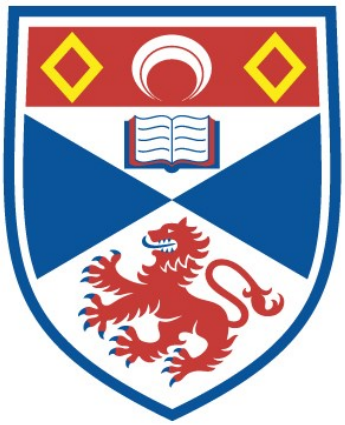
POTENTIOMETRIC AND MODEL STUDIES OF SOME CADMIUM COMPLEXES

Marjorie D. Sanderson

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



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POTENTIOMETRIC AND MODEL STUDIES

of

SOME CADMIUM COMPLEXES

A Thesis

presented for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Science of the

University of St. Andrews

by

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ABSTRACT

The Stability constants of some cadmium (II) ligand complexes have been measured by glass electrode potentiometry. These constants were used in computer model systems in order to assess the ligand's suitability as a cadmium sequestering agent *in vivo*. It is suggested that nitrogen and oxygen containing amino-acids complex zinc preferentially with respect to cadmium, and that the existing cadmium therapeutical, EDTA, is highly unspecific. Glutathionate is proposed as the most promising ligand for the treatment of cadmiumism.

An investigation was undertaken to assess the reliability and accuracy of a solid state cadmium-ion selective electrode. A new system of metal buffers was introduced into the calibration but it was found impossible to obtain reproducible results with this electrode. The electrode was used in a study of the cadmium asparaginate and serinate systems and checked against a computer simulation. The best 'fit' was obtained from a value of mV/pCd far from the theoretical value of -29.586 and it was concluded that the glass electrode was the more reliable means of measuring titration data.

The partition coefficient of the cadmium(II) glutathionate system was calculated for octanol/water as a model for cell membranes. This suggested a maximum of 2% of cadmium inside a cell being partitioned out by glutathionate and that ratios of ligand:metal greater than 1:1 would be the most efficient for complexing the metal.

The Belousov-Zhabotinsky oscillating reaction was taken as a primitive model of biochemistry and the qualitative effect of introducing different materials to the system was noted.

The computer program COMICS was modified for loading on to the St. Andrews IBM 360/44 computer and adapted to include three plotter routines.

D E C L A R A T I O N

I declare that this thesis is based on the results of experiments carried out by me, that it is my own composition and that it has not previously been presented for a Higher Degree.

The work was carried out in the Department of Chemistry of the University of St. Andrews, under the direction of Dr. D.R. Williams, between October 1972 and September 1975.

C E R T I F I C A T E

I hereby certify that Marjorie Douglas Sanderson 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.

10 · 02 · 76

David R. Williams,
Department of Chemistry,
University of St. Andrews.

A C K N O W L E D G E M E N T S

I would like to thank Dr. David R. Williams for suggesting this line of research and for his advice and encouragement throughout.

My thanks also go to members of the Chemistry and Computational Science Departments who have given assistance, especially to Mr. M.L.D. Touche for the use of his computer program THESIS in obtaining all the computer plots, and also to Miss E.M. Paton for the careful typing of this thesis.

Finally, I gratefully acknowledge a Research Studentship from the University of St. Andrews.

C O N T E N T S

CHAPTER I

I N T R O D U C T I O N

INTRODUCTION

A. GENERAL

Today it is realised that life is very much 'inorganic' in as much as living organisms would find it impossible to survive in the absence of certain metal ions. For a healthy human life there are eighteen elements considered to be essential (1) and ten of these are metals. These may be further divided into the main group metals, sodium, potassium, magnesium and calcium, and the transition metals, manganese, iron, cobalt, copper, zinc and molybdenum. These latter six are often referred to as the 'trace metals' but although they are present in the human body in only small amounts they play a vital role as catalysts in enzyme systems.

In this industrial age with the pollution of the environment by heavy metals, especially lead, cadmium and mercury, it is necessary to determine how such pollutants react in the body, the sites where they accumulate, the essential metals with which they compete, and from these facts to endeavour to design more highly specific drugs for their removal.

Over the years it has become apparent that the strict divisions of chemistry into physical, inorganic and organic is a gross over-simplification of natural science. Now it is being established that not only are there areas of overlap between the different branches of this one subject but also between chemistry and other disciplines, and so it is now the 'bio-inorganic' chemist who undertakes the search for new drugs for the treatment of, for example, heavy metal poisoning.

By studying the co-ordination chemistry of metal-ligand complexes in solution and determining their stability constants it is possible to indicate which ligands would be the most efficient for the complexing of certain metal ions *in vivo*. Of course these *in vitro* studies can in no way replace those carried out *in vivo* as it is not possible to

reproduce *in vitro* the many complex equilibria taking place *in vivo*. However, such studies do produce valuable data in suggesting which compounds it would be advantageous to study further, because although there are many competing equilibria in the body, which will necessarily reduce the percentage of metal complexed by the therapeutical, the effect will be the same on all the recommended ligands and, therefore, their order of effectiveness will remain unchanged.

CADMIUM POISONING AND EXISTING TREATMENTS.

Cadmium, although a relatively rare element, averaging only 0.5 g/ton of the earth's crust, has become one of the most dangerously polluting of the toxic heavy metals in the environment. It occurs in Group IIb of the periodic table along with zinc and mercury and since man first started to refine zinc our planet has been polluted with cadmium because the major source of this element is the zinc ores.

Mines, metal smelters and industries using cadmium in the manufacture of products such as alloys, paints, ceramics and plastics, all discharge cadmium into the atmosphere. Over the past few decades there has been a dramatic rise in this utilization of cadmium and with it has come an inevitable increase in its environmental levels. This, either directly (through inhalation), or indirectly (through pollution of soil and water and hence food), can be absorbed by man in whom it accumulates in the liver and kidneys (2, 3, 4).

This accumulation is related to the presence of the protein metallothionein, first shown to be present in equine renal cortex (5) and later demonstrated to be present in human renal cortex (6). This protein binds both zinc and cadmium but the former more firmly and to a greater extent, indeed, it can contain as much as 5% cadmium. The free form of the protein, thionein, was found to comprise about 9% sulphur due to a high percentage of cysteine residues, and U.V. absorption studies have confirmed that it is via these sulphydryl groups

that the cadmium is bound.

The formation of metallothionein has been shown, experimentally in rats, to be stimulated by the administration of low doses of cadmium (7) and this may provide protection against the effects of subsequent more acute poisoning (8, 9, 10). However, a specific drug has yet to be found for the treatment of cadmium poisoning in man.

To date this has been undertaken by the use of chelating agents such as 2, 3 - dimercaptopropanol (British Anti-Lewisite, BAL), and ethylenediaminetetraacetic acid (EDTA). Hydroxyethylenediaminetriacetic acid (HEDTA), diethylenetriaminepentaacetic acid (DTPA) and nitrilotriacetic acid (NTA) have also been used in treatment. BAL has been shown to increase the uptake of cadmium in the kidneys (11,12), EDTA to increase its nephrotoxicity (13) after repeated exposure and NTA to increase its acute toxicity in rats (14). From these findings it can be seen that such treatments are unsatisfactory and also, it must be said that these chelating agents tend to be unspecific and deplete the body of its essential metals such as zinc.

CO-ORDINATION.

Cadmium which has the electronic structure $(Kr)4d^{10}5s^2$ shows a filled d shell. There has been no evidence to suggest the existence of oxidation states higher than II, indeed, this would seem unlikely from the ionisation potentials (15).

Table 1. Ionisation Potentials of Cadmium in eV.

1st	2nd	3rd
8.99	16.84	38.0

Univalent cadmium has only been obtained as Cd_2^{2+} in melts (16,17), therefore, in considering the aqueous co-ordination chemistry of cadmium we are dealing with the divalent cation which has a filled outer d shell. This precludes it from being considered as a transition metal, although its complex forming ability does bestow upon cadmium some measure of similarity with the members of the transition series. Due to the presence of this filled

d shell there is no ligand field stabilisation to take into account, therefore, the stereochemistry of cadmium complexes is dependent entirely upon size, electrostatic and covalent bonding forces.

The most important co-ordination numbers for cadmium are four, five and six (15, 18). X-ray crystallographic studies of cadmium-amino-acid salts (19, 20, 21) have shown that for this type of compound the co-ordination number six is the most important with the geometry being octahedral, albeit sometimes distorted. However, one cannot always extrapolate such results to the situation in solution, although n.m.r. studies have agreed with the X-ray crystallography as to the binding sites of the amino-acids (22).

HSAB

The stability of co-ordination compounds is dependent upon the nature of the metal ion and of the ligand, and the basic concepts of this are defined in the theory of hard and soft acids and bases (HSAB).

This theory arose after it had been noted that for the complexing reactions of Fe^{3+} and Hg^{+} with the halides, the order in which the stability constants decreased was reversed. For the former it was found to be $F^- > Cl^- > Br^- > I^-$ whereas for the latter it was $I^- > Br^- > Cl^- > F^-$. From these and other observations it was concluded (23, 24) that metal ions could be classified as behaving either as Fe^{3+} (hard or class a) or as Hg^{+} (soft or class b) with some being borderline.

The class a metals are defined as having no unshared electrons in their valence shells, small radii and high charge density resulting in low polarizability, hence the definition 'hard'. Class b or soft metals obviously exhibit the converse of these properties.

Ligands also may be so categorized with those containing donor atoms such as N, O or F which are small, highly electronegative, difficult to oxidise and of low polarizability being hard, while those containing donor atoms such as P, S and I being considered as soft.

The terms acid and base are used due to the metals and ligands being considered as Lewis acids and bases, i.e. electron acceptors and electron donors.

It has been shown that hard metal ions form stronger bonds with hard ligands and soft metal ions with soft ligands, with hard-soft bonds being weak. For the soft metal ions, of which cadmium is one, the order of complex stability of different donor atoms has been shown to be

$$N \ll P > As > Sb$$

$$O \ll S < Se \sim Te$$

$$F \ll Cl < Br < I$$

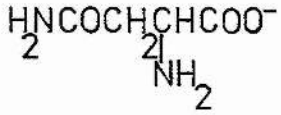
and this can form a useful basis on which to start a search for a specific complexing agent.

CHOICE OF LIGANDS

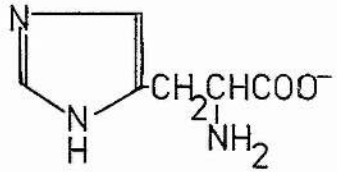
In choosing ligands for the sequestering of a metal ion *in vivo* one must first decide with which essential metal ions it is likely to compete as this is going to affect the desirable properties. Cadmium has a chemistry similar to that of the other Group IIb metal, zinc, and it is reasonable to expect that cadmium will be in competition with zinc which is important in enzyme systems such as carboxypeptidase A and alcohol dehydrogenase.

To gain knowledge of how efficiently existing therapies work, formation constants for complexes of cadmium with naturally occurring ligands - amino-acids - must be available.

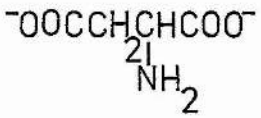
Eight representative amino-acids were chosen displaying a number of different features such as the presence of aromatic groupings, a sulphydryl group or a second carboxyl group in the side chain. These amino-acids had been studied with zinc (25-28) and it was expected that from the formation constants it would be possible, with the aid of computer programs, to demonstrate how inefficiently existing therapeuticals cope with the problem, and from these results to design a compound,



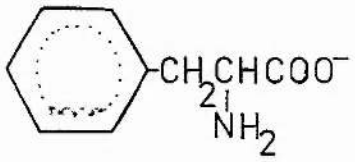
L-asparaginate



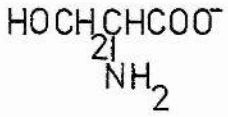
L-histidinate



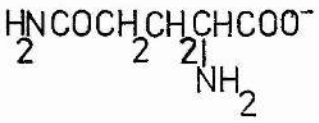
L-aspartate



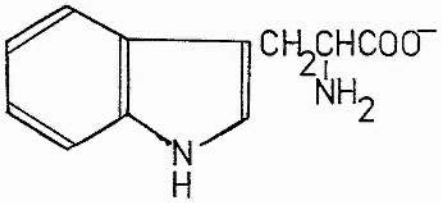
L-phenylalaninate



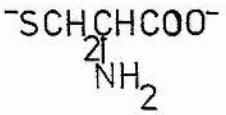
L-serinate



L-glutaminate



L-tryptophanate

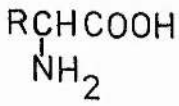


L-cysteinate

Figure 1

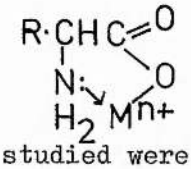
perhaps a peptide, with improved specificity.

Amino-acids contain a carboxylic acid grouping and an amino-group in the α position and can be represented by the general formula



although at physiological pHs it usually occurs as the zwitterion. There are twenty of these naturally occurring and apart from glycine (R = H) they are all optically active with the L-configuration, exclusively, being found *in vivo*.

It is the amino and carboxylate groups which can co-ordinate with metal ions, as indeed can other groups in the side chain, and stable chelate ring structures can be formed.

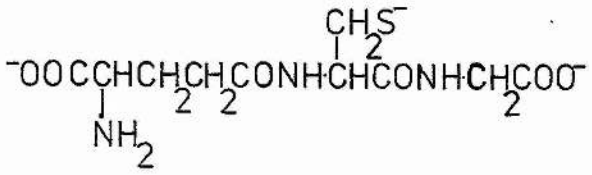


The amino-acids studied were asparagine, aspartic acid, cysteine, glutamine, histidine, phenylalanine, serine and tryptophan, and their structures are shown in Figure 1.

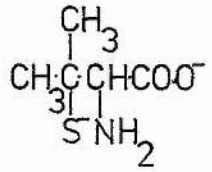
By comparison of the results of this study with those obtained in the aforementioned zinc studies it became evident that by altering the side chain, R,of the amino-acid it would be possible to discriminate between the two metals.

This led to a search for an amino-acid - containing compound, preferably incorporating cysteine. As interest had already been focused on glutathione (L-γ-glutamyl L - cysteinyl glycine) with respect to lead poisoning (29) it was decided to study this with cadmium.

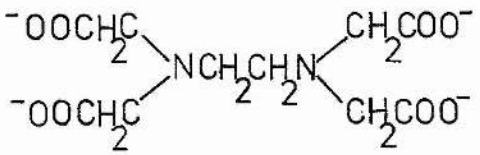
For the sake of completeness it was felt necessary to obtain constants for cadmium with EDTA which is used as a sequestering agent *in vivo* and also to ascertain what change there would be in the relative stabilities



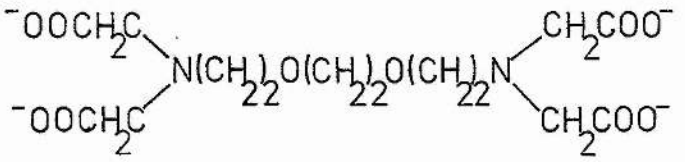
Glutathionate



D-penicillaminate



Ethylenediaminetetraacetate
(EDTA)



1,2-Di(2-aminoethoxy)ethanetetraacetate
(EGTA)

Figure 2

of the zinc and cadmium complexes on using a related aminopolycarboxylic acid. The acid chosen was EGTA (1,2-di(2-aminoethoxy) ethane tetraacetic acid) which differs from EDTA by the inclusion of two ethoxy groups as can be seen in Figure 2.

Another therapeutical, D-penicillamine, which is used extensively in the treatment of Wilson's disease, was also studied because due to its relation to cysteine (D-penicillamine is dimethyl cysteine) it would be expected to complex cadmium preferentially, yet clinical trials have shown that its administration increases the toxicity of cadmium and does not promote its urinary excretion (30).

GLASS AND METAL ION SELECTIVE ELECTRODE POTENTIOMETRY

The complexing reactions of the ligands with cadmium were studied by glass electrode potentiometry and two, serinate and asparaginate, were studied further by means of a solid-state cadmium ion selective electrode with a saturated sodium chloride calomel being used as reference in both cases.

Although the sensitivity of a glass electrode to the acidity of the surrounding medium was first noted by Cramer in 1906 (31) and the quantitative aspects discovered three years later by Haber and Klemensiewicz (32), it was Jacques who, in 1914 (33), was the first to point out that a series of electrode potential measurements could lead to a full description of a system in terms of compositions, dissociation constants and concentrations of all complexes present in solution. However, it was not until the work of Bjerrum (34) that this method came into its own and this led to the great interest shown in this technique in the 1940's and 1950's resulting in the publication of the first volume of "Stability Constants' in 1957 and 1958 (35). This interest has continued to flourish and a second volume was published in 1964 (36).

Although solid-state cadmium ion selective electrodes are a comparatively recent development (37, 38) other types of metal ion

sensitive electrodes have been used previously. The cadmium cyanide system was studied using a cadmium metal electrode (39) and the cadmium halide systems using a cadmium amalgam electrode (40).

It is evident that as a complexing reaction proceeds the concentration of free metal ion will change and that this can be calculated from the electrode potential using the Nernst equation:

$$E_{Cd} = E^{o}_{Cd} - \frac{RT}{zF} \ln b$$

As the conjugate acids of most ligands are weak, there is a competitive reaction between protons and metal ions for the ligand, hence the complexing reactions are pH dependent and can be followed by monitoring pH or electrode potential using a glass electrode. These two quantities are also related by the Nernst equation:

$$E_g = E^{0}_{g} - \frac{RT}{zF} \ln h$$

Results from such studies of simple systems can subsequently be used to build large computer models containing many equilibria. If the data for all the essential metals with a range of biological ligands are available the effect of introducing a polluting metal may be assessed. Furthermore, the introduction of a therapeutical to such a system can lead to figures on its efficiency and selectivity. Perhaps even more important, as no drug is going to be 100% selective *in vivo*, it provides an indication as to which essential metals are going to be complexed by the therapeutical and by what extent, thus leading to the requirements of replacement therapy.

Although this is inevitably an over-simplification of the situation *in vivo* it has been shown that including human serum albumin in the Cu, Zn, amino-acid model systems has little effect (41).

COMPUTING

Without the availability of computers the use of such model systems would be severely restricted. One program in use is COMICS (42), developed by Perrin and Sayce, which calculates equilibrium concentrations of all species in a multi-metal - multi-ligand system from the pH of the solution,

the total concentrations of each ligand and metal and the stability constants of all the species present. This program was modified slightly for loading on the St. Andrews IBM 360/44 computer. To enable more rapid assessment of the results three plotter routines were added, two of them on the on-line printer.

B. There are two other phenomena which have a bearing on the effectiveness of a drug and not only on those designed as heavy metal therapeuticals. One is the existence of circadian rhythms which in effect can alter the effectiveness of a drug depending on what time of day it is administered. The other is the ability of drugs to be transported across cell membranes.

Two lesser projects were carried out in an endeavour to develop methods of modelling these reactions *in vitro*..

CIRCADIAN RHYTHMS AND CHRONOTHERAPY

It is now established that the majority of reactions occurring *in vivo* are oscillatory in nature with a range of periodicities, for example, the plasma concentrations of metals exhibit a daily cycle whereas the ovarian cycle is monthly (43,44). These cycles play an important part in determining the response to a drug, which can show a marked increase or decrease in effectiveness which is dependent upon the time of the cycle at which it is administered, with perhaps an even more important point being that this is also true of the drug's toxicity.

Although this was first observed as early as 1931 (45), it is only comparatively recently that much systematic work has been undertaken in this field.

Such temporal behaviour suggests models involving kinetic rate constants, however, no chemical, oscillating reaction is completely understood in such terms. Nevertheless, it is possible by selecting such a system and observing the effects of certain substances on the period and amplitude to illustrate such phenomena in chemical terms.

The reaction chosen was the Belousov- Zhabotinsky reaction (46,47)

which is the oxidation of organic compounds containing an active methylene group, in this case malonic acid, catalysed by one-electron redox couples having a redox potential greater than 1V. In this study tris, (1,10 - phenanthroline) - iron (II)/tris (1,10 - phenanthroline)-iron (III) was used.

It is true that in a closed system oscillations are necessarily damped with the approach to equilibrium, however, when some reactants are present initially as a large excess (e.g. malonic acid) and the reaction rate not too rapid the oscillations can approach those of an open system where reagents are in continual supply.

The overall reaction is as shown in reaction 1:

$$3BrO_3^- + 5CH_2(COOH)_2 + 3H^+ \xrightarrow{\text{catalyst}} 3BrCH(COOH)_2 + HCOOH + 4CO_2 + 5H_2O \quad .1.$$

which is composed of four basic reactions:

$$BrO_3^- + 2Br^- + 3CH_2(COOH)_2 + 3H^+ \rightleftharpoons 3BrCH(COOH)_2 + 3H_2O \quad .2.$$

$$BrO_3^- + 4Fe^{2+} + CH_2(COOH)_2 + 5H^+ \rightleftharpoons BrCH(COOH)_2 + 4Fe^{3+} + 3H_2O \quad .3.$$

$$6Fe^{3+} + CH_2(COOH)_2 + 2H_2O \rightleftharpoons 6Fe^{2+} + HCOOH + 2CO_2 + 6H^+ \quad .4.$$

$$4Fe^{3+} + BrCH(COOH)_2 + 2H_2O \rightleftharpoons 4Fe^{2+} + 2CO_2 + 5H^+ + Br^- + HCOOH \quad .5.$$

The reaction commences with an induction period during which there is a build up of bromomalonic acid. Thereafter the Br^- and Fe^{2+} and Fe^{3+} concentrations oscillate whilst those of BrO_3^-, $CH_2(COOH)_2$, H^+ and CO_2 vary non-monotonically.

Thus, although the conditions are somewhat extreme, this can be taken to represent the situation in man where the reagent in continual supply is food. Indeed, the relationship between the oscillating concentrations and those varying non-monotonically as shown in Figure 3 do resemble those of the living system because not only do the oscillating reactions continue indefinitely <u>in vivo</u> but also the free energy of the system decreases continuously throughout the living process.

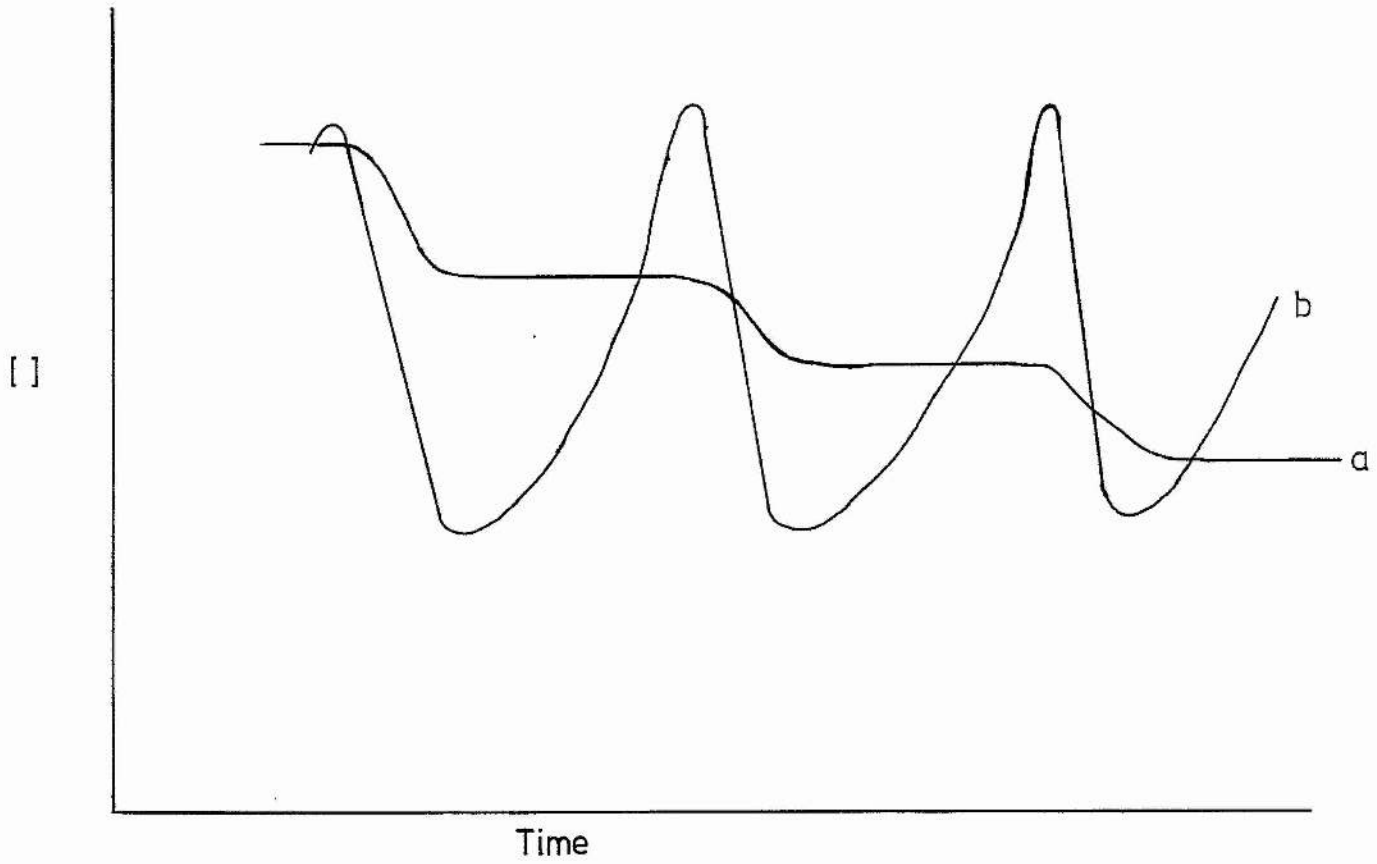


Figure 3 The temporal concentration patterns (a) varying non-monotonically and (b) oscillating

MEMBRANE PERMEABILITIES AND PARTITION COEFFICIENTS

Due to the transport of cadmium across cell membranes it is necessary that some of the complexed cadmium should be membrane soluble. However it is impossible to measure the partition coefficients of substances between cell membranes and intracellular fluid or plasma in vivo. Because of this it has become the practice to measure the partition coefficients between two liquid phases, usually water and an organic layer with a dielectric constant similar to that of an organic membrane.

In the Hansch approach (48,49) this method, with 1-octanol as the organic phase, has been used to obtain lipophilicities of many compounds based on computed structure-activity relationships, so that changing functions on a parent molecule may be accompanied by predictions as to the effect these will have on its biological activity, however, such a theoretical approach can not, as yet, be extended to embrace metal complexes.

It was, therefore, decided to measure the partition coefficients for the cadmium-glutathionate system experimentally in an endeavour to assess its lipophilicity. This particular system was chosen as from the previous potentiometric studies it had been proposed that this ligand could prove to be an effective cadmium therapeutical. Measuring partition coefficients for metal complexes has the distinct advantage that due to the presence of the metal, techniques such as atomic absorption spectrophotometry may be used to analyse for the metal in the organic medium.

Atomic absorption techniques have developed greatly since its commercial introduction in 1963 and is now a widely used method in the analysis of trace metals. In this method the metal is dissociated from its chemical bonds to an unionised ground state and as such is capable of absorbing radiation from discrete lines of narrow band width, the emitting lines being provided by a hollow cathode lamp.

The choice of method for studying biological systems is finely balanced. Should one study the living processes themselves and try to cope with the inherent difficulties such as the insolubility of many of the metal complexes of biological macromolecules or should one study the reactions of simpler systems such as amino-acids and small peptides and try to extrapolate these studies to those more complicated systems?

Both have their drawbacks but the latter method, the one chosen for this thesis, is being used more and more frequently, and as the mass of data accumulates alongside the development of more sophisticated computer programs this becomes more meaningful.

However, science is still a long way from producing the ultimate biological model so no matter how much the bio-inorganic chemist can do in measuring stability constants and proposing effective therapeuticals it is still the clinical trials which provide the final judgment.

CHAPTER II

EXPERIMENTAL TECHNIQUES

EXPERIMENTAL TECHNIQUES

PART I : POTENTIOMETRIC STUDIES

Apparatus

All potentiometric studies were carried out at 25.0°C in 3.00M sodium perchlorate as the ionic background medium.

The hydrogen ion concentration was followed using the cell

$$Hg, Hg_2Cl_2 \,|\, \text{saturated NaCl} \,|\, \text{test solution} \,|\, \text{glass electrode}$$

Sodium chloride had to be used in preference to potassium chloride due to the insolubility of potassium perchlorate which could be formed due to potassium chloride leaking into the test solution through the porous glass plug of the calomel electrode.

The electrode pair (Russell pH Ltd.; glass SF75/B14, calomel CR4/5/Na/B14) was used in conjunction with either a Solartron digital voltmeter LM1867 or a Radiometer pHm4b pHmeter to give readings reproducible to 0.1 m V.

In the studies using a cadmium ion selective electrode, the cadmium ion concentration was monitored using a Radiometer Ruzicka Selectrode F3003Cd (38) in conjunction with a Radiometer pHm4b pH meter using a calomel reference electrode as described above.

All titrations were carried out in a Pye Ingold double walled titration vessel (innerwall pyrex glass, outer wall plastic) which was thermostatted at 25.0°C. The vessel was closed by a plastic cover through which the electrodes, burette inlet and nitrogen inlet were let into the solution.

The titrant was added from a piston burette of 10 ml capacity (Metrohm E274) and mixing of the solution was achieved by slow magnetic stirring. A diagram of the apparatus is shown in Figure 4.

Water

All solutions were prepared using de-ionised water ('Elgastat'), boiled, and cooled under oxygen free nitrogen. The resistivity of the

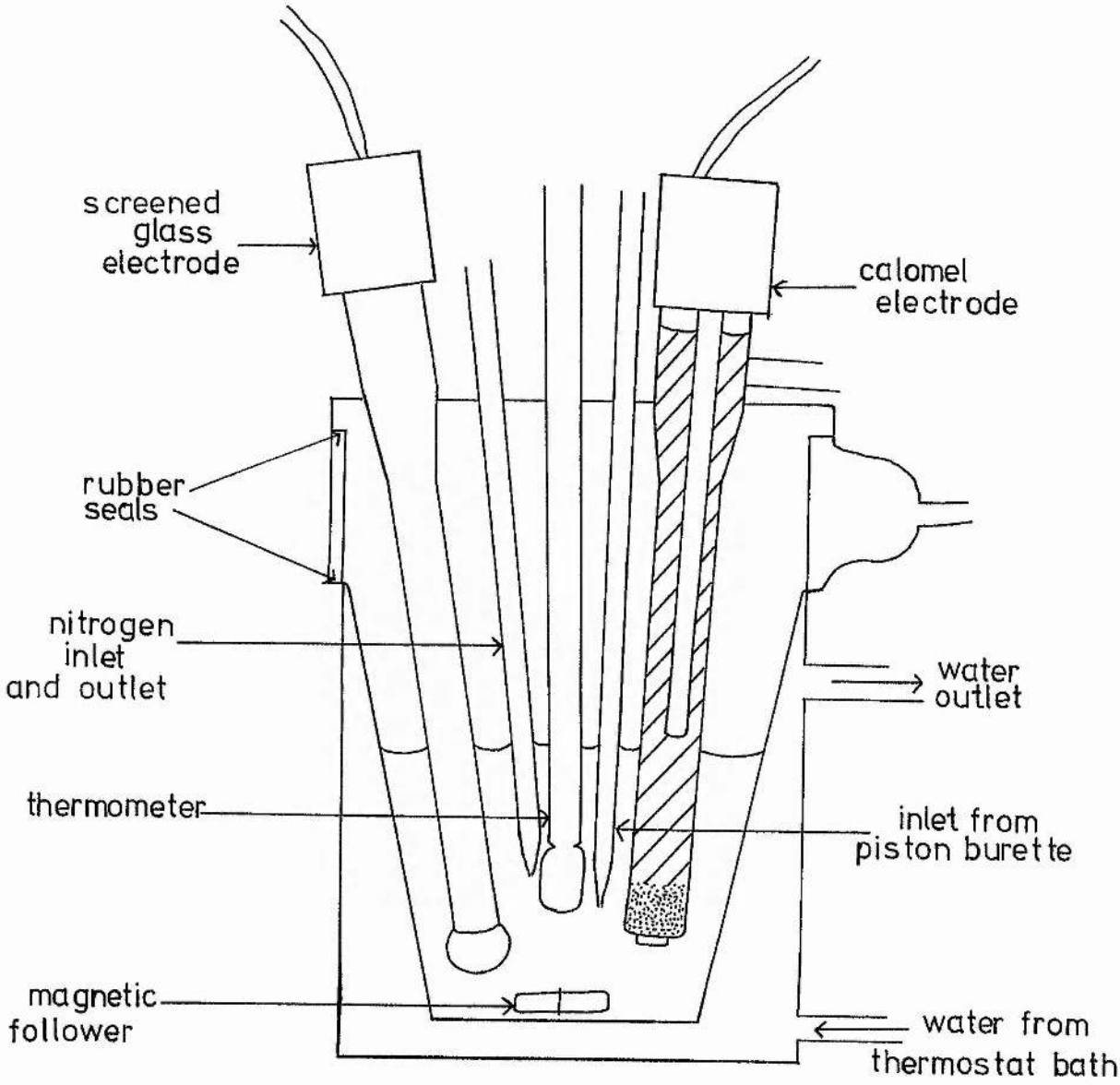


Figure 4: Titration apparatus

water was always greater than 0.5M Ω cm

Sodium Perchlorate

This was prepared by dissolving the monohydrate (B.D.H. AnalaR or Merck "Puriss"). The resulting solution was made alkaline, pH9-10, by the addition of sodium hydroxide (B.D.H. AnalaR) and was then allowed to stand for seven days during which time any silicon or heavy metals were precipitated either as their oxides or hydroxides. These were removed by filtering through micropore (5000 nm and 450 nm) filters (Millipore Ltd.). The solution was then made acid, pH2, by the addition of perchloric acid (Fisons A.R.) and boiled to remove any carbon dioxide. The solution was then cooled under nitrogen and standardised by bringing to pH7 and analysing by cation exchange (50a) and flame photometry (50b).

Perchloric Acid

A stock solution of approximately 3M was prepared by dilution of concentrated perchloric acid (60-62% W/V, Fisons A.R.). This was standardised by titration with sodium carbonate (Fisons A.R.) which had been heated at 260-270°C for half an hour and dried in a desiccator, using methyl orange as indicator (50c) and checked against standard sodium hydroxide (50d).

Sodium Hydroxide

1.00M and 0.100M solutions were prepared from ampoules (B.D.H. concentrated volumetric solutions).

Metal Ion Solutions

Cadmium (II) perchlorate was prepared by dissolving cadmium oxide (B.D.H.) in perchloric acid. The solution was filtered through micropores and analysed using EDTA with xylenol orange as indicator (50e)

Zinc (II) perchlorate was prepared by dissolving zinc perchlorate (G.F. Smith, Chemical Co.) in perchloric acid and filtering through micropores. It was then standardised using quinaldinate (50f) and EDTA (50g) with Eriochrome black T as indicator.

Lanthanum (III) perchlorate was prepared by dissolving lanthanum oxide (American Potash Chemical Corporation) in perchloric acid. The solution was filtered and standardised against EDTA (51) using Erbochrome black T as indicator.

The hydrogen ion concentration of these solutions was obtained by means of Gran plots (52).

EDTA

The disodium salt of ethylenediaminetetraacetic acid (B.D.H. AnalaR) is a primary standard and, therefore, solutions were prepared by direct weighing (50h).

LIGANDS

L-asparagine, L-aspartic acid, L-glutamine, L-phenylalanine, L-serine, L-tryptophan (B.D.H., Biochemical grade); L-histidine (Koch-Light "Puriss"), L-cysteine (E. Merck, Biochemical grade), ethylenediaminetetraacetic acid disodium salt dihydrate (B.D.H.AnalaR), 1,2-di(2-aminoethoxyethane) tetraacetic acid (B.D.H.), glutathione (Sigma) and D-penicillamine (Koch Light) were dried and analysed.

(a) L-asparagine H_2O (m.p.233-235°C; lit.,235°C).

Found: C,31.9; H,7.0; N,18.5. Calc. for $C_4H_{10}N_2O_4$: C,32.0; H,6.7; N,18.7%

(b) L-aspartic acid (m.p.270°C; lit.,270-271°C).

Found: C,35.6; H,5.1; N,10.2. Calc. for $C_4H_7NO_4$: C,36.1; H,5.3; N,10.5%

(c) L-glutamine (m.p.180-185°C; lit., 185-186°C(decomp.)).

Found: C,41.0; H,6.8; N,19.2. Calc. for $C_5H_{10}N_2O_3$: C,41.1; H,6.9; N,19.2%

(d) L-Serine (m.p.210-220°C; lit., 228°C(decomp.)).

Found: C,34.2; H,6.7; N,13.3. Calc. for $C_3H_7NO_3$: C, 34.3; H,6.7; N,13.3%

(e) L-tryptophan (m.p.281°C; lit., 281-282°C).

Found: N,13.67; Calc., N,13.71%

(f) L-histidine (m.p. 285-286°C; lit., 287°C(decomp.)).

Found: C,46.2; H,6.0; N,27.2. Calc. for $C_4H_9N_3O_2$:

C,46.4; H,5.8; N,27.1%

(g) L-cysteine (m.p.220°C; lit., 217-228°C).

Found: C,29.6; H,5.9; N,11.2. Calc. for $C_3H_7NO_2S$:

C,29.7; H,5.8; N,11.6%

(h) L-phenylalanine (m.p.284°C; lit., 283-284°C).

Found: C,65.2; H,6.9; N,8.5. Calc. for $C_9H_{11}NO_2$:

C,65.4; H,6.7; N,8.5%

(i) Ethylenediaminetetraacetic acid disodium salt dihydrate.

Found: C,32.46; H,5.18; N,7.53. Calc. for $C_{10}H_{18}N_2O_{10}Na_2$:

C,32.26; H,4.87; N,7.53%

(j) 1,2-Di(2-aminoethoxyethane) tetraacetic acid.

Found: C,43.48; H,6.64; N,7.07. Calc. for $C_{14}H_{24}N_2O_{10}$:

C,44.17; H,6.36; N,7.36%

(k) Glutathione.

Found: C,38.80; H,5.77; N,13.50. Calc. for $C_{10}H_{17}N_3O_6S$:

C,39.10; H,5.58; N,13.70%

(l) D-penicillamine.

Found: C,40.30; H,7.78; N,9.17. Calc. for $C_5H_{11}NO_2S$:

C,40.23; H,7.43; N,9.42%

These ligands were used without further purification.

NITROGEN

Oxygen-free nitrogen (British Oxygen) was further deoxygenated by passage through chromous chloride, any resulting acid was removed by passage through sodium hydroxide, and finally passed through 3.00 M sodium perchlorate. All these solutions were thermostatted at 25°C.

GLASSWARE

All graduated flasks and pipettes (Technico Grade A) were provided with calibration certificates. All glassware was cleaned regularly with "Quadralene" (Quadralene Chemical Products) and alcoholic potassium hydroxide and then rinsed with deionised water.

Before use, all glassware was thoroughly washed with demineralised water and "Elgastat" deionised water.

PART II STUDIES OF OSCILLATING REACTIONS

APPARATUS

All studies were carried out at 25°C in the vessel described in Part I of this chapter, under nitrogen.

The oscillations in the Fe^{2+}/Fe^{3+} concentrations were monitored using a platinum bright spade electrode (Russell pH Ltd., UMP 5/130) with a sodium sulphate calomel electrode (Activion Glass Ltd., 17 SR/B14) in conjunction with a Radiometer pH meter 26. The changes in potential were recorded using a Heath servo-recorder (Model EUW-20A).

REAGENTS

Potassium bromate, sulphuric acid (Fisons A.R), malonic acid and ferroin (B.D.H.) were used without further purification. Stock solutions of potassium bromate (approx.0.25M), malonic acid (approx.1.0M) and sulphuric acid (approx. 5.0M) were prepared using deionised water ('Elgastat'). Ferroin solution (2.5×10^{-2}M) was used as supplied.

PART III PARTITION STUDIES

APPARATUS

All aqueous solutions were prepared at pH7 in 3.00M sodium perchlorate. The aqueous solution and octanol were shaken mechanically for one hour using a mechanical shaker (Griffin and George flask shaker). Cadmium analysis

of the octanol layer was carried out on an atomic absorption spectrophotometer (Perkin Elmer 360) using a Cathodeon 4QAY/Cd lamp.

REAGENTS

Cadmium (II) perchlorate, sodium perchlorate and glutathione, were as described in Part I. 1-Octanol (Koch Light 'Pure') was used without any further purification.

Cadmium standards in octanol for the atomic absorption spectrophotometer were prepared from 4-cyclohexylbutyric acid - cadmium salt (B.D.H.).

All solutions were prepared using glassware as described in Part I.

CHAPTER III

THEORETICAL CONSIDERATIONS

THEORETICAL CONSIDERATIONS

Since the work of Bodländer and his associates (53-56) and von Euler (57-60) on the stability constants of metal complexes this field of study has progressed rapidly during this century.

Much of the success of this work is due to the development of the constant ionic medium method, which was first reported as early as 1905 (61), whereby the activity coefficient of all solutes, present as small fractions of the total electrolyte concentration, is constant at constant total electrolyte concentration. This principle was first put forward by Brønsted (62) and later developed by Biedermann and Sillén (63).

In studying the complexing reactions of metal ions and ligands in aqueous solution it is necessary to realise that the metal ion,B, exists as the aquo ion,$B(H_2O)_x$, and that the donor sites on the ligands may also attract water molecules, therefore, the reaction between a metal ion,(B), and a ligand,(A), may be represented by:

$$pA(H_2O)_w + qB(H_2O)_x + rH(H_2O)_y \rightleftharpoons ApBqHr(H_2O)_z + (pw+qx+ry-z)\ H_2O \qquad .1.$$

In practice the activity of free water is assumed constant in aqueous solutions and thus the water of hydration is omitted from the equation, therefore:

$$pA + qB + rH \rightleftharpoons ApBqHr \qquad .2.$$

As the law of mass action is only true when activities and not concentrations are being considered, the overall formation constant, β_{pqr} of the complex formed from the individual species is given by:

$$\beta_{pqr} = \frac{(ApBqHr)}{(A)^p(B)^q(H)^r} \qquad .3.$$

which is related to the concentrations of the species by the following equation:

$$\beta_{pqr} = \frac{[ApBqHr]}{[A]^p[B]^q[H]^r} \cdot \frac{fApBqHr}{p^f_a \cdot q^f_b \cdot r^f_h} \qquad .4.$$

where f denotes an activity coefficient. Hence, if all activities are held constant a new formation constant can be defined in terms of molar concentrations:-

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

From these equations it can be seen that the use of equation (4) and the 'thermodynamic' formation constant is restricted to systems where the activity coefficients can be measured or else the results extrapolated to zero ionic strength. Equation (5) also presents limitations in that the results obtained from it can only be compared with those from other systems with a similar ionic background.

CHOICE OF EXPERIMENTAL CONDITIONS

Activity coefficients may be held constant by working in a solution of an inert electrolyte but certain conditions must be met. The electrolyte must be strong with its anion and cation not associating with either the metal ion or the ligand respectively, and neither associating with the complex species. No redox reaction must occur between the ions of the electrolyte and the metal or ligand, and its contribution to the measured properties must be negligible.

One of the salts which satisfies these conditions is sodium perchlorate, but there are differences of opinion as to which set of experimental conditions should be used (64). The two most widely used are 3 M $NaClO_4$ at 25°C and 0.15 M $NaClO_4$ at 37°C. Obviously if one is considering results in terms of a biological model, 3M, 25°C is far removed from blood plasma conditions, also at this concentration any impurities in the ionic background will be emphasised as would any tendency of ion pairing (65). It has been found that cadmium can form weak complexes with perchlorate

ions (66) but even at 3M ClO_4^- the amount of complex formation is not appreciable.

The major disadvantages of working at 0.15M and 37°C are that most of the standard volumetric glassware requires recalibration and unless the complete titration apparatus is thermostatted, condensation occurs on the cooler parts and the resulting electrothermal effect in the electrodes can cause an error of up to 3mV(67). Also this low concentration of ionic background permits only an 0.008M change in concentration without significantly altering the activity coefficients whereas in 3M background there is a tolerance of 10% or 0.3M (63,68).

After consideration of these facts and also taking into account that most of the potentiometric work at St. Andrews had been carried out at 3M-25°C it was felt justifiable to carry out the work in this thesis under these conditions.

MATHEMATICAL RELATIONSHIPS INVOLVING STABILITY CONSTANTS

The use of potentiometric methods involves the Nernst equation, which for the reaction

$$kK + lL \rightleftharpoons mM + nN \qquad .6.$$

is given by

$$E = E^o + \frac{RT}{zF} \ln \frac{(K)^k(L)^l}{(M)^m(N)^n} \qquad .7.$$

which requires that the unit activity or standard state of the species present should be defined. For the glass electrode this is given by:

$$E = E^o + \frac{RT}{F} \ln(h) \qquad .8.$$

In determining the stability constants of metal complexes from potentiometric titration data the first step is to obtain formation curves in terms of $\bar{n}$, the average number of ligands bound to each metal ion, and the logarithm of the free ligand concentration.

$\bar{n}$ is defined as follows:

$$\bar{n} = \frac{[AB] + 2[A_2B] + \ldots\ldots\ldots\ldots N[A_NB]}{[B] + [AB] + [A_2B] + \ldots\ldots\ldots\ldots [A_NB]} \quad .9.$$

$$= \frac{\sum_{1}^{N} n\beta_n a^n}{\sum_{0}^{N} \beta_n a^n} \quad .10.$$

From equation (9) it can be seen that

$$\bar{n} = \frac{\text{bound ligand concentration}}{\text{total metal concentration}}$$

In potentiometric studies this can be determined from a knowledge of the initial total concentrations of metal, ligand and mineral acid, the pH of the solution and the stability constants for any protonated ligand species.

As the concentration of bound ligand must equal the total ligand concentration minus the sum of the concentrations of free ligand and protonated ligand it follows that:

$$\bar{n} = \frac{A - a - (\beta_{101}ah + \beta_{102}ah^2 + \ldots\ldots\ldots\ldots \beta_{10N}ah^N)}{B} \quad .11.$$

$$= \frac{A - a(1 + \sum_{1}^{N} \beta_{10n}h^n)}{B} \quad .12.$$

The free ligand concentration, a, maybe determined as follows:

$$H = h - \frac{Kw}{h} + a(\beta_{101}h + 2\beta_{102}h^2 + \ldots\ldots\ldots\ldots N\beta_{10N}h^N) \quad .13.$$

$$= h - \frac{Kw}{h} + a\sum_{1}^{N} n\beta_{10n}h^n \quad .14.$$

i.e. $$a = \frac{H - h + Kw/h}{\sum_{1}^{N} n\beta_{10n}h^n} \quad .15.$$

These relationships assume that neither protonated nor hydroxy metal complexes occur, therefore the curves would be expected to have points of inflexion at each integral $\bar{n}$ value. Furthermore, if there are no polynuclear complexes the curves should be completely superimposable over a range of ligand: metal ratios at varying total metal concentrations. However, this situation seldom exists in practice.

The existence of protonated metal species results in falsely high values for $\bar{n}$ and a (i.e. low values of pa), whereas the presence of hydroxy species has the converse effect.

An initial estimate of the stability constants may be made by considering the steps of the complex formations as being distinct, but this is only valid where $\log\beta_n - \log\beta_{n-1} > 2$. If this is so it can be assumed that at any point only two complexes exist simultaneously in any considerable concentration.

If $\bar{n} = n - 1/2$, $\quad [A_{n-1}B] = [A_nB]$

and $$K_n = \frac{[A_nB]}{[A_{n-1}B]\,a}$$

where a is the free ligand concentration, therefore

$$K_n = \frac{1}{a} \quad \text{or} \quad \log K_n = -\log a$$

These first approximations may then be refined by iterative procedures based on numerical approximations for which the use of computers has become invaluable.

CHAPTER IV

COMPUTER PROGRAMS

COMPUTER PROGRAMS

The use of computers has greatly facilitated the study of stability constants (69,70) enabling data to be processed very much more quickly than would otherwise be possible.

In the studies reported in this thesis the computing can be divided into three sections; first, the calculation of $\bar{n}$ and -log a enabling/

the plotting of formation curves; second, the refinement of the constants and the back calculation of theoretical formation curves from the computed constants; third, the use of these constants in computer models simulating biological systems.

For the calculation of $\bar{n}$ and - log a the program RWZPLOT was used. The stability constants were refined using either SCOGS or MINIQUAD and the calculation of theoretical formation curves by RWPSEUDOPLOT. Finally the model systems were generated using either HALTAFALL or RWCOMPLOT.

RWZPLOT

This program calculates $\bar{z}$ and -log a for every experimental point in the titration, the mathematics of which have been described in the preceding chapter. The input was arranged to be the same as for SCOGS making for ease of data interchange between the two programs. In addition RWZPLOT contains a plotter routine thus allowing a quick visual appraisal of the system, which with experience can be used to postulate the existence of protonated or hydroxy complexes.

SCOGS (71, 72).

Much of the work reported here used SCOGS, but for some of the more complex systems SCOGS could not be used due to exponent overflow.

This program which refines the stability constants of generalised species employs the non-linear least squares approach.

First this program estimates the concentration of free metal and free ligand for the first point making the approximation that the free metal concentration is equal to that of the total metal i.e. that no complexing has taken place. Using this assumption the concentration of free ligand can be calculated from the total concentration and the acid association constants.

These first approximations are then improved iteratively in a subroutine using the Newton-Raphson method until they are satisfactory roots in the simultaneous equation for the total metal and total ligand. These improved free concentrations are then used to calculate the concentrations of all the complex species present and hence the analytical hydrogen ion concentration.

This quantity is subsequently used in the main program to calculate the titre value which leads to a residual R_i where

R_i = actual titre - calculated titre

This is carried out for each experimental point building up the least squares equations which are solved by matrix inversion to give the shifts in the constants. The improved constants are then calculated and the whole cycle is repeated for a specified number of times.

MINIQUAD (73)

This program, which is based on an earlier program LETAGROP (74-76) is also used to refine stability constants,

Unlike SCOGS which varies sets of $\log\beta$ this program varies sets of β To prevent exponent overflow, which has occurred in the use of SCOGS, the formation constants are stored as mantissa and exponent and during the refinement only the mantissa is changed, the exponent being kept constant. The method of refinement used in MINIQUAD is the Gauss-Newton which has been shown to be preferable to the Newton-Raphson method which is used in SCOGS (77).

Instead of minimising the sum of the squares of residuals in titre volumes, MINIQUAD minimises the sum of the squares of residuals in analytical concentrations. The least-squares minima are approached using the Fletcher-Powell steepest descent method rather than a Jacobean matrix.

HALTAFALL (78)

This program calculates the equilibrium concentrations of species in mixtures of any number of components forming any number of complexes. It can cope with systems containing two fluid phases, either two liquids or one liquid and one gaseous, and also with systems containing solids. HALTAFALL can, therefore, be used in obtaining distribution data.

This program has since been modified in our laboratory to incorporate the Z PLOT program thus allowing the quick calculation of theoretical

formation curves using the computed formation constants and the experimental titration data (64). The graphs thus obtained allow a quick visual comparison to be made between the experimentally and theoretically obtained formation curves which gives an indication as to how well the constants describe the system.

COMPLOT

This is the St. Andrews version of COMICS (41), a program which calculates the equilibrium concentrations of species in multi-metal, multi-ligand systems, which was published by Perrin and Sayce in 1967. It was modified slightly to enable loading on the St. Andrews IBM 360/44 computer and also to allow the inclusion of three plotter routines. It can handle up to ten metals, ten ligands and one hundred complex species including protonated, hydrolysed, mixed and polynuclear complexes.

The program considers the metal ions B^a, B^b, B^c.... and the ligands A^r, A^s, A^t ..... as being capable of forming a number of different complexes of the type $(B^a)_\alpha (B^b)_\beta$ .... $(A^r)_\rho (A^s)_\sigma$ ... $(OH)_\omega$ where $\alpha, \beta \ldots\ldots \rho, \sigma$ are either positive integers or zero and where ω can be a positive integer (a hydrolysed species), a negative integer (a protonated species), or zero.

The concentration of one of these species may, therefore be given by:

$$C_j = \beta_j [B^a]^\alpha [B^b]^\beta \ldots\ldots [A^r]^\rho [A^s]^\sigma \ldots\ldots [OH]^\omega \qquad .1.$$

where β_j is the overall formation constant. From this the total concentration of the metal, i, is shown by the following expression

$$[B^i]_T = [B^i] + \sum_{j=1}^{j=n} P_{ij} C_j \qquad .2.$$

where p_{ij} is the number of metal ions of metal i in the species j.

Initially it is assumed that $[B^i] = [B^i]_T$, that is, that the amount of complex formation is negligible. As in

MINIQUAD this allows the free ligand concentration to be calculated from a knowledge of the total ligand concentration and the acid association constants.

From this the quantity on the right hand side of equation (2) is calculated to give the quantity $\left[B^i\right]_T^{calc}$. The initial value is then replaced by $\left[B^i\right]$ ($\left[B^i\right]_T / \left[B^i\right]_T^{calc})^{\frac{1}{2}}$ and this is repeated until $\left[B^i\right]_T$ and $\left[B^i\right]_T^{calc}$ agree to within 0.001% of $\left[B^i\right]_T$. A similar operation is carried out for the ligand concentrations.

- - - - - - - - - - - - -

The advantages to the chemist in using computers in such systems are obviously considerable. In the study of stability constants much time is saved from being spent on tedious calculations, thus allowing a greater number of systems to be studied than would otherwise be possible. Also, without the availability of programs such as HALTAFALL and COMICS, it is a moot point whether or not model systems would be studied in any great detail.

However, useful though it is, the computer is not indispensible, and certainly its use does not make ones chemical knowledge redundant as interpretation and discrimination are still vital when assessing results.

CHAPTER V

POTENTIOMETRY

PART I - GLASS ELECTRODE

PART II - SOLID STATE ELECTRODE

P O T E N T I O M E T R Y

PART I: GLASS ELECTRODE POTENTIOMETRY

All studies were carried out at 25°C, I = 3.00 M.Na ClO_4 in the apparatus described in Chapter II.

For ligand protonations the total concentration of ligand in the titrate (S) and titrant (T) was kept constant, only the hydrogen ion concentration being varied. For the metal-ligand systems the ratio of ligand to metal was varied thus allowing the investigation of polynuclear, protonated or hydroxy species where non-superimposable sets of formation curves occurred.

Before each titration the E^o was measured using a standard acid solution.

ELECTRODE CALIBRATION

The electrode pair was calibrated by titrating perchloric acid with sodium hydroxide, the experimental results of which are shown in Table 2.

TABLE 2

Experimental results for the calibration of the electrode pair.

Titrate 25.01ml, 0.00779 M $HClO_4$
Titrant 0.02502 MNaOH

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.00	342.0	7.00	274.3	7.78	-24.2	9.00	-178.8
0.50	340.3	7.50	237.7	7.80	-42.0	10.00	-196.2
1.50	336.2	7.60	213.8	7.85	-67.0	11.00	-206.3
2.50	331.0	7.65	187.3	7.90	-81.8	13.00	-218.0
3.50	324.9	7.70	128.0	8.00	-104.7	15.00	-225.5
4.50	316.7	7.72	98.1	8.10	-123.2	17.00	-230.7
5.50	306.1	7.74	56.2	8.30	-146.4		
6.50	289.4	7.76	4.3	8.50	-160.2		

The pH of the solution was then calculated from the volumes and concentrations using a value of $-\log K_w = 14.22$ (79).

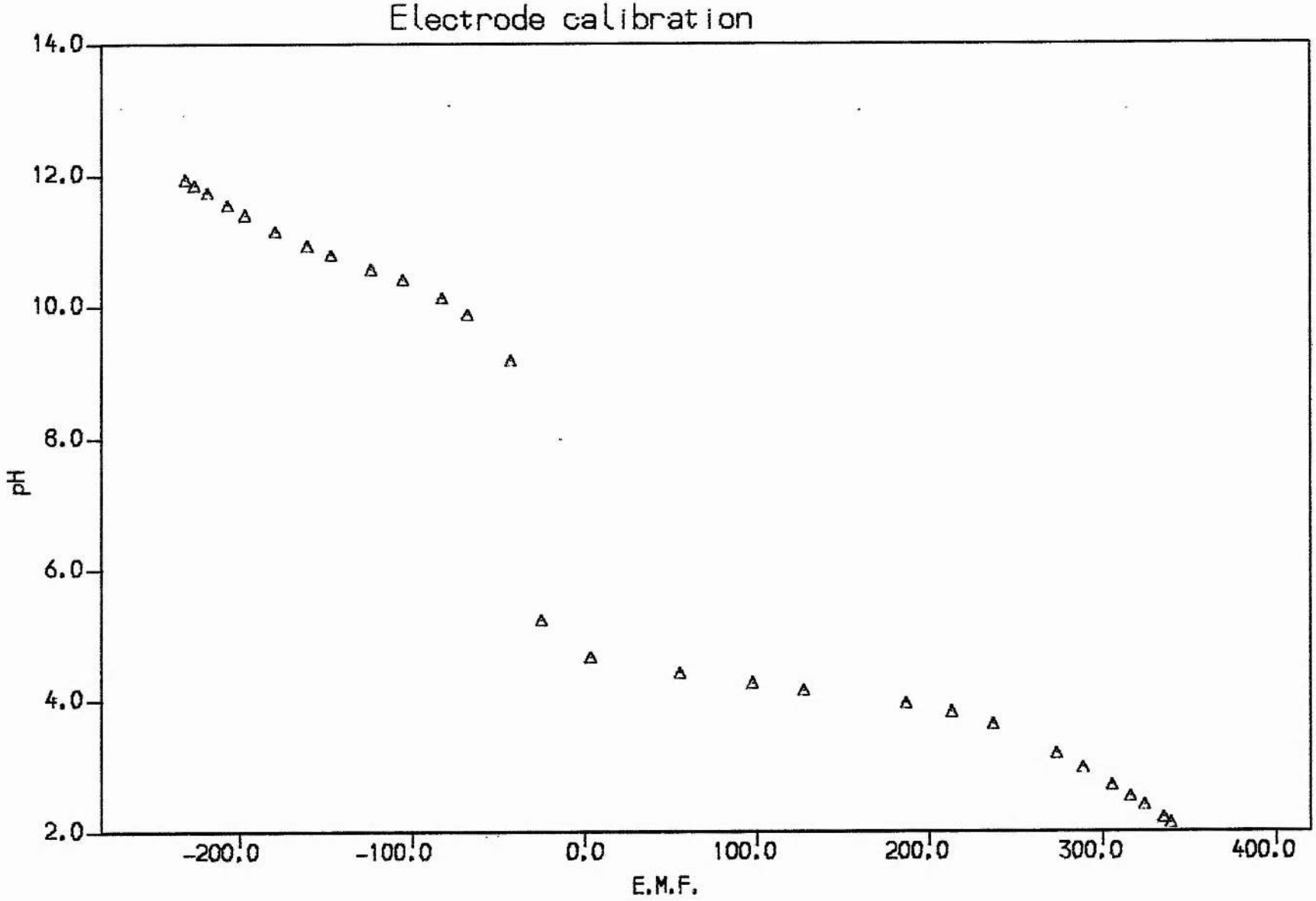

Electrode calibration
14.0
12.0
10.0
8.0
6.0
4.0
2.0
pH
-200.0
-100.0
0.0
100.0
200.0
300.0
400.0
E.M.F.

Figure5

From the equation

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

it can be seen that where E° and E_j, the liquid junction potential, are constant a plot of pH versus e.m.f. should give a straight line of gradient $-\frac{zF}{2.303RT}$. As can be seen from Figure 5 an S-shaped curve was obtained between pH3.0 and 10.0 which can be attributed to the fact that the solution is unbuffered. However, the linear portions at either end of the curve do allow a measurement of electrode response.

TABLE 3

Experimental results for the formation of cadmium (II) - asparaginate.

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.05280	0.00820	0.002083	0.0000	0.0000	-0.10010	30.01	453.6
2.	0.090130	0.021070	0.005219	0.0000	0.0000	-0.100100	35.01	454.2
3.	0.12090	0.0000	-0.02003	0.0000	0.024580	0.006094	25.01	453.3

b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.20	217.2	1.60	86.4	4.40	19.1	7.50	-49.0
0.40	205.5	2.00	73.9	4.80	9.7	8.00	-56.4
0.60	183.4	2.40	63.4	5.20	-0.3	8.50	-63.1
0.80	146.3	2.80	54.1	5.60	-10.3	9.00	-68.1
1.00	119.1	3.20	45.4	6.00	-19.3		
1.20	104.3	3.60	36.7	6.50	-31.0		
1.40	94.5	4.00	28.0	7.00	-40.6		

TABLE 3b contd.

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.50	228.4	4.00	107.4	10.00	47.6	17.00	-18.1
1.00	219.4	4.50	99.6	11.00	39.5	18.00	-28.4
1.50	206.4	5.00	93.1	11.90	32.0	19.00	-38.3
2.00	184.2	5.50	87.3	12.50	26.7	20.00	-47.4
2.30	164.5	6.00	82.0	13.50	17.7	21.00	-55.7
2.60	146.2	7.00	72.6	14.50	8.1	22.00	-63.7
3.00	130.2	8.00	64.0	15.50	2.1		
3.50	116.8	9.00	55.7	16.00	-7.3		

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
5.00	-30.6	9.00	12.6	13.00	44.0	17.00	58.9
6.00	-21.8	10.00	23.4	14.00	48.6	18.00	61.7
7.00	-11.3	11.02	32.0	15.00	52.5	19.00	64.4
8.00	0.5	12.00	38.7	16.00	55.9	20.00	66.8

TABLE 4

Experimental results for the formation of cadmium (II) - aspartate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV.
	A	B	H	A	B	H		
1.	0.024151	0.00985	0.002322	0.0000	0.0000	-0.10000	25.01	456.6
2.	0.011616	0.00190	0.000447	0.0000	0.0000	-0.1000	26.00	456.6
3.	0.012075	0.0000	-0.020008	0.0000	0.04921	0.011615	25.01	457.7
4.	0.020112	0.00820	0.002196	0.0000	0.0000	-0.10000	30.02	458.9
5.	0.005919	0.00096	0.000228	0.0000	0.0000	-0.10000	25.51	458.9
6.	0.012068	0.0000	-0.020008	0.0000	0.04921	0.11615	25.01	458.6

TABLE 4b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	277.3	5.00	199.8	7.50	66.3	10.90	-54.8
0.50	271.2	5.50	188.6	7.70	58.3	11.10	-64.7
1.00	263.4	5.90	176.8	8.00	47.5	11.30	-75.3
1.40	257.0	6.20	164.4	8.30	37.5	11.50	-87.2
1.80	250.8	6.40	152.8	8.60	27.8	11.70	-99.3
2.00	247.8	6.60	135.6	9.00	15.0	11.90	-111.6
2.50	240.1	6.70	125.0	9.30	5.3	12.10	-124.1
3.00	232.8	6.80	113.2	9.60	-4.7	12.30	-136.8
3.50	225.2	6.90	102.7	10.00	-18.8	12.50	-148.9
4.00	217.5	7.10	87.2	10.30	-29.7		
4.50	209.2	7.30	75.7	10.60	-41.7		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	263.4	1.50	221.1	3.10	105.4	3.90	-33.9
0.30	257.1	1.70	214.9	3.30	47.3	4.00	-48.9
0.50	251.0	1.90	208.6	3.40	31.6	4.10	-63.5
0.70	244.8	2.10	201.8	3.50	18.1	4.20	-76.5
0.90	238.6	2.30	194.0	3.60	5.2	4.30	-87.5
1.10	232.8	2.70	173.0	3.70	-7.3		
1.30	226.9	2.90	154.4	3.80	-20.3		

TABLE 4b cont'd.

titration 3.

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.20	-116.7	2.80	-13.0	7.00	50.8	13.00	114.0
1.30	-110.1	3.00	-6.9	7.50	55.4	13.30	119.6
1.40	-104.1	3.30	0.7	8.00	59.8	13.60	125.5
1.50	-95.9	3.60	7.3	8.50	64.2	13.80	129.7
1.60	-86.5	4.00	14.8	9.00	68.6	14.00	134.0
1.70	-76.3	4.30	19.7	9.50	73.1	14.20	138.4
1.80	-66.2	4.60	24.2	10.00	77.8	14.40	142.7
1.90	-57.5	5.00	29.5	10.50	82.4	14.60	146.8
2.00	-50.0	5.30	33.2	11.00	87.4	15.00	154.4
2.20	-37.6	5.60	36.7	11.50	93.0		
2.40	-28.0	6.00	41.0	12.00	99.2		
2.60	-19.9	6.50	46.1	12.50	106.1		

titration 4.

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.50	273.4	5.50	190.8	7.10	90.3	8.60	28.2
1.00	265.5	6.00	176.0	7.20	83.6	8.80	21.9
1.50	257.6	6.20	167.9	7.30	77.6	9.00	15.6
2.00	249.7	6.40	157.0	7.40	72.4	9.20	9.0
2.50	242.1	6.50	149.9	7.50	67.6	9.40	2.3
3.00	234.5	6.60	141.5	7.60	63.2	9.60	-4.2
3.50	227.0	6.70	131.0	7.80	55.3	9.80	-11.0
4.00	219.3	6.80	119.6	8.00	48.0	10.00	-18.1
4.50	211.1	6.90	108.3	8.20	41.2		
5.00	201.8	7.00	98.7	8.40	34.6		

TABLE 4b cont'd

titration 5

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.20	252.3	1.10	197.3	1.80	-14.1	2.10	-84.6
0.40	240.8	1.20	188.7	1.85	-26.7	2.15	-93.6
0.60	229.5	1.65	27.4	1.90	-38.8	2.20	-101.6
0.80	217.7	1.70	12.2	1.95	-51.2		
1.00	205.0	1.75	-1.4	2.00	-63.3		

titration 6

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.30	-111.0	2.20	-38.8	4.80	26.8	8.00	59.7
1.40	-104.5	2.40	-28.8	5.30	33.0	8.50	64.1
1.50	-96.5	2.60	-20.6	5.60	36.5	9.00	68.4
1.60	-87.9	2.80	-13.7	6.00	40.8	9.50	72.8
1.70	-77.7	3.20	- 2.2	6.30	44.0	10.00	77.2
1.80	-68.0	3.60	6.9	6.60	46.9		
1.90	-59.4	4.00	14.7	7.00	50.7		
2.00	-51.4	4.40	21.0	7.50	55.3		

TABLE 5

Experimental results for the formation of cadmium (II) - cysteinate

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml.	E° mV
	A	B	H	A	B	H		
1.	0.001912	0.000946	0.000223	0.0000	0.0000	-0.10000	26.01	456.0
2.	0.003682	0.001822	0.000430	0.0000	0.0000	-0.10000	27.01	456.0

TABLE 5b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	197.8	0.35	157.6	0.65	63.9	0.95	-67.4
0.10	188.7	0.40	145.9	0.70	35.3	1.00	-88.3
0.15	183.9	0.45	128.4	0.75	6.2	1.05	-117.4
0.20	179.0	0.50	95.1	0.80	-15.4		
0.25	173.1	0.55	82.3	0.85	-33.8		
0.30	166.4	0.60	74.7	0.90	-50.6		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	236.5	0.35	205.3	0.90	151.4	1.60	-4.9
0.10	223.4	0.40	202.3	1.00	117.0	1.70	-26.1
0.15	215.7	0.50	196.0	1.10	88.4	1.80	-45.5
0.20	212.9	0.60	188.9	1.20	77.0	1.90	-63.9
0.25	210.6	0.70	180.5	1.40	52.1	2.00	-85.2
0.30	207.8	0.80	168.7	1.50	2.5	2.10	-115.2

TABLE 6

Experimental results for the formation of cadmium (II) - glutaminate

a.

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.04744	0.008201	0.001936	0.0000	0.0000	-0.100000	30.01	450.3
2.	0.08133	0.02107	0.004974	0.0000	0.0000	-0.10000	35.01	450.3
3.	0.11380	0.0000	-0.02003	0.0000	0.02458	0.005804	25.01	450.3

TABLE 6b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.60	123.4	2.00	43.5	5.00	-20.1	8.00	-78.2
0.70	104.7	2.50	31.9	5.50	-31.3	8.50	-85.0
0.90	85.6	3.00	21.5	6.00	-42.3	9.00	-91.6
1.00	79.2	3.50	11.4	6.50	-52.6	9.50	-97.9
1.30	64.8	4.00	1.2	7.00	-61.8	10.00	-104.0
1.60	54.5	4.50	-9.1	7.50	-70.2		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
2.00	144.9	5.00	64.7	11.00	12.5	17.00	-43.6
2.30	122.0	6.00	54.3	12.00	4.0	18.00	-53.3
2.60	108.0	7.00	45.3	13.00	-4.6	19.00	-62.2
3.00	96.1	8.00	36.8	14.00	-13.7	20.00	-72.4
3.50	85.5	9.00	28.7	15.00	-23.5		
4.00	77.4	10.00	20.6	16.00	-33.5		

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
5.00	-46.9	9.00	-4.4	13.00	26.0	17.00	39.5
6.00	-37.2	10.00	8.3	14.00	30.2	18.00	41.9
7.00	-26.8	11.00	15.5	15.00	33.6	19.00	44.2
8.00	-15.3	12.00	21.2	16.00	36.7	20.00	46.3

TABLE 7

Experimental results for the formation of cadmium(II)-histidinate

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.03994	0.009846	0.002324	0.0000	0.0000	-0.10000	25.01	458.6
2.	0.03994	0.01968	0.03358	0.0000	0.0000	-0.10000	25.01	458.6
3.	0.03994	0.009846	0.06016	0.0000	0.0000	-0.10000	25.01	457.8
4.	0.019980	0.009844	0.03125	0.0000	0.0000	-0.10000	25.02	457.8

TABLE 7b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	55.5	3.00	24.8	5.60	-36.6	7.00	-86.8
0.50	51.6	3.50	18.0	5.80	-45.9	7.30	-93.9
1.00	46.8	4.00	9.9	6.00	-54.8	7.60	-100.2
1.50	41.7	4.50	0.1	6.20	-63.0	7.90	-106.2
2.00	36.5	5.00	-13.0	6.50	-73.3		
2.50	30.9	5.30	-23.6	6.80	-81.8		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	154.0	3.00	118.3	8.00	82.7	12.50	50.4
0.50	146.8	4.00	110.3	9.00	76.0	13.00	46.2
1.00	139.4	5.00	102.9	10.00	69.3	13.50	41.7
1.50	133.2	6.00	96.0	11.00	62.0	14.10	35.9
2.00	127.7	7.00	89.3	12.00	54.4	14.50	31.8

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
4.60	248.0	5.70	160.7	8.50	107.9	12.00	75.0
4.90	229.5	6.00	149.9	9.00	102.6	12.50	70.6
5.00	220.0	6.30	141.5	9.50	97.6	13.00	66.5
5.10	208.4	6.60	135.0	10.00	92.9	13.50	62.1
5.20	196.7	7.00	127.8	10.50	88.3	14.00	57.8
5.30	186.2	7.50	120.2	11.00	83.9		
5.50	171.2	8.00	113.7	11.50	79.4		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
3.00	187.0	3.60	141.0	6.00	94.7	9.00	56.9
3.05	179.0	4.00	128.6	6.50	88.3	9.50	49.8
3.10	172.3	4.50	117.5	7.00	82.2	10.00	41.8
3.20	162.3	5.00	108.8	7.50	76.2		
3.40	149.8	5.50	101.3	8.00	70.0		

TABLE 8

Experimental results for the formation of cadmium (II) - phenylalaninate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1	0.04116	0.008206	0.001937	0.0000	0.0000	-0.05003	30.01	452.1
2.	0.04116	0.0000	-0.00834	0.0000	0.04923	0.01162	30.01	452.1
3.	0.004555	0.0000	-0.003704	0.0000	0.04923	0.01162	27.01	452.3
4.	0.011710	0.002343	0.000553	0.0000	0.0000	-0.05003	21.01	450.7

TABLE 8b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.40	208.3	1.20	138.0	1.80	86.4	3.50	50.6
0.60	200.5	1.30	121.4	2.00	79.4	4.00	44.3
0.80	189.9	1.40	110.2	2.20	73.8		
1.00	173.1	1.50	102.0	2.50	67.0		
1.10	156.5	1.60	95.5	3.10	56.5		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.80	-62.9	1.20	-49.9	1.50	-37.6	1.70	-28.8
1.00	-56.9	1.40	-42.0	1.60	-33.2		

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.60	-104.5	2.00	-27.1	5.00	29.6	6.70	75.5
0.70	-94.5	2.40	-19.5	5.20	33.6	6.80	80.2
0.80	-85.0	2.80	-11.1	5.40	37.8	6.90	86.0
0.90	-76.9	3.20	-3.1	5.60	42.0	7.00	92.6
1.00	-69.1	3.60	4.1	5.80	46.7	7.10	101.8
1.20	-57.2	4.00	11.2	6.00	51.8	7.20	113.5
1.40	-48.2	4.40	18.4	6.20	57.4	7.30	132.6
1.60	-40.9	4.60	22.1	6.40	63.7	7.35	145.7
1.80	-34.4	4.80	25.8	6.50	67.2		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.15	180.7	0.30	73.1	0.70	23.0	2.10	-42.7
0.18	168.1	0.33	64.4	0.90	11.4	2.30	-52.2
0.20	155.1	0.36	57.7	1.10	1.5	2.50	-61.4
0.22	135.8	0.40	51.0	1.30	-7.3	2.70	-70.1
0.24	109.2	0.45	44.5	1.50	-15.9	2.90	-78.6
0.25	99.1	0.50	39.0	1.70	-24.6		
0.27	86.0	0.60	30.2	1.90	-33.5		

TABLE 9

Experimental results for the formation of cadmium (II) - serinate

a

Titration	Titrate (S) Molarity			Titrant (T) molarity			Volume ml	E° mV
	A	B	H	A	B	H		
1.	0.07628	0.02107	0.004974	0.0000	0.0000	-0.10000	35.01	453.1
2.	0.1067	0.0000	-0.02003	0.0000	0.02458	0.005804	25.00	454.0
3.	0.04245	0.008201	0.001936	0.0000	0.0000	-0.10010	30.01	451.6

TABLE 9b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.30	203.8	3.50	92.3	10.00	30.3	17.00	-36.3
1.80	167.3	4.00	84.6	11.00	22.3	18.00	-47.5
2.00	146.5	5.00	72.5	12.00	14.0	19.00	-58.4
2.20	131.4	6.00	62.7	13.00	5.3	20.00	-69.0
2.40	121.0	7.00	54.0	14.00	-4.1		
2.70	110.0	8.00	45.9	15.00	-14.3		
2.00	102.1	9.00	38.1	16.00	-25.1		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
4.00	-50.3	9.00	5.4	14.10	39.8	19.00	53.7
5.00	-41.9	10.00	15.7	15.00	43.1	20.00	55.8
6.00	-31.7	11.00	23.7	16.00	46.2		
7.00	-19.9	12.00	30.0	17.00	48.9		
8.00	-7.0	13.00	35.1	18.00	51.4		

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.80	97.9	3.00	29.0	5.50	-28.4	8.00	-80.1
1.00	84.8	3.50	18.2	6.00	-40.6	8.50	-87.9
1.50	64.8	4.00	7.9	6.50	-52.2	9.00	-95.4
2.00	51.0	4.50	-3.5	7.00	-62.5	9.50	-102.5
2.50	39.4	5.00	-15.7	7.50	-71.7	10.00	-109.8

TABLE 10

Experimental results for the formation of cadmium (II) - tryptophanate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.010133	0.0000	-0.001667	0.0000	0.02461	0.005808	30.01	456.5
2.	0.003379	0.0000	-0.001667	0.0000	0.02461	0.05808	30.02	458.5
3.	0.004056	0.000985	0.000232	0.0000	0.0000	0.10000	25.01	459.0
4.	0.04220	0.008199	0.001936	0.0000	0.0000	-0.050030	30.01	452.3
5.	0.03798	0.01230	0.002905	0.0000	0.0000	-0.050030	20.00	452.3
6.	0.022910	0.004096	0.000968	0.0000	0.0000	-0.05003	30.01	451.9

TABLE 10b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.30	-71.8	0.95	-23.6	1.90	10.6	6.00	70.8
0.40	-66.2	1.00	-20.4	2.10	14.5	6.50	81.5
0.50	-59.8	1.05	-17.4	2.40	19.8	7.00	96.9
0.60	-52.3	1.10	-14.6	2.70	24.3	7.20	106.0
0.65	-48.2	1.20	-9.9	3.00	28.5	7.40	117.8
0.70	-44.0	1.30	-5.7	3.50	35.3	7.60	133.9
0.75	-39.6	1.40	-2.2	4.00	41.5	7.80	152.4
0.80	-35.4	1.50	0.8	4.50	48.0	8.00	165.3
0.85	-31.2	1.60	3.7	5.00	54.8		
0.90	-27.4	1.70	6.2	5.50	62.3		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.40	-101.7	1.20	-44.0	4.00	12.7	6.80	75.6
0.50	-94.5	1.40	-36.4	4.50	20.3	6.90	81.2
0.60	-86.4	1.60	-30.2	5.00	28.4	7.00	87.2
0.70	-77.7	1.80	-25.0	5.50	37.6	7.10	96.0
0.80	-68.9	2.00	-20.6	6.00	48.6	7.20	108.2
0.90	-61.0	2.50	-10.8	6.30	56.7	7.30	126.1
1.00	-54.6	3.00	-2.5	6.60	67.0		
1.10	-48.9	3.50	5.1	6.70	71.1		

TABLE 10b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.60	83.3	1.20	21.0	2.80	-17.8	5.00	-54.6
0.65	68.0	1.30	17.1	3.00	-21.2	5.30	-59.7
0.70	58.7	1.40	13.6	3.20	-24.7	5.60	-65.0
0.75	51.2	1.50	10.5	3.40	-28.1	6.00	-72.0
0.80	45.7	1.60	7.7	3.60	-31.3	6.30	-77.2
0.85	41.2	1.80	2.5	3.80	-34.6	6.60	-82.3
0.90	37.2	2.00	-2.0	4.00	-37.8	7.00	-89.0
0.95	33.7	2.20	-6.4	4.20	-41.2	7.30	-93.9
1.00	30.8	2.40	-10.4	4.40	-44.6	7.60	-98.6
1.10	25.5	2.60	-14.1	4.60	-47.9	8.00	-104.7

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.60	195.7	1.00	173.6	1.40	124.0	2.00	87.3
0.70	191.5	1.10	163.6	1.50	113.2	2.20	81.1
0.80	186.8	1.20	151.0	1.60	106.0	2.40	76.1
0.90	180.8	1.30	136.5	1.80	95.1	2.60	72.0

titration 5

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.00	188.7	1.40	129.6	1.60	106.6	2.00	85.5
1.20	165.7	1.50	116.1	1.80	94.0	2.20	78.8

titration 6

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.60	101.9	0.80	76.2	1.00	63.5	1.40	48.8
0.70	85.4	0.90	68.8	1.20	55.3	1.60	44.4

TABLE 11

Experimental results for the protonation of glutathionate

a

Titration	Titrate (S) Molarity		Titrant (T) Molarity		Volume ml	E^{o} mV
	A	H	A	H		
1.	0.009986	0.01246	0.009986	-0.03004	20.01	466.4
2.	0.004967	0.01246	0.004986	-0.06008	20.01	466.4
3.	0.008039	0.02494	0.008010	-0.1501	20.01	466.0
4.	0.001998	0.01246	0.002002	-0.06008	20.01	466.0

TABLE 11b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.00	327.1	15.00	229.4	21.80	145.8	29.00	-46.8
2.00	319.8	16.00	223.1	22.00	133.8	30.00	-50.4
3.00	312.1	17.00	216.0	22.20	113.8	31.00	-53.4
4.00	304.7	18.00	208.3	22.40	71.2	32.00	-56.4
5.00	297.0	18.50	204.1	22.50	52.3	33.00	-59.1
6.00	289.6	19.00	199.3	22.70	31.5	34.00	-61.5
7.00	282.0	19.40	195.2	23.00	15.2	35.00	-63.7
8.00	274.9	19.70	191.8	23.20	8.0	36.00	-65.8
9.00	267.8	20.00	188.0	23.60	-2.3		
10.00	261.0	20.30	183.6	24.00	-9.7		
11.00	254.2	20.60	178.9	25.00	-22.2		
12.00	248.0	21.00	171.2	26.00	-30.9		
13.00	241.7	21.20	166.4	27.00	-37.2		
14.00	235.7	21.60	154.5	28.00	-42.3		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	343.1	4.20	266.1	6.20	143.7	10.00	-115.9
0.50	339.0	4.40	259.9	6.25	94.5	11.00	-137.0
1.00	333.1	4.60	253.6	6.30	27.7	12.00	-168.6
1.50	326.3	4.80	246.9	6.35	7.8	12.10	-172.6
1.80	321.7	5.00	240.0	6.40	-3.8	12.20	-176.4
2.20	314.8	5.20	232.6	6.50	-18.0	12.40	-183.8
2.50	309.1	5.40	224.3	6.60	-27.3	12.70	-193.2
2.80	303.0	5.60	215.1	6.80	-40.3		
3.00	298.4	5.70	209.7	7.00	-49.8		
3.20	293.7	5.80	203.4	7.50	-66.1		
3.40	288.7	5.90	195.6	8.00	-78.0		
3.60	283.6	6.00	186.1	8.50	-88.3		
3.80	278.0	6.05	179.3	9.00	-97.5		
4.00	272.1	6.10	171.2	9.50	-106.6		

TABLE 11b cont'd.

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	361.8	3.60	252.2	4.58	24.0	6.30	-105.8
0.50	356.3	3.80	240.4	4.60	10.4	6.70	-120.0
1.00	348.0	3.90	234.5	4.65	-7.4	7.00	-132.3
1.40	339.6	4.10	220.2	4.70	-18.8	7.20	-142.4
1.70	332.2	4.20	211.9	4.75	-27.3	7.40	-155.3
2.00	323.1	4.30	201.2	4.80	-34.1	7.60	-172.0
2.30	313.3	4.40	186.2	4.90	-44.0	7.70	-181.8
2.60	301.8	4.45	174.8	5.10	-58.2	7.80	-191.1
2.80	293.2	4.48	164.5	5.30	-68.7	7.90	-199.2
3.00	283.8	4.52	140.4	5.50	-77.2	8.10	-211.6
3.20	273.8	4.54	112.0	5.70	-84.9	8.30	-220.1
3.40	263.2	4.56	49.6	6.00	-95.4		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	348.7	4.30	242.7	4.88	9.7	6.20	-120.7
0.50	345.1	4.40	234.0	4.90	-3.7	6.40	-134.2
1.00	339.8	4.50	224.3	4.95	-24.0	6.50	-141.3
2.00	326.7	4.60	213.2	5.00	-34.5	6.60	-149.8
2.50	317.6	4.65	206.0	5.05	-42.8	6.70	-158.5
3.00	305.8	4.70	196.7	5.10	-49.5	6.80	-167.2
3.40	292.8	4.75	183.8	5.20	-59.9	6.90	-175.4
3.60	284.9	4.78	173.0	5.30	-68.0	7.00	-182.3
3.80	275.3	4.80	162.8	5.40	-75.1	7.20	-193.3
4.00	264.0	4.82	145.2	5.60	-87.3	7.40	-201.6
4.10	257.6	4.84	103.5	5.80	-98.3		
4.20	250.2	4.86	32.3	6.00	-109.2		

TABLE 12

Experimental results for the protonation of D-penicillaminate

a

Titration	Titrate (S) Molarity		Titrant (T) Molarity		Volume ml	E^{o} mV
	A	H	A	H		
1.	0.01028	0.025051	0.009766	-0.10009	20.01	461.4
2.	0.005104	0.025051	0.005181	-0.05005	20.01	460.7
3.	0.008049	0.025051	0.008113	-0.05005	20.01	460.8

TABLE 12b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	356.0	4.83	219.7	5.10	22.9	7.70	-111.6
1.00	347.2	4.85	210.3	5.20	10.8	7.85	-122.9
1.80	337.5	4.87	196.9	5.30	2.3	8.00	-133.0
2.50	327.0	4.88	186.5	5.50	-10.5	8.20	-144.7
3.10	315.8	4.89	169.3	5.70	-20.4	8.40	-153.9
3.60	304.2	4.90	146.2	6.00	-32.7	8.70	-164.9
4.00	291.9	4.91	113.6	6.30	-44.0	9.00	-173.8
4.20	284.0	4.92	90.5	6.60	-54.7	9.30	-181.7
4.40	274.0	4.93	78.7	6.90	-66.1	9.60	-188.7
4.55	263.7	4.94	68.8	7.10	-74.7	10.00	-197.2
4.65	254.3	4.96	57.6	7.30	-84.9		
4.73	243.7	5.00	43.0	7.50	-97.1		
4.80	229.4	5.05	31.2	7.60	-104.1		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	361.0	9.80	207.8	10.05	32.8	13.70	-132.1
2.00	351.9	9.82	198.4	10.12	22.3	13.90	-141.3
3.50	343.1	9.84	186.7	10.20	14.1	14.20	-152.2
5.00	332.7	9.85	178.0	10.35	2.7	14.60	-163.4
6.00	324.0	9.86	167.5	10.55	-8.3	15.00	-172.3
7.00	313.3	9.87	154.1	10.80	-19.0	15.50	-181.7
8.00	299.0	9.88	135.6	11.10	-29.7	16.00	-189.5
8.60	286.9	9.89	116.0	11.50	-41.9	17.00	-202.5
9.00	275.8	9.90	99.2	11.90	-53.4	18.00	-212.8
9.30	263.6	9.91	86.9	12.30	-65.9	19.00	-220.8
9.50	251.7	9.92	76.8	12.70	-80.3	20.00	-227.7
9.60	242.8	9.94	64.3	13.00	-93.7		
9.70	230.3	9.97	51.9	13.30	-110.2		
9.75	221.3	10.00	43.1	13.50	-121.8		

TABLE 12b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	358.2	9.75	199.2	10.10	30.3	16.30	-137.3
2.00	347.9	9.78	185.2	10.25	17.8	16.70	-147.1
3.50	338.3	9.79	178.3	10.40	9.2	17.20	-156.7
5.00	326.7	9.80	170.6	10.60	0.3	18.00	-168.7
6.20	315.0	9.81	159.8	10.90	-9.9	19.00	-180.3
7.20	303.3	9.82	145.3	11.40	-22.6	20.10	-190.7
7.90	292.9	9.83	128.3	11.90	-32.9	21.00	-198.0
8.40	283.2	9.84	113.5	12.40	-42.2	22.00	-205.6
8.80	273.2	9.85	100.9	13.00	-52.9	23.00	-212.0
9.10	263.0	9.86	90.4	13.80	-67.3	24.50	-220.4
9.30	253.8	9.88	76.9	14.30	-78.0	26.00	-227.3
9.45	244.4	9.90	68.3	14.80	-90.2	28.00	-234.1
9.60	230.3	9.93	58.2	15.30	-105.3	30.00	-239.7
9.68	218.0	9.96	50.8	15.60	-115.8		
9.72	209.0	10.00	43.6	15.90	-125.6		

TABLE 13

Experimental results for the protonation of ethylenediaminetetraacetate

Titration	Titrate (S) Molarity		Titrant (T) Molarity		Volume ml	E° mV
	A	H	A	H		
1.	0.009997	0.025051	0.010053	-0.10006	20.01	461.6
2.	0.004998	0.025051	0.004992	-0.10006	20.01	462.9
3.	0.015038	0.025051	0.014932	-0.10006	20.01	461.2
4.	0.019988	-0.10006	0.0000	0.31142	20.01	459.0

TABLE 13b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	339.0	4.70	237.3	5.20	99.1	8.40	-44.7
1.00	329.7	4.75	227.9	5.30	91.4	8.70	-54.8
1.50	324.3	4.78	219.6	5.50	78.9	9.00	-63.8
2.00	318.3	4.81	209.7	5.70	69.7	9.50	-78.2
2.50	311.7	4.84	194.0	6.00	57.7	10.00	-92.9
3.00	304.2	4.86	181.3	6.30	46.8	10.50	-101.5
3.50	294.4	4.88	168.0	6.60	36.0	10.80	-127.4
3.80	287.3	4.90	156.7	7.00	20.7	11.00	-142.9
4.10	278.0	4.92	147.3	7.30	7.0	11.10	-153.7
4.30	269.7	4.95	137.3	7.50	-3.0	11.20	-165.1
4.40	264.3	5.00	125.3	7.70	-13.6	11.30	-176.0
4.50	257.8	5.05	116.3	7.90	-23.7	11.40	-185.3
4.60	249.6	5.12	107.4	8.10	-32.9	11.50	-192.7

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	353.8	4.80	230.7	5.10	91.2	6.60	-48.8
1.00	345.2	4.83	218.0	5.20	79.2	6.75	-59.4
2.00	333.6	4.85	205.1	5.30	69.3	6.95	-72.6
2.80	322.1	4.87	181.2	5.45	56.9	7.15	-86.0
3.50	308.9	4.88	172.7	5.60	45.4	7.35	-100.8
4.00	295.6	4.89	161.5	5.80	30.3	7.55	-120.7
4.30	283.8	4.91	143.8	5.95	17.2	7.65	-134.8
4.50	272.2	4.93	133.0	6.10	1.3	7.70	-143.4
4.60	263.9	4.96	120.0	6.25	-15.3	7.75	-153.0
4.70	252.2	4.99	111.3	6.35	-26.3	7.80	-162.3
4.75	243.3	5.03	102.8	6.45	-36.2	7.90	-178.4

TABLE 13b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	327.3	4.91	171.7	6.70	54.9	12.50	-76.9
1.00	318.8	4.94	158.1	7.20	43.3	13.20	-87.7
2.00	308.0	4.96	151.2	7.60	34.7	13.80	-97.7
2.80	297.0	4.99	142.3	8.00	25.6	14.30	-107.6
3.60	282.7	5.02	135.8	8.40	15.7	14.70	-116.5
4.00	272.3	5.06	128.4	8.80	4.7	15.10	-128.3
4.30	261.0	5.11	121.2	9.20	-7.8	15.40	-140.0
4.40	256.2	5.20	111.9	9.50	-17.0	15.60	-150.3
4.50	250.0	5.35	100.7	9.80	-25.7	15.70	-156.3
4.80	215.1	5.50	92.4	10.20	-36.2	15.80	-162.9
4.85	199.9	5.70	83.7	10.60	-45.1		
4.87	190.7	6.00	73.2	11.10	-54.8		
4.89	180.9	6.30	64.7	11.70	-64.9		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
3.87	-157.4	5.15	-24.9	6.49	111.6	6.85	262.3
3.90	-146.7	5.22	-15.3	6.52	123.2	6.95	270.7
3.93	-138.1	5.30	-7.6	6.54	133.0	7.10	280.4
3.98	-127.6	5.38	1.0	6.56	149.3	7.30	289.7
4.03	-119.1	5.48	10.7	6.57	162.1	7.60	300.0
4.10	-110.0	5.60	20.8	6.58	175.8	8.00	310.2
4.20	-99.7	5.75	32.3	6.59	189.6	8.50	320.3
4.32	-89.8	5.90	43.3	6.60	199.2	9.00	328.9
4.45	-80.1	6.05	54.5	6.61	207.7	10.00	342.4
4.60	-70.3	6.20	67.4	6.63	218.9	11.00	353.0
4.80	-56.4	6.30	78.0	6.65	227.0		
4.95	-44.7	6.38	88.4	6.70	241.0		
5.05	-35.3	6.45	101.7	6.75	249.7		

TABLE 14

Experimental results for the protonation of 1,2-di(2-amineothoxy) ethanetetraacetate

Titration	Titrate (S) Molarity		Titrant (T) Molarity		Volume ml	E^o mV
	A	H	A	H		
1.	0.010033	0.0061213	0.010015	-0.10006	20.01	463.0
2.	0.004994	-0.004530	0.004986	-0.10006	20.01	462.3
3.	0.0150113	-0.01458	0.014986	-0.10006	20.015	462.3
4.	0.0020397	0.005190	0.002024	-0.10006	20.01	461.1

TABLE 14b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	330.7	5.90	231.4	6.45	35.4	13.50	-111.1
0.50	326.3	6.00	223.6	6.50	27.0	14.00	-122.6
1.00	321.4	6.10	211.9	6.60	15.9	14.30	-131.6
1.50	316.2	6.15	203.8	6.80	2.1	14.60	-143.3
2.00	310.9	6.20	191.4	7.00	-7.7	14.90	-159.8
2.50	305.3	6.23	180.2	7.30	-18.0	15.00	-166.2
3.00	299.2	6.25	170.0	7.60	-25.8	15.20	-178.9
3.50	292.6	6.27	154.1	8.00	-34.2	15.40	-189.7
4.00	285.4	6.29	128.9	8.50	-43.0	15.60	-196.1
4.50	276.8	6.30	112.3	9.00	-50.3	15.80	-202.5
5.00	266.4	6.31	94.2	10.00	-63.1	16.00	-207.8
5.30	258.1	6.32	82.4	11.00	-75.1	16.50	-217.6
5.50	251.7	6.33	74.1	12.00	-87.6		
5.70	243.1	6.35	64.7	12.50	-94.4		
5.80	238.0	6.40	46.3	13.00	-102.2		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	287.2	1.06	198.7	1.23	16.1	3.50	-120.2
0.20	282.2	1.08	182.2	1.30	2.5	3.60	-130.2
0.40	274.4	1.09	170.1	1.40	-10.9	3.70	-143.2
0.60	264.5	1.10	150.8	1.50	-20.0	3.80	-159.6
0.70	258.2	1.11	119.6	1.70	-33.0	3.90	-175.5
0.80	250.4	1.12	88.0	1.90	-43.5	3.95	-182.2
0.90	239.6	1.13	69.4	2.20	-56.7	4.00	-187.4
0.93	235.0	1.14	60.1	2.50	-68.7	4.10	-196.8
0.96	230.3	1.16	43.5	2.80	-80.4		
0.99	224.1	1.18	33.1	3.10	-94.5		
1.02	216.0	1.20	25.4	3.30	-105.6		

TABLE 14b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	294.2	4.07	170.7	4.50	23.3	14.00	-85.3
0.50	289.6	4.10	155.3	4.60	16.7	15.00	-91.6
1.00	283.9	4.12	140.3	4.80	6.8	16.00	-98.3
1.50	277.6	4.13	130.7	5.00	-0.8	17.00	-106.0
2.00	270.3	4.14	118.8	5.30	-9.1	18.00	-114.6
2.50	261.8	4.15	106.1	5.70	-17.6	18.70	-120.7
3.00	250.7	4.16	96.0	6.20	-25.6	19.30	-129.0
3.30	241.6	4.18	83.0	7.00	-35.4	20.00	-139.5
3.50	233.9	4.20	72.7	8.00	-45.2	20.50	-150.2
3.70	223.1	4.22	64.9	9.00	-53.2	21.00	-163.3
3.80	216.0	4.25	56.1	10.00	-60.2	21.50	-178.7
3.90	206.1	4.30	45.3	11.00	-66.9	22.00	-192.2
3.95	199.2	4.35	38.0	12.00	-72.9		
4.00	190.7	4.40	32.0	13.00	-79.0		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	325.4	1.90	207.2	2.00	12.8	2.70	-93.4
0.50	316.1	1.91	197.0	2.02	2.8	2.75	-100.8
1.00	301.8	1.92	180.9	2.05	-8.2	2.80	-108.8
1.30	289.3	1.93	147.0	2.10	-20.3	2.85	-118.6
1.50	277.8	1.94	103.8	2.15	-29.7	2.90	-130.6
1.60	270.2	1.95	64.3	2.20	-37.1	2.95	-145.3
1.70	260.3	1.96	45.7	2.30	-49.8	2.98	-154.3
1.80	245.0	1.97	35.8	2.40	-60.4	3.01	-162.7
1.85	232.5	1.98	26.3	2.50	-70.7	3.05	-172.4
1.88	219.9	1.99	18.7	2.60	-81.4		

TABLE 15

Experimental results for the formation of cadmium (II)-glutathionate

a

Titration	Titrate (S) molarity			Titrant (T) molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.010066	0.003167	0.007119	0.000	0.000	-0.05003	20.01	465.1
2.	0.015204	0.004749	0.007549	0.000	0.000	-0.05003	20.01	465.5
3.	0.003326	0.003167	0.007119	0.000	0.000	-0.05003	20.01	464.9
4.	0.006050	0.003167	0.007119	0.000	0.000	-0.05003	20.01	464.3

TABLE 15b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
2.00	285.3	6.00	221.1	8.90	112.0	10.80	-39.7
2.30	280.9	6.30	216.6	9.00	101.3	11.00	-48.8
2.60	276.2	6.60	211.4	9.10	89.7	11.20	-57.1
2.90	271.2	7.00	203.9	9.20	77.3	11.40	-64.3
3.20	266.4	7.30	196.8	9.30	67.1	11.70	-74.0
3.50	261.4	7.60	188.7	9.40	57.1	12.00	-82.8
3.80	256.3	7.80	182.2	9.50	47.0	12.30	-91.0
4.10	251.2	8.00	174.8	9.60	38.0	12.60	-98.3
4.50	244.4	8.20	165.6	9.80	21.7	13.00	-108.3
4.80	239.4	8.40	154.1	10.00	7.1	13.40	-118.2
5.10	234.7	8.60	140.1	10.20	-6.3		
5.40	230.1	8.70	131.7	10.40	-18.7		
5.70	225.8	8.80	122.2	10.60	-29.4		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
2.00	285.6	8.00	226.0	12.40	107.1	15.20	-41.1
2.50	280.2	8.50	221.7	12.60	91.9	15.60	-53.0
3.00	275.0	9.00	214.8	12.80	77.0	16.00	-63.2
3.50	269.7	9.50	208.1	13.00	62.7	16.50	-74.2
4.00	264.1	10.00	200.1	13.20	48.9	17.00	-83.6
4.50	258.7	10.50	190.2	13.40	36.7	17.50	-92.6
5.00	253.4	10.80	183.1	13.60	25.7	18.00	-100.7
5.50	248.8	11.10	174.4	13.80	15.3	18.50	-108.8
6.00	244.0	11.40	163.8	14.00	6.0		
6.50	239.8	11.70	150.7	14.20	-3.2		
7.00	235.2	12.00	134.3	14.50	-15.8		
7.50	230.8	12.20	121.3	14.80	-27.2		

TABLE 15b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.20	327.3	2.70	270.5	3.90	214.4	5.00	160.3
1.00	315.2	2.90	262.4	4.10	206.2	5.10	152.2
1.50	305.6	3.10	253.7	4.30	197.9	5.20	143.2
1.80	298.4	3.30	244.4	4.50	189.3	5.30	133.1
2.10	290.5	3.50	234.5	4.70	179.2	5.40	121.9
2.40	281.3	3.70	223.9	4.90	167.2		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.20	318.3	5.30	206.1	7.60	47.0	9.60	-110.8
0.50	314.4	5.70	195.2	7.70	34.3	9.80	-124.0
1.00	307.0	6.10	181.3	7.80	22.2	10.00	-138.5
1.50	298.2	6.30	172.3	7.90	11.4		
2.00	288.8	6.50	161.9	8.00	2.1		
2.50	277.7	6.70	149.1	8.10	-7.0		
3.00	265.8	6.90	133.8	8.20	-15.4		
3.40	255.3	7.00	124.6	8.40	-30.2		
3.70	247.1	7.10	113.7	8.60	-43.9		
4.00	238.9	7.20	102.0	8.80	-57.2		
4.30	230.3	7.30	88.9	9.00	-70.7		
4.60	222.7	7.40	74.7	9.20	-84.4		
4.90	215.7	7.50	60.7	9.40	-97.5		

TABLE 16

Experimental results for the formation of cadmium (II) penicillaminate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.009129	0.003154	0.008652	0.0000	0.0000	-0.05006	15.00	461.9
2.	0.003225	0.003154	0.008652	0.0000	0.0000	-0.05006	20.01	461.9
3.	0.006184	0.003154	0.008652	0.0000	0.0000	-0.05006	20.01	461.7
4.	0.018243	0.003154	0.008652	0.0000	0.0000	-0.05006	20.01	462.4
5.	0.015231	0.005258	0.009224	0.0000	0.0000	-0.05006	20.01	461.2

TABLE 16b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	321.2	4.00	174.1	5.45	31.9	7.25	-103.9
0.60	312.3	4.10	165.4	5.60	22.1	7.30	-112.8
1.20	299.8	4.20	155.2	5.80	10.9	7.35	-120.3
1.60	289.0	4.30	143.6	6.00	0.4	7.45	-135.9
1.90	278.3	4.48	123.7	6.20	-9.7	7.55	-148.4
2.10	268.8	4.55	116.0	6.40	-20.3	7.65	-158.4
2.40	246.6	4.65	105.8	6.60	-33.3	7.75	-166.7
2.50	238.1	4.75	95.5	6.75	-45.0	7.90	-176.8
2.65	229.0	4.85	85.3	6.85	-53.7	8.10	-187.8
2.90	219.6	4.95	75.6	6.95	-63.1	8.30	-195.8
3.20	210.1	5.05	65.9	7.05	-74.3	8.60	-205.7
3.55	197.9	5.15	56.6	7.15	-87.8		
3.80	186.7	5.30	43.3	7.20	-95.3		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
3.35	233.9	4.90	166.7	5.68	98.6	5.92	16.9
3.40	226.1	5.10	155.8	5.75	87.3	5.98	-28.2
3.45	220.2	5.30	141.2	5.80	76.1	5.99	-38.0
4.00	197.9	5.40	132.0	5.85	60.6	6.01	-51.2
4.40	185.6	5.50	121.6	5.88	45.3	6.03	-59.7
4.70	175.0	5.60	109.7	5.90	30.3		

TABLE 16b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	326.7	3.50	226.7	6.03	113.4	7.45	-7.5
0.80	318.0	3.75	217.2	6.15	103.5	7.85	-19.2
1.50	307.8	4.20	206.0	6.27	93.6	8.20	-45.7
2.00	298.3	4.60	195.3	6.40	82.3	8.30	-58.7
2.40	288.4	5.20	173.9	6.50	73.4	8.35	-67.4
2.70	278.9	5.40	163.3	6.62	63.1	8.40	-77.4
2.95	268.0	5.50	153.1	6.75	52.0	8.45	-91.4
3.15	255.2	5.65	145.1	6.90	40.1		
3.25	246.9	5.75	136.7	7.05	29.2		
3.35	237.3	5.90	124.3	7.25	15.9		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	305.4	5.75	158.7	8.30	17.1	13.50	-115.1
0.80	296.7	5.90	145.7	8.70	6.1	13.80	-127.4
1.50	287.1	6.05	133.5	9.50	-14.1	14.05	-136.7
2.10	276.3	6.20	122.3	10.00	-25.3	14.30	-144.8
2.60	264.4	6.35	112.2	10.50	-35.7	14.60	-152.9
3.00	252.0	6.55	98.9	11.00	-45.7	15.00	-162.2
3.30	242.3	6.75	86.3	11.50	-55.4	15.50	-171.9
4.30	220.8	6.95	73.7	12.00	-66.4	16.10	-181.8
4.80	208.8	7.15	61.8	12.40	-76.3	16.80	-191.8
5.20	194.5	7.35	51.3	12.70	-85.0		
5.50	178.2	7.60	40.3	13.00	-95.0		
5.65	167.2	7.90	29.3	13.20	-102.9		

TABLE 16b cont'd

titration 5

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	312.8	6.90	183.4	9.45	57.9	13.60	-75.2
0.90	303.1	7.20	170.1	9.70	47.3	13.80	-87.0
1.60	293.3	7.40	159.0	10.00	35.9	13.95	-99.8
2.20	283.4	7.60	147.1	10.30	25.8	14.20	-122.1
2.80	270.0	7.75	138.6	10.70	14.2	14.35	-134.7
3.20	257.8	7.95	127.8	11.20	2.0	14.50	-145.3
3.50	249.1	8.20	115.0	11.70	-9.8	14.70	-156.3
4.00	239.4	8.45	103.8	12.20	-21.2	15.00	-168.9
4.70	229.0	8.70	91.6	12.70	-36.9	15.40	-182,2
5.40	218.4	8.95	80.3	13.10	-51.4	15.90	-194.7
6.00	208.0	9.20	68.9	13.40	-65.8	16.50	-206.2

TABLE 17

Experimental results for the formation of zinc (II)–penicillaminate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1.	0.009276	0.002996	0.006252	0.0000	0.0000	-0.05004	20.01	464.3
2.	0.006117	0.002996	0.003160	0.0000	0.0000	-0.05004	20.01	467.4
3.	0.003132	0.002996	0.003160	0.0000	0.0000	-0.05004	20.01	467.7
4.	0.018257	0.002996	0.003160	0.0000	0.0000	-0.05004	20.01	468.1
5.	0.010155	0.004995	0.005224	0.0000	0.0000	-0.05004	20.01	468.6
6.	0.018126	0.006019	0.006296	0.0000	0.0000	-0.05004	20.01	468.7

TABLE 17b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	310.4	5.50	110.0	7.35	12.4	8.55	-94.5
0.60	301.1	6.00	97.2	7.45	1.0	8.75	-120.1
2.55	184.5	6.40	84.7	7.55	-10.3	8.90	-136.3
2.70	173.9	6.70	72.3	7.85	-33.2	9.05	-148.4
2.90	165.4	6.90	61.0	8.00	-44.0	9.20	-158.9
3.20	155.8	7.05	49.2	8.15	-55.4	9.40	-168.8
4.30	133.7	7.15	37.8	8.30	-68.5	9.70	-180.8
4.90	122.6	7.25	25.4	8.45	-82.4		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	300.0	1.40	177.9	5.15	73.7	6.06	-39.8
0.30	292.5	1.55	167.6	5.40	62.7	6.08	-55.4
0.55	283.6	1.80	157.2	5.60	51.1	6.10	-71.9
0.75	274.1	2.10	148.6	5.75	39.6	6.11	-79.1
0.90	264.3	2.60	137.5	5.85	28.3	6.12	-85.8
1.00	255.0	3.10	127.7	5.92	16.3	6.14	-97.3
1.20	217.8	3.60	117.1	5.97	4.8	6.16	-100.5
1.23	207.7	4.50	94.9	6.00	-5.7		
1.27	196.8	4.85	84.3	6.02	-14.9		

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	308.9	1.29	181.6	2.70	116.0	3.60	68.7
0.40	297.8	1.35	170.1	3.00	106.1	3.68	55.0
0.70	285.3	1.45	159.9	3.30	92.7	3.73	42.4
1.26	191.2	1.95	137.4	3.50	79.3		

TABLE 17b cont'd

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	279.9	2.50	166.0	6.05	46.5	9.40	-54.0
0.40	269.2	3.10	154.0	6.15	39.4	9.80	-63.8
0.70	257.3	3.60	143.7	6.35	27.0	10.40	-81.4
0.90	245.8	4.00	134.4	6.55	16.9	10.70	-92.7
1.03	235.3	4.40	123.2	6.80	6.8	10.90	-101.1
1.13	225.2	5.10	98.7	7.10	-3.0	11.10	-110.3
1.35	204.6	5.35	88.2	7.50	-13.7	11.40	-121.4
1.50	195.3	5.55	78.4	7.90	-22.8	11.70	-132.9
1.70	186.7	5.75	67.1	8.40	-33.2	12.00	-142.3
2.00	177.6	5.90	57.3	8.90	-43.3	12.40	-152.7

titration 5

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	309.2	2.18	205.0	5.80	128.3	9.95	17.7
0.50	301.0	2.30	194.2	7.00	109.4	10.00	10.8
1.00	289.7	2.45	185.9	7.60	98.7	10.05	1.0
1.70	261.1	2.70	176.8	8.20	86.7	10.10	-12.4
1.85	249.3	3.10	167.0	8.70	75.1	10.13	-24.8
1.95	238.2	3.70	156.7	9.10	64.0	10.15	-34.8
2.03	226.6	4.40	145.9	9.40	53.4	10.17	-46.1
2.10	215.3	5.10	137.8	9.85	27.9		

TABLE 17b cont'd

titration 6

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	302.4	4.20	172.8	11.95	39.8	14.60	-102.3
0.70	292.3	5.00	162.9	12.10	29.9	14.70	-110.8
1.20	282.6	6.00	152.1	12.25	19.6	14.85	-122.3
1.60	272.7	7.00	140.8	12.40	9.7	15.00	-132.6
1.90	261.8	7.80	131.1	12.55	0.6	15.20	-143.7
2.10	251.9	8.40	122.8	12.75	-9.7	15.40	-152.4
2.25	242.5	9.00	114.2	13.00	-20.7	15.70	-162.8
2.40	230.6	9.60	104.7	13.30	-33.0	16.00	-171.3
2.50	222.1	10.20	94.1	13.60	-45.0	16.40	-181.1
2.85	202.1	11.10	74.3	13.90	-58.0	16.90	-191.3
3.15	192.1	11.40	65.1	14.10	-67.5	17.50	-201.0
3.60	182.3	11.70	53.3	14.45	-90.1		

TABLE 18

Experimental results for the formation of cadmium (II) - ethylenediaminetetraacetate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^o mV
	A	B	H	A	B	H		
1.	0.009934	0.003154	0.000854	0.0000	0.0000	-0.050090	20.01	459.3
2.	0.009934	0.003154	0.000854	0.0000	0.0000	0.077883	20.01	461.6
3.	0.003057	0.003154	-0.004155	0.0000	0.0000	0.077883	20.01	460.6
4.	0.003057	0.003154	-0.004155	0.0000	0.0000	-0.050090	20.01	460.2
5.	0.017973	0.003154	-0.004155	0.0000	0.0000	-0.050090	20.01	460.2
6.	0.017973	0.003154	-0.004155	0.0000	0.0000	0.077929	20.01	458.3
7.	0.006033	0.003154	-0.004155	0.0000	0.0000	-0.050090	20.01	461.6
8.	0.006028	0.003154	-0.004155	0.0000	0.0000	0.077929	20.01	461.0
9.	0.013518	0.003154	-0.004155	0.0000	0.0000	0.077929	20.01	461.5
10.	0.013518	0.003154	-0.004155	0.0000	0.0000	-0.05006	20.01	461.5

TABLE 18b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	294.1	2.80	196.9	3.20	120.3	5.60	-10.0
0.50	288.8	2.83	187.4	3.30	94.2	5.80	-21.5
1.00	281.6	2.85	181.1	3.45	84.7	6.00	-32.3
1.40	274.2	2.87	174.5	3.65	74.4	6.20	-42.3
1.80	264.9	2.89	166.3	3.90	64.0	6.40	-51.1
2.10	255.6	2.92	158.0	4.20	52.7	6.70	-62.7
2.30	247.2	2.94	150.3	4.50	42.0	7.00	-73.0
2.50	235.9	2.96	143.1	4.80	30.9	7.30	-83.3
2.60	227.0	3.00	132.7	5.00	23.0	7.60	-94.6
2.70	215.3	3.05	121.5	5.20	14.1		
2.75	207.1	3.10	113.8	5.40	4.1		

Titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	296.9	1.70	316.7	4.50	336.7	10.00	356.3
0.30	301.0	2.40	322.6	5.50	341.7		
0.70	306.0	3.10	327.7	6.80	346.9		
1.20	311.9	3.80	332.8	8.00	350.9		

Titration 3

titre (ml)	e.m.f. (MV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.05	277.5	1.20	311.4	4.00	340.9	11.00	364.1
0.20	284.3	1.60	317.9	5.00	346.2	13.00	367.4
0.40	291.9	2.00	323.8	6.00	350.6		
0.60	298.0	2.50	329.1	7.50	355.9		
0.90	305.2	3.20	335.3	9.00	359.9		

TABLE 18b cont'd

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	273.2	0.50	246.3	0.65	228.0	0.73	207.1
0.30	260.6	0.60	235.8	0.70	217.2	0.76	192.6

titration 5

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	248.3	0.87	166.7	2.10	83.5	6.80	-13.1
0.20	241.7	0.91	157.3	2.50	74.2	7.10	-21.3
0.40	231.4	0.95	149.3	3.00	64.7	7.40	-29.1
0.50	224.0	1.00	141.1	3.70	53.6	7.80	-38.8
0.60	215.2	1.10	128.3	4.50	39.2	8.30	-49.1
0.70	202.9	1.20	119.7	5.10	28.8	8.80	-58.2
0.75	194.4	1.35	110.2	5.60	19.0	9.40	-68.3
0.80	184.0	1.55	101.0	6.00	10.2	10.00	-77.9
0.83	176.7	1.85	90.4	6.40	0.6	10.60	-87.9

titration 6

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	247.5	1.40	281.7	5.50	316.2	13.00	345.0
0.20	255.1	2.00	289.3	7.00	323.8	15.00	349.7
0.50	264.2	3.00	299.2	9.00	332.6	17.00	353.9
0.90	273.1	4.00	307.0	11.00	339.4	20.00	358.7

TABLE 18b cont'd

titration 7

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.20	262.6	0.87	163.9	1.10	90.0	2.10	-17.1
0.40	252.3	0.88	158.3	1.20	77.9	2.15	-24.0
0.55	241.3	0.89	151.7	1.30	67.4	2.20	-30.4
0.65	231.0	0.90	146.1	1.40	58.7	2.30	-41.9
0.70	223.0	0.92	135.0	1.55	46.0	2.40	-51.7
0.75	213.2	0.94	125.8	1.70	33.0	2.55	-64.6
0.80	199.2	0.97	116.0	1.80	23.4	2.70	-76.3
0.83	185.6	1.00	108.0	1.90	12.4	2.85	-88.7
0.85	175.7	1.05	98.0	2.00	0.1		

titration 8

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	270.3	0.70	293.2	3.00	326.0	8.00	352.6
0.10	274.2	1.00	299.8	4.00	334.0	10.00	358.0
0.20	278.2	1.50	308.6	5.00	340.4	12.00	362.1
0.40	285.1	2.00	315.4	6.00	345.2	14.00	365.3

titration 9

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	257.0	1.40	291.2	5.50	326.9	14.00	356.8
0.20	264.3	2.10	300.3	7.00	335.1	17.00	362.1
0.50	273.5	3.00	309.4	9.00	343.1		
0.90	282.6	4.00	317.3	11.00	349.6		

TABLE 18b cont'd

titration 10

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.02	255.1	0.86	173.4	1.80	83.3	5.10	-15.0
0.20	248.5	0.89	162.9	2.10	73.9	5.35	-24.4
0.40	238.8	0.93	152.1	2.50	63.0	5.65	-35.0
0.55	227.3	0.98	141.2	3.00	51.0	6.00	-45.8
0.65	217.2	1.04	131.2	3.50	39.0	6.40	-56.6
0.70	210.3	1.11	122.0	3.90	28.9	6.80	-66.2
0.75	201.9	1.20	113.6	4.25	18.8	7.20	-75.4
0.80	190.3	1.35	103.1	4.60	7.2	7.70	-86.9
0.83	182.2	1.55	93.1	4.85	-5.0		

TABLE 19

Experimental results for the formation of cadmium(II)-1,2-di(2-aminoethoxy)ethanetetraacetate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E° mV
	A	B	H	A	B	H		
1	0.002489	0.002630	-0.001494	0.0000	0.0000	-0.02003	20.02	462.0
2	0.004997	0.002603	0.000181	0.0000	0.0000	-0.02003	20.015	462.7
3	0.007535	0.002603	0.003713	0.0000	0.0000	-0.05008	20.015	461.6
4	0.015062	0.002603	-0.005080	0.0000	0.0000	-0.05008	20.015	462.4
5	0.006030	0.006309	0.007240	0.0000	0.0000	-0.05008	25.01	461.6

TABLE 19b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	303.3	7.30	230.2	7.99	146.9	8.09	-35.9
1.00	298.8	7.50	221.6	8.00	140.4	8.10	-46.3
2.00	293.4	7.60	215.9	8.01	130.0	8.11	-54.4
3.00	287.2	7.70	208.8	8.02	117.8	8.13	-64.4
4.00	279.9	7.80	198.4	8.03	91.7	8.15	-71.6
4.80	273.0	7.85	191.2	8.04	68.7	8.18	-78.7
5.60	264.3	7.90	181.7	8.05	31.1	8.25	-84.6
6.20	255.7	7.93	173.5	8.06	7.3	8.35	-93.7
6.60	248.7	7.96	163.0	8.07	-10.2	8.45	-109.7
7.00	239.7	7.98	153.7	8.08	-23.2	8.50	-120.0

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	321.3	13.00	249.9	14.72	133.9	15.10	12.3
1.00	318.4	13.50	239.9	14.75	122.7	15.20	2.7
2.00	315.2	13.80	231.9	14.77	113.3	15.30	-4.7
3.00	312.9	14.00	224.8	14.79	102.1	15.50	-15.6
4.00	308.3	14.20	215.4	14.81	87.3	15.70	-24.0
5.00	304.8	14.40	201.0	14.82	79.1	16.00	-34.3
6.00	300.7	14.50	190.2	14.84	67.8	16.30	-42.6
7.00	296.3	14.55	181.9	14.86	59.4	16.70	-52.4
8.00	291.6	14.60	171.7	14.88	52.9	17.20	-63.4
9.00	286.3	14.63	163.8	14.91	44.6	17.70	-74.3
10.00	280.1	14.66	154.3	14.95	35.0	18.00	-81.0
11.00	272.7	14.68	147.2	15.00	26.2	18.40	-90.6
12.00	263.0	14.70	140.3	15.05	18.6		

TABLE 19b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.00	325.1	9.00	225.6	9.39	111.1	9.80	-6.1
2.00	319.7	9.10	216.3	9.40	94.6	10.00	-18.7
3.00	314.0	9.15	210.7	9.41	81.4	10.20	-28.2
4.00	307.7	9.20	203.3	9.42	70.8	10.45	-37.7
5.00	300.3	9.25	193.6	9.43	63.6	10.70	-45.7
6.00	291.4	9.30	178.9	9.45	52.0	11.00	-54.3
7.00	279.9	9.32	169.7	9.47	44.3	11.40	-65.3
7.50	272.3	9.34	157.7	9.49	36.8	11.80	-76.3
8.00	263.0	9.35	150.7	9.52	29.7	12.30	-91.7
8.30	255.6	9.36	143.4	9.55	23.0	12.50	-98.9
8.60	246.0	9.37	134.9	9.60	14.7		
8.80	237.4	9.38	123.8	9.70	2.3		

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
0.10	320.9	10.80	238.3	11.85	90.4	13.70	-26.1
1.00	317.3	11.10	228.1	11.87	79.0	14.20	-34.0
2.00	313.2	11.30	218.7	11.90	66.7	14.80	-42.1
3.00	308.8	11.40	212.4	11.93	57.4	15.50	-50.6
4.00	304.0	11.50	204.4	11.97	48.3	16.50	-61.3
5.00	298.7	11.60	193.1	12.00	43.2	17.50	-72.1
6.00	292.6	11.70	174.7	12.05	35.6	18.50	-83.3
7.00	286.1	11.75	158.3	12.15	25.3	19.50	-96.4
8.00	278.2	11.77	149.9	12.25	17.4	20.00	-104.8
9.00	268.6	11.79	139.2	12.40	9.0	20.40	-112.3
9.50	262.5	11.81	124.6	12.60	0.3		
10.00	255.3	11.83	106.9	12.90	-8.9		
10.50	245.6	11.84	97.7	13.30	-18.4		

TABLE 19b cont'd

titration 5

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.00	335.6	12.00	276.7	15.14	155.2	15.26	-28.6
2.00	331.8	13.00	264.5	15.16	141.8	15.27	-36.0
3.00	328.3	13.50	256.3	15.17	133.3	15.29	-50.5
4.00	324.3	14.00	246.3	15.18	122.8	15.31	-58.2
5.00	320.2	14.40	234.6	15.19	107.7	15.35	-62.0
6.00	315.9	14.70	220.9	15.20	89.3	15.40	-64.7
7.10	310.7	14.90	206.0	15.21	65.7	15.50	-74.8
8.00	306.2	15.00	193.9	15.22	37.6	15.60	-91.3
9.00	300.3	15.05	184.3	15.23	16.7	15.65	-104.4
10.00	293.7	15.08	176.9	15.24	-0.9		
11.00	286.0	15.10	167.5	15.25	-16.3		

TABLE 20

Experimental results for the formation of zinc (II)-1,2-di(2-aminoethoxy)ethanetetraacetate

a

Titration	Titrate (S) Molarity			Titrant (T) Molarity			Volume ml	E^{o} mV
	A	B	H	A	B	H		
1	0.005009	0.002499	0.002074	0.0000	0.0000	-0.02002	20.015	461.2
2	0.007521	0.002498	0.005612	0.0000	0.0000	-0.050075	20.015	461.2
3	0.015035	0.002499	-0.003051	0.0000	0.0000	-0.05005	20.015	461.6
4	0.006030	0.005993	0.005759	0.0000	0.0000	-0.05005	25.01	459.7

TABLE 20b

titration 1

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
2.00	311.9	13.80	192.6	16.50	52.2	19.70	-76.7
3.50	305.8	14.00	182.7	16.55	44.9	20.20	-87.1
5.00	299.8	14.20	172.6	16.60	37.3	20.70	-99.0
6.50	291.7	14.50	159.3	16.70	23.4	21.10	-110.2
8.00	283.0	14.80	147.5	16.80	12.1	21.40	-120.1
9.00	276.2	15.10	136.7	16.90	3.5	21.70	-131.3
10.00	268.3	15.40	125.8	17.00	-3.3	22.00	-144.2
11.00	258.2	15.70	114.0	17.20	-14.4	22.20	-152.7
12.00	244.8	15.90	104.8	17.40	-22.7	22.40	-160.3
12.60	233.4	16.10	92.9	17.70	-32.6	22.60	-167.0
13.00	223.7	16.25	81.5	18.00	-40.7		
13.30	214.0	16.35	72.7	18.50	-52.3		
13.60	202.1	16.45	59.1	19.00	-62.7		

titration 2

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
3.70	305.7	8.95	189.2	9.94	68.0	11.00	-36.3
4.90	295.4	9.00	182.7	9.97	59.8	11.30	-45.9
6.00	284.3	9.05	176.2	10.00	51.1	11.70	-57.0
6.80	274.2	9.15	164.2	10.05	36.9	12.10	-67.4
7.50	262.1	9.25	153.8	10.08	29.9	12.50	-77.7
8.00	249.9	9.35	144.2	10.11	24.2	12.90	-89.5
8.30	239.4	9.45	135.2	10.20	10.7	13.30	-103.7
8.50	229.8	9.60	121.0	10.30	0.1	13.60	-117.2
8.70	216.2	9.75	103.9	10.40	-7.9	13.80	-129.1
8.80	207.0	9.85	88.0	10.55	-17.1		
8.90	195.4	9.90	78.1	10.75	-26.7		

TABLE 20b cont'd

titration 3

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
1.50	311.8	11.20	210.4	12.40	86.8	14.00	-20.3
2.80	306.0	11.35	198.8	12.45	77.4	14.50	-29.7
4.00	300.1	11.45	189.2	12.50	66.9	15.20	-39.8
5.00	294.8	11.55	178.7	12.55	56.9	16.00	-49.7
6.00	289.0	11.65	167.9	12.60	47.4	17.00	-60.4
7.00	282.2	11.75	157.7	12.65	40.0	18.00	-70.8
8.00	274.4	11.85	147.8	12.75	28.3	19.00	-81.8
9.00	264.6	11.95	138.9	12.85	19.9	20.00	-94.3
10.00	250.1	12.10	124.7	13.00	10.6	20.50	-102.0
10.50	239.2	12.25	108.4	13.20	1.3	21.00	-111.0
10.90	226.3	12.35	95.0	13.50	-8.7	21.50	-122.8

titration 4

titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)	titre (ml)	e.m.f. (mV)
2.70	314.7	11.30	199.0	14.20	79.4	14.69	-37.2
4.00	307.2	11.50	189.3	14.30	72.0	14.70	-45.2
5.00	300.6	11.70	179.3	14.40	62.9	14.71	-49.2
6.00	293.3	11.90	169.8	14.50	49.9	14.73	-61.1
7.00	285.1	12.10	160.7	14.55	40.3	14.75	-71.7
8.00	275.2	12.40	148.8	14.60	26.1	14.77	-81.4
9.00	262.7	12.70	138.5	14.63	13.1	14.79	-88.9
9.70	250.7	13.00	128.7	14.65	-0.3	14.82	-98.7
10.30	236.8	13.40	115.0	14.66	-8.8	14.85	-106.8
10.70	223.3	13.70	104.3	14.67	-18.2	14.90	-119.3
11.00	212.9	14.00	91.0	14.68	-28.6	14.95	-129.4

In discussing the results from the glass electrode potentiometry COMPLOT has been used to calculate the specificity of the ligand in question for cadmium with respect to zinc.

COMPLOT calculates the free metal and complex concentrations as pH is varied and the results are, in all cases, portrayed graphically. This allows a rapid appraisal of the specificity of the ligand and also reveals the complexes in which the metals are distributed.

In all systems, the total concentration of zinc is taken as 45.88 μM, its concentration in blood plasma. The total cadmium concentration is taken as 10 μM, the concentration at which clinical symptoms would be expected to become evident in vivo.

The ligand concentrations vary depending on the ratio of ligand to cadmium in the highest complex.

Thus in the cadmium asparaginate system where the highest complex is the 310 the ligand concentration is 30 μM, but, in the cadmium-histidinate system where the highest complex is the 210 the ligand concentration is 20 μM.

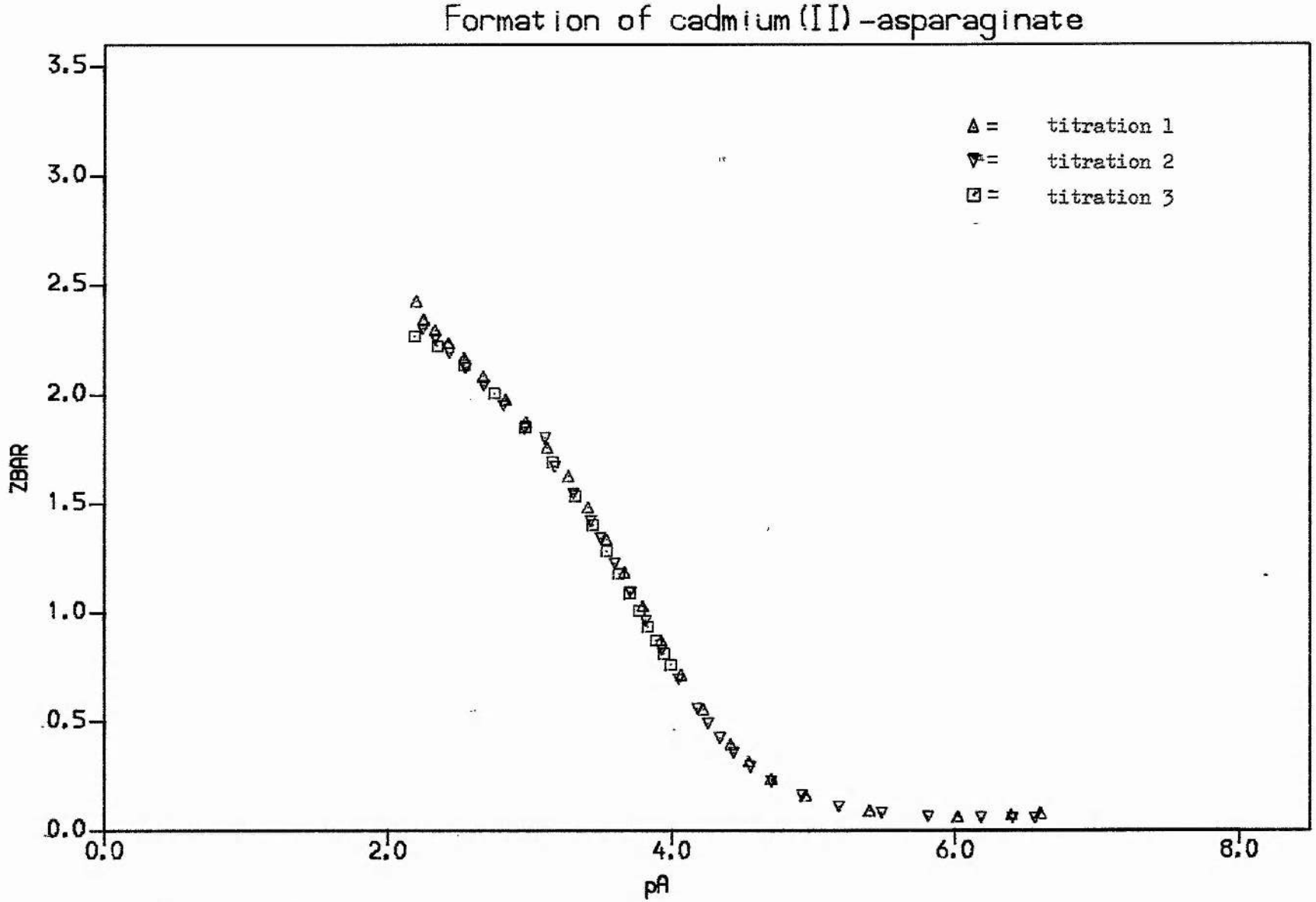

Formation of cadmium(II)-asparaginate
Δ = titration 1
▽ = titration 2
⊡ = titration 3
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
pA

Figure 6

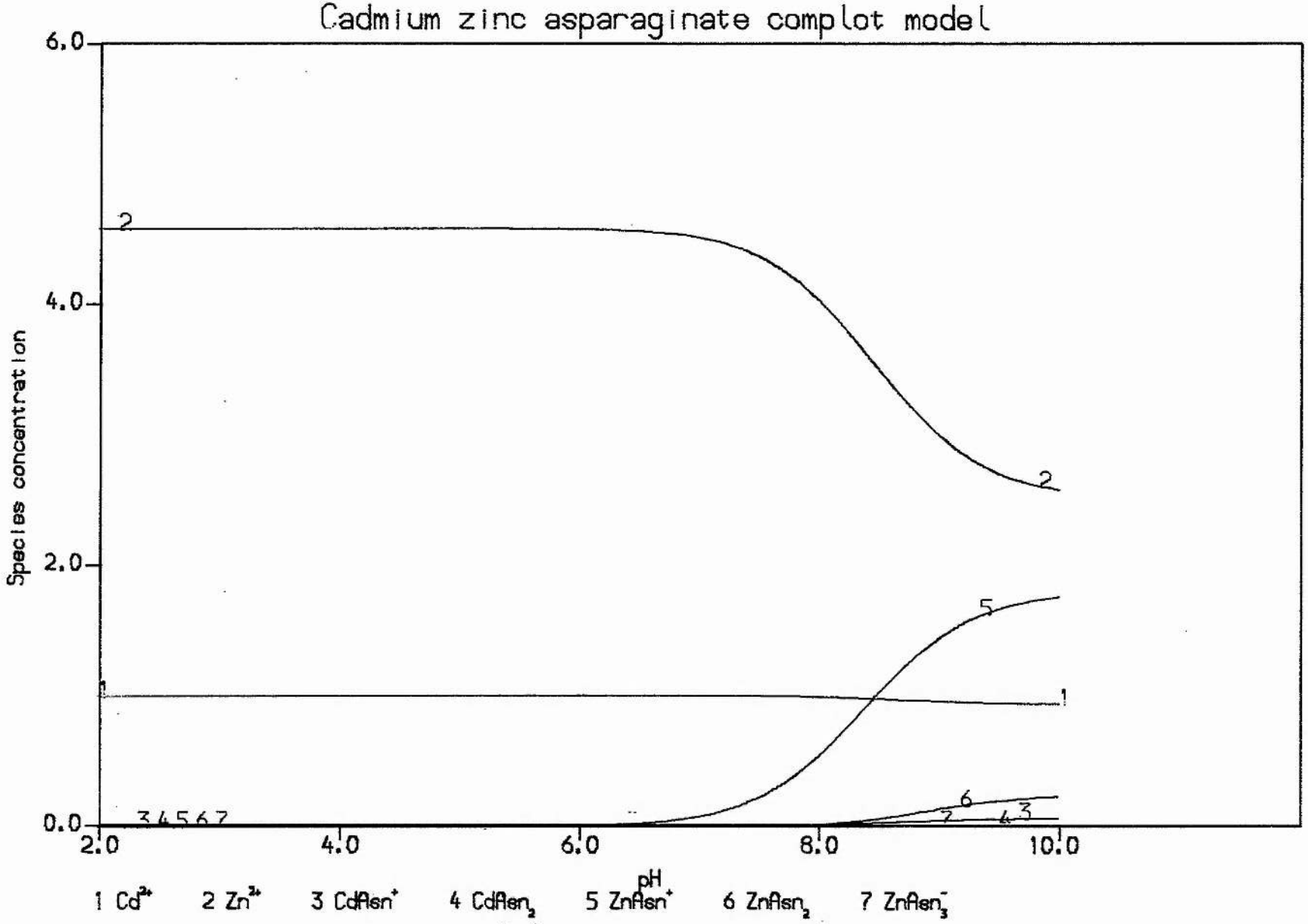


Figure 7 Total Cd(II) = 10, Zn(I) = 45.88 and Asn = 30.00 μM.

CADMIUM ASPARAGINATE

As can be seen from table 21, three binary complexes were obtained for this system. The formation curves shown in figure 6 are superimposable thus indicating the presence of the three complexes found and the absence of protonated and hydroxy species.

It is probable that the asparaginate binds through both the amino and carboxyl groupings with the structure being six - co-ordinate octahedral, but, from stability constant measurements alone, it is not possible to formulate any definite conclusions about structures.

The constants obtained are similar to those found for the corresponding zinc system (27) where $\log \beta_{110} = 5.070$, $\log \beta_{210} = 9.426$ and $\log \beta_{310} = 12.300$. This gives a value of -1.00 for $\log \beta^{Cd}_{110} - \log \beta^{Zn}_{110}$, which implies that asparaginate would not be an effective sequestering agent for cadmium *in vivo*. Indeed, from the COMPLOT study which is shown in figure 7 it can be seen that it is almost exclusively the zinc which is complexed. This is not completely unexpected as from the HSAB approach the nitrogen and oxygen donors would be expected to complex the 'harder' metal ion in preference.

Comparison of these results with those of other workers reveals a dearth of data on the 110 complex with $\log \beta$ values for the 210 ranging from 6.8 (80) to 7.1(81). These are lower than the figure reported here, however, these studies were carried out under different conditions, I = 0.01M, 20°C and I = 0.005M $CdSO_4$, 15°C respectively.

TABLE 21

Log formation constants for cadmium (II)- aminoacid anion complexes at 25°, I = 3.00M($NaClO_4$)

pqr =	Log β pqr 110	111	210	211	212	310	n[a]	SDT[b]
Asn[1]	4.071±0.012		7.581±0.007			9.610±0.018	71	0.0932
Asp[1]	4.851±0.086	12.013±0.165	10.131±0.112	18.303±0.137	23.494±0.808		192	0.6154
Cys[1]	12.875±0.057		19.627±0.136				45	0.0690
Gln[2]	4.099±0.023		7.664±0.012			9.999±0.030	61	0.1612
His[3]	6.484±0.021		11.105±0.029				86	0.2030
Phe[1]	4.363±0.025		7.935±0.099			11.090±0.093	85	0.5091
Ser[2]	4.154±0.025		7.863±0.012			10.221±0.033	62	0.1813
Try[3]	4.482±0.008		8.582±0.014			12.028±0.023	126	0.3214

a, n= number of experimental points; b, SDT = standard deviation in titre.

(1) Protonation constants taken from reference 117.

(2) Protonation constants taken from reference 26

(3) Protonation constants taken from reference 25

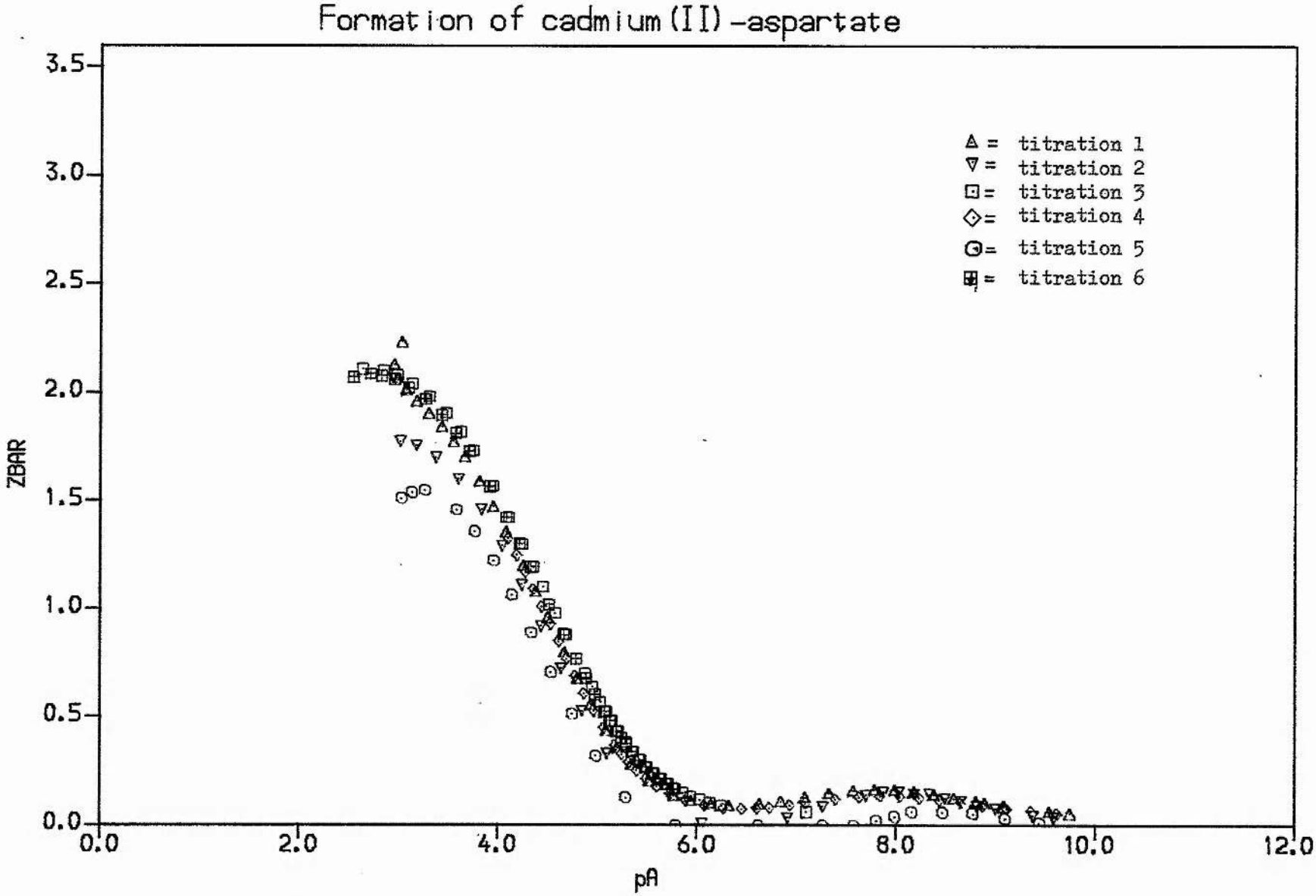

Formation of cadmium(II)-aspartate
ZBAR
pA
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
△ = titration 1
▽ = titration 2
⊡ = titration 3
◇ = titration 4
⊙ = titration 5
⊞ = titration 6

Figure 8

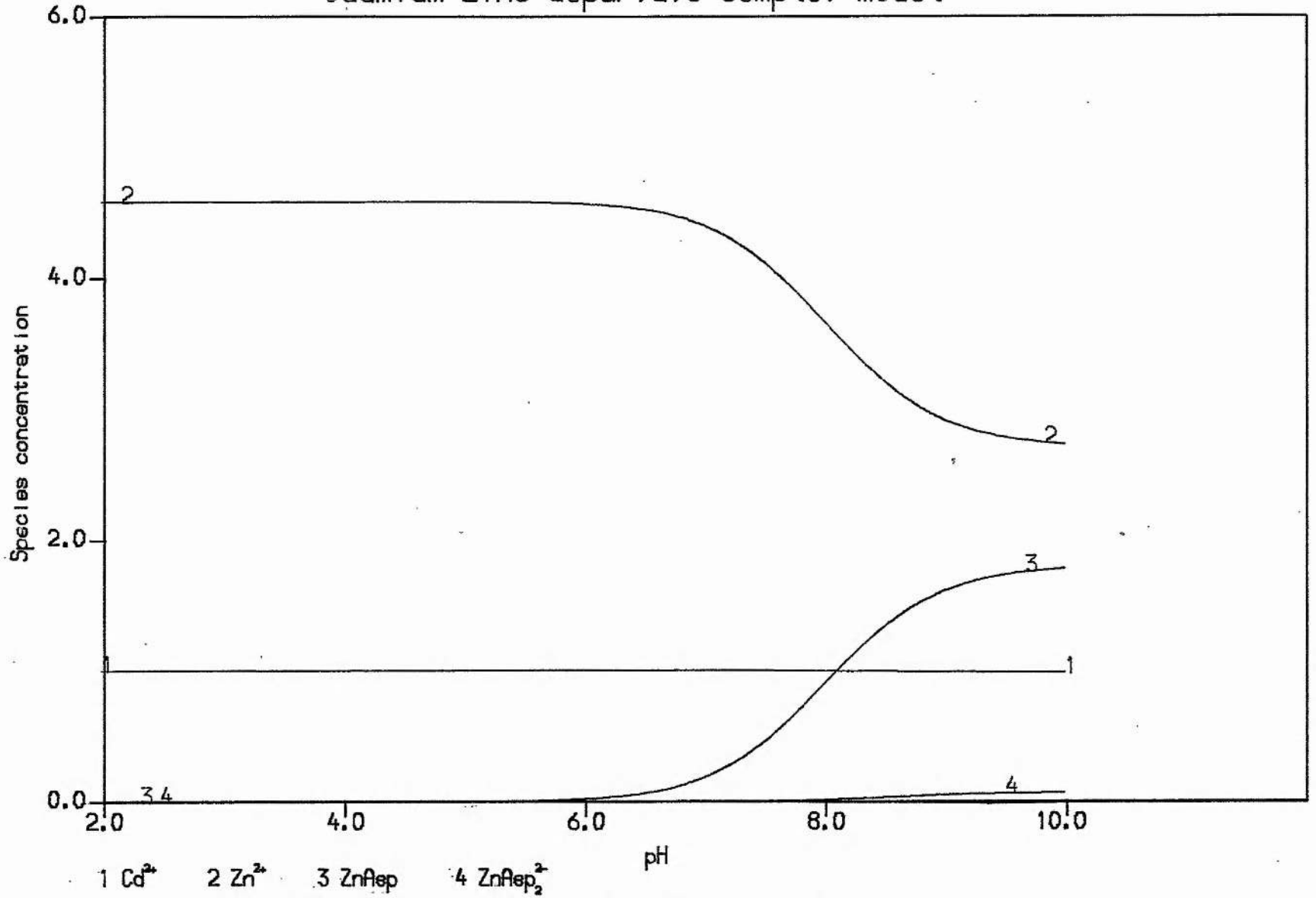


Figure 9 Total Cd(II) = 10.00, Zn(II) = 45.88 and Asp = 20.00 μM

CADMIUM ASPARTATE

Initial formation constants reported for this system were $\log \beta_{110} = 5.013$, $\log \beta_{210} = 9.120$. However, upon re-examination a set of non-superimposable curves was revealed which are shown in figure 8, and from this it was possible to refine to five constants which are shown in table 21.

Here, the highest ratio of ligand: metal in the complexes is 2:1 with a possibility of binding through an amino and two carboxyl groupings, although for stereochemical reasons the probability of all three binding simultaneously would seem remote

The constants reported for zinc only include $\log \beta_{110} = 6.379$, $\log \beta_{210} = 11.539$ and $\log \beta_{111} = 11.927$ (28). Once again $\log \beta^{Zn}_{110} > \log \beta^{Cd}_{110}$, and this leads to a similar picture from the COMPLOT studies, shown in figure 9, as was obtained for the asparaginate system.

Other workers results have not included any protonated species, but some (82,83) have included values for $\log \beta_{310}$ of 10.30 and 10.31 respectively at I = 1.0MKNO$_3$, 30°C. From the formation curves shown in figure 8 it can be seen that there is a levelling off at $\bar{Z} = 2.0$ and, therefore, the presence of the 310 complex is extremely unlikely. Other reported values for the formation constant of the 110 complex are 4.37(84) and 4.39(85), but once again these studies were undertaken at different experimental conditions, I = 0.1MKCl, 30°C and I = 0.1MKCl, 25°C respectively.

CADMIUM CYSTEINATE

Although this sytem was known to precipitate (86) it was investigated in order to estimate the order of magnitude of a sulphur containing amino acid binding with a cadmium ion. From the formation curves shown in figure 10, an approximate value of $\log \beta_{110} = 12.8$ was obtained which compared

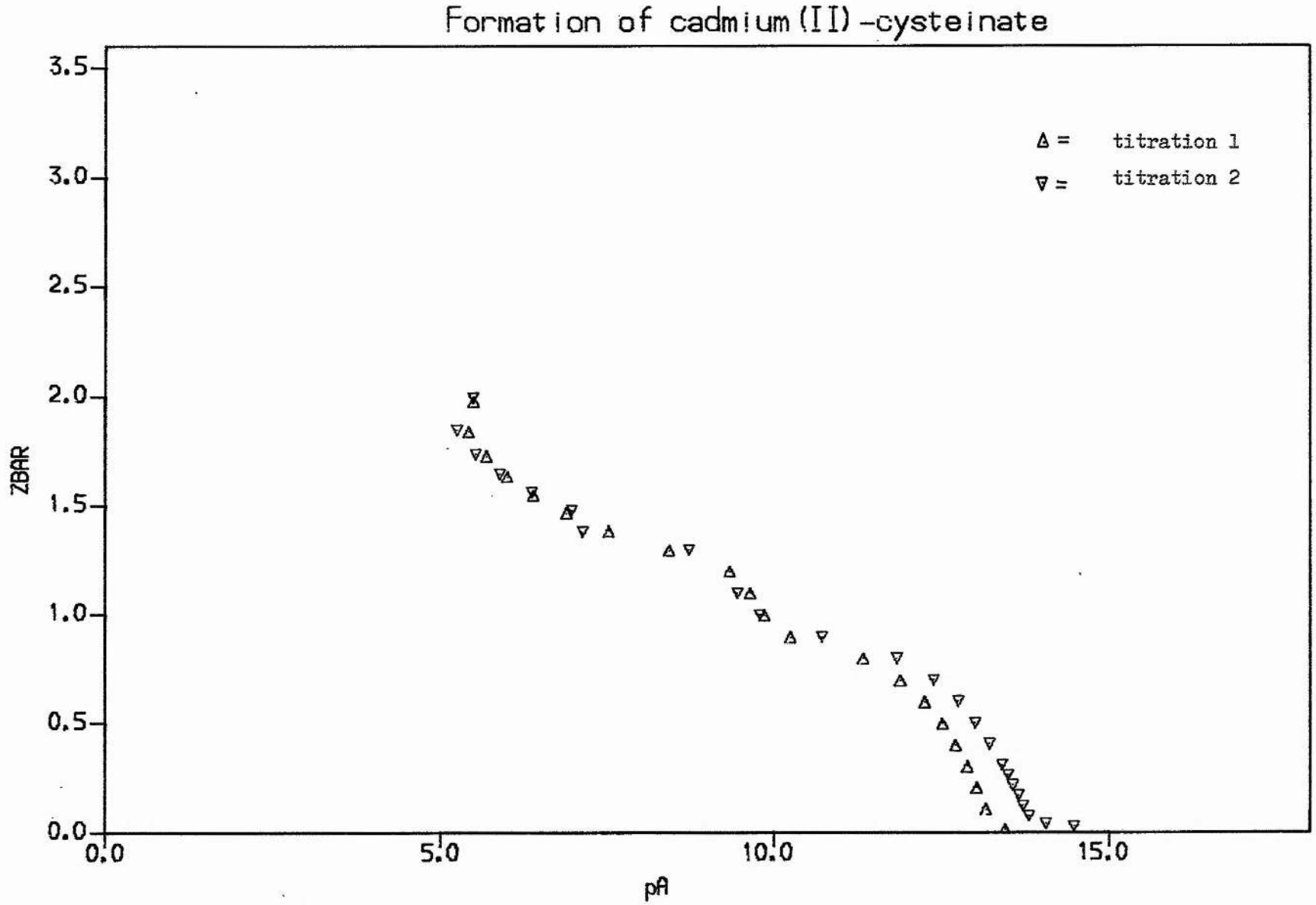

Formation of cadmium(II)-cysteinate
Δ = titration 1
∇ = titration 2
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
pA

Figure 10

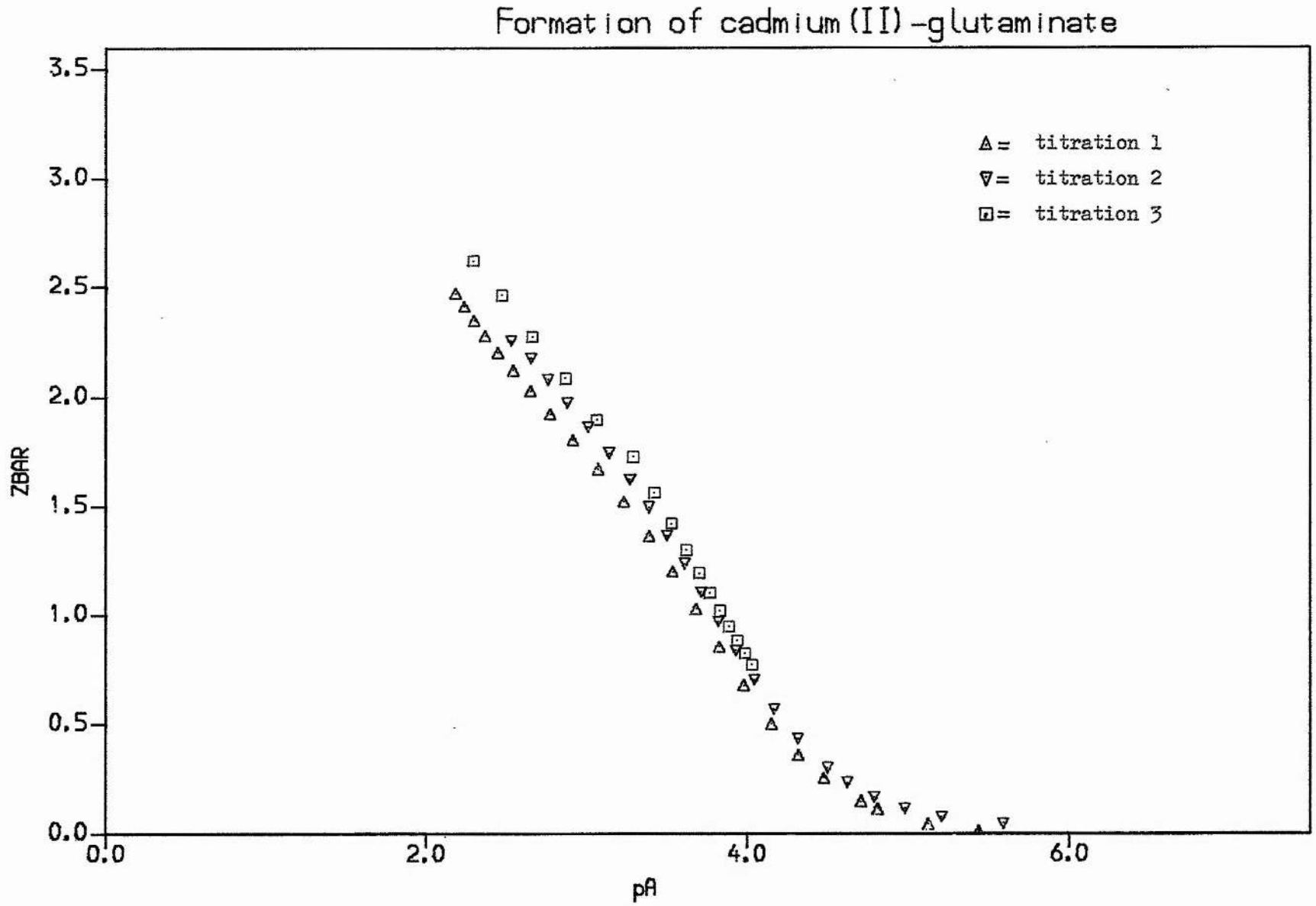

Formation of cadmium(II)-glutaminate
△= titration 1
▽= titration 2
⊡= titration 3
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
pA

Figure11

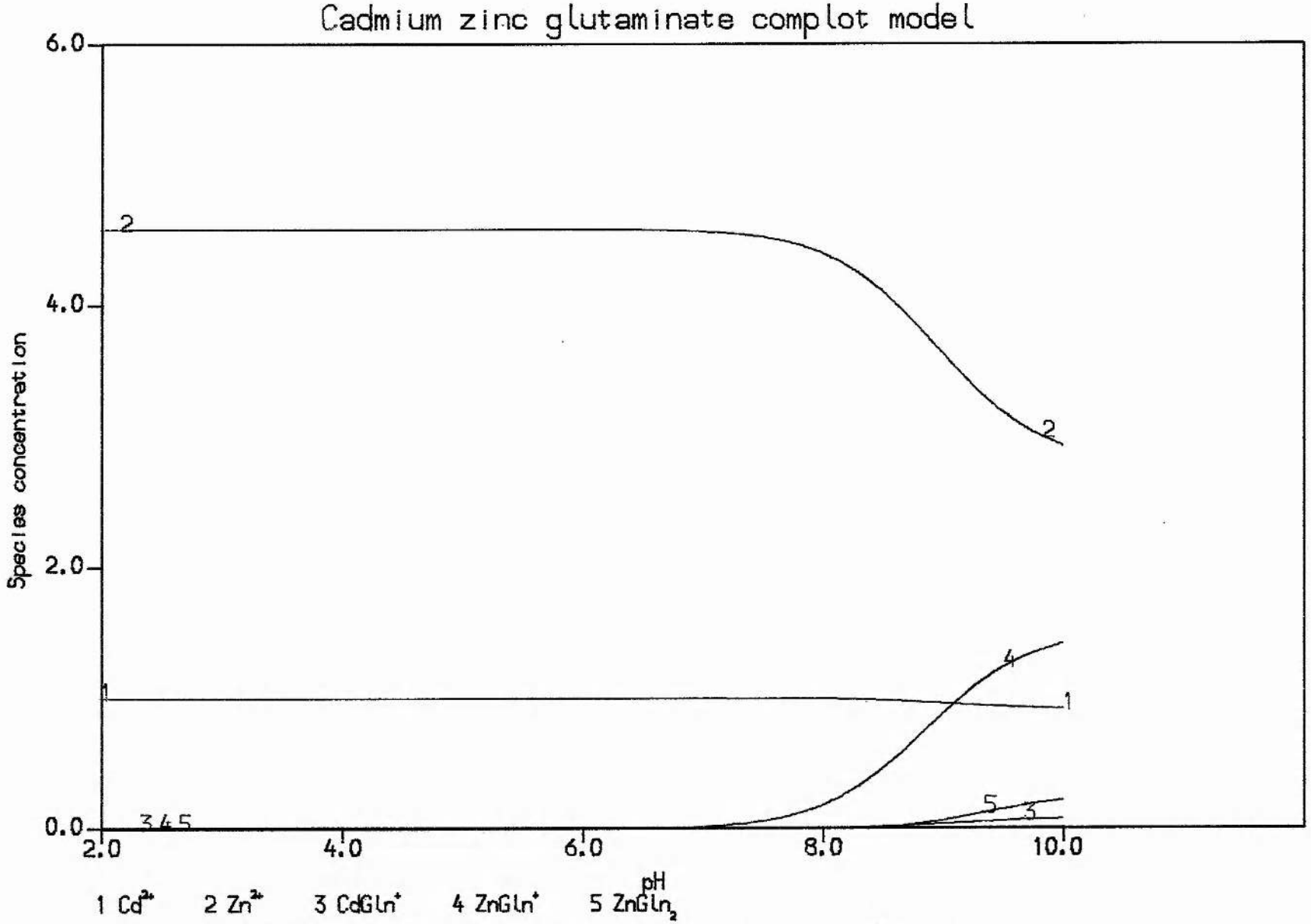


Figure 12 Total Cd(II) = 10.00, Zn(II) = 45.88, Gln = 30.00 μM

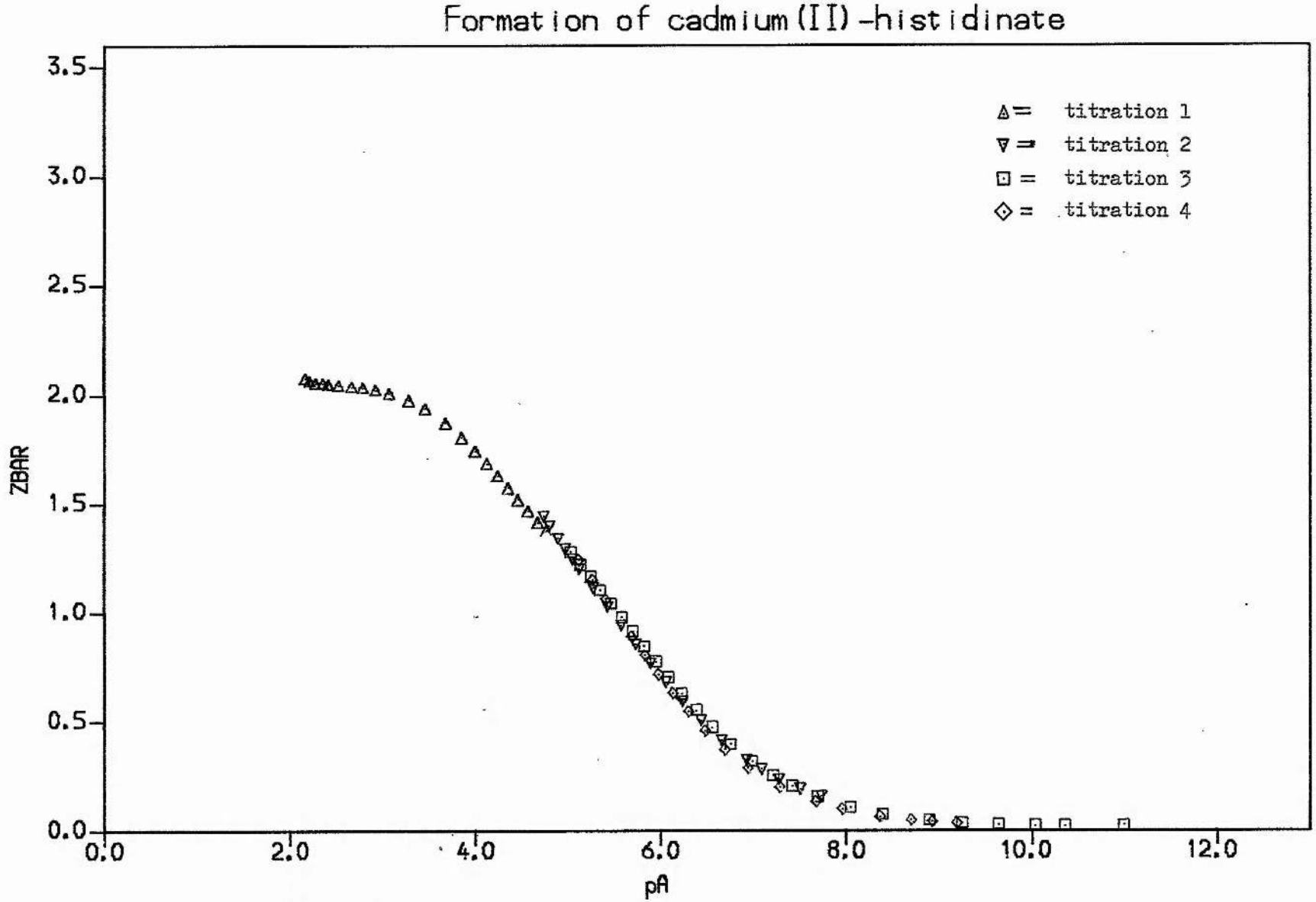

Formation of cadmium(II)-histidinate
△= titration 1
▽= titration 2
□= titration 3
◇= titration 4
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
pA

Figure 13

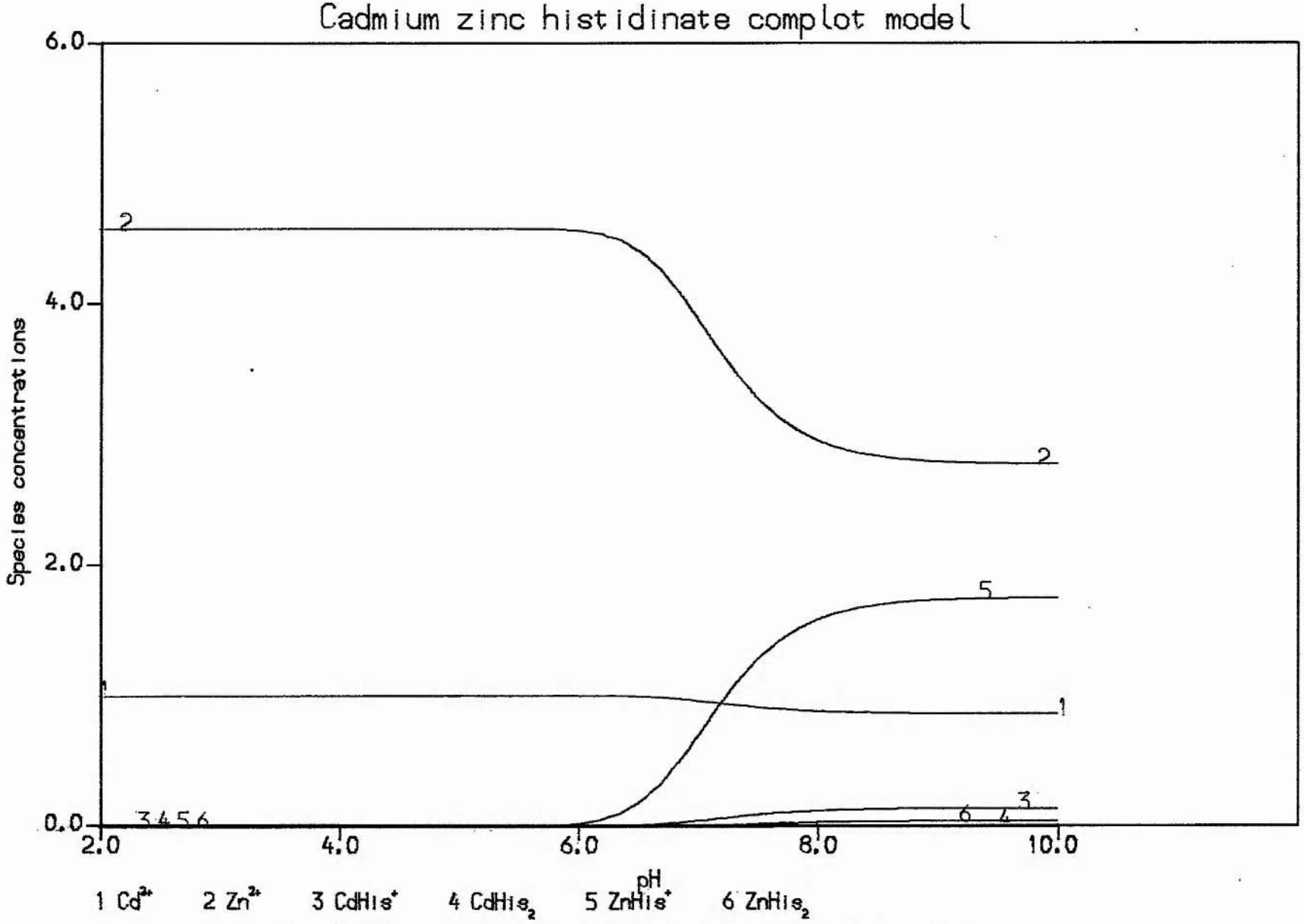


Figure 14 Total Cd(II) = 10.00, Zn(II) = 45.88, His = 20.00 μM

favourably with the figures for zinc thus indicating that a sulphur containing compound should provide a more selective sequestering agent *in vivo*.

CADMIUM GLUTAMINATE

For this system three constants, for the 110,210 and 310 complexes, were found, once again indicating binding through the amine nitrogen and the carboxyl group. The formation curves are superimposable as is shown in figure 11.

The constant for the 210 complex is similar to that reported by Perkins where $\log \beta_{210} = 7.64$(81)

Once again the constants are lower than those obtained for the corresponding zinc system (26) and the usual pattern of curves is obtained from COMPLOT studies (figure 12) with a preferential complexing of zinc.

CADMIUM HISTIDINATE

For this system only two constants were obtained, β_{110} and β_{210}. $\log \beta_{110}$ at 6.484 is about 1.5 log units higher than the corresponding formation constants for the other non-sulphur containing amino-acids which is perhaps due to binding through the imidazole nitrogen - indeed this has been shown to be the case in the crystal state (87). The superimposable formation curves are shown in figure 13.

Once again the cadmium constants are less than those for the corresponding zinc system where $\log \beta_{110} = 7.068$ and $\log \beta_{210} = 12.741$(25), which leads to the familiar pattern of curves from COMPLOT studies shown in figure 14.

Figures for $\log \beta_{210}$ from the work of Perkins (81) and Li and Manning (86) are in good agreement with the figure reported here, with $\log \beta_{210} = 11.10$ in both cases. The work of Vallasdas-Dubois (88) reports a value of 5.65 for $\log \beta_{110}$ which is lower than that reported here, but his value for $\log \beta_{210}$ is also much lower at 9.65.

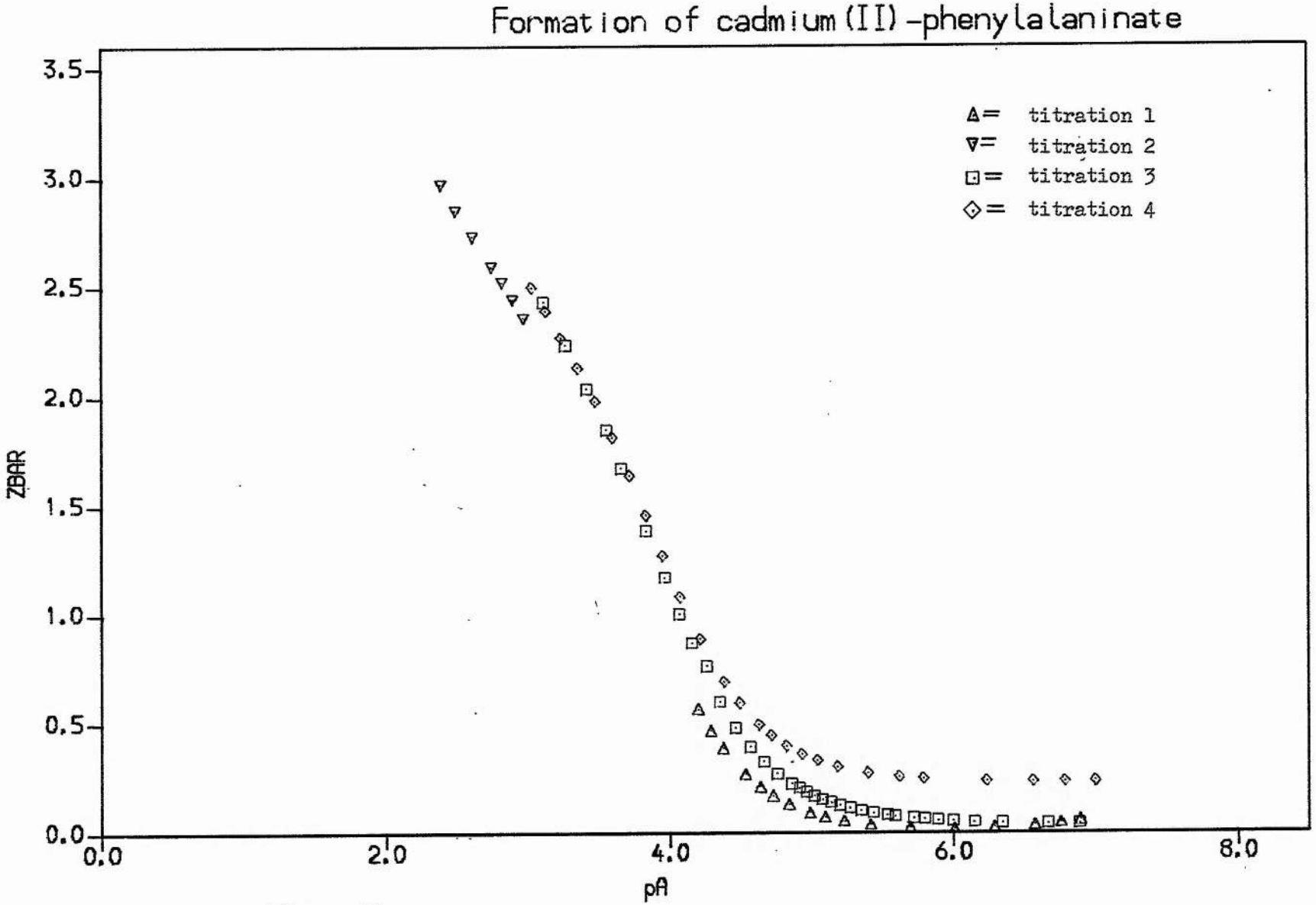

Formation of cadmium(II)-phenylalaninate
Δ= titration 1
▽= titration 2
⊡= titration 3
◇= titration 4
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
pA

Figure 15

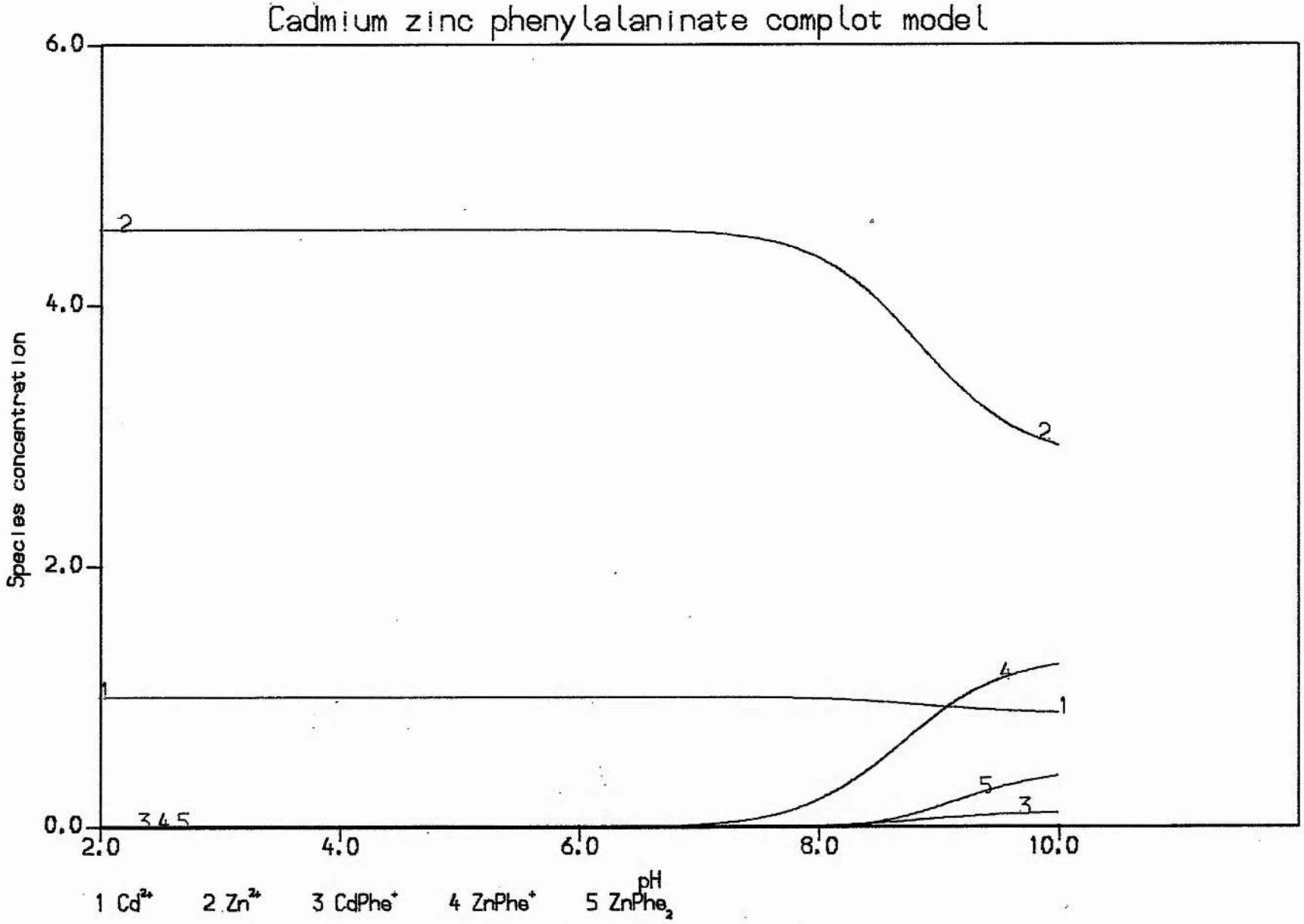


Figure 16 Total Cd(II) = 10, Zn(II) = 45.88, Phe = 30.00 μM

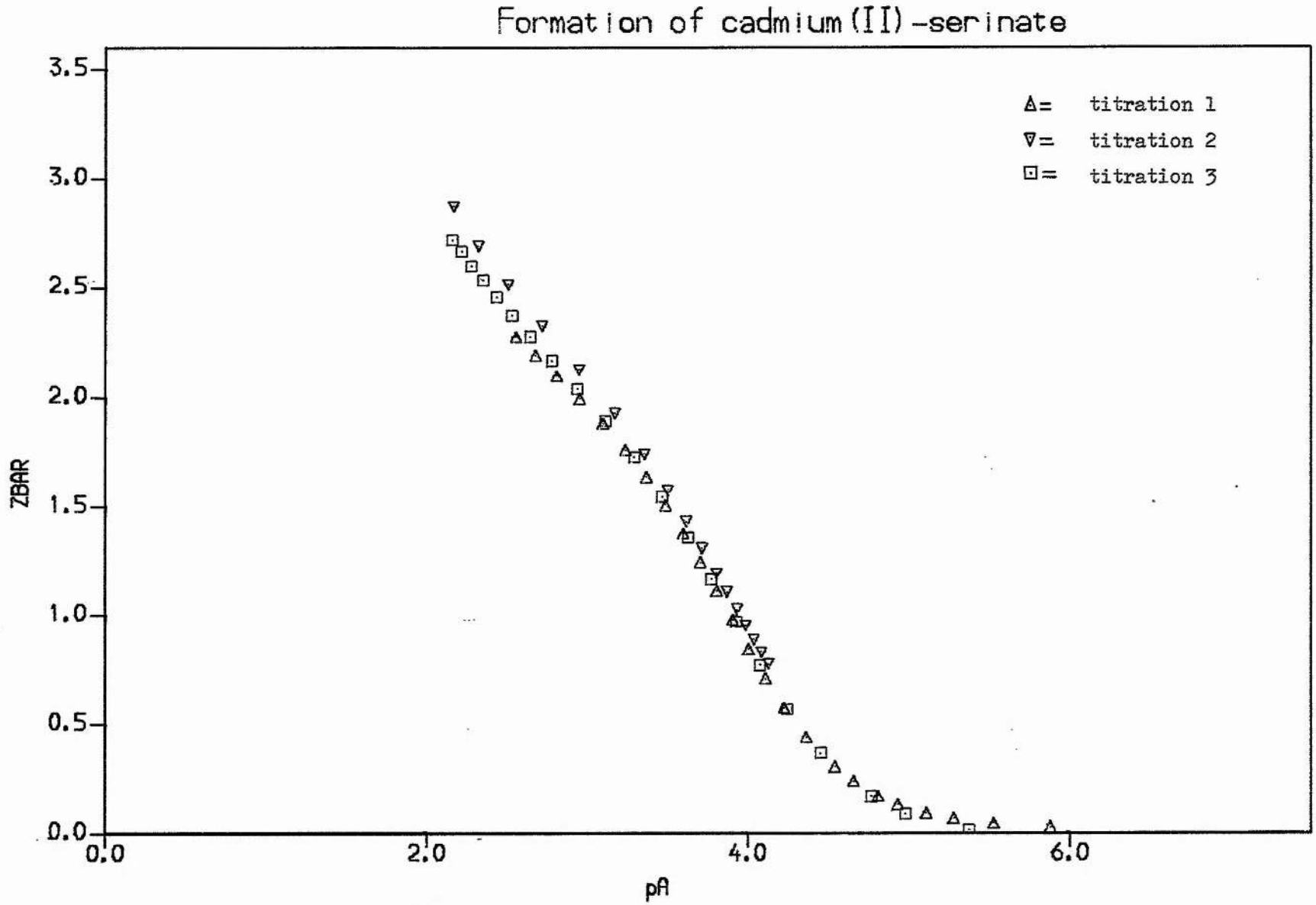

Formation of cadmium(II)-serinate
Δ= titration 1
▽= titration 2
⊡= titration 3
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
pA

Figure 17

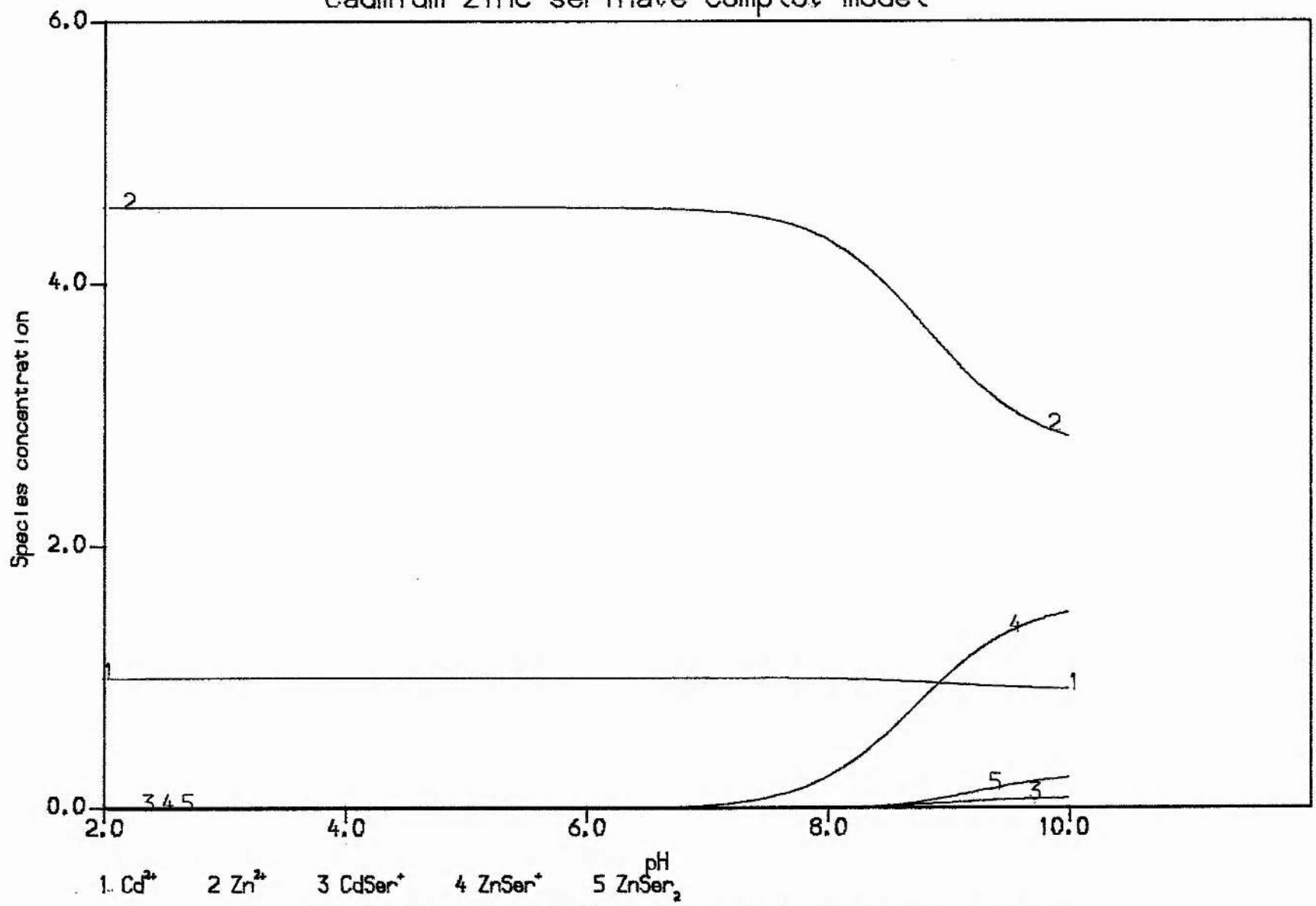


Figure 18 Total CD(II) = 10.00, Zn(II) = 45.88, Ser = 30.00 μM

CADMIUM PHENYLALANINATE

For this system three constants were obtained, for the 110, 210 and 310 complexes whereas only two, the 110 and 210 have been reported for zinc (28). This may be due either to the size of the ligand or the preference of zinc for tetrahedral co-ordination.

With phenylalaninate there was difficulty in overcoming problems of complex insolubility, but by attacking from both the acid and alkaline end of the formation curve a complete curve was obtained (figure 15).

Although the curves are not superimposable it was found impossible to refine any protonated or hydroxy species.

The COMPLOT study of this system with zinc - see figure 16 - shows results having the usual pattern with the zinc being complexed at the expense of the cadmium.

CADMIUM SERINATE

This hydroxy containing amino-acid forms three complexes with cadmium, the 110, 210 and 310.

Once again the comparison with zinc, $\log \beta_{110} = 4.898$, $\log \beta_{210} = 9.279$ and $\log \beta_{310} = 11.909$ (26), gives a negative value for $\log \beta^{Cd}_{110} - \log \beta^{Zn}_{110}$ indicating preferential binding of the ligand to zinc, which is indeed the case as can be seen from the COMPLOT model shown in figure 18.

In comparison with other workers results, the $\log \beta_{210}$ of 7.14 at $I = 0.005M CdSO_4$, $20^{\circ}C$ reported by Perkins (81) is slightly lower but no values are given for the 110 and 210 complexes.

CADMIUM TRYPTOPHANATE

This amino-acid containing an indole ring system gives constants for the 110, 210 and 310 complexes, once again indicating co-ordination at the carboxyl group and the amino nitrogen.

In this system there was difficulty in obtaining the region of the curve at low pA and high $\bar{Z}$, see figure 19, but this was achieved by working

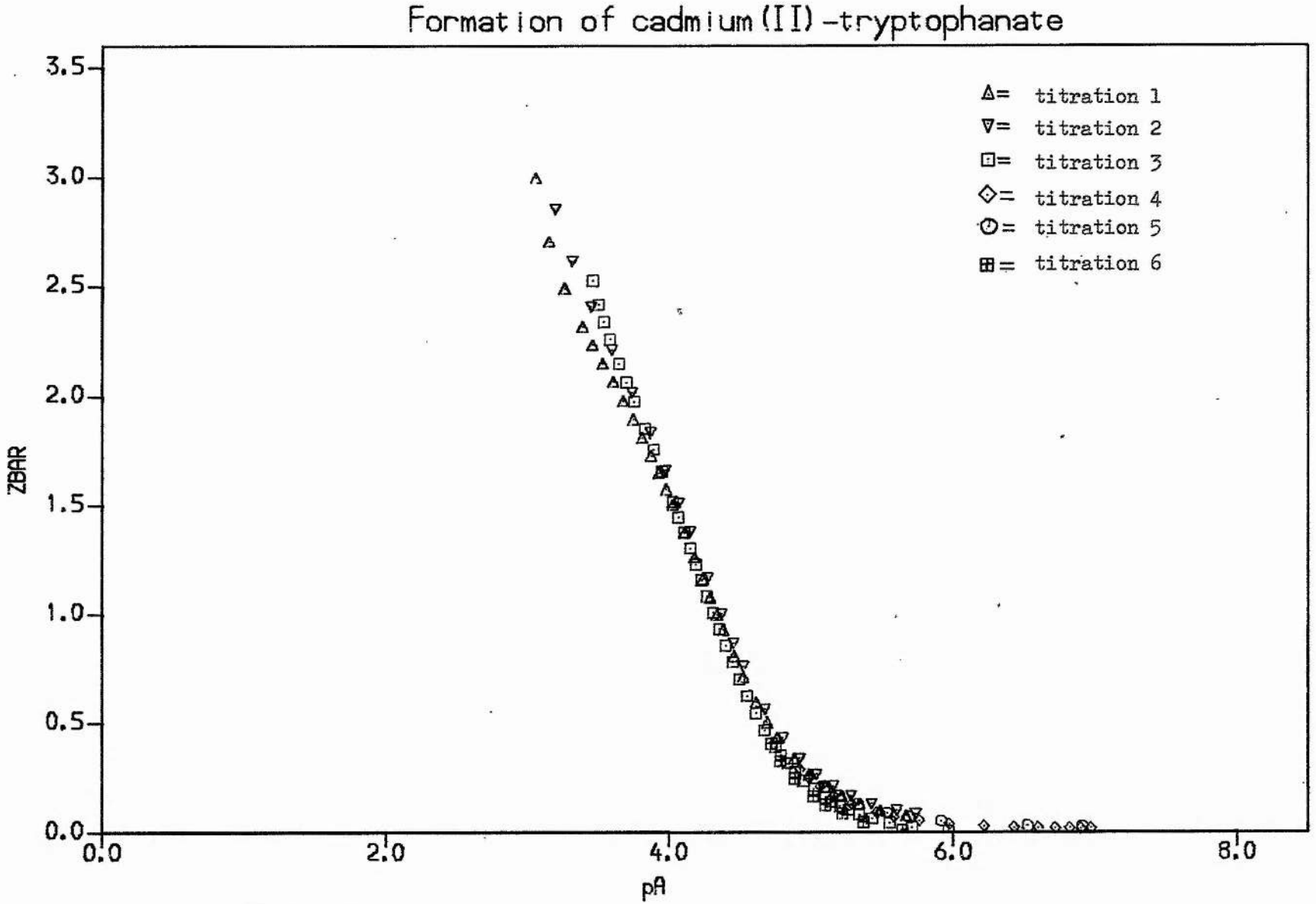

Formation of cadmium(II)-tryptophanate
△= titration 1
▽= titration 2
⊡= titration 3
◇= titration 4
⊙= titration 5
⊞= titration 6
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
pA

Figure 19

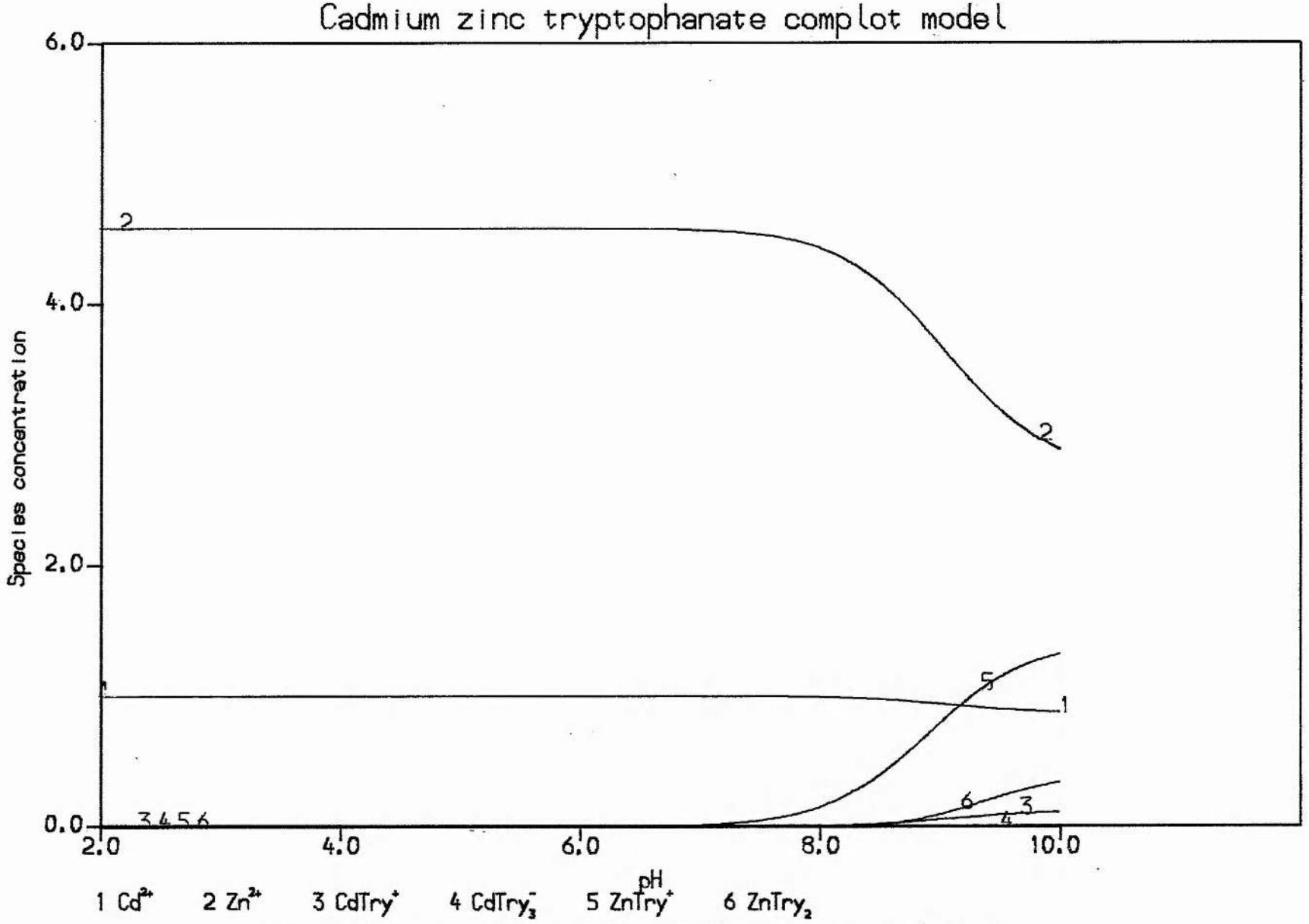


Figure 20 Total Cd(II) = 10.00, Zn(II) = 45.88, Try = 30.00 μM

at low concentration.

However, computational studies showed this region to be highly sensitive to small changes in total concentration of any of the three components of the system and also to a small variation in E^{o}. With this in mind, it is felt that the formation constant for the 310 complex should be viewed with some reservation.

Other workers have already reported constants for the 210 complex and that of Perkins (81) is much lower at 7.0, however from other systems it can be seen that $\log \beta_{210} - \log \beta_{110}$ should give a value of 3.5 to 4.0 log units which would give Perkins rather a small value for $\log \beta_{110}$.

As with the other amino-acids, apart from cysteine, the zinc-tryptophanate constants are higher than those for cadmium and thus the COMPLOT study shown in figure 20 shows the familiar pattern of preferential complexing of zinc.

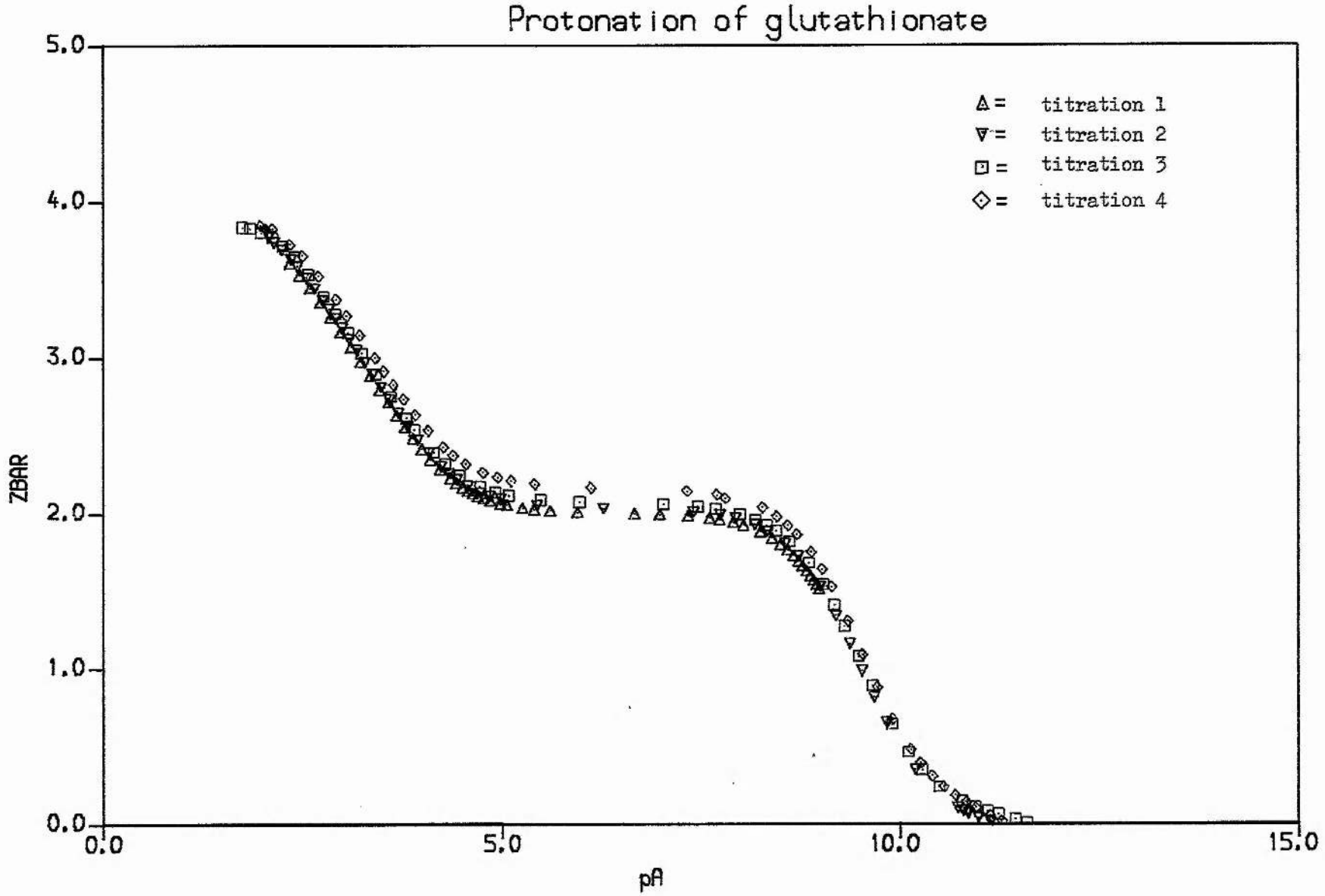

Protonation of glutathionate
5.0
4.0
3.0
2.0
1.0
0.0
ZBAR
0.0
5.0
10.0
15.0
pH
Δ = titration 1
▽ = titration 2
□ = titration 3
◇ = titration 4

Figure 21

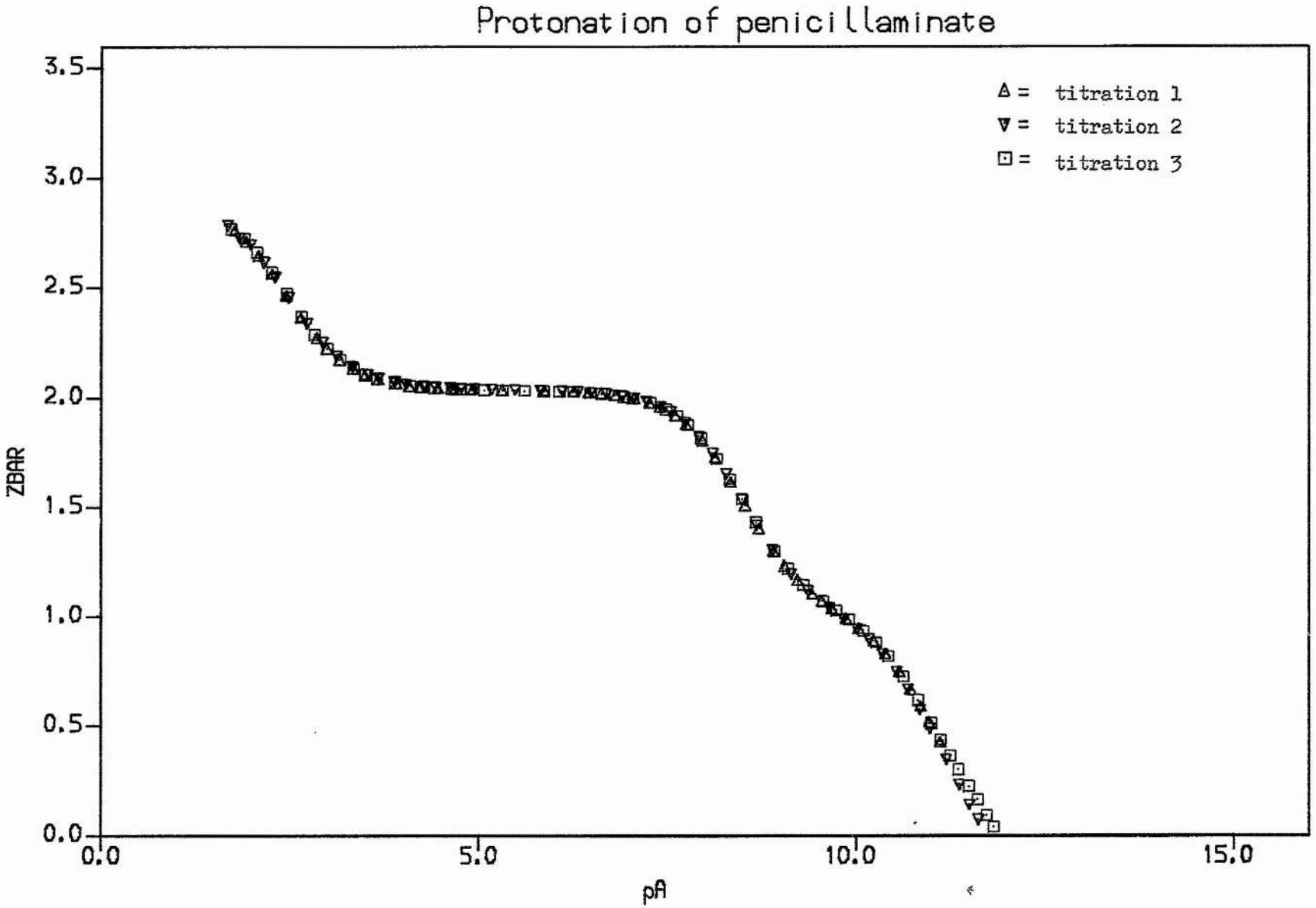

Protonation of penicillaminate
Δ = titration 1
▽ = titration 2
⊡ = titration 3
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
pA

Figure 22

PROTONATION OF GLUTATHIONATE, D-PENICILLAMINATE, ETHYLENEDIAMINETETRAACETATE and 1, 2-DI(2-AMINOETHOXY)ETHANETETRAACETATE

All protonations should give superimposable formation curves similar to those obtained for simple metal-ligand systems. However, as will be discussed later, this is not always the case with the aminopolycarboxylic acids, although this problem can be overcome.

GLUTATHIONATE

The results of the protonation of glutathionate are shown in table 22 and the formation curves in figure 21. These yield pKa values of 9.874, 9.153, 3.719 and 2.599 of which the first two may be assigned to the protonation of the sulphydryl and amino groups respectively while the latter two may be assigned to the two carboxyl groups. The fully protonated ligand is of the form H_4A with a residual positive charge.

The constants reported here are in good agreement with those of other workers taking into consideration that all other work has been carried out under different experimental conditions (86, 89 - 92)

D-PENICILLAMINATE

The protonation of D-penicillaminate, A^{2-} gives three constants from the formation curves shown in figure 22. These yield pK values of 11.010, 8.602 and 2.432. Once again these may be assigned in the order of sulphydryl, amino and carboxylate protonation respectively.

These results are in general agreement with those of other workers (93-96) taking into consideration that the work was carried out under different experimental conditions.

EGTA and EDTA

These both presented problems initially due to the non-superimposability of the formation curves, some appearing to be displaced vertically. However, this was overcome by attacking both ends of the curve from the centre, the problem only occurring when the initial solution of ligand was very acid.

TABLE 22

Log formation constants for ligand protonation at 25°, I = 3.00M $(Na)CLO_4$.

pqr =	Log β pqr				n[a]	SDT[b]
	101	102	103	104		
Glutathionate	9.874±0.014	19.037±0.011	22.856±0.011	25.455±0.013	192	0.0774
D-penicillaminate	11.010±0.008	19.612±0.014	22.044±0.023		160	0.0161
EDTA	9.060±0.005	16.100±0.007	18.680±0.017	20.953±0.014	194	0.1289
EGTA	9.360±0.014	17.973±0.015	20.970±0.049	23.697±0.039	191	0.2304

a, n = number of experimental observations; b, SDT = standard deviation in titre

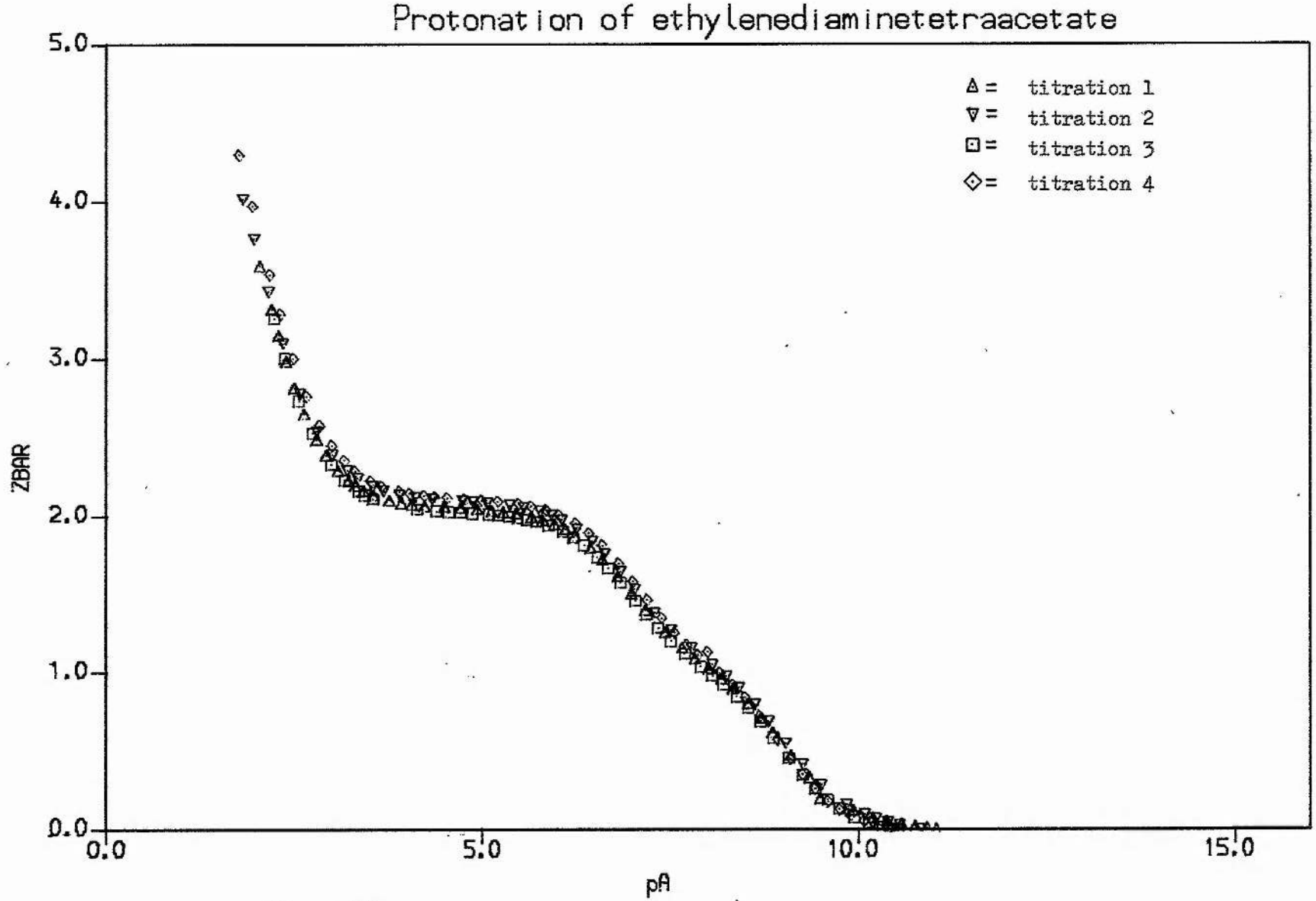

Protonation of ethylenediaminetetraacetate
△ = titration 1
▽ = titration 2
□ = titration 3
◇ = titration 4
ZBAR
5.0
4.0
3.0
2.0
1.0
0.0
0.0
5.0
10.0
15.0
pA

Figure 23

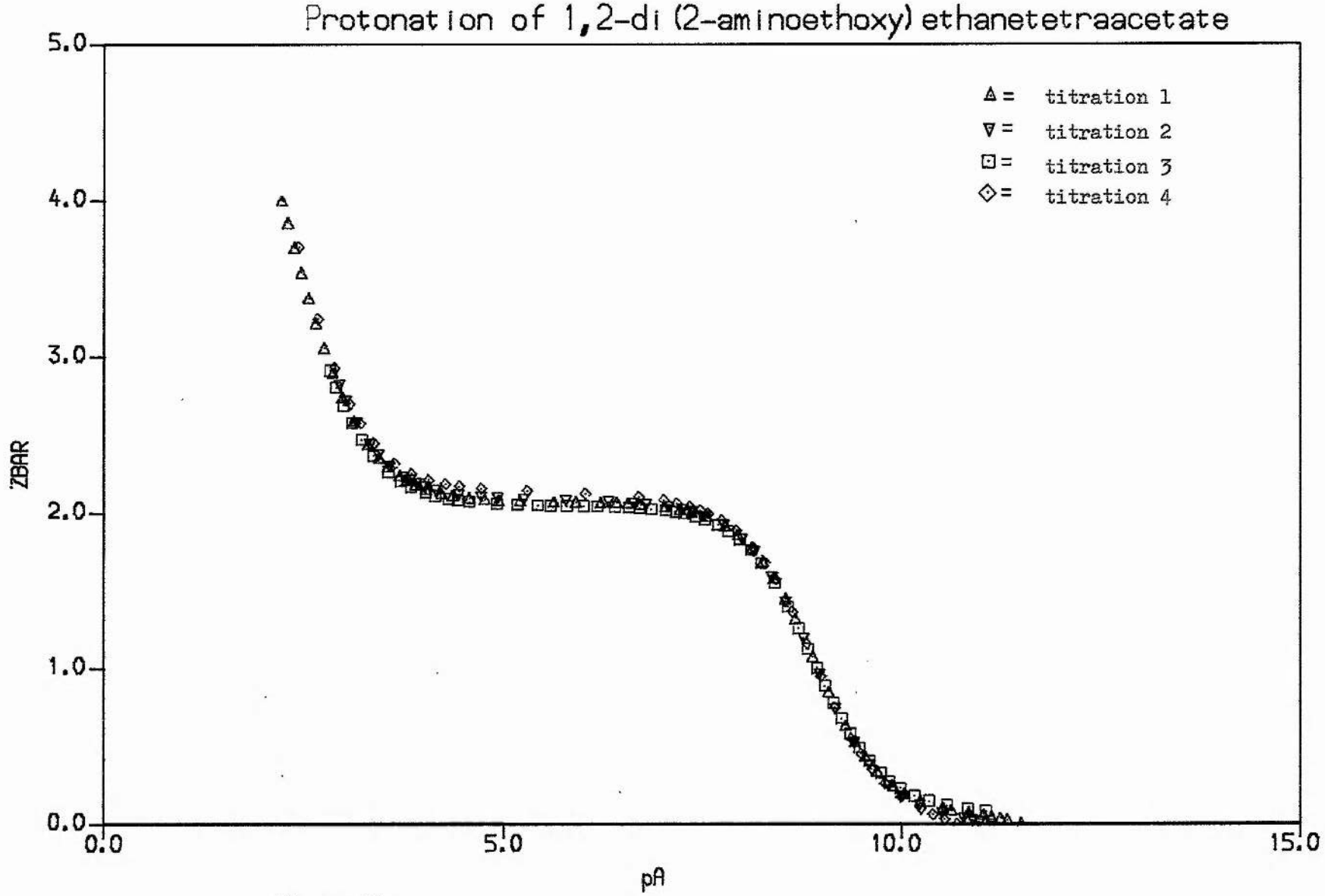

Protonation of 1,2-di (2-aminoethoxy) ethanetetraacetate
△ = titration 1
▽ = titration 2
⊡ = titration 3
◇ = titration 4
ZBAR
5.0
4.0
3.0
2.0
1.0
0.0
0.0
5.0
10.0
15.0
pA

Figure 24

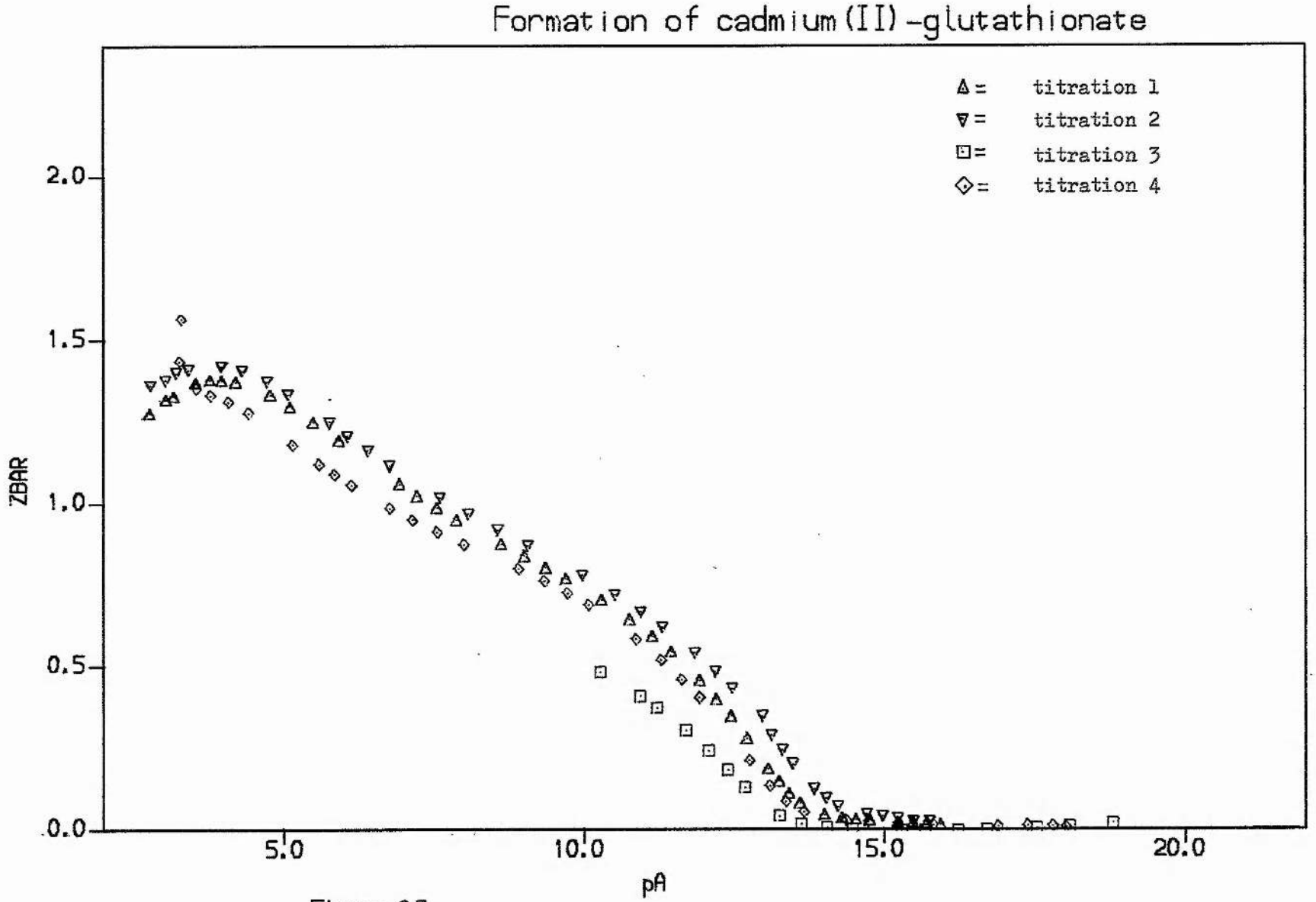

Formation of cadmium(II)-glutathionate
Δ = titration 1
▽ = titration 2
□ = titration 3
◇ = titration 4
ZBAR
2.0
1.5
1.0
0.5
0.0
5.0
10.0
15.0
20.0
pA

Figure 25

The problem could well have been due to the amino nitrogens being protonated in highly acid conditions and their setting up a non-equilibrium state. Indeed when this occurred there was continual drifting to higher pH.

This phenomenon of the protonated amino nitrogen of aminopolycarboxylic acids has been much discussed, especially in relation to EDTA. The concept was proposed as early as 1947 by Schwarzenbach and Ackermann (97) and more recently by Carini and Martell (98) and Sawyer and Tackett (99) although infra-red studies discount this (100).

The superimposable curves for the protonations of these two ligands, shown in figures 23 and 24, gave the $\log\beta$ values shownin table 22.

The results for the protonation of EGTA are in good agreement with those of Anderegg (101), whereas those for EDTA are lower than those of other workers (101 - 103). This difference, however, is not great, being of the order of one log unit which can be accounted for by the differing experimental conditions with most other work being carried out at 20°C in 0.1 M ionic background solution.

CADMIUM GLUTATHIONATE

As can be seen from figure 25, the formation curves for this system are non-superimposable. This indicates the presence of species other than simple binary complexes, possibly protonated, hydroxy or polynuclear. The final set of constants obtained are shown in table 23 and include three protonated and two hydroxy species.

Li and Manning (86) found $\log\beta_{110} = 10.5$ which is in general agreement with the corresponding constant reported here, but no other constants were reported.

Perrin and Watt (92) reported a similar set of constants although theirs differ by the addition of the 120 complex and the absence of the 21-1.

In this work it was found impossible to refine together all the constants using the computer program SCOGS (71, 72) due to exponent overflow and the more sophisticated program MINIQUAD (73) was used.

The high standard deviations of the hydroxy species is due to the fact that they are of only minor importance and then only at high pH.

The structures suggested from these constants are similar to those proposed by Perrin and Watt (92) but this disagrees with the C^{13} analysis of cadmium glutathionate reported by Fuhr and Rabenstein (22). The former suggest binding at the glutamyl terminal carboxyl, the glutamyl amino nitrogen, the cysteinyl peptide nitrogen and sulphydryl for the 110 complex with similar binding but excluding the amino nitrogen for the 210. However, the C^{13} n.m.r. shows no evidence of binding to the peptide linkage. Without this the possibility of the sulphydryl binding simultaneously with the glutamyl residue would seem remote as this would involve a ten membered ring as is shownin figure 26. However, from potentiometric data alone it is not possible to deduce definite structures without other information such as enthalpies of formation.

From the constants obtained for the zinc glutathionate system

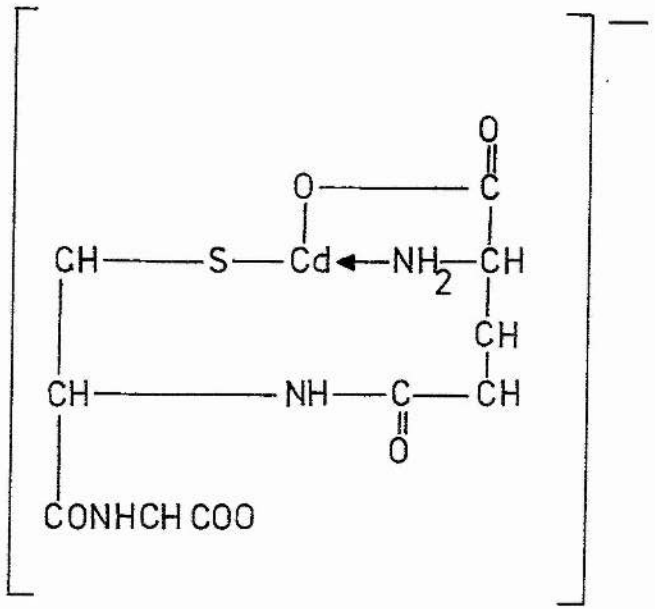


Figure 26

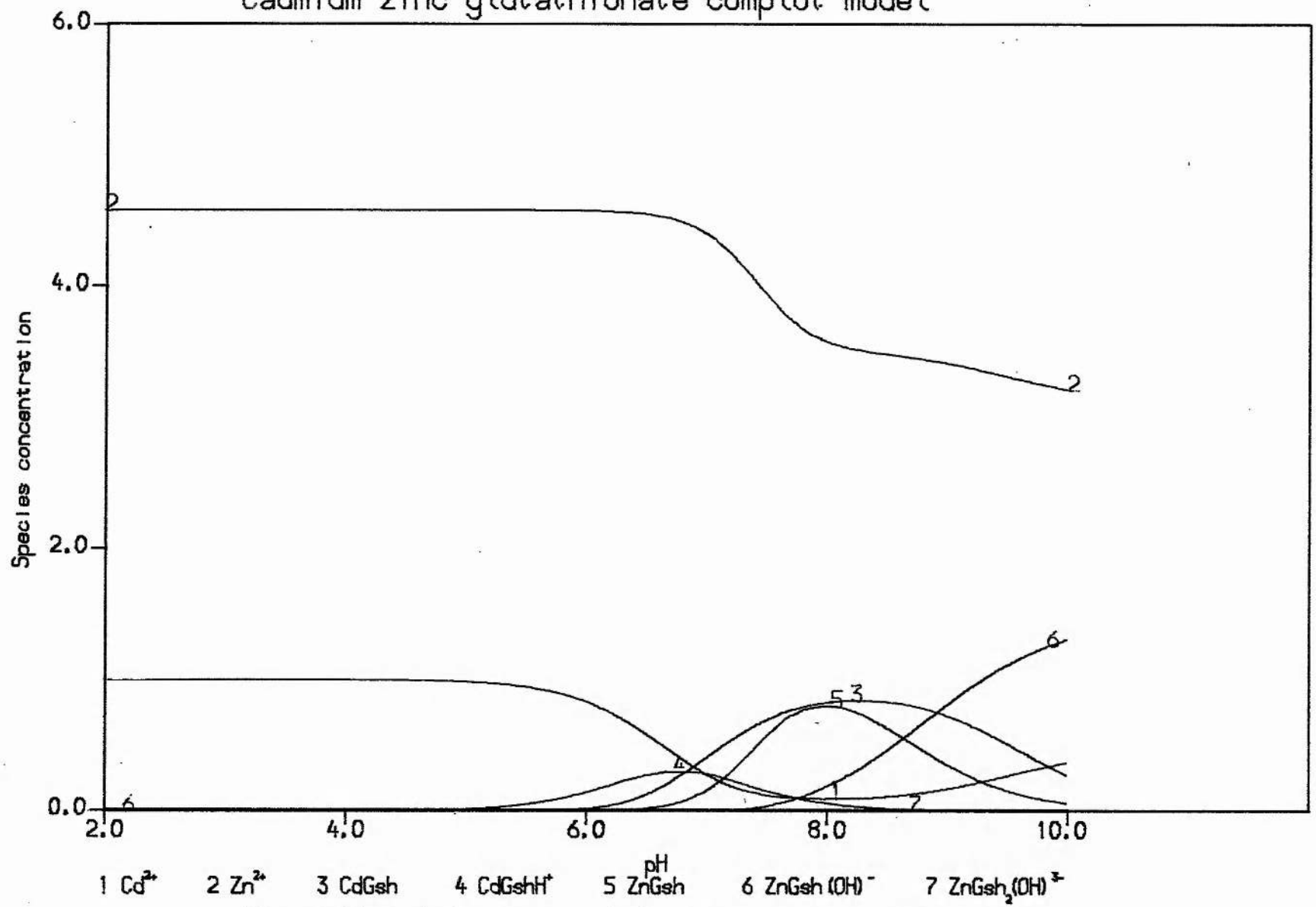


Figure 27 Cd(II) = 10.00, Zn(II) = 45.88, glutathionate = 20.00 μM

TABLE 23

Log formation constants for cadmium (II) - ligand anion complexes at 25°, I = 300M(Na) ClO_4

	Log β pqr							a_n
	110	111	11-1	210	211	212	21-1	
Glutathionate	10.180±0.245	17.024±0.021	0.291±0.631	15.353±0.064	25.086±0.052	33.032±0.040	3.169±5.382	158
D-penicillaminate	12.681±0.047	17.152±0.075		20.683±0.057	28.306±0.056	34.533±0.074	9.138±0.079	200
[b] EDTA	14.677±0.055	17.427±0.032(SDT = 0.2863)						232
EGTA	15.020±0.057	18.670±0.055						240

Log formation constants for zinc (II) - ligand anion complexes at 25°, I = 3.00M(Na) ClO_4

	Log β pqr								a_n
	110	111	210	211	212	21-1	430	431	
D-penicillaminate			20.521±0.019	26.794±0.040	32.724±0.018	8.563±0.057	47.582±0.086	53.826±0.084	198
EGTA	11.485±0.042	17.345±0.032							181

a, n = number of experimental observations.

b, constants used in models. A second set was refined which made no improvement in the PSEUDO PLOT..

$$\left.\begin{array}{l} \text{Log } \beta_{110} = 14.811 \pm 0.050 \\ \text{Log } \beta_{111} = 17.066 \pm 0.067 \\ \text{Log } \beta_{120} = 18.429 \pm 0.110 \end{array}\right) \quad \text{SDT} = 0.2647$$

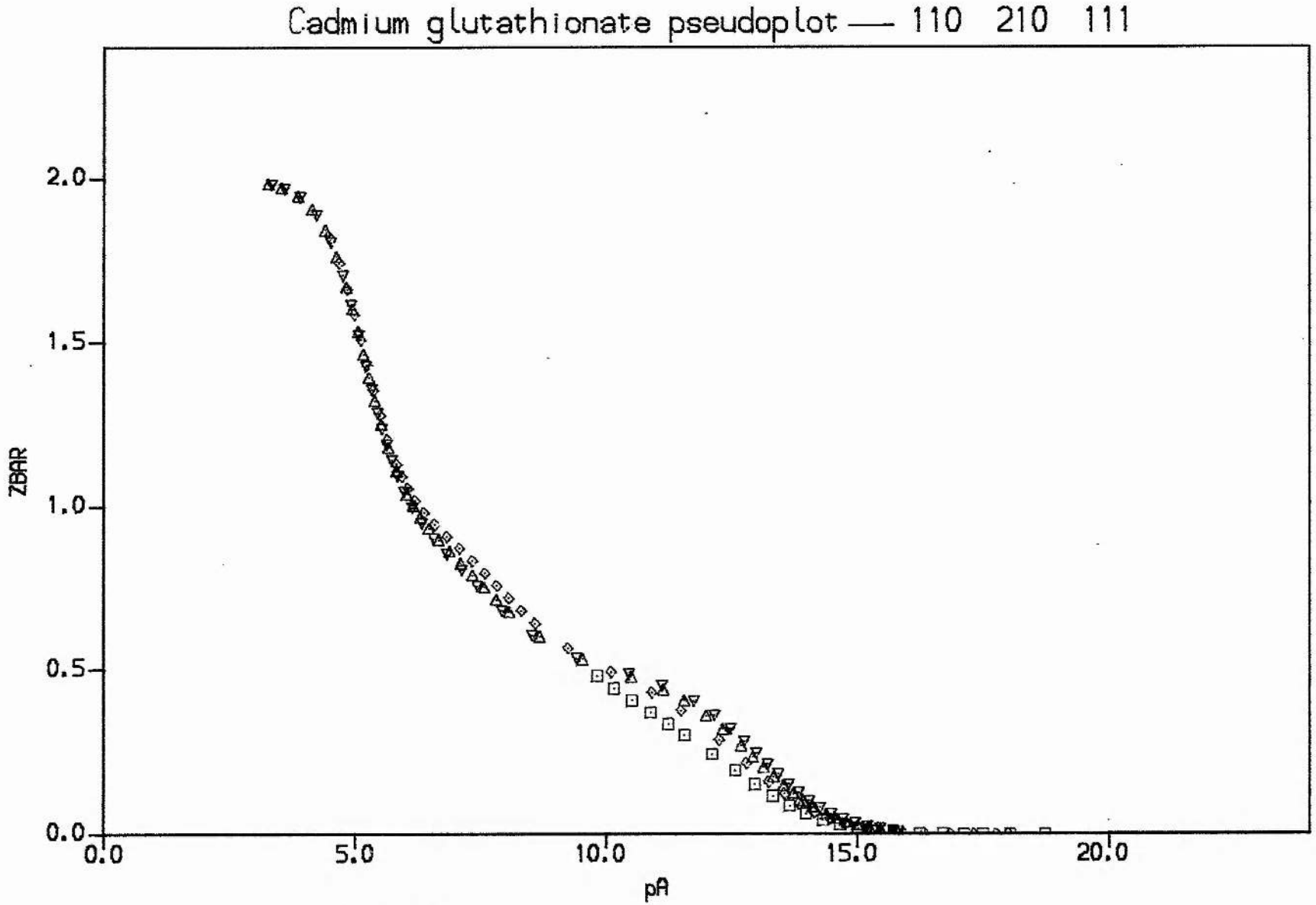

Cadmium glutathionate pseudoplot — 110 210 111
2.0
1.5
1.0
0.5
0.0
ZBAR
0.0
5.0
10.0
15.0
20.0
pA

Figure 28

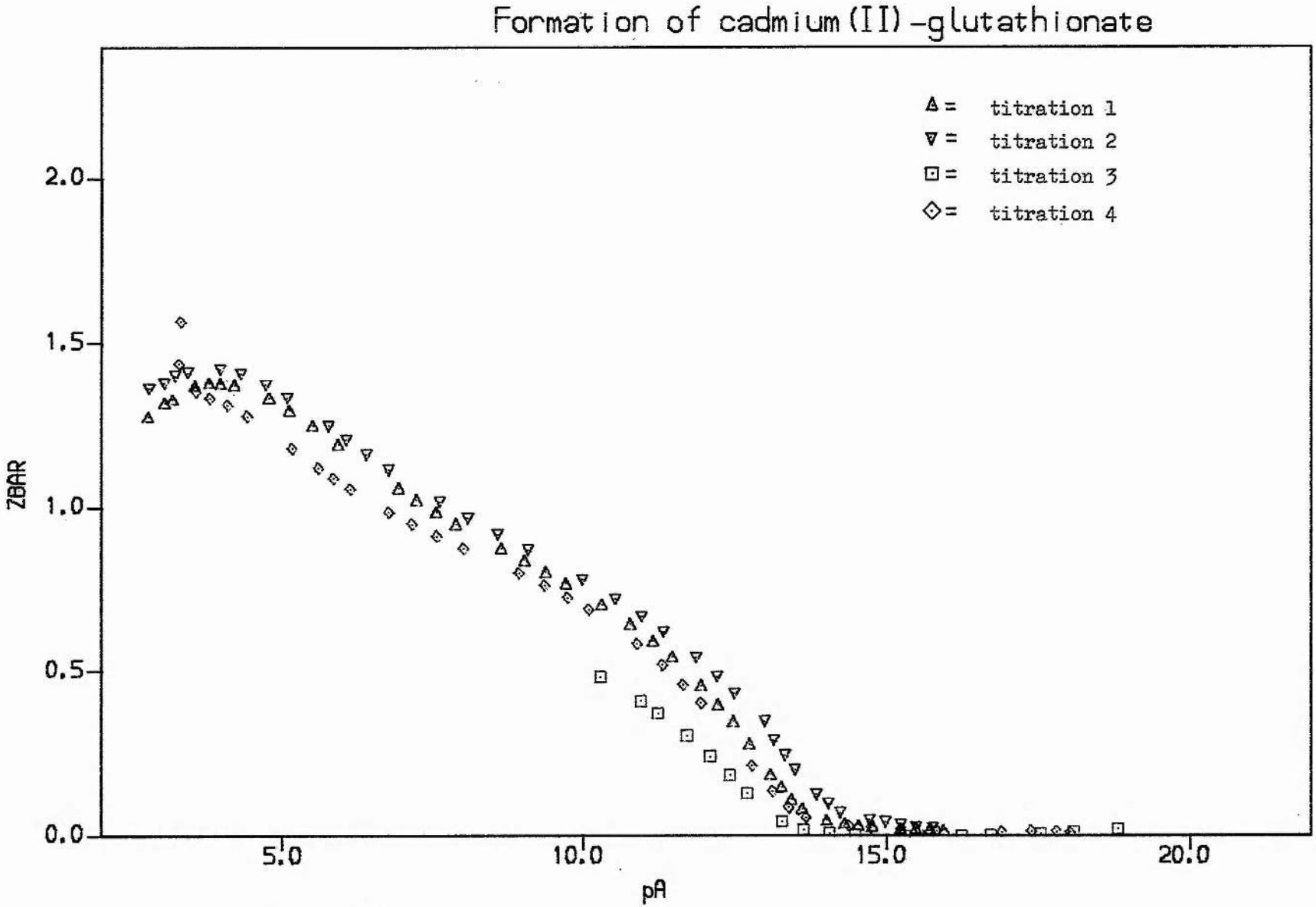

Formation of cadmium(II)-glutathionate
Δ = titration 1
▽ = titration 2
⊡ = titration 3
◇ = titration 4
ZBAR
2.0
1.5
1.0
0.5
0.0
5.0
10.0
15.0
20.0
pA

Figure 25

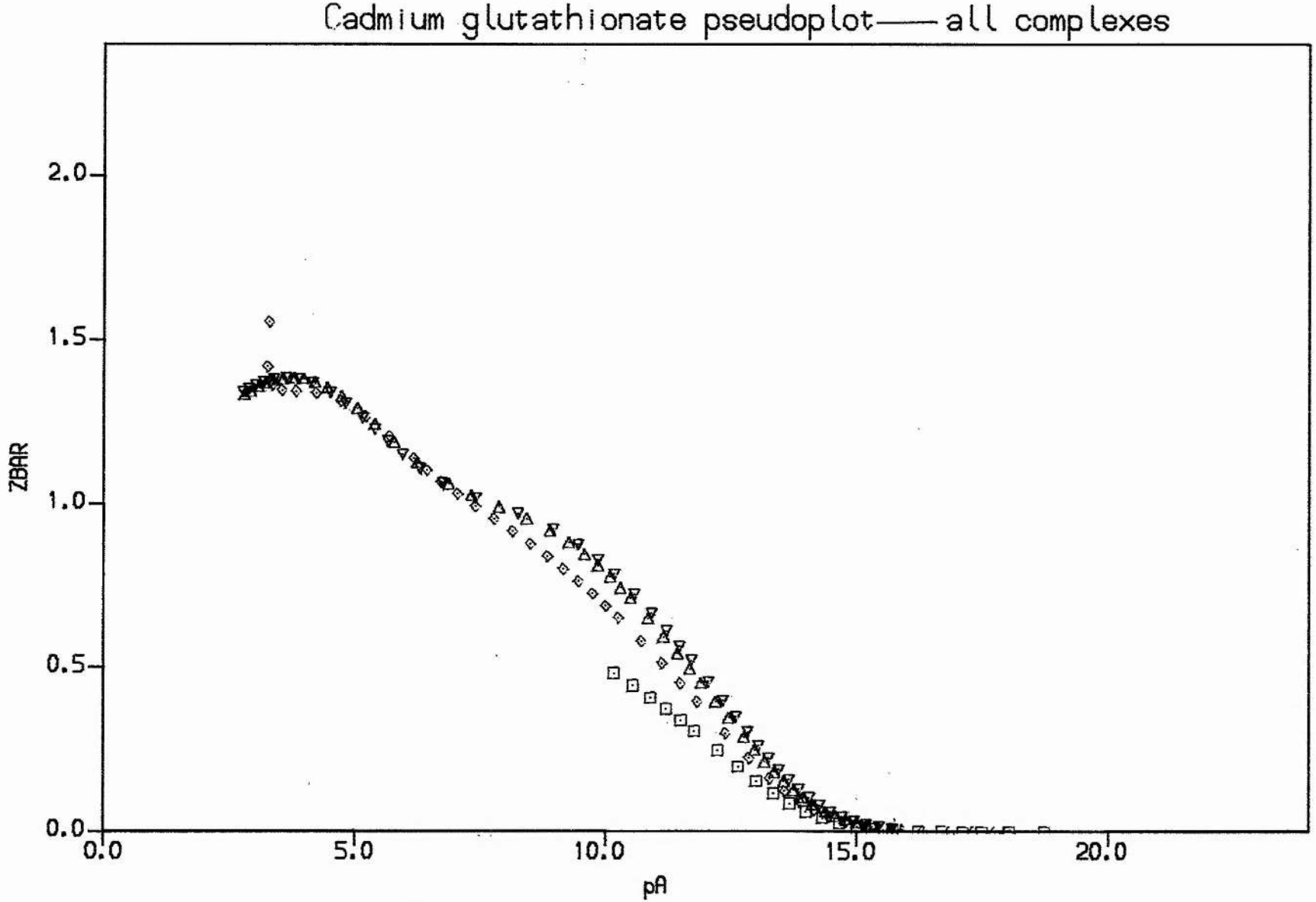

Cadmium glutathionate pseudoplot—— all complexes
2.0
1.5
1.0
0.5
0.0
ZBAR
0.0
5.0
10.0
15.0
20.0
pA

Figure 29

($\log \beta_{110} = 8.568$, $\log \beta_{111} = 14.762$, $\log \beta_{11-1} = -0.074$, $\log \beta_{210} = 13.586$, $\log \beta_{211} = 23.271$, $\log \beta_{212} = 30.616$, $\log \beta_{21-1} = 3.634$(104)) it can be seen that this ligand should be much more effective as a sequestering agent than the previously mentioned amino-acids since $\log \beta^{Cd}_{110} - \log \beta^{Zn}_{110} = 1.612$.

From the COMPLOT model studies shown in figure 27 it can be seen that for $Cd^{2+} = 10\ \mu M$, $Zn^{2+} = 45.88\ \mu M$ and glutathionate^{3-} = 20.00 μM, approximately 90% of the cadmium is complexed at a biological pH of 7.4

This system is used here to illustrate the value of the program PSEUDOPLOT (64). Figure 28 shows the PSEUDOPLOT where only the 110, 210 and 111 complexes have been used. Figure 29 shows the PSEUDOPLOT obtained by using all the constants obtained from MINIQUAD. When these are compared with figure 25, the experimental formation curves, an improvement can be discerned. If this was not available it might be that one would be satisfied with a set of constants which could be much improved. It is still noticeable that the final set of constants does not furnish a set of curves from PSEUDOPLOT which are completely superimposable on the experimental set. This is especially noticeable at the lowpA, high $\bar{Z}$ region which might be accounted for by being at the limit of electrode sensitivity.

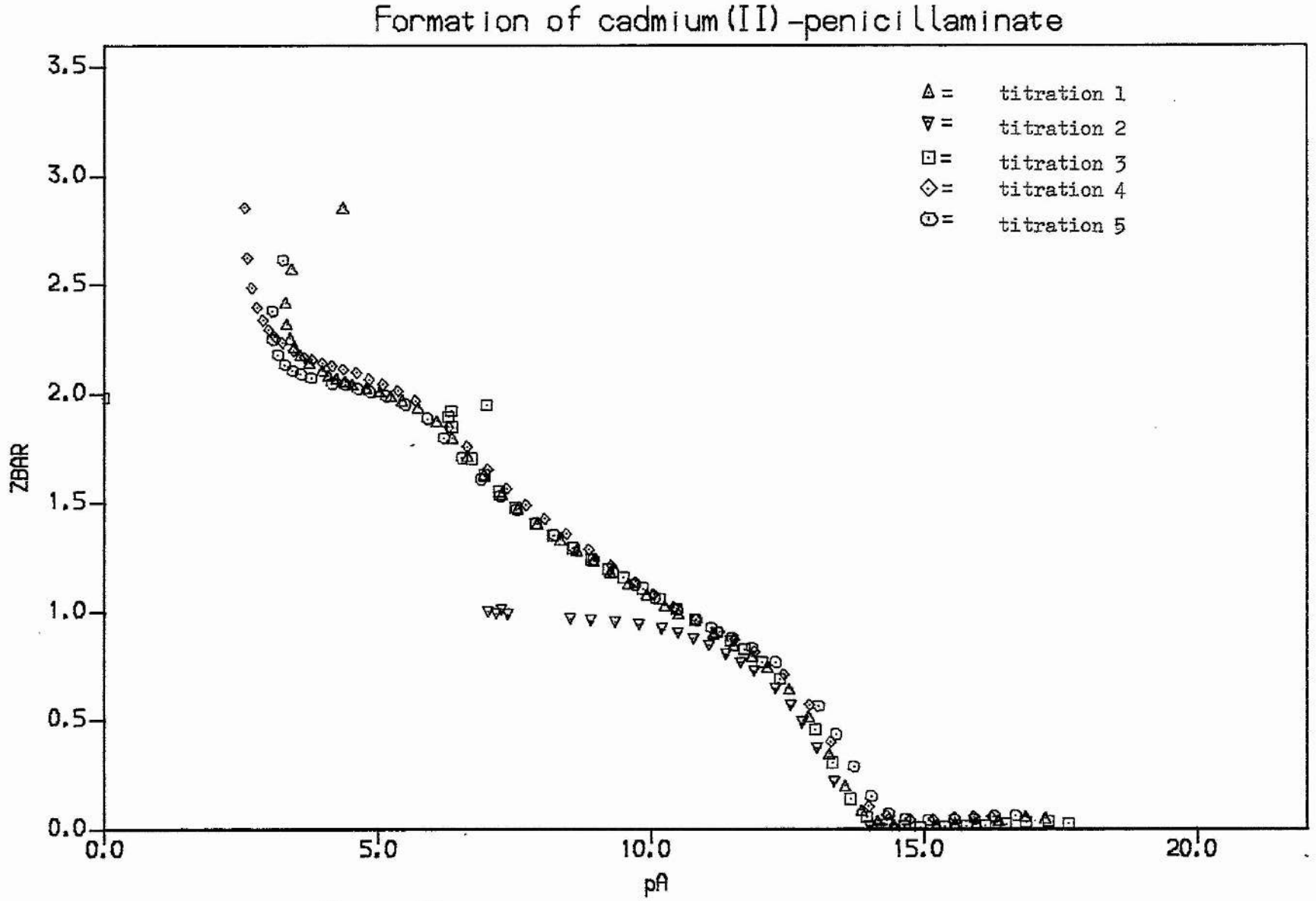

Formation of cadmium(II)-penicillaminate
△ = titration 1
▽ = titration 2
□ = titration 3
◇ = titration 4
○ = titration 5
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
pA

Figure 30

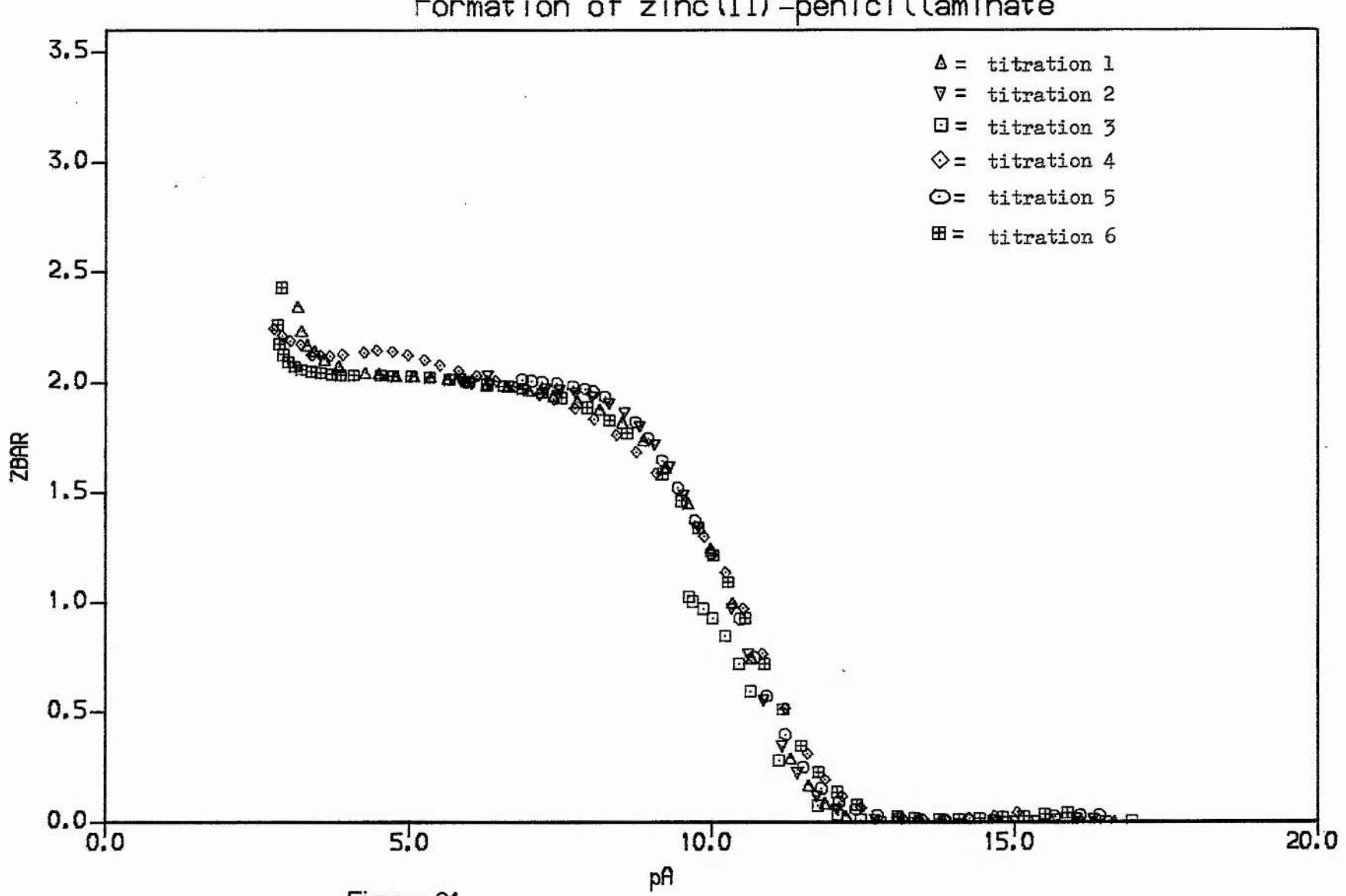

Formation of zinc(II)-penicillaminate
Δ = titration 1
∇ = titration 2
⊡ = titration 3
◇ = titration 4
⊙ = titration 5
⊞ = titration 6
ZBAR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
pA

Figure 31

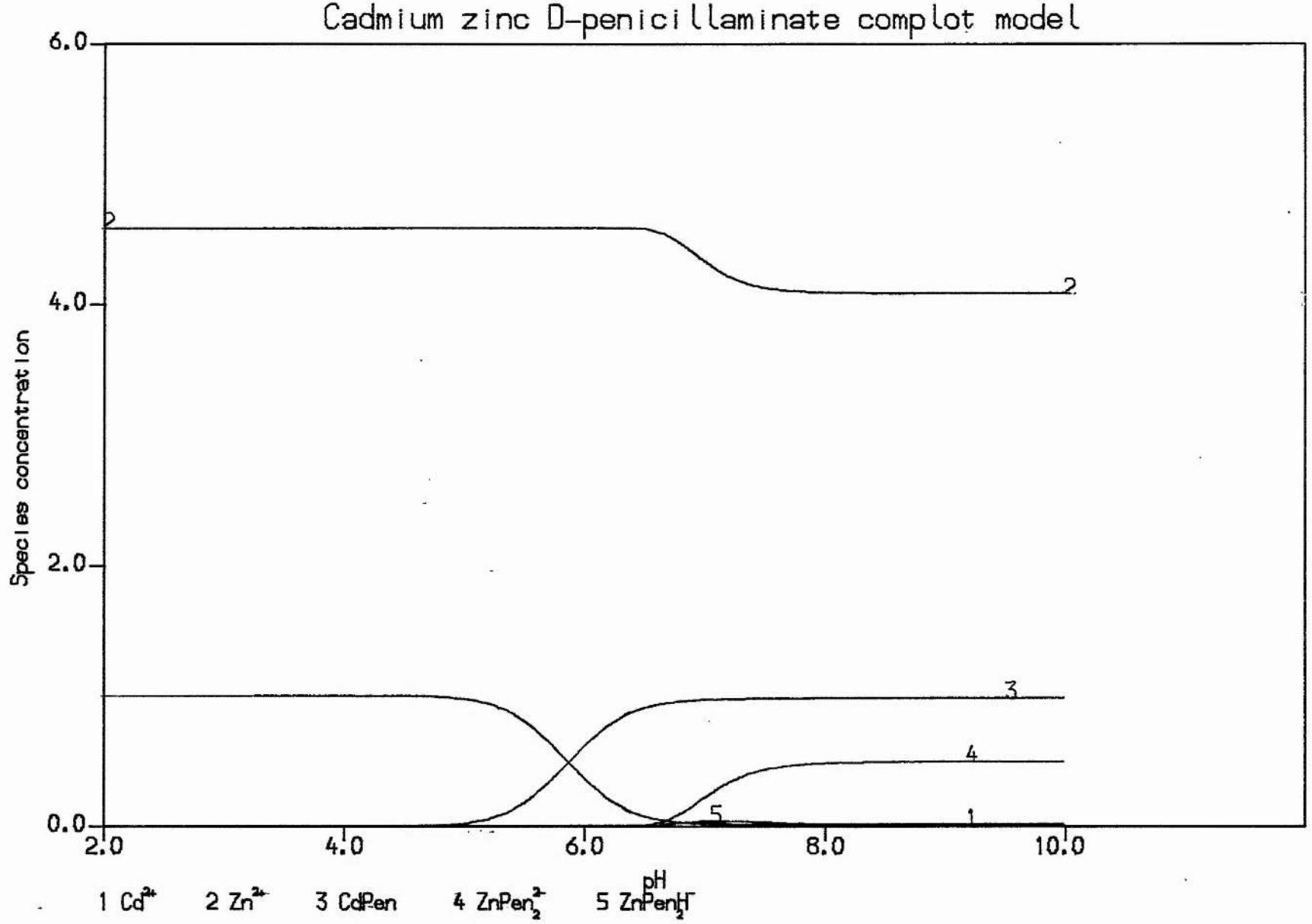


Figure 32 Total Cd(II) = 10.00, Zn(II) = 45.88, D-penicillaminate = 20.00 μM.

CADMIUM AND ZINC PENICILLAMINATE

Similarly, the cadmium penicillaminate system exhibits a series of non-superimposable formation curves (see figure 30) and once again a set of constants was refined which included a number of protonated and hydroxy species - these are listed in table 23.

This set of constants included a $\log\beta$ value for the 110 complex. The zinc system, for which the formation curves are shown in figure 31, exhibited a similar pattern but in this case a significantly improved value of the sum of squares was obtained by discarding the 110 complex. This was included in the set reported by Perrin and Sayce (95), which also differs in that these authors did not report constants for the 21-1, 430 and 43-1 species.

Structurally it is likely that the metals bind *via* the sulphydryl, amino and carboxyl groups. COMPLOT studies show that D-penicillaminate is an extremely efficient sequestering agent for cadmium with respect to zinc. This is shown in figure 32 where it can be seen that nearly 100% of the cadmium present is complexed at pH7.4 where $[Cd^{2+}] = 10\ \mu M$, $[Zn^{2+}] = 20\mu M$ and $[\text{glutathionate}^{3-}] = 45.88\mu M$. However, clinical studies have shown that this ligand does not promote the urinary excretion of this metal (30,105) and can in fact increase its toxicity, perhaps by the stable complex accumulating in the kidney.

It is difficult to compare this work with that of other workers, except Perrin and Sayce, due to the fact that only values for $\log\beta_{110}$ or at most $\log\beta_{110}$ and $\log\beta_{210}$ are reported (93, 94, 106).

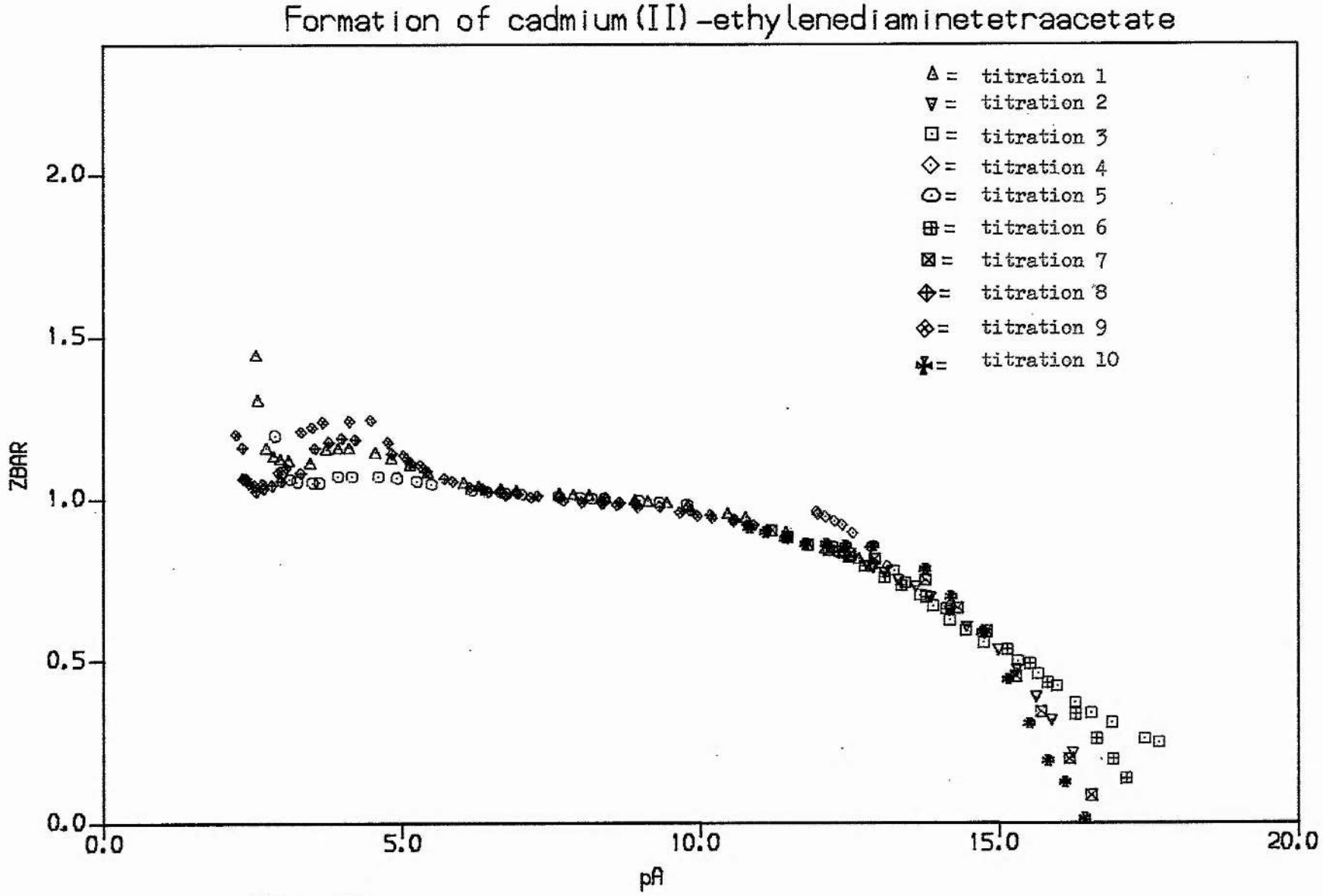

Formation of cadmium(II)-ethylenediaminetetraacetate
titration 1
titration 2
titration 3
titration 4
titration 5
titration 6
titration 7
titration 8
titration 9
titration 10
ZBAR
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
pA

Figure 33

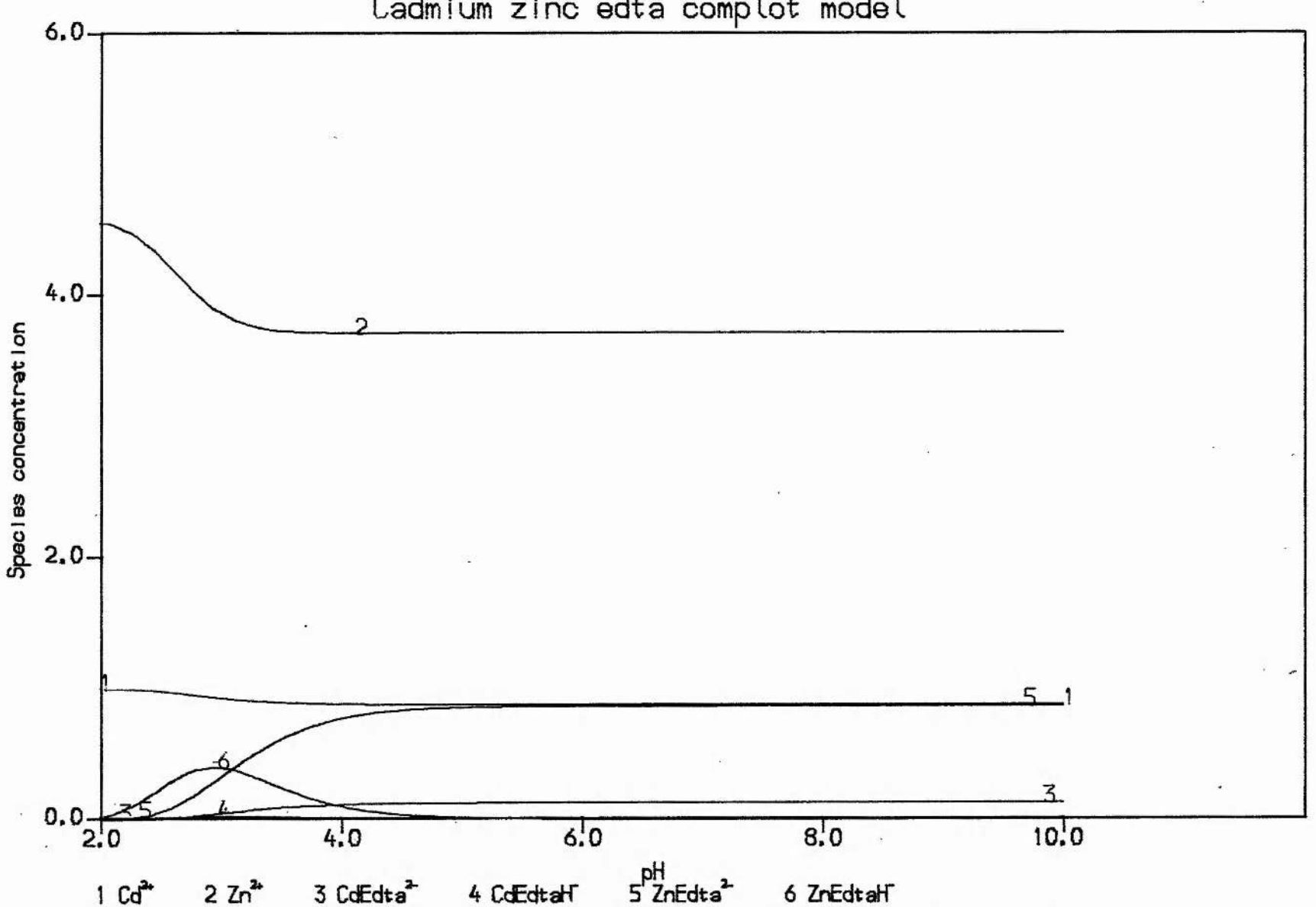


Figure 34 Total Cd(II) = 10.00, Zn(II) = 45.88, EDTA = 10.00 μM

CADMIUM ETHYLENEDIAMINETETRAACETATE

The formation curves for this sytem exhibit non-superimposability (see figure 33) and so the presence of protonated species was suspected. In SCOGS a 110 and a 111 complex were refined which gave a standard deviation in titre of 0.2863. A 120 complex converged and reduced the standard deviation to 0.2647 but gave no significant improvement in the PSEUDOPLOT fit. Previous studies have only reported the 110 and 111 (101) and the significance of the 120 complex is doubtful. Other protonated and polynuclear species were attempted and none would converge although the poor fit of the PSEUDOPLOT curves with the experimental formation curves would indicate the presence of other species.

The constants obtained are lower than those obtained by previous workers (101-103, 107), but comparing the cadmium figures reported here with those obtained by Corrie for zinc (104), where $\log \beta_{110} = 14.873$ and $\log \beta_{111} = 17.965$, which show a corresponding difference, this could be explained by the different working conditions.

EDTA is used as a cadmium sequestering agent *in vivo* but as may be seen from the COMPLOT model shown in figure 34, only about 35% of the cadmium is complexed, with, perhaps even more important, 36% of the total zinc being complexed also.

CADMIUM AND ZINC 1,2-DI(2-AMINOETHOXY) ETHANETETRAACETATES

These systems are similar to that of cadmium ethylenediaminetetraacetate. For the zinc EGTA system, the formation curves of which are shown in figure 36, it was possible to refine together in SCOGS constants for the 110, 111 and 112 complexes which give a standard deviation in titre of 0.1872 compared to 0.2004 when the 112 was excluded. However in MINIQUAD it was only possible to refine together the 110 and 111. As the 112 gave no improvement in the PSEUDOPLOT fit it was discarded.

Similarly for the cadmium system, (see figure 35), constants for the

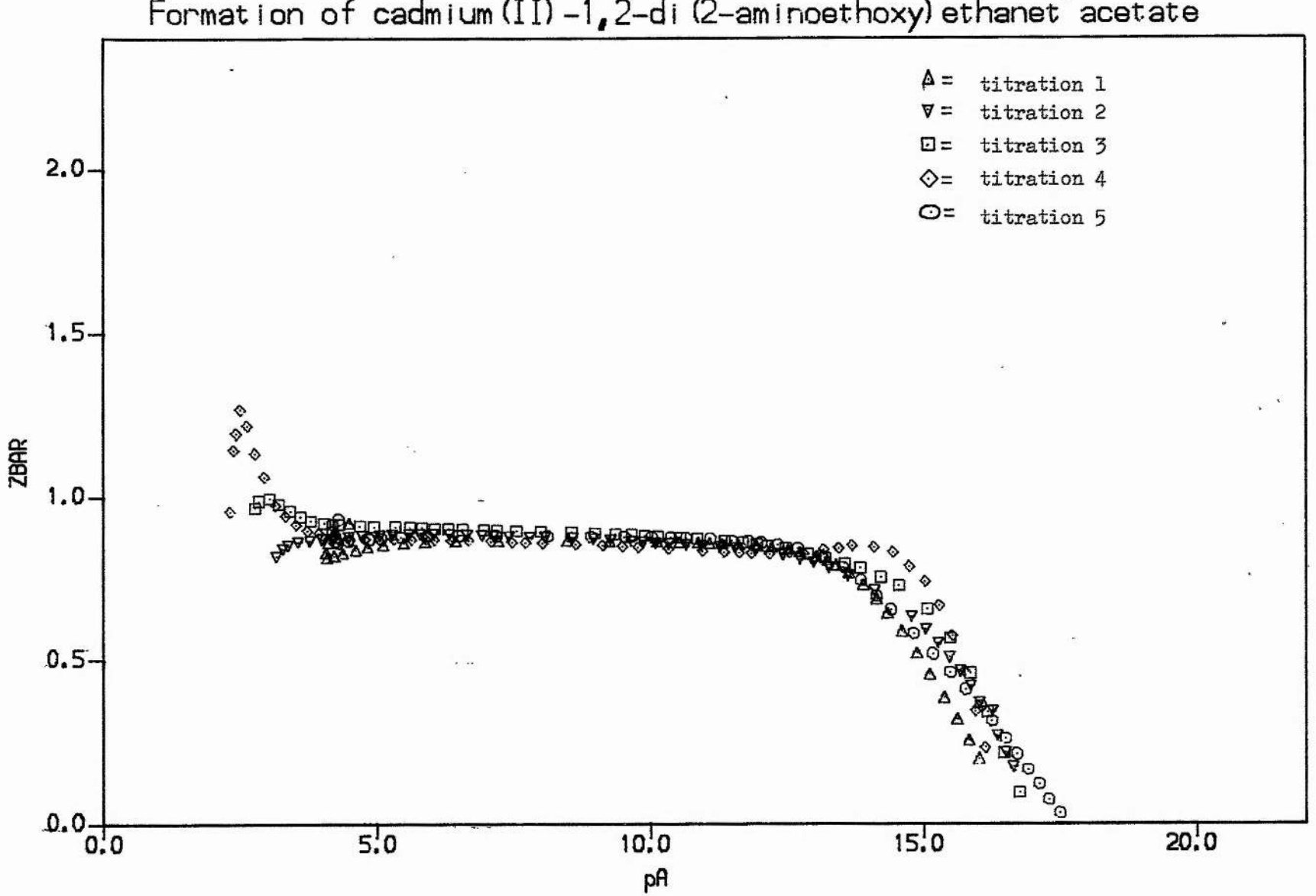

Formation of cadmium(II)-1,2-di(2-aminoethoxy)ethanet' acetate
titration 1
titration 2
titration 3
titration 4
titration 5
ZBAR
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
pA

Figure 35

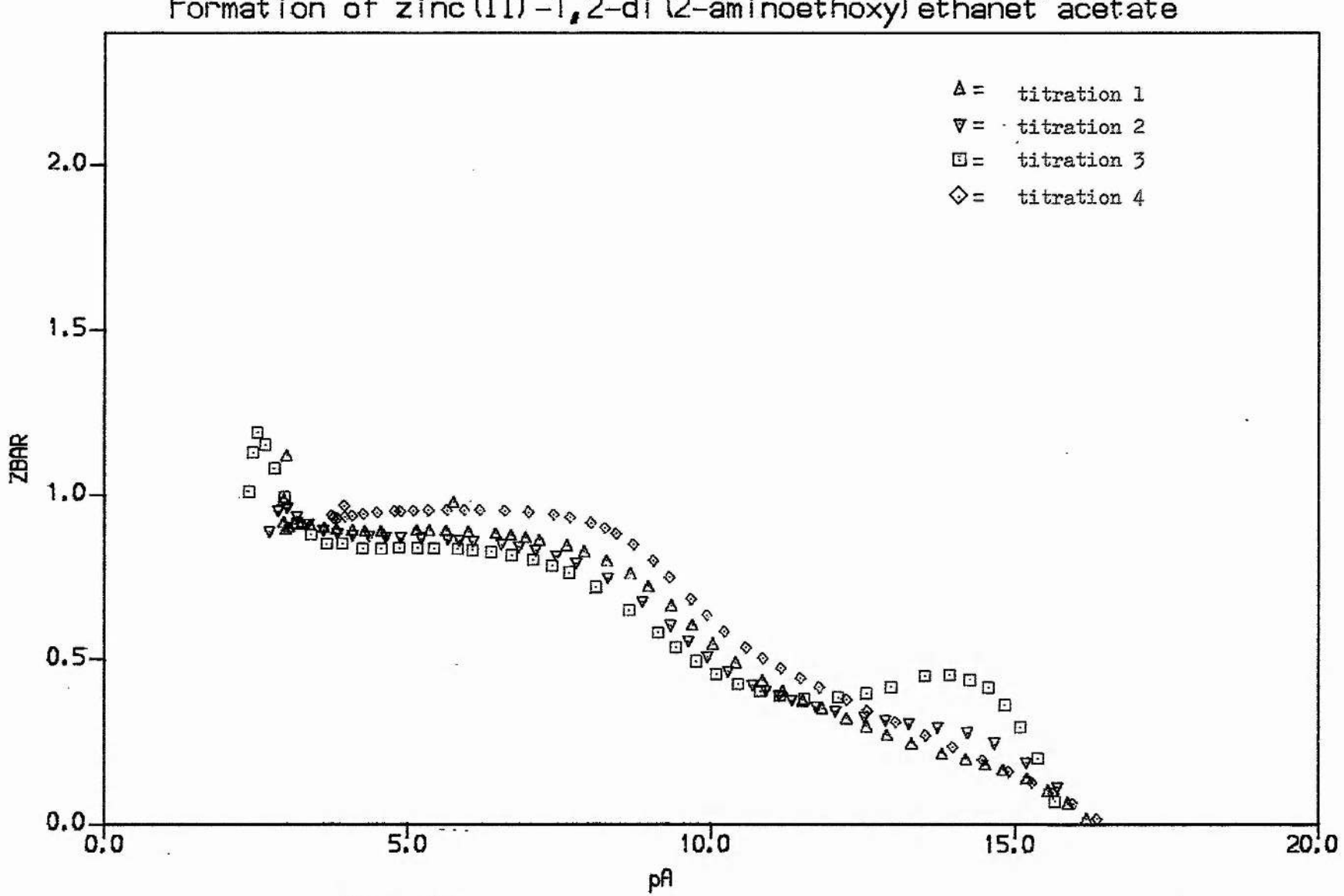

Formation of zinc(II)-1,2-di(2-aminoethoxy)ethanet' acetate
Δ = titration 1
▽ = titration 2
⊡ = titration 3
◇ = titration 4
ZBAR
2.0
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
pA

Figure 36

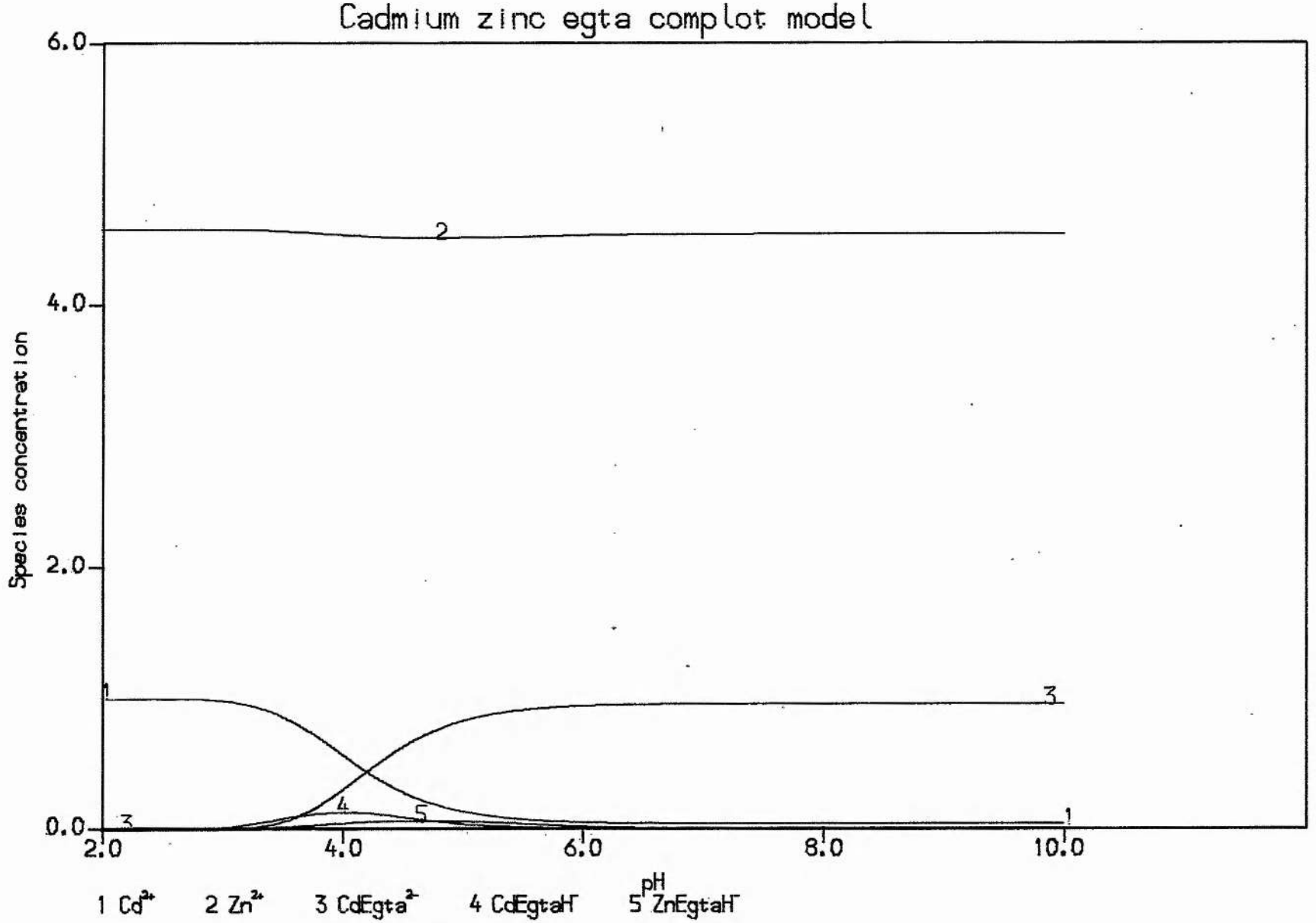


Figure 37 Total Cd(II) = 10.00, Zn(II) = 45.88, EGTA = 10.00 μM.

110 and 111 complexes could be refined using SCOGS but in MINIQUAD only the log β_{110} constant would converge.

As in the case of EDTA, the PSEUDOPLOTS of these systems did not provide a good fit for the formation curves but no other species could be accounted for.

Results reported previously for cadmium show log β_{110} (6.1(108), 16.73(109) and 16.7(110,111)), however these were all carried out under different experimental conditions.

As can be seen from table 23, the constants for the cadmium system are approximately 3.6 log units higher than those for zinc so it is not surprising that 100% of the cadmium is complexed as is shown in the COMPLOT model in figure 37.

This is unexpected as this ligand is a nitrogen oxygen donor and as such would be expected to prefer the harder metal ion zinc. This may be due to the size differences of the two metal ions with the larger ion being complexed more successfully by the large ligand.

SOLID STATE ELECTRODE POTENTIOMETRY

PART II
CALIBRATION

This electrode was calibrated with reference to a saturated sodium chloride calomel electrode, the emf, E_{Cd} being given by

$$E_{Cd} = E^{o}_{Cd} - 29.586 \log b \qquad mV$$

where $b = [Cd^{2+}]$

Thus, measurement of E_{Cd} for a series of solutions of varying b ought to produce a straight line of intercept $E_{Cd} = E^{o}_{Cd}$ at log b = 0 and slope= 29.586 mV$(\log b)^{-1}$ from a plot of E_{Cd} versus log b. Practically, this is only reliable for solutions containing $b = 10^{-1}$-10^{-3}M, and as with glass electrode calibration, problems arise in the range of concentrations encountered in the cadmium-amino-acid complexing studies due to the absence of any buffering.

In order to overcome this problem a series of standard solutions were prepared involving a system of metal buffers (112). This system involves using an excess of a second metal ion which complexes less strongly with the ligand in solution than does the metal ion under consideration, which results in the free metal concentration being held constant over a range of pH.

This is only achieved if the 110 is the only complex formed and because of this the ligand used was EDTA and the second metal, lanthanum $^{3+}$.

It must be said that this work was carried out before the protonation and cadmium constants for EDTA were calculated and this would slightly alter the results reported here. The theory of this buffer system is as follows: if $\beta_{Cd\text{-}EDTA} > \beta_{La\text{-}EDTA}$ and $T_{Cd} > T_{EDTA} > T_{La}$ (where T is the total initial concentration) it can be seen that

$$[Cd\text{-}EDTA^{2-}] \cong T_{Cd^{2+}} \quad \text{and} \quad [La^{3+}] \cong T_{La} - (T_{EDTA} - T_{Cd}).$$

$$\beta_{Cd\text{-}EDTA} = \frac{[Cd\text{-}EDTA^{2-}]}{[Cd^{2+}]\,[EDTA^{4-}]} \quad \text{and} \quad \beta_{LaEDTA} = \frac{[La\text{-}EDTA^{-}]}{[La^{3+}]\,[EDTA^{4-}]}$$

As $[La\text{-}EDTA^{-}] \cong T_{EDTA} - T_{Cd}$ it can be seen that

$$\beta_{La\text{-}EDTA} = \frac{T_{EDTA} - T_{Cd}}{(T_{La} - T_{EDTA} + T_{Cd})\ [EDTA^{4-}]}$$

$$\text{Thus } EDTA^{4-} = \frac{T_{EDTA} - T_{Cd}}{\beta_{La\text{-}EDTA}\ (T_{La} - T_{EDTA} + T_{Cd})}$$

so that the free ligand concentration can be defined in terms that are independent of pH.
Furthermore,

$$[Cd^{2+}] = \frac{[Cd\text{-}EDTA^{2-}]}{\beta_{Cd\text{-}EDTA}\ [EDTA^{4-}]} \cong \frac{T_{Cd}}{\beta_{Cd\text{-}EDTA}\ [EDTA^{4-}]}$$

which is also pH independent.

This approach is capable of providing pH independent buffered standard b solutions down to 10^{-7}M which is the limiting sensitivity of the electrode.

The assumptions involved and the effect of metal hydrolysis were all proved negligible from COMPLOT computations of b and -log h. From this it was found that a similar pH independent free metal ion concentration could be obtained by having Cd^{2+} and EDTA alone with the metal in excess. This, presumably, can be accounted for by the stability of the 110 cadmium EDTA complex as can be seen from the formation curves in figure 33.

Experimental

TABLE 24

Experimental results for the calibration of the solid state ion-selective electrode.

Calibration 1 (Buffered)

T_{Cd} = 1.476mM	T_{La} = 2.936mM	T_{EDTA} = 2.235mM	
pCd = 3.66			
E_{Cd} (mV)	Eg (mV)	E_{Cd}(mV)	Eg(mV)
51.4	316.0	31.1	86.6
45.5	285.1	30.3	59.1
44.5	263.6	30.1	48.1
41.5	234.1	29.7	39.6
39.8	188.0	29.3	28.4
37.6	161.5	16.0	28.6
33.4	138.4		

Calibration 2 (buffered)

T_{Cd} = 0.492mM	T_{La} = 0.979mM	T_{EDTA} = 0.745mM	
pCd = 4.14			
E_{Cd}(mV)	Eg (mV)	E_{Cd}(mV)	Eg(mV)
16.9	279.0	10.9	84.3
12.8	207.2	10.8	74.8
12.1	172.0	10.6	61.0
11.7	150.3	10.2	36.8
11.5	131.5	10.3	28.6
11.2	107.1		

TABLE 24 cont'd

Calibration 3 (buffered)

T_{Cd} = 0.148mM		T_{La} = 0.294 mM	T_{EDTA} = 0.223mM
pCd = 4.66			
E_{Cd}(mV)	E_g(mV)	E_{Cd}(mV)	E_g(mV)
19.6	290.4	11.1	86.2
14.1	268.5	9.9	76.2
11.5	243.5	9.4	63.2
11.0	223.8	8.2	42.4
17.7	197.6	7.3	26.0
14.1	144.0	6.3	9.2
12.7	117.2	4.3	-18.9
11.3	106.2	1.5	-31.8

Calibration 4 (buffered)

T_{Cd} = 4.92×10^{-5}M		T_{La} = 9.79×10^{-5}M	T_{EDTA} = 7.45×10^{-5}M
pCd = 5.13			
E_{Cd}(mV)	E_g(mV)	E_{Cd}(mV)	E_g(mV)
0.9	286.8	-19.0	100.2
-11.0	233.6	-19.5	63.5
-13.7	211.8	-20.4	48.2
-15.4	186.8	-21.8	25.5
-16.8	164.1	-22.7	20.0
-17.9	132.2	-24.6	6.0

TABLE 24 cont'd

Calibration 5 (buffered)

$T_{Cd} = 4.92 \times 10^{-7}M$	$T_{La} = 9.79 \times 10^{-7}M$	$T_{EDTA} = 7.45 \times 10^{-7}M$	
pCd = 7.14			
E_{Cd}(mV)	E_g(mV)	E_{Cd}(mV)	E_g(mV)
-8.4	263.2	-26.9	115.5
-13.8	248.9	-27.6	99.6
-17.7	234.0	-28.1	86.6
-20.8	206.2	-28.7	72.1
-22.7	173.2	-29.4	61.6
-23.8	157.0	-28.1	52.2
-25.2	139.2	-28.2	45.8

Calibration 6 (unbuffered)

$T_{Cd} = 9.845$mM		$T_{EDTA} = 7.455$mM	
pCd = 2.6			
E_{Cd}(mV)	E_g(mV)	E_{Cd}(mV)	E_g(mV)
93.5	343.0	77.4	125.7
87.0	307.8	77.1	96.2
84.5	291.0	75.2	73.2
81.6	269.1	75.2	58.9
79.0	243.8	74.8	45.8
78.8	215.6	75.0	27.4
77.8	168.8	75.4	23.4
77.8	147.9	74.6	15.0

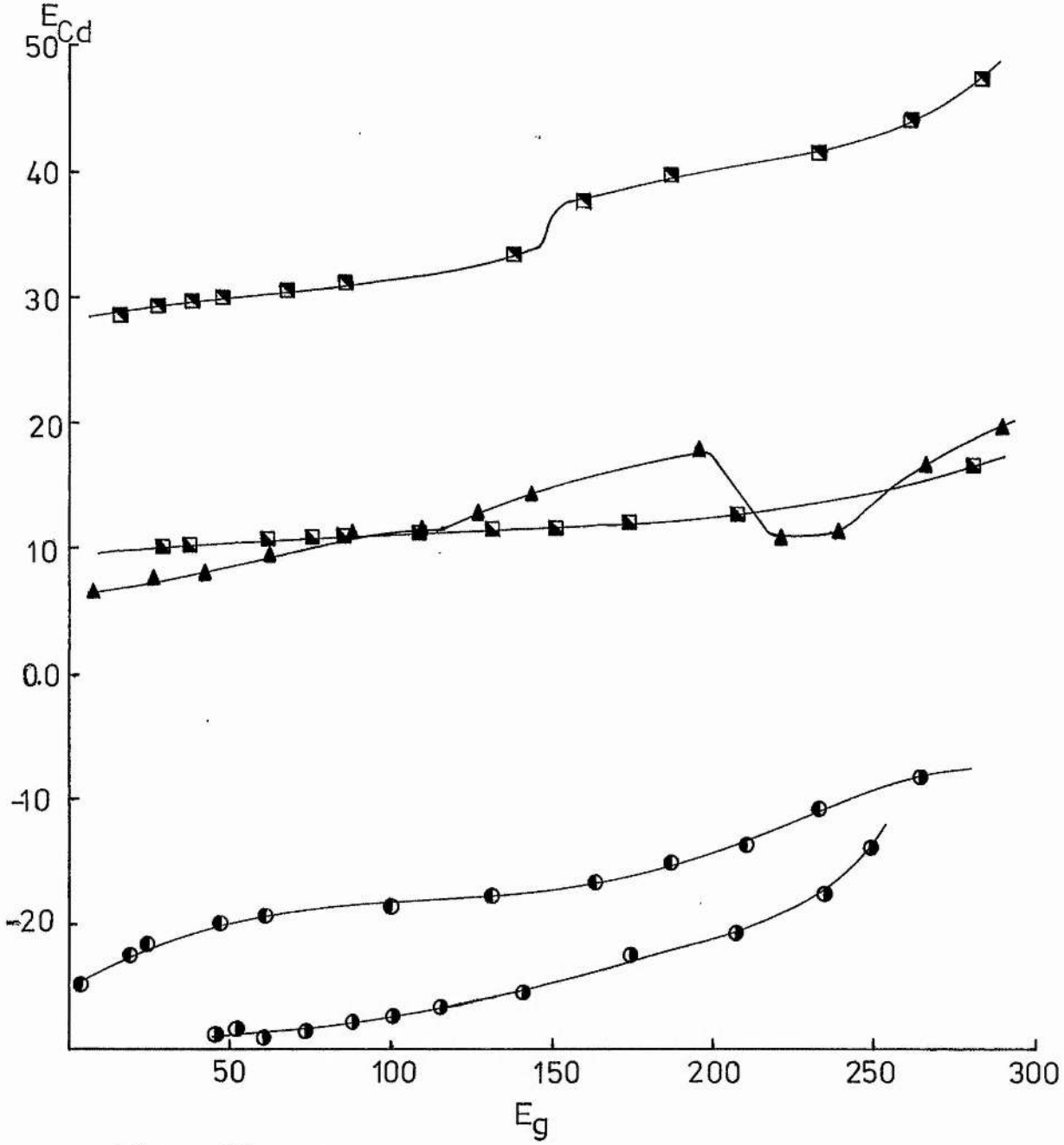


Figure 38 Calibration curves for the solid state electrode (buffered systems)

◪ = calibration 1, ◪ = calibration 2, ▲ = calibration 3,

◐ = calibration 4, ◑ = calibration 5.

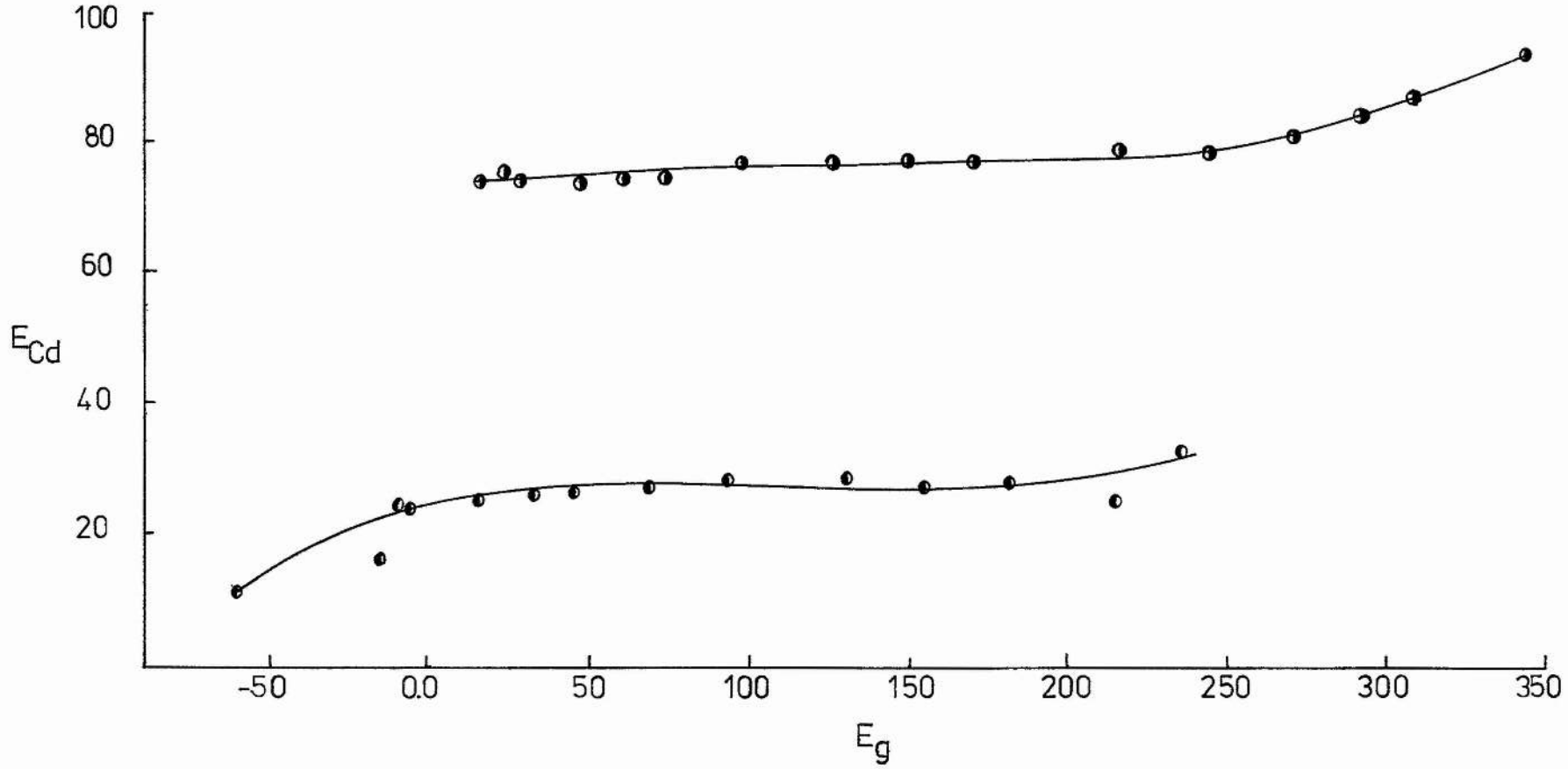


Figure 39 Calibration curves for the solid state electrode (unbuffered system)

◑ = calibration 6, ◐ = calibration 7.

TABLE 24 cont'd

Calibration 7 (unbuffered)

$T_{Cd} = 9.845 \times 10^{-5}M$		$T_{EDTA} = 7.455 \times 10^{-5}M$	
pCd = 4.6			
E_{Cd}(mV)	E_g(mV)	E_{Cd}(mV)	E_g(mV)
33.0	234.8	27.0	31.6
25.3	214.8	26.8	13.9
28.4	181.1	25.4	-7.1
27.5	154.4	24.4	-10.2
29.2	130.0	25.1	-16.4
28.5	92.7	16.3	-62.0

From the calibration curves which are shown in figures 38 and 39, it can be seen that there is a region over which the free metal ion concentration (in terms of E_{cd}) changes little with respect to pH (in terms of Eg). However, this is more apparent in the cadmium - EDTA systems (figure 39) than in those containing lanthanum.

From these curves values of pCd were taken at Eg = 100 mV (approx.pH6) which lies in the middle of this region. From these a number of lines can be drawn as is shown in figure 40. Of the values obtained only two, -23.7 and-29.8 mV/pCD seemed reasonable, but the inability to obtain consistent results was disturbing.

In an effort to check the accuracy of the electrode, two systems which had been studied using glass electrode potentiometry were studied. This was carried out by keeping the pH and the total cadmium concentration constant and changing the total ligand concentration. The titration set up was similar to that used previously, with the pH being kept constant by the addition of acid and alkali from an "Agla" syringe, the pH being monitored by a glass electrode.

The two systems studied were the serinate and asparaginate. From the titration data shown in tables 25 and 26 it is possible to obtain log $\frac{B}{b}$

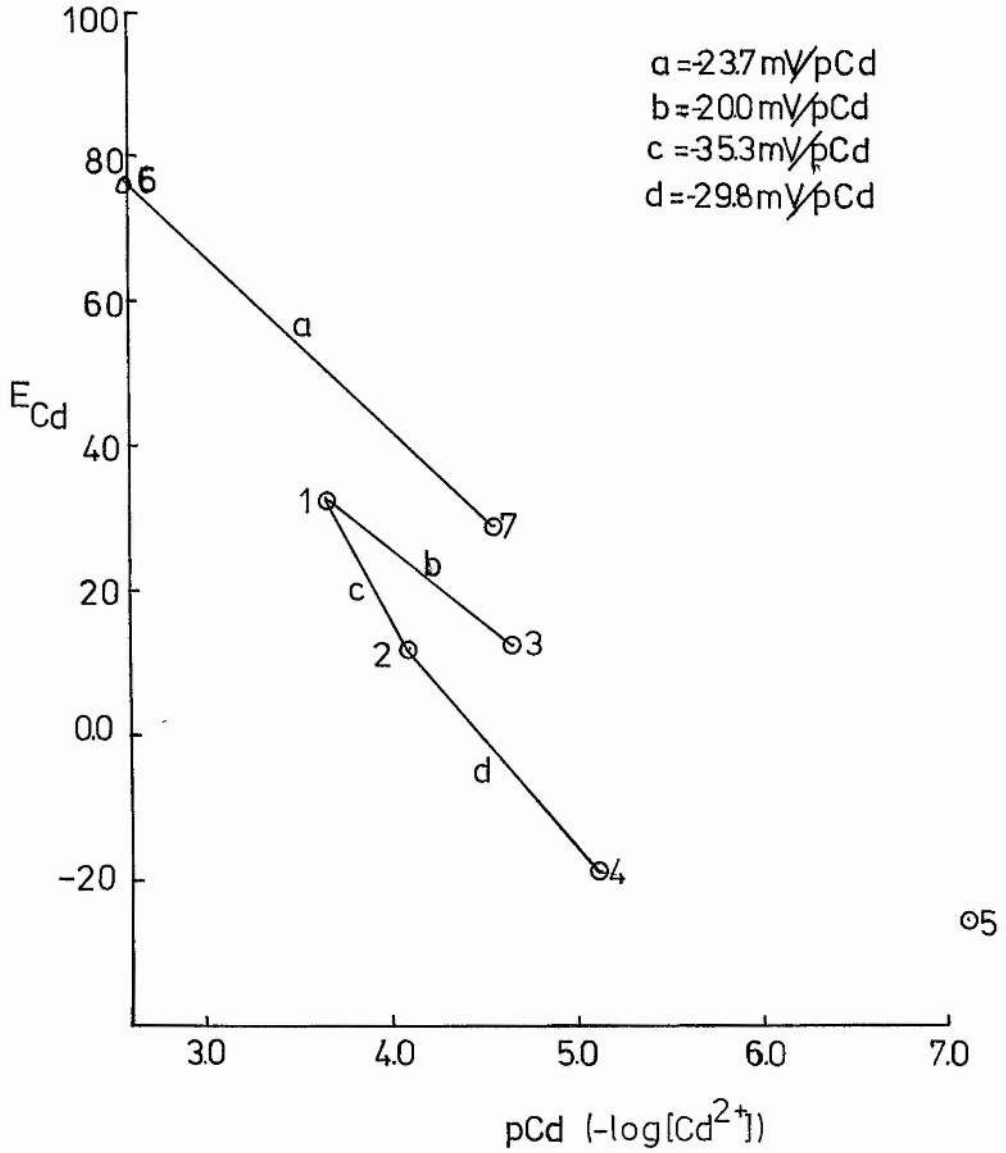


Figure 40 The various constants calculated from the calibration curves (figures 38 and 39) at Eg = 100mV (approximately pH

and log A values. These values were calculated using both the constants mentioned above not forgetting the consequent difference in E^{o}_{Cd}.

TABLE 25

Experimental data for the formation of cadmium (II) - asparaginate at pH8.

Titrate mM		Titrant mM	Vol (ml.)
B	A	B	
9.852	70.50	9.852	20.01

Titre ml(of Cd^{2+})	total volume (titre + acid)	e.m.f. mV
3.0	4.11	39.9
6.0	7.21	44.0
8.0	9.283	44.4
10.0	11.373	44.4
13.0	14.446	48.3
17.0	18.576	49.4
20.0	21.666	51.5
24.0	25.756	55.8
28.0	29.846	58.0
30.0	31.896	58.8
34.0	35.986	60.6
38.0	40.076	62.3

TABLE 26

Experimental data for the formation of cadmium(II) serinate at pH8

Titrate (mM)		Titrant (mM)	Volume ml
B	A	B	
9.852	72.39	9.852	20.01

Titre ml	total titre ml	e.m.f. mV
3.0	3.78	44.2
6.0	6.86	47.0
10.0	10.97	50.9
13.0	14.03	54.4
16.0	17.09	57.3
20.0	21.17	59.6
23.0	24.21	62.2
25.0	26.25	63.6
28.0	29.29	65.7

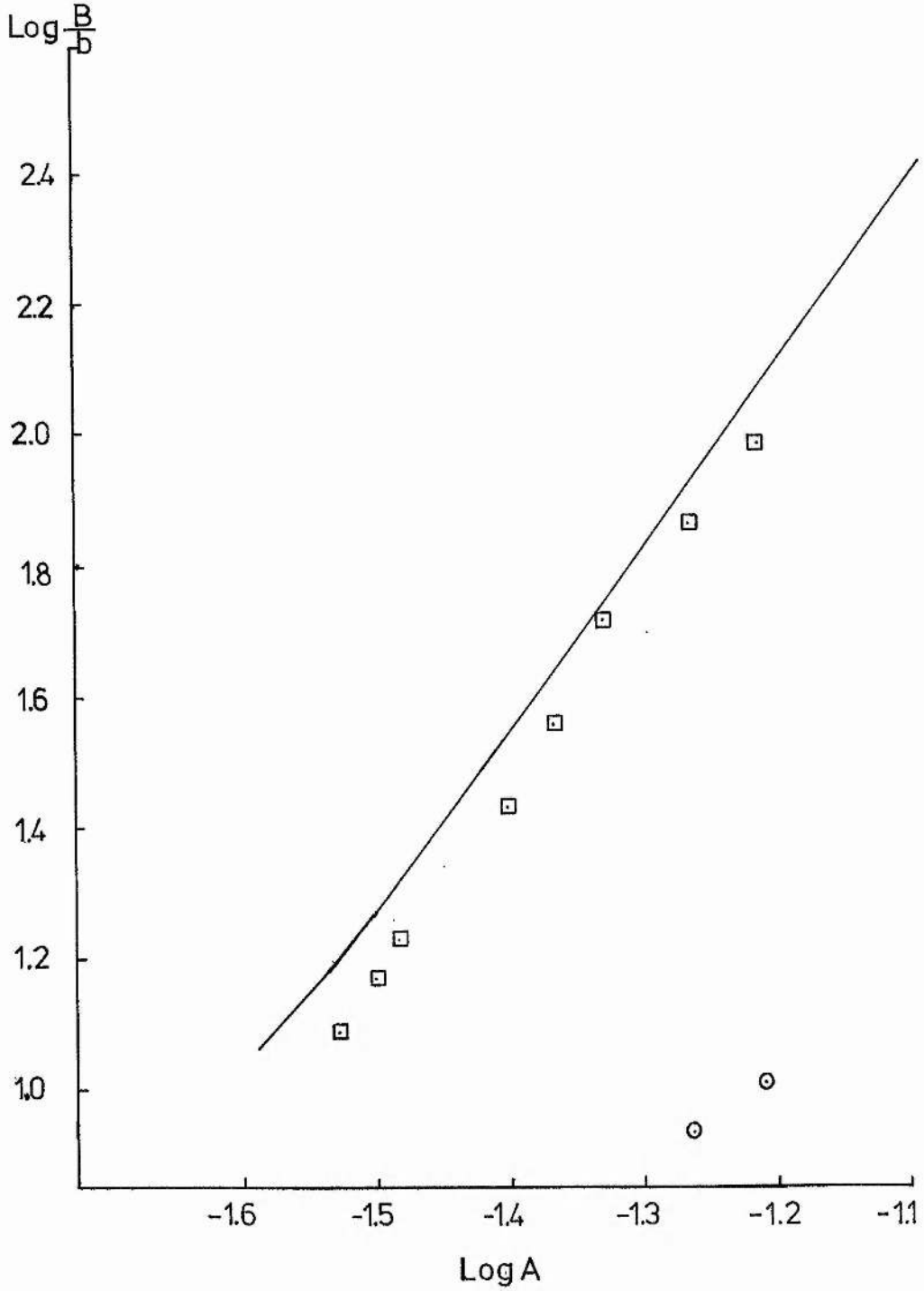


Figure 41 The cadmium serinate experimental points showing the relative position of the HALTAFALL simulated log B/b against log A curve. ——— = HALTAFALL simulation
⊡ =-23.7 mV/pCd, ⊙ = -29.8mV/pCd.

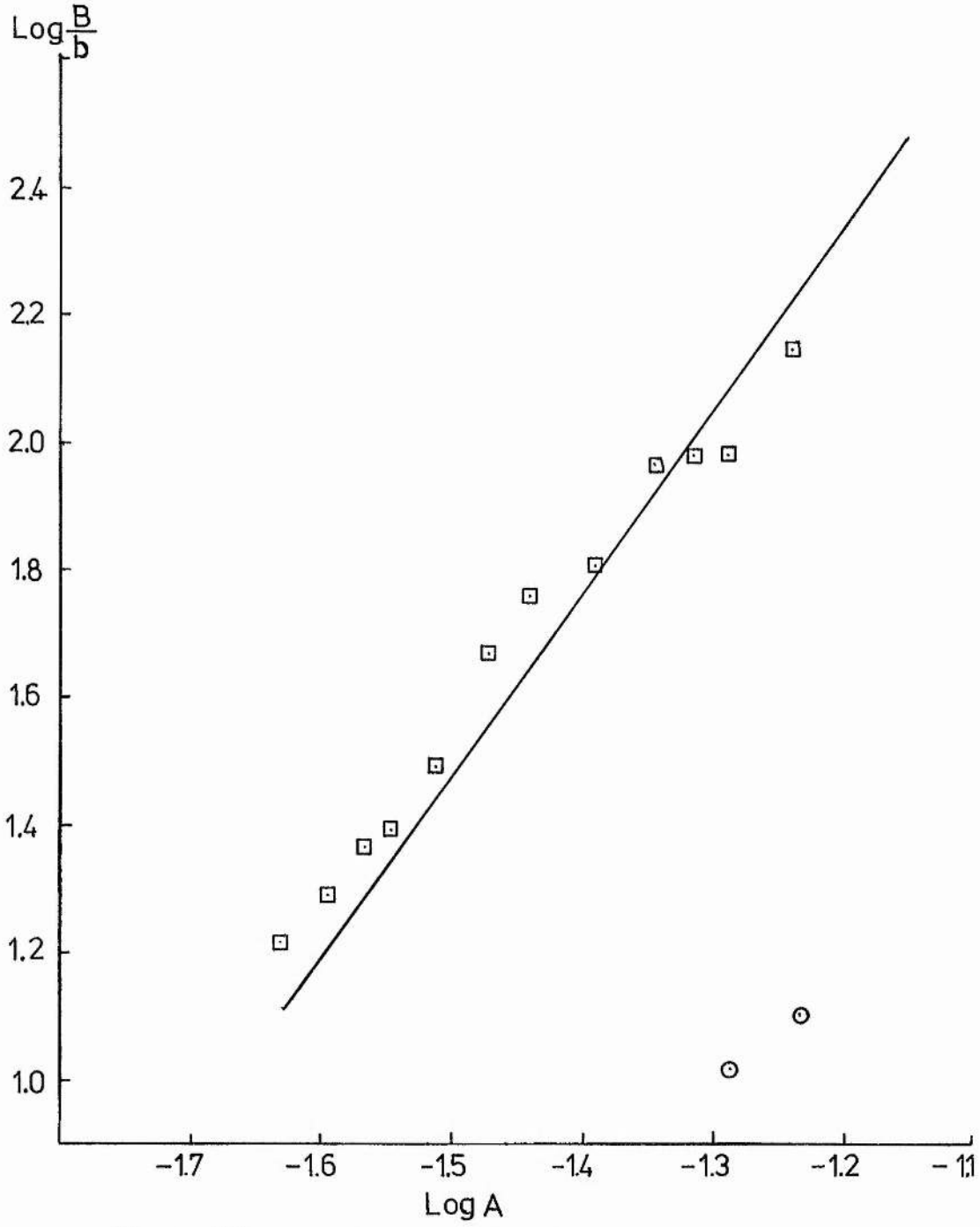


Figure 42 The Cadmium asparaginate experimental points showing the relative position of the HALTAFALL simulated log B/b against log A curve. ——— = HALTAFALL simulation. ⊡ =-23.7 mV/pCD, ⊙ = -29.8 mV/pCd.

Similar log B/b and log A data was simulated in HALTAFALL using the previously calculated formation constants from the glass electrode work and this was then used as a standard by which to evaluate the results from the solid state electrode.

As can be seen in figures 41 and 42 the value of-23.7 mv/pCd gives much better correlation with the HALTAFALL simulation than does the value of-29.8 mV/pCd. This is surprising as the latter value is much nearer the theoretical value of 29.586.

Because of this and the difficulty in obtaining consistent and reproducible results the glass electrode is felt to be still the more reliable of the two electrodes.

CHAPTER VI

OSCILLATING REACTIONS

OSCILLATING REACTIONS

An initial study was carried out to determine the effect, if any, of changing the initial concentrations of the reactants involved in the Belousov-Zhabotinsky reaction with reference to a standard state. The effect looked for being changes in amplitude, oscillation period and period of initiation.

The standard state contained malonic acid (0.4M), potassium bromate (0.10M), sulphuric acid (0.2M) and ferroin (10^{-4}M). The results of this study are shown in table 27.

TABLE 27

Qualitative observations on the effects of reactants on the amplitude and period of the standard reaction. s= standard concentration as in text, + = increase, - = decrease.

$CH_2(COOH)_2$	$KBrO_3$	H_2SO_4	Ferroin	Amplitude	Oscillating Period	Initiation Period
-	S	S	S	0	0	0
+	S	S	S	-	+?	0
S	-	S	S	+	++	+
S	+	S	S	0	-	0
S	S	-	S	+	++	+
S	S	+	S	-	-	++
S	S	S	-	--	-	++
S	S	S	+	+	+	0
-	-	-	-	0	++	-
+	+	+	+	-	-	+

As an extension of this study, the effect of the introduction of different impurities into the system was investigated. These impurities embraced such groups as amino-acids, metal ions, drugs and poisons.

For the metal ions and amino-acids it was originally planned to work at an [iron] to [metal] or [amino-acid] ratio similar to that found *in vivo*. However, such low concentrations did not have any measurable effect on the system, so higher concentrations had to be used.

The results of this study are shown in table 28.

TABLE 28

Qualitative observations on the effect of adding certain impurities to the standard reaction.

	period	amplitude
ethanol	-	-
EDTA	-	-
phenyalanine	+	+
$MnSO_4$	++	++
soluble aspirin	+	0 and peak broadening
$ZnSO_4$	+	0
Aspartic acid	+	0
Malic acid	0	0

Further systematic studies were then undertaken with EDTA, ethanol and also with theobromine which has been reported as having an effect on the periodicity of leaf movements of phaseolus multiflorus (113, 114). It is interesting to note that this period lengthening observed in vivo is mimiked in the in vitro studies reported here.

TABLE 29

Percentage changes in amplitude and period caused by adding ethanol (to give a final % Vol/Vol concentration as listed) to the standard oscillating reaction.

% $[C_2H_5OH]$	Amplitude	Period
0.04	-13.5	-9.6
0.08	-24.0	-44.8
0.12	-34.0	-64.8
0.16	-40.7	-61.0
0.20	-53.9	-76.0
0.24	-70.4	-58.4
0.28	-95.5	-60.0

TABLE 30

Percentage changes in amplitude and period caused by the addition of EDTA to the standard oscillating reaction.

[EDTA]	Amplitude	Period
9.24×10^{-4}M	-13.0	-35.3
1.25×10^{-3}	-30.4	-58.9
1.56×10^{-3}	-21.7	-43.8
1.65×10^{-3}	-24.0	-37.5
2.88×10^{-3}	-34.7	-58.9
3.34×10^{-3}	-45.8	-56.0
3.61×10^{-3}	-34.7	-64.5
4.54×10^{-3}	-64.0	-58.9
5.19×10^{-3}	-76.0	-64.5
6.56×10^{-3}	-96.0	-70.6
8.55×10^{-3}	-91.3	-76.5

TABLE 31

Percentage changes in amplitude and period caused by the addition of theobromine to the standard oscillating reaction

[Theobromine]	Amplitude	Period
1.11×10^{-4}M	-2.7	+11.1
2.48×10^{-4}	-10.7	+19.1
3.55×10^{-4}	-15.8	+25.9
5.71×10^{-4}	-47.2	+38.5
6.66×10^{-4}	-68.4	+48.1

There have been many suggestions put forward as to why oscillations exist in nature, but the complexity of biochemical control circuits and feedback mechanisms *in vivo* do make them almost inevitable. However, in this thesis the reason for their existence is of little concern, but the fact that they do exist and that they are of great importance in the medical field is of more interest.

It is important to recognise that such rhythms can account for increases in drug tolerance and response *in vivo* and that, in extremes, this can mean the difference between failure and success of a therapy.

The conditions used in the above experiments are, it must be said, physiologically 'freakish' but further research is needed for the development of more acceptable models, especially in the field of the trace metals as this should facilitate further work in the pharmacological field.

CHAPTER VII

PARTITION STUDIES OF THE CADMIUM (II)-GLUTATHIONATE SYSTEM

PARTITION STUDIES OF THE CADMIUM (II)-GLUTATHIONATE SYSTEM

Earlier in this thesis it was proposed that glutathionate should be an effective therapeutical for the removal of cadmium ions *in vivo*. However for it to be really effective it must be able to remove the metal ions from cells and to facilitate this it is necessary for some of the complexed cadmium to be organic membrane soluble. In practice this usually means in the form of an uncharged complex, therefore, in order to determine the potential of a drug the partition coefficients between cell membranes and intracellular fluid or plasma should be determined.

As has been mentioned previously, this is impossible *in vivo*, so once again we must look to model systems.

In this work the octanol/water partition coefficient (K_D) for cadmium(II)-glutathionate was determined at 25°, I = 3.00M(Na^+)ClO_4^- in the aqueous phase, thus enabling the use of the previously determined stability constants.

In such a system the cadmium ions are distributed not only between a number of complexes but also between two phases. To facilitate the measurements two assumptions are made; (i) the amount of neutral ligand partitioning into octanol is small enough to be neglected and (ii) of the various cadmium complexes present in the aqueous phase only the neutral ABH (where A = glutathionate $^{3-}$. B = Cd^{2+} and H = proton) partitions into the octanol.

The first assumption is felt to be justified since COMPLOT studies have shown that for pure aqueous solutions of the concentrations of metal and ligand being used, no more than 0.033% of the total glutathionate would be in the neutral form at pH7. The second assumption is justified on the grounds of octanol having a lower dielectric constant than water and so being unable to solvate and dissolve charged species.

K_D(expt.), the experimentally measured distribution coefficient is

dependent upon the total ligand concentration and hence upon the free ligand concentration, a, in the aqueous phase.

Equations of the form

$$\log K_D = \log K_{D(expt.)} + f \log a$$

have been produced by Dyrssen et al (115) and Rossotti and Rossotti (116). Clearly

as $a \longrightarrow 0$ $\quad \log K_D = \log K_D$ (expt.)

K_D(expt) was calculated according to the following relationship:

$$K_D(expt) \cong \frac{[Cd]\ octanol}{[ABH]_{COMPLOT} - [Cd]\ octanol}$$

and from a plot of log K_D(expt) versus log a, log K_D is taken as log $K_D(expt)_{Max}$ where K_D is defined as

$$K_D = \frac{[ABH]\ octanol}{[ABH]\ aqueous}$$

EXPERIMENTAL

Seven solutions were prepared at pH7. The total cadmium concentration was held constant at 7.8822×10^{-5}M with the glutathionate concentration being varied as follows:

a) 7.883×10^{-5}M; b) 9.197×10^{-5}M; c) 1.1819×10^{-4}M;

d) 1.3788×10^{-4}M; e) 1.5766×10^{-5}M; f) 1.8394×10^{-4}M;

g) 1.9706×10^{-4}M.

Equal volumes of these solutions (5 ml) were shaken with equal volumes of sodium perchlorate saturated octanol for one hour. After the layers settled, the organic phase was analysed by atomic absorption spectrophotometry. This leads directly to the concentration of cadmium in p.p.m. taking into account the density of octanol = 0.827 gcm^{-3}. The results of this analysis are shown in table 32.

Figure 43 shows the plot of log K_D(expt) versus log a and from this it can be seen that log K_D = -1.4

This value was then used in HALTAFALL to determine

1) the percentage distribution of cadmium between the different complexes

TABLE 32

Result of the partitioning of the cadmium (II)-glutathionate system between water/1-octanol

	ppm Cd^{2+} in octan.	Moles l^{-1} Cd^{2+} in octanol	Moles l^{-1} ABH from COMPLOT	$K_D = \frac{ABH\ oct}{ABH\ aq}$	log K_D	A^{3-} Molarity	log a
a	0.123	9.048×10^{-7}	2.375×10^{-5}	3.5997×10^{-2}	-1.4023	1.283×10^{-10}	-9.8918
b	0.09	6.619×10^{-7}	2.499×10^{-5}	2.7160×10^{-2}	-1.5661	1.870×10^{-10}	-9.7282
c	0.076	5.587×10^{-7}	2.544×10^{-5}	2.2455×10^{-2}	-1.6486	3.360×10^{-10}	-9.4737
d	0.053	3.896×10^{-7}	2.489×10^{-5}	2.5902×10^{-2}	-1.7986	4.651×10^{-10}	-9.3324
e	0.033	2.420×10^{-7}	2.405×10^{-5}	1.0163×10^{-2}	-1.9931	6.034×10^{-10}	-9.2194
f	0.03	2.206×10^{-7}	2.280×10^{-5}	9.7690×10^{-3}	-2.0102	7.959×10^{-10}	-9.0991
g	0.01	7.358×10^{-8}	2.216×10^{-5}	3.3309×10^{-3}	-2.4775	8.948×10^{-10}	-9.0483

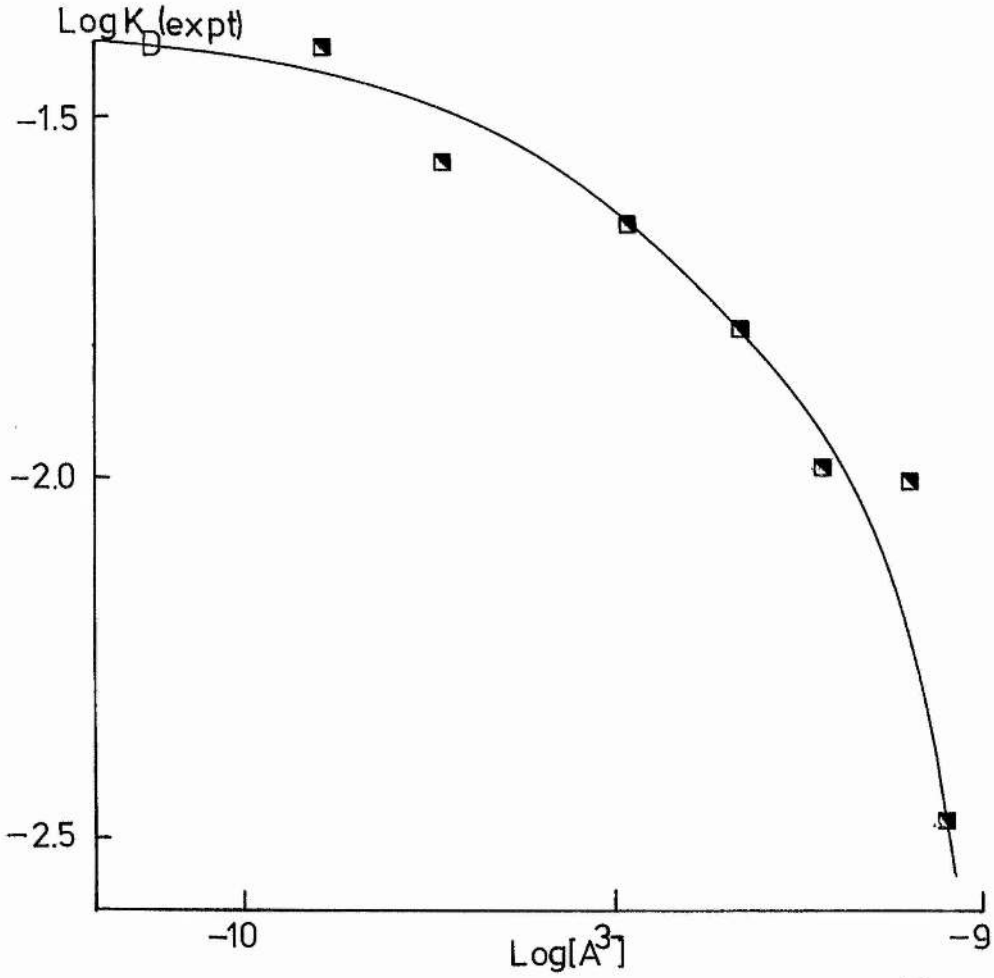


Figure 43 A plot of log K_D(expt.) against log [glutathionate^{3-}]

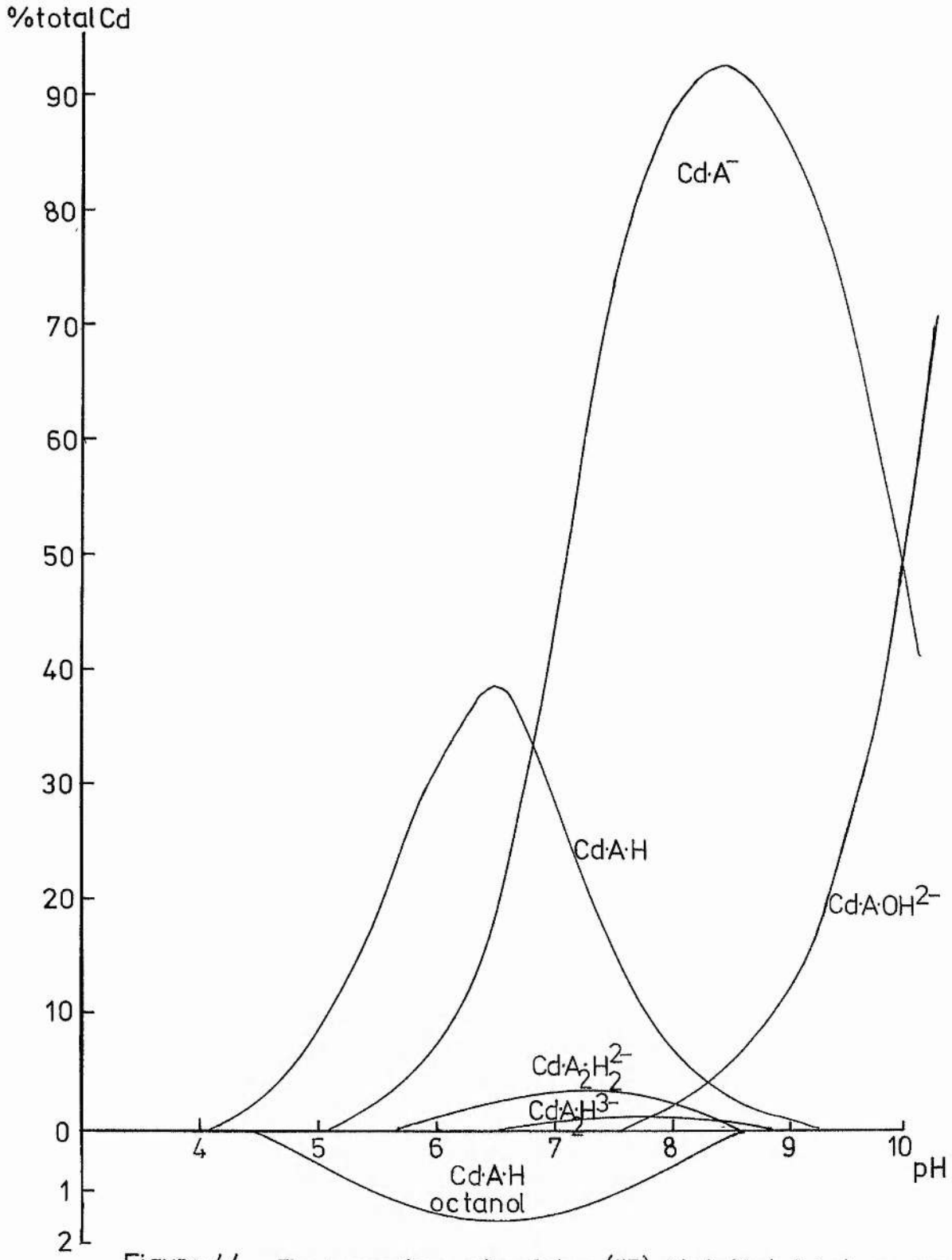


Figure 44 The percentage of cadmium (II) distributed between various complexes in the aqueous (upper plots) and octanol (lower plot) phases at different pHs calculated using the HALTAFALL computer program.

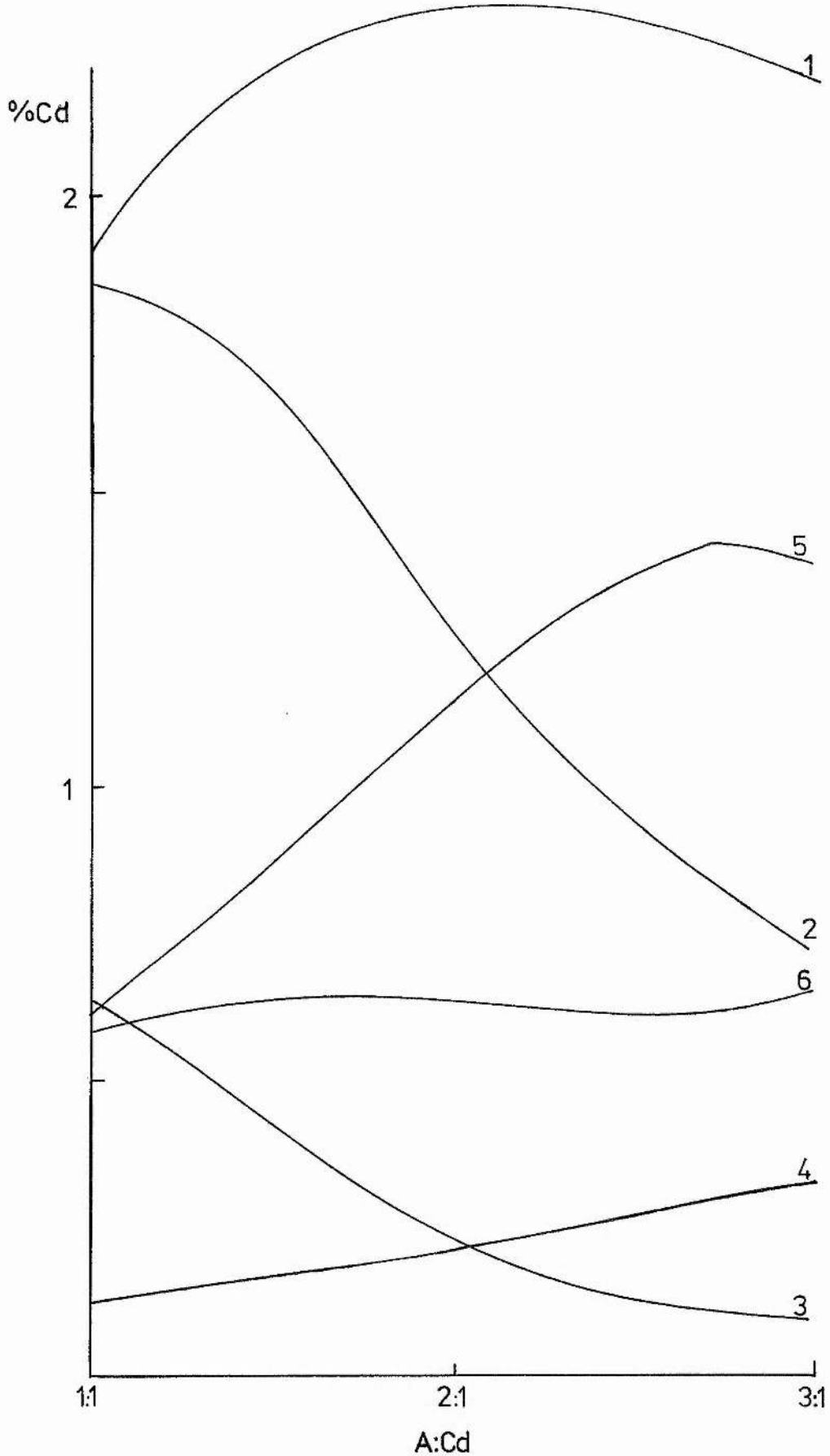


Figure 45 Plots of the percentage of cadmium (II) present in the A.Cd.H complex in the octanol layer at various pHs and total cadmium concentrations. 1,2,3 -10^{-3}M Cd at pH = 5.5,6.5 and 7.5 respectively; 4,5,6 = 10^{-5}M Cd at pH = 5.5, 6.5, 7.5 respectively.

and phases over a range of pH.

and

2) the percentage of total cadmium partitioned into octanol at varying ligand: metal ratios and the effect of change in total concentration and pH on these values.

The results of these are shown in figures 44 and 45. This data suggests a maximum of 2% of the total cadmium being transported through the cell membranes.

This figure, although small, is not as hopeless as it may seem at first as even this small a loss could, due to life being a continuous process, eventually deplete the cell of cadmium. In addition, much of the cadmium and cadmium glutathionate chemistry occurs in the aqueous phase since in vivo biochemistry is mainly aqueous. One factor which must be remembered in this case is that existing cadmiumism treatment involves EDTA, a drug whose activity is strictly extracellular and thus relies solely on the natural wastage of cadmium from decaying cells since neither EDTA nor its cadmium complex can penetrate a lipid protein cell membrane.

From figure 45 it can be seen that there is no easy answer to what is the optimum glutathionate dosage for the removal of cadmium from cells although ratios of glutathionate:cadmium greater than 1:1 are suggested. From previous COMPLOT studies a ratio of 2:1 was proposed, however, this only takes account of the aqueous chemistry. Where transport across cell membranes is concerned the factors of pH and total cadmium concentration must be taken into account as they play a more important role.

Such studies still require considerable refinement. Further partition studies with the biotrace metals would provide vital information as to the amount of 'topping up' of essential bio-metals required during cadmiumism therapy. Similarly, better model systems could perhaps be found, for example, chloroform might be preferable to octanol, being more lipophilic and thus more similar to human enythrocyte membrane and to the blood brain barrier.

Another consideration is the aqueous phase on either side of the membrane. The aqueous environment inside the cell and its pH must be accounted for in future models.

CONCLUSION

The treatment of cadmiumism to date has been undertaken by the use of BAL, NTA and EDTA and its derivatives. Computer models have shown EDTA to be unspecific for cadmium with zinc being preferentially complexed.

Two ligands have been shown to be more efficient in the sequestering of cadmium with respect to zinc, namely glutathionate and EGTA. However, glutathionate is felt to be the more suitable for *in vivo* studies as due to its being naturally occurring its degradation and excretion should present no problem.

One problem with a ligand such as glutathionate is that to be effective it must be kept in the reduced form, which may perhaps be achieved by administering it together with a suitable reducing agent such as ascorbic acid. Another important factor to be taken into account is the toxicity of such ligands and LD_{90} measurements of glutathione are being determined.

Obviously computer models cannot always be extrapolated to the situation *in vivo* as has been seen in the case of penicillamine and so clinical trials would be of valuable assistance in assessing the worth of these ligands.

Further work on finding more cadmium specific ligands which would be non-toxic *in vivo* would be of value. Perhaps studies on various short peptides containing cysteine would provide a fruitful area of research. Further, it could be taken into consideration the co-ordination of cadmium. Whereas zinc ions are usually bonded tetrahedrally in aqueous solution those of cadmium are usually octahedral or square planar and so by designing a cryptate like compound it should be possible to have a highly specific ligand which would exclude zinc ions. It must be noted, however, that the ionic radius of cadmium is similar to that of calcium

(Cd^{2+} = 97, Ca^{2+} = 99. Zn^{2+} = 74 pm) (18) but this could be overcome by the choice of 'soft' donor atoms.

An extension of the partition work could also provide helpful information. Measurement of the partition coefficients for glutathionate - essential metal ions, which could be used in conjunction with already determined formation constants to calculate the extent of topping up of essential metals, would provide a useful comparison with figures for the aqueous phase. Also, as has been mentioned previously other solvents, more lipophilic than octanol, might provide a superior membrane model.

The oscillating reactions are more difficult to assess in terms of their potential usefulness as model systems. It is obvious that the chemicals and concentrations used in this model are not applicable to physiological conditions. However, this is not to minimise the importance of oscillations in vivo. Any future research should aim at more relevant models, perhaps involving the biotrace elements. This may lead to 'identikit' pictures of the effects of drugs and poisons, perhaps in the form of a large kinetic model.

As was stated in the introduction, science is far from the ultimate biological model, indeed, it is most unlikely that this will or could be achieved. However, this must not deter future researchers from aiming in this direction as the saving in time and animal trials that may be realised in drug screening and development of new treatments must make it a valuable addition to the scientists armory.

APPENDIX

Input data and listing of the computer program

COMPLOT

This is the version of COMICS (41) used on the St. Andrews IBM 360/44 computer. The program 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, hydrolysed, protonated and polynuclear species.

The input has been modified from the published version as has the output which 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.

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

INPUT

The program is stored under the 44 MFT system and is called using the following cards

```
1. // Job name  JØB,  TO5P100
2. //SYSRDR     ACCESS CLØPT(PLØTTER)
3. /*
4. //SYSOOO     ACCESS  RWCØMPLT,'DISK' = SA45V1
5. //␣EXEC   CLOADER(INCLUDE)
6. /*
                    D A T A
7. /*
8. /&
```

If no plotter is required, i.e. only one of the printer plotter routines, cards 2 and 3 are not included.

The experimental data is as follows

Item		Format
1.	Number of jobs	12
2.	Number of experiments (number of systems containing the same complexes)	12
3.	Concentration/p concentration option 1 = concentration, 0 = pconcentration. This refers to both output listing and plotter routines. Printing option, 1 = results not printed, 0 = results printed.	212
4.	Total number of ligands; total number of metals; total number of complexes; total number of pH values	2I2, 2I3
5.	Complex composition: for each complex a card carrying number of A_1, A_2, ... A_{10}; number of B_1, B_2.... B_{10}; number of OH(proton-ve) and logarithm of the formation constant (include water as OH, protonated ligand and all hydroxy species).	2I2, 8X, F8.0
6.	pH values; 15 values on a card, if more than 15 are required then use a second card. If 15 or less are required blank cards are unnecessary as the number of pH values to be read has already been fed in.	15 F5.0
7.	Title (for the first system.)	
8.	Total concentration of A_1, A_2 .....A_{10} (if more than eight use a second card)	8E10.4
9.	Total concentration of B_1, B_2 .....B_{10}	8E10.4
10.	Graph option 0 = compounded printer plot (C) 1 = separate printer plot (S) 2 = Plotter 3 = Plotter +(S)	I2
	Number of graphs required and the power of 10 by	2I3

which concentration must be raised to bring to a unit number. This is only needed for the plotter and then only if the concentration option is used. (C automatically plots all complexes and if more than 26 are present only the first 26 will be plotted).

11. Maximum and minimum value of concentration or p concentration for the printer plotter (S). (No card is required if (S) is not called). 2E10.4

12. If plotter required one card carrying minimum and maximum X values, length of X axis, minimum and maximum Y values and length of Y axis (X = pH: Y = conc. or pconc. N.B. concentration has been raised by a power of 10. No card is needed if the plotter is not required). 6F10.0

13. Plotting data for the plotter and/or printer plotter(S)

Species option	1 = metal	I 2
	2 = ligand	
	3 = complex	
Species index		I 4

e.g. if complex 6 is to be plotted the card should read 030006. One card is required for each graph to be plotted.

Items 12 and 13 are not required for the printer plotter (C).

Return to item 7 until all experiments have been punched.

Return to item 2 until all jobs have been punched.

```
C                    PROGRAMME COMICS
C
      INTEGER A1/'META'/,A2/'L   '/,A3/'LIGA'/,A4/'ND  '/,A5/'COMP'/,A6/
     1'LEX '/,A7/'+'/,A8/'X'/,A9/'*'/
      INTEGER GR
      DIMENSION C(100),Y1(10),Y2(10),Y3(10),Y4(10),BTOT(10),CLTOT(10),TX
     1(10),VX(10),ML(10,100),MM(10,100),MN(100),AL(10,100),AM(10,100),AN
     2(100),B(100),E(100),DM(10),DMY(10),TITLE(20),AC(50,100),ATX(50,10)
     3,AVX(50,10),APH(50),X(50),LABX(2)
      DIMENSION SYM(26),PT(117)
C
      COMMON C,Y1,Y2,Y3,Y4,BTOT,CLTOT,TX,VX,ML,MM,MN,AL,AM,AN,NL,NM,N,B,
     1UX,IPT
C
      DATA PLUS,BLANK,SYM/'+',' ','A','B','C','D','E','F','G','H','I','J
     1','K','L','M','N','O','P','Q','R','S','T','U','V','W','X','Y','Z'/
     2
   10 FORMAT(2I2)
   20 FORMAT(2I2,8X,F8.0)
   30 FORMAT(1X,I3,3X,10(1X,I2),1X,10(1X,I2),2X,I2,F8.4)
   40 FORMAT(8E10.4)
   50 FORMAT(' ','TOTAL CONC.OF METAL(',I2,')=',E10.3)
   60 FORMAT(' ','TOTAL CONC.OF LIGAND(',I2,')=',E10.3)
   70 FORMAT(13X,2HC1,9X,2HC2,9X,2HC3,9X,2HC4,9X,2HC5,9X,2HC6,9X,2HC7,9X
     1,2HC8,9X,2HC9,8X,3HC10)
   80 FORMAT(2I2,2I3)
   90 FORMAT(15F5.0)
  100 FORMAT('0',/,'0','PH=',F6.3)
  110 FORMAT(8X,'L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 M1 M2 M3 M4 M5 M6 M7 M8
     1M9 M10 OH LOG.BETA',/)
  120 FORMAT(' ','FREE METALS')
  130 FORMAT(8X,10(1X,F10.4))
  140 FORMAT(' ','FREE LIGANDS')
  150 FORMAT(' ','COMPLEX SPECIES')
  160 FORMAT(1X,I3,1H-,I3,10(1X,F10.4))
  170 FORMAT(20A4)
  180 FORMAT(1X,20A4//)
  190 FORMAT(1H1)
```

```
  200 FORMAT('0')
  210 FORMAT(' ',120A1)
C
      READ(5,10) NJJ
      DO 890 JOBS=1,NJJ
      READ(5,10) NJ
      READ(5,10) LC,IPR
      WRITE(6,190)
      WRITE(6,220)JOBS
      WRITE(6,200)
  220 FORMAT('0',20X,'JOB ',I2)
      WRITE(6,110)
      READ(5,80) NL,NM,N,NPH
      DO 240 J=1,N
  230 READ(5,20) (ML(I,J),I=1,10),(MM(I,J),I=1,10),MN(J),E(J)
  240 WRITE(6,30)J,(ML(I,J),I=1,10),(MM(I,J),I=1,10),MN(J),E(J)
      READ(5,90) (APH(I),I=1,NPH)
      NJD=0
      GY=0.0
  250 READ(5,170) (TITLE(I),I=1,20)
      WRITE(6,190)
      WRITE(6,180)(TITLE(I),I=1,20)
      READ(5,40) (CLTOT(I),I=1,NL)
      READ(5,40) (BTOT(I),I=1,NM)
      WRITE(6,200)
      WRITE(6,60)(I,CLTOT(I),I=1,NL)
      WRITE(6,50)(I,BTOT(I),I=1,NM)
      WRITE(6,200)
      IF(LC.EQ.1) GO TO 270
      WRITE(6,260)
  260 FORMAT('0','VALUES GIVEN BELOW ARE PCONCS.(I.E. -LOG(CONC.))'/)
      GO TO 290
  270 CONTINUE
      WRITE(6,280)
  280 FORMAT('0','VALUES GIVEN BELOW ARE CONCS.'/)
  290 CONTINUE
      HX=ALOG(10.0)
      IPT=1
```

```
      DO 300 J=1,N
      AN(J)=MN(J)
      DO 300 I=1,10
      AL(I,J)=ML(I,J)
  300 AM(I,J)=MM(I,J)
      DO 310 I=1,N
  310 B(I)=EXP(HX*E(I))
      DO 320 I=1,NM
  320 Y1(I)=BTOT(I)*0.00001
      DO 330 I=1,NL
  330 Y3(I)=CLTOT(I)*0.00001
      L=0
  340 L=L+1
      WRITE(6,100)APH(L)
      UX=EXP(HX*APH(L))
      IF(IPT-1) 350,350,420
  350 DO 360 I=1,NM
  360 VX(I)=BTOT(I)
      DO 400 I=1,NL
      DMY(I)=1.0
      DO 390 J=1,N
      IF(ML(I,J)) 390,390,370
  370 DO 380 K=1,NM
      IF(MM(K,J)) 390,380,390
  380 CONTINUE
      DM(I)=(EXP(HX*E(J)))*UX**MN(J)
      DMY(I)=DMY(I)+DM(I)
  390 CONTINUE
  400 CONTINUE
      DO 410 I=1,NL
  410 TX(I)=CLTOT(I)/DMY(I)
C
  420 CALL COGS
C
      IF(LC.EQ.1) GO TO 500
      DO 430 I=1,NM
  430 AVX(L,I)=-ALOG10(VX(I))
      DO 440 I=1,NL
```

```
440 ATX(L,I)=-ALOG10(TX(I))
    DO 450 I=1,N
    AC(L,I)=-ALOG10(C(I))
    XY=AC(L,I)
    IF(XY.GT.GY) GY=XY
450 CONTINUE
    IF(IPR.EQ.1) GO TO 490
    WRITE(6,70)
    WRITE(6,120)
    WRITE(6,130)(AVX(L,I),I=1,NM)
    WRITE(6,140)
    WRITE(6,130)(ATX(L,I),I=1,NL)
    WRITE(6,150)
    KP=0
460 KP=KP+1
    KN=10*KP
    KM=KN-9
    IF(KN-N) 470,480,480
470 WRITE(6,160)KM,KN,(AC(L,I),I=KM,KN)
    GO TO 460
480 WRITE(6,160)KM,N,(AC(L,I),I=KM,N)
490 CONTINUE
    GO TO 600
500 CONTINUE
    DO 510 I=1,NM
510 AVX(L,I)=VX(I)
    DO 520 I=1,NL
520 ATX(L,I)=TX(I)
    DO 530 I=1,N
    AC(L,I)=C(I)
    XY=AC(L,I)
    IF(XY.GT.GY) GY=XY
530 CONTINUE
    IF(IPR.EQ.1) GO TO 590
    WRITE(6,70)
    WRITE(6,120)
    WRITE(6,570)(AVX(L,I),I=1,NM)
    WRITE(6,140)
```

```
      WRITE(6,570)(ATX(L,I),I=1,NL)
      WRITE(6,150)
      KP=0
  540 KP=KP+1
      KN=10*KP
      KM=KN-9
      IF(KN-N) 550,560,560
  550 WRITE(6,580)KM,KN,(AC(L,I),I=KM,KN)
      GO TO 540
  560 WRITE(6,580)KM,N,(AC(L,I),I=KM,N)
  570 FORMAT(8X,10(1X,E10.4))
  580 FORMAT(1X,I3,1H-,I3,10(1X,E10.4))
  590 CONTINUE
  600 CONTINUE
      IF(L.LT.NPH) GO TO 340
      NJD=NJD+1
      READ(5,620) GR,NG,IPOW
      IF(GR.NE.1.AND.GR.NE.3) GO TO 610
      READ(5,40) YMIN,YMAX
  610 CONTINUE
  620 FORMAT(I2,2I3)
      IZ=1
      IF(GR.EQ.0) GO TO 800
      POW=10.0**IPOW
      IF(GR.EQ.1) GO TO 640
      READ(5,630) XM,XG,XSG,YM,YG,YSG
  630 FORMAT(6F10.0)
      CALL ZAPIN(NG,XG,XSG,YG,YSG,XM,YM)
  640 KK=0
  650 READ(5,660) II,LL
  660 FORMAT(I2,I4)
      KK=KK+1
      GO TO (670,690,710),II
  670 DO 680 I=1,L
  680 X(I)=AVX(I,LL)*POW
      LABX(1)=A1
      LABX(2)=A2
      IP=A7
```

```
      GO TO 730
  690 DO 700 I=1,L
  700 X(I)=ATX(I,LL)*POW
      LABX(1)=A3
      LABX(2)=A4
      IP=A8
      GO TO 730
  710 DO 720 I=1,L
  720 X(I)=AC(I,LL)*POW
      LABX(1)=A5
      LABX(2)=A6
      IP=A9
  730 CONTINUE
      IF(GR.EQ.2) GO TO 740
      CALL PLTT(L,APH,X,LABX,APH(L),APH(1),YMAX,YMIN,IP,LL,KK)
      IF(GR.EQ.3) GO TO 740
      IF(KK.LT.NG) GO TO 650
      GO TO 800
  740 CONTINUE
  750 CALL ZAPA(YG,YSG,APH,X,NPH,IZ,XG,XSG,XM,YM)
      IF(GR.NE.2) GO TO 790
      IF(KK.NE.1) GO TO 770
      WRITE(6,760)
  760 FORMAT('1','PLOTTER INFORMATION'/)
  770 WRITE(6,780)KK,LABX(1),LABX(2),LL
  780 FORMAT(' ','GRAPH(',I2,') IS ',2A4,'(',I3,')')
  790 CONTINUE
      IF(KK.LT.NG) GO TO 650
      CALL PLOT(7)
  800 CONTINUE
      IF(GR.NE.0) GO TO 880
      TS=GY
      IF(LC.EQ.1) TS=TS/20.
      WRITE(6,190)
      WRITE(6,810)APH(1),APH(NPH),TS
  810 FORMAT(' ','VERTICAL IS PH,HORIZONTAL IS CONC./PCONC.',/,' ','VAL.
     1AT TOP IS ',F5.2,' VAL.AT BOTTOM IS ',F5.2,/,' ','VAL.AT LEFT IS 0
     2.0 VAL.AT RIGHT IS ',E8.2)
```

```
      WRITE(6,820)
  820 FORMAT(' ',' *+++++++++|+++++++++|+++++++++|+++++++++|+++++++++***
     1+++++++|+++++++++|+++++++++|+++++++++|++++++++*++++++++++++++++')
      DO 870 IP=1,NPH
      DO 830 K=1,117
  830 PT(K)=BLANK
      PT(2)=PLUS
      PT(102)=PLUS
      NN=N
      IF(NN.GT.26) NN=26
      DO 850 J=1,NN
      XX=(AC(IP,J)*100.)/TS
      K=IFIX(XX+0.5)
      IF(K.LT.115) GO TO 840
      K=115
  840 IF(K.LE.0) GO TO 850
      PT(K+2)=SYM(J)
  850 CONTINUE
      WRITE(6,210)PT
      WRITE(6,860)
  860 FORMAT(' ',' +',99X,'+')
  870 CONTINUE
      WRITE(6,820)
  880 CONTINUE
      IF(NJD.LT.NJ) GO TO 250
  890 CONTINUE
      STOP
      END
      SUBROUTINE COGS
C
      DIMENSION TERM(100),TERN(100),C(100),Y1(10),Y2(10),Y3(10),Y4(10),B
     1TOT(10),CLTOT(10),TX(10),VX(10),ALO(10),BO(10),TY(10),VY(10),ML(10
     2,100),MM(10,100),MN(100),AL(10,100),AM(10,100),AN(100),B(100)
C
      COMMON C,Y1,Y2,Y3,Y4,BTOT,CLTOT,TX,VX,ML,MM,MN,AL,AM,AN,NL,NM,N,B,
     1UX,IPT
C
   10 FORMAT('0','NUMBER OF ITERATIONS=',I4)
```

```
   20 FORMAT(' ','ITERATION DID NOT CONVERGE')
C
      NIT=0
      DO 30 K=1,N
   30 TERM(K)=B(K)*UX**MN(K)
   40 DO 50 K=1,N
   50 TERN(K)=TERM(K)
      DO 60 K=1,N
      DO 60 J=1,NM
   60 TERN(K)=TERN(K)*VX(J)**MM(J,K)
      DO 80 K=1,N
      DO 70 J=1,NL
   70 TERN(K)=TERN(K)*TX(J)**ML(J,K)
   80 C(K)=TERN(K)
      NIT=NIT+1
      DO 100 I=1,NM
      BO(I)=VX(I)
      DO 90 K=1,N
   90 BO(I)=BO(I)+AM(I,K)*C(K)
      RATIO=BO(I)/BTOT(I)
      VY(I)=VX(I)/SQRT(RATIO)
  100 Y2(I)=ABS(BO(I)-BTOT(I))
      DO 120 I=1,NL
      ALO(I)=TX(I)
      DO 110 K=1,N
  110 ALO(I)=ALO(I)+AL(I,K)*C(K)
      RATIO=ALO(I)/CLTOT(I)
      TY(I)=TX(I)/SQRT(RATIO)
  120 Y4(I)=ABS(ALO(I)-CLTOT(I))
      IF(NIT-999) 130,130,190
  130 DO 140 I=1,NM
      IF(Y1(I)-Y2(I)) 160,140,140
  140 CONTINUE
      DO 150 I=1,NL
      IF(Y3(I)-Y4(I)) 160,150,150
  150 CONTINUE
      IPT=IPT+1
      WRITE(6,10)NIT
```

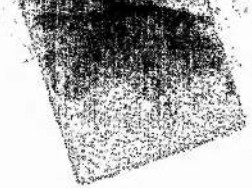
```
      RETURN
  160 DO 170 I=1,NL
  170 TX(I)=TY(I)
      DO 180 I=1,NM
  180 VX(I)=VY(I)
      GO TO 40
  190 WRITE(6,20)
      IPT=1
      RETURN
      END
      SUBROUTINE PLTT(N,X,Y,LABX,XMAX,XMIN,YMAX,YMIN,IY,LL,KK)
      DIMENSION X(2),Y(2),LABX(2),IPL(75),KPL(75),LPL(75)
      INTEGER IB/' '/
      XINC=(XMAX-XMIN)/74.
      YINC=(YMAX-YMIN)/39.
      WRITE(6,110)XMIN,XMAX,XINC,YMAX,YMIN,YINC
      WRITE(6,120)
      IF(XINC*YINC.EQ.0.) GO TO 90
      DO 10 I=1,75
      IPL(I)=0
      KPL(I)=0
   10 CONTINUE
      DO 20 I=1,N
      IK=(Y(I)-YMIN)/YINC
      II=40-IK
      IPL(I)=II
      IK=(X(I)-XMIN)/XINC
      II=IK+1
      KPL(I)=II
   20 CONTINUE
      L=0
      M=0
      DO 90 I=1,40
      L=L+1
      M=M+1
      DO 30 K=1,75
      LPL(K)=0
   30 CONTINUE
```

```
      DO 40 J=1,N
      IF(IPL(J).NE.I) GO TO 40
      JJ=KPL(J)
      LPL(JJ)=1
   40 CONTINUE
      DO 60 J=1,75
      IF(LPL(J).NE.0) GO TO 50
      LPL(J)=IB
      GO TO 60
   50 LPL(J)=IY
   60 CONTINUE
      IF(M.EQ.5) GO TO 70
      WRITE(6,130)(LPL(J),J=1,75)
      GO TO 90
   70 IF(L.EQ.15) GO TO 80
      WRITE(6,150)(LPL(J),J=1,75)
      M=0
      GO TO 90
   80 WRITE(6,140)(LPL(J),J=1,75),LABX(1),LABX(2),LL
      M=0
   90 CONTINUE
      WRITE(6,120)
      WRITE(6,100)KK
  100 FORMAT(' ',30X,'GRAPH(',I2,')')
  110 FORMAT('1','VAL.AT LEFT=',F6.2,' VAL.AT RIGHT=',F6.2,' INC.=',F6.2
     1,/,' ','VAL.AT TOP=',E8.2,' VAL.AT BOTTOM=',E8.2,' INC.=',E8.2,/,'
     2 ','HORIZONTAL IS PH,VERTICAL IS CONCENTRATION')
  120 FORMAT(' ',1X,'A+----+----+----+----+----+----+----+----+----+----
     1+----+----+----+----+----A')
  130 FORMAT(' ',1X,'|',75A1,'|')
  140 FORMAT(' ',1X,'+',75A1,'+',5X,2A4,'(',I3,')')
  150 FORMAT(' ',1X,'+',75A1,'+')
      RETURN
      END
C
      SUBROUTINE ZAPIN(M,XG,XSC,YG,YSC,XM,YM)
      XSC=XSC*0.3937
      IF(YSC.LE.30.0) GO TO 20
```

```
      YSC=30.0
      WRITE(6,10)
   10 FORMAT(20X,' ***** GIVEN Y-AXIS LENGTH IS TOO GREAT FOR PLOTTER; R
     1ESET TO 30.0 CM. *****')
   20 YSC=YSC*0.3937
      CALL PLOT(1,XM,XG,XSC,1.0,YM,YG,YSC,0.1)
      XIS=(XG-XM)/XSC
      YIS=(YG-YX)/YSC
      XLAX=0.5*(XG+XM)
      YLAX=YM-0.4*YIS
      YLAY=0.5*(YG+YM)
      XLAY=XM-0.7*XIS
      CALL PLOT(91,XLAX,YLAX)
      WRITE(3,30)
   30 FORMAT(' PH',100X)
      CALL CHAR(0.1,0)
      CALL PLOT(99)
      CALL PLOT(91,XLAY,YLAY)
      WRITE(3,40)
   40 FORMAT(' CONC',100X)
      CALL CHAR(0.1,1)
      CALL PLOT(99)
      XLAY=XM-0.5*XIS
      YLAX=YM-0.2*YIS
      YINC=YM
      IY=YG+1.5-YM
      DO 60 I=1,IY
      CALL PLOT(91,XLAY,YINC)
      WRITE(3,50)YINC
   50 FORMAT(F4.1,100X)
      CALL CHAR(0.1,10)
      CALL PLOT(99)
   60 YINC=YINC+1.0
      XLAY=XM-0.2*XIS
      XINC=XLAY
      IX=XG+1.5-XM
      AINC=XM
      DO 70 I=1,IX
```

```
      CALL PLOT(91,XINC,YLAX)
      WRITE(3,50)AINC
      CALL CHAR(0.1,10)
      CALL PLOT(99)
      AINC=AINC+1.0
   70 XINC=XINC+1.0
      IF(M.GT.10) GO TO 100
      XLEG=XG-1.6*XIS
      YLEG=YG-0.3*YIS
      XLEP=XG-0.3*XIS
      DO 90 I=1,M
      CALL PLOT(91,XLEG,YLEG)
      WRITE(3,80)I
   80 FORMAT(' GRAPH (',I2,') =  ',100X)
      CALL CHAR(0.1,10)
      CALL PLOT(99)
      YLEP=YLEG+0.04*YIS
      CALL PLOT(91,XLEP,YLEP)
      CALL POINT(I,0.1,1,0)
      CALL PLOT(99)
   90 YLEG=YLEG-0.15*YIS
  100 CONTINUE
      RETURN
      END
C
      SUBROUTINE ZAPA(YG,YSC,PH,CN,IN,IZ,XG,XSC,XM,YM)
      REAL PH(2),CN(2)
      IF(IZ.EQ.11) IZ=1
      GIGY=11.0*(YG-YM)/YSC+YM
      X=1.
      DO 20 I=1,IN
      AN=CN(I)
      BN=PH(I)
      IF(AN.LT.GIGY.AND.AN.GE.YM) GO TO 10
      AN=GIGY-(X/25.)
      BN=XG+1.
      X=X+1.
      IF(X.EQ.50.) X=1.
```

```
      10 CALL PLOT(91,BN,AN)
         CALL POINT(IZ,0.1,1,0)
      20 CONTINUE
         CALL PLOT(8)
         IZ=IZ+1
         RETURN
         END
// FORMS CHANGE BACK TO NORMAL PAPER AND CONTROL TAPE                    20.51.55
```



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