calculated for a solution of mercaptoacetic acid (0.01M) and zinc(II) ions (0.0025M) from pH 3-11. The results, plotted in Fig. 6.01 show that in this system, where the stepwise formation constant $K_2$ is greater than $K_1$, the species ZnL is less important than previous work suggested. $^{35,38,42}$ On the other hand, $\text{Zn}_2\text{L}_3^{2-}$, $\text{Zn}_3\text{L}_4^{2-}$, and $\text{ZnL}_3^{4-}$ are all significant species, although none has previously been reported. The concentrations of $\text{Zn}_2\text{L}_3^{2-}$ are comparatively low in the stability constant titration, giving rise to a somewhat high standard deviation in the value of $\log \beta_{32}$. However, $\text{Zn}_2\text{L}_3^{2-}$ appears to be the predominant polynuclear species under the conditions of the e.m.f. studies. An analogous species, $\text{Cd}_2\text{L}_3^{2-}$, has been shown to exist in solutions of mercaptoacetic acid containing cadmium(II) ions. $^{26}$ The structure of the binuclear complex $\text{Zn}_2\text{L}_3^{2-}$ is suggested to be similar to structure VII (Fig. 1.03), while that of the trinuclear species is probably similar to that formed by 2-mercaptopoethylamine and nickel (structure IV, Fig. 1.02).
Fig. 6.01. Variation with pH of the composition of solutions of mercaptoacetic acid (0.01M) and (a) zinc(II) ions (0.0025M), (b) nickel(II) ions (0.0025M).

(a) Curves 1-6 show the percentage of total zinc present as free metal, ZnL, ZnL₂⁺, ZnL₃⁻, ZnL₄⁻, and ZnL₅⁻, respectively.

(b) Curves 1-6 show the percentage of total nickel present as free metal, NiL, NiL₂⁺, NiL₃⁻, NiL₄⁻, and NiL₅⁻, respectively.
With the inclusion of these species, the standard deviation in titre was reduced to the magnitude found in the refinement of data for the $pK_a$ titrations, i.e. the error caused by the neglect of further species is of the order of the experimental error. Thus the accuracy of the titration data, and the use of computed best fit as the main criterion for the existence of species, do not justify the inclusion of any further species, although these may exist and are especially likely at higher concentrations.

Consistent with these results, are those for nickel with mercaptoacetic acid, which extend the conclusions of Leussing et al.\(^ {39}\) that $\text{NiL}_2^{2-}$ and $\text{Ni}_4\text{L}_6^{4-}$ are predominant species together with small amounts of $\text{Ni}_3\text{L}_4^{2-}$. Interpretation of the results in terms of these species alone, gave a moderately good fit, but it is now possible to observe the effect of including further complexes. The addition of the species $\text{Ni}_2\text{L}_3^{2-}$ significantly improved the fit to the data, whereas $\text{NiL}$, $\text{Ni}_2\text{L}_2$, and $\text{Ni}_6\text{L}_{12}^{12-}$ appeared to be unimportant. The last complex was tried because Gould and Taylor have recently proposed such a species from X-ray data for the complex of 2-mercaptoethanol with nickel(II).\(^ {19}\) Woodward et al. have suggested a similar structure for the nickel(II) complex of ethanethiol,\(^ {18}\) and several more complexes of this type have since been
The structures of the species Ni₂L₃²⁻ and Ni₃L₄²⁻ are probably similar to IX (Fig. 1.03) and IV (Fig. 1.02) respectively. That of Ni₄L₆⁴⁻ is probably VIII (Fig. 1.03) as previously suggested by Leussing et al. 39

The formation of Ni₄L₆⁴⁻ suggests that the polynuclear chain may well be extended, especially at higher metal and ligand concentrations. Its stability is surprising because the most probable structure involves bonding of one sulphur atom to two metal ions (with the carboxyl group free) in preference to the more common bidentate chelation. 39 However polynuclear complexes have been shown to be formed by monothiols, and this type of bonding is believed to be involved. 19

The stability constants of the nickel(II) complexes are somewhat smaller than those of the corresponding zinc(II) species, and β₁₁ is so small relative to the other constants that the species NiL may be neglected. Using the remaining constants (Table 6.01) the concentrations of all species have been calculated from pH 4.4 to 12 for a solution of mercaptoacetic acid (0.01M) and nickel(II) ions (0.0025M). The values are plotted in Fig. 6.01. Too much significance should not be attached to the relative values of the concentrations, because it is apparent that several polynuclear species exist over the same pH range, so that precise values of their
stability constants (and hence the calculated values of the respective concentrations) are very sensitive to the precision of the pH data. In this connection, it was found that with the nickel(II)-mercaptoacetate system, the data could be fitted moderately well by two different sets of species. Thus the variances obtained using the two sets of complexes (a) NiL$_2^{2-}$, NiL$_3^{4-}$, Ni$_2$L$_3^{2-}$, Ni$_3$L$_4^{2-}$, and (b) NiL, NiL$_2^{2-}$, NiL$_3^{4-}$, Ni$_4$L$_6^{4-}$, were comparable, although less satisfactory than the final set described above.

This fact emphasises the need for caution in using such calculations as the only method for determining the nature of complexes in a mixture of polynuclear species, particularly when these are likely to exist over similar pH regions. The significance of this limitation has not been widely recognized, and it is important that it should not be overlooked as these computer methods are developed. It is for this reason that when dealing with more complicated systems, it is not possible to provide a complete description of the species involved, and an approximation is then all that can be expected.

The complexes of manganese(II) with mercaptoacetic acid are very much weaker than those of nickel(II) and zinc(II), in agreement with the Irving-Williams order. The relative magnitudes of the stepwise formation constants, $K_1$ and $K_2$, are reversed, $K_1$ now being the
greater, as would be predicted from most considerations (probability, neutralisation of charge etc.), and as found in the majority of metal complex equilibria. Further, manganese(II) shows no evidence of forming polynuclear species. These differences in behaviour from the later metals of the transition series bear out Leussing's suggestion that the number of $d$ electrons on the metal ion may be of importance in determining the precise nature of the metal-ligand bond. 64

Again in accordance with the Irving-Williams order, the complexes of cobalt(II) with mercaptoacetic acid are more stable than those of manganese(II) but with cobalt(II), $K_2$ is greater than $K_1$. Although it must be noted that the data for cobalt(II) were affected by side reactions, the approximate constants obtained were used to calculate the concentrations of the various species in a mixture of mercaptoacetic acid (0.01M) and cobalt(II) ions (0.005M) from pH 5 to 10. The results, plotted in Fig. 6.02, show that CoL is of limited importance, but $\text{Co}_2\text{L}_3^{2-}$ (which was assumed to be the predominant polynuclear complex) and CoL$_2^{2-}$ are major species. Although it is not certain that $\text{Co}_2\text{L}_3^{2-}$ is the only polynuclear complex, it is evident that polynuclear complexes of some form are of great importance in this system.
Fig. 6.02. Variation with pH of the composition of a solution of mercaptoacetic acid (0.01M) and cobalt(II) ions (0.005M). Curves 1-4 show the percentage of total cobalt present as free metal, CoL, \( \text{Co}_2\text{L}_3^{2-} \), and \( \text{CoL}_2^{2-} \), respectively.
Little can be said about the complex-forming tendencies of cadmium(II) and mercury(II) with mercaptacetic acid, because of the lack of data for the former metal, and the inability of present techniques to interpret the data for the latter. It is clear however, that the stability of the complexes is very high, and it would appear that the order Zn < Cd < Hg is followed. This is to be expected because the heavier metals have a more diffuse electron cloud, and therefore take part in back-donation and π-bonding more readily. This being the case, polynuclear complex formation should be especially likely to occur with cadmium and mercury, and the fact that mercury(II) has been shown to form polynuclear species linked by chloro-bridges, bears this out. Anomalous effects were observed in the mercury(II) titrations. These are not likely to be a result of reduction of the metal ion to the mercurous state, since most reductions of mercuric ions result in the precipitation of metallic mercury. Also very few mercury(I) complexes are known. It is more probable that the effects observed were due to the simultaneous formation of protonated and of polynuclear complexes, examples of both of which have been reported. The presence of both would render further interpretation of the data impossible at present.
2-Mercaptoethylamine

Although the poor solubility of 2-mercaptoethylamine complexes made it impossible to employ concentrations as high as those used with other ligands, it was shown that polynuclear complexes were formed by nickel(II) ions. With zinc(II) ions, on the other hand, no polynuclear species were detected at the concentrations used, and apart from $\text{ZnL}_2$, the predominant species was the protonated complex $\text{ZnHL}_2^+$. Thus the behaviour of the two metals with 2-mercaptoethylamine differed from that with mercaptoacetic acid, with which both metals were shown to form polynuclear species, although zinc did so to a lesser degree. Another difference is also apparent. Zinc(II) complexes of mercaptoacetic acid were more stable than those of nickel(II), but with 2-mercaptoethylamine the opposite order occurred. The two ligands show one important similarity however, in that with both metals $K_2$ was unusually high relative to $K_1$, and the latter constant was so small as to be negligible in the complexes of 2-mercaptoethylamine.

It is at present possible only to speculate on the reasons for such behaviour, but it is likely that some of these effects are due to the $\pi$-bonding ability of sulphur. Craig and Nyholm have shown that the stability of metal carbonyls depends on the extent of $d_{\pi}-p_{\pi}$ overlap,\textsuperscript{10}
This is at a maximum in the region near manganese and iron, and decreases thereafter, because although, for example, nickel has more dπ electrons, these are more tightly bound and overlap is thus reduced. Little overlap would be expected with the dipositive zinc(II) ion unless the bond length was very short, unless charge had been added to the metal ion to enable the d orbitals to expand (as for instance when the ligand was a di-anion), or finally unless sufficiently electronegative groups were attached to the donor atom of the ligand so as to increase its π-acceptor ability.

These remarks may be extended to the dπ-dπ overlap which is a possible feature of the systems considered in the present study. π-Bonding is likely to be quite important in nickel(II) complexes, and may play a part in the binding of zinc(II) complexes in favourable circumstances. As discussed in Chapter 1, the occurrence of π-bonding would encourage a high value of $K_2$ and would favour polynuclear complex formation.

π-Bonding is likely to occur in complexes of both metals with mercaptoacetic acid (a di-anion). Bonding of zinc(II) to the carboxyl group results in transfer of negative charge to the zinc ion. This in turn causes expansion of the d-orbitals on the metal ion, permitting dπ-dπ overlap as well as π-bonding to the sulphydryl ion. The back-donation leaves the sulphur atom with sufficient
charge to enable it to donate electrons to a second metal ion, and leaves the metal ion sufficiently positively charged to bind a second ligand molecule readily. Nickel(II), binding d electrons less tightly than zinc(II), may be expected to form π-bonds more readily, and to have a greater tendency to form polynuclear species.

It is interesting to note that in all the previously reported studies with sulphur-containing chelating agents, those which are di-anionic give rise to the order of stability $\text{Zn}^{2+} > \text{Ni}^{2+}$, whereas those which are mono-anionic show the reverse order. With the mono-anionic ligand 2-mercaptoethylamine π-bonding will still occur with nickel(II) but is unlikely to be important with zinc(II). Further, bis(2-mercaptoethylamine)nickel(II) has been shown to be diamagnetic, and presumably square-planar. The spin-pairing which thus occurs will lead to an increased stability as a result of the crystal field stabilization energy. This effect cannot occur with the zinc(II) ion ($d^{10}$).

As Leussing and Tischer have observed, in a tetragonal field high-spin nickel(II) can lose up to one third of the stabilization energy which was obtained in an octahedral field. Spin-pairing results in a gain in energy, but this will only occur with a very highly polarisable ligand, or when π-bonding contributes appreciably. However only in
such cases will the stability of a square-planar nickel(II) complex be greater than a comparable zinc(II) species. This behaviour is found to occur in the present studies of aminothiol complexes.

Zinc(II) does not tend to form polynuclear species with 2-mercaptoethylamine, because of the absence of sufficient π-bonding. Zinc(II) does however form a protonated complex, while nickel(II) forms no comparable species. The formation of ZnHL$_2$ may be regarded as the combination of the species ZnL with the undissociated aminothiol in a "thio-ether" type of bond. The pK$_a$ value for the loss of the proton from the complex is 7.210 (i.e. log β$_{21}$-1-log β$_{21}$), considerably lower than either of the pK$_a$ values of the free ligand. The pK$_a$ value for the corresponding nickel(II) species is likely to be even lower because the complex is more stable, and protonation of the bis-complex might well result in loss of spin-pairing, with a corresponding loss of stabilization energy. This would account for the lack of any significant concentration of protonated complex species in the nickel(II)-2-mercaptoethylamine system.

Penicillamine

The nickel(II)-penicillamine system was the simplest of all those studied, and no new species were
observed. All the methods employed, spectrophotometric studies, Irving-Rossotti plot, formation curves, and GAUSS refinement, indicated the formation of only NiL and NiL₂²⁻. The constants (Table 6.01) differ from those of previous workers (Table 1.04) in that $K_2$ is significantly greater than $K_1$. The calculation method of Kuchinskas and Rosen⁵⁵ could not have revealed this order, and that of Lenz and Martell⁵³ also assumed that $K_1$ was much greater than $K_2$, and that the two constants could therefore be calculated independently.

As mentioned in Chapter 1, this reversal of the usual order of stabilities probably arises from the back-donation through π-bonding, which may occur with these sulphur-containing ligands. The result is the decreased importance of the species NiL, as illustrated in Figure 6.03 which shows the concentration of species in a solution of penicillamine (0.01M) and nickel(II) ions (0.005M) at various pH values. The assumption, implicit in the calculation methods of previous workers, that formation of NiL is complete before the formation of NiL₂²⁻ commences, is evidently quite unfounded.
The stability constants of zinc(II) with penicillamine ion suggest that back-donation occurs, the value of $\beta$ being greater than $E_1$. Two points of interest arise. Firstly, complexes of nickel(II) with penicillamine are more stable than those of zinc(II), the opposite order to that observed with peroxoeteric acid, but to the same degree as that found with 2-mercaptoethanol acid. Secondly, zinc(II) forms protonated species with penicillamine, while nickel(II) does not.

An explanation of these facts could be provided in terms of the ability of nickel(II) to employ tridentate octahedral bonding, while zinc(II) prefers tetrahedral, or square-planar, bonding and can thus only bind two groups at a time. Such an explanation has been proposed to account for a similar effect in the complexes of nickel(II) and zinc(II) with histidine.[26] However, it is significant that, in particular to those with 2-mercaptoethanol, and it is likely that similar considerations apply.

Fig. 6.03. Variation with pH of the composition of solutions of penicillamine (0.01M) and (a) nickel(II) ions (0.005M), (b) zinc(II) ions (0.005M).
(a) Curves 1-3 show the percentage of total nickel present as free metal, NiL, and NiL$_2$$^-$, respectively.
(b) Curves 1-5 show the percentage of total zinc present as free metal, ZnHL$_2$, ZnHL$_2$$^-$, ZnL, and ZnL$_2$$^-$, respectively.
The stability constants of zinc(II) with penicillamine again suggest that back-donation occurs, the value of $K_2$ being greater than $K_1$. Two points of interest arise. Firstly, complexes of nickel(II) with penicillamine are more stable than those of zinc(II), the opposite order to that observed with mercaptoacetic acid, but the same order as that found with 2-mercaptoethylamine, and secondly, zinc(II) forms protonated species with penicillamine, while nickel(II) does not.

An explanation of these facts could be provided in terms of the ability of nickel(II) to employ tridentate octahedral bonding while zinc(II) prefers tetrahedral, or square-planar, bonding and can thus only bind two groups of the ligand at a time. Such an explanation has been proposed to account for a similar effect in the complexes of nickel(II) and zinc(II) with histidine. However it is significant that the observations are similar to those with 2-mercaptoethylamine, and it is likely that similar considerations apply.

Nickel(II) readily forms amino-thiol complexes which are likely to be stabilized by $\pi$-bonding and crystal field stabilization energy. In protonated species the metal ion is unlikely to be in the stabilized low-spin state, and
such species are therefore not favoured. Zinc(II) on the other hand is stabilized more by π-bonding in its complexes with di-anionic ligands and therefore forms protonated species binding by the sulphydryl and carboxyl groups. At higher pH values deprotonation of the amino group will occur and then binding will be by the amino-thiol chelate, with possible participation by the carboxyl group. The latter possibility is indicated by the large stability constants of these complexes relative to those of 2-mercaptoethylamine.

Using the calculated constants (Table 6.01) the concentrations of all species were calculated over the pH range 4 to 8. These are plotted in Figure 6.03. Once again the fact that $K_2$ is greater than $K_1$ means that $\text{ZnL}_2^{2-}$ is a more important species than $\text{ZnL}$. This effect is also observed in the protonated species, where it is found that $\text{ZnHL}^+$ is of negligible importance, while $\text{ZnH}_2\text{L}_2$ is present in significant amounts.

Cysteine

The stability constants of nickel(II) complexes of cysteine were lower than those of the nickel(II)-penicillamine species (Table 6.01). The concentrations of the various complex species present in a solution of cysteine (0.01M) and nickel(II) ions (0.005M) have been calculated for pH
values of 4 to 8 using these constants, and the results are plotted in Figure 6.04.

Although other effects must also be present, the decreased donating ability of cysteine is to some extent reflected in the \( pK_a \) values of the ligands. Cysteine and penicillamine exhibit three acidic dissociations of which the first (that of the carboxyl group) is unimportant in complex formation. The third \( pK_a \) of cysteine is 0.2 pH below that of penicillamine, thus the complexes \( \text{NiL} \) and \( \text{NiL}_2^{2-} \) of cysteine would be expected to be the less stable. Thus it is found that cysteine forms a negligible amount of the \( \text{NiL} \) species, and the value of \( \log \beta_{21} \) is considerably lower than that for the penicillamine complex. On the other hand the second dissociation of cysteine has a \( pK_a \) value 0.8 pH above that of penicillamine, and, in keeping with this, the complex \( \text{NiHL}^+ \) of cysteine may be moderately stable, although a species of this type does not appear to be formed by penicillamine.

A more important difference between the two ligands is the ability of cysteine to form polynuclear complexes with nickel(II). This tendency, already observed with mercaptoacetic acid and 2-mercaptoethylamine is absent in penicillamine presumably because steric hindrance between the methyl groups of the ligand prevents the approach of neighbouring species to form a polynuclear complex.
The inability of penicillamine to form polynuclear species also provides an explanation for the observation that copper(II) oxidised penicillamine to form an almost colourless solution, while with cysteine, a transient black colouration was observed, and this faded rapidly with the formation of a pale green precipitate. The black colouration suggests charge transfer in a polynuclear species. This would be followed by oxidation of the ligand and the formation of a copper(II) complex. The copper ion (aq$^{10+}$) will form very few species, and the tendency to form polynuclear species will be high.

Polynuclear cuprous cysteinate complexes may well be poorly soluble, and the mononuclear penicillamine species will remain in solution. This (and the more rapid metabolic turnover of cysteine) may be the main reasons why penicillamine is so useful for mobilising copper in vivo while penicillamine is much less effective.

Fig. 6.04. Variation with pH of the composition of solutions of cysteine (0.01M) and (a) nickel(II) ions (0.005M), (b) zinc(II) ions (0.005M).

(a) Curves 1-5 show the percentage of total nickel present as free metal, NiHL$^+$, Ni$_2$L$^2-$, Ni$_3$L$^4-$, and NiL$^2-$.

(b) Curves 1-5 show the percentage of total zinc present as free metal, ZnHL$^+$, Zn$_2$L$^2-$, Zn$_3$L$^4-$, and ZnL$^2-$, respectively.
The inability of penicillamine to form polynuclear species also provides an explanation for the observation that copper(II) oxidised penicillamine to form an almost colourless solution while with cysteine a transient black colouration was observed, and this faded rapidly with the formation of a pale green precipitate. The black colouration suggests charge-transfer in a polynuclear species. This would be followed by oxidation of the ligand and the formation of a cuprous complex. The cuprous ion (d$^{10}$) will form very strong complexes with sulphydryl ligands, and the tendency to form polynuclear species will be high. Polynuclear cuprous cysteinate complexes may well be poorly soluble whereas the mononuclear penicillamine species will remain in solution. This (and the more rapid metabolic turnover of cysteine) are probably the main reasons why penicillamine is a useful ligand for mobilising copper in the body, while cysteine is much less effective.\textsuperscript{80}

It was shown that protonated species were of only minor importance in the nickel(II)-cysteine system. As expected from the above consideration of penicillamine, these species are more important in complex formation between cysteine and zinc(II). The set of species listed in Table 6.01, represents the best set of those tested, but is probably only an approximation to that which exists in these solutions. Using the computed constants,
concentrations of the relevant species have been calculated from pH 5 to 9 for a solution of cysteine (0.01M) containing zinc(II) ions (0.005M). These are plotted in Figure 6.04. It is evident that protonated and polynuclear species exist over the same range. Thus it is to be expected that protonated polynuclear species will be present, and evidence for such species exists. No method is at present available for providing a complete description for such a complicated system, and for this reason no attempt was made to fit protonated polynuclear species to the data. It does however appear that the simple species ZnL is unimportant relative to protonated species, and ZnL$_2^{2-}$ is again a major component. As observed with penicillamine, the zinc(II) complexes of cysteine are less stable than the comparable nickel(II) species, confirming this as the order of stability for amino-thiol complexes.

2,3-Dimercaptopropane-1-sulphonic Acid.

Little can be said about the complexes formed by 2,3-dimercaptopropane-1-sulphonic acid with nickel(II) and zinc(II) ions. The approximate constants reported (Table 6.01) for both metals assumed negligible polynuclear complex formation. This assumption was not justified and both constants are therefore too high, that
for the nickel(II) species by several log units. These figures thus represent upper limits of the stability constants, and reliable values must await a much more detailed investigation. There is no doubt that, with this ligand, long chain polynuclear species are formed with both metals, and the system may well be further complicated by the presence of protonated complexes. The very high apparent stability constants observed with these metals, suggest that this ligand may well prove to be useful in the treatment of poisoning by heavy metals.

Conclusion

It is of interest to consider further the nature of the bonding in the complexes proposed above, and the types of species likely to be formed by sulphur-containing ligands in general. There is little doubt that the sulphur-metal bonds in complexes of sulphydryl ions with the later (b) class metals will have a large degree of covalent character, but the importance of π-bonding in accounting for polynuclear complex formation and the high values of $K_2$ relative to $K_1$ is more debatable.

Bonding by $d_{π}-d_{π}$ overlap is not essential for the existence of bridging through a ligand atom. With acetylacetone, nickel(II) forms a trinuclear species$^{131}$ whose structure persists in solutions in non-coordinating solvents.$^{132}$ Bridging in this molecule is through
oxygen, so that $\pi$-$\pi$ bonding cannot be responsible, however where it can occur it will undoubtedly aid this type of reaction.

There is insufficient X-ray crystallographic evidence to make possible unambiguous comparison of the $\pi$-bonding abilities of nickel(II) and zinc(II), but the short bond lengths observed indicate the existence of some $\pi$-bonding in complexes of both metals with thiosemicarbazide.\textsuperscript{133,134} $\pi$-Bonding has even been proposed for the nickel(III) complex of 1,2-dicyanoethylene-1,2-dithiol, which was shown to have the Ni-S bond length of $2.146 \pm 0.001 \text{Å}.\textsuperscript{135}$ It was suggested that simple bonding would have resulted in a distance of $2.20 \text{Å}$.

A less speculative description of the bonding in these complexes must await further studies in the field, but it appears at present very likely that some degree of multiple bonding exists, and that this will account for some of the unusual properties observed. These effects are likely to be more marked in the complexes of the heavier (b) class metals.

The present work does not represent the first study of sulphur-containing ligands in which the unusual order of formation constants $K_2 > K_1$ has been demonstrated. A similar order was found by Irving and Fernelius in studies of complex formation by various sulphur-containing acids,
chelating through carboxyl and thio-ether groups, and exemplified by S-ethylmercaptopropanoic acid with cadmium(II), nickel(II), and zinc(II).\(^1\) It is significant that these authors were among the few who have used a rigorous calculation method.\(^{136}\) These results, and the present work on cobalt(II), nickel(II), and zinc(II), suggest that the phenomenon of \(K_2 > K_1\) may be found to be quite widespread in the complexes of sulphur-containing ligands with (b) class metals.

Several further points arise from the present study. It is evident that thiol ligands can form very stable complexes with the (b) class metals, and it is clear that many of the systems for which stability constants have been reported require closer study, with a view to identifying the most important species, and obtaining more meaningful results.

It is to be expected that all \(\alpha\)- and \(\beta\)-dithiol ligands will show a marked tendency to form polynuclear species with the (b) class metals, except when this is precluded by steric factors. 2,3-Dimercaptopropan-1-sulphonic acid formed very soluble polymeric species, and in this respect dihydrolipoic acid (Fig. 1.04) is likely to be very similar. The carboxyl group of this ligand is too remote to take part in chelation, but will aid solubility.

Thio-ethers have a much lower proton basicity than
alkyl thiolate ions and would not be expected to form such stable complexes. However as "soft" bases, these ligands should form stable complexes with the more polarisable metal ions, particularly if the latter are capable of donating \( \pi \)-electrons. Thus thio-ether complexes are formed by mercury(II) and platinum(II), but not by platinum(IV).\(^7\)

As a weaker donor than the sulphydryl ion, and with greater steric requirements, thio-ethers are unlikely to act as bridging groups in forming polynuclear species, but, as noted above, values of \( K_2 \) greater than \( K_1 \) have been observed with these ligands.\(^1\)

The possibility of polynuclear species must also be considered in the solution studies of other sulphur-containing ligands. For instance, thioureas, dithiocarbamates etc., might be expected to form polynuclear species, and some already have been observed in crystals by X-ray studies.\(^137,138,139\) The solution chemistry of the new range of complexes of ligands of the type of toluene-3,4-dithiol would also be of interest with regard to the effect on the nature and stability of the complexes of the delocalised electrons in the chelate ring. It is notable that solid specimens of polynuclear complexes have been prepared with some of these ligands.\(^33,34\)

In order to obtain meaningful results in stability constant studies with such complexing agents, it would be
necessary to employ soluble ligands and to study their complex formation over as wide a range of concentrations as possible. Volumetric and analytical methods would have to be of the highest attainable accuracy, and the calculation methods would have to be adequate. It appears that large computer programmes of the type of GAUSS and LETAGROP would be vital to this work, and the availability of larger and faster computers will enable the use of such methods to become more widespread. Development of these programmes is in progress, and it may soon be possible to provide the computer with an extensive range of data, and a list of all possible complex species, and then to allow the computer to search for the best set of complexes subject to certain conditions. Such a procedure should not be employed without regard to the intrinsic limitations discussed above, or to the chemical requirements of the system, and other methods should be employed where possible to confirm the nature of the proposed species. It is, however, clear that this type of approach will prove very powerful in future studies in this field.
REFERENCES

2. L. J. Harris, Biochem. J., 1922, 16, 739.
15 C. K. Jørgensen, Prog. in Inorg. Chem., 1962, 4, 73.
26 A. M. Cabrera and T. S. West, Talanta, 1962, 9, 730.


M. Cefola, A. Tompa, A. V. Celiano, and P. S. Gentile, 

R. E. Laramy and D. L. Leussing, Unpublished results quoted in ref. 8.

Q. Fernando and H. Freiser, J. Amer. Chem. Soc., 
1958, 80, 4928.

E. Felder, A. Rescigno, and R. Radica, Gazzetta, 
1955, 85, 453.

E. C. Knoblock and W. C. Purdy, Radiation Res., 
1961, 15, 94.

A. Corsini, Q. Fernando, and H. Freiser, Talanta, 
1964, 11, 63.

E. J. Cohn and J. D. Edsall, "Proteins, Amino Acids, 
and Peptides," Reinhold Publishing Corporation, 

L. K. Ryklan and C. L. A. Schmidt, Arch. Biochem., 
1944, 5, 89.

W. Stricks and I. M. Kolthoff, J. Amer. Chem. Soc., 
1951, 73, 1723.

W. Stricks and I. M. Kolthoff, J. Amer. Chem. Soc., 
1953, 75, 5673.

G. R. Lenz and A. E. Martell, Biochem., 1964, 3, 745.


J. H. van't Hoff, Ber., 1877, 10, 669.


J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.


R. S. Tobias, personal communication.


Z. Z. Hugus, quoted in ref. 96.


122 W. W. Cleland, Biochem., 1964, 3, 480.


accepted for publication.

127 P. J. Antikainen and V. M. K. Rossi, Suomen Kem.,
1963, B36, 132.

128 A. T. Pilipenko and A. P. Kostyshina, Izv. Vysshikh

1966, 1377.

130 J. Aveston, E. W. Anacker, and J. S. Johnson,

131 G. J. Bullen, R. Mason, and P. Pauling,


133 L. Cavalca, M. Nardelli and G. Fava,

134 L. Cavalca, M. Nardelli, and G. Branchi,


136 B. P. Block and G. H. McIntyre, J. Amer. Chem. Soc.,
1953, 75, 5667.


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(ii) Initial conditions: $[Zn^{2+}] = 0.002562 M$; $[L] = 0.009993 M$; $[Acid] = 0.000051 M$; volume = 50.00 ml.; titrant KOH = 1.000 M.

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(iii) Initial conditions: $[Zn^{2+}] = 0.001025 M$; $[L] = 0.001999 M$; $[Acid] = 0.000020 M$; volume = 50.00 ml.; titrant KOH = 0.4000 M.

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<td>4.591</td>
<td>0.34</td>
<td>5.318</td>
<td>0.41</td>
<td>6.044</td>
<td>0.48</td>
<td>7.566</td>
</tr>
<tr>
<td>0.28</td>
<td>4.783</td>
<td>0.35</td>
<td>5.408</td>
<td>0.42</td>
<td>6.182</td>
<td>0.49</td>
<td>8.195</td>
</tr>
</tbody>
</table>
APPENDIX I

Tables of Experimental pH Titration Data

Table A.01

Complete data for pH titrations of mercaptoacetic acid solutions containing zinc(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Zn}^{2+}]=0.005124\text{M}; \ [\text{H}_2\text{L}]=0.009993\text{M}; \)

\([\text{Acid}]=0.000102\text{M}; \) volume=50.00 ml.; titrant KOH=1.000M.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.48</td>
<td>4.051</td>
<td>0.63</td>
<td>4.405</td>
<td>0.78</td>
<td>5.008</td>
<td>0.93</td>
<td>6.605</td>
</tr>
<tr>
<td>0.51</td>
<td>4.118</td>
<td>0.66</td>
<td>4.492</td>
<td>0.81</td>
<td>5.218</td>
<td>0.96</td>
<td>7.152</td>
</tr>
<tr>
<td>0.54</td>
<td>4.185</td>
<td>0.69</td>
<td>4.591</td>
<td>0.84</td>
<td>5.482</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.57</td>
<td>4.255</td>
<td>0.72</td>
<td>4.705</td>
<td>0.87</td>
<td>5.806</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>4.327</td>
<td>0.75</td>
<td>4.841</td>
<td>0.90</td>
<td>6.179</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(ii) Initial conditions: \([\text{Zn}^{2+}]=0.002562\text{M}; \ [\text{H}_2\text{L}]=0.009993\text{M}; \)

\([\text{Acid}]=0.000051\text{M}; \) volume=50.00 ml.; titrant KOH=1.000M.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.46</td>
<td>4.150</td>
<td>0.55</td>
<td>4.471</td>
<td>0.62</td>
<td>4.818</td>
<td>0.69</td>
<td>5.460</td>
</tr>
<tr>
<td>0.48</td>
<td>4.215</td>
<td>0.56</td>
<td>4.510</td>
<td>0.63</td>
<td>4.885</td>
<td>0.70</td>
<td>5.592</td>
</tr>
<tr>
<td>0.50</td>
<td>4.285</td>
<td>0.57</td>
<td>4.554</td>
<td>0.64</td>
<td>4.959</td>
<td>0.71</td>
<td>5.739</td>
</tr>
<tr>
<td>0.51</td>
<td>4.320</td>
<td>0.58</td>
<td>4.599</td>
<td>0.65</td>
<td>5.041</td>
<td>0.72</td>
<td>5.900</td>
</tr>
<tr>
<td>0.52</td>
<td>4.356</td>
<td>0.59</td>
<td>4.649</td>
<td>0.66</td>
<td>5.130</td>
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<td></td>
</tr>
<tr>
<td>0.53</td>
<td>4.392</td>
<td>0.60</td>
<td>4.699</td>
<td>0.67</td>
<td>5.230</td>
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<td></td>
</tr>
<tr>
<td>0.54</td>
<td>4.431</td>
<td>0.61</td>
<td>4.757</td>
<td>0.68</td>
<td>5.340</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(iii) Initial conditions: \([\text{Zn}^{2+}]=0.001025\text{M}; \ [\text{H}_2\text{L}]=0.001999\text{M}; \)

\([\text{Acid}]=0.000020\text{M}; \) volume=50.00 ml.; titrant KOH=0.4000M.

<table>
<thead>
<tr>
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<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>4.510</td>
<td>0.32</td>
<td>5.141</td>
<td>0.39</td>
<td>5.805</td>
<td>0.46</td>
<td>6.914</td>
</tr>
<tr>
<td>0.26</td>
<td>4.600</td>
<td>0.33</td>
<td>5.229</td>
<td>0.40</td>
<td>5.921</td>
<td>0.47</td>
<td>7.198</td>
</tr>
<tr>
<td>0.27</td>
<td>4.691</td>
<td>0.34</td>
<td>5.318</td>
<td>0.41</td>
<td>6.044</td>
<td>0.48</td>
<td>7.586</td>
</tr>
<tr>
<td>0.28</td>
<td>4.783</td>
<td>0.35</td>
<td>5.408</td>
<td>0.42</td>
<td>6.182</td>
<td>0.49</td>
<td>8.195</td>
</tr>
</tbody>
</table>
### Table A.02

Complete data for pH titrations of mercaptoacetic acid solutions containing nickel(II) ions. Titres in ml.

#### (i) Initial conditions: $[\text{Ni}^{2+}]=0.005025\text{M}$; $[\text{H}_2\text{L}]=0.009833\text{M}$; $[\text{Acid}]=0.000157\text{M}$; volume=50.00 ml.; titrant KOH=1.000 M.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56</td>
<td>4.900</td>
<td>0.64</td>
<td>5.125</td>
<td>0.72</td>
<td>5.403</td>
<td>0.80</td>
<td>5.828</td>
</tr>
<tr>
<td>0.57</td>
<td>4.927</td>
<td>0.65</td>
<td>5.155</td>
<td>0.73</td>
<td>5.446</td>
<td>0.81</td>
<td>5.901</td>
</tr>
<tr>
<td>0.58</td>
<td>4.955</td>
<td>0.66</td>
<td>5.186</td>
<td>0.74</td>
<td>5.490</td>
<td>0.82</td>
<td>5.985</td>
</tr>
</tbody>
</table>

#### (iv) Initial conditions: $[\text{Zn}^{2+}]=0.0005124\text{M}$; $[\text{H}_2\text{L}]=0.001999\text{M}$; $[\text{Acid}]=0.000010\text{M}$; volume=50.00 ml.; titrant KOH=0.4000 M.

<table>
<thead>
<tr>
<th>Titre</th>
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<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.205</td>
<td>4.214</td>
<td>0.250</td>
<td>4.689</td>
<td>0.295</td>
<td>5.282</td>
<td>0.340</td>
<td>5.990</td>
</tr>
<tr>
<td>0.210</td>
<td>4.257</td>
<td>0.255</td>
<td>4.752</td>
<td>0.300</td>
<td>5.353</td>
<td>0.345</td>
<td>6.092</td>
</tr>
<tr>
<td>0.215</td>
<td>4.302</td>
<td>0.260</td>
<td>4.817</td>
<td>0.305</td>
<td>5.422</td>
<td>0.350</td>
<td>6.212</td>
</tr>
<tr>
<td>0.220</td>
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<td>0.265</td>
<td>4.881</td>
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<td>0.270</td>
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<td>5.567</td>
<td>0.360</td>
<td>6.510</td>
</tr>
<tr>
<td>0.230</td>
<td>4.454</td>
<td>0.275</td>
<td>5.014</td>
<td>0.320</td>
<td>5.644</td>
<td>0.365</td>
<td>6.726</td>
</tr>
<tr>
<td>0.235</td>
<td>4.509</td>
<td>0.280</td>
<td>5.080</td>
<td>0.325</td>
<td>5.722</td>
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<td></td>
</tr>
<tr>
<td>0.240</td>
<td>4.567</td>
<td>0.285</td>
<td>5.147</td>
<td>0.330</td>
<td>5.805</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.245</td>
<td>4.627</td>
<td>0.290</td>
<td>5.216</td>
<td>0.335</td>
<td>5.893</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### (v) Initial conditions: $[\text{Zn}^{2+}]=0.002492\text{M}$; $[\text{H}_2\text{L}]=0.009964\text{M}$; $[\text{Acid}]=0.000058\text{M}$; volume=50.00 ml.; titrant KOH=1.0134 M.

<table>
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<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.775</td>
<td>8.906</td>
<td>0.810</td>
<td>9.424</td>
<td>0.870</td>
<td>9.965</td>
<td>0.910</td>
<td>10.221</td>
</tr>
<tr>
<td>0.780</td>
<td>9.028</td>
<td>0.820</td>
<td>9.581</td>
<td>0.880</td>
<td>10.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.790</td>
<td>9.218</td>
<td>0.855</td>
<td>9.833</td>
<td>0.890</td>
<td>10.092</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.800</td>
<td>9.360</td>
<td>0.860</td>
<td>9.897</td>
<td>0.900</td>
<td>10.158</td>
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</tbody>
</table>
(ii) Initial conditions: $[\text{Ni}^{2+}]=0.002513\text{M}$; $[\text{H}_2\text{L}]=0.009833\text{M}$; $[\text{Acid}]=0.000078\text{M}$; volume=50.00 ml.; titrant KOH=1.000M.

<table>
<thead>
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<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.510</td>
<td>4.958</td>
<td>0.575</td>
<td>5.245</td>
<td>0.640</td>
<td>5.630</td>
<td>0.705</td>
<td>7.122</td>
</tr>
<tr>
<td>0.505</td>
<td>4.982</td>
<td>0.580</td>
<td>5.269</td>
<td>0.645</td>
<td>5.672</td>
<td>0.710</td>
<td>7.327</td>
</tr>
<tr>
<td>0.520</td>
<td>5.004</td>
<td>0.585</td>
<td>5.292</td>
<td>0.650</td>
<td>5.718</td>
<td>0.715</td>
<td>7.509</td>
</tr>
<tr>
<td>0.525</td>
<td>5.027</td>
<td>0.590</td>
<td>5.317</td>
<td>0.655</td>
<td>5.768</td>
<td>0.720</td>
<td>7.675</td>
</tr>
<tr>
<td>0.530</td>
<td>5.049</td>
<td>0.595</td>
<td>5.342</td>
<td>0.660</td>
<td>5.825</td>
<td>0.725</td>
<td>7.833</td>
</tr>
<tr>
<td>0.535</td>
<td>5.070</td>
<td>0.600</td>
<td>5.368</td>
<td>0.665</td>
<td>5.888</td>
<td>0.730</td>
<td>7.983</td>
</tr>
<tr>
<td>0.540</td>
<td>5.092</td>
<td>0.605</td>
<td>5.396</td>
<td>0.670</td>
<td>5.963</td>
<td>0.735</td>
<td>8.128</td>
</tr>
<tr>
<td>0.545</td>
<td>5.113</td>
<td>0.610</td>
<td>5.425</td>
<td>0.675</td>
<td>6.050</td>
<td>0.740</td>
<td>8.273</td>
</tr>
<tr>
<td>0.550</td>
<td>5.135</td>
<td>0.615</td>
<td>5.455</td>
<td>0.680</td>
<td>6.156</td>
<td>0.745</td>
<td>8.433</td>
</tr>
<tr>
<td>0.555</td>
<td>5.157</td>
<td>0.620</td>
<td>5.485</td>
<td>0.685</td>
<td>6.286</td>
<td>0.750</td>
<td>8.603</td>
</tr>
<tr>
<td>0.560</td>
<td>5.179</td>
<td>0.625</td>
<td>5.518</td>
<td>0.690</td>
<td>6.458</td>
<td>0.755</td>
<td>8.763</td>
</tr>
<tr>
<td>0.565</td>
<td>5.200</td>
<td>0.630</td>
<td>5.554</td>
<td>0.695</td>
<td>6.673</td>
<td>0.760</td>
<td>8.997</td>
</tr>
</tbody>
</table>

(iii) Initial conditions: $[\text{Ni}^{2+}]=0.001002\text{M}$; $[\text{H}_2\text{L}]=0.001954\text{M}$; $[\text{Acid}]=0.000031\text{M}$; volume=50.00 ml.; titrant KOH=0.4000M.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.250</td>
<td>5.270</td>
<td>0.310</td>
<td>6.049</td>
<td>0.370</td>
<td>6.473</td>
<td>0.435</td>
<td>7.310</td>
</tr>
<tr>
<td>0.255</td>
<td>5.446</td>
<td>0.315</td>
<td>6.082</td>
<td>0.375</td>
<td>6.515</td>
<td>0.440</td>
<td>7.428</td>
</tr>
<tr>
<td>0.260</td>
<td>5.568</td>
<td>0.320</td>
<td>6.114</td>
<td>0.380</td>
<td>6.560</td>
<td>0.445</td>
<td>7.562</td>
</tr>
<tr>
<td>0.265</td>
<td>5.656</td>
<td>0.325</td>
<td>6.147</td>
<td>0.385</td>
<td>6.606</td>
<td>0.450</td>
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<tr>
<td>0.270</td>
<td>5.724</td>
<td>0.330</td>
<td>6.181</td>
<td>0.390</td>
<td>6.656</td>
<td>0.455</td>
<td>7.879</td>
</tr>
<tr>
<td>0.275</td>
<td>5.780</td>
<td>0.335</td>
<td>6.214</td>
<td>0.395</td>
<td>6.708</td>
<td>0.460</td>
<td>8.062</td>
</tr>
<tr>
<td>0.280</td>
<td>5.829</td>
<td>0.340</td>
<td>6.249</td>
<td>0.400</td>
<td>6.763</td>
<td>0.465</td>
<td>8.254</td>
</tr>
<tr>
<td>0.285</td>
<td>5.872</td>
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<td>6.283</td>
<td>0.405</td>
<td>6.821</td>
<td>0.470</td>
<td>8.455</td>
</tr>
<tr>
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<td>5.910</td>
<td>0.350</td>
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<td>6.886</td>
<td>0.475</td>
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<tr>
<td>0.295</td>
<td>5.948</td>
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<td>6.355</td>
<td>0.420</td>
<td>7.033</td>
<td>0.480</td>
<td>8.864</td>
</tr>
<tr>
<td>0.300</td>
<td>5.982</td>
<td>0.360</td>
<td>6.393</td>
<td>0.425</td>
<td>7.113</td>
<td>0.485</td>
<td>9.070</td>
</tr>
<tr>
<td>0.305</td>
<td>6.015</td>
<td>0.365</td>
<td>6.432</td>
<td>0.430</td>
<td>7.206</td>
<td>0.490</td>
<td>9.274</td>
</tr>
</tbody>
</table>
(iv) Initial conditions: $[\text{Ni}^{2+}] = 0.0005015 \text{M}; [\text{H}_2\text{L}] = 0.001954 \text{M}; [\text{Acid}] = 0.000016 \text{M}; \text{volume} = 50.00 \text{ ml.}; \text{titrant KOH} = 0.4000 \text{M}.$

<table>
<thead>
<tr>
<th>Titre pH</th>
<th>Titre pH</th>
<th>Titre pH</th>
<th>Titre pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2550  5.710</td>
<td>0.2850  6.169</td>
<td>0.3151  6.496</td>
<td>0.3450  7.012</td>
</tr>
<tr>
<td>0.2575  5.776</td>
<td>0.2875  6.195</td>
<td>0.3175  6.527</td>
<td>0.3475  7.080</td>
</tr>
<tr>
<td>0.2600  5.830</td>
<td>0.2900  6.222</td>
<td>0.3200  6.560</td>
<td>0.3500  7.159</td>
</tr>
<tr>
<td>0.2625  5.876</td>
<td>0.2925  6.248</td>
<td>0.3225  6.593</td>
<td>0.3525  7.243</td>
</tr>
<tr>
<td>0.2650  5.919</td>
<td>0.2950  6.273</td>
<td>0.3250  6.629</td>
<td>0.3550  7.336</td>
</tr>
<tr>
<td>0.2675  5.958</td>
<td>0.2975  6.299</td>
<td>0.3275  6.666</td>
<td>0.3575  7.439</td>
</tr>
<tr>
<td>0.2700  5.993</td>
<td>0.3000  6.326</td>
<td>0.3300  6.704</td>
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</tr>
<tr>
<td>0.2725  6.025</td>
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<td>0.3325  6.746</td>
<td>0.3625  7.674</td>
</tr>
<tr>
<td>0.2750  6.056</td>
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<td>0.3350  6.790</td>
<td>0.3650  7.800</td>
</tr>
<tr>
<td>0.2775  6.086</td>
<td>0.3077  6.409</td>
<td>0.3375  6.840</td>
<td>0.3675  7.961</td>
</tr>
<tr>
<td>0.2800  6.114</td>
<td>0.3100  6.438</td>
<td>0.3400  6.891</td>
<td>0.3700  8.130</td>
</tr>
<tr>
<td>0.2827  6.145</td>
<td>0.3125  6.465</td>
<td>0.3425  6.950</td>
<td></td>
</tr>
</tbody>
</table>

(v) Initial conditions: $[\text{Ni}^{2+}] = 0.0025125 \text{M}; [\text{H}_2\text{L}] = 0.009964 \text{M}; [\text{Acid}] = 0.000078 \text{M}; \text{volume} = 50.00 \text{ ml.}; \text{titrant KOH} = 1.0134 \text{M}.$

<table>
<thead>
<tr>
<th>Titre pH</th>
<th>Titre pH</th>
<th>Titre pH</th>
<th>Titre pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.70  6.834</td>
<td>0.76  8.885</td>
<td>0.83  9.852</td>
<td>0.89  10.248</td>
</tr>
<tr>
<td>0.71  7.249</td>
<td>0.78  9.327</td>
<td>0.84  9.924</td>
<td>0.90  10.307</td>
</tr>
<tr>
<td>0.72  7.610</td>
<td>0.79  9.470</td>
<td>0.85  9.995</td>
<td>0.91  10.367</td>
</tr>
<tr>
<td>0.73  7.915</td>
<td>0.80  9.583</td>
<td>0.86  10.059</td>
<td>0.92  10.426</td>
</tr>
<tr>
<td>0.74  8.214</td>
<td>0.81  9.682</td>
<td>0.87  10.121</td>
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</tr>
<tr>
<td>0.75  8.541</td>
<td>0.82  9.771</td>
<td>0.88  10.185</td>
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</tr>
</tbody>
</table>

Table A.03

Representative data for pH titrations of mercaptoacetic acid solutions containing manganese(II) ions. Titres in ml.

(i) Initial conditions: $[\text{Mn}^{2+}] = 0.005072 \text{M}; [\text{H}_2\text{L}] = 0.009964 \text{M}; [\text{Acid}] = 0.000101 \text{M}; \text{volume} = 50.00 \text{ ml.}; \text{titrant KOH} = 1.0134 \text{M}.$

<table>
<thead>
<tr>
<th>Titre pH</th>
<th>Titre pH</th>
<th>Titre pH</th>
<th>Titre pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.54  7.229</td>
<td>0.64  8.098</td>
<td>0.74  8.731</td>
<td>0.84  9.376</td>
</tr>
<tr>
<td>0.56  7.475</td>
<td>0.66  8.225</td>
<td>0.76  8.858</td>
<td>0.86  9.509</td>
</tr>
<tr>
<td>0.58  7.661</td>
<td>0.68  8.353</td>
<td>0.78  8.986</td>
<td>0.88  9.648</td>
</tr>
<tr>
<td>0.60  7.819</td>
<td>0.70  8.478</td>
<td>0.80  9.115</td>
<td>0.90  9.718</td>
</tr>
<tr>
<td>0.62  7.964</td>
<td>0.72  8.603</td>
<td>0.82  9.243</td>
<td>0.92  9.864</td>
</tr>
</tbody>
</table>
(ii) Initial conditions: \([\text{Mn}^{2+}] = 0.002536\text{M}; \ [\text{H}_2\text{L}] = 0.009964\text{M}; \ [\text{Acid}] = 0.00005\text{M}; \text{volume} = 50.00\text{ ml.}; \text{titrant KOH} = 1.0134\text{M}.\]

<table>
<thead>
<tr>
<th>Titre</th>
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<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.51</td>
<td>6.784</td>
<td>0.57</td>
<td>7.985</td>
<td>0.63</td>
<td>8.604</td>
<td>0.69</td>
<td>9.127</td>
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<tr>
<td>0.52</td>
<td>7.174</td>
<td>0.58</td>
<td>8.096</td>
<td>0.64</td>
<td>8.700</td>
<td>0.70</td>
<td>9.202</td>
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<tr>
<td>0.53</td>
<td>7.407</td>
<td>0.59</td>
<td>8.205</td>
<td>0.65</td>
<td>8.786</td>
<td>0.71</td>
<td>9.277</td>
</tr>
<tr>
<td>0.54</td>
<td>7.585</td>
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<td>8.316</td>
<td>0.66</td>
<td>8.878</td>
<td>0.72</td>
<td>9.349</td>
</tr>
<tr>
<td>0.55</td>
<td>7.734</td>
<td>0.61</td>
<td>8.408</td>
<td>0.67</td>
<td>8.965</td>
<td>0.73</td>
<td>9.417</td>
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<tr>
<td>0.56</td>
<td>7.865</td>
<td>0.62</td>
<td>8.508</td>
<td>0.68</td>
<td>9.048</td>
<td>0.74</td>
<td>9.484</td>
</tr>
</tbody>
</table>

(iii) Initial conditions: \([\text{Mn}^{2+}] = 0.001012\text{M}; \ [\text{H}_2\text{L}] = 0.001989\text{M}; \ [\text{Acid}] = 0.0000203\text{M}; \text{volume} = 50.00\text{ ml.}; \text{titrant KOH} = 0.4054\text{M}.\]

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
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<th>pH</th>
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</thead>
<tbody>
<tr>
<td>0.26</td>
<td>7.456</td>
<td>0.32</td>
<td>8.696</td>
<td>0.38</td>
<td>9.333</td>
<td>0.44</td>
<td>9.889</td>
</tr>
<tr>
<td>0.27</td>
<td>7.851</td>
<td>0.33</td>
<td>8.810</td>
<td>0.39</td>
<td>9.431</td>
<td>0.45</td>
<td>9.977</td>
</tr>
<tr>
<td>0.28</td>
<td>8.097</td>
<td>0.34</td>
<td>8.923</td>
<td>0.40</td>
<td>9.518</td>
<td>0.46</td>
<td>10.065</td>
</tr>
<tr>
<td>0.29</td>
<td>8.278</td>
<td>0.35</td>
<td>9.031</td>
<td>0.41</td>
<td>9.620</td>
<td>0.47</td>
<td>10.147</td>
</tr>
<tr>
<td>0.30</td>
<td>8.434</td>
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<td>0.42</td>
<td>9.710</td>
<td>0.48</td>
<td>10.231</td>
</tr>
<tr>
<td>0.31</td>
<td>8.596</td>
<td>0.37</td>
<td>9.238</td>
<td>0.43</td>
<td>9.800</td>
<td>0.49</td>
<td>10.307</td>
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</table>

(iv) Initial conditions: \([\text{Mn}^{2+}] = 0.0005062\text{M}; \ [\text{H}_2\text{L}] = 0.001989\text{M}; \ [\text{Acid}] = 0.000010\text{M}; \text{volume} = 50.00\text{ ml.}; \text{titrant KOH} = 0.4054\text{M}.\]

<table>
<thead>
<tr>
<th>Titre</th>
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<th>pH</th>
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<th>pH</th>
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</thead>
<tbody>
<tr>
<td>0.25</td>
<td>6.050</td>
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<td>8.841</td>
<td>0.35</td>
<td>9.480</td>
<td>0.40</td>
<td>9.910</td>
</tr>
<tr>
<td>0.26</td>
<td>7.802</td>
<td>0.31</td>
<td>8.997</td>
<td>0.36</td>
<td>9.580</td>
<td>0.41</td>
<td>9.987</td>
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<tr>
<td>0.27</td>
<td>8.197</td>
<td>0.32</td>
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<td>9.671</td>
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<tr>
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<td>9.375</td>
<td>0.39</td>
<td>9.837</td>
<td>0.44</td>
<td>10.192</td>
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</tbody>
</table>
Table A.04

Representative data for pH titrations of mercaptoacetic acid solutions containing cobalt(II) ions. Titres in ml.

(i) Initial conditions: $[\text{Co}^{2+}]=0.004977\text{M}; \quad [\text{H}_2\text{L}]=0.009905\text{M};$

$[\text{Acid}]=0.0000995\text{M}; \quad \text{volume}=50.00\text{ml.}; \quad \text{titrant KOH}=1.0134\text{M}.$

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
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<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.52</td>
<td>5.372</td>
<td>0.60</td>
<td>5.805</td>
<td>0.68</td>
<td>6.087</td>
<td>0.76</td>
<td>6.423</td>
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<tr>
<td>0.54</td>
<td>5.380</td>
<td>0.62</td>
<td>5.877</td>
<td>0.70</td>
<td>6.162</td>
<td>0.78</td>
<td>6.527</td>
</tr>
<tr>
<td>0.56</td>
<td>5.646</td>
<td>0.64</td>
<td>5.946</td>
<td>0.72</td>
<td>6.243</td>
<td>0.80</td>
<td>6.646</td>
</tr>
<tr>
<td>0.58</td>
<td>5.732</td>
<td>0.66</td>
<td>6.015</td>
<td>0.74</td>
<td>6.333</td>
<td>0.82</td>
<td>6.779</td>
</tr>
</tbody>
</table>

(ii) Initial conditions: $[\text{Co}^{2+}]=0.002488\text{M}; \quad [\text{H}_2\text{L}]=0.009964\text{M};$

$[\text{Acid}]=0.0000499\text{M}; \quad \text{volume}=50.00\text{ml.}; \quad \text{titrant KOH}=1.0134\text{M}.$

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.51</td>
<td>5.457</td>
<td>0.55</td>
<td>5.858</td>
<td>0.59</td>
<td>6.092</td>
<td>0.63</td>
<td>6.333</td>
</tr>
<tr>
<td>0.52</td>
<td>5.600</td>
<td>0.56</td>
<td>5.920</td>
<td>0.60</td>
<td>6.149</td>
<td>0.64</td>
<td>6.400</td>
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<tr>
<td>0.53</td>
<td>5.703</td>
<td>0.57</td>
<td>5.957</td>
<td>0.61</td>
<td>6.207</td>
<td>0.65</td>
<td>6.478</td>
</tr>
<tr>
<td>0.54</td>
<td>5.787</td>
<td>0.58</td>
<td>6.036</td>
<td>0.62</td>
<td>6.267</td>
<td>0.66</td>
<td>6.561</td>
</tr>
</tbody>
</table>

(iii) Initial conditions: $[\text{Co}^{2+}]=0.0009954\text{M}; \quad [\text{H}_2\text{L}]=0.001981\text{M};$

$[\text{Acid}]=0.0000199\text{M}; \quad \text{volume}=50.00\text{ml.}; \quad \text{titrant KOH}=1.0134\text{M}.$

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.100</td>
<td>5.420</td>
<td>0.115</td>
<td>6.516</td>
<td>0.130</td>
<td>6.843</td>
<td>0.145</td>
<td>7.129</td>
</tr>
<tr>
<td>0.105</td>
<td>6.092</td>
<td>0.120</td>
<td>6.638</td>
<td>0.135</td>
<td>6.938</td>
<td>0.150</td>
<td>7.231</td>
</tr>
<tr>
<td>0.110</td>
<td>6.363</td>
<td>0.125</td>
<td>6.745</td>
<td>0.140</td>
<td>7.032</td>
<td>0.155</td>
<td>7.339</td>
</tr>
</tbody>
</table>
**Table A.05**

Representative data for pH titrations of mercaptoacetic acid solutions containing cadmium(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Cd}^{2+}]=0.001038\text{M}; \ [\text{H}_2\text{L}]=0.001993\text{M};\) 
\([\text{Acid}]=0.0000244\text{M}; \ \text{volume}=50.00\ \text{ml.}; \ \text{titrant KOH}=1.0134\text{M}.\)

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.004</td>
<td>3.158</td>
<td>0.004</td>
<td>3.225</td>
<td>0.004</td>
<td>3.301</td>
<td>0.004</td>
<td>6.189</td>
</tr>
<tr>
<td>0.008</td>
<td>3.171</td>
<td>0.008</td>
<td>3.240</td>
<td>0.008</td>
<td>3.318</td>
<td>0.008</td>
<td>6.344</td>
</tr>
<tr>
<td>0.012</td>
<td>3.184</td>
<td>0.012</td>
<td>3.257</td>
<td>0.012</td>
<td>3.337</td>
<td>0.012</td>
<td>6.636</td>
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<tr>
<td>0.016</td>
<td>3.196</td>
<td>0.016</td>
<td>3.270</td>
<td>0.016</td>
<td>5.869</td>
<td>0.016</td>
<td>6.803</td>
</tr>
<tr>
<td>0.020</td>
<td>3.210</td>
<td>0.020</td>
<td>3.287</td>
<td>0.020</td>
<td>6.031</td>
<td>0.020</td>
<td>7.041</td>
</tr>
</tbody>
</table>

(ii) Initial conditions: \([\text{Cd}^{2+}]=0.00519\text{M}; \ [\text{H}_2\text{L}]=0.0009964\text{M};\) 
\([\text{Acid}]=0.0000122\text{M}; \ \text{volume}=50.00\ \text{ml.}; \ \text{titrant KOH}=0.20268\text{M}.\)

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>3.438</td>
<td>0.02</td>
<td>3.513</td>
<td>0.02</td>
<td>3.564</td>
<td>0.02</td>
<td>6.785</td>
</tr>
<tr>
<td>0.03</td>
<td>3.463</td>
<td>0.03</td>
<td>3.538</td>
<td>0.03</td>
<td>3.575</td>
<td>0.03</td>
<td>7.120</td>
</tr>
<tr>
<td>0.04</td>
<td>3.488</td>
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<td>3.550</td>
<td>0.04</td>
<td>3.594</td>
<td>0.04</td>
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</tr>
</tbody>
</table>

**Table A.06**

Representative data for pH titrations of mercaptoacetic acid solutions containing mercury(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Hg}^{2+}]=0.002039\text{M}; \ [\text{H}_2\text{L}]=0.004982\text{M};\) 
\([\text{Acid}]=0.0001166\text{M}; \ \text{volume}=50.00\ \text{ml.}; \ \text{titrant KOH}=1.0134\text{M}.\)

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
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</thead>
<tbody>
<tr>
<td>0.38</td>
<td>3.723</td>
<td>0.38</td>
<td>3.882</td>
<td>0.38</td>
<td>4.113</td>
<td>0.38</td>
<td>4.419</td>
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<tr>
<td>0.39</td>
<td>3.757</td>
<td>0.39</td>
<td>3.955</td>
<td>0.39</td>
<td>4.205</td>
<td>0.39</td>
<td>4.557</td>
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<tr>
<td>0.40</td>
<td>3.810</td>
<td>0.40</td>
<td>4.032</td>
<td>0.40</td>
<td>4.303</td>
<td>0.40</td>
<td>4.731</td>
</tr>
</tbody>
</table>
(ii) Initial conditions: \([Hg^{2+}]=0.0008154\text{M}; [H_2L]=0.001993\text{M}; [Acid]=0.0004662\text{M};\) volume=50.00 ml.; titrant KOH=1.0134M.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.080</td>
<td>3.082</td>
<td>0.115</td>
<td>3.331</td>
<td>0.145</td>
<td>3.645</td>
<td>0.175</td>
<td>4.095</td>
<td></td>
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<tr>
<td>0.090</td>
<td>3.153</td>
<td>0.120</td>
<td>3.375</td>
<td>0.150</td>
<td>3.708</td>
<td>0.180</td>
<td>4.195</td>
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</tr>
<tr>
<td>0.095</td>
<td>3.189</td>
<td>0.125</td>
<td>3.424</td>
<td>0.155</td>
<td>3.774</td>
<td>0.185</td>
<td>4.313</td>
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<tr>
<td>0.100</td>
<td>3.225</td>
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<td>3.842</td>
<td>0.190</td>
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<td>3.527</td>
<td>0.165</td>
<td>3.922</td>
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(iii) Initial conditions: \([Hg^{2+}]=0.0004077\text{M}; [H_2L]=0.0009964\text{M}; [Acid]=0.0002331\text{M};\) volume=50.00 ml.; titrant KOH=0.2027M.

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(iv) Initial conditions: \([Hg^{2+}]=0.0004077\text{M}; [H_2L]=0.0009964\text{M}; [Acid]=0.0002331\text{M};\) volume=50.00 ml.; titrant KOH=0.2027M.

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Table A.07

Representative data for pH titrations of 2-mercaptoethylamine solutions containing nickel(II) ions. Titres in ml.

(i) Initial conditions: \([Ni^{2+}]=0.002988\text{M}; [HL]=0.004080\text{M}; [Acid]=0.004120\text{M};\) volume=50.00 ml.; titrant KOH=1.000M.
(ii) Initial conditions: \([\text{Ni}^{2+}]=0.00201\text{M}; \ [\text{HL}]=0.004193\text{M};\]
\[\text{[Acid]}=0.004592\text{M}; \ \text{volume}=50.00 \text{ml.}; \ \text{titrant KOH}=1.006\text{M}.\]

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(iii) Initial conditions: \([\text{Ni}^{2+}]=0.001002\text{M}; \ [\text{HL}]=0.004193\text{M};\]
\[\text{[Acid]}=0.004562\text{M}; \ \text{volume}=50.00 \text{ml.}; \ \text{titrant KOH}=1.006\text{M}.\]

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(iv) Initial conditions: \([\text{Ni}^{2+}]=0.001002\text{M}; \ [\text{HL}]=0.002090\text{M};\]
\[\text{[Acid]}=0.002296\text{M}; \ \text{volume}=50.00 \text{ml.}; \ \text{titrant KOH}=0.4024\text{M}.\]

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(v) Initial conditions: \([\text{Ni}^{2+}] = 0.005025 \text{M}; [\text{HL}] = 0.002090 \text{M}; [\text{Acid}] = 0.00228 \text{M}; \) volume = 50.00 ml.; titrant KOH = 1.006 M.

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Table A.08

Representative data for pH titrations of 2-mercaptoethylamine solutions containing zinc(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Zn}^{2+}] = 0.002002 \text{M}; [\text{HL}] = 0.004193 \text{M}; [\text{Acid}] = 0.004574 \text{M}; \) volume = 50.00 ml.; titrant KOH = 1.006 M.

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(ii) Initial conditions: \([\text{Zn}^{2+}] = 0.0009980 \text{M}; [\text{HL}] = 0.004193 \text{M}; [\text{Acid}] = 0.004552 \text{M}; \) volume = 50.00 ml.; titrant KOH = 1.006 M.

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(iii) Initial conditions: \( [\text{Zn}^{2+}] = 0.0009980\text{M} \); \( [\text{HL}] = 0.002090\text{M} \);  
\( [\text{Acid}] = 0.002267\text{M} \); volume = 50.00 ml.; titrant KOH = 0.4024M.

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(iv) Initial conditions: \( [\text{Zn}^{2+}] = 0.0004995\text{M} \); \( [\text{HL}] = 0.002086\text{M} \);  
\( [\text{Acid}] = 0.002271\text{M} \); volume = 50.10 ml.; titrant KOH = 0.4024M.

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Table A.09

Representative data for pH titrations of penicillamine solutions containing nickel(II) ions. Titres in ml.

(i) Initial conditions: \( [\text{Ni}^{2+}] = 0.005025\text{M} \); \( [\text{H}_2\text{L}] = 0.009964\text{M} \);  
\( [\text{Acid}] = 0.000157\text{M} \); volume = 50.00 ml.; titrant KOH = 1.0134M.

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(ii) Initial conditions: \([\text{Ni}^{2+}]=0.002010\text{M}; \ [\text{H}_2\text{L}]=0.009964\text{M};\)  
\([\text{Acid}]=0.0000628\text{M}; \ \text{volume}=50.00\text{ ml.}; \ \text{titrant KOH}=1.0134\text{M}.\)

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(iii) Initial conditions: \([\text{Ni}^{2+}]=0.001005\text{M}; \ [\text{H}_2\text{L}]=0.0019928\text{M};\)  
\([\text{Acid}]=0.0000314\text{M}; \ \text{volume}=50.00\text{ ml.}; \ \text{titrant KOH}=1.0134\text{M}.\)

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(iv) Initial conditions: \([\text{Ni}^{2+}]=0.0005025\text{M}; \ [\text{H}_2\text{L}]=0.001993\text{M};\)  
\([\text{Acid}]=0.0000157\text{M}; \ \text{volume}=50.00\text{ ml.}; \ \text{titrant KOH}=0.4054\text{M}.\)

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Table A.10

Representative data for pH titrations of penicillamine solutions containing zinc(II) ions. Titres in ml.

(i) Initial conditions: $[\text{Zn}^{2+}] = 0.005005 \text{M}; [\text{H}_2\text{L}] = 0.01000 \text{M}; [\text{Acid}] = 0.000112 \text{M}; \text{volume} = 50.00 \text{ml.}; \text{titrant KOH} = 1.0134 \text{M}.$

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(ii) Initial conditions: $[\text{Zn}^{2+}] = 0.002002 \text{M}; [\text{H}_2\text{L}] = 0.01000 \text{M}; [\text{Acid}] = 0.000045 \text{M}; \text{volume} = 50.00 \text{ml.}; \text{titrant KOH} = 1.0134 \text{M}.$

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(iii) Initial conditions: $[\text{Zn}^{2+}] = 0.001001 \text{M}; [\text{H}_2\text{L}] = 0.002000 \text{M}; [\text{Acid}] = 0.000022 \text{M}; \text{volume} = 50.00 \text{ml.}; \text{titrant KOH} = 1.0134 \text{M}.$

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(iv) Initial conditions: $[\text{Zn}^{2+}] = 0.0005005\text{M}$; $[\text{H}_2\text{L}] = 0.002000\text{M}$; $[\text{Acid}] = 0.000011\text{M}$; volume $= 50.00\text{ ml.}$; titrant KOH $= 0.4054\text{M}$.

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Table A.11

Representative data for pH titrations of cysteine solutions containing nickel(II) ions. Titres in ml.

(i) Initial conditions: $[\text{Ni}^{2+}] = 0.005025\text{M}$; $[\text{H}_2\text{L}] = 0.01002\text{M}$; $[\text{Acid}] = 0.000100\text{M}$; volume $= 50.00\text{ ml.}$; titrant KOH $= 1.0134\text{M}$.

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(ii) Initial conditions: $[\text{Ni}^{2+}] = 0.002513\text{M}$; $[\text{H}_2\text{L}] = 0.01002\text{M}$; $[\text{Acid}] = 0.000050\text{M}$; volume $= 50.00\text{ ml.}$; titrant KOH $= 1.0134\text{M}$.

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(iii) Initial conditions: \([\text{Ni}^{2+}]=0.001005\, \text{M}; \quad [\text{H}_2\text{L}]=0.002004\, \text{M};\]
\([\text{Acid}]=0.000020\, \text{M}; \quad \text{volume}=50.00\, \text{ml.}; \quad \text{titrant KOH}=0.4054\, \text{M}.\]

<table>
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<td>5.411</td>
</tr>
<tr>
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<td>5.489</td>
</tr>
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<td>5.545</td>
</tr>
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<tr>
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<td>5.592</td>
</tr>
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<td>5.636</td>
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(ii) Initial conditions: \([\text{Ni}^{2+}]=0.0005025\, \text{M}; \quad [\text{H}_2\text{L}]=0.002004\, \text{M};\]
\([\text{Acid}]=0.000010\, \text{M}; \quad \text{volume}=50.00\, \text{ml.}; \quad \text{titrant KOH}=0.4054\, \text{M}.\]

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<tbody>
<tr>
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<td>5.282</td>
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<td>0.02</td>
<td>5.410</td>
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<td>5.578</td>
</tr>
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<td>5.614</td>
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</table>

(iv) Initial conditions: \([\text{Zn}^{2+}]=0.004984\, \text{M}; \quad [\text{H}_2\text{L}]=0.01002\, \text{M};\]
\([\text{Acid}]=0.000010\, \text{M}; \quad \text{volume}=50.00\, \text{ml.}; \quad \text{titrant KOH}=1.000\, \text{M}.\]

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<tr>
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<tr>
<td>0.12</td>
<td>5.237</td>
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<tr>
<td>0.16</td>
<td>5.320</td>
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</table>

<table>
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<tbody>
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<tr>
<td>0.08</td>
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<td>5.237</td>
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<td>5.320</td>
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Table A.12

Representative data for pH titrations of cysteine solutions containing zinc(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Zn}^{2+}]=0.004984\, \text{M}; \quad [\text{H}_2\text{L}]=0.01002\, \text{M};\]
\([\text{Acid}]=0.000010\, \text{M}; \quad \text{volume}=50.00\, \text{ml.}; \quad \text{titrant KOH}=1.000\, \text{M}.\]

<table>
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<tr>
<td>0.08</td>
<td>5.124</td>
</tr>
<tr>
<td>0.12</td>
<td>5.237</td>
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<td>0.16</td>
<td>5.320</td>
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<tr>
<td>0.08</td>
<td>5.124</td>
</tr>
<tr>
<td>0.12</td>
<td>5.237</td>
</tr>
<tr>
<td>0.16</td>
<td>5.320</td>
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(ii) Initial conditions: \([\text{Zn}^{2+}] = 0.002447 \text{M}; [\text{H}_2\text{L}] = 0.01002 \text{M}; [\text{Acid}] = 0.000050 \text{M}; \text{volume} = 50.00 \text{ ml.}; \text{titrant KOH} = 1.000 \text{M}.

<table>
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(iii) Initial conditions: \([\text{Zn}^{2+}] = 0.0009968 \text{M}; [\text{H}_2\text{L}] = 0.002004 \text{M}; [\text{Acid}] = 0.000020 \text{M}; \text{volume} = 50.00 \text{ ml.}; \text{titrant KOH} = 0.4000 \text{M}.

<table>
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<th>pH</th>
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<td>7.737</td>
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(iv) Initial conditions: \([\text{Zn}^{2+}] = 0.0004984 \text{M}; [\text{H}_2\text{L}] = 0.002004 \text{M}; [\text{Acid}] = 0.000010 \text{M}; \text{volume} = 50.00 \text{ ml.}; \text{titrant KOH} = 0.4000 \text{M}.

<table>
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<th>Titre</th>
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Table A.13

Representative data for pH titrations of 2,3-dimercapto-propan-1-sulphonic acid containing nickel(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Ni}^{2+}]=0.0020\text{M}; [\text{H}_2\text{L}]=0.0100\text{M}; [\text{Acid}]=0.000040\text{M}; \text{volume}=50.00 \text{ml.}; \text{titrant KOH}=1.000\text{M}.

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<td>3.557</td>
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(ii) Initial conditions: \([\text{Ni}^{2+}]=0.0020\text{M}; [\text{H}_2\text{L}]=0.00100\text{M}; [\text{Acid}]=0.000040\text{M}; \text{volume}=50.00 \text{ml.}; \text{titrant KOH}=0.2500\text{M}.

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Table A.14

Representative data for pH titrations of 2,3-dimercaptopropane-1-sulphonic acid containing zinc(II) ions. Titres in ml.

(i) Initial conditions: \([\text{Zn}^{2+}]=0.01000\text{M}; \ [\text{H}_2\text{L}]=0.004000\text{M};\]
\([\text{Acid}]=0.002000\text{M}; \ \text{volume}=50.00\ \text{ml}; \ \text{titrant KOH}=1.000\text{M}.

<table>
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<tr>
<th>Titre</th>
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<th>pH</th>
<th>Titre</th>
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(ii) Initial conditions: \([\text{Zn}^{2+}]=0.004000\text{M}; \ [\text{H}_2\text{L}]=0.004000\text{M};\]
\([\text{Acid}]=0.000080\text{M}; \ \text{volume}=50.00\ \text{ml}; \ \text{titrant KOH}=1.000\text{M}.

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(iii) Initial conditions: \([\text{Zn}^{2+}]=0.002000\text{M}; \ [\text{H}_2\text{L}]=0.01000\text{M};\]
\([\text{Acid}]=0.00040\text{M}; \ \text{volume}=50.00\ \text{ml}; \ \text{titrant KOH}=1.000\text{M}.

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<td>5.638</td>
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<td>0.34</td>
<td>6.104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>3.938</td>
<td>0.24</td>
<td>4.778</td>
<td>0.36</td>
<td>6.219</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(iv) Initial conditions: \([Zn^{2+}]=0.000500\text{M}; [H_2L]=0.004000\text{M}; [\text{Acid}]=0.000100\text{M}; \text{volume}=50.00\text{ml.}; \text{titrant KOH}=0.2500\text{M}.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
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<tr>
<td>0.00</td>
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<td>4.379</td>
<td>0.24</td>
<td>4.956</td>
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<tr>
<td>0.02</td>
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<td>0.14</td>
<td>4.434</td>
<td>0.26</td>
<td>5.235</td>
<td>0.38</td>
<td>6.229</td>
</tr>
<tr>
<td>0.04</td>
<td>4.190</td>
<td>0.16</td>
<td>4.495</td>
<td>0.28</td>
<td>5.530</td>
<td>0.40</td>
<td>6.421</td>
</tr>
<tr>
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<td>0.18</td>
<td>4.568</td>
<td>0.30</td>
<td>5.728</td>
<td>0.42</td>
<td>7.023</td>
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<tr>
<td>0.08</td>
<td>4.284</td>
<td>0.20</td>
<td>4.659</td>
<td>0.32</td>
<td>5.874</td>
<td>0.44</td>
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</tr>
<tr>
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<td>0.34</td>
<td>5.995</td>
<td>0.46</td>
<td>7.371</td>
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</tbody>
</table>

(v) Initial conditions: \([Zn^{2+}]=0.00020000\text{M}; [H_2L]=0.0008000\text{M}; [\text{Acid}]=0.0000400\text{M}; \text{volume}=50.00\text{ml.}; \text{titrant KOH}=0.2500\text{M}.

<table>
<thead>
<tr>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
<th>Titre</th>
<th>pH</th>
</tr>
</thead>
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<td>0.005</td>
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<td>0.030</td>
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<td>0.055</td>
<td>5.185</td>
<td>0.080</td>
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</tr>
<tr>
<td>0.010</td>
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<td>0.035</td>
<td>5.015</td>
<td>0.060</td>
<td>5.232</td>
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<td>5.567</td>
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<tr>
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<td>0.065</td>
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<td>5.341</td>
<td>0.095</td>
<td>5.799</td>
</tr>
<tr>
<td>0.025</td>
<td>4.939</td>
<td>0.050</td>
<td>5.140</td>
<td>0.075</td>
<td>5.405</td>
<td>0.100</td>
<td>5.911</td>
</tr>
</tbody>
</table>
APPENDIX II

The subroutines employed by programme GAUSS are described below. The three subroutines, C\textsuperscript{O}NC\textsubscript{5}, C\textsuperscript{O}NC\textsubscript{6}, and C\textsuperscript{O}NC\textsubscript{7} have been little altered from the original form of Tobias\textsuperscript{102} but for the sake of completeness they are listed and described here. All involve a Newton-Raphson iteration to find the free metal and/or free ligand concentrations. The analytical hydrogen ion concentration, and the variance in this quantity, are then calculated, and these are returned to the main programme and used in the calculation and weighting of the residuals. The three subroutines employ the same general approach, but C\textsuperscript{O}NC\textsubscript{5} is somewhat more complicated as the equations for total ligand and total metal are solved simultaneously for free metal and free ligand.

A flow chart for this programme is given below. Flow charts are not provided for C\textsuperscript{O}NC\textsubscript{6} (iteration in total ligand equation) or for C\textsuperscript{O}NC\textsubscript{7} (iteration in total metal equation) as the method may be understood from the listing of these programmes read in conjunction with the flow chart for C\textsuperscript{O}NC\textsubscript{5}.

The matrix inversion subroutine (SUB760) is a standard IBM library programme, and is listed at the end of this section. It was used in preference to that employed by Tobias, which was less efficient and could lead to erroneous results.
Significant changes from the original programme have been made only where the programme has been altered to suit GAUSS4, or to increase efficiency. The following changes may be noted. No provision is made in the present versions to return to the main programme at the start of any subroutine, since the main programme is now designed to call only the subroutine appropriate to the system under study. The calculation of free metal and free ligand is now carried out in double precision. This aided convergence when run on an IBM 360/50 computer, but was unnecessary when a CDC 3600 (with greater word length) was used. The activity is now included in the calculation, since practical constants are now calculated. A counter has been included for the number of non-convergent iterations for a point (NNIT). If this is greater than zero the initial values for free metal and free ligand, used to commence the iteration for the next point, are obtained assuming as a starting approximation the absence of any complex formation. If NNIT=0 the initial values for a point are taken as the final values for the previous point. The counter NNCI follows the number of non-convergent iterations per cycle (i.e. per shift of the constants to be refined.) If this exceeds 99 per cycle the data are probably faulty and the job is abandoned. The computer then advances to the next set of data.
In most other respects the programmes are similar to those of Tobias, whose description, derivations and flow chart are reproduced below with only slight alterations.

In the three concentration subroutines free metal and/or free ligand concentrations are calculated by means of a Newton-Raphson iterative procedure. In the iteration for a single mole balance equation, e.g. for total metal (C\textsuperscript{NC}7), the function, \( f(b) = (B_{\text{expt}} - B_{\text{calc}}) \), is expanded about the initial approximation to the equilibrium metal ion concentration, \( b_1 \), where \( B \) here refers to the total metal concentration, and the correct value of \( b \) is that for which \( f(b) = 0 \). Using Taylor's series, and neglecting terms of second and higher orders,

\[
f(b) = f(b_1) + f'(b_1) \text{shift}_b = 0
\]

\[
\text{shift}_b = -\frac{f(b_1)}{f'(b_1)} = b_2 - b_1
\]

The programme is simplified slightly by calculating fractional shifts i.e. \( -\frac{f(b_1)}{b_1 f'(b_1)} \). Such an iteration is demonstrated in subroutine C\textsuperscript{NC}7, and C\textsuperscript{NC}6 is very similar.

In C\textsuperscript{NC}5 the iteration is carried out simultaneously for the free metal and free ligand concentrations using the mole balance equations for total metal and total ligand. Let \( f(b, l) = (B_{\text{expt}} - B_{\text{calc}}) \), where \( b \) refers to free metal,
and $l$ to free ligand, concentration. Similarly let $g(b,l) = (L_{\text{expt}} - L_{\text{calc}})$, where $L$ is the total ligand concentration. For the correct values of $b$ and $l$ we have $f(b,l) = g(b,l) = 0$. Again expanding in Taylor's series and neglecting higher order terms,

$$f(b,l) = f(b_1,l_1) + f'_b(b_1,l_1)\text{shift}_b + f'_l(b_1,l_1)\text{shift}_l = 0 \quad A.02$$

$$g(b,l) = g(b_1,l_1) + g'_b(b_1,l_1)\text{shift}_b + g'_l(b_1,l_1)\text{shift}_l = 0 \quad A.03$$

where $f'_b$, $g'_b$ and $f'_l$, $g'_l$ refer to the first derivatives with respect to free metal and free ligand concentration respectively. These equations are solved simultaneously for shift in free metal concentration ($\text{shift}_b$) and in free ligand concentration ($\text{shift}_l$). This requires solution of the simultaneous equations (in matrix notation)

$$\begin{pmatrix} f'_b & f'_l \\ g'_b & g'_l \end{pmatrix} \begin{pmatrix} \text{shift}_b \\ \text{shift}_l \end{pmatrix} = \begin{pmatrix} -f \\ -g \end{pmatrix} \quad A.04$$

Once again for simplicity in programming the fractional shifts are calculated, and in CÔNC5 the improved values $b_2$ and $l_2$ are obtained from

$$b_2 = b_1 + (\text{negative fractional shift}_b) \times b_1 \quad A.05$$

$$l_2 = l_1 + (\text{negative fractional shift}_l) \times l_1 \quad A.05$$

The weighting procedure used in this programme was devised by Professor Z. Z. Hugus Jr., of the University of Minnesota.
In calculations of this type there are three mole balance equations which must be considered, i.e. the total metal, total ligand and analytical hydrogen ion equations. Since iterations are carried out in the total metal and total ligand mole balance equations, these are regarded as exact equations in the experimental data. The equilibrium constants are then adjusted using the free metal and free ligand concentrations obtained from the iterative procedure, together with the mole balance equation for the analytical hydrogen ion concentration. The constants are adjusted so as to minimize the sum of the squares of the weighted residuals in the analytical hydrogen ion concentration, i.e. the difference between the experimental value and that calculated from the stability constants.

CØNC7

The weights used in the computation are set equal to the reciprocal of the variance in the residual. In the programme CØNC7 there are two mole balance equations, in total metal and in analytical hydrogen ion concentration.

\[ F = -B + b + \sum m^B \sum_{mn} b^m[H^+]^{-n} = 0 \] A.06

\[ G = -H + \frac{[H^+]}{K_w[H^+]} - \sum n^B \sum_{mn} b^m[H^+]^{-n} \] A.07

where \( H \) is the experimental analytical hydrogen ion concentration. In the computation, \( F \) is considered an identity in the (inaccurate) experimental data, because
the iteration is carried out in the total metal mole balance equation. The equilibrium constants are then adjusted to minimize the sum of the squares of $G$. Treating the equations in the standard deviations in $G$ and $F$ as vectors, with $i$, $j$, etc., as unit vectors,

$$\sigma_F = i \sigma_B + j \sigma_H + k \sigma_b + l \sigma_{[H^+]} \sigma_{[H^+]}$$  \hspace{1cm} A.08$$

$$\sigma_G = i \sigma_B + j \sigma_H + k \sigma_b + l \sigma_{[H^+]} \sigma_{[H^+]}$$  \hspace{1cm} A.09$$

where $F_B = \partial F/\partial B$, $G_B = \partial G/\partial B$ etc. Since $F$ is regarded as an identity, $\sigma_F = 0$. Evaluating the derivatives we find $F_B = -1$, $G_H = -1$, $F_H = 0$, and $G_B = 0$. As the quantity $\sigma_b$ is not known the equation in $\sigma_F$ is used to solve for $\sigma_b$ in terms of known standard deviations.

$$k \sigma_b = i \sigma_B - l \sigma_{[H^+]} \sigma_{[H^+]}$$  \hspace{1cm} A.10$$

which on substitution yields

$$\sigma_G = -j \sigma_H + i \sigma_b + \left( G_{[H^+]} - G_b F_{[H^+]} \right) \sigma_{[H^+]}$$  \hspace{1cm} A.11$$

Now, assuming that there is no correlation between the errors in $F$ and $G$, taking the dot product $(\sigma_G \cdot \sigma_G)$ we obtain the variance in $G$, $\sigma_G^2$, in terms of quantities which are calculated from the data for the calculation.
The quantity \( \sigma_G^2 \) is called SIGR in the programme, and is computed in the step

\[
\text{SIGR} = \text{SIGCHX} \times 2 + (\text{SIGBBX} \times \text{CFC/AFA}) \times 2 + (\text{SIGHX} \times (\text{CHC} - \text{CFC} \times \text{CFC/AFA} \times \text{UX}) \times 2
\]

\[A.13\]

**CÖNC6**

The weighting procedure in CÖNC6 is identical with that of CÖNC7 except that the mole balance equation for total ligand replaces that for total metal in all of the calculations. The appropriate mole balance equations are

\[
E = -L + 1 + \sum \beta_n \ln [H^+]^{-n} = 0
\]

\[A.14\]

\[
G = -H + [H^+] - K_w/[H^+] - \sum \beta_n \ln [H^+]^{-n}
\]

\[A.15\]

The equations in standard deviations are

\[
\sigma_E = \frac{1}{2} E_L \sigma_L + \frac{1}{2} E_H \sigma_H + \frac{1}{2} E_1 \sigma_1 + \frac{1}{2} E_1 [H^+] \sigma_{[H^+]} = 0
\]

\[A.16\]

\[
\sigma_G = \frac{1}{2} G_L \sigma_L + \frac{1}{2} G_H \sigma_H + \frac{1}{2} G_1 \sigma_1 + \frac{1}{2} G_1 [H^+] \sigma_{[H^+]} \]

\[A.17\]

From which

\[
k \sigma_1 = \frac{i}{2} \sigma_L - \frac{1}{2} E_1 [H^+] \sigma_{[H^+]} \]

\[A.18\]

Giving on substitution

\[
\sigma_G = \frac{1}{2} \sigma_H + \frac{1}{2} G_1 \sigma_1 + \left( \frac{G [H^+] - G_1 E_1 [H^+]}{E_1} \right) \frac{1}{2} \sigma_H
\]

\[A.19\]
Thus

\[ \sigma_G^2 = \sigma_H^2 + \frac{G_1^2 \sigma_L^2}{E_1^2} + \left( \frac{G[H^+] - G_1 E[H^+]}{E_1} \right)^2 \sigma_{[H^+]}^2 \]

In programme language, this is

\[ SIGR = SIGCHX**2 + (SIGCLX*CGC/BGB)**2 \]

\[ + (SIGHX*(CHC-CGC*BHB/BGB)*UX)**2 \]

**CÔNC5**

In the subroutine CÔNC5 iterations are carried out simultaneously to find the free metal and free ligand concentrations. There are thus three mole balance equations, in total metal, total ligand, and analytical hydrogen ion concentrations.

\[ E = -L + \sum \sum \sum 1_{\beta_{lmn}} b_{lm1} [H^+]^n = 0 \]

\[ F = -B + b + \sum \sum \sum m_{\beta_{lmn}} b_{lm1} [H^+]^n = 0 \]

\[ G = -H + L - K_w/[H^+] - \sum \sum \sum n_{\beta_{lmn}} b_{lm1} [H^+]^n = 0 \]

The first two equations are considered identities in the (inaccurate) experimental data, as the iteration is carried out in the total metal and total ligand equations. The equilibrium constants are then adjusted to minimize the sum of the squares of G. Treating the equations in the standard deviations in E, F, and G as vectors, and with i, j, etc. as unit vectors,
In these equations $E_B = \frac{\partial E}{\partial B}$, $G_1 = \frac{\partial G}{\partial l}$ etc.

Since both $E$ and $F$ are treated as identities, $\sigma_E = 0 = \sigma_F$.

Evaluating the derivatives which are taken with respect to the quantities for which standard deviations can be calculated from the input data,

$E_B = E_H = 0, E_L = -1; F_L = F_H = 0, F_B = -1; G_L = G_B = 0, G_H = -1$.

Substituting we obtain the following equations for the standard deviations:

$$\sigma_E = -j \sigma_L + lE_B \sigma_b + mE_1 \sigma_1 + nE[H^+]\sigma[H^+] = 0$$  \hspace{1cm} \text{(A.27)}

$$\sigma_F = -i \sigma_B + lF_B \sigma_b + mF_1 \sigma_1 + nF[H^+]\sigma[H^+] = 0$$  \hspace{1cm} \text{(A.28)}

$$\sigma_G = k\sigma_H + lG_1 \sigma_1 + mG_1 \sigma_1 + nG[H^+]\sigma[H^+]$$  \hspace{1cm} \text{(A.29)}

The first two of these equations are solved simultaneously for the unknown standard deviations $\sigma_b$ and $\sigma_1$

$$\sigma_b = \frac{iE_1 \sigma_b - jE_1 \sigma_L + (F_1 E[H^+] - F[H^+] E_1) nE[H^+]}{E_B - E_b F_1}$$  \hspace{1cm} \text{(A.30)}
\[ \sigma_1 = \tilde{E}_b \sigma_B - jF_b \sigma_L + (F_b E[H^+] - E_b F[H^+]) \tilde{\sigma}_{[H^+]} \]

\[ (E_b F_1 - F_b E_1) \]

These quantities are then substituted into equation A.29 above.

\[ \sigma_G = -k' \sigma_H + j\sigma_B (G_b E_1 - E_b G_1) - j\sigma_L (G_b F_1 - F_b G_1) + \]

\[ \tilde{\sigma}_{[H^+]} (E_b F_1 - F_b E_1) \]

\[ \tilde{\sigma}_{[H^+]} (E_b F_1 - F_b E_1) \]

Rearranging and taking the dot product \((\sigma_G, \sigma_G)\),

\[ \sigma_G^2 = \sigma_H^2 + \sigma_B^2 (G_b E_1 - E_b G_1)^2 + \sigma_L^2 (G_b F_1 - F_b G_1)^2 + \]

\[ \tilde{\sigma}_{[H^+]} (E_b F_1 - F_b E_1) \]

\[ \tilde{\sigma}_{[H^+]} (E_b F_1 - F_b E_1) \]

which in programme language is:

\[ \text{SIGR} = \text{SIGCHX}**2 + (\text{SIGBBX} \times \text{TEMQ} \times (\text{CFC} \times \text{BGB} - \text{BFB} \times \text{CGC}))**2 \]

\[ + (\text{SIGCLX} \times \text{TEMQ} \times (\text{CFC} \times \text{AGA} - \text{AFA} \times \text{CGC}))**2 \]

\[ + ((\text{SIGHX} \times \text{UX}) \times (\text{CHC} + \text{TEMQ} \times (\text{AHA} \times (\text{BFB} \times \text{CGC} - \text{BGB} \times \text{CFC}) \]

\[ - \text{BHB} \times (\text{AFA} \times \text{AGA} \times \text{CFC}))))**2 \]

where \(\text{TEMQ} = 1.0 / (\text{AFA} \times \text{BGB} - \text{BFB} \times \text{AGA})\).

A flow chart for the subroutine CØNC5, and listings of all the subroutines required by GAUSS4, are shown below.
FLOW CHART FOR SUBROUTINE \( \text{CONCS} \)

START

20 \( \text{NIT}=0 \)

\( J: N \)

30 \( \text{TERM}(J)= \)

\( J: N \)

2 \( C(J)= \)

\( \text{NIT}=\text{NIT}+1 \)

\( B=VX \)

\( ALO=TX \)

\( J: N \)

\( ALO=ALO+AL(J)+C(J) \)

\( B=B+AM(J)+C(J) \)

\( Y2=\text{DABS}(B-BT0T) \)

\( Y4=\text{DABS}(AL0-CLT0T) \)

Exit to 16

Exit to 9 when iteration complete

Sets to zero counter for number of iterations per data point.

Sets up values for quantities to be using in calculation of species concentrations.

Sets up values of species concentrations

\[ C_i = \beta_{1mM} L_i^1 M_i^{m(H^+)^n} \]

Advances iteration counter.

Begins accumulation of total metal and total ligand concentrations.

Accumulates total ligand concentration.

Accumulates total metal concentration.

Quantity to be tested for convergence in total metal.

Quantity to be tested for convergence in total ligand.

Exits here if no convergence after 99 cycles.

Checks if convergence yet complete for total metal.

Checks if convergence yet complete for total ligand.

Begin Newton-Raphson iteration.

Calculates first and second elements in simultaneous equation constant vector.

Begins to accumulate the 2 x 2 matrix for the solution of the two simultaneous equations to find shifts in free metal and free ligand concentrations.
\[ \text{SEM}(l,1) = \text{SEM}(l,2) = \text{SEM}(2,2) = \text{SEM}(2,1) = \text{SEM}(1,2) \]

\[ \text{SEI}(I,J) = \text{SEM}(I,J) \]

\[ \text{SHFT}(I) = 0.0 \]
\[ \text{SHFT}(2) = 0.0 \]

\[ \text{SHFT}(I) = \text{SHFT}(I) + \text{SEI}(J) \cdot \text{SEV}(J) \]

Exit to 16 if both shifts zero

\[ \text{SHFT}(1) = -0.9999 \]
\[ \text{SHFT}(2) = -0.9999 \]

\[ \text{SHFT}(1) = -0.9999 \]
\[ \text{SHFT}(2) = -0.9999 \]

\[ \frac{\partial M_{\text{tot}}}{\partial M_{\text{free}}} \]
\[ \frac{\partial M_{\text{tot}}}{\partial L_{\text{free}}} \]
\[ \frac{\partial L_{\text{tot}}}{\partial L_{\text{free}}} \]
\[ \text{Completes accumulation of matrix elements} \]
\[ \text{Completes matrix by reflection} \]

Preserves matrix before inversion.
Terms will be used in calculation of weights.

Calls matrix inversion subroutine, and
inverts matrix. \( \text{SEI} = \text{SEI}^{-1} \)
Initialises shift in free metal
Initialises shift in free ligand

Solves for shifts

Checks that both shifts are not zero (in which case no convergence possible and exit is used)

Ensures that fractional shift is not more negative than -1 which would lead to negative concentration

Similar check for second shift.
Calculates new free metal concentration
Calculates new free ligand concentration using fractional shift.
Begins calculation of analytical hydrogen ion concentration.
\[
[H^+] - [OH^-] = \frac{[H^+]}{[OH^-]}
\]

Accumulates terms in analytical hydrogen ion concentration.
Checks whether weights were supplied or are to be calculated.
Commences calculation of variance of residual in analytical hydrogen ion concentration from estimated errors in input quantities.

\[
\begin{align*}
&W_b/\beta [H^+] \\
&W_L/\beta [H^+] \\
&W_b H/\alpha [H^+]
\end{align*}
\]

Quantity used in next step.
Calculates variance in analytical hydrogen ion concentration.
Returns to main programme to calculate residuals.
When iteration fails to converge, sets calculated analytical hydrogen ion concentration = experimental value.
Advances counter for non-convergent iterations per point.
Advances counter for non-convergent iterations per cycle.
Returns control to main programme.
LISTING OF GAUSS3
C READS IN DATA
C
S. 0051 100 READ (1,104) NPTC
S. 0052 WRITE (3,900)
S. 0053 NPCD=0
S. 0054 101 READ (1,102) INEXP
S. 0055 107 KK=0
S. 0056 IEC=0
S. 0057 READ (1,1) M,N,NDP,EX1,EX2,1WS,1MS,1NO
S. 0058 READ (1,2) (ML(I),MM(I),MK(I), I=1,N)
S. 0059 DO 3 I=1,N
S. 0060 AL(I)=ML(I)
S. 0061 AM(I)=MM(I)
S. 0062 AN(I)=MK(I)
S. 0063 3 AN(I)=MM(I)
S. 0064 146 IMT=0
S. 0065 ENDP=NDP
S. 0066 WRITE (3,RO00)
C READS TITRATION DATA FOR EACH EXPERIMENT
C
S. 0067 DO 106 KL=1,INEXP
S. 0068 READ (1,130) (TITLE(I),I=1,20)
S. 0069 WRITE (3,131) (TITLE(I),I=1,20)
S. 0070 175 READ (1,5) TM,TL,ACID,BASE,VOL
S. 0071 WRITE (3,720) TM,TL,ACID,BASE,VOL
S. 0072 IF (1WS) 179,179,147
S. 0073 147 READ (1,122) DM,DL,ACID,BASE,UV,DET,DPH
S. 0074 WRITE (3,721) DM,DL,ACID,BASE,UV,DET,DPH
S. 0075 179 I=I+1
S. 0076 READ (1,10) TITRE(I),ZU11,IINDEX
S. 0077 IF (INDEX) 180,179,180
S. 0078 180 NOR=I
S. 0079 WRITE (3,181) NOR
C CORRELATES DATA WITH THAT FOR OTHER EXPERIMENTS OF SET
C
S. 0080 N=19 I=1,NOR
S. 0081 ZAC(I)=ACID
S. 0082 ZBA(I)=BASE
S. 0083 ZW(I)=VOL
S. 0084 ZB(I)=1M*VOL/(VOL+TITRE(I))
S. 0085 ZL(I)=TL/(VOL+TITRE(I))
S. 0086 19 ZM(I)=ZL(I)+(ENDP+ACID)*VOL/(VOL+TITRE(I))
S. 0087 IF (INDEX) 180,179,180
C CALCULATES ERRORS AT EACH POINT FROM INPUT ERRORS
C
S. 0088 DEM=TM*DM/100.
S. 0089 DEL=TL*DL/100.
S. 0090 DAE=ACID*DACID/100.
S. 0091 DER=BASE*DBASE/100.
S. 0092 DEV=VOL*DV/100.
S. 0093 DO 140 I=1,NOR
S. 0094 GN=VOL*TITRE(I)
S. 0095 FN=VOL/GN
S. 0096 ZIGCH(I)=SORT ((FA*DEM)**2+(GN*TM-TX*VOL)*DEV/GN)**2+(-TM*FN*)
S. 0097 ZIGC(I)=SORT ((FA*DEL)**2+(GN*TL*VOL)*DEV/GN)**2+(-TL*FN*)
S. 0098 DEAC50=(FA*DEA)**2+(GN*ACID-ACOD*VOL)*DEV/GN)**2+(-ACID*FN*)
S. 0099 DET/GN)**2
S. 0100 DEASU(TITRE(I))=2*(GN*BASE-BASE*TITRE(I))
S. 0101 IF (INDEX) 180,179,180
S. 0102 180 MMC=1+MOR
S. 0103 MMC=1+MOR
S. 0104 MMC=1+MOR
S. 0105 MMC=1+MOR
S. 0106 MMC=1+MOR
S. 0107 MMC=1+MOR
S. 0108 MMC=1+MOR
S. 0109 MMC=1+MOR
S. 0110 MMC=1+MOR
S. 0111 MMC=1+MOR
S. 0112 MMC=1+MOR
S. 0113 MMC=1+MOR
IF (IKV) 172,172,173
SIGBR(I)=SIGR(MMT)
SIGCL(I)=SIGCL(MMT)
SIGPH(I)=SIGPH(MMT)
SIGCH(I)=SIGCH(MMT)
CONTINUE
MT=MT+1
READ (1,4) CKWL,F,(F(I),I=1,N)
WRITE (3,1730) NDP,CKWL,F
WRITE (3,1739) N,MT
READ (1,7) NCD,NCV
WRITE (3,142) NCD
IF (IKV) 710,710,711
710 WRITE (3,145)
GO TO 712
711 WRITE (3,141)
712 WRITE (3,144)
WRITE (3,143) (I,ML(I),MH(I),MM(I),E(I),I=1,N)
IF (IEC) 7120,7120,7121
7120 WRITE (3,148)
READ (1,8) (IG(I),H(I),I=1,NCV)
DO 269 I=1,NCV
269 WRITE(I,H(I))
BEGINS REFINEMENT
HX=ALOG(10.0)
NCC=0
ICC=0
CKW=EXP(HX*CKWL)
IF (TM) 221,221,222
221 UB=0
GO TO 225
222 IF (TL) 223,223,224
223 SUM=1
GO TO 225
224 SUM=-1
225 DO 9 I=1,MT
9 UX(I)=EXP(HX*U(I))
IF (IKV) 11,11,110
110 DO 12 I=1,MT
12 SIGH(I)=SIGPH(I)*HX/UX(I)
11 NCC=NCC+1
MCI=0
SQR=0.0
SRC=0.0
SRC=0.0
INC=0
712 DO 713 I=1,MT
713 NICH(I)=0
DO 7127 I=1,N
7127 EORIG(I)=T(I)
DO 48 J=1,20
48 CC(1,J)=0.0
L=0
49 K=1
37 RIOT=BR(K)
CLTOT=CL(K)
NNIT=0
J=0
M=0
1F (IKV) 13,13,14
14 SIGX=SIGX(K)
SIGRX=SIGR(K)
SIGCLX=SIGCL(K)
SIGCHX=SIGCH(K)
13 CHI=CH(K)
UX=UX(K)
Y1=EX1*RIOT
Y3=EX2*CLTOT
DETERSIMLES INITIAL ESTIMATES OF FREE METAL AND FREE LIGAND CNCM.
1F (ICC-1) 60,15,15
60 IF (K=1) 62,62,63
63 IF (NIT=09) 16,62,62
62 VX=BR(K)
DMY=1.0
DO 51 I=1,N
IF (ISUB) 226, 227, 228
226 CALL CONC5
   GO TO 126
227 CALL CONC6
   GO TO 126
228 CALL CONC7
126 IF (NWC (> 30)) 25, 71, 71
25 IF (ICC = 1) 18, 21, 21
18 VX = TVX(K)
21 TX = TTX(K)
22 SIG(X = 1, 0)
23 WT = L0/SORT(SIGX)
24 RUM(=H+CH(K))
25 R = T R U M
26 IF (KK) 24, 24, 70
24 IF (L = 1) 32, 31, 31
32 N =1
27 GO TO (250, 251, 252), M
250 SOR() = S OR() + R
251 GO TO 16
252 H02 = H01
253 E(IS) = EORIG(IS) + H(J)
260 M =1
261 H(J) = H(J) * 5.0
262 INCCH =1
263 ICH(J) =1
264 NINCCH(K) = 1
265 GO TO 263
266 DE(J) = (WTO*(H02-H03) )/(2*H(J))
267 H(J) = H01
270 IF (J = 1, 1, NCX) 73, 50, 50
273 J = J + 1
275 M =1
276 IS = I G(J)
277 E(IS) = EORIG(IS) + H(J)
280 GO TO 16
30 DO 36 11 = 1, NCV
31 CK(11) = CK(11) - R0*DF(I1)
32 DO 36 JJ = 1, NCV
33 CC(JJ) = CC(JJ) + DF(I1)*E(JJ)
34 IF (NNIT) 700, 700, 701
35 K = K + 1
36 DO 951 I = 1, NCV
37 DO 951 J = 1, NCV
38 RC(I, J) = CC(I, J)
39 IF (I CH(J) ) 265, 265, 266
40 WRJ T (3, ?62) I
A-36
2650 CONTINUE
   NICH=0
   DO 7140 I=1,MT
       NICH=NICH+INCH(I)
   7140
   WRITE (3,7141) NICH
C CALLS MATRIX INVERSION SUBROUTINE
C
265 CALL SUB760 (NCV,BC)
   DO 39 I=1,NCV
       X(I)=0.0
   DO 40 I=1,NCV
       DO 40 J=1,NCV
           X(I)=X(I)+BC(I,J)*X(J)
   40
   IEOS=0
   DO 490 I=1,NCV
       IF (1.0-X(I)) 492,490,492
   490 IEOS=IEOS+1
   492 DO 491 I=1,NCV
       IF (0.5-XAR) 510,510,515
   502 IF (1.0-XAR) 515,515,520
   510 X(I)=0.5
   515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 46
   493 DO 494 I=1,NCV
       XAB=DABS(X(I))
   494 IF (1.0-XAR) 510,510,515
       515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   495 DO 496 I=1,NCV
       IF (0.5-XAR) 510,510,515
       510 X(I)=0.5
   515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   497 DO 498 I=1,NCV
       IF (0.5-XAR) 510,510,515
       510 X(I)=0.5
   515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   499 DO 500 I=1,NCV
       IF (0.5-XAR) 510,510,515
       515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   501 DO 502 I=1,NCV
       IF (0.5-XAR) 510,510,515
       515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   503 DO 504 I=1,NCV
       IF (0.5-XAR) 510,510,515
       515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   505 DO 506 I=1,NCV
       IF (0.5-XAR) 510,510,515
       515 WRITE (3,516) I,X(I)
   520 CONTINUE
   GO TO 44
   507 DO 508 I=1,NCV
       IF (0.5-XAR) 510,510,515
       515 WRITE (3,516) I,X(I)
   520 CONTINUE
   156 WRITE (3,152) SORO
   157 L=L+1
   158 IF (L-15) 152,153,154
   159 WRITE (3,155) I,X(I)
   160 X=0.5
   161 X=0.5
   PRINTS IMPROVED CONSTANTS, E.S.D. IN CONSTANT, AND SHIFT FROM LAST VALUE
   WRITE (3,162) IS, E(IS), X(I), X(I)
   CONTINUE
   IF (IS=I) 156,157,158
   159 WRITE (3,155) I,X(I)
   160 X=0.5
   161 X=0.5
   PRINTS IMPROVED CONSTANTS, E.S.D. IN CONSTANT, AND SHIFT FROM LAST VALUE
   WRITE (3,162) IS, E(IS), X(I), X(I)
   CONTINUE
   IF (IS=I) 156,157,158
   159 WRITE (3,155) I,X(I)
   160 X=0.5
   161 X=0.5
   PRINTS IMPROVED CONSTANTS, E.S.D. IN CONSTANT, AND SHIFT FROM LAST VALUE
   WRITE (3,162) IS, E(IS), X(I), X(I)
   CONTINUE
   IF (IS=I) 156,157,158
   159 WRITE (3,155) I,X(I)
   160 X=0.5
   161 X=0.5
   PRINTS IMPROVED CONSTANTS, E.S.D. IN CONSTANT, AND SHIFT FROM LAST VALUE
   WRITE (3,162) IS, E(IS), X(I), X(I)
   CONTINUE
   IF (IS=I) 156,157,158
   159 WRITE (3,155) I,X(I)
   160 X=0.5
   161 X=0.5
   PRINTS IMPROVED CONSTANTS, E.S.D. IN CONSTANT, AND SHIFT FROM LAST VALUE
   WRITE (3,162) IS, E(IS), X(I), X(I)
   CONTINUE
   IF (IS=I) 156,157,158
   159 WRITE (3,155) I,X(I)
READ (1,9951) I,E(I)
K=0
GO TO 712
200 K=1
WRITE (3,27)
WRITE (3,26)
GO TO 49

FINAL BACK CALCULATION TO GIVE TABLE

70 COMPL=0.0
IF (SUB) 1022,1020,1020
1020 ENPAR=0.0
GO TO 1021
76 CONTINUE
ENPAR=COMPL/BTOT
ALGL=ALOG(TX)/ALOG(10.)
1021 WRITE (3,89) K,COMPL(1),CL(K),III(K),HO,RW,MT,VX,TX,ENBAR,ALGL
DO 790 J=1,R
IF (C(J)-10.**(-20)) 751,750,750
751 C(J)=0.0
750 CONTINUE
CITR(K)+=CL(K)*EMD(AK)+AC(K)*HO-BK*VA(K)-CL(K)*EMD
ERROR(K)=(CITR(K)-CTITR(K))
WRITE (3,329) K,(C(J),J=1,R),CTITR(K),ERROR(K)
K=K+1
IF (K-MT) 37,37,371
NPCO=NPO+1
SIGERR=0.0
DO 1100 K=1,MT
1100 SIGERR=SIGERR+ERROR(K)**2
SDTIT=SQRT(SIGERR/(MT-NCV))
WRITE (3,1101) SDTIT
DO 72 J=1,R
72 C(J)=0.0
WRITE (3,900)
WRITE (3,1000)
NO 902 J=1,NCV
902 WRITE (3,903) (CC(I,J),J=1,NCV)
WRITE (3,305) (CK(I,J),J=1,NCV)
906 WRITE (3,906)
907 WRITE (3,903) (BC(I,J),J=1,NCV)
908 WRITE (3,310) (XI(I),I=1,NCV)
WRITE (3,911)
NO 915 J=1,1
915 CC(I,1)=FC(I,1)/SQRT(CC(I,1))
IF (TEMP(I,1)) 916,915,915
916 TEMP(I,1)=FC(I,1)
919 CONTINUE
NO 913 J=1,NCV
913 CC(I,J)=FC(I,J)/SQRT(TEMP(I,J))
WRITE (3,903) (CC(I,J),J=1,1)
END OF MATRIX PRINT OUT

IF (NPCD-PTC) 150,150,150
STOP
END OF MATRIX PRINT OUT

WRITE (3,900)
2000 IF (NPCD-NPC) 150,150,150
150 STOP
END
SUBROUTINE CONC5

SUBROUTINE USED FOR METAL COMPLEX FORMATION

Solves simultaneously equations for total metal and total ligand concn.

DOUBLE PRECISION H0, HO1, HO2, HI3, R, RUW, RO, TX, AL0, BD, Y1, Y2, Y3, Y4,

1AD, ANU,

2CH(200), CK(20), 3C(20, 20), CC(20, 20), DE(20), X(20), TERM(20), C(20),

3SEV(2), SEM(2, 2), SEI(20, 20), SHFT(2)

DIMENSION AL(20), AM(20), AN(20), BB(200), BC(200), CC(200), DE(20),

2G(20), ML(20), MM(20), MN(20), B(20),

3SIGBR(200), SIGCL(200), SIGCH(200), SIGPH(200), SIGH(200)

COMMON H0, HO, VX, TX, CC, RC, CK, X, Y1, Y2, Y3, Y4, CH, AL0, SEM, SEI, SHFT,

1SEV, TERM,

2ML, MM, MN, UX, BTOT, CLTOT, N, NCV, UXL, AL, AN, B, F, K, NIT,

3SIGB, SIGC, SIGD, SIGE, SIGF, SIGG, ANCI, NNT

20 NIT=0

500 DO 30 J = 1, N

30 TERM(J) = H(J) * UX ** MN(J)

1 DO 2 J = 1, N

2 C(J) = TERM(J) * TX ** ML(J) * VX ** MM(J)

NIT = NIT + 1

80 = VX

10 AL0 = TX

DO 3 J = 1, N

3 AL0 = AL0 + AL(J) * C(J)

3 BO = RO + AM(J) * C(J)

111 Y2 = DABS(BO - BTOT)

Y4 = DABS(AL0 - CLTOT)

IF (NIT <= 99) 15, 15, 16

15 IF (Y1 - Y2) <= 0.0004, 8, 8

8 IF (Y3 - Y4) <= 0.0004, 9, 9

4 SEV(1) = BTOT + 0.0

2 SEV(2) = CLTOT + 0.0

3 SEM(1, 1) = VX

2 SEM(1, 2) = 0.0

5 SEM(2, 1) = TX

2 SEM(2, 2) = 0.0

DO 5 J = 1, N

5 SEM(1, 1) = SEM(1, 1) - C(J) * AM(J) * AM(J)

2 SEM(1, 2) = SEM(1, 2) - C(J) * AM(J) * AL(J)

5 SEM(2, 1) = SEM(2, 1) - C(J) * AL(J) * AL(J)

DO 950 J = 1, 2

950 SEV(I, J) = SEM(I, J)

CALL MATRIX INVERSION SUBROUTINE

CALL SUB7AO(2, SEV1)

SHFT(I) = O.0

SHFT(2) = O.0

DO 6 I = 1, 2

6 DO 6 J = 1, 2

SHFT(I) = SHFT(I) + SEV(I, J)

IF (SHFT(I) <= 0.0001) 40, 39, 40

39 IF (SHFT(I) >= 0.9999) 10, 11, 10

11 SHFT(I) = -0.9999

10 VX = VX + VX * SHFT(I)

TX = TX + TX * SHFT(2)

GO TO 1

9 HO = 1.0 / UX - CKW = UX

H0 = HO / F

DO 14 J = 1, N

14 HO = HO - AN(J) * RC(J)

14 CONTINUE

IF (IWS) 18, 14, 12

18 AHA = 0.0

14 RHR = 0.0

18 CHC = 1.0 / UX - CKW = UX

14 CHC = CHC / F

DO 31 J = 1, N

31 AHA = 0.0

18 RHR = 0.0

14 CHC = 1.0 / UX - CKW = UX

14 CHC = CHC / F

DO 31 J = 1, N
S.0060  AHA=AHA+AM(I)AN(I)*C(I)
S.0061  RHR=RHR+AL(I)AN(I)*C(I)
S.0062  CMC=CMC+AM(I)AN(I)*C(I)
S.0063  CFC=AHA
S.0064  GCC=RHR
S.0065  AF=SEM(1,1)
S.0066  RFR=SEM(1,2)
S.0067  AGA=SEM(2,1)
S.0068  RGR=SEM(2,2)
S.0069  TENG=1,0((AF=ARR-RR=AGA)
S.0070  SIGC=SIGCHX**2+(SIGCLX*TEMQ)(CFC*AGB-BFB*CGC)**2+(SIGCLX*TEMQ*
2-RR=ARR=AGC=AGA*SEM(C))**2}
S.0071  18 RETURN
S.0072  16 M=0
S.0073  NNIT=NIT+1
S.0074  UXL=ALNG(UX)**4,342945
S.0075  MNI=MCI+1
S.0076  RETURN
S.0077  END

S.0001  SUBROUTINE CONCA
C
S.0002  SUBROUTINE USED FOR PKA CALCULATION
C
S.0003  SOLVES EQUATION FOR TOTAL LIGAND CONCENTRATION
C
S.0004  DOUBLE PRECISION HO,MO1,HO2,H03,R,RW,U,X,TX,AL06,DO,Y1,Y2,Y3,Y4,
1ADF,ANH,
3SEV12),SEM(2,7),SET(20,20),SHFT(2)
C
2IG(20),ML(20),MN(20),1120),,E(20),
3SIG(200),SIGG(200),SIGCH(200),SIGPH(200),SIGH(200)
C
S.0006  COMMON BO,HO,UX,TX,CC,BC,CK,X,Y1,Y2,Y3,Y4,CH,C,AL0,SEM,SEI,SHFT,
1SEV,TERM,
2ML,MM,NN,UX,BTOT,CLTOT,N,MCV,UXL,AL,AM,AN,B,F,K,NIT,
3CKW,CMO,STK,1WS,STGCHX,STGCLX,STGBX,STGX,NNCI,NNT
C
S.0007  17 NIT=0
S.0008  DO I=1,N
S.0009  C(J)=TERM(J)*UX**MN(J)
S.0010  NIT=NIT+1
S.0011  ALN=TX
S.0012  DO 4 J=1,N
S.0013  ALN=ALN+AL(J)*C(J)
S.0014  4 YJ=DARS(ALN,CLTOT)
S.0015  5 IF(NIT-99)5,5,13
S.0016  5 IF(YJ-Y47,11,11
S.0017  7 AN=CLTOT-ALN
S.0018  8 ADF=-TX
S.0019  DO 8 J=1,N
S.0020  8 ADE=ADE-C(J)*AL(J)*AL(J)
S.0021  SHFT=AN/ADJ
S.0022  9 IF(SHFT=0,9999)9,9,10
S.0023  10 SHFT=0,9999
S.0024  10 TX=TX-TX*SHFT
S.0025  G0 TO 2
S.0026  11 HO=1,UX-CKW=UX
S.0027  11 HO=HO/F
S.0028  DO 12 J=1,N
S.0029  12 HO=HO-AN(J)*C(J)
S.0030  12 IF(TWIS20,20,21
C
C
S.0031  20 RETURN
S.0032  21 RHR=RR*0
S.0033  CHC=1,UX+CKW=UX
S.0034  CHC=CHC/F
S.0035  DO 22 I=1,N
S.0036  RHR=RHR+AL(I)*AN(I)*C(I)
C
C
SURROUNTE CONC

SURROUNTE USED FOR METAL HYDROLYSIS
SOLVES EQUATION FOR TOTAL METAL CONCENTRATION

DOUBLE PRECISION HO, HO1, HO2, HR, RT, VO, VX, ALU, RU, Y1, Y2, Y3, Y4,
ADEF, ANU,
2CH(201), CK(201), RC(20, 20), CC(20, 20), D(E1(201), X(201), TERN(20), C(20),
3SEV(2), SEM(2), SHFT(2)

DIMENSION AL(20), AM(20), AN(20), RH(200), CL(200), U(2001, F(201),
2IG(20), M(20), MM(20), MNI(20), K(20),
3SIG(200), SIGCL(200), SIGPH(200), SIGH(200)

COMMON BD, HO, VX, TX, CC, RC, CK, X, Y1, Y2, Y3, Y4, CH, ALU, SEM, SEI, SHFT,
1SEV, TERM,
2M, MR, RN, U, RTOT, CTOT, M, NCV, UX, AL, AM, AS, F, K, NIT,
3CK, EMO, SIGK, IN, SIGCHX, SIGCLX, SIGPHX, SIGX, SIGH, NNCI, NMC

17 NIT=0
S. 0006
DO 1 J=1, N
S. 0007
1 TERM(J)=R(J)*UX**MN(J)
S. 0008
2 DO 3 J=1, N
S. 0009
3 CJ=TERM(J)*UX**MN(J)
S. 0010
NIT=NIT+1
S. 0011
R=VX
S. 0012
DO 4 J=1, N
S. 0013
4 R=R+AM(J)*C(J)
S. 0014
Y2=DA55(TM-RTOT)
S. 0015
IF(NIT=30) 5, 5.13
S. 0016
5 IF(Y1-Y2) 7, 7, 11
S. 0017
7 ANU=RTOT-RD
S. 0018
ADEF=VX
S. 0019
DO 8 J=1, N
S. 0020
8 ADEF=ANF-C(J)*AM(J)*AX(J)
S. 0021
SHIFT=ANU/ADE
S. 0022
IF(SHIFT=0.9999) 9, 9, 10
S. 0023
10 SHIFT=0.9999
S. 0024
9 VX=VX-VX*SHIFT
S. 0025
GO TO 2
S. 0026
11 M=1./UX-CKW*UX
S. 0027
HJ=HO/F
S. 0028
AL=UX/RTOT
S. 0029
DO 12 J=1, N
S. 0030
12 M=M+AM(J)*C(J)
S. 0031
IF(ISW120) 20, 21
S. 0032
20 RETURN
S. 0033
C CALCULATES VARIANCE OF RESIDUAL
S. 0034
21 AHA=0.0
S. 0035
CHC=1./UX*CKW*UX
S. 0036
CHC=CHC/F
S.0036)
S.0037)
S.0038)
S.0039)
S.0040)
S.0041)
S.0042)
S.0043)
S.0044)
S.0045)
S.0046)
S.0047)
S.0048)
S.0001)
S.0002)
S.0003)
S.0004)
S.0005)
S.0006)
S.0007)
S.0008)
S.0009)
S.0010)
S.0011)
S.0012)
S.0013)
S.0014)
S.0015)
S.0016)