ELECTROLYTE SYSTEMS RELATING TO MILK

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at Massey University.

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ABSTRACT

A pH titration method using a glass electrode/saturated calomel electrode cell has been applied to the determination of acidity and stability constants in dilute aqueous solutions at 25°C. Two computer programs in FORTRAN have been written and used to calculate the constants from the titration data.

Acidity constants for the homologous series of aliphatic α,ω-dicarboxylic acids (succinic acid to sebacic acid inclusive) have been determined. Acidity constants for tricarballylic, citric and a number of other carboxylic acids have also been determined and the values obtained are in good agreement with values reported by other workers. A new set of micro acidity constants, differing from those reported by other workers, has been obtained for citric acid from a pH titration study of various methyl esters of citric acid. The stability constants for the magnesium and calcium complexes of citric acid have been redetermined.

The method of calculating acidity constants from substituent effects has been refined to distinguish between macro and micro acidity constants and has been used with some success in the prediction of both micro and macro acidity constants. Good values have been obtained for the first and second but not the third acidity constants for citric acid using this technique. An analogous method for calculating stability constants from substituent effects has been tested and found promising but its application is

hampered by the lack of suitable experimental data.

The thermodynamic basis of the cation exchange resin method of determining cation activities in solution has been described and a new method of resin calibration using two parameter equations developed. The ion exchange resin method has been applied to studies of the seasonal variation of milk composition and to brief studies of the effects of milk pH adjustment, the factors affecting the renneting time of milk and the determination of cation activities in non bovine milks.

Some of the problems associated with calculating cation activities in milk have been briefly discussed. In a preliminary study of synthetic whey, comparisons have been made between cation activities determined experimentally and those calculated from a knowledge of composition and of the relevant acidity and stability constants.

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CHAPTER 1.

GENERAL INTRODUCTION

Dairy products constitute a large proportion of the European dietary intake and are of considerable economic importance, especially to New Zealand where approximately 20% of export earnings are from the sale of dairy products. Milk and dairy products are the subject of much scientific study. Some of this study is aimed at solving particular problems of the dairy industry while other work is aimed at increasing our knowledge of milk generally. In this thesis some of the more fundamental properties of milk as an electrolyte system have been studied.

1.1 A DESCRIPTION OF MILK AND SOME OF ITS PROPERTIES

Bovine milk is a complex fluid and many of its properties are poorly understood. Composition is fairly variable. Climate, stage of lactation, nature and level of feed, breed, age and health of the cow all affect composition (Kirchgessner et al., 1967). Whole milk contains milkfat, usually in the range 4-5%, which exists as an oil-in-water type emulsion with the fat globules having a surface membrane and averaging 2-5 μm in diameter. The composition of a typical sample of factory supply raw skim (fat-free) milk is given in Table 1.1. Only the major constituents are listed.

The group of proteins known as the caseins exist in milk mainly in the form of rather large colloidal particles

Table 1.1 The Composition of Typical Bovine Skim Milk

	Total Conc.	
Sodium	20	mmol/l
Potassium	41	"
Magnesium	5	"
Calcium	34	"
Citrate	9	"
Inorganic phosphate	23	"
Chloride	28	"
Lactose	5	g/l
Casein	25	"
Whey proteins	6	"

Ionic Strength (1, 2) 0.073, 0.085

pH 6.68 - 6.72 (25°C)

Buffer Value 0.016 g equivalents/liter/pH unit.

Ref. 1. R. Nordbo, (1939). J. Biol. Chem. 128, 745.

2. E.O. Whittier, (1929). J. Dairy Science 12, 405.

or micelles containing the protein and also calcium, magnesium, inorganic phosphate, citrate and water. There is evidence (McKenzie, 1971) that the micelles have a complex ordered structure. Typical micelle diameters are in the range 30-300 nm.

Skim milk can be considered as a two phase system, one phase being the disperse micelles and the second phase continuous serum. The volume occupied by the disperse micelle phase is probably dependent on the temperature, pH and method of defining the phase boundary but is estimated to be about 10% of the total volume of the skim milk (see Appendix I). In normal milk the colloidal dispersion is remarkably stable to extremes of temperature and concentration. It can sustain boiling, freezing and concentration to one-third volume. It can be dried to a powder and substantially recovers its normal dispersion upon mixing with water. Under sterile conditions the integrity of the colloidal dispersion is maintained over protracted storage periods. The addition of low concentrations of divalent salts to milk may cause a marked change in the stability of the dispersion.

A number of different methods have been used to obtain separation or partial separation of the two phases of skim milk. Among the methods used are ultra-centrifugation, ultrafiltration, rennet coagulation of the casein micelles and equilibrium dialysis. Only the last of these methods involves an equilibrium process. Ultrafiltration and dialysis also result in the separation of the serum proteins from the continuous phase. Table 1.2 indicates the approximate compositions of the two

Table 1.2 Approximate Composition of the Serum and Micelle Phases of Typical Bovine Skim Milk at 20°C.

	Serum	Micelles	
Sodium	21.4	9	mmol/l
Potassium	42.5	28	"
Magnesium	3.8	16	"
Calcium	12.6	226	"
Citrate	9.3	5	"
Inorganic phosphate	13.3	110	"
Chloride	31.0	?	"
Lactose	5.6	?	g/l
Casein	?	250	"
Whey proteins	6.7	?	"

Notes: Data have been calculated using experimental results reported in the literature (Davies and White, 1960). The figures for the micelle phase have been found by difference and are less reliable. The micelle phase has been assumed to occupy 10% of skim milk volume. The composition of the skim milk is the same as given in Table 1.1.

phases.

Chlorides, phosphates, citrates and bicarbonates of sodium, potassium, magnesium and calcium are generally referred to as the milk salts. They influence the condition and stability of the milk proteins, particularly casein, and therefore greatly affect milk properties and manufacturing characteristics. Such phenomena as rennet curdling of milk during cheese or rennet casein making, coagulation of evaporated milk during heat sterilization and the age thickening of sweetened condensed milk are all known to be markedly influenced by the concentration of milk salts present. Pyne (1962) has reviewed some aspects of the physical chemistry of the milk salts.

1.2 AN INTRODUCTION TO THE PRESENT WORK

There is not yet sufficient information about milk as a chemical system to enable the prediction of manufacturing properties from a knowledge of its composition. Micelle structure and stability, the effect of heat treatments on the heat stability of milk and the stabilizing (and destabilizing) effects of adding salts to milk are aspects of milk chemistry which are poorly understood. Milk protein stability is of practical importance in dairy manufacturing and successful processing of milk generally depends on either maintenance of stability as in the manufacture of condensed and powdered milks or deliberate destabilization as in the manufacture of cheese and casein.

In the past many of the dairy chemistry studies related to the milk salts have involved the determination of ion concentrations rather than activities. But the positions of chemical equilibrium reactions and also the rates of chemical reactions are more accurately described using the activities of the reacting species rather than their concentrations. In fluids as complex as milk with many possible types of ion-ion interactions, activities and concentrations are often very different. A knowledge of milk solute activities and the way in which they vary with pH and temperature would allow a more complete understanding of milk properties. This in turn, would help in the clearer understanding of many milk processing phenomena.

The aim of the present work has been to obtain basic fundamental knowledge of milk as an electrolyte system and also of simpler systems that simulate in part the milk

system. Effort has been concentrated mainly on the cations of milk because of their known importance and their amenability to study. Under favourable circumstances the activities of a system can be either measured directly or can be calculated from a knowledge of the composition of the system and of the relevant equilibrium constants. Both approaches have been attempted in the present work.

The thesis work is divided into three sections. In part I the measurement of equilibrium dissociation constants for citric acid and other acids and the measurement of equilibrium stability constants for citrate complexes and a number of other related compounds are reported and the results discussed.

In part II the cation exchange resin method for determining cation activities is developed and discussed and its application is described.

Part III deals with calculation of activities in milk, whey and synthetic whey using equilibrium constants and total concentrations, and the comparison of the calculated activities with measured activities.

A general discussion follows and deals with the results and conclusions of the earlier parts.

PART I

ACIDITY AND STABILITY CONSTANTS

CHAPTER 2.

INTRODUCTION

2.1 DEFINITIONS

In this work the Bronsted concept of an acid as a proton donor has been adopted. In aqueous solutions, which are the only solutions of interest in this work, the equilibrium protolytic reaction of the Bronsted acid HX, can be represented by

$$HX + H_2O \rightleftharpoons X^- + H_3O^+ \qquad (1)$$

The strength of the acid can be expressed by the thermodynamic equilibrium constant, K_T, for this reaction, which is defined as

$$K_T = \frac{A_{X^-} \cdot A_{H_3O^+}}{A_{HX} \cdot A_{H_2O}} \qquad (2)$$

where the A's are the activities of the indicated species. In dilute solutions the activity of water is virtually constant so that the term, A_{H_2O}, can be combined with K_T to give a new constant, K_a. Furthermore for reasons of convenience of notation, the hydrated proton will be denoted by the symbol H^+. Thus equation (2) becomes

$$K_a = K_T . A_{H_2O} = \frac{A_{X^-} \cdot A_{H^+}}{A_{HX}} \qquad (3)$$

K_a is the thermodynamic acidity constant or acid dissociation constant for the acid HX. Strong acids are those which have large K_a values; weak acids are those which have small K_a values.

Two other acidity constants are commonly used in cases where it is difficult to determine the necessary activities. These are the "mixed", "practical" or Bronsted acidity constant defined by

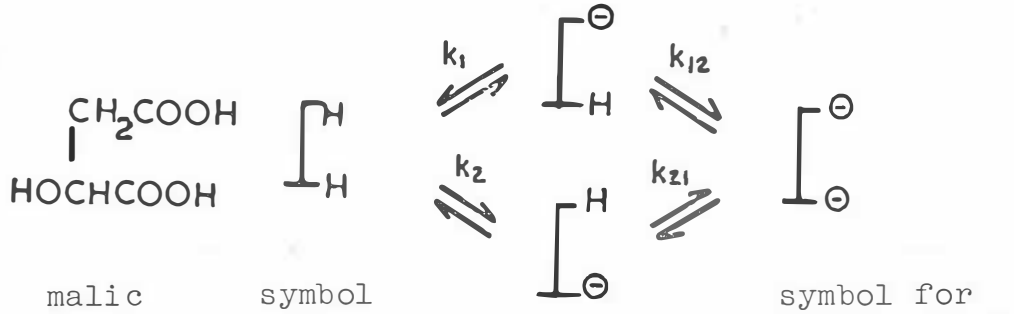
$$K_B = \frac{[X^-]}{[HX]} \cdot 10^{-pH} \qquad (4)$$

and the stoichiometric or concentration acidity constant defined by

$$K_C = \frac{[X^-]\,[H^+]}{[HX]} \qquad (5)$$

where the brackets denote concentrations. Bronsted and concentration "constants" are only true constants under conditions of constant ionic strength.

Polybasic acids (acids with more than one acidic proton) can dissociate by more than one pathway if the acidic protons are in distinguishable positions in the molecule. This can be conveniently illustrated using dibasic malic acid as an example.



malic acid — symbol for malic acid — symbol for malate anion

The acidity constants for each individual step are called intrinsic, microscopic or <u>micro acidity constants</u> and will in general be denoted by a lower case "k" to distinguish them from the <u>macro acidity constants</u>, denoted by an upper case "K". The macro acidity constants of a polybasic acid are normally referred to just as 'acidity constants' and these are the constants which are normally measured and reported. If the two monohydrogen malate anions are denoted by HX' and HX'' respectively, the ionic charge being omitted for clarity, then the macro concentration acidity constants for malic acid are given by

$$K_1 = \frac{([HX'] + [HX'']) \quad [H]}{[H_2X]}$$

$$K_2 = \frac{[X] \, [H]}{[HX'] + [HX'']}$$

By comparing the expressions for the macro and micro acidity constants for malic it can be shown that

$$K_1 = k_1 + k_2$$

$$1/K_2 = 1/k_{12} + 1/k_{21}$$

Micro acidity constants cannot be measured by direct experimental methods. An N-basic acid with none of the acid groups equivalent has N macro acidity constants and $N2^{N-1}$ micro acidity constants.

Weak acids, according to the Bronsted concept, yield on protolysis a strong base. Such bases frequently form complexes with metal ions, particularly polyvalent metal ions. The strength of the complex or its stability in the thermodynamic sense is expressed by the formation or <u>stability constant</u> for the equilibrium involved in its formation, as shown in the following example.

$$X + M \rightleftharpoons MX$$

$$K = \frac{A_{MX}}{A_X . A_M} \qquad (6)$$

Ionic charges have been omitted for clarity. X is an acid anion, M is a metal ion and K is the <u>thermodynamic stability constant</u>. The <u>concentration stability constant</u>, K_c, is defined by -

$$K_c = \frac{[MX]}{[M] \cdot [X]} \qquad (7)$$

For a polybasic acid, $H_N A$,

$$K_n = \frac{[MH_{N-n}A]}{[M]\ [H_{N-n}A]} \qquad (8)$$

where K_n is the n'th concentration stability constant.

A strong electrolyte, such as $NaNO_3$, $NaClO_4$, $(CH_3)_4NCl$ or KCl, is often added to a titration solution to increase the ionic strength of the solution. In the present work the electrolyte added for this purpose (KCl) is referred to as the supporting electrolyte.

2.2 A BRIEF SURVEY OF THE METHODS AVAILABLE FOR DETERMINING ACIDITY AND STABILITY CONSTANTS

The most precise methods of determining the acidity constants of weak acids in dilute aqueous solution are conductance measurements and electromotive force (emf) measurements on cells without liquid junctions. Less precise but often more convenient methods include emf measurements on approximately symmetric cells with liquid junctions and on asymmetric cells with liquid junctions. There are a great many other methods available (King, 1965). These are usually of limited applicability and of lower precision than the best conductance and emf methods.

There is an even wider range of methods available for the determination of the stability constants of complexes of metal ions with weak acids (Beck, 1970).

pH titrations using glass electrodes have been widely used for reasons of convenience. The same experimental set up can be used to determine acidity constants and complex stability constants and the method can be readily applied to polybasic acids. The chief difficulties are caused by uncertainty over liquid junction potentials and single ion activities. The pH titration method was chosen for the present work, chiefly for reasons of convenience.

2.3 THE MICRO ACIDITY CONSTANTS FOR CITRIC ACID

The complete dissociation scheme for citric acid is given and the micro acidity constants are defined in Figure 2.1.

The relationships between the micro and macro acidity constants for citric acid are given in the following equations which may be deduced from the definitions of the respective constants:

$$K_1 = 2k_1 + k_2 \quad (9)$$

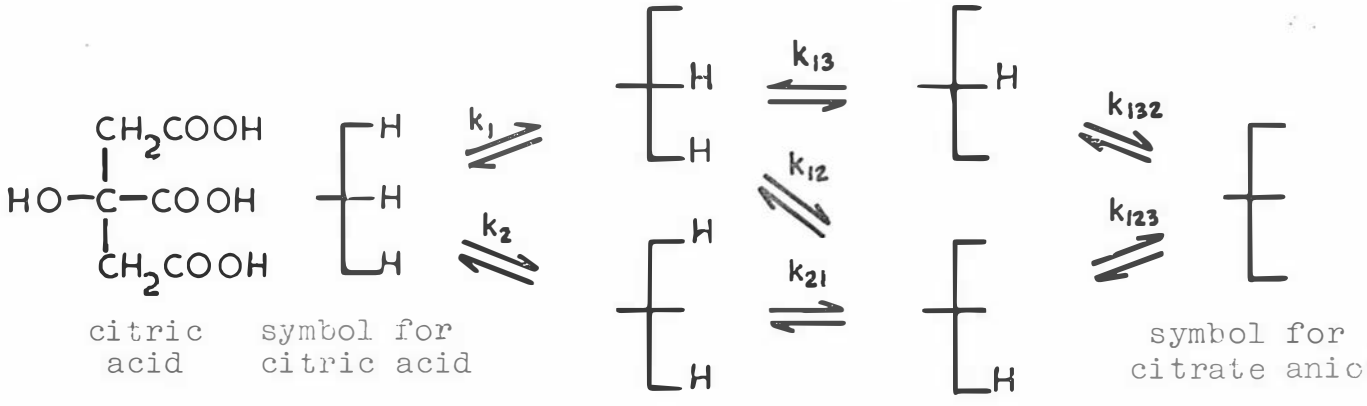
$$K_1K_2 = k_1k_{13} + 2k_2k_{21} = k_1k_{13} + 2k_1k_{12} \quad (10)$$

$$K_1K_2K_3 = k_1k_{12}k_{123} = k_1k_{13}k_{132} = k_2k_{21}k_{123} \quad (11)$$

$$1/K_3 = 2/K_{123} + 1/K_{132} \quad (12)$$

In the general case of a tribasic acid having non-equivalent acidic groups a minimum of four of the micro constants must be independently determined to enable calculation of all of the remaining micro constants (King, 1965). In the case of citric acid the equivalence of the terminal carboxylic groups reduces the minimum number of micro constants which must be independently determined to

Figure 2.1 The Complete Dissociation Scheme for Citric Acid.



two. The choice of the two constants is subject to the constraint that they must apply to species of different charge. Thus the choice of say k_1, k_{13} is acceptable while the choice of k_1, k_2 is not.

There have been a number of studies of the acidity of citric acid in aqueous solution reported in the literature. Loewenstein and Roberts (1960) used a nuclear magnetic resonance technique on comparatively concentrated solutions of citric acid and of several of its methyl esters to obtain a measure of the concentrations of the various species present in citric acid solutions at different pH's. The method is not an accurate one, because of the similarity of the spectra of the different citrate species and the inherent limitations of the n.m.r. technique.

Later Martin (1961) carried out some pH titrations on aqueous solutions of various methyl esters of citric acid. The esterification is used to block selected protolytic reactions thus permiting the micro acidity constants of the unblocked groups to be determined. The values obtained for the micro acidity constants were at variance with the results of Loewenstein and Roberts. However, recalculation of Loewenstein and Roberts data using values for the macro acidity constants for citric acid more appropriate to the ionic strengths used, brought the two sets of results into closer accord.

An X-ray diffraction study (Glusker et al., 1965) demonstrated that in the crystalline state it is the central carboxylic acid group which is ionized in sodium dihydrogen citrate and in lithium dihydrogen citrate.

The Methyl Citrates as Model Compounds

A problem with the use of the methyl citrates as model compounds is that only trimethyl citrate and symmetrical dimethyl citrate can be prepared as pure crystalline solids. The other esters are prepared by saponification and have not been isolated from solution. Previously the only evidence concerning the completeness of saponification and the purity of the solutions was from nuclear magnetic resonance measurements. Recent kinetic studies (see Appendix II) now allow calculation of the approximate composition of solutions after saponification.

There is no direct method of testing the suitability of the methyl citrates as model compounds for the determination of citric acid micro acidity constants. Ideally, substitution of a methyl group for an acidic proton should not alter the acidity of any of the remaining acidic protons. The following points are noted as indicating the general suitability of the methyl citrates as model compounds.

1. Nuclear magnetic resonance studies (Loewenstein and Roberts, 1960) of aqueous solutions of citric acid and of the methyl citrates show that methyl ester formation has no observable effect on the chemical shift of the methylene protons of citric acid.

2. The acidity constant for methyl succinate is equal, within experimental error, to the first micro acidity constant for succinic acid (see Table 5.2).

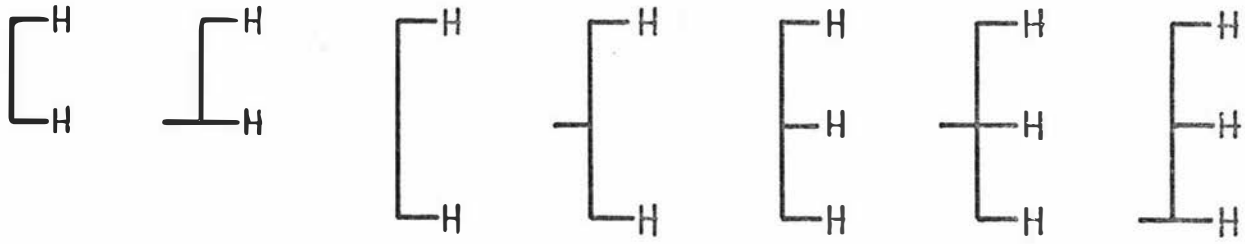
These results do not exclude possible steric or hydrogen

bonding effects resulting from esterification.

New titration studies of the methyl citrates have been made both at very low ionic strengths and at an ionic strength of 0.1 and are reported in this work. In addition an attempt is made to calculate the micro acidity constants from empirical structural considerations.

2.4 STRUCTURAL FORMULAE OF ACIDS RELATED TO CITRIC ACID

The convenient notation already used in this chapter for denoting the structures of malic and citric acids will also be used for denoting the structures of the other acids of interest.



succinic malic glutaric β hydroxy glutaric tricarballylic citric isocitric

The main carbon chain backbone is denoted by the vertical line, a carboxylate by a horizontal line lying to the right and a hydroxyl by a horizontal line lying to the left.

CHAPTER 3.

THEORY AND COMPUTER PROGRAMS

3.1 DERIVATION OF GENERAL FORMULAE

Suppose that a weak N-basic acid, H_NA, can form complexes with a p-valent metal ion, M^{p+}, of the type MH_nA^{p+n-N} (n = 0, 1, ..., N-1) then the following relations hold for the titration of the acid with strong monovalent cation base in the presence of strong salt MCl_p at constant ionic strength and constant temperature. Charges have been omitted for clarity. Concentrations are denoted by square brackets.

(1) Overall acid association constants.

$$\beta_n = [HnA] / ([A].[H]^n) \qquad n = 1, 2, ----N$$

$$\beta_o = 1 \text{ by definition.}$$

The β's are related to the stepwise acid dissociation constants K , K_2---K_N by —

$$\beta_n = 1/(K_N.K_{N-1}.-----K_{N+1-n})$$

(2) Metal complex stability constants.

$$K_{N-n} = [MH_nA]/([M].[H_nA]) \qquad n = o, 1, ----N-1$$

(3) Total ligand concentration.

$$A = \sum_{n=o}^{N} [HnA] + \sum_{n=o}^{N-1} [MHnA]$$

(4) Total metal concentration

$$B = [M] + \sum_{n=0}^{N-1} [MHnA]$$

(5) Electroneutrality

$$[H] + T + p.[M] = \sum_{n=0}^{N} (N-n).[HnA] + \sum_{n=0}^{N-1} (N-n-p).[MHnA] + [OH] + pB$$

where T is the concentration of alkali metal cation resulting from the addition of titrant base.

(6) Ionic product of water

$$K_w = [H].[OH]$$

By substituting for B and for OH in equation (5) using equations (4) and (6) we obtain, after rearrangement -

$$(7) \quad [H] + T - K_w/[H] = \sum_{n=0}^{N} (N-n).[HnA] + \sum_{n=0}^{N-1} (N-n).[MHnA]$$

Substituting equations (1) and (2) in (3), (4) and (7)

$$(8) \quad A = \sum_{n=0}^{N} \beta n.[H]^n.[A] + [M] \sum_{n=0}^{N-1} K_{N-n}.\beta n.[H]^n.[A]$$

$$(9) \quad B = [M] \left(1 + \sum_{n=0}^{N-1} K_{N-n}.\beta n.[H]^n [A] \right)$$

$$(10) \quad [H] + T - K_w/[H] = \sum_{n=0}^{N} (N-n)\beta n [H]^n[A] + [M] \sum_{n=0}^{N-1} (N-n)K_{N-n}\beta_n [H]^n[A$$

Substituting for [A] from equation (8) in (9)

$$B = [M]\left\{1 + \frac{\sum_{N=o}^{N+1} K_{N-n\beta n} [H]^n . A}{\sum_{n=o}^{N} \beta_n [H]^n + [M] \sum_{n=o}^{N-1} K_{N-n\beta_n} . [H]^n}\right\}$$

which can be expanded to

$$\sum_{n=o}^{N} \beta_n [H]^n + B[M]\sum_{n=o}^{N-1} K_{N-n\beta n} [H]^n = [M]\left(\sum_{n=o}^{N} \beta_n [H]^n + [M]\sum_{n=o}^{N-1} K_{N-n\beta n} [H]^n\right)$$

$$+ [M] \sum_{n=o}^{N-1} K_{N-n\beta_n} [H]^n A$$

and rearranged to give

$$[M]^2 \sum_{n=o}^{N-1} K_{N-n\beta n} [H]^n + [M]\left(\sum_{n=o}^{N} \beta_n [H]^n + \sum_{n=o}^{N-1} K_{N-n\beta_n} [H]^n (A-B)\right)$$

$$- B \sum_{n=o}^{N} \beta_n [H]^n = 0 \qquad (11)$$

Substituting for [A] from equation (8) in equation (10) we obtain

$$[H] + T - K_w/[H] = \frac{\sum_{n=o}^{N} (N-n)\beta_n [H]^n A + [M] \sum_{n=o}^{N-1} (N-n) K_{N-n\beta_n} [H]^n A}{\sum_{n=o}^{N} \beta_n [H]^n + [M] \sum_{n=o}^{N-1} K_{N-n\beta n} [H]^n}$$

which can be expanded to

$$\left([H] + T - K_w/[H]\right) \sum_{n=o}^{N} \beta_n [H]^n + \left([H] + T - K_w/[H]\right) [M] \sum_{n=o}^{N-1} K_{N-n} \beta_n [H]^n$$

$$= \sum_{n=o}^{N} (N-n) \beta_n [H]^n A + [M] \sum_{n=o}^{N-1} (N-n) K_{N-n} \beta_n [H]^n . A$$

and rearranged to give

$$\sum_{n=o}^{N} \left(\beta_n [H]^n \left[[H] + T - K_w/[H] - (N-n). A \right] \right)$$

$$+ \sum_{n=o}^{N-1} \left(K_{N-n} [M] \beta_n [H]^n \left[[H] + T - K_w/[H] - (N-n) \cdot A \right] \right) = 0$$

(12)

which can be conveniently denoted by

$$f = \sum_{n=o}^{N} g_n + \sum_{n=o}^{N-1} K_{N-n} . h_n = 0$$

In the special case when no complex forming metal ion is present, i.e. $B = [M] = 0$, equation (11) vanishes and equation (12) reduces to -

$$(13) \qquad \sum_{n=0}^{N} \beta_n [H]^n \left[[H] + T - K_w/[H] - (N-n)\, A \right] = 0$$

which can be conveniently denoted by

$$f = \sum_{n=0}^{N} \beta_n . \mathcal{G}_n = 0$$

Equation (13) provides a functional relationship between the variables $[H]$, T, A with the β's as parameters. If N different sets of values of the variables (denoted by $[H]_i$, T_i, A_i) are known then the values of the N parameters, β_1, β_2, ---- β_N, can be found by solving the N simultaneous linear equations —

$$f_i = 0 \quad i = 1, 2, \text{---- } N$$

where $f_i \equiv f$ for the particular set of values of the variables, $[H]_i$, T_i and A_i. Experimentally measured values (or the observed values) of the variables will not in general be equal to the true values because of experimental error and thus it is not possible to find the true values of the parameters. If the observed values of the variables and estimated values of the parameters are substituted into equation (13) then

$$f_i = p_i$$

where p_i is the residual. In general $p_i \neq 0$. The best estimates of the parameters are given by the method of least squares in which the weighted sum of the squares of the residuals is minimised.

3.2 THE METHOD OF LEAST SQUARES (Wolberg, 1967; Brownlee, 1960)

The method of least squares requires that the observed values of the variables are distributed (not necessarily normally) about the 'true' values. In other words, if the measurements of the variables are repeated N times and the average values calculated, these average values approach the true values as N approaches infinity. This requirement is satisfied if the measurements are free from systematic error. The values of the parameters calculated by the least squares analysis are normally distributed about the true values of the parameters and have the least possible standard deviation. They are therefore the best possible estimates of the parameters.

The first step of the least squares method is to write a functional relationship between the variables, parameters and constants in the form

$$F = 0$$

where F is a function of the variables, parameters and constants. Estimates are made of the standard deviations of the various variables. The derivatives of F with respect to each of the variables and each of the parameters are then calculated using (if necessary) preliminary estimates of the parameters. These preliminary estimates can be guessed or found graphically or, in special cases, can be found by a method to be discussed later. Values of the function F are also calculated by substituting observed values of the variables and the preliminary values of the parameters.

Solution of a matrix equation (the so-called 'normal equation') by matrix inversion gives adjustments to the preliminary values of the parameters and also gives estimates of the standard deviations of the parameters. Using the adjusted values of the parameters the above procedure is repeated until convergence is obtained.

The matrix equation which is solved in the least squares method can be denoted by

$$CA = V$$

where

$$C_{kl} \equiv \sum_i \frac{F_k F_l}{\sum_j (F_j \cdot \rho_j)^2}$$ the (kl)th element of the coefficient matrix C

F_k, F_l are the values of the derivatives of the function F with respect to the k and l th parameters respectively at the ith titration point. F_j is the value of the derivative of the function F with respect to the j th variable at the ith titration point.

ρ_j is the standard deviation of the j th variable.

$$V_k \equiv \sum_i \frac{F_k F_o}{\sum_j (F_j \rho_j)^2},$$ the k th element of the vector V

F_o is the value of the function F obtained by substituting in the observed values of the variables and the estimated values of the parameters at the ith titration point.

$A_k \equiv \Delta_k$, the k th element of the vector A,
the adjustment for the k th parameter

$a_{k,p+1} = a_{k,p} - \Delta_k$

where $a_{k,p}$ is the p th estimate of the value of the k th parameter.

Calculation of the Preliminary Estimate of the Parameters

In the case of functional relationships which are linear with respect to the parameters, the preliminary estimate of the values for the parameters can be found by solving the matrix equation -

$$C^{\bullet} a = V^{\bullet}$$

where $C^{\bullet}_{k,l} = \sum_i F_k . F_l$

$$V^{\bullet}_k = \sum_i F_k . F_*$$

F_* is the value of constant term(s) of the function F.
a_k is used as the preliminary estimate of the k th parameter.

This method of finding a preliminary estimate of the parameters involves minimising the unweighted sum of the squares of the residuals.

Estimation of Error

The uncertainty (or standard deviation) of the least squares parameters is of considerable importance. If the errors in the variables are not too large and are not

correlated with each other then an unbiased estimate of the Variance (= standard deviation squared) of the k th parameter is given by

$$S_k = \frac{R}{n-p} \cdot C^{-1}_{kk} \quad , \quad k = 1,2,----,p$$

and the covariance of the l th and k th parameters is given by

$$S_{lk} = \frac{R}{n-p} \cdot C^{-1}_{lk} \qquad l \neq k$$

where R is the weighted sum of the squares of the residuals

n is the number of data points

p is the number of parameters

and C^{-1}_{lk} is the l,k th element of the inverted coefficient matric C.

The Variance of a function, f, of the parameters can be calculated from

$$S_f = \frac{R}{n-p} \sum_{j=1}^{p} \sum_{k=1}^{p} f_j . f_k . C^{-1}_{jk}$$

where f_j, f_k are the derivatives of the function, f, with respect to the j th and k th parameters respectively.

Application of the Method of Least Squares

The method of least squares is readily applied in the special case when no complexing metal ions are present and equation (13) above applies. Equation (13) is linear in the β's which are the parameters to be determined. In the more general case of metal complex formation equations

(11) and (12) apply. The β's are considered as known constants and the K's are the parameters to be determined. Both equations (11) and (12) are linear in the K's but contain the unknown variable $[M]$, the concentration of uncomplexed metal ion. An iterative procedure is required to find the $[M]$'s and the K's.

The procedure used was as follows. A preliminary estimate or guess of the values of the K's is made and these values are substituted into equation (11) which is then solved as a quadratic in $[M]$. The value for $[M]$ obtained is fed into equation (12) to which the unweighted least squares method is applied to obtain new estimates of the K's. The process is continued iteratively until convergence is obtained.

3.3 COMPUTER PROGRAMS

All computer programs were written in Fortran II D and were run on the Massey University IBM 1620 II computer which is equipped with disc drive, line printer and card reader.

Two separate programs were used, (Figures 3.1, 3.2) the first to calculate acidity constants and the second to calculate metal ion complex stability constants. Each program is in two parts, the main program and a matrix inversion subroutine (MATINV, Wiberg, 1965) which is used to solve simultaneous linear equations. The calculations begin with 'initialization' or the clearing of certain computer memory areas and the setting of various indexes to initial values. Certain data, such as the name and basicity of the acid, the concentration

Figure 3.1. Simplified Flow Chart for Computer Program to Calculate Acidity Constants from pH Titration Data

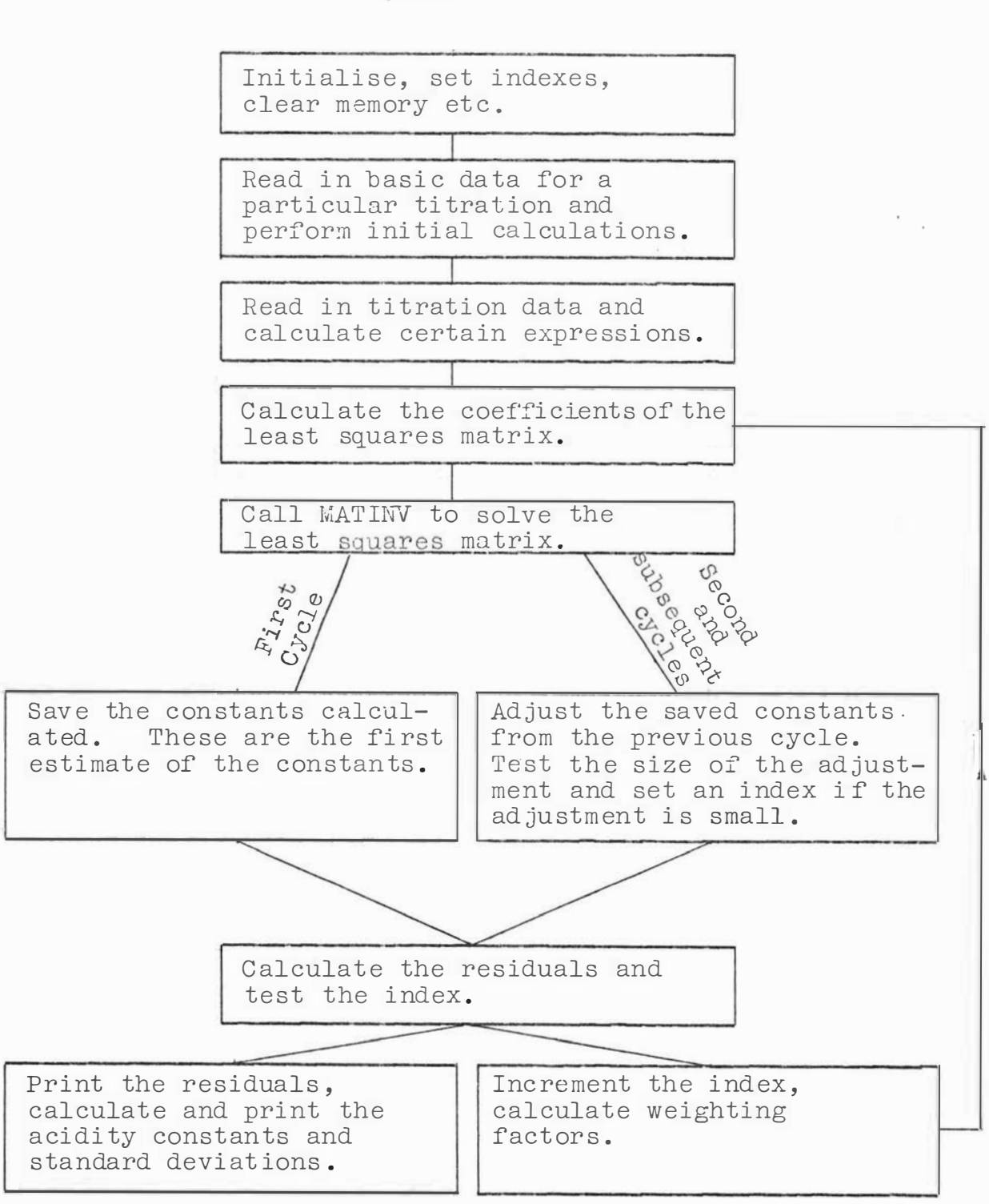
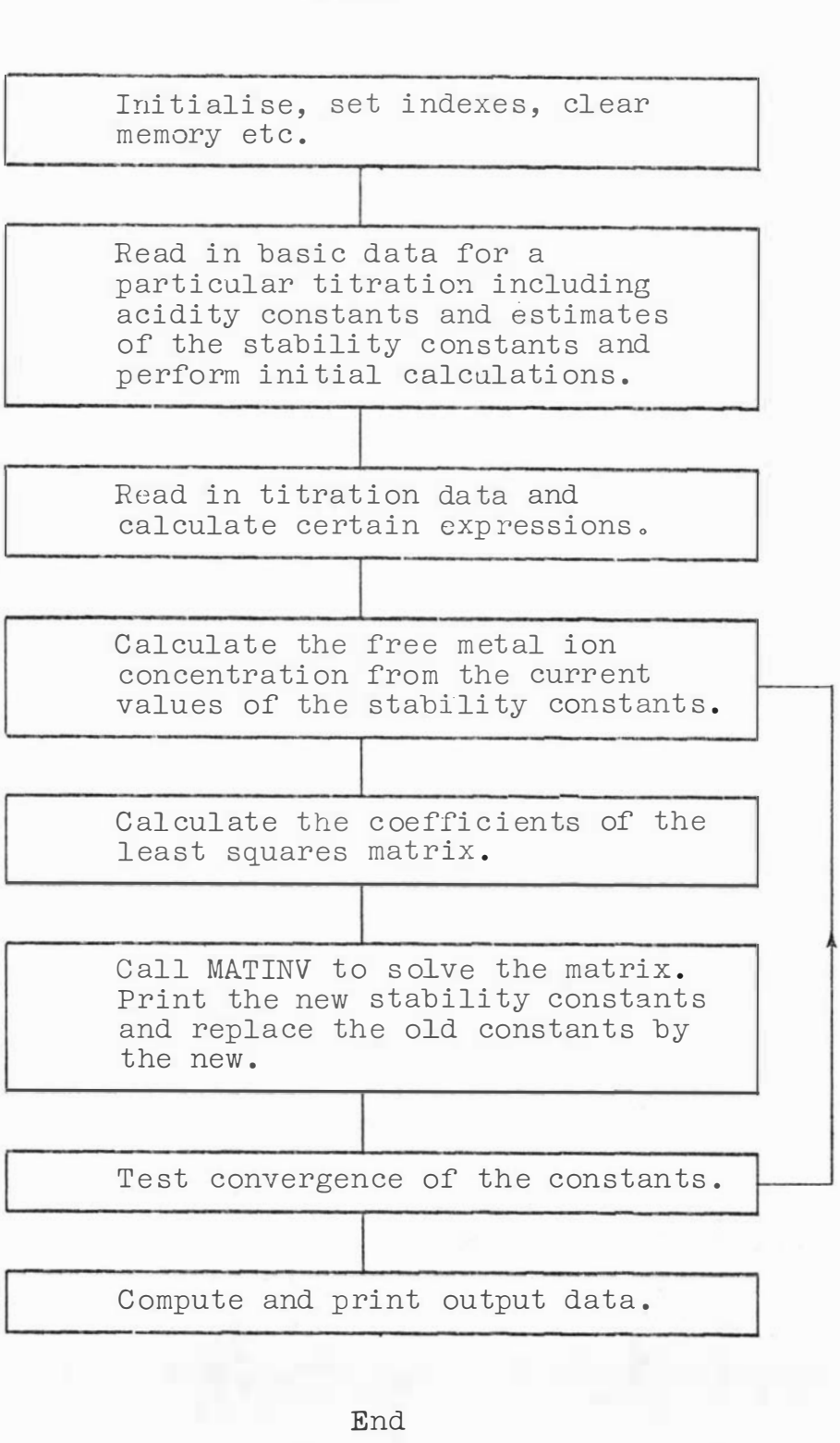


Figure 3.2. Simplified Flow Chart for Computer Program to Calculate Stability Constants of Metal Ion Complexes from pH Titration Data



of the titrant base, the ionic strength and various constants etc, are read in from cards and a number of preliminary calculations made. The titration data (pH versus titre) is then read in. Limited computer memory and computational time considerations limit the amount of data which can be conveniently handled. An arbitrary limit of 20 titration data for an acid with not more than five acidic protons has been imposed. The hydrogen ion concentration, the concentration of alkali metal ion added as base, the stoichiometric concentration of acid and, in the case of the second program, the stoichiometric concentration of complexing metal ions are all calculated. Corrections are made for the dilution caused by the addition of base. Certain expressions which have constant values and are used repeatedly in the calculations are evaluated and the results stored. The programs then proceed to iterate, a check being maintained that the iterations lead to convergence. In the event of divergence or of slow convergence the calculations are terminated.

The first step of the iteration in the first program is the calculation of the elements of the unweighted least squares matrix which is then inverted by calling the matrix inversion subroutine (MATINV). In the second and subsequent iteration cycles, the elements of the weighted least squares matrix are calculated. The standard deviation of the pH and titre being taken as 0.005 pH units and 0.2% of total syringe volume, respectively. After a satisfactory convergence has been obtained or after four iteration cycles, the titration data, residuals, acidity constants and standard deviations are printed out and the

program re-initializes ready for the processing of further data.

The first step of the iteration cycle of the program for the calculation of metal ion complex stability constants is the calculation of the free metal ion concentration. The elements of the unweighted least squares matrix are calculated and the matrix inverted. The values obtained for the stability constants are then used in the next iteration cycle. Iteration proceeds until a satisfactory convergence is obtained or for a maximum of six cycles. Titration data, residuals, stability constants and standard deviations are printed and the program re-initializes ready for the processing of further data.

CHAPTER 4.

EXPERIMENTAL

4.1 DESCRIPTION OF APPARATUS

pH Meter. All pH measurements were made using a Radiometer pH Meter 4d. This is a battery-powered, transistorised, null balance meter and has a built-in standard cell. Scale readability is 0.001 pH units. The meter has no provision for compensating for electrodes with electromotive efficiencies of less than 100%. The manufacturer states that overall instrument accuracy is $\pm$ 0.005 pH units.

pH Electrode. Radiometer combined type C glass/saturated calomel electrodes were used for all pH measurements. Type C electrodes have rugged glass membranes, may be used over the temperature range 0-60^{o}C, and can be used up to pH 12 without serious sodium ion interference. Contact between the calomel electrode and solution to be investigated is by a small sintered glass plug. Generally in this work combined glass/calomel pH electrodes will be loosely referred to as glass electrodes.

Generally grade A pipettes and grade B standard flasks were used in all standard volumetric work.

4.2 REAGENTS

When commercially available, analytical grade reagents were used, generally without further purification or assay.

Carbonate-free standard potassium hydroxide solution was prepared according to the method of Albert and Sergeant, (1962). Excess barium hydroxide was added to a solution of potassium hydroxide to remove carbonate by precipitation as barium carbonate. The excess barium ions were removed from the solution by passage down a column of strong cation exchange resin (Amberlite IR 120) in the potassium form. The resulting potassium hydroxide solution was standardised against a solution of potassium hydrogen phthalate using a glass electrode to detect the end point. Generally approximately 0.2 M potassium hydroxide was used.

pH Standards. An 0.05 M solution of British National Physics Laboratory certified potassium hydrogen phthalate was used as a primary pH standard. (In some early work analytical reagent grade potassium hydrogen phthalate was used). Radiometer S 1001 concentrated buffer solution (pH 6.50 $\pm$ 0.02 at 20^{o}C) was used as a secondary standard.

Water. Distilled water was available on tap. This was deionised by passage down a mixed bed ion exchange resin column shortly before it was required. The conductivity of the deionised water was less than 1×10^{-6} S. The distilled, deionised water was stored in small polythene tanks.

Trimethyl Citrate was prepared by the method of Donaldson

et al. (1934). Anhydrous citric acid was dissolved in an excess of methanol and hydrogen chloride gas was bubbled into the refluxing solution over a period of four hours. Crystals of trimethyl citrate separated out on cooling of the reaction mixture and were collected on a filter and washed with cold methanol. The trimethyl citrate was re-crystallised from water and from methanol. Melting point, found 74.5-75°C, literature 76°C (Donaldson et al., 1934), 73-73.5°C (Loewenstein and Roberts, 1960), 75-76°C (Martin, 1961).

Symmetric Dimethyl Citrate was prepared by the method given by Schroeter and Schmitz (1902). Citric acid monohydrate was dissolved in excess methanol, a catalytic quantity of sulphuric acid added and the mixture refluxed for one hour. The reaction mixture was neutralised with calcium carbonate, filtered and the filtrate concentrated by evaporation under vacuum. The resulting solid was dissolved in hot water, the solution filtered and the ester precipitated from the solution with concentrated hydrochloric acid. The ester was then twice re-crystallised from water. Melting point, found 118-123°C, literature 125-126°C (Schroeter and Schmitz,1902), 115-117°C (Loewenstein and Roberts,1960), 116-118°C (Martin, 1961). Equivalent wt, calculated for dihydrate 256, calculated for the monohydrate 238, found 254, literature 236, (Schroeter and Schmitz,1902), 257 (Martin,1961).

Other Methyl Citrates were prepared by the method of Loewenstein and Roberts (1960) using partial saponification of either trimethyl citrate or symmetric dimethyl citrate

and were not isolated from solution. The saponifications were carried out by the addition of a measured quantity of KOH shortly before pH titration with standard hydrochloric acid. The yields of the various esters have been calculated from the saponification rate constants (see Appendix II) and are given in Table 4.1.

4.3 EXPERIMENTAL PROCEDURE

Known weights (or volumes of standard solutions) of the various reagents were quantitatively transferred to a standard 100 ml flask, dissolved, and made up to the mark. The contents of the standard flask were then poured into a titration vessel consisting of a water-jacketed conical glass flask with a capacity of about 125 ml. Water was continuously pumped through the jacket from a thermostat bath, (Grant Instruments, thermostat/pump, type SU2). Temperatures did not fluctuate more than 0.5°C. The contents of the vessel were stirred magnetically using a teflon encased follower. Oxygen-free nitrogen gas (available commercially in cylinders) was bubbled through a sintered glass disc into water at the temperature of the thermostat bath and was then admitted to the bottom of the titration flask. The gas flow was adjusted at the cylinder outlet. The solution was stirred and nitrogen bubbled through it for 15-30 minutes. The glass electrode was carefully standardised against potassium hydrogen phthalate buffer, rinsed with water and transferred to the solution in the titration vessel.

Titrant base was added to the titration flask in convenient aliquots from a precision micrometer glass

Table 4.1 Approximate Yields of Methyl Citrates Produced by Saponification. Calculated from the ratio of the rate constants at 25°C in dilute aqueous solution. Yield %.

Starting Ester - Trimethyl Citrate

Moles of base per mole of Ester	Trimethyl	Asym Dimethyl	Sym Monomethyl	Citrate
0.5	51.5	47.1	1.4	0
1.0	10.4	79.2	10.4	0
2.0	0	1.9	97.1	1.0

Starting Ester - Sym Dimethyl Citrate

Moles of base per mole of Ester	Sym Dimethyl	Asym Monomethyl	Citrate
2.0	8.1	83.9	8.0

syringe burette with the tip of the burette (bore 0.2 mm) immersed in the solution contained in the titration vessel. Volumes of titrant could be measured to 0.1% of the total syringe capacity. Total volumes of added titrant were in the range 0.2-10 ml. The pH was measured after each addition of titrant. Stirring and bubbling of nitrogen gas was continuous throughout the titration unless it was found to disturb the pH measurement. In such a case stirring and bubbling were stopped during the pH measurement but were restarted before the addition of further titrant. After the end point was reached the glass electrode was again checked against the phthalate buffer. The electromotive efficiency of the electrode was occasionally checked by measuring the pH of a second standard buffer.

CHAPTER 5.

RESULTS

Aqueous solutions of various acids were titrated in the absence and in the presence of various complex forming metal ions. Citric acid and its magnesium and calcium complexes were the compounds of chief interest in the present work because of their importance in milk. The methods developed for these determinations were tested using acids with reliably known acidity constants. A number of acids bearing a structural relationship to citric acid were also studied as a knowledge of the constants of these acids is helpful in elucidating and understanding the factors affecting the acidity of citric acid and the stability of citrate complexes. Stability constants of citrate with other cations of the alkaline earth series (barium, strontium) were also measured.

A study of the homologous series of aliphatic $\alpha\,\omega$ dicarboxylic acids was made. Succinic acid was chosen as a polybasic acid with reliably known acidity constants and glutaric acid as being structurally related to citric acid (see Section 2.4). Higher members of the homologous series (all available commercially in high purity) were included for study, although not directly related to milk salt systems, because of the importance of the series in studies of the effect of electrically charged substituents on acidity. At the time of measurement there was only one known study of this series of acids in aqueous solution (Gane and Ingold, 1931). Recently a second study has been

reported (Ninomiya and Toei, 1969) in which the acidity constants of the acids have been determined in 0.1M KNO_3 solution at 25°C by pH titration using a glass electrode.

Some of the results calculated from pH titration data using the methods given in Chapter 3 are tabulated in this chapter. Results reported by other workers together with references are included for comparison.

5.1 THE $\alpha\omega$ DICARBOXYLIC ACIDS

Table 5.1 gives results for the homologous series of aliphatic $\alpha\omega$ dicarboxylic acids of general formula $C_nH_{2n}(CO_2H)_2$. The first two members of the series, oxalic acid ($n=0$), and malonic acid ($n=1$) have been excluded because their acid strength is too great to allow accurate determination of the first acidity constants from glass electrode titrations and further they are not typical members of the series.

The solubility of the acids in water decreases with increasing n. Thus a saturated aqueous solution of sebacic acid ($n=8$) at 25°C is approximately 0.0013M and is the last member of the series which can be studied readily in aqueous solution.

The close agreement between the results of this work and the results of Pinching and Bates (1950) for succinic acid allows some confidence in the results for the remaining acids.

The notes at the foot of Table 5.1 concerning methods and media also apply to subsequent tables in this Chapter.

Table 5.1 Thermodynamic and Concentration Acidity Constants for the $\alpha\omega$ Dicarboxylic Acids

pK_1	pK_2		Method[a]	Temp/°C	Medium[b]	Ref.[c]
Succinic Acid						
4.193	5.477	E2		25	0	G
4.2066	5.636	E1		25	0	P
4.25	5.63	gl		25	~ 0	this work
4.00	5.21	gl	$p\gamma$ = 0.081	25	0.1 $NaClO_4$	Y
4.00	5.23	gl	$p\gamma$ = 0.084	25	0.1 KCl	this work
Glutaric Acid						
4.344	5.420	E2		25	0	G
4.39	5.40	gl		25	~ 0	this work
4.14	5.01	gl	$p\gamma$ = 0.081	25	0.1 $NaClO_4$	Y
4.15	5.02	gl	$p\gamma$ = 0.084	25	0.1 KCl	this work
Adipic Acid						
4.418	5.412	E2		25	0	G
4.48	5.39	gl		25	~ 0	this work
4.28	5.00	gl	$p\gamma$ = 0.081	25	0.1 $NaClO_4$	Y
4.24	5.02	gl	$p\gamma$ = 0.084	25	0.1 KCl	this work
Pimelic Acid						
4.484	5.424	E2		25	0	G
4.53	5.41	gl		25	~ 0	this work
4.30	5.06	gl	$p\gamma$ = 0.084	25	0.1 KCl	this work
Suberic Acid						
4.517	5.403	E2		25	0	G
4.56	5.42	gl		25	~ 0	this work
4.31	5.08	gl	$p\gamma$ = 0.084	25	0.1 KCl	this work

pK_1	pK_2	Method[a]	Temp/°C	Medium[b]	Ref.[c]
Azelaic Acid					
4.551	5.415	E2	25	0	G
4.59	5.39	gl	25	~0	this work
4.36	5.07	gl $p\gamma = 0.084$	25	0.1 KCl	this work
Sebacic Acid					
4.62	5.43	gl	25	~0	this work
4.40	5.22	gl ?	20	0.1 ?	W
4.41	5.11	gl $p\gamma = 0.084$	25	0.1 KCl	this work

Notes

(a) E1 E.m.f. measurements in cells without liquid junction.

E2 E.m.f. measurements using cell with hydrogen and saturated calomel electrodes.

gl Glass electrode titration using a cell with salt bridge.

$p\gamma$ Correction applied to convert pH meter readings to negative logarithms of hydrogen ion concentrations (see Section 6.3).

(b) The medium was in all cases dilute aqueous solution. The supporting electrolyte and the ionic strength are given (mol/l).

0, constants extrapolated to zero ionic strength.

~0, constants determined at very low ionic strength.

(c) G R. Gane and C.K. Ingold, (1931). J. Chem. Soc. (1931) 2153.

P G.D. Pinching and R.G. Bates, (1950). J. Res. Nat. Bur. Stand. 45, 322 and 444.

W P.E. Wenger and I. Kopetanidis, (1960). Rec. Trav. Chem. 79, 569.

Y M. Yasuda et al., (1960). Bull. Chem. Soc. Japan 33, 1067.

5.2 ACIDITY CONSTANTS FOR VARIOUS CARBOXYLIC ACIDS

Table 5.2 contains data for a variety of acids. The results for acetic acid are in excellent agreement with the reliable values found by Harned and Hickey (1937) while those for monomethyl succinate show that the ester is a satisfactory model compound for determining the micro acidity constants for succinic acid. Tricarballylic acid is of particular interest as a model compound for citric acid differing only in having no hydroxyl group (see Section 2.4). Glass electrode titrations do not lend themselves to the accurate determination of the thermodynamic acidity constants of polybasic acids (see Chapter 6.1) so that the results given need to be interpreted with caution.

5.3 ACIDITY CONSTANTS FOR CITRIC ACID

Measurements of the acidity constants for citric acid were made at a number of temperatures and ionic strengths. The results for a series of experiments, performed in random order, are given in Table 5.3. Although the concentration of citric acid was increased over a forty-seven fold concentration range the acidity constants remained essentially constant.

The data of experiment 167 (Table 5.3) was also processed using the computer program SCOGS* (Sayce, 1968) and the Bronsted constants obtained, when converted to concentration constants (2.87, 4.34, 5.68 respectively) are in agreement with those given in Table 5.3.

The program SCOGS gave the standard deviation of the titre, which is used as a test of "goodness of fit",

* processing was arranged by Professor W.A.E. McBryde, University of Waterloo, Ontario.

Table 5.2 Acidity Constants for Various Carboxylic Acids

pK_1	pK_2	pK_3	Method	Temp/°C	Medium	Ref.
Monomethyl Succinate						
4.52			gl	25	~0	this work
4.51	calculated from pK_1 for succinic acid					P
4.29			gl $p\gamma = 0.084$	25	0.1 KCl	this work
4.30	calculated from pK_1 for succinic acid					this work
Benzoic Acid						
4.07			gl $p\gamma = 0.084$	25	0.1 KCl	this work
4.01			gl $p\gamma = 0.081$	25	0.1 $NaClO_4$	Y
Acetic Acid						
4.55			gl $p\gamma = 0.084$	25	0.1 KCl	this work
4.546			E1 HCl	25	0.1 KCl	H
Malic Acid						
3.21	4.70		gl $p\gamma = 0.084$	25	0.1 KCl	this work
3.26	4.68		E2 $p = 0.100$	?	0.2 KCl	Ca
Tricarballylic Acid						
3.73	4.90	6.39	gl	25	~0	this work
3.73	4.95	6.42	gl	25	cor 0	this work*
3.49	4.53	5.85	gl $p\gamma = 0.084$	25	0.1 KCl	this work
3.47	4.54	5.89	gl [H]	25	0.1	C

Notes

P G.D. Pinching and R.G. Bates, (1950). J. Res. Nat. Bur. Stand. 45, 444.

Y M. Yasuda et al., (1960). Bull. Chem. Soc. Japan 33, 1067.

H H.S. Harned and F.C. Hickey, (1937). J. Am. Chem. Soc. 59, 2303.

C E. Campi et al., (1964). J. Inorg. Nucl. Chem. 26, 553.

Ca R.K. Cannan and A. Kibrick, (1938). J. Am. Chem. Soc. 60, 2314.

* Calculated from values at ionic strength = 0.1, using activity coefficient functions found for citric acid.

as 5×10^{-4} ml, corresponding to a standard deviation in the individual acidity constants of the order of 0.002 pK units. The end point titre for the experiment was of the order of 1 ml.

The results given in Table 5.3 were combined to give a weighted mean, and a correction for the formation of the species $KCit^{=}$ was applied to pK_3. The resulting values are compared with values from the literature in Table 5.4. The agreement for the results at 25°C is excellent.

5.4. ACIDITY CONSTANTS FOR THE METHYL CITRATES

Table 5.5 contains the results for the methyl esters of citric acid. There are significant differences between the results of this work and the results reported by Martin (1961). The results obtained for the two ionic strengths (I=0, 0.1) are compatible except in the case of symmetric monomethyl citrate. A survey of a number of carboxylic acids whose acidity constants are well known shows that in general the differences $pK_1(I=0) - pK_1(I=0.1)$ and $pK_2(I=0) - pK_2(I=0.1)$ are in the ranges 0.20 - 0.25 and 0.38 - 0.43 respectively. The differences for symmetric monomethyl citrate (0.14 and 0.22 respectively) are well below the normal values and experimental error rather than some special property associated with the particular ester is probably the cause. In general the precision, as indicated by the standard deviations, of data at ionic strength 0.1 is greater than that at approximately zero ionic strength. Thus the error is more likely

Table 5.3

Acidity Constants and Standard Deviations for Citric Acid as a Function of Acid Concentration in 0.1 mmol/l KCl Solution at 25°C

Expt. No.	pK_1		pK_2		pK_3		Approx Citric Acid Conc.
		(a)		(a)		(a)	
172	2.841	0.043	4.421	0.015	5.819	0.007	0.1 mmol/l
166	2.912	0.007	4.358	0.007	5.720	0.003	0.3
167	2.877	0.002	4.338	0.002	5.690	0.001	0.6
170	2.916	0.005	4.353	0.005	5.702	0.002	0.9
173	2.925	0.010	4.340	0.010	5.683	0.005	1.5
169	2.891	0.008	4.337	0.007	5.675	0.003	1.9
174	2.893	0.005	4.330	0.004	5.663	0.002	2.4
171	2.895	0.004	4.337	0.004	5.673	0.002	2.9
175	2.888	0.009	4.325	0.009	5.659	0.005	3.8
168	2.920	0.004	4.345	0.003	5.685	0.002	4.7
		(b)		(b)		(b)	
mean	2.896	0.025	4.348	0.027	5.697	0.047	
weighted mean (c)	2.891		4.340		5.688		

Notes

(a) The figures in these columns are the standard deviations (= S) of the pK values obtained from single titration experiments.

(b) These figures are the standard deviations of the mean pK values obtained from the 10 separate titration experiments.

(c) The weighted means have been calculated according to the formula:

$$\overline{pK} = \frac{\sum pK/s^2}{\sum 1/s^2}$$

Table 5.4

Concentration Acidity Constants for Citric Acid at I = 0.1

pK_1	pK_2	pK_3[a]	Method[b]	Temp/°C	Medium[c]	Ref[d]
2.88	4.33	5.85	E pf_Hf_{Cl} = 0.228	25	~0.055 KCl	B
2.84	4.34	5.94	gl $p\gamma_H$ = 0.10	30	$NaNO_3$	W
2.87	4.35	5.90	gl [H]	20	0.1 $NaClO_4$	C
2.88	4.36	5.84	gl $p\gamma_H$ = 0.0985	25	Me_4NCl	T
2.89	4.34	5.83	gl $p\gamma_H$ = 0.084	25	0.1 KCl	this work*

Notes:

(a) Where appropriate a correction for $NaCit^=$ or $KCit^=$ (see Section 6.2) has been applied to the pK_3 values reported in the original paper.

(b) E. E.m.f. measurements in cells without liquid junction.

gl Glass electrode titration using a cell with salt bridge. The electrode was standardised against standard pH buffers.

gl [H] Glass electrode titration using a cell with salt bridge. The electrode was standardised by titration of acetic acid solutions of the same ionic strength, the hydrogen ion concentrations of which were calculated from the known (pK = 4.546 (Harned and Hickey, 1937)) concentration acidity constant for acetic acid.

pf_Hf_{Cl}, $p\gamma_H$ Corrections applied to convert the hydrogen ion activity to concentration.

(c) The medium was in all cases dilute aqueous solution. The supporting electrolyte is given together with its concentration (mol/l) in the cases where this was constant.

(d) B R.G. Bates and G.D. Pinching, (1949). J. Am. Chem. Soc. 71, 1274. Mixed acidity constants at I = 0.1 were read from the extrapolation graphs used to find the thermodynamic acidity constants.

W R.C. Warner and J. Weber, (1953). J. Am. Chem. Soc. 75, 5086. Mixed acidity constants are given in this paper.

C E. Campi et al., (1964). J. Inorg. Nucl. Chem. 26, 553.

T S.S. Tate et al., (1965). J. Chem. Soc. (1965) 3905.

* From Table 5.3 with a correction for $KCit^=$.

Table 5.5 Acidity Constants for the Methyl Citrates

All values are for 25°C and were determined from glass electrode titrations.

pK_1 (a)		pK_2 (a)		Medium	Ref. (b)
Asym Dimethyl Citrate					
3.99	0.01			~0	this work
3.78	0.01			0.1 KCl	" "
3.85				~0	M
Sym Dimethyl Citrate					
3.21	0.002			~0	this work
3.02	0.004			0.1 KCl	" "
3.35				~0	M
Asym Monomethyl Citrate					
3.0	0.11	5.05	0.02	~0	this work
2.7	0.08	4.66	0.01	0.1 KCl	" "
		5.25		~0	M
Sym Monomethyl Citrate					
3.54	0.01	4.74	0.003	~0	this work
3.40	0.01	4.51	0.003	0.1 KCl	" "
3.55		4.70		~0	M

Notes

(a) The figures in these columns are the standard deviations of the pK values.

(b) M. R.B. Martin, (1961). J. Phys. Chem. 65, 2053.

associated with the constants for zero ionic strength rather than for those at I=0.1.

The standard deviations of the pK_1's found for asymmetric monomethyl citrate were very large and probably reflected the contamination by symmetric dimethyl citrate and citric acid (see Table 4.1). Martin (1961) does not report a value for this constant.

The micro acidity constants for citric acid derived from the data in Table 5.5 using the relationships given in Section 2.3 are given in Table 6.6.

5.5 STABILITY CONSTANTS FOR VARIOUS CITRATE COMPLEXES

Table 5.6 contains values reported in the literature for the stability constants for calcium complexes of citric acid. There is good agreement between the different workers for log K_3 at I=0.15 - 0.16. The data for other ionic strengths and for the other constants is less satisfactory.

Campi et al. (1964) investigated citric and tricarballylic acid complexes of calcium and a number of other divalent metal ions, including magnesium but with particular attention to copper. They report that they used hydrogen ion concentration titrations in solutions of ionic strength, I=0.1, at 20°C. They give apparent acidity constants for the acids in the presence of large excesses of the metal ions and also the stability constants of the complexes, computed from the known metal ion concentrations and the true acidity constants, using equations similar to 6.1. In most cases they have not reported the metal ion concentration but these can be

Table 5.6

A Summary of Values Reported for the Stability Constants of Citric Acid/Calcium Complexes

Log K_1	Log K_2	Log K_3	Method[a]	Temp/°C	Medium[b]	Ref[c]
		4.85	sol	25	0	B
		3.22	frog heart	22-23	0.16 NaCl	Ha
		3.22			0.16 NaCl	M
		3.17	E pCa	25	0.15 NaCl	J
	3.29	4.84	gl	25	cor 0	He
		3.15	ix	25	0.16 NaCl	S
1.10	3.09	4.68	ix, sol($Ca(IO_3)_2$)	25	cor 0	D & S
		3.22	col	20	0.15 NaCl	Ra
		3.20	gl	28	0.15 NaCl	L
		3.19	col	25	0.16 Me_4NCl	W
		3.62	gl	33	~0.25 $NaClO_4$	P
1.05	2.10	3.55	gl	20	0.10 $NaClO_4$	C
		3.67	Ca electrode	25	0.10 $NaClO_4$	Re

Notes:

(a) sol. The metal ion concentration determined by measuring the solubility of a sparingly soluble salt.

frog heart. Calcium ion concentration determined by comparing and matching with known solutions, the equal amplitude of contraction of the ventricle of an isolated frog's heart being taken as indicating equal calcium ion concentration.

E E.m.f. measurement in cell with liquid junction to determine metal ion activity.

gl Glass electrode titration.

ix Ion exchange resin method.

col Colorimetric determination of calcium ion concentration using murexide or Eriochrome Black T indicator.

(b) The medium was in all cases dilute aqueous solution.

0 Formation constant extrapolated to zero ionic strength.

cor 0 Formation constant corrected to given ionic strength using calculated activity coefficients.

In other cases the ionic strength and the supporting electrolyte are given.

(c) References and Notes

B N. Bjerrum and A. Unmack, (1929). Data reported by L.G. Sillen and A.E. Martell, "Stability Constants of Metal-Ion Complexes", Special Publication No. 17, Chemical Society, London (1964).

Ha A.B. Hastings et al., (1934). J. Biol. Chem. 107, 351.

M J. Muus and H. Lebel, (1936). Data reported by L.G. Sillen and A.E. Martell, (loc cit).

J N.R. Joseph, (1946). J. Biol. Chem. 164, 529.

He E. Heinz, (1951). Biochem. Z. 321, 314. The values given are of low reliability because of the approximate method of calculation. Only one complex species at a time was allowed for.

S J. Schubert and A. Lindenbaum, (1952). J. Am. Chem. Soc. 74, 3529.

D C.W. Davies and B.E. Hoyle, (1953). J. Chem.Soc. (1953) 4134, (1955) 1038.

R J. Raaflaub, (1956). Methods of Biochemical Analysis 3, 301.

L J. Lefebrue, (1957). J. Chim. Phys.(1957). Data reported by L.G. Sillen and A.E. Martell (loc cit).

W M. Walser, (1961). J. Phys. Chem. 65, 159.

P R.K. Patnaik and S. Pani, (1961). J. Indian Chem. Soc. 38, 229.

C E. Campi et al., (1964). J. Inorg. & Nucl. Chem. 26, 553.

Re G.A. Rechnitz and T.M. Hseu, (1969). Anal. Chem. 41, 111.

calculated from the constants given using the equations given. Some of the results of these calculations are given in Table 5.7.

Two points are apparent. (1) The three stability constants reported for a particular ion generally give considerably different calculated metal ion concentrations when they should in fact be identical.

(2) Some of the metal ion concentrations calculated are too large for the solutions to have had ionic strengths of 0.1. (The ionic strength contribution of a divalent metal perchlorate is equal to three times its molar concentration).

The authors do not give details of their calculations so that it is difficult to reconcile the above findings with those given in their paper.

Values for the stability constants of the calcium citrate complexes obtained in this work are given in Table 5.8. The agreement between the different experiments is much less satisfactory than was obtained for the acidity constants of citric acid (Table 5.3). This is particularly so for the constant K_1. The accuracy is much less than that indicated by the standard deviation as the uncertainty associated with the acidity constants has not been included in the calculation. In several experiments particularly those in which the mole ratio of total metal concentration to total acid concentration was close to unity, negative values were obtained for one or both of the constants, K_1 and K_2. Such negative values are meaningless and the reason for their occurrence is not known but may be the result of small systematic errors in pH measurements.

Table 5.7 Metal Ion Concentrations Calculated from Constants Given by Campi et al., for Citric Acid. I = 0.1, 20°C

Complexing Metal	Apparent Acidity Constants.			Stability Constants			Calculated Metal Concentrations (mmol/l)		
	pK_1	pK_2	pK_3	$\log K_1$	$\log K_2$	$\log K_3$			
Ni	2.56	2.82	3.49	1.75	3.30	5.40	19	34	43
Mg	2.78	3.82	4.20	0.84	1.84	3.40	33	46	50
Ca	2.63	3.74	4.15	1.05	2.10	3.55	66	48	66
Ba	2.68	3.98	4.58	0.79	1.75	2.89	89	47	74

Table 5.8 Stability Constants for Citric Acid/Calcium Complexes 25°C, I = 0.1

Expt No	Log K_1	(a)	Log K_2	(a)	Log K_3 (d)	(a)	$(Cit)_t$ mmol/l	$(Ca)_t$ mmol/l
81	1.14	0.03	2.118	0.002	3.598	0.006	0.57	14.4
121	1.10	0.02	1.997	0.003	3.536	0.009	0.83	14.4
122	1.34	0.04	2.107	0.005	3.70	0.02	0.55	24.0
123	0.92	0.01	2.007	0.001	3.614	0.002	1.0	24.0
177	0.83	0.02	1.935	0.004	3.57	0.01	0.90	22.0
242	1.70	0.08	1.75	0.11	3.50	0.09	0.50	1.0
245	1.17	0.01	1.92	0.02	3.87	0.05	5.0	30.0
248	1.14	0.01	1.83	0.05	3.82	0.08	5.0	10.0
250	1.10	0.05	2.025	0.005	3.74	0.02	4.8	30.0
251	1.18	0.04	1.984	0.006	3.58	0.01	0.5	10.0
252	1.95	0.05	1.49	0.19	3.69	0.06	0.5	1.0
256	1.11	0.26	1.63	0.07	3.55	0.04	0.5	2.0
257	1.63	0.05	1.91	0.03	3.63	0.04	0.5	5.0
Mean (b)	1.25	0.32	1.90	0.18	3.65	0.11		
WtMean (c)	1.09		2.02		3.61			

Notes (a) Standard deviation of the estimate for log K (=S).

(b) Mean of 13 values of log K and the standard deviation from the mean.

(c) Weighted mean, calculated from

$$\overline{\log K} = \frac{\sum \frac{\log K}{S^2}}{\sum \frac{1}{S^2}}$$

(d) Constant has been corrected for KCit=.

There are only small differences between the titration curves of acid only and acid plus a low concentration of metal ion, particularly at the lower pH's. Accurate determination of this small difference is difficult. A second possible cause of the negative values may be the neglect of complexes such as $Ca(H_2Cit)_2$ and $Ca(HCit)_2^{=}$. It is not known whether such complexes are in fact important as no systematic trend was observed when the ratio of metal to acid concentration was varied.

A series of titrations of solutions of citric acid and of citric acid plus calcium chloride were performed at different ionic strengths. The stability constants obtained together with the acidity constants used in their calculation are given in Table 5.9. The value obtained for $\log K_3$ at I = 0.15 compares well with previously reported results (Table 5.6).

In the penultimate line of the table the stability constants after extrapolation to zero ionic strength are given. Linear regressions of log K against various functions of ionic strength were used, each datum being given equal weight. The standard deviations given are the estimated standard deviations from the regression at zero ionic strength. A meaningful value for $\log K_1$ at zero ionic strength cannot be found by extrapolation because of the paucity of data and the large uncertainty associated with $\log K_1$ at I = 0.1. A negative value was obtained for K_1 at I = 0.15, indicating a large error.

In the case of $\log K_3$ equally good fits and equal values for $\log K_3$ were obtained for the functions $\sqrt{I}/(1 + 1.5\ \sqrt{I})$ and $\sqrt{I}/(1 + 2.5\ \sqrt{I})$ while for $\log K_2$,

Table 5.9 Variation with Ionic Strength of the Stability Constants for Citric Acid/Calcium Complexes. 25°C

I	$[KCl]_t$	$[Ca]_t$	$[Cit]_t$	pK_1	pK_2	pK_3	log K_1	S	log K_2	S	log K_3	S
	mmol/l	mmol/l	mmol/l									
0.15	0.12	10	0.55	2.83	4.28	5.61			1.714	0.005	3.23	0.01
0.10	0.07	10	0.55	2.88	4.35	5.69	0.2	0.2	1.930	0.003	3.45	0.01
0.06	0.03	10	0.55	2.94	4.41	5.77	1.18	0.02	2.169	0.002	3.685	0.006
0.025	0.02	5	0.25	3.01	4.52	5.94	1.61	0.04	2.424	0.004	4.02	0.01
0							?		2.92	0.02	4.88	0.02
Cor 0							1.48		2.86		4.76	

$\sqrt{I}$ gave a better fit than either of the other two functions.

In the last line of the table the weighted mean stability constants from Table 5.8 after correction to zero ionic strength are given. The following activity coefficients were used in the correction to zero ionic strength: $\gamma_{Ca} = 0.405$, γ_{H_2Cit}, γ_{CaCit}, $\gamma_{CaH_2Cit} = 0.76$, $\gamma_{HCit} = 0.36$, $\gamma_{Cit} = 0.115$.

Table 5.10 contains a summary of values reported in the literature for the stability constants of the magnesium citrate complexes. The agreement between the different workers is not good.

Table 5.11 contains the results obtained in this work with mean values and weighted mean values given at the bottom of the table. The spread of values is greater than that obtained for the calcium citrate complex and the value obtained for log K_1 must be considered as very approximate.

Table 5.12 contains values for the stability constants of various complexes of citric and tricarballylic acids. The order of stability of the complexes of the different metal ions with the same ligand is the commonly observed order of Mn > Ca > Mg > Sr > Ba. Tricarballylic acid forms weaker complexes than citric acid in accord with the known complex strengthening effect of the hydroxyl substituent (Campi et al., 1964).

Table 5.10 A Summary of Values Reported for the Stability Constants of Citric Acid/Magnesium Complexes

log K_1	log K_2	log K_3	Method[a]	Temp/°C	Medium[b]	Ref[c]
		3.22	frog heart	22-23	0.16 NaCl	H
		3.2				N
	1.60	3.29	polarography gl.	25	0.09 $NaNO_3$	L
		3.55	col.	25	0.16 Me_3NCl	W
0.84	1.84	3.40	gl.	20	0.10 $NaClO_4$	C
		3.16	ix	25	0.10 NH_4Cl	T1
		3.96	ix	25	0 NaCl	T2
	1.85	3.73	gl	25	0.10 Me_4NCl	Ta

Notes

(a))
) See Table 5.6
(b))

(c) References

H A.B. Hastings et al., (1934). J. Biol. Chem. 107, 351.

N R. Nordbo, (1938). Skand. Arch. Physiol. 80, 341.

L N.C. Li et al., (1959). J. Inorg. Nucl. Chem. 12, 122.

W M. Walser, (1961). J. Phys. Chem. 65, 159.

C E. Campi et al., (1964). J. Inorg. Nucl. Chem. 26, 553.

T1 S.K. Tobia and Milad, (1963). J. Chem. Soc. (1963), 734.

T2 S.K. Tobia and Milad, (1964). J. Chem. Soc. (1964), 1915.

Ta S.S. Tate et al., (1965). J. Chem. Soc. (1965), 3905.

Table 5.11 Stability Constants for Citric Acid/Magnesium Complexes 25°C, I = 0.1

Expt No.	log K_1	S	log K_2	S	log K_3	S	$(Cit)_t$ mmol/l	$(Mg)_t$ mmol/l
80	0.85	0.09	1.86	0.005	3.56	0.01	0.56	13.8
128	1.06	0.01	1.815	0.002	3.586	0.004	0.85	13.8
129	0.19	0.31	1.72	0.005	3.56	0.01	0.56	22
229	1.51	0.03	1.94	0.02	3.65	0.02	0.50	10
235	0.51	0.11	1.64	0.01	3.49	0.01	0.50	10
258	1.70	0.10	1.11	0.50	3.68	0.08	0.50	5
mean	0.77	0.58	1.68	0.30	3.60	0.07		
wt mean	1.10		1.81		3.57			

Table 5.12 Stability Constants for Various Complexes

25°C, I = 0.1

Complex	log K_1	log K_2	log K_3	Notes
Magnesium/citric acid	1.10	1.81	3.57	a
calcium "	1.09	2.02	3.61	b
strontium "	1.13	1.78	3.21	c
barium "	0.91	1.74	2.96	d
manganese "	1.15	2.10	3.85	e
Magnesium/tricarballylic acid	0.86	1.21	1.94	f
calcium "	0.78	1.22	2.05	g

Notes

(a) From Table 5.10

(b) From Table 5.7

(c) Joseph, (1946) found log K_3 = 2.90 at 25°C I = 0.15

(d) Campi et al., (1964) found 0.79, 1.75, 289 respectively

(e) Li et al., (1959) found log K_2 = 2.08 and log K_3 = 3.67 at I = 0.15

(f) Campi et al., (1964) found 0.77, 1.20, 2.06 respectively

(g) Campi et al., " " 0.88, 1.46, 2.17 respectively

CHAPTER 6.

DISCUSSION

6.1 ERROR

Errors in experimental measurements may be divided into two classes:

(a) systematic errors and

(b) random errors.

Every experimental measurement is subject to random errors and the uncertainty introduced by them may be minimised by taking the mean value of repeated measurements. An estimate of the magnitude of the random error is given by the standard deviation of the measurements from the mean.

There are many possible sources of error in determining equilibrium constants from glass electrode titrations. Some of these sources, which are not necessarily independent, are listed below. Many of them are expected to be insignificantly small and will not be discussed.

Sources of Error in Determining Equilibrium Constants from Glass Electrode Titrations

1. Error in standardising KOH used for the titrations.
2. Contamination of KOH with carbonate.
3. Error in the micrometer syringe burette calibration.
4. Error in the quantity of KCl added and therefore in ionic strength.
5. Variation of ionic strength during the titration not completely swamped by the supporting electrolyte.
6. Error in the pH of the standard buffer.
7. Error in standardising the pH meter against the buffer.

8. Error within the pH meter, nonlinearity.
9. Drift in the glass electrode response.
10. Non-Nernstian glass electrode response.
11. Electrode streaming potential caused by stirring of solution.
12. Error in calculating hydrogen ion concentration from pH meter reading.
13. Liquid junction potentials.
14. Error in total volume of the solution.
15. Error in the complexing metal ion concentration.
16. Effects of impurities.
17. Error in reading pH at a titration point.
18. Error in reading titre at a titration point.
19. Error in determining the end point titre.
20. Temperature fluctuations during the titration.
21. Incorrect or inappropriate value for the ionisation constant of water.
22. Incorrect or inappropriate values for the acidity constants used to calculate stability constants.
23. Formation of complexes between the supporting electrolyte and the acid or the complexing metal cation.
24. Formation of complexes between the acid and the metal ion of a type not allowed for.

Pseudo-Systematic Errors

Each titration in the present work involves single measurements of such properties as the end point titre and the total volume of the solution. These measurements are subject to random errors but as they are in effect constants for a particular titration they make a contribution to the

systematic error of equilibrium constants calculated from the titration data. Thus, for a series of titrations, the standard deviation of the constants from the means calculated for the series is greater than the estimated standard deviations of the constants computed from a single titration. This difference is illustrated by the data of Table 5.3.

In principle the data of several titrations can be combined before equilibrium constants are calculated. This requires that the data from different titrations be properly weighted according to the variances of the various experimental quantities. Limited computer memory would have made such a combination of data difficult in the present case and it was not attempted.

In the case of stability constants an important source of error in their calculation is the value of the acidity constants used in their calculation. For a polybasic acid such as citric acid the uncertainty associated with the first acidity constant (Table 5.3) is carried through into the values calculated for all of the stability constants. The first stability constant is the most affected. For the polybasic acids and the metal ions of interest in this present work the first constant is small and can only be calculated with low precision. The precision is less than that indicated by the standard deviation of the constant as the standard error deviation of the acidity constants are not included in its calculation.

Thermodynamic Acidity Constants of Polybasic Acids

The method of determining acidity constants from titration curves does not lend itself to accurate determination

of the thermodynamic acidity constants of polybasic acids. In order to obtain constants with a small standard deviation it is necessary for the supporting electrolyte to be present in large excess so that there is no appreciable change in ionic strength during the course of a titration. As the acidity constants involving polyvalent ions are markedly dependent on ionic strength, measurements must be made at low ionic strengths to allow accurate extrapolation to zero ionic strength. These two requirements can only be met by the use of very low acid concentrations. Unfortunately this in itself reduces precision. Thus measurements at low ionic strength are invariably less precise than those at higher ionic strengths and extrapolations to zero ionic strength are correspondingly less precise.

6.2 INTERACTION BETWEEN THE SUPPORTING ELECTROLYTE AND THE ACID ANION

The citrate triple negative anion is known to form complexes with sodium and with potassium (Jandetzky and Wertz, 1956; Walser, 1961) and the following stability constants at 25°C and I = 0.16 have been reported by Walser (1961).

$$K_{NaCit^{=}} = 5.0$$

$$K_{KCit^{=}} = 2.7$$

Other acid anions may also form complexes with the alkali metal cations. In cases where the acid anion in general forms weaker complexes than the citrate anion these alkali metal complexes are expected to be weak and unimportant. Anions which in general form complexes of comparable or greater stability than the citrate anion are expected to

form comparatively strong alkali metal complexes.

In the derivations in Chapter 3 no allowance was made for alkali metal complexes. The necessary corrections are now discussed.

Consider equation 3.12 (page 22)
for the titration of a weak polybasic acid in the presence of a complexing metal ion. If the complexing metal ion is present in large excess, as in the case for the supporting electrolyte, its concentration will not alter sensibly during the course of the titration, and the approximation $[M] = B$ can be made. The equation can then be written in the same form as equation 3.13,

$$\sum_{n=0}^{N} \beta^{\bullet}_{n} [H]^{n} \left([H] + T - Kw/[H] - (N-n)A \right) = 0 \qquad (1)$$

where $\beta^{\bullet}_{n} = (K_{N-n}.B + 1)\beta_{n}$, a constant

and $\beta^{\bullet}_{o} = (K_{N}.B + 1) \neq \beta_{o} = 1$

If there is complex formation between the supporting electrolyte and the acid anion the application of equation 3.13 to determine the acidity constants will yield apparent rather than true acidity constants. It can be shown that the following relationships hold

$$pK_{n} = pK^{\bullet}_{n} - \log \frac{K_{n-1}.B + 1}{K_{n}.B + 1} \qquad n = 1, \ldots\ldots, N$$

For the binding of citric acid and alkali metal ion only the citrate triple negative ion is important so that

$$K_oB < K_1B < K_2B << 1$$

and

$$pK_1 \sim pK_1^\bullet$$

$$pK_2 \sim pK_2^\bullet$$

$$pK_3 \sim pK_3^\bullet + \log\left(K_{MCit^-}[M] + 1\right)$$

This correction has been applied to the acidity constants for citric acid reported in Table 5.4.

When a complexing ion such as calcium is present in addition to the supporting electrolyte the situation is more complex. In general the concentration of supporting electrolyte is reduced when complexing metal ion is added in order to maintain a constant ionic strength. It is necessary to use the apparent acidity constants corresponding to the actual supporting electrolyte concentration when computing the stability constants. Some workers use a tetra alkyl ammonium salt as the supporting electrolyte in order to minimise complex formation.

A second possible interaction that has not been allowed for is interaction of the complex cation with the supporting electrolyte anion, possibly though ion pair formation. Interactions of this type are expected to be weak in the dilute aqueous solutions of interest in this present work.

6.3 THE HYDROGEN ION ACTIVITY COEFFICIENT

In order to determine concentration acidity constants it is necessary to convert pH meter readings into hydrogen ion concentrations. This conversion is also necessary in the calculation of the thermodynamic acidity constants of stronger acids when the hydrogen ion concentration is a significant fraction of the total ionic concentration of the solution.

The pH scale is defined operationally by (Bates, 1964a) —

$$pH_x = pH_s + (E_x - E_s).\ F/(RT\ln 10) \qquad (1)$$

where pH_s is the assigned pH of the standard buffer solution and E_x and E_s are the e.m.f. values of the cell —

glass electrode//solution x or s/KCl(satd), $Hg_2Cl_{2(s)}$; $Hg_{(l)}$

when containing unknown solution x and standard buffer s, respectively. The pH of the standard buffer is found from

$$pH_s \equiv p\left(A_H\ \gamma_{Cl}\right)^{\circ} + \log\ \gamma_{Cl} \qquad (2)$$

where $p\left(A_H\ \gamma_{Cl}\right)^{\circ}$ is an experimentally determined quantity and $\log\ \gamma_{Cl}$ is calculated from the Bates-Guggenheim convention (Bates, 1964b) —

$$\log\ \gamma_{Cl} = A\sqrt{I}\Big/\left(1 + 1.5\ \sqrt{I}\right)$$

The e.m.f. of the pH cell when containing the standard buffer can be written as

$$E_s = E^{\circ} + E_j^s - RT\ln 10/F.\ \log\left(C_H^s.\ \gamma_?^s\right) \qquad (3)$$

where E^{O} is the reference electrode potential

E_j^s is the liquid junction potential

$\gamma_?^s$ is an activity coefficient of indefinite nature.

This division of the experimentally determinable cell e.m.f. into three components from a strict thermodynamic view point is arbitrary, as has been shown by Guggenheim (1967). Substituting for E^{O} from equation (3) into the corresponding equation for the unknown solution — and rearranging

$$-\log C_H^x = pC_H^x = \frac{F}{R.T.\ln 10}\left[E_x - E_s - (E_j^x - E_j^s)\right] + p\left(C_H^s . \frac{\gamma_?^s}{\gamma_?^x}\right) \quad (4)$$

If the standard buffer and the unknown solution have very similar compositions then equation (4) reduces to

$$pC_H^x = pC_H^s + (E_x - E_s)\, F./(RT\ln 10) \quad (5)$$

as the liquid junction potentials and the indefinite activity coefficients in the two cells will be equal.

If $E_j^x = E_j^s$ (equality expected for dilute solutions only) then equation (4) becomes, after rearrangement and substitution

$$pC_H^x = p(A_H \gamma_{Cl})^s + \log \gamma_{Cl}^s + \frac{F}{RT\ln 10}(E_x - E_s) + p\left(\frac{\gamma_{Cl.}^s}{\gamma_{HCl}^{s\,2}} \frac{\gamma_?^s}{\gamma_?^x}\right) \quad (6)$$

$$= pH^x + p\Gamma$$

where $\Gamma = \dfrac{\gamma_{Cl}^s}{\gamma_{HCl}^{s\,2}} \cdot \dfrac{\gamma_?^s}{\gamma_?^x}$

γ_{HCl}^s is the mean activity coefficient for HCl at infinite dilution in the standard buffer. At sufficiently low ionic

strengths γ^{s}_{HCl} can be taken as the activity coefficient of a solution of hydrochloric acid at the same ionic strength as the standard buffer.

We will now attempt to evaluate the activity coefficient term, $p\Gamma$ for the standard pH buffers 0.05 M potassium hydrogen phthalate (I = 0.0533) and equimolar 0.025 M phosphate (I = 0.10) where the unknown solution is 0.10 M in KCl.

The conventional chloride ion activity coefficient has values 0.8173 and 0.7770 at ionic strengths 0.0533 and 0.10, respectively. The hydrochloric acid mean activity coefficient at these ionic strengths has values 0.8266 and 0.796, respectively (Robinson and Stokes, 1959). Some of the procedures which can be adopted for the evaluation of the activity coefficient term of equation (6) are outlined in Table 6.0.

TABLE 6.0 pH TO pC_H CORRECTIONS

	γ ?	$-p\Gamma$	
		phthalate	phosphate
1	$\gamma^{2}_{HCl}/\gamma_{Cl}$	0.089	0.089
2	γ_{HCl}	0.094	0.088
3	γ_{KCl}	0.101	0.089

A commonly adopted procedure is to interpret the pH of a solution as equal to the hydrogen ion activity for the solution and to take the activity coefficient of the hydrogen ion as equal to the mean activity coefficient of

hydrochloric acid at the same ionic strength. Thus —

$$p\left(C_H^x\right) = pH_x - p\left(\gamma_{HCl}\right)$$

For I = 0.10 $\quad p\gamma_{HCl} = 0.10.$

Studies with specific ion electrodes including the glass electrode show that for solutions of low ionic strength useful approximately self consistent sets of individual ion activity coefficients can be calculated. Such calculations involve a single extrathermodynamic assumption or the acceptance of some arbitrary convention in order to assign values to the activity coefficient of one particular reference ion. Such a convention is an essential part of the pH scale for which the chloride ion has been taken as the reference ion. It is logical to use the same convention in the calculation of C_H values from pH values as is done in procedure 1 of Table 6.0. Such a procedure yields a value for $p\Gamma$ which is independent of the nature of the standard buffer. Procedures 2 and 3 are not satisfactory in this respect.

All experimentally determined pH's in this work were converted to pC_H's using the relationship

$$pC_H = pH - 0.512\sqrt{I}/(1+2.96\sqrt{I})$$

for I = 0.1 $\quad pC_H = pH - 0.084$

The value obtained for $p\Gamma$ by this procedure is not significantly different from that given by procedure 1 of Table 6.0.

pH Versus pC_H Standards

The standardisation of a glass electrode against a pC_H rather than a pH standard buffer using equation 5 above avoids the difficulties concerning activity coefficients. Acidity constants can be determined in solutions of very nearly constant composition. However, the determination of metal complex stability constants requires a range of solution compositions in which a varying proportion of the supporting electrolyte is replaced by a salt of the complexing metal. This range of solution compositions cannot easily be matched by suitable pC_H standards. A further difficulty concerning pC_H standards is that their buffer capacity is necessarily small as the concentration must be similar to that of the unknown acid. Thus pC_H standards are not as attractive as they appear to be on first sight and were not used in the present work.

If pH standards are used careful specification of the solution composition and of the activity coefficient adopted is necessary to allow comparison of the results of different workers. McBryde (1969 and 1971) and Hedwig and Powell (1971) have obtained empirical linear relationships between pH and pC_H.

6.4. CALCULATION OF ACIDITY CONSTANTS FROM THE SUBSTITUENT EFFECT

Substitution in an organic acid has an effect on the strength of the acid as measured by its pK values where K is the acidity constant.

It is well known (see for example Barlin and Perrin, 1966) that the effect on the pK of an acid of a particular substituent is approximately constant for a wide range of different acids and that the magnitude of the effect is a

characteristic of the particular substituent and the position of substitution relative to the acid group. In the case of aliphatic acids the effect of successive substitutions is approximately cumulative at least in cases where not more than one substituent having a large effect is present at each chain carbon. In cases where experimental results are not available useful estimates of approximate pK values for organic acids in aqueuous solution have been made by consideration of substituent effects. (Barlin and Perrin, 1966; Brown et al., 1955). In the case of polybasic acids care is needed to distinguish between micro and macro acidity constants. In this section the micro and macro acidity constants for various esters of citric acid and for citric acid and other acids have been calculated using the substituent effect. A test has also been made to see if an analogous substituent effect operates for metal complex formation reactions and the results are reported in Section 6.6.

The acid strengthening effect of a substituent is found by subtracting the pK value for a substituted acid from the value for the unsubstituted or 'parent' acid. In cases where more than one carboxylic acid group is present the appropriate micro constant calculated from the macro constants must be used. The method of calculating substituent effects is illustrated in Table 6.1. The choice of a single value for a particular substituent effect is somewhat arbitrary as different values are obtained from different pairs of parent and substituted acids. Values for various substituent effects are collected in Table 6.2. All values are for infinitely dilute aqueous solutions of aliphatic carboxylic

Table 6.1 Illustrations of the Method of Calculating the Effects of Substituents on Acid Strengths

Substituent	Acid	pK	ΔpK	Ref.
	propionic	4.874		Harned, 1933 a
α-OH	lactic	3.858	1.016	Martin, 1937
	acetic	4.756		Harned, 1933 b
α-OH	glycollic	3.831	0.925	Nims, 1936
	propionic	4.784		
β-COOH	succinic pk_1	4.508	0.366	Pinching, 1950
	propionic	4.784		
β-COO	succinic pk_2	5.335	-0.461	

Table 6.2 Acid Strengthening Effect of Substituents for Aliphatic Carboxylic Acids in Aqueous Solution

	α	β	γ
-OH	1.00	0.16	0
-COOH	1.60	0.36	0.18
$-COO^-$	-0.64	-0.46	-0.29

(cf. Barlin and Perrin, 1966)

acids at or near 25°C. (The methyl carboxylate group, $-COOCH_3$, is assumed to have the same substituent effect as the carboxylic group, -COOH).

Table 6.3 shows the calculation of the micro acidity constants for citric acid. Propionic acid has in all cases been taken as the parent acid and values for the substituent effect have been taken from Table 6.2.

Table 6.4 shows the calculation of the macro acidity constants for citric acid from the calculated micro acidity constants of Table 6.3. The relationships between the micro and macro acidity constants have been given in Section 2.3 and the ones used in the calculation are repeated at the right hand side of the table. The calculated micro acidity constants are consistent with a single set of values for the macro acidity constants.

Calculated pK values for a number of different acids, some of whose pK values are reliably known from experiment, are given in Table 6.5. No reliable experimental value is available for the pK of glyceric acid at zero ionic strength nor for the hydroxyglutaric acids. In the case of malic and (+)-tartaric acids the agreement between calculated and experimental values of the acidity constants is satisfactory. The method of calculating acidity constants from substituent effects does not provide for differences between diastereomers and the poor agreement between the calculated and experimental values of the acidity constants for mesotartaric acid indicates that spatial factors, such as steric interference or intramolecular hydrogen bonding, may be important. Such spatial factors may also be important in other highly

Table 6.3 Calculation of the Micro Acidity Constants for Citric Acid

pk_1		pk_2	
propionic	4.84	propionic	4.84
β-OH	-0.16	α-OH	-1.00
β-COOH	-0.36	2 β-COOH	-0.72
γ-COOH	-0.18		3.12
	4.14		

pk_{12}		pk_{21}		pk_{13}	
propionic	4.84	propionic	4.84	propionic	4.84
α-OH	-1.00	β-OH	-0.16	β-OH	-0.16
β-COOH	-0.36	β-COO^-	0.46	γ-COO^-	0.29
β-COO^-	0.46	γ-COOH	-0.18	β-COOH	-0.36
	3.92		4.96		4.61

pk_{132}		pk_{123}	
propionic	4.84	propionic	4.84
α-OH	-1.00	β-OH	-0.16
2 β-COO^-	0.92	β-COO^-	0.46
	4.76	γ-COO^-	0.29
			5.43

Table 6.4 Calculation of Macro Acidity Constants for Citric Acid From Calculated Micro Acidity Constants

	pK	K	
$2k_1$	3.84	1.445×10^{-4}	$K_1 = 2k_1 + k_2$
k_2	3.12	7.590 "	
K_1	3.04	9.035 "	
$k_1 k_{13}$	8.75	0.178×10^{-8}	$K_1K_2 = k_1k_{13} + 2k_2k_{21}$
$2k_2k_{21}$	7.78	1.659	
K_1K_2	7.74	1.837	
K_2	4.70		
$k_1k_{12}k_{123}$	13.51		$K_1K_2K_3 = k_1k_{12}k_{123}$
K_3	5.77		

Table 6.5 Calculated pK's for Several Acids

Acid	Calculated pK's		Experimental pK's	Ref.
Glyceric	3.68		3.52 (μ=0.20)	C
Malic	3.42, 5.20		3.46, 5.10	E
Tartaric	3.02, 4.44	dl	3.04, 4.37	B1
		meso	3.22, 4.82	J
α-hydroxy glutaric	3.62, 5.17			
β-hydroxy glutaric	4.20, 5.27			
Tri-carballylic	3.75, 4.95, 6.13		3.73, 4.95, 6.42	*
Isocitric	3.18, 4.57, 5.92	Threo D_s	3.29, 4.71, 6.39	H
Citric	3.04, 4.70, 5.77[x]		3.13, 4.76, 6.40	B2

Ref.

C Cannan and Kibrick, (1938) J. Am. Chem. Soc. 60, 2314.

E Eden and Bates, (1959) J. Research N.B.S. 62, 161.

B1 Bates and Canham, (1951) J. Res. Nat. Bur. Standards 47, No. 5.

J. Jones and Soper, (1934). J. Chem. Soc. (1934), 1836.

* This work (Table 5.2).

H Hitchcock, D.I., (1958) J. Phys. Chem. 62, 1337.

B2 Bates and Pinching, (1949) J. Am. Chem. Soc. 71, 1274.

x Data from Table 6.3.

substituted acids such as isocitric acid.

Tricarballylic, citric and isocitric acids form a family of acids of particular interest because of the similarity of structure (see Section 2.4). The agreement between the calculated and experimental values of the first and second acidity constants for these acids is satisfactory and especially so for the first two of these acids. In the case of the third acidity constant, the calculated values of the constant are considerably greater than the experimental values, with the greatest difference in the case of citric acid.

The mechanisms by which substituents influence acid strength are not clearly understood. A distinction is sometimes made between field effects, which act directly across space and are electrostatic in nature, and inductive effects, which act through the bonding electrons of the molecule, but in practice, it has so far been found impossible to disentangle the two effects (Roberts and Caserio, 1965).

Calculations of field effects, such as those made by Kirkwood and Westheimer (1938), are of limited usefulness because of the large number of empirical parameters, related to the size and shape of the molecule, the location of charges and the orientation of dipoles, to which the calculations are sensitive. Because of the poor understanding of the substituent effect, discussion of the failures to adequately predict the third acidity constants of tricarballylic, citric and isocitric acids is necessarily speculative. Any correction applied (e.g. such as an allowance for dielectric saturation of

the solvent) must affect the micro acidity constants in a manner such that they remain consistent with a single set of macro acidity constants.

6.5 MICRO ACIDITY CONSTANTS FOR CITRIC ACID

It has been explained in Section 2.3 that a minimum of two micro acidity constants must be independently determined to enable the calculation of the remaining five micro constants for citric acid. Independent determination of more than two micro constants provides a means of checking both the method and the suitability of the methyl esters as model compounds.

In the present work a total of six macro acidity constants have been determined for the methyl esters of citric acid. These are - one acidity constant for each of the monobasic dimethyl citrates and two acidity constants for each of the dibasic monomethyl citrates. Because of experimental error and/or non-ideality of the methyl citrates as model compounds, the values obtained for the macro constants are not consistent with a single set of values for the micro acidity constants of citric acid. For example, if pk_1 and pk_{13} are calculated from the experimentally observed macro acidity constants for symmetric monomethyl citrate (Table 5.5) the following set of micro constants is obtained: pk_1 3.84, pk_2 3.34, pk_{12} 4.58, pk_{13} 4.44, pk_{21} 5.08, pk_{123} 5.87, pk_{132} 6.00. The alternative of equating pk_2 with the acidity constant for symmetric dimethyl citrate and calculating pk_{13} from the second macro acidity constant for symmetric monomethyl citrate yields: pk_1 4.19, pk_2 3.21, pk_{12}

4.09, pk_{13} 4.44, pk_{21} 5.07, pk_{123} 6.00, pk_{132} 5.66. The first set of values are in reasonable agreement with the values found by Martin (1961) but are significantly different from the second set of values. The choice of values for the two independent micro acidity constants is important as it affects the values obtained for all of the micro constants and some criterion is necessary to judge the "goodness" of the choice of values.

As the precision of the various macro constants, as indicated by the standard deviations, varies widely it is inappropriate to give equal weight to each. In the absence of a more satisfactory procedure the following statistic was used as a measure of the "goodness" of a particular choice of values for the two independent micro acidity constants:

$$P = \sum \left(\frac{pk_i - pk_i^{\bullet}}{\rho_i} \right)^2$$

where pk_i is the acidity constant for a methyl citrate calculated from the values chosen for the micro acidity constants for citric acid, $pk_i^{\bullet}$ is the experimentally observed constant for the methyl citrate and ρ_i the standard deviation. The summation is for all six of the macro constants for the methyl citrates. The P statistic makes no allowance for systematic errors.

In principle it is possible to find values for the independent micro acidity constants giving the smallest possible value for P and therefore the "best" values for the micro constants. In practice such a procedure is difficult and is, in fact, not necessary in the present

case. The only methyl ester macro acidity constant which is significantly dependent on the micro constant, pk_{13}, is the second constant for symmetric monomethyl citrate. Thus there is no difficulty in choosing the "best" value for pk_{13}. The choice of the "best" value for the second independent micro constant is simplified by the great weight which must be given to the macro constant for symmetric dimethyl citrate by virtue of its very small standard deviation. Thus the best value for pk_2 is very close to the value found for the constant for symmetric dimethyl citrate. It is important to note that the "best" value is not necessarily the "right" value.

As has been explained in Chapter 5 the results obtained for symmetric dimethyl citrate at zero ionic strength are suspect because of the smaller than usual difference from the acidity constants found at 0.1 ionic strength. If the second acidity constant for symmetric dimethyl citrate at zero ionic strength is adjusted from 4.74 to 4.94 then the micro acidity constants for citric acid become those given in Table 6.6. Also given in the table are the micro acidity constants for citric acid at 0.1 ionic strength.

The agreement between the micro acidity constants calculated from the substituent effect and the experimental values is very satisfactory except in the case of pk_{123} and pk_{132} but even here the order of acid strength is the same. The agreement between the results of this work and those of Martin is poor.

The relative amounts of the different citrate species can be calculated from the micro acidity constants for

Table 6.6 A Comparison of Calculated and Experimental Acidity Constants for Citric Acid

	Calculated		Experimental		
				This work	
			Martin (1961)	I = 0	0.1
pk_1	4.14	data from Table 6.3	3.85	4.19	3.74
pk_2	3.12		3.35	3.21	3.02
pk_{12}	3.94		4.60	4.05	3.85
pk_{21}	4.96		5.10	5.03	4.57
pk_{13}	4.61		4.40	4.64	4.24
pk_{123}	5.43		5.85	6.05	5.47
pk_{132}	4.76		6.05	5.46	5.08
			Bates and Pinching (1949)		
pK_1	3.04	data from Table 6.4	3.13		
pK_2	4.70		4.76		
pK_3	5.77		6.40		

Table 6.7 Comparison of Calculated and Experimental pK's for the Mono Methyl Citrates

		Calculated	Experimental	
			Martin	This Work
Sym Mono Methyl Citrate	pK_1	3.84	3.55	3.54
	pK_2	4.91	4.70	4.73
Asym Mono Methyl Citrate	pK_1	3.08	-	3.03
	pK_2	5.00	5.25	5.02

citric acid. If the experimental constants for zero ionic strength are used in the calculation it is found that at any pH in a solution containing citric acid 83% of the monovalent citrate species (H_2Cit^-) have the central carboxylic acid group ionized. The experimental acidity constants at 0.1 ionic strength yield a corresponding figure of 72% and the calculated constants of Table 6.6 84%. For comparison, Martin found 60%.

For the divalent citrate species ($HCit^=$) the experimental acidity constants for zero and 0.1 ionic strengths and the calculated acidity constants yield, respectively, 89%, 83% and 90% asymmetric species compared with 55% found by Martin.

The results of this work indicate that the central carboxylic acid group of citric acid is the most acidic of the groups in the neutral, monovalent and divalent citric acid species.

6.6 CALCULATION OF STABILITY CONSTANTS FROM SUBSTITUENT EFFECTS

The success of using the substituent effect in predicting acidity constants has been demonstrated in Section 6.4. An analogous prediction of metal complex stability constants from substituent effects does not appear to have been reported and it was considered worthwhile to attempt such calculations. Stability constants for complexes of five metals with five acids (both dibasic and tribasic) have been calculated and the results are given and are discussed in this section.

Substituent effects for various metals have been

calculated in a manner analogous to that used for acidity constants and the results are given in Table 6.8.

The relationships between micro and macro stability constants for dibasic and tribasic acids are:

Dibasic $$K_1 = k_{11} \frac{k_{a1}}{K_{a1}} + k_{22} \frac{k_{a2}}{K_{a1}}$$

$$K_2 = k_{121} + k_{122}$$

Tribasic $$K_1 = k_{11} \frac{k_{a1}}{K_{a1}} + k_{22} \frac{k_{a2}}{K_{a1}} + k_{33} \frac{k_{a3}}{K_{a1}}$$

$$K_2 = (k_{121}+k_{122}) \frac{k_{a12}\, k_{a1}}{K_{a1}\, K_{a2}} + (k_{131}+k_{133}) \frac{k_{a13}\, k_{a1}}{K_{a1}\, K_{a2}} + (k_{232}+k_{233}) \frac{k_{a23}\, k_{a2}}{K_{a1}\, K_{a2}}$$

$$K_3 = k_{1231} + k_{1232} + k_{1233}$$

Lower-case letters denote micro constants while the upper-case letters denote macro constants. The acidity constants are subscripted 'a'. The last numerical subscript of the micro stability constants denotes the position of the metal ion on the acid anion.

The calculation of macro constants for citric acid are given in Table 6.9 and experimental and calculated values for a number of different acids are compared in Table 6.10.

The results of Table 6.10 indicate that the method for predicting stability constants using substituent effects is apparently much less successful than the method for predicting acidity constants (Section 6.4) on which it is modelled. There are several possible reasons for this. In general the data from which the stability constant

Table 6.8 Complex Strengthening Effect of Substituents

Calculated from the data of Cannan and Kibrick (1938) and applies to ionic strength I = 0.2. The temperature of measurement was not given but is assumed to be 20-25°C.

	Zn	Mg	Ca	Sr	Ba
α-OH	0.87	0.40	0.58	0.32	0.24
β-OH	0	0	0.10	0.15	0.18
α-COOH	-0.19	-0.04	-0.06	-0.02	0.05
β-COOH	-0.11	-0.02	0.02	0.05	0.11
γ-COOH	-0.16	-0.01	-0.01	0	0.05
α-COO^-	1.45	1.10	0.63	0.52	0.54
β-COO^-	0.47	0.36	0.40	0.33	0.39
γ-COO^-	0.30	0.25	0.25	0.25	0.25

Table 6.9 Calculation of Stability Constants for Various Citrate Complexes

	Zn	Mg	Ca	Sr	Ba
Butyric	1.00	0.53	0.51	0.36	0.31
β-OH	0	0	0.10	0.15	0.18
β-COOH	-0.11	-0.02	0.02	0.05	0.11
γ-COOH	-0.16	-0.01	-0.01	0	-0.05
$p(2k_{a1}/K_{a1})$	-0.80	-0.80	-0.80	-0.80	-0.80
$Log(2k_{a1}/K_{a1}.k_{11})$	-0.07	-0.30	-0.18	-0.24	-0.25
Butyric	1.00	0.53	0.51	0.36	0.31
α-OH	0.87	0.40	0.58	0.32	0.24
2 β-COOH	-0.22	-0.04	0.04	0.05	0.22
$p(k_{a2}/K_{a1})$	-0.08	-0.08	-0.08	-0.08	-0.08
	1.57	0.81	1.05	0.65	0.69
Calculated log K_1	1.58	0.84	1.08	0.70	0.74
Butyric	1.00	0.53	0.51	0.36	0.31
β-OH	0	0	0.10	0.15	0.18
β-COO^-	0.47	0.36	0.40	0.33	0.39
γ-COOH	-0.16	-0.01	0.01	0	0.05
Log k_{121}, log k_{233}	1.31	0.88	1.00	0.84	0.93
Butyric	1.00	0.53	0.51	0.36	0.31
α-OH	0.87	0.40	0.58	0.32	0.24
β-COO^-	0.47	0.36	0.40	0.33	0.39
β-COOH	-0.11	-0.02	0.02	0.05	0.11
Log k_{122}, log k_{232}	2.23	1.27	1.51	1.06	1.05
Log $(k_{121}+k_{122})$	2.28	1.42	1.63	1.26	1.29
$p(2k_{a2}k_{a21}/K_{a1}/K_{a2})$	-0.04	-0.04	-0.04	-0.04	-0.04
	2.24	1.38	1.59	1.22	1.25
Butyric	1.00	0.53	0.51	0.36	0.31
β-OH	0	0	0.10	0.15	0.18
β-COOH	-0.11-	-0.02	0.02	0.05	0.11
γ-COO^-	0.30	0.25	0.25	0.25	0.25
$p(2k_{a13}.k_{a1}/K_{a1}/K_{a2})$	-0.71	-0.71	-0.71	-0.71	-0.71
Log	0.48	0.05	0.17	0.10	0.14
Calculated log K_2	2.25	1.40	1.61	1.25	1.28

	Zn	Mg	Ca	Sr	Ba
Butyric	1.00	0.53	0.51	0.36	0.31
α-OH	0.87	0.40	0.58	0.32	0.26
2 β-COO	0.94	0.72	0.80	0.66	0.78
log k_{1232}	2.81	1.65	1.89	1.34	1.33
Butyric	1.00	0.53	0.51	0.36	0.31
β-OH	0	0	0.10	0.15	0.18
β-COO^-	0.47	0.36	0.40	0.33	0.39
γ-COO^-	0.30	0.25	0.25	0.25	0.25
log k_{1231}	1.77	1.14	1.26	1.09	1.13
Calculated log K_3	2.88	1.86	2.06	1.66	1.68

Table 6.10 A Comparison of Calculated and Experimental log Stability Constants

		Zn		Mg		Ca		Sr		Ba	
		Calc	Expt	Calc	Expt	Calc	Expt	Calc	Expt	Calc	Expt
Malic Acid	K_1	1.72	1.57	0.88	0.77	1.06	1.02	0.78	0.72	0.68	0.67
	K_2	2.40	2.80	1.45	1.55	1.60	1.80	1.30	1.45	1.24	1.30
Tartaric Acid	K_1	1.77	1.44	0.92	0.92	1.20	1.11	0.95	0.91	0.87	0.88
	K_2	2.65	2.68	1.60	1.36	1.88	1.80	1.53	1.65	1.45	1.62
Tricarballylic	K_2	0.75	0.94	0.49	0.77	0.53	0.88	0.43		0.69	0.73
	K_2	1.45	1.61	1.00	1.20	1.04	1.46	0.84		0.90	1.15
	K_3	2.31	2.43	1.66	2.06	1.69	2.17	1.45		1.48	1.95
Citric	K_1	1.58	1.25	0.84	0.84	1.08	1.05	0.70	1.13	0.74	0.79
	K_2	2.25	2.98	1.40	1.84	1.61	2.10	1.25	1.78	1.28	1.75
	K_3	2.88	4.98	1.86	3.40	2.06	3.55	1.66	3.21	1.68	2.89
Isocitric	K_1	1.50		0.83		1.02		0.69		0.70	
	K_2	2.16		1.36		1.33		1.20		1.18	
	K_3	2.76		1.82		1.98	2.47	1.62	2.02	1.63	

Notes

1. Experimental data in this table has been taken from the following sources -

   Malic, dl Tartaric: Zn, Mg, Ca, Sr, Ba (I = 0.2) Cannan and Kibrick, 1938.

   Tricarballylic, Citric: Zn, Mg, Ca, Ba (I = 0.1) Campi et al., 1964.

   Isocitric: Ca, Sr (I = 0.16) Schubert and Lindenbaum, 1952.

2. Calculated values are for I = 0.2.

substituent effects were derived and the data with which the calculated constants are compared, are not as reliable as the corresponding data used in the calculation and comparisons of acidity constants and, in addition, the data apply to several different ionic strengths. A further possibility is that empirical relationships of the type found for acidity constants do not hold for stability constants.

The data of Cannan and Kibrick (1938) was chosen for the calculation of substituent effects even though it was obtained at the inconvenient ionic strength of 0.2 because their study was the only one in which all of the necessary stability constants were measured. It was considered that as the same methods and conditions were used in all of their determinations, systematic errors would tend to cancel in the subsequent calculations.

Correction of stability constants to a constant ionic strength is difficult in the ionic strength range $I = 0.1 \text{ —— } 0.2$, particularly for equilibria involving polyvalent ions. If the extrapolations used to obtain values for $\log K_2$ and $\log K_3$ for the calcium citrate complexes in Table 5.9 are extended to $I = 0.2$ then $\log K_2 = 1.53$ and $\log K_3 = 3.09$ are obtained compared with values of 2.10 and 3.55 respectively at ionic strength 0.1. If corrections of the same magnitude are applied to $\log K_2$ and $\log K_3$ of the tricarballylic and citric acid complexes there is a general improvement in the agreement between the calculated and the (corrected) experimental values of these constants. However, the agreement is still very poor for the $\log K_3$ of citric

acid complexes.

An X-ray diffraction study of crystals of magnesium citrate decahydrate (Johnson, 1965) has shown that the hydroxyl group of the citrate is involved in the chelation of magnesium ions and that citrate acts as a tridentate ligand with one five member and one six member ring respectively, the hydroxyl being common to both rings. A study of molecular models indicates that the isocitrate ion can also act as a tridentate ligand whereas the tricarballylate ion can only act as a bidentate ligand. The X-ray data (Johnson, 1965) indicates intramolecular hydrogen bonding between the hydroxyl hydrogen and the oxygens of the non-chelating carbonyl of the citrate ion. Similar intramolecular hydrogen bonding could also occur in aqueous solution although there would be competition from intramolecular hydrogen bonding involving solvent water. Studies of molecular models indicate that intramolecular hydrogen bonding, with a seven member hydrogen bonded ring, is possible in threo but not erythro isocitric acid.

The differences in the strengths of the complexes of tricarballylic, isocitric and citric acids (e.g. for the calcium complexes, log K_3 = 2.17, 2.47 and 3.55 respectively) may be due to these differences in chelation and intramolecular hydrogen bonding.

It is considered that the results of this work on the calculation of cation complex stability are sufficiently promising for further work to be done if and when more reliable values for the relevant stability constants become available.

PART II

CATION ACTIVITIES IN MILK, MILK PRODUCTS AND OTHER FLUIDS

CHAPTER 7.

INTRODUCTION

In most previous studies of cation activities in milk and milk products the results have been reported as concentrations of "ionized calcium" (Christianson et al., 1954), or "ionic calcium" (Demott, 1968), or "free ions" (Van Kreveld and Van Minnen, 1955) etc, rather than as activities. The usual procedure has been to find a solution of the chlorides of sodium, potassium, magnesium and calcium having the same magnesium and calcium activities as the milk and in addition having either the same ionic strength as predicted for the milk or the same sodium and potassium concentrations. The concentrations of magnesium and calcium in this solution are then reported as a measure of the activities. Thus care is needed when using results from the literature.

Early studies of cation activities in milk involved the measurement of the solubility of sparingly soluble salts in milk ultrafiltrates. Harnapp (1938) used calcium picrolonate and found levels of 1.2 - 1.4 mM for calcium at 30°C and Nordbo (1939) used magnesium tropaeolin OO and found a level of 0.5 mM for magnesium at 37°C.

Pyne (1948, also Pyne and McHenry, 1955) used a rennet coagulation time method to determine the "effective calcium ion concentration" in milks. It was recognised that "effective concentration" was a composite quantity related to the "true concentration" but affected to some

degree by the presence of other ions. Values in the range 3.5 - 5.5 mM were obtained and a correlation with the heat stability of the milks was found.

Christianson and coworkers (1952) were the first to use a cation exchange resin method. They reported levels of 1.0 - 1.1 mM for calcium but completely neglected the effects of magnesium. This neglect was later corrected and the method was used to study the effect of milk pH, addition of citrate and heat treatment on calcium ion activities (1954). They found values of 2.0 - 2.3 mM for calcium and 0.82 - 0.85 mM for magnesium in raw milk at temperatures of 22 - 25°C. Milk pH was found to have a marked effect on calcium ion activity.

Van Kreveld and Van Minnen (1955) independently devised a cation exchange resin method and found that raw skim milk was 2.2 mM in calcium and 0.8 mM in magnesium. They found that pasteurization caused a reduction in calcium and magnesium ion activities. They also made measurements on reconstituted skim milk powder and on condensed milks.

Belec and Jenness (1963) adapted the cation exchange resin method to rapid routine use by introducing batch equilibration of milk and resin in the place of column equilibration. They found values of 2.25 mM for calcium and 0.77 - 1.15 mM for magnesium for skim milk.

Seekles and Smeets (1954; also Smeets, 1955) applied the murexide method for calcium (Raaflaub, 1951) to the investigation of the "Utrecht instability" in milk which is caused by a dietary mineral imbalance and characterised by an increased tendency for the casein to

flocculate. They found levels in the vicinity of 2.75 mM for normal milks while unstable milks had levels of 4.0 mM or greater.

White and Davies (1958) applied the murexide method to studies of milk stability and found values of 2.6 - 3.2 mM for milks and values of 3. 8 - 5.1 mM for colostrum. The stability of milks to ethanol, and to a lesser extent, rennet, was found to correlate with the calcium ion activity.

Tessier and Rose (1958) modified the murexide method to give increased sensitivity. They found calcium levels in skim milk in the range 2.5 - 3.4 mM and investigated the effect of adding calcium, phosphate and citrate to milk and the effect of heat treatment. They also studied milk concentrated by low temperature evaporation.

A recent development has been the use of calcium ion specific electrodes. Demott (1968) investigated the effect of heat treatments on milk and found values for calcium in the range 1.4 - 2.5 mM for raw milk. The effect of addition of milk powder and of water to milk and the effect of treatment of milk with anion exchange resin was also studied.

Kramer and Lagoni (1969) determined calcium ion activities in fresh milk, milk reconstituted from skim milk powder, evaporated milk and diluted evaporated milk. They report a value of 0.89 mM for raw milk.

Muldoon and Liska (1969) compared the calcium ion specific electrode and cation exchange resin methods using raw, pasteurized and sterilized skim milks. They found the two methods gave comparable results and reported values

for calcium in the range 2.24 - 2.77 mM for raw skim milk.

Of all the methods which have been used to study cation activities in milk and in milk products only the cation exchange resin method is capable of giving the activities of all of the cations. The murexide indicator method (Raaflaub, 1951) is limited to the determination of calcium in non-turbid fluids at pH's of less than 7.5. Generally milk ultrafiltrates are used to avoid the problem of turbidity. Ion specific electrodes are potentially promising for the rapid non-destructive measurement of negative logarithms of ion activities by methods analogous to pH measurements using glass electrodes. Electrodes specific to Na^+, K^+ and Ca^{++} but not Mg^{++} are available (Durst, 1969) but unfortunately these electrodes tend to be unstable and easily poisoned in biological systems. The specificity of the potassium electrode is poor in the presence of sodium.

The cation exchange resin method can be applied to milk directly and because it can give the activities of all the cations it was the method of choice in the present work. In the following Chapters the cation exchange resin method is developed as a very general method applicable to fluids of diverse compositions. The theory of the method is developed and a criticism of earlier work is made. The experimental procedures are briefly described and the application of the method to the study of milk is described and discussed.

CHAPTER 8.

THEORY AND APPLICATION OF THE ION EXCHANGE RESIN METHOD FOR DETERMINING CATION ACTIVITIES

In this Chapter the thermodynamic basis of the cation exchange resin method for determining cation activities in solution is developed. Such a treatment of the method does not appear to have been reported previously. The application of the method to milk is discussed.

8.1 GENERAL THEORY OF THE ION EXCHANGE RESIN METHOD FOR DETERMINING CATION ACTIVITIES

Consider a hypothetical system consisting of two electrolyte solutions separated by an ideal membrane of strongly acidic cation exchange resin. The resin membrane is homogeneous and is totally impervious to anions, to hydrogen ions and to the solvent. Solute cations A, B, - - -, can exchange freely between the various phases. Temperature and pressure are constant and the system is in a state of equilibrium.

Phase I	Phase II	Phase III
solution	cation exchange resin	solution

The following symbols will be used

μ_A^I electrochemical potential of cation A in phase I

A_A^I activity of cation A in phase I

Z_A valency of cation A

X^I electrical potential of phase I

F Faraday constant

T thermodynamic temperature

Under conditions of equilibrium -

$$\mu_A^I = \mu_A^{II} = \mu_A^{III}$$

$$\mu_B^I = \mu_B^{II} = \mu_B^{III}$$

etc.

Expanding

$$\mu_A^I = \mu_A^{I^\circ} + RT\ln\left(\frac{A_A^I}{A_A^{I^\circ}}\right) + Z_A F X^I = \mu_A^{III^\circ} + RT\ln\left(\frac{A_A^{III}}{A_A^{III^\circ}}\right) + Z_A F X^{III}$$

Rearranging

$$RT\ln\left(\frac{A_A^I}{A_A^{III}}\right) = \mu_A^{III^\circ} - \mu_A^{I^\circ} + RT\ln\left(\frac{A_A^{I^\circ}}{A_A^{III^\circ}}\right) + Z_A F\left(X^{III} - X^I\right)$$

If the standard states in phases I and III are similarly defined this equation can be simplified to

$$RT\ln\left(\frac{A_A^I}{A_A^{III}}\right) = Z_A F\left(X^{III} - X^I\right) \qquad (1)$$

Similarly for component B

$$RT\ln\left(\frac{A_B^I}{A_B^{III}}\right) = Z_B F\left(X^{III} - X^I\right) \qquad (2)$$

Dividing equation (1) by equation (2)

$$Z_B \ln\left(\frac{A_A^I}{A_A^{III}}\right) = Z_A \ln\left(\frac{A_B^I}{A_B^{III}}\right)$$

or

$$\frac{\left(A_A^I\right)^{Z_B}}{\left(A_B^I\right)^{Z_A}} = \frac{\left(A_A^{III}\right)^{Z_B}}{\left(A_B^{III}\right)^{Z_A}} \qquad (3)$$

Equation 3 states that the activity quotient for the pair of cations A, B has the same value in phases I and III. This result has been derived without need for detailed knowledge of the resin phase.

In a system of N cations there are a total of N(N-1) different activity quotients only N-1 of which are independent. That is, if N-1 activity quotients are specified then all activity quotients are specified.

Equation 3 is the basis of the Ion Exchange Method for Determining Cation activities in solution. If solution I is the solution under investigation and solution III is a simple dilute solution of known composition whose cation activities can be found by calculation or by other means then the activity quotients for solutions III and I can be calculated. It is necessary to have an independent assessment of the activity of just one cation in solution I before all the cation activities can be calculated from the activity quotients.

8.2 APPLICATION

In order to apply the Ion Exchange Method the hypothetical experiment described above must be replaced by an equivalent set of experiments. These are:

(1) From a series of equilibrations of beads of ion exchange resin with different standard solutions find empirical relationships between resin composition and activity quotients.

(2) Equilibrate an excess of the unknown solution with ion exchange resin and determine resin composition.

(3) Use the empirical relationships and the equilibrium resin composition to calculate the activity quotients for the unknown solution.

These experiments are now discussed in more detail.

1. Resin Calibration

Activity quotients for dilute aqueous solutions of strong electrolytes of known composition can be calculated using the Debye-Huckel equation for activity coefficients:

$$\log \gamma_i = \frac{-A Z_i^2 \sqrt{I}}{1 + Ba_i\sqrt{I}}$$

where the symbols have their usual meanings. Values of the closest approach parameter, a_i for various cations have been given by Kielland (1937). For this reason such solutions are useful for 'calibrating' the ion exchange resin. A series of such solutions are equilibrated with different portions of ion exchange resin after which solution and resin are separated and are analysed. Functional relationships between activity quotients and

resin composition are then found empirically. It is not necessary for the calibrating solutions all to have the same ionic strength although ionic strength must be sufficiently low for the Debye-Huckel equation to apply.

2. Unknown Solution Equilibration

A large volume of the solution under investigation is equilibrated with a small portion of ion exchange resin. Because the solution is present in excess its composition is not sensibly altered by the equilibration. After the equilibration the resin has a composition which is characteristic of the activity quotients of the solution.

3. Calculation of the Activity Quotients for the Unknown Solution

The activity quotients for the solution under investigation are found by substituting the equilibrium resin composition into the empirical relationships for the activity quotients.

CHAPTER 9.

A CRITICAL DISCUSSION OF THE WORK OF VAN KREVELD AND VAN MINNEN (1955)

The cation exchange resin method for determining cation activity quotients has been applied to milk several times (see Chapter 7) since its introduction by Christiansen, Jenness and Coulter in 1952 and has recently been applied to the intestinal contents of ruminants (Molloy and Richards, 1971). The theory behind the method does not appear to have been discussed in the literature and there seems to have been little appreciation of the generality of the method. It has been shown in Chapter 8 that the cation exchange resin method gives cation activity quotients. The problem remains of estimating single cation activities from these quotients. Van Kreveld and Van Minnen (1955) have discussed parts of this problem in some detail and a critical discussion and extension of their arguments is given in this Chapter.

Validity of Single Ion Activities

The activity of a single ionic species cannot be determined from thermodynamic study alone but requires the adoption of some extrathermodynamic assumption or convention. The significance and meaning of single ion activities is the subject of differences of opinion with many thermodynamic purists continuing to oppose the

concept (Bates, 1964c; Bates and Alfenaar, 1969). The justification for the use of single ion activities is a pragmatic one. Consistent scales of single ion activities, such as the pH scale, can be constructed and have been found useful in the understanding of diverse aspects of chemistry.

Use of the thermodynamically definable quantities A_{Na}/γ_K, A_{Mg}/γ_K^2, A_{Ca}/γ_K^2 avoids the difficulties with single ion activities and these quantities can probably be considered as being approximately equal to the "concentration of free ions" used by some workers (see Chapter 7) as $\gamma_{Na} \sim \gamma_K$ and $\gamma_{Mg} \sim \gamma_{Ca} \sim \gamma_K^2$ so that $[Na^+] \sim A_{Na}/\gamma_K$, $[Mg^{++}] \sim A_{Mg}/\gamma_K^2$ and $[Ca^{++}] \sim A_{Ca}/\gamma_K^2$. In this case the square brackets denote concentration of 'free ions' and not the total stoichiometric concentration.

Equation 8.3, which gives the relationships between the cation activities, applies to milk and the corresponding isoionic solution of Van Kreveld and Van Minnen (1955). It is apparent that if an activity can be assigned to one of the cations of milk and the values for all of the activity quotients are known, then single ion activities can be easily calculated for all of the cations. Van Kreveld and Van Minnen (1955) chose to assign a value to the potassium ion activity. (It should be noted that it is not necessary for the potassium ion activity to be the same in milk and in the calibrating solution in order to allow calculation of cation activities in milk from the activity quotients).

The Relationship Between Potassium Ion Concentration and Potassium Ion Activity in Milk. Factors Affecting Potassium Ion Activity

A source of error which was not discussed by Van Kreveld and Van Minnen (1955) is the difference between the potassium ion concentration in milk and the concentration in the serum phase of milk. Typical concentrations for milk and milk serum are given in Tables 1.1 and 1.2. The difference for potassium is small so that only a small error is introduced by the use of potassium ion concentration in milk in the place of potassium ion concentration in the serum phase.

1. Binding by Protein

The serum phase of skim milk contains about 0.6% protein, about half of which is β lactoglobulin. If the number average weight of the serum protein is taken as 30,000 then the serum is about 0.2 mM in protein. There will be interaction between the positively charged potassium ions and the negatively charged serum proteins (Carr, 1956; Basch and Timashebb, 1967). If the average charge number per protein molecule is, say, -15 then the anion concentration due to the protein will be about 3 milli equivalents per liter. The potassium ion concentration in the serum phase is about 35 milli equivalents per liter. If it is assumed that potassium ions alone balance the negative charge of the serum proteins and that the potassium ions needed for charge balance are tightly bound to the protein then the potassium ion activity will be reduced by about 3 mM or less than 10% because of the protein interaction. There is expected to be preferential interaction of divalent cations with the

protein and also competitive interaction from sodium ions. Further, the potassium ions are not expected to be so tightly bound that none are available to contribute to the potassium activity of the solution. Thus protein - potassium ion interactions in the serum phase are expected to cause only a small reduction in potassium ion activity. At lower pH's average protein charge number will be less than -15 and potassium ion activity will be correspondingly affected even less by serum protein interaction.

2. Lactose

Lactose, present in milk at about 5% or 150 mM, might be expected to affect activity coefficients of ions even if only by diluting the solvent water or by affecting its dielectric constant. Experiments (Robinson and Stokes, 1962; Kelly et al., 1961) with mixtures of the sugar alcohol D-mannitol and KCl (and also NaCl) show that mannitol has only a small effect on the activity coefficient for KCl. No analogous data is available for lactose or for any disaccharide or aldose. There is some evidence for interaction between lactose and salts in aqueous solution (Herrington, 1934). Salts affect the equilibrium specific rotation of aqueous solutions of lactose and also affect the kinetics of mutarotation. The effects are small in dilute salt solutions. Smeets (1955) found no evidence for lactose - calcium ion interaction when studying calcium ion activities in milk serum by the murexide calcium indication method. The effect of lactose on the activity coefficient of potassium is expected to be small.

3. Electrostatic Interactions

Debye and Huckel have shown how to calculate activity coefficients for dilute electrolyte solutions by consideration of the electrostatic interaction energy of impenetrable spherical ions.

Attempts to apply the Debye-Huckel equation to solutions containing protein poses problems especially as regards the concepts of ionic strength and closest approach distances. Protein ions are amphoteric. The net charge, Z, of a protein is the algebraic sum of the positive and negative charges due to such groups as $-NH_3^+$ and $-COO^-$. Even when the net charge is zero there is expected to be some electrostatic interaction of the protein with ions in the surrounding solution. Treatment of the protein molecule as a large impenetrable spherical ion of charge Z would give comparatively large calculated values for the ionic strength of milk serum at normal milk pH's. The closest approach parameter for interactions involving the protein would be much greater than for interactions not involving the protein, contrary to the requirement of the Debye-Huckel model that the closest approach distances be equal or approximately equal for all pairs of ions. Thus the treatment of the protein molecule as multicharged sphere is not very realistic.

An alternative is to treat the protein ion in its contribution to the ionic strength as being equivalent to Z independent univalent ions. This procedure is more reasonable for long chain polyelectrolytes (the charges of which may be expected to be more capable of independent motion) than it is for the more rigid protein ions.

Van Kreveld and Van Minnen (1955) refer to experimental data for flexible polyelectrolytes in support of their assumption that as far as the serum proteins of milk are concerned "the negative charges are so far apart that they should be considered as monovalent in the ionic atmosphere theory" and thus contribute negligibly to the ionic strength of milk. Low angle X-ray scattering studies (Witz et al., 1964) and pH titration studies (Tanford, 1962) indicate a rigid compact structure for β lactoglobulin, the principal serum protein.

Boulet and Marier (1960) have measured the ionic strength of some milk ultrafiltrates. They determined the solubility product for calcium citrate (Ca_3Cit_2) in the ultrafiltrates and substituted the values obtained into a previously found empirical equation for the ionic strength dependence of the solubility product. Solution of the equation for ionic strength yielded values close to 0.08, in good agreement with caclulated values (Whittier, 1929; Nordbo, 1939). However, as milk ultrafiltrates are practically free of protein this figure cannot be applied directly to the serum phase of milk.

At ionic strengths in the vicinity of 0.08 the activity coefficient for potassium ions is not very sensitive to changes in the ionic strength or in the closest approach distance. Although it is not easy and is possibly not meaningful to calculate or to measure the contribution of protein to the ionic strength of milk serum it is not necessary to assume as have Van Kreveld and Van Minnen that any such contribution is negligible in order to calculate the potassium ion activity coefficient in milk

to a reasonable approximation.

Summary

The potassium ion activity of milk is dependent on the potassium ion concentration of the serum phase of milk and is probably little affected by lactose or by binding by serum proteins. A reasonable value for the activity coefficient for the potassium ion can be calculated from the Debye-Huckel equation if the contribution of the protein to the ionic strength is ignored.

CHAPTER 10.

EXPERIMENTAL

10.1 DESCRIPTION OF APPARATUS

Flame Photometer

A Zeiss PMQII spectrophotometer fitted with a 'FA2' flame attachment was used for all flame photometry. The flame was acetylene/air and was produced at a slit burner. A Sargeant SRL recorder was coupled to the spectrophotometer through a resistor-capacitor integrating circuit with a time constant of 2.5 seconds. Magnesium was determined by flame absorption, the light source being a modulated 'Perkin-Elmer' magnesium hollow cathode lamp.

Columns

Glass tubes of internal diameter 0.5 cm and length 8 cm drawn to a tip at the bottom and plugged with glass wool were used for elution of resin. The columns were supported by rubber discs. The discs sat on the rims of the flasks used to collect the column effluent and the columns resided in holes in the discs. Generally about 0.2 g of resin was eluted at a time. Elutant was added to the column in small aliquots from a wash bottle. Surface tension prevented the columns from running dry between additions.

Generally grade A pipettes and grade B standard flasks were used in all standard volumetric work.

10.2 REAGENTS

Commercially available reagents of analytical grade were used without further purification or assay. Distilled water was deionised by passage down a mixed bed ion exchange column shortly before it was required. The distilled water and the deionised water were stored in polythene tanks.

Ion Exchange Resin

Strong cation exchange resin 'Amberlite' IR-120 of analytical reagent grade was used. This bead-like granular material is a sulphonic resin based on the polymerisation of styrene containing about 8% of divinyl benzene. Bead size was in the range 16-50 mesh. All the exchange resin used came from bottles with the same batch number.

Flame Photometry Standards

Analytical reagent grade NaCl, KCl, Mg metal and $CaCO_3$ were used. Accurately weighed quantities of these materials were dissolved in water or a small excess of hydrochloric acid, as appropriate, and made up to standard volume. The resultant standard stock solutions contained about 1,000 ppm of the cation. Standards having concentrations similar to the unknowns were prepared daily by dilution of the standard stock solutions.

10.3 PROCEDURES

Resin Conditioning

The cation exchange resin, initially in the potassium form, was treated with a series of solutions containing NaCl, KCl, $MgCl_2$ and $CaCl_2$ to produce a resin composition similar to that found after equilibration of a portion of the resin with milk. The resin was then thoroughly washed, the fines removed by decanting and the resin dried in air at room temperature. The conditioned resin was then stored for later use.

Resin Calibration

Approximately 0.2 g portions of the conditioned resin were gently shaken with 100 ml portions of solutions containing approximately known concentrations of NaCl, KCl, $MgCl_2$ and $CaCl_2$ for periods of not less than four hours at constant temperature. The resin and solution were then separated by decanting and the resin drained and washed with distilled water on a sintered glass filter. After being transferred to small columns the resin was eluted with 10 ml of 3 M hydrochloric acid over a period of about $2\frac{1}{2}$ hours. The resulting column effluents and the equilibrated solutions were subsequently analysed by flame photometry.

Resin Equilibration with Milk

Resin equilibration of milk was achieved by two different methods.

(1) 3-4 liters of skim milk were passed down a column containing about 10 g of conditioned resin over a period

of 4-5 hours;

(2) approximately 0.2 g portions of conditioned resin were shaken for 15 minutes or longer with three or four successive portions (approximately 100 ml) of skim milk.

After equilibration, the resin was thoroughly drained and washed with cold distilled water. The resin was then eluted with HCl as for the calibration procedure.

Flame Photometric Analysis

After suitable dilution the milk and solutions were analysed as outlined in Table 10.1.

Table 10.1 Analysis Methods

	column effluent, calibrating solution	milk
flame emission photometry	Na, K, Ca	Na, K
flame absorption photometry	Mg	Mg
E.D.T.A. titration using eriochrome Black, T	-	Mg+Ca

A standard was run between each unknown determined by flame photometry. Total divalent cation (Magnesium + Calcium) in milk was determined by titration with a standard solution (approximately 0.01 M) of ethylene diamine tetraacetic acid disodium salt, (E.D.T.A.) using the coloured indicator, Eriochrome Black T.

CHAPTER 11.

RESULTS

11.1 THE EFFECT OF TEMPERATURE ON RESIN COMPOSITION

Changes in temperature might be expected to change the composition of ion exchange resin in equilibrium with solution.

Portions of conditioned ion exchange resin were equilibrated overnight with different portions of a solution of the chlorides of sodium, potassium, magnesium and calcium, at temperatures of 4, 16.6 and 30°C. The resulting resin compositions were then determined and linear regressions between mole fraction quotients and temperature were calculated. A small but statistically significant effect was found for magnesium and calcium but not for sodium (e.g. Figure 11.1). The temperature coefficients and standard deviations were as follows:

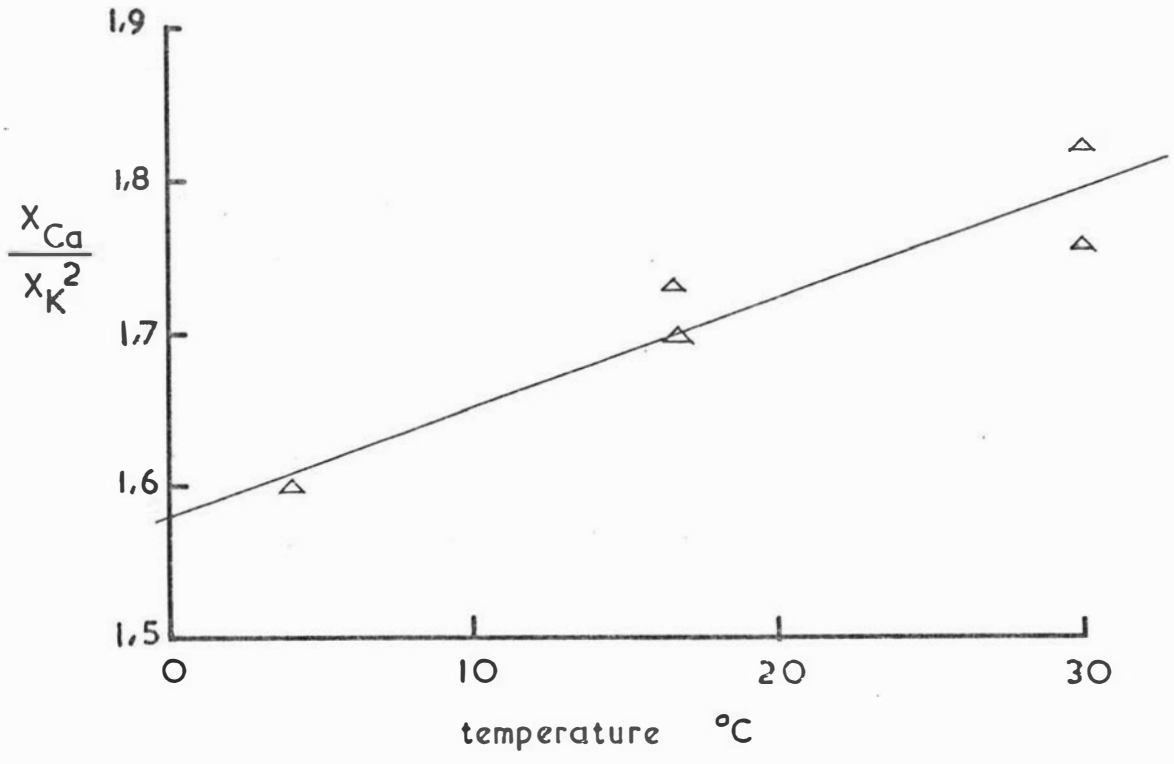
$$\frac{d(X_{Na}/X_K)}{dT} = -0.0006 \pm 0.0006$$

$$\frac{d(X_{Ca}/X_K^2)}{dT} = 0.0071 \pm 0.0015$$

$$\frac{d(X_{Mg}/X_K^2)}{dT} = 0.0022 \pm 0.0007$$

The cation activity quotients for the equilibrated solution are assumed to be almost invariant with temperature.

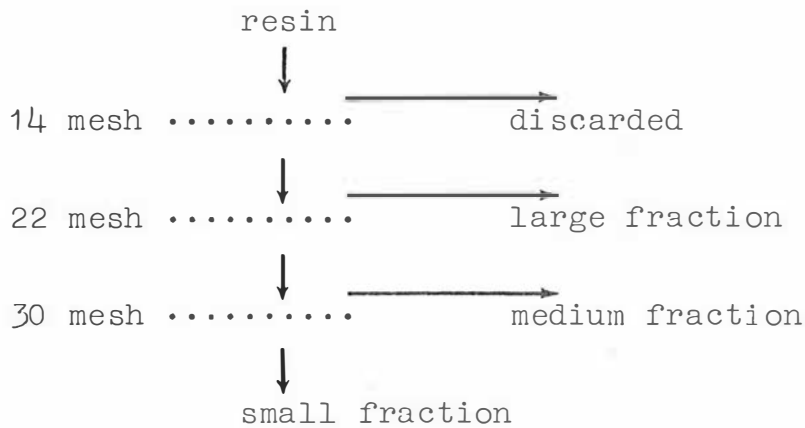
Figure 11.1 Effect of Temperature on [illegible]



11.2 THE EFFECT OF RESIN BEAD SIZE

As some of the processes of the ion exchange method involving manipulation of small quantities of resin beads might result in size fractionation a test for this was made. Air dry conditioned IR120 resin was passed through a series of standard sieves according to the scheme outlined in Figure 11.2.

Fig. 11.2 Resin Bead Fractionation Scheme Using Standard Sieves



0.2 g portions of the various resin fractions were transferred to columns, eluted with HCl, the elutents analysed and the resin composition calculated.

It was assumed that the standard deviation of the mole fraction of a particular ion was the same for all bead sizes and the students t test was applied to test for a difference in the means for various pairs of size fractions. No significant difference was found and it is therefore concluded that there is no significant bead size effect. As the smaller beads might be expected to equilibrate more quickly than the larger beads any apparent size effect observed during an experiment is likely to indicate incomplete equilibration or elution.

11.3 ELUTION KINETICS

0.2 g portions of conditioned resin were placed in small columns and were eluted with approximately 3 mol/l HCl. 0.25 ml aliquots of acid were added every 5 minutes and successive 1 ml fractions of column effluent were collected and analysed for Na and Ca. The logarithm of the concentrations decreased approximately linearly with time or with total volume of effluent. Sodium was eluted more rapidly than calcium and elution was more than 98% complete after the passage of 5 ml of HCl, (100 minutes).

11.4 CALIBRATION OF THE ION EXCHANGE RESIN

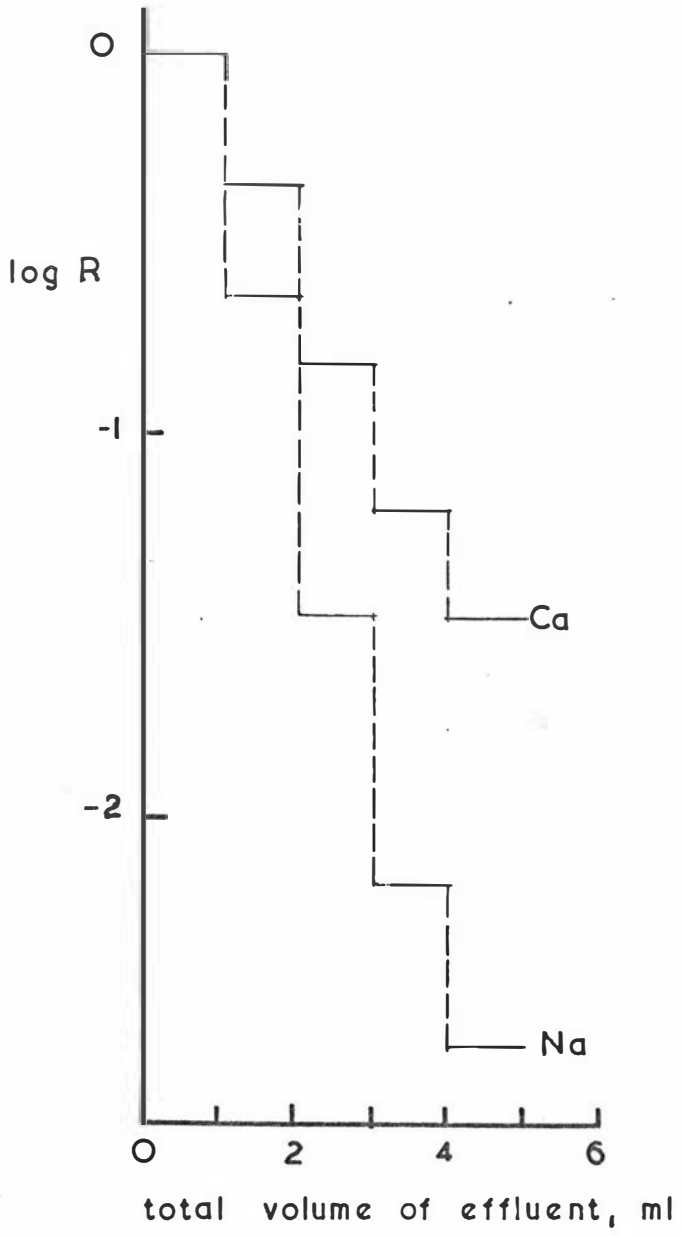
Flame photometric analysis of diluted column effluents and equilibrated solutions for 'Amberlite IR-120' resin at 12°C yielded the results given in Table 11.1. The results are given to four figures to avoid arithmetic rounding errors in subsequent calculations. Four figure accuracy is not implied.

Cation activity quotients for the equilibrated solutions and mole fraction quotients for the equilibrated resin were calculated from the data of Table 11.1 and the results of these calculations are given in Table 11.2. The activities were calculated using the Debye-Huckel equation:

$$-\log \gamma_i = \frac{0.507 . Z_i^2 . \sqrt{I}}{1 + 0.3282 . a_i . \sqrt{I}}$$

and the following values for Z and a_i, respectively:
Na 1, 4; K 1, 3; Mg 2, 8; Ca 2, 6 (Kielland, 1937).

Figure 11.3 Variation of Column Effluent C with Total Volume of Effluent. Elution f. Amberlite IR-120



$$R = \frac{\text{Concentration in nth fraction}}{\text{Concentration in 1st fraction}}$$

elutent 3 mol/l HCl, flow rate 3 ml per hour
1 ml fractions collected and analysed.

Table 11.1 Flame Photometry Results from the Calibration of IR-120 Resin at 12°C

Sample No.	Composition of diluted equilibrated solutions mg/l				Composition of diluted column effluents mg/l			
	Na	K	Ca	Mg	Na	K	Ca	Mg
Dilution	1 in 250	1 in 25	1 in 25	1 in 25	1 in 10	1 in 10	1 in 10	1 in 10
1	2.703	6.830	3.469	0.7176	1.139	7.723	5.879	0.5180
2	1.302	7.280	3.092	0.6861	0.5470	6.927	5.624	0.4771
3	3.506	7.172	3.633	0.7592	1.716	7.193	5.637	0.4776
4	3.126	3.525	2.814	0.6662	1.368	5.426	7.247	0.6476
5	2.810	12.02	3.837	0.7644	1.096	11.10	5.332	0.4123
6	2.898	7.343	3.099	0.4444	1.138	8.280	6.813	0.3520
7	2.190	7.506	3.498	1.248	1.063	7.304	5.688	0.7861
8	1.786	6.927	1.964	0.6437	1.176	8.869	5.532	0.6814
9	1.807	7.038	6.120	0.7650	0.9410	7.224	7.113	0.3845
10	3.596	12.71	4.139	0.7704	1.273	8.798	4.034	0.3109
11	3.548	7.268	3.659	1.250	1.759	7.237	5.390	0.7926
12	3.541	7.153	6.167	0.7752	1.178	5.141	5.446	0.3087
13	1.904	12.72	4.043	1.347	0.6963	8.680	4.039	0.5191
14	1.883	12.86	6.701	0.8133	0.6706	8.983	5.758	0.2688
15	1.863	7.238	6.256	1.337	0.5768	4.686	5.518	0.4847

Table 11.2 Calibration Data for Amberlite IR-120 at 12°C

Sample No.	Equilibration Solution activity quotients mmol/l				Resin mole fraction quotients		
	I	A_{Na}/A_K	A_{Ca}/A_K^2 x 10^4	A_{Mg}/A_K^2 x 10^4	X_{Na}/X_K	X_{Ca}/X_K^2	X_{Mg}/X_K^2
1	0.0818	0.6875	8.129	3.036	0.2507	1.560	0.2265
2	.0686	.3099	6.463	2.567	.1342	1.613	.2257
3	.0931	.8512	7.657	2.911	.4055	1.741	.2431
4	.0638	1.5348	25.218	10.636	.4285	3.808	.5605
5	.1169	.4089	2.837	1.043	.1679	0.7948	.1013
6	.0856	.6865	6.270	1.627	.2336	1.690	.1440
7	.0822	.5069	6.790	4.375	.2474	1.656	.3774
8	.0694	.4467	4.526	2.658	.2255	1.191	.2417
9	.0785	.4459	13.552	3.050	.2215	2.178	.1938
10	.1305	.4960	2.719	0.9393	.2461	0.783	.0995
11	.0957	.8507	7.493	4.665	.4132	1.683	.4076
12	.0982	.8629	13.021	2.986	.3895	2.604	.2431
13	.1138	.2616	2.673	1.641	.1364	0.7655	.1621
14	.1177	.2561	4.326	0.9691	.1269	1.126	.0865
15	.0824	.4473	13.053	5.039	.2093	2.901	.4200

The following regressions and correlation coefficients were found:

$$A_{Na}/A_K = 2.745\ (X_{Na}/X_K)^{1.134} \qquad R = 0.9409$$

$$A_{Ca}/A_K^2 = 0.000\ 3725\ (X_{Ca}/X_K^2)^{1.342} \qquad R = 0.9837$$

$$A_{Mg}/A_K^2 = 0.001\ 577\ (X_{Mg}/X_K^2)^{1.181} \qquad R = 0.9774$$

A second resin calibration experiment was performed using the same batch of resin but covering a wider range of values for the activity quotients. The data obtained from this experiment are given in Table 11.3.

The following regressions and correlation coefficients were found:

$$A_{Na}/A_K = 2.353\ (X_{Na}/X_K)^{0.9948} \qquad R = 0.9938$$

$$A_{Ca}/A_K^2 = 0.000\ 3303\ (X_{Ca}/X_K^2)^{1.374} \qquad R = 0.9961$$

$$A_{Mg}/A_K^2 = 0.001\ 890\ (X_{Mg}/X_K^2)^{1.32} \qquad R = 0.9880$$

Combination of the data of the first experiment, at 12°C (excluding point number 4) with that of the second experiment at 23°C (excluding point number 6) yielded the least squares regressions given in Table 11.4.

Regressions calculated from the data of Van Kreveld and Van Minnen (1955) for Duolite C-20 cation exchange resin are given in Table 11.5 for comparison.

Table 11.3 Calibration Data from Amberlite IR-120 at 23°C

Sample No.	Equilibration Solution activity quotients mmol/l				Resin mole fraction quotients		
	I	A_{Na}/A_K	A_{Ca}/A_K^2 x 10^3	A_{Mg}/A_K^2 x 10^3	X_{Na}/X_K	X_{Ca}/X_K^2	X_{Mg}/X_K^2
1	0.0398	0.4961	1.096	0.4436	0.2141	2.683	0.3984
2	.0391	.4424	2.604	1.0378	.1958	4.315	.6479
3	.0481	.4407	5.374	2.1931	.1821	7.746	1.135
4	.0720	.4541	13.772	5.5613	.1813	14.68	2.126
5	.0678	.2597	14.501	5.8468	.1090	14.44	2.087
6	.0610	.8028	47.325	19.1629	.3351	41.63	6.183
7	.0531	.4560	6.016	5.6223	.1878	7.857	2.601
8	.0644	.4177	14.084	2.3588	.1822	14.79	0.9267

Table 11.4 Amberlite IR-120 Resin Calibration Parameters

$$\frac{A_M}{A_K^{Zm}} = A\left(\frac{X_M}{X_K^{Zm}}\right)^B \qquad \text{(21 data points)}$$

M	A	σ_A	B	σ_B	R
Na	2.14	0.24	0.969	0.071	0.953
Ca	0.000 366	0.000 013	1.344	0.026	0.996
Mg	0.001 870	0.000 099	1.290	0.037	0.992

Table 11.5 Duolite C-20 Resin Calibration Parameters. Calculated from the data of Van Kreveld & Van Minnen (1955). (16 data points)

M	A	σ_A	B	σ_B	R
Na	1.59	0.11	1.273	0.071	0.979
Ca	0.000 281	0.000 019	1.259	0.059	0.985
Mg	0.000 703	0.000 018	1.181	0.059	0.983

11.5 CATION ACTIVITIES IN BOVINE RAW SKIM MILK. SEASONAL TRENDS

In this and in following sections some of the applications of the cation exchange resin method for determining cation activities are described.

As part of a larger study of seasonal variation of milk composition and properties involving a number of workers weekly or fortnightly analyses were carried out on sample of factory supply bulk raw milk collected from the Linton area near Palmerston North, New Zealand, during the 1968/1969 dairying season. Data obtained for the milk is given graphically in Figures 11.4 and 11.5. It is important to note that the graphs consist of discrete points, each point corresponding to a single bulk milk sample. The points have been joined by straight lines to aid in interpretation of the graphs. A continuous variation of properties between points is not implied. In some cases where data is lacking points present in one graph have been omitted from other graphs. Such omissions may in some cases give a false impression of a difference in trend.

The following points are noted:

1. The volume of milk produced varied through the season. Peak production was reached in early November with a rapid rise during September. Production declined after November, falling to almost zero in May, June and July. Production began to rise again during August.
2. Comparison of sodium concentration, C_{Na}, and A_{Na}/γ_K provides a useful check of the cation exchange resin

Figure 11.4 Seasonal Variation of Sodium and Potassium Concentrations and pH in Bulk Raw Skim Milk from Linton Area, 1968/69.

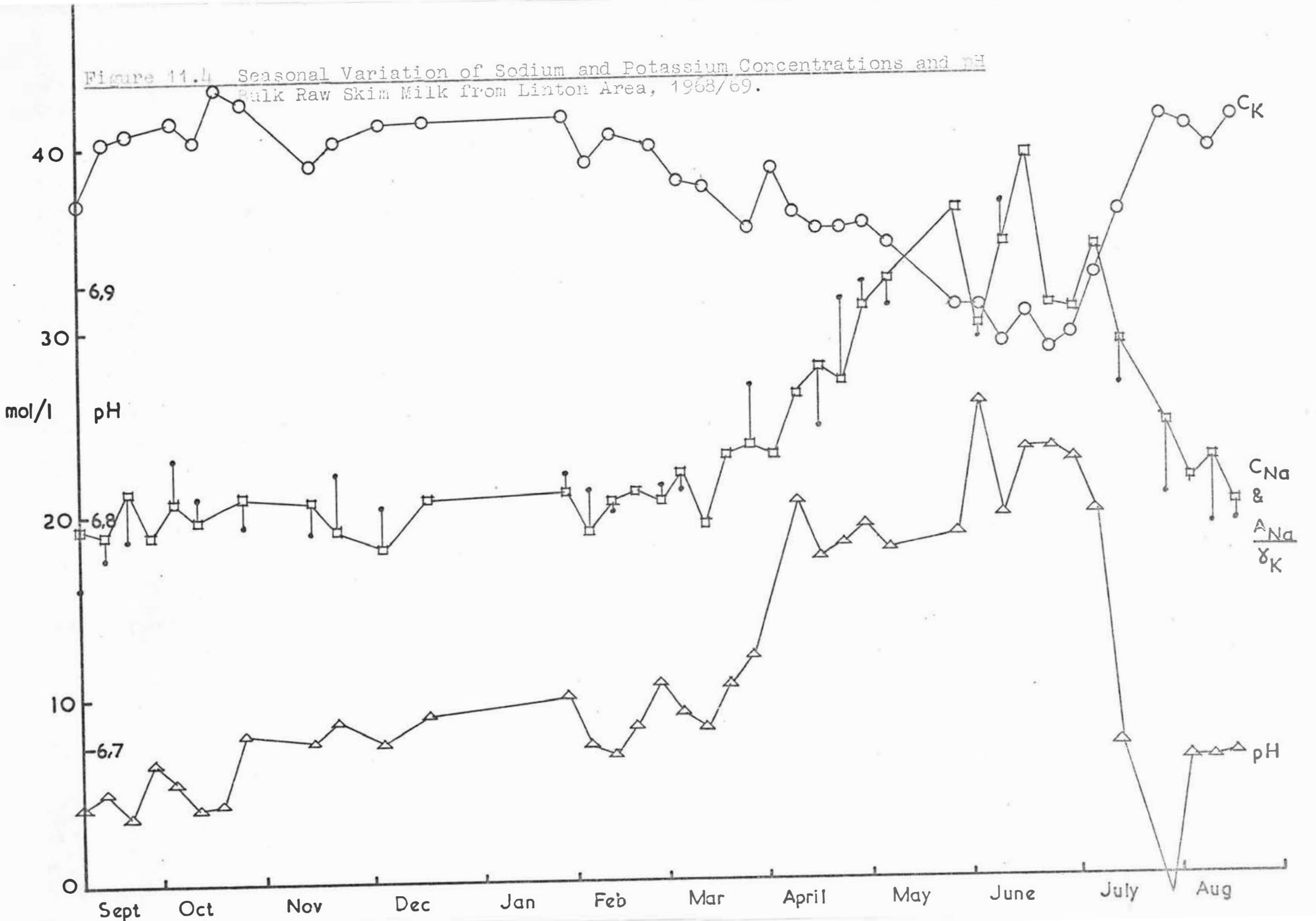
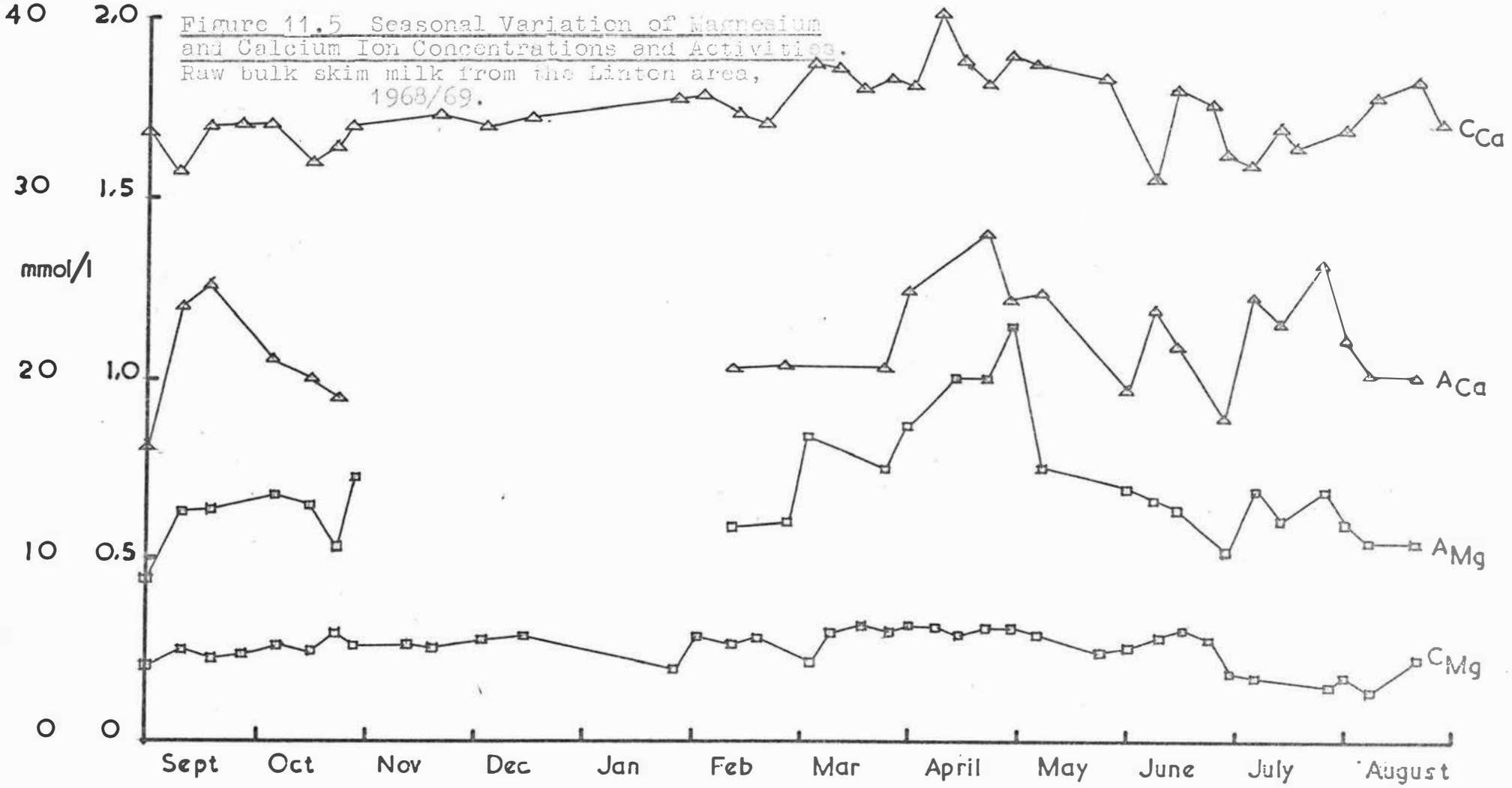


Figure 11.5 Seasonal Variation of Magnesium and Calcium Ion Concentrations and Activities. Raw bulk skim milk from the Linton area, 1968/69.



method of determining cation activities. Ideally the two parameters should follow each other closely as $C_{Na} = A_{Na} / \gamma_{Na}$ and γ_{Na} is expected to be very nearly equal to γ_K. The standard deviation between C_{Na} and A_{Na} / γ_K was found to be about 2.5 mmol/l.

3. The sodium and potassium concentrations show the well known 'end of season' trends (Dawes, 1970). Towards the end of the season the fall in potassium concentration is matched by an approximately equal rise in sodium concentration. The changes in sodium and potassium concentration are sufficiently large for the cation exchange resin method of determining cation activities to become impractical if the resin is calibrated for fixed sodium and potassium concentrations only (as was done by Christianson et al., 1954). Many such calibrations of the resin would be required to match the range of sodium and potassium concentrations. The more general method of calibration used in this work avoids the difficulty.

4. Up to the end of February the concentrations and activities of all ions are approximately constant or show only slight trends.

5. From March until July there are marked changes as the electrolyte composition of the milk tends to become more like that of bovine blood. Magnesium and calcium ion activities, however, show little change.

6. During July and August electrolyte composition shows a rapid change back towards the 'normal' or early season (1968) levels.

7. The pH of the milk was measured at 25°C after the milk had been aged at this temperature for at least one

hour. pH appears to correlate with the sodium concentration.

Results obtained by Van Kreveld and Van Minnen and results calculated from their data are given in Table 11.6 together with typical results for milk from the Linton area and it can be seen that their results are very similar to those reported here.

11.6 THE VARIATION OF CATION ACTIVITIES IN MILK WITH TEMPERATURE

A sample of bulk raw skim milk was divided into four portions and each portion was stored at a different temperature for about four hours and the cation activities were then determined at the different temperatures using the cation exchange resin method. No attempt was made to determine whether the cation activities had attained steady values characteristic of an equilibrium condition. As the cation exchange resin selectivity is known to be only slightly affected by temperature (see Section 11.1) any differences in the resin compositions were assigned solely to differences in the cation activities in the milk. Calcium ion activity showed the greatest temperature effect, increasing from 0.9 mmol/l at 6°C to 1.4 mmol/l at 37°C. The corresponding change for magnesium ion activity was from 0.4 mmol/l to 0.5 mmol/l. The change in the activities would be less, but would still be significant if a correction had been applied for changes in the resin selectivity.

The variation of the calcium ion activity of milk with temperature has been studied by Rose and Tessier (1959) who prepared ultrafiltrates from hot milks (27-110°C) and then

Table 11.6 Cation Activities in Raw Bovine Milk

Note	Na		K		Ca		Mg	
	C	A	C	A	C	A	C	A
1	17.5	14	43	35	2.2	0.9	0.8	0.3
2	17.9	14.2	43	33.8	2.2	1.2	0.84	0.45
3	20	15.9	39	30.6	2.1	1.2	0.79	0.42

C concentration in the isoionic solution, mmol/l.

A activity in the milk, mmol/l.

Notes

1. Figures published by Van Kreveld and Van Minnen (1955). The activities were calculated from the concentrations in the isoionic solution using the following activity coefficients: γ_{Na}, $\gamma_K = 0.8$, γ_{Ca}, $\gamma_{Mg} = 0.4$.

2. Figures calculated using the method described in this work and the original data of Van Kreveld and Van Minnen. The concentrations in the isoionic solution were calculated from the activities using the following activity coefficients: $\gamma_{Na} = 0.793$, $\gamma_K = 0.785$, γ_{Ca}, $\gamma_{Mg} = 0.536$ (milk assumed to have an ionic strength of 0.08).

3. Typical figures from milk from the Linton area of New Zealand. The activity coefficients used were the same as in 2 above.

analysed the ultrafiltrates after they had cooled. Calcium ion activity was determined by the murexide method (see Section 7.1) and pH, total calcium, magnesium, sodium, potassium, phosphate and citrate were also determined. It was found that the calcium ion activity in the cooled ultrafiltrate decreased with increasing temperature of ultrafiltration. A little later, Davies and White (1960) prepared milk dialysates and ultrafiltrates at 20°C and at 4°C, the milk having been stored at these temperatures for some time before the separation. They also used the murexide method to determine calcium ion activity and they report a small reversible increase in calcium ion activity on cooling of the milk. The temperature of the dialysates and ultrafiltrates at the time of the calcium ion activity determination is not stated.

Both reports indicate decreasing calcium ion activity with increasing milk temperature contrary to the findings of the present work. The difference may be due to the difference in temperature at which the measurements of calcium ion activity were made. Rose and Tessier (1959) and probably Davies and White (1960) made their measurements at room temperature whereas the measurements of the present work were made over a range of temperatures. Only one bulk skim milk sample was studied in the present work and further studies are desirable to confirm the findings. Study of the variation of calcium ion activity in milk ultrafiltrates with temperature also appears desirable.

11.7 MILK pH AND CALCIUM ION ACTIVITY

The pH's of different portions of a sample of raw skim milk were adjusted by the addition of differing amounts of hydrochloric acid to give a range of pH's from 6.34 to 6.69. The cation activities were then determined by the cation exchange resin method and the following regressions were found for 14 datum points

$$A_{Ca}/\gamma_K^2 = 18.64 - 2.569\ pH \qquad R = -0.9775$$

$$A_{Mg}/\gamma_K^2 = 4.399 - 0.585\ pH \qquad R = -0.9859$$

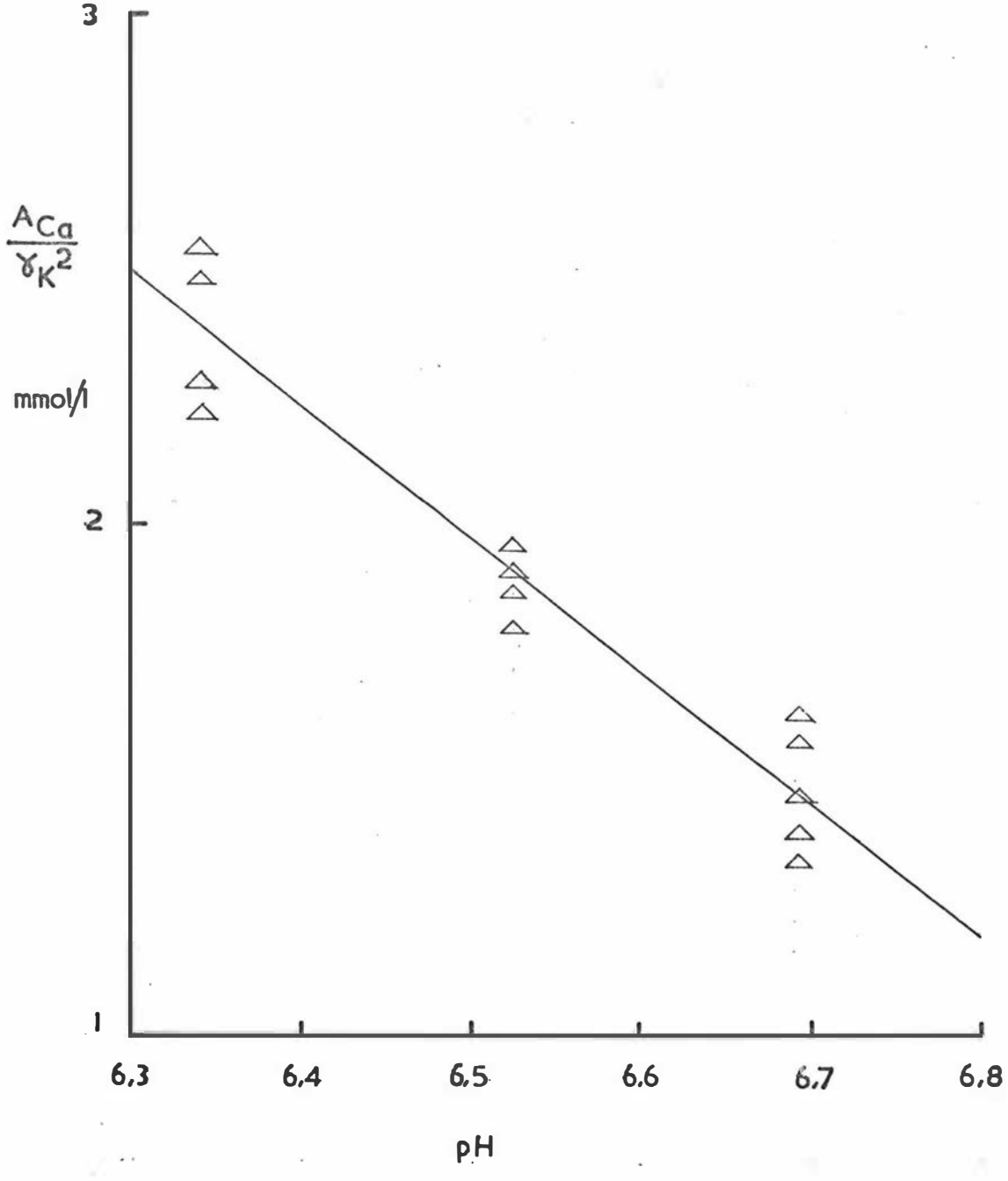
A plot of the data for A_{Ca}/γ_K^2 is given in Figure 11.6.

As the binding of calcium and magnesium by citrate, phosphate and protein is dependent on pH these regressions probably reflect a direct causal relationship. The effect of pH is sufficiently large for accidental and natural differences in milk pH's to be significant in determining the calcium ion activity of a particular milk sample. The dependence of calcium ion activity on milk pH was reported by Christianson et al., (1954).

11.8 RENNETING TIME AND CALCIUM ION ACTIVITY

It is widely accepted (Pyne, 1955) that calcium is important in the rennet coagulation of milk. A correlation between renneting time (the time taken for rennet to coagulate milk under certain standard conditions) and milk pH has been observed (McDowell et al., 1969) and in view of the dependence of calcium ion activity on milk pH described above a study of renneting time as a function of calcium ion activity was made.

Figure 11.6 Calcium Ion Activities in Milk with Adjusted pH's.



A sample of raw skim milk was divided into six portions. The calcium ion activities (and also pH's) of various of the portions was adjusted by the addition of either sodium oxalate, calcium chloride, hydrochloric acid or sodium hydroxide. The pH, calcium ion activities and renneting times were then determined. The results are given in Table 11.7 and a plot of the reciprocal of the renneting time versus calcium ion activity was approximately linear (Figure 11.7). The following regression equation was found for the renneting time, t, expressed in minutes:

$$1/t = 0.23 \pm 0.07 + (0.25 \pm 0.04)\, A_{Ca}/\gamma_K^2$$

$$R = 0.961$$

There is also a significant correlation between renneting time and pH in this case (see Figure 7.8). It seems probable that changes in renneting time brought about by adjustment of milk pH are due to the consequential changes in calcium ion activity rather than being a direct effect of pH on say rennin activity.

11.9 CATION ACTIVITIES IN GOAT, COW AND SHEEP MILKS

The cation exchange resin method was applied to the determination of cation activities in single samples of goat, cow and sheep milks which were to be used in a study of micelle structure (B.C. Richardson, work in progress). The raw skim milks were cooled to 16.6°C prior to the determination and the cation activities and pH's were determined at this temperature. Total cation concentrations were also determined and the results are given in Table 11.8.

Figure 11.7 Variation of the Rennet Coagulation Time (t) of Milk with Calcium Ion Activity

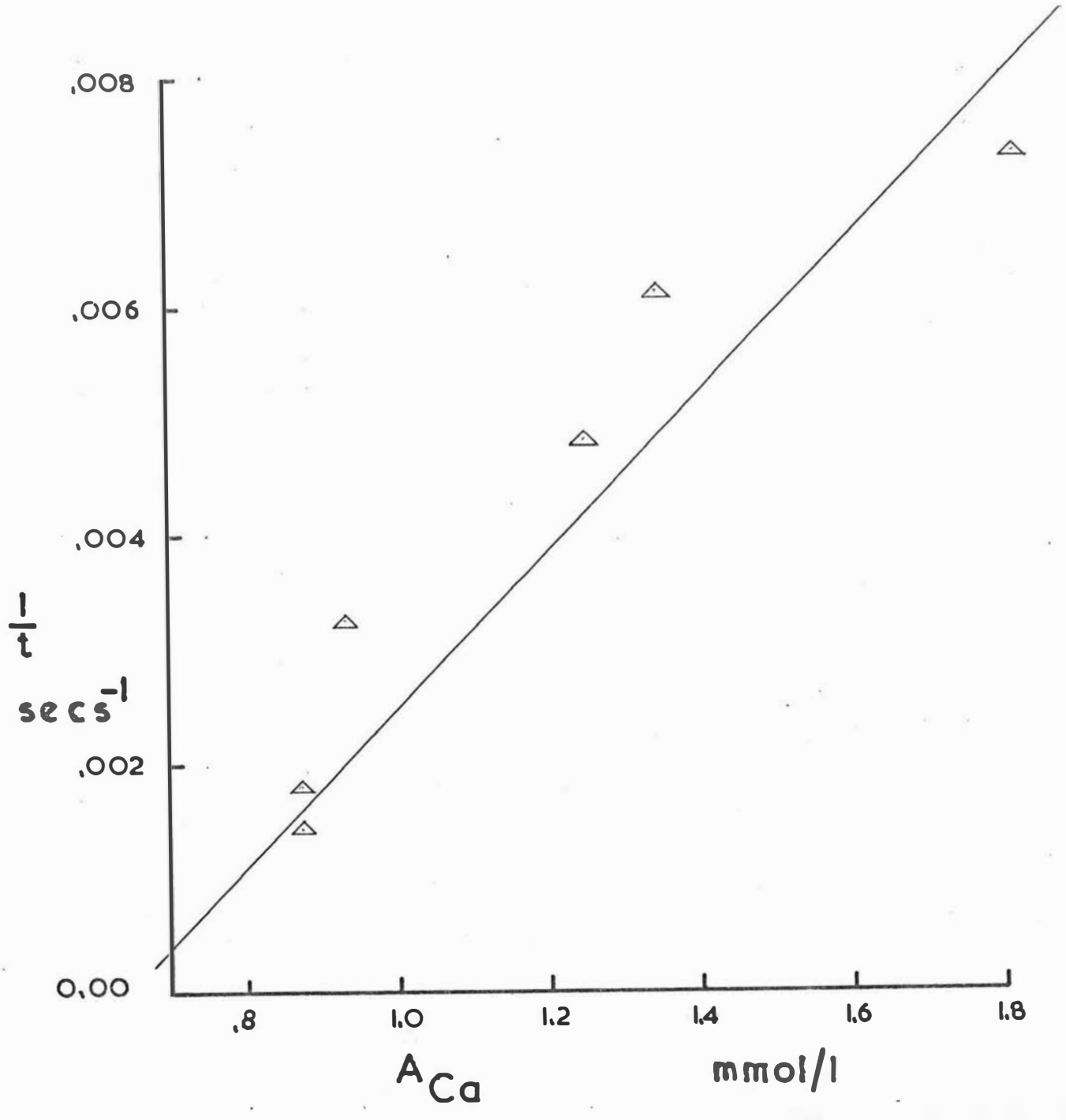


Figure 11.8 Variation of the Rennet Coagulation Time (t) of Milk with pH.

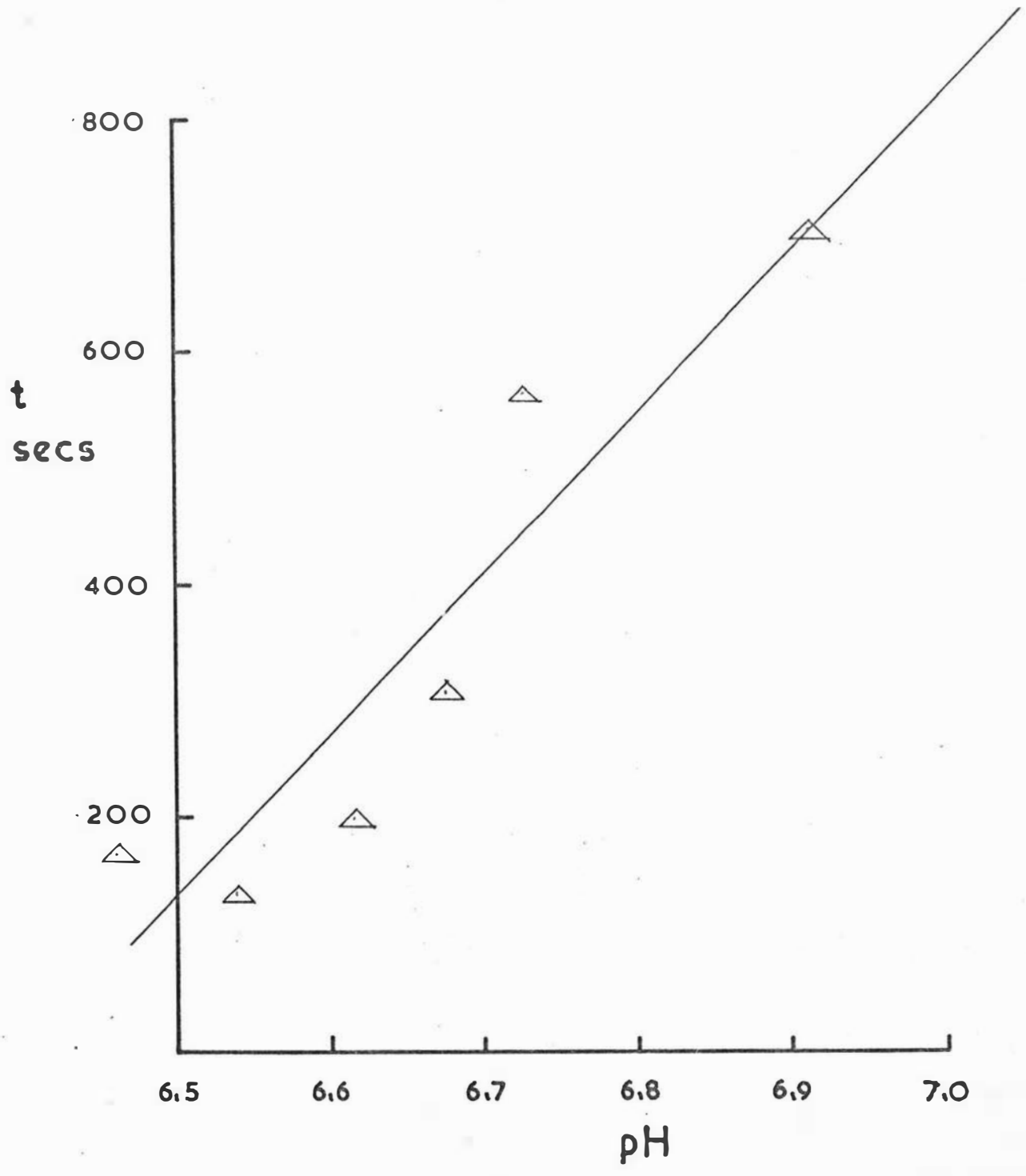


Table 11.7 Effects of Additives on Skim Milk

Additive	mmol/l	Renneting time seconds	pH	A_{Ca} mmol/l	A_{Mg} mmol/l
Nil		309	6.67	0.93	0.37
$Na_2C_2O_4$	2	567	6.72	0.88	0.35
$CaCl_2$	2	205	6.61	1.25	0.41
$CaCl_2$	5	134	6.53	1.81	0.47
HCl	4	161	6.47	1.35	0.43
NaOH	4	713	6.91	0.88	0.34

Table 11.8 Cation Concentrations and Activities in Various Milks mmol/l 16.6°C

Milk	pH	[Na]	A_{Na}/γ_K	[K]	[Mg]	A_{Mg}	[Ca]	A_{Ca}
Goat	6.90	13.6	18	48.1	3.76	0.41	27.0	1.00
Single Cow	6.64	16.7	26	46.6	4.03	0.33	27.6	1.04
Bulk Cow	6.69	18.4	21	41.0	4.27	0.33	31.7	0.93
Sheep	7.00	35.1	45	33.6	15.8	0.99	87.2	0.76

The differences between the cation activities in the various milks are proportionately smaller than the differences between the cation concentrations. With the exception of magnesium ion activity in sheep's milk which is markedly greater than in the other milks studied, there is little difference between the respective cation activities of the milks. Cation activities in non-bovine milks do not appear to have been previously studied.

CHAPTER 12.

DISCUSSION

12.1 FUNCTIONAL RELATIONSHIPS AND REGRESSIONS BETWEEN EQUILIBRIUM SOLUTION CATION ACTIVITIES AND RESIN COMPOSITION

If a small quantity of cation exchange resin is equilibrated with a large volume of solution containing cations then there is a causal relationship between the activities of the cations in the solution and the resin composition. For a resin with ideal properties this relationship would have the same simple form as equation (8.3).

$$\frac{A_A^{Z_B}}{A_B^{Z_A}} = K. \frac{X_A^{Z_B}}{X_B^{Z_A}}$$

where X_A is the mole fraction of cation A having change Z_A, in the resin. Real resins are expected to behave in a more complex manner as it is known that ion exchange resin selectivity coefficients are dependent on resin composition (Reichenberg, 1966)

$$\frac{A_A^{Z_B}}{A_B^{Z_A}} = \text{function}\left(X_A, \text{----}\right)$$

Ideally any functional relationship can be approximated over a specific interval by a polynomial of the independent variables having sufficient degree. The Taylor's expansion provides a convenient method of finding the

coefficients of the polynomial. This is the basis of the method used by Van Kreveld and Van Minnen (1955) who use a first degree polynomial. In general the greater the degree of the polynomial and the greater the number of terms included in the polynomial the better is the approximation to the original function.

Initially the relationships between equilibration solution cation activities and resin composition were approximated by polynomials in three variables and of degree two, found by multiple linear regression. The intention was to reject all terms the analysis showed to be of low significance and to find a new regression not containing these terms. However, it was found that all terms were of low significance and that the polynomials were "ill conditioned". That is, some of the terms of the polynomial were very large compared with the value of the polynomial so that a small change in the value of one dependent variable caused a very large change in the value of the polynomial.

Later, much simpler two parameter regression equations containing the appropriate mole fraction quotient as the only resin composition variable were found to be satisfactory. The behaviour of the resin in a solution of the four cations is adequately and conveniently described using a total of six parameters such as given in Table 11.4. In contrast Van Kreveld and Van Minnen's calibration procedure requires 12 parameters yet does not yield results significantly different from those given by the six parameter procedure described.

The empirical resin calibration equations of this work (Tables 11.4 and 11.5) are equivalent to the widely observed empirical relationship governing the ion exchange equilibrium constants of so-called "n type" ion exchanges (Hogfeldt 1955, Karreman and Eisenman, 1962). The non-ideality of the exchangers which is indicated by the departure of the "B" (or "n") parameter from unity can be explained in a simple qualitative manner.

Consider a cation exchange resin in equilibrium with a solution containing monovalent potassium ions and divalent calcium ions. The resin will contain both potassium and calcium ions. As the calcium-potassium activity quotient for the solution is increased the calcium-potassium mole fraction quotient for the resin will also increase. The increase will be less than proportionate for the following reason.

It is probable that the ion exchange sites of the resin are not all equivalent (Reichenberg, 1966). The calcium ions will tend to occupy those pairs of exchange sites giving the most favourable energy change. As more and more pairs of exchange sites are occupied by calcium ions the energy changes will become less and less favourable. The greatest departures from ideality will be at high mole fractions of calcium. This prediction is in accord with the empirical relationships. Arguments similar to those above would predict a departure from ideality for monovalent-monovalent ion exchange only when the two ions are markedly dissimilar in properties.

12.2 LIMITATIONS OF THE CATION EXCHANGE RESIN METHOD OF DETERMINING CATION ACTIVITIES

The cation exchange resin method of determining cation activities is, in principle, an extremely general method suitable for the study of fluids of diverse nature, containing two or more cations. (An analogous anion exchange resin method of determining anion activities probably could be developed). The method is, however, subject to a number of limitations some of which are now discussed.

The 'selectivity' of ion exchange resins is known to be dependent on the composition of the resin (Reichenberg, 1966). Satisfactory regressions between activity quotients in solution and mole fraction quotients in the resin have been found for limited ranges of composition without the need for consideration of other resin composition factors. Such simple regression relationships cannot be expected to be adequate for larger ranges of composition. Consequently the regressions should only be used in the range of compositions for which they were derived and extrapolation to other ranges of composition should be avoided.

The theory of the cation exchange resin method has been given for an ideal resin in Chapter 8. We now consider departures from ideality. The ideal resin membrane is impervious to hydrogen ions. This is a requirement that the resin should not exchange hydrogen ions. Sulphonic resins based on polymerisation of styrene probably meet this requirement at all except very low pH's. (This restriction on the permeability of the resin to hydrogen ions is not necessary in the most general case

as hydrogen ions need not be distinguished from the general cation. In practice, Van Kreveld and Van Minnen (1955) found the hydrogen ion exchanging weakly acid ion exchange resin Amberlite IRC-50 to be unsatisfactory. Weakly acid resins suffer a loss of cations during washing with water because of hydrolysis, are slow to reach equilibrium and have a very great affinity for hydrogen ions (Samuelson, 1963)).

A second requirement was that the ideal resin membrane be totally impervious to anions. Sulphonic polystyrene resins probably conform with this requirement except when the resin is equilibrated with solutions of high ionic strength (Samuelson, 1963). Such solutions should be avoided as should strongly acidic solutions.

The requirement in the hypothetical experiment that the resin be impervious to solvent was necessary to prevent the transport of water through the membrane in a process of water activity equalization. Such transport of water is not possible in the practical version of the experiment. However some changes in the swelling of the resin may occur when it is equilibrated with different solutions. It is necessary that such changes should not significantly affect the resin selectivity. This condition can probably be met by the choice of a resin with a suitable degree of cross linking and by the avoidance of solutions with greatly differing solvent activities.

The same solvent must be used for all calibrating and test solutions as the standard states have been taken as identical in all solutions.

Measurement of cation activities in very dilute solutions may be difficult because of the requirement that solutions should not be significantly altered by equilibration with resin. Careful "conditioning" of the resin and the use of a minimum of resin may overcome the problem provided a sufficient volume of the solution is available. Solutions whichare comparatively concentrated or which are buffered with respect to one or more of the cations are particularly easy to handle and only small volumes of such solutions may be needed.

Viscous fluids such as condensed milk present special problems. Such fluids are often relatively concentrated and because of their viscosity it is difficult to completely free the resin of entrapped fluid. A small quantity of entrapped fluid may result in the resin composition determined being significantly different from the true composition. Washing of the resin with water causes dilution of the entrapped fluid and may cause a change in the activity quotients of the fluid and a shift in resin composition towards a new equilibrium. Centrifugation or rapid draining and washing on a filter pump may be used to separate resin and fluid thereby minimising such errors. A further possible difficulty with viscous fluids is slow equilibration with the ion exchange resin because of slow transport of ions through the viscous medium.

Special problems may occur with inherently unstable fluids such as blood as contact with the resin may be all that is necessary to induce protein precipitation. Addition of stabilizers (such as citrate to blood) cannot be used as such additives often act by modifying the cation

activities. Careful conditioning of the resin to closely match the cation activity quotients of the unstable fluid or pretreatment of the unstable fluid to remove unstable components (centrifugation, ultrafiltration, enzyme action) may be possible remedies.

In solutions of high ionic strength and in solutions lacking a suitable reference cation, calculation of single ion activities from the activity quotients may be difficult. Monovalent cations of the alkali metals, especially Na^+ and K^+ are the preferred reference ions because of the known weakness of complexes of these ions in comparison with most other cations. There is also much information on the activity coefficients of these ions in solution.

Although no test has been made it is anticipated that the cation exchange resin method may not be suited to the study of solutions containing such cations as Al^{+++}, Fe^{+++} because of the great affinity of the resin for such ions. The activity of the ions in solution would have to be low to avoid almost complete occupancy of all exchange sites. Large departures from ideality and difficulty in achieving complete elution could be expected.

12.3 PRECISION AND ACCURACY OF THE CATION EXCHANGE RESIN METHOD FOR DETERMINING CATION ACTIVITIES

An indication of the precision of the method for determining cation activities is given by the standard deviations of the regression parameters given in Table 11.4. If the standard deviations of the regression parameters, σ_A, σ_B, are independent then it can be shown from the theory of propagation of error (Deming, 1943) that

$$\left(\frac{\sigma_y}{y}\right)^2 = \left(\frac{\sigma_A}{A}\right)^2 + \left(\sigma_B \log_e x\right)^2$$

where y is the cation activity quotient for the solution and x is the cation mole fraction quotient for the resin. If values for the parameters are taken from Table 11.4 and are substituted into this equation together with typical values for x the following results are obtained:

y	x	σ_y/y
A_{Na}/A_K	0.2	0.16
A_{Mg}/A_K^2	0.3	0.069
A_{Ca}/A_K^2	2	0.040

These results for the precision are comparable with the results obtained from six independent determinations of activity quotients for a single milk sample;

y	σ_y/y
A_{Na}/A_K	0.086
A_{Mg}/A_K^2	0.054
A_{Ca}/A_K^2	0.031

Figures of this order were common. The greater spread of sodium values is probably due to contamination of the dilute (1-2 ppm) solutions used in the flame photometric determinations and to dust which contained sodium being carried into the flame. Such dust particles caused small orange-yellow flashes which were easily visible to the eye.

It is difficult to assess the accuracy of the method. Comparison of results from ion exchange resin determinations

with other methods is complicated by the different standardization procedures used by different workers. Often these standardization procedures are not given or are only briefly stated so that it is not possible to recompute the results to a consistent standard. A further problem concerning the comparison of results is the diversity of the temperature histories of the milks studied. It is also clear that the pH of the milk studied is important and that bacterial deterioration and other processes which affect milk pH also affect the cation activities.

PART III

PREDICTION OF CATION ACTIVITIES IN WHEY AND SYNTHETIC WHEY

CHAPTER 13.

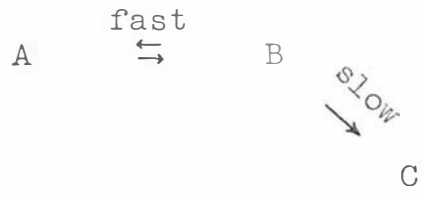
THEORETICAL PREDICTION AND EXPERIMENTAL DETERMINATION OF CATION ACTIVITIES IN SYNTHETIC WHEY

Given a knowledge of the stoichiometric composition of a solution and of the relevant equilibrium constants it is, in principle, possible to calculate concentrations and activities of all of the various species present and the way in which they vary with, say pH. In the case of skim milk which is composed of two phases, the situation is more complex and calculations of the above type may be possible only if the two phases are in equilibrium. The question of whether the micelle and serum phases of milk are in equilibrium is also important in understanding the structure of the micelle (McKenzie, 1967).

13.1 SKIM MILK AS AN EQUILIBRIUM SYSTEM (McMeekin and Groves, 1964)

Systems which are stable in that they show no apparent change with time are not necessarily at equilibrium. It may be that the rate of change is very small and not observable during the period of observation. Apparently stable systems can be described as either thermodynamically stable or kinetically stable depending on whether they are in thermodynamic equilibrium or are kinetically non-labile. Only equilibrium systems are amenable to classical thermodynamic treatment. A condition of equilibrium may exist between various components of a system even though

the system as a whole is undergoing slow change. Such a system can be represented by:



where A and B remain in equilibrium with each other even though the system as a whole is undergoing slow change. Such equilibria follow the normal laws of thermodynamics.

An essential characteristic of a dynamic equilibrium system is reversibility. Pertubations cause reversible change in such systems. A test for equilibrium can be made by testing for reversibility.

Casein

The level of casein in the serum phase of milk is known to be quite variable with large differences between individual cows (Rose, 1968). Changes in temperature and pH and the addition of calcium, phosphate and E.D.T.A. all produce apparently reversible changes in the serum casein level and therefore indicate that micelle and serum casein are in equilibrium. However, Rose (1968) found that if a sample of milk was diluted with essentially protein-free milk ultrafiltrate prepared from a different sample of the same milk, there was no detectable increase in the total amount of casein in the serum phase. Rose concluded therefore that the micelle and serum casein did not form an equilibrium system controlled by the solubility of the caseins. Great care is needed in applying the results obtained for synthetic milks to natural milk.

Heat Treatment and Temperature Effects

There have been a number of studies of the effect of heat treatment on milk and on the partition of milk salts between the serum and micelle phases.

These studies do not give direct information on the status of milk as regards equilibrium.

Consider a sample of milk the components of which may or may not be in equilibrium with each other. It is rapidly heated to pasteurization temperature (62.5°C). The milk will tend to undergo changes towards a condition of equilibrium characteristic of the pasteurization temperature and the changes are not necessarily of the same kind as would occur for a lesser increase in temperature. After being held at the pasteurization temperature for the required time the milk is rapidly cooled. Again there will be a tendency for the milk to undergo changes towards a condition of equilibrium characteristic of the new temperature. This equilibrium condition may not be the same as for the unheated milk as irreversible changes may have been induced by the high temperature. Any differences between the cooled pasteurized milk and the untreated milk are not necessarily indicative of the changes that would occur for a small increase in temperature. For example pasteurization causes a decrease in the calcium ion activity of milk (Van Kreveld and Van Minnen, 1955; Tessier and Rose, 1958; Demott, 1968) whereas a small increase in temperature may cause an increase in the calcium ion activity of raw milk (Section 11.6).

Jenness and Patton (1959) report a transfer of both calcium and phosphate to the colloidal state during heating

of milk, the transfer being slowly reversed upon holding the milk cold after the heating. They found considerable variations between milk samples as to the effect of heat and cool ageing. However, when Verma and Sommer (1958) and Verma (1965) studied the effect of pasteurization and cool ageing on the salt balance of various milk samples they found no clear-cut trends and very large variations between different milk samples.

In studies of the partition of milk salts between micelle and serum phases using ultrafiltration and dialysis, Davies and White (1960) found that there was a small reversible increase in total calcium, total phosphate and calcium ion activity in the serum phase when milk was cooled from 20°C and stored at 3°C for 24 hours and longer. They attributed the differences to a change in the partition of calcium phosphate which has a negative temperature coefficient of solubility.

Rose and Tessier (1959) found that on heating milk and returning it to room temperature the original concentrations and activities in the serum phase were not completely restored.

Partition of Added Salts

In a number of different studies (Tessier and Rose, 1958; Rose and Tessier, 1959; Verma, 1965; Demott, 1968) it has been found that when HCl, NaOH, on salts such as $CaCl_2$, Na_2HPO_4, Na_2 Oxalate or Na_3Cit are added to milk both micelle and serum phases undergo changes in composition. Thus addition of calcium to milk causes an increase in calcium ion activity and also of total calcium in the serum

and in the micelles. Addition of phosphate causes a decrease of calcium ion activity and also of total serum calcium whereas addition of citrate to skim milk causes a decrease in calcium ion activity but increases total calcium and total phosphate in the serum phase. The simultaneous changes in both serum and micelle phases is indicative of equilibrium.

Conclusions

From a consideration of the above it seems probable that the two phases of milk are in a condition of equilibrium as far as the milk salts are concerned. The position concerning the protein is less certain. In any event too little is known of the micelle-serum system and its reactions to allow calculations of concentrations and activities of the type discussed in the preamble to this Chapter.

The serum phase of milk is a rather simpler system than milk but still contains lactose and whey proteins. Synthetic solutions have been devised (Jenness and Koops, 1962) to simulate the behaviour of the milk salts in milk serum and these provide a convenient and suitably simple system for initial study. Milk ultrafiltrate, being free of protein, is another system which would be suitable for initial studies.

13.2 CALCULATION OF CATION ACTIVITIES IN SYNTHETIC WHEY

A computer program (Creamer, 1969) has been used to calculate concentrations and activities in synthetic whey solutions. Total concentrations of sodium, potassium, magnesium, calcium, citrate, phosphate, sulphate and lactate together with pH and the necessary acidity and stability constants are read in. The approximate concentrations of the various species are calculated and an estimate is made of ionic strength. The acidity and stability constants are then adjusted to the new ionic strength using the Davies equation for activity coefficients (Davies, 1938) and the calculations are repeated. Iteration is continued until satisfactory convergence is obtained and the activities and concentrations are then printed out.

13.3 EXPERIMENTAL DETERMINATION OF CATION ACTIVITIES IN SYNTHETIC WHEY

A synthetic protein-free and lactose-free whey was prepared according to the method of Jenness and Koops (1962). The pH's of different samples of whey were adjusted by addition of either NaOH or HCl solution (0.1M) and the cation activities were determined at 16.6^{o}C by the cation exchange resin method developed in Part II of this work.

The synthetic whey had the following composition - K 37.4 mM, Mg 2.8 mM, Ca 9.0 mM, phosphate 11.6 mM, citrate 9.6 mM, sulphate 1.0 mM, carbonate 2.2 mM. The sodium and chloride concentrations varied according to whether NaOH or HCl was used to adjust pH. Experimental results for the whey are given in Table 13.1.

Table 13.1 Experimental Results for Synthetic Whey

pH	Titre (a)	[Na] mM	A_{Na}/γ_k mM	A_{Mg} mM	A_{Ca} mM
6.98	-5.0	23.3	26	0.29	0.68
6.57	-1.8	20.1	26	0.35	0.81
6.33	0	18.3	22	0.39	0.99
6.00	2.0	18.3	21	0.39	1.00
5.40	5.0	18.3	21	0.50	1.19
4.72	10.0	18.3	20	0.70	1.78

Note

(a) Negative sign indicates base added. Units are mmoles of acid (HCl) (or base (NaOH)) added per liter of synthetic whey.

Experimental and calculated activities are compared in Figure 13.1. The agreement for magnesium is good but for the calcium ion activity the calculated values are consistently lower than the measured values. The experimental and calculated pH titration curves are compared in Figure 13.2. A small difference in the slopes of the two curves is apparent.

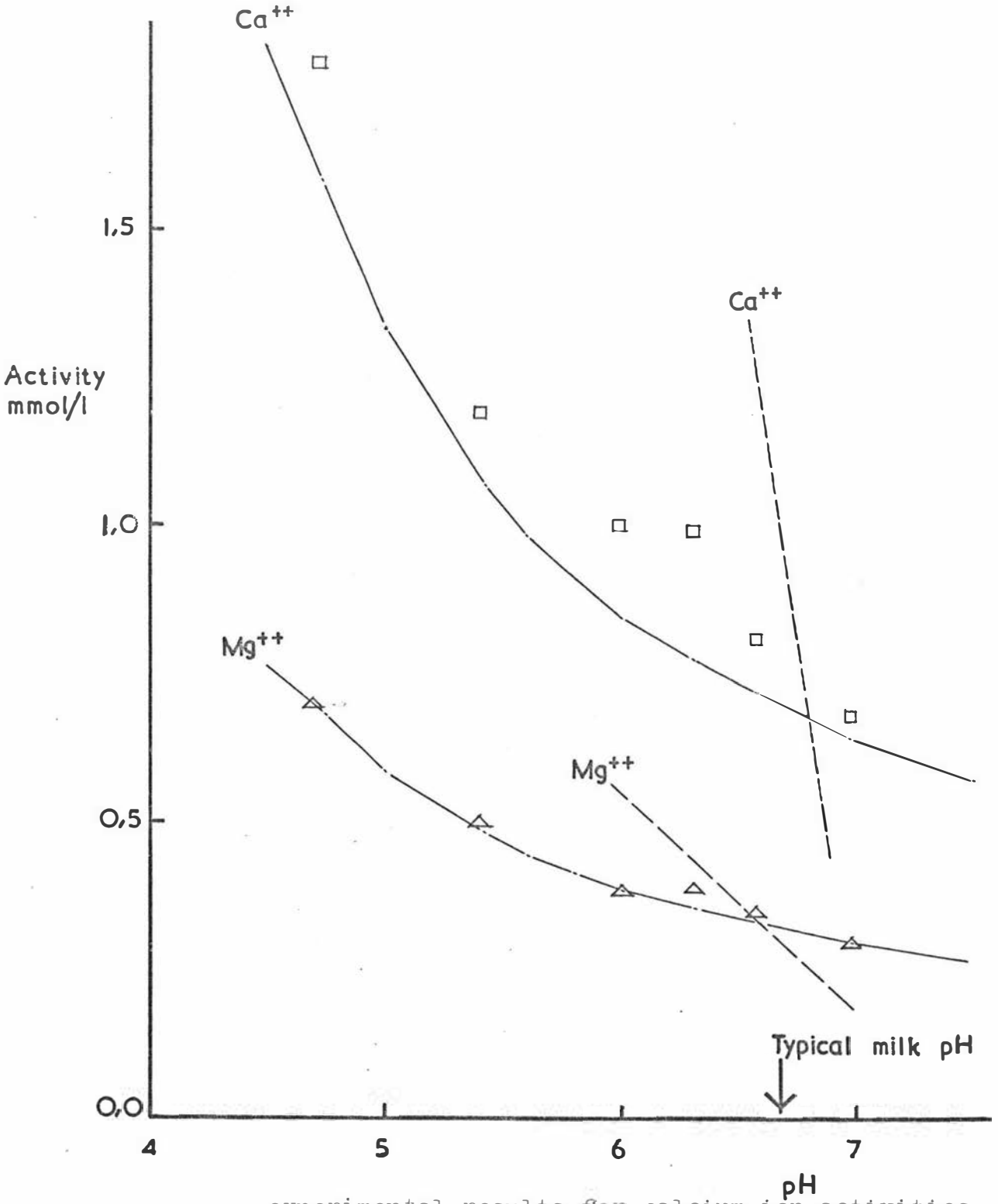
The broken lines in Figure 13.1 show the variation of calcium and magnesium ion activities with pH, found for skim milk (Section 11.7). The much greater pH dependence of the cation activities of the milk compared to those of the whey can be explained by reference to the reservoirs of magnesium and calcium in the milk micelles (Table 1.2). The capacity of the reservoirs is pH dependent. As milk pH is decreased (say by the addition of HCl) more and more magnesium and calcium is released into the whey. Approximately two-thirds of the total calcium of the milk and one-third of the total magnesium is contained in the micelle phase and the effect of pH change is greater for calcium than for magnesium.

The broken line in Figure 13.2 is the pH titration curve of a sample of skim milk. The large difference between the curve for synthetic whey and the curve for the milk reflects the importance of the micelle phase of milk in determining milk properties.

Solubility Products

Milk is either saturated or close to saturation with calcium phosphate and with calcium citrate (Boulet and Marier, 1961; Boulet and Marier, 1960; Tessier and Rose,

Figure 13.1 Comparison of Calculated and Experimental Data for Synthetic Whey



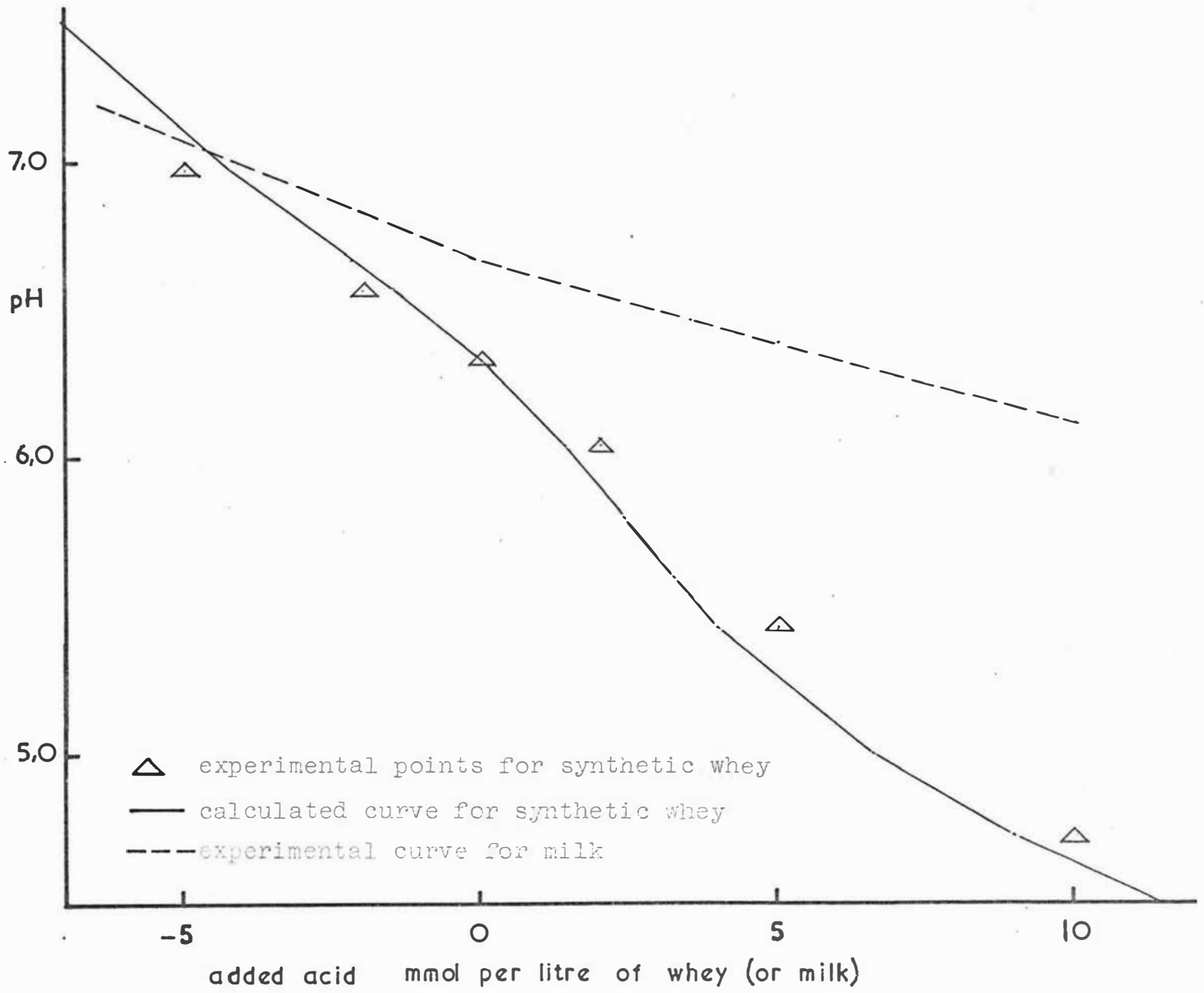
□ experimental results for calcium ion activities in synthetic whey.

△ experimental results for magnesium ion activities in synthetic whey.

—— calculated curve for synthetic whey.

– – – typical variation for skim milk.

Figure 13.2 pH Titration Curves for Synthetic Whey and Skim Milk



1958) but the composition and crystal form of the solid state of the calcium phosphate in milk is uncertain (McMeekin and Groves, 1964). (The crystal form and solubility of calcium phosphate is also of interest in studies of soil, bone and tooth).

Various ion activity products (denoted by Q) were calculated for the synthetic whey. Comparison of the negative logarithms of these values (denoted by pQ) with literature values for the negative logarithms of the corresponding solubility products (pK_S) allows the degree of saturation of the whey to be determined.

If $pQ > pK_S$ the whey is undersaturated.

$pQ = pK_S$ " " " just saturated.

$pQ < pK_S$ " " " supersaturated.

Values for pQ ($CaHPO_4$) (where $Q = A_{Ca}.A_{HPO_4}$) are given in Table 13.2 and they indicate that the synthetic whey is supersaturated with respect to $CaHPO_4 2H_2O$ at pH values greater than about 5.7. No precipitation was observed in the whey samples at the higher pH values during the course of the experiment. It is known that calcium phosphate forms stable supersaturated solutions (Chughtai et al., 1968) and this is probably the explanation for the lack of precipitation.

Values for pQ ($Ca_3\ Cit_2$), ($Q = A_{Ca}^{3}.\ A_{Cit}^{2}$) varied between 17.28 and 17.61 compared with pK_S (Ca_3Cit_2) = 17.63 (Boulet and Marier, 1960) indicating that the synthetic whey was close to the saturation point throughout the pH range studied. Values for pQ ($Ca_3(PO_4)_2$) ($Q = A_{Ca}^{3}.A_{PO_4}^{2}$) were greater than 30.4 throughout the pH range studied and

Table 13.2 Calculated Results for Synthetic Whey

pH	Ionic Strength	$[HCit^{=}]$	$[CaCit^{-}]$	$[MgCit^{-}]$	$[H_2PO_4^{-}]$	$[HPO_4^{=}]$	$[CaHPO_4]$	pQ
6.98	0.076	0.06	6.46	2.13	4.77	5.60	0.81	5.83
6.57	0.071	0.15	6.49	2.12	7.35	3.31	0.55	6.00
6.33	0.069	0.23	6.46	2.10	8.60	2.21	0.40	6.14
6.00	0.068	0.40	6.34	2.05	9.65	1.30	0.26	6.33
5.40	0.070	1.16	5.53	1.76	10.71	0.35	0.09	6.80
4.72	0.076	2.30	3.19	0.97	10.80	0.07	0.03	7.33

Notes. Concentrations are in mmol/l, only dominant species are shown. $Q = A_{ca} \cdot A_{HPO_4^{-}}$

c.f. pK_S ($CaHPO_4 2H_2O$) = 6.55 at 25°C (Moreno et al., 1966).

as $pK_S(Ca_3(PO_4)_2)$ has a value in the vicinity of 26 (Sillen and Martell, 1964) it is concluded that the whey was, at all times, less than saturated with $Ca_3(PO_4)_2$.

Table 13.2 gives calculated concentrations of some of the major species present in the synthetic whey. The whey is supersaturated with respect to $CaHPO_4 2H_2O$ (as has been shown above) and changes in solution composition would occur on precipitation of the excess salt.

The following conclusions can be drawn concerning the supersaturated synthetic whey in the pH range 4.7 - 7.0:

(1) Calcium citrate is the major calcium species and magnesium citrate is the major magnesium species.

(2) Phosphate binds little calcium or magnesium.

(3) Phosphate provides the major part of the buffering at the higher pH's, citrate becomes important at lower pH's.

(4) Ionic strength does not vary greatly.

Investigation of the composition of the saturated solution after precipitation of the supersaturated salts would be of interest.

CHAPTER 14.

SUMMARY AND GENERAL CONCLUSIONS

14.1 SUMMARY

The aim of the present work was to obtain basic fundamental knowledge of milk as an ionic or electrolyte system and also of simpler systems that simulate some of the properties of the milk system.

A pH titration method has been applied to the determination of acidity and stability constants and specially written computer programs have been used to calculate the constants from the titration data. A new set of micro acidity constants differing from those reported by other workers has been obtained for citric acid and the stability constants of magnesium and calcium citrate have also been redetermined. The method of calculating acidity constants from substituent effects has been refined to distinguish between macro and micro acidity constants and has been used with some success in the prediction of both macro and micro acidity constants. An analogous method for calculating stability constants shows some promise as a useful technique but its application is hampered by the lack of suitable data.

The thermodynamic basis of the cation exchange resin method has been described and a new method of resin calibration developed. The ion exchange resin method has been applied to studies of the seasonal variation of milk composition and to brief studies of the effects of milk pH adjustment, the factors affecting the renneting

time of milk and the determination of cation activities in non-bovine milks.

In a preliminary study of synthetic whey, comparisons have been made between cation activities determined experimentally and those calculated from a knowledge of composition and of the necessary acidity and stability constants.

14.2 SUGGESTIONS FOR FURTHER WORK

Protein-Cation Interactions

The synthetic whey system of Chapter 13 was chosen for initial study to avoid the complications of protein-cation interactions. The first refinement of the model is to allow for the addition of serum proteins. There have been a number of studies of the binding of calcium (Zittle et al., 1957), sodium (Barker and Saroff, 1965) and potassium (Basch and Timasheff, 1967) to β-lactoglobulin, the chief whey protein. Empirical expressions can be derived to describe such protein-cation interactions and it should be possible to predict the variation of cation activities with pH in such solutions by extending the present computer program. As an approximation, the minor whey proteins (α-lactalbumin, serum albumin, immunoglobulins, etc) could probably be considered as a single entity and their relevant properties described by a single set of empirical equations and constants.

The addition of casein micelles to the protein containing whey, as a further refinement of the model system, represents a marked complication, as little is

known of the micelle-serum equilibrium although there have been studies of the binding of cations by the caseins (Waugh et al., 1971; Dickson and Perkins, 1971). Experimental studies of the way in which micelle composition varies with changes in the cation activities (including pH) of the serum are probably necessary. The micelles may be considered to have some of the characteristics of a moderately cross-linked, low-capacity mixed weak acid/phosphoric acid cation exchange resin and it may be possible for empirical relationships to be derived based on a model of this sort. The presence of phosphate in the casein micelle is a complication.

Studies of Renneting Time

The results of this work (Section 11.8) coupled with previously published results indicate that calcium ion activity may have a major influence on the renneting time of a milk sample. Separation of the effect of pH on calcium ion activity from other pH effects requires that pH and calcium ion activity be varied independently. The addition of $CaCl_2$ or, conversely, of a calcium chelating compound such as citrate to milk followed by titration back to the original pH of the milk should allow regressions between renneting time and calcium ion activity to be found. The corresponding experiment of maintaining calcium ion activity constant but varying pH is much more difficult to perform as calcium ion activity is more difficult to monitor than is pH. However, if both pH and calcium ion activity are varied simultaneously multiple linear regressions could be found.

A second field of study would be to compare the relative effectiveness of magnesium and calcium ion activities in influencing renneting times. Addition of KMgCit to milk would cause a partial replacement of calcium by magnesium with a minimum of other changes. Treatments with cation exchange resin could also be used to effect changes in the ratio of calcium to magnesium ion activity.

The methods which have been developed in this work are at present being applied to the study of the renneting of milk.

Studies of Cheese

Cheddar cheese contains approximately 35% water and has a water activity in the range of 0.95-0.96 (Creamer, 1971). The aqueous phase of cheese is a concentrated electrolyte solution approximately one molar in NaCl but also containing many other ions. The processes which occur during the maturing of cheese, such as the formation of calcium lactate crystals (responsible for the defect of 'white spots') and the breakdown of protein are probably dependent on the conditions in the aqueous phase. Knowledge of the composition, ionic strength and cation activities of the aqueous phase of cheese would be helpful in understanding the chemistry of cheese.

Cheese water has been isolated by pressing mixtures of cheese and sand (Mabbitt, 1955) and can probably be considered as representative of the aqueous phase of cheese. The cation exchange resin method for determining cation activity quotients can probably be extended to solutions of

high ionic strength provided the range of ionic strengths is kept small. If this is so it would provide a possible method of determining cation activities in cheese.

APPENDIX I. VOLUME OF THE MICELLE PHASE OF MILK.

Milk micelles are envisaged as swollen porous sponge-like spheres (McKenzie, 1971) and the volume occupied by the micelle phase of milk will depend on the location chosen for the boundary with the serum phase. If the serum phase is considered to penetrate the porous micelle, micelle volume will be comparatively small. Some of the methods which give information on micelle volume are now briefly discussed.

1. VISCOSITY MEASUREMENTS

Einstein (1906) derived the following expression for a dilute dispersion in liquid of electrically neutral hard spherical particles:

$$\eta_S = \eta_O (1 + 2.5\varphi)$$

where η_S is the viscosity of the suspension

η_O is the viscosity of the pure liquid

φ is the fraction of the total volume occupied by the dispersed spheres.

Various extensions of Einstein's treatment have been proposed (see for example Kruyt, 1952) to allow for higher concentrations of particles and for electric charges on the particles but these treatments have had little quantitative success. Application of the Einstein equation to milk and whey can be considered as yielding the 'effective' volume fraction occupied by the micelle phase. If there

is little interaction between micelles and between the micelles and the surrounding serum then the effective volume will be a reasonable approximation to the true volume. Viscosity data from the literature is given in Figure AI.1. The most important point arising from this data is the indication that the volume of the micelle phase may be markedly dependent on temperature in the temperature range 0 - 30°C.

2. LACTOSE AND CHLORIDE DISTRIBUTION

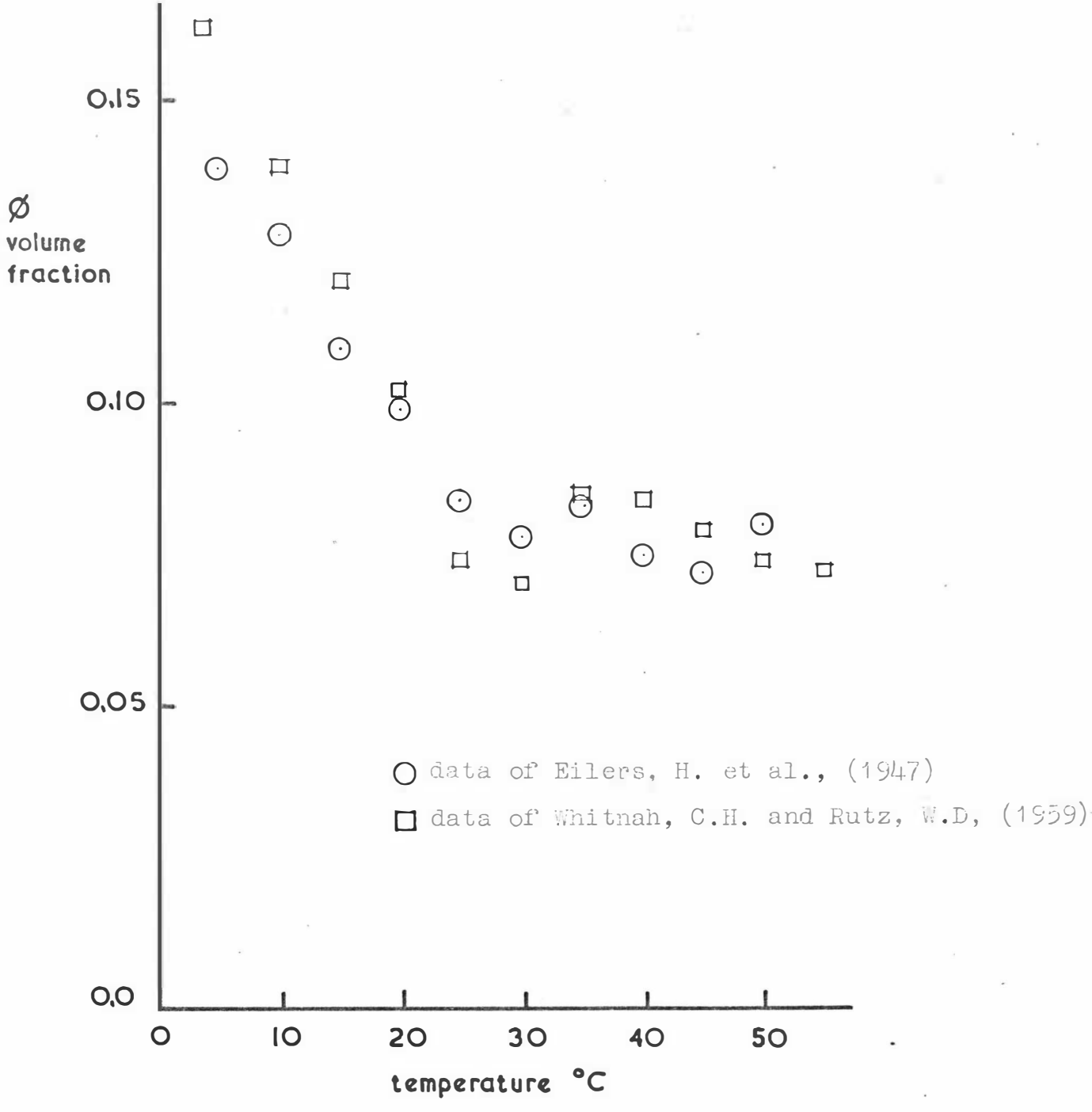
The lactose and chloride concentrations in milk sera prepared by dialysis, centrifugation, ultrafiltration or rennet action are higher than the concentrations in the original skim milk. The difference may be accounted for if part (or all) of the water of the micelle phase is protein bound and has reduced solvent powers so that lactose and chloride are excluded or partly excluded. A lower limit for the micelle phase volume fraction can be found from the equation:

$$\varphi = 1 - C_m/C_s$$

where C_m is the concentration of lactose (or chloride) in the skim milk and C_s is the concentration in the serum.

Recalculation of the data of Davies and White (1960) yields values for φ in the range 0.040-0.061 for a temperature of 20°C.

Figure A1.1 Volume Fraction of the Micelle Phase of Milk. Calculated from dynamic viscosity data for skim milk and rennet whey using the Einstein Equation.



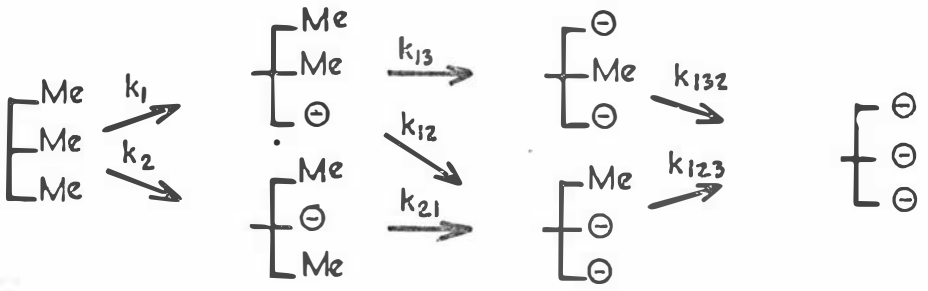
3. ULTRACENTRIFUGATION AND DIRECT MEASUREMENT ON THE SEDIMENT

This is apparently a simple method but results depend on both the gravitational field strength and the length of time it is applied. No sharp boundary is formed between the sediment and the serum when milk alone is centrifuged. Newstead (unpublished work) used an organic solvent layer of specific gravity 1.055 in order to obtain a cleaner separation. The organic layer is originally at the bottom of the centrifuge tube and the micelle phase sediments through it to form a pellet at the bottom of the tube. At room temperatures Newstead found pellet volume fractions in the vicinity of 0.14. It is clear from the viscosity measurements that accurate temperature control is necessary in all determinations of the volume of the micelle phase of milk.

APPENDIX II. THE KINETICS OF METHYL CITRATE SAPONIFICATION

The methyl esters of citric acid are important in the determination of the micro acidity constants of citric acid (see Section 2.3). Methods are available for the preparation of trimethyl citrate (Donaldson et al., 1934) and of symmetric dimethyl citrate (Schroeter and Schmitz, 1902) in pure crystalline form, but the remaining esters have not been isolated from solution (see Section 4.2). Figure AII.1 shows the complete saponification scheme for the methyl esters of citric acid.

Figure AII.1 Saponification Scheme for the Methyl Citrates
The k's denote rate constants.



Nuclear magnetic resonance experiments (Loewenstein and Roberts, 1960) (briefly described in Section 2.3) indicate that k_1 is large compared to k_2 and that k_{13} is large compared with k_{12}. In this study of the kinetics of methyl citrate saponification it has been assumed that k_2 and k_{12} are negligible compared with k_1 and k_{13}, that the saponification reactions are second order and consecutive, and that there are no significant parallel reactions. This is in accord with the experimental results for ethyl citrate saponification (Pinnow, 1918). Because

of the large differences in the rates of the consecutive saponification steps resolution of the rate constants is easy. Throughout much of the total reaction time only a single saponification step is dominant. Approximate values for the rate constants k_1, k_{13}, k_{21}, k_{132} and k_{123} have been obtained. Knowledge of these allows quantitative discussion of the composition of solutions of methyl citrates following saponification (Table 4.1).

Two experimental techniques were used to determine the various rate constants. In the first, which was suited to the study of faster reactions, a pH stat technique was used in which the (pseudo first order) reaction was followed by the continuous addition of KOH solution to the reaction mixture at the rate necessary to maintain constant pH. In the second technique, equal molar quantities of ester and KOH were mixed in solution and the reaction was followed by acid titration of aliquots of the reaction mixture. Results for dilute aqueous solutions, 0.1M in KCl, and for three temperatures are given in Table AII.1.

Table AII.1. Reaction Rate Constants for the Saponification of the Methyl Esters of Citric Acid

	Temperature °C	Rate Constants liter. mole^{-1} second^{-1}	Method
k_1	10	2.35, 2.8	A
	25	4.2, 4.0, 5.4	A
	40	12, 13	A
k_{13}	5	0.086, 0.077	B
	25	0.27, 0.45, 0.38	A
	40	0.86, 0.94	A
k_{132}	5	0.00022	B
	25	0.0012, 0.0013	B
	40	0.0035, 0.0031	B
k_{21}	5	0.033, 0.029	B
	25	0.24, 0.25	A
	40	0.84, 0.91, 0.77	A
k_{123}	5	0.0021	B
	25	0.013, 0.013	B
	40	0.052	B
	40	0.11, 0.15	A

Notes

(1) The rate constants are second order constants and are defined in Figure AII.1.

(2) The rate constants are for dilute aqueous solutions of the esters, 0.1 M in KCl.

(3) A = pH stat method.
B = Acid titration method.

APPENDIX III. SYMBOLS AND UNITS.

The International System of Symbols and Units has been used in the present work with a few exceptions. Concentrations of solutions have been given in terms of molarity (moles of solute per liter of solution) denoted by the symbol M and electrical conductance in terms of siemens, denoted by the symbol S. This last unit is equivalent to a reciprocal ohm, and is under consideration for inclusion as a S.I. unit. Molarity and the single letter symbol M have been retained for the convenience of a brief notation.

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