PHENYLALANINE AS A COMPLEXING AGENT

by

PAUL A. YEO

A SUMMARY

The complexing power of phenylalanine with protons and transition metal ions is examined. The relevance of phenylananine and the transition metals to biological systems is discussed. Computational methods of calculation were used to determine the stability constants of the complexes and the two main programs, SCOGS and LETAGROP VRID are compared. A program, RWCALCOR, was developed for use in the calorimetric work and the mathematics of this program are given. The experimental details and results are then given and finally discussed.

Phenylalanine is a diet essential amino-acid for homo sapiens and features prominently in the genetic disorder phenylketoneuria. It is also an important precursor to the C_6H_5-C-C-C group of compounds. The transition metal ions are important features of many enzyme systems and have been found to be bound to a variety of amino-acids in the blood stream.

This work was carried out using the constant medium method where the concentration of a background salt (3.00M $NaClO_4$) is so high that the activities of the reacting species are kept constant. The Gibbs free energy of the complexation reaction is calculated by determining the stability constant of the reaction and then using the reaction isochore

$$\Delta G^{\circ} = -RT\ln K.$$

The stability constant is obtained by following the hydrogen ion concentration with an electrode system of a glass electrode and a standard calomel electrode. The enthalpy of the reaction was determined directly by using an isothermal titration calorimeter, whence the entropy of the reaction could be found from the Gibbs-Helmholtz relationship

$$\Delta G^{\circ} - \Delta H^{\circ} = -T\Delta S^{\circ}$$

The calculation of stability constants from titration data was achieved by means of computer programs. The main program used was SCOGS which uses a non-linear least squares technique to minimise the function

$$U = \sum (\text{Titre}_{calc} - \text{Titre}_{expt})^2$$

This was compared, using both experimental and hypothetical data, with LETAGROP VRID - mathematically a more rigorous program.

The protonation results were compared with (i) results for the same ligand in different media and (ii) results for similar ligands in the same medium. This was done quantitatively for (i) using an extension of the Debye-Hückel equation, and qualitatively for the similar ligands tryptophan and histidine.

The metal ions studied were Cu(II), Ni(II), Co(II), Fe(II) and Fe(III). These were found to obey the Irving-Williams series with phenylalanine and for the ions studied calorimetrically, Cu(II), Ni(II), Co(II), the enthalpy was found to be the governing factor. The nickel and cobalt systems formed an insoluble hydrated 1:2 complex due to its high lattice energy, but iron(II) formed a 1:3 complex. Hydrolysis was seen to be an important factor for copper(II) and even more so for iron(III) as a previously unreported type of complex, $Fe_2(OH)_2aa_2$, was found.

The calorimetric study of iron(II) was precluded due to oxidation of the system. The calorimetric study of the iron(III) system was abandoned due to difficulties in calculation.

A further study of iron(II) and iron(III) with amino-acids is considered to be a logical extension of the work in this thesis.

To

Mum and Dad

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Acknowledgements

During the course of this work I have incurred the debt of several people and I would like to take this opportunity to thank them.

I would like to thank the Department of Chemistry of the University of St. Andrews for providing the laboratory and library facilities where this work was carried out and the University for providing a maintenance grant to myself.

I would thank the members of staff of the Department for providing both a happy and a stimulating environment for research and particularly Mrs. Patricia Cooper who, with great patience typed this thesis.

My greatest debt, however, is to my supervisor, David R. Williams, who, over three years, has been a mentor, supervisor, and teacher, but most of all a friend.

NOMENCLATURE

[ ]	concentrations
( )	activities
A & A'	total concentrations of ligands A & A'
a & a'	concentrations of free A & A'
B & B'	total concentrations of metals B & B'
b & b'	concentrations of free B & B'
E	electromotive force
E_j	liquid junction potential
E^o	standard electrode potential of glass/calomel electrode pair
F	faraday
f	activity coefficient
H	total concentration of hydrogen ions
h	concentration of free hydrogen ions
I	ionic strength
K_{a_n}	stepwise formation constant for a protonation reaction
K_n	stepwise formation constant for complexation reaction
K_w	ionic product of water
p & p'	number of ligands A and A' in a complex
q & q'	number of central groups (metals) B & B' in a complex
r	number of hydrogen ions in a complex
R	gas constant
s	standard deviation
T	absolute temperature

z	number of electrons involved in a redox process
$\bar{Z}$	average number of ligands A bound to one central group B
β^{o}_{pqr}	thermodynamic overall formation constant of $A_pB_qH_r$
β_{pqr}	concentration overall formation constant of $A_pB_qH_r$
$\triangle G^{o}_{o}$	thermodynamic standard Gibbs free energy change
$\triangle H^{o}_{o}$	thermodynamic standard enthalpy change
$\triangle S^{o}_{o}$	thermodynamic standard entropy change
	where thermodynamic refers to water as the standard solvent
$\triangle G^{o}$	standard Gibbs free energy change
$\triangle H^{o}$	standard enthalpy change
$\triangle S^{o}$	standard entropy change
	with 3.00 M $(Na)ClO_4$ as the standard solvent

C O N T E N T S

DECLARATION

I declare that this thesis is my own composition, that the work of which it is a record has been carried out by myself and that it has not been submitted in any previous application for a Higher Degree.

This thesis describes the results of research carried out at the Chemistry Department, United College of St. Salvator and St. Leonard, University of St. Andrews, under the supervision of Dr. David R. Williams since 1st of October, 1968.

PAUL A. YEO

CERTIFICATE

I hereby certify that Paul Yeo has spent eleven terms of research work under my supervision, has fulfilled the conditions of Ordinance No. 16 (St. Andrews) and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

DAVID R. WILLIAMS

CHAPTER I INTRODUCTION

The acquisition of knowledge about living systems has proceeded remarkably in the last decade. New techniques have played a large part in these advances but the interplay of the various disciplines of co-ordination chemistry, inorganic biochemistry, organometallic chemistry, enzymology and molecular biology, has made a significant contribution. The theoretical advances of the thirties, culminating in Pauling's "The Nature of the Chemical Bond"[1], inspired Hedges to write "Inorganic Chemistry is again coming into its own"[2]. This comment is relevant again today, but now the renaissance is due to a more balanced advance in both theoretical and experimental fields. The discovery of new experimental spectroscopic techniques, combined with an increased sophistication of instrumentation generally, has revealed new areas of study and has extended existing areas beyond recognition. The development of high speed digital computers has had a profound effect on science and technology, and chemistry is no exception.

The division of chemistry into physical, inorganic and organic has always been recognised as an oversimplification and these divisions, in recent times, have become increasingly less meaningful. Today, this is shown by the vast field of organometallic chemistry, and by the fact that entire journals are devoted to physical organic chemistry. And thus, the unification of scientific disciplines has produced a series of bastardised names for these new fields of research (e.g. organometallic chemistry and inorganic biochemistry). Inorganic

biochemistry is a term that was coined by Williams[3] to describe the study of inorganic, particularly metal, compounds in biological systems and the use of metal co-ordination compounds as models for metalloenzymes.

The importance of metals in the human metabolism was recognised as long ago as 1500 B.C., when a Prince Iphyclus was cured of his impotence by the administration of a suspension of rust in wine[4], but it is only in the last twelve years that inorganic biochemistry has emerged as a field in its own right. Of the eighty-seven naturally occuring elements, twenty can be considered as essential to the human body and, of these, three classes exist; (a) the non-metals, H, C, O, N, S, P, Cl, Br, F and I; (b) the bulk metals, Na, K, Ca, Mg; and (c) the trace metals, Mn, Fe, Co, Cu, Zn and Mo. The remaining metals, particularly the heavy metals Hg and Pb, are becoming increasingly important due to the pollution of the environment by modern day living. Knowledge of the role of the above classes of elements in the body is quite well established;

class (a) form (i) simple organic molecules - the amino acids, which polymerise to peptides which in turn help to form highly complicated structures such as DNA and proteins, and, (ii) the more complicated structures steroids, lipids and carbohydrates;

class (b) is also subdivided, (i) the charge carriers, K^+ and Na^+, these will almost certainly be aquated cations, and (ii) $Mg^{2+}_{(aq)}$ and $Ca^{2+}_{(aq)}$, which are of prime importance in the action of adenosine triphosphate (ATP), the most important store of energy in the human body, and also act as structure formers and triggers in the body[3];

class (c) has divisions that are not as well defined but, essentially, Cu, Fe, Mo, and to some extent Mn, act as catalysts in redox reactions whereas Zn controls pH by hydrolysis. The rôle of cobalt is not yet clearly understood at the molecular level except for its action in corrinoid enzymes[5]. When the complexing power and the donating ligands are examined, the chemistry of the two classes of metals are contrasted and the reasons for their differing biological rôles are revealed.

Table 1.

Classification of metal ions in vivo

	Na, K	Mg, Cu, (Mn)	Zn, (Co)	Cu, Fe, Mo, (Mn)
Ligand atoms	O	O	N & S	N & S
Strength of bond*	5.85	13.12	23.41	36.24
i.e.	weak	moderate	strong	strong

*These figures refer to $-\Delta H$, (enthalpy kJ mol^{-1}) for the formation of the metal -EDTA complex. In each group the figure for the first metal is quoted[6]..

At the pH of blood (7.35-7.42), the extent of complexation of class (b) is small and relatively unimportant; conversely, class (c) forms strong complexes at this pH and therefore the study of these complexes and their formation is of great biological importance.

The importance of stereochemistry to biological systems cannot be underestimated; the reproduction of genetic material is dependent upon the double-helix structure of DNA, and the action of most metalloenzymes is dependent upon the geometry of the metal[7-10]. As a particular example, the complexes of iron show a remarkable variation in co-ordination and reactivity in the body. Nature equips iron with a stereochemistry that is irreproducible in solution, but one of the most wide spread in mammals, i.e. haemoglobins, although the formation of a macromolecule which can reversibly complex molecular oxygen has been imitated using a polystyrene film[11]. In nature's macromolecule, iron has, essentially, octahedral co-ordination where four planar positions are occupied by the donor nitrogens of a porphyrin ring and an axial position by an imidazole nitrogen from a histidyl residue[12].

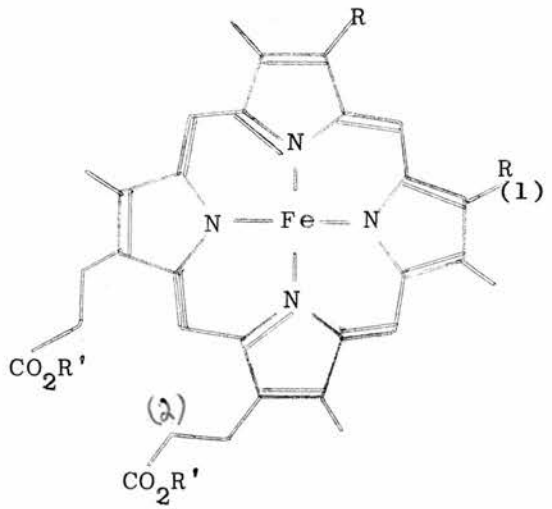


Figure 1.

The iron-porphyrin ring system.

The function of the sixth co-ordination site is to reversibly bind and release molecular oxygen. Cytochrome C has the same basic structure, but having histidyl residues occupying both of the axial sites. These histidines are portions of peptide chains which contain cysteine residues which are in turn attached to carbons (1) and (2). Cytochrome C is an electron-transferring protein and is intimately linked with the interconversions between the ferrous and ferric states of the haem iron[12]. The third type of complex is peroxidase, where the sixth position is used for complexing hydrogen peroxide. Here, as in the haemoglobins, the bond between substrate and iron is continuously made and broken but, unlike the haemoglobins, the iron is oxidised to the iron (III) state, although there is evidence that it is iron (IV)[13].

Whilst kinetics and stereochemistry play a large part in biological systems, thermodynamics has its place, as is shown by the importance of ATP and the frequency with which it is invoked as taking a part in biological reactions.

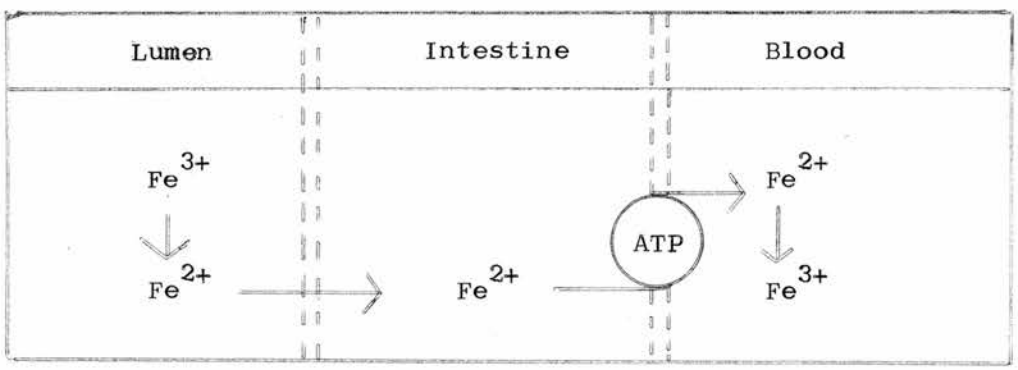


Figure 2

The active transport mechanism for intestinal regulation and control of iron uptake.

ATP is also essential to the biosynthesis of squalene, the precursor of the steroid system[14].

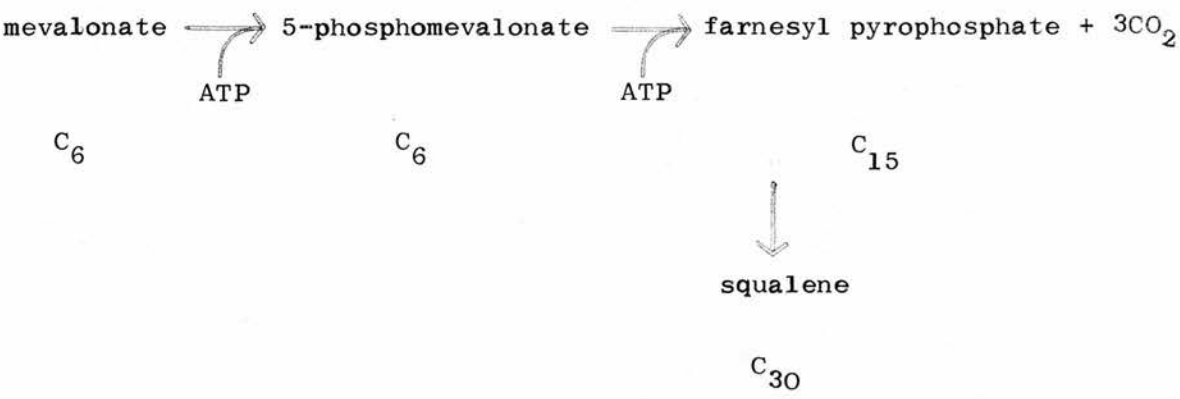


Figure 3
The biosynthesis of squalene.

The conventional view of the mode of action of ATP is that the hydrolysis of one phosphate group provides a large amount of energy to drive other reactions. Recently, however, there has been much discussion concerning the validity of this theory[15-17]. Unfortunately, the possibility of catalysis by magnesium ions was omitted from the discussion[18].

More recently, a "breakthrough" in the action of aspirin was reported[19]. Again the possible importance of a metal ion was omitted from consideration even though speculation has been rife for many years[20]. The understanding of rôle of aspirin is that it inhibits the formation of prostaglandins but in 1954[20] Schubert suggested that the rôle of aspirin was to "... remove or inactivate excess copper present in an intracellular site," and, as yet, these two theories have not been linked.

Even though the field of inorganic biochemistry is expanding rapidly,

its influence is not yet as widespread as its importance due to the need for more relevant experimental data.

The methods currently available for studying the formation, electronic structure and three-dimensional structure are numerous but can conveniently be classified into spectroscopic, potentiometric and calorimetric techniques.

Spectroscopy

(a) Mössbauer:- The use of this nuclear gamma-ray resonance spectroscopy has been extensive for iron containing species, and for samples in the solid state but, due to the widespread abundance of iron in biological systems, this is no real constraint as it has several advantages over other methods of resonance spectroscopy. Firstly, there are no interfering signals from other atomic species and secondly, the Mössbauer nuclide does not perturb the electronic environment being studied. Mössbauer has been used to study the haemoproteins, haem prosthetic groups and iron-sulphur proteins[21,22] usually, in association with other techniques.

(b) Nuclear Magnetic Resonance:- This technique can be applied to any compound containing a nucleus with a spin, but is usually applied to the simplest nucleus, that of hydrogen, and this yields volumes of information about any species containing this nucleus. Both complex molecules[23] and simple complexes[24] of biological importance have been studied by nmr.

(c) Nuclear Quadrupole Resonance:- For a nucleus to show a nqr signal it must have a nuclear spin of one or greater (i.e. $I > 1$). The main nuclei studied have been the halogens, except fluorine-19 which has no nqr, and cobalt-59[25]; this means that nqr has had no significant effect on the study of biological complexes as yet, although it may be possible to apply the technique with some success.

(d) Electron Paramagnetic Resonance:- Complexes with unpaired electrons will give an epr signal, hence, Cu(II), Fe(III), Co(II) and Mo(V) can be studied by this technique. The elucidation of the interaction between molybdenum and riboflavin was carried out by using epr and its importance emphasised. "... the case of adjacent radicals is one of great importance in biological systems. Electron paramagnetic resonance is almost the only method which will detect such a situation."[18]

(e) Crystallography:- X-ray spectroscopy is by far the most important branch of crystallography with the more precise neutron and electron diffraction playing only a minor part. The elucidation of the structure of complex molecules has played a great part in the advances made in the biological sciences in the last twenty-five years. This has been recognised by the award of the Nobel Prize several times to crystallographers, including to Hodgkin for the elucidiation of the structure of insulin and vitamin B_{12}[26].

(f) Optical Rotatory Dispersion and Circular Dichroism:- These techniques are used with optically active complexes and hence involve a degree of preparative difficulty. They have been applied with success to complexes of transition metals and optically active amino acids[27,28].

(g) Infra-Red and Ultraviolet Spectroscopy:- These more conventional forms of spectroscopy continue to yield a great deal of information about the electronic configuration of the central metal ion, the type of bonds formed and the extent of formation of complexes[29].

Potentiometry

This is a method of determining the activity of a species by means of an electrode pair and when applied to complex chemistry it is possible to evaluate the formation constants of complexes in solution. The glass electrode is by far the most important as this determines the hydrogen ion activity in solution; this in turn means that a reaction dependent upon hydrogen ion activity can be followed in solution. Ligands that complex with metal ions also associate with protons in solution and so the complex formation reaction, being competitive, is pH dependent. This will be dealt with in greater detail in Chapter II.

There are several other ion-sensitive electrodes commercially available but as yet they do not have the applicability, stability and sensitivity of the glass electrode. The most widely used of these electrodes are the fluoride electrode and the calcium electrode although new electrodes, such as amino acid electrodes[30], are becoming more widely used.

Calorimetry

This technique gives the enthalpy of, i.e. the strength, of a metal-substrate bond being formed in solution, and is usually used

conjunction with potentiometry. Together these methods give the Gibbs free energy, ΔG^{o}, the entropy, ΔS^{o} and the enthalpy, ΔH^{o} changes involved in the formation of complexes. This will be given more consideration in Chapter II.

The methods chosen to study complexes, depend upon the complexes chosen for study. Either natural systems can be examined, which involves such difficulties as arise from the complexes involving high molecular weight proteins and, in general, the higher the molecular weight the more limited are the solution studies, so that a wider range of techniques are necessary, or simple, complexes may be researched and the results extrapolated to apply to biological systems. The latter has been the approach used by Perrin's school (Canberra) and the collection of large quantities of data on simple systems have been combined to produce important biological conclusions[31].

This also, has been the approach used in the present study, where the complexing between phenylalanine (an important biological amino acid) and iron (II and III), cobalt (II), nickel (II), copper (II) and protons (Fe, Co & Cu are trace metals *in vivo*) is examined using *in vitro* potentiometry and calorimetry. For aqueous complex studies, these two techniques are the most economical in terms of conclusions yielded per given amount of data and time. This economy is further enhanced by analysing the results using powerful computer programs.

The in vivo roles of the metals used have already been mentioned, phenylalanine's importance is also worth consideration. It is diet essential amino acid for homo sapiens, where it has two main reaction paths (figure 5)

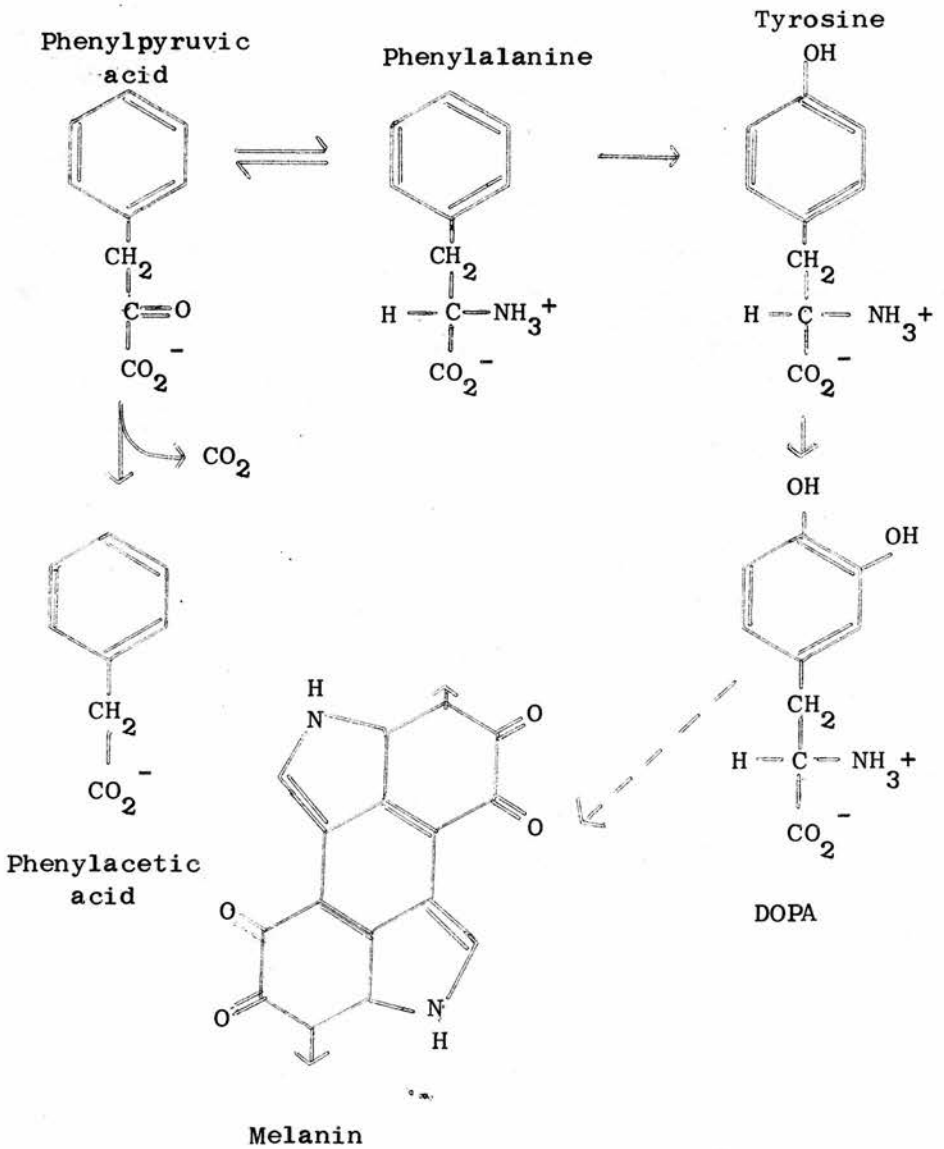


Figure 5

Phenylalanine in vivo

To the right hand side, tyrosine is formed by the action of phenylalanine hydroxylase, and from this the important pigment melanin. To the left hand side, phenylpyruvic acid and phenylacetic acid are formed by transamination; normally this reaction occurs only to a minor extent but if there is a lack of phenylalanine hydroxylase this reaction predominates and produces an excess of the acids which cause brain damage. This disease is called phenylketonuria and is an inborn disorder, hence, all newborn children in Britain are tested for an excess of phenylalanine which, if present, is treated by giving a phenylalanine-free diet to the child. In plant life, too, phenylalanine has an important role, as it stands at the head of the biosynthetic sequence that leads to the C_6-C-C-C class of compounds, e.g. cinnamic acids, and is also the precursor of terphenyls and numerous alkaloids including mescalin[32].

CHAPTER II THEORETICAL CONSIDERATIONS

The study of complex formation in solution, in common with most branches of chemistry, has a series of milestones, represented by the names of scientists, who contributed to the field. Werner was the founder of modern co-ordination chemistry and his paper on the stereochemistry of complexes[35] inspired Bodländer[36] and N. Bjerrum[37], early workers in solution chemistry. The next big advances were, the acceptance of the constant ionic media method (see later) and the work of J. Bjerrum and Schwarzenbach in the late 1930's and early 1940's, which was closely followed by the work of Leden, the theoretical work of the Rossotti's[38] and the incalculable contribution of Sillén, whose sad death was announced recently[39].

Consider the formation of a complex $A_pB_qH_r$. In aqueous solution; the complex will be associated with a number of water molecules and the formation can be represented by

$$pA(H_2O)_w + qB(H_2O)_x + rH(H_2O)_y \rightleftharpoons A_pB_qH_r(H_2O)_z + (pw + qx + ry - z)H_2O \qquad [1]^*$$

In practice the activity of free water is assumed constant and the water of hydration is omitted from equations

Hence $$pA + qB + rH \rightleftharpoons A_pB_qH_r \qquad (2)$$

* Nomenclature is given on page i.

The values of p, q and r are integers, positive or zero for p and q, but possibly negative for r as hydrolysed species are formed. The constant used to define the system in terms of activities is called the thermodynamic formation constant.

$$\beta^{o}_{pqr} = \frac{(A_pB_qH_r)}{(A)^p.(B)^q.(H)^r} \qquad [3]$$

$$= \frac{[A_pB_qH_r]}{[A]^p.[B]^q[H]^r} \cdot \frac{f_{A_pB_qH_r}}{pf_A.qf_B.rf_H} \qquad [4]$$

If the activity coefficients of all species are held constant then a new constant can be defined in terms of molar concentrations

$$\beta_{pqr} = \frac{[A_pB_qH_r]}{[A]^p.[B]^q.[H]^r} \qquad [5]$$

A third constant sometimes encountered is the mixed constant, so called because some terms are concentrations and some are activities (usually only the hydrogen ion is quoted as an activity).

The simplicity of these definitions does not extend into practice and many problems arise. The use of equation [4] is limited to systems where, either the activity coefficients can be calculated, and in systems where there are numerous stepwise species this is difficult, or where the results can be extrapolated to zero ionic strength. Equation [5] is used frequently, but the thermodynamics of the system can only be compared to other systems with a similar ionic background. Activity coefficients may be held constant by working in a solution of sufficiently

high ionic background such that ion pair effects are constant. This can be achieved by using a salt which contains ions having no complexing tendencies, and so the usual choice is sodium, or sometimes lithium, perchlorate. In monitoring the reaction by potentiometry, use is usually made of the Nernst equation

$$E = E^{o} + \frac{RT}{zF} \ln \frac{(K)^{k} (L)^{l}}{(M)^{n} (N)^{n}} \qquad [6]^{40}$$

(For reaction $kK + lL \rightleftharpoons mM + nN$)

This calls for a definition of unit activity (i.e. the standard state) of the species present.

For the glass electrode

$$E = E^{o} + \frac{RT}{zF} \ln (H) \qquad [7]$$

The choice of standard state is arbitrary and related to the selection of a standard solvent.

For a cell

$$- \text{Pt}(H_2) \left| \begin{matrix} X^- & ClO_4^- \\ H^+ & Na^+ \end{matrix} \right| \text{Ag, AgX} \quad +$$

If the standard solvent is water then

$$E = E^{o}_{aq} - \frac{RT}{F} \ln [H^+][X^-] - \frac{2RT}{F} \ln f$$

$$E^{o}_{aq} = \lim_{[H^+][X^-]\, C_{tot} \to 0} \left(E + \frac{RT}{F} \ln [H^+][X^-] \right) \qquad [8]$$

This implies,

$$\lim_{[H^+][X^-]\, C_{tot} \to 0} f = 1$$

(where CTOT = $\frac{1}{2}\sum_i Ci|Zi| = [X] + [ClO_4] = [H] + [Na]$ and

where f is the mean activity coefficient).

But, if the standard state is chosen as a solvent with a defined composition then

$$E = E_{NaX^o} - \frac{RT}{F} \ln [H^+][X^-] - \frac{2RT}{F} \ln f$$

$$E_{NaX^o} = \lim_{\substack{[H^+][ClO_4] \to 0 \\ [X^-][Na^+] \to C_{tot}}} \left(E + \frac{RT}{F} \ln [H^+][X^-]\right) \quad [9]$$

This implies,

$$\lim_{\substack{[H^+][ClO_4^-] \to 0 \\ [X^-][Na^+] \to C_{tot}}} f' = 1$$

where f' is the mean activity coefficient.

Hence these results obtained may be extrapolated to the standard solvent, but Biedermann[41] has shown this to be unnecessary as in 3.00 M $NaClO_4$, the sum of the concentrations of the ions in a reaction ($A^- + B^+ \rightleftharpoons AB$) does not exceed 0.15 M then the error in a potentiometric reading does not exceed $\pm$ 0.1 mV. This latter approach is the one used in this work.

The thermodynamic quantity obtainable from the stability constant is the Gibbs free energy of the reaction, which is related to the stability constant by the reaction isotherm[40].

$$\Delta G^o = - RT \ln \beta, \quad [10]$$

where ΔG^{o} is the standard Gibbs free energy, with 3.00 M sodium perchlorate as standard solvent. Further thermodynamic parameters can be obtained by determining β_{pqr} at different temperatures and then invoking the reaction isochore[40],

$$\left(\frac{\partial \ln \beta}{\partial T} \right)_{P} = \frac{\Delta H^{o}}{RT^{2}} \qquad [11]$$

$$\text{Integrating} \quad \ln \frac{\beta_2}{\beta_1} = - \frac{\Delta H^{o}}{R} \left(\frac{1}{T_2^{2}} - \frac{1}{T_1^{2}} \right) \qquad [12]$$

but this is assuming that ΔH^{o} does not vary with temperature. This is very often a false assumption[42], and so, the method is not reliable.

The enthalpy of formation can also be obtained, directly, by calorimetry, and by then using the Gibbs-Helmholtz equation[40],

$$\Delta G^{o} = \Delta H^{o} - T \Delta S^{o} \qquad [13]$$

values of the entropy of the reaction can be calculated. The enthalpy change is related to the difference in the bond and solvation energies of the products and reactants, whereas the entropy change is related to the change in randomness which accompanies the reaction.

Influence of the medium on the thermodynamic quantities.

The relationship between the quantities obtained in 3.00 M sodium perchlorate and the quantities, ΔG_{o}^{o}, ΔH_{o}^{o} and ΔS_{o}^{o}, referring to the more usual thermodynamic standard state, pure water, is worthy of consideration, if, for no other reason, at least to compare results for the same reaction in different media. The thermodynamics of the

formation of cadmium (II)-halide and -acetate complexes have been studied at various ionic strengths[43] (I) and it was found that the thermodynamic quantities are only slightly affected by changes of I in the range 0.3 - 3.0 M. The heat of ionisation of water (ΔH_W) has also been determined at various ionic strenths[44,45] and this quantity varies linearly with I in the range 0.5 - 3.0 M. ΔG^o, however passes through a minimum at I≈0.5, which is due to a sharp increase in the ΔS^o term up to .5M (fig.6a). This minimum has also been noted by Dyrssen et al for several other systems[46] (Figure 6b).

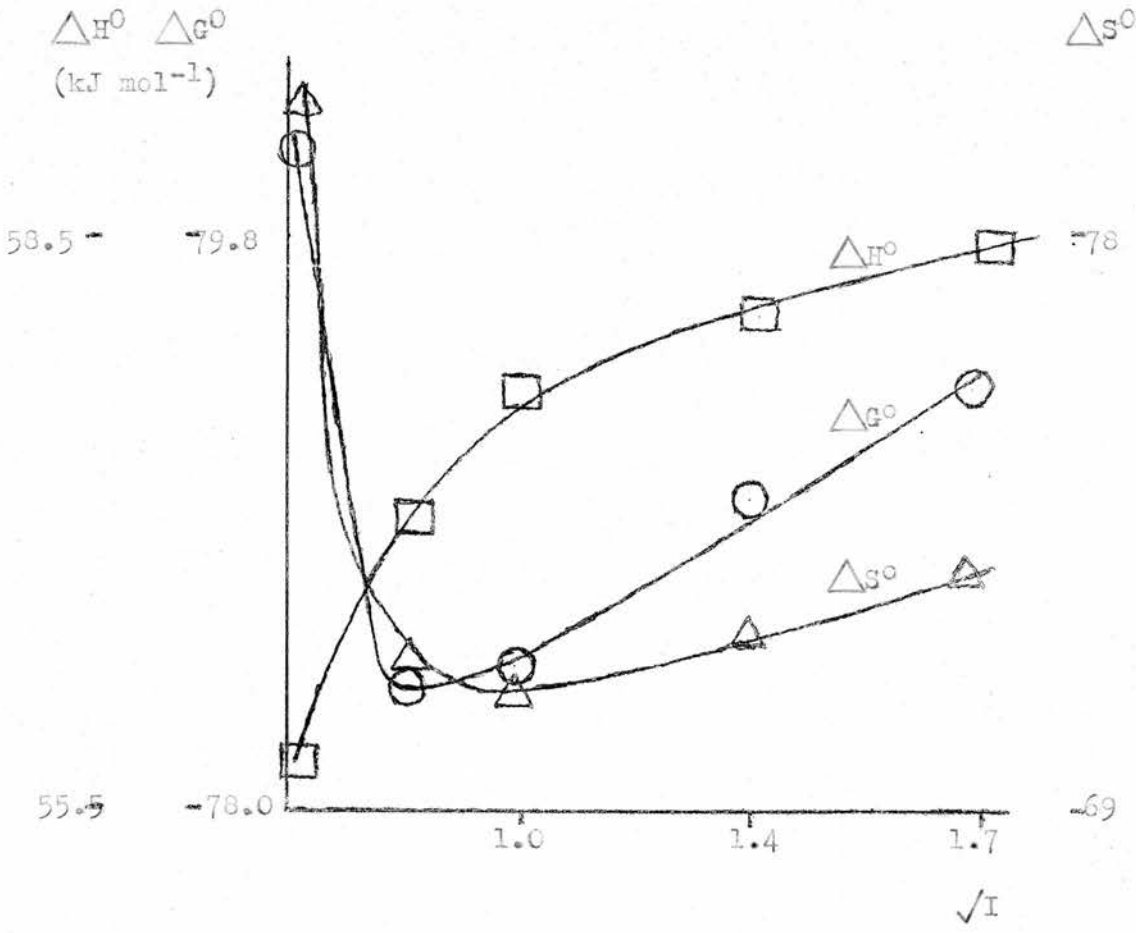


Figure 6a.
Variation of ΔG^o_W, ΔH^o_W and ΔS^o_W with ionic strength.

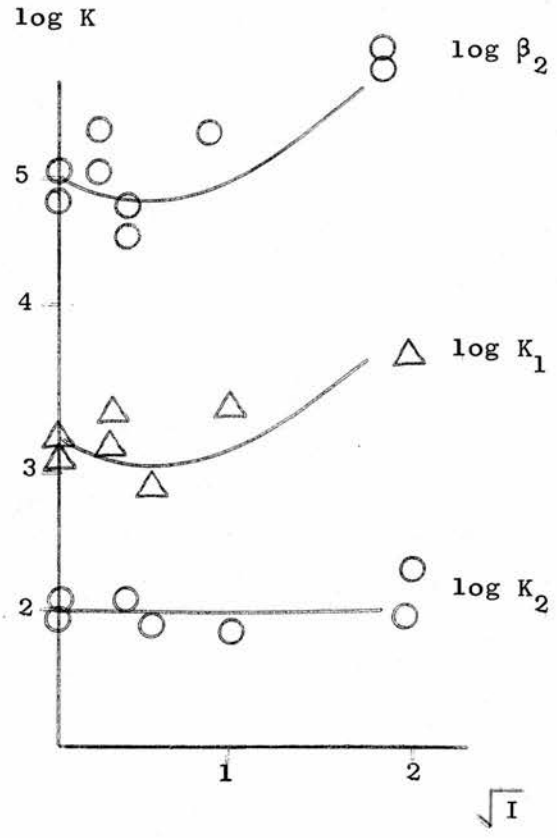


Figure 6b.
Variation of $\triangle G^o$, $\triangle H^o$ and $\triangle S^o$ with $\sqrt{I}$ for Ag^+ Cl^- system

The significance of the thermodynamic quantities:-

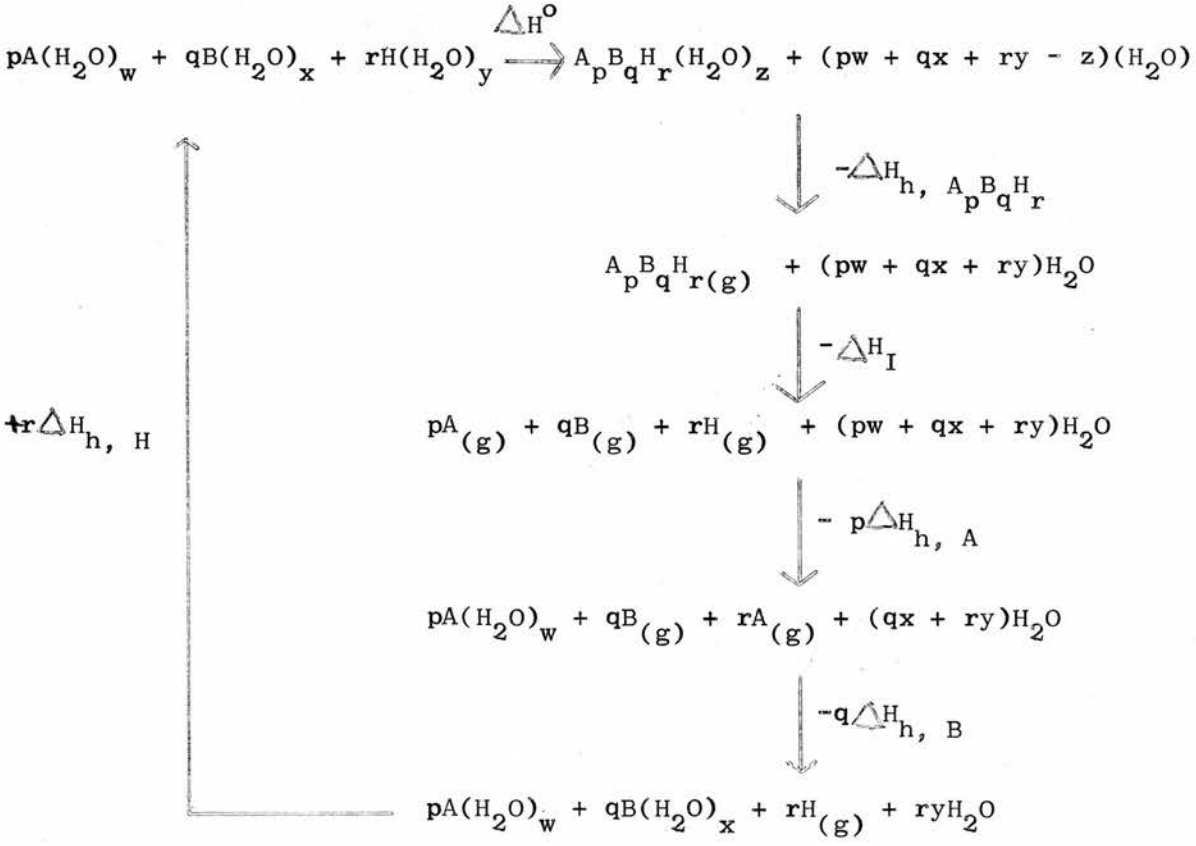
Enthalpy.

Complex formation is favoured by negative heat changes. Enthalpy changes, which are independent of the composition as a whole[47], in aqueous solution are the heat changes which accompany the replacement of water by other ligands. Large ($\sim$ -40 kJ mol^{-1}) heat changes are attributed to the formation of, essentially, covalent bonds. The change

in standard enthalpy, $\triangle H^{o}$, can be considered to consist of two portions, $\triangle H_{I}$, the internal part, and $\triangle H_{E}$, the environmental part. $\triangle H_{I}$ can be considered to be the heat change for the reaction

$$pA_{(g)} + qB_{(g)} + rH_{(g)} \rightleftharpoons A_pB_qH_{r(g)} \qquad [14]$$

To evaluate $\triangle H_{I}$ it is necessary to determine the heats of hydration of the ligand, the metal ion, the proton and the complex as is in figure 7.



Where $\triangle H_{h,\ A_pB_qH_r}$, $\triangle H_{h,\ B}$, $\triangle H_{h,\ A}$ and $\triangle H_{h,\ H}$ are the heats of hydration of $A_pB_qH_r$, B, A and H respectively.

Figure 7
Determination of the internal part of $\triangle H^{o}$.

ΔH_I is then given by Hess' law

$$\Delta H_I = \Delta H^o + q\Delta H_{h,\,B} + p\Delta H_{h,\,A} + r\Delta H_{h,\,H} - \Delta H_{h,\,A_pB_qH_r} \qquad [15]$$

and the environmental part by

$$\Delta H_E = \Delta H^o - \Delta H_I \qquad [16]$$

The heats of hydration of several metal ions have been reported[48], but values of the heats of hydration of ligands, and of complexes, are much more difficult to obtain. Values for ligands can be obtained from thermodynamic cycles, which involve dissolving the ligand in acid solution. Unfortunately, an experimental value of the electron affinity of the ligand is required[49], and few values exist. At the present time there is no satisfactory way of evaluating the heats of hydration of complex ions, however, values can be calculated using the Born charging equation[49]

$$\Delta H_h = - \frac{10.Z^2.e^2}{2.R} \left(1 - \frac{1}{D} + \frac{T}{D^2} \cdot \left(\frac{\partial D}{\partial T} \right)_P \right) \qquad [17]$$

where Ze = product of charge on ion and electronic charge

R = radius of central ion plus diameter of water molecule, nm,

D = dielectric constant of medium

T = temperature, K.

The values obtained from using equation [17] should be considered approximate, but can give a guide to trends along a series of metal ions or of similar ligands.

Entropy.

The change in entropy on complexation of a metal ion is very dependent upon the ligand. If the ligand is uncharged, then solvent will be less ordered around the complex than the metal ion, but if the ligand is charged then, on complexation, there will be a decrease in the number of ions, neutralisation of the electrical charge, attenuation of the remaining charge, and displacement of water from the hydration spheres of the reactants. For a chelating ligand, further factors, such as loss of configurational entropy, must be taken into account. Reactions are favoured by an increase in entropy and so the factors favouring complexation are a decrease in the number of ions, neutralisation of charge, and a less ordered solvent. Those factors inhibiting the formation of complexes are the loss of translational entropy to vibrational and rotational entropy, and the loss of rotational entropy for polyatomic ligands. For most complex formation reactions, however, the ligational entropy changes are positive[50,51].

Gibbs Free Energy

The Gibbs free energy is the criterion for the thermodynamic feasibility of a reaction and, for a reaction to proceed, ΔG^{o} must be negative. Its dependence on the enthalpy and entropy is shown in equation [13] and so a reaction is possible with either unfavourable entropy or unfavourable enthalpy as long as the favourable variable is predominant.

Table II[50]

Dependence of ΔG^{o} upon ΔH^{o} and ΔS^{o}.*

AB	$-\Delta G^{o}$	ΔH^{o}	ΔS^{o}
$[CaOOCH]^{+}$	8.11	4.17	41.8
$[CaOOCCH_3]^{+}$	7.07	3.76	37.6
$[CeSO_4]^{+}$	19.20	19.66	129.6
$[MgOOCH]^{+}$	8.11	-7.5	4.2
$[MgOOCCH_3]^{+}$	7.07	-6.27	4.2
$[CeClO_4]^{2+}$	10.87	-49.35	-129.6

*ΔG^{o} and ΔH^{o} in kJ mol^{-1}; ΔS^{o} in J K^{-1} mol^{-1}

It can be seen from Table II that a negative ΔG^{o} is not sufficient to describe a reaction and ΔH^{o} and ΔS^{o} are valuable parameters to determine.

Methods of Calculation

In considering the formation of the complex $A_pB_qH_r$ (Equations [1] and [2]) the Gibbs free energy change is given by equation [10].

$$\text{i.e. } \Delta G^{o} = -2.303\ RT \log \beta_{pqr}$$

Hence ΔG^{o} can be obtained directly from a knowledge of the formation constant. In this study, the formation constants were obtained by following the hydrogen ion concentration during a titration of the

reactants A and B. For initial calculations, the function $\bar{Z}$, defined as the number of ligands bound to each central group[38], was plotted against -log a, which is known as the "formation curve". This treatment of data is only useful for mononuclear complexes as, for polynuclear complexes, (including protonated species) the function $\bar{Z}$ has little signficance. Mathematically $\bar{Z}$ is defined as

$$\bar{Z} = \frac{[AB] + 2[A_2B] + ...}{[B] + [AB] + [A_2B] + ...} = \frac{\sum_1^N n\beta_n a^n}{\sum_0^N \beta_n a^n} \qquad [18]$$

Practically, $\bar{Z}$ can be determined from a knowledge of the total concentrations of A, B and H in solution, the values of the formation constants of any species AH_r and the free hydrogen ion concentration, h.

$$\text{From [18] } \bar{Z} = \frac{\text{Bound ligand concentration}}{\text{Total metal concentration}}$$

Bound ligand concentration = Total ligand - (Free ligand + protonated ligand

$$= A-(a + \sum_1^N a\beta_n h^n)$$

$$= A-a(1 + \sum_1^N \beta_n h^n) \qquad [19]$$

But the free ligand concentration is obtained from a mass balance equation

$$H = h - \frac{h}{W_k} + \sum_1^N n[AH_n]$$

$$= h - \frac{h}{W_k} + \sum_1^N an\beta_n h^n$$

$$\text{i.e. } a = (H - h + h/W_k)/\sum_1^N n\beta_n h^n \qquad [20]$$

hence from [19] and [20] $\bar{Z}$ can be obtained.

The transformation of $\bar{Z}$, -log a plots into formation constants, by graphical means, has been extensively reviewed by Rossotti and Rossotti[38]. The methods used in this study were numerical methods using high speed digital computers (see Chapter III). The aim was to reproduce the experimental $\bar{Z}$, -log a curve using constants obtained from the computer programs.

The change in enthalpy, ΔH^{o}, was measured directly by calorimetry. The concentrations of the species in the calorimeter were determined by using HALTAFALL[52], a computer program, and then changes in concentration, together with the corrections necessary for the heats of formation of water, of deprotonation and of hydrolysis were calculated using RWCALCOR (Chapter III and Appendix 1).

This treatment yields the experimental parameters, heat evolved and change in concentration of species under study. From this the change in enthalpy can be calculated numerically (Chapter III) or graphically. A plot of heat evolved per mole of metal against the degree of formation, $\bar{Z}$, will yield ΔH_1^{o} at $\bar{Z} = 1$, ΔH_2^{o} at $\bar{Z} = 2$ etc., for simple systems with well separated heats.

ΔS^{o} can now be calculated from equation [13].

CHAPTER III COMPUTATIONAL ASPECTS

The advent of the computer has had a profound effect on the chemists' approach to numerical problems. It would not be an exaggeration to say that crystallography has been revolutionised, and even menial tasks, such as drawing mass spectrometry spectra, are lending themselves to programing[53].

Crystallography was the first branch of chemistry to use the computer nearly twenty-years ago[54] as the type of calculation carried out was fairly straight forward, but long and tedious. Perhaps the biggest strides taken in chemistry, using a computer, have been in the field of quantum mechanics. The large storage space and the speed at which calculations are performed in the latest generation of computers have enabled quantum chemists to tackle hitherto impossible calculations[55].

Computers are used extensively in analytical chemistry[56] for processing data obtained by physical methods. Chromatography, mass spectrometry, nmr and chemical literature lend themselves to the setting up of libraries of information for future comparison and retrieval. Phase equilibria[52], nmr[57], esr[58] and calorimetry[58] have prompted programs where trial parameters are used to produce simulated distribution curves, spectra and thermograms respectively. General curve analysis is also employed in spectroscopy and electro-analytical chemistry. Another branch of chemistry where the computer has had a large impact is in the calculation of formation constants by various numerical approaches. These computations have been the

subject of two recent reviews[60,61] and have inspired a great deal of interest in solution chemistry in recent years.

Many programs, of varying complexity, have been written to determine formation constants, and the type of program chosen depends upon several factors. For simple systems, A_pB or H_rA, non statistical programs are adequate[62], although, if the constants are overlapping a linear least squares technique is more applicable. For more general systems, i.e. those involving polynuclear, hydrolysed or protonated species, a more general non-linear least squares program must be used. The availability of a high speed digital computer with a large storage capacity is a criterion for the use of the latter type of program, as also, is the necessary time needed for familiarisation with the use of the program. As the present work included a search for protonated and hydrolysed species, a general program was required to calculate the stability constants. Two such programs, SCOGS[63] and LETAGROP VRID[64-68], have been described in the literature and both were available for use. LETAGROP was stored on the Atlas computer at Chilton and SCOGS on the St. Andrews IBM 360/44, hence, for geographical reasons, most of the present work used SCOGS.

Both programs depend up Taylor's series, a mathematical method of expressing a function in terms of its derivatives.

If $y = f(k_r; a_r)$ where $r = 1$ to M [1]

where y = a measured quantity

a_r = variable, but accurately known, quantities.

k_r = unknown constants.

Then Taylor's series gives an expression

$$U = U_o + \sum_1^M \left(\frac{\partial U}{\partial k_r}\right) \delta k_r + \sum_1^m \left(\frac{\partial \left(\sum_1^M \left(\frac{\partial U}{\partial k_r}\right) \delta k_r \right)}{\partial k_r} \right) \qquad [2]$$

where U is the error square sum

$$U = \sum_i \left(y - f\left(\sum_{r=1}^M k_{r,i} \; ; \; \sum_{r=1}^M a_{r,i} \right) \right)^2$$

The "best" values are those which minimise this sum i.e. U_o. At this point the two programs begin to differ in rigour.

LETAGROP VRID

In this program equation [2] is the equation of a "pit" with U_o the minimum point. The values of k_r are incremented to give not only the minimum point, U_o, but also a map of the surroundings. The function y is $\bar{Z}$ and so the quantity minimised is

$$U = \sum_i (\bar{Z}_{calc} - \bar{Z}_{expt})^2 \qquad [3.L]$$

Equation [2] represents a generalised ellipsoid in (M + 1) dimensional space. A measure of the spread of data around U_o is enclosed in the "D boundary"[64].

$$U = U_o + \frac{U_o}{n-M} \qquad [4]$$

When n is the number of experimental points and as generally n>>M

$$U \approx U_o + \frac{U_o}{n} \qquad [5]$$

The D boundary is, again, a generalised ellipsoid but this time in M dimensional space with the "least squares" point (k_r") at its centre. The "standard deviation" can be calculated from the D boundary because it is identical with the range of values each k_r can assume on the boundary. The VRID block[67] was added to overcome the problem of a "skew pit", which arises from correlation of shifts and causes incorrect convergence. The shifts are altered by a "twist matrix" so that they are made along the axes of the skew pit rather than the co-ordinate axes.

SCOGS (appendix 2)

This is a more general program than LETAGROP VRID, when applied to stability constant work, because it can deal with up to twenty complexes of the type $A_pA'_{p'},B_qB'_{q'},H_r$. For the jth complex this leads to a stability constant

$$\beta_j = \frac{[A_{p_j}.A'_{p'_j}.B_{q_j}.B'_{q'_j}.H_{r_j}]}{[A]^{p_j}[A']^{p'_j}[B]^{q_j}[B']^{q'_j}[H]^{r_j}} \qquad [6]$$

$$\text{or } C_j = \beta_j.a^{p_j}.a'^{p'_j}.b^{q_j}.b'^{q'_j}.h^{r_j} \qquad [7]$$

(NOTE:- There is provision for the use of the activity coefficient of the hydrogen ion and hence SCOGS can give mixed constants.)

For N complexes

$$A = \sum_1^N (a + p_j\beta_jC_j) \qquad [8]$$

$$A' = \sum_1^N (a' + p'_j\beta_jC_j) \qquad [9]$$

$$B = \sum_{1}^{N} (b + q_j \beta_j C_j) \qquad [10]$$

$$B' = \sum_{1}^{N} (b' + q'_j \beta_j C_j) \qquad [11]$$

If a, a', b and b' were known then A, A', B and B' would be the experimental values. But, as they are not known, let

$$f(a) = A_{expt} - A \quad \text{(equal to zero for true values of a, a', b and b')}$$

$$= A_{expt} - (a + \sum_{1}^{N} p_j \beta_j C_j) \qquad [12]$$

similarly

$$f(a') = A'_{expt} - (a' + \sum_{1}^{N} p'_j \beta_j C_j) \qquad [13]$$

$$f(b) = B_{expt} - (b + \sum_{1}^{N} q_j \beta_j C_j) \qquad [14]$$

$$f(b') = B'_{expt} - (b' + \sum_{1}^{N} q'_j \beta_j C_j) \qquad [15]$$

Values of a, a', b and b' (and hence C_j; equation [7]) are now obtained by a Newton-Rapheson iterative method. This minimises the functions [12]-[15] by calculating shifts that will simultaneously minimise all four functions. Progressing through each point, least squares equations are built up and solved, by matrix inversion, to yield shifts in constants. These shifts are obtained from Taylor's series but unlike the "pit mapping" procedure, only the first order terms are used. The quantity minimised is

$$U = \sum_{i} (Titre_{expt} - Titre_{calc})^2 \qquad [3.S]$$

The calculated titre is obtained from the current set of constants and the experimental value of the hydrogen ion concentration.

The original SCOGS has been amended somewhat (Appendix 2), mainly in input and output. The input has been generalised to deal with any titrant, and the output contains several calculated functions, such as $\bar{Z}$. The size of the program has been increased to simultaneously refine up to thirty individual experiments or a total of five hundred readings.

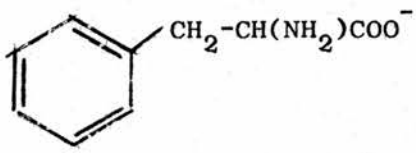
SCOGS or LETAGROP

Although both the Gaussian and the "pit mapping" approaches have been in use for several years, no conclusions have been reached as to which method is "best". One criterion used to differentiate between them, is the range of the initial guess of each β required. Naturally Sillén favoured "pit mapping" and Perrin favours Gaussian[60], but Tobias[70], who could be assumed to be unbiased, as he has used both methods, favours the Gaussian approach. Further criteria are the rigour of the mathematics, the ability to refine difficult systems and the avoidance of the false minimums, but the ultimate criterion must be the reproduction of experimental parameters from the constants obtained. LETAGROP was devised as a "supplement to graphical methods"[65], but since 1962 it has become a method in its own right; whereas SCOGS was conceived as a numerical method and, if it is used correctly, should be the faster method. It must not be forgotton, however, that LETAGROP has a wide applicability that extends far beyond potentiometry.

The two programs were compared with two sets of data of a different nature.

(a) Phenylalanine

This data was obtained from a study of the protonation of the ligand



The collection of the data is discussed in Chapter V. The data obtained was (-log h, titre) and for input into LETAGROP this was processed, by RWZASCOG (Appendix 2) to ($\bar{Z}$, - log h). The same ninety-five readings were used in the two programs to determine constants for the following equilibria

$$H + A \rightleftharpoons HA \qquad K_{a_1} = \frac{[HA]}{[H][A]}$$

$$H + HA \rightleftharpoons H_2A \qquad K_{a_2} = \frac{[H_2A]}{[H][HA]}$$

The results obtained are given in Table III.

Table III

Formation constants of Phenylalanine

	SCOGS	LETAGROP
pK_{a_1}	9.610 ± 0.005	9.583 ± 0.011
pK_{a_2}	2.754 ± 0.011	2.728 ± 0.016

As a further comparison, the values of $\bar{Z}$ calculated from the SCOGS "constants" were run, with the experimental -log h values, on LETAGROP

and the following constants were obtained

$$pK_{a_1} = 9.587 \pm 0.011$$

$$pK_{a_2} = 2.684 \pm 0.016$$

The results obtained from LETAGROP, for both the experimental $\bar{Z}$ and the values obtained from the SCOGS "constants", show no significant difference. It must be concluded, therefore, that the two programs give consistent results for this data with an error of $\pm$ 0.016.

(b) Acetic acid

The data used is a potentiometric study of the dimerisation of acetate[71]. The following are the equilibria involved and the published results

$$[H] + [A] \rightleftharpoons [HA] \quad B_{11} = 9.234 \times 10^{6} \ M^{-1}$$

$$[H] + 2[A] \rightleftharpoons [HA]_2 \quad B_{12} = 1.111 \times 10^{7} \ M^{-2}$$

$$2[H] + 2[A] \rightleftharpoons [H_2A_2] \quad B_{22} = 2.49 \times 10^{13} \ M^{-3}$$

These results were obtained by both graphical methods and by using LETAGROP[71]. When the same experimental data was used in SCOGS no convergence could be obtained despite using a variety of values for the input constants. Convergence was obtained when only two constants were used, but the standard deviation was so large that the results were meaningless.

This lack of convergence was examined more closely by using a simulated set of data obtained from HALTAFALL. This data was designed such that a metal-ligand interaction was examined, as in SCOGS' original

conception, and that two of the constants should lie close to one another.

(c) Simulated Data

The hypothetical system consisted of five equilibria including the phenylalanine equilibria shown in (a). The remaining equilibria and constants were

$$A + B \rightleftharpoons AB \quad \log \beta_1 = 6.990$$

$$2A + B \rightleftharpoons A_2B \quad \log \beta_2 = 7.041$$

$$3A + B \rightleftharpoons A_3B \quad \log \beta_3 = 13.792$$

Nine experiments were simulated giving a total of ninety-nine readings to be refined together.

Table IV

Simulated data for the testing of SCOGS. Initial concentrations

Metal concentration*	Ligand Concentration*
20.0	30.0
20.0	20.0
20.0	10.0
10.0	30.0
10.0	20.0
10.0	10.0
5.0	30.0
5.0	20.0
5.0	10.0

*concentration in mM.

For all simulated titrations, the initial volume was 24.94 ml, the

titrant was sodium hydroxide (0.100 M) and each addition was of 2.00 ml stepwise up to a total of 20.00 ml.

Convergence was unobtainable using SCOGS demonstrating that the program is suspect when formation constants lie close together.

A FORTRAN version of LETAGROP[72], LGVRID, became available towards the end of this work and, although the formation constants obtained were the same as from SCOGS, the program seems particularly sensitive to the parameter stegbyt[73] as this parameter controls the search for a minimum. The program must still be considered to be under development.

LETAGROP cannot be faulted in any way except in the complexity of its use and the understanding of its mechanics. This complexity is caused by its wide applicability to solution chemistry problems, and as such cannot be a valid criticism. SCOGS, however, can be faulted in its inability to refine constants which are close to each other, and when dealing with systems, such as the formation constants of D- and L-amino acid - metal complexes, then SCOGS should not be used.

HALTAFALL

This program has been used for predicting concentrations of species, from formation constants, previously in this laboratory[74].

RWCALCOR (Appendix 1)

From an input consisting of the concentrations of the complexes under study, obtained from SCOGS or HALTAFALL, and the experimental heat

this program calculates the change in concentration of each species and heat corrections. Up to twenty complexes of the type $A_pB_qH_r$, where p, q and r are positive or zero, but r can also be negative, can be handled, but the core capacity of the I.B.M. 360/44, limits the number of points per experiment to twenty-five. The program can be used to determine heats of protonation, heats of hydrolysis and heats of formation of $A_pB_qH_r$ simultaneously but this is not regarded as the best use of the program. Experiments should be designed to obtain the heats of formation of AH_r and B_qH_r (where r is -ve) separately, whence, these values can be used to calculate corrections for experiments to obtain the heats of formation of the more complex, $A_pB_qH_r$, species. The output, pertinent to the calculation of heats of formation, is the corrected experimental heat, Q, associated with the formation of s complexes, and the change in concentrations, n_s, of these complexes. For the Nth point

$$Q_N = \sum_{1}^{s} (\Delta H_s . \Delta n_s)_N$$

where Q_N is in joules

ΔH_s is in J mol^{-1}

Δn_s is in mol

For s complexes, s values of N are needed to solve for the heats of formation but, generally, more values than this are determined experimentally and then the following "least squares" method can be used.

Suppose the "best" heats are $\Delta H'_1, \Delta H'_2 \ldots \Delta H'_3$

$$\text{i.e.} \quad \sum_{1}^{s} (\Delta H'_s . \Delta n_s)_N - Q_N = 0$$

Then let $F = \sum_{1}^{M} (Q_N - \sum_{1}^{s} (\Delta H'_s \Delta n_s)_N)^2$

The "best" values of the heats are obtained by minimising the function

$$\frac{\partial F}{\partial (\Delta H'_s)} = 0 = - \sum_{1}^{M} (2\Delta n_s . (Q_N - \sum_{1}^{s} \Delta H'_s . \Delta n_s)).$$

There will be s functions of this type that can be solved as s simultaneous equations, to give values of $\Delta H'_s$ which are the "best" constants for the set of experimental results, 1 to M.

A program RWSOLV (appendix 3) was available to calculate ΔH_s's in this manner, it was also added on to the end of RWCALCOR to facilitate speedy calculation. The three versions of RWCALCOR that were used in this work were RWCALCR1, which does not contain RWSOLV, RWCALCR2, which calculates the heats of formation for each experiment individually and RWCALCR3, which gives heats of formation for all the experiments combined together.

RWZASCOG (Appendix 2)

This, too, was an established program but the input was altered to make it consistent with that of SCOGS.

Whilst considerable advantages can be ascribed to the use of a computer, there are numerous pitfalls. A degree of knowledge of computer languages is necessary to avoid simple, time consuming errors such as format errors. Access to the computer should be good, so as

to be able to take advantage of the peripheral facilities and so evade unnecessary duplication of punching, program language and system knowledge. The use of "trial parameter" programs, such as SCOGS and LETAGROP, is a technique in its own right and has to be mastered. The usual criterion in this type of program is that, the set of constants giving the smallest standard deviation is the "best" set of constants to describe the experimental data. But, this criterion must not be imposed too rigourously as the possibility of obtaining a constant that describes a chemically non-sensical species or a constant that gives concentrations of species that have no physical significance are all too obvious. A knowledge of the program combined with a degree of chemical intuition and knowledge can give meaningful results, although the danger always exists of misinterpretation of data due to a bias either way. The editing of data is a problem for all experimenters and the recognition of poor results is as important as the interpretation of good results. Editing should be confined to pruning results from areas of low experimental accuracy.

To chemists the computer has become as important a tool as some spectroscopic techniques. The speed of calculation and the use of iterative procedures are useful and relevant to chemical calculations and whilst the computer is not indispensible it is certainly a great boon.

CHAPTER IV EXPERIMENTAL TECHNIQUES

Potentiometry and calorimetry were carried out in special vessels (Chapters V and VI respectively), and using solutions prepared and analysed by the following methods.

Water:-

All the water used for solutions was 'Elgastat' deionised, boiled and cooled by the passage of oxygen-free nitrogen. The resistivity of water was then better than 2 $M\Omega cm^{-1}$.

Sodium Perchlorate:-

This had to be used in pure state, as an impurity of 1 part in 10^5 could give an error of 1%, when dealing with 1 mM metal ions. Solutions of sodium perchlorate were made by, either dissolving the monohydrate (Merck 'Puriss') in water, or by neutralising perchloric acid (Fisons A.R.) with sodium carbonate (Fisons A.R.). The solution (> 6.00 M) was then filtered through a sintered glass funnel (porosity 4), its pH adjusted to 9-10 and allowed to stand for a minimum of seven days. The precipitate of silica and heavy metal oxides and hydroxides was removed by filtration through a micropore (450 nm pore diameter) filter (Millipore Ltd.). Carbon dioxide was removed by making the solution acidic ($\sim$pH 2) and boiling then cooling under nitrogen. From this point two alternatives were possible i.e. either crystals of $NaClO_4$ were made or a standard solution was prepared and analysed.

(i) Crystals:- These were prepared from the above solution by adjusting its pH to 7 and heating in an evaporating basin to 140°C. After cooling to 105°C, the slurry was filtered through a sintered glass funnel (porosity 3) and dried in an oven at 110°C. The pH of

a 3.00 M solution was in the range 5.5 to 7.0.

(ii) Standard solution:- After adjusting the pH to 7 the solution was analysed by cation exchange[75a] and flame photometry[75b].

Perchloric acid:-

Concentrated perchloric acid (Fisons A.R.) was diluted to make a primary stock solution of ~1 M, which was further diluted to 0.1 M to be used as a working stock solution. Both solutions were analysed by titrating against sodium carbonate (Fisons A.R.) (methyl orange as indicator)[75c], and checked with standard sodium hydroxide solution[75d].

Sodium hydroxide:-

1.00 M and 0.100 M solutions were obtained from ampoules (B.D.H. concentrated volumetric solutions) and their molarity was checked against standard acid solution[75d] and standard potassium hydrogen phthalate (Fisons A.R.)[75e].

Metal Solutions:-

Metal perchlorates (G. Frederick Smith, Chemical Co.) were dissolved, allowed to stand for several days, filtered through micropore filters (450 nm) and analysed by two independent methods.

Copper (II): Electrodeposition[75f] and EDTA[75g] (Fast sulphon black F.)

Nickel (II): Electrodeposition[75h] and EDTA[75i] (murexide)

Cobalt (II): Electrodeposition[75j] and EDTA[75k] (xylenol orange)

Iron (III): Jones reductor[75l] and sulphurous acid[75m] followed by titration with standard potassium dichromate (Fisons A.R.)[75n] (sodium diphenylamine as indicator).

Commercial Iron (II) perchlorate was analysed for purity and was found to contain up to 4% iron (III), hence, iron (II) perchlorate was prepared by dissolving iron sponge (Johnson Matthey Chemicals 'Specpure') in standard perchloric acid[76]. The solution was analysed by oxidation with potassium dichromate[75n] and the results obtained by this method agreed with those obtained from the weight of iron dissolved. Solutions prepared in this manner were stable for up to a week but when made 3.00 M in $(Na)ClO_4$, significant oxidation had occured in 24 hours.

The concentration of hydrogen ions in the metal solutions was obtained by means of Gran plots[77].

EDTA:-

Solutions were made up from ampoules (B.D.H. concentrated volumetric solutions) and their molarities checked against magnesium chloride (Fisons A.R.)[75m]

Phenylalanine:-

DL-β-phenylalanine was obtained commercially (B.D.H. Biochemical grade), dried, and subjected to analysis C, 65.25; H, 6.92; N, 8.47 (Calculated C, 65.45; H, 6.67; N, 8.57%) mp. 284°C. It was, thus, used without further purification.

Nitrogen:-

Oxygen-free nitrogen (British Oxygen) was further de-oxygenated by passage through chromous chloride and then "scrubbed" in 3.00 M sodium perchlorate, both of which were thermostated at 25°C.

Solutions were analysed prior to the ionic background being made 3.00 M due to difficulties of analysis in this medium. All solutions were stored under nitrogen and sealed with "Parafilm" (Gallenkamp).

Apparatus:-

Volumetric apparatus ('E-mil (Green line)' M.J. Elliott) was provided with calibration certificates. Several calibrations were checked but all were found to agree with the certificates. The apparatus was calibrated at 20°C hence all solutions were stored at this temperature before use.

All apparatus was cleaned regularly with 'Quadralene' (Quadralene Chemical Products) and alcoholic potassium hydroxide. Before use, apparatus was washed with demineralised water, 'Elgastat' water, alcohol and anaesthetic ether and then dried by suction.

CHAPTER V POTENTIOMETRY

The determinations were carried out at 25.0°C and with an ionic background of 3.00 M in (sodium) perchlorate. The hydrogen ion concentration was followed by using a cell of the type

$$Hg, Hg_2Cl_2 \;\big|\; \text{satd. NaCl} \;\big|\; \text{test solution} \;\big|\; \text{glass electrode}$$

A sodium chloride salt bridge was employed because potassium ions form insoluble potassium perchlorate in the porous plug of the calomel electrode. The electrode pair (Activion; glass, 17SB; calomel, RCB) and a Beckman research pH meter were used, to give readings reproducible to 0.1 mV. A toughened glass electrode (Activion 27SB) was tested but the linear response to -log h was limited to the range 2-9, which was too narrow for the present study.

The reaction cell, figure 8, contained ligand, acid, metal perchlorate and sufficient sodium perchlorate to produce an ionic background of 3.00 M (Na) ClO_4. Polynuclear formation and hydrolysis were investigated by varying -log h, titrimetrically, for different concentrations and ligand to cation ratios.

The electrode pair was calibrated by a titrimetric procedure, whence the ionisation constant of water, K_w could be checked.

The emf of the above cell is given by

$$E = E^o + \frac{RT}{zF} \ln h + E_j$$

$$\text{i.e } E = E^o + 59.162 \log h + E_j \text{ at } 25^oC \qquad (1)^{41}$$

Where E is the emf

E^o is a constant

and E_j is the liquid junction potential.

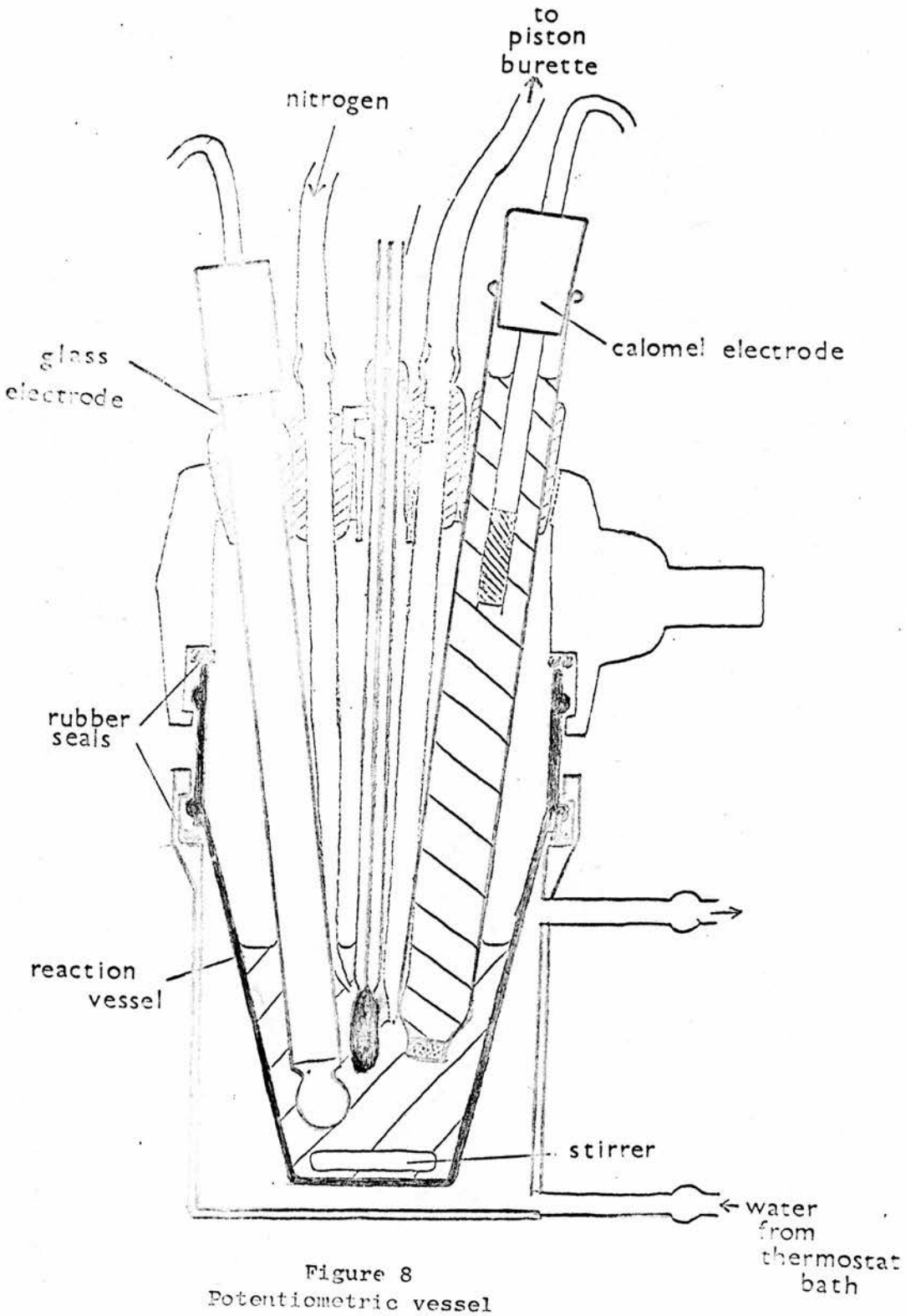


Figure 8
Potentiometric vessel

If E_j is constant then a plot of E vs -log h ought to give a straight line of slope -59.162 mV$(-\log h)^{-1}$. Values of -log h > 7 were calculated using a value of -log K_w = 14.22[78].

Table V. Electrode calibration

24.98 ml perchloric acid (7.245×10^{-3} M) titrated with sodium hydroxide (0.5000 M) using an 'Agla' syringe.

ml added	mV	-log h
0.000	309.9	2.140
0.270	268.1	2.740
0.325	233.2	3.137
0.340	200.1	3.363
0.350	69.9	3.628
0.355	-101.5	3.863
0.360	-148.4	4.417
0.370	-183.6	10.411
0.390	-209.3	10.939
0.460	-239.3	11.495
0.500	-246.9	11.647
Standard solutions of h		
	364.5	1.142
	329.4	1.744
	240.4	3.251

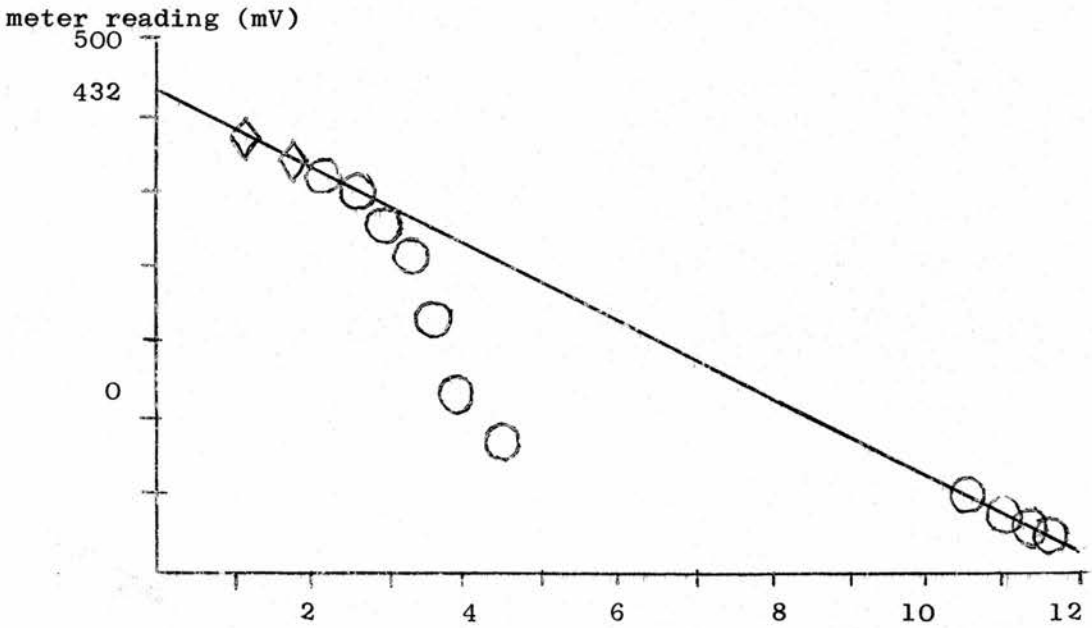


Figure 9. Electrode calibration curve.

As can be seen from figure 9 this plot is linear in the range -log h = 1.2 to 3.0 and again from 10.0 to 11.6. The deviation from linearity in the region 3.0 to 10 is due to unbuffered solutions, and has been observed previously by Williams and Williams[79]; a linear response was obtained in buffered solutions. E_j was assumed constant in the range -log h = 1.2 to 11.6 due to the linearity of the plot in this region.

Formation constants of phenylalanine

As the accuracy of the subsequent metal-ligand complex formation study was to depend upon the accuracy of the pK's of the ligand the following procedure was adopted.

(1) All titrations were performed at constant ligand concentration (i.e. A = constant)

(2) Each protonation was studied independently.

(3) Only values of $\pm$ 0.25 either side of $\bar{Z}$ = 0.5 or 1.5 were used for calculation of pK's[80].

The formation curve (Figure 10) was constructed using a method, similar to that for $\bar{Z}$ (Chapter III), as in the computer program in appendix 3. The formation curve can be seen to be independent of the ligand concentration and polynuclear species were therefore assumed absent.

The data was then analysed using SCOGS (appendix 2) and the results obtained were

$$pK_{a_1} = 9.6097 \pm 0.0018$$

$$pK_{a_2} = 2.7544 \pm 0.011$$

s (in titre) = 0.196 (95 readings)

The effect of experimental error on the pK_a's was investigated and the results summarized in Table VII.

Table VI

Experimental results for the protonation of phenylalanine

(a)

Titration	Titrate (S) molarity		Titrant (T) molarity		Volume ml	E^{o} mV
	A	H	A	H		
1	0.0500	-0.0100	0.0500	0.0500	14.98	433.7
2	0.0500	0.0500	0.0500	0.0195	14.98	433.7
3	0.0500	0.0500	0.0500	0.0195	15.00	444.6
4	0.02498	0.02498	0.02498	0.01448	15.00	433.7
5	0.02498	0.02498	0.02498	-0.00998	15.00	433.7
6	0.00999	0.00999	0.00999	-0.01179	15.00	433.7
7	0.00999	-0.01179	0.00999	0.01149	15.00	444.6
8	0.00999	0.00999	0.00999	-0.01149	15.00	444.6
9	0.00499	0.00499	0.00499	0.01047	15.00	433.7
10	0.00499	0.00499	0.00499	-0.01089	15.00	433.7

Table VI

(b)

1		2		3		4	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
8.00	-168.4	1.50	243.6	1.50	254.1	1.00	248.9
9.00	-163.2	2.00	253.0	2.00	263.4	1.50	263.0
10.00	-159.4	3.00	267.3	3.00	277.5	2.00	273.7
11.00	-155.9	4.00	278.6	4.00	288.6	3.00	290.6
13.00	-150.0	5.00	288.7	5.00	298.2	4.00	303.5
15.00	-145.4	6.00	296.6	6.00	306.9	5.00	313.6
17.00	-141.3	7.00	304.3	7.00	314.6	7.00	327.1
20.00	-136.2	8.00	310.9	8.00	321.3		
23.00	-132.1			9.00	327.2		
26.00	-128.4						

5		6		7	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.00	-76.3	.75	-79.6	2.00	-125.7
1.50	-87.7	1.00	-87.5	2.20	-114.6
2.00	-95.1	1.50	-98.7	2.40	-103.0
3.00	-106.0	2.00	-107.1	2.60	-90.2
4.00	-114.0	3.00	-119.7	2.80	-73.0
5.00	-120.2	4.00	-129.5	3.60	244.0
6.00	-125.5	6.00	-145.5	3.80	258.2
8.00	-134.2	8.00	-159.9	4.00	267.9
10.00	-141.5			4.50	285.5
12.50	-149.3			5.00	298.1
15.00	-156.4			6.00	315.2
20.00	-170.2			7.00	326.4
				8.50	337.3
				10.00	344.3

8		9		10	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
.40	250.5	.30	244.3	.50	-82.1
.50	257.8	.40	254.1	.75	-92.9
.70	269.6	.50	261.6	1.00	-100.8
.90	278.4	.75	275.7	1.50	-113.0
1.20	289.0	1.00	285.7	2.00	-122.3
1.50	297.5	1.60	301.7	3.00	-137.6
1.80	304.5	2.00	308.8	4.00	-150.9
2.30	313.8	3.00	320.9	5.00	-164.2
3.00	323.6	4.00	328.6		
4.00	333.2				

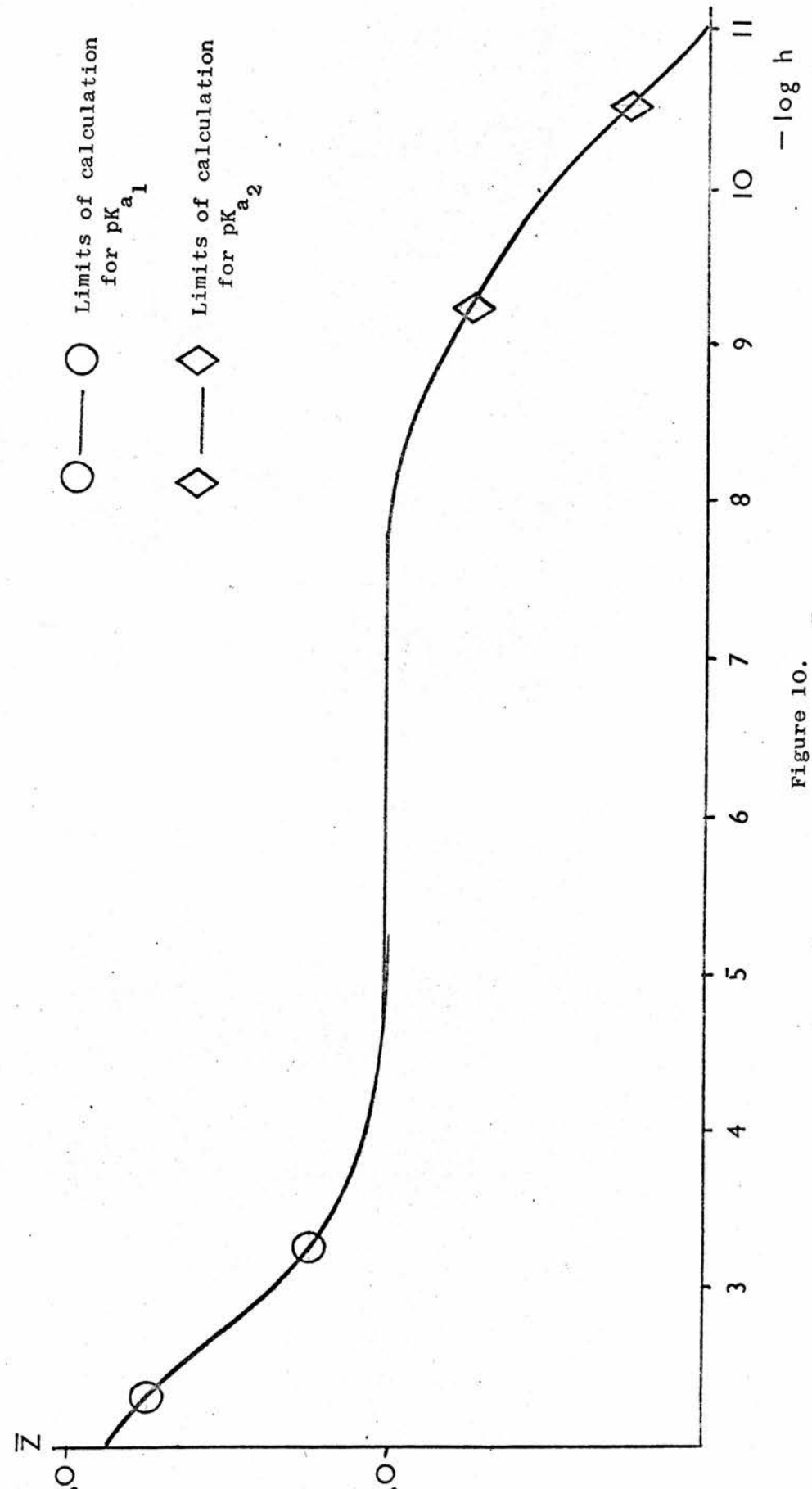


Figure 10.
Formation curve of phenylalanyl$^-$ ion with protons.
Solid line is the total experimental curve.

Table VII

The significance of various experimental errors.

variable	variation	pK_a obtained for + ve variation	change
Temperature	$\pm$ 0.25°C	9.618	0.008
E^o	$\pm$ 1 mV	9.627	0.016
Ligand concentration	$\pm$ 1%	9.620	0.01
Initial volume	$\pm$ 1%	9.607	0.003
Base concentration	$\pm$ 1%	9.600	0.01
Each A analysed independently	0.05 M	9.611	0.001
	0.025 M	9.606	0.004
	0.010 M	9.580	0.03
	0.005 M	9.607	0.003

All the variations are within the range of the SCOGS-LETAGROP variation and are thus acceptable. Although the low value of pK_{a_1}, obtained from the 10 mM results, could be attributed to a systematic error, the computer output does not give substance to this theory. It can be seen that E^o is the most sensitive variable and, as such, care must be exercised in its determination.

Formation constants for copper (II) - phenylalanine complexes.

The hydrogen ion concentration in the vessel was varied by titrating with sodium hydroxide solution (0.100 M) whence precipitation heralded the end of the titration. A formation curve was constructed from the results in Table VIII.

Table VIII

Experimental results for the Cu(II)-phenylalanine system.

(a)

Titration	Initial concentrations (M)			Volume (ml)
	Metal	ligand	acid	
1	0.01896	0.04009	2×10^{-4}	24.93
2	0.00948	0.04010	1×10^{-4}	24.92
3	0.00473	0.04009	5×10^{-5}	24.93
4	0.01898	0.02004	2×10^{-4}	24.91
5	0.0955	0.02004	1×10^{-4}	24.91
6	0.0473	0.02004	5×10^{-5}	24.91
7	0.01894	0.00994	2×10^{-4}	24.90
8	0.00955	0.00994	1×10^{-4}	24.91
9	0.00474	0.00994	5×10^{-5}	24.90
10	0.01898	0.00497	2×10^{-4}	24.90
11	0.00955	0.00497	1×10^{-4}	24.90
12	0.00474	0.00497	5×10^{-5}	24.90

E^{o} = 444.6 mV for all titrations

Table VIII

(b)

1		2		4		5	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	266.4	0.0	245.6	0.0	266.5	0.0	251.6
0.50	262.8	0.50	240.5	0.50	261.2	0.50	244.4
1.00	259.3	1.00	235.2	1.00	255.4	1.00	236.2
1,50	255.6	1.50	229.1	1.50	249.0	1.50	226.9
2.00	251.7	2.00	222.2	2.00	241.9	2.00	215.8
2,50	251.7	2.50	214.3	2.50	234.0	2.50	203.8
3.00	242.8	3.00	204.6	3.00	224.7	3.00	186.8
3.50	238.0	3.50	192.6	3.50	213.5	3.50	166.6
4.00	232.7	4.00	175.4	4.00	199.0	4.00	138.1
4.50	227.0	4.50	141.8	4.50	177.4	4.50	86.8
5.00	220.5			5.00	118.3		
5.50	213.6						
6.02	205.3	3		6		7	
6.50	196.7	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
7.00	186.4						
7.50	174.3						
8.00	159.2	0.0	231.8	0.0	238.5	0.0	265.4
8.50	139.7	0.50	224.0	0.50	227.2	0.50	256.0
9.00	110.2	1.00	213.7	1.00	212.8	1.00	244.9
9.50	18.8	1.50	199.5	1.55	190.7	1.50	230.7
		2.00	174.3	2.00	159.8	2.00	208.9
						2.50	137.6
						3.00	91.4

8		9		10		11	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	250.3	0.0	237.8	0.0	257.3	0.0	248.2
0.50	238.2	0.50	220.6	0.50	239.7	0.50	225.7
1.00	223.4	1.00	197.6	1.00	208.0	1.00	185.6
1.50	204.2	1.50	165.6				
2.00	175.1	2.00	112.4				
2.50	78.4						

12	
titre (ml)	e.m.f. (mV)
0.0	238.0
0.50	207.6
1.00	153.7

Total number of readings = 91

Figure 11 shows that the curves are not coincident as $\bar{z}$ approaches 2.0. The experimental data was processed in SCOGS and an extensive survey of possible species was made with particular reference to hydrolysed species, which have been previously reported[81]. For $(Phe^-)_p Cu_q^{(II)} H_r$ species with the values p q r, 1 1 0, 2 1 0, 3 1 0, 1 1 1, 2 1 1, 2 1 2, 1 1 -1, 1 1 -2, and 2 2 -2 were searched for; the species 1 0 1, 1 0 2 and 0 2 -2 were assumed present and their formation constants kept constant[82].

The results were best described by three constants

$$\log \beta_{110} = 8.247 \pm 0.022$$

$$\log \beta_{210} = 15.549 \pm 0.022$$

$$\log \beta_{22-2} = 4.6 \pm 0.4$$

These constants gave a standard deviation in titre of 0.2174. For just β_{110} and β_{210} the same constants were obtained but the standard deviation was 0.2178. Additional data that had been obtained near precipitation, but had been rejected, was also processed and was best described by

$$\log \beta_{110} = 8.247 \pm 0.0211$$

$$\log \beta_{210} = 15.549 \pm 0.0211$$

$$\log \beta_{22-2} = 3.55 \pm 0.2$$

$$s \text{ (108 readings)} = 0.207$$

The system can thus be described by the two complexes $Cu^{II}(phe^-)$ and $Cu^{II}(phe^-)_2$

$$\log \beta_{110} = 8.247$$

$$\log \beta_{210} = 15.549$$

but evidence is shown for the hydrolysis of $Cu^{II}(phe^{-})_2$ at high -log h giving a species $Cu^{II}{}_2(OH)_2(phe^{-})_2$ with a formation constant $\log \beta_{22-2} \approx 4.0$.

Formation constants for nickel (II)-phenylalanine complexes.

Hydrogen ion concentration was varied by titration with sodium hydroxide (0.04999 M) using the same electrode pair (E^{o} = 446.7 mV) for all the titrations. A formation curve (Figure 11) was constructed from the results given in table IX.

Table IX

Results for the nickel-phenylalanine system

(a)

Titration	Initial concentrations (M) ligand	metal	Initial volume (ml)
1	0.00994	0.00951	19.96
2	0.00995	0.00475	19.94
3	0.01170	0.00224	16.96
4	0.00497	0.00952	19.96
5	0.00497	0.00475	19.96
6	0.00450	0.00173	21.98
7	0.00234	0.01120	16.96
8	0.00234	0.00559	16.96
9	0.00209	0.00200	19.00

Table IX

(b)

1		2		3		4	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	163.1	0.0	147.7	0.0	133.2	0.0	150.1
0.25	136.9	0.10	134.8	0.10	117.9	0.10	141.1
0.40	127.7	0.20	125.6	0.20	106.5	0.20	131.1
0.55	119.9	0.30	117.9	0.30	96.9	0.30	121.8
0.70	113.1	0.40	111.2	0.40	88.1	0.40	113.7
1.00	101.3	0.50	105.2	0.50	82.4	0.50	107.1
1.25	92.7	0.75	92.4			0.60	100.8
1.50	84.7	1.00	81.5			0.70	94.7
1.75	76.5					0.80	89.3
2.00	68.9					0.90	83.7
						1.00	77.9
						1.10	72.8
						1.20	66.9
						1.30	61.6
						1.40	55.9
						1.50	50.1
						1.60	43.8
						1.70	37.1
						1.80	29.7
						1.90	20.6

5		6		7	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	132.2	0.0	106.6	0.0	137.3
0.10	117.5	0.10	91.7	0.10	115.7
0.20	107.3	0.20	80.5	0.20	102.0
0.30	99.5	0.30	70.5	0.30	89.6
0.40	92.5	0.40	61.6	0.40	75.2
0.50	85.8	0.50	54.1	0.50	63.6
0.60	80.1	0.60	48.1	0.60	47.5
0.70	74.3				
0.80	69.2				
0.90	64.3				
1.00	60.2				

8		9	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	120.8	0.0	97.2
0.10	99.5	0.10	76.3
0.20	86.4	0.20	60.1
0.30	72.9	0.30	47.7
0.40	58.7	0.40	32.5
0.50	46.3	0.50	19.5
0.60	31.7	0.60	3.7
		0.70	-14.9

It can be seen from figure 11 that the formation curves are coincident, hence the system is described by simple stepwise species. The data was analysed using SCOGS and the following results were obtained

$$\log \beta_{110} = 5.353 \pm 0.026$$

$$\log \beta_{210} = 10.487 \pm 0.050$$

s (84 readings) in titre = 0.063.

Evidence for a tris complex was not found as the 2:1 species was insoluble in the ionic medium used. The solid was filtered, washed with water, alcohol, and ether, air dried and subject to analysis. $Ni^{II}(C_9H_{10}O_2N)_2.2H_2O$ C, 51.29; H, 5.99; N, 6.57 (calculated C, 51.09; H, 5.72; N, 6.62%). Similar compounds have been reported in the literature[83]. An attempt to remove the complexed water by heating in a vacuum oven to 150°C but a small percentage (~10%) of the water remained; NiA_2, C, 55.33; H, 5.41; N, 6.98 (calculated C, 55.85; H, 5.21; N, 7.24%). Infra-red spectra of the two complexes were run but conclusions could not be drawn from them.

Formation constants of the cobalt (II)-phenylalanine system.

Hydrogen ion concentration was varied by titration with sodium hydroxide (0.04999 M) using the same electrode pair (E^o = 446.7 mV) for all the titrations. A formation curve was constructed from the results given in table X.

Table X

Results for the cobalt (II)-phenylalanine system

(a)

Titration	Initial concentrations (M) ligand	metal	Initial volume (ml)
1	0.00662	0.00751	14.96
2	0.00993	0.00563	19.96
3	0.00995	0.00281	19.94
4	0.00397	0.00901	24.96
5	0.00497	0.00563	19.94
6	0.00663	0.00375	14.94
7	0.00234	0.00662	16.96

(b)

1		2		3		4	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	112.0	0.0	110.1	0.00	1.5	0.00	8.2
0.0	109.0	0.10	87.1	0.10	71.7	0.10	84.7
0.10	85.4	0.20	6.0	0.20	58.1	0.20	72.0
0.20	71.4	0.30	66.8	0.30	48.3	0.30	62.0
0.30	59.4	0.40	59.3	0.40	40.3	0.40	44.3
0.40	50.3	0.50	53.0	0.50	33.3	0.50	47.3
0.50	42.6	0.60	47.5	0.60	26.0	0.60	40.8
0.60	35.6	0.70	42.3	0.70	21.3	0.70	35.0
0.70	29.1	0.80	37.8	0.80	16.5	0.80	29.9
0.80	22.9	0.90	33.6	0.90	11.5	0.90	24.7
0.90	16.3	1.00	29.5			1.00	19.7
1.00	11.4	1.10	25.7			1.10	15.9
1.10	5.6	1.20	22.2			1.20	10.0
1.20	0.3	1.30	19.0			1.30	5.1

5		6		7	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	94.6	0.0	86.9	0.0	85.7
0.10	73.2	0.10	60.3	0.10	53.8
0.20	59.9	0.20	46.7	0.20	37.0
0.30	49.9	0.30	35.9	0.30	23.9
0.40	41.3	0.40	27.5	0.40	12.7
0.50	33.3	0.50	18.3	0.50	1.3
0.60	27.5	0.60	13.0		
0.70	1.5	0.70	6.0		
0.80	15.9				
0.90	10.5				
1.00	5.4				
1.10	0.2				

Total number of readings = 78

It can be seen from figure 11 that the formation curves are coincident, hence the system is described by simple stepwise species. The data was analysed using SCOGS and the following results were obtained

$$\log \beta_{110} = 4.449 \pm 0.014$$

$$\log \beta_{210} = 8.439 \pm 0.037$$

s (78 readings) in titre = 0.041

No evidence of a tris complex was obtained due to the formation terminating at $\bar{Z} = 0.8$.

Formation constants of the iron (II)-phenylalanine system

Hydrogen ion concentration was varied by titration with sodium hydroxide (0.04997 M) using the same electrode pair (E^{o} = 446.7 mV) for all the titrations. Particular care was exercised to exclude oxygen from the system as oxidation readily occured. On completion of each titration the solution in the vessel was tested for oxidation by the addition of potassium thiocyanate; half of the titrations were rejected because of oxidation. A formation curve was constructed from the results given in table XI.

Table XI

Results for the iron (II)-phenylalanine system

(a)

Titration	Initial concentrations (M) ligand	metal	Initial volume (ml)
1	0.01013	0.01111	19.94
2	0.01065	0.00469	18.96
3	0.00675	0.00741	29.92

Table XI

(b)

1		2		3	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
20.20	72.6	8.20	52.4	20.10	71.6
20.30	50.2	8.30	30.1	20.20	47.5
20.40	36.6	8.40	17.6	20.30	33.0
20.50	26.9	8.50	8.3	20.40	23.4
20.60	19.2	8.60	1.2	20.50	15.2
20.70	13.1	8.70	-4.5	20.60	9.3
20.80	8.3	8.80	-9.3		
20.90	4.4	8.90	-13.6		
21.00	0.7	9.00	-17.2		
21.10	-2.5	9.10	-20.3		
		9.20	-23.3		

The results were analysed using SCOGS and the system was best described by three constants

$$\log \beta_{110} = 3.763 \pm 0.016$$

$$\log \beta_{210} = 6.882 \pm 0.244$$

$$\log \beta_{310} = 10.627 \pm 0.232$$

s (29 readings) in titre = 0.018

for only two constants the following was obtained

$$\log \beta_{110} = 3.736 \pm 0.009$$

$$\log \beta_{210} = 7.192 \pm 0.025$$

s (29 readings) in titre = 0.019

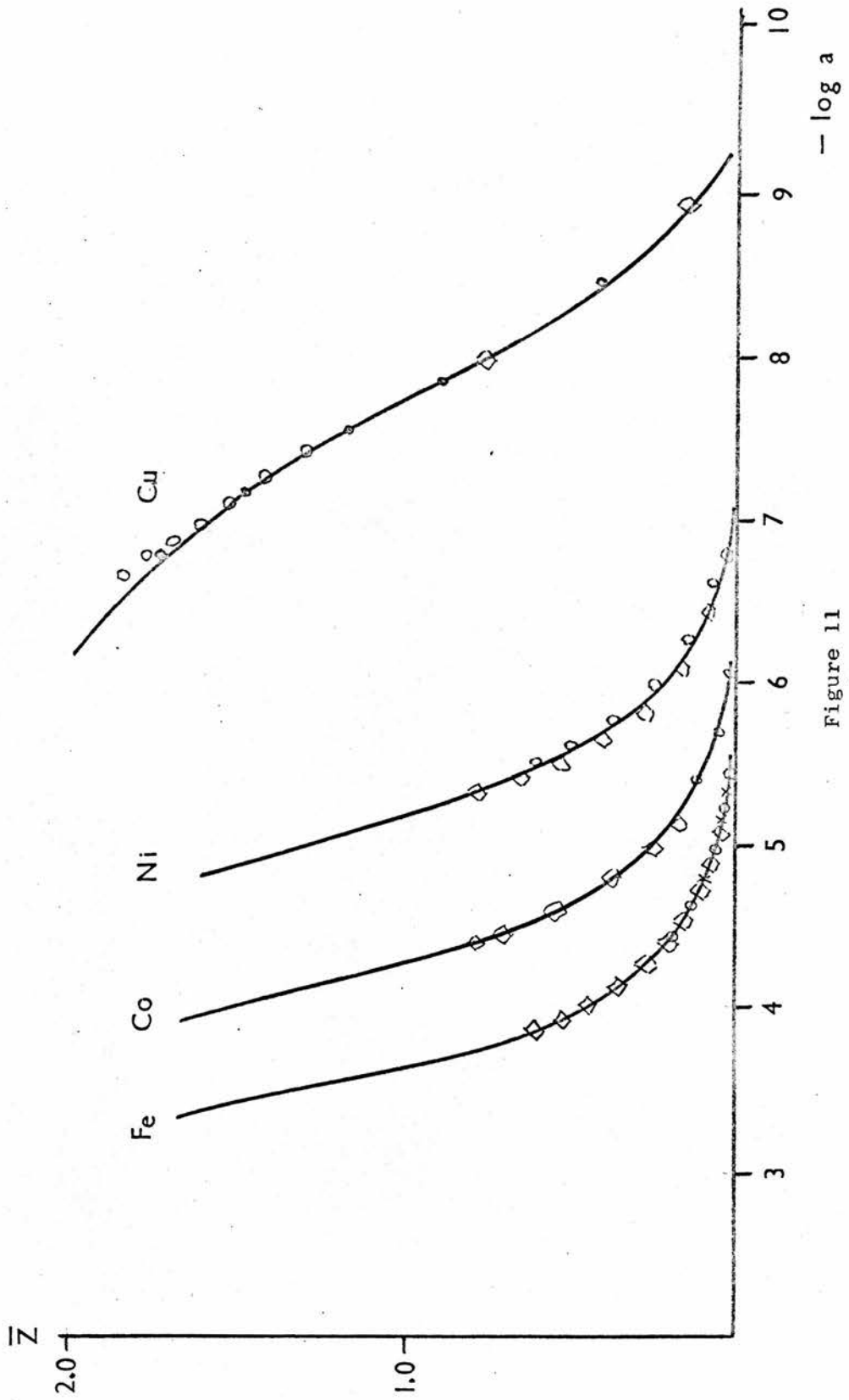


Figure 11

Formation curves of M^{2+}_{aq} with phenylalanine.
The points shown are experimental; the curves are theoretical.

As $\bar{Z}$ did not exceed 0.6 the contribution made by β_{310} must be small and, hence, accounts for the large errors in log β_{210} and log β_{310}. The values obtained by the convergence of two constants will be the "best" constants and the value obtained for log β_3 a guide to the value of this constant i.e.

$$\log \beta_{110} = 3.736 \pm 0.009$$

$$\log \beta_{210} = 7.192 \pm 0.025$$

$$\log \beta_{310} = 10.7 \pm 0.2$$

s (29 readings) in titre = 0.02.

It will be difficult to obtain log β_{310} more accurately in this medium as oxidation occurs so readily at pH 7.

Formation constants of the iron (III)-phenylalanine system

Due to the formation of an insoluble precipitate during titrations with alkali[98] this investigation was carried out by titrating iron (III)-phe solutions with perchloric acid (0.0538 M) using the same electrode pair (E^o = 446.7 mV).

Table XII

Results for the iron (III)-phenylalanine system

(a)

Titration	Initial concentrations (M)			Initial volume (ml)
	ligand	metal	acid	
1	0.01483	0.00628	3×10^{-4}	25.00
2	0.02060	0.00262	1×10^{-4}	23.98
3	0.01235	0.00786	3×10^{-4}	19.98

Table XII

(b)

1		2		3	
titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.0	284.2	0.0	245.6	0.0	293.9
0.50	286.7	0.25	248.7	0.50	297.1
1.00	288.8	0.50	251.7	1.00	299.8
1.50	291.0	0.75	254.5	2.00	302.6
2.00	293.0	1.00	257.5	2.50	307.6
2.50	295.1	1.25	260.0	3.00	310.0
3.00	297.0	1.50	262.3	3.50	312.1
3.50	298.9	1.75	264.4	4.00	314.4
4.00	300.7	2.00	266.6		
4.50	302.6	2.50	270.5		
5.00	304.4	3.00	274.1		
5.50	306.1	3.50	277.4		
6.00	307.7	4.00	280.6		
7.00	310.9	4.50	283.6		
8.00	313.7	5.00	286.4		
		5.50	289.2		
		6.00	292.0		
		6.51	294.6		
		7.00	297.0		
		7.50	299.4		
		8.00	301.6		

The aqueous iron (III) system is complicated by hydrolysis but this has been investigated by Hedström[98] for the 3.00 M $(Na)ClO_4$ medium and the constants he obtained have been used in the calculations for this system.

$$\log \beta_{011} = -3.045$$

$$\log \beta_{012} = -6.310$$

$$\log \beta_{022} = -2.914$$

SCOGS was used to find the "best" constants that describe the experimental data. For the simple 1:1 and 2:1 species the following

was obtained

$$\log \beta_{110} = 10.412 \pm 0.04$$
$$\log \beta_{210} = 19.225 \pm 0.066$$

$$s = 0.341 \text{ (44 readings)}$$

Hydrolysis of these two species was also investigated and a constant for the following system was found

$$2Fe^{III}phe \rightleftharpoons 2Fe^{III}phe(OH) \rightleftharpoons Fe_2^{III}(OH)_2phe_2$$

no evidence of $Fe_2^{III}(OH)_2phe_4$ was found.

$$\log \beta_{110} = 10.388 \pm 0.04$$
$$\log \beta_{210} = 19.113 \pm 0.13$$
$$\log \beta_{22\text{-}2} = 16.92 \pm 0.34$$

$$s = 0.338 \text{ (44 readings)}$$

A search for the 3:1 complex was also made

$$\log \beta_{110} = 10.390 \pm 0.04$$
$$\log \beta_{210} = 19.087 \pm 0.18$$
$$\log \beta_{310} = 26.13 \pm 1.77$$
$$\log \beta_{22\text{-}2} = 16.95 \pm 0.37$$

$$s = 0.342 \text{ (44 readings)}$$

The difference in the standard deviation is small for the three descriptions of the system, hence, the "best" constants can be a combination.

$$\log \beta_{110} = 10.39 \pm 0.04$$

$$\log \beta_{210} = 19.11 \pm 0.10$$

$$\log \beta_{310} \approx 26.0$$

$$\log \beta_{22-2} = 16.9 \pm 0.30$$

$$s = 0.34 \text{ (44 readings)}.$$

The high errors in $\log \beta_{310}$ and $\log \beta_{22}$ can be attributed to the small degree of formation of the complexes in the present study. At the highest -log h value the degree of formation of the above complexes was 23%, 60%, 3% and 12% of the metal respectively.

Comparison with other workers results

The formation constants obtained in 3.00 $(Na)ClO_4$ are higher than those obtained at lower ionic strengths[99] and this has been found for all the systems studied when compared with published results (Tables XIII and XIV). Figure 12 shows the $\log \beta_2$ s for Cu(II)-phe, obtained by other workers, plotted against $\sqrt{I}$. A smooth curve can be drawn, with a minimum at $\sqrt{I} \approx 0.7$, through most of the points, showing that this system is similar to those discussed in Chapter III, and that the present work is in good agreement with much of the previously published work. The comparisons for Ni(II)-phe and Co(II)-phe are similar to that for Cu(II)-phe but not as extensive. Previous work on the two main oxidation states of iron is scanty and the constants obtained are not well characterised.

Table XIII

Protonation of phenylalanine

pK_{a_1}	pK_{a_2}	temp °C	method*	I	Reference
9.610	2.754	25	gl	3.00	This work
9.31	2.20	25	gl	→ 0	84
9.13	1.83	25	emf	0.06?	85
9.35	2.41	25	gl	0.37	96
9.18	2.21	20	gl	1.00	97
9.08	2.09	25	gl	0.05	88
9.02	-	25	gl	0.16	89

* gl = glass electrode

emf = electromotive force method

Table XIV

Metal phenylalanine complexes

a comparison of results

Metal	t °C	Method	I	log β_1	log β_2	Ref.
Cu(II)	25	gl	3.00	8.247	15.549*	This work
	25	gl	0.05	7.92	13.76	88
	25	gl	→ 0	8.18	15.18	88
	25	gl	0.16	7.51	14.25	89
	25	gl	→ 0	8.25	15.38	84
	20	gl	0.37	8.23	15.14	86
	25		1.00	.	14.92	92
	25	gl	0.01	7.38	14.24	93
	25	c	→ 0	7.87	14.77	90
	25	c	0.027	7.44	14.64	90
	25	p	0.06	.	14.22	85
	20	gl	0.01	.	14.90	91
Ni(II)	25	gl	3.00	5.353	10.487	This work
	25	gl	0.05	5.11	9.43	88
	25	gl	→ 0	5.46	9.99	88
	25	gl	→ 0	5.56	10.22	94
	20	gl	0.37	5.19	9.66	86
	25	gl	0.01	4.73	10.02	93

Table XIV continued

Co(II)	25	gl	3.00	4.449	8.439	This work
	25	gl	0.05	4.03	7.47	88
	25	gl	0.01	4.00	8.08	93
	20	gl	0.01		7.9	91
Fe(II)	25	gl	3.00	3.736	7.192*	This work
	20	gl	1.00	3.26		87
	20	gl	0.01		6.3	91
Fe(III)	25	gl	3.00	10.39	19.11*	This work
	20	emf	1.00	8.9		87

gl - glass electrode

c - conductivity

p - polarography

* - system described by more than two constants

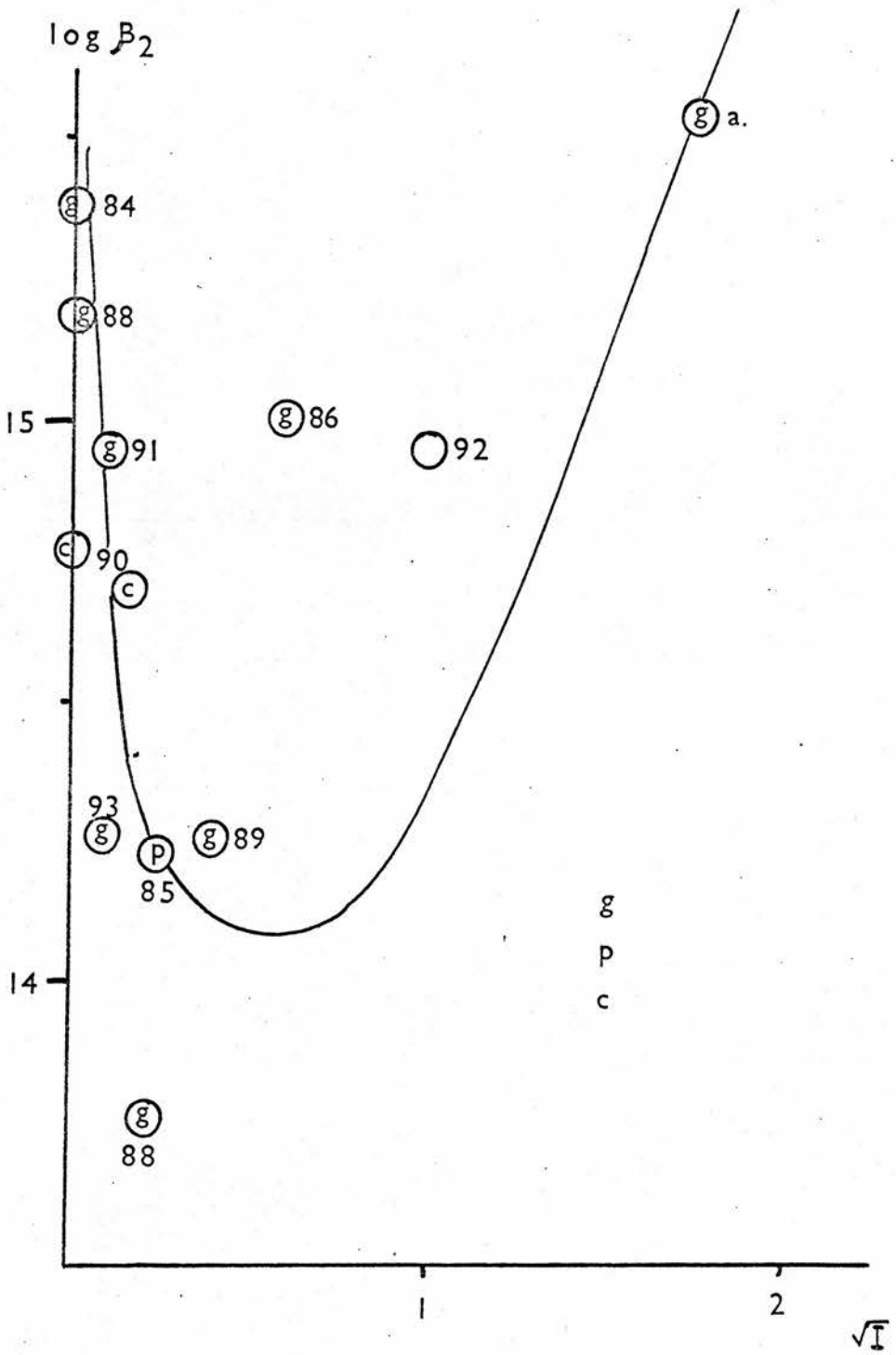


Figure 12

Published results for β_2 for
Copper (II)-phenylalanine system

g = glass electrode

c = conductivity

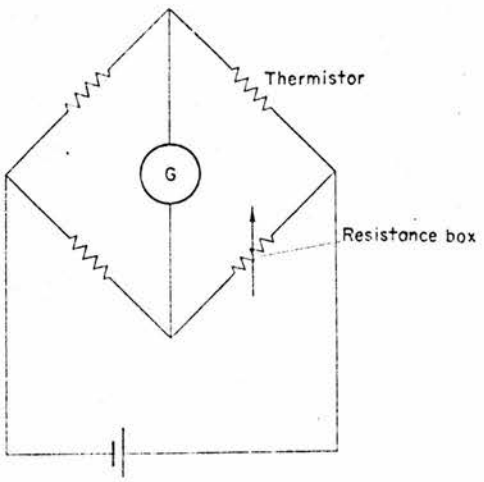
p = polarography

CHAPTER VI CALORIMETRY

The calorimeter was of the Gerding, Leden and Sunner[95] design, as shown in figure 13, and is similar to another calorimeter used in this laboratory[96]. The unit comprises of an inner reaction vessel of glass and an outer shielding vessel partially of glass and partially of copper. The two vessels are attached to the "lid" of the calorimeter; the reaction vessel by two springs and the outer vessel by an O-ring seal (O) to prevent leakage. The chimneys provide the means of introducing probes into the reaction vessel: (a) a burette tip, (b) a stirrer which also acts as a cooler, (c) a heat detector, (d) a heater, and (e) an electrode pair.

(a) The burette, (B). The titrant was added through a glass burette tip protected from back diffusion by a polytetrafluoroethylene (PTFE) cap held on a gold spring. The titrant was prewarmed, to the temperature of the bath in a spiral of nylon tube (P) (Portex SFD Nylon C, 8 ml capacity) on top of the vessel, by immersion in the water of the bath. The free end of the nylon tube was then attached to a piston burette (Metrohm AG, E 274, 10 ml.).

(b) The stirrer, (S). Vibro-stirring was used because it appears to have smaller heat of stirring corrections[96], but primarily it was used to minimise the effects of inefficient stirring and pressure upon the heat detecting system. The stirrer disc was a flat circular plate 1 mm thick PTFE (2.5 cm diameter) containing 10 holes of 1 mm diameter. This was screwed onto a hollow stainless steel tube held in nylon impregnated with molybdenum disulphide to reduce friction. The tube was connected directly to the vibro-motor (Chemap AP, E1) using an L-shaped bar. Heat conduction from the



The Wheatstone bridge circuit for measuring thermistor resistance. G is a galvanometer.

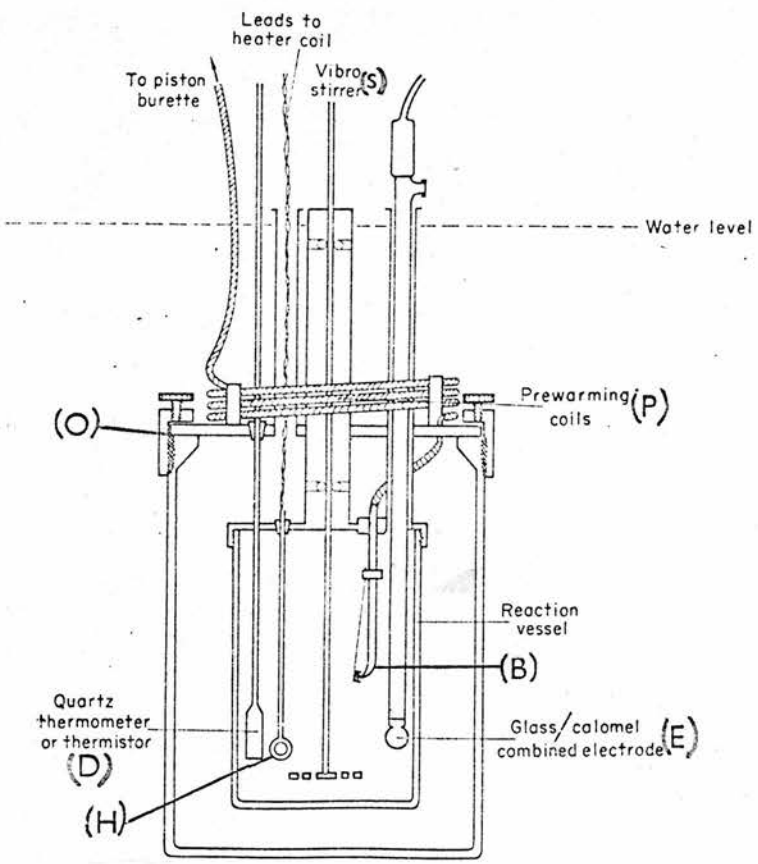


Figure 13.

The isothermal calorimeter.

motor to the calorimeter *via* the bar was prevented by wrapping the bar in asbestos tape at the connection to the motor. The stirrer tube was hollow to allow cooled nitrogen to be passed into it from a long hollow needle.

(c) The heat detector, (D). The temperature change was measured by using a thermistor (Stantel F23), a conventional DC-Wheatstone bridge, a preamplifier (Pye, 11330) and a Scalamp galvanometer (Pye, 420). For a temperature rise of less than $0.1^{o}C$ the temperature can be assumed to be inversely proportional to the resistance[95] ($-60\,\Omega\ deg^{-1}$ at $25^{o}C$).

(d) The heater, (H). To convert the change in resistance to energy, a calibration experiment is required. This was done electrically by means of a heater coil of non-inductively wound resistance wire (20.83 Ω) coated with a chemically resistant epoxy resin (Araldite). The voltage across the heater was measured on a digital voltmeter (Solartron LM 1420.2). The current flowing was passed through the heater resistor and also through a 10.000 Ω standard resistance; the voltage across this resistance was measured to give the current in the heater circuit. The time for which the heating current flowed was automatically recorded to within 0.02 s using a stopwatch (Jaquet 309 e).

(e) Electrode pair, (E). Although there is provision for the use of a combination electrode (Activion T1N7DB/180) in the calorimeter it was not used in this work as more accurate results can be obtained by direct potentiometry.

The complete system was suspended in a thermostat bath controlled to 25.0000 ± 0.0005°C (LKB 7602 controller on 7603A bath) which was located in a thermostatted room (22°C ± 0.5°C).

Experimental procedure.

For each titration the vessel was charged by either, directly pipetting the solutions into the reaction vessel or, making up the titrate in a siliconed (Beckman Desicote) flask and pouring the contents (99.57 ml) into the reaction vessel. The calorimeter was assembled and immersed in the water bath. After a minimum of eight hours, the stirring and electronic system were switched on and after two hours reached steady state conditions whence the titration could proceed.

For each point the following procedure was adopted.

(1) 8 minute 'fore' period.

(2) 6 minute reaction period in which 0.5 - 2 ml of titrant were added (or electrical energy for a calibration point).

(3) 8 minute 'aft' period.

(4) Nitrogen cooling and a 10 minute pause.

This is shown in figure 14.

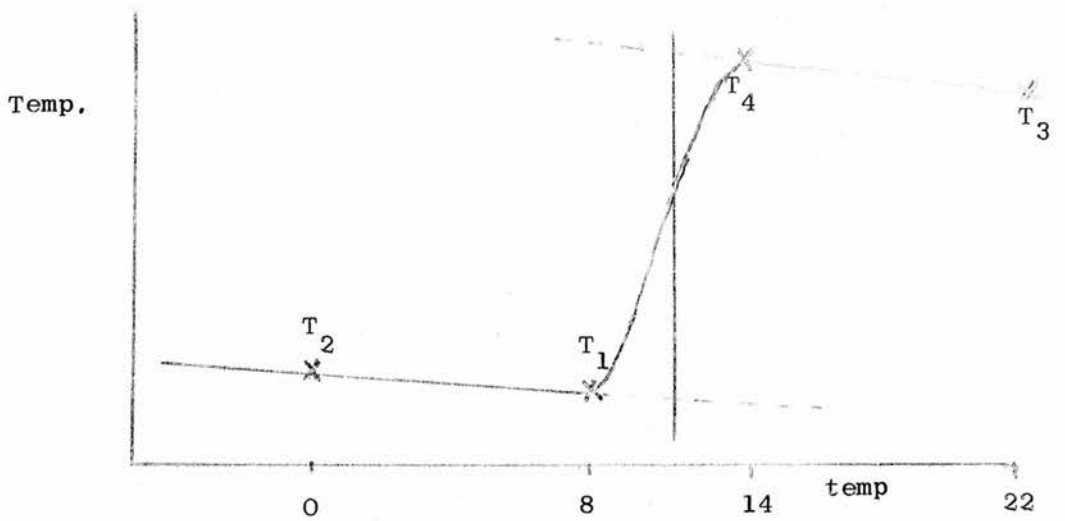


Figure 14. Temperature-Time plot for the reaction period of the calorimeter.

The heat corrections are calculated as follows

$$\triangle T = \frac{3(T_2-T_1)}{8} + \frac{3(T_4-T_3)}{8} + (T_4-T_1)$$

The values of 6, 8 and 10 minutes were arrived at by plots of resistance versus time and the above values were the times required to attain steady state. Each point would, thus, take 32 minutes making it possible to complete about 15 points per day.

Calibrations:- These were performed during the course of titrations, and a body of data built up to construct a calibration curve. The electrical energy supplied to the system is calculated from

$$J = VIt$$

J in joules

I in amperes

t in seconds

The calibration constant is calculated

$$= \frac{J}{\triangle R} \qquad \text{i.e. } J\Omega^{-1}$$

and plotted against the total volume of solution in the reaction vessel.

The heat of ionisation of water, $\triangle H_w$.

This was determined to assess the accuracy and reproducibility of the calorimeter. The conditions used in the calorimeter for these experiments were similar to those used in subsequent heat of formation determinations i.e. alkali was titrated into acid. The results obtained were for the formation of 2×10^{-4} moles of water and were as follows

(i) $-55.913\ kJ\ mol^{-1}$ $(-13.364\ kcal\ mol^{-1})$

(ii) $-55.774\ kJ\ mol^{-1}$ $(-13.330\ kcal\ mol^{-1})$

(iii) $-55.879\ kJ\ mol^{-1}$ $(-13.355\ kcal\ mol^{-1})$

average $-55.522 \pm 0.024\ kJ\ mol^{-1}$ $(-13.350 \pm 0.008\ kcal\ mol^{-1})$

This value agrees well with the literature values where the "best" calorimetric value is given as "close to -13.34 kcal mol^{-1}"[97]

The heats of protonation of phenylalanine.

The following results were obtained by the titration of phenylalanine solutions with sodium hydroxide (0.100 M). Seven "runs" totalling 49 readings were completed.

Table XV

Results for the heats of protonation of phenylalanine

Initial ligand, mol	0.02510	0.02510	0.02510	0.02521	0.02514	0.04820
Initial mineral acid,	0.01464	0.01464	0.000	0.000	0.000	0.000
Initial volume, ml	99.57	99.57	99.57	99.57	99.57	51.73
ml added			Heat evolved (Joules)			
2.0	9.788	9.282	0.368	1.125	1.384	
4.0	19.558	19.043	0.913	2.039	1.900	0.828
6.0	28.971	28.428	2.298	3.058	2.603	2.211
8.0	37.333	38.663	3.302	4.518	4.153	3.761
10.0	47.309	45.922	4.342	5.853	5.104	5.443
12.0	55.261		5.00	7.041	6.184	6.535
14.0			6.162	7.948	6.994	7.725
16.0			7.397	9.185	8.015	9.475
18.0			8.148	9.379	9.324	10.947
20.0				9.869	10.376	11.938

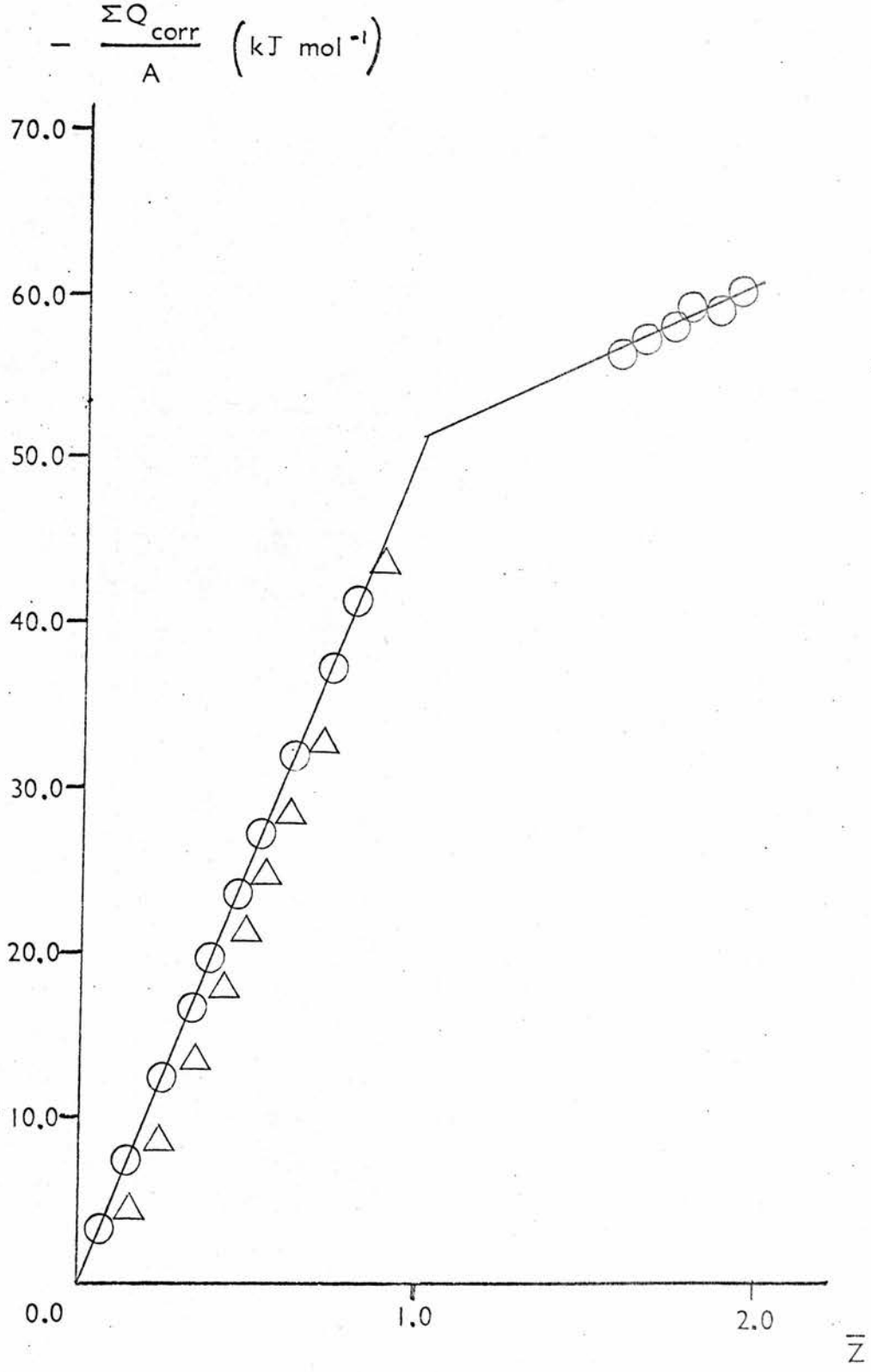


Fig. 15 Enthalpic Curve for Phe^{-}/H^{+} system.

An enthalpic curve was plotted (figure 15) but the values for the heats of protonation were obtained using the "least squares" method described in Chapter III (page 35). The results obtained were

$$\triangle H_1 = -50.405 \pm 0.13 \text{ kJ mol}^{-1}$$

$$\triangle H_2 = -60.14 \pm 0.5 \text{ kJ mol}^{-1}$$

The heats of formation of Cu(II)-phe$^-$ complexes

The results were obtained by the titration of Cu(II)-phe solutions with sodium hydroxide (0.100 M) solution. Six "runs" totalling 47 readings were completed.

Table XVI

Results for the heats of formation of Cu(II)-phe complexes

Initial Cu(II) (M)	0.0191	0.0191	0.0192	0.0192	0.0192	0.00955
Initial phe (M)	0.0397	0.0397	0.0201	0.0201	0.0201	0.0200
Initial min.acid	0.000	0.000	0.000	0.000	0.000	0.0001
Initial volume (ml)	99.90	99.90	99.57	99.57	99.57	100.04
ml added	Heat evolved (Joules)					
2.0	8.389	8.389	6.784	6.402	5.693	
4.0	9.656	8.768	6.176	6.469	5.918	16.641
6.0	8.914	8.348	7.036	6.717	6.281	8.274
8.0	9.408	9.940	7.456	6.927	6.839	8.409
10.0	8.967	8.998	8.197	7.630	7.063	7.694
12.0	8.372	9.178	7.034	7.603	6.143	
14.0	9.141	8.834	6.355	7.087	7.492	15.246
16.0	8.701	8.728	6.371	7.076	3.783	10.473
18.0		7.802	5.192	5.861		9.417
20.0		10.222	5.635	4.947		
22.0		8.815				
24.0		6.139				

An enthalpic curve was plotted (figure 16) but the values of $\triangle H_1$ and $\triangle H_2$ were obtained by using the method described in chapter III. The results were: $\triangle H_1 = -19.179 \pm 1.8$ kJ mol^{-1} $\triangle H_2 = -58.352 \pm 4.0$ kJ mol^{-1}

s (47 readings) = 1.17

Even though evidence for a hydrolysed species was found in the potentiometry, no account of it was taken during the above calculations due to the conditions employed in the experiments i.e. -log h was too low for $Cu_2^{II}(OH)_2(phe^-)_2$ to be formed.

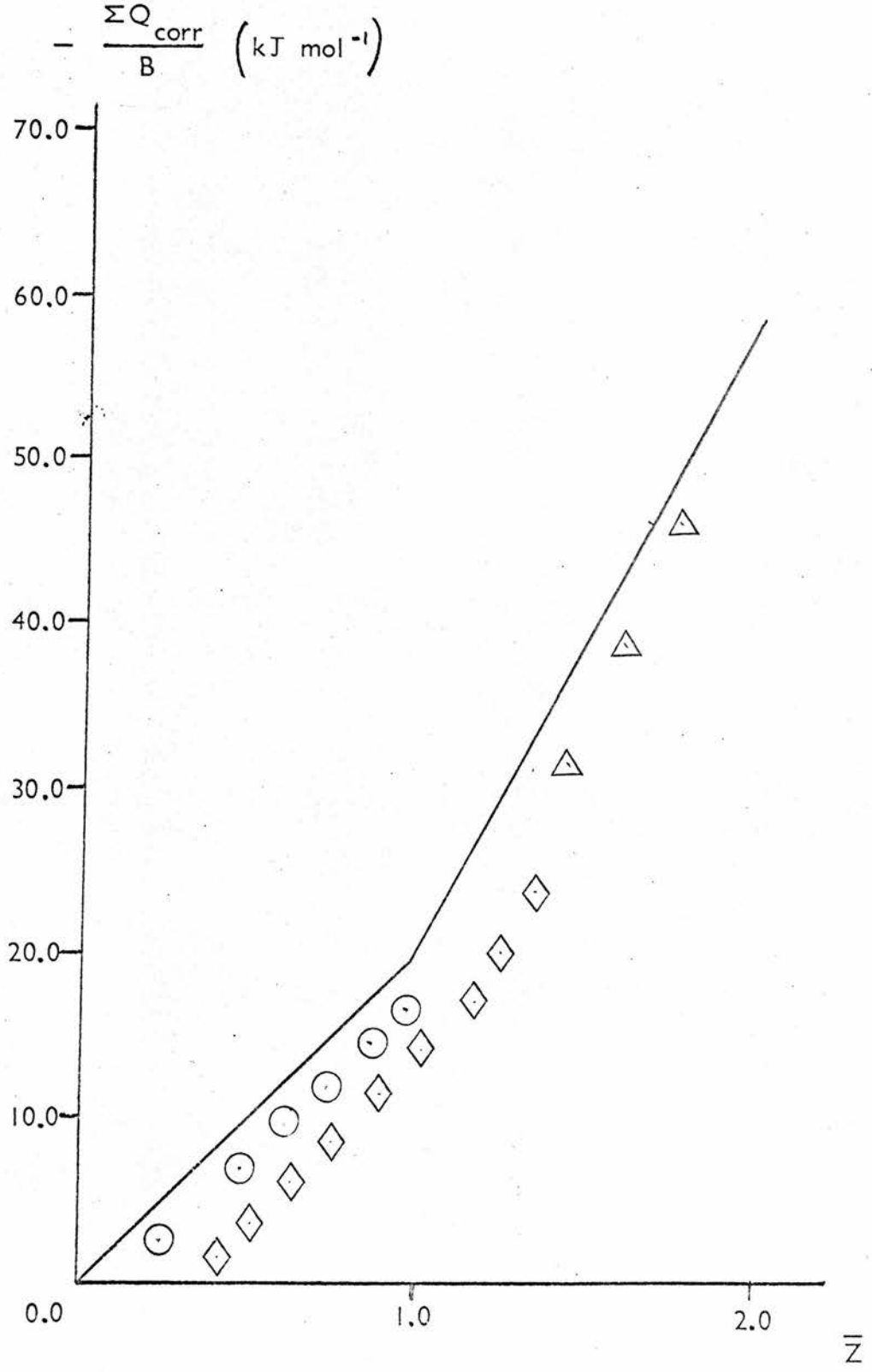


Fig. 16 - Enthalpic Curve for Phe^{-}/Cu^{++} system.

The heats of formation of Ni(II)-phe$^-$ complexes

The results were obtained by the titration of copper(II)-phenylalanine solutions with sodium hydroxide solutions. Five "runs" totalling thirty readings were completed.

Table XVII

Results for the heats of formation of Ni(II)-phe complexes.

	a	b	c	d	e
Initial nickel(II) (mM)	1.903	9.514	5.286	18.99	9.495
Initial phenylalanine (mM)	2.024	10.120	8.996	29.82	9.900
Sodium hydroxide (mM) (Titrant)	49.97	49.97	49.97	100.00	100.00
Initial volume (ml)	100.00	100.00	90.00	99.90	99.90

ml added	Heat evolved (Joules)				
	a	b	c	d	e
0.5	0.401				
1.0	1.425	1.587	1.478		2.611
1.5	1.679				
2.0	2.328	2.353	2.182	4.726	3.835
2.5	2.807				
3.0	2.995	2.578	3.138		5.600
4.0		3.332	4.029	8.185	7.557
5.0		4.285	4.333		
6.0		5.825			9.847
7.0		7.526			
8.0		9.060		18.251	11.880
10.0				23.721	

An enthalpic curve was plotted (figure 17) but the values of ΔH_1^o and ΔH_2^o quoted below were obtained using the method described in Chapter III.

These results were

$$\Delta H_1^o = -9.815 \pm 0.6 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -24.537 \pm 1.2 \text{ kJ mol}^{-1}$$

s(30 readings) = 0.6

Under the conditions of the experiment sufficient of the A_2B complex was formed to be able to calculate a value of ΔH_2^o but nevertheless it was insufficient to be able to draw a complete enthalpic curve and line AB is an extrapolation. Measurements near the precipitation point were unreliable due to various heats of solution etc.

The heats of formation of Co(II)-phe$^-$ complexes

The results were obtained by the titration of cobalt(II)-phenylalanine solutions with sodium hydroxide solutions (0.1 M). Four "runs" totaling 28 readings were completed.

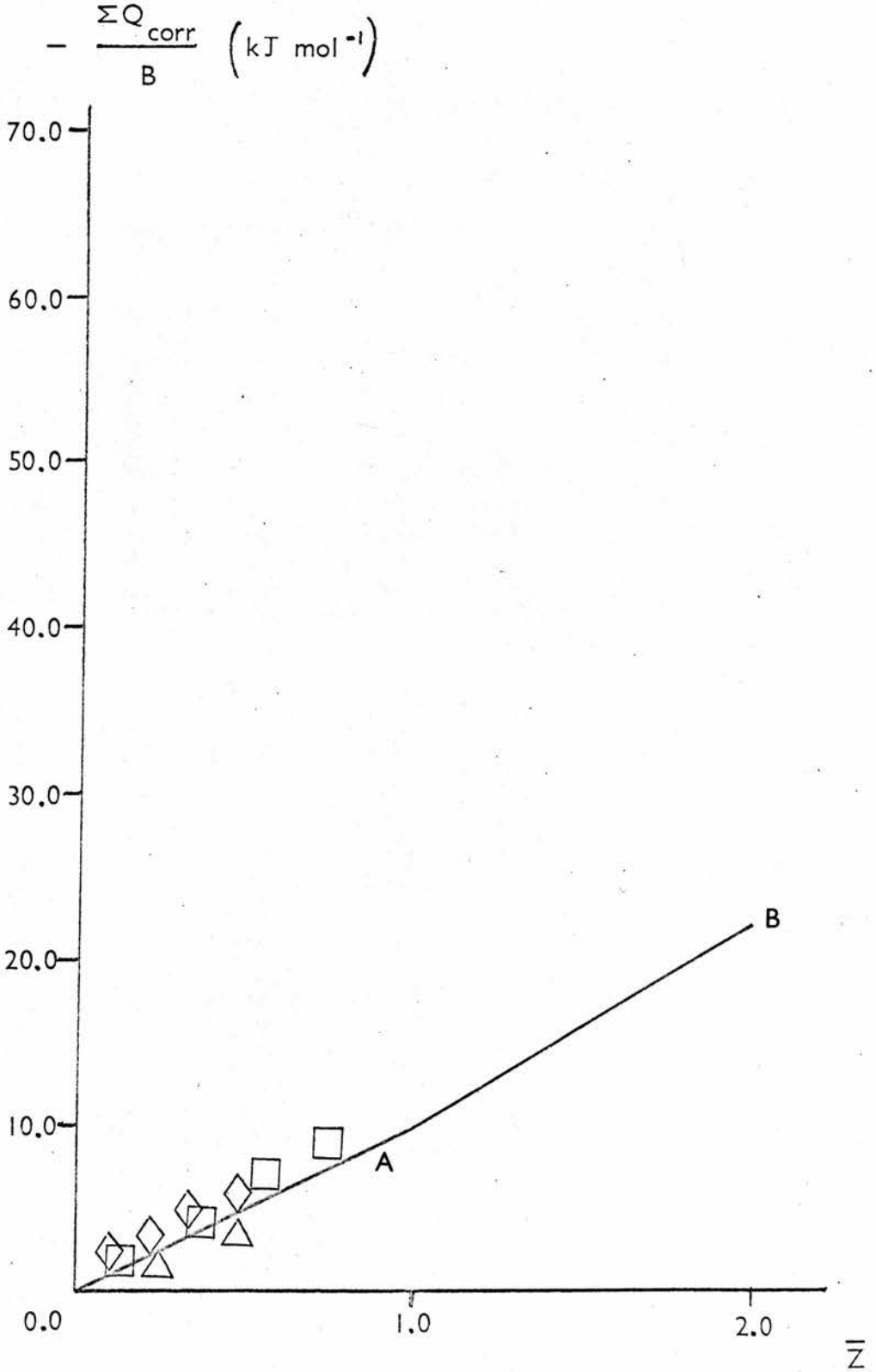


Fig. 17 Enthalpic Curve for Phe^{-}/Ni^{++} system.

Table XVIII

Results for the heat of formation of Co(II) complexes

	1	2	3	4
Initial cobalt(II) (mM)	7.880	7.880	2.253	2.253
Initial phenylalanine (mM)	6.556	6.556	10.92	10.92
Initial volume (ml)	100.00	100.00	102.00	102.00
ml added	heat evolved (Joules)			
0.5			0.497	0.497
1.0	0.691	0.691	0.300	0.273
1.5			0.300	0.304
2.0	0.512	0.534	0.298	0.292
2.5			0.290	0.298
3.0	0.522	0.504		
3.5			0.581	0.578
4.0	0.518	0.519	0.293	0.288
4.5			0.297	0.301
5.0	0.517	0.517		
6.0	0.549	0.540		

An enthalpic curve was plotted (figure 18) and the values of ΔH_1^o and ΔH_2^o were calculated by the method described in chapter III.

The results were

$$\Delta H_1^o = -5.297 \pm 0.8 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -13.361 \pm 1.6 \text{ kJ mol}^{-1}$$

s (28 readings) = 0.8

The enthalpic curve shown is partly extrapolated for reasons identical to those mentioned for nickel in the previous section.

The heats of formation of Fe(II)-phe complexes

Values of ΔH^{o} were not obtained because of the oxidative instability of the system. The calorimeter described in Chapter VI was not designed to incorporate an inert atmosphere and so it was impossible to maintain anoxic conditions for sixteen hours in the present apparatus. However, I consider the study of iron(II)-amino acid systems to be sufficiently important to warrant a closer scrutiny of the experimental difficulties with a view to either adapting the present apparatus or to constructing a new calorimeter for determining the heats of formation of such complexes.

The heats of formation of Fe(III)-phe complexes

Although a reasonable amount of data was collected for this system, it was not possible to express it in terms of ΔH^{o} values. Due to hydrolysis and subsequent precipitation in this system when titrated with alkali, the method used for all the previous systems had to be adapted. Solutions of iron(III) perchlorate and phenylalanyl^{-} were titrated with perchloric acid. This gave a pH range of 2.2 to 2.8 and unfortunately the concentration changes over this range are small. Thus, heat changes are correspondingly small and difficult to measure. Thermistor resistance is the experimental quantity that was actually measured and for the ferric system such measurements were only about twice the minimum measurable quantity and furthermore, except for the first few points, the reaction was endothermic and this renders the heat corrections excessive. Thus it was impossible to determine heats of formation of the iron(III)-phenylanine system.

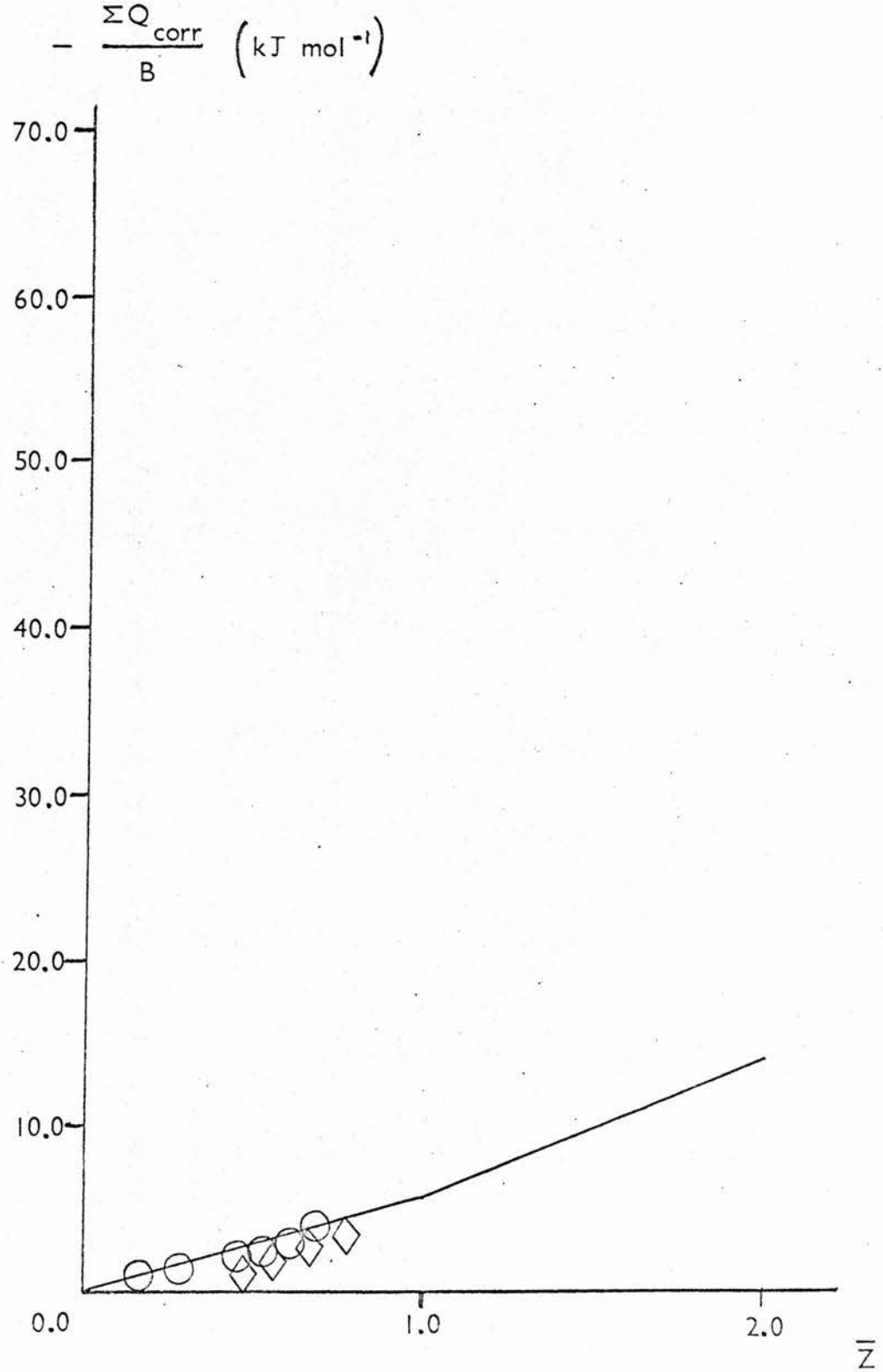


Fig. 18. Enthalpic Curve for Phe^{-}/Co^{++} system.

Comparison with other workers' results

No direct comparisons may be made because of different background media, different methods of calculation and different types of calorimeter but, primarily, because the accuracy of some published work leaves something to be desired. Some comparative results are shown in table XIX. The ligand protonations and copper(II) systems have been well studied, but research into the cobalt(II) and nickel(II) systems is less extensive.

Table XIX

Heats of formation of phenylalanyl complexes

Metal	°C	Method	ΔH_1^o	ΔH_2^o	Ref.
H^+	25	Cal	-50.405	-60.140	This work
	25	Cal	-44.601	-	84
	25	Temp.	-42.921	-	84
	25	Cal	-	-	88
	25	Cal	-43.138	-	89
Cu^{++}		Cal	-19.18	-58.35	This work
		Cal	-19.7	-48.5	88
		Cal	-21.41	-45.55	89
		Cal	-22.16	-48.93	84
Ni^{++}		Cal	-9.815	-24.537	This work
		Cal	-11.3	-19.3	88
		Cal	-13.38	-27.10	84
Co^{2+}		Cal	-5.297	-13.361	This work
		Cal	-6.28	-7.53	88

The accuracy of the results listed are naturally dependent upon the precision with which various experimental parameters can be measured. The error in analytical techniques was estimated as being < 0.3% but, by far the largest, and hence the governing, error was the observed temperature change. This was measured by recording the change in thermistor resistance ($\pm$ 0.01Ω). This gave a maximum error of 8% in the derived enthalpies. Errors as high as this probably only occured for the cobalt system and were much smaller for the other systems because these evolved more heat.

CHAPTER VII DISCUSSION

The results can be most efficiently discussed in three sections· (a) the protonation of the phenylalanyl anion, (b) chelation trends occurring across the transition series, and (c) the individual complexing reactions of each metal ion.

(a) The Protonation of the Phenylalanyl anion.

Several workers have reported[84-89] pKs for the phenylalanyl anion referring to a variety of temperatures and ionic strengths.

Activity coefficients can be calculated from a relationship suggested by Davis[40]

$$-f_{\pm} = \frac{A z_+ z_- \sqrt{I}}{1 + \sqrt{I}} - bI$$

where $z_{\pm}$ are the charges on the cation and anion and A and b are constants. For aqueous systems,

$$A = 1.825 \times 10^6 (\epsilon T)^{-3/2}$$

$$= 0.509 \text{ at } 25^{\circ}C$$

(ϵ is the dielectric constant of water).

Constant b encompasses corrections for the ionic strength variation of the dielectric constant of the medium and the effective sizes of the hydrated ions.

The activity coefficient can then be used to calculate the concentration pKs over a range of ionic strengths and these values can then be compared with those obtained experimentally. This is illustrated in figure 19, where the value of b giving the "best" fit was chosen as 0.398.

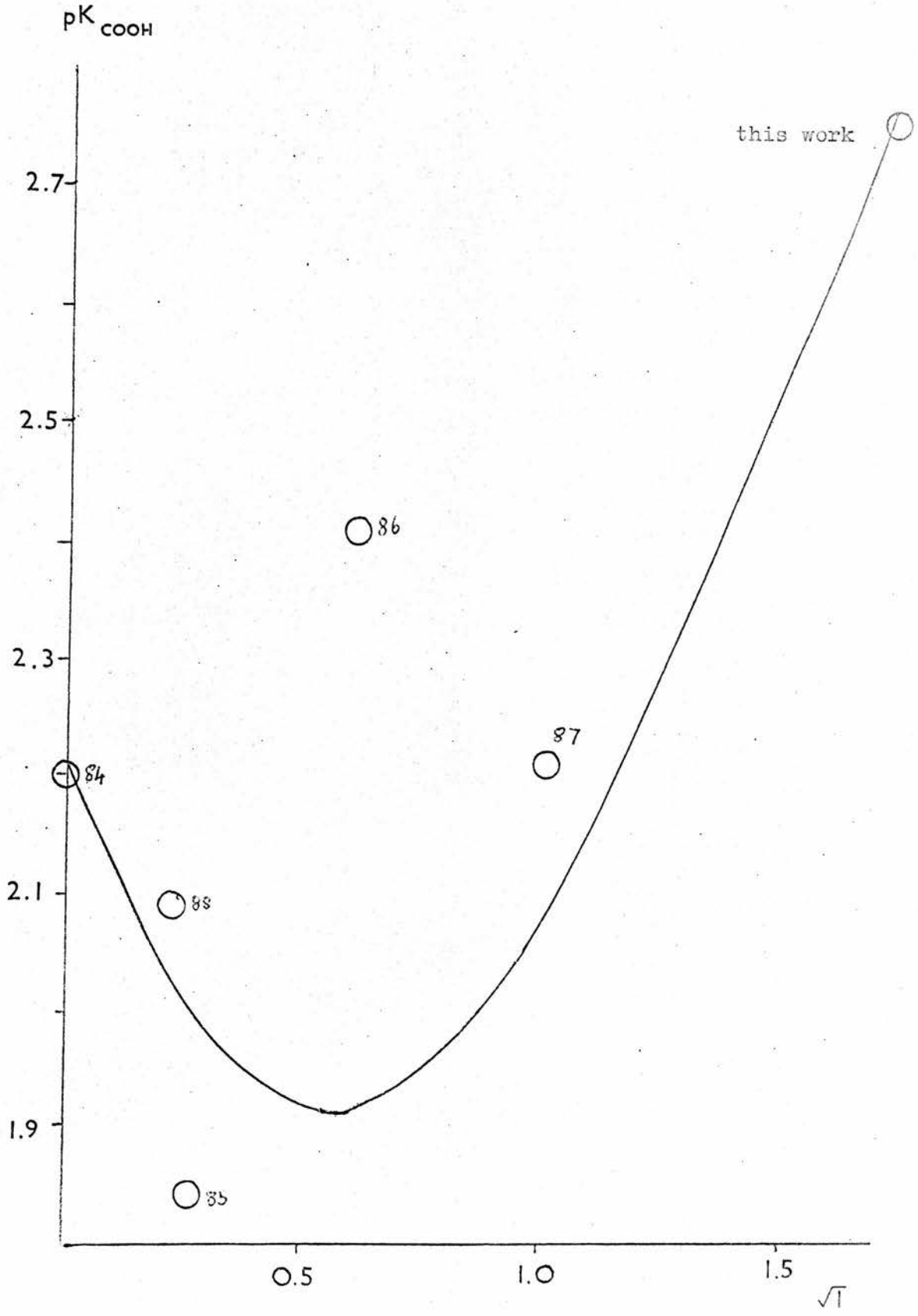


Fig. 19. Variation of pK with ionic strength.

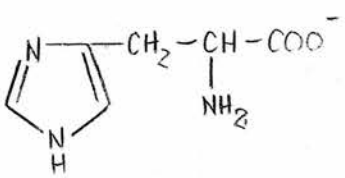
Simon and Weber have noted their result as being high but have offered no explanation[86]. The other five results, which included the present work, all lie near the curve and thus indicat that the results are consistent.

Table XX

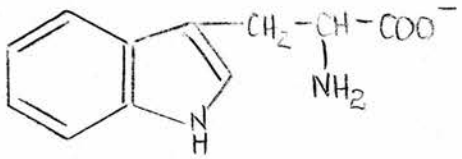
Thermodynamic parameters for the protonation of the phenylalanyl anion in 3.00 M $(Na)ClO_4$ at 25°C

	ΔG^o (kJ mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J mol^{-1} K^{-1})
$-NH_2$	-54.87	-50.41	14.99
$-COO^-$	-15.73	-9.73	20.02
Overall values	-70.60	-60.14	35.01

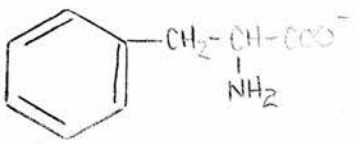
The $\Delta H^o_{-COO^-}$ value is somewhat higher than those reported[99] for other amino acid carboxyl groups in this media. For example, consider:



I (histidyl)



II (tryptophyl)



III (phenylalanyl)

$\Delta H^o_{-COO^-}$	-1.25	-3.35	-9.64 (kJ mol^{-1})

The governing factor appears to be the electron withdrawing power of the group attached to the β-carbon of the amino acid since no possibility exists for conjugation to occur over two saturated carbon atoms. An electron withdrawing group will have the effect of stabilising the negative charge on the carboxyl group and thus rendering the enthalpy of protonation less negative. The electron withdrawing power of the aromatic ring can be enhanced by more electron withdrawing groups or atoms, such as substituted nitro groups or a heterocyclic ring as in II. There are other factors which are also important, for example, the availability of the lone pairs on the nitrogen atoms. Hence a quantitative treatment must necessarily be very complex. The ratio of ΔH^{o}_{I}: ΔH^{o}_{II}: ΔH^{o}_{III} is 1:3:9 but considering the complexity of electron withdrawal this cannot really be simplified to claim that each additional nitrogen has an equivalent effect, i.e. the numerical ratio 1:3:9 is considered to be coincidental.

For $\Delta H^{o}_{-NH_2}$, I = -40.5; II = -38.4; III = -50.4 (kJ mol^{-1}) but no comparison can be made because of the added complication arising from protonating the imidazole ring of histidyl.

The entropy terms for phenylalanyl's two protonations are less positive than those for the other two amino-acids under discussion.

	$\Delta S^{o}_{-NH_2}$	$\Delta S^{o}_{-COO^-}$
Histidyl	48.5	40.2
Tryptophyl	61.1	41.4
Phenylalanyl	15.0	20.4

(kJ mol^{-1} K^{-1})

These values arise mainly from the decrease in the hydration sheaths

upon protonation. The two related molecules, I and II have similar entropy changes whereas III is less polar and so has a smaller value.

(b) The Chelation of the Metal Ions

The increasing importance of the +II oxidation state as one crosses the first transition series is an important aspect of transition metal chemistry. This increased stability to the eventual exclusion of all other oxidation states at zinc arises because the 3d orbitals change from being diffuse excited orbitals into tightly-bound core orbitals. The +II state becomes most important from manganese onwards and, except for copper(I) and iron(III), the aqueous chemistry of these transition metals is wholly that of the +II state. Iron(III) has great importance not only because of its aqueous chemistry but also because of its biological role and thus it merits special attention. This +III oxidation state for iron occurs against a trend of decreasing occurrence of oxidation states higher than +II because of the comparatively low third ionisation potential for iron which in turn arises from the metal's electronic configuration. A similar reason explains the stability of copper(I). These points will be dealt with more fully in a subsequent section.

The +II oxidation state occurs for the metals manganese through to zinc by ionisation of the two 4s electrons to produce an outer electronic configuration of $3d^n$ (n is 5 for Mn and 10 for Zn). All the ions form complexes and are hydrated in aqueous solution. Small ligands usually produce octahedral complexes and so the aquated ions are written $[B(H_2O)_6]^{2+}$. Copper deviates from this stereochemistry and gives Jahn-Teller distorted octahedral coordination, which in extreme cases becomes square planar.[102]

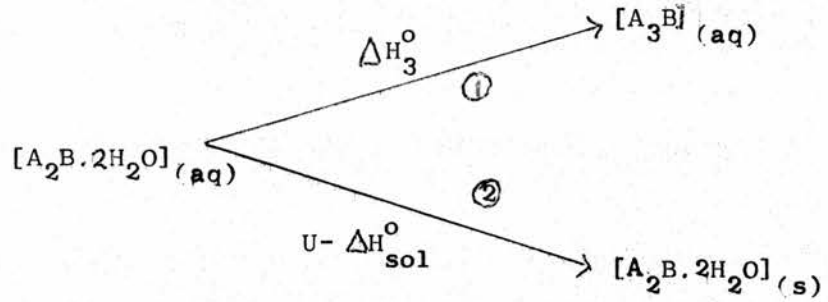
Complexes have been reported having up to three amino acids anions bound per divalent central metal ion[99] but in this study, this situation arose only for iron(II). Copper, as might be expected, formed $Cu^{II}phe_2$ (where phe is the phenylalanyl anion) but not $Cu^{II}phe_3$; Nickel and cobalt, however, could be expected to form A_3B complexes quite readily, but we found no evidence for these complexes because precipitation occurred at $\bar{Z} = 0.8$. This was due to the formation of an insoluble, aquated, 2:1 complex $A_2B.2H_2O$. The nickel complex was identified by analysis and similar complexes have been studied by infra-red spectroscopy[83]. In fact both nickel- and cobalt-phenylalanine complexes had been reported previously and their insolubility had been commented upon[88].

Table XXI

Thermodynamic parameters for metal-phenylalanyl complexes in 3.00 M $(Na)ClO_4$ at 25°C.

		ΔG^o (kJ mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J $mol^{-1} K^{-1}$)
Cu^{2+}	β_1	-47.09	-19.18	93.60
	K_2	-41.69	-39.17	8.48
	β_2	-88.78	-58.35	102.1
Ni^{2+}	β_1	-30.57	-9.82	69.59
	K_2	-29.26	-14.72	48.76
	β_2	-59.83	-24.54	118.4
Co^{2+}	β_1	-25.40	-5.30	67.45
	K_2	-22.78	-8.06	49.81
	β_2	-48.19	-13.36	116.85
Fe^{2+}	β_1	-21.33		
	K_2	-19.73		
	β_2	-41.07		
Fe^{3+}	β_1	-59.33		
	K_2	-49.79		
	β_2	109.12		

The formation of the solid $A_2B.2H_2O$ in preference to the soluble complex A_3B is due to the high lattice energy of $A_2B.2H_2O$.



where ΔH_3^o is the heat of formation of A_3B

U is the lattice energy of $A_2B.2H_2O_{(s)}$

ΔH_{sol}^o is the heat of solution of $A_2B.2H_2O_{(s)}$

However, the reaction path followed is dependent upon the Gibbs free energy of each reaction and so the entropy terms ought also to be examined in addition to these enthalpy terms.

Complexes have a greater freedom of movement in solution compared to the solid state and so the entropy of precipitation ought to be negative. The neutrality of the complex will minimise the hydration sheath which for most charged ions is large enough to make the entropy term positive and enhance precipitation.

The entropy term for forming A_3B is a small positive quantity[99] and so even though the signs of the entropy changes are opposite this will produce only a small numerically difference compared to the large enthalpic differences.

$\Delta H_{(sol)}^o$ is small compared to the lattice energy and so the latter is the more important term in dictating the choice between paths ① and ②.

$$U = - \frac{NA^2 z^2}{r} \left(1 - \frac{1}{n} \right) 103$$

U is lattice energy (ergs)

N is Avogadro's number (6.024×10^{23})

A is Madelung constant

e is electronic charge (4.802×10^{-10} e.s.u.)

r is ionic radius (cm.)

n is a constant for the system.

Thus, the lattice energy is dependent upon the two parameters r and n, (n has been experimentally determined from compressibility measurements and has values in the range 5-12[103]). Upon inspection, the B-O distance in $B(H_2O)_6^{2+}$ when B = Fe^{2+} is different from when B = Ni^{2+} and Co^{2+}.

Table XXII

Bond distances in $B(H_2O)_6^{2+}$ "octahedra."[100]

	H_2O - B(nm)			B-O (nm) (oxide)
B	1*	2*	3*	
Fe^{2+}	0.217	0.214	0.210	0.217
Co^{2+}	0.211	0.209	0.208	0.212
Ni^{2+}	0.209	0.208	0.204	0.208

* (There are three different B-O distances in all the aquo ions)

Hence this difference in ionic radii quite probably explains Fe^{2+} following reaction scheme (1) and Co^{2+} and Ni^{2+} following scheme (2) since the lattice energies for the latter ions become larger (more negative) then ΔH_3^os.

For ions in the series from Mn^{2+} though to Zn^{2+}, the general stability sequence for the replacement of water by more polarizable ligands is given

by the Irving-Williams series:

$$Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}.$$

This reflects the changes in heats of complex formation along the series[100] and as can be seen from table XXI, the results obtained in this study obey the Irving-Williams order.

Assuming that ionisation potential sums are a measure of the tendency of a metal ion to draw electrons to itself by the formation of covalent bonds, then some correlation between these sums and the stability of the complexes of the metal ions would be expected. Figure 20 shows this correlation for the four divalent metal ions studied complexing with phenylalanine.

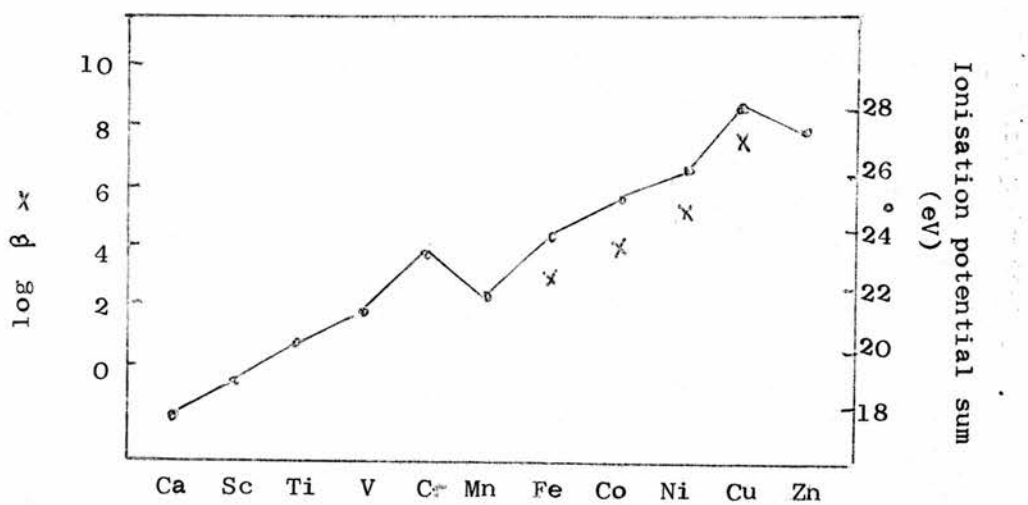


Figure 20

Log B_1 for phenylalanyl complexes compared to the ionisation energies of the metal atoms giving the divalent cations.

From Table XXI it can be seen (i) that the enthalpy contribution to the Gibbs free energy makes this correlation an enthalpy effect and (ii) that for all the metals studied $-\Delta H_1^o < -\Delta H_{1,2}^o$, an effect that has been previously reported by Izatt et al[42] and ascribed to the large difference in hydrational energies between $B^{2+}_{(aq)}$ and $AB^{+}_{(aq)}$ ΔS_1^o, however, is consistently larger than ΔS_2^o and this is mainly due to statistical factors. Thus, the overall effect is $-\Delta G_1^o > -\Delta G_{1,2}^o$ a fact uniformly true for all literature values of metal-amino acid interactions with just one exception (Sychev and Migal's[93], phenylalanine work; their experimental approach has been questioned by Gergely et al[88]).

The trends in ΔS^o show an interesting inversion in that ΔS_1^o decreases from Cu^{2+} to Ni^{2+} to Co^{2+} whereas $\Delta S_{1,2}^o$ increases along this series. This inversion can be ascribed to the number of water molecules released in the two reactions

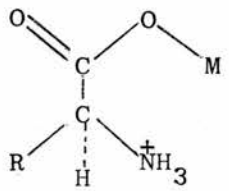
$$B^{2+}_{(aq)} + A^{-}_{(aq)} \longrightarrow BA^{+}_{(aq)}$$

$$\text{and } BA^{+}_{(aq)} + A^{-}_{(aq)} \longrightarrow BA_{2(aq)}$$

Cu^{2+} has the smallest ionic radius[102] and hence will have the largest hydrated radius (cf. the lanthanides[102]) and similarly $Ni^{2+} < Co^{2+}$. For BA_2, however, the size order will be reversed and so the overall entropy values will take the order $Cu^{2+} > Ni^{2+} > Co^{2+}$ (see table XXI). The trend in $\Delta S_{1,2}^o$ suggests that the number of water molecules released in the second reaction is $Cu^{2+} < Ni^{2+} < Co^{2+}$ and indicates that the size of the hydrated complex BA has the same order.

Metal-amino acid systems in solution have been studied by several schools[84,89,91,104,96] and the more recent publications have included

various protonated and hydrolysed species. Whilst the major complexes formed are the simple A_pB species, protonated, hydrolysed and polynuclear species have been found to exist[106,107] for ligands related to amino acids and for a few amino acids themselves. Perrin[105] obtained constants for the protonated species ABH and A_2BH involving a series of divalent metal ions and aliphatic amino acids. These complexes were said to be analogous to acetate complexes and hence the amine group was protonated and the metal-ligand bond utilises the carboxyl group. Jones and Williams[96] have reported the formation of protonated species with the lanthanide^{3+} ions and histidyl but here the protonation is on the imidazole nitrogen. In this present study, we found no protonated species for the aromatic phenylalanine-metal system thus confirming the results of Izatt et al[34] and Curchod[90]. Clearly, the formation of complexes such as



is dependent upon the type of R group and so for aliphatic groups that are electron repelling (e.g. $-CH_3$) then the positive charge on the nitrogen will undoubtedly be stabilised whereas for electron withdrawing R groups (e.g. aromatic rings) the charged nitrogen will be destabilised and hence protonation inhibited thus encouraging complex formation. This extra stability of aromatic amino acid complexes has been noted by other authors[88,84].

Although protonated complexes were not present, hydrolysis was detected in the copper(II) and iron(III) systems. Hydrolysis in these systems is well authenticated[108], although formation constants have not been reported for hydrolysis (leading to polynuclearity) amongst simple amino acid complexes.

(c) The Individual Complexing Reactions

(i) Copper(II)

The +II oxidation state is dominant in the aqueous solution chemistry of copper becuase of the elements low second ionisation potential and the high energy of hydration for the Cu^{2+} ion. Chelating agents also encourage the +II oxidation state; for example ethylenediamine (en) reacts with copper(I) chloride in potassium chloride solution to give

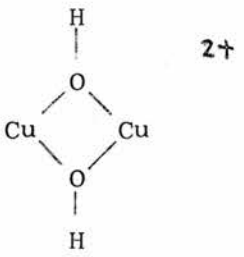
$$2CuCl + 2\ en = [Cu(en)_2]^{2+} + 2Cl^- + Cu$$

Hence complications, from varying oxidation states are unlikely to arise in our interpretation of the results obtained for the copper-phenylalanine system.

The formation of only Cuphe and $Cuphe_2$ may be expected under our experimental conditions since ethylenediamine ('log $\beta_1 \approx 9$) forms a 3:1 complex "only at extremely high concentrations of en."[102]

The copper phenylalanyl complexes were deep blue indicating a shift in absorption from the far red to the middle of the red region because of the stronger ligand field produced by the nitrogen donors.

In the presence of oxygen donors, copper forms a series of polynuclear complexes, the simplest of which is the hydroxy complex reported by Biedermann[41]. This as a dimer of structure



Hence, the formation of $Cu_2(phe^-)_2(OH)_2$ is not surprising, other analogous structures are found in the acetate and diazoaminobenzene complexes (both dimeric), and the formate and benzoate (both polymeric) complexes[102]. Of

these the acetate is the nearest analogue and also the most studied. The Cu-Cu distance is sufficiently short to permit some metal-metal interaction and this has been detected from magnetic studies of the solid complex.

Dimerisation has thus been recognised as an important feature of Cu(II) chemistry when copper is in an "oxygen environment" and, for this reason, the $Cu_2(phe^-)_2(OH)_2$ complex merits further study.

Figure 21 shows the distribution of complexes as a function of pH for blood plasma copper and ligand conditions. Values of these concentrations were obtained from "The Biochemist's Handbook". The predominent species at the pH of blood (7.38) are Cuphe and $Cuphe_2$ and the concentration of the dimeric hydroxy species is present as 2% of the total copper. Interpretations of the biological role of copper and its compounds ought to take into account such hydrolysed species if we are to learn from the analogy of the importance of hydrolysed iron species in liver storage.

(ii) Nickel(II)

Nickel forms, almost exclusively, divalent compounds in solution but these can have a variety of stereochemistries, of which the most important are the octahedral, tetrahedral and square planar configurations. In the solid state the formation of polymers through ligand sharing is important[102].

The 1:1 and 2:1 complexes with the phenylalanyl anion are formed by substituting the water of the hexaquo ion $[Ni(H_2O)_6]^{2+}$, by the chelating phenylalanyl ion to give octahedral complexes having four and two water ligands respectively.

The solid diaquobisphenylalinato-nickel(II) will, almost certainly, have a structure analogous to that of the glycine complex - a distorted

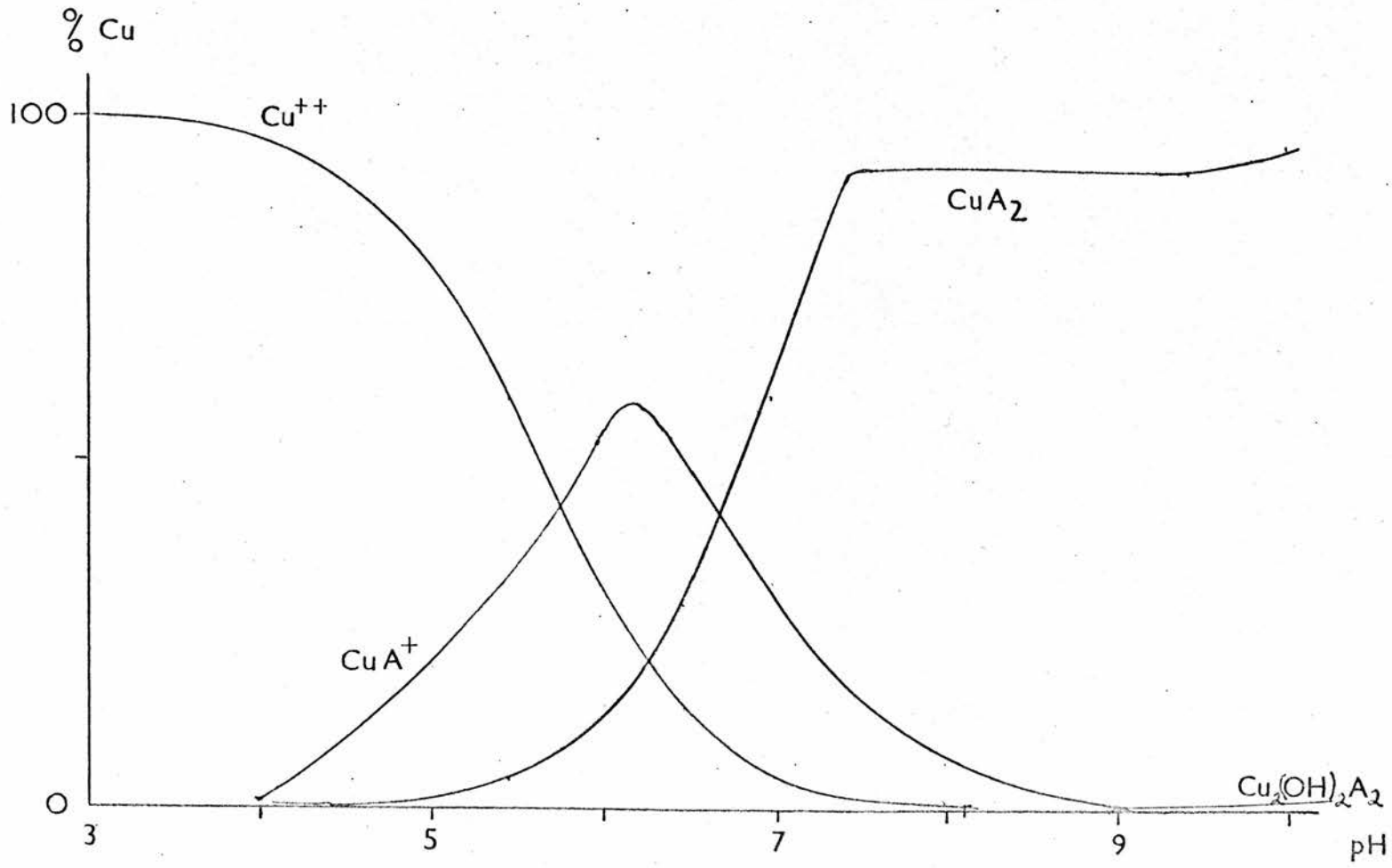


Fig.21. Species distribution for the Cu^{++}/Phe^{-} system in blood.

octahedra with two glycinate anions acting as chelating ligands in a plane and two water molecules occupying the trans-axial positions[109]. When heated in vacuo, this complex gives the anhydrous solid which is six-coordinate and contains terdentate ligands in which the carboxylate group is both bidendate and bridging[83]. There are other amino acids that behave in a like manner: α-alanine, β-alanine and α-amino-n-butyric acid. Hence, there is no apparent reason why phenylalanine ought not to be considered to form analogous compounds.

Although nickel is not an essential element for life in humans, iron, cobalt and nickel constitute group VIII of the first transition series and complexes of metals from this group are currently undergoing clinical trials as anticancer therapeuticals.[108]

(iii) Cobalt(II)

Cobalt has two main oxidation states and, in the absence of complexing ligands, the +II is the most stable state in aqueous solution. Complexation particularly with nitrogen donor ligands, makes relatively easy

$$[Co(NH_3)_6]^{3+} + e^- \rightarrow [Co(NH_3)_6]^{2+} \quad E^o = 0.1V$$

This stability of cobalt(III) complexes leads to an extensive solution chemistry and also, introduces the redox properties of the cobalt(II)/cobalt(III) couple, which biologically is very important.[28]

The simple aqueous chemistry of cobalt(II), however, is similar to that of nickel. The major species exist as simple salts and in solution as $[Co(H_2O)_6]^{2+}$. These mononuclear complexes are usually octahedral[102]. The data obtained for the cobalt(II)-phenylalanine system was most similar to that for nickel, hence it is reasonable to assume that the insoluble complex

formed had the same composition and structure.

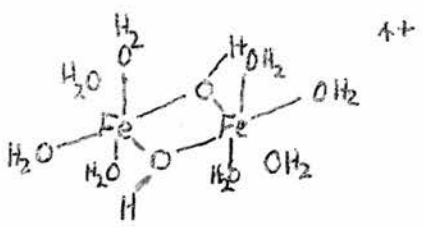
Several oxygen donor complexes of cobalt(II) are similar to those of nickel(II), the most striking examples being the polymeric nature of the acetylacetonates; the cobalt complex is tetrameric[110] and the nickel complex trimeric[111].

The biological importance of cobalt has not been fully characterised and much more work, both *in vivo* and *in vitro*, is necessary before the full implications of the Co(II)/Co(III) couple, and the unusual stereochemistries of the complexes, are determined and understood.

(iv) Iron(II and III)

The iron(II) and iron(III) results are considered together since any discussion of either must necessarily account for the redox couple between them. Both ions form octahedral hexaquo ions in solution and simple octahedral complexes. The hexaquo iron(II) is unstable, with respect to iron(III), in the presence of an oxidising agent, for example atmospheric oxygen. The hydrolysis of iron(III) is a major feature of its aqueous chemistry.

Although the data that we obtained for the iron(II)-phenylalanine system is limited, it is more comprehensive than any comparable study published by other schools for the amino acid system. The iron(III) hydrolysed species is analogous to that of the copper complex and thus the following structure has been suggested for the dimer[102]:



It is presumed that the $Fe_2^{III}(phe^-)_2(OH)_2$ will have a structure related to this hydroxy dimer and to the copper dimer discussed on page 96.

The Fe(II)/Fe(III) couple in 3.00 M $(Na)ClO_4$ was investigated but the oxidising power of the media was such that there was considerable potential drift rendering results unreliable. We intended to measure redox potentials for a range of complexes and thus describe the complete iron(II) iron(III)/phenylalanine system. This is a major project and would be the logical extension of the present work in addition to the calorimetric investigation of this iron system. The Fe(II)/Fe(III) couple has been extensively studied in the presence of a wide variety of ligands and most recently by Irving and Sharpe[112]. The multitude of complexes that occur in these systems demand a computer and a specially designed program to calculate the results.

Conclusions and the Future

The human body is but 3% metals and yet life depends upon these elements far more than this figure suggests. For example, *in vivo* the transition metal ions usually occur in the active centres of enzymes which control the marshalling of amino acids into peptides. Chemical investigations of transition metal ion - amino acid interactions have been reported for over twenty years and there is still ample scope for further studies.

The present thesis presents thermodynamic parameters for the protonation and complex formation of phenylalanine, by *in vivo* standards a relatively simple ligand, and yet an intriguing range of polynuclear and hydrolysis species are soon encountered at biological pHs. Such complexes demand further investigation and especially the iron systems which compared to

their *in vivo* importance have received but occasional glances in this thesis. Knowledge of the *in vitro* chemistries of such systems does not always directly reveal *in vivo* mechanisms but it certainly helps!

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APPENDIX I

RWCALCOR --- Instruction sheets(2).
sample output
Program

Program RWCALCOR

This program calculates the heat corrections for the formation of water, hydrolysed species and heats of protonation. The heat corrections together with the change in concentrations of each complex are then solved to give the heats of formation of the complexes.

INPUT

The program is stored under the 44PS system in three versions:

1. gives normal output explained later.

2. gives the normal output plus the heats of formation obtained from the experimental points for each experiment.

3. gives normal output plus the heats of formation for the total job;

and is called as follows

Card 1. `//  JOB  , name  CHEM  005`

(positions 1, 11, 19, 36, 52)

Card 2. `//bEXECbRWCALCR1`
or 2
or 3

DATA

`/*`
`/&`

The experimental input is as follows:-

ITEM 1 (I2) No. of experiments (CARE:- can only deal with one SCOOS job at a time)

Item 2 (20A4) Title

Item 3 (5I2) No. of AH_r complexes, no. of BH_r (r-ve) complexes, no. of A_pB complexes, no. of A_pBH_r complexes. Integer for reference point i.e. M = 0 for every point refered back to first point (additive) M > 0 for point to point.

Item 3a (I2) Value of r in A_pBH_r complexes (omit of no A_pBH_r complexes)

Item 4 (7E11.4) Heat of formation of water, heats of hydrolysis and heats of protonation. (Secure out if not formed - no blanks)

Item 5 Output from SCOGS*. This consists of N (the no. of points).

Then for each point a card containing pH and total volume and cards with the concentrations of all species formed.

Item 6 (2F10.3) (N-1) cards each having $\Delta^{o}C$ (or$\Delta\Omega$) and calibration ($J\ deg^{-1}$ or $J\ ohm^{-1}$)

For two experiments items 2 to 6 are repeated

(*The input for SCOGS must be in the same order as for Item 2

i.e. (i) Protonated species
(ii) Hydrolysed species
(iii) Complexes

OUTPUT

This is both printed and punched (Punched data for use in RWSOLV). The punched data consists of (N-1) cards for each experiments, the first point being omitted as it is a reference point. Each card shows the corrected heat change and the change in concentration of each metal-ligand complex.

CALORIMETRIC RUN C/A1

THE NUMBER OF LIGAND COMPLEXES = 2
THE NUMBER OF HYDROLYSED SPECIES = 0
THE HEAT OF FORMATION OF WATER = 5.5689E 04

HEATS OF HYDROLYSIS
0.0
0.0

HEATS OF PROTONATION
5.1469E 04
6.1000E 04
0.0
0.0

PH	VOLUME	Q-MEASURED	HEAT CORRECTIONS WATER	HEAT CORRECTIONS PROTONATION	HEAT CORRECTIONS HYDROLYSIS	Q-CORRECTED	CHANGE IN CONCENTRATION
3.0970	101.9000	8.0798D 00	1.1146D 01	-5.2948D 00	-0.0	2.2289D 00	
							COMPLEX(1) = 8.3877D-04 MOLES
							COMPLEX(2) = 1.0668D-04 MOLES
3.1557	103.9000	1.7421D 01	2.2295D 01	-1.0774D 01	-0.0	5.8961D 00	
							COMPLEX(1) = 8.8496D-04 MOLES
							COMPLEX(2) = 1.2732D-04 MOLES
3.2173	105.9000	2.6015D 01	3.3426D 01	-1.6446D 01	-0.0	9.0345D 00	
							COMPLEX(1) = 9.1908D-04 MOLES
							COMPLEX(2) = 1.5018D-04 MOLES
3.2837	107.9000	3.5096D 01	4.4527D 01	-2.2299D 01	-0.0	1.2868D 01	
							COMPLEX(1) = 9.5081D-04 MOLES
							COMPLEX(2) = 1.7745D-04 MOLES
3.3536	109.9000	4.3731D 01	5.5698D 01	-2.8464D 01	-0.0	1.6497D 01	
							COMPLEX(1) = 9.7794D-04 MOLES
							COMPLEX(2) = 2.0944D-04 MOLES
3.4296	111.9000	5.1765D 01	6.6828D 01	-3.4870D 01	-0.0	1.9807D 01	
							COMPLEX(1) = 9.9899D-04 MOLES
							COMPLEX(2) = 2.4733D-04 MOLES
3.5122	113.9000	6.0563D 01	7.7990D 01	-4.1615D 01	-0.0	2.4188D 01	
							COMPLEX(1) = 1.0126D-03 MOLES
							COMPLEX(2) = 2.9204D-04 MOLES
3.6026	115.9000	6.8914D 01	8.9134D 01	-4.8688D 01	-0.0	2.8469D 01	
							COMPLEX(1) = 1.0169D-03 MOLES
							COMPLEX(2) = 3.4492D-04 MOLES
3.8130	119.9000	8.4195D 01	1.1142D 02	-6.3982D 01	-0.0	3.6756D 01	
							COMPLEX(1) = 9.8942D-04 MOLES
							COMPLEX(2) = 4.8878D-04 MOLES

END OF JOB

```
C            CALCR1
C  CALCULATION OF HEAT CORRECTIONS & CHANGE IN CONCENTRATIONS OF COMPLEXES
C
C
      DOUBLE PRECISION   QCOR(25)  , DCI(25,25)   ,HHYD(20),
     1          XC%20,25)  , DC%20,25) ,HYD(20,25)    , SIGMAC%25)  ,
     2 CALIB%25)  , QMEAS%25)  , QCORR%25)  , CORPRO%25)  , CORH20%25)
     3 , CORHYD%25)  , ZU(25)  ,VOLF%25)  , H%25) ,TITLE(20),HH(20)
      REAL*8 LIG(20,25)
      DIMENSION NA(4),NB(4)      ,NH(10)
      DOUBLE PRECISION  SUMP%4<,SUMQ%4<,SUMR%4<,SUMS%4<,P%4<,Q%4<,R%4<,
     1T%4<,A%50,5<,AC%50<,V%4<,SUMT%4<,QCALC%50<,PCENT%50<
      DOUBLE PRECISION C(4,4),AC(4,4),PP(4),CC(5,4),DSQRT
C  ..................................................................
C PARAMETERS USED
C                    ZU(I)      THE PH OF THE SYSTEM AT POINT 'I'
C                    VOLF(I)    TOTAL VOLUME
C                    H(I)       CONC. OF FREE ACID
C                    XC(J,I)    CONC. OF SPECIES(J =)1 TO NCX
C                    LIG(J,I)   CONC. OF PROTONATED SPECIES 1 TO NLC
C                    HYD(J,I)   CONC. OF METAL HYDROLYSED SPECIES
C                    XC(JJ,I)   METAL-LIGAND COMPLEX CONCS.
C                    DC(J,I)    CHANGE IN CONCENTRATION FROM M TO I OF THE SPECIES.
C                               UNDER INVESTIGATION. (HEATS OF PROTONATION FOR NCX
C                               = 0).
C                    SIGMAC(I)  CHANGE IN TEMP(RESISTANCE) FROM I TO I+1
C                    CALIB(I)   CALIBRATION FOR CALORIMETER AT POINT 'I'.
C                    QMEAS(I)   HEAT (IN JOULES) EVOLVED IN THE REACTION FROM M TOI
C                    CORPRO(I)  HEAT CORRECTION FOR THE PROTONATION OF THE LIGAND.
C                    CORH2O(I)  HEAT CORRECTION FOR THE FORMATION OF WATER.
C                    CORHYD(I)  HEAT CORRECTION FOR THE HYDROLYSIS OF THE METAL.
C                    QCORR(I)   HEAT EVOLVED(TAKE IN) BY THE REACTION OF SPECIES
C                               UNDER INVESTIGATION.
C  ..................................................................
C        ** OUTPUT -- NEW PAGE
      WRITE(6,99)
C
```

```
   99 FORMAT('1')
C        *  INPUT -- ONE CARD  (ITEM 1)
      READ(5,10)KKKK
C
   10 FORMAT(I2)
      DO 1090 KKK= 1,KKKK
C        *  INPUT -- ONE CARD  (ITEM 2)
      READ(5,20)  TITLE
C
   20 FORMAT(20A4)
C
C
C    NLC IS THE NUMBER OF LIGAND COMPLEXES.
C    NHYDR IS THE NUMBER OF HYDROLYSIS COMPLEXES.
C     NABH IS THE NUMBER OF ABH SPECIES
C
C     MM IS AN OPTION. IF = 0 THEN ALL CALCULATIONS REFER TO POINT 1 AS BASE
C                      ALL OTHER VALUES GIVE 'POINT BY POINT'.
C
C     HW   IS THE HEAT OF FORMATION OF WATER
C     HHYD(L)    HEATS OF HYDROLYSIS
C     HH(L)L)    HEATS OF PROTONATION
C
C        *  INPUT -- ONE CARD  (ITEM 3)
      READ(5, 30)NLC  , NHYDR ,NCX ,NABH ,MM
C        *  INPUT -- IF ANY ABH SPECIES ONE CARD  (ITEM 3A)
      IF(NABH.GT.0)READ(5,30) (      NH(L),L = 1,NABH)
      IF(NHYDR.EQ.0)GO TO 41
C
   30 FORMAT(5I2)
C        *  INPUT -- ONE CARD  (ITEM 4)
      READ(5,40)HW,(HHYD(L),L = 1,  NHYDR   ),(HH(L) ,L = 1,NLC)
   41 READ(5,40)HW,                          (HH(L) ,L = 1,NLC)
C
   40 FORMAT(7E11.4)
C
C        ***  INTEGER - NHYD  VALUE OF J IN XC(J,I) TO GIVE 2ND. HYDROLYSIS CX.
```

```
      NHYD = NHYDR+ NLC
C
C
C        ***  INTEGER - NDP2  VALUE OF J IN XC(J,I) TO GIVE 1ST. HYDROLYSIS CX.
      NDP2 = NLC + 1
C
C
C        ***  INTEGER - NHYD2 VALUE OF J IN XC(J,I) TO GIVE 1ST. M-L CX.
      NHYD2 = NHYD + 1
C
C
C        ***  INTEGER - NCX  BECOMES THE TOTAL NUMBER OF ALL SPECIES PRESENT
      NCX = NCX + NHYD
      IF(NABH.GT.0) NCX = NCX + NABH
C
C        ** OUTPUT -- NEW PAGE AND TITLE OF EXPT. (ITEM 2 OF INPUT)
      WRITE(6,100) TITLE
C
  100 FORMAT('1',43X,20A4,/43X,'************************************'//)
C        ** OUTPUT -- INPUT ITEMS 3 & 4
      WRITE(6,105) NLC  ,NHYDR, HHYD(1) ,HH(1) , HW ,HHYD(2) ,HH(2),HH(3
     2),HH(4)
  105 FORMAT(1X,'THE NUMBER OF LIGAND COMPLEXES =    'I1,
     240X, 'HEATS OF HYDROLYSIS',  10X, 'HEATS OF PROTONATION'/, 1X,
     2  'THE NUMBER OF HYDROLYSED SPECIES = ',I1,
     4 45X, 1PE11.4,  15X, 1PE11.4, /1X, 'THE HEAT OF FORMATION OF WATER
     5 ='1PE11.4,38X,1PE11.4,15X,1PE11.4/,108X,1PE11.4/,
     6108X,1PE11.4//)
C
      WRITE(6,110)
  110 FORMAT(' '/6X,'PH', 8X,'VOLUME', 5X,'Q-MEASURED',2X,'********** H
     1EAT CORRECTIONS **********',
     2 4X,'Q-CORRECTED',6X,'CHANGE IN NO. OF MOLES',/43X,'WATER',5X,'
     3PROTONATION',4X,'HYDROLYSIS'/)
C
C   DATA AS DIRECTLY FROM SCOGS(3).
C
```

```
C         *  INPUT -- ONE CARD  (ITEM 5)
      READ(5,10)N
C
      DO 1060 I = 1,N
C         ***  INTEGER - M IS A VALUE ONE BEHIND I IN THE 'DO' LOOP
      M = I - 1
      IF(MM.EQ.0) M = 1
C
C         *  INPUT -- '2N' CARDS  (ITEM 5). (IF TOTAL NO. OF CXS.> 7 THEN'3N'CARD
      READ(5,50) ZU(I), VOLF(I)
      H(I)=DEXP(-2.3026*ZU(I))
C
   50 FORMAT(2E11.4)
      READ(5,40)(XC(J,I),J=1,NCX)
      DO 1000 J = 1,NLG
 1000 LIG(J,I) =XC(J,I)
C          IF  NO  HYDROLYSED SPECIES GO TO STATEMENT 1030
      IF(NHYDR.EQ.0)GO TO 1030
C
C
      GO TO(1020,1010),NHYDR
 1010 HYD(2,I) =XC(NHYD,I)
 1020 HYD(1,I) =XC(NDP2,I)
 1030 CONTINUE
C          FOR LIGAND PROTONATION HEATS CONTINUE BUT FOR M-L CXS. GO TO 2000
      IF(NCX.NE.NHYD)GO TO 2000
C
      JJ = 0
      DO 2050  J = 1, NLC
      JJ = JJ + 1
C
C          POINT 'I' NOW MEANS THE CHANGE ASSOCIATED WITH THE REACTION FROM M TOI
C
 2050 DC(JJ,I) =(VOLF(I)*LIG(J,I)  - VOLF(M)*LIG(J,M))*C.001
 2060 CONTINUE
      GO TO 1060
 2000  JJ = 0
```

```
      DO 1040 J = NHYD2,NCX
      JJ = JJ + 1
 1040 XC(JJ,I) =XC(J,I)
      DO 1050 J = 1,JJ
 1050 DC( J,I) = (VOLF(I)*XC( J,I) - VOLF(M)*XC( J,M))*0.001
 1060 CONTINUE
C
C  READ CALORIMETRY DATA IN,
C
C        *  INPUT -- 'N-1' CARDS (ITEM 6)
      READ(5, 51)(SIGMAC(I),CALIB(I),I = 2,N)
C
   51 FORMAT(2F10.3)
      M = 0
      DO 1070 I = 1,N
      M = I - 1
      IF(MM.EQ.0) M = 1
      QMEAS(I)=SIGMAC(I)*CALIB(I)
      IF(MM.EQ.0) QMEAS(I) = QMEAS(I) + QMEAS(I-1)
C
C   CORRECTIONS FOR WATER, HYDROLYSIS &PROTONATION
C
      IF(NCX.EQ.NHYD)GO TO 2070
      DO 1073 L = 1,NLC
 1073 CORPRO(I) = CORPRO(I) + 0.001 * HH(L) *(VOLF(I) * LIG(L,I) - VOLF(
     2M) * LIG(L,M))
C
C
C   FOR EITHER ACID INTO ACID OR BASE INTO BASE TITRATIONS, NO WATER IS FORMED.
C   HENCE, THE HEAT CORRECTION IS MISSED OUT. (GO TO 1071)
C
C
      IF(ZU(I).GT.ZU(M)) GO TO 2071
      IF(ZU(I).LT.ZU(M)) GO TO 2072
 2072 IF(NHYD.GT.0)GO TO 2070
      IF(ZU(I).LT.7.00)  GO TO 1071
      GO TO 2070
```

```
 2071 IF(NLC.GT.0)GO TO 2070
      IF(ZU(I).GT.7.00) GO TO  1071
 2070 CORH2O(I) = -(0.001*(VOLF(I)*(LIG(1,I) + 2.0*LIG(2,I) + 3.0*LIG(3,
     2I) + 4.0*LIG(4,I) + H(I)) -
     3                       VOLF(M)*(LIG(1,M) + 2.0*LIG(2,M) + 3.0*LIG(3,
     4M) + 4.0*LIG(4,M) + H(M))) )*HW
C
C
C     THIS STATEMENT CALCULATES THE LOSS OF PROTONS FOR A BASE INTO ACID
C     TITRATION , HENCE THE WATER PRODUCED IS THE REVERSE QUANTITY .I.E. -
C
C
      IF(NABH.EQ.0) GO TO 1071
       DO 1072 L = 1,NABH
 1072 CORH2O(I) = CORH2O(I) - NH(L) *(VOLF(I) * XC(L,I) - VOLF(M)*XC(L,M
     2))  * HW * 0.001
 1071 CONTINUE
C
C
      DO 1069  L = 1,NHYDR
      CORH2O(I) = CORH2O(I) - 0.001 * HHYD(L) * (VOLF(I) * HYD(L,I) -
     2 VOLF(M) * HW
 1069 CORHYD(I) = CORHYD(I) + 0.001 * HHYD(L) * (VOLF(I) * HYD(L,I) -
     2 VOLF(M) * HYD(L,M))
      QCORR(I)  =  QMEAS(I) - (CORPRO(I)+CORH2O(I)+ CORHYD(I))
 1070 CONTINUE
      DO 1080 I = 2,N
      IF(H(I))119,119,118
  118 ZU(I) = -DLOG10(H(I))
C        ** OUTPUT -- VALUES OF QCORR(I),ZU(I),VOLF(I),QMEAS(I) & HEAT CORRECTIO
C                     NS FOR I = 2,N
  119 WRITE(6,120)ZU(I) , VOLF(I) , QMEAS(I) , CORH2O(I) , CORPRO(I) ,
     2CORHYD(I) , QCORR(I)
C
  120 FORMAT(2(3X,F8.4),4(3X,1PE11.4),6X,1PE11.4)
C        ** OUTPUT -- VALUES OF DC(J,I)
      WRITE(6,130)(J,DC(J ,I),J = 1,JJ)
```

```
C
  130 FORMAT(101X,'COMPLEX('I1,') ='1PE11.4, '  MOLES')
 1080 CONTINUE
      WRITE(6,150)
  150 FORMAT(1X,///40X,'    END OF JOB')
 1090 CONTINUE
      STOP
      END
```

APPENDIX II

RWSCOGSY --- Instruction sheets(3)
Program

RWZASCOG --- Instruction sheet
Program

Program RWSCOGSY

This program is the version of SCOGS[1] used on the St. Andrews I.B.M. 360/44 computer. The program refines estimates of the Stability Constants for systems containing up to two metals and two ligands and can deal with protonated and mixed species. pK's are considered complexes of A and H^+.

Several modifications have been made to the imput and output, so care ought to be exercised if this version is to be compared with the published program.

INPUT

The program is stored under the 44PS system and is called using the following cards:-

```
Card 1.     //      JOB      , name     CHEM     010
            ↑1      ↑11      ↑19        ↑36      ↑52

Card 2.     //bEXECbRWSCOGSY          (Where b is a blank)
            ↑1
                    DATA

            /*
            /&
```

The experimental data is as follows

ITEM 1 (I2) Number of Jobs

ITEM 2 - (I2) Type of output required:-

01 Normal Iteration procedure

02 Normal Iteration procedure plus punched output

03 No iterations with punched output

NOTE: The punched output is for use in RWCALCR

ITEM 3 (I2) The number of experiments to be refined together.

ITEM 4 (3I2) Total number of ligands, total number of metals and total number of complexes (including protonated ligands)

ITEM 5 (5I2,F8.4) For each complex a card carrying: No. of A_1, no. of A_2, no. of B_1, no. of B_2, no. of hydroxyl ions (-ve sign for protons), contained in the complex, the logarithm of the Formation Constant of the corresponding species (β_j)

ITEM 6 (2I2) No. of dissociable protons on ligand 1, no. of protons on ligand 2, in the forms in which they were added to the solution (e.g.(1) histamine is zero but histamine dinitrate is two e.g.(2) phenylalanine is one)

ITEM 7 (20A4) Title of the experiment.

ITEM 8 (7F10.3) Initial concentrations of B_1, B_2, A_1, A_2, acid* and volume (Titrate (S))

ITEM 9 (7F10.3) Concentrations in titrate (T) of B_1, B_2, A_1, A_2, acid*, EO (in mV), FN (no. of mV/pH i.e. 59.162 at 25°)

ITEM 10 (2F10.3,4x,I1) One card for each experimental point bearing the titre in ml, Emf and Index (zero for all cards but the last)

ITEM 11 (2F8.4) $-pK_w$ and activity coefficient

ITEM 12 (2I2) No. of cycles of refinement desired (about 5) and no. of constants to be varied.

ITEM 13 (I2,F10.3) One card for each constant to be varied carrying the no. of the constant (in order of listing in Item 4) and the amount by which it is to be varied each time (0.0004 is usually enough)

When two sets of experiments are refined together the sequence of cards is: ITEMS 1,2,3,4,5,6,7,8,9,10 (for 1st expt.), 7,8,9,10 (for 2nd expt.), 11,12,13

(When two experiments are refined together it is considered as one job)..

For separate jobs, Items 2-13 are repeated for each job until the number in Item 1 is reached.

(* Acid is mineral acid)

OUTPUT

The output as far as the end of refinements should be self explanatory. Next there appears a long list of values of interest. These are calculated from the final formation constants, and are again self explanatory except for YH and YB. These are as defined in the Österberg[2] paper.

CARE:- All the output quantities have been calculated and are not the experimental (For experimental values use RWZASCOG (ZBAR) and RWYLOGH (YH and YB))

References

1. Sayce I.G., Talanta, 1968, 145, 1397.

2. Österberg R. and Sjöberg B., J. Biol. Chem., 1968, 3038.

```
C      SCOGS WITH A GENERAL INPU    JUNE 5TH 1970.
C
C
C      THIS PROGRAM IS WRITTEN IN FORTRAN IV(E)
C
       DOUBLE PRECISION CK(20),BC(20,20),CC(20,20),BTOT(2),CLTOT(2),
      1Y1(2),Y2(2),Y3(2),Y4(2),E(20),B(20),EORIG(20),TX(2),VX(2),C(20),          3
      2DE(20),X(20),TURM(500),CTITR,DTITR,R,RO,CT1,CT2,CT3,HO ,EL(20)
C
       DIMENSION NORC(30)  ,SIGMAM(2)  ,SIGMAL(2)  , ZBAR(2,2)  ,YBB(2,2)
      6,Y(2)  ,AMN(20), VOLF(500)  ,TLT(2)  ,TMT(2)  ,AML(2,20), AMM(2,20
      7)
       DIMENSION TITLE(20),ML(2,20),MM(2,20),MN(20),AL(2,20),AM(2,20),           5
      1AN(20),NDP(2),ENDP(2),TM(2),TL(2),TITRE(60),ZU(60),ZB(2,60),             6
      2ZL(2,60),U(500),AC(500),BA(500),V(500),TITR(500),TEMP(20,20),            7
      3BB(2,500),CL(2,500),IG(20),H(20),HORIG(20),UXS(500),NINCCH(500),         8
      4ICH(20),DM(2),DMY(2),IFR(30)
C
       COMMON C,Y1,Y2,Y3,Y4,BTOT,CLTOT,TX,VX,HO,B,                              10
      1ML,MM,MN,AL,AM,AN,NL,NM,N,UX,F,CKW,NIT,NNCI,KJ                           11
C
       INTEGER TYPE
C
   99 FORMAT(2I2)
  101 FORMAT (I2)
  102 FORMAT (20A4)                                                             34
  103 FORMAT (1X,20A4)                                                          36
  104 FORMAT('1'8X,'L1  L2  M1  M2  OH  LOG.BETA   ( BETA      )'/)
  105 FORMAT (3I2)                                                              18
  107 FORMAT (5I2,F8.4)                                                         21
  108 FORMAT (1X,I3,2X,5(2X,I2),3X,F8.4 ,3X, '(',1PE11.4,')')
  110 FORMAT (2I2)                                                              28
  111 FORMAT(/' NDP(',I1,') = ',I2)                                             74
  113 FORMAT (7F10.3)                                                           38
  114 FORMAT(' ',10X,'TITRATE  (S)    M1 = '  F9.6,'  M2 = ',  F9.6'  L1
     2 = ',  F9.6,'  L2 = ', F9.6,'  ACID = '  F9.6, '  INITIAL VOLUME =
     3 ', F7.3/
```

```
     4          ,11X,'TITRANT  (T)    M1 = '  F9.6,'  M2 = ',  F9.6'  L1
     5 = ',  F9.6,'  L2 = ', F9.6,'  ACID = '  F9.6, '  E0 = ', F7.2)
   116 FORMAT (2F10.5,4X,I1)                                                47
   118 FORMAT(' (',I3,' READINGS)'/)                                        51
   122 FORMAT (2F8.4)                                                       81
   123 FORMAT (/' CKWL=',F7.3,5X,'F=',F5.2/)                                83
   124 FORMAT (/' NUMBER OF PARAMETERS  ',I2/' TOTAL NUMBER OF READINGS     85
     1 ',I3/)                                                               86
   126 FORMAT (I2,E10.3)                                                    91
   173 FORMAT (/' INCREMENT FOR VARIABLE CONSTANT ',I2,' RAISED')          208
   175 FORMAT (/' INCREMENTS CHANGED FOR ',I3,' POINT(S)')                 215
   182 FORMAT (/' OVERSHIFT, VARIABLE CONSTANT NO. ',I2,' X(I)=',1PE10.3)  230
   183 FORMAT (/' EXTREME OVERSHIFT, VARIABLE CONSTANT NO. ',I2,' X(I)=',  234
     11PE10.3)                                                              235
   196 FORMAT (/' THE STANDARD DEVIATION IN TITRE WITH THE INPUT CONSTANT
     1S IS ',1PE11.4/)
   197 FORMAT (/' THE STANDARD DEVIATION IN TITRE IS ',1PE11.4)            266
   201 FORMAT (/' X1 NEGATIVE')                                            273
   202 FORMAT (1X,I2,F12.4,F8.4,2X,'SHIFT=',F7.4)                          276
   206 FORMAT (/' HALF SHIFTS APPLIED FOR NEXT CYCLE')                     283
   207 FORMAT (/' CYCLES CALCULATED ',I2)                                  285
   209 FORMAT (//2X,'K',3X,'PH',5X,'TITRE',5X,'RESID',6X,'TM1',8X,'TM2',8  293
     1X,'TL1',8X,'TL2',8X,'FM1',8X,'FM2',8X,'FL1',8X,'FL2',12X,'YH'/,
     2         12X,'C1',9X,'C2',9X,'C3',9X,'C4',9X,'C5',9X,'C6',9X,'C7',9
     3X,'C8',9X,'C9',8X,'C10',12X,'YB1,1'/,
     4         8X,'C11',8X,'C12',8X,'C13',8X,'C14',8X,'C15',8X,'C16',8X,'
     5C17',8X,'C18',8X,'C19',8X,'C20',12X,'ZBAR1,1'/)
   212 FORMAT (1X,I3/4X,F6.3,2F11.6,8(1X,1PE10.3),2X, OPF7.4)
   999 FORMAT (1H1)                                                        13
C
C      READS IN DATA
C
       READ (5,99) NJ
       CALL CLEUND
       NJD=0
     1 WRITE (6,999)
       READ(5,99) TYPE
```

```
  100 READ (5,101) INEXP
      READ (5,105) NL,NM,N
      DO 106 J=1,N                                                          19
  106 READ (5,107)   (ML(I,J),I=1,2),(MM(I,J),I=1,2),MN(J),E(J)             20
      DO 109 J=1,N                                                          22
      EL(J) = 10.**E(J)
      AMN(J) = -MN(J)
      AN(J)=MN(J)                                                           23
      DO 109 I=1,2                                                          24
      AML(I,J) = ML(I,J)
      AMM(I,J) = MM(I,J)
      AL(I,J)=ML(I,J)                                                       25
  109 AM(I,J)=MM(I,J)                                                       26
      READ (5,110) (NDP(I),I=1,2)                                           27
      DO 112 I=1,2                                                          29
  112 ENDP(I)=NDP(I)                                                        30
      ITN=0                                                                 31
      DO 231 I=1,15
  231 IFR(I)=0
       KKK = 0
      DO 121 NEXP=1,INEXP                                                   32
C
C     READS IN TITRATION DATA FOR INDIVIDUAL EXPERIMENT
C
      READ (5,102) TITLE
      WRITE (6,103) TITLE
      READ (5,113) TM(1),TM(2),TL(1),TL(2),ACID,     VOL
      READ(5,113) TMT(1)  ,TMT(2)  , TLT(1)  ,TLT(2)  ,ACIDT,EO ,FN
      BASE = -ACIDT
      WRITE(6,114) TM(1)  ,TM(2)  ,TL(1)  ,TL(2)  ,ACID  ,VOL  ,TMT(1) ,
     2TMT(2)  ,TLT(1)  ,TLT(2)  ,ACIDT ,EO
      K=0                                                                   43
      KK = 0
      IF(TYPE.EQ.3)KK = 1
  115 K=K+1                                                                 45
      READ (5,116) TITRE(K),ZU(K),INDEX                                     46
      IF (INDEX) 117,115,117                                                48
```

```
  117 NOR=K                                                                  49
      KKK = KKK + 1
      NORC(KKK) = NOR
      WRITE (6,118) NOR
      DO 119 K=1,NOR
      ZU%K<#%E0-ZU%K<</FN
      VOL1=VOL+TITRE(K)                                                      53
      DO 119 I=1,2                                                           54
      ZB(I,K) = (TM(I)*VOL + TMT(I)*TITRE(K))/VOL1
  119 ZL(I,K) = (TL(I)*VOL + TLT(I)*TITRE(K))/VOL1
 5003 KTN=ITN+1
      IFR(NEXP)=KTN                                                          58
      LTN=ITN+NOR                                                            59
C
C     COMBINES DATA FOR INDIVIDUAL EXPERIMENTS
C
      DO 120 K=KTN,LTN                                                       60
      MTN=K-ITN                                                              61
      U(K)=ZU(MTN)                                                           62
      AC(K)=ACID
      BA(K)=BASE                                                             64
      V(K)=VOL                                                               65
      TITR(K)=TITRE(MTN)                                                     66
      DO 120 I=1,2                                                           67
      BB(I,K)=ZB(I,MTN)                                                      68
  120 CL(I,K)=ZL(I,MTN)                                                      69
  121 ITN=ITN+NOR                                                            70
      IF(TYPE.NE.1) WRITE(7,99) NORC(1)
      DO 1210 K=1,ITN                                                        71
 1210 TURM(K)=CL(1,K)*ENDP(1)+CL(2,K)*ENDP(2)                                72
      WRITE (6,111) (I,NDP(I),I=1,NL)
      WRITE (6,104)
      DO 301 J=1,N                                                           77
  301 WRITE (6,108) J,(ML(I,J),I=1,2),(MM(I,J),I=1,2),MN(J),E(J) ,EL(J)
      READ (5,122) CKWL,F                                                    80
      WRITE (6,123) CKWL,F
      WRITE (6,124) N,ITN
```

```
      IF(TYPE.EQ.3) WRITE(6,209)
      READ (5,110) NCD,NCV
      DO 127 I=1,NCV                                                    89
      READ (5,126) IG(I),H(I)                                           90
  127 HORIG(I)=H(I)                                                     92
C
C     BEGINS REFINEMENT
C
      NCC=0                                                             93
      ICC=0                                                             94
      CKW=10.**CKWL                                                     95
      DO 129 K=1,ITN                                                    96
  129 UXS(K)=10.**U(K)                                                  97
      DO 1290 I=1,2                                                     98
      TX(I)=0.0                                                         99
 1290 VX(I)=0.0                                                        100
  130 NCC=NCC+1                                                        101
      NNCI=0                                                           102
      SQR=0.0                                                          103
      SQRO=0.0                                                         104
      INCCH=0                                                          105
      DO 131 K=1,ITN                                                   106
  131 NINCCH(K)=0                                                      107
      DO 132 J=1,N                                                     108
  132 EORIG(J)=E(J)                                                    109
      DO 133 J=1,20                                                    110
      C(J) = 0.0
      ICH(J)=0                                                         111
      CK(J)=0.0                                                        112
      DO 133 J1=1,20                                                   113
  133 CC(J,J1)=0.0                                                     114
      L=0                                                              115
  134 K=1                                                              116
      NEXP=1                                                           117
  135 DO 136 I=1,2                                                     118
      BTOT(I)=BB(I,K)                                                  119
  136 CLTOT(I)=CL(I,K)                                                 120
```

```
      J=0                                                                  122
      M=0                                                                  123
      UX=UXS(K)                                                            124
      DO 138 I=1,2                                                         125
      Y1(I)=BTOT(I)*0.0000001                                              126
  138 Y3(I)=CLTOT(I)*0.0C00001                                             127
C
C     DETERMINES INITIAL ESTIMATE FOR FREE METAL AND FREE LIGAND CONCENTRATIONS
C
      IF (ICC-1) 139,140,140                                               128
  139 IF (K-IFR(NEXP)) 142,143,142                                         129
  142 IF (NIT-99) 140,141,141                                              130
  143 NEXP=NEXP+1                                                          131
  141 DO 144 I=1,NM                                                        132
  144 VX(I)=BTOT(I)                                                        133
      DO 146 I=1,NL                                                        134
      DMY(I)=1.0                                                           135
      DO 146 J1=1,N                                                        136
      IF (ML(I,J1))146,146,147                                             137
  147 DO 148 K1=1,NM                                                       138
      IF (MM(K1,J1)) 146,148,146                                           139
  148 CONTINUE                                                             140
      DM(I)=10.**E(J1)*UX**MN(J1)                                          141
      DMY(I)=DMY(I)+DM(I)                                                  142
  146 CONTINUE                                                             143
      DO 145 I=1,NL                                                        144
  145 TX(I)=CLTOT(I)/DMY(I)                                                145
  140 DO 149 I=1,N                                                         146
  149 B(I)=10.**E(I)                                                       147
      KJ=K                                                                 148
C     CALLS SUBROUTINE FOR CALCULATION OF ALL FREE METAL, FREE LIGAND, AND
C     SPECIES CONCENTRATIONS
C
      CALL COGSNR
C
      IF (NNCI-30) 150,215,215                                             150
  150 CTITR=V(K)*(TURM(K)+AC(K)-HO)/(BA(K)-TURM(K)+HO)                     151
```

```
      CT=CTITR                                                          152
      DTITR=TITR(K)                                                     153
      R=CTITR-DTITR                                                     154
      IF (NIT-100) 220,220,221                                          155
  221 R=0.0                                                             156
  220 IF (KK) 152,152,153                                               157
  152 IF (L-1) 154,216,216                                              158
  154 M=M+1                                                             159
      GO TO (155,156,157),M                                             160
  155 SQRO=SQRO+R*R                                                     161
      CT1=CTITR                                                         162
      RO=R                                                              163
C
C     BEGINS NUMERICAL DIFFERENTIATION
C
      J=J+1                                                             164
      IS=IG(J)                                                          165
  160 E(IS)=EORIG(IS)+H(J)                                              166
      ICC=ICC+1                                                         167
      GO TO 140                                                         168
  156 CT2=CTITR                                                         169
      E(IS)=EORIG(IS)-H(J)                                              170
      GO TO 140                                                         171
  157 CT3=CTITR                                                         172
      E(IS)=EORIG(IS)                                                   173
      IF (CT2-CT3) 158,159,158                                          174
  159 M=1
C
C     INCREASES INCREMENT USED IN NUMERICAL DIFFERENTIATION, IF THIS WAS TOO
C     SMALL
C
      H(J)=H(J)*5.0                                                     177
      INCCH=1                                                           178
      ICH(J)=1                                                          179
      NINCCH(K)=1                                                       180
      GO TO 160                                                         181
  158 DE(J)=(CT2-CT3)/(2.*H(J))                                         182
```

```
      H(J)=HORIG(J)                                                     183
      IF (J-NCV) 161,162,162                                            184
  161 J=J+1                                                             185
      M=1                                                               186
      IS=IG(J)                                                          187
      E(IS)=EORIG(IS)+H(J)                                              188
      GO TO 140                                                         189
C
C     SETS UP MATRIX
C
  162 DO 163 II=1,NCV                                                   190
      CK(II)=CK(II)-RO*DE(II)                                           191
      DO 163 JJ=1,NCV                                                   192
  163 CC(II,JJ)=CC(II,JJ)+DE(II)*DE(JJ)                                 193
  164 K=K+1                                                             198
      ICC=0                                                             199
      IF (K-ITN)135,135,167                                             200
  167 DO 168 I=1,NCV                                                    201
      DO 168 J=1,NCV                                                    202
  168 BC(I,J)=CC(I,J)                                                   203
      IF (INCCH) 169,169,170                                            204
  170 DO 171 I=1,NCV                                                    205
      IF (ICH(I)) 171,171,172                                           206
  172 WRITE (6,173) I
  171 CONTINUE                                                          209
      NICH=0                                                            210
      DO 174 I=1,ITN                                                    211
      NICH=NICH+NINCCH(I)                                               212
  174 NINCCH(I)=0                                                       213
      WRITE (6,175) NICH
C
C      CALLS MATRIX INVERSION SUBROUTINE, AND SOLVES FOR SHIFTS
C
  169 CALL SUB760(BC,NCV)
C
      DO 176 I=1,NCV                                                    217
  176 X(I)=0.0                                                          218
```

```
      DO 177 I=1,NCV                                                    219
      DO 177 J=1,NCV                                                    220
  177 X(I)=X(I)+BC(I,J)*CK(J)                                           221
      IEOS=0                                                            222
      IOS=0                                                             223
      DO 178 I=1,NCV                                                    224
      XAB=DABS(X(I))                                                    225
C
C     CHECKS SIZE OF SHIFT AND IF GREATER THAN 1 LOG UNIT REDUCES SHIFT TO
C     0.5. IF ANY SHIFT WAS GREATER THAN 0.5 BUT NONE WERE GREATER THAN 1
C     REDUCES ALL SHIFTS TO HALF VALUE. PRINTS APPROPRIATE MESSAGE
C
      IF (1.-XAB) 179,180,180                                           226
  180 IF (0.5-XAB) 181,178,178                                          227
  181 IOS=1                                                             228
      WRITE (6,182) I,X(I)
      GO TO 178                                                         231
  179 IEOS=1                                                            232
      WRITE (6,183) I,X(I)
  178 CONTINUE                                                          236
      IF (IEOS) 184,184,185                                             237
  185 DO 186 I=1,NCV                                                    238
      IF (1.-X(I)) 187,187,188                                          239
  188 IF (1.+X(I)) 189,189,186                                          240
  187 X(I)=0.5                                                          241
      GO TO 186                                                         242
  189 X(I)=-0.5                                                         243
  186 CONTINUE                                                          244
      GO TO 190                                                         245
  184 IF (IOS) 190,190,191                                              246
  191 DO 192 I=1,NCV                                                    247
  192 X(I)=0.5*X(I)                                                     248
  190 SQRO=SQRO/FLOAT(ITN-NCV)                                          249
      DO 193 I=1,NCV                                                    250
      IS=IG(I)                                                          251
  193 E(IS)=EORIG(IS)+X(I)                                              252
      IF (NCC-1) 194,194,195                                            253
```

```
  194 SQRP=SQRT(SQRD)
C
C     PRINTS STANDARD DEVIATION IN TITRE WITH INITIAL CONSTANTS
C
      WRITE (6,196) SQRP
  195 L=L+1                                                              257
      ICC=0                                                              258
      GO TO 134                                                          259
  216 SQR=SQR+R*R                                                        260
      K=K+1                                                              261
      IF (K-ITN) 135,135,217                                             262
  217  YA=SQR/FLOAT(ITN-NCV)
      YB=SQRT(YA)
C
C     PRINTS STANDARD DEVIATION IN TITRE WITH NEW CONSTANTS
C
      WRITE (6,197) YB
      DO 198 I=1,NCV                                                     267
      IS=IG(I)                                                           268
      X1=BC(I,1)*YA                                                      269
      IF (X1) 199,200,200                                                270
  199 X1=-X1                                                             271
      WRITE (6,201)
  200 X1=SQRT(X1)                                                        274
C
C     PRINTS IMPROVED CONSTANTS, ESTIMATED STANDARD DEVIATIONS, AND SHIFTS
C     FROM FORMER VALUES
C
  198 WRITE (6,202) IS,E(IS),X1,X(I)
      IF (SQRD-YA) 203,204,204                                           277
  203 DO 205 I=1,NCV                                                     278
      IS=IG(I)                                                           279
      X(I)=0.5*X(I)                                                      280
  205 E(IS)=E(IS)-X(I)                                                   281
      WRITE (6,206)
  204 WRITE (6,207) NCC
      IF (NCC-NCD) 130,208,208                                           286
```

```
C
C      AFTER FINAL CYCLE OF REFINEMENT CALCULATES AND PRINTS TABULATION OF
C      QUANTITIES OF INTEREST USING FINAL VALUES OF CONSTANTS
C
  208 KK=1                                                                287
      DO 230 I=1,20                                                       288
  230 C(I)=0.0                                                            289
      WRITE (6,209)
      GO TO 134                                                           299
  153 IF(TYPE.EQ.1)GO TO 6000
 6001 VOLF(K) = V(K) + TITR(K)                                            52
      WRITE(7,778) U(K), VOLF(K)
  778 FORMAT(7E11.4)
      N1 = N + 1
      DO 6101 J = N1,10
 6101 C(J) = 0.0
      WRITE(7,778)(C(J),J=1,N )
      NORS # 0
      DO 6011 KKK = 1,30
      NORS # NORS & NORC%KKK<
      IF(NORS.EQ.ITN)GO TO 6000
      IF(K.EQ.NORS)WRITE(7,99) NORC(KKK+1)
 6011 CONTINUE
 6000 DO 5998  I =1,2
      SIGMAL(I)  = 0.
      SIGMAM(I)  = 0.
      SIGMAN     = 0.
      DO 5999 J = 1,N
      SIGMAN     = SIGMAN    +AMN(J)*C(J)
      SIGMAM(I)  = SIGMAM(I)  +AMM(I,J)*C(J)
      IF(AMM(1,J))5989,5989,5988
 5989 IF(AMM(2,J))5999,5999,5988
 5988 SIGMAL(I)  = SIGMAL(I)  +AML(I,J)*C(J)
 5999 CONTINUE
      DO 5998 II =1,2
      IF(BB(II,K).EQ.0.0)GO TO 5998
      IF(CL(II,K).EQ.0.0)GO TO 5998
```

```
      ZBAR(I,II) = SIGMAL(I) /BB(II,K)
      YBB(I,II)= SIGMAM(I) /CL(II,K)
      Y(II) = SIGMAN /CL(II,K)
 5998 CONTINUE
      WRITE (6,212) K,U(K),TITR(K), R,BB(1,K),BB(2,K),CL(1,K),CL(2,K),        3
     1VX(1),VX(2),TX(1),TX(2)  ,Y(1)                                          301
      WRITE (6,213) (C(J),J=1,10)  ,YBB(1,1)
      WRITE (6,214) (C(J),J=11,20) ,ZBAR(1,1)
      DO 333 J = 1,N
      IF(ML(2,J))331,331,332
  331 IF(MM(2,J))333,333,332
  333 CONTINUE
      GO TO 335
  332 WRITE(6,334) ZBAR(1,2)  , (ZBAR(2,I),I=1,2) ,YBB(1,2) ,(YBB(2,I),I
     2=1,2) ,  Y(2)
  335 K=K+1
  334 FORMAT(' ', 5X, 'ZBAR1,2 =',F7.4,2X,'2,1 =',  F7.4, 2X,  '2,2 =',
     2F7.4,'****  YB1,2 =',  F7.4, 2X, '2,1 =',  F7.4,2X,  '2,2 =', F7.4
     3,  2X,'Y2 =', F7.4)
  213 FORMAT (8X,10(1X,1PE10.3),4X,0PF7.3)
  214 FORMAT (5X,10(1X,1PE10.3), 5X,0PF7.3)
      IF (K-ITN) 135,135,215                                                   308
  215 CONTINUE
  250 NJD=NJD+1
      IF (NJD-NJ) 1,1000,1000
 1000 STOP
      END                                                                      343
      SUBROUTINE COGSNR
C
C
      DOUBLE PRECISION TERM(20),TERN(20),C(20),Y1(2),Y2(2),Y3(2),Y4(2),
     1BTOT(2),CLTOT(2),TX(2),VX(2),BO(2),ALO(2),SEM(20,20),SEI(20,20),
     2SEV(20),SHFT(20),B(20),HO
      DIMENSION ML(2,20),MM(2,20),MN(20),AL(2,20),AM(2,20),AN(20)
C
      COMMON C,Y1,Y2,Y3,Y4,BTOT,CLTOT,TX,VX,HO,B,                              349
     1ML,MM,MN,AL,AM,AN,NL,NM,N,UX,F,CKW,NIT,NNCI,KJ                           350
```

```
C
  998 FORMAT (' ITERATION DID NOT CONVERGE, POINT NUMBER ',I3)                351
C
C     CALCULATES CONCENTRATION OF EACH SPECIES
C
      NIT=0
      DO 1 K=1,N                                                            353
    1 TERM(K)=B(K)*UX**MN(K)                                                354
    2 DO 3 K=1,N                                                            355
    3 TERN(K)=TERM(K)                                                       356
      IF (NM) 42,42,41
   41 DO 4 K=1,N
      DO 4 J=1,NM                                                           358
    4 TERN(K)=TERN(K)*VX(J)**MM(J,K)
   42 DO 5 K=1,N
      IF (NL) 5,5,50
   50 DO 6 J=1,NL
    6 TERN(K)=TERN(K)*TX(J)**ML(J,K)
    5 C(K)=TERN(K)                                                          373
  100 NIT=NIT+1                                                             374
C
C     CALCULATES EACH TOTAL METAL AND TOTAL LIGAND CONCENTRATION
C
      DO 7 I=1,NM                                                           375
      BO(I)=VX(I)                                                           376
      DO 8 K=1,N                                                            377
    8 BO(I)=BO(I)+AM(I,K)*C(K)                                              378
    7 Y2(I)=DABS(BO(I)-BTOT(I))                                             379
      DO 9 I=1,NL                                                           380
      ALO(I)=TX(I)                                                          381
      DO 10 K=1,N                                                           382
   10 ALO(I)=ALO(I)+AL(I,K)*C(K)                                            383
    9 Y4(I)=DABS(ALO(I)-CLTOT(I))                                           384
      IF (NIT-100) 11,11,999                                                385
C
C     CHECKS DEGREE OF CONVERGENCE
C
```

```
   11 DO 12 I=1,NM                                                      386
      IF (Y1(I)-Y2(I)) 14,12,12                                         387
   12 CONTINUE                                                          388
      DO 13 I=1,NL                                                      389
      IF (Y3(I)-Y4(I)) 14,13,13                                         390
   13 CONTINUE                                                          391
 2000 HO=1./UX-CKW*UX                                                   392
      HO=HO/F                                                           393
      DO 2001 J=1,N                                                     394
 2001 HO=HO-AN(J)*C(J)                                                  395
      IPT=IPT+1                                                         396
      RETURN                                                            397
C
C     IF CONVERGENCE INSUFFICIENT SETS UP, AND SOLVES, MATRIX FOR IMP-
C     ROVED VALUES OF EACH FREE METAL AND FREE LIGAND CONCENTRATION
C
   14 M1=NM+1                                                           399
      M2=NM+NL                                                          400
 1070 DO 1001 I=1,M2                                                    401
      DO 1001 J=1,M2                                                    402
 1001 SEM(I,J)=0.0                                                      403
      DO 1002 I=1,NM                                                    404
 1002 SEM(I,I)=-VX(I)                                                   405
      DO 1003 I=M1,M2                                                   406
      IMNM=I-NM                                                         407
 1003 SEM(I,I)=-TX(IMNM)                                                408
      DO 1004 I=1,NM                                                    409
      DO 1004 J=1,NM                                                    410
      DO 1004 K=1,N                                                     411
 1004 SEM(I,J)=SEM(I,J)-C(K)*AM(I,K)*AM(J,K)                            412
      DO 1005 I=M1,M2                                                   413
      DO 1005 J=1,NM                                                    414
      DO 1005 K=1,N                                                     415
      IMNM=I-NM                                                         416
      IMNM=I-NM                                                         416
 1005 SEM(I,J)=SEM(I,J)-C(K)*AM(J,K)*AL(IMNM,K)                         417
      DO 1006 I=1,NM                                                    418
```

```
      DO 1006 J=M1,M2                                                   419
      DO 1006 K=1,N                                                     420
      JMNM=J-NM                                                         421
 1006 SEM(I,J)=SEM(I,J)-C(K)*AM(I,K)*AL(JMNM,K)                         422
      DO 1007 I=M1,M2                                                   423
      DO 1007 J=M1,M2                                                   424
      DO 1007 K=1,N                                                     425
      IMNM=I-NM                                                         426
      JMNM=J-NM                                                         427
 1007 SEM(I,J)=SEM(I,J)-C(K)*AL(IMNM,K)*AL(JMNM,K)                      428
      DO 1008 I=1,NM                                                    429
 1008 SEV(I)=-BTOT(I)+BO(I)                                             430
      DO 1009 I=M1,M2                                                   431
      IMNM=I-NM                                                         432
 1009 SEV(I)=-CLTOT(IMNM)+ALO(IMNM)                                     433
      DO 1010 I=1,M2                                                    434
      DO 1010 J=1,M2                                                    435
 1010 SEI(I,J)=SEM(I,J)                                                 436
C
C     CALLS MATRIX INVERSION SUBROUTINE
C
      CALL SUB760(SEI,M2)
C
      DO 1011 I=1,M2                                                    438
 1011 SHFT(I)=0.0                                                       439
      DO 1012 I=1,M2                                                    440
      DO 1012 J=1,M2                                                    441
 1012 SHFT(I)=SHFT(I)+SEI(I,J)*SEV(J)                                   442
      DO 1013 I=1,M2                                                    443
      IF (SHFT(I)+0.9999) 1014,1013,1013                                444
 1014 SHFT(I)=-0.9999                                                   445
 1013 CONTINUE                                                          446
      DO 1015 I=1,NM                                                    447
 1015 VX(I)=VX(I)+VX(I)*SHFT(I)                                         448
      DO 1016 I=M1,M2                                                   449
      K=I-NM                                                            450
 1016 TX(K)=TX(K)+TX(K)*SHFT(I)                                         451
```

```
      GO TO 2                                                          452
C
C     EXITS IF NO CONVERGENCE AFTER 100 ITERATIONS
C
  999 WRITE(6,998) KJ
      IPT=1                                                            454
      NNCI=NNCI+1                                                      455
      RETURN                                                           456
      END                                                              457
      SUBROUTINE SUB760(A760,N760)
C
      DOUBLE PRECISION A760(20,20)                                     459
C
    3 DO 764 K760=1,N760
      CM760=A760(K760,K760)                                            461
      A760(K760,K760)=1.0                                              462
      DO 765 J760=1,N760
  765 A760(K760,J760)=A760(K760,J760)/CM760
      DO 764 I760=1,N760
      IF(I760-K760)762,764,762
  762 CM760=A760(I760,K760)
      A760(I760,K760)=0.0
      DO 763 J760=1,N760
  763 A760(I760,J760)=A760(I760,J760)-CM760*A760(K760,J760)
  764 CONTINUE
      RETURN
       END
```

Program RWZASCOG

This program is used for calculating formation curves of metal-ligand interactions from experimental results that have been prepared as data for input into RWSCOGSY.

INPUT

The program is stored under the 44PS system on the St. Andrews I.B.M. 360/44 computer and is called using the following cards

```
Card 1.   //      JOB      , Name     CHEM     005
          ↑       ↑        ↑          ↑        ↑
          1       11       19         36       52

Card 2.   //bEXECbRWZASCOG

                   DATA

          /*
          /&
```

The experimental DATA is identical to the input of RWSCOGSY + a few blank cards as item 14 of data.

OUTPUT

This is self explanatory. A formation curve is a plot of ZBAR versus pA.

```
C            RWZASCOG
C
C
C
C
      DOUBLE PRECISION DEXP,     DLOG
      DIMENSION TITLE(20,30),BS(30),ASI(30),HSI(30),VINIT(30),HTI(30),EO
     2(30)        ,VADD(30,40),E(30,40),AT(30),BT(30),V(30,40),TH(30,40),
     3AA(30,40),PH(30,40),BB(30,40),FLIG(30,40),ZBAR(30,40),PA(30,40),ZO
     3NA(30,40),H(30,40),B(5),BETA(5),Z(5),FN(30) ,NUM(30)
      READ(5,65) MM
      DO 144 MMM = 1,MM
      READ(5,2000)
      READ(5,65)M
 2000 FORMAT(20I2)
      READ(5,2099) NLXS
 2099 FORMAT(4X,I2)
      DO 2098 II = 1,NLXS
      READ(5,2001) BETA(II)
 2001 FORMAT(10X,F10.0)
 2098 CONTINUE
      READ%5,65) NDP
      DPN = NDP
      DO 14 J=1,M
 5001 FORMAT(20A4)
      READ(5,5001)(TITLE(I,J),I = 1,20)
      READ(5,2003) BS(J)  , ASI(J)  , HSI(J),  VINIT(J)
 2003 FORMAT(F10.3,10X,F10.3,10X,2(F10.3))
      READ(5,5100) BT(J)  ,AT(J)  ,HTI(J)  , EO(J)   ,FN(J)
 5100 FORMAT(F10.3,10X, F10.3,       10X,3(F10.3))
      NUM(J) = 0
      I = 1
   15 READ(5,40) VADD(J,I), E(J,I)  , INDEX
   40 FORMAT(2(F10.3),4X,I1)
      NUM(J) = NUM(J) + 1
      I = I + 1
      IF(INDEX.EQ.0)GO TO 15
      I = 0
```

```
  14 CONTINUE
     READ(5,2004) WK
2004 FORMAT(F10.3)
     WK = 10.0**WK
     READ(5,64) NBETA
  64 FORMAT(2X,I2)
     NBETA = NLXS - NBETA
     READ(5,2002)(Z(I) ,I=1,NBETA    )
2002 FORMAT( F10.0 )
     DO 2097 II = 1,NBETA
2097 B(II) = 10.0**BETA(II)
     DO 144 J = 1,M
     WRITE(6,2009) (TITLE(I,J) , I= 1,20)
2009 FORMAT('1' ,40X ,20A4//<
     WRITE(6,30) HSI(J)  ,BS(J)  ,ASI(J)  , VINIT(J),   B(1) ,HTI(J)  ,
    2BT(J)  ,AT(J)  ,EO(J)  ,   B(2)  ,   B(3)
  30 FORMAT(' ',  5X, 'INITIAL SOLUTIONS .... ACID =',F9.6,3X,'METAL ='
    2,F9.6,3X,'LIGAND =',F9.6,3X,    'VOLUME =', F7.2    ,5X, 'BETA1 ='
    3  ,1PE11.4 /
    4 , 6X, 'TITRATING    "    .... ACID =',0PF9.6,3X,'METAL =',F9.6,3X
    5 ,     'LIGAND =' ,  F9.6,3X,   'EO     =', F7.2    ,5X, 'BETA2 ='
    6  ,1PE11.4   /     106X,  'BETA3 ='1PE11.4/)
     WRITE(6,60)
  60 FORMAT(19X,'VADD'6X,1HE,7X,2HPH,7X,4HZBAR,6X,2HPA,7X,4HFLIG,
    18X,4HZONA//)
     NUMJ = NUM(J)
     HSI(J)  = HSI(J) + ASI(J) * DPN
     HTI(J) = HTI(J) + AT(J)  * DPN
     DO 144 I = 1,NUMJ
     V(J,I) = VINIT(J)   + VADD(J,I)
     TH(J,I) = ((HSI(J)*VINIT(J))/V(J,I)) +(HTI(J)*VADD(J,I)/V(J,I))
     AA(J,I) = (ASI(J)*VINIT(J)/V(J,I))+(AT(J)*VADD(J,I)/V(J,I) )
     PH(J,I) =(EO(J) - E(J,I))/FN(J)
     H(J,I) = EXP(-2.3026*PH(J,I))
     BB(J,I) = ( BS(J)*VINIT(J)      + BT(J) * VADD(J,I))/ V(J,I)
     FLIG(J,I) = (TH(J,I) - H(J,I) + WK/H(J,I)) / (B(1)*H(J,I) + 2.0*B(
    22)*H(J,I)**2.0    + 3.0*B(3) *H(J,I)**3.0)
```

```
      ZBAR(J,I) =(AA(J,I) - FLIG(J,I)*(1.0 + B(1)*H(J,I) + B(2)*H(J,I)**
     22.0 + B(3) *H(J,I)**3.0))/BB(J,I)
      IF(FLIG(J,I))143,143,142
  142 PA(J,I) = -ALOG10(FLIG(J,I))
  143 ZONA(J,I) = ZBAR(J,I)/FLIG(J,I)
  144 WRITE(6,50) VADD(J,I)  ,E(J,I)  ,PH(J,I)  ,ZBAR(J,I)  ,PA(J,I)  ,F
     2LIG(J,I)  ,ZONA(J,I)
   50 FORMAT(' '15X,F6.2,3X,F6.1,3H   F7.4,3H   F7.4,
     13H   F7.4,3H   E11.4,3H   E11.4/)
   65 FORMAT(I2)
      CALL EXIT
      END
```

APPENDIX III

RWSOLV

```
C            RWSOLV
C
C
C
C
      DOUBLE PRECISION    NA(4) ,NB(4) ,     DEL,
     1           SUMP%4<,SUMQ%4<,SUMR%4<,SUMS%4<,P%4<,Q%4<,R%4<,
     1T%4<,A%90,5<,AO%90<,V%4<,SUMT%4<,QCALC%90<,PCENT%90<
      DOUBLE PRECISION CC(4,4) ,AC(4,4) ,PP(4),   C(5,4)
      READ%5,20<M
      DO 28 L#1,M
      READ%5,20< N, NX
      NY#NX&1
      DO 17 I#1,N
   17 READ%5,30< A%I,NY< , %A%I,J<,J#1,NX<
   30 FORMAT(E11.4, 6  E11.4)
      DO 11 J#1,NY
      DO 11 K#1,NX
      C%J,K<#0.0
      DO 11 I#1,N
   11 C%J,K<#C%J,K<&A%I,K<*A%I,J<
      DO 12 I#1,NX
      DO 13 J#1,NX
      CC%I,J<#C%I,J<
 13   AC%I,J<#C%I,J<
 12   PP%I<#C%NY,I<
      CALL   MINV(AC,4,DEL,NA,NB)
    5 FORMAT %6E15.8<
      XN#NX
      XMAX#XN&0.5
      XMIN#XN-0.5
      SUM#0.0
      DO 32 I#1,NX
      DO 32 J#1,NX
      S#0.
      DO 31 K#1,NX
   31 S#S&CC%1,K<*AC%K,J<
   32 SUM#SUM&S
```

```
      IF%SUM-XMAX<35,40,40
   35 IF%SUM-XMIN<40,40,50
   40 WRITE(6,45) SUM ,DEL
   45 FORMAT('1','COEFFICIENTS MATRIX SINGULAR',2(E16.8)///)
   50 DO 52 I#1,NX
      CX#0.0
      DO 51 J#1,NX
   51 CX#CX&AC%I,J<*PP%J<
   52 V%I<#CX
      WRITE%6,100< %V%I<,I#1,NX<
  100 FORMAT('1',10X,'H1 = ',1PE11.4,10X,'H2 = ',1PE11.4,10X,'H3 = ',1PE
     211.4,10X,'H4 = ',1PE11.4////)
      WRITE%6,60<
   60 FORMAT(' ',9X,'QEXPT',11X,'QCALC',            16X,'COEFFICIENTS 1,
     22,3 & 4   **********'//)
      SQ = 0.0
      DO 27 I#1,N
      S#0.
      DO 54 J#1,NX
   54 S#S&A%I,J<*V%J<
      SQ = SQ + (S - A(I,NY))**2
      QCALC%I<#S
   27 WRITE(6,80) A(I,NY),   QCALC(I)  ,              (A(I,J),J=1,NX)
   80 FORMAT(' ',2(6X,1PE11.4),          4(6X,1PE11.4)//)
      G#N
      SIGMA =  SQRT(SQ/(G - (4.0 - XN))          )
   28 WRITE%6,70<       SIGMA
   70 FORMAT(' ',30X, 'THE STANDARD DEVIATION IS' , F7.4)
   20 FORMAT%212)
      STOP
      END
C                                                                      MINV  10
C     ..................................................................MINV  20
C                                                                      MINV  30
C        SUBROUTINE MINV                                               MINV  40
C                                                                      MINV  50
C        PURPOSE                                                       MINV  60
```

```
C           INVERT A MATRIX                                                 MINV  70
C                                                                           MINV  80
C       USAGE                                                               MINV  90
C          CALL MINV(A,N,D,L,M)                                             MINV 100
C                                                                           MINV 110
C       DESCRIPTION OF PARAMETERS                                           MINV 120
C          A - INPUT MATRIX, DESTROYED IN COMPUTATION AND REPLACED BY       MINV 130
C              RESULTANT INVERSE.                                           MINV 140
C          N - ORDER OF MATRIX A                                            MINV 150
C          D - RESULTANT DETERMINANT                                        MINV 160
C          L - WORK VECTOR OF LENGTH N                                      MINV 170
C          M - WORK VECTOR OF LENGTH N                                      MINV 180
C                                                                           MINV 190
C       REMARKS                                                             MINV 200
C          MATRIX A MUST BE A GENERAL MATRIX                                MINV 210
C                                                                           MINV 220
C       SUBROUTINES AND FUNCTION SUBPROGRAMS REQUIRED                       MINV 230
C          NONE                                                             MINV 240
C                                                                           MINV 250
C       METHOD                                                              MINV 260
C          THE STANDARD GAUSS-JORDAN METHOD IS USED. THE DETERMINANT        MINV 270
C          IS ALSO CALCULATED. A DETERMINANT OF ZERO INDICATES THAT         MINV 280
C          THE MATRIX IS SINGULAR.                                          MINV 290
C                                                                           MINV 300
C     .....................................................................MINV 310
C                                                                           MINV 320
      SUBROUTINE MINV(A,N,D,L,M)                                            MINV 330
```