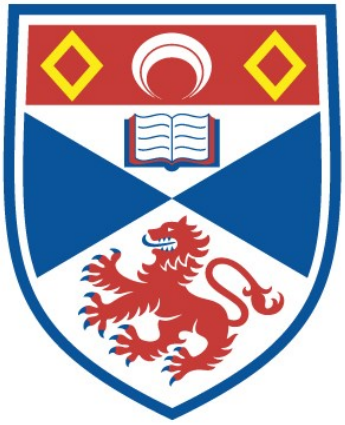
A POTENTIOMETRIC STUDY OF SOME SOLUTION EQUILIBRIA INVOLVING BIOLOGICAL LIGANDS AND TRANSITION METAL IONS

George Kamel Rizkalla Makar

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1976

A POTENTIOMETRIC STUDY OF SOME SOLUTION EQUILIBRIA INVOLVING BIOLOGICAL LIGANDS AND TRANSITION METAL IONS

A Thesis

presented for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Science of the

University of St. Andrews

by

George Kamel Rizkalla Makar

To my FATHER and my MOTHER

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

i

Th 8811

"A Potentiometric Study of Some Solution Equilibria Involving Biological Ligands and Transition Metal Ions" by George K.R. Makar.

A thesis for the Degree of Ph.D. at the University of St. Andrews.

ABSTRACT

The formation constants for several metal ion - ligand complexes have been measured by glass electrode potentiometry in aqueous solution at 37°C using an ionic background of 150mM sodium perchlorate.

The three topics comprising this thesis are (i) a study of the reaction of several metal ions, namely Co(II), Ni(II), Cu(II) and Zn(II), with ligands such as adeninate, cyclohexylamine and cyclopentylamine. These were studied in order to gain experience in the techniques of potentiometry and computation. (ii) The second topic, which comprises the major portion of the thesis, involved the *in vitro* study of zinc complexes with a series of ligands which can be divided into two groups; those containing only oxygen donor groups (acetate, galacturonate, β-hydroxybutyrate, malate, malonate, oxalate, salicylate and tartarate) and those which contain oxygen and nitrogen donor groups (glycinate, glycylglycinate and glycylglycylglycinate); the purpose of this investigation being to suggest the best zinc supplementing drug for treating zinc deficiency conditions. β-hydroxybutyrate and galacturonate are suggested to be the most promising ligands for zinc absorption. (iii) Finally, computer simulation models of equilibria involving zinc and ligands in intestinal solution were used to correlate the rate of growth of turkey poults with the type of metal-ligand complexing occurring in intestinal fluid.

ACKNOWLEDGEMENTS

I wish to express my gratitude and thanks to Dr. DAVID R. WILLIAMS for his cooperation, guidance and supervision throughout this work. His kindness and readiness to offer sincere help are greatly appreciated.

Thanks are also due to my colleagues Miss ANNA M. CORRIE and Mr. MURRAY L.D. TOUCHE for their valuable ideas and particularly to Mrs. MAUREEN SANDERS who, with great patience, typed this thesis.

I thank the Department of Chemistry of the University of St. Andrews, for the facilities offered.

Special thanks to my parents for providing a maintenance grant for myself.

CERTIFICATE

I hereby certify that GEORGE KAMEL RIZKALLA MAKAR has researched under my supervision and has fulfilled the conditions of Ordinance General Number 12 and Resolution of the University Court 1967, number 1 and is qualified to submit this thesis in application for the degree of Doctor of Philosophy.

DAVID R. WILLIAMS

Department of Chemistry
University of St. Andrews

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.

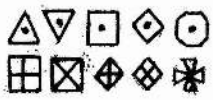
The thesis describes the results of research performed in the Chemistry Department, University of St. Andrews, under the supervision of Dr. David R. Williams since October 1972.

GEORGE K.R. MAKAR

CONTENTS

NOMENCLATURE

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
TI	Therapeutic index
LD_{50}	Lethal dose (for 50% of the animals)
ID_{90}	Inhibitory dose (The ID_{90} is the minimum dose that causes complete tumour regression for 90% of the animals)
E	Electrode potential
E_g	Glass electrode potential
E^o	Standard electrode potential
R	Gas constant
T	Absolute temperature
F	Faraday
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'
H	Total concentration of hydrogen ions
h	Concentration of free hydrogen ions
ph	$-\log h$
pa	$-\log a$
pH	$-\log \underline{a}_{H^+}$ (where $\underline{a}$ stands for activity)
p & p'	Number of ligands A & A' in a complex
q & q'	Number of metals B & B' in a complex
r	Number of hydrogen ions H in a complex
θ_c	Temperature in degrees centigrade

$\bar{Z}$	Average number of ligands bound per metal ion
$\bar{Z}_h$	Average number of protons bound per ligand
I	Ionic strength
ΔG^o	Standard Gibbs free energy change
ΔH^o	Standard enthalpy change
ΔS^o	Standard entropy change
β_{pqr}	Overall formation constant for complex $A_pB_qH_r$
K	Stepwise formation constant
K_w	Ionic product of water
S	Standard deviation (in ml)
n	Number of experimental observations
	Graphical representations of titrations 1 to 10.

CHAPTER 1 - INTRODUCTION

CONTENTS

CHAPTER 1

INTRODUCTION

General Introduction

Metal ions play a vital rôle in many biological processes. In the last few years, new techniques have been developed and these have accelerated studies involving both inorganic chemistry and biological sciences, an interdisciplinary area which is now considered to be one of the most rapidly expanding research fields.

It is well known that eighteen elements are essential for a healthy human life [1-9]: ten of these are metals. These elements fall into three broad classes:-

a) the main group metals - sodium, potassium, magnesium, and calcium which are ionic and mobile *in vivo*;

b) the trace metals - manganese, iron, cobalt, copper, zinc, and molybdenum which are usually covalently bonded to the same donor groups;

c) the main group non-metals - hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, chlorine, and iodine which are the molecular building blocks for *in vivo* matter.

The Importance of the Bio-inorganic Study

The most likely area in which one might be able to contribute to man's knowledge of biochemistry appears to be in the field of trace element dietetics and pharmacology. Seventy per cent of our body weight is water and so it seems advisable to study the aqueous chemistry of

bio-metal-ligand interactions rather than undertake, for example, solid state investigations.

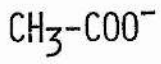
Projects Reported in this Thesis

Three topics comprise this thesis. The first topic, involving the reaction of several metal ions, namely Co(II), Ni(II), Cu(II), and Zn(II) with ligands such as adeninate, cyclohexylamine and cyclopentylamine, was studied in order to gain experience in the techniques of potentiometry and computer programming. However, the ligands investigated also have biological functions which will be discussed later in this chapter. The second topic, comprising the major portion of the thesis, involved the *in vitro* study of zinc complexes with a series of ligands which can be divided into two groups; those containing only oxygen donor groups and those which contain oxygen and nitrogen donor groups. The purpose of this investigation being to suggest the best zinc supplementing drug for treating zinc deficiency conditions. Finally, computer simulated models of the equilibria involving zinc and ligands in intestinal solution were used to correlate the rate of growth of turkey poults with the type of metal-ligand complexing occurring.

The structures of the ligands investigated by the potentiometric method in the first two topics are listed in figure 1. The ligands investigated in the final topic are listed in figure 2 along with their structures and abbreviated names [10].

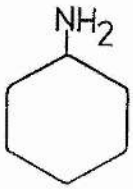
FIGURE 1

LIGANDS AND THEIR STRUCTURAL FORMULAE

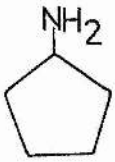


Acetate

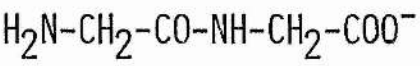
Adeninate



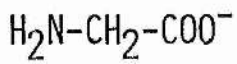
Cyclohexylamine



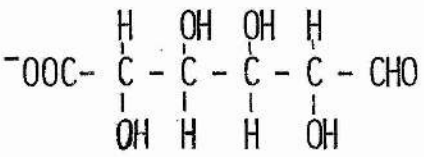
Cyclopentylamine



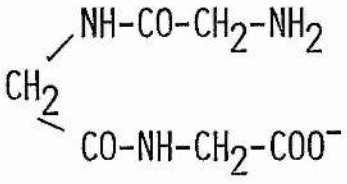
Glycylglycinate



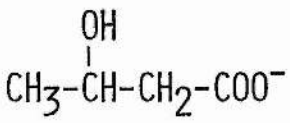
Glycinate



Galacturonate (aldehydo form)

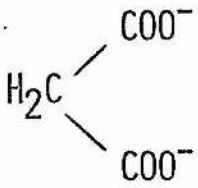


Glycylglycylglycinate

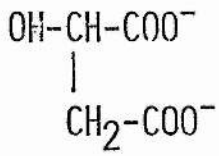


β-Hydroxybutyrate

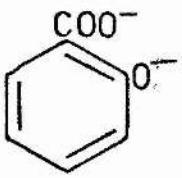
FIGURE 1 continued



Malonate

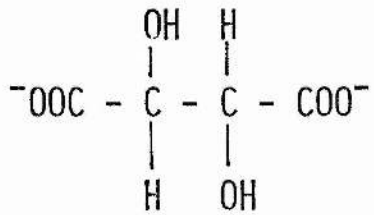


Malate



Salicylate

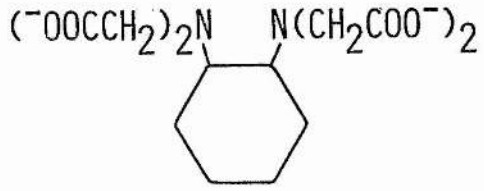
Oxalate



Tartarate

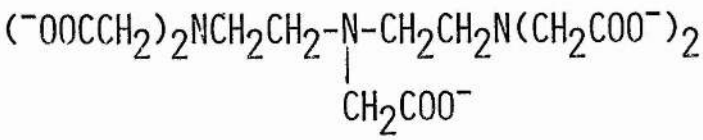
FIGURE 2

LIGANDS THEIR STRUCTURES AND ABBREVIATED NAMES



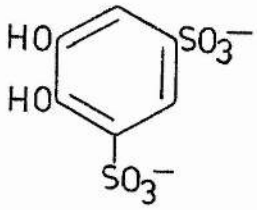
1,2-Diaminocyclohexanetetraacetate

(CDTA)



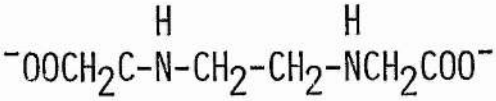
Diethylenetriaminepentaacetate

(DTPA)



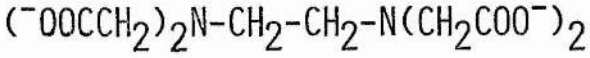
4,5-Dihydroxy-m-benzenedisulphonate

(DHBDS)



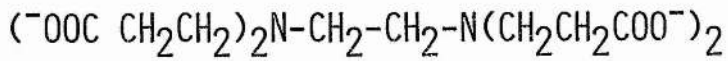
Ethylenediamine-N,N'-diacetate

(EDDA)



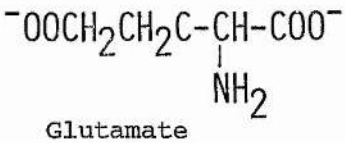
Ethylenediaminetetraacetate

(EDTA)



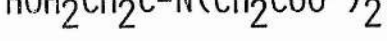
Ethylenediaminetetrapropionate

(EDTP)



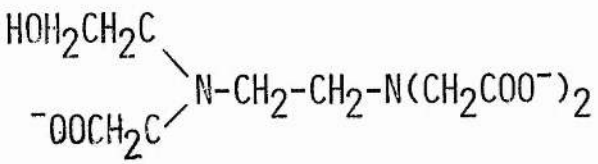
Glutamate

(GA)



2-Hydroxyethyliminodiacetate

(HEIDA)



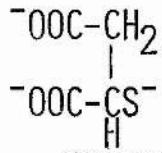
Hydroxyethylethylenediaminetriacetate

(HEDTA)

FIGURE 2 continued

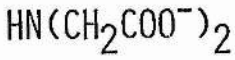
8-Hydroxy-5-quinolinesulphonate

(HQS)



Mercaptosuccinate

(MSA)

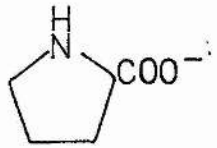


Iminodiacetate

(IDA)

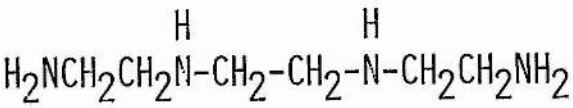
Nitrilotriacetate

(NTA)



Prolinate

(P)



Triethylenetetraamine

(TETA)

Metals Investigated [11]

1. Cobalt

In aqueous solution, cobalt has two common oxidation states, II and III. When complexed Co(II) usually has octahedral or tetrahedral ligand-metal bonds. Many Co(II) complexes, e.g. $Co(NH_3)_6^{2+}$, are readily oxidized by O_2 to give Co(III) complexes as the ultimate products, e.g., $Co(NH_3)_6^{3+}$. Complexes of Co(III), like those of Co(II) are numerous. Co(III) has a strong affinity for nitrogen donors and most of its complexes contain either amines, nitro groups, water molecules or halide ions.

Co(II) is needed for enzyme reactions, for example, it is needed as an enzyme activator for carbonic anhydrase and carboxypeptidase activity.

The term vitamin B_{12} generally means cyanocobalamin which has a Co(III) - CN group, and is involved in the manufacture of the red corpuscles of the blood. Vitamin B_{12} can be used for the treatment of pernicious anaemia, and perhaps it is the most potent substance known in its physiological activity; one microgram per day of vitamin B_{12} being effective in the control of the condition [12]. This is the only compound of cobalt that is known to be essential for the human body.

2. Nickel

In its aqueous chemistry, as well as its non-aqueous chemistry, nickel has its most important oxidation state as II.

Nickel is not an essential metal ion, but Ni(II) is included in the investigation as a model of Pd(II) and Pt(II) because it stands at the top of group VIII [13]. The latter two metals have complexes with

anti-cancer properties[14]. Graham and Williams [15] have reported formation constants for mixed ligand complexes of Ni(II) and Pd(II) with asparaginate and chloride which have anti-cancer activity. However, palladium complexes are considerably more active against carcinoma than are nickel complexes.

3. Copper

Copper like cobalt is found in two different oxidation states, I and II. In aqueous solutions the relative stabilities of Cu(I) and Cu(II) depend strongly on the nature of anions or other ligands present. Oxidation state II is the most important one. Cu(I) is easily oxidized to Cu(II) and is thus only found in solution when complexed or in solids, for example, CuCl.

Copper is stored in the liver, and is found in many metalloenzymes, for example, phenolase or haemocyanin, which are both capable of carrying oxygen like haemoglobin. Actually copper is required in the production of haemoglobin. It plays a part in the incorporation of iron into haem in the synthesis of haemoglobin and also in other aspects of the metabolism of iron.

Copper deficiency anaemia is often hypochromic and microcytic which suggests that iron deficiency is responsible and that the haemoglobin content of red cells is reduced. To copper deficient animals, the administration of copper causes a reduction in the iron content of the liver and an increase in the iron contained in haemoglobin. Animals deficient in iron and copper have more iron stored in the liver than animals which are only deficient in iron. These results show that copper is necessary for the release of iron from storage sites.

In serum, copper is loosely bound to albumin and tightly bound to the α_2 globulin, ceruloplasmin. This protein has a molecular weight of approximately 151,000 and contains 8 atoms of copper per molecule.

There are four possible biological activities of ceruloplasmin that have occupied investigators;

a) enzymatic activity
b) relation to psychiatric disorders
c) erythropoietin activity and
d) its possible role in the regulation of copper balance.

4. Zinc

This metal ion will be discussed in more detail, because this thesis reports more studies involving zinc than any other of the metals of the transition series.

Zinc has been estimated to rank twenty fifth in terms of abundance in the hydrosphere [16], and to make up 0.004 to 0.01% of the Earth's crust [16,17]. The human body contains about 1.4 - 2.3g of zinc [18]. Nevertheless, there is an increasing awareness that zinc deficiency

is quite frequently found in malnourished populations and even in Western countries during diseases or injuries.

Reports indicating zinc deficiency have been presented by Prasad and co-workers [19,20,21] in Egypt and by Halsted and co-workers [22,23] in Iran. In 1960 and 1961 Prasad and co-workers [19] and Halsted and Prasad [24] studied a group of Iranian dwarfs with extreme iron deficiency anaemia. They suggested that zinc deficiency might be the reason for the growth and sexual retardation observed in these dwarfs. They later confirmed that a daily oral administration of zinc sulphate resulted in a significantly rapid growth and sexual development.

Neldner and Hambidge [25] reported severe zinc deficiency in an adult woman with acrodermatitis enteropathica* in whom the plasma zinc concentration, serum alkaline phosphatase and urine zinc excretion rates were extremely low. Following an oral administration of zinc sulphate, the above parameters rapidly returned to normal. Thus, the beneficial effects of zinc therapy in this patient confirm the efficacy of oral zinc in the treatment of acrodermatitis enteropathica. However, zinc sulphate has side effects [26]. Vomiting is one of the several symptoms of zinc toxicity which occurs after the ingestion of large quantities. In fact, an oral dose of 2g of zinc sulphate (454 mg of zinc) has been recommended as an emetic [27]. Some of the other symptoms of zinc toxicity in humans include dehydration, stomach pain, and dizziness. Reports have shown that death may result after the ingestion of 45 g of zinc sulphate [28,29].

---

* "Acrodermatitis enteropathica, an autosomal recessive inherited disorder of unknown causes characterized by severe diarrhoea, dermatitis, and alopecia, begins in infancy, often coincident with the change from breast milk to cow's milk" [25].

As already mentioned, several conditions, including pregnancy, stress, and oral contraception, induce the lowering of the plasma zinc concentration [30,31]. It is not clear whether this is due to a zinc deficiency or a redistribution of zinc between amino-acids, free zinc, and albumin.

Pories and Strain [32,33] in 1966, reported that healing time was apparently decreased with oral administration of zinc sulphate heptahydrate, and suggested that the patient's initial zinc condition may affect his response to zinc therapy. For example, Hallböök and Lanner [34] "observed significant differences in healing time between zinc treated and untreated patients in a group whose individual serum zinc levels were less than 110 μg $(100\ ml)^{-1}$ but not in a group whose serum zinc levels were 110 μg $(100\ ml)^{-1}$ or higher".

In the United States, Hambidge and co-workers [35], reported cases of low hair zinc concentration and low taste acuity that responded to zinc supplementation.

The biochemical functions in which zinc has been considered as indispensable include

a) protein synthesis

b) enzymes and enzymatic function

c) carbohydrate metabolism

Several studies have been published on the distribution of zinc in tissues. The data is summarized in table 1 [28].

In the periodic table, zinc stands at the top of group IIb. It has an atomic number of 30 and an atomic weight of 65.4. The electronic configuration of zinc is $3d^{10}4s^2$ and the two electrons in the $4s$ shell are conveniently lost to give the Zn (II), $3d^{10}$ state. Metallic zinc

TABLE 1

Zinc Concentrations in human tissues [28]

(mg/kg dry weight)*

		Reference
Liver	141-245	(36, 37, 38, 39, 40)
Kidney	184-230	(36, 37, 38, 39, 40)
Lung	67-86	(36, 37, 39, 40)
Muscle	197-226	(37, 41)
Pancreas	115-135	(37, 41)
Heart	100	(37)
Bone	218	(41)
Prostates		
normal	520	(42)
hyperplasia	2330	(42)
cancer	285	(42)
Eye		
retina	571	(38)
choroid	562	(38)
ciliary body	288	(38)
Testes		
Esophagus		

* data are expressed as range of published mean values

is amphoteric and will dissolve in mineral acids and strong bases, and is also a good reducing agent.

The chemistries of Zn and Cd are very similar, some of their physical properties are listed in table 2.

TABLE 2

Some properties of Zn and Cd elements

	Zinc	Cadmium
Electronic configuration	$3d^{10}\ 4s^2$	$4d^{10}\ 5s^2$
Melting point, ^{o}C	419	321
Boiling point, ^{o}C	907	767
Radii of divalent ions, Å	0.69	0.92

Because of their completed *d* shells, there are no ligand field stabilisation effects in the Zn(II) and Cd(II) ions. Thus, the stereochemistry of their compounds is determined solely by ion sizes and bond strengths.

In their complexes, Zn and Cd commonly have coordination numbers four, five and six, with five especially common for zinc [11,43].

Ligands Investigated

The choice of ligands depends on the specific bio-inorganic aspect being investigated.

Adeninate (6-aminopurinate)

Among the outstanding achievements of this century has been the establishment of the structures of DNA and RNA, where the substituted purines and pyrimidines constitute the backbone bases of these two molecules. Several purine derivatives have been found to be efficient in humans for cancer therapy [44], for example, 6-mercaptopurine and various other derivatives. Structurally, 6-aminopurine is similar to 6-mercaptopurine, the only difference being the amino group in the former and the mercapto group in the latter. It is known that these compounds possess chelating properties and some relationship between chelation and cancer is obvious [45,46]. Therefore, the chelation reactions of 6-aminopurine with the metal ion Zn(II) and the carcinogenic metal ions [47] Co(II), Cu(II) and Ni(II) have been investigated.

Cyclohexylamine (hex) and Cyclopentylamine (pent)

These two ligands have been studied because of the high therapeutic indices of their complexes * [48], compared to *cis* - $Pt(NH_3)_2Cl_2$ which is

---

* The therapeutic index (T.I.) is the ratio of the dose which kills 50 percent of the animals (LD_{50}) to that which causes tumour regression in 90 percent of the animals (ID_{90}).

active against the ADJ/PC 6A murine plasma cell tumour (compare the therapeutic indices of *cis* - $Pt(NH_3)_2Cl_2$, 8.1; *cis* - $Pt(pent)_2Cl_2$, 235.7; and *cis* - $Pt(hex)_2Cl_2$, >267) [49]. This potentiometric investigation is aimed at elucidating the changes brought about when a coordinated ammonia is substituted by a cyclopentylamine or cyclohexylamine ligand. Unfortunately due to the insolubilities of the Pd(II) and Pt(II) cyclopentylamine or cyclohexylamine systems (less than 1 mM), we could not perform any potentiometric investigation. We have investigated Ni(II) as a model of the Pd(II) and Pt(II) group and also Co(II), Cu(II), and Zn(II) which are the nearest neighbours to Ni(II) in the first transition series.

Glycinate, Glycylglycinate, and Glycylglycylglycinate

Amino-acids and peptides are indispensable components of living systems. The interactions between metals and amino-acids and peptides have become of considerable interest as models for biological systems. Some interesting examples of metal complexes involving amino-acids and peptides as ligands appear throughout Martell and Calvin's book [50] and Nakamoto and McCarthy's "Spectroscopy and Structure of Metal Chelate Compounds" [51].

Acetate, Galacturonate, β-Hydroxybutyrate, Malate, Malonate, Oxalate, Salicylate, and Tartarate

The formation of some carbohydrate and carboxylate compounds of zinc were studied in order to compare these results with those of iron complexes, the latter having been found to be effective reagents for treatment of iron deficiency [52].

The Relationships Between the Metals and Ligands Investigated

For the first two topics, the approach used was the study of the ligands mentioned previously reacting with each of the metal ions and then using computer calculations in order to simulate the complex *in vivo* system. In particular we require the presence of neutral complexes for effective lipid-protein membrane solubility and permeability[53]. The four metal ions that have been investigated are classified as acid acceptors of borderline hard/soft character and as such are vulnerable to symbiosis effects. This phenomenon of symbiosis is actually an advantage because, for the metal ions listed as borderline it means that the metal ions can be converted into hard or soft depending on whether their environment is hard or soft. For example, zinc in carbonic anhydrase binds halide ions $I^- > Br^- > Cl^- > F^-$ and in aqueous solution, the hydrated zinc ion binds $F^- > Cl^- > Br^- > I^-$. Clearly the enzyme environment has symbiotically rendered the borderline zinc ion soft whereas in water the hard solution sphere has rendered the zinc ion hard[18].

As for the last topic, we are dealing with the publication of Kratzer and co-workers[10] in which were reported several observations concerning the absorption of zinc ions from soya bean protein (as reflected in poultry growth rates) with, and without, the presence of zinc chelating agents related to EDTA. Our approach was to compute the complex species concentrations *versus* ph profiles for Kratzer's ligands by using formation constants available in the chemical literature and trying to identify the principle controlling maximum zinc uptake. In Kratzer's study the ligand donor groups were varied successively (e.g. the number of carboxylates in a series of ligands) until a

general order of magnitude for the formation constant giving good biological response was found. However, ligands producing neutral complexes with metal ions can promote absorption [53].

Justification for Equilibrium Studies [9]

Until recently, only qualitative remarks have been made about the distribution of metal ions among competing ligands in biological systems. This was due to the difficulties of taking quantitative account of the numerous equilibria involved and of solving the resulting set of simultaneous equations.

A biological system is never truly in a state of equilibrium. Instead, the components have a continuous movement across cell membranes. Materials enter the system, and as they are transported through the various compartments they undergo a series of transformations, and finally the waste products are excreted. There is a delicate balancing of synthesis and degradation, so that the reaction processes and the kinetics of diffusion must also be taken into account. However, in order to achieve high efficiencies of energy conversion, most biological systems operate near to reversible equilibria and the rates of complex formation and dissociation are usually very fast.

Potentiometric Measurements

Many techniques are available for the determination of formation constants. In this work, potentiometry, which is widely applicable

to the study of ionic equilibria and is one of the most precise techniques, was the chosen method. By potentiometric measurements, both the metal ion and ligand concentrations could be determined. For the study of ionic equilibria, the electrodes usually employed are reversible to metal ions, protons, or anions. In this manner the activity of the species in question can be calculated from the measured potential by means of the Nernst equation:-

$$E = E^{o} + \frac{RT}{nF} \ln \underline{a}_{B}{}^{n+}$$

Furthermore, the potentiometric technique may be used in the determination of the Gibbs free energy, which is related to the formation constant by the reaction isotherm [54].

$$\Delta G^{o} = - RT \ln \beta$$

The best method for determining the enthalpy of reaction is by direct calorimetry, however, there are problems associated with this technique. For example, solubility difficulties, hydrolysis and oxidation. Galacturonic acid, for example, is not stable in the presence of hydroxyl ions and air, being oxidised to the dicarboxylic acid [55]. Fortunately, another method is available, i.e. that of van't Hoff. This uses formation constants at different temperatures to calculate ΔH^{o} from the equation [54]

$$\left(\frac{\partial \ln \beta}{\partial T}\right)_{p} = \frac{\Delta H^{o}}{RT^{2}}$$

where P is the total pressure

This equation is known as the reaction isochore.

By integrating $$\ln \frac{\beta_{T_2}}{\beta_{T_1}} = -\frac{\Delta H^\circ}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

However, this method is usually of low accuracy because the formation constants do not vary substantially over the working temperature range for aqueous solutions.

ΔH° and ΔG° having been determined, ΔS° can be found from the equation

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ$$

Where ΔH° is available it is a measure of bond strength and ΔS° is an indication of the change in the number of particles in the complexation reaction.

However, the three projects about to be described in subsequent chapters involve only the formation constant aspects of ΔG° and use them in a variety of computed models of concentration and biological response relationships *in vivo*.

CHAPTER 2 - THEORETICAL CONSIDERATIONS

CONTENTS

CHAPTER 2

THEORETICAL CONSIDERATIONS

Complex formation between metal ions and ligands is conveniently researched using glass electrode ph (which is defined as $-\log h$) potentiometry. The premier aim of such work is to completely define the system in terms of formation constants (β) for all metal-ligand-proton complexes present. The steps that are usually followed for such work are : firstly, the calibration of the electrodes to respond to concentrations, then the measurement of the protonation constants for the parent ligand by the observation of emf *versus* titrant (ml)added data. Then this data is fed into the computer program ZPLOT in order to obtain the formation curves, which help to reveal the compositions of the salient complexes present, and finally, several other computer programs are used in turn (namely PSEUDOPLOT, SCOGS and MINIQUAD) to determine the formation constants (β), which can then be used to generate models of species distribution using the COMPLOT program. These programs will be discussed in more detail in both this chapter and in chapter 4.

The Electrode System [56]

ph measurements are accomplished by determining the potential developed by an electrochemical cell containing an electrode that responds to a_h The experimental measurements are performed in an

ionic background of I = 150 mM sodium perchlorate; this medium having been shown to be a suitable one for minimising variations in activity coefficients arising from opposite charges being neutralized during protonation and complexation [57]. The cell consists of two electrodes - glass electrode and reference electrode — immersed in the same test solution. This electrode system is shown in figure 3.

The glass electrode is the measuring element of the system, it consists of an internal sealed tube with a metallic termination (typically of silver-silver chloride) and an external tube which contains a buffered chloride solution. The tip of the electrode which is immersed in the solution is a ph-sensitive glass bulb. A potential is developed across the glass when the hydrogen-ion concentration in the solutions on the two sides of the glass is different. The potential of the glass electrode is proportional to the ph of the solution in which it is immersed.

The reference electrode provides a constant electric potential relative to which the potential of the glass electrode can be measured. This reference electrode consists of a metallic internal element [typically of mercury-mercurous chloride (calomel) or silver-silver chloride] immersed in an electrolyte, in our case, a saturated solution of sodium chloride. The electrolyte solution forms a salt bridge between the metallic element and the sample solution in which the glass and reference electrodes are immersed. A small but constant flow of electrolyte solution is maintained through a liquid junction in the tip of the outer body of the reference electrode in order to maintain electrical contact between the internal metallic element and the sample

THE ELECTRODE SYSTEM

FIGURE 3

solution.

The potential developed at the glass electrode is measured using a digital millivolt meter.

Calibration of the Electrode Pair [58] (for the data refer to table 2a)

The glass electrode is calibrated with reference to a saturated sodium chloride calomel electrode, the emf of this pair E, at 37°C is given by the equation:

$$E = E^{o} + 61.54 \log h \text{ mV; where } h = [H^{+}] \text{ in mol dm}^{-3}$$

A plot of E *versus* - log h ought to give a straight line of slope 61.54 mV$(-\log h)^{-1}$. In practice, when pure ionic background solution was titrated with acid or alkali from an 'Agla' micrometer syringe an S shaped curve was obtained (figure 4). As can be seen from figure 4, the plot is linear in the acid range -log h = 2.2 to 3.3 then deviates and then becomes linear again in the alkaline region. The deviation from linearity in the intermediate region occurs only in unbuffered solutions and has been observed previously by Williams and Williams [59]. A linear response was obtained in buffered solutions.

Sources of contamination such as impurities in the ionic background salt or in the deionised water were searched for in order to explain the phenomenon of the S shaped curve, but these possible causes were eliminated after performing several purification tests [59]. It was later found that contamination occurred from the glass of the electrodes and possibly the titration vessel. These results obtained by Williams and Williams also show that the calibration was more linear when a plastic vessel and stirrer were used, and the electrodes were kept out of the solution except for measurements.

FIGURE 4

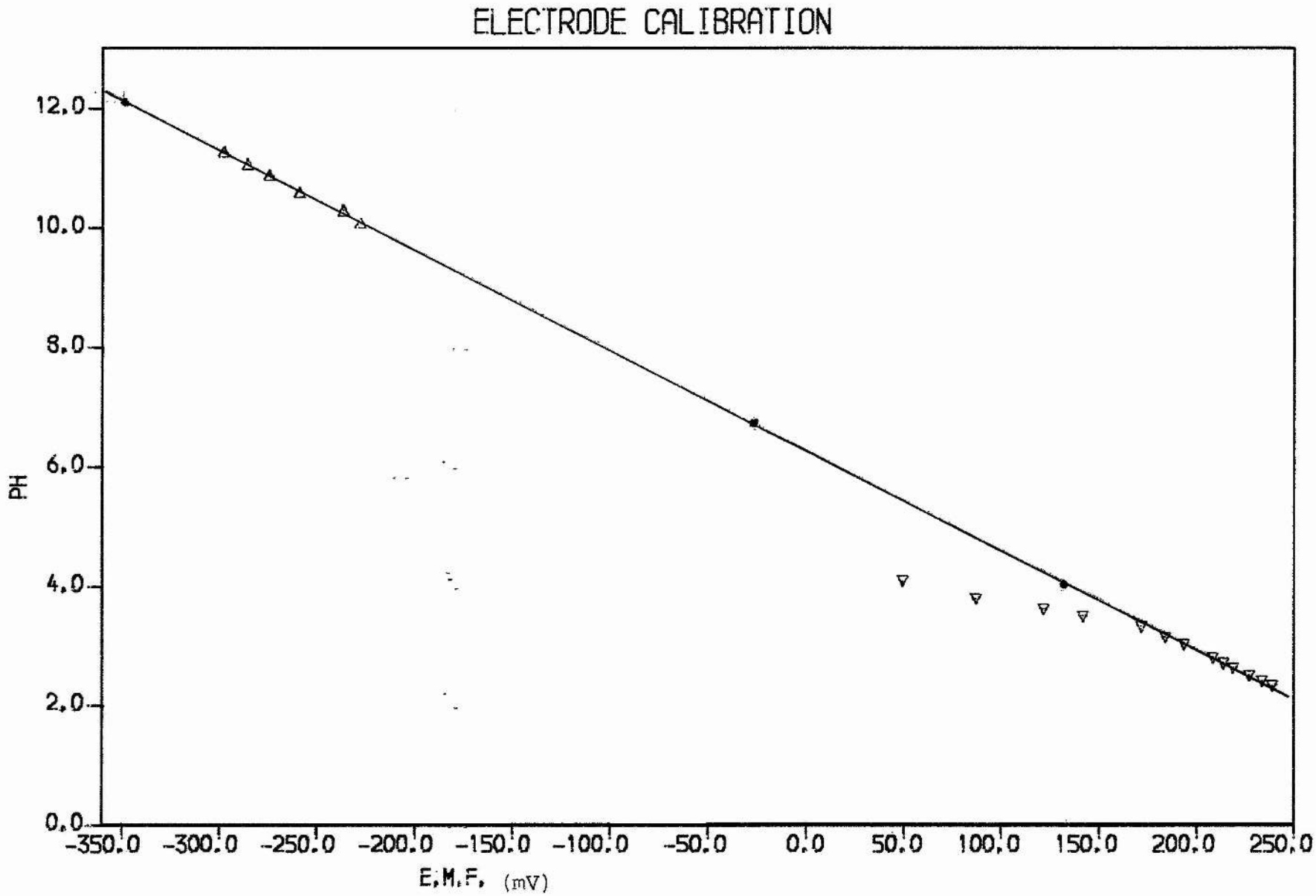

ELECTRODE CALIBRATION
12.0
10.0
8.0
6.0
4.0
2.0
0.0
PH
-350.0
-300.0
-250.0
-200.0
-150.0
-100.0
-50.0
0.0
50.0
100.0
150.0
200.0
250.0
E.M.F. (mV)

TABLE 2a

Electrode Calibration

25.00 ml sodium perchlorate (150 mM) titrated with sodium hydroxide (220.0 mM) using an 'Agla' syringe

ml added	mV	$-\log[H^+]$ calculated
0.03	-229.3	10.05
0.05	-238.1	10.27
0.10	-260.4	10.57
0.20	-275.9	10.87
0.30	-287.1	11.05
0.50	-299.2	11.26

TABLE 2a continued

25.00 ml sodium perchlorate (150 mM) titrated with perchloric acid (214.6 mM) using an 'Agla' syringe

ml added	mV	$-\log[H^+]$ calculated
0.01	47.8	4.07
0.02	85.7	3.76
0.03	120.2	3.58
0.04	140.3	3.46
0.06	170.1	3.29
0.09	182.4	3.11
0.12	191.8	2.99
0.20	206.8	2.77
0.25	212.3	2.67
0.30	217.2	2.59
0.40	225.5	2.47
0.50	232.1	2.37
0.60	237.4	2.30

Buffer solutions	mV	$-\log \underline{a}_{H^+}$
	130.3	4.02
	-30.2	6.84
	-350.6	12.14

It is important to note that such deviations from linearity occur only in completely unbuffered solutions. It is, however, prudent to check that the electrode pair does respond in a linear manner in buffered solutions and so, at the beginning of a series of titrations, linearity of E (which is the potential of the electrode system) response was checked by a buffer line (using pH buffers at pH = 4.02, 6.84, 12.14); where pH = $-\log a_{H^+}$. The single purpose of this buffer line is to choose the glass electrode with the most linear response. Next, E° is measured by the Nernst equation and this value is checked before and after a titration by measuring E of a solution of known hydrogen-ion concentration in the ionic background I.

5a,60

Choice of Experimental Conditions

The two problems to be decided are at what ionic strength to work and which ionic background salt to use to maintain this strength. The strengths of ion pair bonds lie in the order $SO_4^{=} > Cl^- > Br^- > I^- > NO_3^- > ClO_4^-$. So, in order to avoid the complications of ion pairing (which could lead to an error in the values of the formation constants) perchlorate or nitrate are considered to be the best anions. The ionic mobility of potassium is almost equal to that of nitrate, whereas sodium has a much lower mobility. Thus potassium nitrate is a widely used background salt. For similar reasons potassium perchlorate could be recommended but unfortunately this salt is insoluble, so the sodium or lithium perchlorates are used instead.

We have given much thought to the choice of an ideal background salt and temperature for bio-potentiometric studies, and in particular we have considered 3M * $NaClO_4$ at 25°C *versus* 150 mM $NaClO_4$ at 37°C. We concluded that the major disadvantages of using I = 3M $NaClO_4$ and T = 25°C are:-

a) these are far removed from biological blood-plasma conditions (which approximate to 37°C and I = 150 mM Cl^-)

b) the final traces of impurity remaining in the sodium perchlorate are emphasized when the background salt is 3M

c) even though the perchlorate ion has little tendency to ion pairing, at 3M concentration some may still occur.

The disadvantages of using I = 150 mM $NaClO_4$ and T = 37°C are:-

a) a considerable quantity of volumetric glassware needs recalibrating

b) unless the complete system of vessel and electrodes are thermostatted at 37°C, condensation occurs in the cooler parts of the system and the electro-thermal effect in the electrodes can cause an error of up to 3mV

c) the tubing linking the burette to the titration vessel also needs to be maintained at 37°C to minimise temperature fluctuations in the vessel

d) I = 150 mM permits only a 0.008 M change in ion concentration without significantly changing the activity coefficients [57].

Clearly, there is no ideal medium, each set of conditions having certain merits and problems. However, this listing has two uses:-

a) conditions ought to be specifically selected for each investigation to minimise as many of these disadvantages as possible

b) being aware of these difficulties, a more realistic consideration of experimental errors is possible.

---

* 1 M = 1 mol dm^{-3}

Derivation of $\bar{Z}$ and Methods of Calculation [61]

For a system of mononuclear complexes, the total concentrations A and B of the ligand and the metal, respectively, are given by:-

$$A = [A] + [BA] + 2[BA_2] + \ldots + N[BA_N] = a + b\sum_{1}^{N} n\beta_n a^n \quad \text{——— (1)}$$

$$B = [B] + [BA] + [BA_2] + \ldots + [BA_N] = b\sum_{0}^{N} \beta_n a^n \quad \text{———— (2)}$$

The terms a and b are the free concentrations of the ligand and metal respectively. If corresponding values of both a and b have been calculated, the formation constants can be measured directly from equations (1) or (2), provided that either A or B is known. If, values of only one of the variables a and b have been determined as a function of A and B, the data may be treated by the $\bar{Z}$ method.

$$\bar{Z} = \frac{[BA] + 2[BA_2] + \ldots\ldots}{[B] + [BA] + [BA_2] + \ldots\ldots} = \frac{\sum_{1}^{N} n\beta_n a^n}{\sum_{0}^{N} \beta_n a^n} \quad \text{————(3)}$$

Equation (3) shows that $\bar{Z}$ is a function of a only, provided that the system is entirely mononuclear. If polynuclear complexes are present, $\bar{Z}$ will also depend on the total concentration of B.

$$\bar{Z} = \frac{\text{Bound ligand concentration}}{\text{Total metal concentration}}$$

Bound ligand concentration = Total ligand - (Free ligand + Protonated ligand)

$$= A - (a + \sum_{1}^{N} a\beta_n h^n)$$

$$= A - a(1 + \sum_{1}^{N} \beta_n h^n) \quad \text{———} \quad (4)$$

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

$$H = h - \frac{K_w}{h} + a \sum_{1}^{N} n\beta_n h^n \qquad \text{(5)}$$

where K_w is the ionic product of water, and from equation (5)

$$a = \frac{H-h+K_w/h}{\sum_{1}^{N} n\beta_n h^n} \qquad \text{(6)}$$

hence from equations (4) and (6), $\bar{Z}$ can be determined.

Methods treating the resulting data are explained in great detail by Whewell, Rossotti and Rossotti [62].

The Selection of Formation Constants and their Use in Describing an Equilibrium System in which Several Complexes are Present [60]

The formation constant, β, of the resulting complex between a ligand, A, and a metal ion, B, is the most obvious quantity for determining the extent of complex formation. Further, most ligands readily accept protons to form HA and so on to more highly protonated species. In general, titrations are performed whilst keeping constant as many parameters as possible. Our systematic searching is known as the "grid approach" and it was applied (where possible) for both protonation and metal complexing studies. For example, rather than titrating a solution of A and B with alkali, the concentrations of A and B in the titrant ought to equal those in the titrate, the sole variant being the mineral acid content of these two solutions. This produces constant ligand and constant metal ion concentration titrations. Next, formation curves were plotted $\bar{Z}_h$ (the average number of protons per ligand) *versus* ph, or $\bar{Z}$ (the

average number of ligands per metal ion) *versus* pa (-log [free ligand])[61,63] If only mononuclear metal-ligand species are present, the curves recorded at different total metal and ligand concentrations will be superimposable. If protonated complexes, hydroxy complexes, or polynuclear species are present these formation curves form a complicated pattern (i.e. they are definitely not superimposable).

We have developed a computer program, PSEUDOPLOT, in order to interpret these patterns of formation curves. In this program it is assumed that titrations have been carried out and the experimental data plotted as $\bar{Z}_h$ *versus* ph, or $\bar{Z}$ *versus* pa. The PSEUDOPLOT program is a combination of Sillén and his co-workers'[64] HALTAFALL program and our ZPLOT program [65, 66]. The HALTAFALL portion uses, as input, the experimental conditions [i.e. concentrations in titrate, titrant, volumes (all except the ph readings)] and a selected set of β values. The output is simulated titrant (ml) added *versus* ph data that could have been obtained had such a system been titrated experimentally, and assuming that the β selection were exact. In the ZPLOT portion the program uses this data to produce $\bar{Z}_h$ *versus* ph or $\bar{Z}$ *versus* pa curves. These simulated titration curves are then compared with the experimental data and then additional sets of βs are tried until the 'best' fit is obtained.

In non-simple mononuclear systems we are faced with two difficulties:

a) the qualitative problem of finding the best set of βs and

b) the quantitative task of assigning values to these βs.

Qualitatively, the possible βs are limited by the denticity of the ligands and by the coordination numbers of the metal ions. The concentration dependence of complexing is also a useful guide to approximate values of

βs: $\bar{Z}$ *versus* pa curves have characteristic *mono*, *bis*, and *tris* complex pa regions (ca. $\bar{Z}$ = 0.5, 1.5 and 2.5 respectively); low metal and ligand concentrations encourage mononuclearity, high concentrations encourage polynuclearity, titrations at acid phs encourage protonated complex formation, while alkaline phs encourage hydroxy complexes. Thus, lists of βs can be suggested and used to generate PSEUDOPLOT patterns of curves.

The best set of βs, as judged from the best fit of PSEUDOPLOT curves to the experimental data, can then be carried forward to the more quantitative aspects in which the least-squares program SCOGS [67,68], (which has been published by Sayce[67] and generalised by Jones and Williams[68]) or MINIQUAD [69] (which is based upon LETAGROP [70] and has been developed by Sabatini, Vacca and Gans) refines suggested values of βs to better values. The SCOGS program varies sets of βs until the relationship

$$\left(\text{Titre}_{calc}-\text{Titre}_{exp}\right)=\left[\text{Initial volume}\left\{\frac{\left(H_{\text{initial in vessel}}-H_{calc}\right)}{H_{calc}-H_{\text{titrant}}}\right\}-\text{Exp. volume}\right]$$

is minimised using a least squares approach. H refers to total analytical concentrations and the expression for H_{calc} uses all the measured emf values. In the MINIQUAD program, formation constants are stored as mantissa and exponents, only the mantissa being varied, thus avoiding the possible overflow or underflow, which may be observed in the SCOGS program.

The program COMPLOT [71,72] (which will also be discussed in more detail in Chapter 4) produces computer simulated models of species distribution in solutions at different phs. These models require the total concentrations of each metal and ligand, the ph of the solution and the logarithm of the formation constant for each complex as input data as just described.

The use of such computer programs has permitted the design of more realistic models for some of the biochemical aspects of physiological problems.

CHAPTER 3 - EXPERIMENTAL TECHNIQUES

CONTENTS

CHAPTER 3

EXPERIMENTAL TECHNIQUES

Potentiometry was performed in a special vessel (figure 5), using solutions and glassware prepared and analysed by the methods described below.

Water

All the water used was "Elgastat" de-ionised, boiled and cooled by the passage of oxygen-free nitrogen. The resistivity of water was then better than 2M Ω cm.

Perchloric Acid

Concentrated perchloric acid (60-62% W/V, Fisons A.R.) was diluted to make a stock solution of approximately 3M. This solution was then analysed by titrating against sodium carbonate (Fisons A.R.) using methyl orange as indicator [73a], and checked with standard sodium hydroxide solution [73b].

FIGURE 5

TITRATION VESSEL

Sodium Hydroxide

This solution was made up as required from ampoules (B.D.H. concentrated volumetric solutions) and the molarity was checked against standard acid solution [73b] and standard potassium hydrogen phthalate (Fisons A.R.) [73c]. No solutions more than a month old were used.

Sodium Perchlorate

Solutions of sodium perchlorate were made by either neutralising perchloric acid (Fisons A.R.) with sodium carbonate or by dissolving the monohydrate (Merck "Puriss" or B.D.H. Analar) in de-ionised water. The solution ($\simeq$ 7.0 M) was then made alkaline (ph 9-10) by addition of sodium hydroxide (B.D.H. Analar) and allowed to stand for a minimum of seven days. Silica, heavy metal oxides and hydroxides precipitated during this time and were removed by filtration through micropore filters (Millipore Ltd) (average pore diameter 5×10^{-6}m and 0.45×10^{-6}m respectively). Carbon dioxide was then removed by making the solution acidic (ph $\simeq$ 2.0) and boiling and eventually cooling under nitrogen. The ph was adjusted as closely as possible to 7.0 using a digital pH meter to check the progress of neutralisation. The exact molarity was checked by passing samples through a cation exchange column [73d] and titrating the acid produced against standard sodium hydroxide, using bromothymol blue as indicator. The solution was also analysed by the flame photometric technique [73e].

Metal Ion Solutions

Metal perchlorates (G.F. Smith, Chemical Co.) were dissolved in perchloric acid, to prevent hydrolysis, allowed to stand for several days, filtered through micropore filters and analysed as follows.

Zn(II) : Quinaldinate [73f] and EDTA (Eriochrome Black T) [73g]

Cu(II) : Electrodeposition [73h] and EDTA (Fast Sulphon Black F) [73i]

Ni(II) : Electrodeposition [73j] and EDTA (Murexide) [73k]

Co(II) : Electrodeposition [73l] and EDTA (Xylenol Orange) [73m]

The hydrogen ion concentration in the metal solution was obtained by means of Gran plots [74].

EDTA

The disodium salt of ethylenediaminetetra-acetic acid is available as a primary standard and so solutions of it could be prepared by direct weighing [73n].

Ligands

The ligands used and their suppliers are listed below:-

Adenine (B.D.H.), cyclopentylamine (Koch-Light, pure grade), cyclohexylamine (Koch-Light, puriss. grade), glycine (Fisons A.R.), glycylglycine (Koch-Light, puriss. grade), glycylglycylglycine (Koch-Light, puriss. grade), α-D-galacturonic acid monohydrate (Sigma Chemical Company),

D,L-β-hydroxybutyric acid sodium salt (Boehringer Mannheim GmbH), L-malic acid (Koch-Light, puriss. grade), malonic acid (Koch-Light, puriss. grade), oxalic acid hydrated (B.D.H. Analar), salicylic acid (B.D.H. Analar), sodium acetate hydrated (Fisons A.R.), L-tartaric acid (Koch-Light, puriss. A.R. grade).

These ligands were checked by microanalysis for carbon, hydrogen, and nitrogen, using a Perkin-Elmer Model 240 Automatic Analyser.

1) Adenine (m.p. 361-3°C; lit. (anhydr.) 360-5°C (decomp.))

Found : C, 44.4; H, 3.6; N, 51.8%

Calculated for $C_5H_5N_5$: C, 44.4; H, 3.7; N, 51.8%

2) Cyclopentylamine (liquid at room temperature)

Found : C, 70.1; H, 13.2; N, 16.2%

Calculated for $C_5H_{11}N$: C, 70.6; H, 12.9; N, 16.5%

3) Cyclohexylamine (liquid at room temperature)

Found : C, 72.5; H, 13.2; N, 14.0%

Calculated for $C_6H_{13}N$: C, 72.0; H, 13.4; N, 13.8%

4) Glycine (m.p. 260°C; lit. 262°C (decomp.), turns brown at 228°C).

Found : C, 32.1; H, 6.5; N, 18.5%

Calculated for $C_2H_5NO_2$: C, 32.0; H, 6.7; N, 18.6%

5) Glycylglycine (m.p. 214°C; lit. 215°C (decomp.))

Found : C, 36.3; H, 6.4; N, 21.1%

Calculated for $C_4H_8N_2O_3$: C, 36.4; H, 6.1; N, 21.2%

6) Glycylglycylglycine (m.p. 243-5°C; lit. 246°C (decomp.))

Found : C, 38.0; H, 6.1; N, 22.1%

Calculated for $C_6H_{11}N_3O_4$: C, 38.1; H, 5.9; N, 22.3%

7) α-D-Galacturonic acid monohydrate (m.p. 156°C; lit. 156-9°C (decomp.))

Found : C, 33.9; H, 5.7%

Calculated for $C_6H_{12}O_8$: C, 34.0; H, 5.7%

8) D,L-β-Hydroxybutyric acid sodium salt(ionic salt; m.p. too high to measure)

Found : C, 38.0; H, 5.6%

Calculated for $C_4H_7O_3Na$: C, 38.1; H, 5.6%

9) L-Malic acid (m.p. 100°C; lit. 99-102°C)

Found : C, 35.5; H, 4.3%

Calculated for $C_4H_6O_5$: C, 35.8; H, 4.5%

10) Malonic acid (m.p. 135°C; lit.135.6°C (slightly sublimes))

Found : C, 34.5; H, 3.9%

Calculated for $C_3H_4O_4$: C, 34.6; H, 3.8%

11) Oxalic acid hydrated (m.p. 100-1°C; lit. (hydr.) 101.5°C)

Found : C, 19.0; H, 4.8%

Calculated for $C_2H_6O_6$: C, 19.1; H, 4.8%

12) Salicylic acid (m.p. 158°C; lit. 159°C)

Found : C, 60.8; H, 4.4%

Calculated for $C_7H_6O_3$: C, 60.9; H, 4.4%

13) Sodium acetate hydrated (ionic salt; m.p. too high to measure)

Found : C, 17.6; H, 6.5%

Calculated for $C_2H_9O_5Na$: C, 17.7; H, 6.6%

14) L-Tartaric acid (m.p. 171-2°C; lit. 171-4°C)

Found : C, 32.0; H, 4.1%

Calculated for $C_4H_6O_6$: C, 32.0; H, 4.0%

Nitrogen

Oxygen-free nitrogen (British Oxygen) was further de-oxygenated by passage through chromous chloride and then "scrubbed" in 150 mM sodium perchlorate, all solutions being thermostatted at 37°C.

Glassware

M.J. Elliot's "E-mil (Green Line)" calibrated glassware was used. This glassware was supplied with calibration certificates stating that it conformed to the appropriate British Standards Institution requirements for grade "A" calibrated glassware.

All glassware was cleaned regularly with "Quadralene" (Quadralene Chemical Products) and alcoholic potassium hydroxide (for about a minute). Before use, glassware was washed with demineralised water, "Elgastat" water, alcohol and anaesthetic ether, and then dried by suction.

Accuracy and Precision

In our work accuracy and precision are two important factors that should be considered. Accuracy expresses the correctness of a measurement, while precision the reproducibility of a measurement.

The accuracy and precision of all parameters measured are listed below:

Stock solution concentration	$\pm$ 0.1%
Added volume measurements - burettes	$\pm$ 0.005 ml
- pipettes	$\pm$ 0.1%
Weights	$\pm$ 0.05 mg
Flasks	$\pm$ 0.05%
E (emf)	$\pm$ 0.1 mV
E^{o}	$\pm$ 0.2 mV

In general, we try to keep all errors less than 0.1%.

CHAPTER 4 - COMPUTATIONAL ASPECTS

CONTENTS

CHAPTER 4

COMPUTATIONAL ASPECTS

The trend towards more quantitative interpretation of experimental data has greatly increased the necessity for chemists and other scientists to be acquainted with computer programming.

Many computer programs have been developed for use in the determination of formation constants of metal complexes, from ph titration data. As mentioned in Chapter 2 (page 15), these programs include ZPLOT, SCOGS, MINIQUAD, HALTAFALL, PSEUDOPLOT and COMICS, which are discussed below in greater detail.

ZPLOT [65,66]

As described earlier in Chapter 2 (page 23), this program plots formation curves, $\bar{Z}_h$ (the average number of protons per ligand) *versus* ph, or $\bar{Z}$ (the average number of ligands per metal ion) *versus* pa (-log [free ligand]). The mathematics of the calculation of $\bar{Z}$ are explained in Chapter 2 (refer to page 22). Table 3 lists the input and output data of ZPLOT, and similar tables will also be given for the other programs which will be discussed in the coming pages.

The relationships used in ZPLOT are only valid for single ligand and/or single metal systems.

ZPLOT PROGRAM

TABLE 3

INPUT		OUTPUT	
a)	pK_w and log Ks	a)	titrant added (ml)
b)	number of dissociable protons on each ligand	b)	emf for each point
c)	concentrations of metals, ligands and protons in titrate and titrant.	c)	ph and pa
d)	initial volume in the vessel (ml).	d)	$\bar{Z}$ or $\bar{Z}_h$
e)	E^o(mV), $\frac{RT}{nF}$ = 61.54mV/ph at 37°C.	e)	free ligand concentration
f)	titrant added (ml), and emf for each point.	f)	a plot of $\bar{Z}$ or $\bar{Z}_h$ *versus* . pa or ph

SCOGS [67,68]

SCOGS (Stability Constants Of Generalized Species), which employs the conventional non-linear least-squares approach, is used to calculate formation constants for systems containing up to two metals and two ligands and can deal with protonated and mixed species. This program can deal with up to twenty complexes of the type $A_pA'_{p'}B_qB'_{q'}H_r$, when the overall formation constant β_j is given by the expression

$$\beta_j = \frac{\left[A_{p_j} \cdot A'_{p'_j} \cdot B_{q_j} \cdot B'_{q'_j} \cdot H_{r_j}\right]}{[A]^{p_j}\,[A']^{p'_j}\,[B]^{q_j}\,[B']^{q'_j}\,[H]^{r_j}}$$

where square brackets [ ] denote concentrations in moles dm^{-3}. In SCOGS, refinement consists of minimising the sum of the squares of residuals in titre, ΣR_i^2, where R_i = (actual titre of base) - (titre calculated from the current set of constants and the experimental value of the hydrogen ion concentration). The constitution of each species (i.e. A_j, A'_j, B_j, B'_j and H_j) must be included together with an estimate of the overall formation constant. In the calculation, if the value of the constant is accurately known, then, this value may be retained, otherwise it may be adjusted by the computer to give the best fit to the data.

The original SCOGS has been modified, mainly in respect of input and ouput [75] (see table 4). The size of this program has been increased so that it can refine up to a total of five hundred titration readings simultaneously.

SCOGS PROGRAM

TABLE 4

INPUT	OUTPUT
a) pK_w , log Ks and log βs	a) titrant added (ml).
b) number of dissociable protons on each ligand	b) ph
c) concentrations of metals, ligands and protons in titrate and titrant	c) residual in titre
d) initial volume in the vessel (ml)	d) total ligand and free ligand concentrations
e) E^o (mV), $\frac{RT}{nF}$ = 61.54 mV/ph at 37°C.	e) total metal and free metal concentrations
f) titrant added (ml), and emf for each point	f) log β and its estimated standard deviation.
	g) the standard deviation in titre

The two programs SCOGS and MINIQUAD although they perform the same job (i.e. computing the constants) are used as a check. In one program, SCOGS, a non-existent species may be refined, but, since the other program uses different mathematics, there is little probability of its being refined by both programs.

MINIQUAD is necessary for more complicated systems to avoid the exponent overflow problem which occurs in SCOGS, and is faster.

MINIQUAD [69]

This program accepts potentiometric data and uses it to refine estimates of formation constants. MINIQUAD, from the Italian for least squares, MINImi QUADrati, can treat data for systems containing any number of reactant species and potentiometric electrodes, and all types of complexes, e.g. mononuclear, polynuclear, and hydrolyzed complexes.

Table 5 lists the input and output data for the MINIQUAD program.

MINIQUAD PROGRAM

TABLE 5

INPUT	OUTPUT
a) temperature in degrees centigrade	a) titrant added (ml) and the emf for each point
b) pK_w , log Ks and log βs	b) log β and its estimated standard deviation
c) concentrations of metals, ligands, and protons in titrate and titrant	c) the sum of squared residuals
d) initial volume in the vessel (ml)	
e) E^o (mV)	
f) titrant added (ml) and emf for each point	

HALTAFALL [69]

This program has not been used, but is included in this thesis, since it is a portion of the PSEUDOPLOT program.

HALTAFALL calculates the equilibrium concentrations of the species in mixtures of up to 10 components which can form a maximum of 40 complexes,

provided concentrations can be used in the equilibrium concentrations.

HALTAFALL PROGRAM

TABLE 6

INPUT	OUTPUT
a) pK_w, log Ks and log βs	simulated data for titrant added (ml) *versus* ph, that could have been obtained had such a system been titrated experimentally, and assuming that the β selection was exact.
b) concentrations of metals, ligands, and protons in titrate and titrant	
c) initial volume in the vessel (ml)	
d) titrant added (ml)	
e) selected set of β values	

PSEUDOPLOT

As mentioned earlier in Chapter 2 (page 24) this program is a combination of HALTAFALL and ZPLOT programs. It calculates pseudo-$\bar{Z}$ and pseudo-pa for titrations and will plot any pair of parameters. It is important to note that the mass-balance relations in ZPLOT, and in the ZPLOT part of PSEUDOPLOT, assume mononuclearity and absence of hydroxy and protonated complexes. Thus, when these conditions are not valid, $\bar{Z}$ and pa (or ph) are really pseudo-$\bar{Z}$ and pseudo-pa (or ph), these functions being ideal for showing the degree of variation from mononuclearity, and for comparing experimental with simulated titrations.

The two main advantages of this program are its speed of use and the

visual representation of errors. The speed arises because it is unnecessary to derive different normalised functions for different sets of βs and also the plotting is done mechanically. Although least-squares programs such as SCOGS produce numerical values of 'standard deviations in titres' and 'residuals in tires', (a) the human mind prefers to see such errors in *diagrammatic* form and, once having seen these discrepancies in the PSEUDOPLOT fit, it is then in a position to suggest a β to correct them (from observing the area of the plots where the data and calculated curves are most mismatched), and (b) all calculated 'residuals in titres' etc. are based on the set of βs being offered to the computer program. Until least-squares programs have automatic species selectors included in their functioning, PSEUDOPLOT could be widely used to advantage.

PSEUDOPLOT PROGRAM

TABLE 7

INPUT	OUTPUT
a) pK_w	a simulated plot of $\bar{Z}$ or $\bar{Z}_h$ *versus* pa or ph, that could have been obtained had such a system been titrated experimentally and assuming that the β selection were exact
b) concentrations of metals, ligands, and protons in titrate and titrant	
c) initial volume in the vessel (ml)	
d) number of dissociable protons on each ligand	
e) titrant added (ml)	
f) selected set of β values	

COMPLOT [71,72]

This program is the version of COMICS [71] used on the St. Andrews IBM 360/44 computer.

The program COMICS (Concentrations Of Metal Ions and Complex Species) calculates equilibrium concentrations of all species in multi-metal-multi-ligand mixtures from the ph of the solution, the total concentration of each metal and each complexing agent and the logarithm of the formation constant for each complex. The complexes can comprise mixed, hydrolyzed, protonated and polynuclear species.

The input has been modified from the published version as has the output which now has three plotter routines available. It is recommended that in the first instance the compounded printer plot be used as this gives a good, quick, picture of what is taking place in the system.

COMPLOT PROGRAM

TABLE 8

INPUT	OUTPUT
a) β value for each complex (including the ionic product of water pK_w, protonated ligand, hydroxy species and polynuclear species).	a) concentrations of the free metals, free ligands, and complex species at every ph value
b) ph values	b) a plot of the concentrations *versus* ph
c) total concentrations of ligands and metals.	

This program is limited to systems containing ten metal ions, ten ligands, one hundred formation constants, and fifty ph values.

To chemists the use of electronic digital computers has become as important as some spectroscopic techniques. The speed of calculation is one of the greatest advantages of these computer programs.

CHAPTER 5 - POTENTIOMETRY

CONTENTS

CHAPTER 5

POTENTIOMETRY

All potentiometric determinations were carried out at 37°C and using an ionic background solution of 150 mM sodium perchlorate. The hydrogen ion concentration was followed by using a cell of the type

Hg, Hg_2Cl_2 | saturated NaCl | test solution | glass electrode

The electrodes used were an 'Activion' ph sensitive glass electrode, type 17SR, and a calomel reference electrode, type CR, with a saturated sodium chloride salt bridge. A sodium chloride salt bridge was used because potassium ions form insoluble potassium perchlorate in the porous plug of the calomel electrode. Several glass electrodes were employed throughout the series of titrations performed. The pH meter used was a Radiometer type PHM 52, to give readings reproducible to 0.1 mV. Solutions were contained in an enclosed reaction vessel with an outer water jacket thermostatted at 37°C. Titrant was added from a 'Metrohm' 10 ml piston burette. The solution was stirred using a magnetic stirrer and 'TEFLON' coated magnetic follower. Titrations were performed under an inert atmosphere of nitrogen, with nitrogen bubbling through the solution. The apparatus was set up as shown in Chapter 3 (figure 5).

The following pages of this chapter record tables and figures for all the systems studied. Refer to page 211 for the next chapter.

Protonation Constants of Adeninate

The formation curve (figure 6) was obtained using the ZPLOT computer program (Chapter 4). The formation curve can be seen to be independent of the ligand concentration, therefore polynuclear species were assumed to be absent.

The data was then fed into SCOGS (Chapter 4) and the results obtained were:

$\log K_{101} = 3.83 \pm 0.01$ $\quad S(\text{titre}) = 0.28$ (19 readings)

$\log K_{10-1} = -9.26 \pm 0.01$ $\quad S(\text{titre}) = 0.20$ (18 readings)

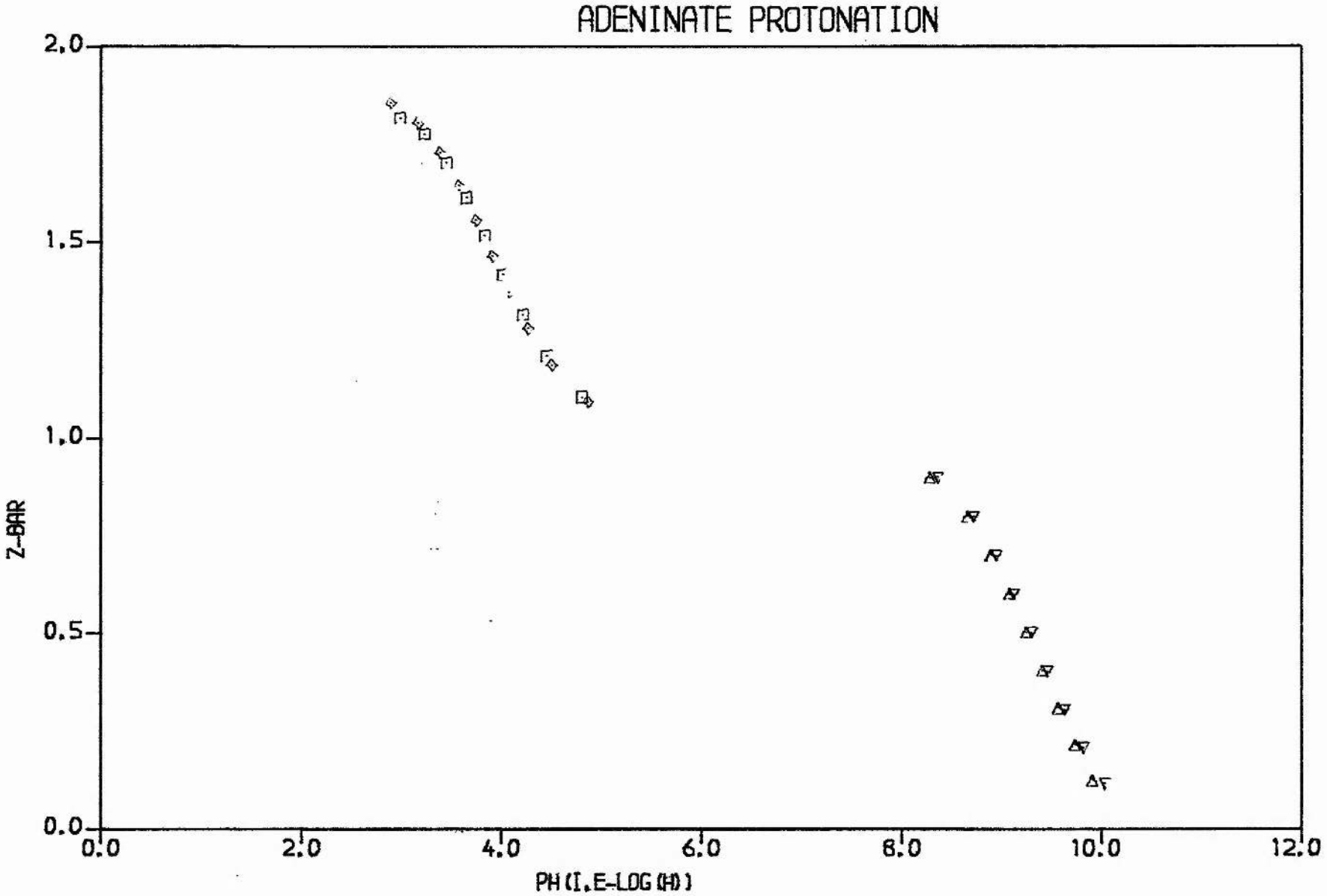
where S is the standard deviation.

TABLE 9

Experimental results for the protonation of adeninate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E° (mV)
	A	H	A	H		
1	10.00	0	0	21.46	20.00	411.1
2	20.00	0	0	47.27	25.00	411.1
3	10.00	0	0	-20.04	20.00	411.1
4	20.00	0	0	-50.00	25.00	411.1

FIGURE 6


ADENINATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH (I.E-LOG (H))

Titration number 1

volume added (ml)	emf (mV)
1.00	115.3
2.00	137.0
3.00	151.7
4.00	164.0
5.00	175.2
6.00	186.5
7.00	198.6
8.00	212.1
9.00	227.0

Titration number 2

volume added (ml)	emf (mV)
1.00	111.7
2.00	134.1
3.00	148.4
4.00	160.0
5.00	170.3
6.00	180.5
7.00	191.0
8.00	202.5
9.00	216.2
10.00	232.9

Titration number 3

volume added (ml)	emf (mV)
1.00	-98.8
2.00	-122.2
3.00	-136.6
4.00	-148.0
5.00	-158.2
6.00	-168.0
7.00	-177.8
8.00	-188.2
9.00	-199.0

Titration number 4

volume added (ml)	emf (mV)
1.00	-104.6
2.00	-126.4
3.00	-140.5
4.00	-151.2
5.00	-162.1
6.00	-172.0
7.00	-182.5
8.00	-194.1
9.00	-207.5

Formation Constants for Cu(II)-Adeninate Complexes

The formation curve (figure 7) was obtained from the results in table 10.

TABLE 10

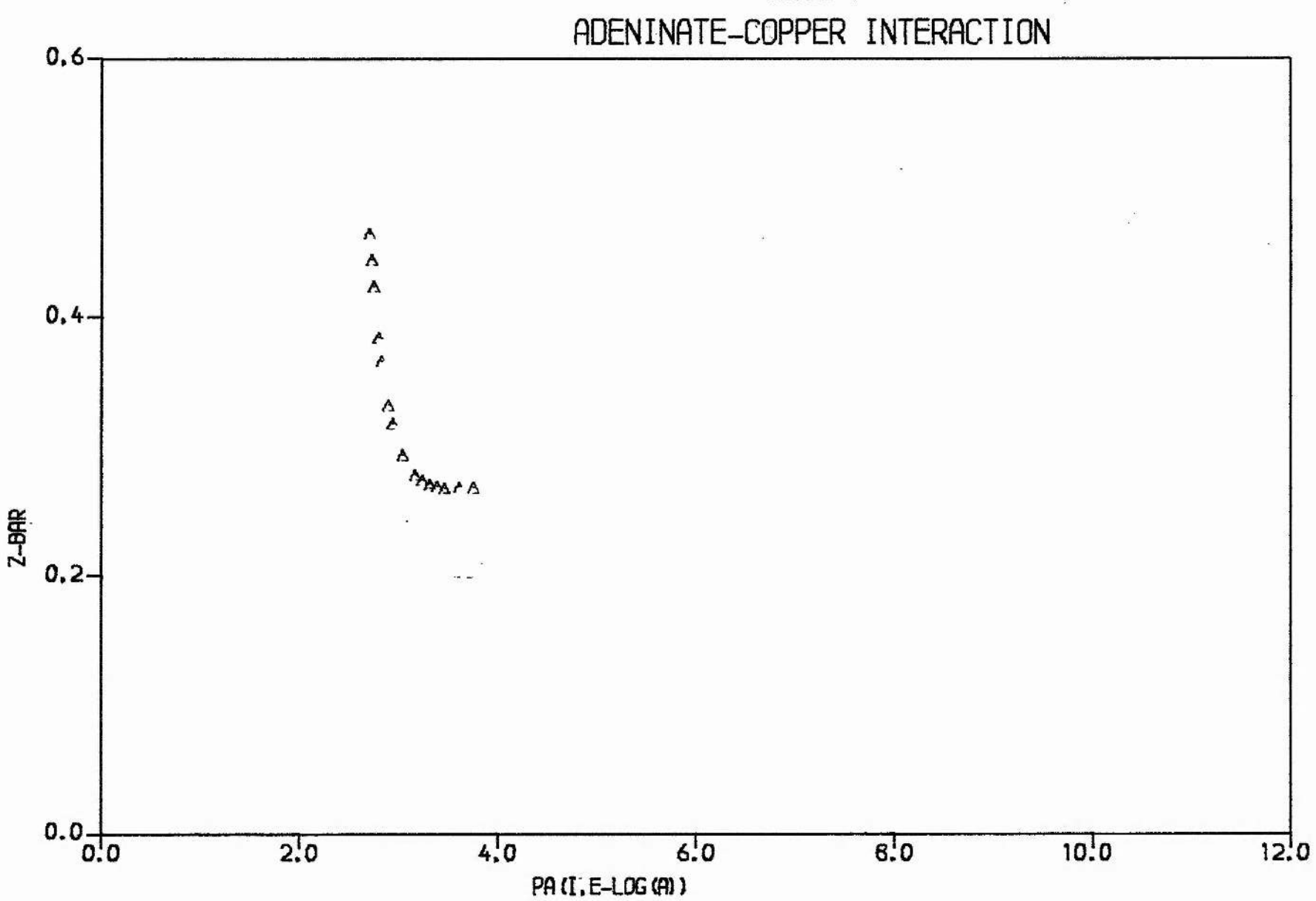
Experimental results for the Cu(II) - adeninate system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	20.00	21.55	0	18.18	0	25.00	363.6

Titration number 1

volume added (ml)	emf (mV)
20.00	219.8
22.00	213.5
24.00	206.2
25.00	202.4
26.00	198.4
27.00	194.3
28.00	190.2
30.00	182.6
32.00	176.2
33.00	173.4
35.00	168.7
36.00	166.6
38.00	163.0
39.00	161.4
40.00	159.8

FIGURE 7


ADENINATE-COPPER INTERACTION
0.6
0.4
0.2
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I,E-LOG(A))

Total number of readings = 15.

Metal hydroxy precipitation restricted our working range of -log h to 2.1 - 3.3. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Cu_2(OH)_2$,[76] the β values for the latter being constant. The 'best' log constant obtained was

$$\log \beta_{110} = 2.68 \pm 0.04 \qquad S\,(\text{titre}) = 3.67$$

The system can then be described by the complex Cu(adeninate)$^+$.

Formation Constants for Ni(II) - Adeninate Complexes

The formation curve was obtained from the results in table 11.

TABLE 11

Experimental results for the Ni(II) - adeninate system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E^o (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	15.00	10.34	0	9.15	0	25.00	411.1

Titration number 1

volume added (ml)	emf (mV)
6.00	220.0
7.00	216.2

Total number of readings = 2.

Metal hydroxy precipitation restricted our working range of $-\log h$ to 2.3 - 2.4. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Ni(OH)^{77}$, the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 1.47 \pm 0.31 \qquad S.(\text{titre}) = 0.10$$

The system can then be described by the complex $Ni(\text{adeninate})^{+}$.

Formation Constants for Co(II)- Adeninate Complexes

The formation curve (figure 8) was obtained from the results in table 12.

TABLE 12

Experimental results for the Co(II) - adeninate system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	24.35	0	31.92	0	25.00	355.4
2	12.50	0	12.17	0	15.95	-10.00	30.00	355.4

Titration number 1

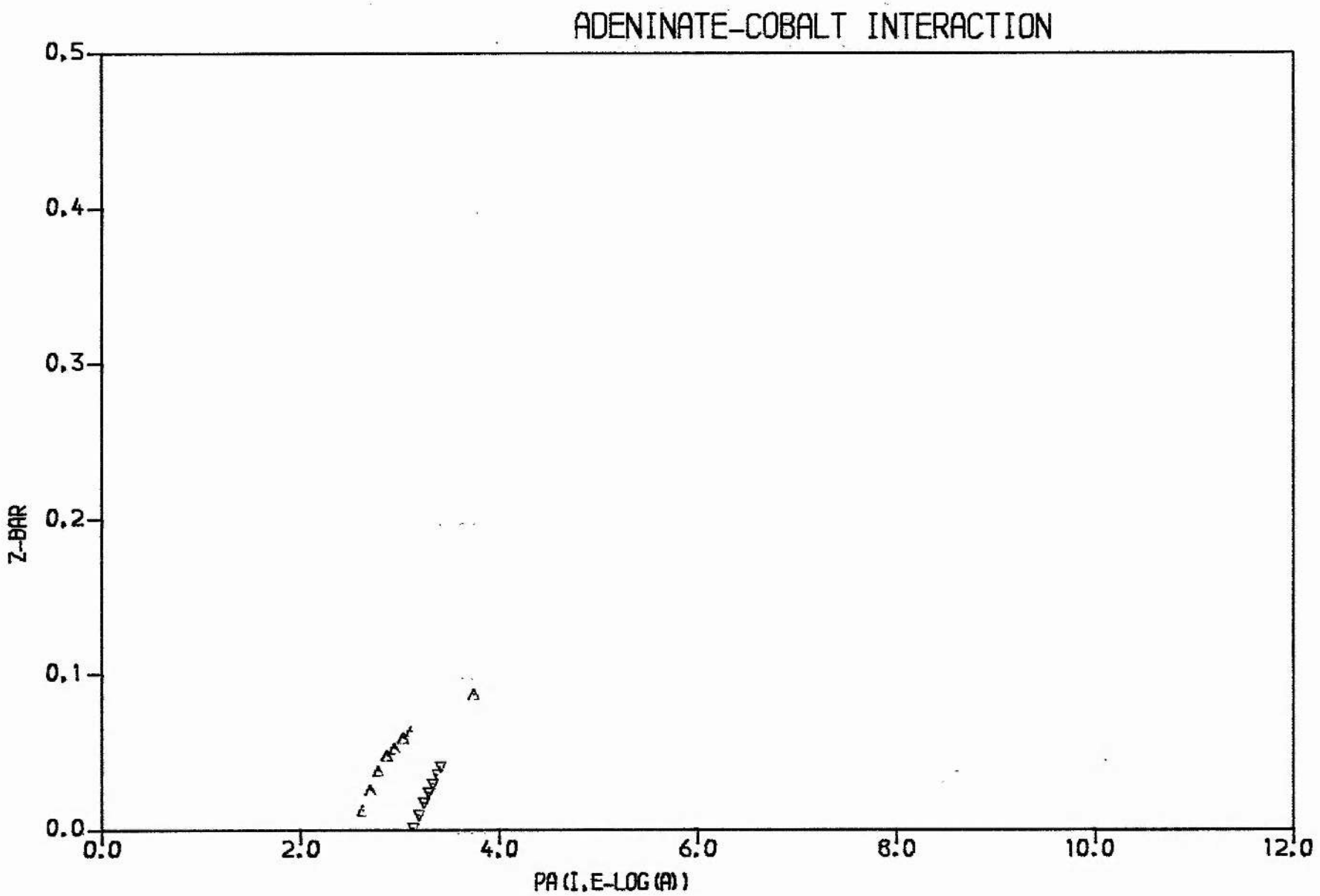
volume added (ml)	emf (mV)
20.00	226.3
30.00	193.5
31.00	188.9
32.00	183.9
33.00	179.1
34.00	173.9
35.00	168.8
36.00	164.0

Titration number 2

volume added (ml)	emf (mV)
0.00	210.0
1.00	206.9
2.00	203.6
3.00	200.2
4.00	196.6
5.00	192.7
6.00	188.6

Total number of readings = 15.

FIGURE 8


ADENINATE-COBALT INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Metal hydroxy precipitation restricted our working range of -log[h] 2.1 - 3.1. The data was analysed using SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Co(OH)^{78}$, the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 1.38 \pm 0.11 \qquad S(\text{titre}) = 0.88$$

The system can then be described by the complex Co(adeninate)$^{+}$.

Formation Constants for Zn(II) - Adeninate Complexes

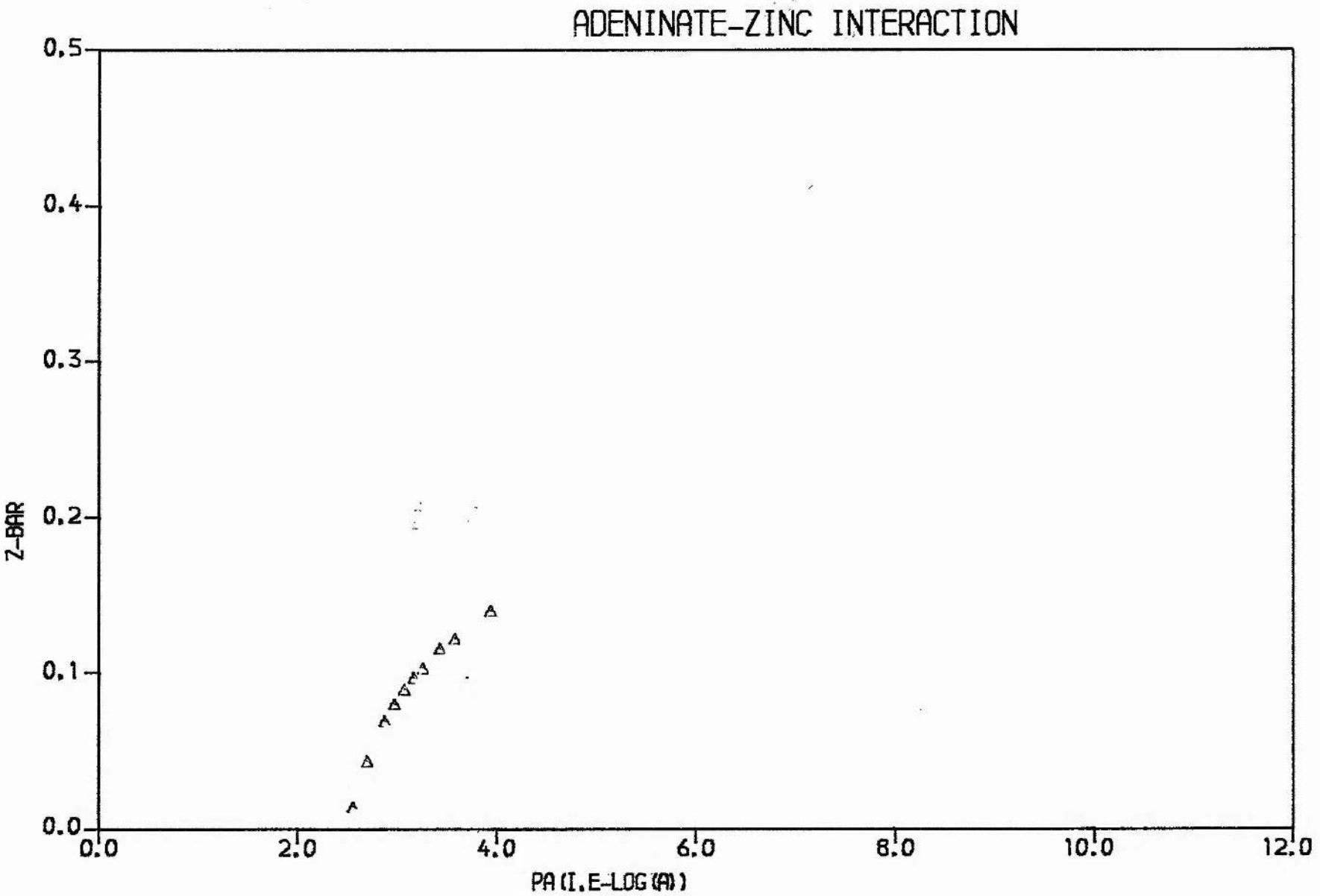
The formation curve (figure 9) was obtained from the results in table 13.

TABLE 13

Experimental results for the Zn(II) - adeninate system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	20.00	21.36	0	20.19	0	25.00	363.6

FIGURE 9


ADENINATE-ZINC INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
15.00	231.4
20.00	216.3
22.00	208.9
24.00	199.8
25.00	194.8
26.00	189.4
27.00	183.7
28.00	177.9
30.00	167.1
32.00	158.0

Total number of readings = 12.

Metal hydroxy precipitation restricted our working range of -log h 2.1 - 3.3. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Zn(OH)_2$[79], the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 1.62 \pm 0.23 \qquad S(\text{titre}) = 3.33$$

The system can then be described by the complex Zn(adeninate)$^+$.

Table 14 below lists our formation constants of the complex formation of adeninate.

TABLE 14

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$

B	p	q	r	Present work	S	n	
	1	0	-1	-9.26	0.01	18	(8.2 - 10.0)
	1	0	1	3.83	0.01	19	(2.9 - 4.8)
Cu	1	1	0	2.68	0.04	14	(2.1 - 3.3)
Ni	1	1	0	1.47	0.31	2	(2.3 - 2.4)
Co	1	1	0	1.38	0.11	15	(2.1 - 3.1)
Zn	1	1	0	1.62	0.23	12	(2.1 - 3.3)

A = adeninate B = metal ion^{2+}, H = H^+, S = standard deviation in log constants, n = number of titration readings for each series. Parentheses refer to ph range studied.

Comparison with other workers results

The formation constants obtained in 150mM $NaClO_4$ are lower than those obtained at higher ionic strengths and this has been found for all the systems studied when compared with published results (tables15 and 16).

TABLE 15

Protonation of adeninate

$\log K_{101}$	$\log K_{101 \rightarrow 102}$	$\theta_c/^oC$	Method*	Medium	Reference
3.83	-9.26†	37	gl	0.15	This work
9.83	4.25	20	gl	0.1	80
10.65	3.54	25	gl	0.01 50 vol% dioxan	81

† - 9.26 $\log K_{10-1}$ * gl = glass electrode

TABLE 16

Metal-adeninate complexes

Metal	$\theta_c/^oC$	Method	Medium	$\log \beta_1$	$\log \beta_2$	Reference
Cu(II)	20	gl	0.1		14.22	80
	25	gl	0.01 50 vol% dioxan	8.94		81
Ni(II)	20	gl	0.1	4.37		80
	25	gl	0.01 50 vol% dioxan	6.18		81
Zn(II)	25	gl	0.01 50 vol% dioxan	6.42		81

As mentioned earlier in chapter 1 (page 2), these systems have been studied in order to gain experience in the techniques of potentiometry and computer programming.

Protonation Constants of Cyclohexylamine

The formation curve (figure 10) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using SCOGS (chapter 4) and the results obtained were

$$\log K_{101} = 9.93 \pm 0.01 \qquad S(\text{titre}) = 4.03 \quad (227 \text{ readings})$$

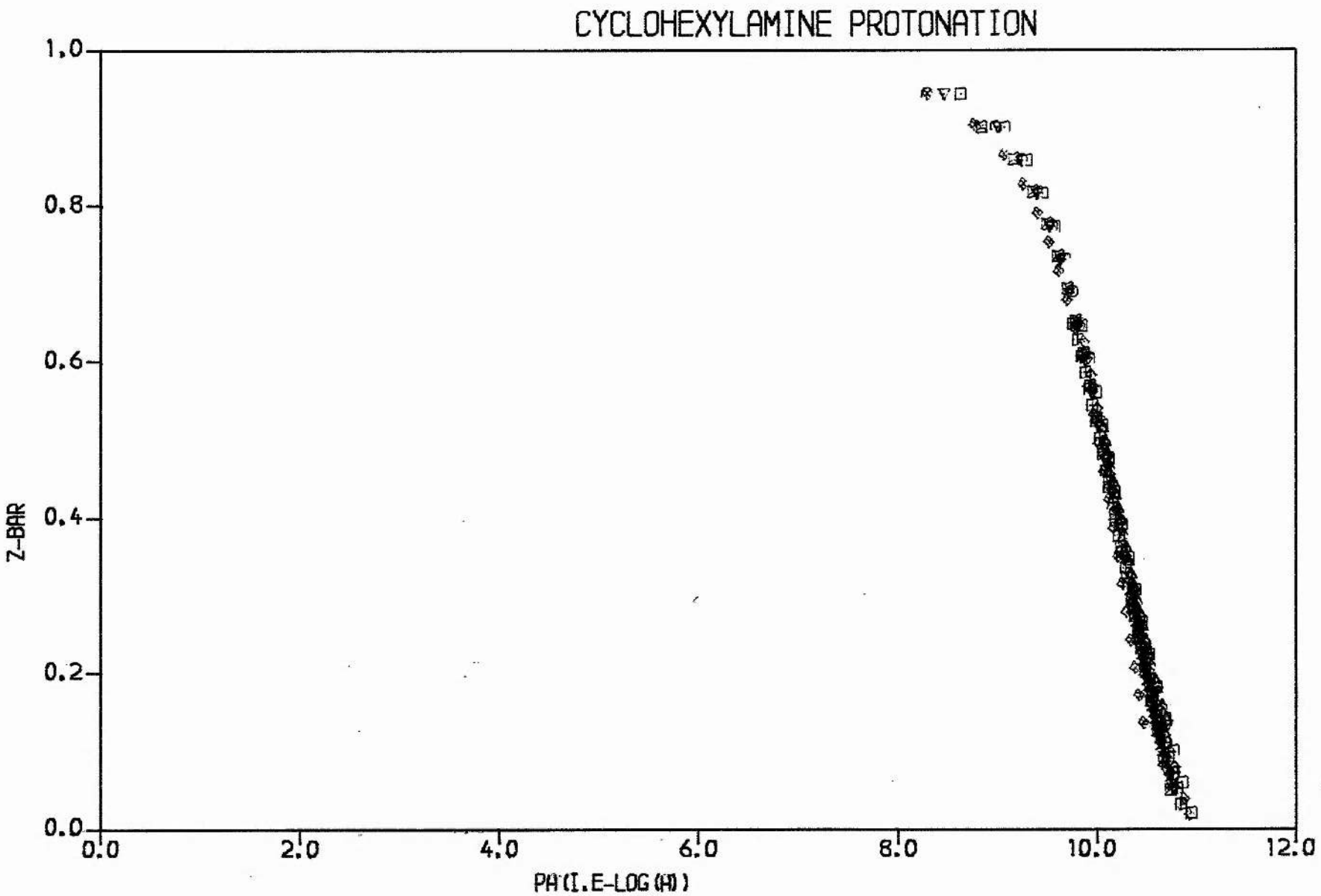
where S is the standard deviation.

TABLE 17

Experimental results for the protonation of cyclohexylamine

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^{o} (mV)
	A	H	A	H		
1	250.00	0	0	107.30	25.00	360.0
2	250.00	0	0	268.30	25.00	360.0
3	100.00	0	0	107.30	25.00	362.0
4	100.00	0	0	53.66	25.00	361.8
5	50.00	0	0	53.66	25.00	362.6
6	50.00	0	0	26.83	25.00	362.4
7	25.00	0	0	26.83	25.00	364.2
8	25.00	0	0	5.36	25.00	365.8
9	5.00	0	0	5.36	25.00	378.0

FIGURE 10


CYCLOHEXYLAMINE PROTONATION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(H))

Titration number 1

volume added (ml)	emf (mV)
8.00	-298.5
9.00	-296.0
10.00	-293.7
11.00	-291.5
12.00	-289.4
13.00	-287.4
14.00	-285.6
15.00	-283.9
16.00	-282.1
17.00	-280.4
18.00	-278.6
19.00	-276.8
20.00	-275.0
21.00	-273.5
22.00	-272.0
23.00	-270.4
24.00	-268.6
25.00	-267.0
26.00	-265.5
27.00	-263.9
28.00	-262.3
29.00	-260.7
30.00	-259.0

Titration number 2

volume added (ml)	emf (mV)
3.00	-299.0
4.00	-292.7
5.00	-287.0
6.00	-282.0
7.00	-277.4
8.00	-272.9
9.00	-268.8
10.00	-264.5
11.00	-260.4
12.00	-256.3
13.00	-252.1
14.00	-247.6
15.00	-242.9
16.00	-237.8
17.00	-232.2
18.00	-225.7
19.00	-218.0
20.00	-208.3
21.00	-193.7
22.00	-160.5

Titration number 3

volume added (ml)	emf (mV)
0.00	-311.7
1.00	-306.3
2.00	-300.7
3.00	-295.5
4.00	-290.3
5.00	-285.6
6.00	-281.3
7.00	-277.0
8.00	-272.7
9.00	-268.7
10.00	-264.4
11.00	-260.6
12.00	-256.7
13.00	-252.7
14.00	-248.3
15.00	-243.6
16.00	-238.7
17.00	-233.3
18.00	-226.9
19.00	-219.3
20.00	-209.6
21.00	-195.8
22.00	-168.8

Titration number 4

volume added (ml)	emf (mV)
0.00	-310.7
1.00	-307.6
2.00	-304.6
3.00	-301.7
4.00	-298.7
5.00	-295.8
6.00	-292.9
7.00	-290.4
8.00	-288.2
9.00	-285.8
10.00	-283.5
11.00	-281.4
12.00	-279.3
13.00	-277.3
14.00	-275.2
15.00	-273.3
16.00	-271.4
17.00	-269.4
18.00	-267.4
19.00	-265.5
20.00	-263.4
21.00	-261.7
22.00	-259.7
23.00	-257.8
24.00	-255.9
25.00	-253.8
26.00	-251.7
27.00	-249.7
28.00	-247.4
29.00	-245.2
30.00	-242.8

Titration number 5

volume added (ml)	emf (mV)
0.00	-304.8
1.00	-300.6
2.00	-295.8
3.00	-291.6
4.00	-287.4
5.00	-283.0
6.00	-278.9
7.00	-275.1
8.00	-271.0
9.00	-267.1
10.00	-263.2
11.00	-259.4
12.00	-255.5
13.00	-251.4
14.00	-247.0
15.00	-242.4
16.00	-237.3
17.00	-231.5
18.00	-225.0
19.00	-216.9
20.00	-206.2
21.00	-189.9
22.00	-147.4

Titration number 6

volume added (ml)	emf (mV)
0.00	-304.9
1.00	-302.5
2.00	-299.8
3.00	-297.1
4.00	-294.7
5.00	-292.3
6.00	-289.7
7.00	-287.5
8.00	-285.4
9.00	-282.9
10.00	-280.4
11.00	-278.5
12.00	-276.4
13.00	-274.5
14.00	-272.4
15.00	-270.6
16.00	-268.5
17.00	-266.5
18.00	-264.5
19.00	-262.7
20.00	-260.3
21.00	-258.4
22.00	-256.5
23.00	-254.5
24.00	-252.4
25.00	-250.0
26.00	-247.9
27.00	-245.6
28.00	-243.2
29.00	-240.8
30.00	-238.0

Titration number 7

volume added (ml)	emf (mV)
0.00	-296.7
1.00	-292.4
2.00	-288.7
3.00	-284.8
4.00	-281.0
5.00	-277.3
6.00	-273.6
7.00	-269.6
8.00	-265.9
9.00	-262.1
10.00	-258.6
11.00	-254.7
12.00	-250.9
13.00	-246.7
14.00	-242.6
15.00	-237.8
16.00	-232.8
17.00	-226.8
18.00	-219.8
19.00	-211.1
20.00	-199.5
21.00	-179.2

Titration number 8

volume added (ml)	emf (mV)
0.00	-296.0
1.00	-295.2
2.00	-294.2
3.00	-292.7
4.00	-291.7
5.00	-290.8
6.00	-289.6
7.00	-288.8
8.00	-287.8
9.00	-286.9
10.00	-285.8
11.00	-285.0
12.00	-284.2
13.00	-283.3
14.00	-282.6
15.00	-281.9
16.00	-281.0
17.00	-280.2
18.00	-279.4
19.00	-278.7
20.00	-277.8
21.00	-277.1
22.00	-276.4
23.00	-275.6
24.00	-274.9
25.00	-274.2
26.00	-273.5
27.00	-272.9
28.00	-272.1
29.00	-271.4
30.00	-270.4

Titration number 9

volume added (ml)	emf (mV)
0.00	-266.1
1.00	-263.5
2.00	-260.9
3.00	-258.2
4.00	-255.6
5.00	-253.1
6.00	-250.2
7.00	-247.4
8.00	-244.5
9.00	-241.5
10.00	-238.1
11.00	-234.9
12.00	-231.2
13.00	-227.2
14.00	-223.1
15.00	-218.4
16.00	-213.2
17.00	-207.2
18.00	-200.1
19.00	-191.2
20.00	-179.5
21.00	-160.9
22.00	-132.1

Formation Constants for Cu(II) - Cyclohexylamine Complexes

The formation curve (figure 11) was obtained from the results in table 18.

TABLE 18

Experimental results for the Cu(II) - cyclohexylamine system

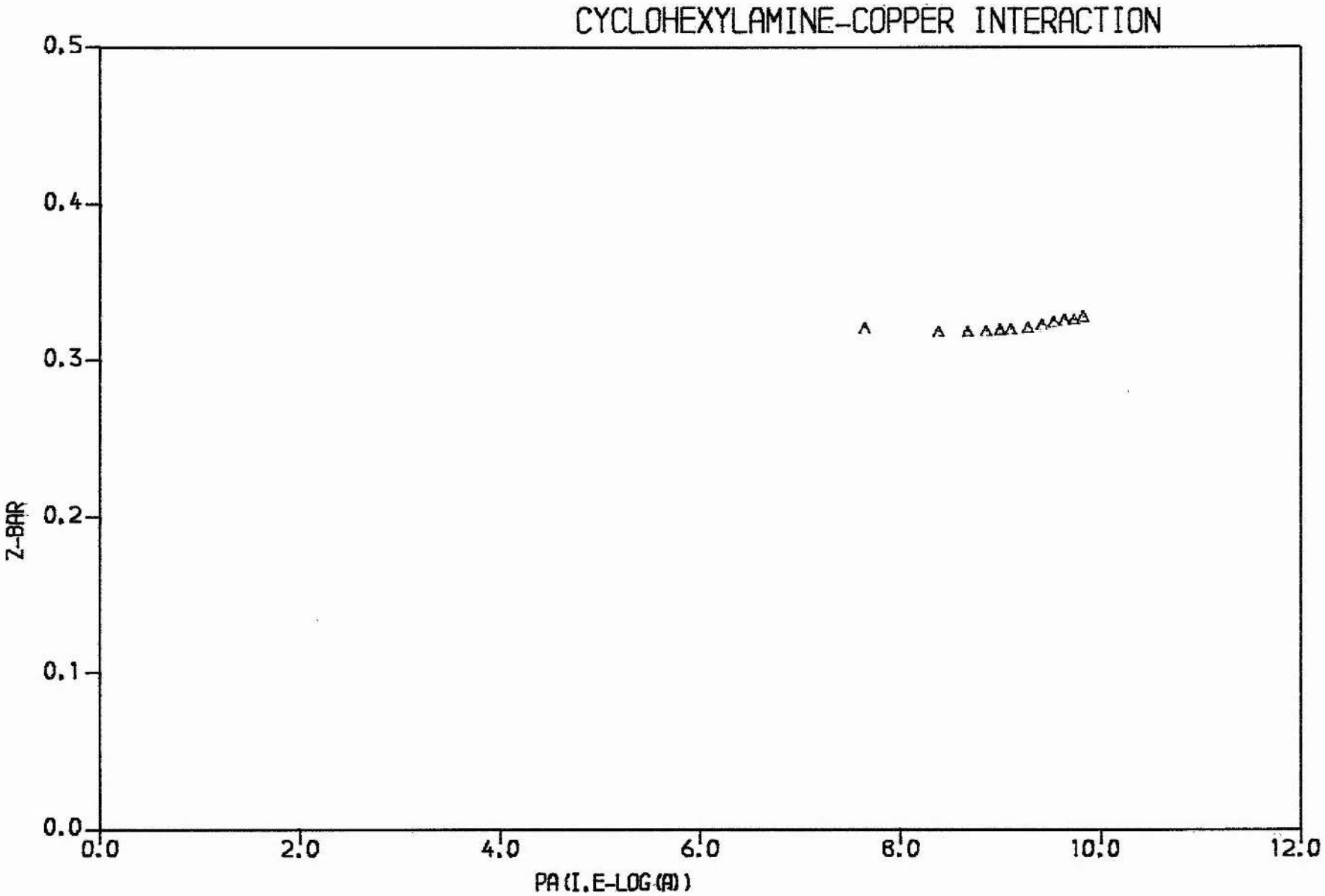
Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial Volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	23.70	0	19.99	0	25.00	362.0

Titration number 1

volume added (ml)	emf (mV)
19.00	220.3
20.00	216.3
21.00	212.0
22.00	207.0
23.00	201.0
24.00	193.7
25.00	184.2
25.50	178.1
26.00	170.1
26.50	159.3
27.00	141.7
27.50	96.4

Total number of readings = 12.

FIGURE 11


CYCLOHEXYLAMINE-COPPER INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Metal hydroxy precipitation restricted our working range of -log h 2.3 - 4.3. The data was analysed using SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Cu_2(OH)_2$ [76], the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 7.67 \pm 0.28 \qquad S(\text{titre}) = 12.1$$

The system can then be described by the complex Cu(cyclohexylamine)$^{2+}$.

Formation Constants for Ni(II) - Cyclohexylamine Complexes

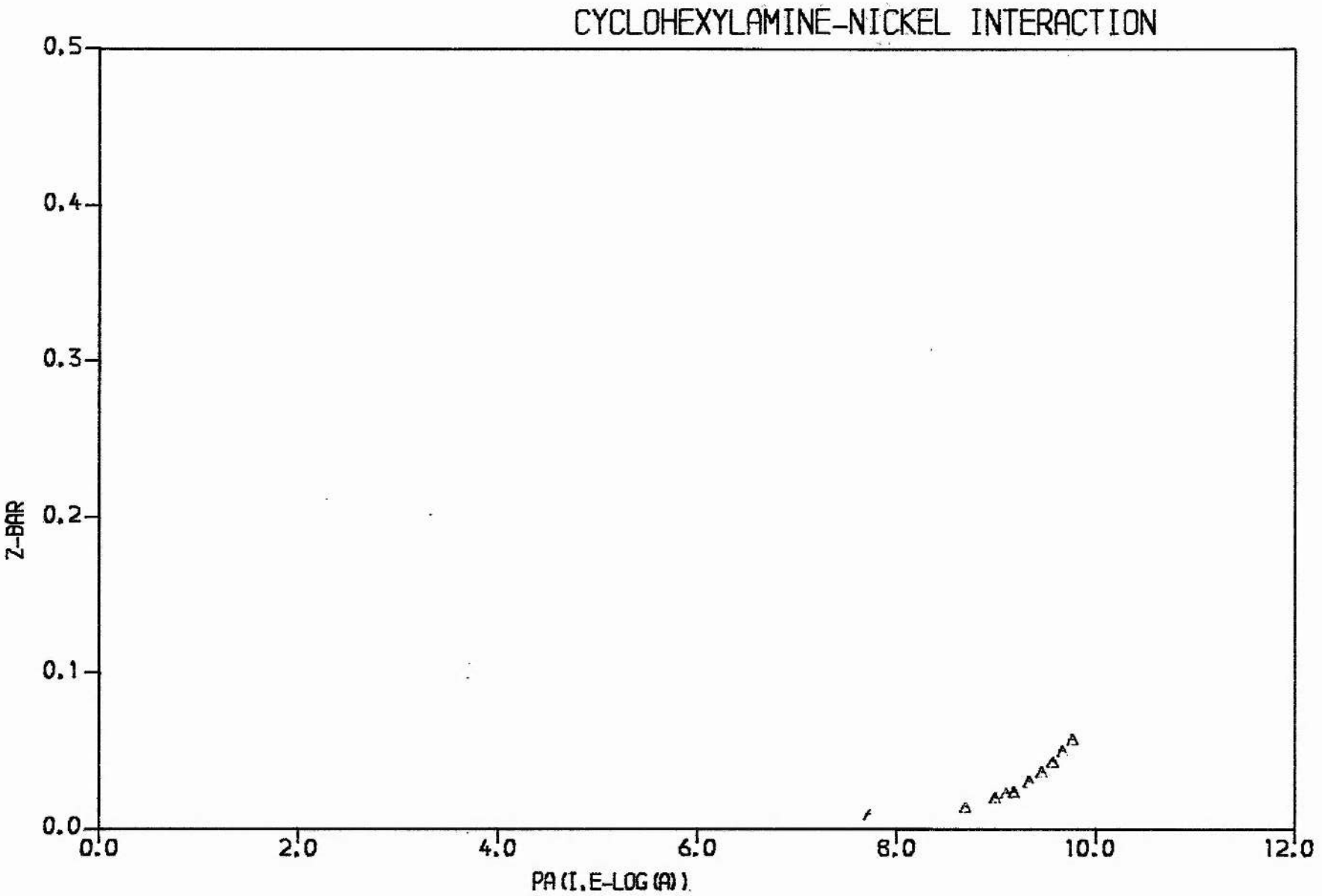
The formation curve (figure 12) was obtained from the results in table 19.

TABLE 19

Experimental results for the Ni(II) - cyclohexylamine system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial Volume (ml)	E^o (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	25.87	0	22.90	0	25.00	355.4

FIGURE 12


CYCLOHEXYLAMINE-NICKEL INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
15.00	218.1
16.00	213.8
17.00	209.0
18.00	203.5
19.00	197.0
20.00	188.7
20.50	184.2
21.00	178.1
22.00	160.4
23.00	101.8

Total number of readings = 10.

Metal hydroxy precipitation restricted our working range of -log h 2.2 - 4.1. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Ni(OH)^{77}$, the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 5.94 \pm 0.84 \qquad S(\text{titre}) = 1.62$$

The system can then be described by the complex Ni(cyclohexylamine)$^{2+}$.

Formation Constants for Co(II) - Cyclohexylamine Complexes

The formation curve (figure 13) was obtained from the results in table 20.

TABLE 20

Experimental results for the Co(II) - cyclohexylamine system

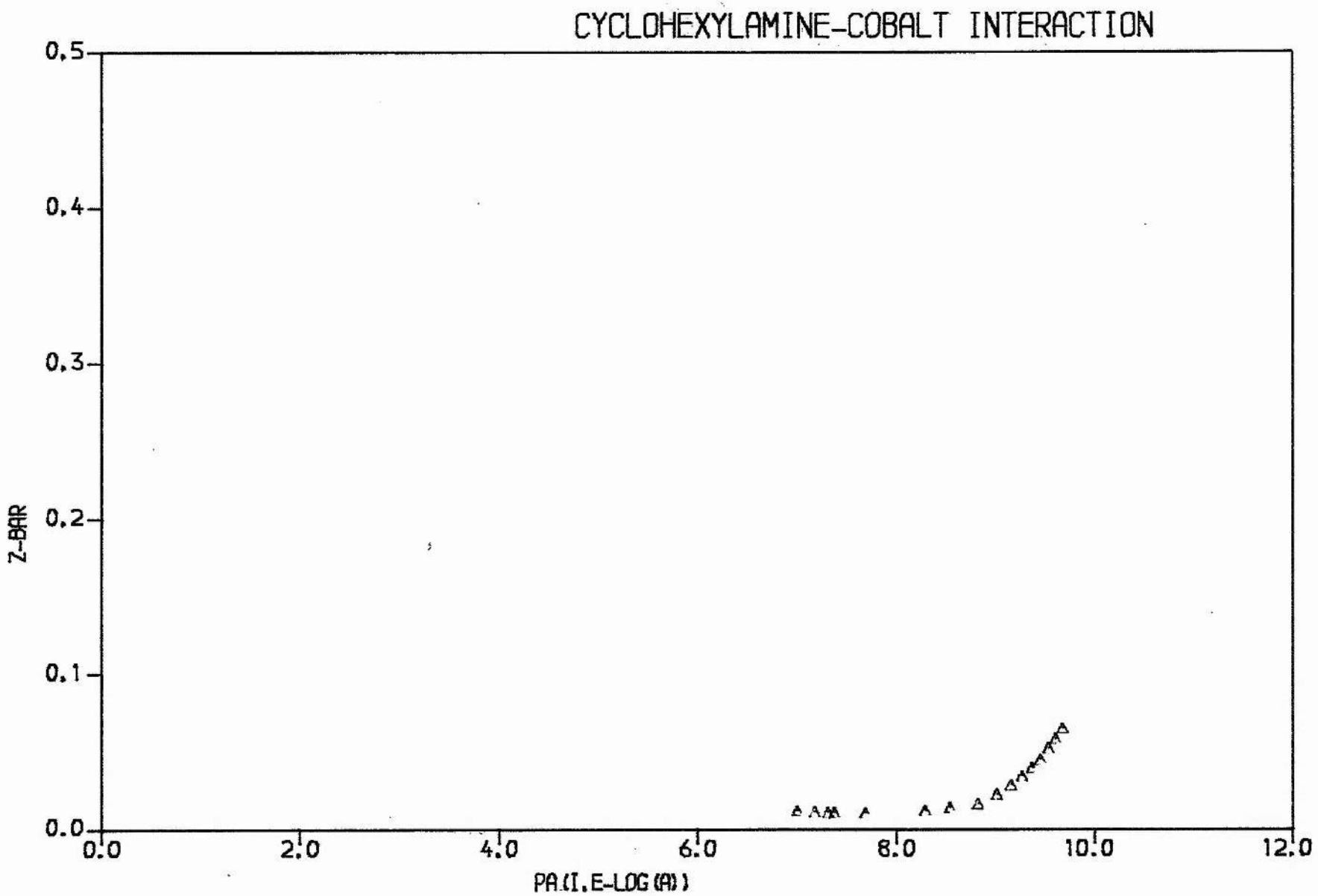
Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial Volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	24.35	0	31.92	0	25.00	355.4

Titration number 1

volume added (ml)	emf (mV)
22.00	219.2
23.00	215.8
24.00	212.1
25.00	207.9
26.00	203.3
27.00	198.0
28.00	191.6
29.00	183.5
30.00	172.3
31.00	155.6
31.50	140.2
32.00	103.5
32.10	84.2
32.12	80.2
32.15	71.9
32.18	61.4

Total number of readings = 16.

FIGURE 13


CYCLOHEXYLAMINE-COBALT INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I,E-LOG(A))

Metal hydroxy precipitation restricted our working range of -log h to 2.2 - 4.7. The data was analysed using SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Co(OH)_2^{78}$, the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 5.28 \pm 0.50 \qquad S(\text{titre}) = 1.23$$

The system can then be described by the complex Co(cyclohexylamine)$^{2+}$.

Formation Constants for Zn(II)- Cyclohexylamine Complexes

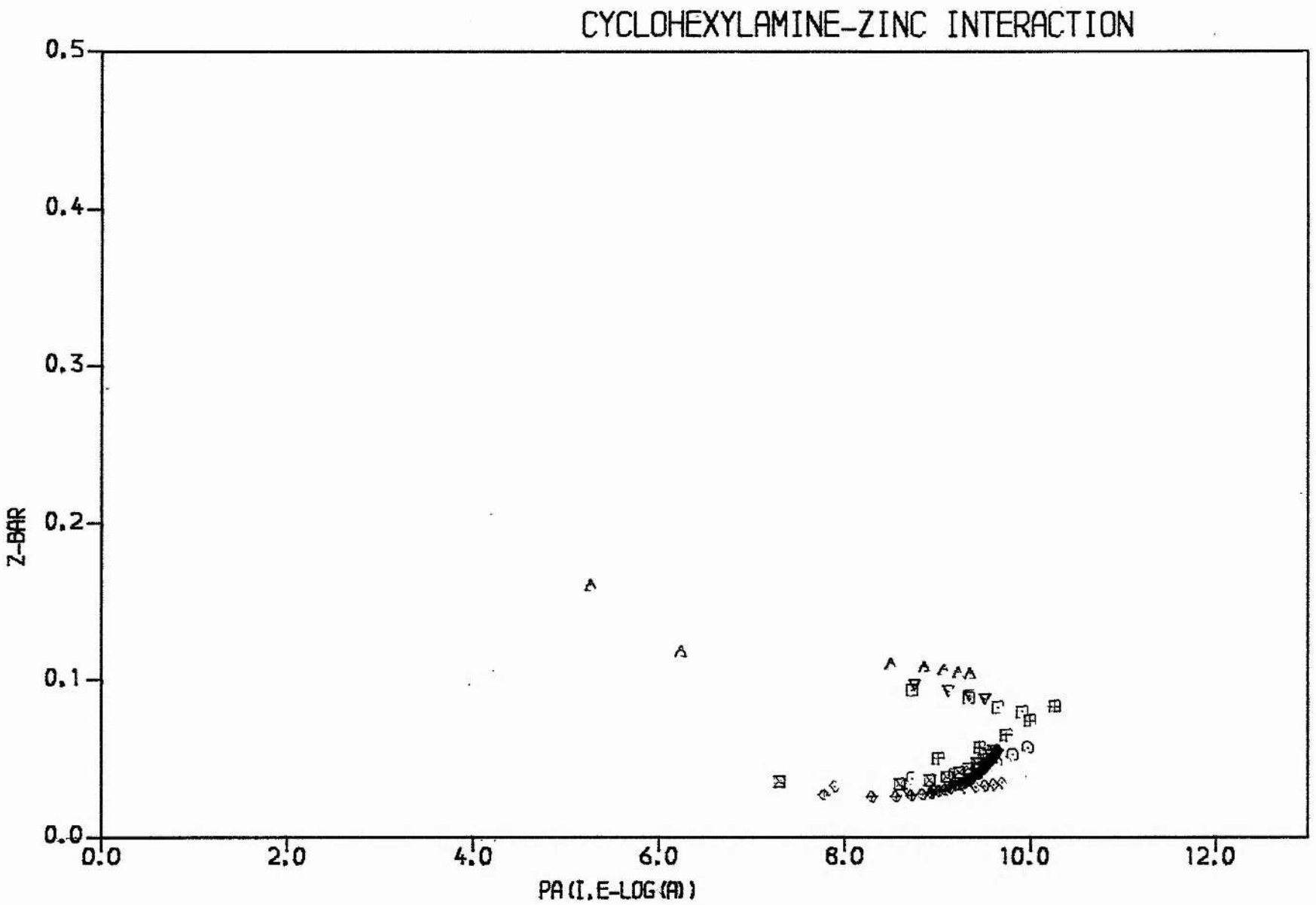
The formation curve (figure 14) was obtained from the results in table 21.

TABLE 21

Experimental results for the Zn(II) - cyclohexylamine system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E° (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	23.50	0	22.21	0	25.00	363.1
2	0	25.00	14.24	0	13.46	0	25.00	363.1
3	0	25.00	7.12	0	6.73	0	25.00	363.1
4	0	15.00	14.24	0	13.46	0	25.00	363.1
5	0	15.00	7.12	0	6.73	0	25.00	361.6
6	0	15.00	4.70	0	4.44	0	25.00	361.6
7	7.50	0	11.75	0	11.10	-10.00	30.00	361.6
8	7.50	0	11.75	0	11.10	- 4.00	30.00	361.6

FIGURE 14


CYCLOHEXYLAMINE-ZINC INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
20.00	203.9
21.00	197.0
22.00	188.3
23.00	176.1
24.00	154.7
25.00	15.9
26.00	-44.5

Titration number 2

volume added (ml)	emf (mV)
11.00	203.8
12.00	195.0
13.00	182.8
14.00	162.0

Titration number 3

volume added (ml)	emf (mV)
4.00	206.4
5.00	196.1
6.00	181.0
7.00	147.2

Titration number 4

volume added (ml)	emf (mV)
15.00	208.7
16.00	204.5
17.00	199.8
18.00	194.4
19.00	188.0
20.00	180.0
21.00	169.1
22.00	152.2
23.00	105.2

Titration number 5

volume added (ml)	emf (mV)
6.00	205.0
7.00	198.9
8.00	191.5
9.00	182.1
10.00	168.5
11.00	142.7

Titration number 6

volume added (ml)	emf (mV)
3.00	203.6
4.00	196.3
5.00	187.0
6.00	174.0
7.00	150.5

Titration number 7

volume added (ml)	emf (mV)
3.00	205.9
4.00	201.9
5.00	197.5
6.00	192.6
7.00	186.8
8.00	180.0
9.00	171.3
10.00	159.4
11.00	139.0
12.00	58.6

Titration number 8

volume added (ml)	emf (mV)
5.00	207.0
6.00	205.1
7.00	203.1
8.00	201.1
9.00	199.1
10.00	197.0
11.00	194.8
12.00	192.6
13.00	190.2
14.00	187.9
15.00	185.5
16.00	182.8
17.00	180.0
18.00	177.1
19.00	174.0
20.00	170.7
21.00	166.9
22.00	162.9
23.00	158.3
24.00	152.9
25.00	146.6
26.00	138.7
27.00	128.1
28.00	111.9
29.00	79.6

Total number of readings = 70.

Metal hydroxy precipitation restricted our working range of -log h 2.5 - 6.6. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Zn(OH)_2$ [79], the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 4.60 \pm 0.13 \qquad S(\text{titre}) = 2.1$$

The system can then be described by the complex Zn(cyclohexylamine)$^{2+}$.

Table 22 below lists our formation constants of the complex formation of cyclohexylamine and the published formation constants for the ammonia complexes as a comparison.

TABLE 22

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$

B	p	q	r	Present work	S	n
	1	0	1	9.93	0.01	227
Cu	1	1	0	7.67	0.28	12 (2.3-4.3)
Ni	1	1	0	5.94	0.84	10 (2.2-4.1)
Co	1	1	0	5.28	0.50	16 (2.2-4.7)
Zn	1	1	0	4.60	0.13	70 (2.5-6.6)

Ammonia has log β_{101} = 9.47 (H^+), log β_{110} = 4.11 (Cu), 2.80 (Ni), 2.11 (Co), and 2.37 (Zn).

A = cyclohexylamine, B = metal ion^{2+}, H = H^+, S = standard deviation in log constants, n = number of titration readings for each series. Parentheses refer to ph range studied.

Comparison with other workers results

The value 10.66 at 24°C, I = 0.001 M [82] for cyclohexylamine protonation is the only one available in the literature. For the formation constants of the metal complexes none are available.

Protonation Constants of Cyclopentylamine

The formation curve (figure 15) was constructed using the ZPLOT computer program (chapter 4). The data was then analysed using SCOGS (chapter 4) and the results obtained were

$$\log K_{101} = 10.03 \pm 0.01 \qquad S(\text{titre}) = 2.61 \qquad (229 \text{ readings})$$

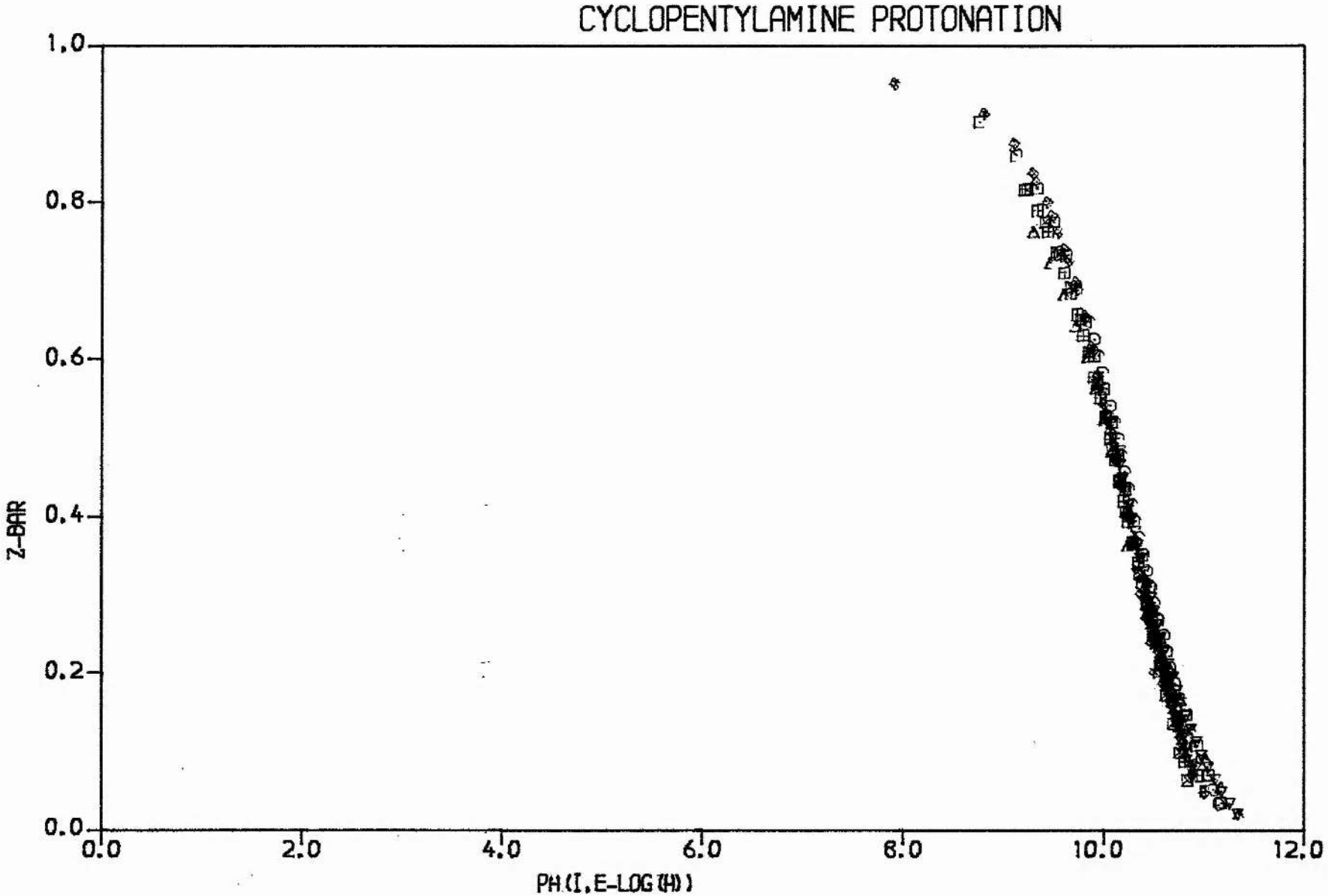
where S is the standard deviation.

TABLE 23

Experimental results for the protonation of cyclopentylamine

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E° (mV)
	A	H	A	H		
1	249.00	0	0	250.10	25.00	388.9
2	249.00	0	0	107.10	25.00	387.9
3	99.60	0	0	107.10	25.00	387.9
4	49.30	0	0	53.50	25.00	387.9
5	99.60	0	0	53.50	25.00	387.9
6	49.30	0	0	26.80	20.00	389.9
7	25.00	0	0	26.80	25.00	388.9
8	25.00	0	0	5.30	25.00	390.9
9	4.90	0	0	5.30	25.00	393.9

FIGURE 15


CYCLOPENTYLAMINE PROTONATION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH(I,E-LOG(H))

Titration number 1

volume added (ml)	emf (mV)
0.00	-310.0
1.00	-299.0
2.00	-289.6
3.00	-281.0
4.00	-274.0
5.00	-267.5
6.00	-261.8
7.00	-256.5
8.00	-251.2
9.00	-246.3
10.00	-241.3
11.00	-236.3
12.00	-231.3
13.00	-226.4
14.00	-221.3
15.00	-216.5
16.00	-209.0
17.00	-202.0
18.00	-193.5
19.00	-183.0

Titration number 2

volume added (ml)	emf (mV)
0.00	-310.0
1.00	-305.0
2.00	-300.0
3.00	-296.0
4.00	-291.6
5.00	-287.5
6.00	-284.5
7.00	-281.2
8.00	-278.0
9.00	-275.2
10.00	-272.5
11.00	-270.0
12.00	-267.2
13.00	-264.8
14.00	-262.2
15.00	-260.5
16.00	-258.2
17.00	-256.2
18.00	-254.1
19.00	-252.2
20.00	-250.0
21.00	-248.0
22.00	-246.5
23.00	-244.4
24.00	-242.5
25.00	-240.7
26.00	-238.9
27.00	-237.0
28.00	-235.0
29.00	-233.1
30.00	-231.1

Titration number 3

volume added (ml)	emf (mV)
0.00	-300.0
1.00	-292.2
2.00	-285.0
3.00	-278.5
4.00	-271.5
5.00	-266.0
6.00	-260.5
7.00	-255.5
8.00	-250.9
9.00	-245.9
10.00	-240.5
11.00	-236.0
12.00	-232.0
13.00	-227.0
14.00	-221.5
15.00	-216.1
16.00	-210.2
17.00	-204.2
18.00	-196.4
19.00	-186.6
20.00	-173.4
21.00	-151.0

Titration number 4

volume added (ml)	emf (mV)
0.00	-289.4
1.00	-284.0
2.00	-278.5
3.00	-272.0
4.00	-267.5
5.00	-262.5
6.00	-258.0
7.00	-253.2
8.00	-248.9
9.00	-244.1
10.00	-240.0
11.00	-235.5
12.00	-231.0
13.00	-226.1
14.00	-221.0
15.00	-215.8
16.00	-210.0
17.00	-202.2
18.00	-195.5
19.00	-185.9
20.00	-172.5

Titration number 5

volume added (ml)	emf (mV)
0.00	-298.5
1.00	-295.2
2.00	-291.5
3.00	-288.0
4.00	-283.5
5.00	-280.5
6.00	-277.3
7.00	-274.5
8.00	-271.5
9.00	-268.8
10.00	-266.4
11.00	-264.5
12.00	-261.0
13.00	-258.7
14.00	-256.5
15.00	-254.0
16.00	-251.8
17.00	-249.5
18.00	-247.0
19.00	-245.0
20.00	-242.5
21.00	-240.1
22.00	-238.0
23.00	-236.0
24.00	-233.8
25.00	-231.0
26.00	-228.6
27.00	-226.3
28.00	-223.7
29.00	-221.3
30.00	-218.5

Titration number 6

volume added (ml)	emf (mV)
0.00	-288.5
1.00	-283.0
2.00	-275.5
4.00	-271.0
5.00	-267.6
6.00	-264.5
7.00	-261.0
8.00	-258.0
9.00	-255.2
10.00	-252.2
11.00	-249.0
12.00	-246.6
13.00	-243.2
14.00	-240.1
15.00	-237.5
16.00	-235.0
17.00	-232.0
18.00	-229.0
19.00	-226.0
20.00	-223.0
21.00	-219.0
22.00	-215.8
23.00	-212.5
24.00	-208.9
25.00	-205.0
26.00	-201.0
27.00	-196.4
28.00	-190.9
29.00	-184.5
30.00	-176.8

Titration number 7

volume added (ml)	emf (mV)
0.00	-278.0
1.00	-273.5
2.00	-269.5
3.00	-265.0
4.00	-261.0
5.00	-256.5
6.00	-252.6
7.00	-248.5
8.00	-244.2
9.00	-240.0
10.00	-236.0
11.00	-231.5
12.00	-227.3
13.00	-222.0
14.00	-217.0
15.00	-212.0
16.00	-206.0
17.00	-199.0
18.00	-190.3
19.00	-179.2

Titration number 8

volume added (ml)	emf (mV)
0.00	-278.8
1.00	-278.3
2.00	-276.5
3.00	-275.2
4.00	-274.0
5.00	-272.9
6.00	-271.9
7.00	-270.9
8.00	-269.9
9.00	-268.8
10.00	-267.8
11.00	-266.8
12.00	-265.8
13.00	-264.8
14.00	-263.8
15.00	-262.8
16.00	-261.7
17.00	-260.9
18.00	-260.0
19.00	-259.1
20.00	-258.2
21.00	-257.0
22.00	-255.8
23.00	-254.8
24.00	-253.8
25.00	-253.0
26.00	-252.1
27.00	-251.5
28.00	-250.8
29.00	-250.0
30.00	-249.2

Titration number 9

volume added (ml)	emf (mV)
0.00	-258.0
1.00	-252.5
2.00	-250.5
3.00	-247.5
4.00	-244.0
5.00	-241.0
6.00	-238.1
7.00	-235.0
8.00	-231.9
9.00	-228.2
10.00	-225.0
11.00	-221.0
12.00	-217.5
13.00	-213.6
14.00	-209.4
15.00	-205.0
16.00	-199.8
17.00	-193.5
18.00	-186.5
19.00	-177.8
20.00	-166.3
21.00	-147.8
22.00	- 93.0

Formation Constants for Cu(II) - Cyclopentylamine Complexes

The formation curve (figure 16) was obtained from the results in table 24.

TABLE 24

Experimental results for the Cu(II) - cyclopentylamine system

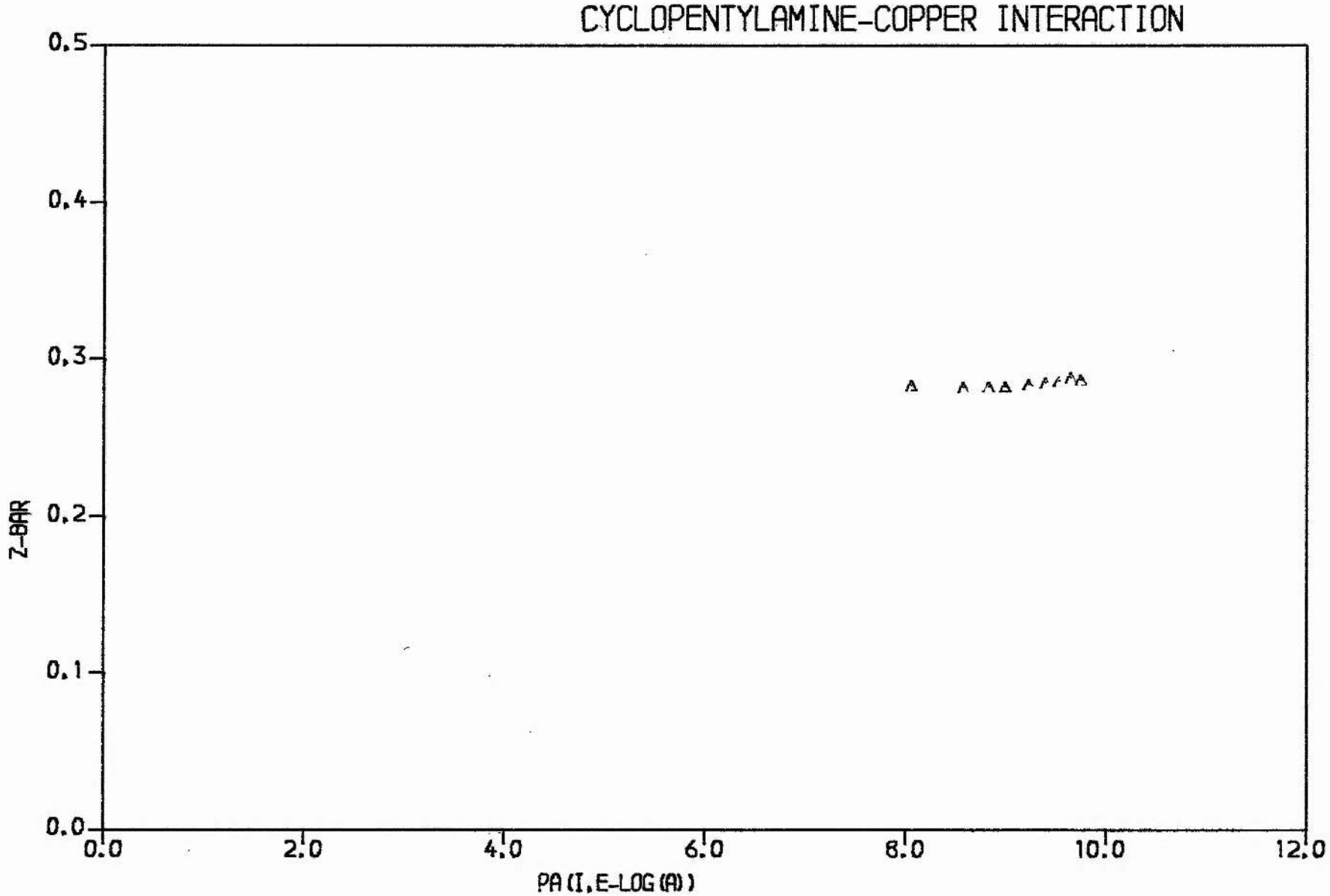
Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	23.70	0	19.99	0	25.00	363.7

Titration number 1

volume added (ml)	emf (mV)
20.00	214.6
21.00	209.9
22.00	204.0
23.00	197.0
24.00	187.9
25.00	174.6
25.50	164.9
26.00	149.7
26.50	118.2

Total number of readings = 9

FIGURE 16


CYCLOPENTYLAMINE-COPPER INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I,E-LOG(A))

Metal hydroxy precipitation restricted our working range of -log h to 2.4 - 3.9. The data was analysed using SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Cu_2(OH)_2$ [76], the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 8.01 \pm 0.23 \qquad S(\text{titre}) = 9.89$$

The system can then be described by the complex Cu(cyclopentylamine)$^{2+}$.

Formation Constants for Ni(II) - Cyclopentylamine Complexes

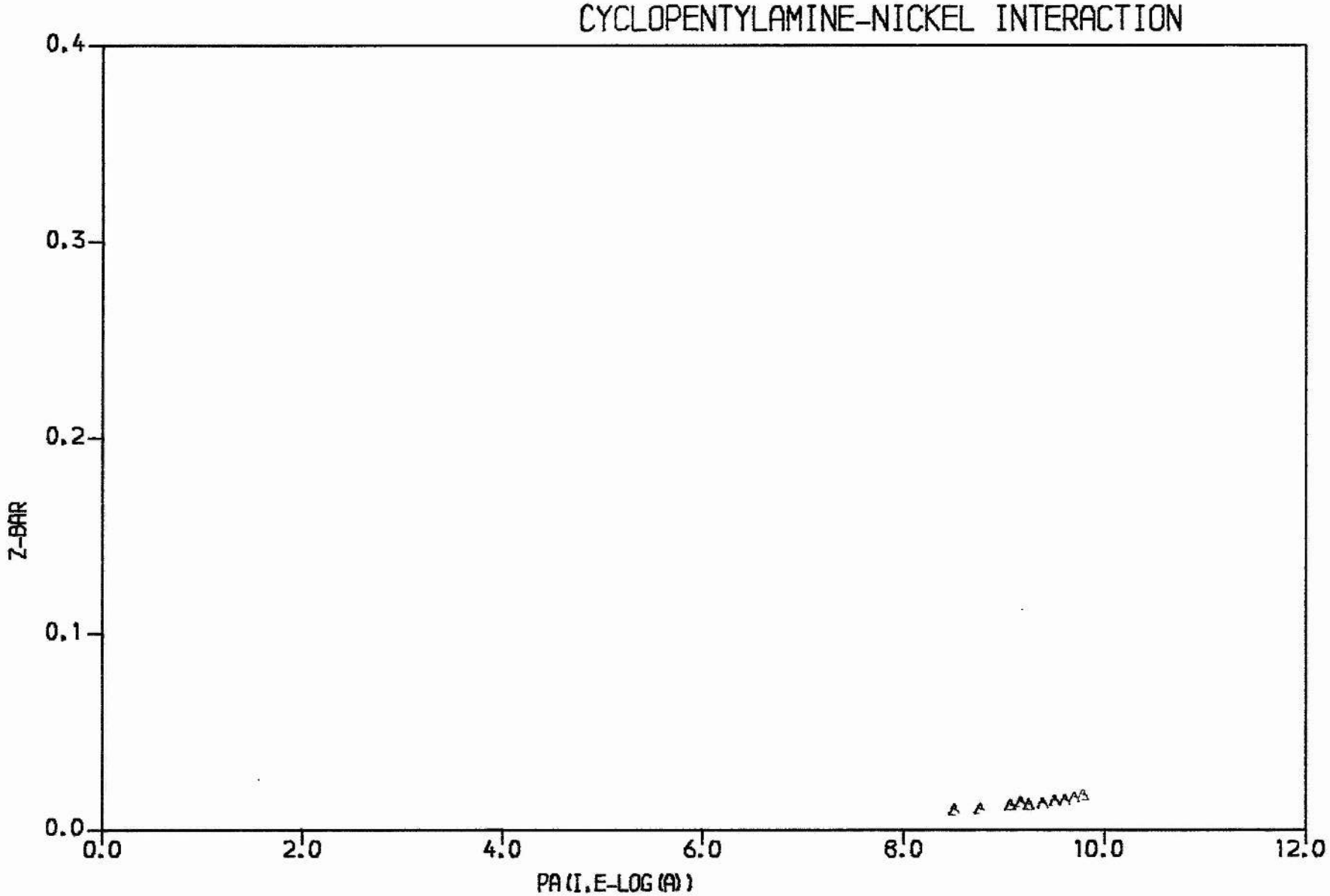
The formation curve (figure 17) was obtained from the results in table 25.

TABLE 25

Experimental results for the Ni(II) - cyclopentylamine system

Titration number	Initial concentrations (mM); Titrate (S) and Titrant (T)						Initial volume (ml)	E^o (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	25.87	0	22.90	0	25.00	361.0

FIGURE 17


CYCLOPENTYLAMINE-NICKEL INTERACTION
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
15.00	220.6
16.00	216.4
17.00	211.7
18.00	206.4
19.00	200.0
20.00	192.1
20.50	187.6
21.00	181.5
22.00	163.9
22.50	148.4

Total number of readings = 10.

Metal hydroxy precipitation restricted our working range of -log h to 2.2 - 3.4. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Ni(OH)^{77}$, the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 6.82 \pm 0.20 \qquad S(\text{titre}) = 0.58$$

The system can then be described by the complex Ni(cyclopentylamine)$^{2+}$.

Formation Constants for Co(II) - Cyclopentylamine Complexes

The formation curve (figure 18) was obtained from the results in table 26.

TABLE 26

Experimental results for the Co(II) - cyclopentylamine system

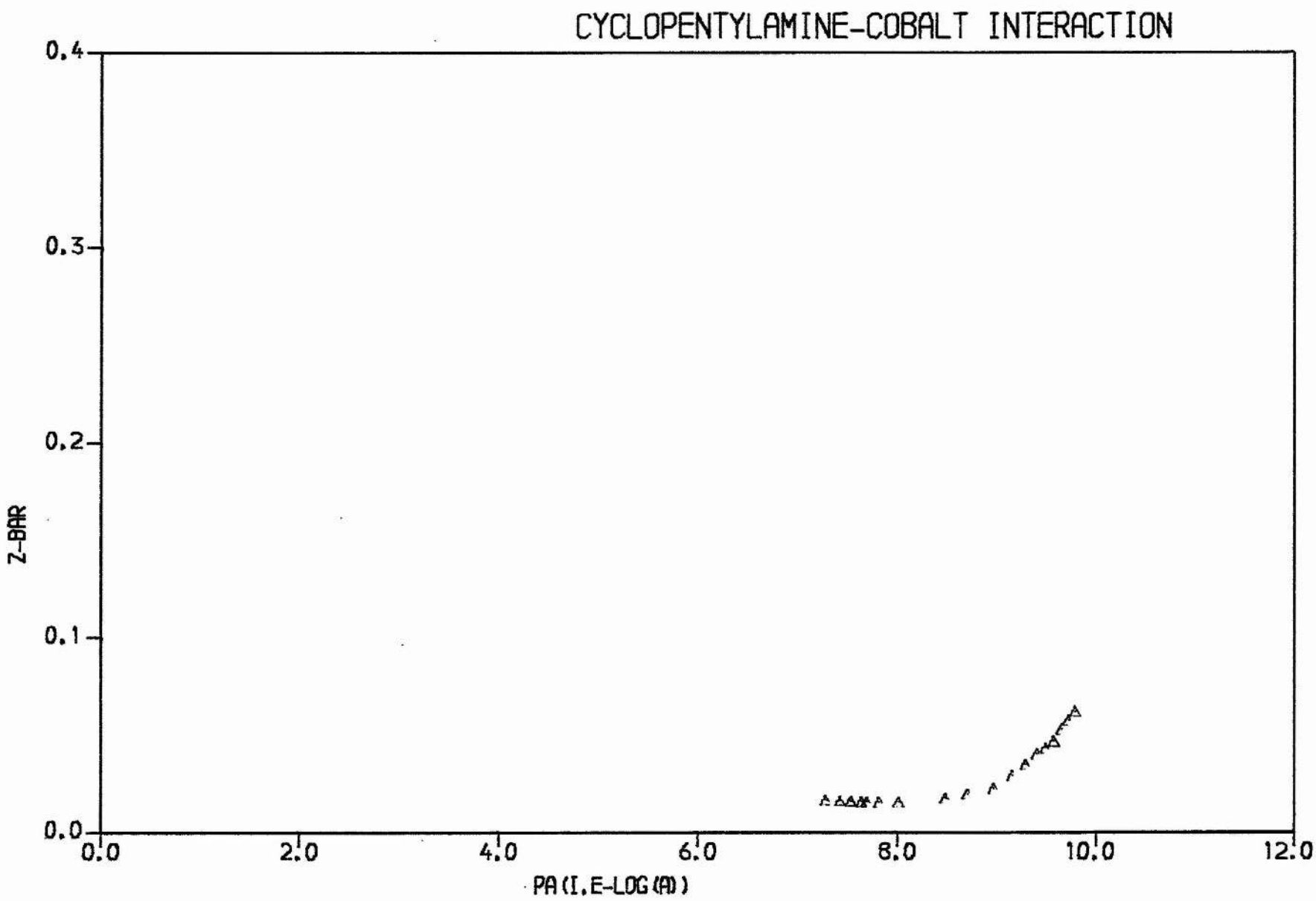
Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	25.00	24.35	0	31.92	0	25.00	355.4

Titration number 1

volume added (ml)	emf (mV)
22.00	219.0
23.00	215.7
24.00	212.1
25.00	208.0
26.00	203.6
27.00	198.6
28.00	192.4
29.00	184.6
30.00	173.8
31.00	158.0
31.50	144.4
32.00	115.8
32.10	104.0
32.15	96.5
32.17	93.2
32.20	86.7
32.23	80.1
32.26	71.1

Total number of readings = 18

FIGURE 18


CYCLOPENTYLAMINE-COBALT INTERACTION
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Metal hydroxy precipitation restricted our working range of -log h to 2.2 - 4.6. The data was analysed using SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Co(OH)^{78}$, the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 5.70 \pm 0.30 \qquad S(\text{titre}) = 1.15$$

The system can then be described by the complex Co(cyclopentylamine)$^{2+}$.

Formation Constants for Zn(II) - Cyclopentylamine Complexes

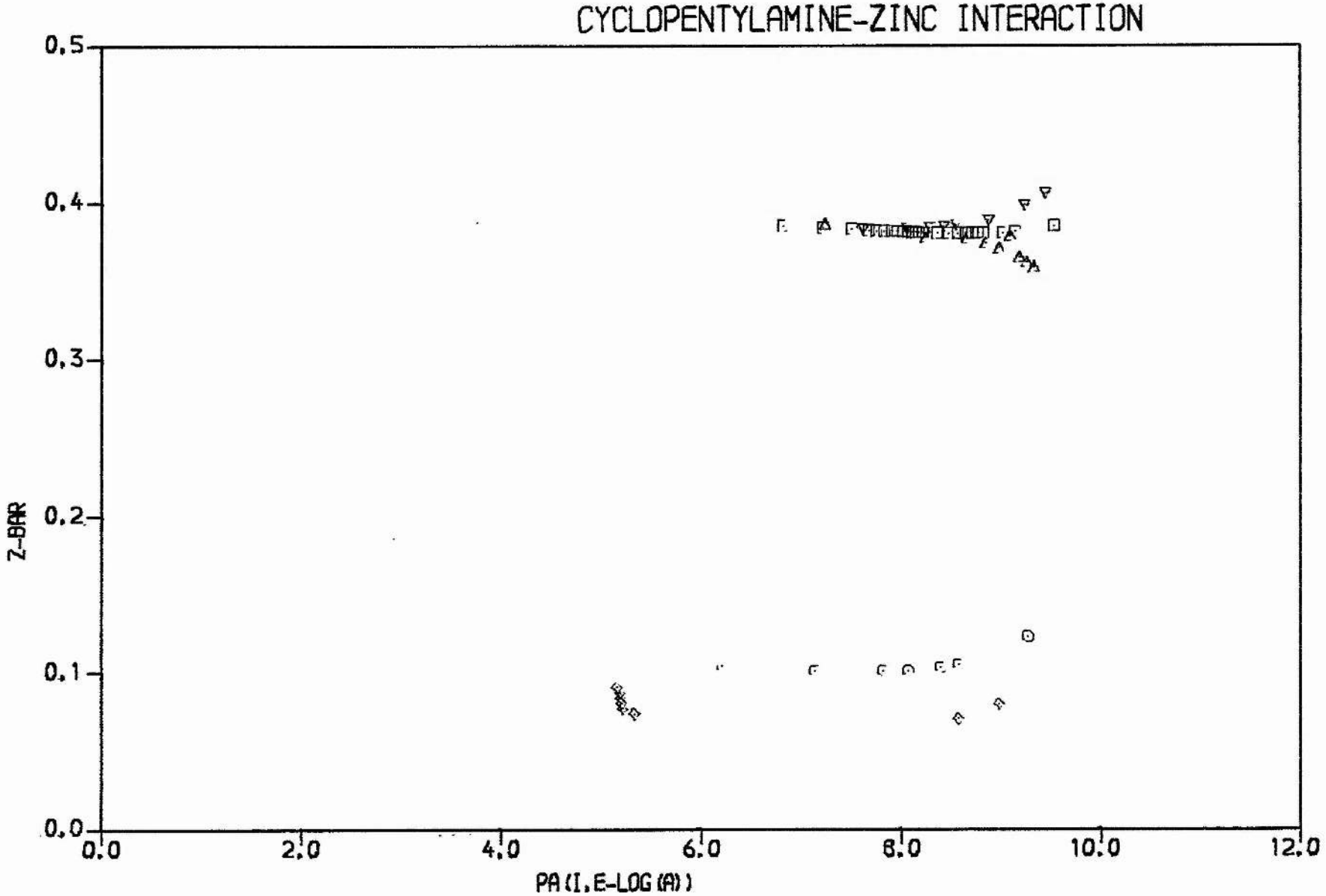
The formation curve (figure 19) was obtained from the results in table 27.

TABLE 27

Experimental results for the Zn(II) - cyclopentylamine system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	65.10	117.50	0	111.00	0	25.00	384.3
2	0	65.10	47.00	0	44.42	0	25.00	384.3
3	0	65.10	23.50	0	22.10	0	25.00	384.3
4	0	99.80	117.50	0	110.00	0	25.00	384.3
5	0	99.80	47.00	0	44.42	0	25.00	384.3

FIGURE 19


CYCLOPENTYLAMINE-ZINC INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I,E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
52.00	246.5
53.00	242.5
54.00	238.0
55.50	232.5
56.00	226.2
57.00	218.0
58.00	206.8
59.00	181.5
60.00	119.5

Titration number 2

volume added (ml)	emf (mV)
21.00	241.5
22.00	230.0
23.00	209.5
23.50	190.0
23.60	182.8
23.70	173.9
23.80	160.0
23.90	133.2

Titration number 3

volume added (ml)	emf (mV)
10.00	234.3
11.00	213.5
11.20	206.6
11.45	195.0
11.50	192.0
11.55	188.5
11.60	184.5
11.65	180.0
11.70	174.5
11.75	167.5
11.80	158.5
11.81	156.2
11.82	154.3
11.83	152.0
11.84	149.5
11.85	146.5
11.86	143.0
11.87	140.0
11.88	135.0
11.89	130.2
11.90	125.5
11.92	115.0
11.94	97.2
11.96	72.9

Titration number 4

volume added (ml)	emf (mV)
28.00	237.4
29.00	213.2
30.00	14.2
30.10	6.8
30.20	6.1
30.30	5.5
30.40	5.0
30.50	3.2

Titration number 5

volume added (ml)	emf (mV)
11.00	239.0
12.00	199.0
12.10	188.0
12.20	168.2
12.25	152.8
12.30	110.8
12.35	53.9

Total number of readings = 56.

Metal hydroxy precipitation restricted our working range of -log h to 2.2 - 6.1. The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Zn(OH)_2$ [79], the β values for the latter being held constant. The 'best' log constant obtained was

$$\log \beta_{110} = 4.16 \quad \pm 0.53 \qquad S(\text{titre}) = 12.87$$

The system can then be described by the complex Zn(cyclopentylamine)$^{2+}$.

Table 28 below lists our formation constants of the complex formation of cyclopentylamine and the published formation constants for the ammonia complexes as a comparison.

TABLE 28

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150 mM $NaClO_4$

B	p	q	r	Present work	S	n	
	1	0	1	10.03	0.01	229	
Cu	1	1	0	8.01	0.23	9	(2.4-3.9)
Ni	1	1	0	6.82	0.20	10	(2.2-3.4)
Co	1	1	0	5.70	0.30	18	(2.2-4.6)
Zn	1	1	0	4.16	0.53	56	(2.2-6.1)

Ammonia has log β_{101} = 9.47 (H^+), log β_{110} = 4.11 (Cu), 2.80 (Ni), 2.11 (Co), and 2.37 (Zn). A = cyclopentylamine, B = metal ion^{2+}, H = H^+, S = standard deviation in log constants, n = number of titration readings for each series, Parentheses refer to ph range studied.

Comparison with other workers results

The value 10.65 at 25°C, I = 0 [83] for cyclopentylamine protonation is the only one available in the literature. For the formation constants of the metal complexes, there have been none reported.

Protonation Constants of Glycinate

The formation curve (figure 20) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using SCOGS (chapter 4) and the results obtained were:

$$\left.\begin{aligned} \log K_{101} &= 9.17 \pm 0.01 \\ \log K_{101 \rightarrow 102} &= 2.34 \pm 0.01 \end{aligned}\right\} \quad S(\text{titre}) = 0.27 \text{ (179 readings)}$$

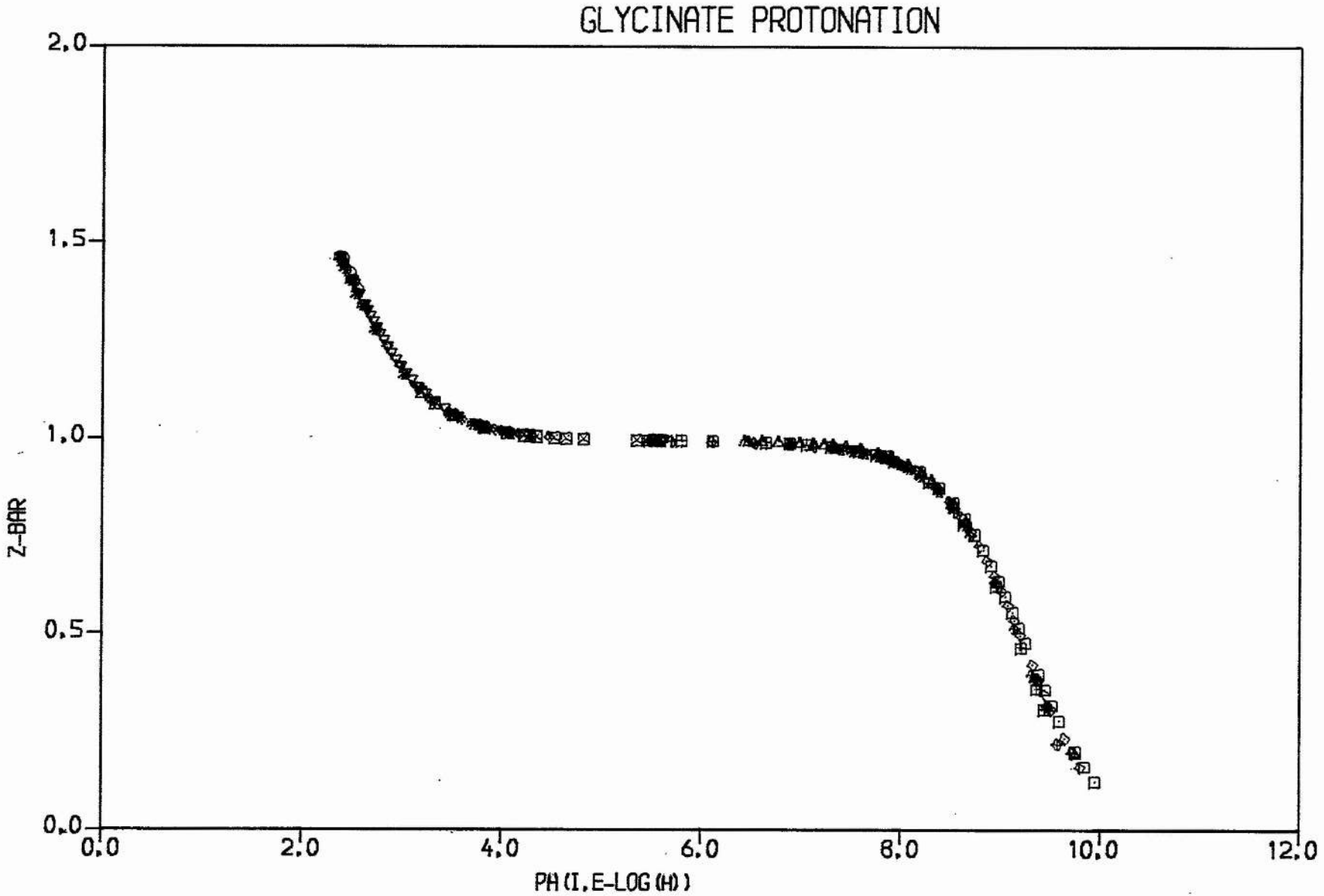
where S is the standard deviation.

TABLE 29

Experimental results for the protonation of glycinate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^{o} (mV)
	A	H	A	H		
1	200.00	0	0	-100.00	25.00	364.5
2	50.00	0	0	26.83	25.00	365.5
3	50.00	0	0	-50.00	25.00	363.8
4	25.00	0	0	-24.03	25.00	363.8
5	25.00	0	0	26.83	25.00	365.5
6	15.00	0	0	-20.00	25.00	364.5
7	15.00	0	0	16.16	25.00	364.5
8	10.00	0	0	-15.15	25.00	367.9
9	10.00	0	0	13.41	25.00	364.3
10	5.00	6.70	5.00	- 8.00	25.00	364.3

FIGURE 20


GLYCINATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH(I.E-LOG(H))

Titration number 1

volume added (ml)	emf (mV)
0.00	10.4
0.01	-31.2
0.02	-33.6
0.05	-41.7
0.10	-51.8
0.20	-65.1
0.30	-73.8
0.40	-80.4
0.50	-85.7
0.70	-93.8
1.00	-103.0
1.50	-113.6
2.00	-121.3
3.00	-132.2
4.00	-140.2
5.00	-146.7
8.00	-160.5
40.00	-235.7

Titration number 2

volume added (ml)	emf (mV)
0.00	20.4
1.00	116.8
2.00	135.5
3.00	146.8
4.00	154.9
5.00	161.3
6.00	166.7
7.00	171.3
8.00	175.3
9.00	178.8
10.00	182.0
11.00	185.0
12.00	187.8
13.00	190.3
14.00	192.6
15.00	194.9
16.00	197.1
17.00	199.0
18.00	201.0
19.00	202.8
20.00	204.5
22.00	207.8
24.00	210.8

Titration number 3

volume added (ml)	emf (mV)
0.00	22.7
1.00	-120.4
2.00	-140.0
3.00	-151.8
4.00	-160.5
5.00	-167.7
6.00	-173.7
7.00	-179.1
8.00	-184.1
9.00	-188.6
10.00	-192.9
11.00	-197.3
12.00	-201.3
13.00	-205.3
15.00	-213.4
16.00	-217.6
17.00	-221.8
18.00	-226.4
20.00	-236.5
21.00	-242.2
22.00	-248.5

Titration number 4

volume added (ml)	emf (mV)
0.00	19.9
1.00	-118.0
2.00	-137.8
3.00	-149.7
4.00	-158.4
5.00	-165.5
6.00	-171.3
7.00	-176.7
8.00	-181.6
9.00	-186.1
10.00	-190.2
11.00	-194.3
12.00	-198.2
13.00	-202.0
15.00	-209.6
16.00	-213.4
17.00	-217.3
18.00	-221.3
20.00	-229.7
21.00	-234.2
22.00	-239.1

Titration number 5

volume added (ml)	emf (mV)
0.00	26.6
1.00	132.7
13.00	208.9
14.00	211.3
15.00	213.6
17.00	217.7

Titration number 6

volume added (ml)	emf (mV)
0.00	27.9
0.02	7.9
0.04	-11.5
0.10	-44.1
0.15	-58.7
0.20	-69.2
0.30	-84.6
0.50	-101.5
0.70	-112.3
1.00	-123.4
2.00	-144.6
4.00	-166.4
7.00	-186.4
10.00	-201.9
12.00	-211.4
13.00	-216.3

Titration number 7

volume added (ml)	emf (mV)
0.00	21.0
0.02	35.1
0.10	68.1
0.15	78.8
0.20	86.3
0.30	97.2
0.35	101.3
0.40	105.0
0.60	115.7
1.00	129.8
2.00	148.9
3.00	160.2
4.00	168.3

Titration number 8

volume added (ml)	emf (mV)
0.00	30.8
0.02	17.7
0.05	-7.6
0.10	-33.6
0.25	-70.0
0.50	-99.0
1.00	-123.7
2.00	-146.1
3.00	-156.9
4.00	-167.1
6.00	-182.3
8.00	-194.3
10.00	-205.1
13.00	-220.9

Titration number 9

volume added (ml)	emf (mV)
0.00	23.2
0.20	89.3
0.30	100.1
0.40	107.7
0.50	113.5
0.60	118.2
0.70	122.5
0.80	126.2
0.90	129.4
1.00	132.3
1.50	143.3
2.00	151.1
3.00	162.3
4.00	170.2
6.00	181.4
8.00	189.4
10.00	195.6
13.00	202.8

Titration number 10

volume added (ml)	emf (mV)
0.00	219.1
0.04	218.9
0.10	218.7
0.60	216.9
1.00	215.5
2.00	211.9
3.00	208.4
4.00	204.6
6.00	196.9
10.00	178.8
14.00	149.7
15.00	136.5
16.00	112.8
17.00	-58.8
17.20	-86.7
17.25	-90.9
17.35	-97.8
17.50	-105.6
17.70	-113.6
17.80	-116.9
18.00	-122.4
18.20	-127.0
18.50	-132.7
19.00	-140.3
20.00	-151.1
21.00	-159.0
22.50	-167.6
40.00	-211.0
45.00	-218.1

Formation Constants for Zn(II) - Glycinate Complexes

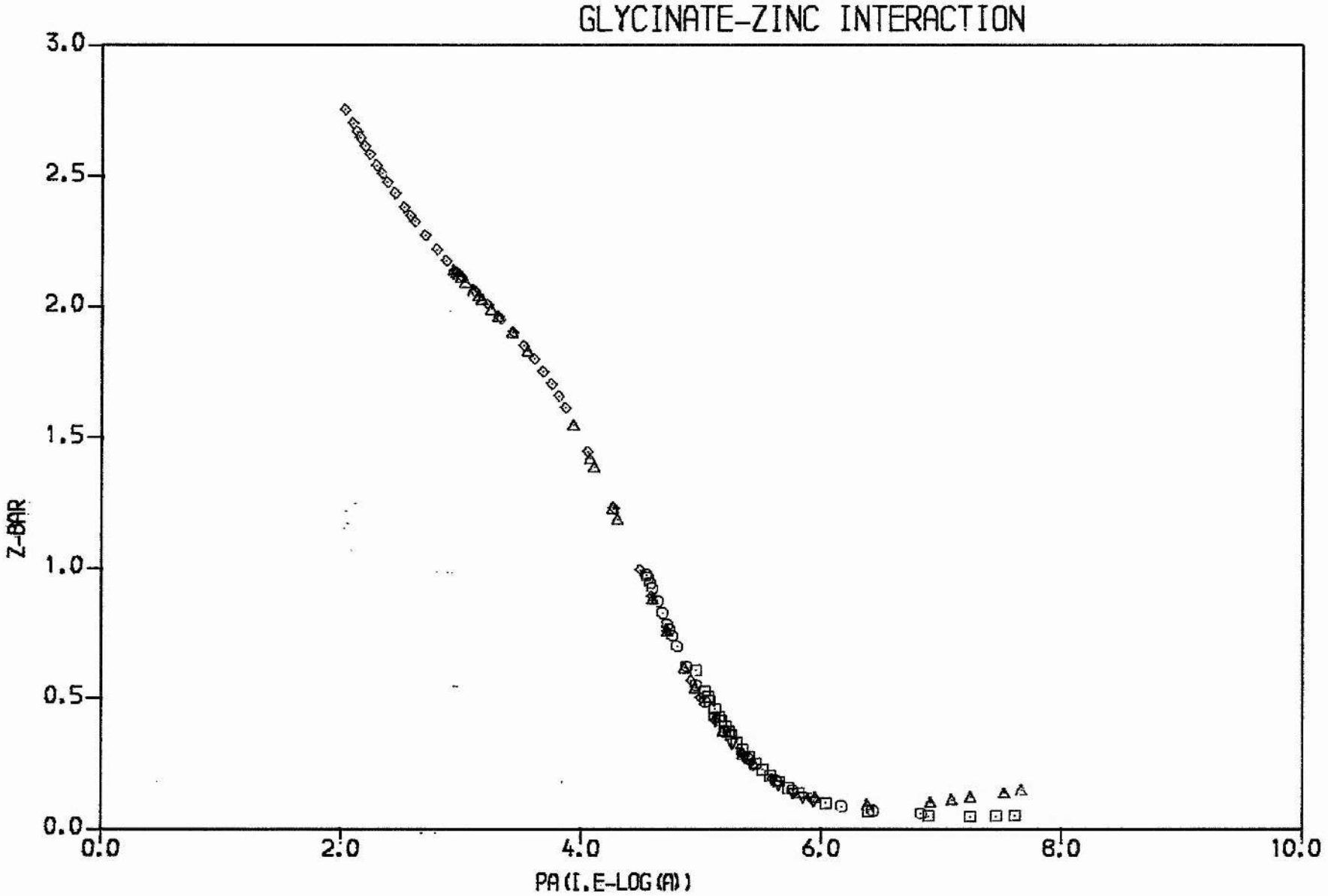
The formation curve (figure 21) was obtained from the results in table 30.

TABLE 30

Experimental results for the Zn(II) - glycinate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	100.00	100.00	24.47	24.47	28.49	-76.87	20.00	362.3
2	50.00	50.00	26.11	26.11	30.04	-25.32	20.00	362.3
3	25.00	25.00	25.87	25.87	-15.54	27.13	20.00	362.3
4	100.00	100.00	24.47	24.47	-76.87	28.49	20.00	362.3
5	50.00	50.00	26.11	26.11	-25.32	30.04	20.00	362.3

FIGURE 21


GLYCINATE-ZINC INTERACTION
Z-BAR
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
10.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
0.00	197.0
1.00	190.3
3.00	176.2
4.00	167.9
5.00	158.1
7.00	127.2
8.00	101.0
10.00	62.9
11.00	52.1
13.00	36.6
14.00	30.5
16.00	20.3
18.00	11.7
24.00	-8.9
25.00	-11.7
29.00	-22.6
30.00	-25.1
34.00	-34.9
46.00	-61.8
50.00	-70.4
54.00	-78.6
56.00	-82.5
59.00	-87.9
60.00	-89.7
62.00	-93.0
65.00	-97.5
67.00	-100.3
68.00	-101.6
69.00	-103.0
70.00	-104.3

Titration number 2

volume added (ml)	emf (mV)
28.00	81.0
29.00	75.2
30.00	70.2
32.00	62.2
38.00	46.7
40.00	43.2
45.00	35.8

Titration number 3

volume added (ml)	emf (mV)
0.00	-22.3
1.00	-12.4
1.30	-9.5
1.50	-7.7
2.00	-3.3
2.40	0.2
2.60	1.9
3.00	5.2
3.30	7.8
3.50	9.4
4.00	13.6
4.50	17.8
5.00	22.1
5.50	26.4
6.00	31.0
6.50	35.8
7.00	40.9
7.50	46.4
8.00	52.5
9.00	67.6
10.00	90.4
11.00	121.3
12.00	142.0
13.00	154.4
14.00	163.0

Titration number 4

volume added (ml)	emf (mV)
0.00	-178.7
0.50	-171.9
0.80	-168.2
1.00	-165.7
1.30	-162.0
1.60	-158.3
2.00	-153.3
2.30	-149.5
2.60	-145.9
3.00	-140.8
3.50	-134.6
3.80	-130.7
4.00	-128.0
4.50	-121.4
5.00	-114.4
5.40	-108.7
5.80	-102.6
6.00	-99.6
6.50	-92.0
7.00	-84.4
7.50	-77.1
8.00	-70.2
8.50	-64.0
9.00	-58.2
9.50	-53.1
10.00	-48.2
10.50	-43.8
11.00	-39.9
13.00	-26.6
16.00	-11.3
20.00	4.5
22.00	11.2
25.00	20.4
30.00	34.4

Titration number 4 continued

volume added (ml)	emf (mV)
32.00	39.8
35.00	47.9
40.00	62.3
42.00	68.5
45.00	78.7
50.00	98.7

Titration number 5

volume added (ml)	emf (mV)
0.00	-22.4
0.10	-21.5
0.30	-19.6
0.50	-18.0
1.00	-14.0
1.50	-10.2
2.00	-6.6
2.20	-5.2
2.50	-3.1
3.00	0.3
4.00	6.8
5.00	13.0
6.00	19.2
7.00	25.3
8.00	31.4
10.00	44.7
12.00	60.4
13.00	69.7
15.00	95.5
16.00	111.5
18.00	135.9

Total number of readings = 123.

The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)_2$ [79], the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 4.91 \pm 0.02$$

$$\log \beta_{210} = 8.97 \pm 0.04$$

$$\log \beta_{310} = 11.30 \pm 0.03$$

$$\log \beta_{111} = 9.30 \pm 0.24$$

$$\log \beta_{11-1} = -2.71 \pm 0.38$$

These constants gave a standard deviation in titre of 0.46. The system can thus be described by the five complexes: $Zn(glycinate)^+$, $Zn(glycinate)^o_2$, $Zn(glycinate)^-_3$, $Zn(glycinate)H^{2+}$, and $Zn(glycinate)OH^o$.

The zinc-glycinate complexes produced a pattern of formation curves as the glycinate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in SCOGS. Then the best PSEUDOPLOT fit was obtained (figure 22).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 23). These models require the total concentrations of zinc and glycinate (5.00 and 10.00 mM respectively) and the formation constants from table 31.

FIGURE 22

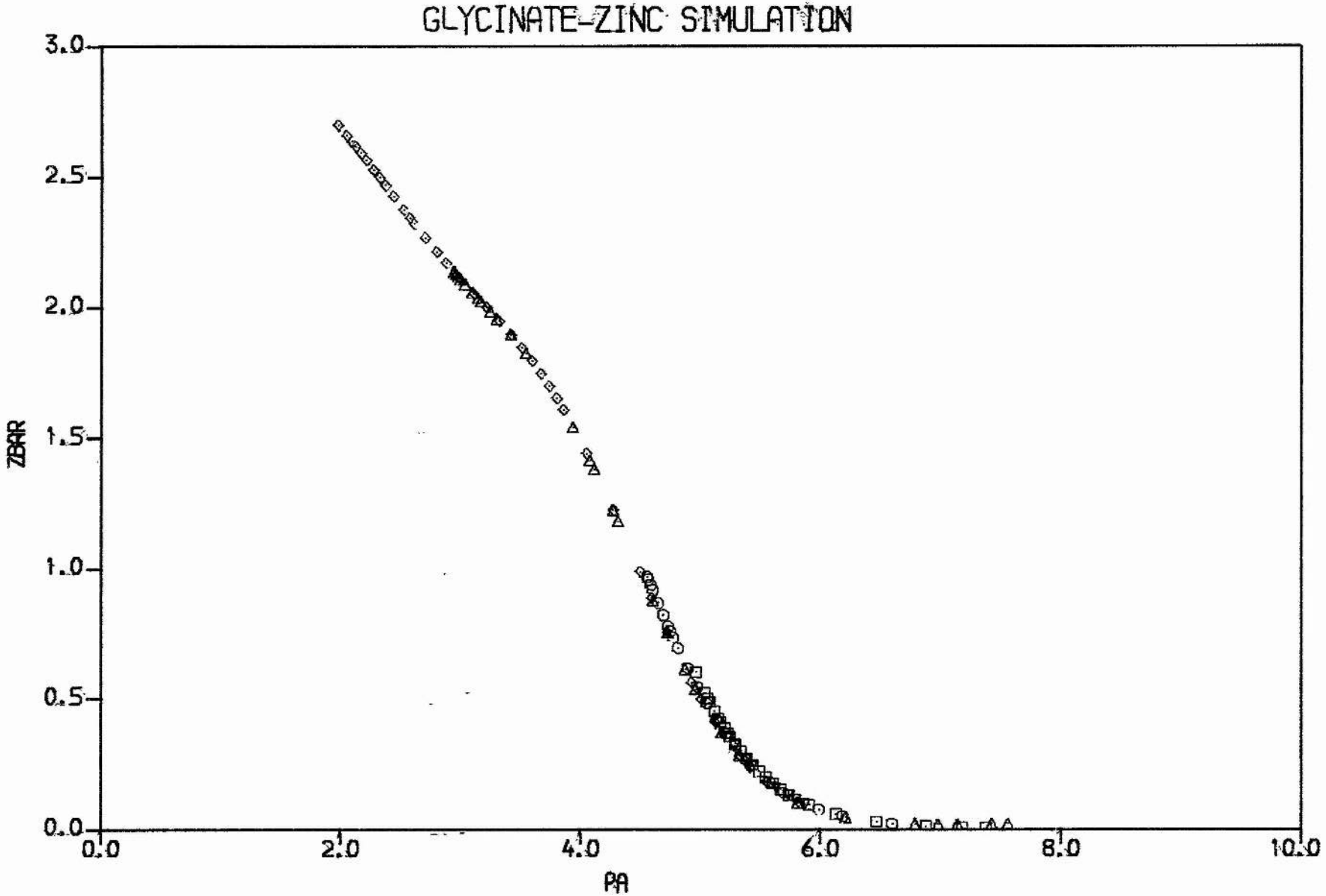

GLYCINATE-ZINC SIMULATION
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ZBAR
0.0
2.0
4.0
6.0
8.0
10.0
PA

FIGURE 23

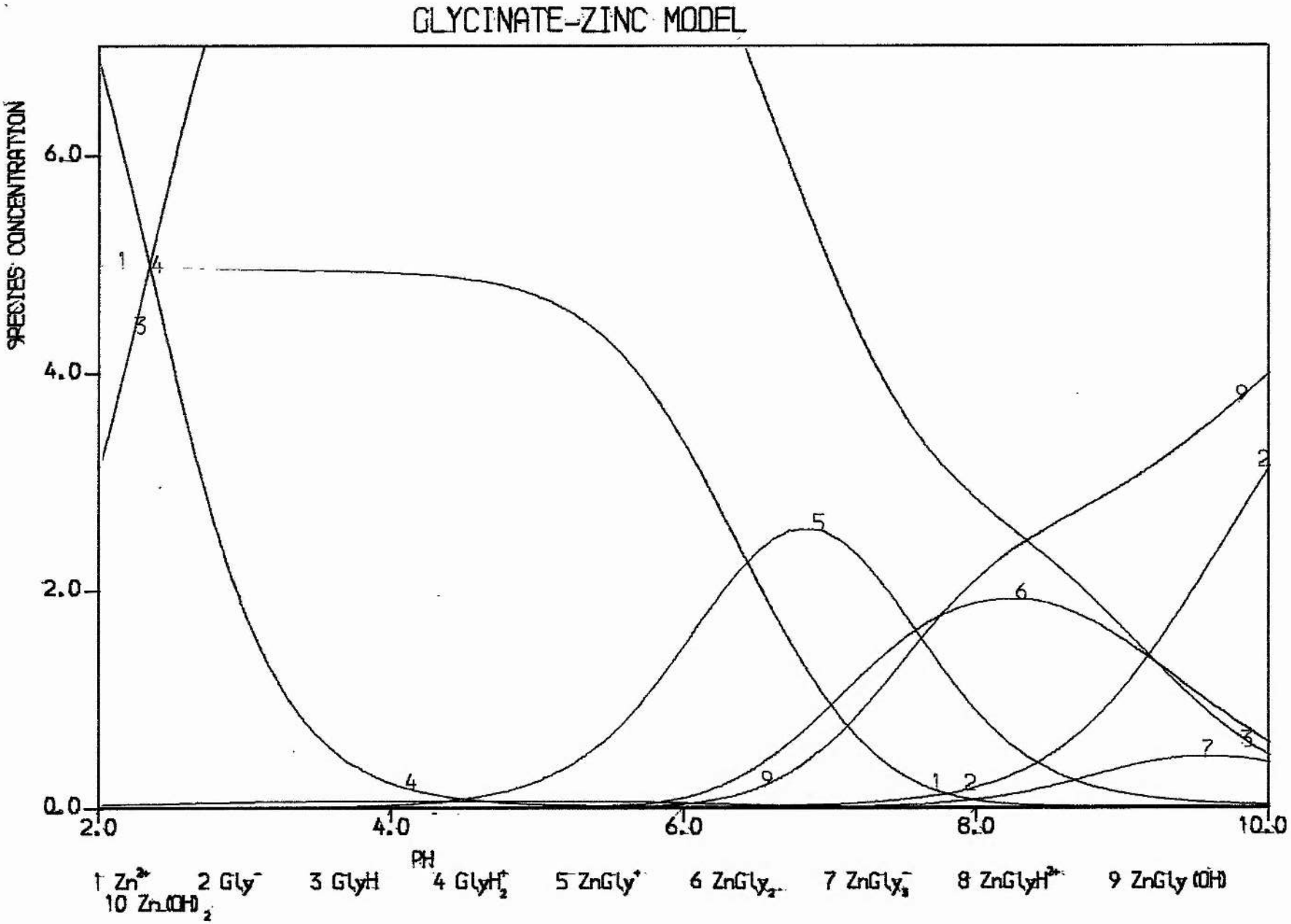

GLYCINATE-ZINC MODEL
SPECIES CONCENTRATION
6.0
4.0
2.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+ 2 Gly- 3 GlyH 4 GlyH2+ 5 ZnGly+ 6 ZnGly2 7 ZnGly3- 8 ZnGlyH3+ 9 ZnGly(OH)
10 Zn(OH)2

Table 31 below lists our formation constants of the complex formation of glycinate and some of the published results from other workers as a comparison.

TABLE 31

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	logβ	S	n	literature data (θ_c/°C, I/M, logβ)	Ref
	1	0	1	9.17	0.01	179	37, 0.15(KNO_3), β_{101} 9.38, β_{102} 11.76	84
	1	0	2	11.51	0.01		25, 0.10($NaClO_4$), β_{101} 9.62, β_{102} 12.05	85
Zn	1	1	0	4.91	0.02	123	37, 0.15(KNO_3), β_1 4.90, β_2 9.01	84
	2	1	0	8.97	0.04		β_3 11.31, β_{11-1} -8.89	
	3	1	0	11.30	0.03		25, 0.10(KNO_3), β_1 5.03, β_2 9.30	86
	1	1	1	9.30	0.24		25, →0, β_1 5.52, β_2 9.96	87
	1	1	-1	-2.71	0.38		20, 0.50(KNO_3), β_1 4.80, β_2 8.94	88
							β_3 11.50	

Protonation constants of Glycylglycinate

The formation curve (figure 24) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using SCOGS (chapter 4) and the results obtained were

$$\left.\begin{aligned} \log K_{101} &= 7.74 \pm 0.01 \\ \log K_{101\rightarrow102} &= 3.10 \pm 0.01 \end{aligned}\right\} \quad S(\text{titre}) = 0.20 \quad (230 \text{ readings})$$

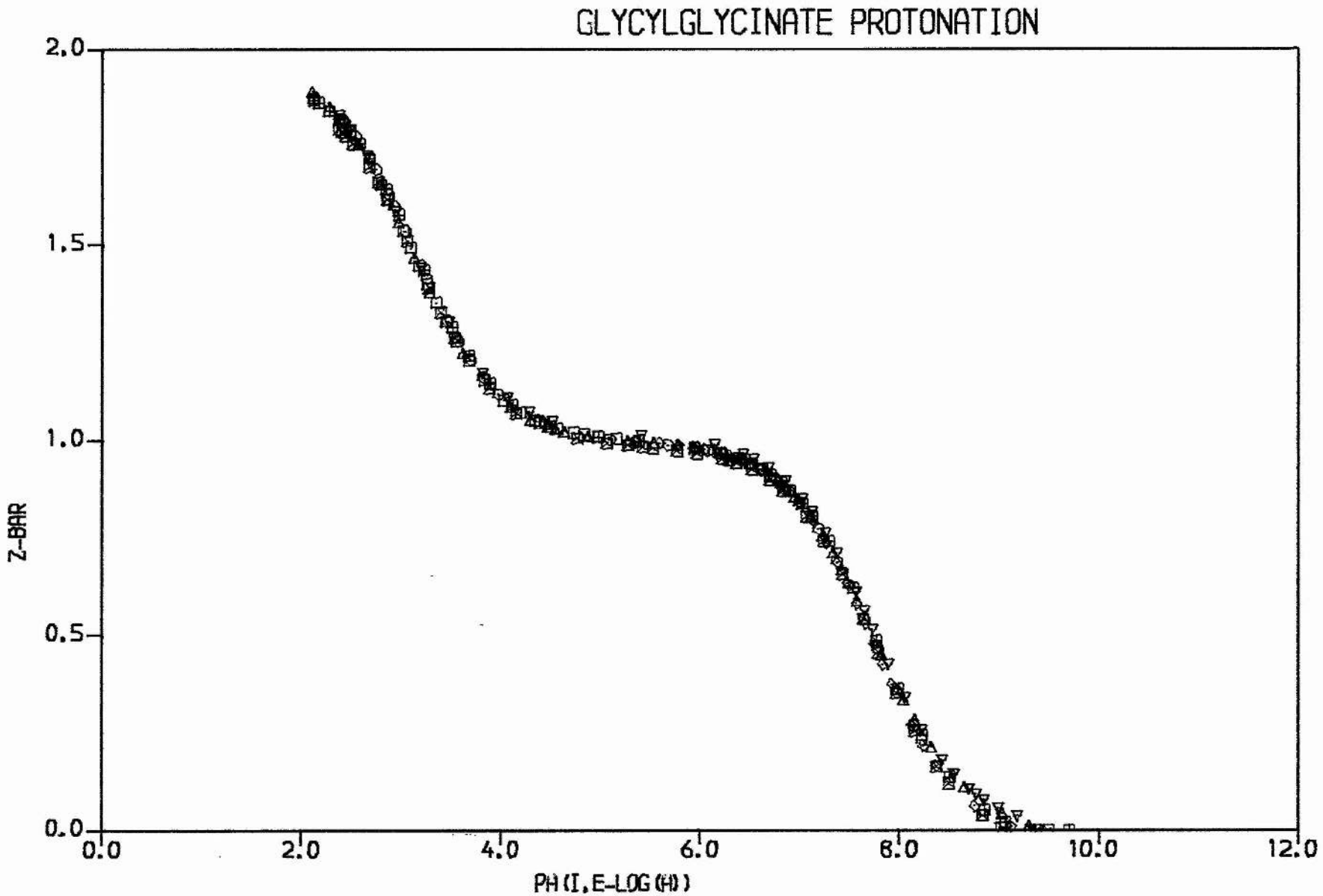
where S is the standard deviation.

TABLE 32

Experimental results for the protonation of glycylglycinate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E° (mV)
	A	H	A	H		
1	100.00	134.10	100.00	-200.00	20.00	364.1
2	50.00	134.10	50.00	-200.00	20.00	364.1
3	25.00	0	0	33.53	25.00	363.8
4	25.00	0	0	-32.57	25.00	363.8
5	15.00	16.16	15.00	-20.00	20.00	364.1
6	10.00	16.16	10.00	-40.00	20.00	364.1
7	5.00	8.08	5.00	-20.00	20.00	364.1

FIGURE 24


GLYCYLGLYCINATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH(I,E-LOG(H))

Titration number 1

volume added (ml)	emf (mV)
2.50	233.4
3.00	222.5
4.00	204.8
5.00	191.3
6.00	180.1
7.00	170.1
8.00	160.6
10.00	140.2
12.00	110.7
12.50	98.2
12.80	87.4
12.90	82.8
13.00	77.4
13.20	62.9
13.40	38.3
13.50	22.2
13.60	7.7
13.70	-2.8
14.00	-22.5
14.40	-37.1
15.00	-50.7
15.50	-58.5
16.00	-64.9
17.00	-74.7
18.00	-82.2
19.00	-88.5
20.00	-94.0
22.00	-103.4
26.00	-118.6
30.00	-132.1
32.00	-138.8
35.00	-149.1
40.00	-169.8
44.00	-193.2
46.00	-209.4

Titration number 2

volume added (ml)	emf (mV)
7.20	216.8
7.50	210.1
8.00	199.7
9.00	182.0
10.00	166.1
11.00	149.1
12.00	128.2
12.50	113.0
12.80	99.1
13.00	84.8
13.30	30.2
13.50	-15.1
13.70	-33.0
13.80	-39.3
14.00	-48.7
14.30	-59.0
14.70	-69.2
15.00	-75.3
15.50	-83.6
16.00	-90.7
17.00	-102.6
17.50	-107.8
18.00	-112.9
19.00	-122.6
20.00	-132.6
21.00	-143.1
22.00	-155.6
22.50	-163.1
23.00	-172.3
23.20	-176.6
23.40	-181.5
23.70	-190.4
24.00	-202.0

Titration number 3

volume added (ml)	emf (mV)
0.00	31.0
0.10	45.4
0.20	56.5
0.30	65.0
0.40	71.4
0.60	81.6
0.70	85.6
0.90	92.3
1.00	95.2
1.40	104.4
2.00	114.7
3.00	126.9
4.00	136.2
5.00	143.9
6.00	150.4
7.00	156.4
8.00	162.0
9.00	167.2
10.00	172.1
11.00	176.8
13.00	186.1
14.00	190.6

Titration number 4

volume added (ml)	emf (mV)
0.00	33.4
0.10	18.1
0.20	6.5
0.30	-2.5
0.40	-9.4
0.60	-20.3
0.70	-24.5
0.90	-31.6
1.00	-34.6
1.40	-44.4
2.00	-54.8
3.00	-67.3
4.00	-76.7
5.00	-84.4
6.00	-91.2
7.00	-97.3
8.00	-103.0
9.00	-108.6
10.00	-113.9
11.00	-119.4
12.00	-125.0
14.00	-127.2
15.00	-144.1
16.00	-152.3
18.00	-176.2
19.00	-198.9

Titration number 5

volume added (ml)	emf (mV)
0.00	215.2
0.05	214.7
0.20	213.5
1.00	206.6
2.00	198.2
2.50	194.0
3.51	186.3
4.00	182.7
5.00	175.8
7.00	162.7
9.00	149.9
10.00	143.1
11.00	135.9
12.00	128.0
13.00	118.7
15.00	89.3
16.00	49.9
16.20	33.2
16.40	13.6
16.65	-3.6
16.80	-10.9
16.90	-15.0
17.00	-18.5
17.50	-31.5
17.80	-37.2
18.30	-44.6
19.00	-52.6
20.00	-61.1
21.00	-67.7
23.50	-79.3
27.00	-90.8
30.00	-98.3

Titration number 6

volume added (ml)	emf (mV)
0.00	232.9
0.10	232.1
0.40	229.1
1.00	222.7
1.50	216.6
2.00	210.0
2.40	204.3
2.80	198.2
3.50	187.7
4.00	179.9
5.00	164.2
6.00	146.9
6.50	136.7
7.00	123.7
7.40	109.5
7.70	92.9
8.00	56.7
8.20	-2.5
8.30	-19.5
8.40	-30.5
8.50	-38.4
8.60	-44.8
8.70	-50.0
8.85	-56.6
9.00	-62.0
9.25	-69.3
9.50	-75.4
10.00	-85.6
11.00	-100.8
12.20	-115.4
13.40	-128.8
14.60	-143.0

Titration number 5 continued

volume added (ml)	emf (mV)
35.00	-107.8
40.00	-115.8
50.00	-128.0
60.00	-138.3
65.00	-143.0

Titration number 6 continued

volume added (ml)	emf (mV)
15.80	-160.4
16.80	-181.9
17.20	-194.9
17.60	-212.8
18.00	-234.2

Titration number 7

volume added (ml)	emf (mV)
0.00	217.1
0.25	215.0
0.50	212.9
1.00	208.3
2.00	198.2
2.50	192.7
3.00	187.0
4.00	174.8
5.00	161.3
5.50	153.9
6.00	145.6
7.00	124.1
7.50	107.2
8.00	69.8
8.10	51.5
8.15	38.3
8.18	29.2
8.20	22.9
8.25	7.8

Titration number 7 continued

volume added (ml)	emf (mV)
8.30	-4.3
8.40	-20.1
8.43	-23.7
8.48	-28.8
8.60	-38.3
8.80	-49.5
9.00	-57.7
9.50	-72.1
10.00	-82.5
10.70	-94.0
11.70	-107.0
12.50	-116.2
13.50	-127.3
14.50	-138.9
15.50	-152.1
16.00	-160.0
17.00	-180.9
17.40	-192.5
17.90	-210.3
18.00	-213.9
18.20	-221.3

Formation Constants for Zn(II) - Glycylglycinate Complexes

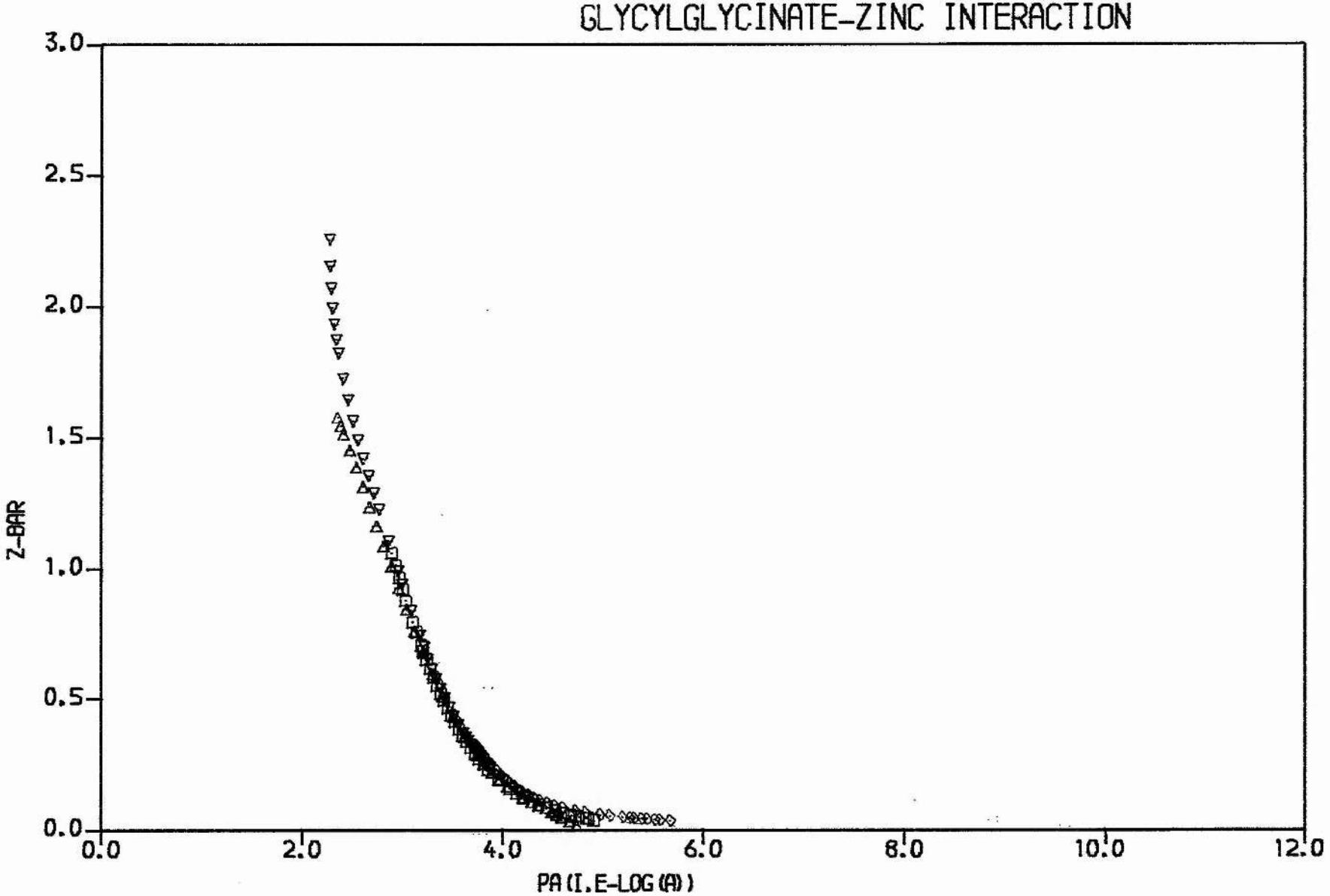
The formation curve (figure 25) was obtained from the results in table 33.

TABLE 33

Experimental results for the Zn(II) - glycylglycinate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	25.00	25.00	1.88	1.88	-7.23	7.13	20.00	364.8
2	25.00	25.00	6.61	6.61	-20.06	8.92	20.00	364.8
3	20.00	20.00	9.40	9.40	-11.20	11.48	20.00	364.8
4	10.00	10.00	9.40	9.40	-3.24	11.48	20.00	364.8

FIGURE 25


GLYCYLGLYCINATE-ZINC INTERACTION
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
0.00	-73.3
0.50	-70.9
1.00	-68.5
2.00	-63.7
3.00	-58.9
4.00	-54.2
5.00	-49.5
6.00	-44.6
7.00	-39.7
8.00	-34.7
9.00	-29.7
10.00	-24.4
11.00	-19.0
12.00	-13.3
13.00	-7.5
14.00	-1.3
15.00	5.5
16.00	12.7
17.00	20.9
17.50	25.2
18.00	29.9
18.50	34.7
19.00	39.7
20.00	49.7

Titration number 2

volume added (ml)	emf (mV)
0.00	-112.4
0.50	-108.7
1.00	-105.1
1.50	-101.7
2.00	-98.3
2.50	-95.1
3.00	-92.0
4.00	-86.2
5.00	-80.5
6.00	-75.4
7.00	-70.3
8.00	-65.5
9.00	-60.8
10.00	-56.5
11.00	-52.1
13.00	-44.2
15.00	-36.7
16.00	-33.4
18.00	-26.8
20.00	-20.5
21.00	-17.4
22.00	-14.9
23.00	-12.0
24.00	-8.9

Titration number 1 continued

volume added (ml)	emf (mV)
20.50	54.7
21.00	59.0
22.00	66.5
22.50	69.7
23.00	72.6
24.00	77.6
25.00	81.9
27.00	88.6
30.00	96.0
35.00	104.3

Titration number 2 continued

volume added (ml)	emf (mV)
25.00	-5.9
26.00	-2.9
27.00	0.3
28.00	3.4
29.00	6.5
30.00	9.8
30.60	11.7
31.00	13.0
31.50	14.7
32.00	16.4
32.60	18.5
33.00	20.0
33.50	21.7
34.00	23.4
34.50	25.3
35.00	27.3
35.15	27.9
36.15	31.7
37.15	36.1
39.15	45.0
40.15	49.8

Titration number 3

volume added (ml)	emf (mV)
0.00	-58.7
0.50	-54.6
1.00	-51.0
1.50	-47.7
2.00	-44.5
3.00	-38.2
3.50	-35.0
4.20	-30.8
4.50	-29.1
5.00	-26.3
5.50	-23.4
6.00	-20.7
6.50	-18.0
7.00	-15.2
7.50	-12.5
8.00	-9.8
8.50	-7.1
9.00	-4.4
9.50	-1.7
10.00	1.1
10.50	3.9
11.00	6.9
11.50	9.8

Titration number 4

volume added (ml)	emf (mV)
0.00	-13.5
0.10	-12.5
0.20	-11.4
0.30	-10.3
0.50	-8.2
0.80	-4.9
1.00	-2.6
1.40	2.1
1.70	5.8
2.00	9.6
2.20	12.3
2.40	15.2
2.70	19.7
3.00	24.7
3.20	28.3
3.40	32.2
3.60	37.1
3.80	41.6
4.00	46.8
4.30	55.2
4.80	70.6
5.00	76.5
5.30	84.4

Titration number 3 continued

volume added (ml)	emf (mV)
12.00	12.8
12.50	15.9
13.00	19.1
14.00	25.9
15.00	33.4
15.50	37.6
16.00	42.0
17.00	51.5
17.50	56.6
18.00	61.9
18.50	67.1
19.00	72.1
19.50	76.7
20.00	81.0
20.20	82.6
20.70	85.8

Titration number 4 continued

volume added (ml)	emf (mV)
5.50	89.0
5.60	91.1
5.80	95.2
6.00	98.6
6.30	103.2
6.50	106.0
7.00	111.9

Total number of readings = 128.

The data was analysed using the SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 3$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)_2$ [79], the β values for the latter being

held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 3.57 \pm 0.04$$

$$\log \beta_{210} = 5.88 \pm 0.05$$

$$\log \beta_{310} = 8.01 \pm 0.14$$

$$\log \beta_{111} = 9.15 \pm 0.10$$

These constants gave a standard deviation in titre of 0.33. The system can thus be described by the four complexes: $Zn(glycylglycinate)^{+}$, $Zn(glycylglycinate)^{o}_{2}$, $Zn(glycylglycinate)^{-}_{3}$, and $Zn(glycylglycinate)H^{2+}$.

The zinc-glycylglycinate complexes produced a pattern of formation curves as the glycylglycinate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in SCOGS. Then the best PSEUDOPLOT fit was obtained (figure 26).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 27). These models require the total concentrations of zinc and glycylglycinate (5.00 and 10.00 mM respectively) and the formation constants from table 34.

Table 34 below lists our formation constants of the complex formation of glycylglycinate and some of the published results from other workers as a comparison.

FIGURE 26

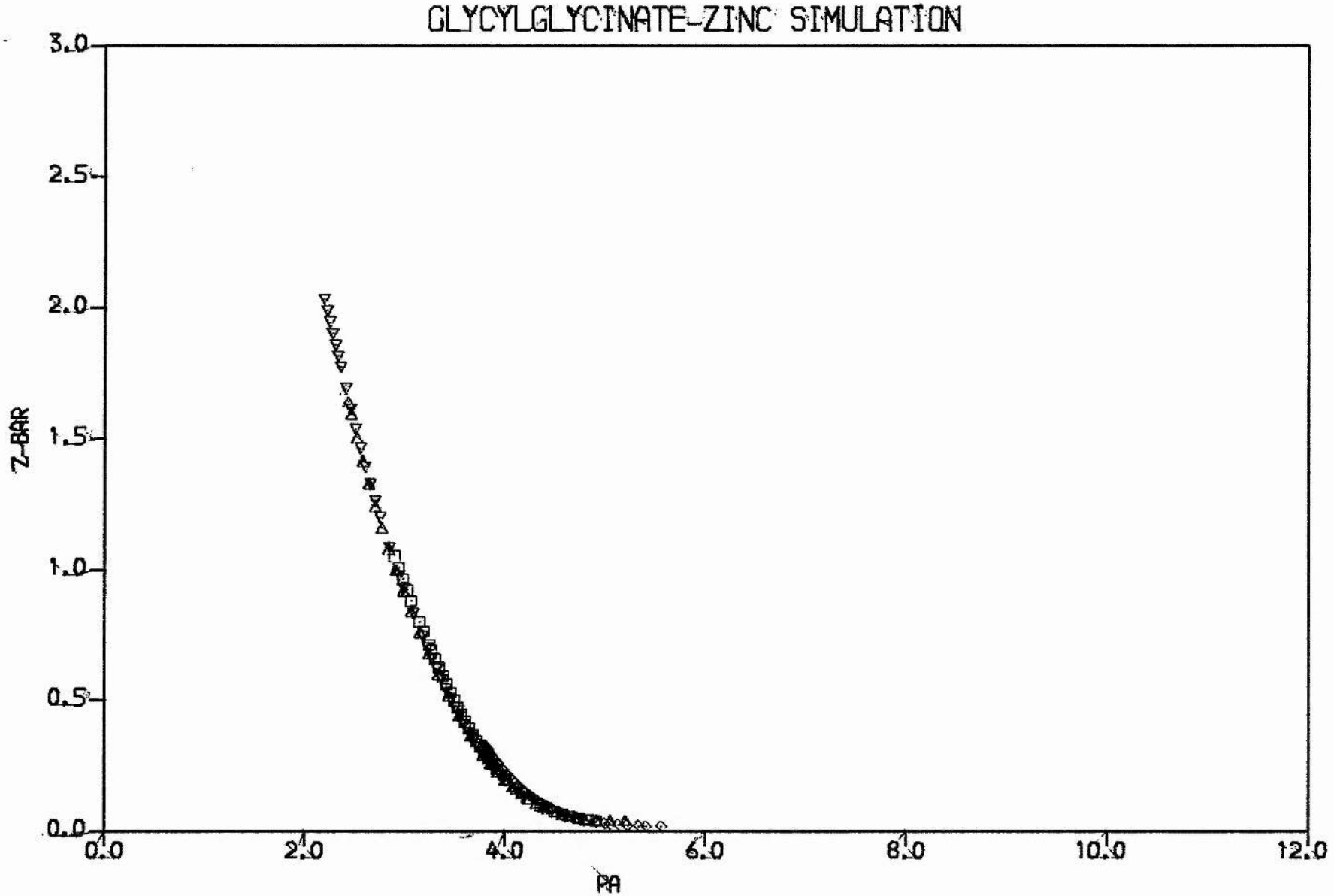

GLYCYLGLYCINATE-ZINC SIMULATION
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA

FIGURE 27

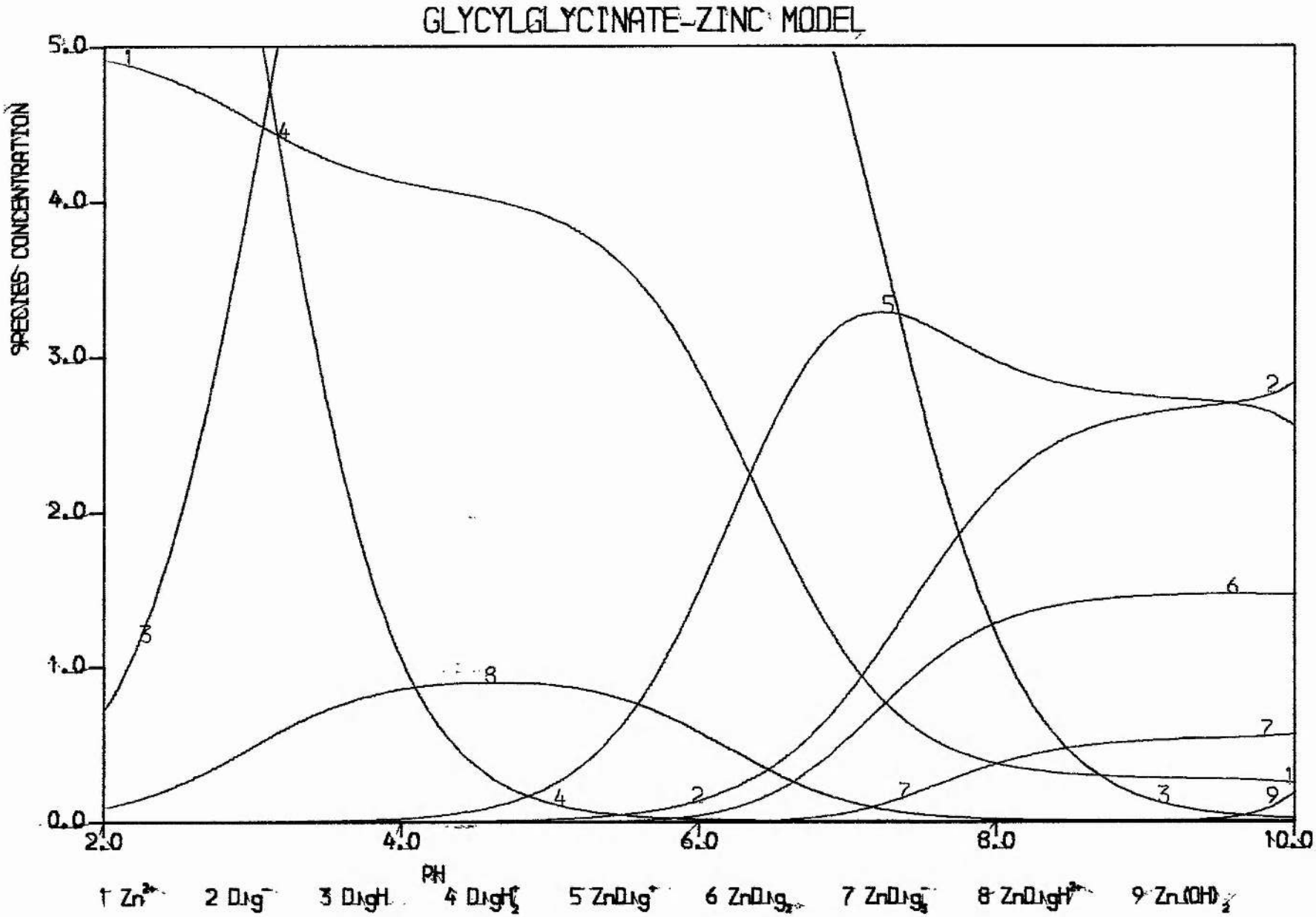

GLYCYLGLYCINATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+ 2 Dig- 3 DigH 4 DigH2 5 ZnDig+ 6 ZnDig2 7 ZnDig3- 8 ZnDigH2+ 9 Zn(OH)2

TABLE 34

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (Θ_c/°C, I/M, logβ)	Ref
	1	0	1	7.74	0.01	230	20,1.0 $NaClO_4$, β_{101} 8.23, β_{102} 11.39	89
	1	0	2	10.84	0.01		25, 0.15, log β_{101} 8.12	90
Zn	1	1	0	3.57	0.04	129	37, 0.15(KNO_3), β_1 3.24, β_2 5.88	91
	2	1	0	5.88	0.05		25, 0.01, β_1 3.6	92
	3	1	0	8.01	0.14		25, → 0, β_1 3.80, β_2 6.57	93
	1	1	1	9.15	0.10		21, 0.01 ($ZnSO_4$), β_2 6.4	94

Protonation Constants of Glycylglycylglycinate

The formation curve (figure 28) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using SCOGS (chapter 4) and the results obtained were

$$\left.\begin{aligned} \log K_{101} &= 7.59 \pm 0.01 \\ \log K_{101 \rightarrow 102} &= 3.11 \pm 0.01 \end{aligned}\right\} \quad S(\text{titre}) = 0.20 \text{ (173 readings)}$$

where S is the standard deviation.

TABLE 35

Experimental results for the protonation of glycylglycylglycinate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^{o} (mV)
	A	H	A	H		
1	50.00	134.10	50.00	-250.00	20.00	364.3
2	25.00	53.66	25.00	-100.00	20.00	364.5
3	15.15	40.65	15.15	-75.75	20.00	365.4
4	9.12	24.48	9.12	-45.63	20.00	366.0
5	5.00	10.73	5.00	-20.00	20.00	364.5

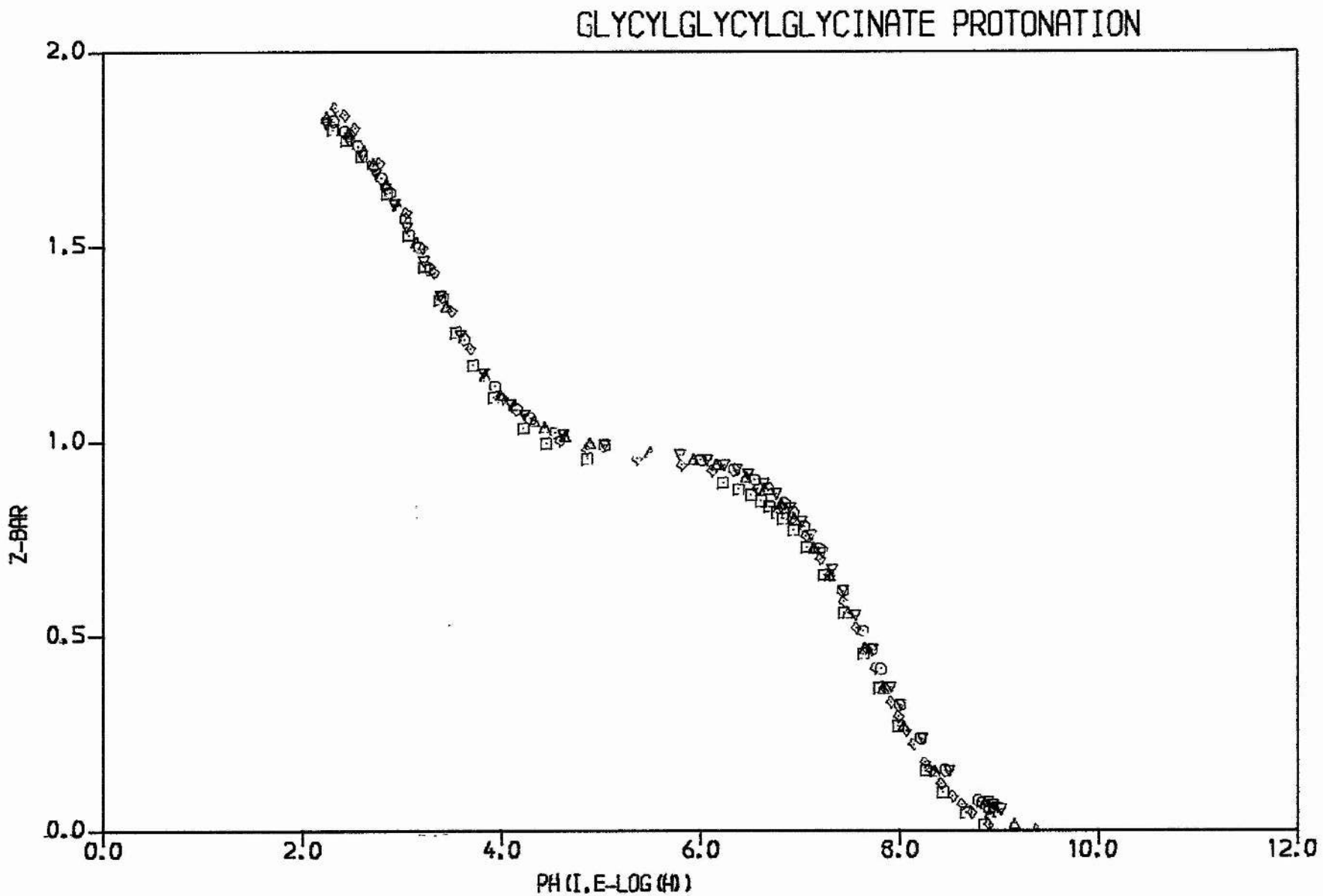
Titration number 1

volume added (ml)	emf (mV)
5.80	227.8
6.20	214.0
6.50	204.0
6.70	198.8
7.00	190.7
7.30	183.4
7.80	172.2
8.70	153.7
9.70	129.6
10.00	120.0
10.15	111.5

Titration number 2

volume added (ml)	emf (mV)
4.30	228.2
5.00	214.3
5.40	205.9
5.80	197.5
6.10	191.5
6.40	185.8
6.80	178.5
7.40	168.1
8.00	157.8
8.70	145.4
9.40	130.9

FIGURE 28


GLYCYLGLYCYLGLYCINATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH(I.E-LOG(H))

Titration number 1 continued

volume added (ml)	emf (mV)
10.40	99.4
10.50	92.8
10.64	80.0
10.75	64.6
10.90	27.5
11.01	0.6
11.10	-13.5
11.30	-31.7
11.50	-43.1
11.75	-53.4
12.00	-61.5
12.50	-73.7
13.00	-83.4
13.70	-94.8
14.40	-105.2
15.20	-116.7
16.00	-129.2
17.00	-148.5
18.00	-182.8
18.25	-198.2
18.40	-211.7

Titration number 2 continued

volume added (ml)	emf (mV)
10.00	114.1
10.20	105.7
10.60	81.5
10.80	55.9
11.00	9.5
11.10	-7.2
11.20	-18.0
11.30	-26.0
11.40	-32.5
11.60	-42.3
11.80	-49.9
12.10	-58.8
12.40	-65.8
12.70	-71.8
13.10	-78.8
13.50	-84.8
14.00	-91.7
14.60	-99.2
15.50	-109.7
16.50	-121.2
17.00	-127.2
18.00	-140.6
19.00	-157.3
20.00	-181.6
20.10	-184.2
20.20	-187.1
20.28	-189.3

Titration number 3

volume added (ml)	emf (mV)
5.00	225.1
5.50	216.8
6.00	207.4
6.80	191.6
7.50	178.0
8.00	168.5
8.50	159.1
9.00	149.3
9.50	138.5
10.00	125.6
10.50	107.3
10.75	93.1
11.00	67.6
11.40	-16.4
11.50	-26.0
11.60	-33.6
11.70	-39.7
11.80	-44.9
11.90	-49.4
12.00	-53.3
12.20	-59.9
12.50	-68.0

Titration number 4

volume added (ml)	emf (mV)
4.00	224.3
4.50	218.2
5.00	211.9
6.00	196.8
7.00	180.1
7.60	170.0
8.00	163.1
8.60	152.4
9.20	140.7
10.00	120.3
10.70	85.1
10.85	68.5
11.00	37.4
11.10	9.9
11.20	-9.1
11.50	-36.5
11.80	-51.3
12.00	-58.4
12.30	-66.9
12.70	-76.1
13.00	-82.0
13.50	-90.5

Titration number 3 continued

volume added (ml)	emf (mV)
13.00	-78.9
13.70	-91.9
14.50	-103.1
15.20	-113.0
16.00	-124.7
17.00	-141.7
17.50	-152.4
18.00	-166.8
18.30	-178.1

Titration number 4 continued

volume added (ml)	emf (mV)
14.00	-98.3
14.40	-104.1
14.80	-109.9
15.20	-115.5
15.50	-119.8
15.80	-124.3
16.10	-128.9
16.40	-133.6
16.80	-140.8
17.00	-144.6
17.30	-150.8
17.60	-158.0
17.80	-163.6
18.00	-169.7
18.30	-180.3

Titration number 5

volume added (ml)	emf (mV)
1.00	223.7
2.00	216.7
3.00	208.6
4.00	199.3
4.50	194.1
5.00	188.6
5.80	179.3
6.50	170.7
7.00	164.2
7.60	156.2
8.50	142.9
9.50	123.9
10.00	110.4
10.20	101.9
10.50	86.6
10.80	56.1
11.10	-3.4
11.30	-24.2
11.50	-36.6
11.70	-45.5
12.00	-55.5
12.20	-60.8
12.50	-67.5

Titration number 5 continued

volume added (ml)	emf (mV)
13.00	-76.8
14.00	-91.4
15.00	-103.7
15.50	-109.2
16.00	-114.9
17.00	-126.5
18.00	-139.3
19.00	-154.8
20.00	-175.3
20.10	-177.6
20.20	-180.0
20.28	-181.9

Formation Constants for Zn(II) - Glycylglycylglycinate Complexes

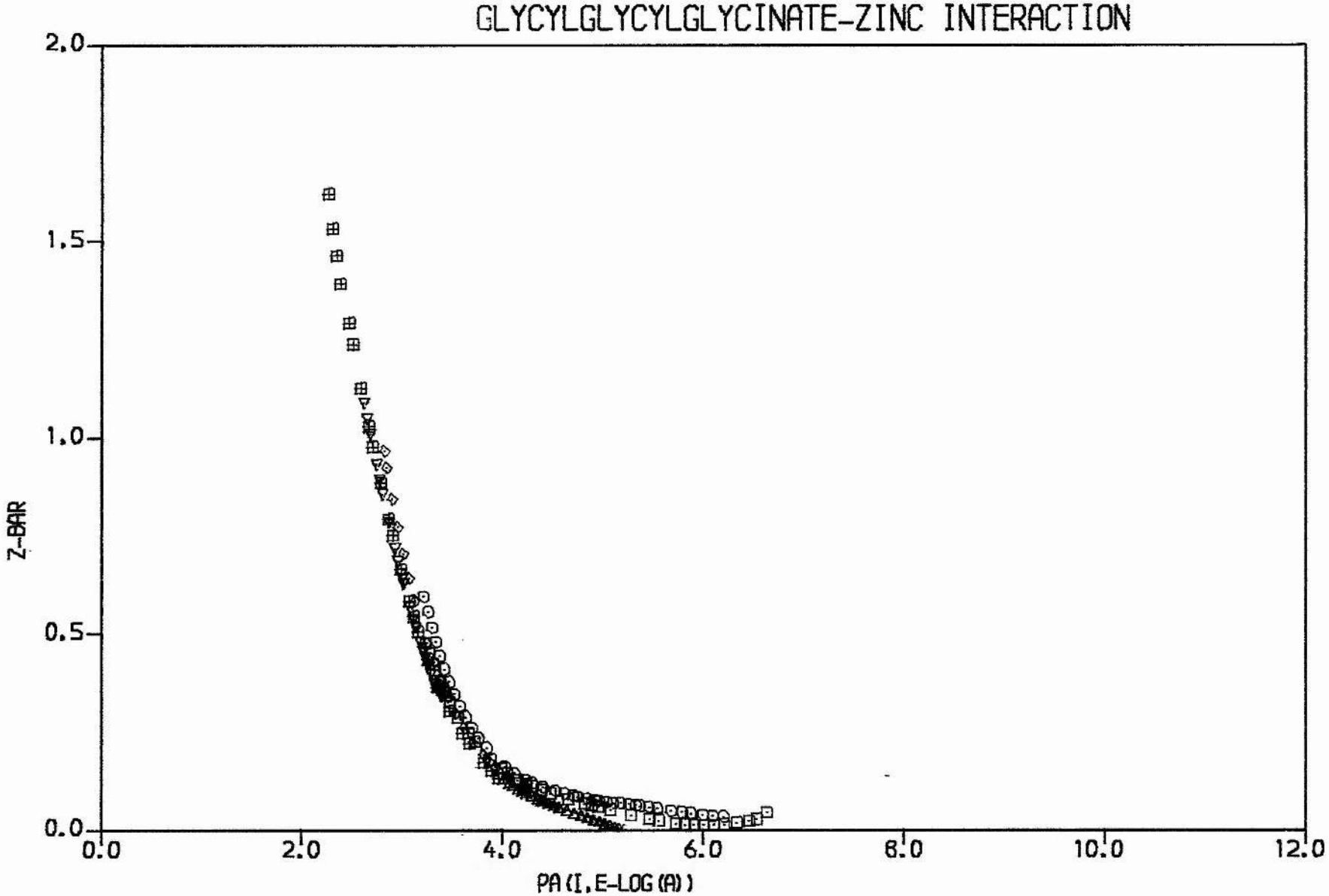
The formation curve (figure 29) was obtained from the results in table 36.

TABLE 36

Experimental results for the Zn(II) - glycylglycylglycinate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	25.00	25.00	6.61	6.61	11.60	-13.76	20.00	362.8
2	25.00	25.00	6.61	6.61	-13.76	11.60	20.00	362.8
3	20.00	20.00	9.40	9.40	11.56	-13.32	20.00	362.8
4	20.00	20.00	9.40	9.40	-13.32	11.56	20.00	362.8
5	10.00	10.00	9.40	9.40	-6.20	11.48	20.00	362.8
6	25.00	25.00	1.88	1.88	-8.23	4.45	20.00	362.8

FIGURE 29


GLYCYLGLYCYLGLYCINATE-ZINC INTERACTION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA(I,E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
10.50	116.1
11.00	113.4
11.50	110.7
12.00	107.9
12.50	105.0
13.00	101.9
13.50	98.6
14.00	95.1
14.50	91.4
15.00	87.2
15.50	82.9
16.00	78.1
16.30	75.1
16.60	71.9
16.90	68.6
17.00	67.5
17.20	65.2
17.50	61.7
17.80	58.1
18.00	55.7
18.20	53.4
18.50	49.9
18.70	47.6
19.00	44.4

Titration number 2

volume added (ml)	emf (mV)
4.00	-53.7
4.50	-51.1
5.00	-48.4
6.00	-43.3
6.50	-40.9
7.00	-38.5
8.00	-33.7
9.00	-29.0
9.50	-26.6
10.00	-24.2
10.20	-23.3
10.40	-22.4
11.40	-17.8
11.91	-15.4
12.30	-13.5
13.00	-10.2
13.40	-8.3
13.70	-6.8
14.00	-5.3
14.50	-2.8
15.00	-0.2
15.30	1.5
15.67	4.1

Titration number 3

volume added (ml)	emf (mV)
0.00	178.1
0.50	174.2
1.00	171.2
2.00	165.9
3.00	160.7
4.00	155.9
5.00	151.3
6.00	146.8
7.00	142.4
8.00	138.0
10.00	129.1
11.00	124.3
13.00	114.1
15.00	102.2
16.00	95.0
16.50	91.1
17.00	86.8
18.00	77.1
19.00	66.2
19.50	60.5
20.00	54.8
20.30	50.9
20.80	45.8

Titration number 4

volume added (ml)	emf (mV)
2.50	-54.2
3.00	-51.4
4.00	-45.8
5.00	-40.3
6.00	-35.0
7.00	-29.8
8.00	-24.8
10.00	-15.3
11.00	-10.0
12.00	-5.1
13.00	-0.1
14.00	5.3
15.00	11.0
16.00	17.2
17.00	24.1
17.50	27.9
18.00	32.0
19.00	41.2
20.00	51.4

Titration number 3 continued

volume added (ml)	emf (mV)
21.50	39.5
22.00	35.5
23.00	28.6
25.00	18.1
26.00	13.9
28.00	7.0
30.00	1.3
31.00	-1.3
32.00	-3.4
33.00	-5.3
35.00	-8.9
36.00	-10.5
38.00	-13.5
40.00	-16.1

Titration number 5

volume added (ml)	emf (mV)
0.00	-54.1
0.50	-48.2
1.00	-43.5
1.50	-39.0
2.00	-34.6
2.50	-30.2
3.00	-25.8
3.50	-21.3
4.00	-16.7
4.50	-12.0
5.00	-7.1
5.50	-2.0
6.00	3.8
7.00	16.8
7.40	23.1
7.80	30.6
8.00	34.8
8.30	41.7
8.60	49.6
8.80	55.1
9.00	60.6
9.10	63.4
9.30	68.8
9.50	73.8
9.60	76.2

Titration number 5 continued

volume added (ml)	emf (mV)
9.80	80.9
10.00	84.8
10.20	89.5
10.50	94.3
10.80	98.4
11.00	100.9
11.50	106.3
12.00	111.0
13.00	118.5
14.00	124.5
15.00	129.4
16.50	135.5
18.00	140.5
20.00	146.0

Titration number 6

volume added (ml)	emf (mV)
0.00	-72.9
1.00	-69.6
2.00	-66.3
3.00	-63.2
5.00	-56.8
6.00	-53.8
8.00	-48.1
10.00	-42.4
11.00	-39.7
13.00	-34.1
15.00	-28.7
16.00	-25.9
18.00	-20.4
20.00	-14.8
21.00	-11.9
22.00	-9.0
24.00	-2.7
26.00	3.8
28.00	11.2
30.00	19.3
31.00	23.7
33.00	32.6
34.00	37.1
35.00	41.5

Total number of readings = 166.

The data was analysed using SCOGS least squares program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)_2$ [79], the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 3.38 \pm 0.03$$

$$\log \beta_{210} = 5.39 \pm 0.15$$

$$\log \beta_{111} = 9.09 \pm 0.08$$

$$\log \beta_{11-1} = -4.68 \pm 0.48$$

These constants gave a standard deviation in titre of 0.68. The system can thus be described by the four complexes Zn(glycylglycylglycinate)$^{+}$, Zn(glycylglycylglycinate)$_2^{o}$, Zn(glycylglycylglycinate)H^{2+}, and Zn(glycylglycylglycinate)OH^{o}.

The zinc-glycylglycylglycinate complexes produced a pattern of formation curves as the glycylglycylglycinate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in SCOGS. Then the best PSEUDOPLOT fit was obtained (figure 30).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 31). These models require the total concentrations of zinc and glycylglycylglycinate (5.00 and 10.00 mM respectively) and the formation constants from table 37.

Table 37 below lists our formation constants of the complex formation of glycylglycylglycinate and some of the published results from other workers as a comparison.

FIGURE 30

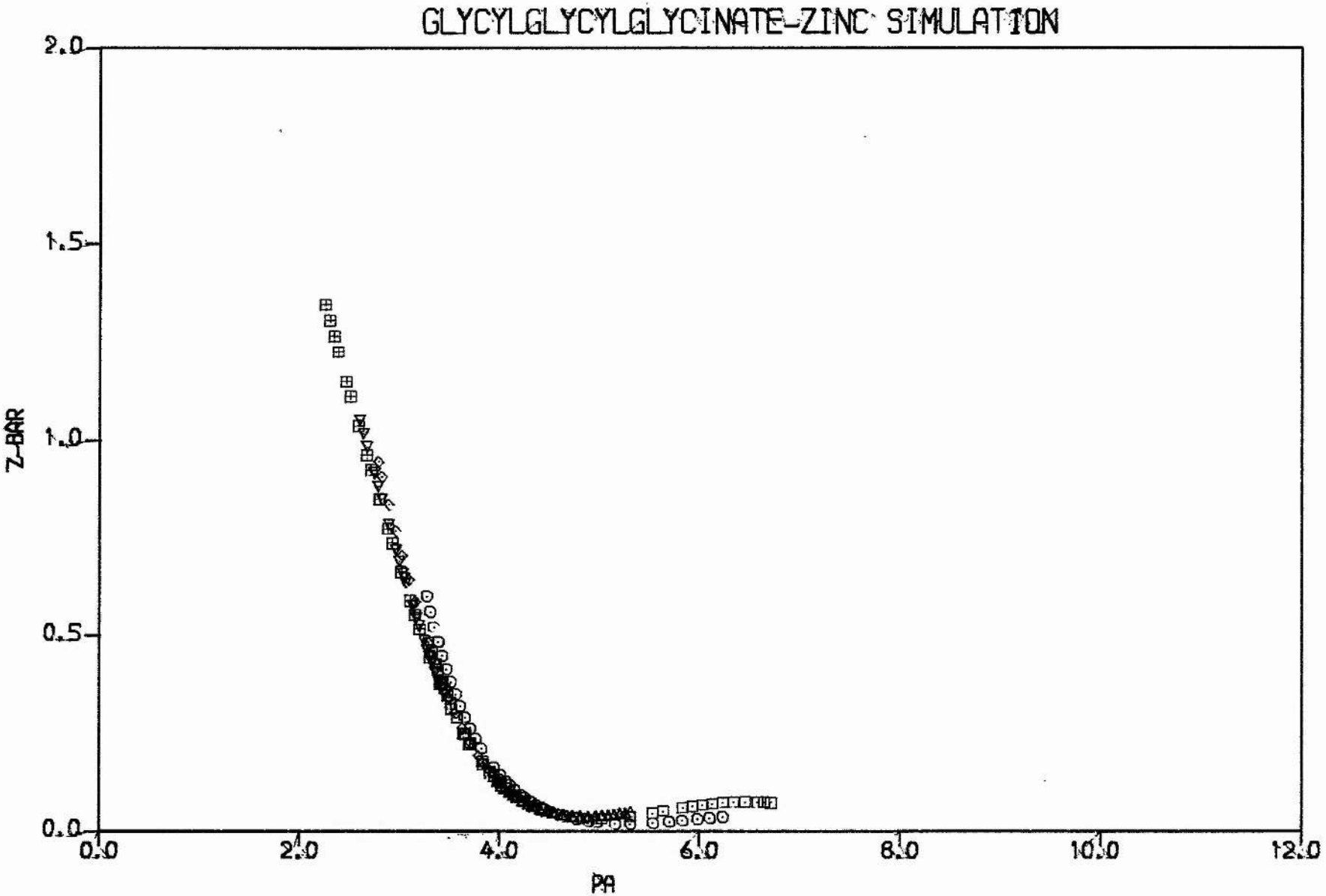

GLYCYLGLYCYLGLYCINATE-ZINC SIMULATION
Z-BAR
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PA

FIGURE 31

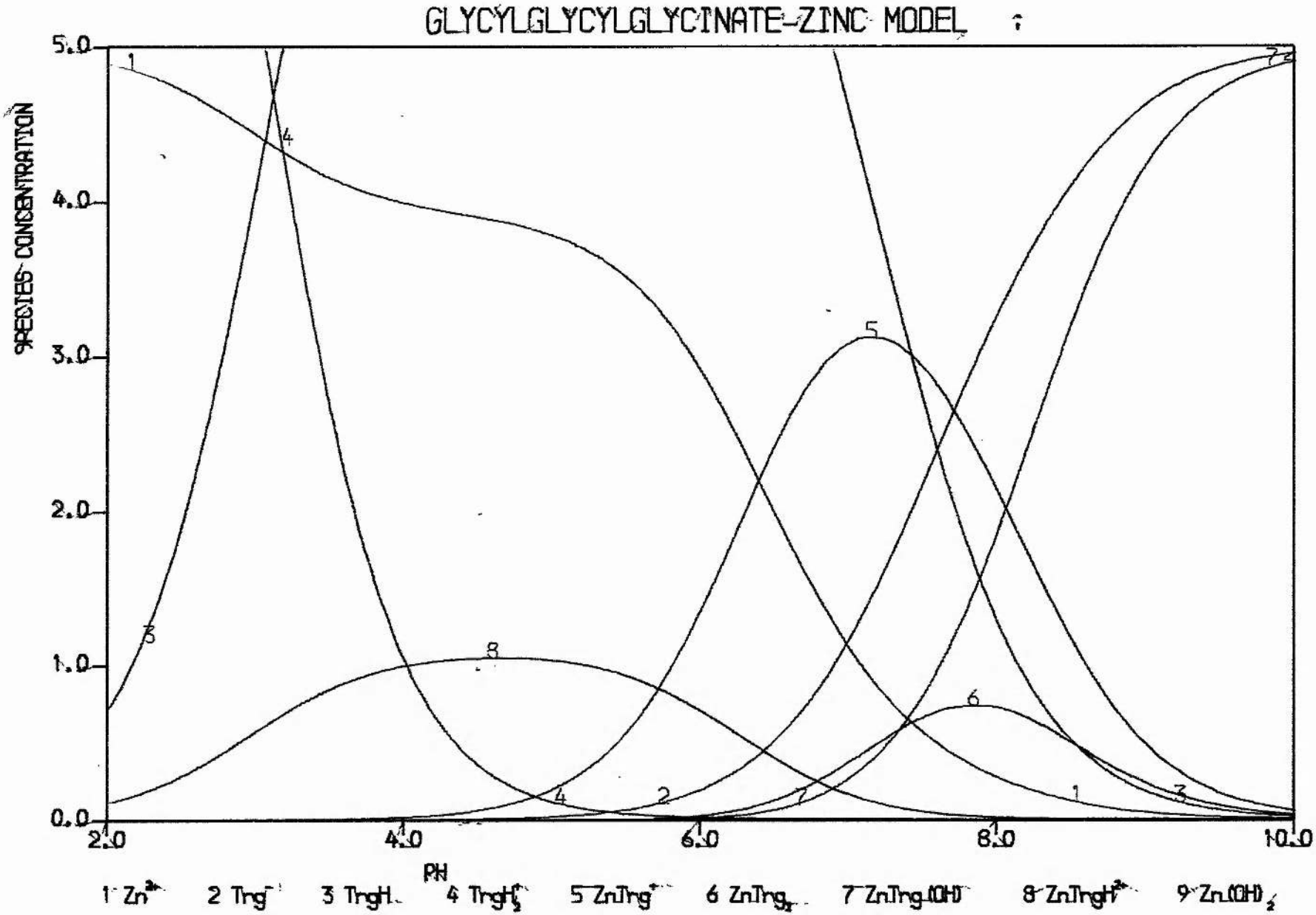

GLYCYLGLYCYLGLYCINATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 Trg-
3 TrgH
4 TrgH2+
5 ZnTrg+
6 ZnTrg2
7 ZnTrg(OH)
8 ZnTrgH2+
9 Zn(OH)2

TABLE 37

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (Θ_c/°C, I/M, log β)	Ref
	1	0	1	7.59	0.01	173	25, 0.15(KNO_3), β_{101} 8.02	90
	1	0	2	10.70	0.01		30, 0.09(KCl), β_{101} 7.74	95
Zn	1	1	0	3.38	0.03	166	37, 0.15(KNO_3), β_1 3.00, β_2 5.34	91
	2	1	0	5.39	0.15		25, 0.15(KNO_3), β_1 3.18	90
	1	1	1	9.09	0.08		25, → 0, β_1, 3.33, β_2 6.32	96
	1	1	-1	-4.68	0.48		25, 0.01 ($ZnSO_4$), β_1 2.6	92

Protonation Constants of Acetate

The formation curve (figure 32) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\log K_{101} = 4.55 \pm 0.01 \quad \text{SUM OF SQUARES} = 2.15 \times 10^{-4} \ (\text{mol}^2\ \text{dm}^{-6})$$

(163 readings).

TABLE 38

Experimental results for the protonation of acetate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^{o} (mV)
	A	H	A	H		
1	100.00	124.52	100.00	-200.10	20.00	356.4
2	50.00	124.52	50.00	-160.10	20.00	356.5
3	25.00	93.76	25.00	-140.91	20.00	356.7
4	10.00	93.76	10.00	-100.05	20.00	356.3

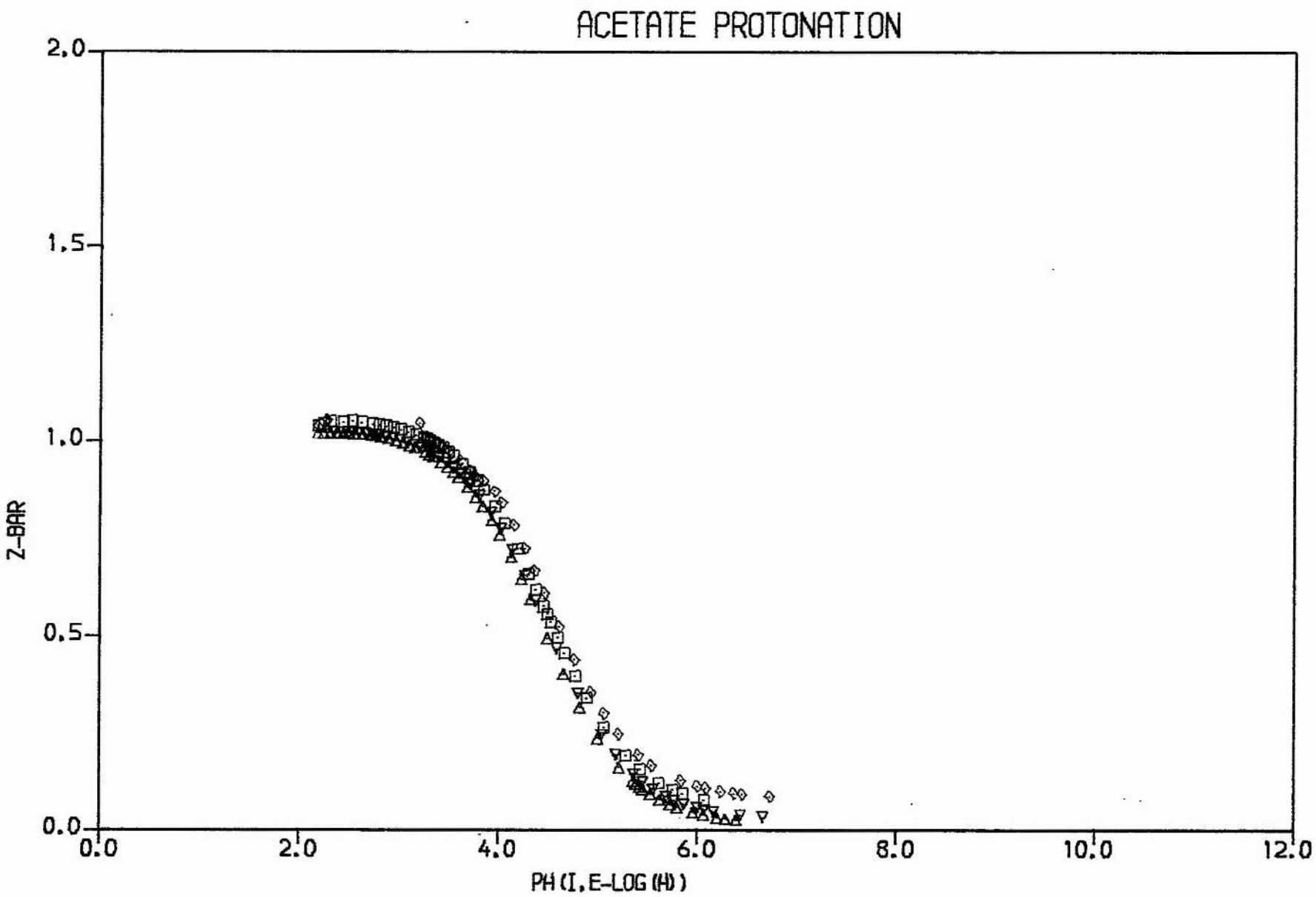
Titration number 1

volume added (ml)	emf (mV)
1.05	220.9
1.10	217.1
1.15	213.2
1.20	208.9
1.24	205.0
1.27	201.7
1.30	198.6
1.35	193.2
1.40	187.6
1.42	185.4
1.45	182.3

Titration number 2

volume added (ml)	emf (mV)
6.40	211.4
6.50	205.8
6.60	199.3
6.65	195.6
6.70	191.7
6.75	187.5
6.80	183.0
6.85	179.1
6.90	174.9
7.00	166.4
7.10	158.6

FIGURE 32.


ACETATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH(I,E-LOG(H))

Titration number 1 continued

volume added (ml)	emf (mV)
1.50	177.4
1.55	172.9
1.60	168.4
1.65	164.5
1.70	161.0
1.80	154.6
1.85	152.2
1.90	149.5
2.00	144.8
2.10	140.8
2.20	137.2
2.30	134.0
2.50	128.3
2.70	123.5
2.90	119.2
3.20	113.4
3.50	108.6
4.00	101.3
4.50	94.9
5.00	89.2
6.00	78.6
7.00	68.6
8.00	58.5
9.00	47.5

Titration number 2 continued

volume added (ml)	emf (mV)
7.20	152.4
7.30	146.5
7.40	141.8
7.50	137.6
7.60	133.7
7.70	130.2
7.80	127.1
8.00	121.4
8.30	114.2
8.60	108.1
9.00	101.0
9.50	92.8
10.00	86.3
11.00	73.1
12.00	60.0
13.00	45.4
13.50	36.5
14.00	25.4
14.20	19.9
14.40	13.3
14.60	5.2
14.70	0.2
14.80	-5.7
14.90	-13.5

Titration number 1 continued

volume added (ml)	emf (mV)
10.00	34.2
10.50	25.5
10.60	23.6
10.70	21.5
10.80	19.3
11.00	14.5
11.20	8.9
11.40	2.4
11.50	-1.7
11.70	-11.4
11.80	-17.9
11.90	-26.2
11.95	-31.5
12.00	-38.3

Titration number 2 continued

volume added (ml)	emf (mV)
14.95	-18.1
15.00	-23.8
15.10	-40.6
15.15	-54.5

Titration number 3

volume added (ml)	emf (mV)
7.10	221.1
7.20	217.6
7.30	213.4
7.50	205.4
7.60	199.8
7.70	194.1
7.80	187.4
7.85	183.4
7.90	179.3
8.00	170.2
8.05	165.4
8.10	160.8
8.15	156.4
8.18	153.6
8.20	151.8
8.23	149.3
8.26	147.0
8.30	144.0
8.35	140.7
8.40	137.5
8.50	132.2
8.60	127.3
8.70	123.1
8.80	119.1

Titration number 4

volume added (ml)	emf (mV)
13.20	220.9
13.50	215.2
14.88	158.0
15.00	156.3
15.05	152.0
15.10	147.9
15.15	143.8
15.20	140.0
15.30	133.0
15.40	126.8
15.45	124.0
15.50	118.8
15.60	111.6
15.70	107.4
15.90	99.8
16.10	92.9
16.30	86.5
16.50	80.4
16.80	71.4
17.10	62.0
17.40	51.7
17.60	43.9
17.80	34.6
18.00	22.2

Titration number 3 continued

volume added (ml)	emf (mV)
9.00	112.1
9.20	105.9
9.50	97.9
9.80	90.8
10.00	86.2
10.20	81.3
10.30	79.0
10.40	76.8
10.60	72.6
10.80	68.4
11.10	61.7
11.40	54.6
11.80	44.1
12.20	30.4
12.40	21.5
12.60	9.8
12.70	1.3
12.75	-4.8
12.85	-17.8

Titration number 4 continued

volume added (ml)	emf (mV)
18.10	14.1
18.25	-4.0
18.30	-14.0
18.32	-19.2
18.35	-28.5
18.37	-36.9
18.38	-41.9
18.40	-59.1

Formation Constants for Zn(II) - Acetate Complexes

The formation curve (figure 33) was obtained from the results in table 39.

TABLE 39

Experimental results for the Zn(II) - acetate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	4.00	2.00	0	14.54	-28.06	20.00	355.8
2	0	4.00	4.00	0	16.63	-28.06	20.00	355.9
3	0	1.00	2.00	0	14.54	-28.06	20.00	355.8

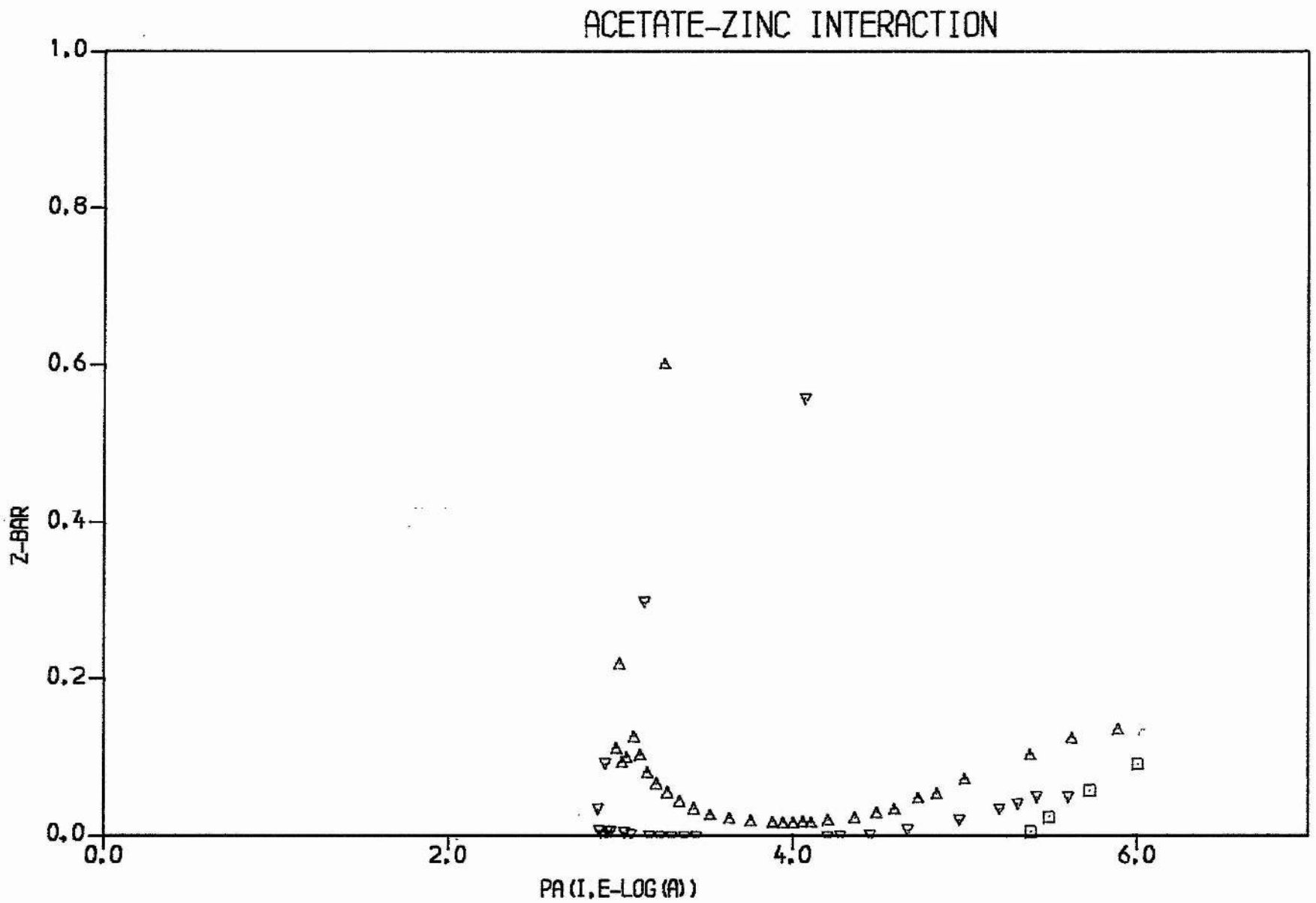
Titration number 1

volume added (ml)	emf (mV)
3.00	229.0
3.50	226.2
4.50	219.9
5.50	212.3
7.00	196.5
7.50	188.7
7.80	183.0
8.10	175.7

Titration number 2

volume added (ml)	emf (mV)
5.00	223.5
6.00	217.1
6.50	213.3
7.00	209.1
8.00	198.7
9.00	183.2
9.50	171.0
9.80	160.6

FIGURE 33


ACETATE-ZINC INTERACTION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
2.0
4.0
6.0
PA(I,E-LOG(A))

Titration number 1 continued

volume added (ml)	emf (mV)
8.30	169.8
8.50	162.4
8.70	153.0
8.80	147.0
8.85	143.8
8.90	140.2
8.95	136.4
9.00	132.4
9.10	123.8
9.20	114.8
9.30	106.0
9.40	97.8
9.50	90.0
9.60	82.5
9.70	75.0
9.80	67.4
9.90	59.6
10.00	50.8
10.05	44.3
10.10	38.0
10.20	23.2
10.30	-1.1
10.35	-27.1

Total number of readings = 59.

Titration number 2 continued

volume added (ml)	emf (mV)
9.90	156.1
10.62	104.2
10.70	98.3
10.80	91.5
10.90	84.8
11.00	78.1
11.20	64.6
11.30	57.5
11.50	40.6
11.55	35.4
11.60	29.1
11.70	13.9
11.80	-15.6
11.82	-26.3
11.84	-40.1
11.85	-47.9

Titration number 3

volume added (ml)	emf (mV)
7.00	203.7
7.50	198.1
8.00	191.3
8.20	188.0

The data was analysed using the MINIQUAD program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 2.69 \pm 0.32$$

$$\log \beta_{121} = 10.02 \pm 0.43$$

These constants gave a sum of squares = 1.60×10^{-7}. The system can thus be described by the two complexes $Zn(acetate)^{+}$ and $Zn_2(acetate)H^{4+}$.

The zinc-acetate complexes produced a pattern of formation curves as the acetate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 34).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 35). These models require the total concentrations of zinc and acetate (7.65 and 15.30 mM respectively) and the formation constants from table 40.

Table 40 below lists our formation constants of the complex formation of acetate and some of the published results from other workers as a comparison.

FIGURE 34

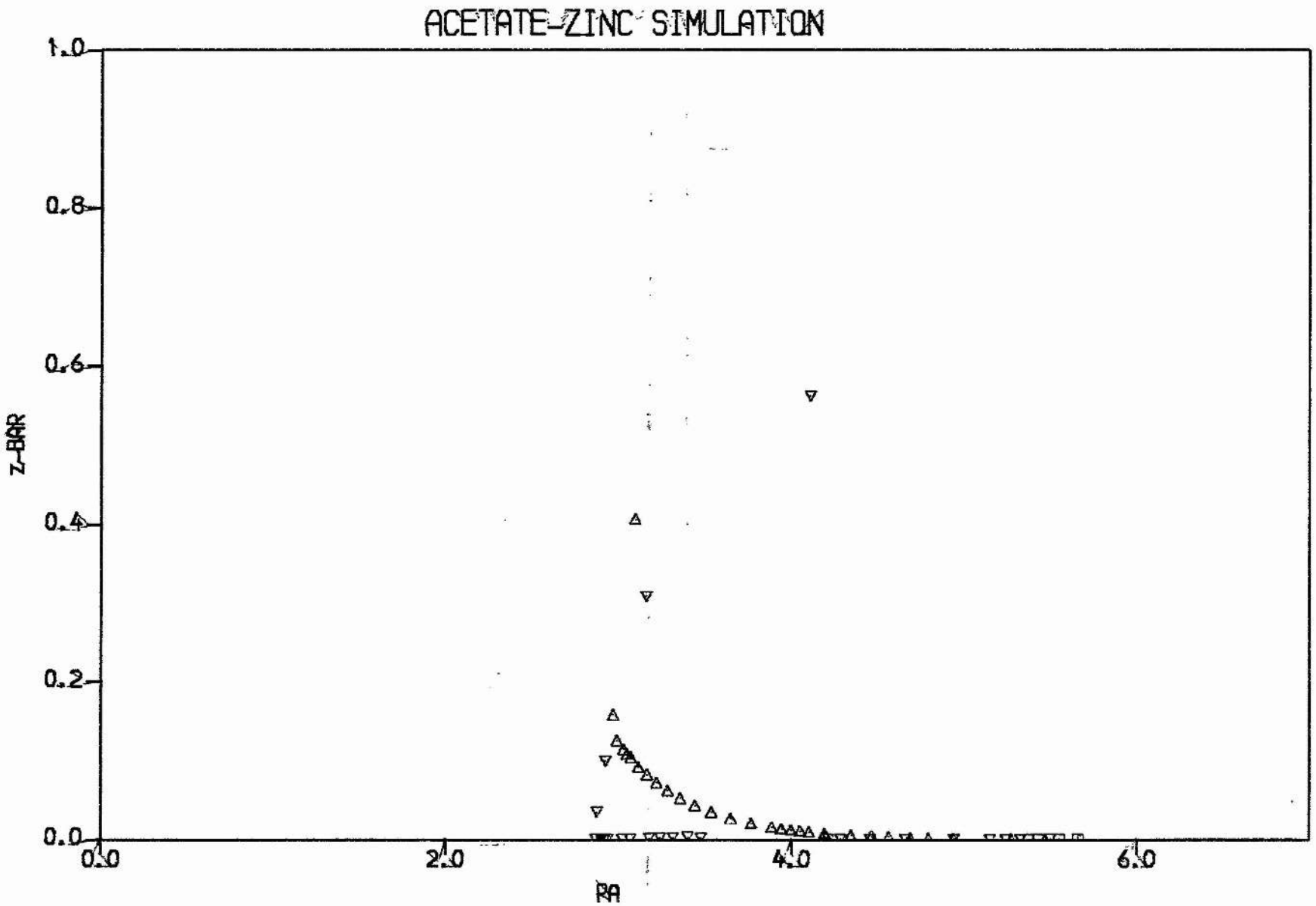


FIGURE 35

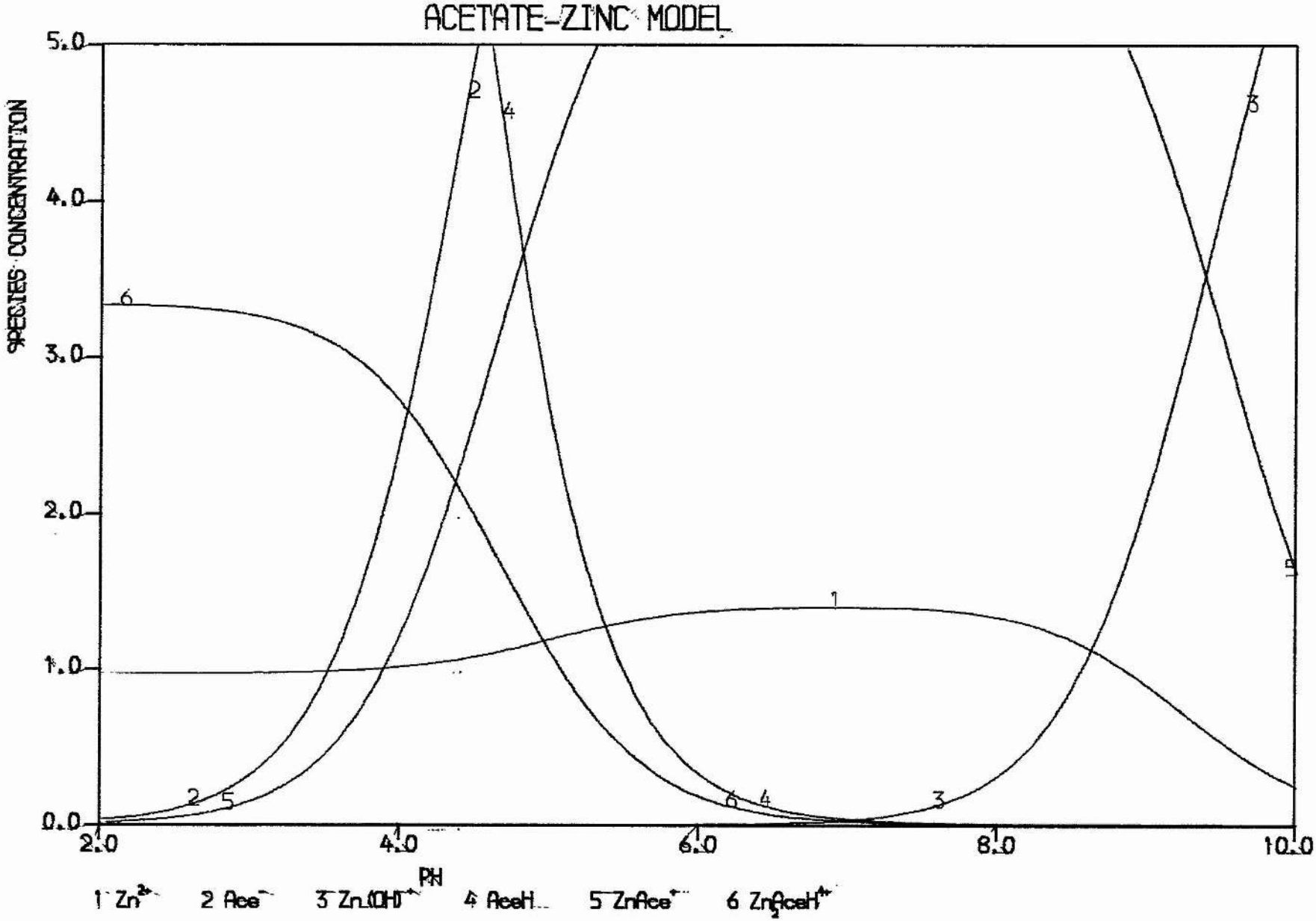

ACETATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 Ace-
3 Zn(OH)+
4 AceH
5 ZnAce+
6 Zn2AceH4+

TABLE 40

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (Θ_c/°C, I/M, log β)	Ref
	1	0	1	4.55	0.01	164	20, 0.10($NaClO_4$), β_{101} 4.55	98
Zn	1	1	0	2.69	0.32	59	20, 0.10($NaClO_4$), β_{110} 1.28, β_{210} 2.09	98
	1	2	1	10.02	0.43		25, 0 corr., β_{110} 1.57	99

Protonation Constants of Galacturonate

The formation curve (figure 36) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\log K_{101} = 3.21 \pm 0.01 \quad \text{SUM OF SQUARES} = 2.84 \times 10^{-6} \ (\text{mol}^2\ \text{dm}^{-6}) \quad (122 \text{ readings})$$

TABLE 41

Experimental results for the protonation of galacturonate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^{o} (mV)
	A	H	A	H		
1	91.45	10.73	0	-100.00	20.00	363.4
2	45.72	5.37	0	-100.00	20.00	363.4
3	22.86	2.68	0	-50.00	20.00	364.2
4	13.73	2.15	0	-40.00	20.00	363.9
5	9.14	1.07	0	-24.03	20.00	363.9
6	4.57	0.53	0	-12.01	20.00	363.9

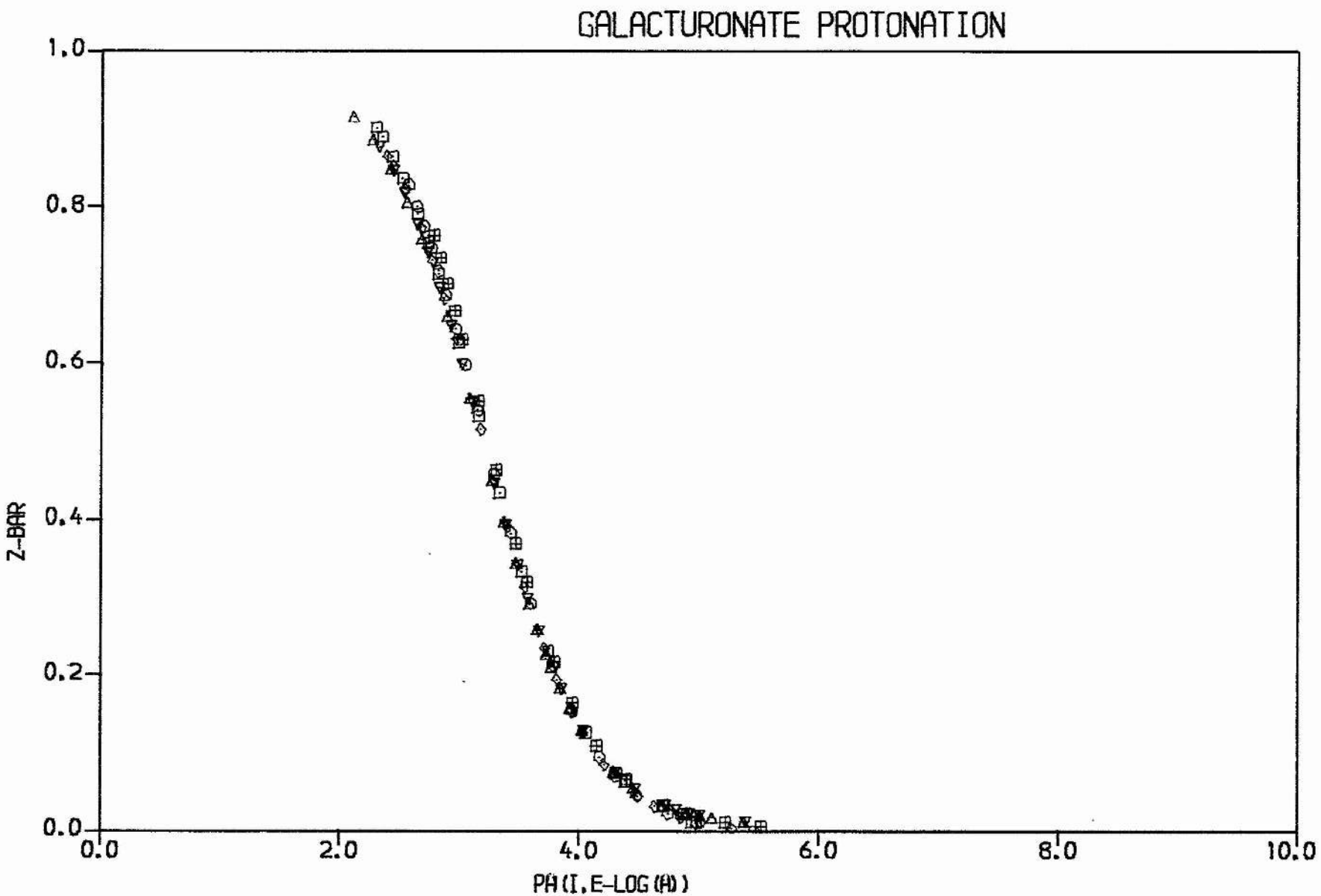
Titration number 1

volume added (ml)	emf (mV)
2.00	232.8
3.00	222.9
4.00	213.6
5.00	205.2
6.00	197.7
8.00	184.4
10.00	172.7
12.00	161.0
13.00	155.0

Titration number 2

volume added (ml)	emf (mV)
1.20	219.8
1.70	212.5
2.10	206.7
2.60	199.9
3.00	194.7
3.50	188.5
4.00	182.5
4.50	176.8
5.00	171.2

FIGURE 36


GALACTURONATE PROTONATION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
PH(I,E-LOG(H))

Titration number 1 continued

volume added (ml)	emf (mV)
14.00	148.9
15.00	142.1
15.60	137.8
16.20	133.1
16.50	130.6
17.00	125.9
17.50	120.8
18.00	114.8
19.00	98.5
19.40	88.5
19.80	73.1
20.00	60.9
20.05	54.1
20.10	47.8
20.20	31.7

Titration number 2 continued

volume added (ml)	emf (mV)
6.00	160.2
6.50	154.4
7.00	148.2
7.40	143.0
7.80	137.2
8.50	125.4
9.00	114.2
9.50	97.7
9.70	87.6
9.90	72.0
9.95	66.4
10.00	59.5
10.02	54.3
10.10	31.0

Titration number 3

volume added (ml)	emf (mV)
0.00	222.0
0.30	218.9
0.80	213.8
1.30	208.4
2.00	200.7
2.50	195.2
3.00	189.8
4.00	179.3
5.00	168.9
6.00	158.3
7.00	146.9
8.00	133.1
9.00	113.7
9.50	97.8
9.60	93.3
9.90	74.0
10.00	63.2
10.10	59.0

Titration number 4

volume added (ml)	emf (mV)
0.00	216.2
0.30	212.8
0.80	207.2
1.50	198.8
2.00	192.7
2.50	186.6
3.00	180.3
4.00	167.6
5.00	154.2
5.60	145.2
6.20	134.6
6.50	128.3
6.80	120.8
7.00	115.0
7.30	104.0
7.60	86.8
7.70	77.9
7.80	64.6
7.85	54.1
7.90	38.5

Titration number 5

volume added (ml)	emf (mV)
0.00	205.1
0.50	200.7
0.90	197.1
1.30	193.4
1.70	189.6
2.10	185.6
2.60	180.7
3.10	175.6
3.70	169.4
4.50	160.7
5.20	152.1
6.00	141.9
6.70	130.6
7.20	120.4
7.70	106.3
7.90	98.3
8.10	87.6
8.30	71.0
8.40	56.5
8.50	26.8

Titration number 6

volume added (ml)	emf (mV)
0.00	192.0
0.50	188.4
1.00	184.8
1.50	181.1
2.00	177.2
3.00	168.8
4.00	159.8
5.00	149.5
5.50	143.7
6.50	129.5
7.00	120.2
7.50	107.8
7.90	92.5
8.20	72.9
8.30	61.1
8.40	41.6
8.45	23.3

Formation Constants for Zn(II) - Galacturonate Complexes

To within the limits of hydrolysis and solubilities, this system was studied using a pattern of titrations where both A and B (the total concentrations of ligand and zinc respectively) were held constant and equal in the titrate and titrant, the sole difference in these solutions being their perchloric acid concentrations.

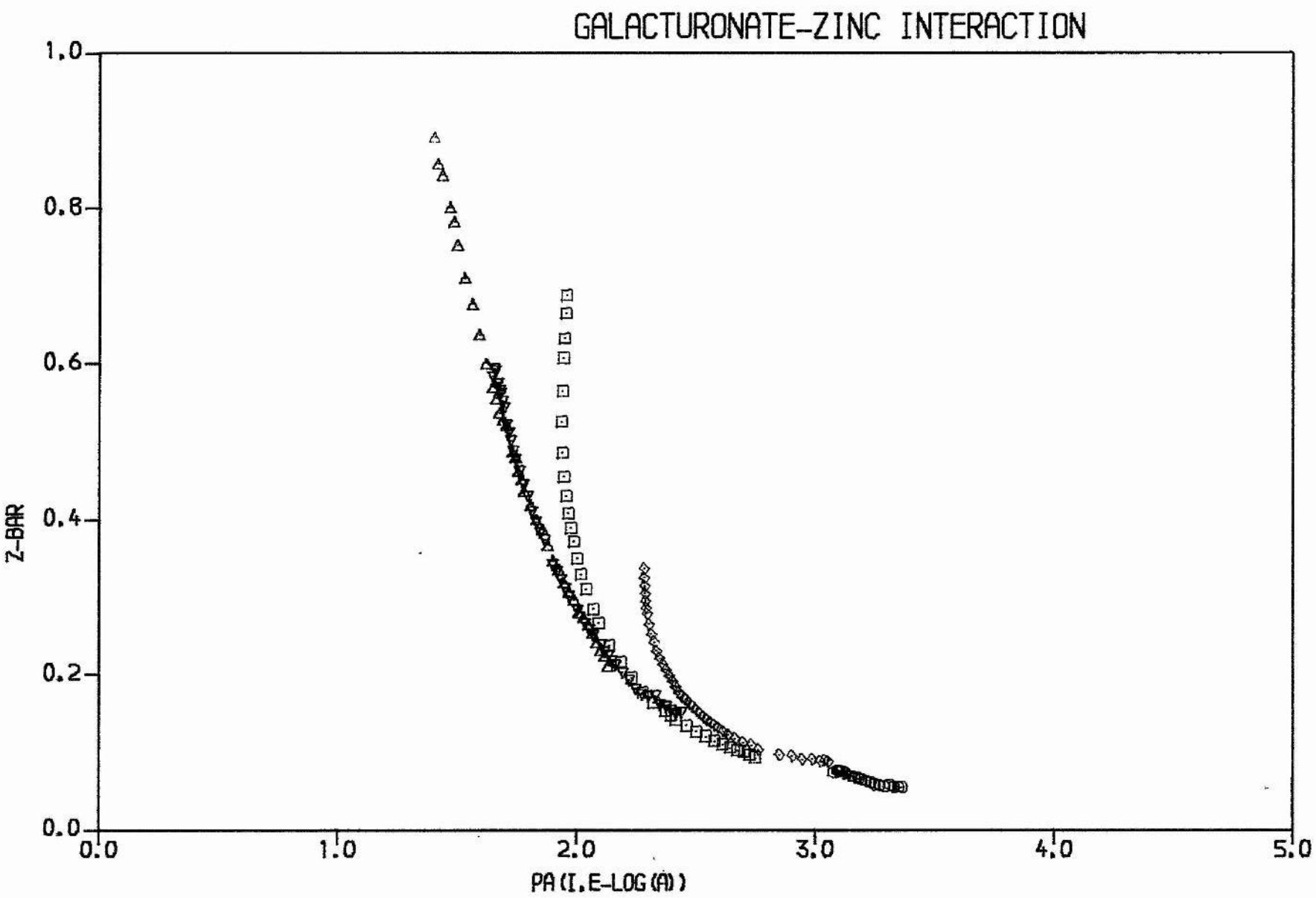
The formation curve (figure 37) was obtained from the results in table 42.

TABLE 42

Experimental results for the Zn(II) - galacturonate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	82.41	82.41	11.75	11.75	-49.13	26.67	20.00	361.4
2	41.20	41.20	11.75	11.75	-28.89	23.56	20.00	361.4
3	20.60	20.60	11.75	11.75	-18.92	23.56	20.00	361.4
4	11.00	11.00	11.75	11.75	-8.89	23.56	20.00	361.4
5	5.50	5.50	11.75	11.75	1.11	23.56	20.00	361.4

FIGURE 37


GALACTURONATE-ZINC INTERACTION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
1.0
2.0
3.0
4.0
5.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
0.00	159.1
0.50	161.5
1.00	163.9
2.00	168.2
2.50	170.2
3.00	172.0
4.00	175.5
5.00	178.8
6.00	181.8
7.00	184.6
8.00	187.3
8.50	188.6
9.00	189.8
9.50	191.1
10.00	192.4
11.00	194.6
11.50	195.8
12.00	196.8
12.50	197.9
13.00	198.9
14.00	201.0
15.00	203.0
16.00	205.0
17.00	206.8
18.00	208.5

Titration number 2

volume added (ml)	emf (mV)
0.00	147.2
0.10	148.2
0.20	149.0
0.40	150.4
0.50	151.2
0.70	152.6
0.80	153.3
1.00	154.6
1.20	155.9
1.50	157.6
1.80	159.4
2.00	160.5
2.30	162.1
2.60	163.7
3.00	165.7
3.50	168.1
4.00	170.4
4.50	172.5
5.00	174.6
5.50	176.6
6.00	178.6
7.00	182.1
7.50	183.9
8.00	185.6
8.50	187.2

Titration number 1 continued

volume added (ml)	emf (mV)
19.00	210.3
20.00	211.9
21.00	213.5
22.00	215.1
23.00	216.5
24.00	218.0
25.00	219.4
26.00	220.7
27.00	221.9
28.00	223.1
29.00	224.3
30.00	225.3

Titration number 2 continued

volume added (ml)	emf (mV)
9.00	188.8
10.00	191.9
11.00	194.8
11.50	196.2
12.00*	197.6
13.00	200.3
13.50	201.5
14.00	202.8
14.50	204.0
15.00	205.2
16.00	207.5
17.00	209.7
18.00	211.7
19.00	213.7
20.00	215.7
21.00	217.7
22.00	219.2
23.00	220.9
24.00	222.4
25.00	223.8
26.00	225.3

Titration number 3

volume added (ml)	emf (mV)
0.00	112.4
0.10	115.4
0.20	117.8
0.30	120.1
0.50	124.2
0.70	127.7
1.00	132.5
1.30	136.7
1.60	140.5
1.90	143.9
2.20	147.0
2.50	149.9
2.90	153.4
3.30	156.6
3.80	160.4
4.50	165.2
5.00	168.3
6.00	174.1
7.00	179.5
8.00	184.4
9.00	188.9
10.00	193.2
11.00	197.3
11.50	199.2
12.00	201.0

Titration number 4

volume added (ml)	emf (mV)
0.00	137.0
0.10	139.0
0.20	140.9
0.30	142.7
0.40	144.4
0.50	146.0
0.60	147.6
0.80	150.6
1.00	153.4
1.20	156.1
1.40	158.5
1.60	160.9
1.80	163.2
2.00	165.4
2.20	167.5
2.40	169.5
2.60	171.4
2.80	173.2
3.00	175.0
3.20	176.9
3.40	178.6
3.60	180.3
3.80	181.9
4.00	183.5
4.20	185.0

Titration number 3 continued

volume added (ml)	emf (mV)
13.00	204.5
14.00	207.7
15.00	210.7
16.00	213.4
17.00	215.9
18.00	218.2
19.00	220.3
20.00	222.3
21.00	224.0
22.00	225.6

Titration number 4 continued

volume added (ml)	emf (mV)
4.40	186.5
4.60	188.0
4.80	189.4
5.00	190.8
5.30	192.8
5.60	194.7
6.00	197.2
6.50	200.1
7.00	202.9
7.50	205.3
9.00	212.0
10.00	215.8
11.00	218.9
12.00	221.8
13.00	224.2
13.50	225.4
14.00	226.4

Titration number 5

volume added (ml)	emf (mV)
0.00	204.5
0.10	205.5
0.15	205.9
0.20	206.3
0.30	207.1
0.50	208.4
0.70	209.6
0.80	210.3
1.00	211.5
1.20	212.7
1.40	213.8
1.70	215.3
2.00	216.8
2.30	218.1
2.60	219.4
3.00	221.0
3.40	222.6
3.80	223.9
4.20	225.2
4.50	226.1

Total number of readings = 180.

The data was analysed using the MINIQUAD program which was offered the range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 1.73 \pm 0.03$$

$$\log \beta_{210} = 2.62 \pm 0.06$$

$$\log \beta_{111} = 3.93 \pm 0.10$$

$$\log \beta_{11-1} = 3.37 \pm 0.10$$

These constants gave a sum of squares = 6.33×10^{-6}. The system can thus be described by the four complexes $Zn(galacturonate)^{+}$, $Zn(galacturonate)^{o}_{2}$, $Zn(galacturonate)H^{2+}$, and $Zn(galacturonate)OH^{o}$.

The zinc-galacturonate complexes produced a pattern of formation curves as the galacturonate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy, or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 38).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 39). These models require the total concentrations of zinc and galacturonate (7.65 and 15.30 mM respectively) and the formation constants from table 43.

Table 43 below lists our formation constants of the complex formation of galacturonate and some of the published results from other workers as a comparison.

FIGURE 38

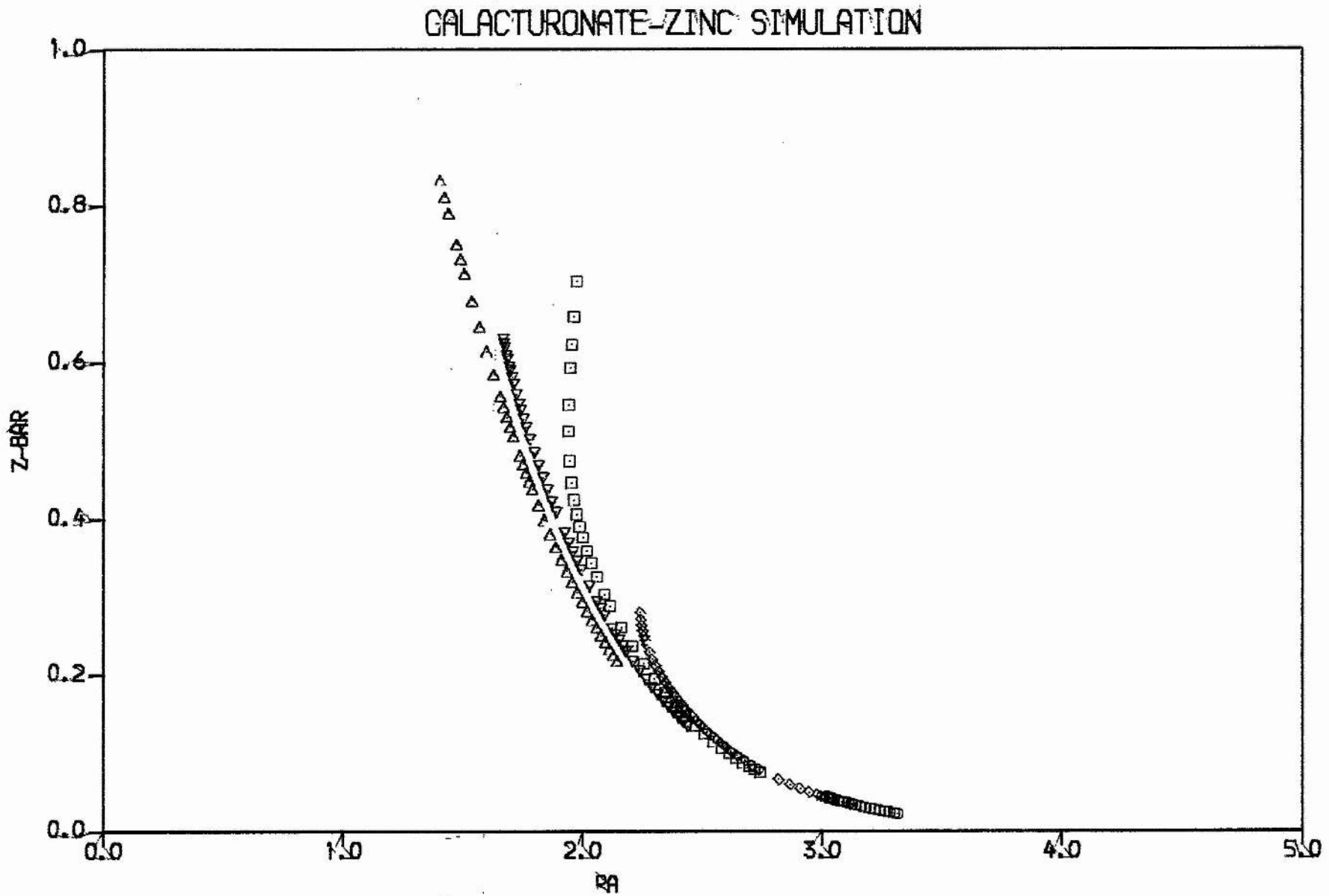

GALACTURONATE-ZINC SIMULATION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
1.0
2.0
3.0
4.0
5.0
RA

FIGURE 39

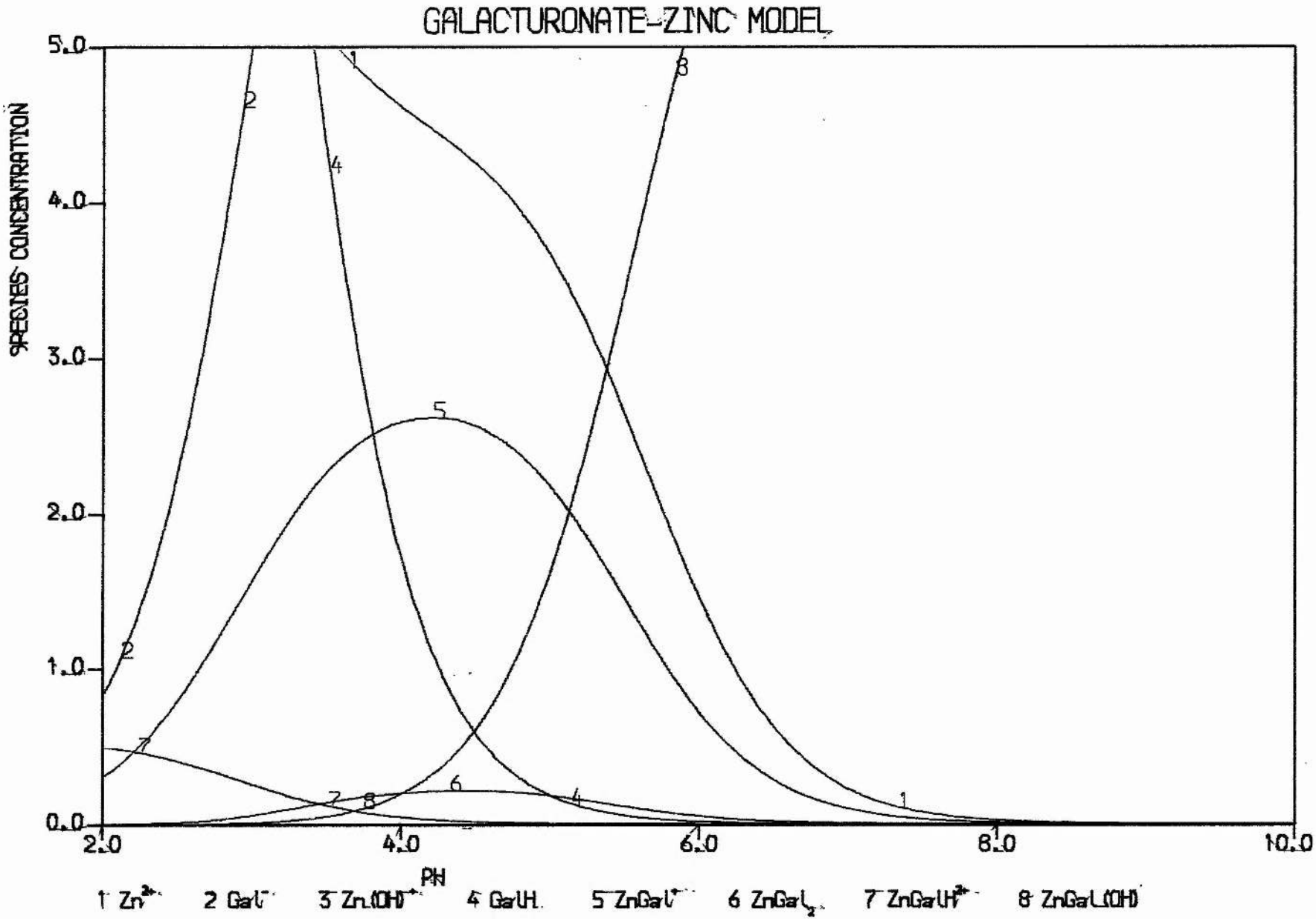

GALACTURONATE-ZINC MODEL
%RESIDUES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 GalA-
3 Zn(OH)+
4 GalH
5 ZnGalA+
6 ZnGalA2
7 ZnGalH2+
8 ZnGalA(OH)

TABLE 43

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (θ_c/°C, I/M, log β)	Ref
	1	0	1	3.21	0.01	122	37, 0.15 $NaClO_4$, β_{101} 11.42, β_{102} 14.65	52
Zn	1	1	0	1.73	0.03	180		
	2	1	0	2.62	0.06			
	1	1	1	3.93	0.10			
	1	1	-1	-3.37	0.10			

Protonation Constants of β-Hydroxybutyrate

The formation curve (figure 40) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\log K_{101} = 4.49 \pm 0.02$$ SUM OF SQUARES = 6.48×10^{-5} ($mol^2\ dm^{-6}$) (147 readings).

TABLE 44

Experimental results for the protonation of β-hydroxybutyrate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E° (mV)
	A	H	A	H		
1	20.00	46.74	20.00	-160.10	20.00	357.9
2	15.00	37.41	15.00	-100.05	20.00	358.9
3	10.00	31.13	10.00	-80.04	20.00	358.1
4	5.00	31.13	5.00	-80.04	20.00	357.0

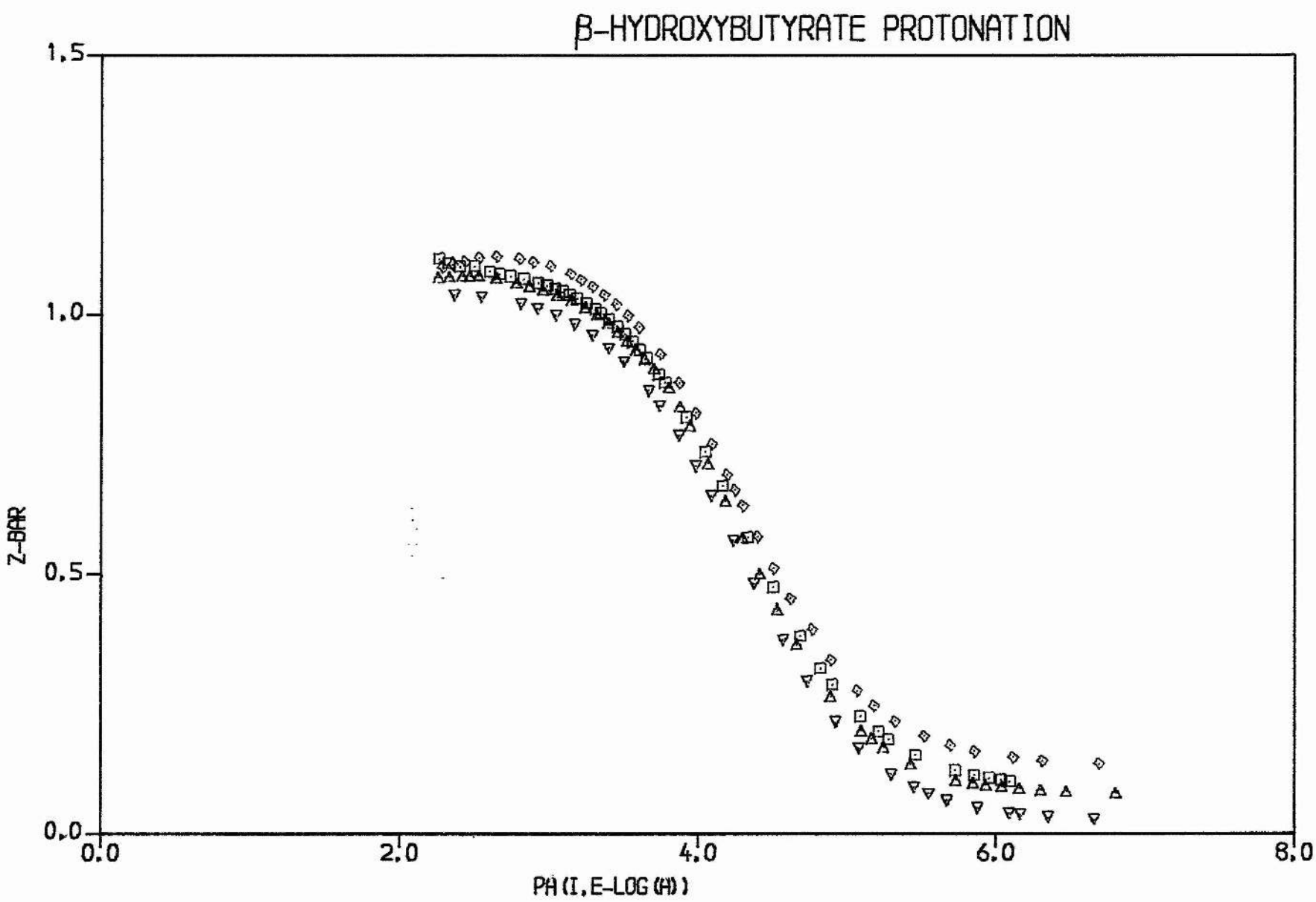
Titration number 1

volume added (ml)	emf (mV)
2.10	219.2
2.20	214.7
2.30	209.4
2.35	206.3
2.40	202.6
2.50	195.3
2.60	186.8
2.65	181.6
2.70	176.0
2.75	170.1
2.80	164.2

Titration number 2

volume added (ml)	emf (mV)
2.90	213.7
3.20	202.5
3.50	186.2
3.60	179.3
3.70	171.7
3.80	164.0
3.90	156.7
4.00	150.0
4.10	143.7
4.30	133.4
4.40	129.0

FIGURE 40


β-HYDROXYBUTYRATE PROTONATION
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
PH(I,E-LOG(H))

Titration number 1 continued

volume added (ml)	emf (mV)
2.85	158.7
2.90	153.8
2.95	149.0
3.00	145.1
3.05	141.3
3.10	137.6
3.15	134.1
3.20	130.2
3.40	119.5
3.50	115.3
3.70	107.8
3.90	100.6
4.10	93.5
4.30	86.3
4.50	79.1
4.70	71.2
5.00	57.1
5.20	44.3
5.25	40.0
5.30	35.1
5.40	23.8
5.50	5.2
5.52	-2.0
5.53	-7.3
5.54	-13.9
5.55	-21.1
5.56	-29.9
5.57	-40.5
5.58	-61.0

Titration number 2 continued

volume added (ml)	emf (mV)
4.60	121.0
4.80	114.0
5.00	107.5
5.30	98.4
5.60	89.8
6.00	77.9
6.30	67.9
6.60	56.2
6.80	46.4
7.00	33.0
7.10	23.6
7.15	17.5
7.20	9.9
7.26	-2.7
7.30	-15.8
7.31	-20.4
7.33	-31.9
7.35	-50.9

Titration number 3

volume added (ml)	emf (mV)
3.00	219.1
3.20	215.2
3.40	210.6
3.60	204.5
3.80	198.1
3.90	194.1
4.00	189.5
4.10	184.1
4.20	178.1
4.25	174.7
4.30	171.2
4.34	168.1
4.38	165.3
4.42	162.2
4.47	158.4
4.52	154.6
4.55	152.5
4.60	149.0
4.65	145.5
4.70	142.4
4.75	139.3
4.80	136.4
4.85	133.5

Titration number 4

volume added (ml)	emf (mV)
4.50	216.6
4.70	212.4
4.90	207.5
5.10	201.5
5.30	194.2
5.50	184.8
5.60	179.1
5.70	172.0
5.80	163.8
5.85	159.4
5.90	154.7
5.95	149.8
6.00	144.8
6.05	140.0
6.10	135.4
6.20	126.8
6.30	119.0
6.40	112.0
6.50	105.3
6.60	99.0
6.65	95.8
6.70	92.6
6.80	86.4

Titration number 3 continued

volume added (ml)	emf (mV)
4.95	128.4
5.00	125.9
5.20	117.0
5.40	109.2
5.60	102.0
5.90	91.5
6.20	81.1
6.50	69.9
6.70	61.5
6.80	56.6
7.00	45.0
7.10	37.5
7.15	33.3
7.25	22.2
7.35	5.7
7.38	-2.3
7.40	-8.4
7.41	-13.3
7.42	-16.9

Titration number 4 continued

volume added (ml)	emf (mV)
6.90	79.8
7.00	72.9
7.10	63.9
7.20	56.2
7.30	45.1
7.35	38.3
7.40	29.5
7.45	17.4
7.48	6.5
7.50	-3.5
7.52	-19.3
7.53	-31.4
7.54	-54.8

Formation Constants for Zn(II) - β-Hydroxybutyrate Complexes

The formation curve (figure 41) was obtained from the results in table 45.

TABLE 45

Experimental results for the Zn(II) - β-hydroxybutyrate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	8.00	2.00	0	14.54	-40.02	20.00	358.1
2	0	16.00	2.00	0	14.54	-40.02	20.00	358.1

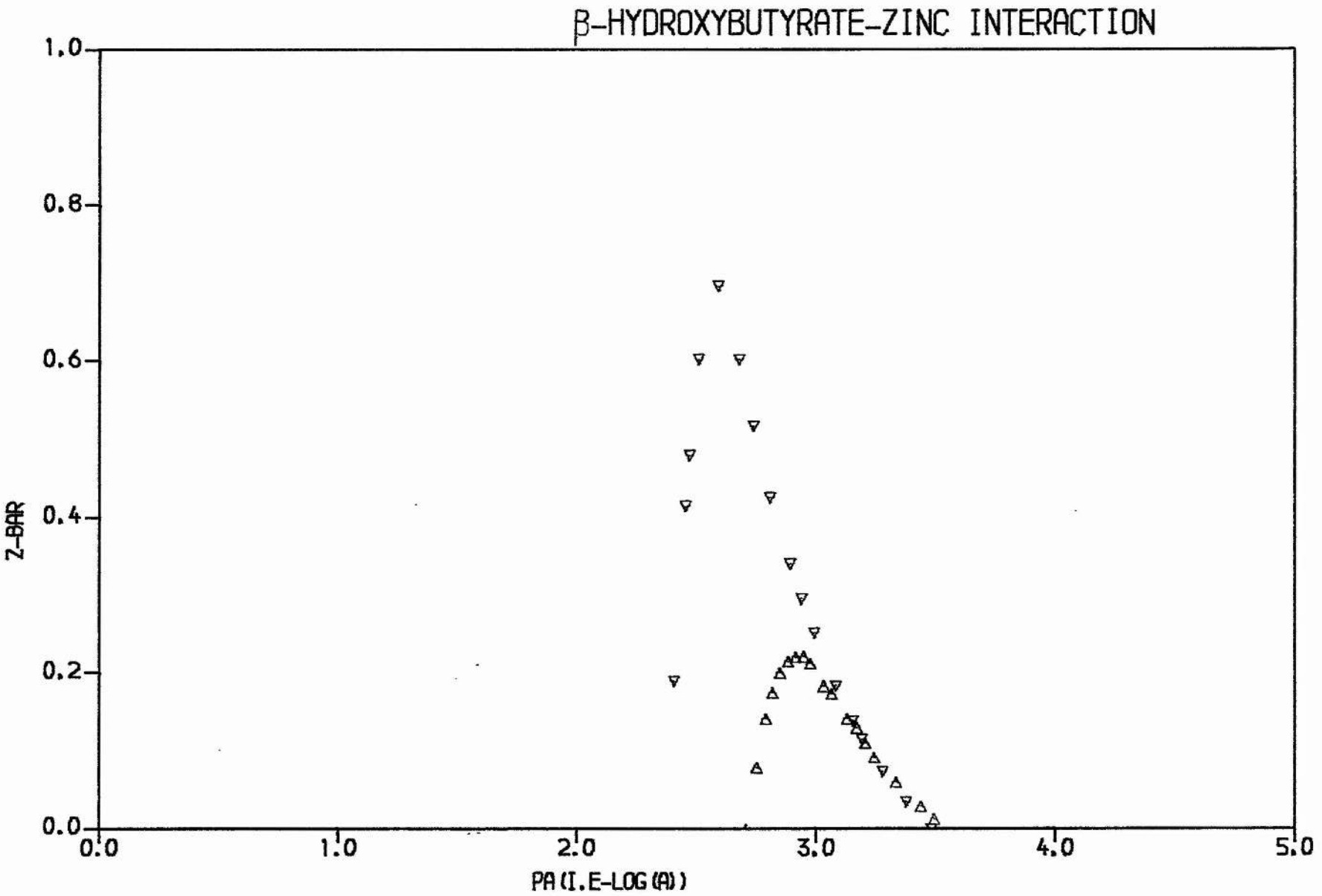
Titration number 1

volume added (ml)	emf (mV)
6.15	124.5
6.20	120.1
6.30	111.4
6.40	103.1
6.45	99.2
6.50	95.4
6.55	91.2

Titration number 2

volume added (ml)	emf (mV)
5.20	141.2
5.30	133.8
5.40	126.6
5.50	119.9
5.55	116.9
5.65	111.0
5.80	102.8

FIGURE 41


β-HYDROXYBUTYRATE-ZINC INTERACTION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
1.0
2.0
3.0
4.0
5.0
PA(I.E-LOG(A))

Titration number 1 continued

volume added (ml)	emf (mV)
6.65	83.2
6.70	78.9
6.80	70.3
6.85	65.5
6.90	60.1
6.95	54.2
7.00	47.5
7.04	41.2
7.07	35.7
7.10	29.0
7.13	21.0

Titration number 2 continued

volume added (ml)	emf (mV)
5.90	97.6
6.00	92.6
6.20	82.7
6.40	72.8
6.60	61.9
6.90	40.4
7.10	14.1
7.15	2.2
7.17	-3.9
7.20	-16.7

Total number of readings = 35.

The data was analysed using the MINIQUAD program which was offered a range of species having $p = 0 \rightarrow 3$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{210} = 5.81 \pm 0.05$$

$$\log \beta_{112} = 9.73 \pm 0.12$$

$$\log \beta_{221} = 13.33 \pm 0.09$$

$$\log \beta_{222} = 17.47 \pm 0.09$$

$$\log \beta_{321} = 16.27 \pm 0.10$$

These constants gave a sum of squares = 2.97×10^{-9}. The system can thus be described by the five complexes $Zn(\beta\text{-hydroxybutyrate})^{o}_{2}$, $Zn(\beta\text{-hydroxybutyrate})H_2^{3+}$, $Zn_2(\beta\text{-hydroxybutyrate})_2H^{3+}$, $Zn_2(\beta\text{-hydroxybutyrate})_2H_2^{4+}$, $Zn_2(\beta\text{-hydroxybutyrate})_3H^{2+}$.

The zinc-β-hydroxybutyrate complexes produced a pattern of formation curves as the β-hydroxybutyrate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy, or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 42).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 43). These models require the total concentrations of zinc and β-hydroxybutyrate (7.65 and 15.30 mM respectively) and the formation constants from table 46.

Table 46 below lists our formation constants of the complex formation of β-hydroxybutyrate and some of the published results from other workers as a comparison.

FIGURE 42

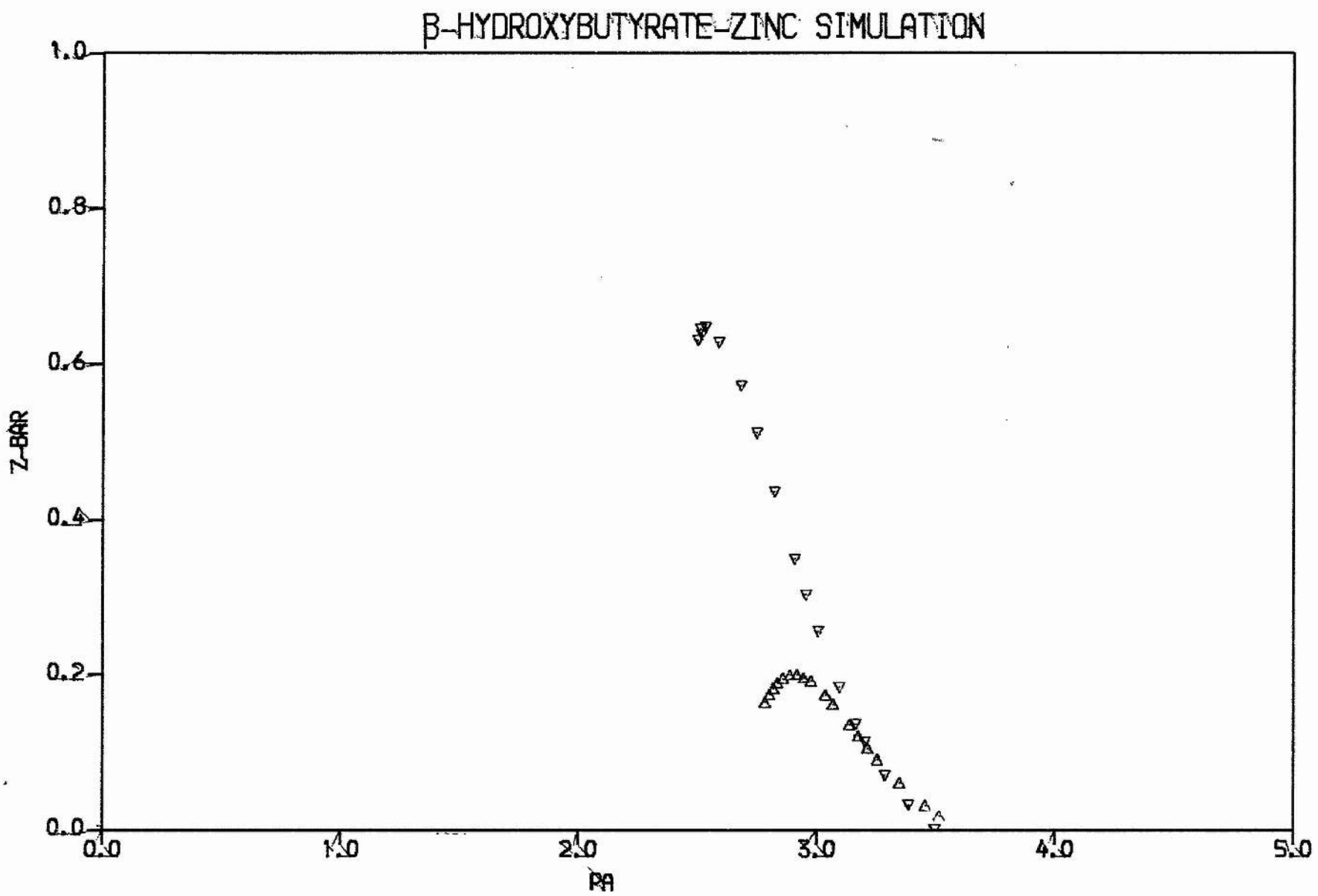

β-HYDROXYBUTYRATE-ZINC SIMULATION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
1.0
2.0
3.0
4.0
5.0
PA

FIGURE 43

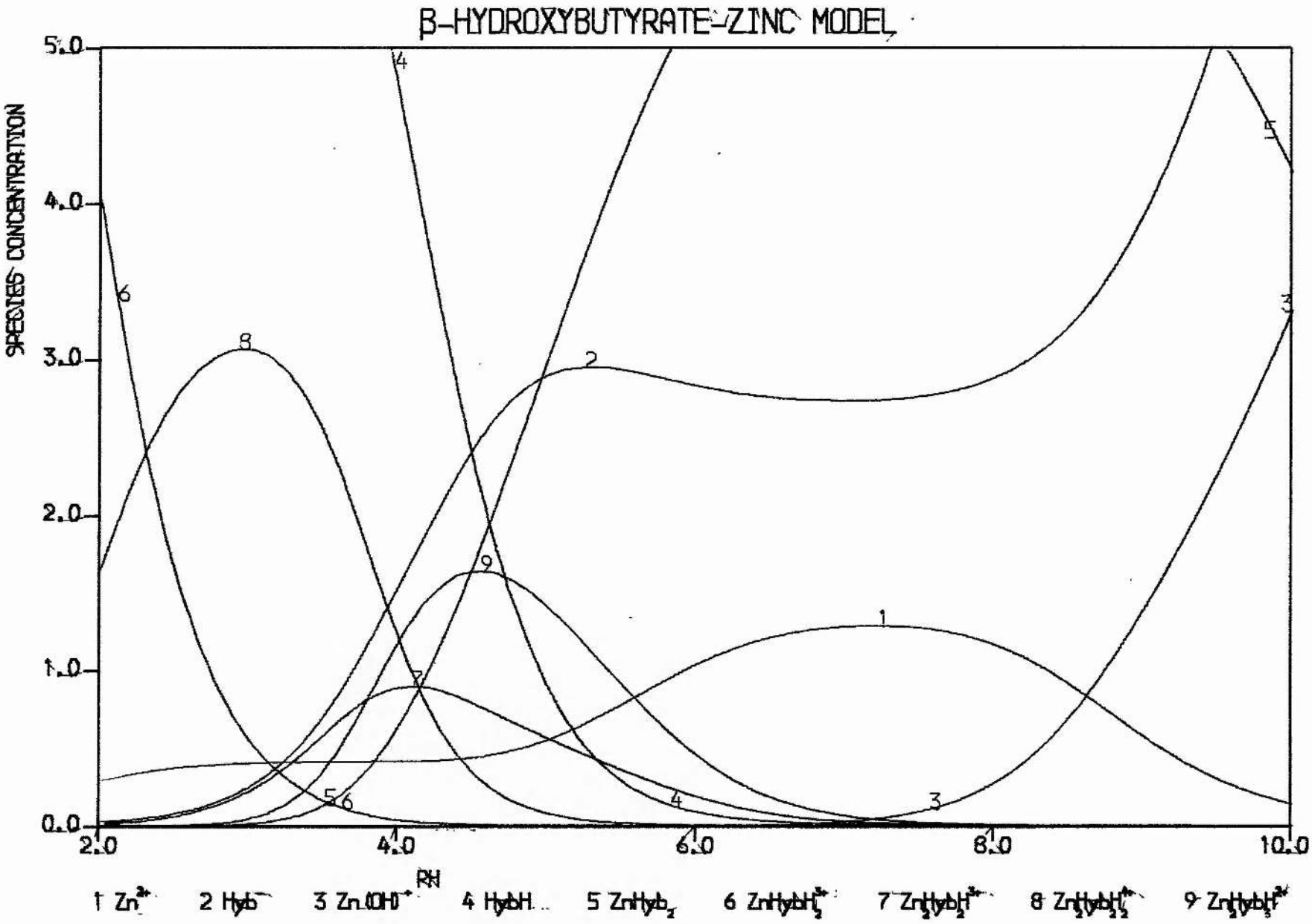

β-HYDROXYBUTYRATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 Hyb-
3 ZnOH+
4 HybH
5 ZnHyb2
6 ZnHybH2+
7 Zn2Hyb2H3+
8 Zn2Hyb2H2+
9 Zn2Hyb3H2+

TABLE 46

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$, n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	Literature data (θ_c/°C, I /M, log β)	Ref
	1	0	1	4.49	0.02	148	31, 0.1($NaClO_4$), β_1 4.40	100
Zn	2	1	0	5.81	0.05	35	25, 0.2(KCl), β_1 1.06	101
	1	1	2	9.73	0.12			
	2	2	1	13.33	0.09			
	2	2	2	17.47	0.09			
	3	2	1	16.27	0.10			

Protonation Constants of Malate

The formation curve (figure 44) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\left.\begin{aligned} \log K_{101} &= 4.48 \pm 0.01 \\ \log K_{101 \rightarrow 102} &= 3.11 \pm 0.01 \end{aligned}\right\} \quad \text{(183 readings)}$$

These constants gave a sum of squares = 1.56×10^{-5} $(mol^2\ dm^{-6})$.

TABLE 47

Experimental results for the protonation of malate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E° (mV)
	A	H	A	H		
1	100.00	5.37	100.00	-200.00	20.00	354.6
2	50.00	5.37	50.00	-151.50	20.00	354.6
3	25.00	5.37	25.00	-151.50	20.00	354.6
4	10.00	1.07	10.00	-30.30	20.00	354.6
5	5.00	5.34	5.00	-25.00	20.00	354.6

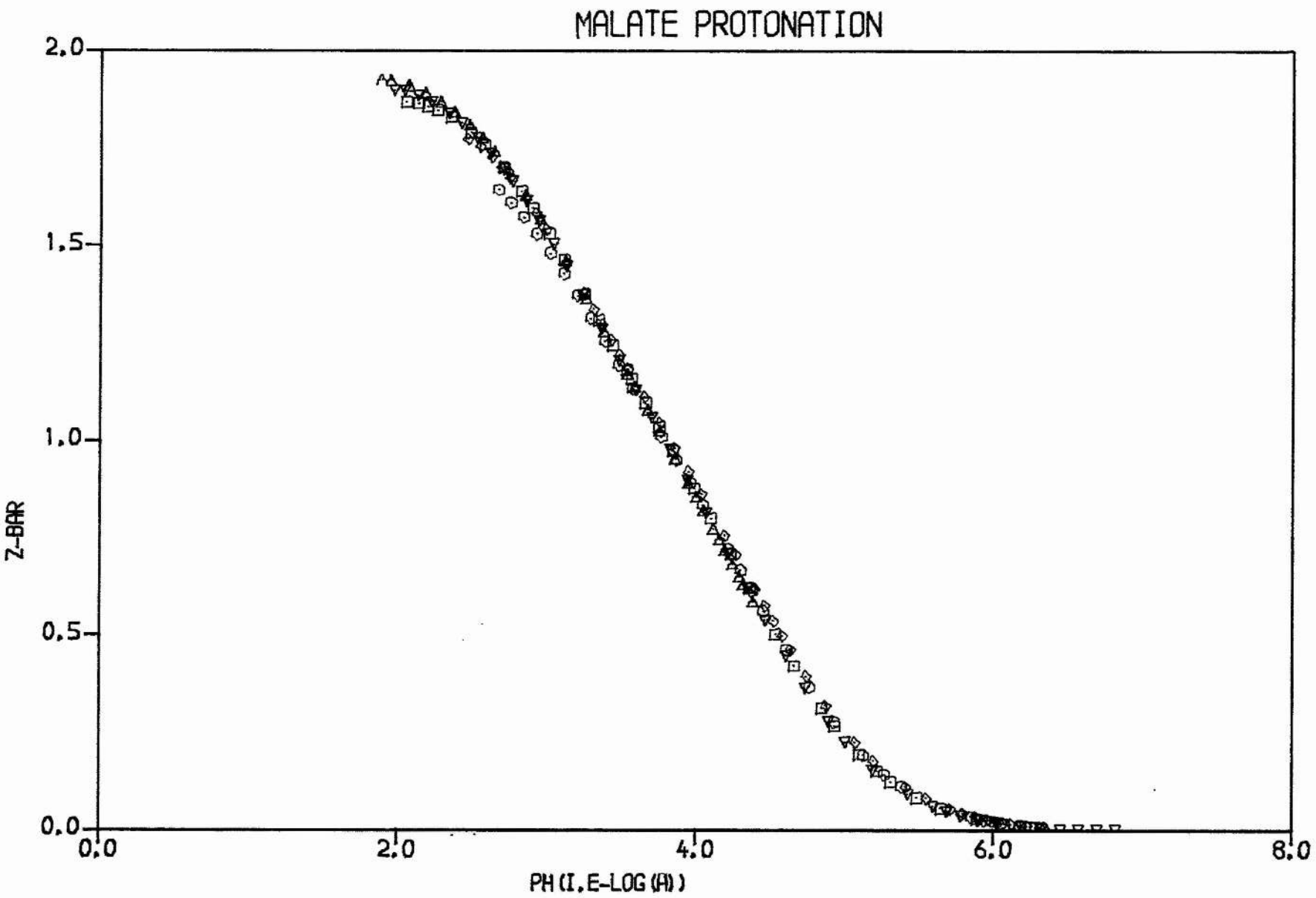
Titration number 1

volume added (ml)	emf (mV)
0.00	238.0
0.20	234.0
0.60	226.7
1.00	219.8
1.40	213.6
1.80	207.9
2.30	201.6
2.80	196.1
3.30	191.2
4.00	185.4
5.00	178.3

Titration number 2

volume added (ml)	emf (mV)
0.00	232.7
0.20	228.5
0.50	223.1
0.80	217.6
1.20	210.2
1.50	205.0
1.90	198.6
2.30	192.8
2.70	187.4
3.00	183.8
3.50	178.1

FIGURE 44



Titration number 1 continued

volume added (ml)	emf (mV)
6.00	172.3
8.00	162.0
10.00	153.4
12.00	146.0
15.00	136.3
16.00	133.3
18.00	127.9
20.00	123.1
23.00	116.6
26.00	111.1
28.00	107.8
30.00	104.8
33.00	100.7
35.00	98.2
37.00	95.9
40.00	92.8
43.00	89.9
45.00	88.2
50.00	84.3

Titration number 2 continued

volume added (ml)	emf (mV)
4.00	172.9
4.60	167.0
5.20	161.6
6.00	155.0
7.00	147.2
8.00	139.9
9.00	133.0
10.00	126.4
11.20	118.9
12.50	111.6
14.00	103.8
16.00	94.7
18.00	86.7
20.00	79.4
22.50	70.8
25.00	62.8
28.00	53.1
30.00	46.2
33.00	34.7
36.00	20.1
37.00	9.6
38.30	4.0
38.90	-1.9
39.50	-9.0

Titration number 2 continued

volume added (ml)	emf (mV)
40.00	-16.7
40.50	-26.9
40.80	-35.5
41.00	-43.0
41.50	-50.5
42.52	-58.1
42.61	-65.6

Titration number 3

volume added (ml)	emf (mV)
0.00	227.8
0.20	222.7
0.35	218.9
0.50	214.9
0.70	209.3
1.00	201.0
1.20	195.3
1.50	187.5
1.80	180.1
2.00	175.4
2.30	168.7
2.60	162.5

Titration number 4

volume added (ml)	emf (mV)
0.00	202.3
0.50	197.2
1.00	192.4
1.40	188.6
1.80	184.9
2.50	178.5
3.00	174.0
3.50	169.8
4.50	161.6
5.50	154.0
6.00	150.3
6.50	146.6
7.00	143.1
7.50	139.6
8.00	136.1
9.00	129.4
10.00	123.0
11.00	116.8
12.00	111.0
13.00	105.6
15.00	95.9
16.00	91.4
18.00	83.3
19.00	79.5

Titration number 3 continued

volume added (ml)	emf (mV)
3.00	154.3
3.30	148.3
3.60	142.5
3.90	136.6
4.00	134.6
4.30	128.9
4.60	123.3
4.90	117.6
5.40	108.6
5.80	101.7
6.30	93.5
6.80	85.7
7.50	75.0
8.00	67.3
8.70	55.8
9.00	50.4
9.50	40.2
9.80	32.9
10.00	27.1
10.30	16.3
10.50	6.2
10.70	-8.6
10.80	-19.7

Titration number 4 continued

volume added (ml)	emf (mV)
20.00	75.9
21.00	72.3
22.00	68.9
24.00	62.4
26.50	54.1
30.00	42.0
32.00	34.5
35.00	21.0
36.50	12.4
37.90	2.4
38.50	-3.0
39.00	-8.3
39.40	-13.2
39.70	-17.5
40.00	-22.7
40.30	-29.1
40.52	-34.6
40.58	-36.3

Titration number 5

volume added (ml)	emf (mV)
4.00	189.4
4.50	184.4
5.00	179.1
5.50	173.7
6.00	168.2
6.50	162.6
7.00	157.0
7.50	151.4
8.00	145.6
8.50	139.9
9.00	134.1
10.00	122.4
10.50	116.3
11.00	110.5
11.50	105.0
12.50	94.5
13.00	89.5
13.50	84.7
14.00	80.0
15.00	70.5
16.00	61.0
17.00	50.7
18.00	38.6
18.60	29.8
19.00	22.7
20.00	-6.3
20.10	-11.9
20.15	-15.0
20.20	-18.4
20.30	-26.4
20.32	-28.5
20.34	-30.7
20.36	-32.9
20.38	-35.6

Formation Constants for Zn(II) - Malate Complexes

To within the limits of hydrolysis and solubilities, this system was studied using a pattern of titrations where both A and B (the total concentrations of malate and zinc respectively) were held constant and equal in the titrate and titrant, the sole difference in these solutions being their perchloric acid concentrations.

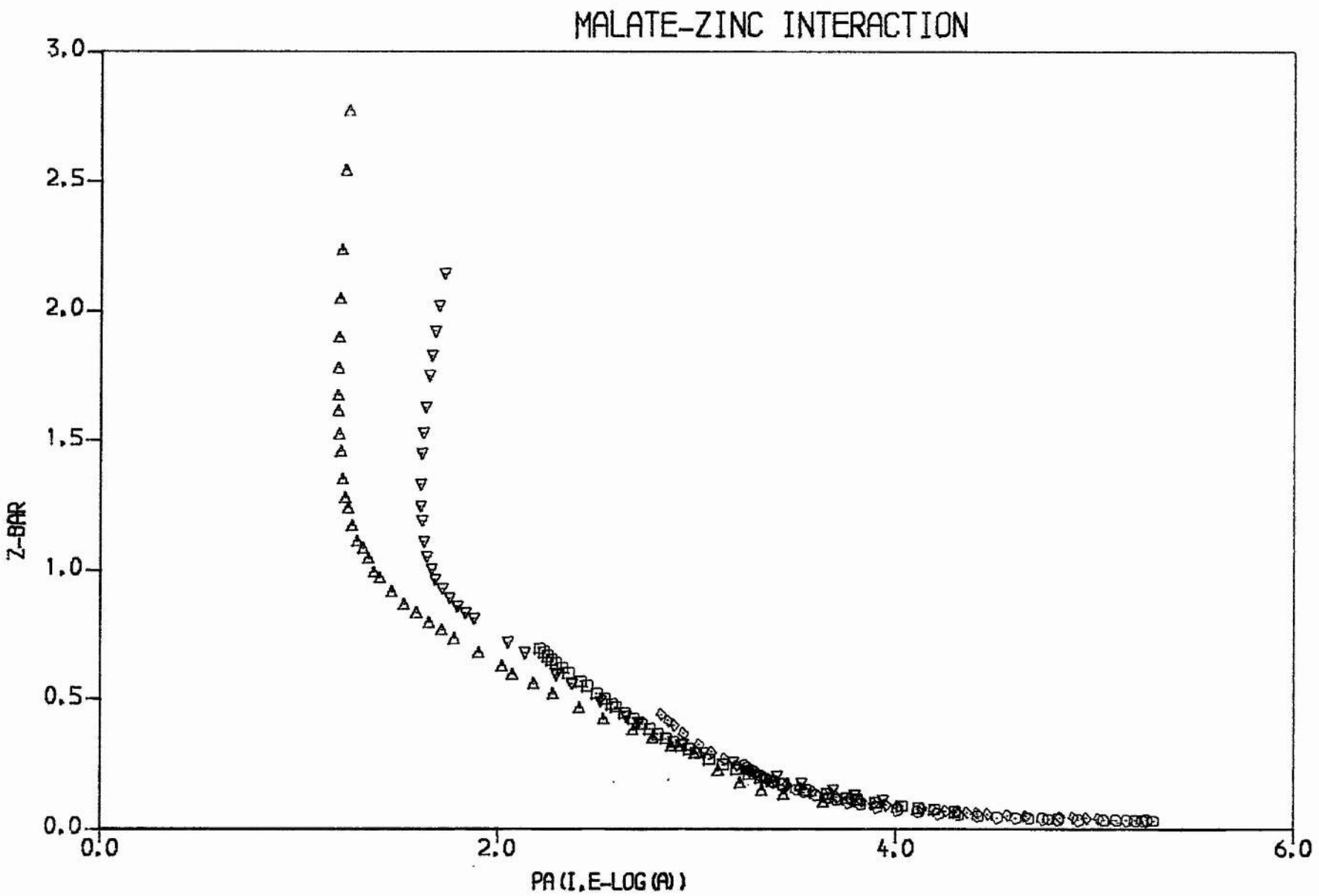
The formation curve (figure 45) was obtained from the results in table 48.

TABLE 48

Experimental results for the Zn(II) - malate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E° (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	90.00	90.00	11.75	11.75	-179.00	26.67	20.00	361.4
2	45.00	45.00	11.75	11.75	-88.89	26.67	20.00	361.4
3	25.00	25.00	11.75	11.75	-38.89	17.33	20.00	361.4
4	12.00	12.00	11.75	11.75	-18.92	23.56	20.00	361.4
5	6.00	6.00	11.75	11.75	-8.89	23.56	20.00	361.4

FIGURE 45


MALATE-ZINC INTERACTION
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
PA(I,E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
0.15	3.5
0.20	7.3
0.30	13.7
0.40	19.0
0.50	23.4
0.60	27.2
0.70	30.5
0.80	33.6
1.00	39.0
1.20	43.6
1.50	49.3
1.80	54.2
2.10	58.6
2.50	63.6
3.00	69.1
3.50	74.1
4.00	78.5
4.50	82.4
5.00	86.2
6.00	93.0
7.00	99.1
8.00	104.8
9.00	110.0
10.00	114.9
11.00	119.4

Titration number 2

volume added (ml)	emf (mV)
0.10	22.0
0.15	24.5
0.20	26.9
0.25	29.0
0.30	31.0
0.40	34.7
0.50	38.0
0.60	41.0
0.80	46.3
1.00	50.9
1.20	55.1
1.50	60.4
1.80	65.1
2.20	70.8
2.50	74.5
3.00	80.4
3.50	85.6
4.00	90.4
4.50	94.9
5.00	99.2
7.00	113.9
8.00	120.3
10.00	131.1
11.00	135.9
13.00	144.1

Titration number 1 continued

volume added (ml)	emf (mV)
13.00	127.6
15.00	134.7
16.00	137.8
18.00	143.7
20.00	148.8
23.00	155.4
26.00	161.1
30.00	167.7
33.00	172.0
36.00	175.8
40.00	180.5
45.00	185.1
50.00	189.3
55.00	193.4
60.00	197.4
65.00	200.9
70.00	204.4

Titration number 2 continued

volume added (ml)	emf (mV)
15.00	151.0
16.00	154.2
18.00	159.8
20.00	164.8
22.00	169.3
25.00	175.6
28.00	181.1
30.00	184.5
33.00	189.2
37.00	195.1
40.00	199.1
44.00	204.1

Titration number 3

volume added (ml)	emf (mV)
0.20	99.9
0.40	102.1
0.60	104.2
0.80	106.2
1.00	108.2
1.30	111.1
1.60	113.8

Titration number 3 continued

volume added (ml)	emf (mV)
2.20	118.9
2.50	121.3
3.00	124.9
3.40	127.7
3.80	130.3
4.00	131.5
4.50	134.5
5.00	137.3
5.50	139.9
6.00	142.4
6.50	144.7
7.00	147.0
7.50	149.1
8.00	151.2
8.50	153.2
9.00	155.0
10.00	158.4
11.00	161.7
12.00	164.8
13.00	167.7
14.00	170.4
15.00	172.9
16.00	175.4

Titration number 4

volume added (ml)	emf (mV)
0.50	117.0
0.80	120.9
1.00	123.4
1.40	127.8
2.00	133.9
2.50	138.5
3.00	142.9
3.50	146.8
4.00	150.6
4.50	154.2
5.00	157.5
5.50	160.7
6.00	163.9
6.50	166.9
7.00	169.7
7.50	172.4
8.00	175.2
8.50	177.8
9.00	180.3
9.50	182.8
10.00	185.2
11.00	189.9
11.50	192.1

Titration number 3 continued

volume added (ml)	emf (mV)
18.00	179.9
20.00	184.2
22.00	188.0
23.00	189.9
25.00	193.4
28.00	198.3
30.00	201.3
32.00	204.1
35.00	207.8

Titration number 4 continued

volume added (ml)	emf (mV)
12.00	194.3
12.50	196.3
13.00	198.3
13.50	200.2
14.00	202.1
15.00	205.6
16.00	208.8
18.05	214.6
19.00	216.9
20.00	219.1
21.00	221.1

Titration number 5

volume added (ml)	emf (mV)
0.00	122.6
0.10	124.4
0.20	126.2
0.30	127.9
0.50	131.0
0.70	134.0
0.90	137.0
1.10	139.9
1.50	145.1
1.70	147.6
2.00	151.2
2.30	154.7
2.50	156.9
2.80	160.1
3.10	163.3
3.50	167.3
4.00	172.1
4.50	176.6
5.00	180.9
5.50	185.0
6.00	188.9
6.50	192.6
7.00	196.0
7.40	198.4

Titration number 5 continued

volume added (ml)	emf (mV)
7.80	200.9
8.00	202.0
8.60	205.3
9.00	207.2
10.00	211.6
10.50	213.7
11.00	215.5
11.40	216.9
11.80	218.1
12.00	218.8
12.40	219.9

Total number of readings = 184.

The data was analysed using the MINIQUAD program which was offered a range of species having $p = 0 \rightarrow 3$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 2.90 \pm 0.02$$

$$\log \beta_{310} = 4.65 \pm 0.04$$

$$\log \beta_{111} = 6.24 \pm 0.03$$

$$\log \beta_{11-1} = -3.64 \pm 0.03$$

$$\log \beta_{112} = 8.87 \pm 0.04$$

These constants gave a sum of squares = 5.68×10^{-6}. The system can thus be described by the five complexes $Zn(malate)^{o}$, $Zn(malate)_3^{4-}$, $Zn(malate)H^{+}$, $Zn(malate)OH^{-}$, and $Zn(malate)H_2^{2+}$.

The zinc-malate complexes produced a pattern of formation curves as the malate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy, or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 46).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 47). These models require the total concentrations of zinc and malate (7.65 and 15.30 mM respectively) and the formation constants from table 49.

FIGURE 46

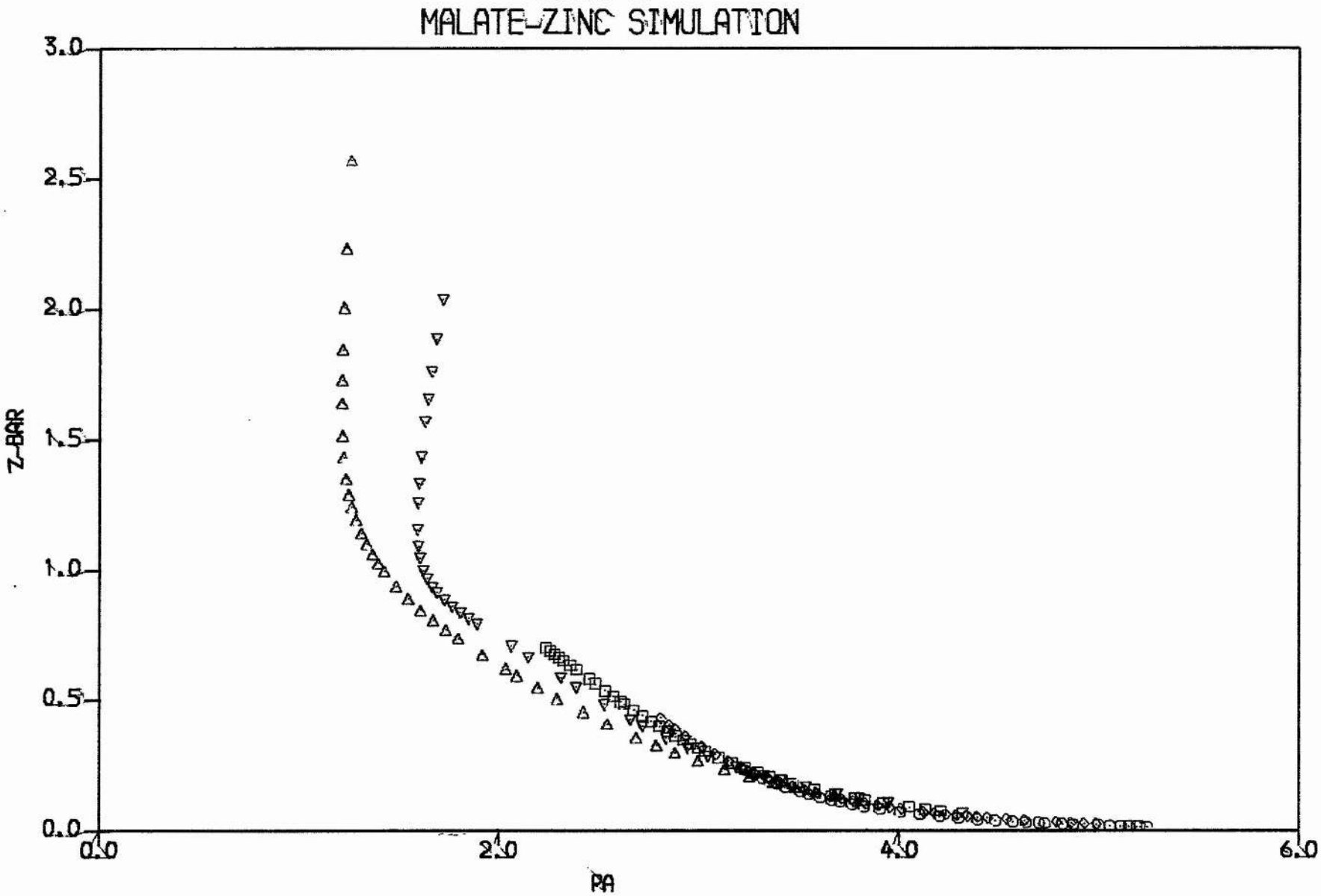


FIGURE 47

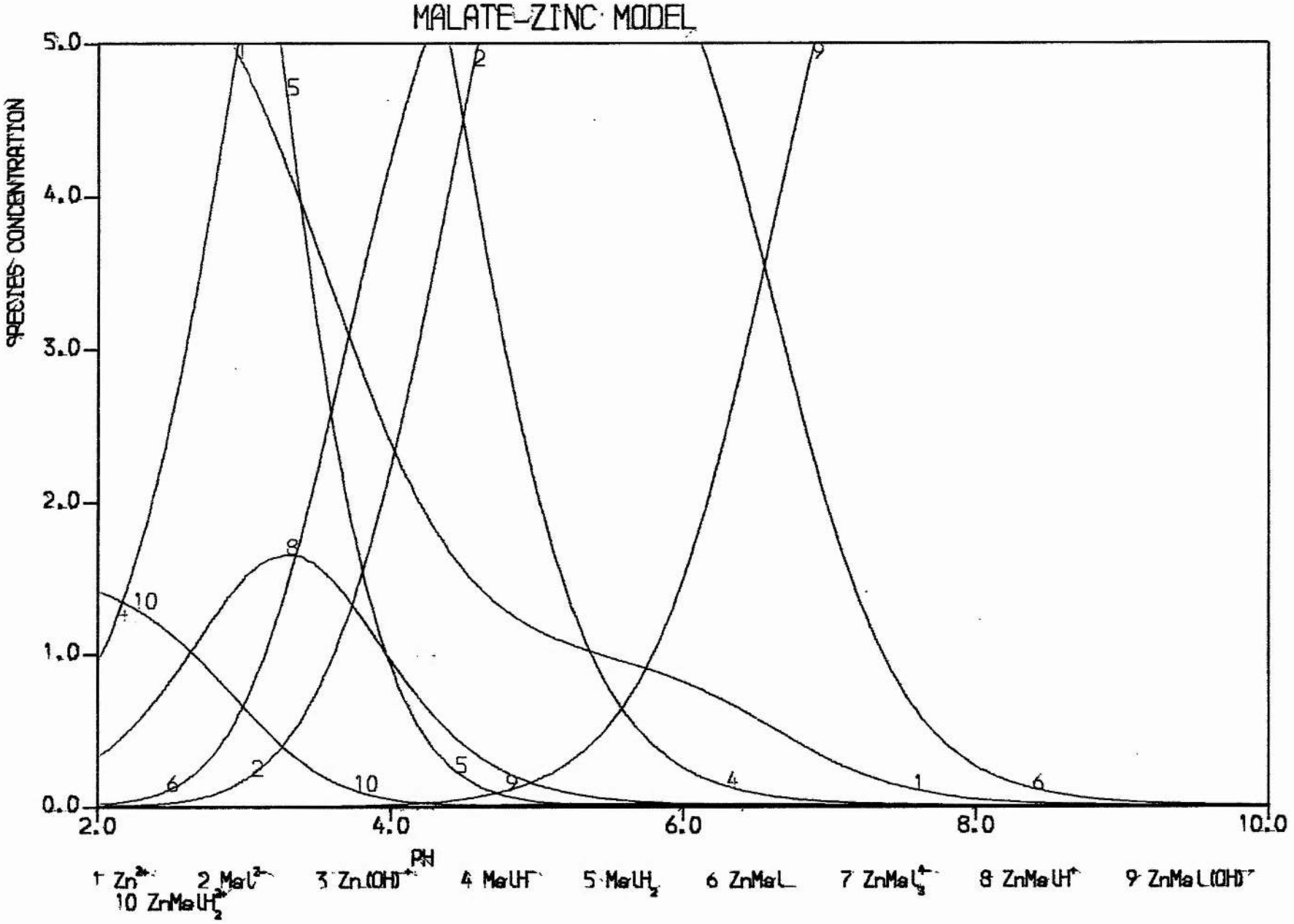

MALATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 MaL2-
3 Zn(OH)+
4 MaLH-
5 MaLH2
6 ZnMaL
7 ZnMaL4-
8 ZnMaLH+
9 ZnMaL(OH)-
10 ZnMaLH2 2+

Table 49 below lists our formation constants of the complex formation of malate and some of the published results from other workers as a comparison.

TABLE 49

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (Θ_c/°C, I/M, log β)	Ref
	1	0	1	4.48	0.01	183	30, 0.1(KCl), β_1 4.78, β_2 8.00	102
	1	0	2	7.59	0.01		20, 0.1($NaClO_4$), β_1 4.72, β_2 8.00	103
Zn	1	1	0	2.90	0.02	184	25, 0.2(KCl), β_1 2.80, $K_{101/111}$ 1.57*	101
	3	1	0	4.65	0.04		25, → 0, β_1 3.32, $K_{101/111}$ 2.00*	104
	1	1	1	6.24	0.03		20, 0.1($NaClO_4$), $K_{102/112}$ 1.66 †	105
	1	1	-1	-3.64	0.03		$K_{101/111}$ 2.93*	
	1	1	2	8.87	0.04			

* $K_{101/111} = K[B^{2+} + HA^{-} \rightleftharpoons BHA^{+}]$

† $K_{102/112} = K[B^{2+} + H_2A \rightleftharpoons BH_2A^{2+}]$

Protonation Constants of Malonate

The formation curve (figure 48) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\left.\begin{aligned} \log K_{101} &= 5.09 \pm 0.01 \\ \log K_{101 \rightarrow 102} &= 2.58 \pm 0.01 \end{aligned}\right\} \quad \text{(200 readings)}$$

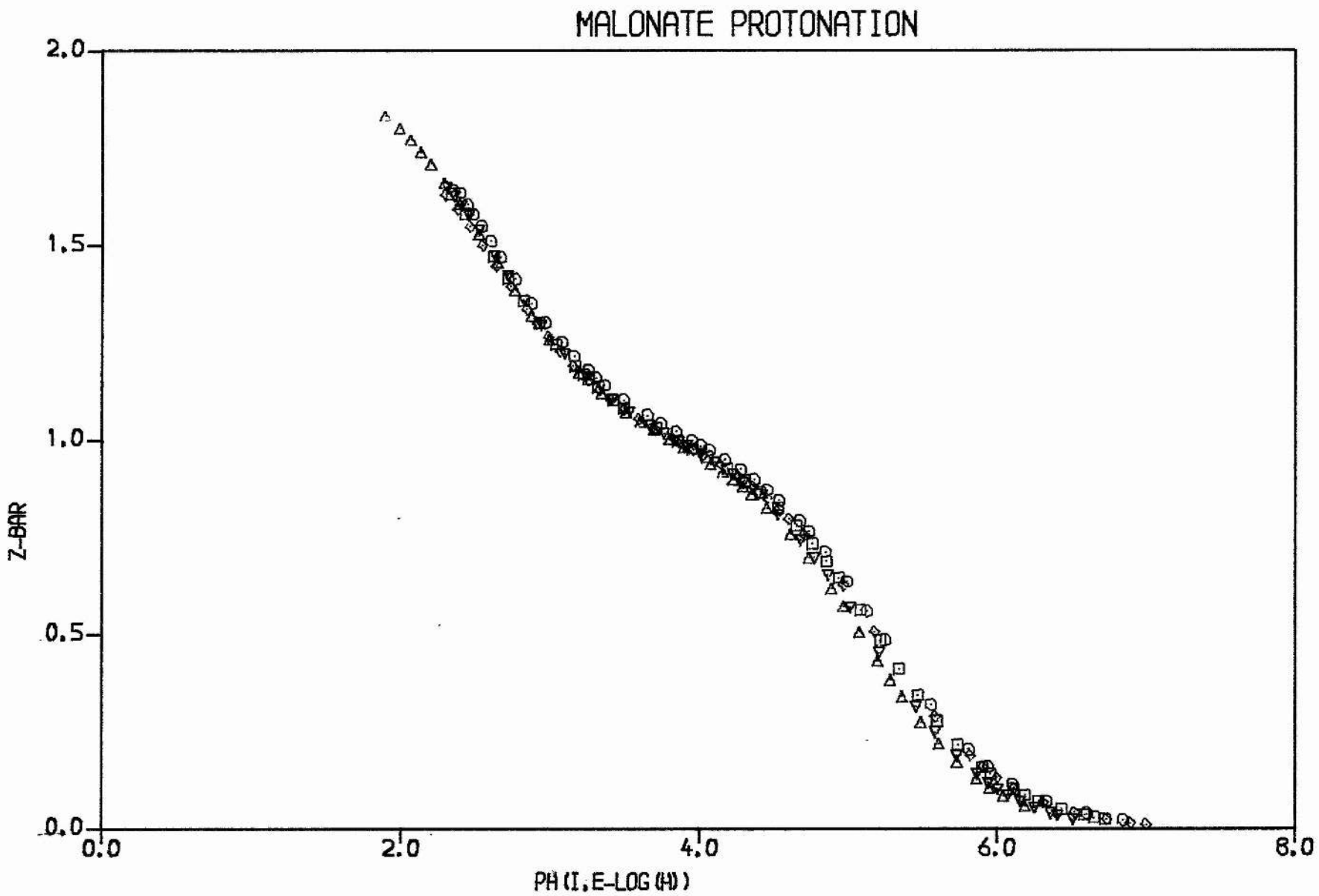
these constants gave a sum of squares = 4.03×10^{-5} ($mol^2\ dm^{-6}$).

TABLE 50

Experimental results for the protonation of malonate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^o (mV)
	A	H	A	H		
1	100.00	2.68	100.00	-250.07	20.00	358.1
2	50.00	2.68	50.00	-200.06	20.00	358.1
3	25.00	2.68	25.00	-100.03	20.00	358.8
4	10.00	2.68	10.00	-50.01	20.00	358.6
5	5.00	2.68	5.00	-40.01	20.00	358.6

FIGURE 48


MALONATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
PH(I,E-LOG(H))

Titration number 1

volume added (ml)	emf (mV)
0.50	241.7
1.00	235.6
1.40	231.2
1.80	227.0
2.20	222.8
2.80	217.2
3.50	211.0
4.50	203.0
5.50	195.3
6.50	188.1
7.50	181.1
8.50	173.7
10.00	161.5
11.00	152.5
12.00	142.4
12.50	136.4
13.00	130.6
13.50	124.4
14.00	118.5
15.00	107.4
15.50	102.4
16.00	98.0
16.50	94.0

Titration number 2

volume added (ml)	emf (mV)
1.60	216.3
1.80	213.3
2.00	210.3
2.20	207.2
2.50	203.0
3.00	196.5
3.40	191.0
3.90	184.3
4.40	177.1
5.00	167.6
5.50	158.7
6.00	148.1
6.30	141.0
6.60	132.7
6.80	126.6
7.00	120.6
7.10	117.4
7.30	111.4
7.50	105.8
7.80	98.3
8.00	93.9
8.30	87.8
8.80	79.5

Titration number 1 continued

volume added (ml)	emf (mV)
17.00	90.4
18.00	84.1
20.00	74.2
22.00	66.8
25.00	57.5
27.00	52.5
30.00	45.9
34.00	38.3
37.00	33.1
40.00	28.3
45.00	20.6
50.00	13.1
55.00	5.5
60.00	-2.6
63.00	-8.0
66.00	-13.8
70.00	-22.7

Titration number 2 continued

volume added (ml)	emf (mV)
9.50	70.3
10.00	64.6
10.50	58.9
11.50	49.8
13.00	37.8
15.00	22.6
16.00	14.7
17.00	5.8
17.80	-2.7
18.20	-7.4
18.50	-11.3
18.80	-15.6
19.10	-20.6
19.40	-26.4
19.70	-33.3
19.80	-36.0
20.00	-42.4

Titration number 3

volume added (ml)	emf (mV)
1.50	215.1
2.00	209.4
3.00	197.7
3.50	191.6
4.00	185.2
4.50	178.7
5.00	171.7
5.50	163.8
5.70	160.4
5.80	158.5
6.00	154.6
6.30	148.5
6.50	143.9
7.00	130.6
7.30	121.7
7.50	115.6
7.70	109.6
8.00	101.2
8.30	93.9
8.60	87.5
9.00	80.2
9.50	72.6
10.00	66.1

Titration number 4

volume added (ml)	emf (mV)
0.50	217.3
1.00	212.4
1.50	207.3
2.00	202.0
2.50	196.3
3.00	190.2
3.50	183.6
3.80	179.4
4.10	174.7
4.40	169.9
4.70	164.6
5.00	158.4
5.20	154.0
5.40	149.1
5.60	143.7
5.80	137.6
6.00	130.6
6.20	122.9
6.30	119.0
6.40	115.0
6.50	111.1
6.70	103.6
6.90	96.5

Titration number 3 continued

volume added (ml)	emf (mV)
10.50	60.3
11.00	55.2
12.00	46.1
13.00	37.9
14.00	30.2
15.00	22.5
16.00	14.5
17.00	5.8
18.00	-4.3
18.30	-7.7
19.00	-17.1
19.30	-21.9
19.60	-27.5
20.00	-37.0
20.30	-46.6
20.40	-50.5
20.50	-55.0

Titration number 4 continued

volume added (ml)	emf (mV)
7.10	90.2
7.30	84.6
7.50	79.7
7.70	75.4
8.00	69.3
9.00	53.2
10.00	40.1
12.00	15.2
13.00	0.6
13.30	-4.7
13.60	-10.6
13.90	-17.6
14.30	-29.6
14.60	-42.6
14.80	-55.8
14.90	-65.8
14.95	-72.3

Titration number 5

volume added (ml)	emf (mV)
0.00	213.9
0.20	211.4
0.50	208.2
0.70	206.0
1.00	202.3
1.30	198.5
1.60	194.5
2.00	188.4
2.40	181.6
2.70	175.9
3.00	169.1
3.20	164.1
3.40	158.4
3.50	155.3
3.60	151.9
3.80	143.8
4.00	134.0
4.10	128.3
4.20	122.1
4.30	115.5
4.35	112.0
4.40	108.5
4.50	102.0

Titration number 5 continued

volume added (ml)	emf (mV)
4.60	95.5
4.70	89.8
4.80	84.5
4.90	79.6
5.10	71.1
5.20	67.3
5.40	60.5
5.70	51.6
6.00	43.3
6.30	35.5
7.00	16.8
7.50	1.1
7.70	-7.0
7.90	-17.1
8.10	-31.0
8.25	-47.7
8.35	-62.6

Formation Constants for Zn(II) - Malonate Complexes

To within the limits of hydrolysis and solubilities, this system was studied using a pattern of titrations where both A and B (the total concentrations of malonate and zinc respectively) were held constant and equal in titrate and titrant, the sole difference in these solutions being their perchloric acid concentrations.

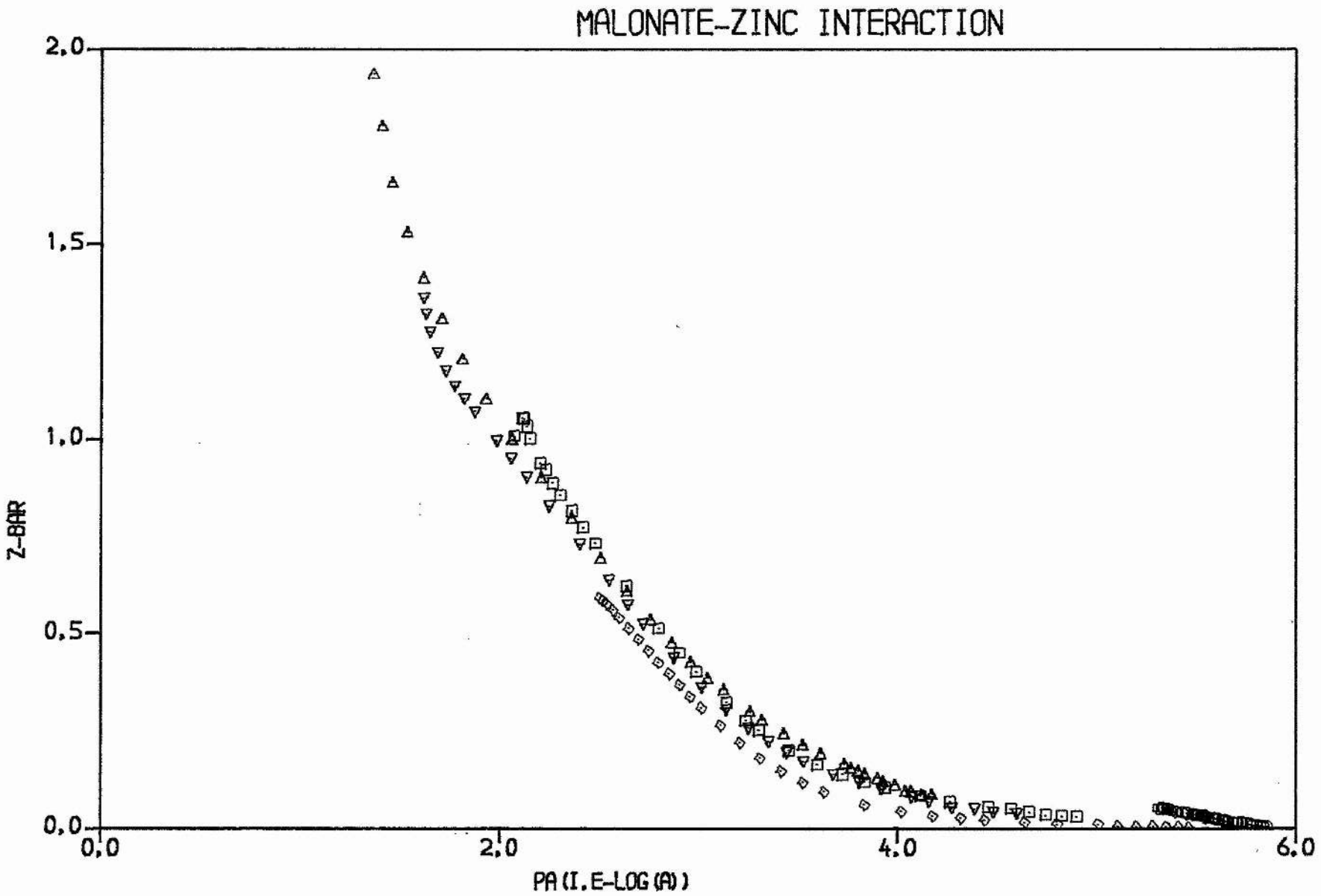
The formation curve (figure 49) was obtained from the results in table 51.

TABLE 51

Experimental results for the Zn(II) - malonate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	85.00	85.00	9.98	9.98	-159.62	26.53	20.00	358.7
2	42.50	42.50	9.98	9.98	-79.68	23.85	20.00	358.7
3	21.25	21.25	9.98	9.98	-39.58	21.17	20.00	358.7
4	10.62	10.62	9.98	9.98	-19.60	19.85	20.00	358.7
5	5.31	5.31	9.98	9.98	0.43	18.49	20.00	358.7

FIGURE 49


MALONATE-ZINC INTERACTION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
PA(I.E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
1.00	22.7
1.50	29.5
2.20	37.8
3.00	45.9
4.00	55.2
5.00	63.9
6.00	72.5
7.00	81.2
8.00	90.3
9.00	99.9
10.00	110.1
11.00	120.1
12.00	129.2
13.00	137.4
14.00	144.5
15.00	150.6
16.00	155.9
17.00	160.5
18.00	164.7
20.00	171.6
21.00	174.6
23.00	179.9
25.00	184.4

Titration number 2

volume added (ml)	emf (mV)
0.00	5.6
0.20	11.0
0.50	17.9
1.00	27.4
1.50	35.3
2.00	42.3
2.50	48.8
3.00	54.9
4.00	66.4
4.60	73.2
5.20	80.0
6.00	89.1
7.00	100.7
7.90	111.0
8.50	117.5
9.00	122.8
10.00	132.7
11.00	141.2
12.00	148.6
13.00	155.0
14.00	160.6
15.00	165.5
16.00	169.8

Titration number 1 continued

volume added (ml)	emf (mV)
27.00	188.4
30.00	193.6
31.00	195.1
32.00	196.6
33.00	198.0
35.00	200.7
36.00	201.9
38.00	204.3
40.00	206.4
41.00	207.5
43.00	209.5
45.00	211.5

Titration number 2 continued

volume added (ml)	emf (mV)
18.00	177.2
20.00	183.4
22.00	188.6
25.00	195.1
27.00	198.8
30.00	203.6
33.00	208.1
36.00	211.8
40.00	216.3

Titration number 3

volume added (ml)	emf (mV)
0.00	17.5
0.10	22.7
0.15	24.4
0.30	28.6
0.50	33.4
1.00	44.0
1.20	48.0
1.50	53.3
1.80	58.4
2.20	65.0
2.60	71.1
3.00	77.2
4.00	91.3
5.00	104.7
5.60	112.4
6.10	118.6
7.00	129.1
7.60	135.5
8.00	139.7
9.00	148.9
10.00	157.0
11.00	164.0

Titration number 4

volume added (ml)	emf (mV)
0.00	28.5
0.10	32.6
0.20	36.3
0.40	42.9
0.60	48.6
0.90	56.3
1.20	63.3
1.50	69.7
1.80	75.5
2.10	81.3
2.40	86.6
2.70	91.7
3.00	96.8
3.50	104.9
4.00	112.8
4.50	120.4
5.00	128.0
5.50	135.2
6.00	142.0
7.00	154.1
8.00	164.2
9.00	172.3

Titration number 3 continued

volume added (ml)	emf (mV)
12.00	169.9
13.00	175.0
15.00	183.5
17.00	190.3
20.00	198.6
22.00	203.3
24.00	207.0
26.00	210.3
28.00	213.4
30.00	216.2

Titration number 4 continued

volume added (ml)	emf (mV)
10.00	179.2
11.00	184.8
13.00	193.8
15.00	200.7
18.00	208.9
20.00	212.8
22.00	216.3
24.00	219.4
26.00	221.9
28.00	224.1
30.00	226.0

Titration number 5

volume added (ml)	emf (mV)
0.00	207.5
0.10	208.1
0.20	208.5
0.40	209.3
0.60	210.0
0.90	211.1
1.30	212.5
1.60	213.5
2.00	214.7
2.10	215.1
2.30	215.7
2.60	216.4
2.80	217.0
3.00	217.4
3.20	217.9
3.50	218.7
3.80	219.3
4.00	219.8
4.40	220.6
4.70	221.2
5.00	221.8
5.50	222.7

Titration number 5 continued

volume added (ml)	emf (mV)
6.00	223.6
6.50	224.5
7.00	225.3
7.50	226.0
8.00	226.6
8.50	227.3
9.00	227.9
9.50	228.5
10.00	229.1

Total number of readings = 168.

The data was analysed using the MINIQUAD program which was offered a range of species having $p = -0 \rightarrow 3$, $q = -0 \rightarrow 2$, and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' constants obtained were

$$\log \beta_{110} = 2.64 \pm 0.08$$

$$\log \beta_{310} = 5.79 \pm 0.03$$

$$\text{Log } \beta_{111} = 5.85 \pm 0.07$$

$$\text{Log } \beta_{311} = 10.62 \pm 0.06$$

$$\log \beta_{31-1} = -0.98 \pm 0.09$$

$$\log \beta_{312} = 14.70 \pm 0.13$$

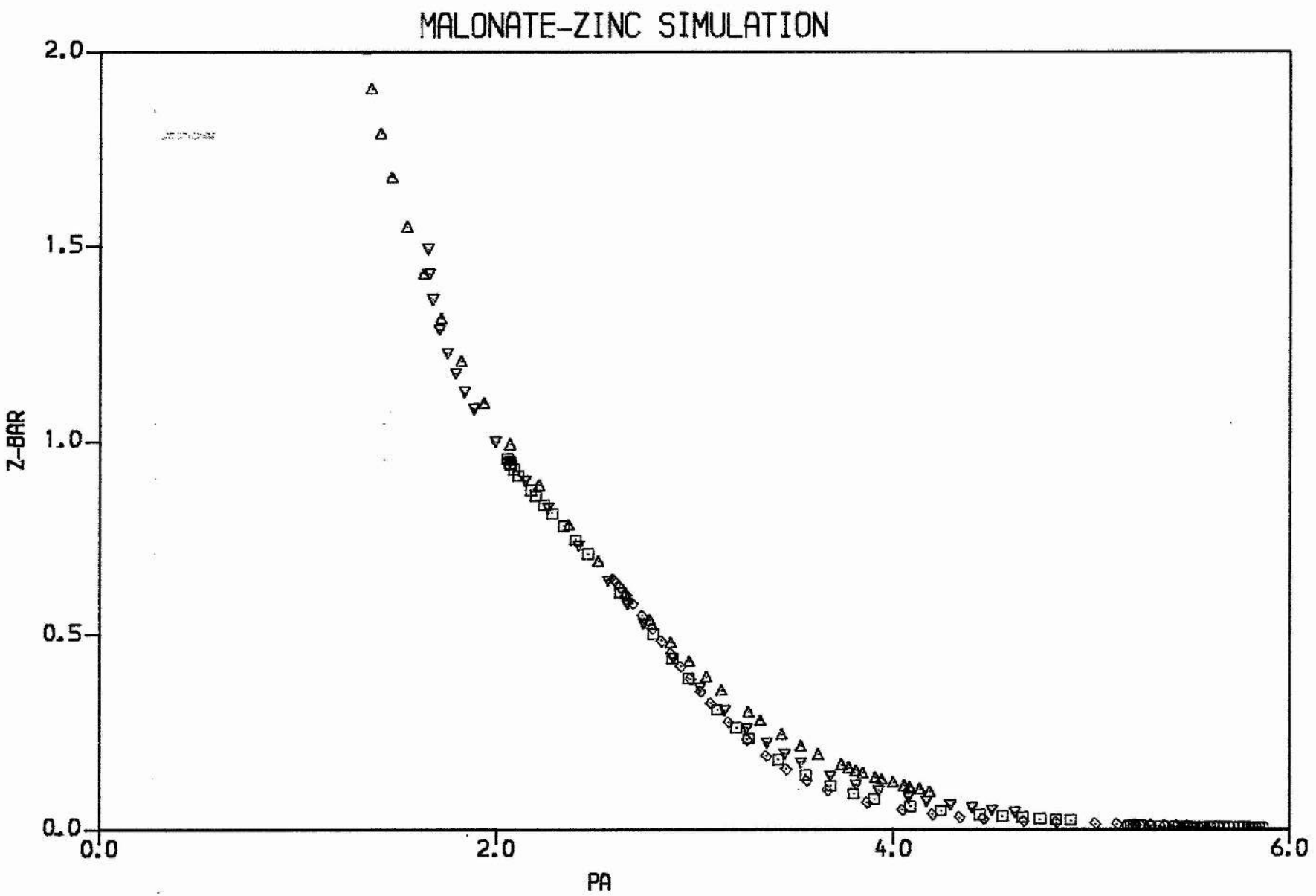
$$\log \beta_{220} = 7.26 \pm 0.17$$

$$\log \beta_{221} = 10.92 \pm 0.19$$

These constants gave a sum of squares = 5.45×10^{-6}. The system can thus be described by eight complexes: $Zn(malonate)^{o}$, $Zn(malonate)_3^{4-}$, $Zn(malonate)H^{+}$, $Zn(malonate)_3H^{3-}$, $Zn(malonate)_3OH^{5-}$, $Zn(malonate)_3H_2^{2-}$, $Zn_2(malonate)_2^{o}$, and $Zn_2(malonate)_2H^{+}$.

The zinc-malonate complexes produced a pattern of formation curves as the malonate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy, or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 50).

FIGURE 50



The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 51). These models require the total concentrations of zinc and malonate (7.65 and 15.30 mM respectively) and the formation constants from table 52.

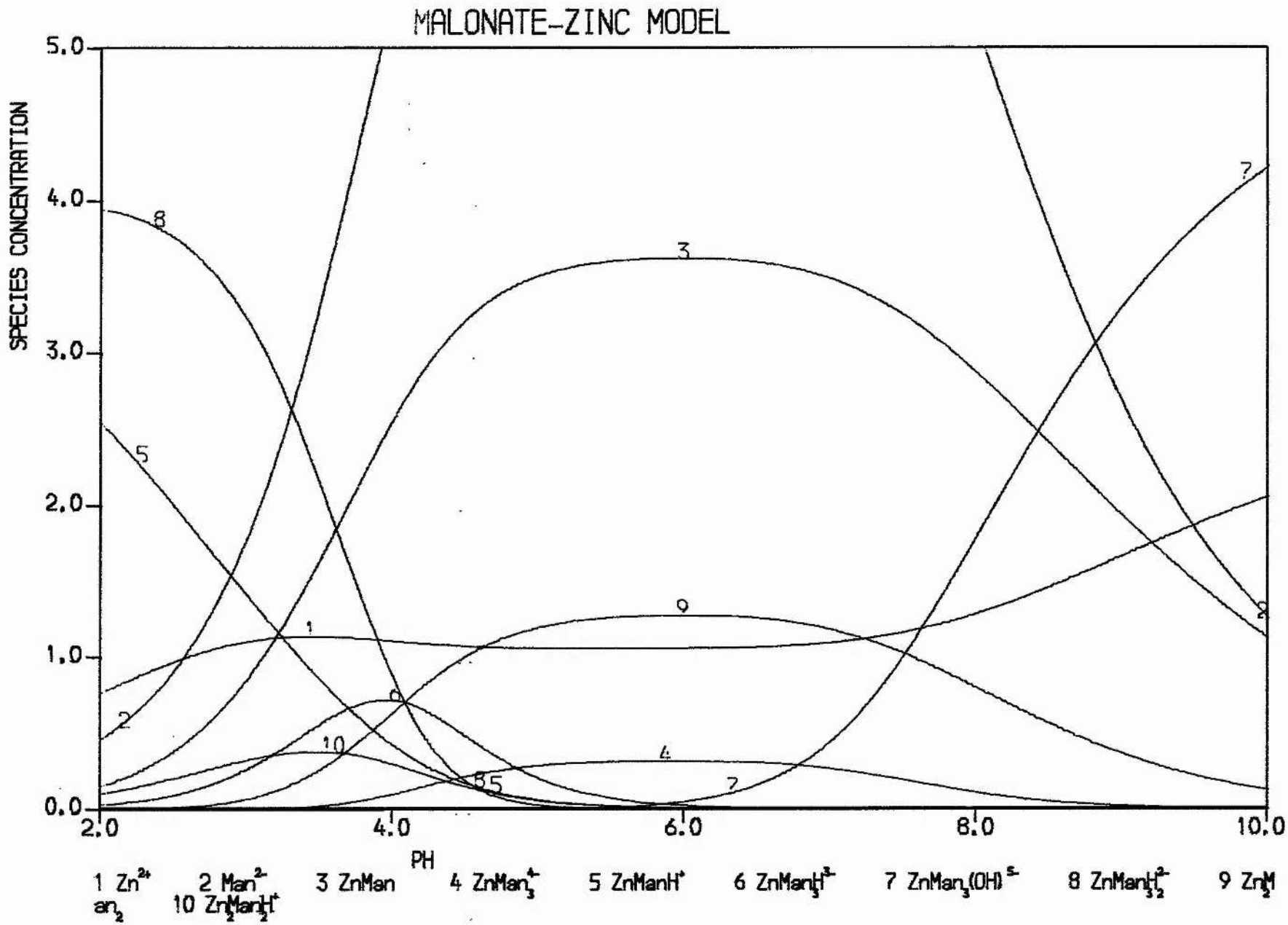
Table 52 below lists our formation constants of the complex formation of malonate and some of the published results from other workers as a comparison.

TABLE 52

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (θ_c/°C, I/M, logβ)	Ref
	1	0	1	5.09	0.01	200	25, 0.15($NaClO_4$), β_1 5.34, β_2 8.19	106
	1	0	2	7.67	0.01		30, 0.2($NaClO_4$), β_1 5.10, β_2 7.70	107
Zn	1	1	0	2.64	0.08	168	20, 0.1($NaClO_4$), β_1 2.97,	105
	3	1	0	5.79	0.03		$K_{101/111}$ 1.24	
	1	1	1	5.85	0.07		25, 0.001, β_1 3.35	108
	3	1	1	10.62	0.06		0-45, 0 corr., β_1(25°) 3.82	109
	3	1	-1	-0.98	0.09		25, 0 corr., β_1 3.85, β_2 5.95	110
	3	1	2	14.70	0.13		25, 0.2(KCl), β_1 2.78,	101
	2	2	0	7.26	0.17		$K_{101/111}$ 0.84	
	2	2	1	10.92	0.19		25, 0.1, β_1 2.7	111

FIGURE 51


MALONATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 Man2-
3 ZnMan
4 ZnMan3 4-
5 ZnManH+
6 ZnMan3H3-
7 ZnMan3(OH)5-
8 ZnMan3H32-
9 Zn2M
an2
10 Zn2Man2H+

Protonation Constants of Oxalate

The formation curve (figure 52) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\log K_{101} = 3.68 \pm 0.01 \qquad \text{(195 readings)}$$

$$\log K_{101 \rightarrow 102} = 1.08 \pm 0.03 \qquad \text{(195 readings)}$$

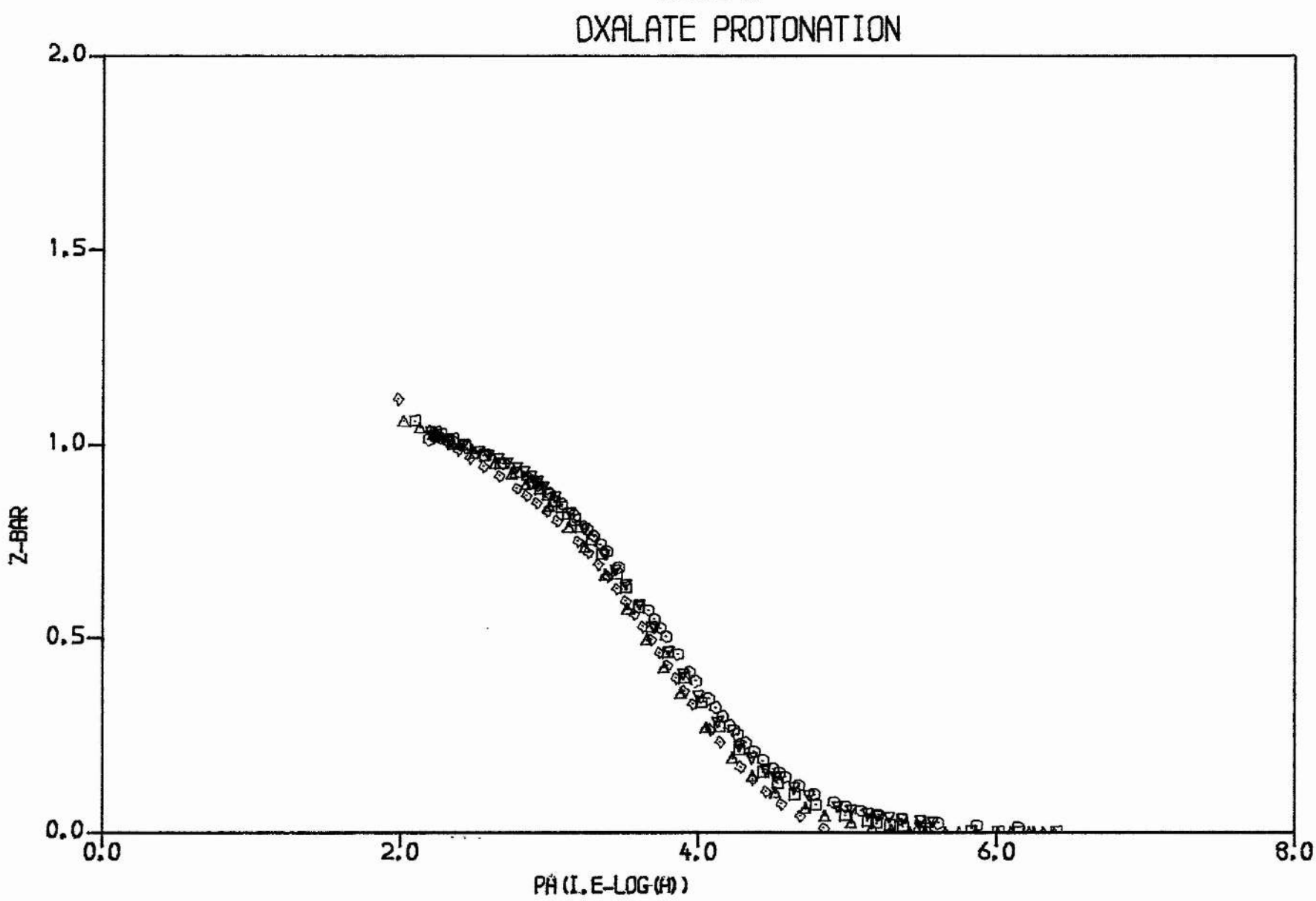
These constants gave a sum of squares = 1.51×10^{-4} $(mol^2\ dm^{-6})$.

TABLE 53

Experimental results for the protonation of oxalate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^o (mV)
	A	H	A	H		
1	100.00	2.68	100.00	-303.12	20.00	359.0
2	50.00	1.34	50.00	-250.07	20.00	358.9
3	25.00	1.34	25.00	-151.56	20.00	358.6
4	10.00	1.34	10.00	-100.03	20.00	360.0
5	5.00	1.34	5.00	-40.01	20.00	358.7

FIGURE 52


OXALATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
PH (I.E-LOG(H))

Titration number 1

volume added (ml)	emf (mV)
8.00	234.0
8.50	227.2
9.00	220.3
9.50	213.3
10.00	204.8
10.50	196.2
11.00	189.3
11.50	183.5
12.50	173.6
13.50	165.6
14.50	159.0
16.00	150.6
18.00	141.4
20.00	133.4
22.00	126.2
24.00	119.2
27.00	108.9
30.00	98.0
32.00	89.7
34.00	80.2
36.00	67.8
37.00	59.8
38.00	49.0

Titration number 2

volume added (ml)	emf (mV)
4.20	222.9
4.50	215.4
4.70	209.5
4.90	202.6
5.00	198.8
5.10	194.8
5.20	191.1
5.30	187.2
5.40	184.2
5.50	181.2
5.60	178.6
5.70	176.1
5.90	171.2
6.20	165.0
6.50	159.2
7.00	150.9
7.30	146.3
7.60	142.0
8.00	136.4
8.50	130.2
9.00	124.2
9.50	118.2
10.00	112.0

Titration number 1 continued

volume added (ml)	emf (mV)
38.60	40.2
39.00	32.4
39.30	24.8
39.50	18.4
39.70	10.0
39.80	4.4
39.90	-2.3
40.00	-11.6
40.05	-16.9
40.10	-24.5
40.11	-26.4
40.13	-30.3
40.15	-34.9

Titration number 2 continued

volume added (ml)	emf (mV)
10.60	104.1
11.20	95.1
11.50	90.0
11.80	84.3
12.00	80.0
12.30	72.5
12.50	66.4
12.80	54.6
12.90	49.0
13.00	42.2
13.05	37.9
13.10	33.2
13.15	27.9
13.20	20.3
13.23	15.2

Titration number 3

volume added (ml)	emf (mV)
2.50	229.1
3.00	220.2
3.10	218.0
3.30	213.2
3.50	208.3
3.80	200.0
4.00	193.6
4.20	187.1
4.40	180.3
4.50	177.1
4.60	174.0
4.70	171.1
4.80	168.2
4.90	165.5
5.00	162.7
5.10	160.3
5.30	155.8
5.50	151.6
5.80	145.6
6.00	141.8
6.30	136.3
6.60	130.9
7.00	124.2
7.40	117.6

Titration number 4

volume added (ml)	emf (mV)
0.00	237.4
1.00	224.0
1.20	220.4
1.40	216.6
1.60	212.3
1.80	207.5
2.00	201.9
2.20	195.4
2.40	188.2
2.50	184.3
2.60	180.0
2.70	175.6
2.80	171.5
3.00	162.9
3.10	158.6
3.20	154.5
3.30	150.6
3.40	146.9
3.50	143.2
3.60	139.7
3.70	136.0
3.80	132.6
3.90	129.1
4.00	125.7

Titration number 3 continued

volume added (ml)	emf (mV)
7.80	110.5
8.20	103.0
8.60	94.7
9.00	84.8
9.20	78.9
9.40	72.0
9.60	63.1
9.80	51.0
9.90	41.8
9.93	38.1
9.97	32.5
10.00	27.2
10.04	18.0
10.10	-1.0
10.12	-12.1
10.13	-20.8
10.14	-36.1

Titration number 4 continued

volume added (ml)	emf (mV)
4.10	122.2
4.20	118.8
4.30	115.3
4.50	108.1
4.60	104.2
4.80	95.6
4.90	90.7
5.00	85.1
5.10	78.7
5.20	71.0
5.30	61.3
5.50	22.8
5.51	18.0

Titration number 5

volume added (ml)	emf (mV)
0.00	223.2
0.20	221.2
0.50	218.4
0.80	215.5
1.00	213.3
1.50	207.5
2.00	200.8
2.50	192.5
3.00	182.6
3.20	178.0
3.40	173.2
3.60	168.2
3.80	163.0
4.00	157.7
4.10	155.0
4.20	152.4
4.30	149.8
4.50	144.7
5.00	132.4
5.10	130.0
5.20	127.5
5.30	125.1
5.50	120.2

Titration number 5 continued

volume added (ml)	emf (mV)
5.70	115.3
5.80	112.8
6.00	107.5
6.10	104.6
6.20	101.7
6.30	98.8
6.35	97.2
6.40	95.6
6.50	92.2
6.60	88.7
6.70	85.0
6.80	80.7
6.85	78.3
6.90	75.9
7.00	70.3
7.10	63.9
7.20	55.8
7.25	50.9
7.30	44.6
7.35	37.5
7.40	27.8
7.43	19.9
7.45	12.7
7.48	-3.1
7.50	-20.1

Formation Constants for Zn(II) - Oxalate Complexes

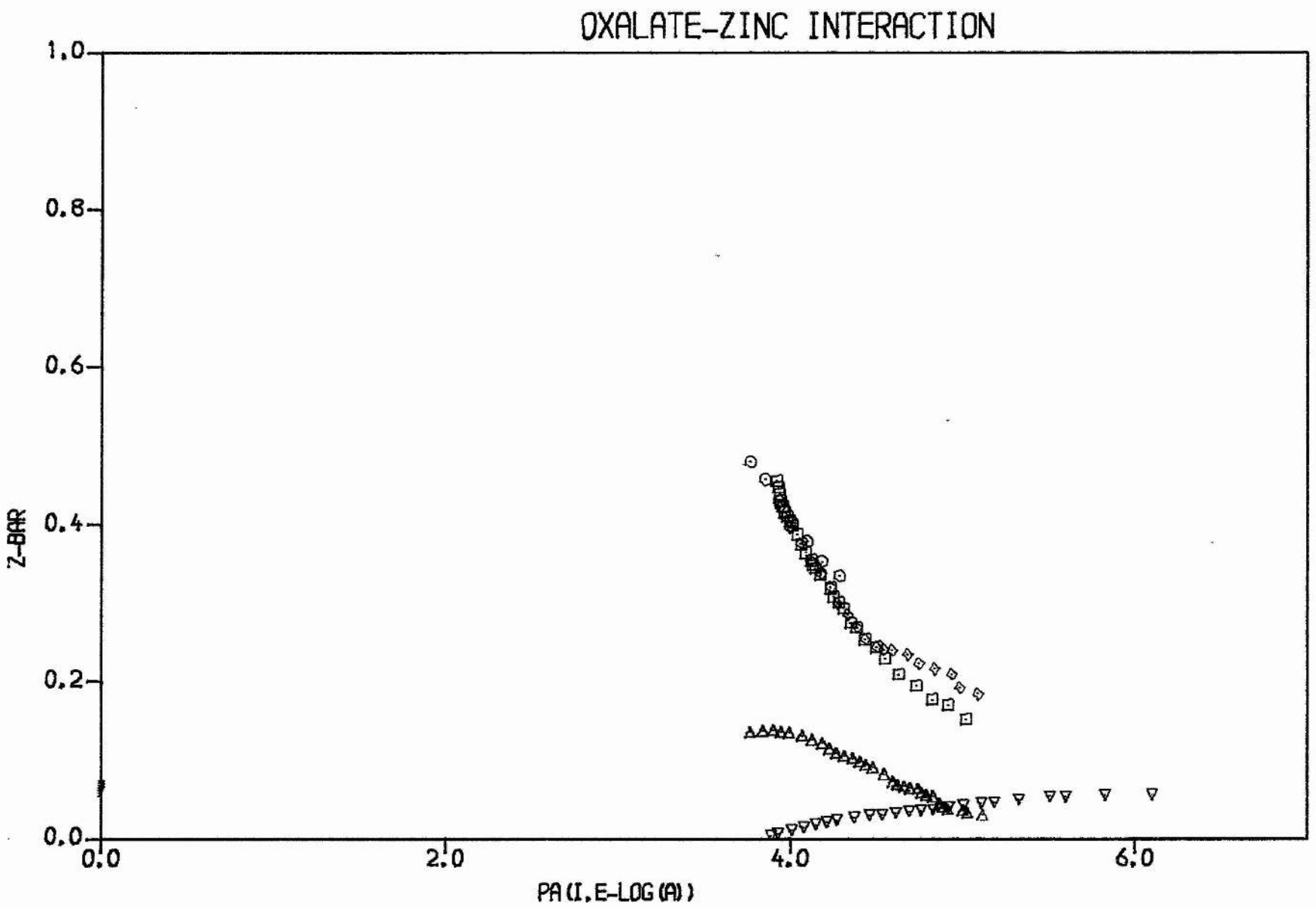
The formation curve (figure 53) was obtained from the results in table 54.

TABLE 54

Experimental results for the Zn(II) - oxalate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	5.00	4.99	0	10.58	-50.00	20.00	359.4
2	0	2.50	4.99	0	10.58	-50.00	20.00	359.4
3	0	10.00	4.99	0	10.58	-50.00	20.00	359.4
4	0	20.00	4.99	0	10.58	-100.00	20.00	357.7
5	0	38.46	4.99	0	10.58	-100.00	20.00	357.4

FIGURE 53


OXALATE-ZINC INTERACTION
1.0
0.8
0.6
0.4
0.2
0.0
Z-BAR
0.0
2.0
4.0
6.0
PA(I,E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
1.70	224.2
1.90	222.2
2.00	221.2
2.20	219.0
2.30	217.9
2.40	216.8
2.60	214.5
2.70	213.2
2.80	211.9
2.90	210.6
3.00	209.1
3.10	207.6
3.20	206.0
3.30	204.4
3.50	201.0
3.70	197.1
3.80	194.9
3.90	192.6
4.00	190.1
4.10	187.3
4.20	184.3
4.30	181.1
4.40	177.6

Titration number 2

volume added (ml)	emf (mV)
1.80	223.1
2.00	220.8
2.20	218.3
2.40	215.6
2.50	214.2
2.60	212.7
2.70	211.2
2.80	209.5
2.90	207.7
3.00	205.9
3.10	203.9
3.20	201.8
3.30	199.5
3.40	197.1
3.50	194.5
3.60	191.6
3.70	188.4
3.80	184.9
3.90	180.8
4.00	176.0
4.05	173.2
4.10	170.1
4.15	166.6

Titration number 1 continued

volume added (ml)	emf (mV)
4.50	173.4
4.60	168.7
4.70	163.0
4.75	159.6
4.80	156.0
4.85	151.6
4.90	146.5

Titration number 2 continued

volume added (ml)	emf (mV)
4.20	162.7
4.25	158.2
4.27	156.1

Titration number 3

volume added (ml)	emf (mV)
2.20	223.7
2.50	221.3
2.70	219.5
3.00	216.8
3.30	213.8
3.60	210.7
3.80	208.5
4.00	206.0
4.20	203.5
4.30	202.1
4.50	199.4
4.60	197.9
4.70	196.3
4.80	194.8
5.00	191.3
5.10	189.4
5.15	188.4
5.20	187.5
5.30	185.4
5.40	183.3
5.50	181.1
5.60	178.7
5.65	177.4
5.70	176.1
5.75	174.7
5.80	173.4
5.85	172.0
5.90	170.7
5.95	169.3
6.00	167.8

Total number of readings = 114.

Titration number 4

volume added (ml)	emf (mV)
1.20	222.3
1.30	220.7
1.40	219.2
1.50	217.4
1.60	215.5
1.70	213.6
1.80	211.4
1.85	210.2
1.90	209.0
2.00	206.6
2.10	204.2
2.20	201.6
2.30	198.7
2.40	195.8
2.50	192.4
2.60	188.6
2.70	184.5
2.80	179.7
2.85	177.2
2.90	174.5

Titration number 5

volume added (ml)	emf (mV)
1.80	221.1
2.00	218.7
2.20	216.3
2.40	213.7
2.60	211.0
2.80	208.1
3.00	204.8
3.10	202.9

The data was analysed using the MINIQUAD program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 4.05 \pm 0.06$$

$$\log \beta_{221} = 13.29 \pm 0.27$$

These constants gave a sum of squares = 1.84×10^{-6}. The system can thus be described by two complexes $Zn(oxalate)^{o}$, and $Zn_2(oxalate)_2H^{+}$.

The zinc-oxalate complexes produced a pattern of formation curves as the oxalate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 54).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 55). These models require the total concentrations of zinc and oxalate (7.65 and 15.30 mM respectively) and the formation constants from table 55.

Table 55 below lists our formation constants of the complex formation of oxalate and some of the published results from other workers as a comparison.

FIGURE 54

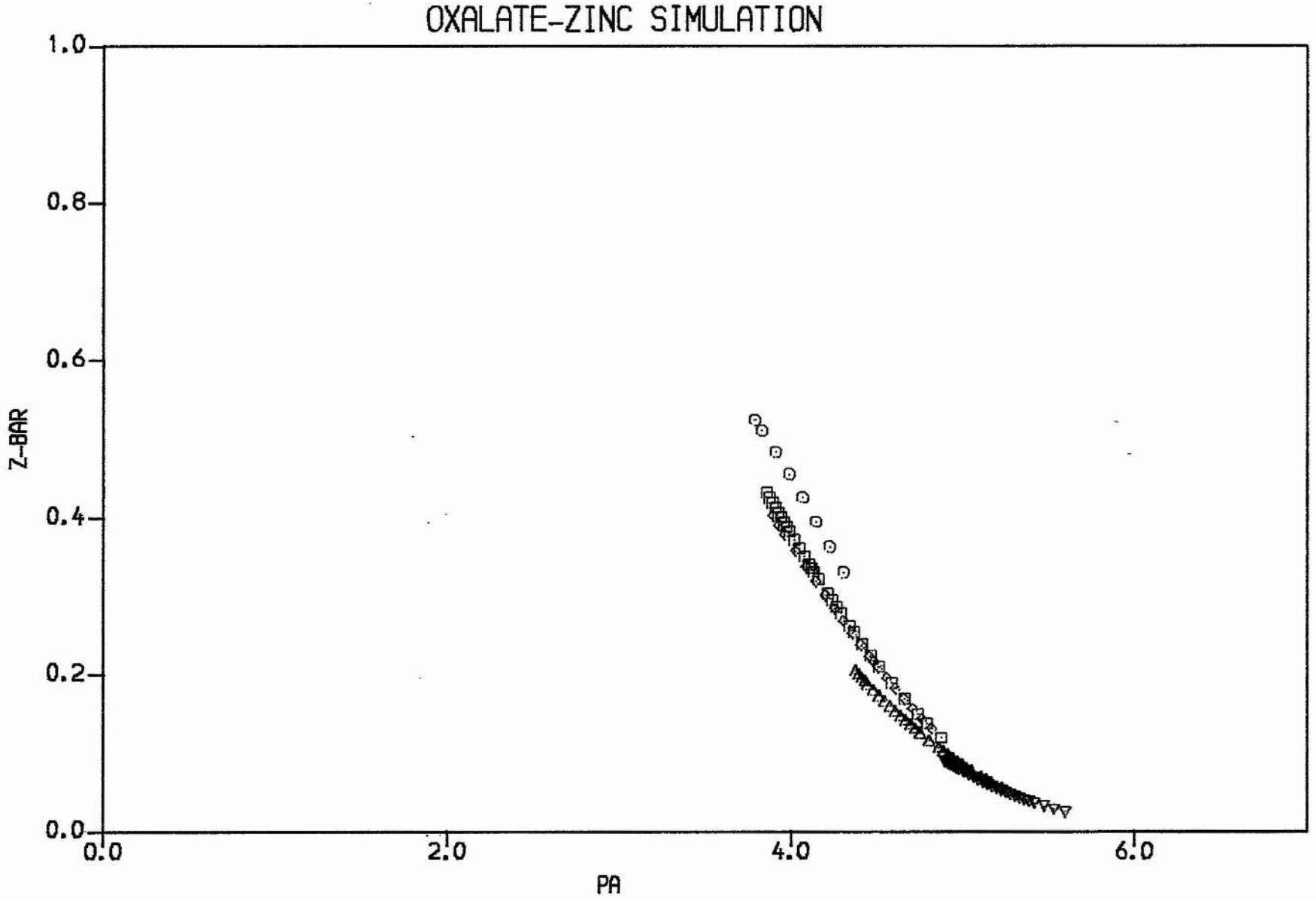


FIGURE 55

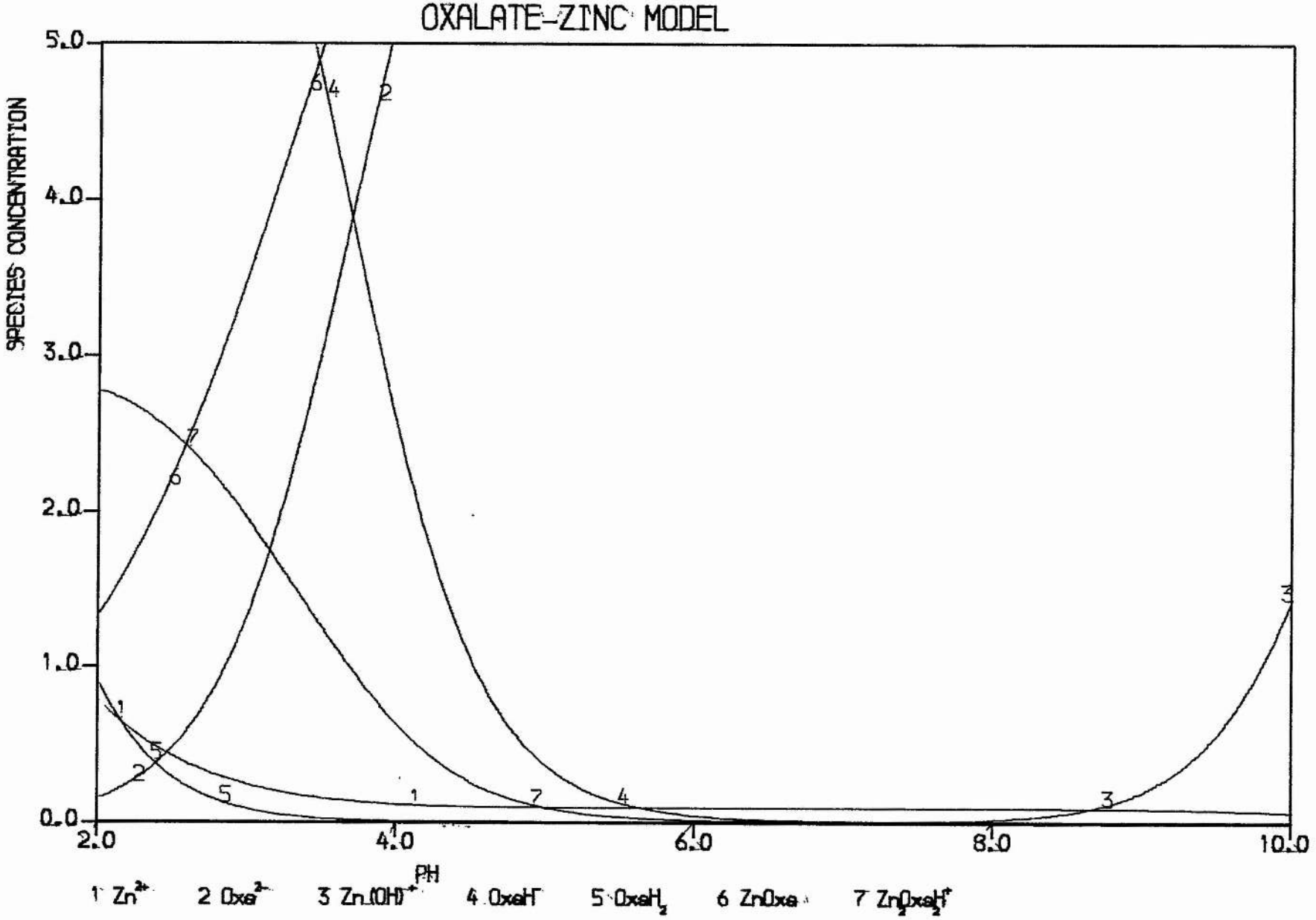

OXALATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 Oxa2-
3 Zn(OH)+
4 OxaH-
5 OxaH2
6 ZnOxa
7 Zn2Oxa2H+

TABLE 55

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data (θ_c/°C, I/M, logβ)	Ref
	1	0	1	3.68	0.01	200	25, 0.15($NaClO_4$), β_1 2.59, β_2 3.92	106
	1	0	2	4.77	0.03		25, 1 $NaClO_4$, β_1 3.54, β_2 ∿ 4.54	112
Zn	1	1	0	4.05	0.06	114	25, 1(KNO_3), β_1 3.44, β_2 6.48, β_3 7.24	113
	2	2	1	13.29	0.27		25, 0.1, β_1 4.9	114

Protonation Constants of Tartarate

The formation curve (figure 56) was obtained using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\left.\begin{aligned} \log K_{101} &= 3.69 \pm 0.01 \\ \log K_{101 \rightarrow 102} &= 2.80 \pm 0.01 \end{aligned}\right\} \quad \text{(197 readings)}$$

These constants gave a sum of squares = 8.79×10^{-5} ($mol^2\ dm^{-6}$).

TABLE 56

Experimental results for the protonation of tartarate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E° (mV)
	A	H	A	H		
1	100.00	-250.00	100.00	2.49	20.00	361.4
2	50.00	6.23	50.00	-151.50	20.00	361.4
3	25.00	6.23	25.00	-151.50	20.00	361.4
4	15.00	6.23	15.00	-151.50	20.00	361.4
5	10.00	6.23	10.00	-100.00	20.00	361.4
6	5.00	3.11	5.00	-50.00	20.00	359.8

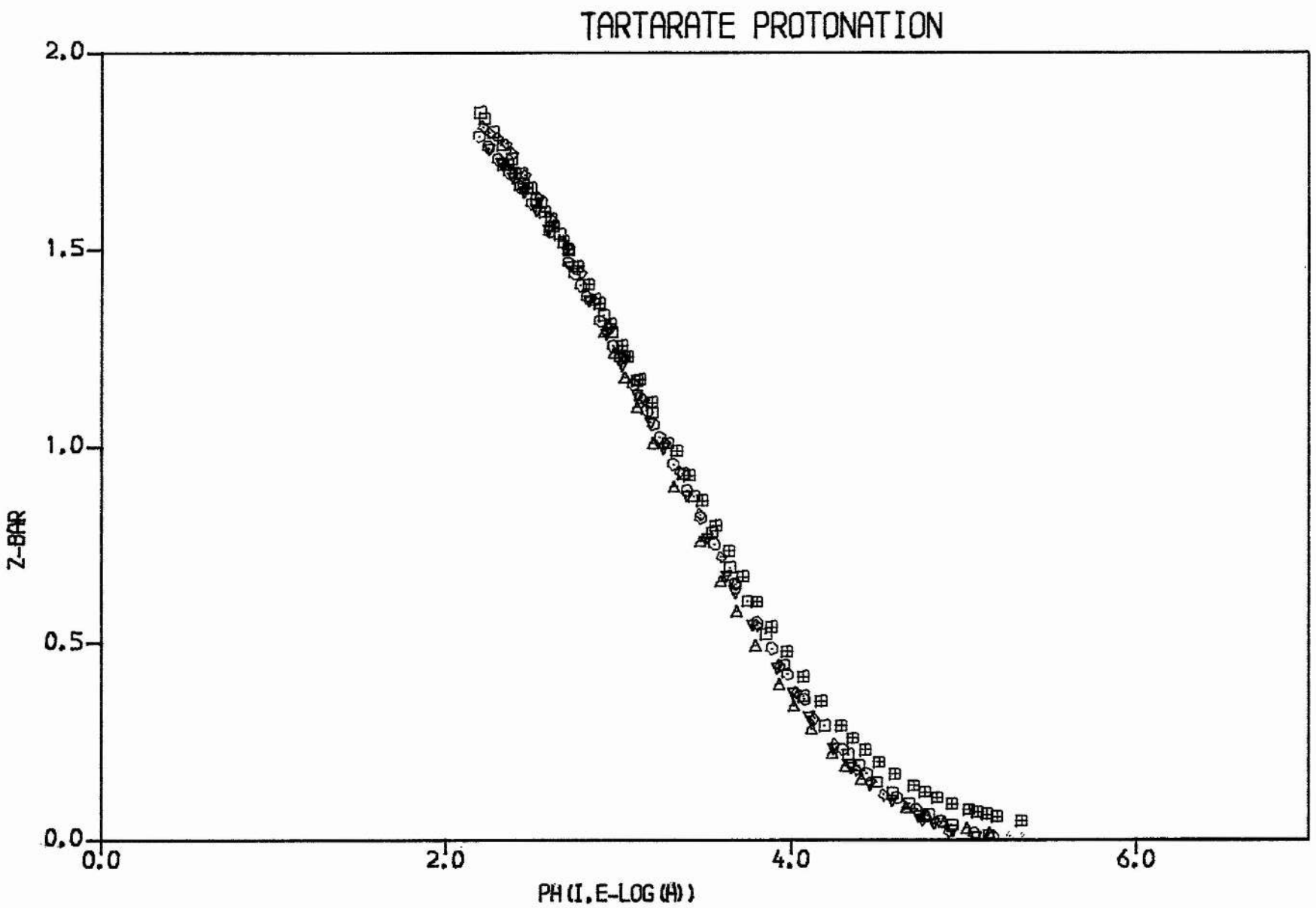
Titration number 1

volume added (ml)	emf (mV)
4.94	25.4
5.00	32.0
5.05	36.4
5.15	43.6
5.30	51.7
5.50	59.8
5.70	65.9
6.00	73.4

Titration number 2

volume added (ml)	emf (mV)
1.80	222.2
2.20	218.0
2.60	213.9
3.00	209.9
3.50	205.4
4.00	201.2
5.00	193.5
6.00	186.6

FIGURE 56


TARTARATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
PH(I,E-LOG(H))

Titration number 1 continued

volume added (ml)	emf (mV)
7.00	89.4
7.50	95.0
8.00	99.7
9.00	107.3
10.00	113.5
11.00	118.7
13.00	127.1
15.00	133.8
17.00	139.5
20.00	146.7
25.00	156.2
30.00	163.7
35.00	169.3
40.00	173.7
45.00	177.5
50.00	181.0

Titration number 2 continued

volume added (ml)	emf (mV)
7.00	180.4
8.00	174.8
9.00	169.5
10.00	164.7
11.00	160.1
13.00	151.7
15.00	144.4
17.00	137.5
18.00	134.4
20.00	128.3
23.00	119.6
25.00	113.9
27.00	108.2
30.00	99.5
32.00	93.2
34.00	86.3
36.00	78.5
38.00	69.1
39.00	63.5
40.00	56.8
41.00	48.5

Titration number 3

volume added (ml)	emf (mV)
0.50	225.4
0.60	223.9
0.80	220.8
1.00	217.5
1.20	214.1
1.40	210.7
1.60	207.3
1.80	203.8
2.00	200.3
2.20	197.0
2.40	193.7
2.60	190.6
3.00	184.4
3.20	181.3
3.40	178.3
3.70	174.0
4.00	169.5
4.40	164.0
4.80	158.5
5.20	153.1
5.50	149.2
6.00	142.7
6.50	136.3
7.00	130.0

Titration number 4

volume added (ml)	emf (mV)
0.40	224.5
0.50	222.0
0.60	219.1
0.70	216.2
0.80	213.8
1.00	209.3
1.20	204.4
1.40	199.4
1.60	194.4
1.80	189.3
2.00	184.4
2.20	179.2
2.40	174.3
2.70	166.9
3.00	159.4
3.20	154.3
3.50	146.9
3.80	139.4
4.00	134.2
4.30	126.6
4.60	118.5
4.80	112.6
5.00	106.2
5.20	99.0

Titration number 3 continued

volume added (ml)	emf (mV)
7.50	123.5
8.00	117.0
8.50	110.1
9.00	102.6
9.50	94.1
9.70	90.3
10.00	84.0
10.20	78.2
10.40	72.3
10.60	65.3
10.80	57.0
11.00	44.0

Titration number 4 continued

volume added (ml)	emf (mV)
5.40	91.3
5.60	81.4
5.80	67.8
5.90	58.3

Titration number 5

volume added (ml)	emf (mV)
0.40	225.8
0.60	222.5
0.80	219.0
1.00	215.2
1.20	211.2
1.40	207.1
1.70	200.8
2.00	194.0
2.10	191.8
2.20	189.5
2.30	187.3
2.50	182.6
2.70	178.0
2.80	175.6
3.00	170.8
3.10	168.4
3.20	166.0
3.30	163.7
3.40	161.3
3.60	156.5
3.80	151.6
4.00	146.7
4.20	141.8
4.50	134.4
4.80	126.6

Titration number 6

volume added (ml)	emf (mV)
0.00	215.6
0.10	214.0
0.30	211.3
0.50	208.5
0.60	207.0
0.80	204.0
1.00	200.8
1.20	197.6
1.40	194.2
1.50	192.4
1.70	188.9
1.90	185.1
2.10	181.2
2.30	177.3
2.50	173.2
2.60	171.3
2.80	167.0
3.00	162.6
3.20	158.2
3.40	153.7
3.60	149.2
3.80	144.6
4.00	139.8
4.20	135.0
4.40	130.2

Titration number 5 continued

volume added (ml)	emf (mV)
5.00	121.3
5.20	115.8
5.40	109.8
5.70	99.9
5.80	96.1
6.00	87.5
6.20	76.7
6.30	69.8
6.40	61.1
6.50	49.2
6.54	42.5

Titration number 6 continued

volume added (ml)	emf (mV)
4.60	125.2
4.80	119.9
5.00	114.4
5.20	108.6
5.40	102.2
5.60	95.0
5.70	91.0
5.80	86.5
5.90	81.5
6.00	75.8
6.10	69.2
6.15	65.2
6.20	60.8
6.25	55.5
6.30	49.4
6.32	46.4
6.34	43.0
6.36	39.4
6.40	30.6

Formation Constants for Zn(II) - Tartarate Complexes

To within the limits of hydrolysis and solubilities, this system was studied using a pattern of titrations where both A and B (the total concentrations of tartarate and zinc respectively) were held constant and equal in the titrate and titrant, the sole difference in these solutions being their perchloric acid concentrations.

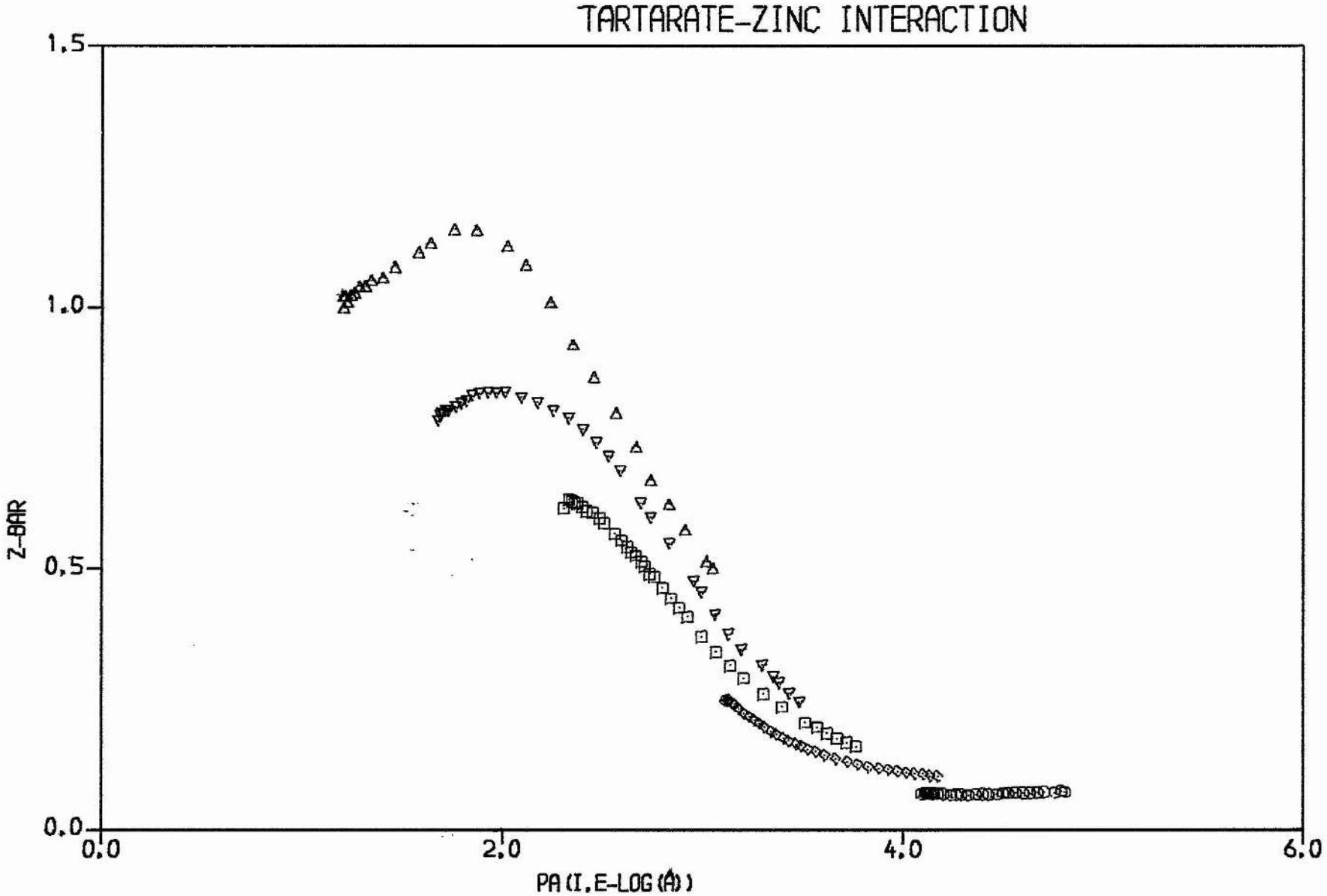
The formation curve (figure 57) was obtained from the results in table 57.

TABLE 57

Experimental results for the Zn(II) - tartarate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	80.00	80.00	9.98	9.98	-159.60	26.53	20.00	357.5
2	40.00	40.00	9.98	9.98	-69.58	23.85	20.00	357.2
3	20.00	20.00	9.98	9.98	-29.57	23.85	20.00	357.2
4	10.00	10.00	9.98	9.98	-9.57	23.85	20.00	357.1
5	5.00	5.00	9.98	9.98	0.43	21.17	20.00	357.1

FIGURE 57


TARTARATE-ZINC INTERACTION
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
PA(I,E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
0.50	61.1
0.60	65.7
0.70	69.6
0.80	73.2
1.00	79.1
1.30	86.3
1.60	92.1
2.00	98.5
2.50	105.0
3.00	110.5
4.00	119.4
5.00	126.7
7.00	138.3
8.00	143.2
10.00	151.7
12.00	158.8
15.00	167.6
17.00	172.5
20.00	178.7
23.00	183.9
26.00	188.6
29.50	192.4
33.00	197.6
36.00	200.6

Titration number 2

volume added (ml)	emf (mV)
0.20	109.8
0.30	111.3
0.40	112.7
0.60	115.3
0.80	117.7
1.20	122.2
1.50	125.3
1.80	128.2
2.10	131.0
2.50	134.4
3.00	138.3
3.50	141.9
4.00	145.3
5.00	151.3
6.00	156.7
7.00	161.5
8.00	165.9
9.00	169.8
10.00	173.3
11.00	176.5
12.00	179.4
14.00	184.4
15.00	186.7
17.00	190.9

Titration number 1 continued

volume added (ml)	emf (mV)
39.50	204.2
43.00	207.3
48.00	211.3
49.50	212.5

Titration number 2 continued

volume added (ml)	emf (mV)
20.00	196.2
21.00	197.8
23.00	200.7
25.00	203.4
27.00	206.0
30.00	209.9
32.00	212.1
33.00	213.1
35.00	215.0
37.00	216.9

Titration number 3

volume added (ml)	emf (mV)
0.00	140.1
0.10	141.9
0.20	143.0
0.30	144.1
0.40	145.2
0.60	147.2
0.80	149.1
1.00	151.1
1.30	153.7
1.50	155.3
2.00	159.1
2.30	161.2
2.60	163.2
2.80	164.4
3.00	165.7
3.30	167.5
3.50	168.6
3.80	170.1
4.00	171.3
4.50	173.7
5.00	176.0
5.50	178.2
6.00	180.3

Titration number 4

volume added (ml)	emf (mV)
0.00	173.6
0.05	174.2
0.10	174.8
0.20	175.5
0.30	176.3
0.50	177.6
0.70	178.9
0.90	180.3
1.10	181.6
1.30	182.9
1.50	184.1
1.80	185.9
2.00	187.1
2.30	188.8
2.50	189.9
2.80	191.6
3.00	192.7
3.30	194.2
3.60	195.8
4.00	197.7
4.50	200.0
5.00	202.3
5.50	204.4

Titration number 3 continued

volume added (ml)	emf (mV)
7.00	183.9
8.00	187.4
9.00	190.7
10.00	193.7
11.50	197.9
13.00	201.8
15.00	206.3
16.00	208.6
17.00	210.5
18.00	212.4
19.00	214.2
20.00	215.9

Titration number 4 continued

volume added (ml)	emf (mV)
6.00	206.4
6.50	208.4
7.00	210.2
7.50	211.9
8.00	213.5
8.50	215.0
9.00	216.5
9.50	217.8
10.00	219.1

Titration number 5

volume added (ml)	emf (mV)
0.00	204.8
0.05	205.3
0.10	205.6
0.15	205.9
0.25	206.4
0.35	207.0
0.50	207.8
0.70	208.8
1.00	210.2
1.20	211.2
1.40	212.1
1.70	213.4
2.00	214.7
2.30	215.9
2.60	216.9
3.00	218.3
3.30	219.4
3.60	220.4
4.00	221.5
4.50	222.8
5.00	224.0
5.50	225.2
6.00	226.3
7.00	228.1
7.50	229.1
8.00	229.8

Total number of readings = 155.

The data was analysed using the MINIQUAD program which was offered a range of species having $p = 0 \rightarrow 2$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 2.58 \pm 0.02$$

$$\log \beta_{210} = 4.49 \pm 0.02$$

$$\log \beta_{111} = 5.59 \pm 0.03$$

$$\log \beta_{211} = 8.22 \pm 0.02$$

These constants gave a sum of squares = 6.56×10^{-6}. The system can thus be described by four complexes $Zn(tartarate)^{o}$, $Zn(tartarate)_2^{2-}$, $Zn(tartarate)H^{+}$, and $Zn(tartarate)_2H^{-}$.

The zinc-tartarate complexes produced a pattern of formation curves as the tartarate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 58).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 59). These models require the total concentrations of zinc and tartarate (7.65 and 15.30 mM respectively) and the formation constants from table 58.

Table 58 below lists our formation constants of the complex formation of tartarate and some of the published results from other workers as a comparison.

FIGURE 58

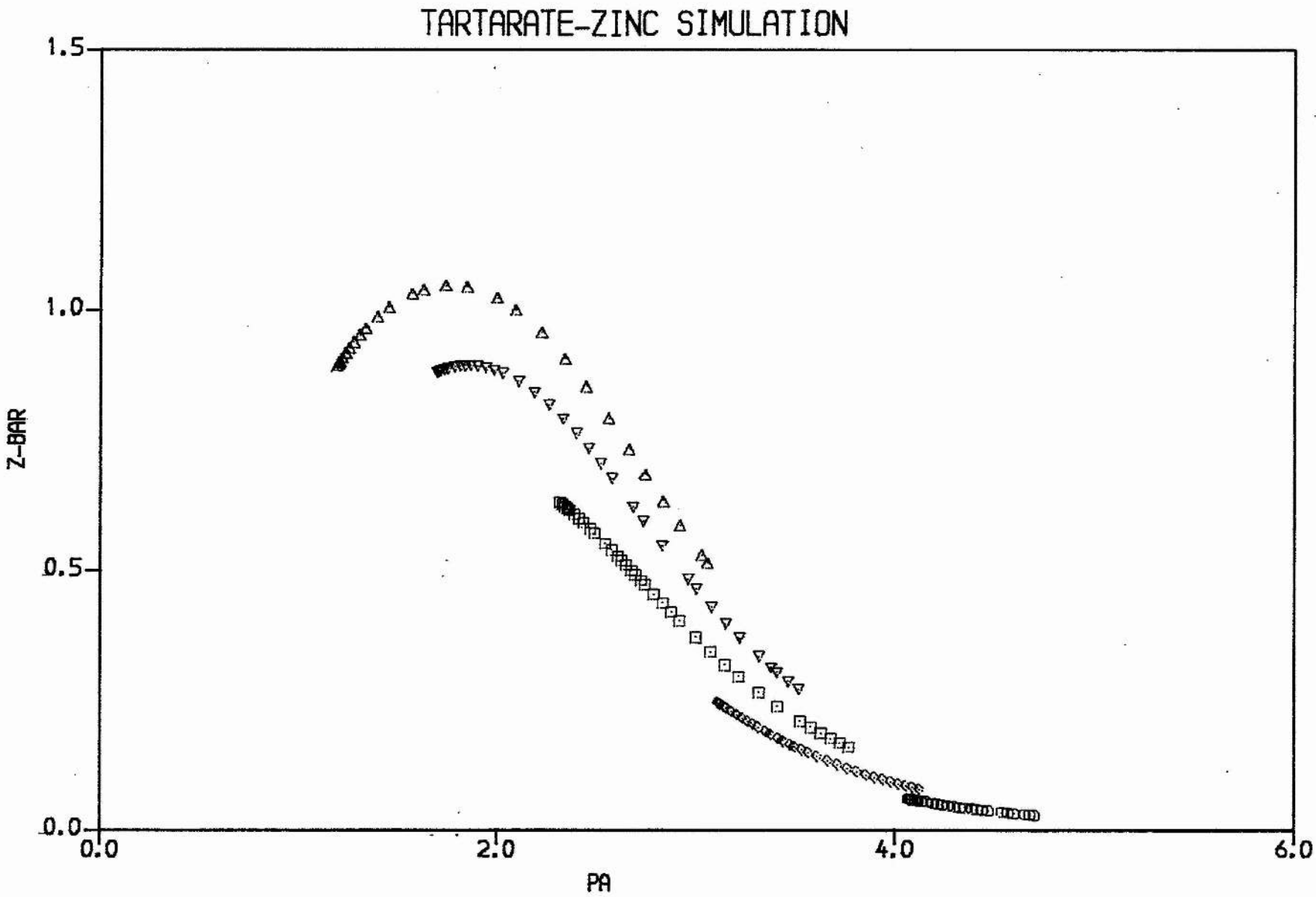


FIGURE 59

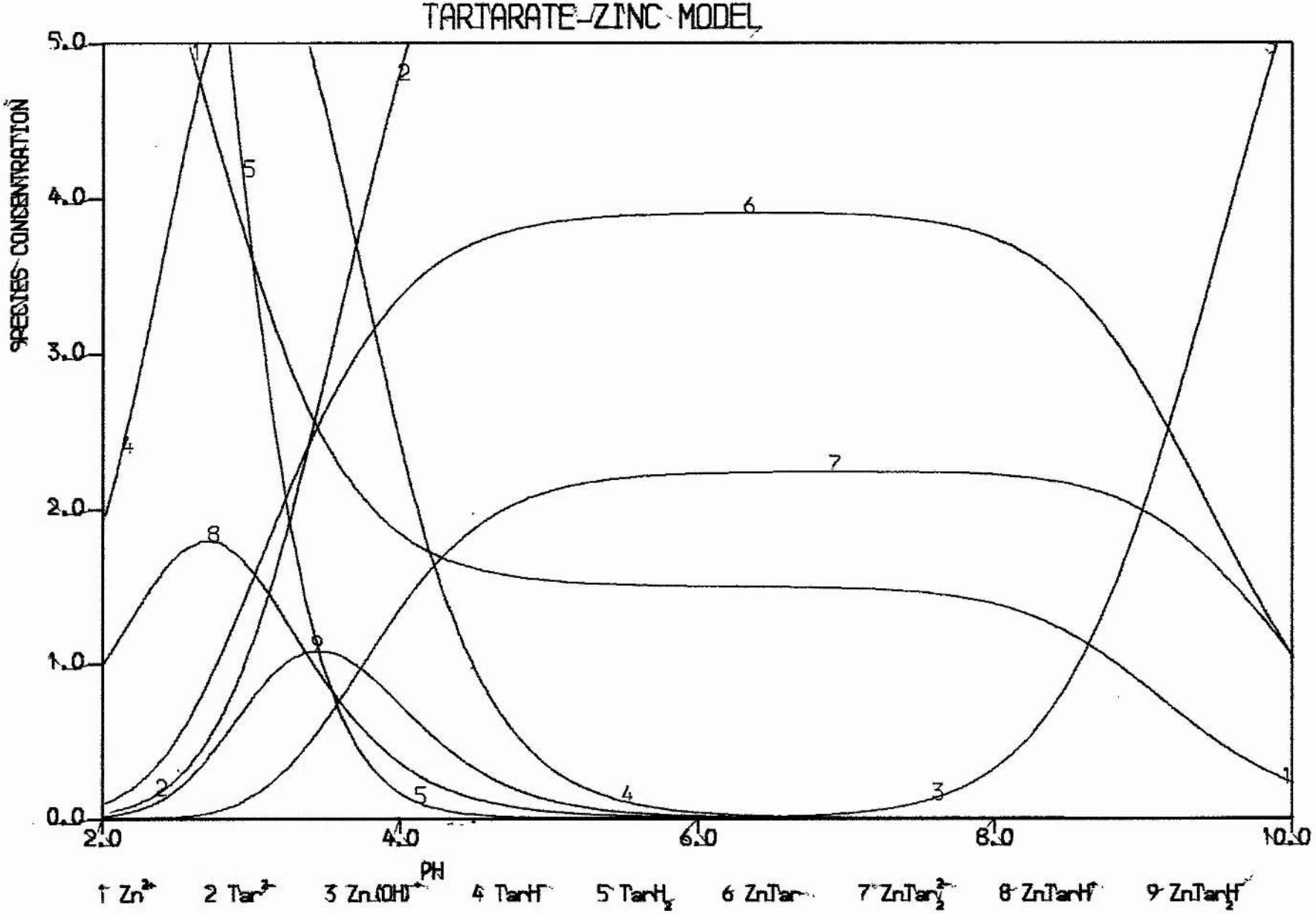

TARTARATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+
2 Tar2-
3 Zn(OH)+
4 TarHf-
5 TarH2
6 ZnTar
7 ZnTar2 2-
8 ZnTarHf-
9 ZnTar2Hf-

TABLE 58

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM : $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	literature data(Θ_c/°C, I/M, logβ)	Ref
	1	0	1	3.69	0.01	197	20, 0.1($NaClO_4$), β_1 3.96, β_2 6.76	103
	1	0	2	6.49	0.01		20, 1.0(KNO_3), β_1 3.77, β_2 6.37	115
Zn	1	1	0	2.58	0.02	155	25, 0.2(KCl), β_1 2.68, $K_{101/111}$ 1.44	101
	2	1	0	4.49	0.02		20, 0.1($KClO_4$), β_1 2.69	116
	1	1	1	5.59	0.03		25, 0 Corr., β_1 3.31, β_2 5.16	110
	2	1	1	8.22	0.02			

Protonation Constants of Salicylate

The formation curve was obtained (figure 60) using the ZPLOT computer program (chapter 4). The data was then analysed using MINIQUAD (chapter 4) and the results obtained were

$$\left.\begin{aligned} \log K_{101} &= 11.02 \pm 0.02 \\ \log K_{101 \rightarrow 102} &= 2.63 \pm 0.05 \end{aligned}\right\} \quad \text{(175 readings)}$$

These constants gave a sum of squares = 3.17×10^{-4} ($mol^2\ dm^{-6}$).

TABLE 59

Experimental results for the protonation of salicylate

Titration number	Titrate (S) (mM)		Titrant (T) (mM)		Initial volume (ml)	E^{o} (mV)
	A	H	A	H		
1	50.00	-100.02	0	134.15	20.00	357.0
2	0	56.71	15.00	-50.03	20.00	358.6
3	0	56.71	10.00	-40.02	20.00	357.6
4	0	28.35	5.00	-30.01	20.00	357.8

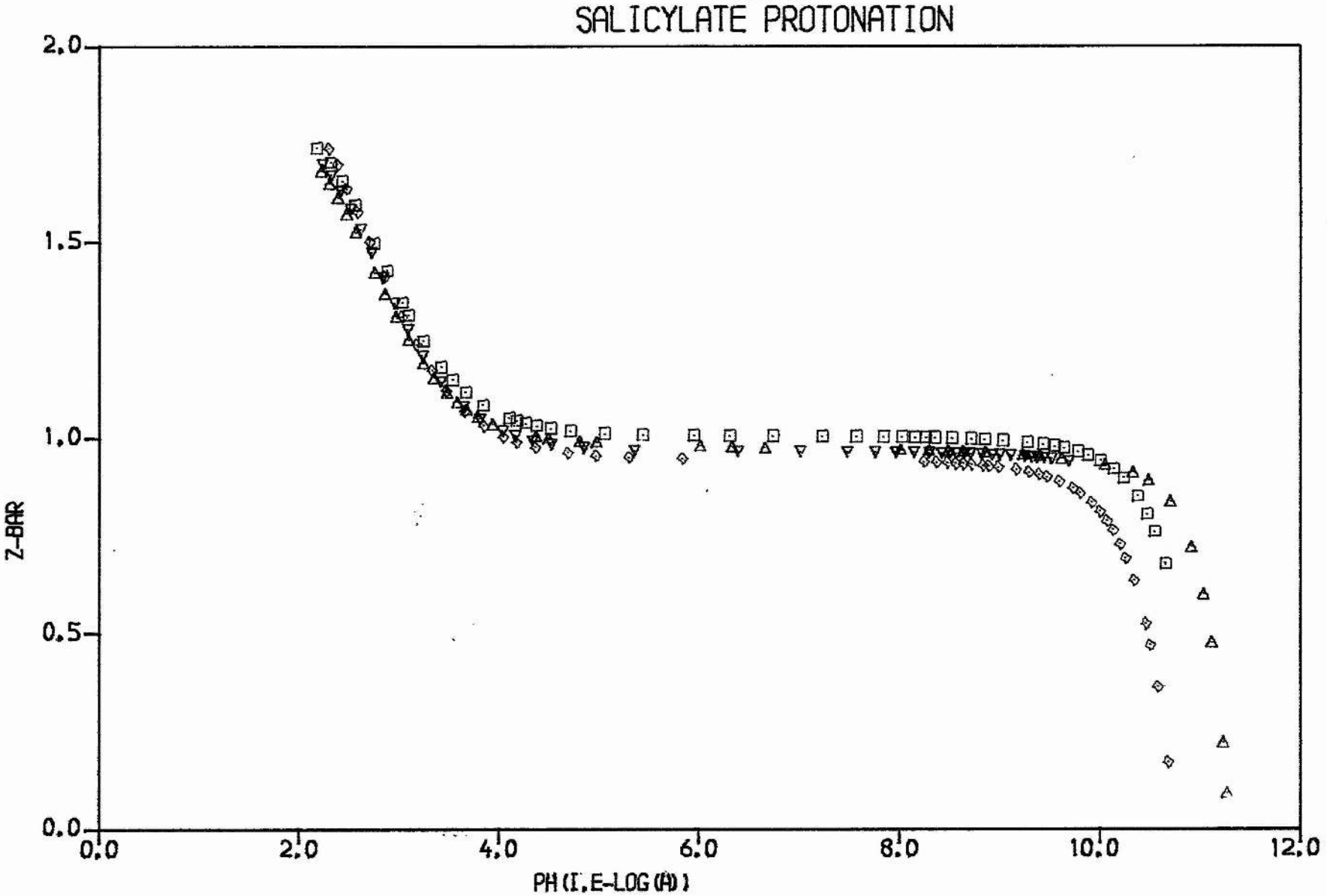
Titration number 1

volume added (ml)	emf (mV)
0.00	-337.7
1.00	-335.2
3.00	-328.3
4.00	-323.2
5.00	-315.8
6.00	-302.8
6.50	-289.5
6.70	-279.9
6.90	-262.8
7.05	-236.3

Titration number 2

volume added (ml)	emf (mV)
20.00	220.0
21.00	214.6
22.00	208.9
23.00	202.9
24.00	196.5
25.00	189.8
26.00	182.8
27.00	175.4
28.00	167.3
29.00	158.3

FIGURE 60


SALICYLATE PROTONATION
2.0
1.5
1.0
0.5
0.0
Z-BAR
0.0
2.0
4.0
6.0
8.0
10.0
12.0
PH (I.E-LOG(H))

Titration number 1 continued

volume added (ml)	emf (mV)
7.10	-222.6
7.13	-212.2
7.18	-189.2
7.20	-175.6
7.21	-166.5
7.22	-154.6
7.23	-137.2
7.27	-54.1
7.29	-33.9
7.31	-14.4
7.38	49.4
7.40	59.8
7.46	78.9
7.50	86.6
7.75	113.3
7.90	122.4
8.05	129.4
8.20	135.2
8.40	141.6
8.70	149.4
9.00	156.0
9.50	165.1

Titration number 2 continued

volume added (ml)	emf (mV)
30.00	147.4
31.00	132.9
31.50	123.1
32.00	109.3
32.20	101.4
32.40	90.7
32.55	79.3
32.70	59.3
32.80	27.8
32.85	-35.6
32.86	-73.9
32.87	-102.7
32.88	-119.8
32.89	-132.7
32.90	-143.7
32.91	-152.9
32.92	-160.5
32.93	-165.3
32.94	-169.6
32.95	-174.7
32.96	-178.2
32.98	-185.4

Titration number 1 continued

volume added (ml)	emf (mV)
10.00	172.8
10.50	179.7
11.00	186.2
12.00	197.9
12.50	203.4
13.00	208.8
13.50	214.0
14.00	219.0

Titration number 2 continued

volume added (ml)	emf (mV)
33.00	-191.6
33.02	-196.2
33.05	-202.9
33.10	-211.6
33.13	-215.8
33.16	-219.3
33.20	-223.5
33.25	-228.1
33.40	-238.6

Titration number 3

volume added (ml)	emf (mV)
23.00	222.4
25.00	213.9
26.50	206.8
28.00	199.0
30.00	187.1
31.20	179.1
32.50	169.6
33.00	165.6
34.00	156.6
35.00	145.7
35.50	138.9
36.00	130.7
36.50	120.0
37.00	103.9
37.10	99.3
37.20	93.8
37.30	87.0
37.40	78.4
37.50	66.3
37.60	45.0
37.65	21.5
37.68	-9.7
37.69	-31.8
37.70	-58.2

Titration number 4

volume added (ml)	emf (mV)
14.00	215.4
15.00	210.1
16.00	204.4
17.00	197.7
18.00	190.1
19.00	181.1
20.00	170.1
20.65	161.0
21.20	151.6
21.60	143.1
22.00	131.8
22.30	119.7
22.50	107.9
22.60	99.7
22.70	88.2
22.80	68.4
22.85	50.8
22.88	30.3
22.90	-2.3
22.95	-150.7
22.96	-158.3
22.97	-164.9
22.98	-170.2
22.99	-174.9

Titration number 3 continued

volume added (ml)	emf (mV)
37.71	-88.4
37.72	-109.5
37.73	-126.1
37.74	-137.4
37.75	-145.4
37.76	-152.0
37.77	-157.5
37.79	-168.2
37.82	-179.9
37.85	-188.2
37.90	-199.3
38.00	-214.4
38.10	-224.1
38.20	-231.3
38.30	-237.0
38.50	-245.5
38.70	-251.8
39.02	-259.4
39.50	-267.5
40.00	-273.6
41.00	-282.1
42.00	-288.0
43.00	-292.5
45.00	-299.3

Titration number 4 continued

volume added (ml)	emf (mV)
23.00	-179.7
23.02	-186.8
23.03	-190.2
23.05	-196.2
23.10	-207.3
23.15	-215.0
23.20	-221.4
23.25	-226.2
23.35	-233.7
23.50	-242.1
23.60	-246.5
23.80	-253.5
24.00	-258.8
24.20	-263.0
24.40	-266.6
24.70	-271.0
25.00	-274.6
25.50	-279.7
26.50	-287.0
27.00	-289.7
28.00	-294.2
30.00	-300.8

Formation Constants of Zn(II) - Salicylate Complexes

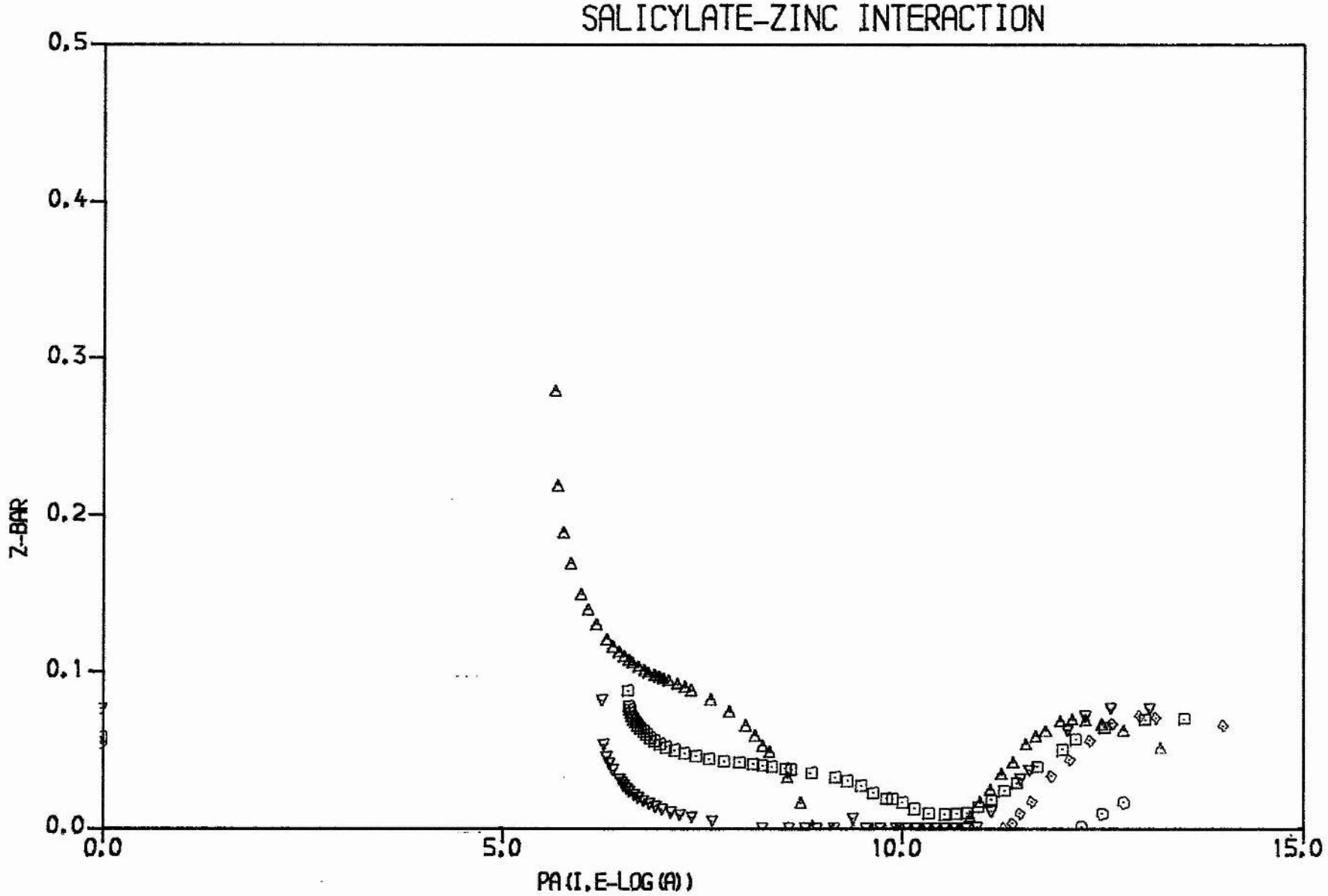
The formation curve (figure 61) was obtained from the results in table 60.

TABLE 60

Experimental results for the Zn(II) - salicylate system

Titration number	Initial concentrations (mM) Titrate (S) and Titrant (T)						Initial volume (ml)	E^{o} (mV)
	Ligand (A)		Metal (B)		Acid (H)			
	S	T	S	T	S	T		
1	0	40.00	4.99	0	10.58	-50.02	20.00	356.6
2	0	20.00	4.99	0	10.58	-40.02	20.00	356.6
3	0	10.00	4.99	0	10.58	-30.04	20.00	356.4
4	0	5.00	4.99	0	10.58	-20.01	20.00	355.4
5	0	2.50	4.99	0	10.58	-20.01	20.00	357.8

FIGURE 61


SALICYLATE-ZINC INTERACTION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
5.0
10.0
15.0
PA(I,E-LOG(A))

Titration number 1

volume added (ml)	emf (mV)
0.30	234.2
0.60	232.4
0.90	230.4
1.20	228.3
1.50	226.1
1.80	223.8
2.20	220.5
2.50	218.0
2.80	215.4
3.20	211.7
3.60	208.1
4.00	204.4
4.40	200.8
4.80	197.2
5.30	192.8
5.70	189.5
6.20	185.5
6.70	181.7
7.20	178.0
8.00	172.5
9.00	166.0
10.00	160.0
11.00	154.1
13.00	142.8

Titration number 2

volume added (ml)	emf (mV)
0.30	234.9
0.80	231.9
1.40	228.1
2.00	223.9
2.50	220.0
3.70	209.4
4.00	206.5
5.00	196.0
5.50	190.3
5.90	185.6
6.30	180.7
6.70	175.6
7.00	171.7
7.40	166.2
7.80	160.4
8.10	155.8
8.50	149.1
9.00	139.3
9.40	129.8
9.80	120.8
10.00	107.8
10.20	95.2
10.30	86.3
10.40	74.1

Titration number 1 continued

volume added (ml)	emf (mV)
15.00	131.1
17.00	117.8
18.00	109.9
19.00	100.4
20.00	87.6
20.30	82.4
20.60	76.8
20.90	70.0
21.30	57.6
21.60	43.6
21.80	28.6
21.85	23.7
21.90	17.9
21.95	11.2
21.98	7.0
22.00	3.9
22.02	0.3
22.05	-4.6
22.07	-7.4
22.10	-12.1
22.13	-16.6
22.15	-19.3
22.18	-23.2
22.21	-26.9
22.25	-31.6

Titration number 2 continued

volume added (ml)	emf (mV)
10.58	54.0
10.59	15.5
10.60	-0.5
10.61	-9.8
10.62	-16.8
10.63	-23.6
10.64	-28.7
10.65	-33.3
10.66	-38.1
10.67	-42.0
10.68	-44.9
10.69	-48.7
10.70	-50.8
10.71	-52.9
10.72	-54.5
10.73	-56.4
10.76	-61.3
10.78	-63.9
10.80	-66.2
10.84	-68.5
10.98	-70.0

Titration number 1 continued

volume added (ml)	emf (mV)
22.30	-36.1
22.40	-44.1
22.50	-50.3
22.60	-55.8
22.80	-63.6
23.00	-69.4
23.30	-73.9
23.90	-76.0

Titration number 3

volume added (ml)	emf (mV)
0.30	234.7
1.00	231.5
1.50	228.9
2.50	223.2
3.50	216.8
4.00	213.2
5.00	205.3
5.80	198.0
6.30	193.0
6.80	187.5
7.30	181.5

Titration number 4

volume added (ml)	emf (mV)
0.50	233.2
1.50	229.7
2.50	225.9
3.00	223.9
4.00	219.5
5.00	214.6
6.00	209.2
7.00	203.3
8.00	196.4
8.60	191.9
9.00	188.6
9.40	185.0

Titration number 3 continued

volume added (ml)	emf (mV)
7.70	176.2
8.10	170.6
8.50	164.3
9.00	155.1
9.40	146.3
9.70	138.3
9.90	131.6
10.00	127.5
10.20	118.2
10.35	109.4
10.47	99.5
10.55	90.4
10.65	72.3
10.70	56.7
10.71	52.2
10.73	41.9
10.74	35.0
10.75	27.5
10.76	17.0
10.77	5.1
10.78	-6.2
10.79	-16.6
10.80	-25.5
10.81	-33.4

Titration number 3 continued

volume added (ml)	emf (mV)
10.82	-39.8
10.83	-44.6
10.84	-48.9
10.85	-52.2
10.86	-55.0
10.87	-57.5
10.88	-59.8
10.89	-62.2
10.90	-63.9
10.91	-65.5
10.92	-66.7
10.93	-67.5
10.94	-68.3
10.95	-68.9
11.00	-69.8

Titration number 5

volume added (ml)	emf (mV)
4.00	219.8
5.00	214.5
6.00	208.5

Total number of readings = 168.

The data was analysed using the MINIQUAD program which was offered a range of species having $p = 0 \rightarrow 3$, $q = 0 \rightarrow 2$ and $r = -2 \rightarrow 3$, in addition to AH, AH_2 and $Zn(OH)^{97}$, the β values for the latter being held constant. The 'best' log constants obtained were

$$\log \beta_{110} = 5.30 \pm 0.20$$

$$\log \beta_{111} = 12.95 \pm 0.12$$

$$\log \beta_{213} = 29.80 \pm 0.15$$

$$\log \beta_{312} = 32.13 \pm 0.21$$

$$\log \beta_{313} = 39.52 \pm 0.21$$

These constants gave a sum of squares = 2.02×10^{-6}. The system can thus be described by the five complexes $Zn(salicylate)^{o}$, $Zn(salicylate)H^{+}$, $Zn(salicylate)_2H_3^{+}$, $Zn(salicylate)_3H_2^{2-}$, and $Zn(salicylate)_3H_3^{-}$.

The zinc-salicylate complexes produced a pattern of formation curves as the salicylate-zinc ratio was varied. This was taken as evidence of protonated, hydroxy or polynuclear complexes being present and some of these were indeed found in MINIQUAD. Then the best PSEUDOPLOT fit was obtained (figure 62).

The next stage involved COMPLOT computer simulation models of species distribution in solutions at different ph (figure 63). These models require the total concentrations of zinc and salicylate (7.65 and 15.30mM respectively) and the formation constants from table 61.

Table 61 below lists our formation constants of the complex formation of salicylate and some of the published results from other workers as a comparison.

FIGURE 62

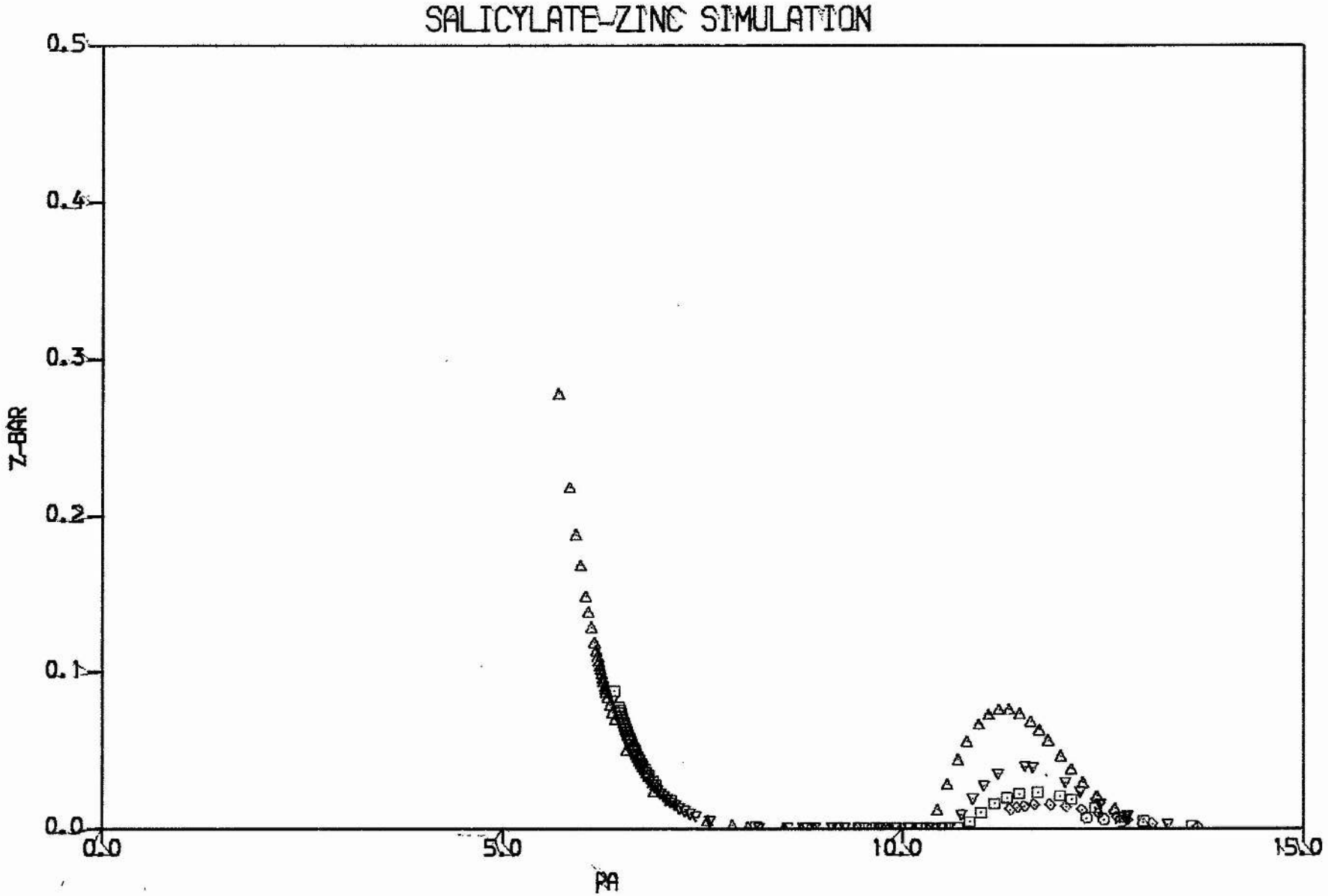

SALICYLATE-ZINC SIMULATION
0.5
0.4
0.3
0.2
0.1
0.0
Z-BAR
0.0
5.0
10.0
15.0
PA

FIGURE 63

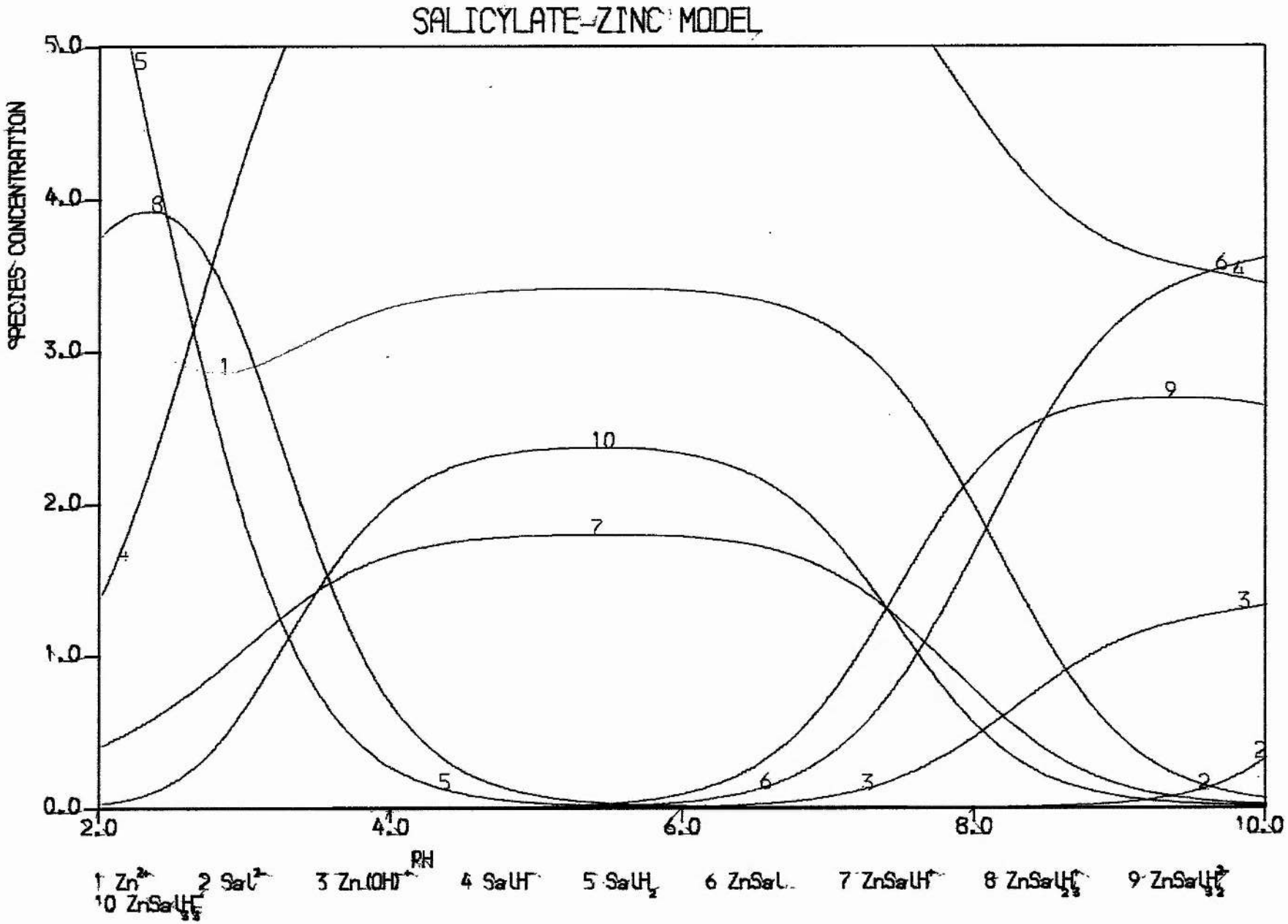

SALICYLATE-ZINC MODEL
SPECIES CONCENTRATION
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
PH
1 Zn2+ 2 Sal2- 3 Zn(OH)+ 4 SalH- 5 SalH2 6 ZnSal 7 ZnSalH+ 8 ZnSalH2 9 ZnSalH2-
10 ZnSalH2

TABLE 61

Log formation constants (β_{pqr}) for the protonation and the metal complexes at 37°C and I = 150mM $NaClO_4$; n = number of experimental observations and S denotes the standard deviation

B	p	q	r	log β	S	n	Literature data (θ_c/°C, I/M, log β)	Ref
	1	0	1	11.02	0.02	175	37, 0.15 KNO_3, β_1 13.00, β_2 15.81	117
	1	0	2	13.65	0.05		20, 0.1 (KNO_3), β_1 13.12, β_2 16.04	118,119
Zn	1	1	0	5.30	0.20	168	20, 0.10-0.15 KCl, β_1 6.85	120
	1	1	1	12.95	0.12		30, 0.1 $NaClO_4$ 75% dioxan, β_1 9.20	121
	2	1	3	29.80	0.15			
	3	1	2	32.13	0.21			
	3	1	3	39.52	0.21			

CHAPTER 6

A STUDY OF THE BIO-AVAILABILITY OF DIETARY ZINC USING COMPUTER SIMULATION MODELS OF THE CO-ORDINATION EQUILIBRIA INVOLVED

CONTENTS

CHAPTER 6

A STUDY OF THE BIO-AVAILABILITY OF DIETARY ZINC USING COMPUTER SIMULATION MODELS OF THE CO-ORDINATION EQUILIBRIA INVOLVED

Introduction

Life and survival involve competition both at the macro, prey-hunting level and at the micro, molecular level. In this latter respect, there are many competitive complexing steps between the metal ions that are contained in our diet and their eventual assimilation and participation *in vivo*. Fortunately, computer simulation can illuminate some steps in this complicated series of mechanisms that occur in the aqueous fluid in the stomach and in the small intestine.

In principle, the biological activity of metal dependent phenomena (for example, the rate of growth) ought to correlate with physical or chemical parameters. If this is possible, then the outcome is that desirable properties of the species present can be optimised by judicious chemical or dose adjustment and simultaneously undesirable side effects can be minimised. In solution, the best method of illustrating such structure and concentration activity relationships is to plot biological effect *versus* concentrations of the species believed to cause the response.

Now that reliable formation constants from sophisticated potentiometric techniques are available in the literature and large computer programs have been developed to simulate (using these constants) solution equilibria, it

is possible to predict the concentration of individual species with reasonable accuracy and to attempt to correlate these concentrations with the biological response. On the other hand, tables of biological response are not so readily available. Fortunately for this study, as mentioned earlier in Chapter 1 (p.11), Kratzer *et al* [10,122-6] have published several tables concerning the absorption of zinc ions from soya bean protein with, and without, the presence of zinc chelating agents related to EDTA. Soya bean protein complexes zinc ions rather firmly and so either extra zinc needs to be added to the diet or alternatively, a similar zinc liberating effect can be achieved by adding EDTA, (the converse of this effect is that phytates bind zinc so firmly that they make it non-available [127]). However, the rôle of the ligand is not restricted to merely winning the zinc from the food, it is also capable of encouraging intestinal absorption and then of releasing its zinc ions to the several processes that assimilate these ions into the components of the animal. Thus, these multifarious rôles are best mutually satisfied by zinc complexing ligands of intermediate complex stability, weak ligands not being able to sequester the dietary zinc, powerful ligands preventing the absorption and release into assimilation mechanisms.

Theoretical Considerations

The salient competitions involve the zinc binding capacity (BC) of food, of the ligand administered and of the tissue protein in the gastrointestinal lining * that is involved in assimilation (subscripts

---

* The actual site of this zinc absorbing tissue protein is open to discussion; the majority of authors take the duodenum as the main absorber whereas other workers suggest that some absorption may occur in the stomach. Thus, this paper reports intestinal fluids bathing these sites at both duodenal and stomach phs (6.5 and 2.0 respectively).

f, l and t respectively). It is exceedingly difficult to quantify BC_f and BC_t but, fortunately, their influences are assumed to have remained constant throughout Kratzer's biological activity determinations. The factor that was varied systematically was BC_l and solution chemists can establish the different contributions which collectively add up to BC_l:-

BC_l is a function of zinc-ligand complex formation constants (β_{ZnA}), proton-ligand constants (β_{HA}), zinc hydrolysis constants ($\beta_{Zn(OH)_2}$), the amount of ligand present, the ratio of total ligand to zinc concentrations, the presence of other competing metal ions such as calcium (β_{CaA}), and the ph of the aqueous intestinal solution from which a zinc species is absorbed into the intestine.

Further, as far as the growth promoting characteristics of BC_l are concerned, it has two influences:- the degree to which the ligand wins over the zinc from the food (clearly $BC_l \geqslant BC_f$), and also the amount of tissue assimilatable, i.e. membrane soluble, species formed. These two factors can sometimes oppose each other. For example, it may be possible to extract extra zinc from the food by increasing the total concentration of ligand but, in turn, this may turn a lipid membrane soluble *mono* complex into a useless *bis* or *tris* complex.

The most convenient means of seeing the effect of these influences is to plot biological activity *versus* concentration of species present at a given ph. These latter concentrations are conveniently displayed using computed models of the equilibria involved by feeding our COMPLOT [128] program with the ligand-proton and ligand-zinc formation constants and the total concentrations of zinc and ligand in a typical diet. Then, if a plot of biological effect *versus* concentration of species present shows a continuous plot this suggests

that that kind of species is the most important in encouraging absorption. It may then be possible to encourage even greater concentrations of the chosen species by varying concentrations or by using other ligands.

Table 62 lists the ligands examined and their formation constants as critically determined from the literature.

Table 63 lists the percentage gain in weight of broad breasted bronze turkey poults on a practical poult starter diet containing isolated soya bean protein compared to their growths without ligand addition [10] *versus* the COMPLOT computed concentrations of species present at stomach and duodenal phs (ph = 2 and 6.5 respectively). A typical ph profile is shown in figure 64 and figures 65 and 66 display relative growth *versus* the degree of zinc complexed. (This is best reflected by plotting the free zinc concentration at equilibrium since this embodies 1:1, 2:1 and protonated ligand:zinc complexes.) Table 63 shows that in this study, as may be anticipated from the fact that we are studying multidentate ligands, the majority of complexing is in the form of 1:1 complexes.

TABLE 62

Ligands and Formation Constants used in this Study

Ligands	*pqr*	$\log\beta_{pqr}$*	Reference
1,2 Diaminocyclohexanetetraacetate (CDTA)	101 =	11.70	129
	102 =	17.84	
	103 =	21.39	
	104 =	23.79	
	110 =	19.32	130
Diethylenetriaminepentaacetate (DTPA)	101 =	10.42	131,132,133
	102 =	19.18	
	103 =	23.60	
	104 =	26.16	
	105 =	27.95	
	110 =	18.3	132
	210 =	22.78	
4,5-dihydroxy-m-benzenedisulphonate (DHBDS)	101 =	12.26	134
	102 =	19.80	
	110 =	10.19	
	210 =	18.52	
Ethylenediamine-N,N'-diacetate (EDDA)	101 =	9.46	135
	102 =	15.88	
	110 =	11.10	
Ethylenediaminetetraacetate (EDTA)	101 =	10.31	136
	102 =	16.52	
	103 =	19.18	
	104 =	21.20	
	110 =	16.26 (Zn)	137,138
	110 =	10.42 (Ca)	139
Ethylenediaminetetrapropionate (EDTP)	101 =	9.60	140
	102 =	16.37	
	103 =	19.80	
	104 =	22.80	
	110 =	7.8	
Glutamate (GA)	101 =	9.41	141
	102 =	13.48	
	110 =	5.45	142
2-hydroxyethyliminodiacetate (HEIDA)	101 =	8.78	143
	102 =	10.74	
	110 =	8.57	
	210 =	12.67	

continued/...

TABLE 62 continued

Hydroxyethylethylenediaminetetraacetate (HEDTA)	101 = 9.73	144
	102 = 15.06	
	103 = 17.70	
	110 = 14.5	
8-Hydroxy-5-quinoline sulphonate (HQS)	101 = 8.43	145
	102 = 12.31	
	110 = 7.59	
	210 = 14.97	
Iminodiacetate (IDA)	101 = 9.40	146
	102 = 11.90	
	110 = 7.27	147
	210 = 12.60	
Mercaptosuccinate (MSA)	101 = 10.37	148
	102 = 15.01	
	103 = 18.65	
	110 = 8.75	149
	210 = 15.57	
Nitrilotriacetate (NTA)	101 = 9.73	150,116
	102 = 12.22	
	103 = 14.11	
	110 = 10.45	151
Prolinate (P)	101 = 10.68	152
	102 = 12.61	
	210 = 10.2	
Triethylenetetraamine (TETA)	101 = 9.92	153
	102 = 19.12	
	103 = 25.79	
	104 = 29.11	
	110 = 12.1	
	111 = 17.22	

* $\log\beta_{p\ q\ r}$ refers to the general Complex $A_p\ Zn_q\ H_r$ where

A = ligand and H = proton

TABLE 63

Kratzer *et al's* results for average percentage gain in weight of broad beasted bronze turkey poults and calculated concentrations of free zinc ions and of 1:1 zinc ligand complexes present at ph = 2.0 and 6.5. *

		$[Zn^{2+}]$		$[Zn.A]^{x-}$	
	% gain	ph = 2	ph = 6.5	ph = 2	ph = 6.5
Ligand	in weight	Concn x 10^{-5}			Concn x 10^{-5}
CDTA	8	7.18	1.43×10^{-14}	2.52×10^{-5}	9.70
DTPA	26	9.70	1.01×10^{-12}	1.21×10^{-8}	9.70
DHBDS	8	9.70	7.29×10^{-5}	4.07×10^{-14}	2.41
EDDA	64	9.70	1.74×10^{-8}	2.75×10^{-9}	9.70
EDTA	100	8.92	7.04×10^{-13}	7.73×10^{-6}	9.70
EDTA-Ca	72	8.92	1.85×10^{-10}	7.73×10^{-6}	9.70
EDTP	0	9.70	3.36×10^{-5}	1.50×10^{-15}	6.34
GA	0	9.70	9.18×10^{-5}	1.54×10^{-12}	0.52
HEIDA	24	9.65	6.66×10^{-7}	5.30×10^{-7}	9.59
HEDTA	120	8.95	7.52×10^{-12}	7.51×10^{-6}	9.70
HQS	4	9.70	4.98×10^{-6}	3.12×10^{-9}	2.35
IDA	10	9.70	2.84×10^{-5}	2.95×10^{-9}	6.68
MSA	8	9.70	1.29×10^{-5}	2.04×10^{-12}	7.85
NTA	68	9.60	7.89×10^{-8}	1.31×10^{-6}	9.69
P	8	9.70	9.70×10^{-5}	-	-
TETA	10	9.70	3.04×10^{-5}	1.55×10^{-17}	9.02

* These models used total concentrations of Zn = 97 μM and ligand = 171 μM

FIGURE 64

COMPLOT species present *versus* ph plots for the zinc - HEIDA system when total zinc = 97μM and the total ligand = 171μM

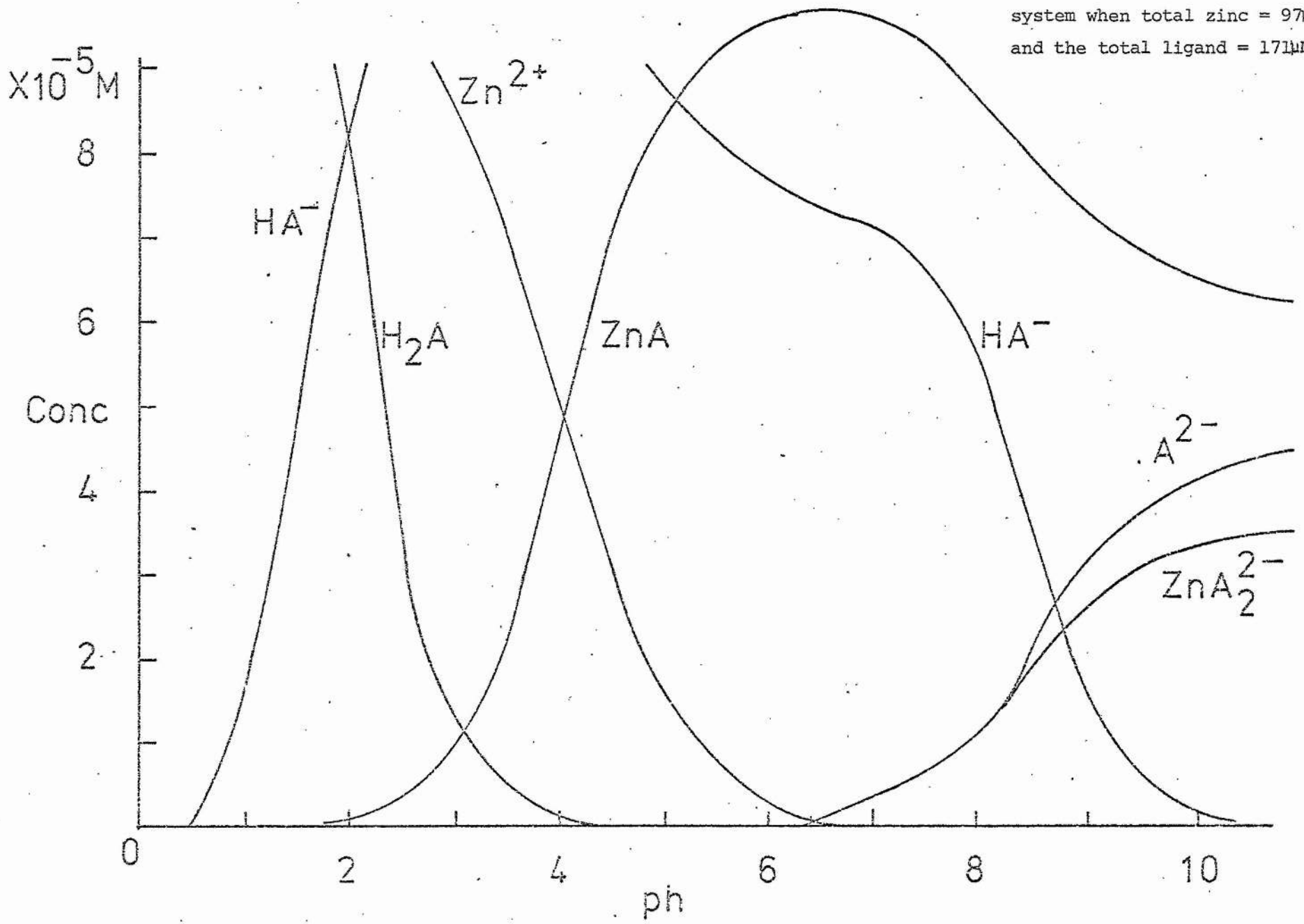
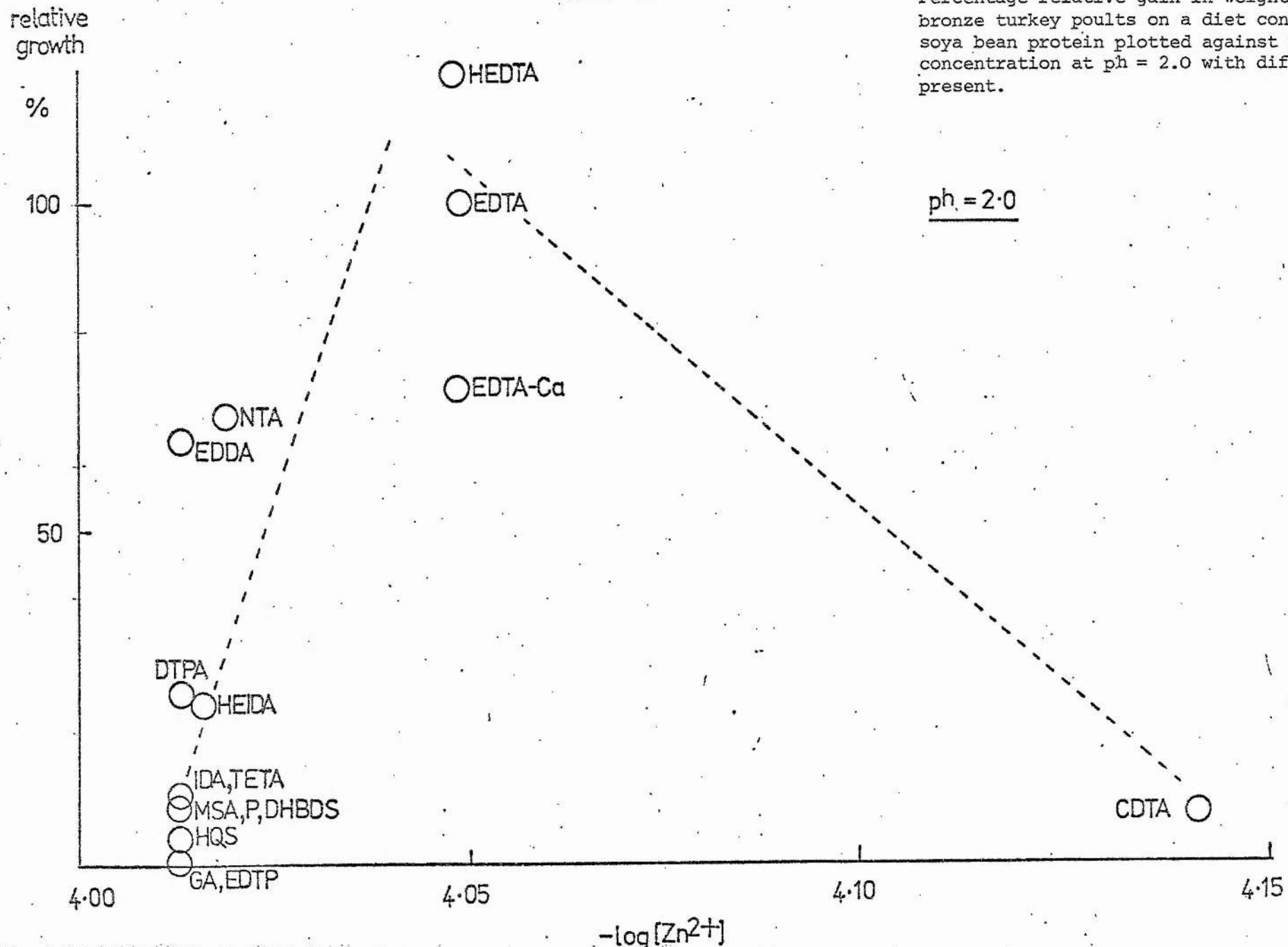


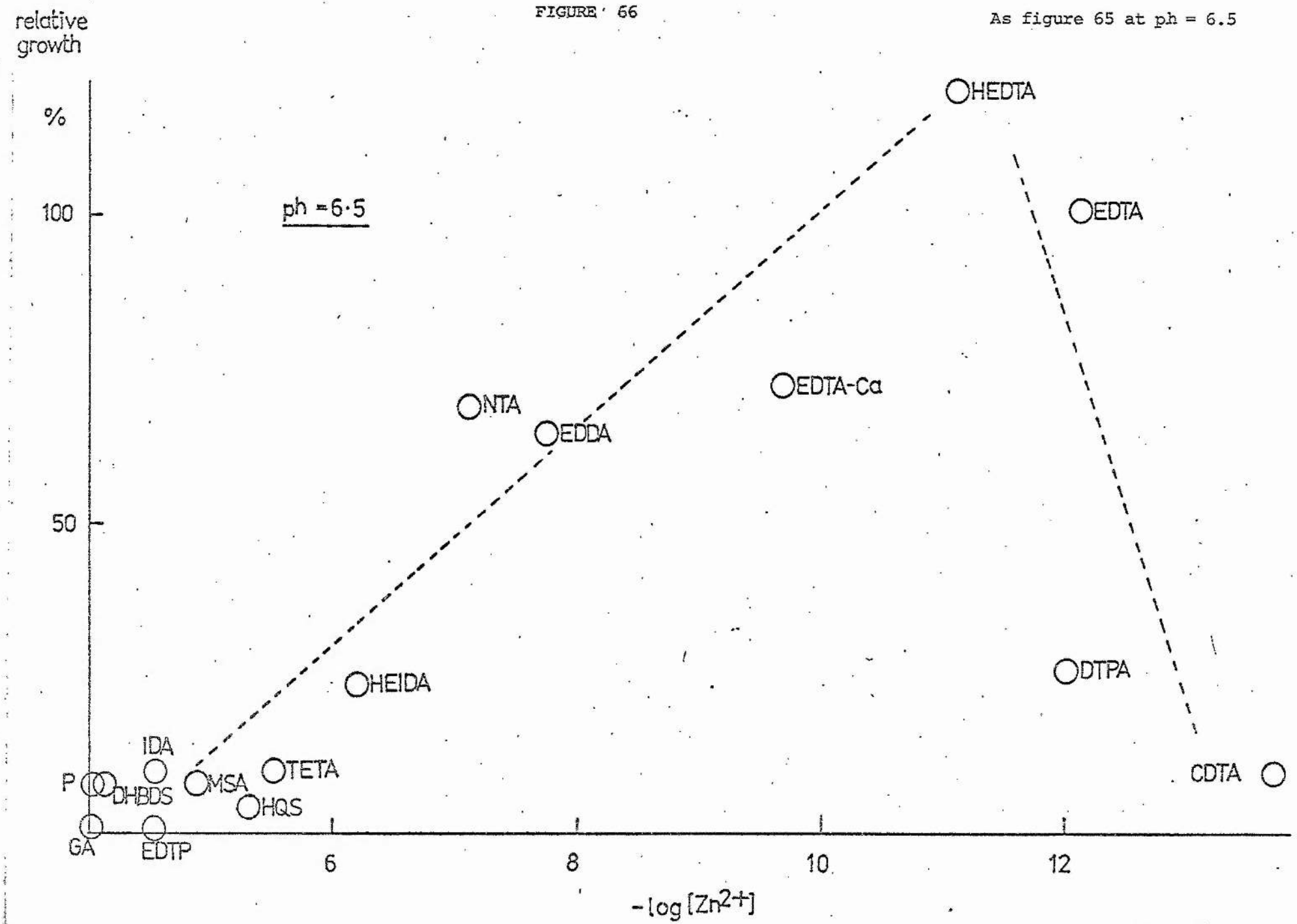
FIGURE 65



Percentage relative gain in weight of broad bre bronze turkey poults on a diet containing isola soya bean protein plotted against free zinc ion concentration at ph = 2.0 with different ligand present.

FIGURE 66

As figure 65 at ph = 6.5



Discussion

This fundamental investigation at the molecular level supports the concepts that a soya bean protein diet requires either supplementary zinc or added ligands to extract the zinc that is firmly encased in the protein. Presumably, the rapid growth characteristics of soya bean requires its roots to have powerful zinc sequestering ligands to win this growth dependent element from the humic acids in soils. Paradoxically, when animals attempt to acquire this zinc from the protein, the plant's sequestering ligands oppose the process because their bond strengths are so high. Further, we suggest that because maximum growth occurs for [HEDTA . Zn] = 1×10^{-4}M and $[Zn^{2+}] = 7 \times 10^{-12}$M at ph = 6.5, very little free zinc is used by the tissue protein but rather that it is the complexed zinc that is assimilated. This agrees with studies reporting the passage of radio-active C^{14} ligand-zinc complexes from intestine into venous blood and then its metabolism to appear in respiratory carbon dioxide and in the urine [122,123].

The explanation of some of the patterns of the published turkey growth rate results (which will be discussed in Chapter 7) enabled us to suggest the principles of improving bioavailability using liberating ligands. Firstly, to aim at a log β of approximately 13 to 17 is not completely foolproof [10] because both bond-strength and concentration dependent terms are included in log β. Secondly, due allowance must be made for the ligand pK influence (protons compete with zinc for the ligand) and for the ratios of metal to ligand used. However, these, sometimes clashing, assorted claims for the zinc can be resolved provided one has access to computer programs which can calculate concentrations *in vivo*.

We suggest the following four-step approach to theoretically choosing ideal ligands for metal ion bioavailability enhancement:-

a) A basic *core* of necessary ligand donor groups is established by successively varying, say, the number of carboxylates in a series of ligands (this is the case for Kratzer's studies) until a general order of magnitude for the formation constant giving good biological response is found ($\log\beta \simeq 13$ to 17 for the present ligands.

b) It is advantageous at this stage to find *naturally occurring ligands* containing this core of donors (EDTA does not occur in nature).

c) This fundamental core of donors can then be modified and improved up to the cut-off binding capacity. This occurs when Zn-ligand $\geqslant$Zinc-tissue protein. (For example, it occurs near HEDTA in the present studies.)

d) Once having reached an optimum position with a naturally occurring ligand, it is sometimes possible to improve the peak height still further by varying the ratios of zinc to ligand in the model. For example, at ph 6.5, a ratio of 1:1 seems ideal (figure 67) whereas at ph 2 a higher ratio improves the concentration of [zinc-ligand].

Conclusion

In conclusion this work suggests that many more biological activity *versus* complex concentrations of species present studies would be useful especially those involving naturally occuring ligands.

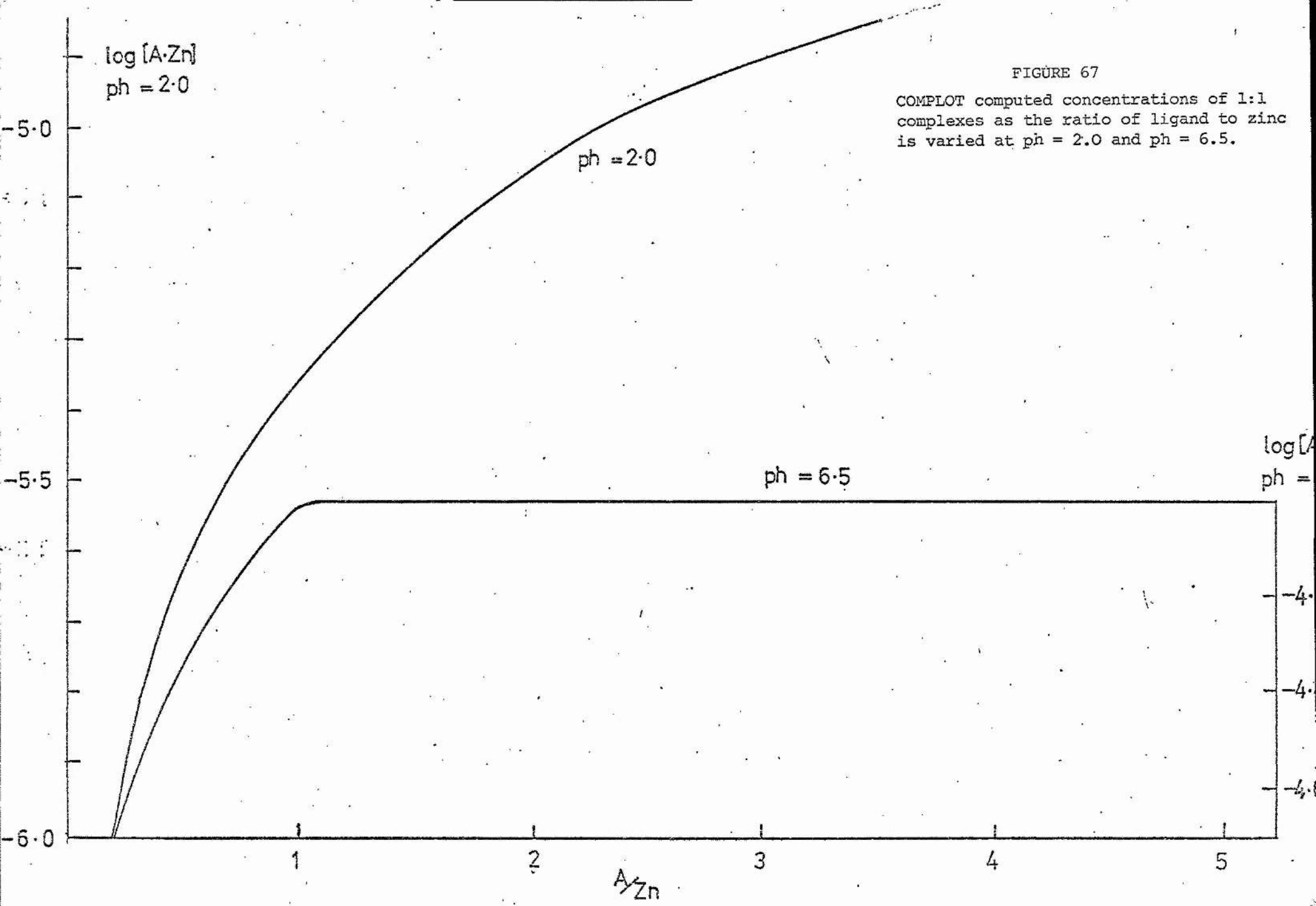


FIGURE 67

COMPLOT computed concentrations of 1:1 complexes as the ratio of ligand to zinc is varied at ph = 2.0 and ph = 6.5.

CHAPTER 7 - DISCUSSION

CONTENTS

CHAPTER 7

DISCUSSION

The results can be most efficiently discussed in five sections:

a) protonation of the ligands containing only nitrogen donor groups

b) protonation of the ligands containing both oxygen and nitrogen donor groups

c) protonation of the ligands containing only oxygen donor groups

d) complexing reactions of Cu(II), Co(II), Ni(II) with the first class of ligands and Zn(II) with the three classes of ligands

e) the study of the bio-availability of dietary zinc using computer simulation models of the coordination equilibria involved.

As mentioned earlier the first four sections have been studied potentiometrically, while in section e) the values of the formation constants of the complexes used were taken from the literature.

Most of the log K values that are published in the literature refer to other temperatures and ionic strengths.

A quantity called the ionic strength was introduced by Lewis and Randall [154] in order that they could represent the variation of activity coefficient with concentration, especially in the presence of added electrolytes. The ionic strength is designated by the symbol I and is

defined as half the sum of the terms obtained by multiplying the concentration of each ion in the solution by the square of its valence.

i.e. $$I = \frac{1}{2} \sum_i C_i Z_i^2$$

where C_i is the ionic concentration in moles per litre of solution and Z_i is the valency of the ion concerned.

Activity coefficients can then be calculated according to the Debye-Hückel equation:-

$$\log f_{\pm} = - \frac{A \mid Z_+ Z_- \mid \sqrt{I}}{1 + Ba \sqrt{I}} + bI \qquad \text{(1)}$$

where A and B are theoretical constants for a given solvent and temperature, a respresents the average distance of approach of two oppositely charged ions which is expressed in Ångstrom units, and b is an empirical constant.

For very dilute aqueous solutions,

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

where ϵ and T represent the dielectric constant of the solvent and the absolute temperature. For a given solvent and temperature, the expression for the ionic activity coefficient is given by

$$\log f_{\pm} = - A \mid Z_+ Z_- \mid \sqrt{I} \qquad \text{(2)}$$

For water as solvent at 25°C, the constant A is then 0.509, so that

equation (2) becomes

$$\log f_{\pm} = - 0.509 |Z_+ Z_-| \sqrt{I} \qquad (3)$$

Equation (3) is called the Debye-Hückel limiting law, which is only applicable to dilute solutions. According to this law the deviation from ideal behaviour in a given solvent is controlled by the ionic strength of the medium and the valences of the ions of the electrolyte, but is independent of their chemical nature. Thus, the activity coefficient of an ionic species in dilute solution depends principally upon its valence and the total ionic strength.

a) Protonation of the Ligands Containing only Nitrogen Donor Groups

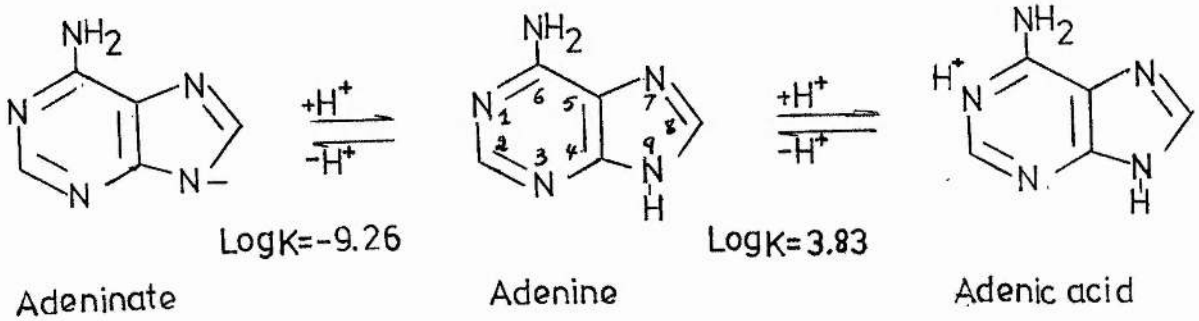
1) Protonation of Adeninate

Several workers [155-160] have the log K values of 3.5 - 4.2 for the proton ionisation from the $C_6H_3N^+$ group. However, there is a fundamental problem of identifying the site protonated. Lewin [161], who summarised the arguments about protonation on N_1H^+ and $C_6H_3N^+$, concluded that C_6H_2N is the protonation site. Pullman, Pullman, and Berthier [162] showed, by molecular orbital calculations, that the C_6H_2N group in adenine has the greatest electron density of any of the nitrogen atoms in the molecule. It was later concluded by Pullman [163] that the most basic site is not necessarily determined by the highest electron density, but rather by the conditions in the transition state. This conclusion led him to express the opinion that in adenine, the

N_1 position is the most likely site of protonation.

There is general agreement that proton ionisation is from the N_9H group (where logK~10)[161].

The log K values are 3.83 ± 0.01 and -9.26 ± 0.01 respectively. The first log K value refers to protonating the NH_2 or N_1 nitrogen (or some zwitterionic intermediate involving both sites). The second log K value refers to deprotonating the imidazole N_9 [164]. In general our figures are in agreement with other literature values [165a,b].



2) Protonation of Cyclohexylamine and Cyclopentylamine [13]

As mentioned earlier (page 10), this potentiometric investigation is aimed at elucidating the changes brought about when a co-ordinated ammonia is replaced by a cyclohexylamine or cyclopentylamine ligand. Our protonation constants, even though they are somewhat lower than the two values available from the literature, (10.66 at 24°C, I = 0.001 M

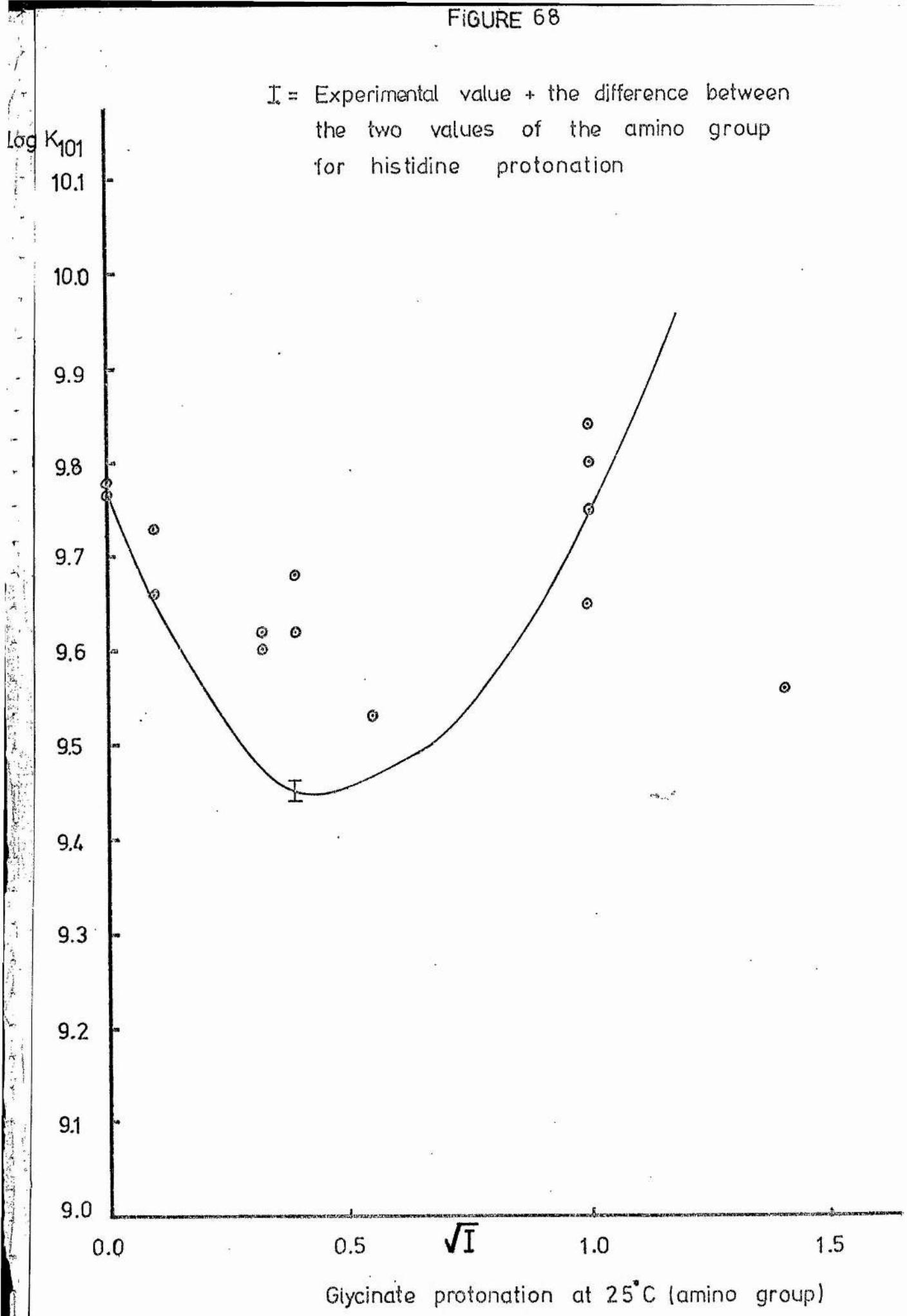
for cyclohexylamine [82] and 10.65 at 25°C, I = 0 for cyclopentylamine[83]) are still larger than the log K of the parent ligand (9.47 for ammonia [166]). This pattern is not easily understood, but such phenomena could be due to entropy effects (large entropy of protonation for cyclohexylamine and cyclopentylamine compared to ammonia) or maybe electrostatic effects (distribution of the electrons in the cyclic ring).

b) Protonation of the Ligands Containing both Oxygen and Nitrogen Donor Group

As mentioned previously in this chapter (page 222), the activity coefficient is dependent on the ionic strength. Our 150 mM - ClO_4^- constants are lower than those values reported in the literature for higher ionic strengths I. Example of a plot of log K *versus* $\sqrt{I}$ (over a range of ionic strengths) is shown in figure 68. These values are reported at 25°C, since only one log K value at 37°C is available in the literature[167].

Our value was taken with comparison of histidine protonations performed at 25°C and 37°C [168,169]. The difference between the two values of the amino (NH_2^-) group protonation was added to our value at 37°C.

FIGURE 68


I = Experimental value + the difference between the two values of the amino group for histidine protonation
Log K_{101}
10.1
10.0
9.9
9.8
9.7
9.6
9.5
9.4
9.3
9.2
9.1
9.0
0.0
0.5
$\sqrt{I}$
1.0
1.5
Glycinate protonation at 25°C (amino group)

1) Glycinate, Glycylglycinate, Glycylglycylglycinate [60]

For each ligand, β_{101} refers to protonating the primary amine group (ph region above 7) whereas K_2 for the second step (β_{102}/β_{101}) refers to protonating the carboxylate group (low ph region).

TABLE 64

Log formation constants (β_{pqr}) for ligand protonation at 37°C and I = 150 mM $NaClO_4$

	log β_{101}	log β_{102}
Glycinate	9.17	11.51
Glycylglycinate	7.74	10.84
Glycylglycylglycinate	7.59	10.70

The proton attracting power of the ligand primary amine group decreases with molecular weight (see β_{101} in table 64). This trend supports a previous observation for substituted ligands [96]. However, the log K values of protonating carboxylate groups increase with peptide size. The origin of this pattern is not apparent from the data, but it might be suggested that this behaviour is due to the increasing distance between the amino and the carboxylate groups or perhaps the presence of the amide group.

C) Protonation of the Ligands Containing only Oxygen Donor Groups

1) Galacturonate

The glycuronic acids (uronic acids) are the tetrahydroxyaldehydo-carboxylic acids of the hexane series. The uronic acids combine the properties of sugars with those of carboxylic acids [170].

Haug [171] reported the log K value (in acidic medium) to be 3.42 (T and I unspecified) by the paper electrophoresis method, which is in agreement with our results (log K 3.21 [172]), this value refers to protonating the carboxylate group.

2) Acetate, β-Hydroxybutyrate, Malate, Malonate, Oxalate and L-Tartarate

Several workers have reported log K values for both carboxylic acids (acetate [98,173,174], β-hydroxybutyrate [100,101]) and dicarboxylic acids (malate [105,102,103], malonate [105,106,107], oxalate [106,175,112] and tartarate [103,115,176,177]).

For carboxylic acids, the log K values obtained refer to protonating the carboxylate group. As for the dicarboxylic acids the log K values refer to protonating both carboxylate groups (these log K figures fall within the accepted values for $-COO^-$ protonation [173,100,103,106]).

3) Salicylate

Solubility problems (salicylic acid is insoluble in acid and slightly soluble in water, but soluble in alkali) and drifting during the period of titration, prevented us from obtaining superimposable formation curves.

This behaviour of the formation curves might suggest that polynuclear species are present. Several attempts were made with the PSEUDOPLOT program to detect this, but unfortunately with no success. In general, in acidic solutions only the carboxylic group dissociates, and in alkaline solutions the phenolic group then dissociates.

d) Complexing Reactions of Cu(II), Co(II), Ni(II) with the First Class of Ligands, and Zn(II) with the Three Classes of Ligands

The metal complexes of adeninate have been investigated by Albert[178], but not enough data is available on these complexes. Harkins and Freiser[179] have studied the complexes of adeninate and first transition series metal ions. Cheney *et al*[159] have investigated the interaction of adeninate with metal ions in mixed water-dioxan solutions, but these are not directly applicable to biological systems. Therefore, it was considered important to investigate the metal complexes of adeninate with Cu(II), Co(II), Ni(II) and Zn(II) ions in aqueous solution.

Our results show that metal complexing is rather weak and so is seriously challenged by metal ion hydrolysis. This hydrolysis restricted our working -log h range from 2.1 to 3.3. The presence of hydroxy complexes prevented us from producing formation curves for the system studied and our obtaining β values for *bis* - and - *tris* complexes. Izatt *et al* [165a] have reviewed the sites of metal ion-adeninate complexing and suggest that Cu(II) is chelated either C_6H_2N - Cu - N_7 or N_3 - Cu - N_9, the latter agreeing with x-ray analysis [180]. Co(II), Ni(II), and Zn(II) could conceivably be C_6H_2N - metal ion - N_7 bonded (from comparison with

Holmes and Williams [181] formation constants for imidazoles, pyridines and amines). It must be emphasized that potentiometric analysis gives only stoichiometric, and not, structural information.

2) Cyclohexylamine and Cyclopentylamine

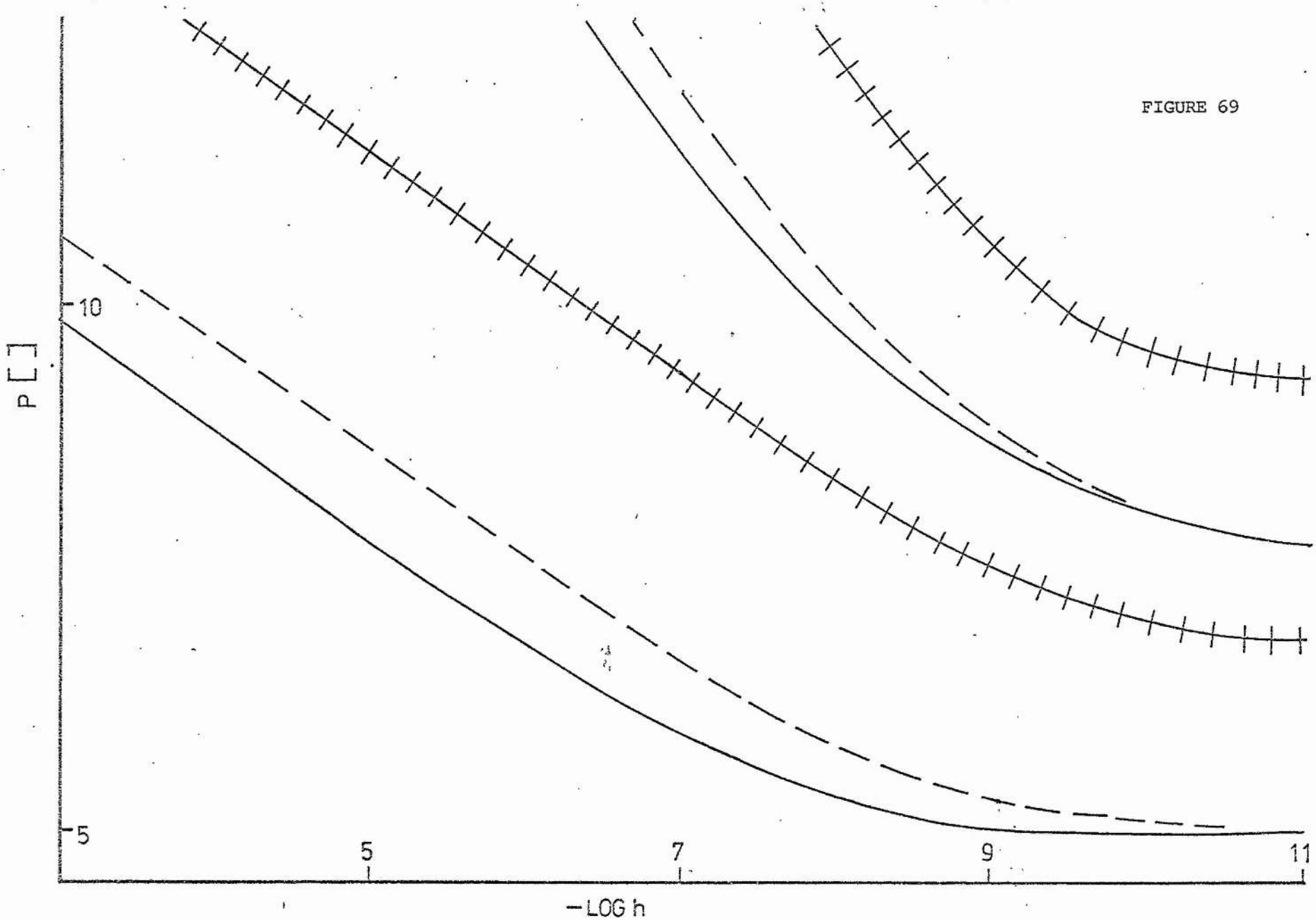
This investigation is the first potentiometric study of the metal complexes formed by cyclohexylamine (hex) and cyclopentylamine (pent) and therefore the tables, (see chapter 5, pages 65 and 79), quote published formation constants for related ammonia complexes as comparison. It may be seen that the complexing order for Co(II), Ni(II) and Cu(II) follows the log β order, cyclopentylamine > cyclohexylamine > ammonia whereas zinc has cyclohexylamine > cyclopentylamine > ammonia.

The complexing reactions are seriously challenged by hydrolysis producing insoluble complexes.

Apart from a general enhancement of the formation of all complexes compared to ammonia, the cyclic rings introduce nothing unusual into the expected complexing order, e.g. from our results, log β_1 values obey the Irving-Williams series.

cis-Dichlorodicyclopentylamineplatinum(II) is a superior anti-cancer drug to *cis*-dichlorodicyclohexylamineplatinum(II) (as far as side reactions in animals are concerned) which in turn is far superior to *cis*-dichlorodiammineplatinum(II) (in terms of therapeutic indices). For the reasons already stated (chpater 1, page 10), formation constants for chloro complexes are not available, nevertheless we set up a COMPLOT computer model [71] of the equilibria involved when $Ni(amine)_2^{2+}$ was allowed to equilibrate at various phs (figure 69). It is important to note that the order of apparent drug effectiveness cyclopentylamine > cyclohexylamine > ammonia coincides with the amount of *mono* and *bis* nickel complexes present at physiological phs. However, more evidence would be desirable before one can be justified in making the sweeping assumption that platinum complexes *in vivo* have solution equilibria analogous to nickel complexes *in vivo*.

FIGURE 69



COMPLOT model of distribution of cyclopentylamine, cyclohexylamine and ammonia (20μM) between H^+ and Ni^{2+} (10μM) Lower curves = BA^{2+} Upper curves = BA_2^{2+}

——— = cyclopentylamine — — — = cyclohexylamine + + + + = ammonia

3) Glycinate, Glycylglycinate and Glycylglycylglycinate

In glycine peptides the following functional groups must be taken into account as potential sites of coordination to metal ions:

a) the terminal amino group,

b) the terminal carboxyl group in its changed form,

c) the peptide oxygen atom

d) the peptide nitrogen atom.

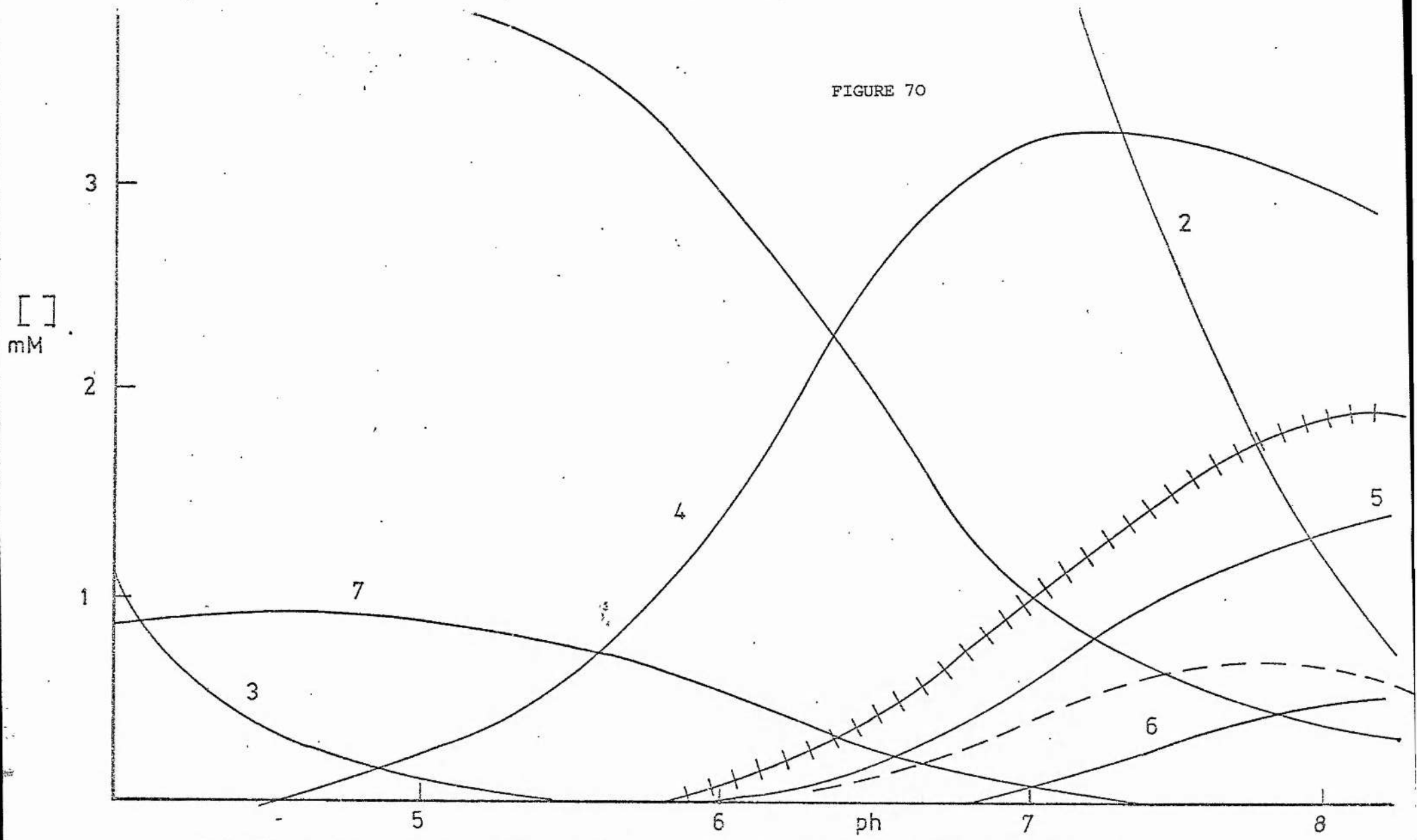
Our results show that complexes formed by Zn(II) and glycinate are more stable than zinc peptide complexes (tables 31,34 & 37). Bidentate glycinate bonds through the amine and carboxylate groups. Glycylglycinate and glycylglycylglycinate zinc complexes involve the primary amine and either the oxygen or possibly the nitrogen atom of the nearest peptide linkage. Whichever the mode of bonding, the similarity between the formation constants for the glycylglycinate and glycylglycylglycinate complexes suggest that the atoms involved in their chelate rings are the same in both instances.

Our models of species distribution show that the amount of uncharged A_2B zinc complex present parallels the size of the peptide (figure 70).

4) Galacturonate

The literature contains no data on Zn(II) or any of the other divalent metal ions with galacturonate. One cannot assign binding sites without further evidence, but from our results Zn(II) could appear to favour the carboxylate groups since the formation constant obtained

FIGURE 70



COMPLOT calculation of ph dependence for complexes present in the zinc-glycylglycinate-proton system when A=10 and B=5 mM: (1), Zn^{2+}; (2), HA; (3), H_2A^+; (4), ZnA^+; (5), ZnA_2; (6), ZnA_3^-; (7), $ZnHA^{2+}$. For comparison purposes the concentrations of bis(glycinate)zinc(+++) and bis(glycylglycylglycinate)zinc(---) are also plotted.

falls within the accepted value for $-COO^-$ complexation.

From the point of view of neutral species, our model studies suggest that α-D-galacturonate could well have a very good zinc absorption promoting characteristic.

5) Acetate, β-Hydroxybutyrate, Malate, Malonate, Oxalate and L-Tartarate

The carboxylate groups are the binding sites of both the carboxylic and the dicarboxylic acids. From this series of ligands our results show that malate and malonate *tris* complexes are more stable than the *bis* complexes, and so formation constants for *bis* complexes were not present. Similarly, the *bis* β-hydroxybutyrate complex is formed in preference to the *mono* complex.

Our models of species distribution show that β-hydroxybutyrate has the best zinc absorption promotion system of neutral complexes. Oxalate also has a high value but it is clearly too toxic for clinical studies.

6) Salicylate

The literature contains a paucity of data on Zn(II) - salicylate in aqueous solutions [167]. Similarly to protonation, formation constants of the complexing reactions are unreliable due to the problems mentioned earlier.

e) A Study of the Bio-availability of Dietary Zinc Using Computer Simulation Models of the Co-ordination Equilibria Involved [53]

Using formation constants available in the chemical literature, we have computed complex species present *versus* ph profiles for the ligands studied *in vivo* by Kratzer *et al*, and attempted to identify the main principle controlling maximum zinc uptake.

We are now able to explain some of the patterns of the published turkey growth rate results:-

a) EDTA - Ca was less effective than EDTA itself. This arises, according to our computed distribution of complexes present, because less EDTA is available for complexing the zinc, some remaining complexed to the calcium ion. Thus, there is effectively a drop in the [Zn.EDTA], an increase in $[Zn^{2+}]$, and these are mirrored in a reduced growth rate (see figures 65, 66 and table 63 in chapter 6),

b) maximum growth, for the diets used, occurs with HEDTA, even though EDTA, DTPA, and CPTA produce more substantial zinc complexing (i.e. $[Zn^{2+}]$ is lower). This, we suggest, arises because in the series up to HEDTA the growth is under the control of the amount of complexed zinc but after the HEDTA cut-off, bond strength control takes over (i.e. as discussed in the theoretical section in chapter 6, $BC_{\underline{t}} \leq BC_{\underline{l}}$ and so the tissue protein is unable to win the zinc from the ligand complex).

In conclusion, we must point out that these ligand-zinc absorption promotion studies refer to a full stomach and intestine. When food protein is absent $BC_{\underline{f}} = 0$ and so powerful ligands of the EDTA-type are unnecessary. However, ligands producing neutral complexes with metal ions can promote absorption [52].

Conclusions and Possible Future Prospects

We have drawn attention to the fact that metal-ions are essential to life, and in this thesis zinc in particular. The concept of possible zinc deficiency in man is relatively new, and the deficiency syndrome is not clearly defined.

Oral administration of zinc sulphate is the usual therapy for zinc deficiency conditions, but certain zinc-ligand mixtures may also increase serum concentrations of zinc more than oral zinc sulphate treatment.

The present thesis presents formation constants for the protonation and complex formation and includes ph *versus* complex species present profiles for a series of ligands, in order to suggest the best zinc supplementing drug for treating zinc deficiency conditions. These ligands were chosen from carboxylate and amino-acids found in man and in particular those known to be, or suspected of being, involved with zinc metabolism (e.g. α-D-galacturonic acid, D,L-β-hydroxybutyric acid, and L-tartaric acid).

The α-D-galacturonate and β-hydroxybutyrate were found to be the best zinc absorption promotion systems having 98.1 and 81.3% Zn^{2+} in potentially lipid/protein soluble form respectively. The complete ph profiles of these systems are shown in figures 39 and 43.

These results are in agreement with those by E. Giroux and N. J. Prakash* (Centre de Recherche Merrell International), where they studied the influence of various salts, chelates and other complexes of zinc given by gavage upon serum concentration of zinc in rats.

In considering future developments, there is a great need for more data on the composition of biological fluids and tissues, in normal and in pathologica states, and also for more formation constant data, determined under physiological conditions ($37^{o}C$ and I = 150mM), of other ligands that are of biological relevance as well as those that are biologically essential.

* Personal Communication

Clearly, as inorganic chemists we must recognise the fact that model computations can only be as reliable as the data used in the model. Also these models are an extreme over-simplification of the *in vivo* process and they give only a tentative suggestion to pharmaceutical researchers of which complexes may be forwarded for animal screening.

Finally, this field of research is poised between the disciplines of biochemistry, inorganic chemistry and medicine. Thus, if researchers are willing to accept the challenge and to expand their interdisciplinary discussion then this balance will lean towards a healthy future.

REFERENCES*

1. D.R. Williams, Chem. Rev., 1972, 72, 203.

2. E. Frieden, Scient. Amer., 1972, 227, 52.

3. R.J.P. Williams, RIC (Roy. Inst. Chem.) Rev., 1968, 1, 13.

4. R.J.P. Williams, Q. Rev. Chem. Soc., 1970, 24, 331.

5a. D.R. Williams, The Metals of Life, Van Nostrand, London, 1971.

5b. D.R. Williams, Ed., An Introduction to Bio-Inorganic Chemistry, Thomas, Springfield, Illinois, 1976.

6. R.J.P. Williams, Endeavour, 1967, 26, 96.

7. G.L.Eichhorn, Ed., Inorganic Biochemistry, Elsevier, Amsterdam, 1973.

8. G.N. Schrauzer, Ed., Bioinorganic Chemistry - an Interdisciplinary Journal, Elsevier, New-York, 1972.

9. H. Sigel, Ed., Metal Ions in Biological Systems, Dekker, New York, 1973.

10. P. Vohra and F.H. Kratzer, J. Nutrition, 1964, 82, 249.

11. F.A. Cotton and G. Wilkinson, Advanced Inorganic Chemistry, 3rd ed., Wiley, London, 1972.

12. L. Pauling, General Chemistry, 2nd ed., Freeman, San Francisco and London, 1953.

13. G.K.R. Makar and D.R. Williams, J.C.S. Dalton, 1974, 1121.

14. B. Rosenberg, L.van Camp, J.E. Trosko and V.H. Mansour, Nature, 1969, 222, 385.

15. R.D. Graham and D.R. Williams, J.C.S. Dalton, 1974, 1123.

16. B.L. Vallee: Biochemistry, physiology and pathology of zinc. Physiol. Rev., 1959, 39, 443-490.

17. G.C. Pimentel, Chemistry, An Experimental Science, W.H. Freeman, San Francis 1963.

* Those references which refer to journals not usually housed in Chemistry Department Libraries have their titles included in this listing.

18. D.R. Williams, Kémiai Kozlemények, 1975, 43, 361.

19. A.S. Prasad, J.A. Halsted and M. Nadimi: Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism, and geophagia. Amer. J. Med., 1961, 31, 532-546.

20. A.S. Prasad, A. Miale, Jr., Z. Farid, H.H. Sandstead, A.R. Schulert and W.J. Darby : Biochemical studies on dwarfism, hypogonadism and anemia. Arch. Intern. Med., 1963, 111, 407-428.

21. A.S. Prasad and D. Oberleas : Zinc; human nutrition and metabolic effects. Ann. Intern. Med., 1970, 73, 631-636.

22. J.A. Halsted : Human zinc deficiency. Trans. Amer. Clin. Climat. Ass., 1970, 82, 170-176.

23. J.A. Halsted, H.A. Ronaghy, P. Abadi, M. Haghshenass, G.H. Amirhakemi, R.M. Barakat and J.G. Reinhold : Zinc deficiency in man; The Shiraz experiment. Amer. J. Med., 1972, 53, 277-284.

24. J.A. Halsted and A.S. Prasad : Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism, and geophagia. Trans. Amer. Clin. Climat. Ass., 1960, 72, 130-149.

25. K.H. Neldner and K.M. Hambidge, N. Engl. J. Med., 1975, 292, 879-882.

26. J.L. Schelling, S. Muller-Hess and F. Thonney: Effect of food on zinc absorption, The Lancet, 1973, October 27, 968.

27. The Merck Index, 7th ed., Merck and Company, Inc., Rahway, 1960, 1118.

28. J.A. Halsted, J.C. Smith, Jr. and M.I. Irwin : A conspectus of research on zinc requirements of man. J. Nutr., 1974, 104, 345-378.

29. A. Osol, G.E. Farrar, Jr. and R. Pratt, Dispensatory of the U.S., 25th ed., Lippincott, Philadelphia, 1955, 1520.

30. I.J.T. Davies, M. Musa and T.L. Dormandy : Measurements of plasma zinc. Part I. In health and disease. J. Clin. Pathol., 1968, 21, 359-365.

31a. J.A. Halsted and J.C. Smith, Jr., : Plasma-zinc in health and disease, Lancet, 1970, 1, 322-324

31b. G.S. Fell : Zinc deficiency in man, Lancet, 1973, February 10, 299.

32. W.J. Pories and W.H. Strain : 'Zinc and wound healing'. Zinc Metabolism, A.S. Prasad, Ed., C.C. Thomas, Springfield, 1966, 378.

33. W.J. Pories and W.H. Strain : 'Zinc sulphate therapy in surgical patients'. Clinical Applications of Zinc Metabolism, W.J. Pories Ed., C.C. Thomas, Springfield, 1974.

34. T. Hallböök and E. Lanner : Serum-zinc and healing of venous leg ulcers, Lancet, 1972, 2, 780-782.

35. K.M. Hambidge, C. Hambidge, M. Jacobs and J.D. Baum : Low levels of zinc in hair, anorexia, poor growth, and hypogensia in children. Pediat. Res., 1972, 6, 868-874.

36. E.M. Butt, R.E. Nusbaum, T.C. Gilmour and S.L. Didio : Use of emission spectrograph for study of inorganic elements in human tissues. Amer. J. clin. Pathol., 1954, 24, 385-394.

37. W.G.E. Eggleton : The zinc and copper contents of the organs and tissues of Chinese subjects. Biochem. J.,1940, 34, 991-997.

38. M.A. Galin, H.D. Nano and T. Hall, Ocular zinc concentration. Invest. Opththalmol., 1962, 1, 142-148.

39. M.G. Netsky, W.W. Harrison, M. Brown and C. Benson : Tissue zinc and human disease, Relation of zinc content of kidney,liver and lung to atherosclerosis and hypertension . Amer. J. Clin. Pathol., 1969, 51, 358-365.

40. I.H. Tipton and M.J. Cook : Trace elements in human tissue. Part II . Adult subjects from the United States. Health Physics, 1963, 9, 103-145.

41. L.D. McBean , J.T. Dove, J.A. Halsted, and J.C. Smith, Jr: Zinc concentrations in human tissues. Amer. J. Clin. Nutr., 1972, 25, 672-676.

42. G.R. Schrodt, T. Hall and W.F. Whitmore, Jr.,: The concentration of zinc in diseased human prostate glands. Cancer, 1964, 17, 1555-1566.

43. D.P. Madden, M.M. da Mota and S.M. Nelson, J. Chem. Soc. (A), 1970, 790.

44. G.B. Brown, Texas Rep. Biol. and Med., 1952, 10, 961.

45. A. Furst, Metal Binding in Medicine, M.J. Seven and L.A. Johnson Eds., Lippincott, Philadelphia, 1960.

46. A. Furst, 'Chemistry of Chelation in Cancer', Amer. Lecture series, Ed. I.N. Kugelmass, Thomas, Illinois, 1963.

47. R. Nayan and A.K. Dey, Z. Naturforsch., 1972, 27b, 688.

48. M.J. Cleare and J.D. Hoeschele, Platinum Metal Rev., 1973, 17, (1), 2.

49. T.A. Connors, M. Jones, W.C.J. Ross, P.D. Braddock, A.R. Khokhar and M.L. Tobe, Chem. Biol. Interactions, 1972, 5, 415.

50. A.E. Martell and M. Calvin, Chemistry of Metal Chelate Compounds, Prentice-Hall, New York, 1962.

51. K. Nakamoto and P.J. McCarthy, Spectroscopy and Structure of Metal Chelate Compounds, Wiley, New York, 1968.

52. J.N. Cape, D.H. Cook and D.R. Williams, J.C.S. Dalton, 1974, 1849.

53. G.K.R. Makar and D.R. Williams, J. Inorg. Nuclear Chem., at press.

54. P.A.H. Wyatt, Energy and Entropy, MacMillan, London, 1967.

55. S. Coffey, Ed., Rodd's Chemistry of Carbon Compounds - Aliphatic Compounds, 1,(F), 2nd Ed., Elsevier, Amsterdam, 1967.

56. Beckman Instruments, Inc., 1963, page 3.

57. G. Biedermann, Svensk Kem. Tidskr., 1964, 76, 1.

58. D.R. Williams, 'Stability Constants for Bio-inorganic Systems', Lectures on Stability Constants. Ed. P. Paoletti, at press.

59. D.R. Williams and G. Williams, Private communication, 1968.

60. A.M. Corrie, G.K. R. Makar, M.L.D. Touche and D.R. Williams, J.C.S. Dalton, 1975, 105.

61. F.J.C. Rossotti and H.S. Rossotti, The Determination of Stability Constants. McGraw-Hill, London, 1961

62. F.J.C. Rossotti, H.S. Rossotti and R.J. Whewell, J. Inorg. Nuclear Chem., 1971, 33, 2051.

63. F.C. Davidson, J.P. Sloan, and D.R. Williams, J. Appl. Chem. Biotechnol., 1971, 21, 300.

64. N. Ingri, W. Kakołowicz, L.G. Sillén and B. Warnqvist, Talanta, 1967, 14, 1261.

65. D.R. Williams, J. Chem. Educ., 1971, 48, 480.

66. D.R. Williams, J.C.S. Dalton, 1973, 1064.

67. I.G. Sayce, Talanta, 1968, 15, 1397.

68. A.D. Jones and D.R. Williams, J. Chem. Soc. (A), 1970, 3138.

69. A. Sabatini, A. Vacca and P. Gans, Talanta, 1974, 21, 53.

70. N. Ingri and L.G. Sillén, Arkiv Kemi, 1965, 23, 97.

71. D.D. Perrin and I.G. Sayce, Talanta, 1967, 14, 833.

72. A.C. Baxter and D.R. Williams, J.C.S. Dalton, 1974, 1117.

73. A.I. Vogel, Quantitative Inorganic Analysis, 3rd ed., Longmans, 1961. Page (a) 235; (b) 242; (c) 243; (d) 714; (e) 885; (f) 535; (g) 433; (h) 608; (i) 441; (j) 613; (k) 435; (l) 614; (m) 443; (n) 432.

74. G. Gran, Analyst, 1952, 77, 661.

75. P.A. Yeo, Ph.D. Thesis, University of St. Andrews, 1972.

76. H. Wenger, dissertation, Eidgenössische Technische Hochschule, Zürich, 1964

77. J. Shankar and B.C. De.Souza, Austral. J. Chem., 1963, 16, 1119.

78. J.A. Bolzan and A.J. Arvia, Electrochim Acta, 1962, 7, 589.

79. T. Sekine, Acta Chem. Scand., 1965, 19, 1526.

80. A. Albert and E.P. Serjeant, Biochem. J., 1960, 76, 621.

81. G.E. Cheney, H. Freiser and Q. Fernando, J. Amer. Chem. Soc., 1959, 81, 261

82. N.F. Hall and M.R. Sprinkle, J. Amer. Chem. Soc., 1932, 54, 3469.

83. J.J. Christensen, R.M. Izatt, D.P. Wrathall and L.D. Hansen. J. Chem. Soc. (A), 1969, 1212.

84. P.S. Hallman, D.D. Perrin, and A.E. Watt, Biochem, J., 1971, 121, 549.

85. F. Basolo and Y.T. Chen, J. Amer. Chem. Soc., 1954, 76, 953.

86. H.A. McKenzie and D.P. Mellor, Austral. J. Chem., 1961, 14, 562.

87. C.B. Monk, Trans. Faraday Soc., 1951, 47, 297.

88. H.V. Flood and V. Lorzs, Tidskr. Kjemi Bergvsen Met. (Kjemi), 1945, 5, 83.

89. D.D. Perrin, J. Chem. Soc., 1959, 290.

90. N.C. Li and M.C.M. Chen, J. Amer. Chem. Soc., 1958, 80, 5678.

91. D.D. Perrin, 'Co-ordination Chemistry in Solution', E. Högfeldt, Ed., Sillén memorial volume, 1972, Swedish Natural Science Research Council, p. 387.

92. D.J. Perkins, Biochem. J., 1954, 57, 702.

93. L.E. Maley and D.P. Mellor, J. Austral. Sci. Res., 1949, A2, 579.

94. D.J. Perkins, Biochem. J., 1952, 51, 487.

95. C.B. Murphy and A.E. Martell, J. Biol. Chem., 1957, 226, 37.

96. W.P. Evans and C.B. Monk, Trans. Faraday Soc., 1955, 51, 1244.

97. D.D. Perrin, J. Chem. Soc., 1962, 4500.

98. R.S. Kolat and J.E. Powell, Inorg. Chem, 1962, 1, 293.

99. D.W. Archer and C.B. Monk, J. Chem Soc., 1964, 3117.

100. C.A. Crutchfield, Jr., W.M. McNabb and J.F. Hazel, J. Inorg,Nuclear Chem., 1962, 24, 291.

101. R.K. Cannan and A. Kibrick, J. Amer. Chem. Soc., 1938, 60, 2314.

102. M. Cefola, A.S. Tompa, A.V. Celiano and P.S. Gentile, Inorg. Chem., 1962, 1, 290.

103. C.F. Timberlake, J. Chem. Soc., 1964, 5078.

104. R.P. Bell and G.M..Waind, J. Chem. Soc., 1951, 2357.

105. E. Campi, Ann. Chim. (Italy), 1963, 53, 96.

106. H.J. DeBruin, D. Kairaitis and R.B. Temple, Austral. J. Chem., 1962, 15 457.

107. V.T. Athavale, N. Mahadevan, P.K. Mathur and R.M. Sathe, J. Inorg. Nuclear Chem., 1967, 29, 1947.

108. D.J.G. Ives and H.L. Riley, J. Chem. Soc., 1931, 1998.

109. V.S.K. Nair, J.Chem. Soc., 1965, 1450.

110. D.L.G. Rowlands and C.B. Monk, Trans. Faraday Soc., 1966, 62, 945.

111. M. Yasada, K. Yamasaki and H. Ohtaki, Bull. Chem. Soc. Japan, 1960, 33, 106

112. T. Sekine, J. Inorg. Nuclear Chem., 1964, 26, 1463 ; idem, Acta Chem. Scand 1965, 19, 1476; R.G. Seys and C.B. Monk, J. Chem Soc., 1965, 2452.

113. Y. Kanemura and J. I. Watters, J. Inorg. Nuclear Chem., 1967, 29, 1701.

114. E. Gelles and R.W. Hay, J. Chem. Soc., 1958, 3673.

115. K.S. Rajan and A.E. Martell, J. Inorg. Nuclear Chem., 1964, 26, 1927.

116. J. Stary, Analyt. Chim. Acta, 1963, 28, 132.

117. D.D. Perrin, I.G. Sayce and V.S. Sharma, J. Chem. Soc. (A), 1967, 1755.

118. M. Bartusek, Coll. Czech. Chem.Comm, 1967, 32, 116.

119. M. Bartusek, and J. Zelinka, Coll. Czech. Chem. Comm,, 1967, 32, 992.

120. D.D. Perrin, Nature, 1958, 182, 741.

121. K. E. Jabalpurwala, K.A. Venkatachalam and M.B. Kabadi, J. Inorg. Nuclear Chem., 1964, 26, 1011, 1027.

122. T.I. Koike, F.H. Kratzer and P. Vohra, Proc. Soc. Exptl. Biol.and Medicine, 1964, 117, 483.

123. F.H. Kratzer, Feedstuffs, 1965, 37:6, 62

124. P. Vohra, E. Krantz and F.H. Kratzer, Proc. Soc. Exptl. Biol. and Medicine, 1966, 121, 422.

125. P. Vohra and F.H. Kratzer, Poultry Science, 1968, XLVII, 699.

126. J. Atkinson and F.H. Kratzer, Proc. Nutr. Soc., 1970, 29, 59A.

127. P. Vohra, G.A. Gray and F.H. Kratzer, Proc. Soc. Exptl . Biol.and Medicine, 1965, 120, 447.

128. M. D. Walker and D.R. Williams, J.C.S. Dalton, 1974, 1186.

129. T. Moeller and T.M. Hsen, J. Inorg. Nuclear Chem., 1962, 24, 1635.

130. G. Anderegg, Helv. Chim. Acta, 1963, 46, 1833.

131. A.E. Frost, Nature, 1956, 178, 322.

132. S. Chaberek, A.E. Frost, M.A. Doran and N.J. Bicknell, J. Inorg. Nuclear Chem., 1959, 11, 184.

133. J. Vandegaer, S. Chaberek and A.E. Frost, J. Inorg. Nuclear Chem., 1959, 11, 210.

134. Y. Murakami, K. Nakamura and M. Tokunaga, Bull. Chem. Soc. Japan, 1963, 36, 669.

135. S. Chaberek Jr. and A.E. Martell, J. Amer. Chem. Soc., 1952, 74, 6228.

136. T. Moeller and S.K. Chu, J. Inorg. Nuclear Chem., 1966, 28, 153.

137. G. Schwarzenbach and E. Freitag, Helv. Chim. Acta, 1951, 34, 1503.

138. G. Schwarzenbach, R. Gut and G. Anderegg, Helv. Chim. Acta, 1954, 37, 937.

139. T.A. Bohigian and A.E. Martell, U.S. Atomic Energy Commission Contract No. AT(30-1) - 1823 Progress Report, 1960.

140. R.C. Courtney, S. Chaberek Jr. and A.E. Martell, J. Amer. Chem. Soc., 1953, 75, 4814.

141. M.H.T. Nyberg and M. Cefola, Arch. Biochem. Biophys., 1965, 111, 321.

142. C. Je-Sia, J. Dolezal and J. Zyka, Coll. Czech. Chem.Comm., 1961, 26, 1768.

143. S. Chaberek Jr., R.C. Courtney and A.E. Martell, J. Amer. Chem. Soc., 1952, 74, 5057.

144. S. Chaberek Jr. and A.E. Martell, J. Amer. Chem. Soc., 1955, 77, 1477.

145. G. Gutnikov and H. Freiser, Analyt. Chem., 1968, 40, 39.

146. K.S. Rajan and A.E. Martell, J. Inorg, Nuclear Chem., 1964, 26, 789

147. G. Anderegg, Helv. Chim. Acta, 1964, 47, 1801.

148. G.R. Lenz and A.E. Martell, Inorg. Chem., 1965, 4, 378.

149. R.S. Saxena, K.C. Gupta and M.L. Mittal, Canad. J. Chem., 1968, 46, 311.

150. W. Noddak and G. Oertel, Z. Electrochem., 1957, 61, 1216.

151. G. Schwarzenbach and E. Freitag, Helv. Chim. Acta, 1951, 34, 1492.

152. A. Albert, Biochem. J., 1950, 47, 531.

153. G. Schwarzenbach, Helv. Chim. Acta, 1950, 33, 974.

154. G.N. Lewis and M. Randall, J. Amer. Chem. Soc., 1921, 43, 1112.

155. P.A. Levene and H.S. Simms, J. Biol, Chem. 1925, 65, 519.

156. H.F.W. Taylor, J. Chem. Soc., 1948, 765.

157. R.A. Alberty, R.M. Smith, and R.M. Bock, J. Biol.Chem., 1951, 193, 425.

158. R.F. Beers and R.F. Steiner, Nature, 1957, 179, 1076.

159. G.E. Cheney, H. Freiser and Q. Fernando, J. Amer. Chem.Soc., 1959, 81, 2611.

160. S. Lewin and N.W. Tann, J. Chem.Soc., 1962, 1466.

161. S. Lewin, J. Chem. Soc., 1964, 792.

162. A. Pullman, B. Pullman, and G. Berthier, C.R. Acad. Sci., 1956, 243, 380.

163. B. Pullman, J. Chem. Soc., 1959, 1621.

164. G.K.R. Makar and D.R. Williams, J. Inorg. Nuclear Chem., 1974, 36, 1675.

165a. R.M. Izatt, J.J. Christensen and J.H. Rytting, Chem.Rev., 1971, 71, 439.

165b. M.M. Taqui Khan and C.R. Krishnamoorthy, J. Inorg. Nuclear Chem., 1971, 33, 1417.

166. T.H. Wirth and N. Davidson, J. Amer. Chem.Soc.,1964, 86, 4325.

167. 'Stability constants of Metal - ion complexes' L.G. Sillén and A.E. Martell Eds., Chem. Soc. spec. Publ. 1964, No. 17; 1971, No. 25.

168. D.R. Williams, J. Chem. Soc. (A), 1970, 1550.

169. A.D. Jones and D. R. Williams, J. Chem. Soc. (A), 1971, 3159.

170. S. Coffey, Ed., Rodd's Chemistry of Carbon Compounds - Aliphatic Compounds, 1 (B), 2nd ed., Elsevier, Amsterdam, 1965.

171. A. Haug and B. Larsen, Acta Chem.Scand., 1961, 15, 1395.

172. G.K.R. Makar, M.L.D. Touche and D.R. Williams, J.C.S. Dalton, DAL 5/1615, at press.

173. I. Feldman and L. Koval, Inorg. Chem., 1963, 2, 145.

174. D. Banerjea and I.P. Singh, Z.Anorg . Chem., 1964, 331, 225.

175. A. McAuley and G.H. Nancollas, Trans. Faraday Soc., 1960, 56, 1165.

176. V. Frei, Coll. Czech. Chem. Comm., 1965, 30, 1402.

177. V. Frei and A. Solcova, Coll. Czech. Chem. Comm., 1965, 30, 961.

178. A. Albert, Biochem. J., 1953, 54, 646.

179. T.R. Harkins and H. Freiser, J. Amer. Chem. Soc., 1958, 80, 1132.

180. E. Sletten, Chem. Comm., 1967, 1119.

181. F. Holmes and D.R. Williams, J. Chem. Soc. (A), 1967, 1256, 1702; 1968, 2965.