

CHEMICAL MODELLING
OF
URINE

A thesis submitted to the
UNIVERSITY OF CAPE TOWN
in fulfilment of the requirements for the degree of
MASTER OF SCIENCE

by

JOHN LITTLE B.Sc. (HONS)

Department of Physical Chemistry
University of Cape Town
Rondebosch
South Africa

March 1984

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TO MY FATHER

My father, I am proud to say
That I am your son, and that I am
Your son, and that I am your son
Your son, and that I am your son
Your son, and that I am your son

My father, I am proud to say
That I am your son, and that I am
Your son, and that I am your son
Your son, and that I am your son
Your son, and that I am your son

Who controls the past controls the future

Who controls the present controls the past

Ignorance is strength

George Orwell

Nineteen Eighty-Four

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Professor P.W.Linder, supervisor of this project. I am indebted for the invaluable support, guidance, encouragement and friendship that he has offered me throughout this project.

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SUMMARY

A urine model has been developed. The model is used in the computation of chemical speciation and degree of precipitation by means of equilibrium constants and solubility products. The urine model was developed in order to investigate some of the factors associated with urolithiasis. A specific model development procedure was used. This procedure was based on iterative construction, validation, refinement and useage of the model. One refinement stage involved the potentiometric characterisation of the solution equilibria of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system. Whereas the subsystems; H-PO_4 , H-NH_3 and $\text{Mg-PO}_4\text{-H}$, were rigorously characterised, the overall system was only tentatively explored. The results obtained, however, indicate that the characterisation of the three subsystems is likely to be sufficient for the purposes of the urine model. The validation of the basic urine model was achieved by comparing the model's predicted precipitation with experimental observations in another project currently being pursued in this department. This validation has indicated that the kinetics of precipitation should be taken into account for some of the important urinary solids. The urine model was nevertheless successfully used to investigate the effect of pH on urinary precipitation, the influence of some urinary components on calcium oxalate precipitation and the role of cadmium in urolithiasis. The results obtained were in general agreement with other observed and predicted results. Finally, a coherent procedure for investigating the problems of urolithiasis by means of such a model is proposed.

LIST OF SYMBOLS

$a(i,j)$	: stoichiometric coefficient of X_j , eqn. (3.13)
a_i	: ionic size parameter of ion i , eqn. (3.3)
c	: empirical parameter, eqn. (3.3)
C_i	: concentration of complex i , eqn. (3.13)
E_{cell}	: measured emf of the cell, eqn. (2.11)
E_o	: collection of all constant potentials, eqn. (2.11)
ΔH°_R	: standard enthalpy of reaction, eqn. (3.1)
ΔH°_S	: standard enthalpy of solution, eqn. (3.1)
i	: ion or complex
I	: ionic strength (units of mol dm^{-3})
j	: component
K_{pqrs}	: stoichiometric stepwise equilibrium constant
K_w	: ionic product of water
Q_y	: activity coefficient quotient, eqn. (2.3)
R	: crystallographic R-factor
S	: Nernstian slope, eqn. (2.11)
T_M	: total concentration of metal, M , eqn. (2.9)
T_L	: total concentration of ligand, L , eqn. (2.8)
T_H	: total concentration of protons, H , eqn. (2.10)
T_j	: total concentration of component j , eqn. (3.14)
X_j	: concentration of free component j , eqn. (3.13)
Y_i	: activity coefficient of ion i , eqn. (2.3)
Y_j	: difference between total analytical and total calculated concentrations of component j , eqn. (3.14)
Z_i	: charge on ion i , eqn. (3.3)
$\bar{Z}$	: average number of ligands bound to metal, eqn. (2.12)

- $\bar{z}_H$: average number of protons bound to ligand
 β_{pqrs}^T : cumulative thermodynamic equilibrium constant, eqn. (2.1)
 β_{pqrs} : cumulative stoichiometric equilibrium constant, eqn. (2.2)
: convergence criterion (a small positive real number),
eqn. (3.15)
 χ^2 : a measure of how Gaussian the distribution of residuals is
{ } : denotes activity
[] : denotes concentration
() : denotes concentration (used in Figures)

Note: In some specific instances, the total concentration is expressed as $[]_T$, the concentration of a solid as $[]_S$, and free concentration as $[]$.

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CHAPTER 1

INTRODUCTION

1.1. UROLITHIASIS

The problem of urinary stone formation has perplexed investigators for many centuries (1). Indeed, students of urolithiasis have not yet reached a consensus about its fundamental causes (2). The simplest hypothesis proposes (1) that the first step in stone formation involves the spontaneous precipitation from a supersaturated urine of a crystal or a number of crystals. These can aggregate forming a particle large enough to become trapped at some narrow part of the urinary tract. This trapped particle is considered to act as a nidus for the formation of a stone.

In general, the most commonly occurring constituents of urinary stones are calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid and cystine (3). Of these, calcium oxalate is the most frequent constituent occurring in about 70% of all cases. Cystine is the most infrequent, its presence being observed in about 1% of all cases. The remaining three are each found to occur at about the same frequency of just below 9%. These five solids therefore account for about 98% of all stones.

The above simple description of the formation of a urinary stone is useful in that it highlights some of the important physical aspects involved in urolithiasis. Supersaturation, nucleation, crystal growth and aggregation are the fundamental steps of stone formation (2). One feature not referred to above is the inhibition of stones. Obviously, inhibition of any one of the four fundamental processes would result in a degree of inhibition of the overall process of stone formation.

Supersaturation is the most fundamental of the above mentioned conditions. If urine were permanently unsaturated with respect to all possible solids, no stone would ever form. The most important recent advances in the understanding of urolithiasis concern the evaluation of urine supersaturation (3). All currently accepted forms of medical therapy for stone disease produce a reduction in urinary supersaturation (2). Thermodynamics indicate that any supersaturated solution must eventually equilibrate by precipitation until such time as the solution is just saturated. Kinetic factors might slow the precipitation process resulting in a supersaturated solution with a precipitate simultaneously present. Metastable solutions might also occur when the solution will remain supersaturated until a seed crystal or other particle allows nucleation to begin. The term supersaturation will therefore be used to encompass metastable solutions which are supersaturated without a precipitate present as well as solutions which are supersaturated and are in the process of precipitating.

Nucleation or the initiation of precipitation is the first event in the life of any stone. The theory of nucleation reconciles the increase in free energy when a new surface is formed with the loss of free energy when a material leaves solution (3). The mechanism of nucleation is important if surface active inhibitors of precipitation are to be considered.

Crystal growth rates in urine, at the levels of supersaturation actually found in practice, appear to be far too low to produce stone formation by crystal growth alone (3). Much work has been done investigating the efficacy of various substances in

inhibiting crystal growth.

Aggregation of small particles to form stones appears to be one of the more commonly recognized processes. There are two well established aggregation mechanisms that apply to particles in urine: aggregation by reduction of zeta potential and aggregation by polymeric bridging (3).

The experimental observations that most stones are conglomerates of different types of solids and that many stones have a specific nucleating solid around which other solids are precipitated appear to support the importance of nucleation, crystal growth and aggregation.

The role of inhibitors in preventing urolithiasis is widely recognized to be of importance. One of the problems associated with the experimental work done in this area is the uncertainty as to which process is indeed being inhibited (3). In other words, it is often shown that a certain substance reduces the occurrence of stone formation, but the experimental procedures do not allow a proposal of the mechanism of inhibition.

Extensive research has been carried out as to the causes of urolithiasis. A vast number of techniques have been used in the course of this research which has generally aimed at investigating one of the main factors outlined above. Supersaturation, nucleation, crystal growth, aggregation and inhibition have all been studied in this way. The techniques used include scanning electron microscopy, X-ray diffraction and stone analysis through to crystal

growth experiments, nucleation studies and the study of phase transformations. The measurement of ionized calcium and the determination of equilibrium constants has also been carried out as well as in vivo studies of dietary restrictions and the use of drugs. One of the more recent techniques has been to model urine in some way. Urinary stone formation has been modelled by assuming the urinary tract to behave like a continuous crystallizer as well as by fast evaporation of urine-like solutions.

More recently, workers in urolithiasis have begun to recognize the value of focussing on the supersaturation of urine. Finlayson (2) has stated that the great clinical utility of assuming that urinary supersaturation is the dominant diagnostic feature, is that this makes it possible to evaluate the effectiveness of therapy by analysing urine. Without this assumption, a long wait would be necessary to determine whether the therapy had caused a reduction in stone occurrence.

The investigation of supersaturation can be approached experimentally by making up solutions of known concentrations and then evaporating these to obtain precipitation. The extent of the precipitation is therefore an indication of the degree of supersaturation in the evaporated solution providing a metastable solution has not been produced. Supersaturation can also be approached by means of a theoretical equilibrium model. In this method, a theoretical "solution" is made up of total concentrations of various important components. Equilibrium constants and solubility products are then used to compute the equilibrium speciation and degree of supersaturation of the equilibrium solution

with respect to various solids. In terms of the program used to perform the calculations, the degree of supersaturation takes on the specific meaning of the amount of solid predicted to precipitate per litre of solution. These calculations will be more fully described in section 3.2. While a great deal of effort might be required in developing such a model for urine, the speed with which urinary supersaturation can ultimately be evaluated is a great advantage.

It was therefore decided to approach the problem of urolithiasis by making the assumption that urinary supersaturation is the dominant diagnostic feature in stone formation. Having made this assumption, the chemical equilibrium method for evaluating urinary supersaturation was chosen because of its ultimate efficiency. Finally, a fully developed model of this nature could be used in the routine evaluation of urinary supersaturation and in the evaluation of various types of therapy to prevent stone formation.

1.2. MODELLING

The role of supersaturation in urolithiasis will be investigated by means of a chemical equilibrium model. This method allows the theoretical calculation of the degree of supersaturation of urine given the total component concentrations and assuming chemical equilibrium.

Similar models have been employed to investigate a wide variety of aqueous systems including sea-water (4, 5), waste water (6), lakes (7), blood plasma (8) and even urine (9, 2, 3). One of the ultimate aims of developing biological models such as those for blood plasma and urine is the incorporation of individual body compartment models into a single, large, multi phase model of the human body (10). While the achievement of such an aim is a long way off, a urine model is one more step towards the chemical model of man.

There are a number of limitations associated with the process of chemical equilibrium modelling and it is important to remember these whilst deciding on how the modelling of a solution is to be approached. Stumm and Morgan (11) have listed a number of reasons why a real system may not be closely approximated by an equilibrium model. The first of these is the inadequacy of the thermodynamic information used. Pertinent chemical equilibria may have been ignored in formulating the model or important species in solid or solution phases not considered. Alternatively, the thermodynamic data of assumed species and phases may be incorrect. The second reason is slow rates of chemical reaction. Equilibrium might only

be slowly attained in the real system when the model has assumed final equilibrium. Another reason is the possibility of irreversible reactions occurring in the real system which have no equilibrium state. Such a reaction would progress towards complete exhaustion of the reactant that is stoichiometrically limiting. Fourthly, it might be the case that one key reaction in a system of coupled reactions is slow while the rest of the reactions are sufficiently rapid that equilibrium is an accurate description of them. In this case, the slow reaction can be modelled kinetically and the output used to adjust the equilibrium model constraints with time. Finally, differences between the real system and the chemical equilibrium model might arise due to incorrect assumptions as to whether the system is open or closed. The closed system is invariant with time while the open system has a flux of material in and out and in this case the steady state is the time-invariant condition.

Applying these considerations to the urine model, it is clear that a number of assumptions will have to be made. Therefore, apart from the assumption that urinary supersaturation is the dominant feature in stone formation, the following further assumptions will be made. Firstly, it will be assumed that all reactions and phase transformations are fast enough for equilibrium to apply. Secondly, the urinary tract will be assumed to be a closed system even though variations do take place with time. It is important to realise that the use of the model might reveal that these assumptions are invalid and force a re-evaluation of the model. This will probably mean that kinetic data will have to be incorporated into the model.

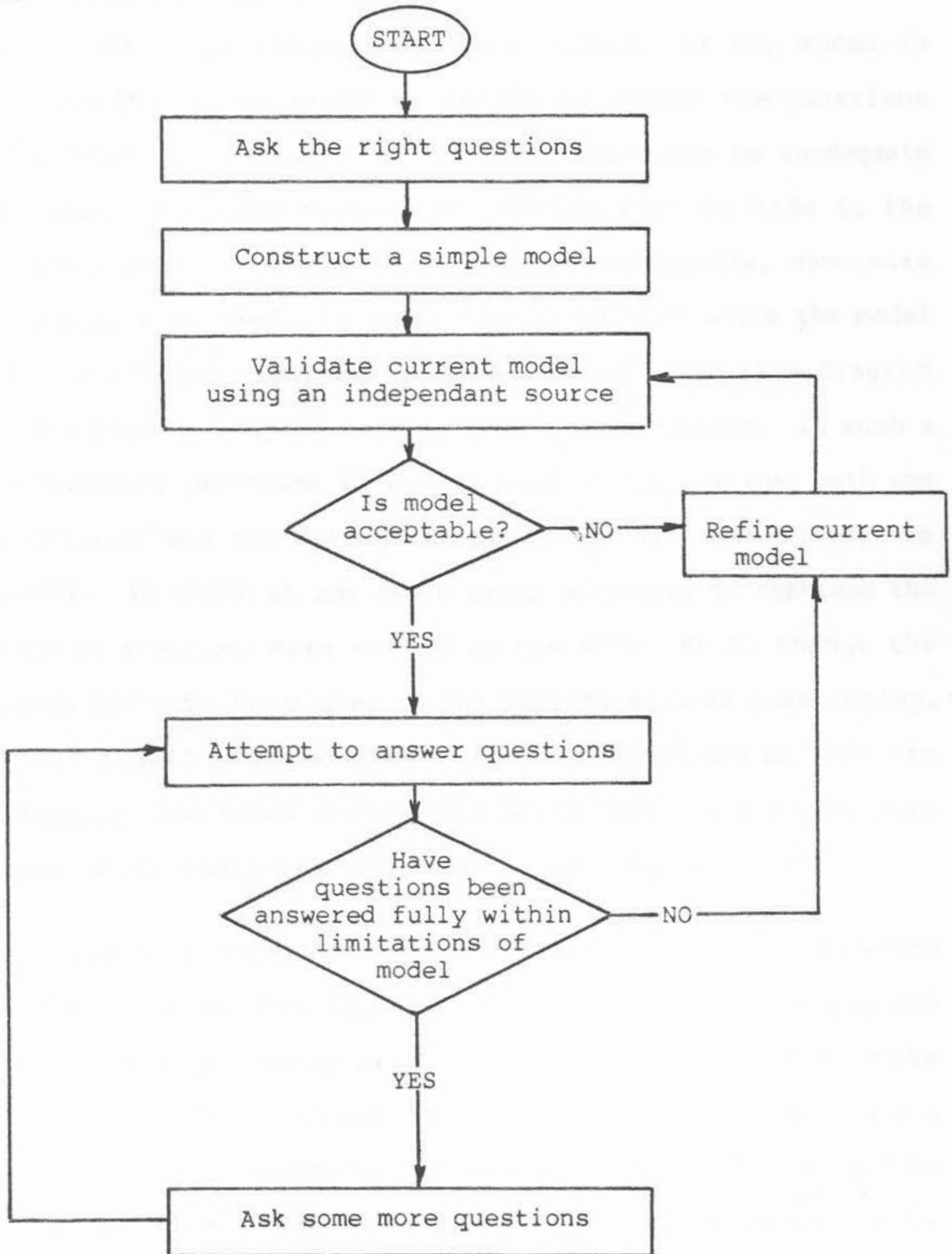
Returning to the problem of urolithiasis, it is specifically hoped that the urine model will be able to answer two types of question. The first is: "What is the extent of supersaturation in urine with respect to the various possible solid phases?" The second is: "What can be said of the role of various inhibitors of stone formation?" The second of these questions might only have negative answers. In other words it might only be possible to conclude that a specific inhibitor does not play a significant thermodynamic role. This would nevertheless direct further research into areas more likely to produce specific answers.

Perhaps the most pertinent comment that has been made on the process of equilibrium modelling is that of Sillén (12) in qualifying the merits of his ocean sediment model: "Finally the model may be proved useless, but this has not yet been done, and perhaps one would learn many interesting things in refuting it".

In reality, the process of chemical modelling does not involve construction of the model followed by the investigation of particular questions and finally discarding the model. The approach to model development to be followed in the present research is intended to be one of iterative construction, validation investigation and refinement. It is felt that in this way the important problem areas are most readily identified and the model suitably refined. Figure 1.1 shows a flow diagram of this iterative model development scheme.

Figure 1.1.

Schematic flow diagram of iterative model development to be followed in the present research.



Essentially, an initial simple model is constructed. This model is then validated using some independent test. If the model does not pass the validation test, it should be refined in some area dictated by the validation procedure and again tested. If the model is thought good enough, an attempt is made to answer the questions initially posed. The answers to the questions might be inadequate and this being so, some further refinement must be made to the model. When, eventually, the answers become acceptable, some more questions should be asked. It might then occur that while the model is good in a certain area, it fails in another. The flow diagram allows for this by looping back to a refinement stage. In such a model development procedure it is important to observe that both the questions asked and the validation procedure are also subject to improvement. It might at any stage prove necessary to rephrase the questions in a manner more suited to the model or to change the validation basis to one closer to the current area of questioning. Finally, it should be noted that the flow diagram has an infinite loop. Ideally, the model development would continue for ever with more areas of the model becoming reliable as time progressed.

It should be mentioned that this model development procedure is designed to reveal quickly the problem areas. Therefore, the guiding principle throughout this research will be to make reasonable assumptions as far as accuracy is concerned. Once a refinement has been deemed to be adequate a validation should be made in order that areas in more urgent need of attention be refined. Hopefully some optimum between speed and accuracy will be obtained and in this way lead to quicker problem identification.

One such problem which arose in one of the early validation tests of the urine model involved the solid, struvite (magnesium ammonium phosphate). It was found that this solid was not predicted to precipitate by the model whereas the experimental validation observations indicated that it should be present as a precipitate. In particular, an equilibrium constant value for the soluble complex of MgNH_4PO_4 which had been used, became recognized as suspect and this accounted erroneously for the solubilization of struvite. Because of the uncertainty of this stability constant and because it is desirable to have as many equilibrium constants in the model determined at model conditions as possible, it was decided to characterise the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system potentiometrically. The equilibrium constants obtained would be included in the urine model and the model development procedure continued.

One final point on the use of models should be made. It is important to realise that a model cannot reflect all features of a real system. If it did this, it would not be a model. The spirit in which modelling should be undertaken is reflected in the words of Henri Theil: "It does require maturity to realize that models are to be used but not to be believed".

1.3. OBJECTIVES OF RESEARCH

The urolithiasis problem is briefly outlined in section 1.1. From a number of possible ways of approaching this problem, the chemical equilibrium modelling approach is chosen. Section 1.2 details this approach and its application to the problem of urolithiasis. A number of assumptions have to be made in order to make the problem tractable. These are:

- a. Urinary supersaturation is the dominant useful feature of urolithiasis.
- b. All the reactions and phase transformations in urine are sufficiently fast to allow the rapid attainment of equilibrium.
- c. The urinary tract represents a closed system.

An iterative model development procedure illustrated in Figure 1.1 is chosen in order to allow efficient problem area identification. In this procedure, desired accuracy in one area must be played off against more pressing attention in another area of the model.

At a certain stage in the procedure, it became necessary to refine the urine model by means of a potentiometric investigation of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ solution equilibria. It was hoped that this would rectify the struvite problem and also enhance the reliability of the model in general.

Two specific areas of investigation have been identified as suitable for study by means of the urine model. These two areas were chosen from the five fundamental factors in urolithiasis:

supersaturation, nucleation, crystal growth, aggregation and inhibition.

The first area of investigation chosen was the supersaturation of urine with respect to the various possible solids. The dependence of urinary stones on pH is a fairly well known phenomenon (3). It was therefore decided to use the urine model to predict urinary supersaturation over the full biological pH range. If successful, this simple test would add to the model's reliability.

The second area of investigation chosen was the inhibition of urinary stones. In an analogous fashion, the promoters of urinary stones may also be investigated. Two urolithiasis problems were investigated in this area. The first was the inhibitory or promotory effect of all the major constituents of urine on calcium oxalate precipitation. This problem has been investigated by similar (3) and other methods and a comparison between the results of this research and previous work would be of value. The second problem in the inhibition/promotion category was the role of cadmium in urolithiasis. Cadmium is a known promoter of urinary stones (13-17) and it would be of value to know in which areas and in what role cadmium acts to cause urinary stones. The efficacy of various drugs in counteracting the promoting influence of cadmium will finally be investigated. The role of cadmium has not yet been investigated by the equilibrium model approach.

The objectives of the present research are therefore:

1. To characterise the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system by potentiometrically studying the solution equilibria involving these components.
2. To construct the urine model by acquiring the relevant equilibrium constants and solubility products.
3. To validate the urine model by comparison with suitable experimental observations.
4. To use the urine model to investigate:
 - a. The effect of pH on urinary supersaturation.
 - b. Inhibitors and promoters of calcium oxalate precipitation.
 - c. The role of cadmium in urolithiasis.

CHAPTER 2

POTENTIOMETRY

2.1 INTRODUCTION

Work had been carried out on the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system as long ago as 1910 when Bube (18) measured the solubility product of $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$. Work on this and other magnesium phosphate solids continued (19-22) until as recently as 1982 when the above solubility product was again determined (23).

The object of the present research, however, is to study the solution equilibria of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system. The literature in this case is divided into the $\text{Mg-PO}_4\text{-H}$ system (24-33) and the $\text{Mg-NH}_3\text{-H}$ system (34-41). A literature survey has shown that no attempt has been made to investigate the combined solution equilibria of these two systems.

In order to investigate the combined system, it would suffice to select as a basis, stability constants from the related subsystems which have already been determined. After correcting these constants for ionic strength and temperature, they could be used as fixed values in the refinement of further constants.

There are, however, a number of disadvantages associated with this procedure. The unreliability of many published constants (42) as well as the difficulties encountered in correcting these constants for ionic strength (43) and temperature (44) are amongst the main disadvantages. It is common practice to determine stability constants with a background electrolyte maintaining the ionic strength sensibly constant (45). This limits the choice of constants from the literature to those determined in the desired

medium.

The problem is further complicated by the proposal of different chemical models to describe the same system. Childs, for example, in his investigation of the $\text{Mg-PO}_4\text{-H}$ system (31) reports five metal complexes while Havel and Högfeldt (32) report two and still others (24-29) report one. These differences are partially the result of varying experimental conditions such as pH and partially due to the way in which the data are refined. Childs' data can support the existence of dimerised complexes while Havel and Högfeldt's data cannot. Which do we choose?

For these reasons it seemed wise to determine all the stability constants pertaining to the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system independently. This could be achieved with initial investigations of the protonation of the two ligands, phosphate and ammonia, followed by the addition of magnesium to these ligands. The accuracy of the protonation constants so determined is imperative since these will be used in the calibration of the glass electrodes (28) used in this study. Furthermore, the experience gained in determining the protonation constants is usually invaluable when it comes to investigation of the metal-ligand equilibria.

Having decided to investigate experimentally the solution equilibria of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system, it remains to choose the background electrolyte, ionic strength and temperature at which this will be done. Urine, a biological fluid with high sodium chloride concentration made the choice of sodium chloride for background electrolyte an obvious one. The level at which this concentration

should be maintained is ideally about 3 mol dm^{-3} (45). This is much higher than the approximate ionic strength of urine and the working ionic strength chosen was $0,20 \text{ mol dm}^{-3}$ (see section 3.3.3). This means that there will be a greater risk of ionic strength variation and hence of activity coefficient variation. However, this risk is balanced (we hope) by the more representative results obtained. These results should be optimally applicable to the biological fluid in which we are interested.

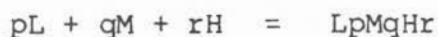
Finally, the temperature chosen was 25°C . The body temperature of 37°C can cause problems with condensation of vapour inside the titration assembly. These problems can be overcome with careful thermostating, perhaps even of the whole room, but it was decided to leave this possibility open to be pursued if time permitted.

2.2 THEORY

The general theory of solution equilibria and the potentiometric determination of the related equilibrium constants is dealt with in the literature (42, 45, 47). Only a broad outline of the principles involved in these determinations will be given here.

The formation of an equilibrium in solution is characterised by its equilibrium constant. Where this refers to the protonation of a ligand, the term protonation constant will be used. Stability constant will be used when referring to the formation of a metal complex.

For a system of three components, represented by L (ligand), M (metal-ion) and H (proton), in which the following generalised equilibrium is established:



the cumulative thermodynamic stability constant is defined as:

$$\beta_{pqr}^T = \{L_pM_qH_r\} / \{L\}^p \{M\}^q \{H\}^r \quad \dots (2.1)$$

where { } denotes activity.

Strictly speaking, both the metal ion and the proton will be hydrated in aqueous solution. The activity of the water molecule should also appear in the formulation of the thermodynamic stability constant. In very dilute solutions, however, the water is effectively in its standard state (45) and its activity is thus negligibly different from unity.

By convention, the equilibria are written as the formation of

a complex from its components. The subscripts of β represent the stoichiometry i.e. coefficients of the complex and the order in which these appear will always be ligand, metal, proton. When the proton subscript is negative, this refers to a proton removed to a water molecule or an hydroxide ligand added.

The problems involved in measuring activity (48) have led to complex equilibria being expressed in terms of concentrations rather than activities. The cumulative stoichiometric stability constant of the above equilibrium is thus defined as:

$$\beta_{pqr} = [L_p M_q H_r] / [L]^p \cdot [M]^q \cdot [H]^r \quad \dots (2.2)$$

where [] denotes concentration.

It is important to realise that this expression is only constant over the course of a titration if the associated activity coefficient quotient is constant.

$$Q_y = y_{LMH} / y_L^p \cdot y_M^q \cdot y_H^r \quad \dots (2.3)$$

where y denotes activity coefficient.

In order to accomplish this, the titration is carried out in the presence of a relatively high concentration of background electrolyte which maintains a constant ionic strength. The activity coefficients and hence the activity coefficient quotient will remain constant if we assume that the activity coefficients are functions of ionic strength (49, 50). The stability constants determined in this way are only applicable to the ionic strength and the medium in which they are measured.

Equilibria can also be characterised by stepwise equilibrium constants. A set of stoichiometric protonation constants could thus be represented as follows:

$$K_{101} = [LH] / [L][H] \quad \dots(2.4)$$

$$K_{102} = [LH_2] / [LH][H] \quad \dots(2.5)$$

$$K_{103} = [LH_3] / [LH_2][H] \quad \dots(2.6)$$

In general, these stepwise constants are related to the cumulative constant in the following way:

$$\beta_n = \prod_{i=1}^n K_i \quad \dots(2.7)$$

The equations on which the determination of stability constants are based are known as the mass balance equations. Thus, at any point in the titration the total concentration of the components can be expressed in terms of the free component concentrations and the stability constants of the complexes. Thus:

$$T_L = [L] + \sum_{r=-R}^R \sum_{q=0}^Q \sum_{p=1}^P p \beta_{pqr} [L]^p [M]^q [H]^r \quad \dots(2.8)$$

$$T_M = [M] + \sum_{r=-R}^R \sum_{q=1}^Q \sum_{p=1}^P q \beta_{pqr} [L]^p [M]^q [H]^r \quad \dots(2.9)$$

$$T_H = [H] + \sum_{r=-R}^R \sum_{q=1}^Q \sum_{p=1}^P r \beta_{pqr} [L]^p [M]^q [H]^r \quad \dots(2.10)$$

In a potentiometric study of these equilibria using a glass electrode in electrical contact with a reference electrode, the measured emf of the cell can be expressed as (46 (eqn. 3))

$$E_{\text{cell}} = E_0 + S \log [H^+] \quad \dots(2.11)$$

where:- E_{cell} is the measured emf of the cell.

S is the Nernstian slope = $RTF^{-1}\ln(10)$.

E_0 is a collection of all constant potentials and includes the hydrogen-ion activity coefficient and factors arising from it.

The aim of electrode calibration in this research is to calibrate the electrodes over the same pH range as they are to be used and also in a similar solution to that in which the measurements are to be carried out. This procedure, though clearly described by May et al (46), will be briefly outlined here.

1. An initial strong acid vs. strong base titration is used to obtain a value for E_0 using the CALIBT subroutine of program MAGEC. This also serves as a useful guide to the electrodes' performance characteristics.
2. The E_0 value is used with ligand-titration data in the program MINQUAD (51, 52) in order to refine the values of the protonation constants of the ligand.
3. The ligand-titration data and protonation constants from step 2 are processed by MAGEC to yield refined E_0 values
4. Steps 2 and 3 are repeated cyclically until convergence is obtained.

The electrodes are thus calibrated "internally" over the same pH range and in the same solution as the protonation constants are determined.

Once the protonation constants have been established, they

can be used to calibrate the electrodes in any subsequent ligand titration with a single execution of the program MAGEC. This will be the case when it comes to determining the metal-complex stability constants. Ideally, a preliminary titration should be carried out with ligand only. This data can then be used to calibrate the electrode. Metal is then added to the same solution and the titration continued. These subsequent data are used together with the refined E_0 to determine the stability constants.

MAGEC makes use of equations 2.8, 2.10 and 2.11 and a sum of squared residuals minimisation by the Nelder and Mead simplex algorithm. MINQUAD on the other hand uses the free proton concentration and equations 2.8, 2.9 and 2.10 to refine both the unknown stability constants and unknown free concentrations. MINQUAD also makes use of a sum of squared residuals minimisation, but uses a generalised Gauss-Newton algorithm.

A useful function in the determination of equilibrium constants is $\bar{Z}$, the average number of ligands bound to the metal.

$$\bar{Z} = \frac{[\text{L bound to M}]}{[\text{M}]} = \frac{[\text{L}]_T - [\text{L}]}{[\text{M}]} \quad \dots(2.12)$$

If we assume that only mononuclear binary complexes form, it can be shown (45) that $\bar{Z}$ is a function of the hydrogen-ion concentration and the protonation constants only. It can similarly be shown that $\bar{Z}$ is independent of the total component concentrations. Thus, the formation curves (plots of $\bar{Z}$ vs. $-\log[\text{L}]$) for different titrations should overlap. Deviations from this general pattern are an indication of protonated or hydroxo complexes

being formed. The experimental formation curve therefore, is a useful tool in analysing the data and deciding on what types of equilibria are present.

In protonated metal-ligand equilibria, the $\bar{Z}$ function can be expressed in different ways. If we take L to be the bare, unprotonated ligand, we are defining $\bar{Z}$ in terms of the ML complex. We could, however, also take HL to be the ligand and focus our attention using the $\bar{Z}$ function on the MHL complex. In order to do this, the protonation constants will have to be adjusted accordingly making the HL ligand the basis rather than L.

While the $\bar{Z}$ function described above is usually suitable in describing equilibria in which there is only one metal, one ligand and protons, the introduction of a further ligand renders this treatment senseless. A similar function was thus sought with which to characterise the two-ligand ternary system under investigation.

Osterberg (53, 54) has developed a procedure using an adaptation of Hedstrom's method (55) which allows the free ligand concentration to be calculated without any special assumptions about the complexes formed. This procedure has been extended by Sarkar and Kruck (56-59) to include measurement of free metal. McBryde (60) has introduced a new theoretical treatment of the method and its extensions and has shown how the functions developed are related to other parameters of complex formation.

McBryde's analysis assumes the formation of mononuclear binary complexes only. This simplifies the derivation, but it is

stated by McBryde that the relationships developed still apply to the formation of protonated complexes and also to the formation of mixed complexes with a second ligand.

Some of the relationships developed will be briefly outlined.

Consider a series of titrations of a weak poly-basic acid in the presence of a fixed total concentration of metal T_M with varying ligand concentrations.

We note the following relationships:

$$F = 1 + \beta_1 [H] + \beta_2 [H]^2 + \beta_3 [H]^3 + \dots \quad \dots (2.13)$$

$$G = \beta_1 [H] + 2\beta_2 [H]^2 + 3\beta_3 [H]^3 + \dots \quad \dots (2.14)$$

$$\bar{Z}_H = G / F \quad \dots (2.15)$$

$$T_H = [H] - [OH] + G[L] \quad \dots (2.16)$$

where β_i refers to cumulative protonation constants of the ligand L. Now, the total concentration of ligand L which varies from titration to titration is given by:

$$T_L = F[L] + \bar{Z} \cdot T_M \quad \dots (2.17)$$

where $\bar{Z}$ is the degree of metal complex formation.

$$\text{Then: } (\partial T_H / \partial [L])_H = G$$

$$\text{and } (\partial T_L / \partial [L])_H = F + T_M (\partial \bar{Z} / \partial [L])_H$$

$$\begin{aligned} \text{Hence: } (\partial T_H / \partial T_L)_H &= G(F + T_M (\partial \bar{Z} / \partial [L])_H)^{-1} \\ &= \bar{Z}_H (1 + T_M \cdot F^{-1} (\partial \bar{Z} / \partial [L])_H)^{-1} \end{aligned} \quad \dots (2.18)$$

Notice that in the absence of metal, equation (2.18) simplifies to:

$$(\partial T_H / \partial T_L)_H = \bar{Z}_H$$

where $\bar{Z}_H$ is the degree of protonation of ligand.

Consider the analagous relationships in a series of titrations of a weak poly-basic acid in the presence of a fixed total concentration of ligand T_L with varying metal concentrations. Equation (2.16) still applies, but (2.17) can be rearranged to give:

$$T_M = (T_L - F \cdot [L]) / \bar{Z} \quad \dots (2.19)$$

Then: $(\partial T_H / \partial [L])_H = G$

and $(\partial T_M / \partial [L])_H = \{-\bar{Z}F - (T_L - F \cdot [L]) (\partial \bar{Z} / \partial [L])_H\} \bar{Z}^{-2}$

$$\begin{aligned} \text{Hence: } (\partial T_H / \partial T_M)_H &= \frac{-\bar{Z} \cdot G}{\bar{Z}F + (T_L - F[L]) (\partial \bar{Z} / \partial [L])_H} \\ &= \frac{-\bar{Z} \cdot G}{F + T_M (\partial \bar{Z} / \partial [L])_H} \\ &= \frac{-\bar{Z} \cdot \bar{Z}_H}{1 + T_M F^{-1} (\partial \bar{Z} / \partial [L])_H} \end{aligned}$$

and $(\partial T_H / \partial T_M)_H = -\bar{Z} (\partial T_H / \partial T_L)_H \quad \dots (2.20)$

While the relationships (2.18) and (2.20) may be extended to expressions for calculating the free ligand and free metal concentrations respectively, they are of most use in this research as they stand. Both are related to the degree of complex formation and both have been used in the characterisation of binary (53, 54, 57) and ternary (58) systems.

A graphical procedure is used to evaluate these functions. The series of titrations performed is depicted as a set of curves of the form T_H vs pH ($\text{pH} \equiv -\log [H^+]$). Lines of constant pH are then constructed cutting these curves. The points of intersection of these lines are displayed as plots of T_H vs T_L (or T_M in the case of

constant ligand).

These plots should comprise a series of straight lines, the slopes of which correspond to the function $(\partial T_H / \partial T_L)_H$ (or $(\partial T_H / \partial T_M)_H$). These slopes can then be plotted against pH and used in cases where the $\bar{Z}$ function cannot equably be used.

Returning to our original intention of obtaining these functions, we now have a set of parameters, $\bar{Z}_H$, $\bar{Z}$, $(\partial T_H / \partial T_L)_H$ and $(\partial T_H / \partial T_M)_H$ which can be determined using only the experimental data and the protonation constants in the case of $\bar{Z}$. These functions all relate to the type of complex formation taking place.

The computational procedure in determining the stability constants is as follows:

1. Electrodes are calibrated using MAGEC.
2. The experimental complex formation function, for example $\bar{Z}$, is calculated.
3. A chemical model comprising the species present in solution is proposed.
4. The β 's are refined using MINQUAD.
5. These β 's and the relevant experimental conditions are used to generate a set of titration data. The program PSEUDOPLOT is used to do this (61).
6. The theoretical titration data are used to calculate the theoretical complex formation function, for example $\bar{Z}_{\text{theor}}$.
7. The "goodness of fit" of the experimental and theoretical complex formation functions indicates how reliable the proposed model and refined β 's are.

An indication of the statistical reliability of the refined β 's and the proposed model is given by the statistical output of MINIQAD. There are two statistical tests performed. The first is in the form of the crystallographic R-factor (62).

$$R = \sqrt{\frac{\sum (f_i^{\text{calc}} - f_i^{\text{obs}})^2}{\sum (f_i^{\text{obs}})^2}}$$

where: the summations are carried out over all components and all data points;

the f_i^{calc} 's are the total component concentrations.

The second is the computed value of χ^2 (chi-squared). This is a measure of how "normal" or Gaussian the distribution of residuals is. A scan of the residuals can then reveal which of the component concentrations are in error.

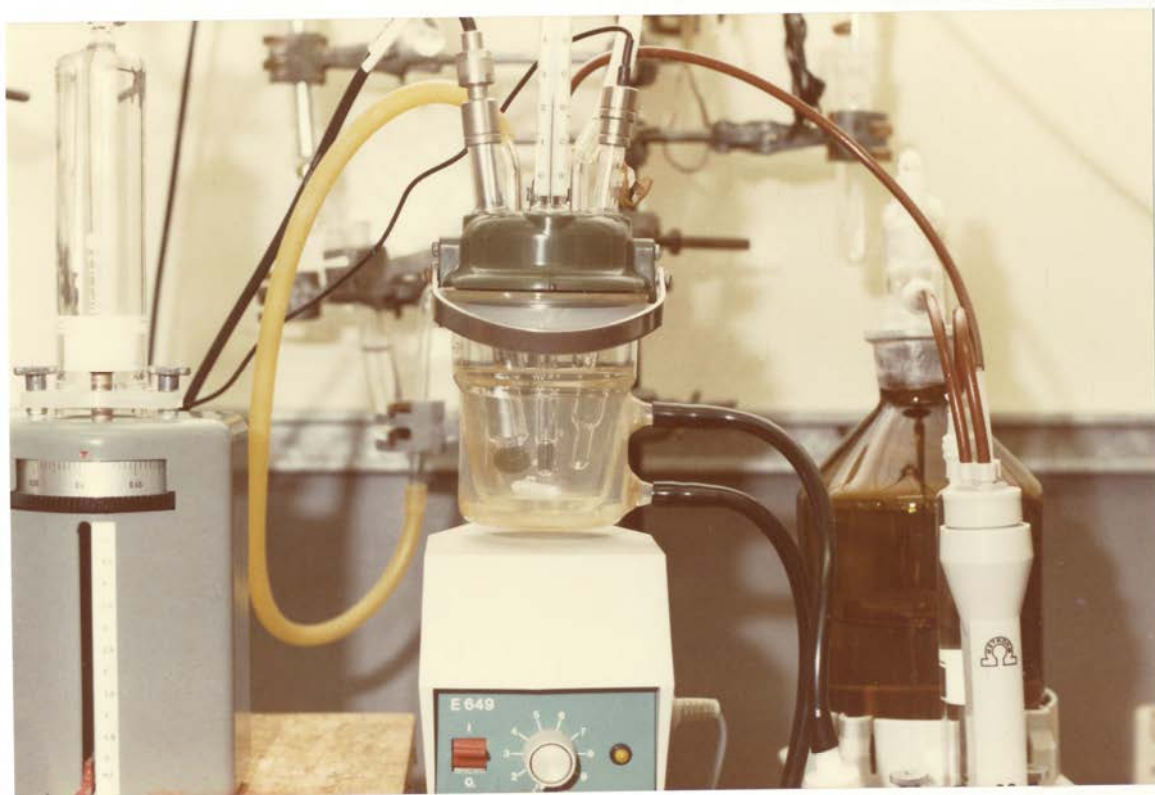
2.3 EXPERIMENTAL

The experimental procedure in this potentiometric study involved the titration of several solutions containing different metal : ligand ratios and concentrations. The protonation constants were determined from solutions containing no metal-ions. The data obtained was in the form of cm^3 , mV and was processed using the computer programs already described.

The chemicals used were all of analytical reagent grade. All the volumetric flasks and pipettes used were calibrated and all the solutions were made up in $0,20 \text{ mol dm}^{-3}$ sodium chloride (Merck ampoules). Sodium hydroxide was prepared (Merck or BDH ampoules) and standardised with hydrogen potassium phthalate (Merck). Ortho-phosphoric acid was prepared from concentrated phosphoric acid (Merck) and standardised by titrating with sodium hydroxide to the second end point. Ammonium chloride (Merck) was accurately prepared by weight and the concentration checked by titration with sodium hydroxide. Hydrochloric acid was prepared from concentrated hydrochloric acid (BDH) and standardized against sodium hydroxide. The magnesium chloride solution was prepared in the following way:

High purity magnesium ribbon (BDH) was cleaned in 20% by volume concentrated hydrochloric acid for a few seconds. The ribbon was then washed in distilled water and initially tissue dried before being placed in a vacuum oven at 40°C to dry fully for 4 hours. The ribbon was then quickly and accurately weighed to six figures before being dissolved in excess hydrochloric acid and made up to volume in a calibrated flask. The acid content of this solution

Figure 2.1 Thermostatted titration vessel and electrode assembly with magnetic stirrer below and automatic burette on the right.



was then standardised with sodium hydroxide. Boiled out glass distilled (carbon dioxide-free) water was used in the preparation of all solutions. Solutions which were not sufficiently acid were protected from carbon dioxide absorption by soda-lime self-indicating granules (BDH) or by a 50% potassium hydroxide solution.

The titrations were performed in a Metrohm (EA876-20) titration vessel using a Metrohm automatic burette (DOSIMAT E635) controlled by a Metrohm automatic titration controller (TITROPROCESSOR E636). A Metrohm glass electrode (EA109) together with a Metrohm calomel reference electrode (EA404) were used to complete the cell with the titroprocessor recording the experimental readings of volume added and emf measured. The calomel electrode's saturated potassium chloride solution was replaced with a saturated sodium chloride solution.

The titration vessel shown in Figure 2.1 was thermostatted at $25^{\circ}\text{C} \pm 0,1^{\circ}\text{C}$ using a HETO thermostat. High purity nitrogen was passed through the titration vessel in order to exclude carbon dioxide and oxygen from the solution. The nitrogen was purified by bubbling through a sequence of gas wash bottles before being admitted to the titration vessel:

- (i) 50% KOH to remove CO_2 ;
- (ii) 15g pyrogallol in 100ml 30% KOH to remove O_2 ;
- (iii) lead wool immersed in an alkaline (200g dm^{-3} KOH) 1% sodium 1,2-naphthaquinone-4-sulphonate solution to remove O_2 ;

Figure 2.2 Nitrogen purification train to remove carbon dioxide and oxygen and to saturate nitrogen with 0,20 mol dm⁻³ sodium chloride. The contents of the train are described in section 2.3. The train runs from right to left in the figure.

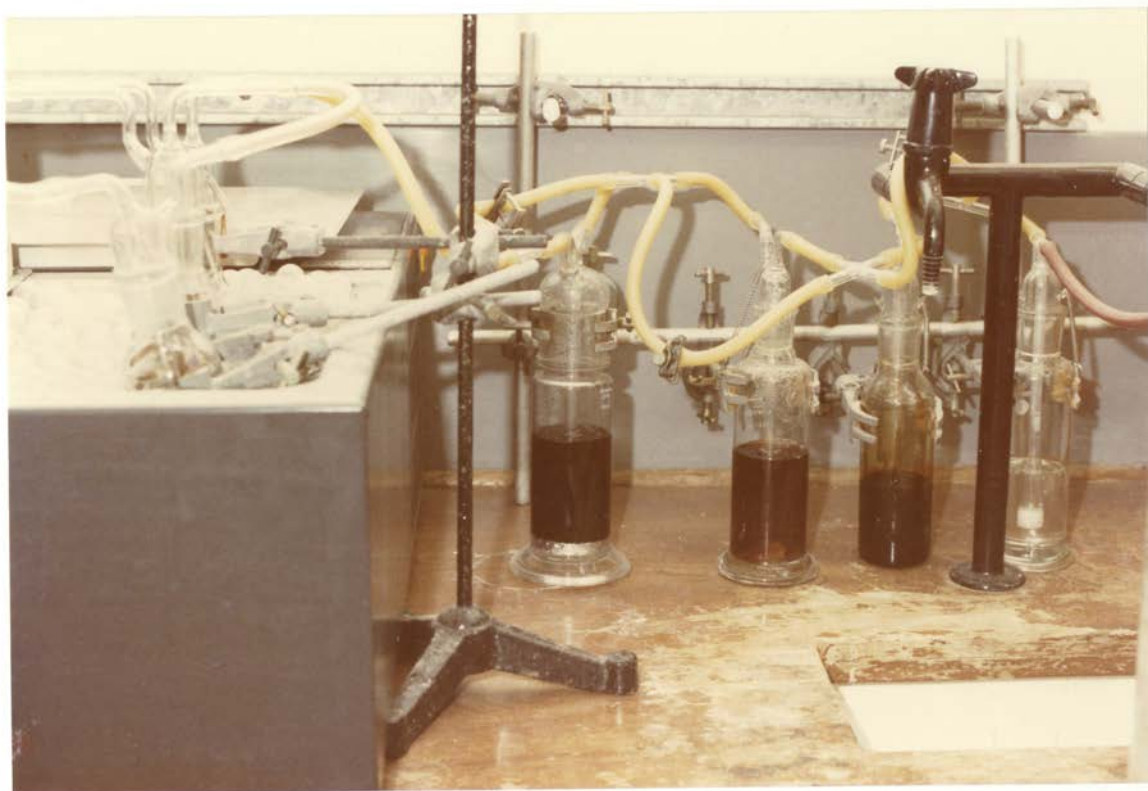


Figure 2.3 Burette and titration assembly showing one automatic and three manual burettes. The functions of the automatic and manual burettes are described in section 2.3.



- (iv) an excess of zinc amalgam in contact with an acidic (50ml dm^{-3} conc. H_2SO_4) chromous/chromic sulphate (300g dm^{-3} $\text{Cr}_2(\text{SO}_4)_3 \cdot 15\text{H}_2\text{O}$) solution to remove O_2 ;
- (v) an empty wash bottle;
- (vi) distilled water thermostatted at 25°C ;
- (vii) a $0,20\text{ mol dm}^{-3}$ NaCl solution thermostatted at 25°C .

Back diffusion after passing through the titration vessel was prevented by releasing the nitrogen to the atmosphere via a $0,20\text{ mol dm}^{-3}$ sodium chloride solution trap. Figure 2.2 shows the nitrogen purification train.

Great flexibility in altering the concentrations of components introduced into the titration vessel was obtained using the burette assembly shown in Figure 2.3. Three Metrohm manual burettes (E274) were calibrated and two of these mounted on jacks. In general, ortho-phosphoric acid was introduced from the unmounted manual burette into the titration vessel. The electrodes were then placed in the solution and sodium hydroxide titrated from the automatic burette with data collection. The titration could then be stopped and quantities of magnesium chloride introduced into the titration vessel without disturbing the contents. This was achieved by lowering the swivel arm of the manual burette into the vessel with the jack, delivering the required volume and raising the arm out again. A solution containing only sodium chloride ($0,2\text{ mol dm}^{-3}$) was kept in the remaining manual burette also mounted on a jack. This was used to adjust the overall concentrations in the vessel before or during the titration.

In this way, initial data collected during the phosphoric acid vs sodium hydroxide titration could be used in electrode calibration and this same solution having had magnesium chloride added could then be used to collect data for metal complexation.

The burette assembly was also extremely useful in meeting the concentration requirements of titrations for analysis via Osterberg's method. This involved titrating with constant metal and varying ligand or constant ligand with varying metal concentrations.

2.4 RESULTS AND OBSERVATIONS

All potentiometric results will be reported here. Observations pertaining to the logical flow of the research will be made while the general discussion of these results will be given in Chapter 4.

The results will be reported in four sections relating to the chemical systems investigated. These are the H-PO_4 , H-NH_3 , $\text{Mg-PO}_4\text{-H}$ and $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ systems. In general, the titrations performed will be listed, followed by electrode calibration results and equilibrium constant refinement data. Finally, the way in which the systems have been characterised will be reported.

Equilibrium constants will be referred to with their stoichiometric coefficients, β_{pqrs} . The order of the coefficients p, q, r, s will be $\text{PO}_4, \text{NH}_3, \text{Mg}, \text{H}$ (or OH). Thus β_{0000-1} is K_w , the ionic product of water.

All reported concentrations will be in units of mol dm^{-3} and represented with square brackets []. Titrations were all duplicated using identical titrating conditions except that two glass electrodes were used, one for each member of the titration pair. Each of these duplicate titration pairs will be referred to using a capital letter. The pH ranges reported are approximate and indicate whether the titration was carried out using hydrochloric acid or sodium hydroxide. The concentration of these titrating solutions was generally about $0,1 \text{ mol dm}^{-3}$ in H^+ or OH^- .

2.4.1 The H-PO₄ System

The titrations performed while investigating this system are listed in Table 2.1.

Table 2.1

Titrations in the H-PO ₄ system		
Titration	[H ₃ PO ₄]	pH Range
A	0,01055	3 - 12
B	0,1070	2 - 12

The electrodes were calibrated over the full pH range of B. Values obtained were:

$$E_O^1 = 410,3 \text{ mV}$$

$$E_O^2 = 416,7 \text{ mV}$$

The logarithms of the overall protonation constants of the H-PO₄ system and K_w are given in Table 2.2.

Table 2.2

Logarithms of overall protonation constants
for the H-PO_4 system and K_w ($0,2 \text{ mol dm}^{-3}$ NaCl
at 25°C).

Titration : B Number of points : 174
R : 0,0007 χ^2 : 23,7
pH range : 2 - 12

Formula	pqrs	$\log \beta$	log std. dev.
HOH	000-1	-13,449	0,001
HPO_4	1001	11,179	0,001
H_2PO_4	1002	17,580	0,001
H_3PO_4	1003	19,264	0,002

All four constants were refined simultaneously from titration B using all points. Titration A yielded similar values, but the pH range was not low enough to allow refinement of a value for 1003. The values obtained in titration A were:

$$\beta_{000-1} = -13,360$$

$$\beta_{1001} = 11,213$$

$$\beta_{1002} = 17,492$$

Higher values for R (0,0058) and χ^2 (102,6) resulted in the choice of the values from titration B.

3.0

Figure 2.5 Experimental complex formation curve for titration B.

Figure 2.4 Theoretical complex formation curve for titration B.

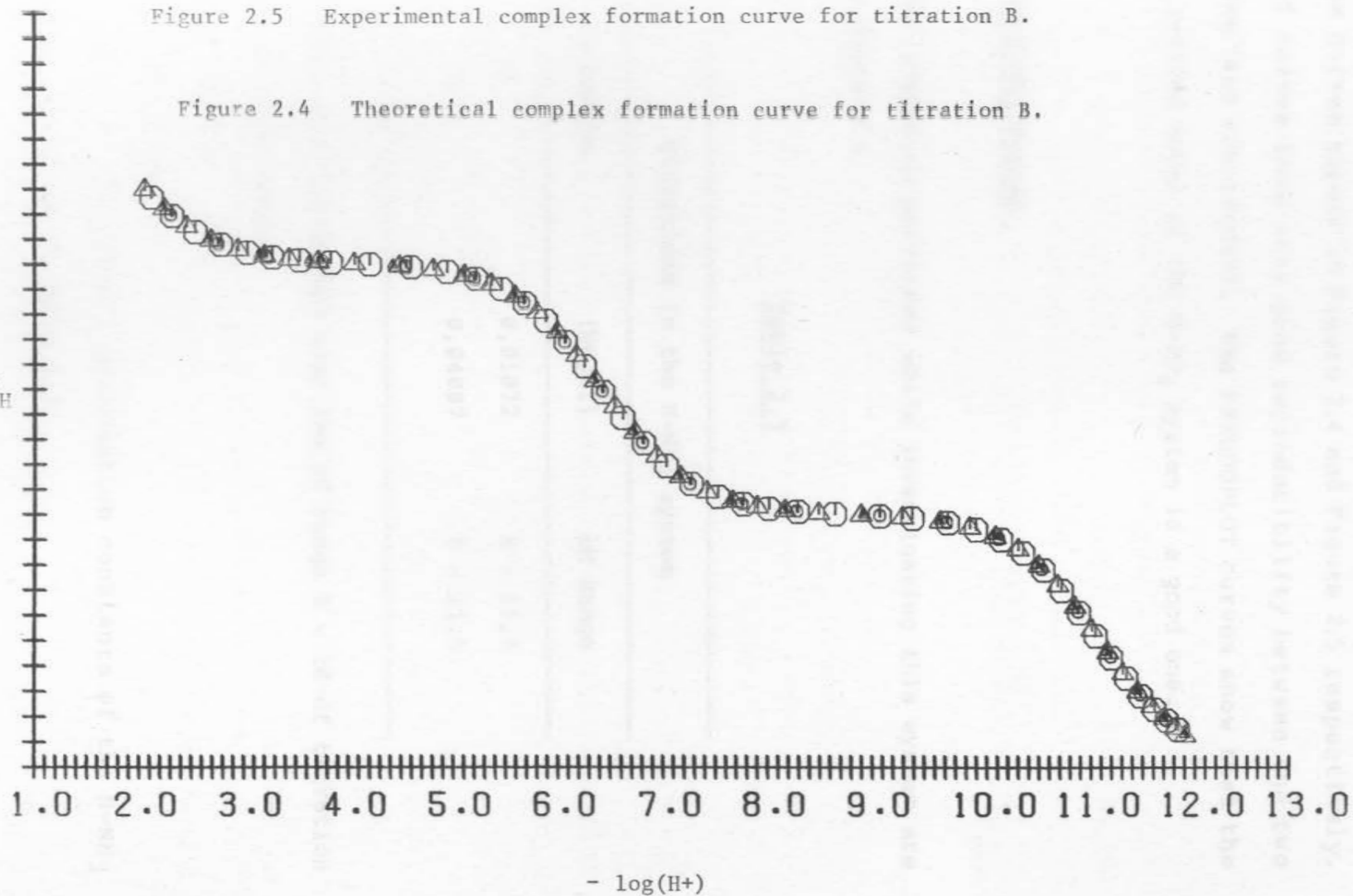
2.0

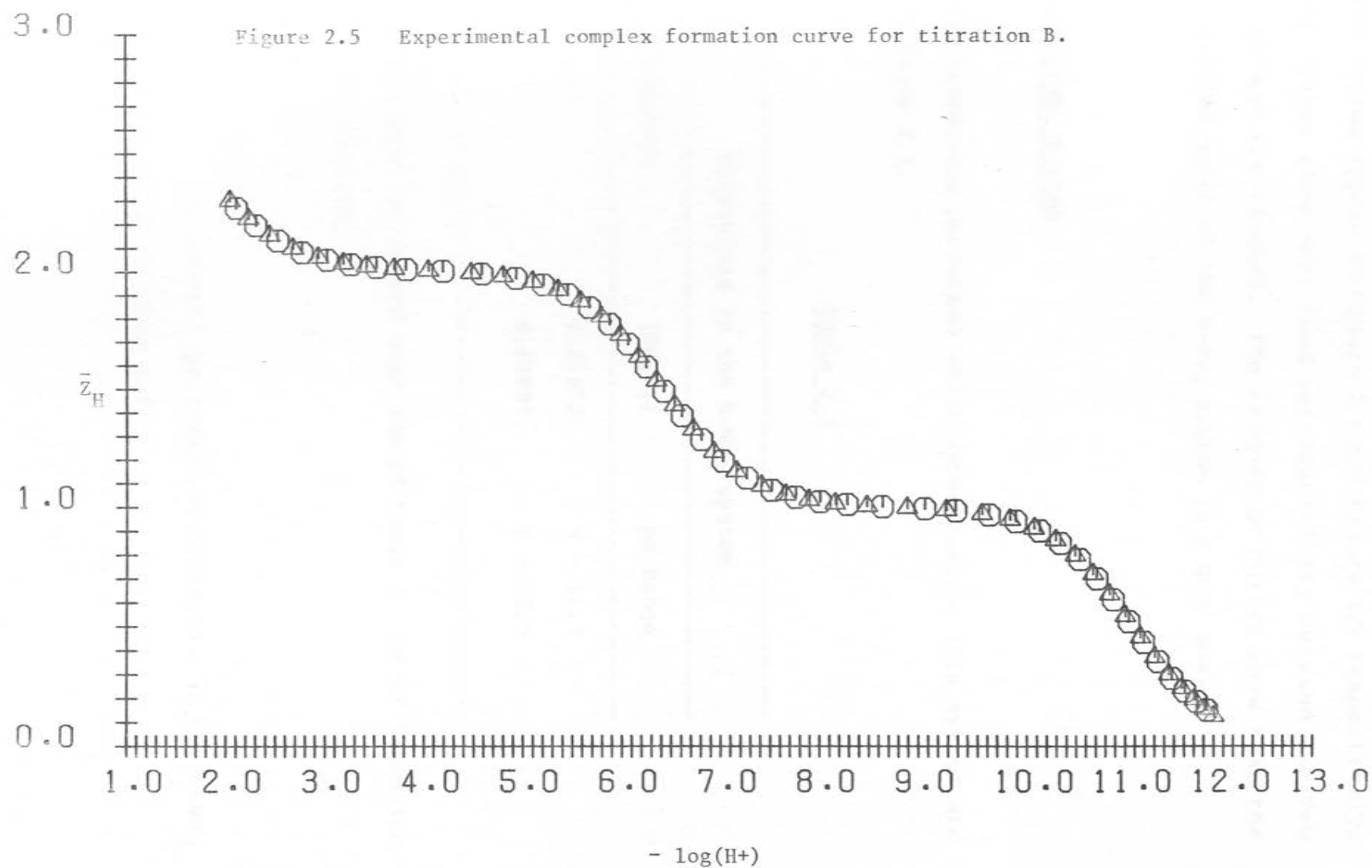
 $\bar{Z}_H$

1.0

0.0

1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0

 $-\log(H^+)$ 



The theoretical (PSEUDOPLOT) and experimental (ZPLOT) formation curves appear in Figure 2.4 and Figure 2.5 respectively. The ZPLOT curves show very good reproducibility between the two titrations (and electrodes). The PSEUDOPLOT curves show that the proposed refined model of the H-PO_4 system is a good one.

2.4.2 The H-NH_3 System

The titrations performed while investigating this system are listed in Table 2.3.

Table 2.3

Titrations in the H-NH_3 system		
Titration	$[\text{NH}_4\text{Cl}]$	pH Range
C	0,01072	8 - 11,5
D	0,04007	8 - 11,5

The electrodes were calibrated over the pH range 8 - 10 of titration D. Values obtained were:

$$E_O^1 = 399,6 \text{ mV}$$

$$E_O^2 = 405,2 \text{ mV}$$

The logarithms of the overall protonation constants of the H-NH_3 system and K_w are given in Table 2.4.

Table 2.4

Logarithms of the overall protonation constants of the H-NH ₃ system and K _w (0,2 mol dm ⁻³ NaCl, at 25°C)			
Titration : D		Number of points : 98	
R : 0,0021		χ^2 : 79,8	
pH Range : 8 - 11,5			
Formula	pqrs	log β	log std. dev.
HOH	000-1	-13,469	0,001
HNH ₃	0101	9,060	0,001

Both constants were refined simultaneously from titration D using all points. Titration C yielded similar values. The values obtained from titration C were:

$$\beta_{0000-1} = -13,480$$

$$\beta_{0101} = 9,058$$

A higher value for R (0,0052) led to the choice of titration D.

The close agreement between log K_w values refined from the two different systems is reassuring and lends confidence to our experimental technique.

$$\log K_w (\text{H-PO}_4) = -13,449$$

$$\log K_w (\text{H-NH}_3) = -13,469$$

Figure 2.7 Experimental complex formation curve for titration D.

Figure 2.6 Theoretical complex formation curve for titration D.

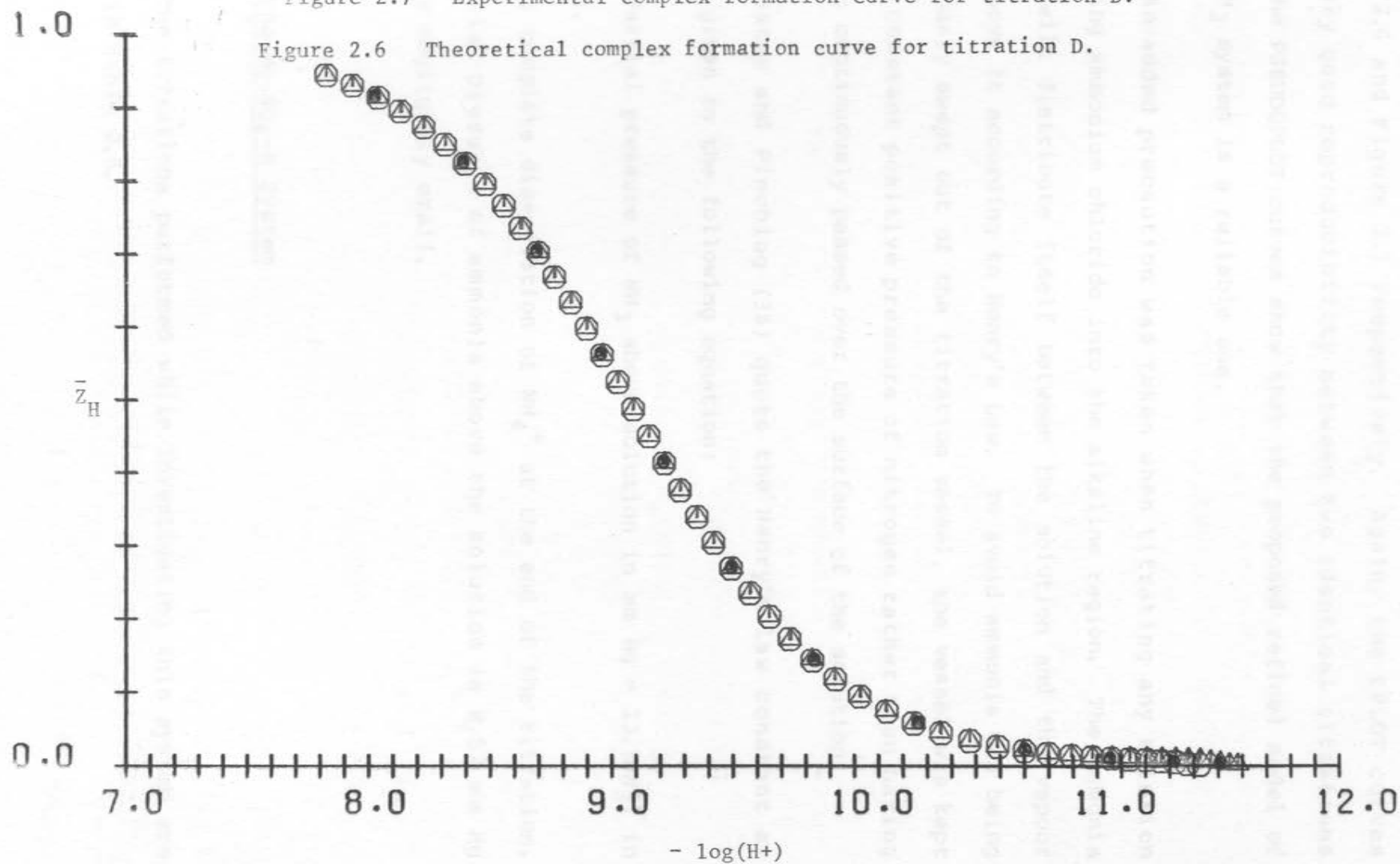
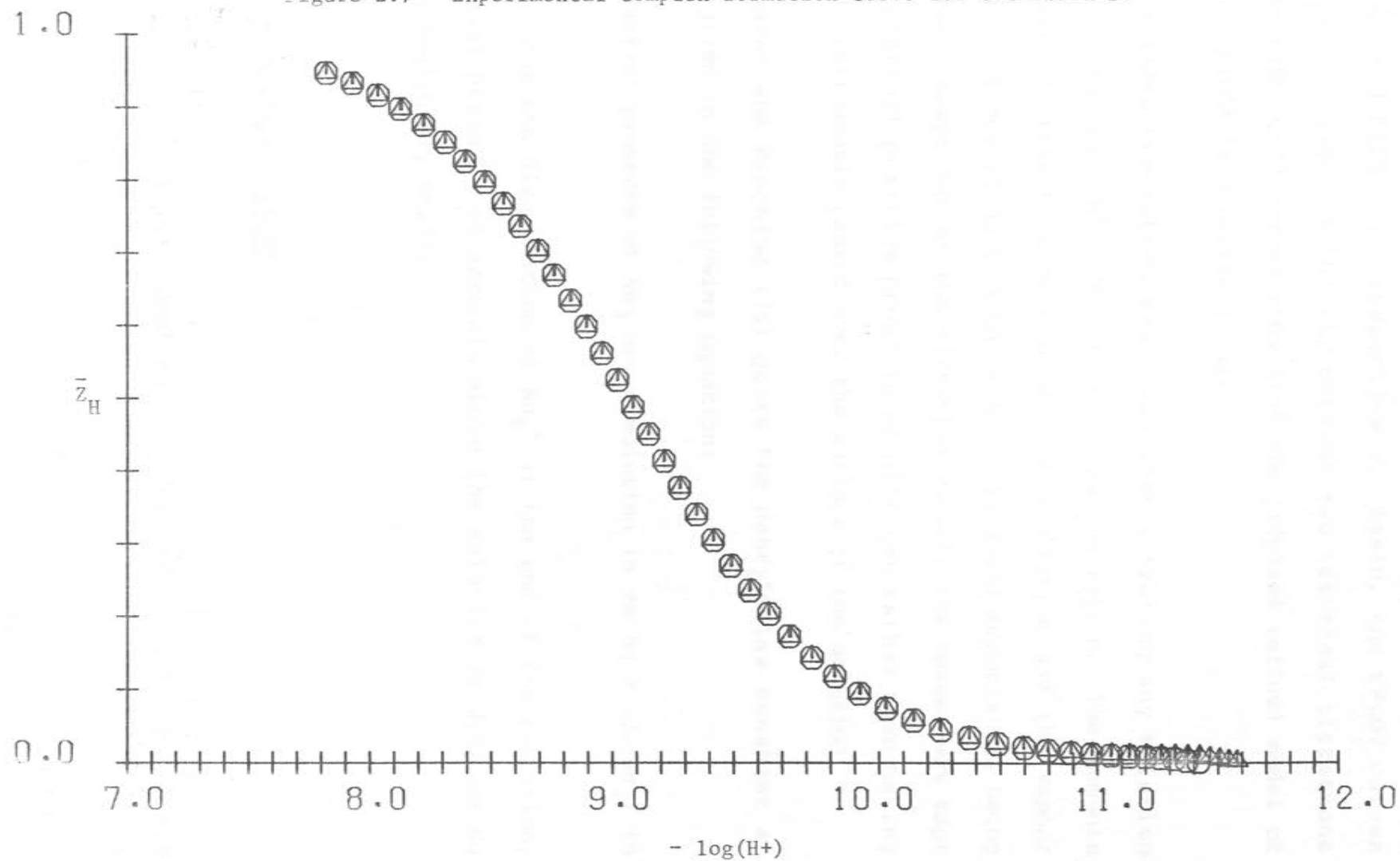


Figure 2.7 Experimental complex formation curve for titration D.



The theoretical and experimental formation curves appear in Figure 2.6 and Figure 2.7 respectively. Again, the ZPLOT curves show very good reproducibility between two identical titrations while the PSEUDOPLOT curves show that the proposed refined model of the H-NH₃ system is a reliable one.

An added precaution was taken when titrating any solution containing ammonium chloride into the alkaline region. The ammonia formed will distribute itself between the solution and the vapour phase above it according to Henry's Law. To avoid ammonia gas being continuously swept out of the titration vessel, the vessel was kept under a constant positive pressure of nitrogen rather than having nitrogen continuously passed over the surface of the solution.

Bates and Pinching (35) quote the Henry's law constant at 25°C as given in the following equation:

Partial pressure of NH₃ above solution in mm Hg = 13.[NH₃] in solution.

Assuming complete dissociation of NH₄⁺ at the end of the titration, the partial pressure of ammonia above the solution is 0,52 mm Hg which is negligibly small.

2.4.3 The Mg-PO₄-H System

The titrations performed while investigating this system are listed in Table 2.5.

Table 2.5

Titrations in the Mg-PO ₄ -H system				
Titration	L:M ratio	[H ₃ PO ₄]	[MgCl ₂]	pH Range
E	1:1	0,004964	0,004727	3 - 9,5
F	2:1	0,006394	0,003044	3 - 9,7
G	5:1	0,007734	0,001473	3 - 9,5
H	0,5:1	0,003429	0,006531	3 - 9,3
I	10:1	0,02252	0,002167	6,8 - 3
J	10:1	0,02252	0,002167	3 - 9,4
K	7:1	0,02252	0,003250	3 - 9,3
L	5:1	0,02252	0,004333	3 - 9,2
M	4:1	0,02252	0,005416	3 - 8,8
N	3:1	0,02252	0,007583	3 - 8,5
P	10:1	0,02252	0,002167	6,8 - 3
Q	7:1	0,01576	0,002167	6,8 - 3
R	5:1	0,01126	0,002167	6,8 - 3
S	4:1	0,009006	0,002167	6,8 - 3
T	3:1	0,006775	0,002167	6,8 - 3

All these titrations were carried out in such a way as to allow a preliminary titration with no metal present between pH 2 and 3. The E₀'s calibrated from this data produced experimental formation curves which became negative between pH 5 and 8. After a

great deal of frustration, it was realised that the E_o 's obtained between pH 2 and 3 were substantially different from E_o 's calibrated between pH 3 and 10.

Fortunately, data was available from phosphoric acid standardisation titrations which covered the pH range between 3 and 10. E_o 's were thus obtained using this data and adjustments made according to the variations in "incorrect" E_o 's obtained for the individual titrations. This allowed for small variations in the standard potential of the glass membrane from time to time (28).

It is important to realise that this "non-linear" response of the electrodes below pH 3 introduces some uncertainty into the value of the protonation constant β_{1003} . The H_3PO_4 species occurs mainly below pH 3. However, this uncertainty should not be carried on to further constants measured above pH 3 even if the β_{1003} constant remains in all subsequent calculations as a fixed value. The equilibrium constant β_{1003} was therefore fixed at the value calculated in section 2.4.1 for all subsequent calculations.

The electrodes were calibrated over the pH range 3 - 10 as follows:

Titration EF GH : $E_o^1 = 406,1 \text{ mV}$

$E_o^2 = 412,0 \text{ mV}$

Titration I to T : $E_o^1 = 406,8 \text{ mV}$

$E_o^2 = 411,6 \text{ mV}$

The logarithms of the overall stability constants of the $Mg-PO_4-H$ system are given in Table 2.6.

Table 2.6

Logarithms of the overall stability constants of the
Mg-PO₄-H system (0,2 mol dm⁻³ NaCl at 25°C)

Titration : EFHLMNPQRS

Number of points : 387

R : 0,0012

χ^2 : 392

pH Range : 3 - 8

Formula	pqrs	log β	log std. dev.
MgHPO ₄	1011	13,12	0,08
MgH ₂ PO ₄	1012	19,09	0,13

Titration : EFHLMN

Number of points : 109

R : 0,0009

χ^2 : 53

pH Range : 8 - 10

Formula	pqrs	log β	log std. dev.
MgPO ₄	1010	3,13	0,01

Titration : LMN

Number of points : 47

R : 0,0004

χ^2 : 19

Formula	pqrs	log β	log std.dev.
MgOH	001-1	-10,38	0,04

Five titration pairs, G, I, J, K and T were rejected on the basis of poor reproducibility of the experimental formation curves. This was in fact, an extremely stringent test since it will be shown that

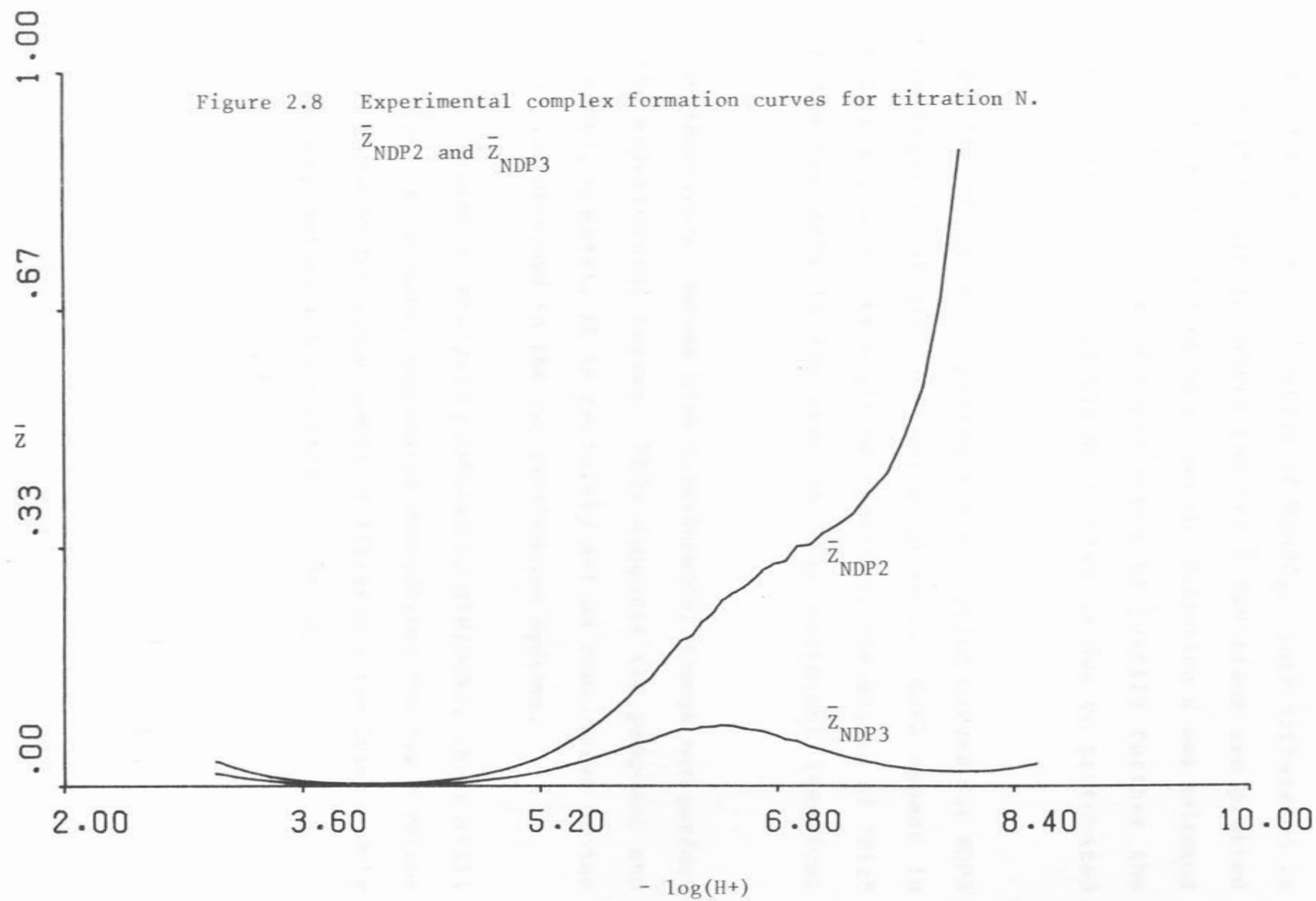
the $\bar{Z}$ function is very sensitive to the value of E_0 . The remaining ten titration pairs formed a large set of data collected over a wide range of experimental conditions. This covered a pH range of 3 to about 10, a $[\text{H}_3\text{PO}_4]$ range of 0,003 to 0,022, a $[\text{MgCl}_2]$ range of 0,002 to 0,008 and a L : M ratio range of 0,5:1 to 10:1.

The data was divided into two sets, that below pH 8 and that above pH 8. This division was made on the basis of the formation curves which showed a hump below pH 8 and a small rise above pH 8. The hump was assumed to be the result of protonated species, a reasonable assumption when we consider the pH ranges involved.

All data below pH 8 was used successfully to refine for the two protonated species MgHPO_4 and MgH_2PO_4 . All data above pH 8 (titrations P, Q, R, S did not extend this far) were used to refine for the species MgPO_4 . A subset of the data above pH 8 was used to refine for the species MgOH . The values of β_{1011} and β_{1012} obtained in the low pH range were fixed in the refinement of β_{1010} . All three of these values were fixed in the refinement of β_{001-1} .

It should be emphasized that an enormous amount of computation was carried out on this data before the final set of stability constants was arrived at. The simplicity of the final refinements in no way reflects the difficulties experienced in achieving this simplicity.

The formation curves for this system are presented in two forms. Setting the number of dissociable protons on H_3PO_4 equal to 3 (NDP3) will focus on the formation of the complex MgPO_4 .



Alternatively, setting the number of dissociable protons equal to 2 (NDP2) will focus of the formation of MgHPO_4 . This difference is highlighted in Figure 2.8 where the two $\bar{Z}$ functions are plotted against pH in order that they be compared. Titration N was selected for this purpose. The NDP2 curve seems to justify further the assumption that the hump in the NDP3 curve is due to protonated species.

The theoretical and experimental formation curves for NDP3 appear in Figures 2.9 and 2.10 while those for NDP2 appear in Figures 2.11 and 2.12. As would be expected, the degree of "fit" between the two sets is the same as they represent identical systems.

The theoretical curves give a reasonable, though not perfect fit to the experimental curves. This supports the proposed and refined model, however, it is certainly not as conclusive as the perfect fitting obtained in the two protonation systems.

The statistical analysis produced by MINQUAD, while still not conclusive, is certainly impressive considering the low R values obtained in spite of the large number of titrations involving widely different concentrations and concentration ratios.

Figure 2.10 Experimental complex formation function, $\bar{Z}_{\text{NDP3}}$, for titrations F, 0; N, Δ and R, +.

Figure 2.9 Theoretical complex formation function, $\bar{Z}_{\text{NDP3}}$, for titrations F, 0; N, Δ and R, +.

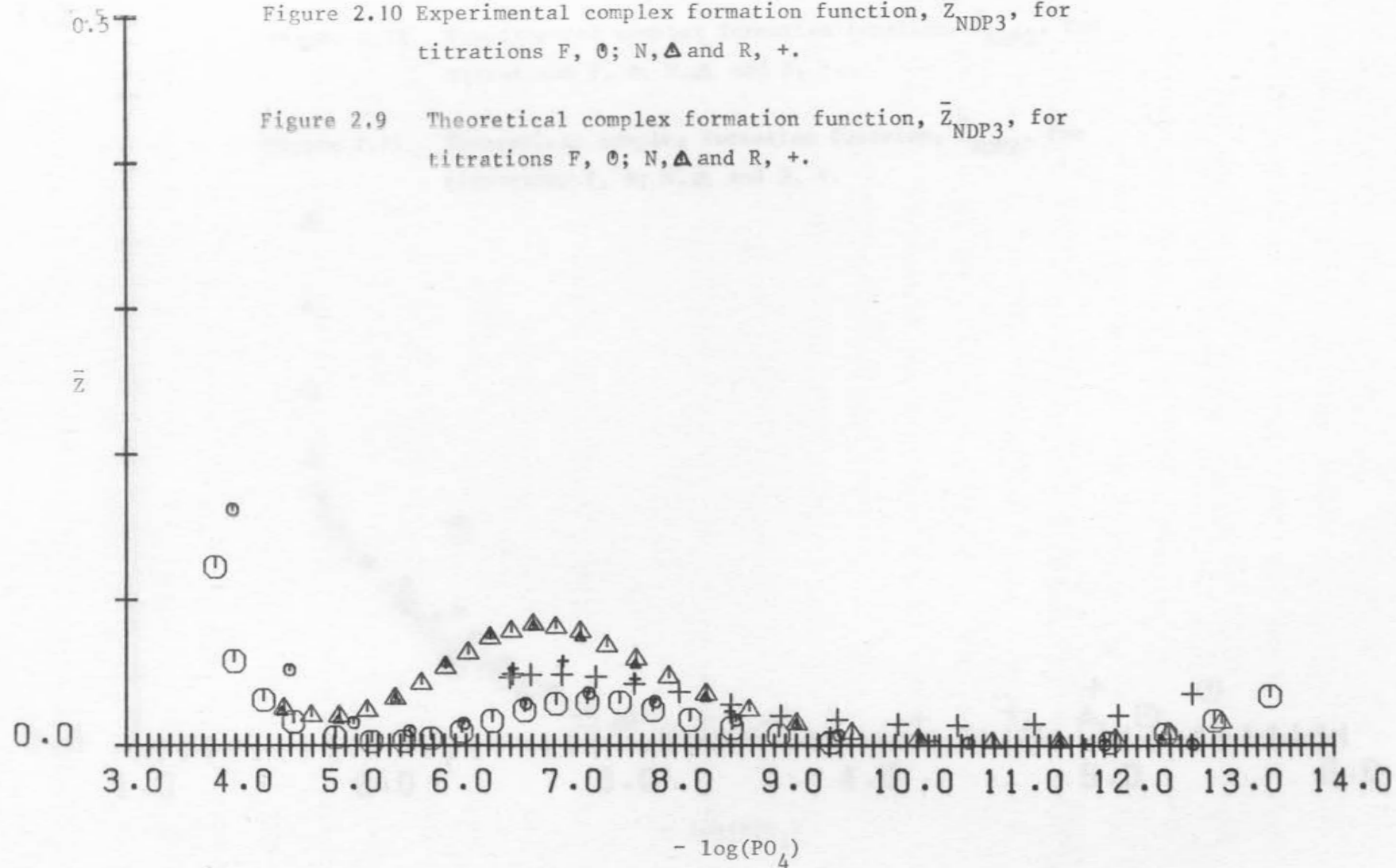
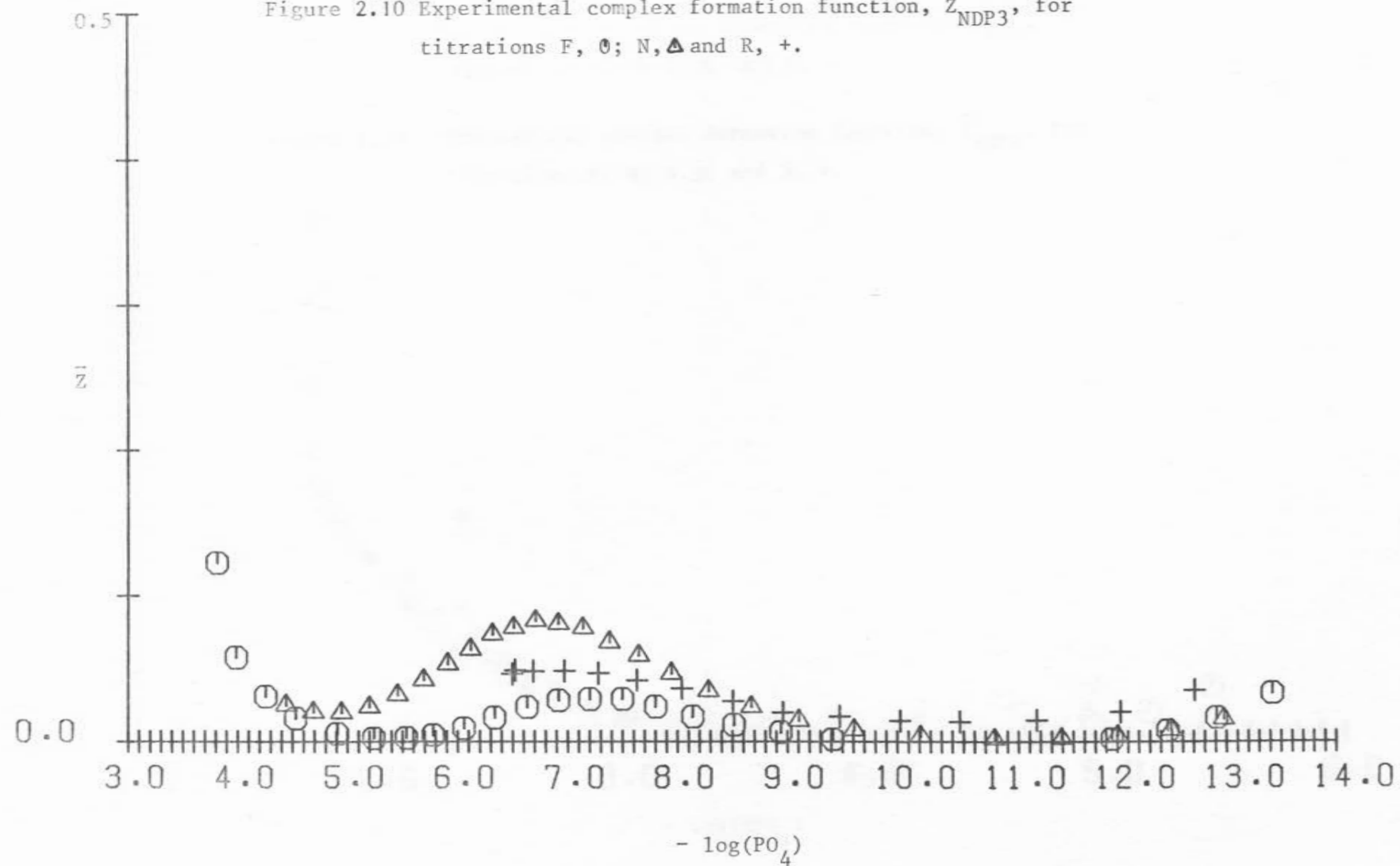


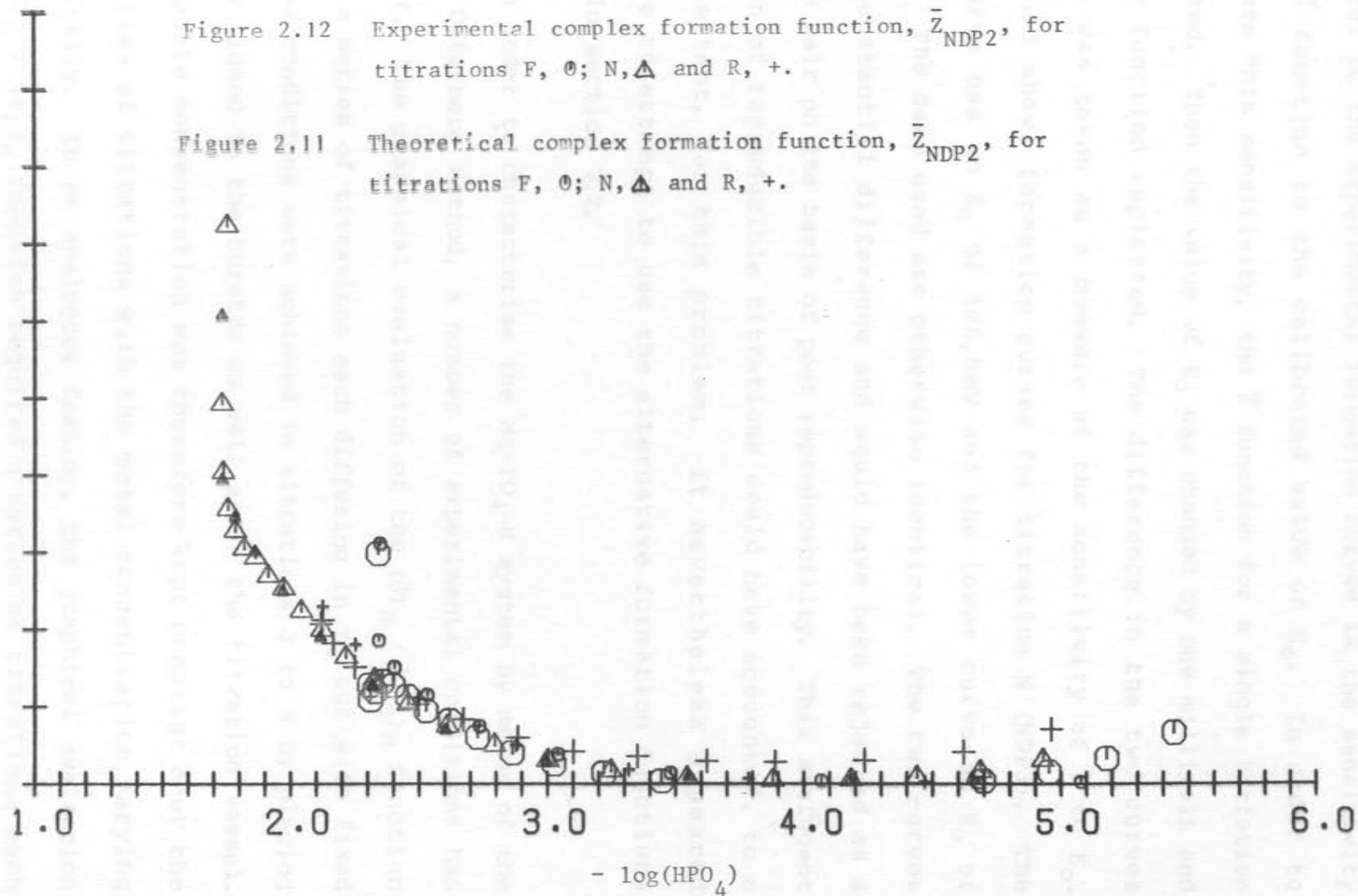
Figure 2.10 Experimental complex formation function, $\bar{z}_{\text{NDP3}}$, for
 titrations F, 0; N, Δ and R, +.



1.0

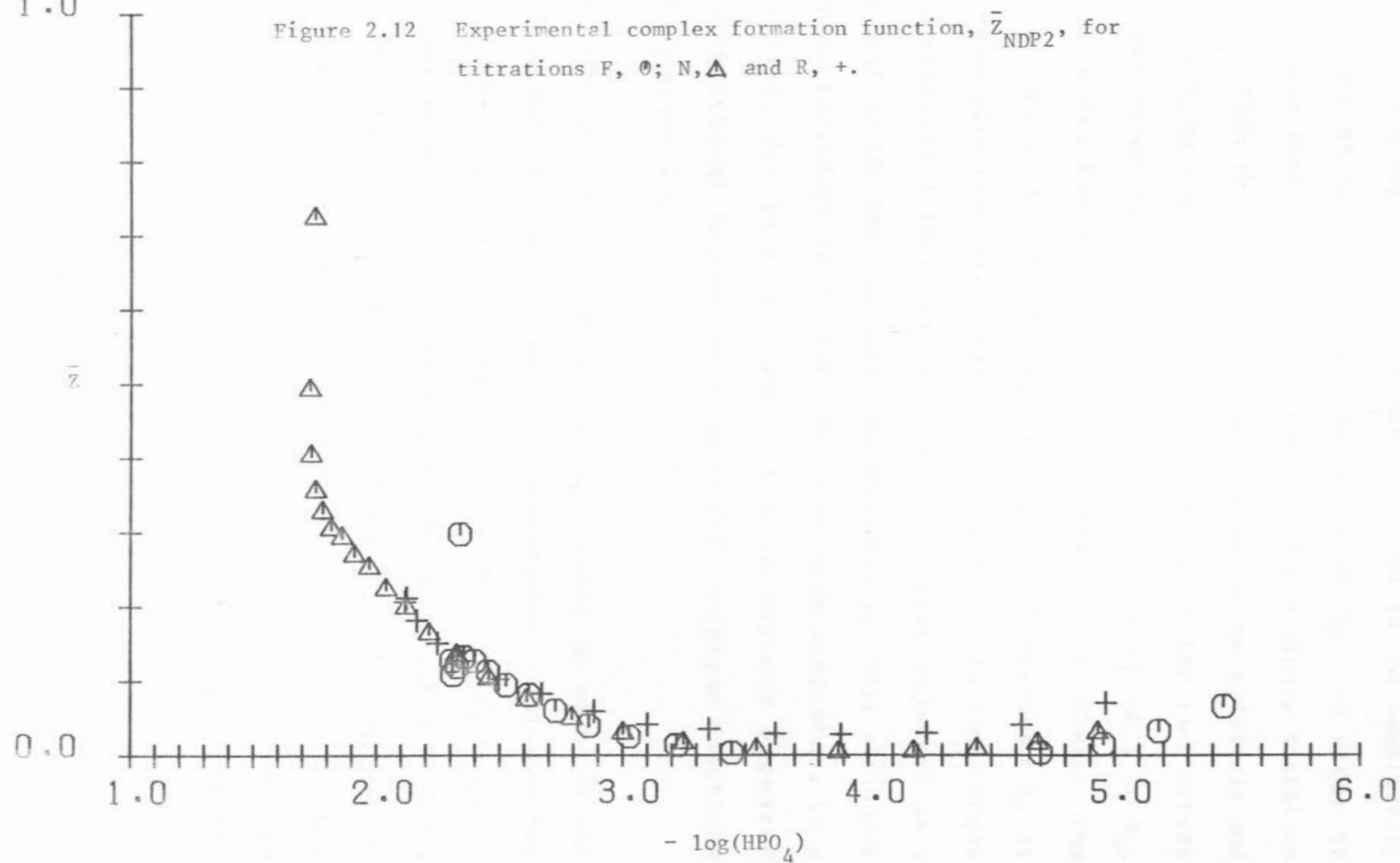
 $\bar{Z}$

0.0

 $-\log(\text{HPO}_4)$

1.0

Figure 2.12 Experimental complex formation function, $\bar{z}_{\text{NDP2}}$, for titrations F, $\circ$; N, Δ and R, $+$.



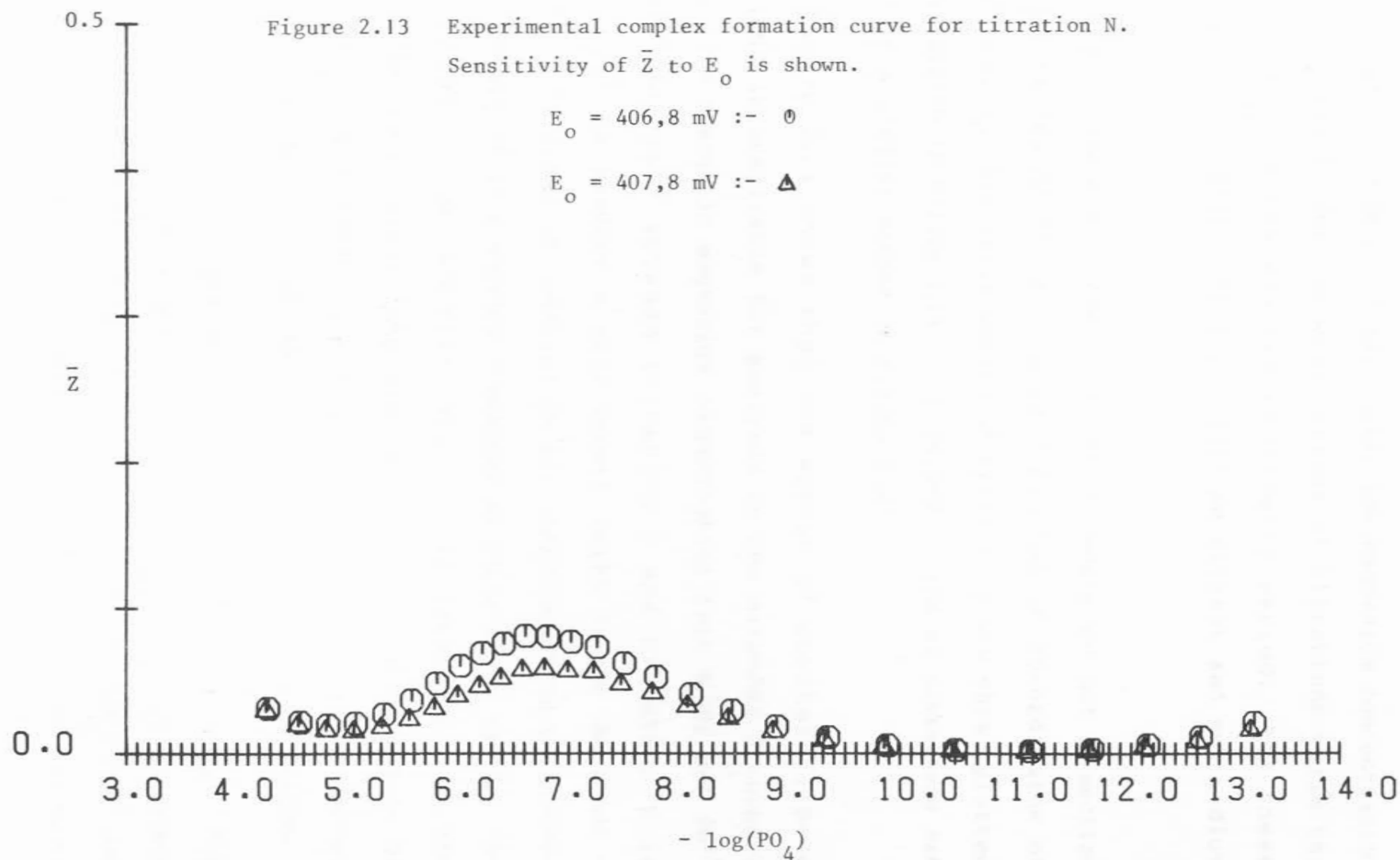
One possible reason for the imperfect fitting of the theoretical to the experimental formation curves is the sensitivity of the $\bar{Z}$ function to the calibrated value of E_0 . In order to investigate this sensitivity, the $\bar{Z}$ function for a single titration was plotted. Then the value of E_0 was changed by one millivolt and the same function replotted. The difference in the two curves obtained was taken as a measure of the sensitivity of $\bar{Z}$ to E_0 . Figure 2.13 shows formation curves for titration N (NDP3). The upper curve has an E_0 of 406,8mV and the lower curve an E_0 of 407,8mV. The data used are otherwise identical. The two curves show a substantial difference and would have been rejected as a titration pair on the basis of poor reproducibility. This stringent selection of reproducible titrations would have accounted, to a certain extent, for this problem. It nevertheless appeared desirable to attempt to use the alternative formation functions derived in section 2.2.

In order to characterise the $Mg-PO_4-H$ system by means of the extended Osterberg method, a number of experimental conditions had to be met. The graphical evaluation of the $(\partial T_H / \partial T_M)_H$ function required a series of titrations each differing in T_M but with fixed T_L . These conditions were achieved in titrations J to N by placing phosphate ligand in the burette as well as in the titration vessel. The phosphate concentration was therefore kept constant over the whole series of titrations with the metal concentration varying systematically. In an analogous fashion, the graphical evaluation of the $(\partial T_H / \partial T_L)_H$ function required a series of titrations each differing in T_L but with fixed T_M . These conditions were achieved

Figure 2.13 Experimental complex formation curve for titration N.
Sensitivity of $\bar{Z}$ to E_o is shown.

$$E_o = 406,8 \text{ mV} :- \bigcirc$$

$$E_o = 407,8 \text{ mV} :- \triangle$$

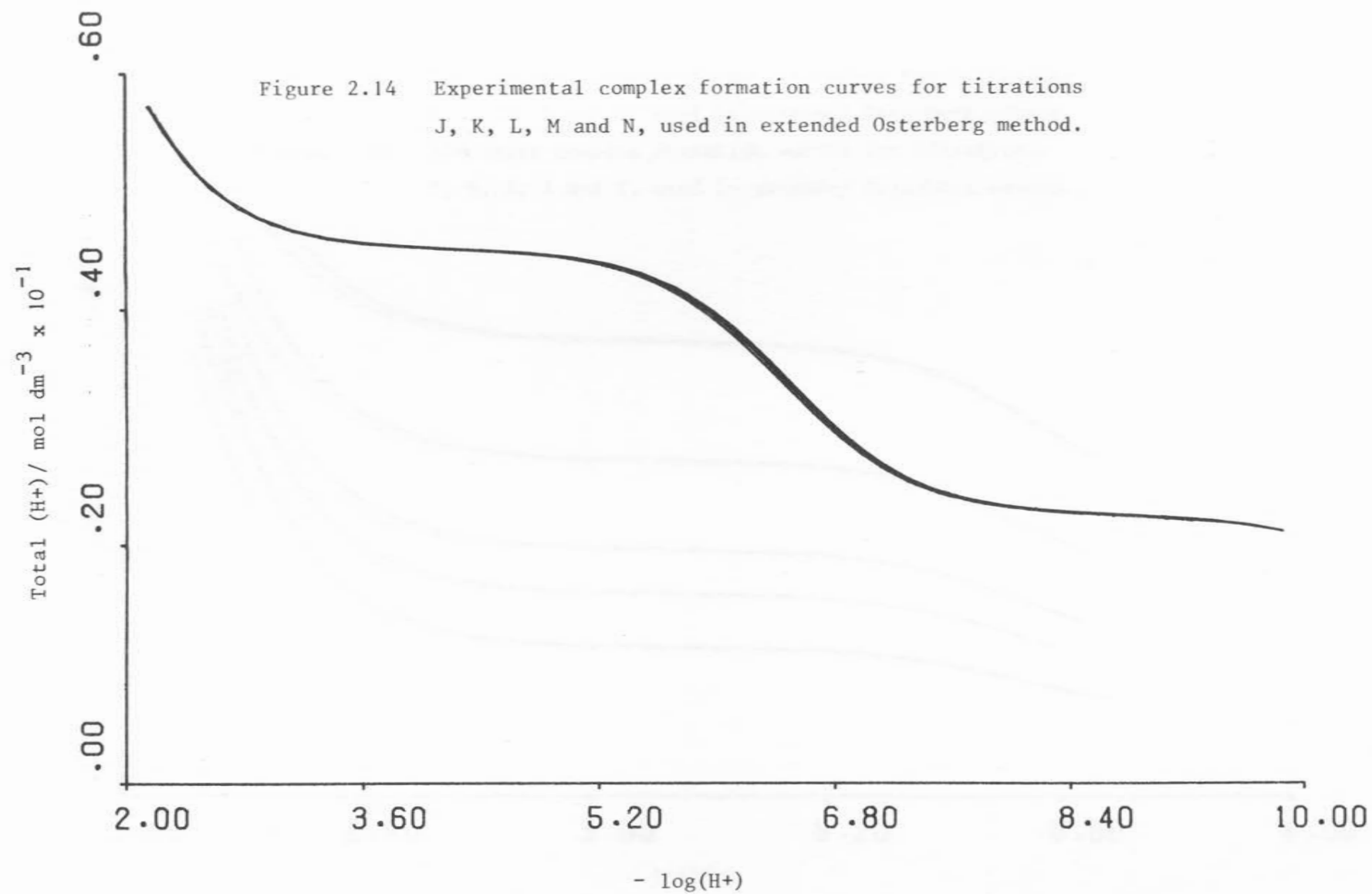


in titrations P to T by placing magnesium in the burette as well as in the titration vessel. In this case, the magnesium concentration was kept constant over the whole series of titrations while the ligand concentration was systematically varied. For these titrations hydrochloric acid was used as titrant and not sodium hydroxide.

In accordance with the graphical procedure set out in section 2.2, both series of titrations were plotted on co-ordinates of $-\log[H^+]$ vs. T_H . The first series of titrations are shown plotted in this fashion in Figure 2.14. The second series of titrations are plotted in a similar manner in Figure 2.16.

Figure 2.14 shows that the series of constant ligand titrations is unsuitable for analysis by the extended Osterberg method. The change in magnesium concentration from $0,002 \text{ mol dm}^{-3}$ to $0,007 \text{ mol dm}^{-3}$ between titration J and titration N is insufficient to produce a significant shift in pH (here $\text{pH} = -\log[H^+]$). If lines of constant pH are constructed on the curves and the points of intersection displayed as plots of T_H vs. T_M , the resulting set of lines are coincident. It appears then, that the changing degree of metal complexation is not large enough to be detected by this method, under the limited range of metal concentrations that could be used without incurring precipitation.

Figure 2.16 at first glance appears more promising. The curves shift substantially as the ligand concentration is decreased from $0,020 \text{ mol dm}^{-3}$ to $0,007 \text{ mol dm}^{-3}$ between titration P and titration T. As shown below, however, the shifts are almost fully



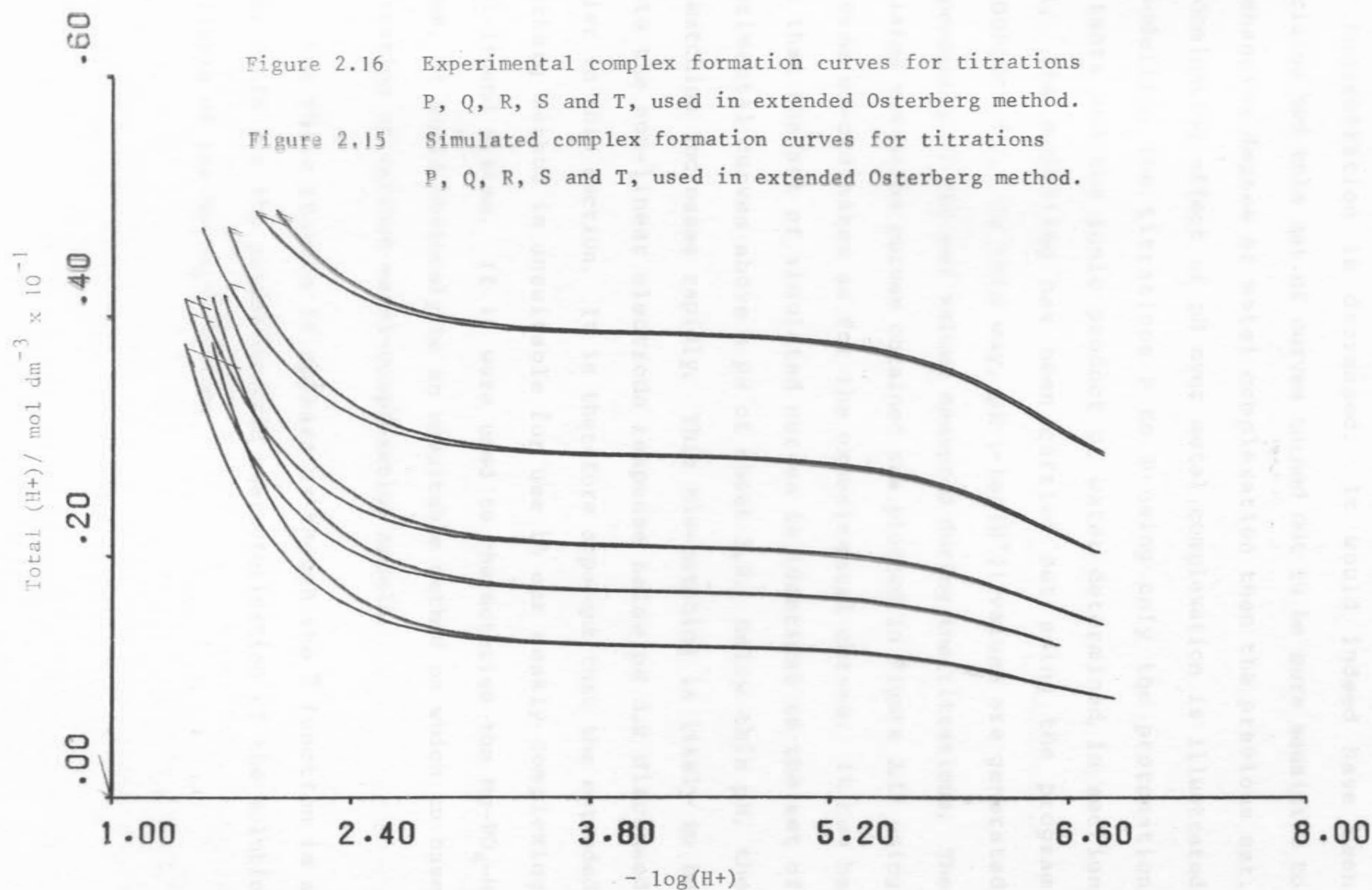
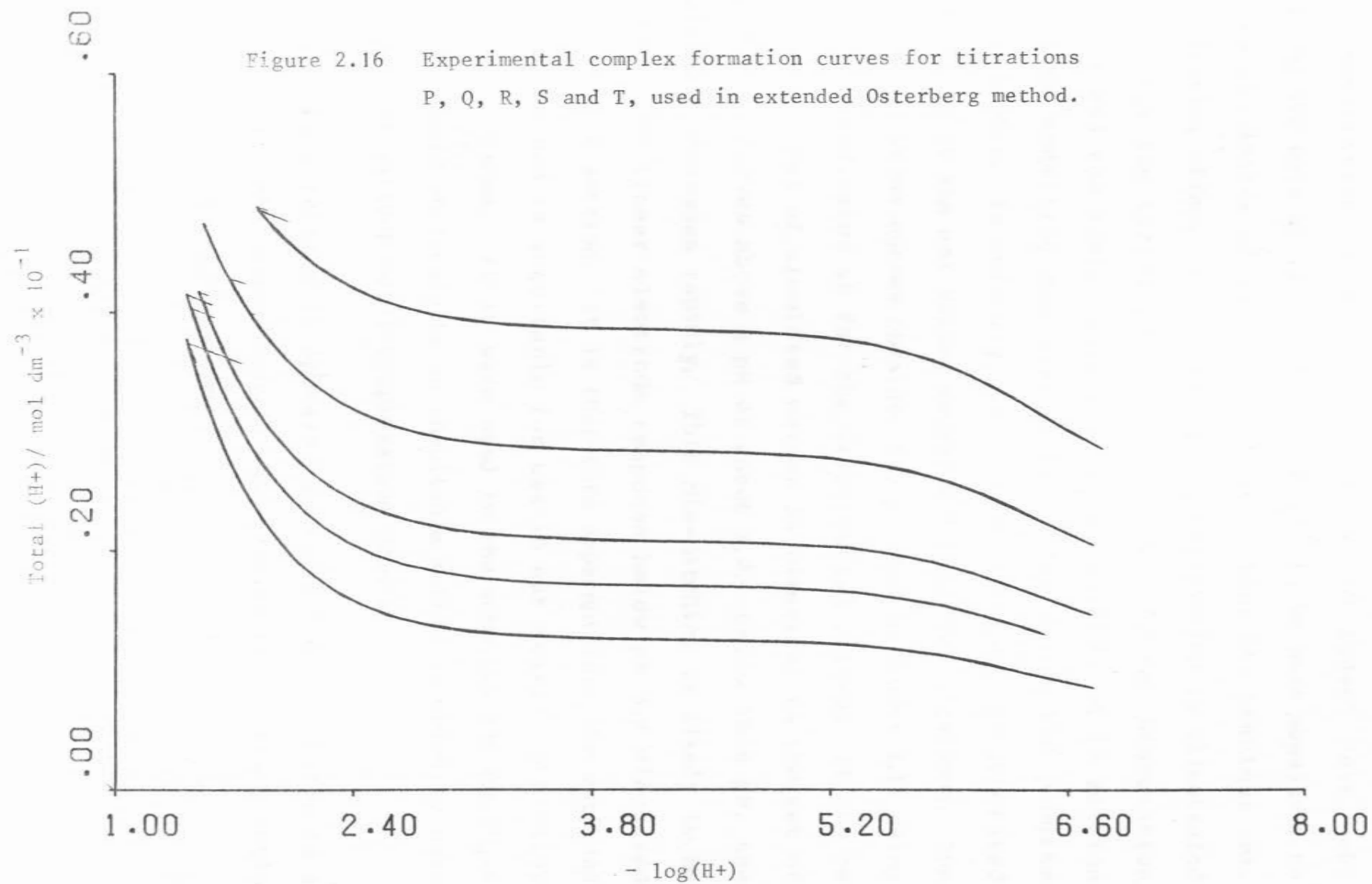


Figure 2.16 Experimental complex formation curves for titrations P, Q, R, S and T, used in extended Osterberg method.



accounted for by the change in acidity experienced as the phosphoric acid concentration is decreased. It would indeed have been surprising had this set of curves turned out to be more sensitive to the changing degree of metal complexation than the previous set. The dominating effect of pH over metal complexation is illustrated by modelling the titrations P to T using only the protonation constants and the ionic product of water determined in section 2.4.1. The modelling has been carried out using the program PSEUDOPLOT (61). In this way, pH ($-\log[H^+]$) values are generated independently of the emf values measured during the titrations. The simulated titration curves obtained are plotted in Figure 2.15 using the same co-ordinates as for the experimental curves. It can be seen that the set of simulated curves is identical to the set of experimental curves above a pH of about 3.0. Below this pH, the mis-matching increases rapidly. This mis-matching is likely to be due to the non-linear electrode response below pH 3.0 discussed earlier in this section. It is therefore apparent that the extended Osterberg method is unsuitable for use in our weakly complexing metal-ligand system. If it were used to characterise the $Mg-PO_4-H$ system, it would obviously be an unsuitable method on which to base the testing of various metal-complexation models.

For these reasons it appears as though the $\bar{Z}$ function is a better basis for the potentiometric characterisation of the solution equilibria of the $Mg-PO_4-H$ system.

2.4.4. The Mg-PO₄-NH₃-H System

The complexation of magnesium with phosphate and its protonated forms has been shown in section 2.4.3. to be fairly weak. This observation is supported by the relatively low values of the stepwise stability constants and the related formation function maxima. These values as presented in section 2.4.3 are reproduced in Table 2.7.

Table 2.7

Measured values of stepwise stability constants of the Mg-PO₄-H system determined in 0,20 mol dm⁻³ NaCl at 25°C, and the related formation function maxima (section 2.4.3.).

Log K ₁₀₁₀ = 3,13	$\bar{z}_{NDP3} < 0,1$
Log K ₁₀₁₁ = 1,94	$\bar{z}_{NDP2} < 0,5$
Log K ₁₀₁₂ = 1,51	

Reported values (34, 39, 63) of the complexation of magnesium with ammonia are considerably smaller than the stepwise stability constants of the Mg-PO₄-H system given in Table 2.7. These values and the conditions at which they were determined are given in Table 2.8. A literature survey revealed no equilibrium constants for the complexation of ammonia with phosphate or the complexation of magnesium with ammonia and phosphate.

Table 2.8

Literature values of stepwise stability constants of the Mg-NH₃-H system determined at various experimental conditions.

Reference	63	34	35
Medium	Dilute	2,0 mol dm ⁻³ NH ₄ NO ₃	2,4 mol dm ⁻³ (NH ₄) ₂ SO ₄
Temperature	25°C	23°C	0°C
log K ₀₁₁₀	-0,10	0,23	0,50
log K ₀₂₁₀		-0,15	-0,04
log K ₀₃₁₀		-0,42	-0,38
log K ₀₄₁₀		-0,70	
log K ₀₅₁₀		-0,95	
log K ₀₆₁₀		-1,30	

In the presence of equal concentrations of two ligands K and L, the mixed complex MKL is statistically more favoured than MK₂ or ML₂ (64). Mixed complexes of magnesium with both phosphate and ammonia could be expected if magnesium formed bis ligand complexes with phosphate and ammonia respectively. However, magnesium has been shown (Table 2.7) to form only mono ligand complexes with phosphate and the bis ligand magnesium ammonia complex has been shown (Table 2.8) to be extremely weak. This complex is not observed in dilute solutions (63), the Mg(NH₃) species being the only one present in solutions which are not very concentrated with respect to ammonia. For these reasons, it seemed unlikely that magnesium would form strong mixed ligand complexes with phosphate

and ammonia. Indeed, it would appear that the addition of ammonia to a magnesium phosphate solution would not significantly alter the magnesium equilibria with phosphate, the only additional equilibrium would be that of ammonia with protons.

For the purposes of the urine model (section 3.3) and due to the lack of a suitable mixed ligand complex formation function (section 2.4.3) it appeared feasible to investigate the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system in an alternate fashion, as decided below.

A series of titrations were performed. The first titration contained magnesium and phosphate. Subsequent titrations contained the same concentrations of magnesium and phosphate but with increasing concentrations of ammonia. In this way it was hoped to show that the equilibrium constants established in sections 2.4.1, 2.4.2 and 2.4.3 could describe the magnesium-ammonia-phosphate titrations as adequately as they could describe the magnesium-phosphate titrations. The MINQUAD R-factor was chosen as the basis for the comparison. Obviously, all nine equilibrium constants from the H-PO_4 , H-NH_3 and $\text{Mg-PO}_4\text{-H}$ systems would be used to model the titrations containing both ammonia and phosphate, while the protonation constant for ammonia would be left out when the magnesium phosphate titration was modelled.

The titrations performed while investigating the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system are listed in Table 2.9.

Table 2.9

Titrations in the Mg-PO ₄ -NH ₃ -H system					
Titration	L:M ratio*	[H ₃ PO ₄]	[NH ₄ Cl]	[MgCl ₂]	pH range
U	-	0,02528	0,02014	-	3 - 8
V	-	0,01264	-	0,007495	3 - 8,5
W	0,67:1	0,01264	0,005034	0,007495	3 - 8
X	1,33:1	0,01264	0,01007	0,007495	3 - 7,5
Y	2:1	0,01264	0,01510	0,007495	3 - 7,4
Z	3:1	0,01264	0,02014	0,007495	3 - 7,3

* L:M ratio here refers to the NH₄Cl : MgCl₂ ratio.

Titration U was used to calibrate the electrodes. The program MAGEC could not be used to calibrate the electrodes based on a two-ligand system and thus an alternative approach was adopted. The protonation constants for both the NH₃ and PO₄ ligands were used by the program PSEUDOPLOT to generate $-\log[H^+]$ data for each titration point using as input the volume of sodium hydroxide added. These $-\log[H^+]$ values were plotted against their corresponding emf values obtained from the actual titration. A straight line of Nernstian slope (59,16) was then fitted to this data using a least squares procedure. The intercept obtained from this was taken to be the value of E_0 . Figure 2.17 is a plot of $-\log[H^+]$ vs. emf data used. In this way, the electrodes were calibrated over the pH range 3 to 8 and the following found:

$$E_O^1 = 401,8 \text{ mV.}$$

$$E_O^2 = 406,7 \text{ mV.}$$

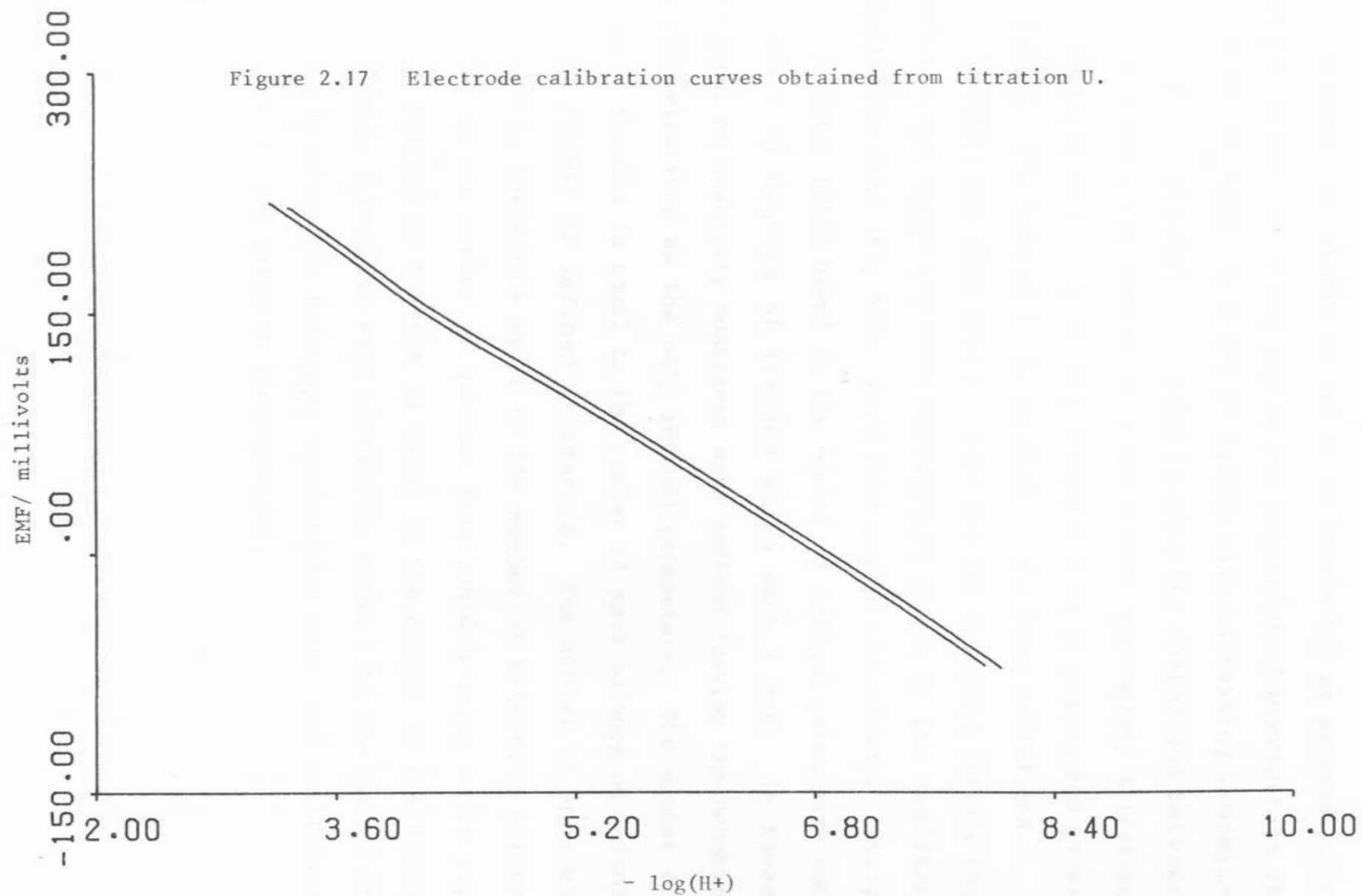


Table 2.9 shows that as the ammonium chloride concentration was increased, the titrations had to be terminated at progressively lower pH values. This was due to the formation of precipitate in the titration vessel at lower pH values with increasing ammonium chloride concentration. In order to make the comparison between titration V and titrations W, X, Y and Z more meaningful, titration V was adjusted in turn to have the same number of points and cover approximately the same pH range as each of the other titrations.

MINIQUAD was then used to calculate the R-factors for all the titrations and these R's then compared by means of the Hamilton significance test (62, 65). This test yields significance levels for an R-factor ratio based on the number of refined parameters and the number of degrees of freedom which each R has. In these titrations, no stability constants were refined leaving the unknown free concentrations as the only refined parameters. The number of degrees of freedom is equal to the number of mass balance equations minus the number of refined parameters. The number of unknown parameters is therefore equal to the number of titration points multiplied by the number of unknown free concentrations while the number of degrees of freedom is equal to the number of titration points. Table 2.10 gives each titration, along with the number of degrees of freedom, the R-factor, the R-factor ratio and confidence level at which this ratio is insignificant.

Table 2.10

Titration, number of points, number of refined parameters, number of degrees of freedom, R-factors and the confidence level obtained for the Hamilton significance test.

Titration	Number of points	Number of refined parameters	Number of degrees of freedom	R factor	R factor ratio & confidence level
W	80	240	80	0,0033	1,182
V _W	80	160	80	0,0039	99,5%
X	73	219	73	0,0026	1,462
V _X	73	146	73	0,0038	99,5%
Y	73	219	73	0,0017	2,235
V _Y	73	146	73	0,0038	< 50%
Z	70	210	70	0,0011	3,364
V _Z	70	140	70	0,0037	< 50%

The Hamilton significance test is applied in the following way. Firstly the null hypothesis is formulated as: "The addition of ammonia does not alter the other complexation equilibria."

Now, each R obtained from MINIQAD is assumed to be an estimate of some true R value, in other words each R is subject to uncertainty. The null hypothesis is equivalent to saying that the R from titration V and the R from any other titration, say W, are equally good estimates of some true R value. Thus the data of V and

the data of W are evidently samples from some common population of data.

In accordance with Hamilton's test (62) the closer the R-factor ratio, R_{VW} / R_W is to unity, the more likely is the null hypothesis to be true. This ratio should always be chosen as greater than unity.

Table 2.10 shows that titrations W and V_W have an R-factor ratio of 1,182 and that this is insignificant (using Hamilton's tables) at the 99,5% confidence level. The null hypothesis cannot be rejected. There is however, a chance of 1 in 200 that this will be the wrong choice. The difference between titrations X and V_X is also insignificant at the 99,5% confidence level. Titrations Y and Z on the other hand are only insignificantly different from titrations V_Y and V_Z at a level of confidence below 50%. This really means that titrations Y and Z are significantly better titrations than V_Y and V_Z respectively and that there is a chance of more than 1 in 2 that this is correct!

The null hypothesis that the addition of ammonia does not alter the other complexation equilibria cannot be rejected for titrations W and X. Titrations Y and Z, however, appear to allow rejection of the null hypothesis and this indicates a significant improvement in R values on addition of ammonia.

This improvement in the "fit" of the data to the proposed model of the nine equilibrium constants established in sections 2.4.1, 2.4.2 and 2.4.3 is surprising. A possible reason might be the variation in glass electrode E_0 values with time. The

alphabetic order of titrations U to Z represents the chronological order in which these titrations were performed. The electrodes were calibrated using titration U and these calibrated values used for all the remaining titrations. While every effort was made to perform these titrations as soon as possible after the calibration titration, this does not preclude the possibility of variation in the glass membrane potential of the electrodes.

As a final measure, attempts were made to refine further constants for the $\text{H-PO}_4\text{-NH}_3$ system and the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system. Using the nine previously established constants as a basis, unsuccessful attempts were made to refine values for β_{1101} , β_{1102} , β_{0110} , β_{0210} , β_{1110} and β_{1111} from different starting values and over different pH ranges. Titrations U and W were used for this purpose.

The $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system has not been as intensively or rigorously characterised as the three subsystems characterised in sections 2.4.1, 2.4.2 and 2.4.3. In particular, the lack of a suitable and sensitive complex formation function is most obvious. This led to an alternate and less rigorous attempt to characterise the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system. An anomalous apparent "improvement" in the fit of titration data to the proposed model has shown the limitations of this method. It therefore appears that further work in this area is warranted. A suitable complex formation function for a ternary system with two ligands appears most desirable.

The reason for investigating the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system was because of problems experienced during urine model validation (sections 1.2 and 3.4). A stability constant for the

$\text{Mg}(\text{NH}_3)(\text{PO}_4)(\text{H})$ soluble complex had been included in the model and this prevented the struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) solid from precipitating when it was expected to. In accordance with the model validation procedure described in section 1.2 it appeared best to return to the model including the equilibrium constants measured and reported in this chapter. All other equilibrium constants for soluble species of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system were removed from the model. Further validation was then performed (section 3.4) and the decision as to where further improvements could best be made left until that stage was complete.

CHAPTER 3

MODELLING

3.1. INTRODUCTION

Jenne (66) has given the following summarising definition of chemical modelling showing the large number of chemical concepts which can be applied to aqueous chemical models.

"Chemical modeling encompasses the aqueous speciation of dissolved cationic elements among organic and inorganic anionic ligands; of anionic elements among their complexes with cations and both cationic and anionic elements among their redox states. Chemical modeling also includes calculation of the degree of saturation of an aqueous media with regard both to metastable and to equilibrium solids and calculation of sorption or desorption. Predictive chemical modeling should also include kinetics". He makes no mention of gaseous or of immiscible non-aqueous liquid phases, both of which could also be incorporated into a chemical model.

The present research is limited to the aqueous speciation of positive metal ions amongst negative ligands, both organic and inorganic. It also incorporates the calculation of the degree of saturation of the aqueous medium with regard to equilibrium solids. It is with these principles that the chemical modelling of urine will be approached.

The ion-association theory is the basis of chemical modelling. There are, however, two different approaches to the problem both subject to the constraints of mass balance and chemical equilibrium. The first of these approaches is the Gibbs Free Energy minimization which involves the following procedure: from an

initial guess of the solution, a positive feasible solution to the mass balance equations is found without consideration of the Gibbs Free Energy of the system; then, subject to the constraint of the mass balance equations, the Gibbs Free Energy is minimized.

The second approach is the so-called equilibrium constant approach. This procedure begins with an initial guess of the free concentrations of a set of components. The minimum Gibbs Free Energy composition is then readily calculated from equilibrium constants. Finally the mass balance equations are solved by iteration.

Zeleznik and Gordon (67) have given a good review of this subject. Morel and co-workers (68) have summed up the two approaches to the problem as follows: "While the former direct minimization of the Gibbs free energy function method seems more elegant in a thermodynamic sense, the latter 'equilibrium constant' method appears to be more an extension of chemists' intuition, thereby more useful and more tractable in many ways". A further reason for selecting the equilibrium constant approach is the fact that there are more reliable and easily available equilibrium constants than Gibbs free energy values. It has been suggested (66) that for relatively simple systems where the free energies are available and reliable, the Gibbs minimization approach is convenient and dependable. For large complex systems the equilibrium constant approach is suggested.

For these reasons, it was decided to adopt the equilibrium constant approach in modelling urine. Having chosen this approach,

the next choice was of a suitable computer program to do the calculations. Some of the criteria were the ability to handle up to about 20 components and 200 complexes as well as about 20 solids. Flexibility in allowing possible extension of the model to include redox and gas phase equilibria was also desirable as was a reliable convergence algorithm.

A literature survey covering the period from 1977 to 1982 was carried out in order to find all the recently published chemical equilibrium programs. The programs found plus some of the well known ones published before 1977 amounted to about 20. Some of these were rejected immediately on the basis of a feature required, but missing. An example here is the inability of many programs to handle solids.

The following programs were amongst those considered: COMICS(69), ECCLES(70), HALTAFALL(71), WATEQ(72), REDEQL2(73), GEOCHEM(74), SUDO6(75) and MINEQL(48). Initially, the program SUDO6 was used because of its availability in this laboratory. However, SUDO6 which is an extension of the HALTAFALL program, could only accommodate five solids at a time and gave problems with non-convergence in a number of instances.

The possibility of using one of the REDEQL family of programs was then considered. The second generation programs of this family are REDEQL2, GEOCHEM and MINEQL. All of these are based on a Newton Raphson iterative convergence algorithm and all have their own database of equilibrium constants. REDEQL2 and GEOCHEM are the "big brothers" of the family and are written to allow for many

specialised modelling capabilities such as adsorption, ion-exchange and ionic-strength calculation and correction. MINEQL, however, is a far more compact program having about 500 lines of FORTRAN code compared to REDEQL2's 2500 lines, and does not have the unnecessary adsorption and ion-exchange sub-routines. It does have the ability to handle as many solids as the user specifies and also iterates on the set of solids until the 'correct' set (section 3.2) in equilibrium with the aqueous solution is obtained. This represents a powerful advantage over SUDO6.

MINEQL was therefore chosen to do the modelling calculations for this project. This does not imply that the programs rejected are 'bad', it is because MINEQL is optimally applicable to the particular chemical system under investigation, urine.

3.2 THEORY

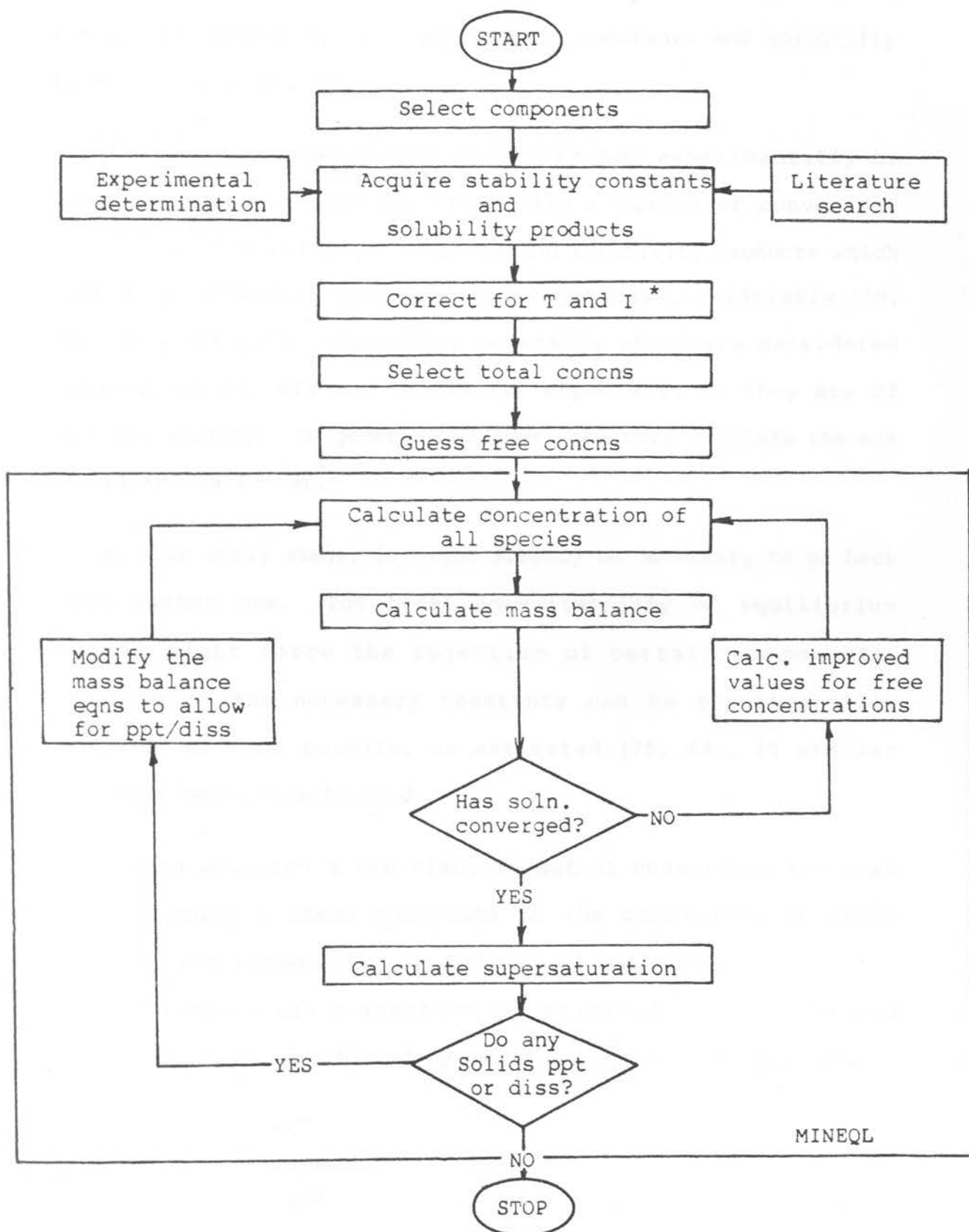
The general theory of chemical modelling is dealt with in the literature (67, 76, 77, 78). Only a broad outline of the principles involved will be given here. The detailed chemical, mathematical and computational procedures used by MINEQL are given in the user's manual (68). Once again, only the salient points of this treatment will be mentioned.

The nature of chemical modelling allows the whole procedure, from selection of components to be considered, to the final output of equilibrium speciation and level of supersaturation, to be represented in a flow diagram. Such a diagram is given in Figure 3.1. It must be emphasized that this is only a schematic representation intended to aid the understanding of the modelling process. It also gives a coherent structure to the general theory discussed. As previously mentioned in section 1.2, this flow diagram represents only part of the overall process of chemical modelling. It should be remembered that, in reality, there are many more "loops" to this diagram involving refinement stages.

To begin with, the components of the proposed chemical model must be selected. In general, only the major components of a solution will be chosen with trace elements being left for addition as a further possible refinement.

The second stage of the process is to acquire the equilibrium constants associated with the chosen components. Equilibrium constant is used here in its most general form, the types of

Figure 3.1: Flow diagram for chemical modelling procedure.



* T = temperature; I = ionic strength

constant being dictated by the nature of the equilibria considered. In the present research, only equilibrium constants and solubility products are required.

The constants may either be determined experimentally or obtained from the literature. There are a number of convenient compilations of stability constants and solubility products which can speed up the search for available constants considerably (79, 80, 81, 82). As with components, constants which are considered negligible can be left out initially, especially if they are of suspect reliability. In general, however, the more complete the set of constants the better.

At this early stage, it might already be necessary to go back to step number one. The total unavailability of equilibrium constants might force the rejection of certain components. Alternatively, the necessary constants can be experimentally determined, if time permits, or estimated (75, 83), if similar systems have been characterised.

Having acquired a satisfactory set of constants, the next step is to correct these constants to the conditions of ionic strength, I , and temperature, T , dictated by the model.

The temperature correction of stability constants and solubility products is achieved by using the van't Hoff equation,

$$\frac{\partial \ln K}{\partial T} = \frac{\Delta H^\circ}{RT^2} \quad \dots (3.1)$$

In the case of equilibrium constants, $\Delta H_R^\ominus$ is the standard enthalpy of reaction, while for solubility products, $\Delta H_S^\ominus$ is the standard enthalpy of solution. If this term $\Delta H^\ominus$ may be assumed constant with respect to temperature, equation 3.1 is easily integrated to give

$$\ln \frac{K_1}{K_2} = \frac{-\Delta H^\ominus}{R} (1/T_1 - 1/T_2) \quad \dots (3.2)$$

Owing to deficiencies in reported data it is frequently necessary to make the approximation of temperature-independent $\Delta H^\ominus$ but since most constants are reported at 25°C, the correction to 37°C involves a range of only 12°C which hopefully is usually sufficiently small for the approximation not to be serious.

The procedure of ionic strength correction for equilibrium constants has been investigated (43). The extended Debye-Hückel equation was used as a basis for the extrapolation of stability constants to different ionic strengths.

$$-\log y_i = \frac{A \cdot Z_i^2 \cdot I^{0,5}}{1 + B \cdot q_i \cdot I^{0,5}} + cI \quad \dots (3.3)$$

where I is the ionic strength of the solution;

y_i is the molar activity coefficient of the ion, i ;

Z_i is the charge on the ion, i ;

A , B are constants characteristic of the solvent;

c is an empirical parameter;

q_i is an ionic size parameter.

Essentially, for a given complexation reaction, the equilibrium constants, K_1 and K_2 , at ionic strengths, I_1 and I_2 are related by:

$$K_2 = (Q_{Y2})^{-1} \cdot (Q_{Y1}) \cdot K_1 \quad \dots (3.4)$$

where each Q_Y is the activity coefficient quotient defined by equation (2.3).

The activity coefficients of these quotients may be calculated from equation (3.3) provided values of the constants, g_i and c are available. Kielland (49) has listed g_i values for a wide range of ions. If the assumption that c has the same value for each ion in the complexation reaction is made, it is shown (43) that this overall value for c may be calculated. This value can then be used in equation (3.3) for predicting the equilibrium constant at the required ionic strength. It was demonstrated that for systems of interest in a soil model at an ionic strength of 0.04, equation 3.3 could be used to extrapolate stability constants using as few as two literature values at higher ionic strengths. The accuracy of the extrapolation was sufficient for the purpose. Where only one stability constant value was available, a value for c in equation 3.3 could not be directly calculated and had to be estimated from a related system. Failing this, a generally recommended value for c had to be used. In both of these cases, the reliability was lower than in the case where two constants were available.

The urine model was to be constructed at an ionic strength of 0.20. While the above procedure was mainly intended for use at low ionic strengths, it was felt that, providing "good" c values could be obtained or calculated, it would give sufficiently accurate extrapolations at this higher ionic strength. A computer program LOGK (43) was used to perform the calculations.

An analogous procedure has been developed by Voyé and Linder in this laboratory for the extrapolation of solubility products to different ionic strengths. While this method has not yet been investigated as thoroughly as the stability constant extrapolation, it is based on the same equation and uses similar assumptions. While realising the need for a proper investigation into the use of the extended Debye-Hückel equation for extrapolation of solubility products to different ionic strengths, it was decided to use the method without such a test. The similarity to the stability constant procedure lent some confidence to the use of this untested procedure. A computer program LOGKSP was used to perform these calculations.

The importance of keeping accuracy in perspective should perhaps be re-emphasised here (section 1.2). It is probably more expedient to use the above untested procedure and then at the model validation stage decide whether the model is good enough or needs further refining. If further refinement is needed, perhaps this could be undertaken in the area of solubility product correction for ionic strength. It is appreciated, however, that some other area of the model may be found to be in even more urgent need of refinement.

The next stage, as shown in the flow diagram Figure 3.1, is the selection of the total concentrations of all the components considered. Also, a guess, to be used as a starting value in the Newton-Raphson refinement, must be made for the free component concentrations in solution at equilibrium.

From here on, all the steps shown on the flow diagram are

handled by the program, MINEQL. The shaded area of the diagram delineates the steps which are performed by the computer.

The basis for the calculation of the chemical speciation at equilibrium is the mass balance equation. The total concentration of each component is equal to the sum of all species containing that component plus the free concentration of the component. The equations so constructed form a set of simultaneous non-linear equations, one for each component. Equations (2.8, 2.9, 2.10) are the mass balance equations for a system of three components, M, L and H. A more specific and very simple example can be used to illustrate the process. Consider two metals, A and B, forming the following complexes with two ligands, K and L:

$$[AK] = \beta_{AK}[A][K] \quad \dots (3.5)$$

$$[AL] = \beta_{AL}[A][L] \quad \dots (3.6)$$

$$[BL] = \beta_{BL}[B][L] \quad \dots (3.7)$$

$$[BL_2] = \beta_{BL2} \cdot [B][L]^2 \quad \dots (3.8)$$

where [A], [B], [K] and [L] represent the free concentrations of the components A, B, K and L respectively.

The following equations represent the mass balances over all four components:

$$T_A = [A] + \beta_{AK}[A][K] + \beta_{AL}[A][L] \quad \dots (3.9)$$

$$T_B = [B] + \beta_{BL}[B][L] + \beta_{BL2}[B][L]^2 \quad \dots (3.10)$$

$$T_K = [K] + \beta_{AK}[A][K] \quad \dots (3.11)$$

$$T_L = [L] + \beta_{AL}[A][L] + \beta_{BL}[B][L] + 2\beta_{BL2}[B][L]^2 \quad \dots (3.12)$$

In general, equations (3.5 - 3.12) can be represented (48) as:

$$C_i = \beta_i \prod_{j=1}^n x_j^{a(i,j)} \quad \dots (3.13)$$

where $i = 1, m$

$$Y_j = \sum_{i=1}^m a(i,j) \cdot C_i - T_j \quad \dots (3.14)$$

where $j = 1, n$.

The notation used in equations 3.13 and 3.14 is as follows:

C_i = concentration of complex i ;

β_i = equilibrium constant of complex i ;

X_j = concentration of free component j ;

$a(i,j)$ = stoichiometric coefficient of X_j in eqn.(3.13)

T_j = total analytical concentration of component j ;

Y_j = difference between the imposed total analytical concentration of component j (T_j), and the amount of component j in all of the complexes as computed from the X_j 's.

The ideal solution to the problem is, of course, the set of X_j 's such that all Y_j 's = 0. The mathematical route to this solution is suggested by the way in which these equations have been developed.

Initially, the concentration of all complexes is calculated using the initial guesses for the free concentrations and equation (3.13). The concentrations calculated are then used to compute the mass balance difference functions defined by equation (3.14). If all these differences (Y_j 's) are equal to zero, then the problem of aqueous speciation is solved.

In practice, however, some criterion must be established such that when each Y_j becomes sufficiently close to zero, the system of equations is said to be "solved". Since each Y_j is the sum of terms which vary widely in order of magnitude, the convergence criterion used in MINEQL reflects the magnitude of Y_j relative to the maximum of the terms of which Y_j is the sum.

The criterion for convergence is therefore:

$$\frac{|Y_j|}{\max(Y_j)} < \epsilon \quad \text{for } j = 1, n \quad \dots (3.15)$$

where $\max(Y_j)$ is the maximum of the absolute values of the set of terms $\{(a(i,j).C_i), i=1,m; T_j\}$ of which Y_j is the sum, and ϵ is a small positive real number.

A specific case of this convergence criterion is when $\max(Y_j)$ is equal to T_j , the total concentration of component j . The criterion then has the physical interpretation that all of component j (within $\epsilon.100\%$) can be accounted for. The convergence criterion is used in the general form given in equation (3.15) due to modification of the mass balance equations when solid phases are taken into account.

If the current set of free concentrations has not resulted in convergence, the Newton-Raphson method for systems of non-linear equations is used to produce better estimates of the free concentrations (68). These new estimates are then used to recalculate the concentrations of all complexes, completing the first loop in the flow diagram. MINEQL will continue looping until either convergence is reached or the maximum number of iterations is

performed. Maximum iterations might mean that the solution to the problem is diverging. If this is the case, it may help to raise the tolerance on the convergence criterion, ϵ . If this allows convergence, the value for ϵ can be lowered and the program restarted using as initial guesses the free concentrations obtained. These improved starting values will hopefully allow convergence using the more stringent convergence criterion.

Once convergence has been obtained, the next stage is to calculate the degree of supersaturation with respect to the various solid phases present. This is accomplished using the solubility products supplied. If the calculated free concentration product exceeds the solubility product for any of the solid phases considered, these solids are deemed to precipitate. This will effectively remove some of the components involved in these solids from the "solution". The mass balance equations are thus modified to take into account the formation of these solid phases. A new computation of the equilibrium speciation is begun using the solution of the preceeding computation as a good guess to start the new one. This completes the second loop of the flow diagram. The first loop is thus "nested" within the second loop.

The program loops around this outer loop until there is effectively no change in the set of solids deemed to have precipitated. Solids, it should be noted, having precipitated, may subsequently dissolve as well.

When no further solid precipitates and no current solid dissolves, the computation of the equilibrium set of solids and

aqueous species is finished. The "concentration" of solid is defined as the number of moles of solid per cubic decimetre of solution. This is indeed a "strange ", but convenient definition and can be used as a measure of the degree of supersaturation of the solution with respect to the set of equilibrium solids.

One final comment with regard to MINEQL is that in order to improve the efficiency of the computation, a transformation of the mathematical basis is performed (68). The result of this is that the Jacobian matrix, produced by the Newton-Raphson method, approaches a diagonal matrix. The solving of this matrix, by Gaussian elimination, is computationally quicker.

Table 1.1

Chemical species for MINEQL	
Species	Formula
Water	H ₂ O
Hydrogen ion	H ⁺
Hydroxide ion	OH ⁻
Carbon dioxide	CO ₂
Carbonic acid	H ₂ CO ₃
Bicarbonate ion	HCO ₃ ⁻
Carbonate ion	CO ₃ ⁻²
Sulfuric acid	H ₂ SO ₄
Sulfate ion	SO ₄ ⁻²
Sulfite ion	SO ₃ ⁻²
Sulfide ion	S ⁻²

3.3 MODEL CONSTRUCTION

3.3.1 Selection of Components

Workers in urolithiasis research have used a wide range of urine-like solutions to investigate various features of stone formation. The composition of these artificial urines has varied from weakly buffered sodium chloride solutions to ones containing a far more sophisticated array of components (84, 85).

In an attempt to introduce a common basis for these investigations, Burns and Finlayson (84) have proposed a standard reference artificial urine. It was decided to choose the components of this artificial urine as the basis of the urine model. The components considered are listed in Table 3.1.

Table 3.1

Components chosen for urine model	
Metal Ions	Ligands
Sodium	Chloride
Potassium	Phosphate
Calcium	Sulphate
Magnesium	Ammonia
(Protons)	Citrate
	Oxalate

The hydrogen ion has been included for completeness under the metal ions. There is no need to list the hydroxide ion as a ligand because its inclusion is implicit from the way in which the equilibrium constants are defined (section 2.2).

3.3.2. Acquisition of Equilibrium Constants and Solubility Products

An extensive literature survey was conducted in order to acquire a reliable set of equilibrium constants and solubility products. Where possible, a number of references to the same constant were found and a critical selection of the "best" constant made.

Amongst the criteria for selection were:

- the journal in which the reference appeared;
- the worker(s) who determined the constant;
- the method of determination;
- the ionic strength, medium and temperature at which the determination was performed.

Where possible, heats of reaction and heats of solution were also obtained from the literature for the equilibrium reactions considered. The references used in the critical evaluation of the constants are listed in Table 3.2. Some of these are general references containing tables of constants while others refer specifically to one ligand. In other words, references to solubility products and equilibrium constants containing phosphate are listed together next to this ligand.

Table 3.2

References considered in the critical selection
of equilibrium constants and solubility products.

Ligand	References
Phosphate	18-32, 86-102
Sulphate	103-111
Ammonia	34-41
Citrate	112-113
Oxalate	114-125
General	72, 79-82, 126-127

A total of 20 equilibrium constants and 14 solubility products was obtained from the literature in this way. A further 9 equilibrium constants were potentiometrically determined in the course of this research (sections 2.4.1, 2.4.2 and 2.4.3). These 29 equilibrium constants and the 14 solubility products, once corrected for temperature and ionic strength, comprised the basic urine model. They are listed in Tables 3.3 and 3.4 respectively. These tables will be more fully discussed in the next section.

3.3.3 Correction for Temperature and Ionic Strength

Having selected 43 critical constants for the urine model, the next stage was to correct these where necessary for temperature and ionic strength.

The human body temperature of 37°C was chosen as the model temperature. All the constants not at 37°C were then adjusted to this temperature according to the procedure described in section 3.2.

The choice of ionic strength for the model was not as easy as the choice of temperature. Ionic strength has been found to vary quite considerably in both normal and stone-forming individuals. Isaacson (128) has determined a mean urinary ionic strength of $0,263 \pm 0,109$ in a group of 48 normal subjects. A group of 15 stone-formers was found to have a mean urinary ionic strength of $0,199 \pm 0,053$.

Initially, an ionic strength of 0,263 was chosen for the model. However, a value of 0,200 was found to be a more convenient ionic strength at which to determine experimentally the equilibrium constants in section 2.4. It was therefore decided to change the ionic strength of the urine model to 0,200. This still appears to be a reasonable value in that it falls well within one standard deviation of the mean for normal subjects. Coincidentally, it also happens to be the mean for the stone-formers investigated.

Having established a reasonable value of ionic strength for the urine model, the computer programs LOGK and LOGKSP were used to correct all the constants. These programs were referred to and are based on the procedures outlined in section 3.2.

Naturally, the equilibrium constants measured in the present research required no correction for ionic strength. It was possible to calculate good c values for all the other protonation constants. This was due to the large number of measured protonation constants

in the literature. It was also possible to calculate c values for six stability constants and four solubility products. Values for the remaining constants were either estimated to be the same as chemically similar systems or taken from a list of c values reported by Truesdell and Jones (72).

Truesdell and Jones calculated mean salt single-ion activity coefficients and then obtained c values from curve fitting using equation (3.3). These values relate only to single ions. They can only be used if it is again assumed that all the ions in the equilibrium reaction have similar c values. The values for calcium (0,165) and magnesium (0,200) were therefore used when no c value could be calculated.

The final values of the equilibrium constants and solubility products at a temperature of 37°C and an ionic strength of 0,200 are listed in Tables 3.3 and 3.4 respectively. The references from which the constants were obtained as well as whether the constant was measured (M) or obtained from the literature (L) are also reported. At the same time, $\Delta H^\ominus$ and c values for the particular equilibria defined in the first column are also given. Again, it is reported whether the $\Delta H^\ominus$ value and c value is from the literature (L) or estimated (E). A c value from the literature means that it has been calculated from the constants in the literature referenced. Gaps in these tables imply that no corrections were necessary. Therefore, all the measured constants have no c values.

Table 3.3

Equilibrium constants used in basic urine model together with $\Delta H_R^\ominus$ (kcal/mol) and c values used to correct these constants for temperature and ionic strength where necessary.

L: literature, E: estimated, M: measured.

Complex	$\Delta H_R^\ominus$	L,E	Ref.	c	L,E	Ref.	log K	M,L	Ref.
H(PO ₄)	-3,50	L	72				11,08	M	
H(HPO ₄)	-0,99	L	72				6,37	M	
H(H ₂ PO ₄)	1,90	L	80				1,74	M	
Ca(PO ₄)	3,10	L	86	0,165	E	72	4,93	L	86
Ca(HPO ₄)	3,30	L	86	0,165	E	72	1,21	L	31
Ca(H ₂ PO ₄)	3,40	L	86	0,165	E	72	0,50	L	31
Mg(PO ₄)	3,10	E	86				3,22	M	
Mg(HPO ₄)	3,30	E	86				2,03	M	
Mg(H ₂ PO ₄)	3,40	E	86				1,61	M	
H(SO ₄)	5,40	L	80	0,051	L	80	1,46	L	80
Ca(SO ₄)	-1,65	L	107	-2,731	L	106	2,26	L	106
Mg(SO ₄)	4,84	L	108	0,876	L	80	1,92	L	108
H(NH ₃)	-12,43	L	41				8,71	M	
H(OH)	-13,55	L	80				13,07	M	
Ca(OH)	-1,19	L	107	0,165	E	72	-11,32	L	31
Mg(OH)	2,14	L	127				-9,94	M	
H(Cit)	0,80	L	80	0,041	L	112	5,49	L	113
H(HCit)	-0,58	L	80	1,704	L	112	4,09	L	113
H(H ₂ Cit)	-1,00	L	80	-1,666	L	112	2,82	L	113
Ca(Cit)	3,17	L	80	0,165	E	72	3,16	L	113

Complex	$\Delta H_R^\ominus$	L,E	Ref.	c	L,E	Ref.	log K	M,L	Ref.
Ca(HCit)	3,17	E	80	0,165	E	72	1,74	L	113
Mg(Cit)	3,19	L	80	-0,190	L	112	3,25	L	113
Mg(HCit)	3,19	E	80	0,200	E	72	1,54	L	113
H(Ox)	1,60	L	80	0,206	L	80	3,76	L	114
H(HOx)	0,90	L	80	0,640	L	80	1,30	L	114
Ca(Ox)				-1,487	L	120	3,40	L	119
Ca(CaOx)				-1,487	E	120	1,98	L	119
Mg(Ox)				-0,286	L	115	3,12	L	115
K(Ox)				-0,760	L	122	1,00	L	122

Table 3.4

Solubility products used in basic urine model together with $H_S^\ominus$ (kcal/mol) and c values used to correct these constants for temperature and ionic strength where necessary.

L: literature, E: estimated, M: measured.

Sol. Prod.	$\Delta H_S^\ominus$	L,E	Ref.	c	L,E	Ref.	log K	M,L,E	Ref.
Ca(HPO ₄)·2H ₂ O	0,63	L	91	-0,257	L	93	-6,60	L	90
Ca ₃ (PO ₄) ₂	10,20	E	129	-0,257	E	93	-24,97	L	92
Ca ₄ H(PO ₄) ₃	17,23	E	72	-0,257	E	93	-41,08	L	94
Ca ₅ OH(PO ₄) ₃	17,23	L	72	-0,257	E	93	-52,77	L	95
Mg(HPO ₄)·3H ₂ O	0,63	E	91	-0,257	E	93	-5,56	L	20
Mg ₃ (PO ₄) ₂	10,20	L	129	-0,257	E	93	-21,58	L	20
MgNH ₄ PO ₄ ·6H ₂ O				-1,257	L	23	-12,42	L	23

Sol. Prod.	ΔH_s°	L,E	Ref.	c	L,E	Ref.	log K	M,L,E	Ref.
Ca(SO ₄).2H ₂ O	0,26	L	72	-0,132	L	103	-3,52	L	104
Ca(OH) ₂	-4,30	L	80	0,165	E	72	-4,38	L	80
Mg(OH) ₂	0,85	L	72	0,200	E	72	-10,21	L	80
Ca(Ox).H ₂ O	5,33	L	121	-0,748	E	124	-8,65	L	125
Ca(Ox).2H ₂ O	5,33	E	121	-0,748	L	124	-8,30	L	124
Ca(Ox).3H ₂ O	5,33	E	121	-0,748	E	124	-8,13	L	125
Mg(Ox)	5,33	E	121	-0,748	E	124	-5,97	L	116

3.3.4 Additional Equilibrium Constants and Solubility Products

In order to investigate uric acid precipitation, the role of cadmium and of various drugs believed to counteract the effect of cadmium, it became necessary to acquire values for the appropriate corresponding constants. The equilibrium constant for the first dissociable proton on uric acid and the solubility product of uric acid were obtained from the literature (130, 131). Both of these constants were corrected for ionic strength using c values of -0,100 which have been generally recommended (43).

An investigation into the role of cadmium in urinary supersaturation meant that cadmium constants were also required. Similarly, constants were required for the drugs to counteract the effect of cadmium. The following drugs were investigated:

1. 1,2-Diaminoethanetetra-acetic acid (EDTA)
2. 2,3-Dimercaptopropane-1-sulphonate (UNI)
3. D-Penicillamine (DPEN)

- | | |
|--|--------|
| 4. 2,3-Dimercapto succinate | (DMSA) |
| 5. 2,3-Dimercapto-1-propanol | (BAL) |
| 6. Diethylenetriaminepenta-acetic acid | (DTPA) |
| 7. Diethyl dithio carbonate | (DDC) |

Fortunately, equilibrium constants of cadmium and the above drugs were made available by Prof. D.R. Williams of UWIST in Cardiff. The constants come from a blood model, constructed at a temperature of 37°C and an ionic strength of 0,150, initially developed at this university (70). Work on the blood model has continued at UWIST where the effects of cadmium in blood plasma has been investigated. The efficacy of the above listed drugs in complexing cadmium has also been studied.

These cadmium and drug equilibrium constants, while at the correct temperature should really be corrected to an ionic strength of 0,200. It was assumed, however, that the difference between the constants at ionic strengths of 0,150 and 0,200 would be negligible. The validity of this assumption was checked by calculating the change in equilibrium constant values for a few constants corrected from an ionic strength of 0,150 to 0,200. The constants selected formed part of the basic urine model listed in Tables 3.3 and 3.4. The values of the constants and the calculated change are given in Table 3.5.

Table 3.5

Change in Equilibrium constants when corrected from ionic strength 0,150 to 0,200 (mol dm ⁻³)			
Constant	log K (I = 0,150)	log K (I = 0,200)	log K
H(Cit)	5,53	5,49	0,04
Ca(Cit)	3,27	3,16	0,11
Mg(Cit)	3,34	3,25	0,09
Ca(SO ₄)	2,21	2,26	-0,05

The change in log K values is not due exclusively to the initial and final ionic strength. It is also determined for example by the values of c , q and Z (section 3.2). The small differences shown in Table 3.5 do however lend some confidence to the assumption that the cadmium and drug constants will not be greatly affected by the relevant change in ionic strength.

All the constants listed in tables 3.3 and 3.4 have, together with the additional constants referred to in the foregoing, been included in Appendix 1 to this thesis. It should finally be pointed out that the constants reported in Tables 3.3 and 3.4 have generally been given as stepwise constants. When listed in Appendix 1, all constants are quoted as overall constants. This is the form in which they are read by the computer program MINEQL.

3.3.5. Selection of Total Concentrations

Burns and Finlayson (84) who suggested the components for the standard reference artificial urine have also proposed concentrations for these components. These concentrations are taken to be the mean values of the respective components as listed in the Documenta Geigy Scientific Tables (132). The synopsis of urine given in these tables is expressed as a mass of component voided per 24 hours. An average volume of urine voided per 24 hours is also given and therefore the average concentration of each component may be calculated. These concentrations are listed in Table 3.6.

Table 3.6

Concentration of Components in Urine Model	
Component	Concentration $\times 10^3 /$ mol dm^{-3}
Sodium	182
Potassium	63,7
Calcium	5,75
Magnesium	3,85
Chloride	208
Phosphate	32,3
Sulphate	20,8
Ammonia	45,5
Citrate	3,21
Oxalate	0,318

The concentrations of sodium and chloride in Table 3.6 indicate that the chosen ionic strength of 0,200 is of the right order of magnitude. If these components were the only components, the ionic strength of the solution would be 0,195.

The range of pH quoted in the Documenta Geigy Tables varies from 5,53 to 6,97. Taking the average of the actual hydrogen ion activities and then converting the average back into logarithmic form, we obtain a mean pH of 5,81. It therefore seems reasonable to assume a pH of 6,0 as a representative value for urine.

In dealing with the phenomenon of hypercalciuria, no typical pathological concentration values for calcium in urine could be found in the literature. The Documenta Geigy Tables give the range of component concentrations into which 95% of the healthy population should fall. It was therefore decided to use values exceeding the upper limit of this range as defining hypercalciuria.

The foregoing considerations provide the desired specification for our basic urine model. The problem of choosing concentrations for the other components, such as cadmium, is dealt with in section 3.5.

3.4 VALIDATION OF THE BASIC URINE MODEL

The urine model has been constructed at a temperature of 37°C and an ionic strength of 0,200. The components of this model are listed in Table 3.1. The equilibrium constants and solubility products are given in Tables 3.3 and 3.4 respectively. In accordance with the objectives of the present research, the urine model has to be validated in some fashion before it can be used with any confidence.

As stated in section 1.2, the validation of the urine model is to be accomplished by comparing the model with appropriate experimental observations. Rodgers and Wandt (133) have carried out investigations into precipitation from an artificial urine. This work was carried out in this department using a fast evaporator and an artificial urine identical to the one chosen in this research. The experimental procedure of these workers was as follows.

1. An artificial urine was made up in the manner described by Burns and Finlayson. The composition of this solution is the same as that in Table 3.6.
2. The pH of this solution was measured using a glass electrode. The pH was always found to be 6,5.
3. The solution was divided into nine fractions.
4. The pH's of these nine fractions were individually adjusted to make up a range of pH values. These were: pH = 3; 4; 5; 5,5; 6; 6,5; 7; 8; 9. Hydrochloric acid was used for the acid region and ammonia solution was used to raise the pH.
5. These nine solutions were initially refrigerated, and then

allowed to equilibrate for an hour at 37°C before being rapidly evaporated to half their volume.

6. The precipitates were filtered using a 0,45 μ m pore size teflon filter. The dried crystals were then subjected to semi-quantitative analysis by X-ray powder diffraction.

Rodgers and Wandt are thus also modelling urine, but experimentally instead of by computer simulation. The nature of their work in making up solutions of precisely known total component concentrations allows comparison with our theoretical urine model. However, in order to validate the theoretical model, we need a definite basis of comparison. There are two obvious features which facilitate this comparison. The first is the pH of the initial solution made up. Rodgers and Wandt find that when the solution of exactly known total concentrations is prepared, the measured pH is 6,5. The second basis of comparison is the observed precipitates. Rodgers and Wandt experimentally observe precipitate, while MINEQL computes precipitation of solids. It should be noted that this calculated precipitation could indeed be interpreted as the degree of supersaturation as defined in section 1.1. As far as MINEQL is concerned, the terms precipitation and supersaturation are synonymous. The total concentrations of the nine solutions are exactly known, as well as the pH. The solutions are evaporated to half their volume, doubling the concentrations. The precipitate from these solutions is equivalent to the calculated level of precipitation in the theoretical model. The concentrations of Table 3.6 are thus doubled and the pH fixed at the appropriate value between 3 and 9. MINEQL may then be run and the calculated

precipitation of the different solids compared with the observed precipitate.

Two points require comment at this stage. First, one of the features of MINEQL allows the free concentration of a component to be fixed. This means that hydrogen ion concentration can be set at any value as required. Secondly, MINEQL computes concentrations and not activities as the equilibrium constants it uses are expressed in terms of concentrations. The pH reported by Rodgers and Wandt is an operational pH while the pH calculated by MINEQL is in terms of concentration.

The activity coefficient reported by Kielland (43) for the hydrogen ion at an ionic strength of 0,200 is equal to 0,83. Kielland unfortunately does not give the medium in which this activity coefficient is determined. If we assume that this activity coefficient applies to the sodium chloride medium of the model, then the hydrogen ion activity will always be 17% lower than the hydrogen ion concentration. Thus if the negative log of hydrogen ion concentration is 6,00, the negative log of the activity is 6,08.

The operational pH measured by Rodgers and Wandt must be assumed to be equivalent to the negative log of the hydrogen ion activity. This pH will have an uncertainty of 0,05 on the pH scale (30). It would therefore appear reasonable, for the present purposes, to assume that the correction from concentration to activity (0,08 log units) is smaller than the uncertainty in interpreting operational pH (+0,05 log units).

Therefore, in reporting the model validation and results, hydrogen ion activity and concentration will be taken as equivalent and will be expressed by the symbol pH. Where accuracy becomes more important, however, this will be clearly stated and the distinction made between activity and concentration.

The first of the validation procedures involves using the same total component concentrations for the theoretical model as were used to make up the initial solution of Rodgers and Wandt. These concentrations are given in Table 3.6. MINEQL is then run and the hydrogen ion concentration calculated. In this particular instant, the distinction between concentration and activity will be made. The hydrogen-ion concentration will be converted to activity and this will be compared with the hydrogen ion activity measured by Rodgers and Wandt. Table 3.7 lists the values obtained.

Table 3.7

Comparison of calculated hydrogen ion activity of theoretical model with the measured hydrogen ion activity of the experimental model.		
Quantity	Theoretical model	Experimental model
Concentration	$1,26 \times 10^{-6}$	
pH	5,9	
Activity	$1,04 \times 10^{-6}$	$0,32 \times 10^{-6}$
pH	6,0	6,5

Table 3.7 shows that the calculated hydrogen ion activity of the theoretical model is about three times higher than the measured experimental value. It is however, at least of the same order of magnitude as the measured value. This difference appears more acceptable when seen on a log scale as pH. It is, nevertheless, the same difference.

The second validation procedure is the comparison of observed precipitation from the experimental model with the calculated supersaturation of the theoretical model. The comparison was made over a range of measured pH values between 3,0 and 9,0.

One feature of this procedure should be stressed. In the experimental method used, the solutions were first corrected for pH by adding acid or base and then evaporated to half their volume. While the total concentrations of all components will double, it is impossible to predict the change in free hydrogen ion concentration. The total concentrations used as input to MINEQL were therefore doubled. The pH was fixed at the value measured at the start of the evaporation.

Table 3.8 gives the observed precipitates as well as the theoretically predicted precipitates together with the value of pH at which these precipitates occur.

Table 3.8

Observed vs. predicted precipitates at varying pH

Precipitate 1: $\text{CaOx} \cdot \text{H}_2\text{O}$	Calcium Oxalate
Precipitate 2: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	Brushite
Precipitate 3: $\text{Ca}_5(\text{OH})(\text{PO}_4)_3$	Hydroxyapatite
Precipitate 4: $\text{Mg}(\text{NH}_4)(\text{PO}_4) \cdot 6\text{H}_2\text{O}$	Struvite

Theoretical Model				pH	Experimental Model			
Predicted precipitates					Observed precipitates			
1				3	1			
1	2			4	1			
1	2			5	1	2		
1	2	4		5,5	1	2		
1		3	4	6	1	2	3	
		3	4	6,5	1	2	3	
		3	4	7		2	3	
		3	4	8		2	3	4
		3	4	9		2	3	4

The agreement between predicted and observed precipitation is fairly good in general. Out of a possible 14 solids, MINEQL predicts only the four solids which are indeed experimentally observed. In particular, the predicted and observed precipitation of calcium oxalate and hydroxyapatite is in remarkably good agreement. The brushite, however, is observed between pH 6 and 9, but not predicted over this range. Struvite on the other hand, is

predicted between pH 5,5 and 9, but only observed between pH 8 and 9.

While the trends shown by brushite and struvite are similar for the predicted and observed cases, some comment should be made about the fairly big differences in these trends. As far as the theoretical model is concerned, the most fundamental assumption made is that of chemical equilibrium. This assumption should always be borne in mind when assessing modelling results. In other words, kinetic limitations in attaining equilibrium might be of significance. In general, the solubility products measured are calculated from data obtained after weeks or months of equilibration. The experimental work by Rodgers and Wandt allowed equilibration over a few hours. Can this difference in time be the cause of the discrepancies in the theoretical model?

The answer to the question is to obtain some reliable kinetic information as to the rate of precipitation and the factors influencing this. Failing this, most comments will be reduced to speculation. From equilibrium considerations alone, however, a predicted precipitate which is not observed can be explained by some form of kinetic inhibition. Struvite, therefore, which is predicted between pH 5,5 and 7, but not observed in this range, might be thermodynamically favourable, but kinetically unfavourable. This implies that the experimental solution could be metastable with respect to struvite in this pH range.

This argument can not be used to explain the difference in the predicted and observed precipitation of brushite. Brown and co-workers have done a great deal of work on the $\text{Ca}(\text{OH})_2\text{-H}_3\text{PO}_4\text{-H}_2\text{O}$ system (90, 93-96). Brown (89) has given an excellent review covering the solubilities of sparingly soluble phosphates of this system. In this review, Brown states that brushite, because of crystal growth kinetics, appears to form very readily. He also states that hydroxyapatite crystal growth appears to be a slow process. Furthermore, the more soluble brushite is often found to form initially with the more stable hydroxyapatite forming subsequently. This process appears to take place through a dissolution step followed by a precipitation step.

In the light of this kinetic information (albeit very qualitative) it appears possible that the difference in time between the experimental equilibration and the "theoretical equilibration" is very significant. If the experimental results are correct in showing brushite as a precipitate, the experimental solution must be supersaturated (at least initially) with respect to brushite. This effect should be apparent right up to pH 9. The theoretical model, however, can be used to test whether this is so. Hydroxyapatite must first be removed from the set of solids in order that this most stable solid does not "use up" all the calcium and phosphate in the equilibrium calculation.

Table 3.9 shows the results of a pH scan identical to that shown in Table 3.8 except for the fact that brushite is the only calcium phosphate solid included in the calculations.

Table 3.9

Observed vs. predicted precipitates at varying pH and the effect
of removing hydroxyapatite from the system
Numbering of solids as in Table 3.8.

Theoretical model (all Ca-PO ₄ solids)	Theoretical model (only CaHPO ₄ ·2H ₂ O)	Intermediate model	pH	Experimental model
1	1	1	3	1
1 2	1 2	1 2	4	1
1 2	1 2	1 2	5	1 2
1 2 4	1 2 4	1 2 4	5,5	1 2
1 3 4	1 2 4	1 2 3 4	6	1 2 3
3 4	1 2 4	1 2 3 4	6,5	1 2 3
3 4	2 4	2 3 4	7	2 3
3 4	2 4	2 3 4	8	2 3 4
3 4	2 4	2 3 4	9	2 3 4

It is immediately apparent that brushite is indeed a possible precipitate in the early stages of the equilibration over almost the entire pH range studied. The theoretical model comprising all the solids can be thought of as a "long term" model and the one having brushite only as a "short term" model. An "intermediate model" would show some short term as well as some long term characteristics and would probably correspond roughly to the experimental situation. The "short term" model shows identical calcium oxalate precipitation to the experimental model as well as the vast improvement in

brushite precipitation characteristics. If we combine the short term and long term models, the "intermediate" theoretical model can be seen to be identical to the experimental model except for struvite precipitation between pH 5,5 and 7.

It has therefore been shown that very good agreement between the theoretical model and the experimental model is obtained if short term kinetic effects are taken into account. These kinetic effects, however, are by no means quantitative and the results should be regarded with circumspection. It appears most desirable, however, that kinetic data on the precipitation of struvite be obtained. The solution equilibria of this system have been characterised to a degree thought adequate for the purposes of this model (section 2.4). It therefore appears that the solid phase solution equilibria require more attention.

The validation procedures lend some confidence to the use of the urine model. While only tentatively explored, it seems likely that short term effects do play a part in the precipitation process. In particular, the brushite - hydroxyapatite system appears to be subject to this kind of kinetic influence. While struvite might also be subject to kinetic effects, no conclusions can be drawn until reliable information regarding the kinetics of precipitation is obtained. In the absence of this information, it appears reasonable to use the urine model as it is and leave the investigation of the struvite problem as a further possible refinement.

3.5 RESULTS AND OBSERVATIONS

In terms of the flow diagram presented in section 1.2, the urine model has been validated using an independent test. This validation procedure has shown the model to be a reasonably good one. In particular, the thermodynamics and kinetics of precipitation of struvite appear to require further investigation. At the same time, some method of taking into account the early precipitation of brushite followed by its subsequent conversion into the more stable hydroxyapatite should also be found. It appears that this latter problem can be accounted for by proposing a short term model which has brushite as the only Ca-PO_4 solid. The results of the short term model combined with the long term equilibrium model seem to give a better fit to the experimental data. This procedure is only made possible by evidence (89) supporting the initial rapid precipitation of brushite followed by a subsequent and slower conversion and precipitation to form hydroxyapatite.

The above comments are directly related to the actual modelling of urine. Solubility products of slow forming solids such as hydroxyapatite are often measured after weeks (95, 97) of equilibration. The retention time of urine in the urinary tract is probably estimated in hours or at most a day. Care should therefore be taken that the theoretical urine model is not used to model something which would only occur long after the urine has passed out of the urinary tract!

While qualitative evidence is often used in modelling where no other evidence is available, it would be preferable to base the

urine model on quantitative and reliable scientific observations. However, in accordance with the modelling procedure outlined in section 1.2, it was decided to use the urine model as presently constructed. Further refinements can be made and might indeed be more correctly formulated after attempts have been made to answer the questions posed at the beginning of the present research.

These questions are:

1. How does pH affect urinary supersaturation?
2. How do the major urinary components affect calcium oxalate supersaturation?
3. What is cadmium's role in urinary supersaturation and how effective are drugs in complexing cadmium?

In investigating the first of these questions, some attempt has been made to take into account the short term precipitation features outlined in section 3.4. The final two questions were investigated using only the long term equilibrium model. All the modelling carried out while studying these three problems was theoretical computer modelling. The component concentrations of normal urine as given in Table 3.6 were used as the basis for these investigations.

3.5.1 pH and Urinary Supersaturation

The Documenta Geigy Tables (132) give the range of normal urinary pH to be between 5,5 and 7. Accordingly, the supersaturation of normal urine was modelled with pH varying from 4,0 to 8,0. It seemed feasible to do the modelling using both the

short and long term models proposed in section 3.4. The reason for this was the improved performance of the model in validation when the qualitative kinetics of brushite and hydroxyapatite precipitation was taken into account.

In addition to the basic set of components as given in Table 3.6, uric acid was also considered. The concentration was calculated as for the other components from the mean value in the Documenta Geigy Tables. The concentration of uric acid used in the modelling was $0,0028 \text{ mol dm}^{-3}$. The results shown in Table 3.10 for the predicted precipitation of solids from normal urine are essentially the same as given in Table 3.9. The difference between the two sets of results is that Table 3.10 shows those of normal urine while Table 3.9 shows the evaporated artificial urine. The results are given again for the sake of completeness and in order to include the precipitation of uric acid. The discussion of these results with respect to calcium oxalate supersaturation will be mainly dealt with in section 3.5.2.

Possibly the most striking feature of the results presented in Table 3.10 is that supersaturation with respect to all the solids is predicted over nearly the whole biological pH range. At pH = 6,0, normal urine is predicted to be supersaturated with respect to five different solids: calcium oxalate, brushite, hydroxyapatite, struvite and uric acid. If this is true, normal urine must either be a metastable supersaturated solution or contain suspended solid particles. It might also be an intermediate combination of these two extremes.

Table 3.10

Predicted precipitation from normal urine with varying pH.

Concentrations as in Table 3.6. Numbering of solids as in

Table 3.8. Except: Precipitate 5: $C_5H_4N_4O_3 \cdot 2H_2O$ Uric acid

Long term model		Short term model		pH	Intermediate model	
1	5	1	5	4,0	1	5
1 2	5	1 2	5	5,0	1 2	5
1 3	5	1 2	5	5,5	1 2 3	5
1 3 4 5	1 2	4 5	6,0	1 2 3 4 5		
3 4	1 2	4	6,5	1 2 3 4		
3 4	1 2	4	7,0	1 2 3 4		
3 4	2	4	8,0	2 3 4		

These features of normal urine were investigated by Robertson (134) in preliminary studies on particle size distribution in urine. Robertson reconfirmed that it is common for most people to pass precipitated particles in their urine. The nature of these particles tended to vary with pH. Uric acid was found at low pH, calcium oxalate at intermediate pH and calcium phosphate at high pH. These general results appear to be reflected in the trends predicted by the urine model and shown in Table 3.10.

More specifically, Finlayson, in a general review of renal lithiasis (3) has stated that calcium phosphates become very soluble below pH 5,5. The predicted pH range for brushite is 5,0 and above

while that for hydroxyapatite is 5,5 and above. This appears to compare favourably with Finlayson's result. Uric acid is predicted by the urine model to precipitate in the pH range of 6,0 and below. Finlayson reports uric acid to become very soluble above pH 6,8. This model result also appears to be similar to the reported value. Finally, struvite is reported by Finlayson to become very soluble below pH 5,5. The model's prediction for struvite is precipitation at pH 6,0 and above. Once again, the range of precipitation appears to compare well with that found experimentally. An important comment with regard to struvite is that it is only found in infected urines. The hydrolysis of urea by urease (a bacterial enzyme) generates high levels of urinary ammonia and alkalinity. These conditions can lead to urinary supersaturation with respect to struvite and also to hydroxyapatite.

Possibly the most pertinent comment to be made at this stage is that normal people don't have either urinary stones or infected urines. The results of the urine model seem to indicate that supersaturation alone is not enough to cause stones. It is possible that the degree of supersaturation is all important in stone formation. For example, only in infected urines where the levels of ammonia and alkalinity are high enough above normal, does struvite precipitate. In this case, the degree of supersaturation with respect to struvite would be much higher than in normal urines. Obviously, other factors might be preventing stones from forming in a normally supersaturated urine. Unfortunately, the role of natural inhibitors of crystallisation and of crystal aggregation are not yet clearly understood (135). Nevertheless, the degree of

supersaturation of stone forming urine is known to be much greater than that of normal urine (3). It would therefore appear worthwhile, to attempt to relate the degree of supersaturation to stone formation.

At the urine model's present stage of development, it would appear unwise to place much confidence in the calculated level of supersaturation. However, if reliable kinetic data could be obtained and incorporated into the model and if this model could then be independently validated against a quantitative experimental procedure, the calculated supersaturation could be used with increasing confidence. The urine model could then be used to establish the relationship between urinary supersaturation and stone formation. If reliable indices could be established, the model could subsequently be used to screen urines for stone forming potential given the total component concentrations.

3.5.2 Calcium Oxalate Supersaturation

The urine model was used to investigate the supersaturation of calcium oxalate. The results of the effect of pH reported in section 3.5.1. will be extended to take into account the formation of the various hydrates of calcium oxalate. These results will be discussed in this section. As well as the effect of pH, the effect of increasing the major component concentrations has also been studied.

Calcium oxalate is the most commonly occurring constituent of urinary stones with a relative rate of occurrence of 75% (63) of all

stone episodes. This is significantly higher than calcium phosphate stones (8%), struvite stones (9%) and uric acid stones (7%). The role of calcium oxalate supersaturation in urine is quite obviously one of the major areas of concern in urolithiasis.

Calcium oxalate precipitates in three different hydrated forms: calcium oxalate monohydrate (COM), dihydrate (COD), and trihydrate (COT). All three of these solids have been included as possible solids in the urine model. Up until now, however, COM has always been the calcium oxalate solid predicted to precipitate. This is not unexpected since COM is the most thermodynamically stable form with COD slightly less stable, and COT the least stable. If the initial solution is found by MINEQL to be supersaturated with respect to any of these three hydrates, COM will always be the chosen precipitate. Once "precipitated" by the model, the remaining solution will be just saturated with respect to COM and thus undersaturated with respect to both COD and COT. These higher hydrates are for these reasons never predicted to precipitate by MINEQL.

This problem is in many ways analogous to the brushite - hydroxyapatite problem explained in section 3.4. Indeed, it has been reported (125) that the precipitation of calcium oxalates takes place via the transformation from a metastable hydrate such as COT, to the thermodynamically stable monohydrate COM. The initial precipitation of COT is rapid with the conversion to COM being a slower process.

In order to determine whether precipitation of these less

stable hydrated forms of calcium oxalate is initially thermodynamically possible, the more stable forms must be removed from the model. This is the same as the procedure followed for the brushite - hydroxyapatite problem. In this manner, COM is removed and the predicted precipitation of COD is observed. Subsequently, COM and COD are both removed and the supersaturation with respect to COT is studied. The results of the supersaturation of calcium oxalate with varying pH are given in Table 3.11.

Table 3.11

Predicted supersaturation with respect to the
various hydrates of calcium oxalate with changing pH

Precipitate 1: Calcium oxalate monohydrate (COM)

Precipitate 2: Calcium oxalate dihydrate (COD)

Precipitate 3: Calcium oxalate trihydrate (COT)

pH	COM	COD	COT
4,0	1	2	3
5,0	1	2	3
5,5	1	2	3
6,0	1	2	
6,5	1		
7,0	1		
8,0			

Table 3.11 shows that both COT and COD are thermodynamically possible precipitates, at least initially. However COM will always

be the final thermodynamic equilibrium solid. COD is only predicted at pH 6,0 and below while COT is predicted to precipitate at pH 5,5 and below. The monohydrate precipitate is thermodynamically stable over the full acid range. It therefore appears that in normal urines, the role of COT as a precursor of COM would only be possible at pH values below 5,5. The pH range of COM precipitation corresponds well to that reported in the literature. An alkaline pH has been shown (137-139) to prevent calcium oxalate precipitation. Alkalinization has in fact been suggested (139) as therapy for calcium oxalate stones.

Once again, these results would seem to indicate that normal people should pass crystals and that stone-formers could be expected to have a higher degree of supersaturation than normal people. As in the previous section (3.5.1), it should again be stated that this supposed importance of the degree of supersaturation might be an oversimplification. It could well be that normal people have present in their urines natural inhibitors of crystallisation and also of crystal aggregation which stone-formers lack. Robertson et al (136) have shown that recurrent stone-formers pass urine which is consistently supersaturated with respect to calcium oxalate. The degree of supersaturation was greater in these stone-formers than in normal people. This was a direct result of higher levels of urinary calcium and oxalate concentration in the stone-formers. Finlayson (3) also supports the existence of the above relationships. As stated previously (3.5.1) it again appears worthwhile to establish indices relating supersaturation to stone formation.

Following the investigation of calcium oxalate

supersaturation with respect to pH, the effect of increasing the total concentrations of the major urinary components was studied. The pH was kept constant at the approximately normal value of 6,0 and the full long term equilibrium model was used throughout these studies. In this case, the calculated level of supersaturation of calcium oxalate monohydrate was used. While not much confidence should be placed in the actual value, the relative increase or decrease will be much more significant. This value was calculated in the following way. Starting with the normal component concentrations of all components, the concentration of the component of interest was increased up to six times its normal value. In this way, the calculated level of calcium oxalate supersaturation could be plotted against the relative increase in total component concentration. The slope of such a plot, shown to be approximately linear, will give the relative increase or decrease in supersaturation. These slopes may then be compared for all components and a quantitative measure obtained of the effect each component has on calcium oxalate supersaturation. These plots are presented in Figure 3.2 and the slopes of the lines together with the associated correlation coefficient are given in Table 3.12.

Table 3.12 and Figure 3.2 show immediately that oxalate is by far the most important factor in producing calcium oxalate supersaturation. A six-fold increase in oxalate concentration from $3,18 \times 10^{-4} \text{ mol dm}^{-3}$ to $1,91 \times 10^{-3} \text{ mol dm}^{-3}$ effectively increases calcium oxalate supersaturation 16 times more than a similar increase in calcium concentration from $5,75 \times 10^{-3} \text{ mol dm}^{-3}$ to $3,45 \times 10^{-2} \text{ mol dm}^{-3}$. Magnesium shows a similar effect to that of

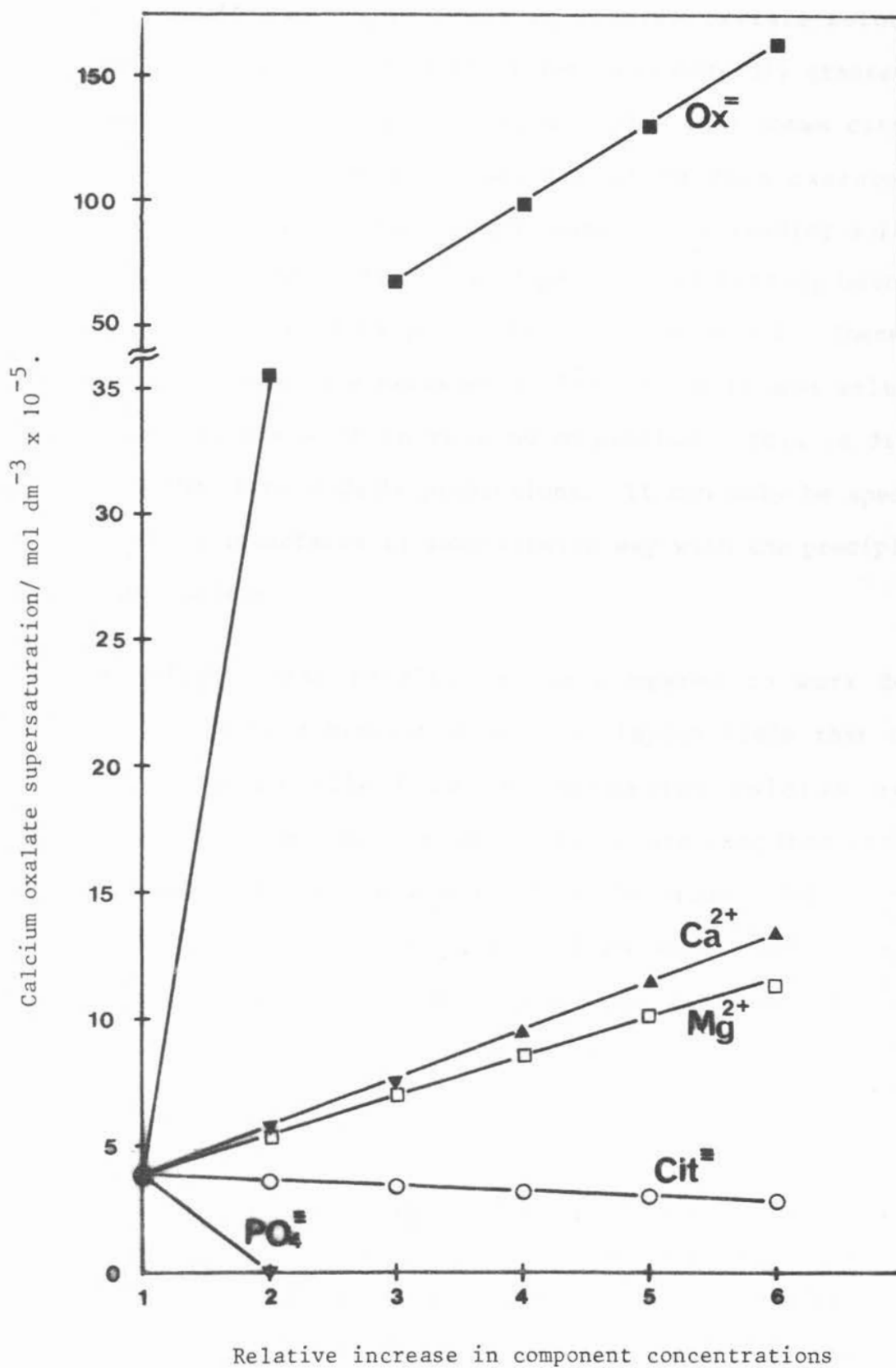
Table 3.12

Relative change in Calcium oxalate supersaturation induced by increasing the concentration of the major urinary components.		
Component	Relative change (slope of line in Figure 3.2)	Correlation Coefficient
Oxalate	31,8	1,000
Calcium	1,93	0,999
Magnesium	1,49	0,993
Citrate	-0,21	0,999
Phosphate	-3,87	1,000

calcium. Citrate and phosphate both decrease calcium oxalate supersaturation. Citrate, however, only produces a marginal decrease, while that of phosphate is far more marked. The effect of increasing phosphate is only based on two observations and not six, however, and should be interpreted cautiously.

These predicted trends show a qualitative similarity to experimental work done. Robertson et al (141) have shown that large increments in dietary calcium cause a small increase in calcium oxalate crystalluria. Increments of dietary oxalate, however, markedly augmented the incidence of calcium oxalate crystalluria. This experimental work was carried out at pH 6,0 which is equivalent to the pH of this investigation. Brigman and Finlayson (142) have shown that orthophosphate causes a 53%

Figure 3.2 Effect of increasing component concentrations on calcium oxalate supersaturation.



reduction in calcium oxalate precipitation relative to control animals. Elliot and Eusebio (143) have shown that citrate and magnesium cause a large increase in calcium oxalate solubility. They also show that the effect of citrate was markedly greater at pH of 7,0 than at pH 5,0. The theoretical model only shows citrate to cause a slight increase in solubility of calcium oxalate. One possible explanation for the marked degree of increasing solubility due to citrate might be the role of pH. It has already been shown that calcium oxalate stops precipitating above pH 7,0. There is no such convenient alternate explanation for the increased solubility of calcium oxalate with increasing magnesium. This is directly opposed to the urine model's predictions. It can only be speculated that magnesium interferes in some kinetic way with the precipitation of calcium oxalate.

Finally, these results can be compared to work done by Finlayson (3) using a similar model. Finlayson finds that oxalate is 15 times more effective in increasing calcium oxalate supersaturation than calcium is. This figure compares extremely well with the factor of 16 obtained from the urine model. Finlayson also found magnesium to increase and phosphate and citrate to decrease the calcium oxalate supersaturation. The results are also in qualitative agreement with work done using another similar model (144).

3.5.3. The Role of Cadmium in Urolithiasis

The final factor examined with the urine model is the influence of cadmium (II) ions on supersaturation. This arises from

reports (13-17) of a significantly higher than average incidence of kidney stones amongst coppersmiths. These coppersmiths use brazing rods with a high cadmium content. The cadmium is thought to upset the calcium metabolism in some way leading to elevated concentrations of calcium in the urine and also to kidney stones.

In preliminary studies of the presence of cadmium in urine, the urine model showed that a large proportion of the cadmium was complexed by citrate. In general, over 50% of the total cadmium was accounted for by soluble cadmium -citrate complexes. Citrate is a well known inhibitor of urinary stones (144-147). This inhibition takes place via the complexation of calcium and also by direct inhibitory influence on the crystallisation of calcium oxalate. It was therefore decided to investigate in more detail the interfering role cadmium plays in complexing urinary citrate.

In order to accomplish this, one further feature of the modelling of urine was used. While citrate has been shown to play a limited role in reducing calcium oxalate supersaturation, it has also been shown to make a significant reduction in struvite supersaturation. These features are explained in terms of the calcium and magnesium complexation by citrate. Because of citrate's far more significant role in struvite supersaturation, it was decided to use this as a measure of the inhibitory power of citrate. This does not take into account the possible direct inhibitory effect of citrate on precipitation. In terms of the urine model it is nevertheless a useful index of citrate's thermodynamic inhibitory role in urine.

The procedure followed in investigating these phenomena began with normal urine at a pH of 6,0. The citrate concentration was then increased until struvite stopped precipitating. Maintaining this citrate concentration, cadmium was then introduced and its concentration increased until the struvite reappeared as a precipitate. Following this, and maintaining the citrate and cadmium concentrations, the various drugs were introduced one at a time and their concentrations increased until the struvite once again stopped precipitating.

In this way, the inhibitory role of citrate is first shown. The interference of cadmium with this inhibition is then revealed. Finally, the efficacy of the drugs in complexing cadmium can be observed. Table 3.13 shows the inhibitory effect of increasing citrate concentration on struvite precipitation. The normal concentration of citrate is $3,21 \times 10^{-3} \text{ mol dm}^{-3}$. At a concentration of $2,77 \times 10^{-2} \text{ mol dm}^{-3}$, struvite stops precipitating. The concentrations given for the two solids, struvite and calcium oxalate, are expressed as the number of moles of precipitate per litre of solution. The use of these units is mathematically convenient for the computation performed by MINEQL and has been described in section 3.2.

Table 3.13

Inhibitory effect of increasing citrate concentration on struvite supersaturation at pH = 6,0.

All concentrations expressed in (mol dm⁻³ x 10⁵)

[Cit] _T	[MgCit]	[CaCit]	[Str] _S	[CaOx] _S
1280	185	17,1	141	3,22
1490	212	19,9	114	3,09
1740	244	23,1	83,1	2,93
2030	280	26,8	48,2	2,76
2370	320	31,1	8,6	2,57
2770	335	36,9	-	2,77

It is clear from Table 3.13 that the decrease in struvite precipitation is due to the increased amount of magnesium complexed by citrate in the MgCit soluble complex. This does not appear to be the case for calcium oxalate precipitation. Only a small decrease in the calcium oxalate precipitate is observed for a much larger increase in the CaCit soluble complex. On the normalised scale used, an increase of about 20 in [CaCit] is accompanied by a decrease of 0,5 in [CaOx]_S. A further 4 is accounted for by the decrease in [hydroxyapatite]_S not shown in this table. It therefore appears as though citrate has a strong thermodynamic inhibitory effect on struvite, but that its observed role in the inhibition of calcium oxalate and hydroxyapatite is not thermodynamic. One may speculate that this role is likely to be a kinetic one.

On introducing cadmium while keeping citrate at the level at which it inhibits struvite precipitation, the citrate is strongly complexed by cadmium. Table 3.14 shows that of the total cadmium concentration, over 50% is bound in citrate complexes.

Table 3.14

The complexation of citrate by cadmium and the resultant promoting effect of struvite supersaturation at pH = 6,0.
All concentrations expressed in (mol dm⁻³ x 10⁵)

[Cd] _T	[CdCit] _T	[MgCit]	[CaCit]	[Str] _S	[CaOx] _S
321	184	328	31,7	-	2,93
375	214	327	31,3	-	2,98
437	250	326	30,9	-	3,02
510	290	325	30,4	0,45	3,08
595	337	322	29,9	2,44	3,17
694	390	319	29,3	5,37	3,26

A cadmium concentration of $5,10 \times 10^{-3}$ mol dm⁻³ is effective in restricting the thermodynamic inhibitory effect of citrate at a concentration of $2,77 \times 10^{-2}$ mol dm⁻³. The effect that cadmium has on the kinetic role of citrate in inhibiting calcium oxalate and hydroxyapatite cannot be directly investigated with this urine model. However, it appears that cadmium could play an important role in this regard by complexing citrate and thus reducing its inhibitory activity.

In order to counteract the complexation of citrate by cadmium, various drugs were introduced. Table 3.15 shows the drug concentrations required to prevent struvite from precipitating in the presence of cadmium.

Table 3.15

Therapeutic effect of cadmium complexing drugs in reducing urinary struvite precipitation at pH = 6,0.

Struvite precipitation is indicated by "ppt".

Full names of drugs given in section 3.3.4.

$\frac{[\text{Drug}]_T}{\text{mol dm}^{-3} \times 10^{-5}}$	EDTA	UNI	PEN	DMSA	BAL	DTPA	DDC
5,0	ppt	ppt	ppt	ppt	ppt	ppt	ppt
10,0	ppt	ppt	ppt	ppt	ppt	ppt	ppt
20,0			ppt				ppt
30,0							ppt
40,0							

Table 3.15 shows that all the drugs have very similar therapeutic effects. DTPA, EDTA, UNI, DMSA and BAL appear to be marginally better than PEN and DDC at complexing cadmium and therefore reducing struvite supersaturation. To give an example of the ability of these drugs to form strong complexes with cadmium, at a total EDTA concentration of $1,00 \times 10^{-4} \text{ mol dm}^{-3}$ in the urine, the CdEDTA soluble complex concentration is $9,99 \times 10^{-5} \text{ mol dm}^{-3}$.

One further comment needs to be made. Cadmium has been related to both urinary stones and to hypercalciuria. The above

results might therefore be true for normal calcium concentrations, but not at elevated calcium concentrations. In order to check that this was not the case, the calcium concentration was increased from $5,75 \times 10^{-3} \text{ mol dm}^{-3}$ to $2,30 \times 10^{-2} \text{ mol dm}^{-3}$ and the MINEQL runs repeated. This increase in calcium concentration took it well outside the normal concentration range given by Documenta Geigy Tables as $3,20 \times 10^{-3} \text{ mol dm}^{-3}$ to $8,13 \times 10^{-3} \text{ mol dm}^{-3}$.

The results obtained showed the same trends observed in Tables 3.13 and 3.14. The only difference in the results was the increased concentration of any species with calcium as a component. As shown in section 3.5.2, calcium causes an increased supersaturation with respect to calcium oxalate. This has also been shown true of hydroxyapatite.

It seems possible, therefore, that the increased level of calcium in the urine stems from some influence cadmium has in another body compartment. The increased urinary calcium will also contribute to the greater degree of supersaturation in urine. Thus it is likely that cadmium has two roles in increasing urinary supersaturation. Firstly, if it is indeed responsible for hypercalciuria, then this effect will result in increased urinary supersaturation with respect to both calcium oxalate and hydroxyapatite. Secondly, it would appear that cadmium complexes citrate fairly strongly and in this way prevents this component's thermodynamic inhibition of struvite supersaturation and kinetic inhibition of calcium oxalate precipitation.

CHAPTER 4

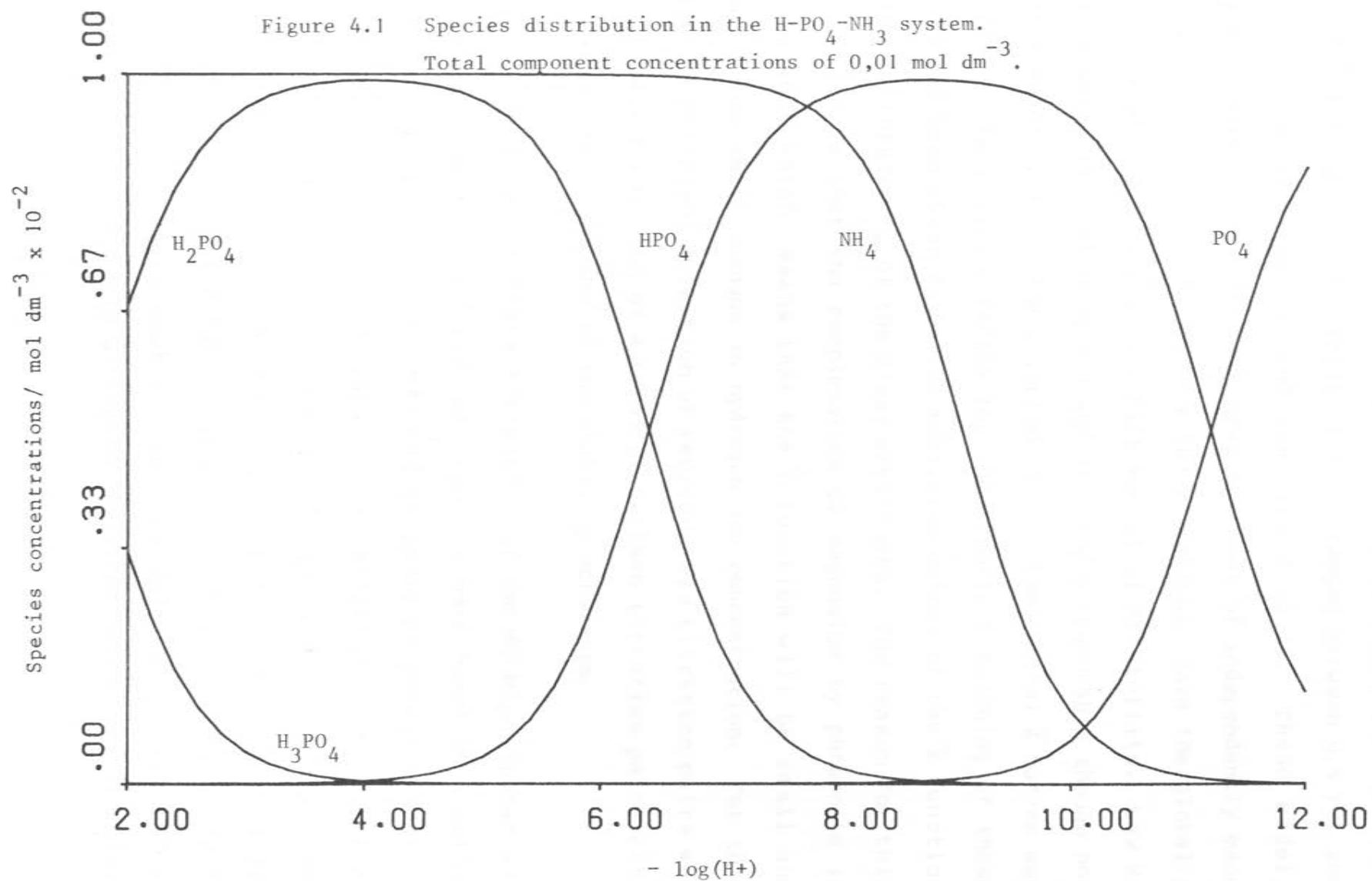
GENERAL DISCUSSION

The general discussion is intended for giving interpretations to the results reported in Chapter 2 and Chapter 3. Ways in which these results could be improved are also mentioned. The extent to which these results meet the objectives of research set out in Chapter 1 is also discussed. Finally, the present research as a whole is evaluated and a proposal made as to the use of the urine model in the future.

The potentiometric characterisation of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system of solution equilibria was partially achieved. The H-PO_4 and H-NH_3 subsystems have been shown to be well characterised by the very good matching of the experimental and theoretical $\bar{Z}_\text{H}$ complex formation curves. The close agreement between the $\log K_\text{w}$ values independently obtained from these two protonation systems was reassuring and lent confidence to the experimental technique employed. A value for $\log K_\text{w}$ of -13,449 was refined for the H-PO_4 system while the H-NH_3 system yielded a refined $\log K_\text{w}$ of -13,469. A species distribution plot for both these systems is given in Figure 4.1. The following species are shown: H_3PO_4 , H_2PO_4 , HPO_4 , PO_4 and NH_4 . The concentration of each species in a solution which is $0,01 \text{ mol dm}^{-3}$ with respect to both phosphate and ammonia is shown plotted against $-\log[\text{H}^+]$.

The agreement between the experimental and theoretical $\bar{Z}$ complex formation function curves for the $\text{Mg-PO}_4\text{-H}$ system was not as good as those for the ligand-proton systems. The stability constants for this system were refined globally from ten pairs of titrations covering a wide range of metal and ligand concentrations and ratios. The phosphate concentration varied between $0,003$ and

Figure 4.1 Species distribution in the $\text{H}-\text{PO}_4-\text{NH}_3$ system.
Total component concentrations of $0,01 \text{ mol dm}^{-3}$.



$0,022 \text{ mol dm}^{-3}$, the magnesium concentration between $0,002$ and $0,008 \text{ mol dm}^{-3}$ and the ligand : metal ratio ranged between $0,5 : 1$ and $10 : 1$. The pH range covered was from 3 to 10. These widely varying conditions and the fact that a number of independently made up solutions were used to perform the titrations, gave the globally refined stability constants a fair amount of credibility. Low R-factors were obtained of $0,0012$ and less and a reasonable though not perfect match between the experimental and theoretical $\bar{Z}$ curves was observed. One possible reason for the imperfect matching of these curves has been shown to be the sensitive nature of the $\bar{Z}$ function to the calibrated E_0 of the glass electrodes. The reason for this sensitivity is that the complexation of magnesium by phosphate is fairly weak which means that the $\bar{Z}$ function will be small and sensitive to small changes in hydrogen ion concentration. For this reason, a stringent selection of reproducible titration pairs was made in which only ten of a total of fifteen titration pairs were chosen for the refinement of the stability constants.

An alternative characterisation of the $\text{Mg-PO}_4\text{-H}$ system was attempted. The complex formation functions were based on a method developed by Osterberg (53, 54) and extended by Sarkar and Kruck (56-59). McBryde (60) has shown in an alternative theoretical development, that the functions $(\partial T_H / \partial T_L)_H$ and $(\partial T_H / \partial T_M)_H$ are related to the conventional $\bar{Z}$ and $\bar{Z}_H$ complex formation functions. It was shown that these functions are not sensitive enough to be used in characterising weakly complexing metal equilibria such as magnesium-phosphate. This attempted characterisation was therefore abandoned.

Species distribution plots for the $\text{Mg-PO}_4\text{-H}$ system are given in Figures 4.2 and 4.3. Figure 4.2 shows the MgH_2PO_4 , MgHPO_4 , MgPO_4 and MgOH species. The concentration of these species in a solution which is $0,01 \text{ mol dm}^{-3}$ with respect to phosphate and magnesium is shown plotted against $-\log[\text{H}^+]$. Notice that the same scale as Figure 4.1 is used in both Figure 4.2 and 4.3. This means that the change in phosphate species from Figure 4.1 to Figure 4.3 is entirely due to the complexation of magnesium and phosphate shown in Figure 4.2. The magnesium-phosphate equilibria were only established between pH 3 and 10 and this is the range of pH in Figure 4.2 and 4.3. Figure 4.2 also shows that over most of the pH range covered, the magnesium accounts for about one third or less of the total phosphate present.

Due to the fairly weak complexation of magnesium with phosphate and also the much weaker complexation of magnesium with ammonia reported in the literature, it was decided to tackle the characterisation of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system in a different fashion. A literature survey had revealed no ternary complexes of magnesium with phosphate and ammonia and also no complexes between phosphate and ammonia. A null hypothesis was formulated in the following way: "the addition of ammonia does not alter the complexation equilibria between magnesium, phosphate and protons". In order to test this hypothesis, a titration was performed using only magnesium, phosphate and protons as components. Further titrations were performed using identical magnesium and phosphate concentrations, but adding increasing concentrations of ammonia. The titrations with ammonia present were compared to the titration with no ammonia

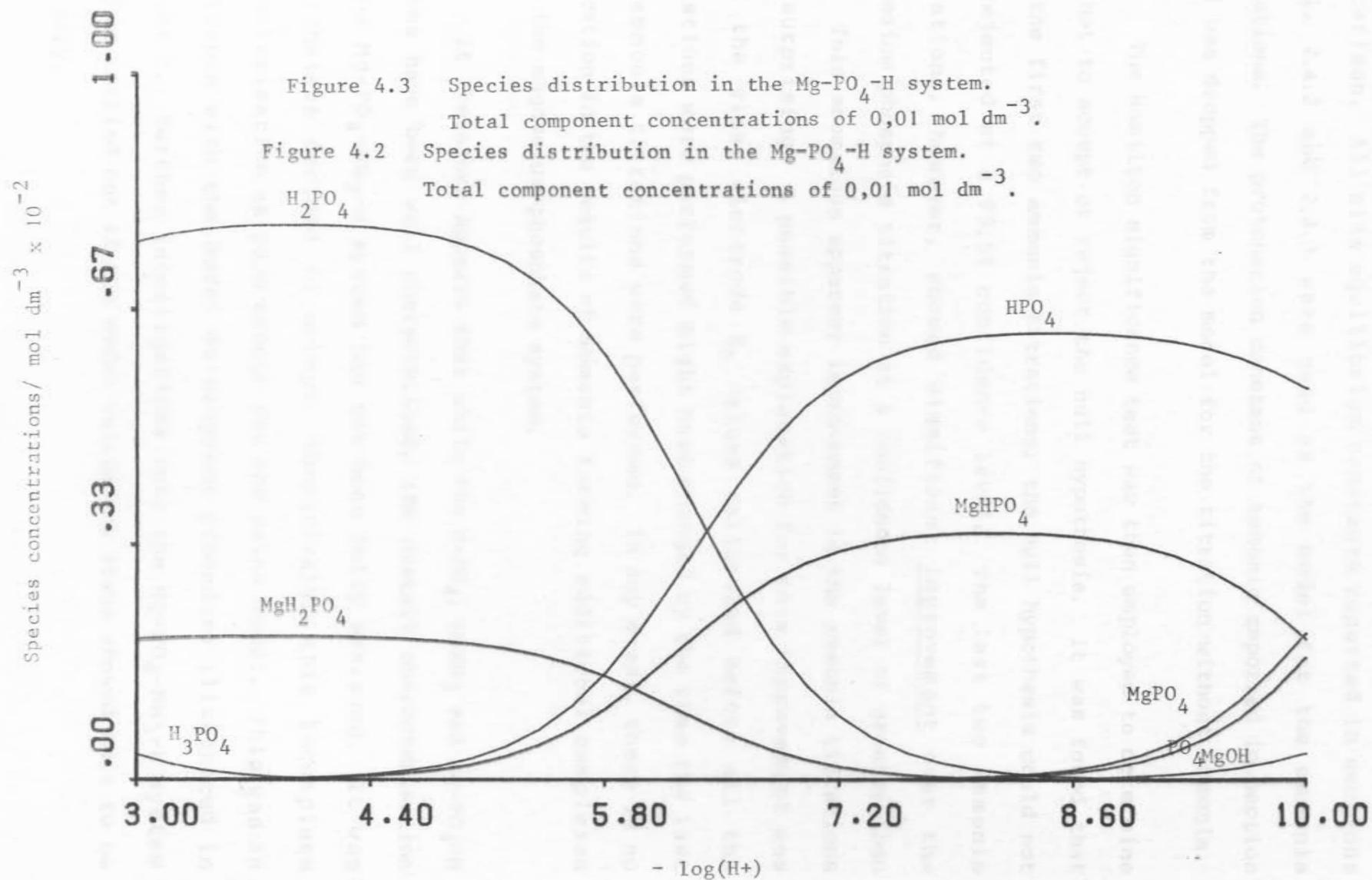
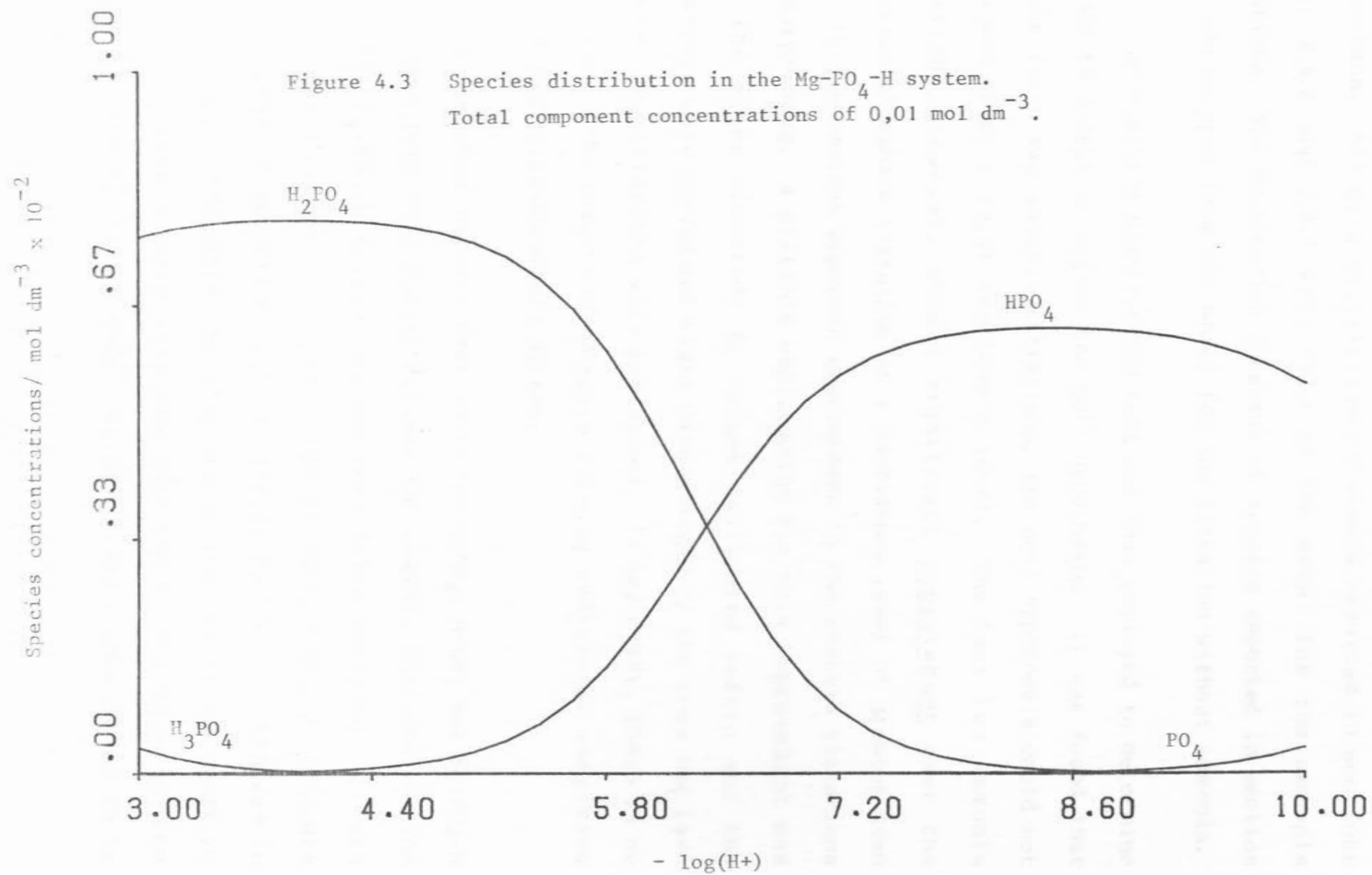


Figure 4.3 Species distribution in the $\text{Mg-PO}_4\text{-H}$ system.
Total component concentrations of $0,01 \text{ mol dm}^{-3}$.



using the R-factor calculated by MINIQUAD as the basis for the comparison. All nine equilibrium constants reported in sections 2.4.1, 2.4.2 and 2.4.3 were used as the model for the ammonia titrations. The protonation constant of ammonia reported in section 2.4.2 was dropped from the model for the titration without ammonia.

The Hamilton significance test was then employed to determine whether to accept or reject the null hypothesis. It was found that for the first two ammonia titrations, the null hypothesis could not be rejected at a 99,5% confidence level. The last two ammonia titrations, however, showed significant improvement over the magnesium phosphate titration at a confidence level of greater than 50%. This anomalous apparent improvement in the ammonia titrations was surprising. A possible explanation for this improvement was that the glass electrode E_0 values calibrated before all the titrations were performed might have changed by the time the last two ammonia titrations were performed. In any event, there is no indication in the results of ammonia forming additional complexes with the magnesium-phosphate system.

It therefore appears that while the $H-PO_4$, $H-NH_3$ and $Mg-PO_4-H$ systems have been well characterised, the overall characterisation of the $Mg-PO_4-NH_3-H$ system has not been fully achieved. It was nevertheless decided to accept, tentatively, this incomplete characterisation as good enough for the urine model. This was in accordance with the model development procedure illustrated in Chapter 1. Further investigations into the $Mg-PO_4-NH_3-H$ system could be carried out if the model validation stage showed this to be necessary.

Table 4.1 lists all the equilibrium constants determined in the present research along with some literature values for the same constants. Although the constants determined are only comparable with constants in the literature measured under similar conditions of medium, ionic strength and temperature, it is of interest to compare the results obtained with those of other workers. As reported constants are often quoted as stepwise and not overall stability constants, the constants measured in the present research have been listed in both overall and stepwise form. The literature values are listed in Table 4.1, in whichever form they have been reported, below the constants determined in the present research. A fair amount of confidence has been placed in the protonation constants and K_w measured in the present research. In general, the protonation constants compare well (allowing for medium, ionic strength and temperature differences) with the literature values quoted in Table 4.1. One interesting point is that our value obtained for $\log K_w$ of -13,449 which does not compare so well with the reported (82) value of -13,72. The latter value is, however, for an ionic strength of 0,100 which differs from the ionic strength for our value. The medium and temperature of the two values are the same. The feature of interest is that when strong acid - strong base titrations were carried out to investigate electrode performance, values of $\log K_w$ varying from -13,68 to -13,71 were obtained in a procedure previously described (46). These values, while far less rigorously established than the measured value given in Table 4.1, are in apparent better agreement with the literature

Table 4.1

Comparison of equilibrium constants measured and reported in sections 2.4.1, 2.4.2 and 2.4.3 with literature values.

Complex	$\log \beta$	$\log K$	Ionic Strength (mol dm ⁻³)	Medium	Temp (°C)	Ref
HOH	-13,449	-13,449	0,20	NaCl	25	this
HOH	-13,72		0,10	NaCl	25	82
HPO ₄	11,179	11,179	0,20	NaCl	25	this
HPO ₄	11,30		0,15	KNO ₃	37	30
H ₂ PO ₄	17,580	6,401	0,20	NaCl	25	this
H ₂ PO ₄	18,00		0,15	KNO ₃	37	30
H ₂ PO ₄		6,279	3,00	NaClO ₄	25	32
H ₃ PO ₄	19,264	1,684	0,20	NaCl	25	this
H ₃ PO ₄	19,95		0,15	KNO ₃	37	30
H ₃ PO ₄		1,879	3,00	NaClO ₄	25	32
HNH ₃	9,060	9,060	0,20	NaCl	25	this
HNH ₃	9,29		0,10	KCl	25	41
HNH ₃	9,24		0,00	-	25	37
HNH ₃	9,54		3,50	NH ₄ NO ₃	25	38
HNH ₃	9,24		0,00	-	25	35
MgPO ₄	3,13	3,13	0,20	NaCl	25	this
MgPO ₄	3,4		0,15	KNO ₃	37	31

Complex	$\log \beta$	$\log K$	Ionic Strength (mol dm ⁻³)	Medium	Temp (°C)	Ref
MgHPO ₄	13,12	1,94	0,20	NaCl	25	this
MgHPO ₄	13,1		0,15	KNO ₃	37	31
MgHPO ₄		1,42	3,00	NaClO ₄	25	32
MgHPO ₄		2,50	0,00	-	25	24
MgHPO ₄		2,87	0,00	-	38	25
MgHPO ₄		1,52	0,20	KCl	30	26
MgHPO ₄		2,74	0,00	-	30	27
MgHPO ₄		1,85	0,20	MgCl	25	28
MgHPO ₄		1,78	0,10	KNO ₃	15	29
MgH ₂ PO ₄	19,09	1,51	0,20	NaCl	25	this
MgH ₂ PO ₄	18,60		0,15	KNO ₃	37	31
MgH ₂ PO ₄		0,16	3,00	NaClO ₄	25	32
MgOH	-10,38	3,07	0,20	NaCl	25	this
MgOH		2,58	0,00	-	25	33

value in Table 4.1 of -13,72. While no confidence may be placed in the values obtained from the sodium hydroxide-hydrochloric acid titrations, it is possible to speculate that K_w may be influenced by the presence of phosphate and ammonia in solution. The values for the complexation of magnesium and phosphate are also in good agreement with the literature considering the differences in temperature, ionic strength and medium. The most commonly reported stability constant of magnesium and phosphate is for the MgHPO₄ complex. Many workers report this complex as the only magnesium

phosphate complex (24-29). This is partially due to the restricted pH range of their investigations and, it appears, partially due to this being the most predominant magnesium phosphate complex as shown by Figure 4.2. The agreement between our value for this complex of $\log \beta_{1011} = 13,12$ and that reported by Childs (31), of 13,1 is good. This however, could be fortuitous considering the differences in medium, ionic strength and temperature. The value of Smith and Alberty (28) measured under very similar conditions is $\log K_{1011} = 1,85$ while the measured value of the present research is 1,94. This does appear to be a good agreement.

The stability constant for MgPO_4 of $\log \beta_{1010} = 3,13$ compares favourably with that of Childs (31) of 3,4 even though there are again differences in experimental conditions. The stability constant of MgH_2PO_4 of $\log \beta_{1012} = 19,09$ is reasonably close to Childs' value of 18,60, but substantially different from Havel and Högfeltdt's value (32). The stepwise constant measured by us is $\log K_{1012} = 1,51$ while Havel and Högfeltdt report a value of 0,16 measured in sodium perchlorate at an ionic strength of 3,0. The least confidence should be placed in the MgOH stability constant value as it was refined over only 47 titration points. The value of $\log K_{001-1} = 3,07$ does show some similarity to the value of 2,58 reported by Stock and Davies (33), however. Not one of the literature equilibrium constants reported in Table 4.1 was subjected to the stringent test of experimental versus theoretical complex function curve matching. This procedure employed in characterising the systems studied in the present research improves the reliability of our values. Also, it should be emphasised that most of the

values shown in Table 4.1 apply to different conditions from ours. Nevertheless, the foregoing comparisons lend confidence to our results.

There are a few ways in which the potentiometric characterisation of the various systems studied could be improved. A computer program, ESTA, is currently being developed by May and Murray at UWIST in Cardiff which will allow simultaneous refinement of stability constants and E_0 values. This could eliminate the problems experienced in electrode calibration which were a general feature of this research. In particular, it might eliminate the anomalous apparent improvement in the ammonia titrations experienced in section 2.4.4. A further improvement might be possible if a magnesium ion selective electrode were used as well as a glass electrode. This would mean one less unknown concentration in the refinement of the stability constants. The performance of the electrode would obviously have to be investigated and shown to be accurate enough for the purpose.

The solution equilibria of the three systems: H-PO_4 , H-NH_3 and $\text{Mg-PO}_4\text{-H}$, appear to have been fairly well characterised. The overall $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system has not been well characterised. However, for the purposes of our urine model, the partial characterisation should suffice. Indeed, model validation undertaken after our measured values were introduced into the model has shown that it is more likely that the solubility product of struvite is now in error and not the solution equilibria associated with this system. Thus the first objective of the present research,

when seen in the context of the overall modelling process, has been met.

The basic urine model has been constructed at an ionic strength of 0,200 and a temperature of 37°C. There are a number of ways in which the urine model could be improved. The amount of effort needed in making these improvements, however, would not necessarily be worthwhile. In terms of the improvement in the quality of the results obtained, a large amount of effort might be required to produce a small improvement. This process is in general exemplified by the "law of diminishing returns". It is nevertheless of importance to be aware of ways in which improvements could be made.

Obviously, the more equilibrium constants and solubility products measured at 37°C and an ionic strength of 0,200 that are included in the model, the better. In particular, all these constants ought, preferably, to be measured in a medium of sodium chloride. If this were the case, no constants involving sodium or chloride would be included in the model. Any small effects of sodium and chloride complexation would be implicit in the values of the constants making up the model.

The validation, on the basis of pH, of the basic urine model lends confidence to the choice of values for the equilibrium constants of the soluble complexes. A pH of 6,5 experimentally observed was approximated by a pH of 6,0 predicted by the model. The predicted pH is largely accounted for by the equilibrium constants of the soluble complexes. In general, it appears as though

critical appraisal of the solubility products requires more attention than for the equilibrium constants. There are two areas in which this attention is required. Firstly, the problem of correcting for ionic strength and secondly, consideration of factors influencing the kinetics of precipitation. The ionic strength correction problem might best be approached by finding literature values of solubility products measured at the same temperature, but at differing ionic strengths. The ability of the program LOGKSP would then be tested by predicting the solubility product at different ionic strengths from each known value at a given ionic strength.

The kinetics of precipitation could be incorporated into the model by obtaining data for the change in apparent solubility product with time. Our computations indicate that the four most important solids are calcium oxalate, brushite, hydroxyapatite and struvite. These solids, in particular, need further investigation as to their kinetics of precipitation. Given a knowledge of the functional relationship of solubility product with time, the urine model could be used to calculate the extent of precipitation with time. This would be achieved by a number of discrete MINEQL runs with the relevant solubility products changed appropriately. This would essentially provide a more detailed picture than has been revealed by the "short term" and "long term" models discussed in section 3.4.

As described in section 1.2, the approach to model development undertaken in the present research is one of iterative model construction, validation and refinement. The results reported

in Chapter 2 and Chapter 3 represent one of these iterations. The potentiometric characterisation of the $\text{Mg-PO}_4\text{-NH}_3\text{-H}$ system represents a stage in which the model was refined by including the relevant equilibrium constants as determined by us in the investigation of the various subsystems. Validation showed the model to be acceptable. The model was then used, with a knowledge of its limitations, to investigate a number of problems. While reasonable answers were obtained, it is obvious that further refinement, especially in the region of solid precipitation, is required. In future developments of this project, this would be the next objective. It, therefore, seems acceptable to claim that the objective of model construction has been achieved. This does not, however, preclude the possibility of making improvements in the future.

The approach undertaken in the validation of the urine model appears to have been a sound one. It should again be stated that the experimental observations used in the validation of the urine model were obtained by Rodgers and Wandt in an independent project. Their work was not undertaken with the specific aim of providing validation data for the urine model. It was indeed fortunate that the data of Rodgers and Wandt were, by chance, available for the validation of the urine model. The successful validation has suggested two procedures which may be used in the future. The urine model may conveniently be divided into two sections: the solution equilibria and the solid phase equilibria with this solution. The equilibrium constants would ideally be validated first and once this had been achieved, the validity of the solubility products

investigated. It is suggested that the validation of the solution equilibria be undertaken by means of a titration. An initial solution containing all the components of the urine model would be prepared. Then, starting at a pH of about 3, this solution could be titrated with sodium hydroxide until pH of about 9. Component concentrations would have to be kept fairly low to prevent the formation of precipitate. A titration curve of volume of sodium hydroxide added versus pH would be obtained. The theoretical urine model could then be employed to simulate this curve at discrete points based on the starting concentrations and the volume and concentration of sodium hydroxide added. The degree of fit obtained would provide some indication of the reliability of the equilibrium constants in the model. This procedure could possibly be undertaken using calcium and magnesium ion selective electrodes at the same time as the glass electrode. The extent of these electrodes' selectivity for calcium and magnesium in the presence of each other would have to be taken into account. The degree of matching between the three experimental and theoretical titration curves so obtained would provide an exceptionally stringent test of the validity of such a model. The next phase of the proposed two stage validation would be to include the solubility products in the partially validated model. In order to test the reliability of these solubility products, a similar procedure to that used in the present research would be used. Solutions of varying pH would be made up. These solutions would have component concentrations greater than those previously used in order to allow precipitation. Ideally, the solutions would be made up in water just below the boiling point and

then cooled to 37°C. On reaching this temperature equilibration would be allowed for whatever time interval it was desired to test the solubility products over. The pH would then be measured and the precipitate rapidly filtered. An XRD analysis would be performed as soon as possible in order to prevent further phase changes in the solids. The XRD analysis is usually a qualitative test of the various solids present in a given sample. It is expected (148) that, with the aid of a computer program, this analysis will become quantitative. In this way, the amount of each precipitate could be expressed as a number of moles per cubic decimetre of supernatant solution. This would be analogous to the degree of supersaturation or precipitation calculated by the urine model. The solubility products could thus be validated at different pH values and over the discrete time intervals for which they apply. Curves of pH versus the degree of precipitation could be constructed for the various time intervals considered. Again, a graphical matching of these experimental and theoretical curves would provide a stringent test of the validity of the model. Finally, it should be realised that this validation is only a measure of how reliable the equilibrium constants and solubility products are. A perfect match obtained between the experimental and theoretical curves, would not necessarily imply that the model constructed is a good model of urine. A model's reliability in modelling urine is only measured by the extent to which it can answer questions about the nature of urine. Once again, it appears acceptable to state that the objective of model validation has been achieved. At the same time, it is recommended that possible improvements suggested by this validation procedure should certainly be undertaken in future

developments of the present research.

The final objective of the present research was to use the urine model to investigate three specific features of urolithiasis. The first of these was the effect of pH on urinary supersaturation. This is a reasonably well understood urolithiasis problem and the model's ability to model urine would be tested here. Secondly, the effect of common inhibitors and promoters of calcium oxalate precipitation was investigated. One of the aims of this section was to compare our findings with those reported by other workers. Finally, the role of cadmium in urolithiasis was investigated. This is a problem which has not been widely researched and it was hoped that the urine model would give some insight into why cadmium appears to cause urinary stones.

The urine model has predicted that a normal urine at a pH of 6.0 will be supersaturated (see definition in section 1.1) with respect to calcium oxalate, brushite, hydroxyapatite, struvite and uric acid. It has been shown that these five solids together account for about 97% of all urinary stones (3). The pH ranges over which these five solids are predicted to precipitate are in general agreement with the pH ranges in which the kidney stones containing these solids are found (3). An interesting feature of these predictions is that the concentrations at which they are being made are normal. This means that most people would have urine which is supersaturated with respect to a number of solids. In other words, most urines will either be metastable solutions or contain suspended solid particles or be an intermediate combination of these two

extremes. This prediction is indeed true, since Robertson (134) has found that it is common for most people to pass precipitated particles in their urine. The nature of these particles tended to vary with pH in a similar way as is predicted by the urine model. It appears that supersaturation of urine with respect to a solid is not enough to cause a stone to form. Indeed, stone-forming urine is found to be more highly supersaturated than normal urine (3). This really means that stone-formers pass more solid particles or pass urine which, if left to equilibrate, would ultimately contain more precipitate than normal people. It was therefore suggested that it would appear worthwhile to relate the degree of supersaturation of urine to stone formation. Hopefully it could be shown that stone formation is more prevalent above particular levels of supersaturation.

Calcium oxalate is the most commonly occurring urinary stone component accounting for about 75% of all stones (3). While calcium oxalate monohydrate is the most thermodynamically stable form, it has been shown by the urine model that the dihydrate and trihydrate are possible kinetic precursors to the monohydrate. This is in agreement with the observation (125) that the precipitation of calcium oxalate takes place via the transformation from the metastable trihydrate to the thermodynamically stable monohydrate.

As far as promoters and inhibitors of calcium oxalate precipitation or supersaturation are concerned, the urine model shows that oxalate is by far the most important thermodynamic promoter. It is 16 times more effective in increasing calcium oxalate supersaturation than calcium is. This observation compares

well with the value of 15 obtained by Finlayson (3) using a similar urine model. Phosphate was found in section 3.4.3 to be the most effective thermodynamic inhibitor of calcium oxalate supersaturation. This agrees well with the observation (142) that phosphate causes a 53% reduction in calcium oxalate precipitation relative to control animals.

Cadmium's role was finally investigated and it appears that cadmium's complexation of citrate may be a factor which allows stones to form. Citrate has been shown by our urine model to be a slight thermodynamic inhibitor of calcium oxalate and a fairly effective thermodynamic inhibitor of struvite. Moreover, citrate has been found to play an important role in the kinetic inhibition of calcium oxalate and calcium phosphate precipitation (145-147). Our urine model has shown that of the total cadmium concentration in urine, 50% is bound to citrate. In this way, cadmium causes the indirect promotion of struvite supersaturation. This is a thermodynamic process. The fact that cadmium complexes citrate so strongly might also influence the inhibitory role of citrate in the kinetics of precipitation of calcium oxalate and calcium phosphate.

The efficacy of a number of drugs in complexing cadmium and thus reducing the promoting effect of cadmium in urinary supersaturation was also investigated. In general, these drugs have been shown to be equally effective.

The urine model has been shown to behave like urine in a number of ways including the precipitation of solids and the inhibition or promotion of this process. The results obtained have

been in general qualitative agreement with experimental work done as well as other theoretical urine models. The urine model was finally used to identify some important features of a specific problem area in urolithiasis. The final objective of the present research has therefore been achieved.

The achievement of all four of the objectives of the present research is tentative and not final. It has already been shown in section 1.2 that the process of model development is, ideally, an eternal iterative procedure. For this reason, the objectives of the present research may be seen to be achieved in the knowledge of the many improvements which the current iteration of the model development procedure have brought to light. In future research on this topic, these improvements would be implemented and validated in a new iteration, hopefully improving the usefulness of the results obtained thereafter.

Apart from the specific achievements reported in Chapters 2 and 3, there has been a general trend of self-improvement associated with the iterative model development procedure followed in the course of this project. Not only has the actual model been refined in a number of iterations, but the overall process of the modelling of urine has also been refined. With reference to the words of Sillén quoted in section 1.2, it seems appropriate to state that the urine model has not yet been proven useless and that many interesting things have indeed been learnt while working with the model. In particular, the validity of the assumptions made has been tested. The first assumption that urinary supersaturation is the

dominant useful feature of urolithiasis appears to be a good one. The chosen approach of chemical modelling has been shown to be fairly successful and is based on this assumption. The assumption that all reactions and phase transformations are fast has been shown to be mainly limited to the solution equilibria. It appears as though the kinetics of precipitation of at least the most important solids identified by the model will have to be taken into account. Finally, it was assumed that the urinary tract is a closed system. This assumption is really forced by the lack of suitable reliable data on the diurnal variation of urinary concentration and the flow characteristics of urine through the urinary tract. It nevertheless appears to be a reasonable assumption. If suitable data become available, this assumption could be disposed of and a more accurate picture built up of how urine behaves with time.

In the light of the foregoing, the following procedure for the chemical modelling of urine with particular reference to the problem of urolithiasis is recommended. This procedure is based on only one major assumption. This is that urinary supersaturation is the dominant useful feature in considering urolithiasis. The proposed procedure is:

1. A set of reliable and reproducible analytical methods for the determination of the total concentrations of the major urinary components will be chosen.
2. This set of analytical methods will be used to determine the diurnal variation in the total component concentrations in normal and stone-forming populations.

3. Tracer or similar suitable methods will be used to establish the time taken for urine to pass through the urinary tract of normal people as well as stone-formers.
4. The proposed two stage validation procedure already discussed will be implemented. The quantitative nature of this procedure will be of most importance and the graphical matching technique will be used in deciding on the reliability of the proposed model.
5. The urine model will be extended to incorporate the kinetics of precipitation of the most important solids and will be refined until both stages of the validation procedure give good matching between experimental and theoretical validation curves.
6. The average degree of supersaturation of both stone-formers and normal people with respect to the various urinary solids will be established by some experimental procedure. Hopefully, quantitative indices of stone-forming potential will be correlated at this stage.
7. The urine model will then be used to confirm these indices of stone-forming potential by using the model on the data collected at stages 2 and 3. The data of stage 3 will be an indication as to what kind of time span to allow for in the kinetic data incorporated into the model.
8. If all the previous stages of this procedure are achieved, the urine model could be used in the routine analysis of urine for stone-forming potential.

9. The urine model could also be used in directing investigations into how the stone-forming potential of urine could be reduced.
10. Finally, if the set of analytical methods could be automatically controlled by a micro-computer capable of handling the MINEQL program, the procedure of routine analysis of urine followed by the prediction of stone-forming potential and suggested treatment could become completely computerised.

This type of procedure might indeed be a long way off. Nevertheless, it is intended to indicate a possible direction in urolithiasis research as well as definite roles for the urine model. The urine model could be used in establishing reliable indices of stone forming potential based on the degree of supersaturation of urine. The routine screening of urine for stone-forming potential would also be facilitated by the urine model. Finally, exploratory tests in evaluating the therapeutic benefit of different treatments and drugs could be conducted with the aid of the urine model. The basis of these three roles for the urine model is the supersaturation assumption. This assumption might indeed be proved useless, but this has not yet been done and perhaps many interesting things would be learnt in refuting it.

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Chemical content of water for various species used in comparison
 of quality.

Species no.	Chemical Abbreviation ^a	Species no.	Chemical Abbreviation ^a
1	Cu	101	Hg
2	A	102	Co
3	X	103	Fe
104	NO ₃	104	NO ₃
105	Cl ⁻	105	Cl ⁻
106	SO ₄ ²⁻	106	SO ₄ ²⁻
107	NO ₂ ⁻	107	NO ₂ ⁻
108	PO ₄ ³⁻	108	PO ₄ ³⁻
109	NO ₃ ⁻	109	NO ₃ ⁻
110	NO ₂ ⁻	110	NO ₂ ⁻
111	NO ₃ ⁻	111	NO ₃ ⁻

APPENDIX 1

^a See text for full description of compounds.

10101	10101	10101	10101	10101	10101
10102	10102	10102	10102	10102	10102
10103	10103	10103	10103	10103	10103
10104	10104	10104	10104	10104	10104
10105	10105	10105	10105	10105	10105
10106	10106	10106	10106	10106	10106
10107	10107	10107	10107	10107	10107
10108	10108	10108	10108	10108	10108
10109	10109	10109	10109	10109	10109
10110	10110	10110	10110	10110	10110
10111	10111	10111	10111	10111	10111
10112	10112	10112	10112	10112	10112
10113	10113	10113	10113	10113	10113
10114	10114	10114	10114	10114	10114
10115	10115	10115	10115	10115	10115
10116	10116	10116	10116	10116	10116
10117	10117	10117	10117	10117	10117
10118	10118	10118	10118	10118	10118
10119	10119	10119	10119	10119	10119
10120	10120	10120	10120	10120	10120

Equilibrium constant database for complex species used in computer modelling.

<u>Component no.</u>	<u>Chemical</u> <u>Abbreviation*</u>	<u>Component no.</u>	<u>Chemical</u> <u>Abbreviation*</u>
1	Ca	2	Mg
4	K	11	Cd
50	H	102	SO ₄
107	NH ₃	109	PO ₄
117	Cit	118	Ox
128	EDTA	158	UH
159	DMP	160	PEN
161	DMS	162	BAL
163	DTPA	164	DDC

* See text for full description of components.

00102									
10001	-13	07	50	-1					
10002	3	76	118	1	50	1			
10003	5	06	118	1	50	2			
10004	3	40	118	1	1	1			
10005	5	38	118	1	1	2			
10006	3	12	118	1	2	1			
10007	1	00	118	1	4	1			
10008	5	49	117	1	50	1			
10009	9	58	117	1	50	2			
10010	12	40	117	1	50	3			
10011	3	16	117	1	1	1			
10012	7	23	117	1	1	1	50	1	
10013	3	25	117	1	2	1			
10014	7	03	117	1	2	1	50	1	
10015	1	46	102	1	50	1			
10016	2	26	102	1	1	1			
10017	1	92	102	1	2	1			
10018	-11	32	50	-1	1	1			
10019	-9	94	50	-1	2	1			
10020	8	71	107	1	50	1			
10021	11	08	109	1	50	1			
10022	17	45	109	1	50	2			
10023	19	19	109	1	50	3			
10024	4	93	109	1	1	1			

10025	12 29	109	1	1	1	50	1
10026	17 95	109	1	1	1	50	2
10027	3 22	109	1	2	1		
10028	13 11	109	1	2	1	50	1
10029	19 06	109	1	2	1	50	2
10030	5 21	158	1	50	1		
10089	-10 50	11	1	50	-1		
10090	-9 50	11	2	50	-1		
10091	2 00	11	1	102	1		
10092	2 60	11	1	107	1		
10093	4 70	11	1	107	2		
10094	6 15	11	1	107	3		
10095	7 00	11	1	107	4		
10096	3 10	11	1	117	1		
10097	7 50	11	1	117	1	50	1
10098	10 00	11	1	117	1	50	2
10099	4 45	11	1	117	2		
10100	2 70	11	1	118	1		
10101	4 50	11	1	118	2		
10102	5 40	11	1	118	3		
10103	12 50	159	1	50	1		
10104	21 06	159	1	50	2		
10105	17 32	159	1	11	1		
10106	61 91	159	3	11	3	50	1
10107	37 72	159	2	11	2		
10108	28 19	159	2	11	1		
10109	35 19	159	2	11	1	50	1
10110	9 12	128	1	50	1		
10111	15 03	128	1	50	2		
10112	17 66	128	1	50	3		
10113	19 66	128	1	50	4		
10114	13 79	128	1	11	1		
10115	16 51	128	1	11	1	50	1
10116	9 36	128	1	1	1		
10117	7 76	128	1	2	1		
10118	10 30	160	1	50	1		
10119	18 10	160	1	50	2		
10120	20 40	160	1	50	3		
10121	10 50	160	1	11	1		
10122	18 00	160	2	11	1		
10123	2 45	160	1	1	1		
10124	2 80	160	2	1	1		
10125	2 55	160	1	2	1		
10126	3 50	160	2	2	1		
10127	12 00	161	1	50	1		
10128	21 42	161	1	50	2		

[illegible]