THE INTERACTION OF URONIC ACIDS AND METAL IONS IN AQUEOUS SOLUTION

by

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Thesis presented for the degree of

Doctor of Philosophy

UNIVERSITY OF EDINBURGH DECEMBER, 1971

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ACKNOWLEDGEMENTS

I am grateful to Dr. R.O. Gould for his advice and encouragement and, in particular, for his assistance in computing problems and in providing crystallographic data for inclusion in this thesis.

Thanks are due to Professor E.A.V. Ebsworth for the provision of laboratory and library facilities.

I would also like to thank the following:-

Dr. D.A. Rees - for advice and helpful discussion on the preparation of some of the ligands.

Dr. B.M. Lowe - for the use of potentiometric equipment and Mr. A.S. Charleston, B.Sc., for providing data for the determination of the stability constant of potassium galacturonate and for some assistance with the investigation into the rate of hydrolysis of lactone.

ABSTRACT

A study has been made of the interaction of some uronic acids and metal ions in aqueous solution. A variety of methods of study were employed for determining stability constants of the complexes formed by such interaction including the use of glass pH electrodes and other ion responsive electrodes, and the study of optical rotation. The complexes studied in greatest detail were those formed between calcium ions and D-glucuronic acid and D-galacturonic acid but a range of uni- and bivalent cation complexes were also studied.

The behaviour of the ligands in both acid and alkaline solution was investigated with particular reference to the effect of pH on mutarotation, lactonisation and hydrolysis of uronolactone. The acidic nature of the ligands was studied and values were determined for the dissociation constants of some uronic acids and uronosides, which confirmed that these ligands function as relatively strong acids.

The methods used in the stability constant determinations included the use of cation responsive electrodes. One type - the liquid membrane electrode recently developed to respond to bivalent cations - was studied in some depth and its behaviour with respect to its response to the test cation and to other uni- and bivalent cations examined. In addition, a suitable method for determining the stability constant of some uronate complexes with this type of electrode was devised.

Another more novel approach to the determination of stability constants was the use of polarimetry. Since all ligands in the present study exhibit optical rotation, it was possible to use this as a means of study and, by observing changes in rotation with increasing concentrations of metal cations present, to determine metal complex stability constants. Furthermore, in conjunction with proton magnetic resonance, it was possible to observe changes in the position of anomeric equilibrium by polarimetry and, from this change, values for the stability constants for the complexes of each anomer was calculated.

The thermodynamic stability constants for the complexes from the different methods and the relative accuracy of the methods were compared.

CHAPTER I

INTRODUCTION

1 . 1 Metal Ions in Biological Systems[3]

Metal ions play an essential role in many biological mechanisms. The metals which are most widespread in nature are sodium, potassium, calcium and magnesium, while certain transition-metals, although present in only trace amounts, also have vital functions. In this work, the interaction of calcium and similar ions with organic ions of a type found in both plant and animal tissue is investigated.

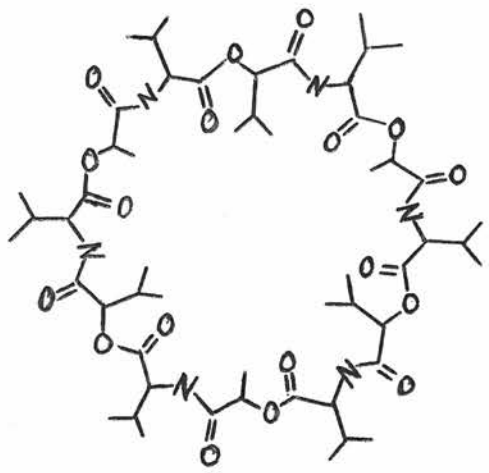
Calcium, which makes up 2% by weight of the human body and 0.7-2.5% of a typical land plant[2], has two primary functions in biological systems. It can be a trigger for the stimulation of the heart, the intestines and the muscles[1], and in both animals and plants it is an important structure former in bone and cell-wall tissue. Most of the functions of metal ions can be associated with their ability to form metal complexes (or at least ion pairs) with organic ligands. These ligands are often highly specific, and the different stabilities of complexes give rise, for example, to the passage of nerve waves through the body and to the release of hormones[3].

The concentrations of some cations and anions are normally much higher inside living cells than outside, while the reverse is true for other ions. Thus, the concentrations of potassium, magnesium and phosphate ions are normally maintained at a relatively high level inside the cell, while those of sodium, calcium and chloride are

maintained relatively low. This distinction is particularly marked for organisms living in sea water, where the concentrations of these cations in the extracellular environment are very different from those in the cell; a concentration gradient opposing the normal osmotic pressure is being maintained.

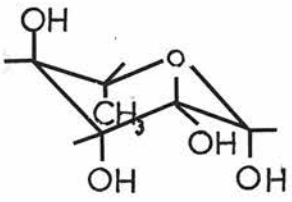
There are three main areas of interest in this apparent concentration imbalance: the selectivity of the membranes, the reason for the differences in internal and external concentrations, and the means of dissipation of the energy stores in the cells by virtue of the concentration gradients. In all of these aspects, the differences can be correlated with the different degrees of stability of the associated species formed between the various cations and organic compounds, primarily proteins and carbohydrates.

The selectivity of cell membranes, both external and internal, is based on the membrane composition. This variation produces environments such that cations are bound more or less strongly depending on their type. The alkali metals, for which specific covalent interactions are unimportant, provide a simple example. A membrane containing compounds such as valinomycin, (Figure 1.1) with many widely spaced hydroxyl groups and no charged centres, will transport potassium in preference to sodium, on account of the difficulty in

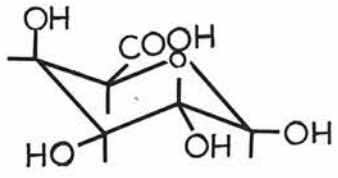


Valinomycin

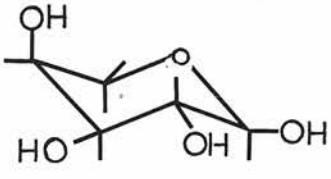
Fig. 1.1



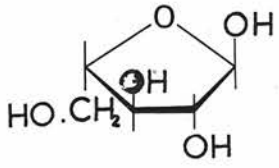
β-L-rhamnose



β-D-galacturonic acid



α-L-arabopyranose



α-L-arabofuranose

Fig. 1.2

achieving a sufficient degree of dehydration of the smaller sodium ions to allow significant interaction with the ligand.

Membranes which show a preference for magnesium ions over calcium are in general those with basic nitrogen-containing groups. Compounds of this type, as well as those containing additional carboxylate and phosphate functions, form more stable complexes with magnesium ions than with calcium and alkali ions. On the other hand, calcium is bound more strongly than magnesium by sites containing multidentate or strongly acidic anions. Compounds of this type include carboxylate, phosphate, and sulphonate groups, but exclude nitrogen bases.

The data summarised for a few model compounds in Table 1.1 illustrate these differences. Although work with any system which is not identical with that found in nature is naturally subject to misinterpretation, such results do indicate that significant correlation of structure with function can be made from studies of relatively simple systems.

Table 1 . 1 Some stability constants for Group IA and 2A Cations.

Ligand	Cation				Ref.
	K^+	Na^+	Mg^{2+}	Ca^{2+}	
Sulphate	0.9*	0.7	2.0	2.3	55
EDTA	0	1.7	8.9	10.7	55
'Football ligand'	5.1	3.6	2.0	4.1	4
Oxalate	-	-	3.4	2.0	55
Glycine	-	-	3.4	1.4	55

* All stabilities are expressed as the logarithm of the stability constant (log K) (see Section 1.3).

The selectivity which allows concentration gradients to be maintained across the cell membrane has also provided a means by which cells of different types could be formed. In higher organisms, on account of the permeability of the membrane, a concentration gradient establishes associated membrane potentials. Since these can be reversed by external factors which control the relative rates of flow of cations into and out of the cell, they have been used in the development of suitable cells for nerve, muscle and brain.

The extensive use of calcium ions in a structure--forming role may also be correlated with this internal/ external concentration imbalance. Calcium ions, normally maintained at higher concentration outside a

cell, are commonly associated with bones, shells and cell-wall structures. The types of compounds to which calcium is observed to bind include polysaccharides, and to a lesser extent proteins, but also important are inorganic salts, especially phosphates, of low solubility; the precipitation of calcium compounds in biological systems is common[3].

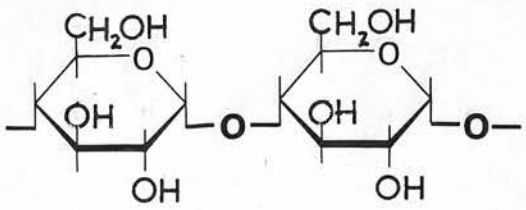
The function of metal ions in biological systems may thus be categorised in terms of their interactions with various ligands. These ligands, which include proteins and polysacchardies, are specific in function. Polysaccharides exist as structure forming and energy storing materials in plants and animals, and in this work, the interaction of some monomer units of many of them with certain metal ions is investigated.

1 . 2 Uronic Acids.

Occurrence and Structure

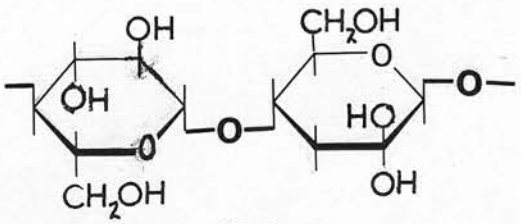
The function of polysaccharides as structure forming materials has been referred to above, and is of particular importance in the cell walls of plants. In fact, the presence of a cell wall outside the enclosing membrane is a characteristic which distinguishes plant cells from those of animals. Polysaccharides are large molecules which may be regarded as being built from units consisting of simple sugars (monosaccharides) and their derivatives by the loss of a water molecule and the formation of an ether linkage between two adjacent sugar units or residues. In nature a great variety of polysaccharides, called homopolysaccharides or heteropolysaccharides as the chains contain identical or different sugar residues respectively.

The most common structural polysaccharide in plants is cellulose, a polymeric chain of D-glucose units (see below). It is, however, not the first cell wall component to be laid down[4]. During the growth stage of higher plants, cell division results in a watery pectin layer. Pectic substances, which make up the primary cell wall, are found in most plants, and are particularly abundant in young tissue. The cellulose, laid down only when the cell ceases growing, is the main component of the secondary wall.



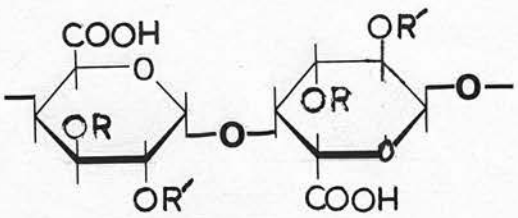
Starch

(α - 1,4 linked glucose units)



Cellulose

(β - 1,4 linked units)

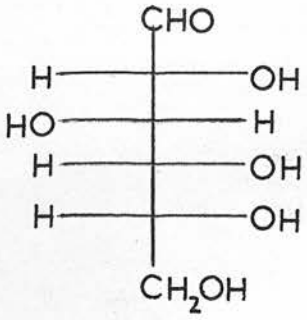


Pectic Acid

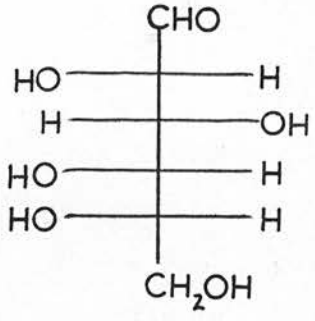
Fig. 1.3

The structure of pectic substances differs in several ways from that of cellulose. They are normally complex heteropolysaccharides, containing such sugar residues as arabinose and rhamnose (see Fig.1.2) but they all have a high proportion of galacturonic acid units. The structure of pectic acid, and idealised homopolysaccharides of galacturonic acid residues is compared with that of starch and cellulose in Fig.1.3.

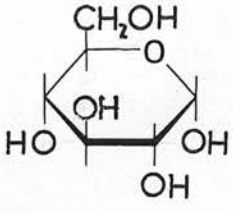
The representation of the structure of a sugar in a way which is both chemically sensible and easy to visualise is not simple, and various systems are in use. In Fig. 1.4 various ways of representing D-glucose and its corresponding uronic acid, D-glucuronic acid are illustrated. (Since uronic acids differ from the parent sugar only in the functional group on the terminal carbon atom, their stereochemistry is simply related to that of the parent sugar). The open chain form represents the structure of only a negligible fraction of sugar molecules in nature, and its adaptation to ring forms clearly does not show the spatial arrangement of the atoms well. It does, however, illustrate the conventional distinction between D- and L-enantiomorphs of sugars which are thus defined without reference to their actual optical rotation. There are two important ring forms, the five-membered furanose type and the six-membered pyranose, which may be considered as the rearrangement of the open chain form to give an internal hemiacetal. These are often represented by the flat rings

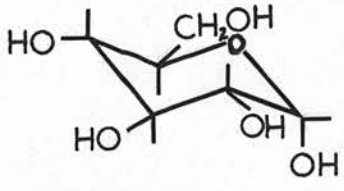


D-glucose

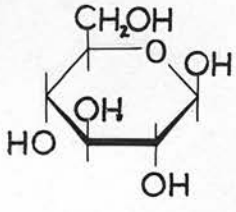


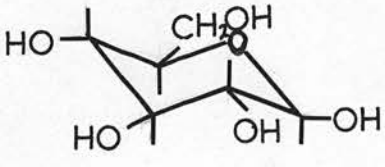
L-glucose



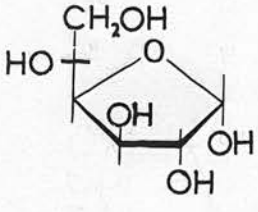


α-D-glucopyranose

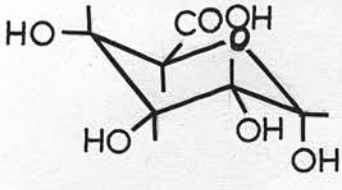




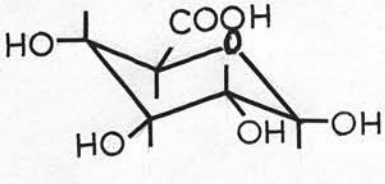
β-D-glucopyranose



α-D-glucofuranose



α-D-glucuronic acid



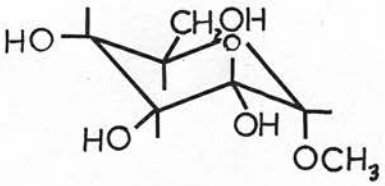
β-D-glucuronic acid

Fig. 1.4

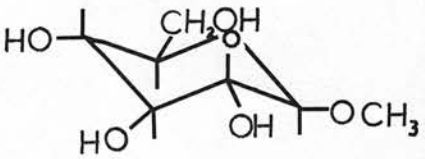
shown, but this is clearly a distortion of the more important pyranose ring. The "chair" structure, which is that normally found in crystal structures of sugars and inferred from n.m.r. studies in solution, is very similar to that of cyclohexane, and shows the distinction between axial and equatorial substituents. The numbering of the atoms is that conventionally used.

If the hydroxyl group at the carbon of the acetal linkage is unsubstituted, the acetal linkage is very labile, and the substance is a "reducing" sugar. The sugars at equilibrium in solution generally consist of various proportions of two isomeric forms called anomers. Conventional usage is defined in a rather complicated way, but, for the substances considered in this work, the α- and β-anomers are the forms with this hydroxyl group axial and equatorial respectively. This establishment of equilibrium is called mutarotation and is discussed below. In polysaccharides, this hydroxyl group is often involved in linkages (cf Fig. 1.3) and mutarotation cannot occur. Important monomeric model substances are thus the glycosides and uronosides, in which the anomer is fixed by substitution. Examples are shown in Fig. 1.5.

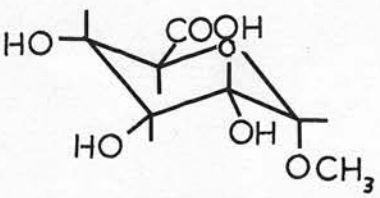
A further possibility resulting from the lability of the acetal linkage is the "flipping" of the ring, converting all axial substituents into equatorial and vice versa. The two possible configurations are called "Cl" and "1C". In the cases considered here,



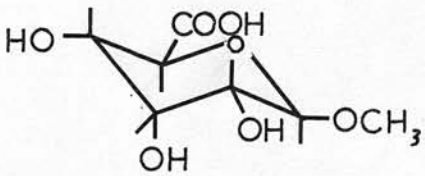
Methyl α-D-glucoside



Methyl β-D-glucoside



Methyl α-D-glucuronoside

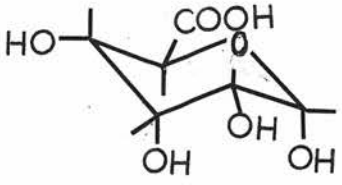


Methyl β-D-galacturonoside

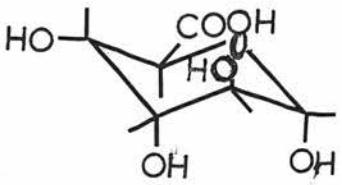
Fig. 1.5

the C1 form is the one with the carboxyl group equatorial and is the only form that need be considered.

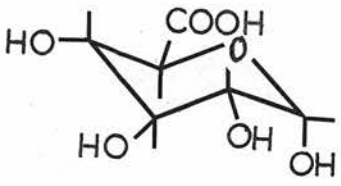
The α-anomers of all the D-hexalduronic acids are shown in Fig. 1.6. They may be regarded as geometrical isomers one of another. In particular, those forms which differ only at the carbon atom adjacent to the acetal carbon are called epimers of one another, as they undergo interconversion reactions relatively readily. The difference between sugars which has received the most study is that in optical rotation, resulting from the five asymmetric carbon atoms in the ring. As it serves both as a means of identification and of following reactions, it will be considered here.



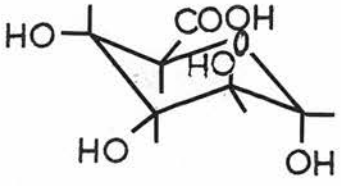
α-D-alluronic acid



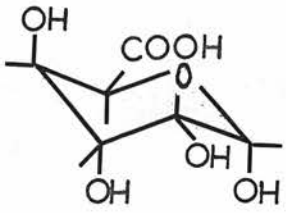
α-D-altruronic acid



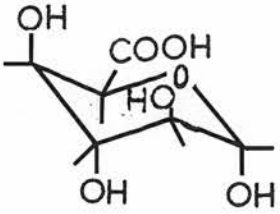
α-D-glucuronic acid



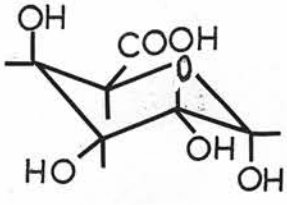
α-D-mannuronic acid



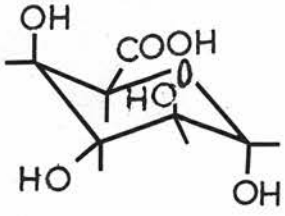
α-D-guluronic acid



α-D-iduronic acid



α-D-galacturonic acid



α-D-taluronic acid

Fig. 1.6

Optical Rotation[6].

When four different atoms or groups are attached to the same tetrahedral centre, in particular a saturated carbon atom, two distinct but equivalent structural arrangements exist. One of these isomeric structures rotates the plane of polarised light to the right, while the second isomer rotates it left to the same extent. Sugar molecules contain several such asymmetric centres, and are therefore optically active compounds.

As the asymmetry exists at the molecular level, solutions of sugars as well as crystalline solids exhibit optical activity, and the angle of rotation for a solution is conveniently measured by a polarimeter. This instrument consists basically of two Nicol prisms, the first acting as a polariser and the second as an analyser for light from a monochromatic source. The sample, enclosed in a tube, is placed between the prisms, and the rotation of the analyser necessary to correct for the effect of the solution is measured.

Optical activity is normally expressed in terms of the specific rotation, $[\alpha]$, which is characteristic of a given compound at a given temperature and wavelength of radiation, and is defined thus:

$$[\alpha] = \frac{100.\alpha}{l.c}$$

where α is the observed rotation (the polarimeter reading), l is the length of the path in decimeters, and c is the

concentration of the solution in g/100cm³.
The temperatures (°C) and wavelength (nm) are given as superscript and subscript, and the value of c and the solvent should also be given thus:

$$[\alpha]_{365}^{20} = +142° \text{ (c 0.3 in } H_2O)$$

Optical activity may be expressed more conveniently for comparisons of compounds of different molecular weight as the molecular rotation, $[M] = [\alpha].M/100$, where M is the molecular weight of the substance.

In this work, however, rotations are recorded as specific rotations, calculated in terms of the total concentration of ligand molecules as if they were in the free acid form, and refer to room temperature (ca. 20°C) unless otherwise stated.

The rotation of a compound is a complex function of the asymmetry of its electron density and of its absorption spectrum. In general, the optical activity increases markedly near an absorption band which is associated with that asymmetry. While it is not convenient to measure the rotation of the solution in a region of high absorption, the presence of " optically active absorption bands" around 300 nm causes the specific rotation for most sugars to be very much greater about 350 nm than it is in the visible region, in particular at the Sodium-D line (589 nm) used for most reported values.

Mutarotation.[7]

As has been mentioned, a characteristic of sugar molecules unsubstituted at O(1) is the equilibration of anomers in solution. As this reaction normally involves a significant change in the optical rotation of the solution, it is called mutarotation. This change in rotation for glucose was first reported by Dubrunfaut[8] in 1846, who also noted its temperature dependence. The term "mutarotation" was introduced by Lowry[9], and is often applied to a variety of isomerisations such as that of nitrocamphor in benzene solution[9].

Several mechanisms have been proposed to explain the change in rotation of solutions of reducing sugars[10,11,12]; the actual reaction was shown by solubility measurements[13] to be the reversible equilibrium between α- and β-forms of the sugar. Kinetic investigations of the mutarotation of various sugars show two distinct reaction paths. The first group, consisting of sugars having the gluco-, manno-, gulo-, or allo- configurations show a simple, first order mutarotation, which can be expressed in the form:

$$[\alpha]_t = Ae^{-k't} + C$$

where $[\alpha]_t$ is the specific rotation at a time t, C is the equilibrium rotation, A the difference between the initial and equilibrium rotations, and k' the "mutarotation constant", or the sum of the rate constants for the forward and back reactions.

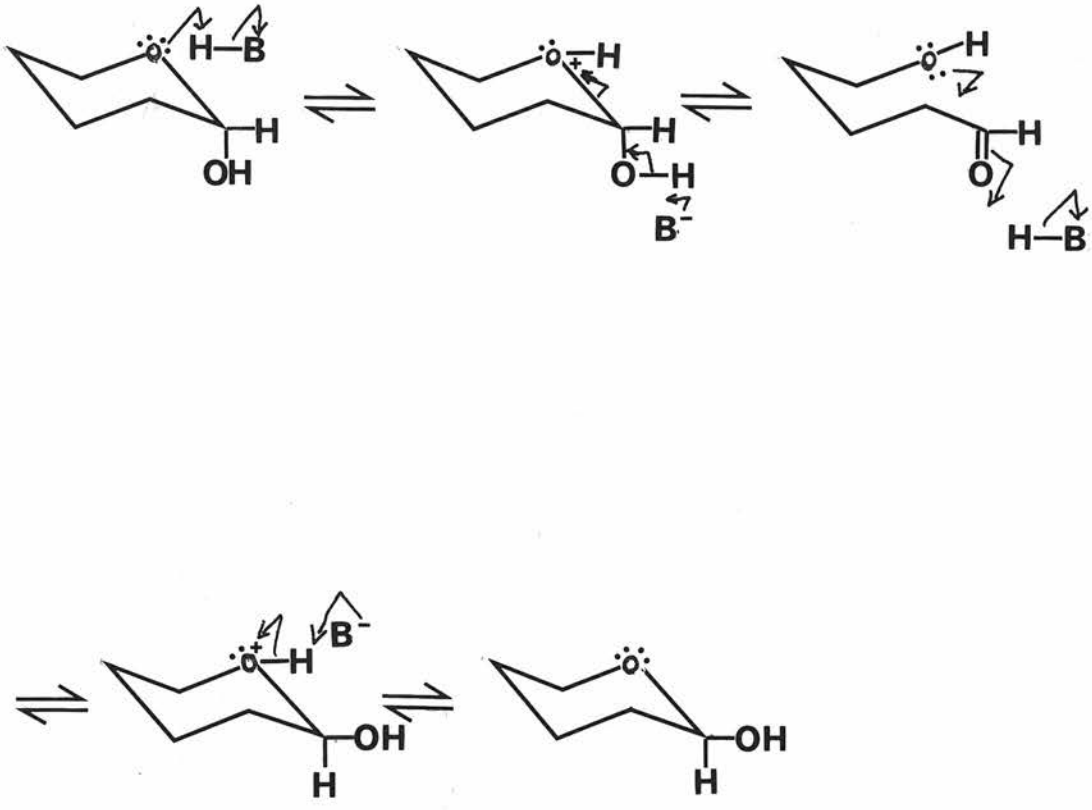
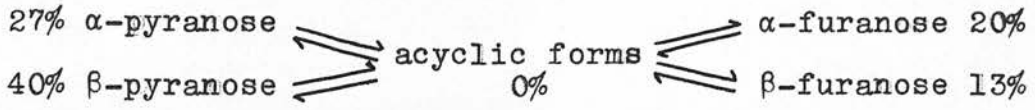


Fig. 1.7 Mutarotation - Acid catalysed

For this series of sugars, the mutarotation constant is constant throughout the time taken for equilibrium to be established, and is identical for the mutarotation of either anomer. From this course of reaction, and from other properties of the sugar solutions[14], the equilibrium solution is shown to contain a mixture of α- and β-pyranose forms with virtually no other species. The accepted mechanism[15] for the mutarotation of these sugars is shown in Fig. 1.7, and involves an open chain form as an intermediate.

It has, however, been found[16] that the value of the mutarotation constant for the α-anomer of D-galactose decreases as the reaction proceeds, while that of the β-anomer increases. The constants do, however, approach the same value in the latter stages of the reaction. This behaviour is typical of the other aldohexoses, those having the galacto-, talo-, altro- and ido- comfigurations.

Attempts have been made[17] to represent these experimental data on the basis of a 3-component equilibrium, but this is not satisfactory, and the equilibrium solution can be shown to contain small but significant amounts of furanose forms. The results for altrose may be represented schematically thus, showing the approximate percentage composition at room temperature[18]: (Figures for galactose are not available, but the proportion of furanose forms appears to be much lower).



An empirical relationship expressing the rate here[17] is:

$$[\alpha]_t = A.10^{-m_1 t} + B.10^{-m_2 t} + C$$

where $\underline{m_1}$ and $\underline{m_2}$ are constants, $\underline{C}$ is the final equilibrium rotation, and $\underline{A} + \underline{B}$ is the difference between the initial and equilibrium rotations. Complex mutarotations of this type in general have two distinct phases[19]. Normally the initial part involves a rapid mutarotation whereas the second part is a slow mutarotation. Separately $\underline{A}$ is the total change in rotation arising from the second slow reaction and $\underline{B}$ is the deviation between the actual initial rotation and that obtained by extrapolation of the slow mutarotation to zero time. In some cases (see *e.g.* Fig. 2.8) the complex mutarotation may even involve a change in the direction of the rotation change.

The position of the conformational equilibrium is controlled in the main by interactions between groups attached to the ring. In general, pyranose forms predominate most completely in those structures with the greatest number of equatorial substituents in this form. For example, in D-glucopyranose, the hydroxyl groups on C(2), C(3), C(4), and the hydroxylmethyl group on C(5) are all equatorial, while in the corresponding galacto-pyranose, the group C(4) is axial. On the other hand, D-galactofuranose is particularly stable, since it

permits all substituents around the ring to be trans to all others. Hence the differences in mutarotation between the two sugars may be correlated with the particularly high stability of pyranose forms in glucose, and the unusually high stability of the less favourable furanose rings in galactose.

Other factors which control the position of the conformational equilibrium are intramolecular hydrogen bonds, as are possible between O(1) and O(3) in the α-pyranose form of altrose and dipole-dipole interactions, such as the so-called Δ-2 effect[20]. This name is given to the unfavourable interaction between axial substituents on C(2) and an equatorial anomeric hydroxyl group. In such cases, equilibrium mixtures contain unusually high proportions of the α-anomer.

Dissociation

Like all carboxylic acids, uronic acids dissociate to a significant extent in aqueous solution giving a carboxylate anion and a proton:

$$HA + H_2O \rightleftharpoons A^- + H_3O^+ \quad \text{or simply} \quad HA \rightleftharpoons A^- + H^+$$

The degree to which this dissociation takes place is a measure of the strength of the acid, and is discussed further in sections 1.3 and 1.4. The degree of dissociation depends on the relative affinities of the anion and the solvent for the proton. The reluctance of an acid to lose a proton is lessened by the presence of electronegative groups in the molecule over which the resulting negative charge can be spread. The hydroxyl groups, which are the main substituents of sugar rings are such groups, with the result that uronic acids are significantly stronger than are unsubstituted carboxylic acids. The relationship of acid strength to conformation is further discussed in section 2.4.

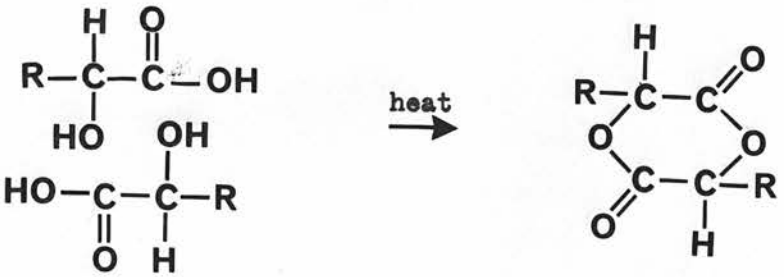
Lactonisation and Hydrolysis

In an aqueous solution of a carboxylic acid with hydroxyl substituents, the free acid can reach equilibrium with a lactone.

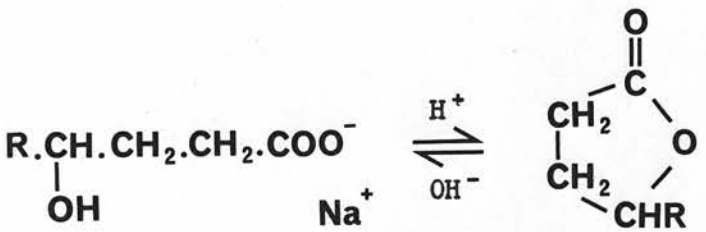
The type of lactone formed varies with the position of the hydroxyl group relative to the acid group. Dimeric species are formed when the hydroxyl group is α- to the carboxyl (see Fig. 1.8) but these will usually be unimportant in dilute solutions. Internal lactones can form with hydroxyl groups γ - to the carboxyl, and these are formed to a significant extent by many uronic acids. Glucuronic and mannuronic acids form stable lactones in the furanose form, and these are shown in Fig. 1.9. Because of the different configuration at C(4) in galacturonic acid, however, a similar lactone cannot form, since the carboxyl group is now on the opposite side of the ring from the hydroxyl substituent at C(3).

Lactones are not stable in alkaline solution, and hydrolyse to give salts of the corresponding uronic acids. There is little published data on the kinetics of this reaction, but the following data, given by Hirsch[21], for the hydrolysis of pure D-glucurone, gives a qualitative picture:

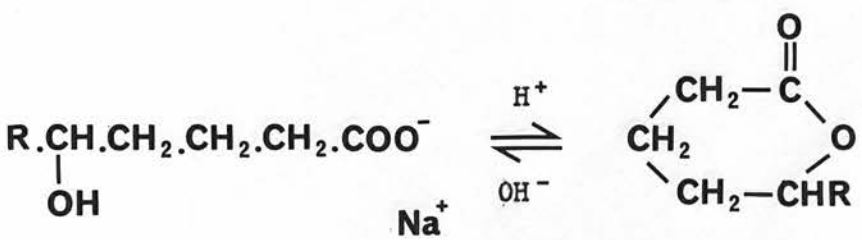
pH = 7 - 7.5	17% hydrolysis after $2\frac{1}{2}$ hours
pH = 7.4 - 7.8	51% hydrolysis after $2\frac{1}{2}$ hours
pH = 8 - 8.5	100% hydrolysis within $1\frac{1}{2}$ hours



α-hydroxy acid lactide

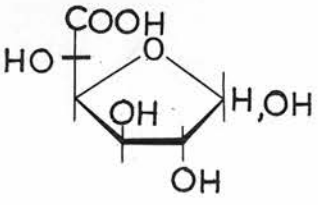


salt of γ-hydroxy acid γ-lactone

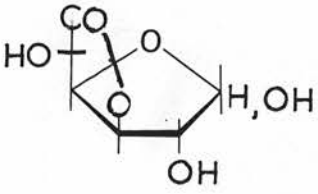


salt of δ-hydroxy acid δ-lactone

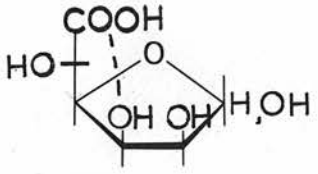
Fig. 1.8



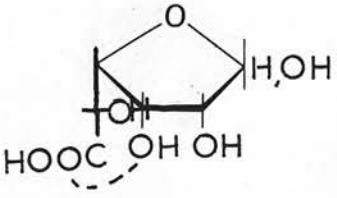
Glucuronic acid



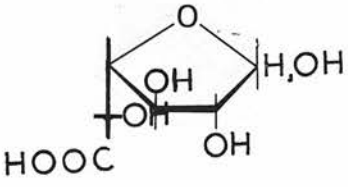
Glucurone



Mannuronic acid



Guluronic acid



Galacturonic acid

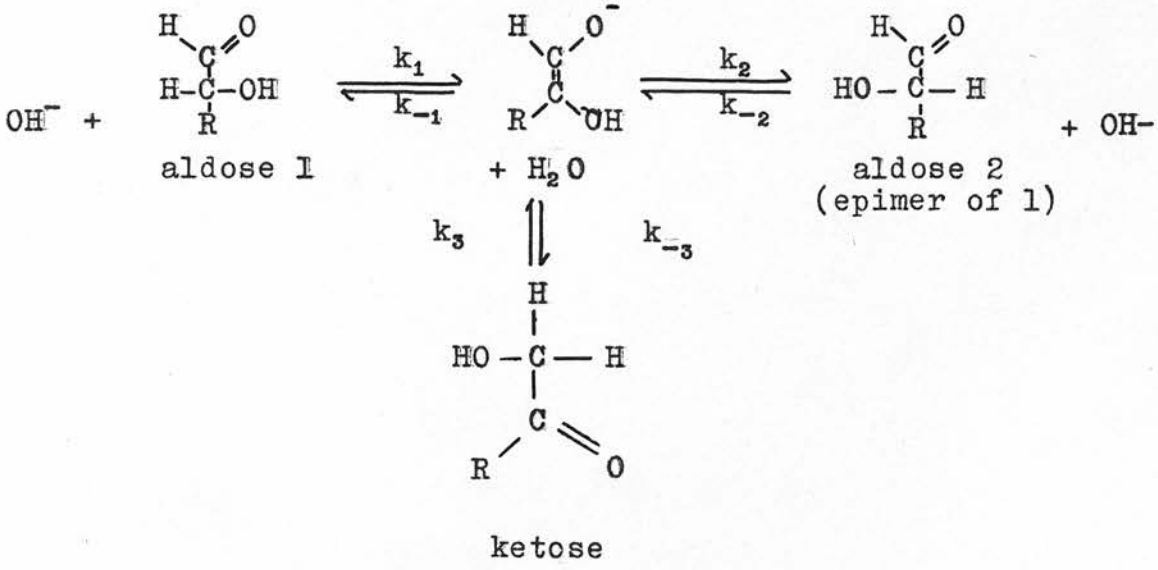
Fig. 1.9

Other workers[22,23] confirm that uronolactones are generally hydrolysed slowly below pH = 7 and much more rapidly at higher pH. Some estimates of the rate of lactonisation of D-glucuronic acid have also been reported[24]. Further experiments in this field are described in section 2.2.

Other reactions in alkaline solution

In addition to causing dissociation, catalysing mutarotation and effecting the hydrolysis of lactones, alkali can, in some circumstances, cause other reactions in sugar and uronic acid solutions. Three important classes of reactions are: 1. reverse aldol condensation (epimerisation), 2. aldose-ketose interconversion, and 3. β-elimination of hydroxyl groups,

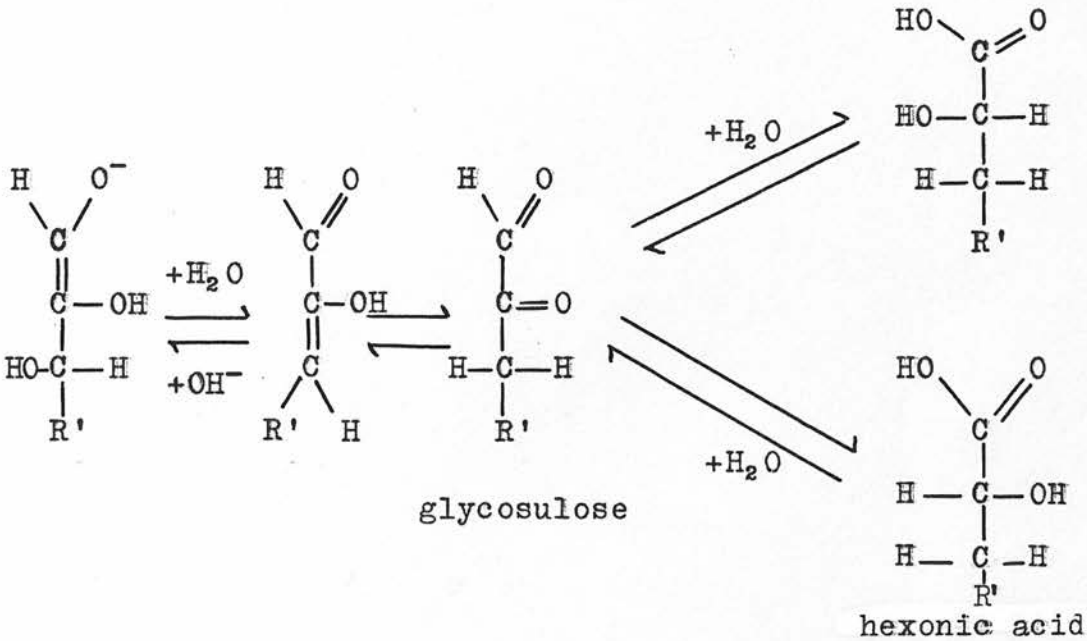
The first two of these normally occur together in the presence of inorganic bases, and were first reported by de Bruyn and van Ekenstein[25] who found that each aldose gave a mixture of substances containing the epimer of the original aldose and the corresponding ketose. The mechanism for the reaction, when the substituent on C(2) is an unblocked hydroxyl group, may be shown thus, representing the sugars in open chain forms:



In the case of D-glucose, the products are D-mannose and D-fructose. A true equilibrium has not been observed,

but the mixture obtained starting with either aldose contains more ketose than epimeric aldose. Also, the initial rates show that $k_3 > k_1 > k_2$, and this initial step is rate determining[26].

Frequently the de Bruyn-van Ekenstein transformation is accompanied by the β-elimination of a hydroxyl group, with the formation of unstable deoxyglycosuloses which are hydrolysed to glyconic acids. This reaction may be shown as follows[27], starting from the same intermediate as above:



An interesting feature of this reaction is that lime water gives a faster reaction than does sodium hydroxide, and that the product ratios depend both on the pH of the solution and on the cation used.

Further degradations may occur in more extreme conditions, and D-glucose gives a number of products after being boiled for three hours in neutral solution[28]. In general, uronic acids undergo the same transformations

and isomerisations as the corresponding aldoses, but the rates of these reactions vary in either direction from those of the aldoses.

In considering the equilibria of associated species between metal ions and uronic acids, all of these side reactions must be minimised or controlled; they are discussed further in Chapter 2. Finally, as uronic acid solutions at near neutral pH are good nutrient media for many types of fungi, precautions must always be taken to store all solutions in sterile conditions or under refrigeration.

1 . 3 Equilibria in Aqueous Solution

The formation of an associated species or complex between two species may be represented by the general equilibrium:

$$x\,A + yB \rightleftharpoons A_xB_y$$

and the equilibrium constant for this reaction, K, is:

$$K = \frac{(A_xB_y)}{(A)^x\ (B)^y}$$

where the round brackets indicate the activity of the enclosed species, or its active concentration relative to the standard state of the species defined for the conditions of measurement. The constant as given is an overall formation or stability constant, and does not explicitly involve the stepwise reactions which take place and the intermediate complexes formed. The study of such general equilibria is very complicated, and fortunately, in the equilibria studied here, x or y is never greater than 1.

Equilibria in aqueous solution are never simple addition reactions such as that shown above, but involve the interaction of solvated species with the resulting partial desolvation of each by the other. Normally B may be taken to represent a cation (including H^+) and A an anion or a molecule with a significant dipole. The solvation of A is generally insignificant compared with that of B, and the formation of 1:1 complex between a univalent metal ion M and an anionic ligand L may be represented thus, assuming that one molecule of water is

displaced from the cation:

$$M(H_2O)_n^+ + L^- \rightleftharpoons M(H_2O)_{n-1}L + H_2O \; ; \; K = \frac{(ML)}{(M^+)(L^-)} \qquad (1.1)$$

The simple form of the equilibrium constant arises because the water may be assumed, in dilute solutions, always to be in its standard state, and it is thus independent of the number of water molecules actually displaced by the ligand. Although the equilibrium between an acid and its anion could be expressed in terms of the stability constant of the proton complex, the usual convention is to give the reciprocal of this value, called the dissociation constant, K_a:

$$H_2O + HL \rightleftharpoons H_3O^+ + L^- \; ; \; k_a = \frac{(H^+)(L^-)}{(HL)}$$

The accepted standard state for an ion or molecule in solution is that concentration whose colligative properties are those of an ideal solution with a concentration of 1 mol/l. (A molal standard is also often used). In very dilute solutions, where interionic interactions are minimal, activities will thus approximate to molar concentrations, and it is convenient even in solutions of much higher concentration to relate activities to concentrations. This ratio is called the activity coefficient (f), and in terms of this, equation 1.1 may be written:

$$K = \frac{(ML)}{(M^+)(L^-)} = \frac{[ML]}{[M^+][L^-]} \times \frac{f_{ML}}{f_+ . f_-}$$

where terms in square brackets represent molar concentrations. It is not possible to measure activity

coefficients for single ions directly; only the mean of the values for a cation and an anion may be obtained by thermodynamic measurements. Since they result from interactions of ions, activity coefficients are generally related to the ionic strength of the solution: $\underline{I} = \frac{1}{2}\sum_i c_i z_i^2$, where $\underline{c}$ and $\underline{z}$ are the molar concentration and charge respectively of each ion in solution. For solutions in which $\underline{I}$ is more than about 0.1 mol/l., interactions between ions of like charge are important, and activity coefficients are complex functions of solution composition. In more dilute solutions, however, the mean activity coefficient may be expressed by the Debye-Hückel formula[29]:

$$-\log f_{\pm} = \frac{A \cdot z_+ z_- \sqrt{I}}{1 + B \cdot a \cdot \sqrt{I}} \qquad (1.2)$$

where $\underline{A}$ and $\underline{B}$ are constants dependent on the dielectric constant and temperature of the solvent, and $\underline{a}$ is a (largely empirical) parameter related to the size of the ions. Neutral molecules may similarly be assumed to have unit activity coefficients.

Measured equilibrium constants differ primarily in the way in which activity coefficients are treated. They fall basically into three categories:

1. "Thermodynamic" constants. These are approximations of true constants expressed in terms of activities. They may be obtained by calculating activity coefficients for the various terms and applying them, or better by measurement of the constants at

various ionic strengths and extrapolating the values to infinite dilution.

2. Stoichiometric or concentration constants. These are values expressed in terms of simple concentrations, and are valid only for the ionic strength at which they were measured and, strictly, only in the precise medium in which they were measured.

3. Mixed constants. The method of measurement (e.g. potentiometry) often gives values for the activities of some species and for the concentration of others. Acid dissociation constants involving the activity of the hydrogen ion and the concentrations of the other species are often reported in this way, and their use is demonstrated in Chapter 2.

Choice of activity coefficients

Although for many purposes the use of concentration and mixed constants is acceptable, it is not always possible or desirable to control the ionic strength at a particular value, and activity coefficients must be calculated or assumed.

In work with any but the simplest systems, it is convenient, although not thermodynamically justified, to use individual ion activity coefficients. This is by no means unusual, as it forms the basis for the National Bureau of Standards definition of the pH scale[30].
In this convention $pH = -\log(H^+) = -\log([H^+].f_+)$.
For dilute solutions, the Debye-Huckel relationship above is used in the form:

$$-\log f_+ = \frac{A.\ z^2\ \sqrt{I}}{1 + B.a.\ \sqrt{I}} \qquad (1.3)$$

In this case, the constant a is a function of the cation only. The British Standards pH scale[31] uses a different convention in which f_+ is approximated by the use of $f_\pm$ for simple 1:1 electrolytes (those having singly charged anions and cations), which may be obtained by the use of equation 1.2. In practice the two scales are virtually identical up to an ionic strength of 0.1 mol/l.

For some 1:1 electrolytes, McInnes[32] has proposed that the values of f_+ and f_- be assumed to be equal, and this convention has been suggested[33] for the establishment of activity standards for use with ion selective electrodes (see Chapter 3). Bivalent ions, however, cannot be

treated so simply, and two different conventions have been used. The first uses equation 1.3[34]. Hence for a 2:1 electrolyte, such as calcium chloride, $\log f_+ = 2\log f_\pm = 4\log f_-$. The second[35] proposes that the approximation $\log f_+ = \log f_\pm = \log f_-$ be made. Comparison has been made of the agreement between the various approximations using a calcium selective electrode[36]. The electrode system was calibrated at $I = 0.01$ mol/l, and mean activity coefficients determined over the range $0 \geq \log I \geq -3$ were equivalent to those determined by thermodynamic measurements. Using the first scheme above, satisfactory agreement was obtained over the range $-1 \geq \log I \geq -3$, but the second scheme gave quite different results. In this work, the first scheme is used for individual activity coefficients of 2:1 electrolytes, and the McInnes convention is used for 1:1.

In making these assumptions, it must be remembered that both activity coefficients and stability constants are expressing degrees of association in solution, and, as solutions become more concentrated or complexes become weaker, some ambiguity of interpretation must result. If activity coefficients appropriate to solutions of metal ions with chloride or nitrate anions are used for solutions containing ligand anions, the stability constants calculated will indicate the extent to which the ligand interacts with the metal in excess of the background electrolyte.

1 . 4 The Scope of the Work

Nature of the Ligands

A problem of some interest in plant physiology is the evaluation of the forces involved in the association of metal cations with the polysaccharide material found in primary plant cell walls. A basis for this very large study is the complexing of metals by the monomer units which make up the polysaccharide chain.
The principal units examined are chemically pure mono--uronic acids. The dissociation constants of these acids and the stability constants of the complexes of their anions with some typical and transition metal ions are measured and discussed. Since these acids have a labile bond between C(1) and O(5), the comparative behaviour of some methyl uronosides is also examined.

Methods of Study

Dissociation constants and stability constants have been calculated from data obtained by a very wide range of methods[37]. The primary essential for such a method is to provide a means for determining accurately the concentration or activity of one of the species in an equilibrium mixture, and to enable the calculation from this of the activities or concentrations of the other species. For the most part, these may be divided into two groups: pL methods in which a direct or indirect measure of the free ligand activity is obtained, and pM methods in which the free metal ion is determined.

A further group, less general in application, determines the amount of one or another complexed species.

By far the most widely applied method is the indirect pL method involving the measurement of the pH of the test solution using a glass electrode[e.g.38]. This method is applicable to any ligand which is the anion of a weak acid, and for which, under experimentally obtainable conditions, protons will compete significantly with the metal ions for the ligand. This method is used here because of its generality and experimental ease (Chapter 4) although its accuracy is severely limited by the fact that it must be used in strongly acid solution when lactonisation of the ligands is a problem (see page 17). More fundamentally it is also unsatisfactory because for strong acids and weak complexes such as are formed here, errors accumulate in the indirect determination of pL from pH.

The potentiometric determination of free metal ion in solution directly has been much less used, except for complexes of such metals as silver, because suitable electrode systems were not available. The recently developed alkali-metal ion responsive glass electrodes[39] and liquid ion-exchange membrane electrodes sensitive to bivalent cations are used here, and their application is discussed in Chapter 3.

In Chapter 5, a novel method of approach using the optical rotation of solutions containing uronic acids is also examined. The three methods of approach are

compared in Chapter 6, and the significance of the results is discussed there.

CHAPTER 2

PREPARATION AND PROPERTIES OF LIGANDS

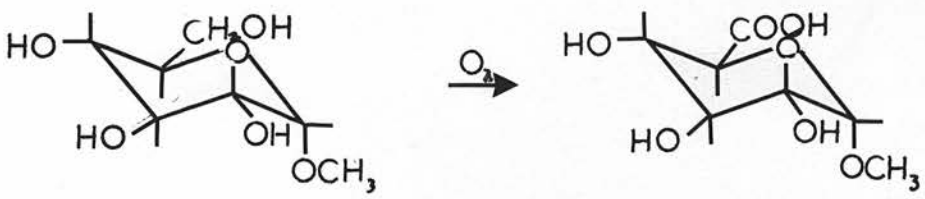
2 . 1 Preparations and Purifications

In this section, brief outlines are given of the methods used to prepare and characterise the principal materials used as ligands in this work. Fuller experimental details are given in section 2.5.

D-Glucuronic acid, as it is readily obtained in a pure form, was the substance most extensively studied. The biochemical grade reagent obtained from BDH Chemicals was used without further purification. (m.p. observed, 163-164°; reported[40], 165°).

D-Galacturonic acid. Monohydrate was also obtained from BDH Chemicals, but required further purification. A method similar to that of Ehrlich and Schubert[41] was used and is described in section 2.5. A yield of about 70% of pure anhydrous D-galacturonic acid was obtained. (m.p. observed, 155-157°; reported[40] 156-158°).

Methyl-α-D-glucuronoside and methyl-β-D-galacturonoside were prepared by catalytic oxidation of the corresponding glucosides, kindly supplied by Dr. A. Rees:



Methyl α-D-glucoside Methyl α-D-glucuronoside

The method, based on that of Marsh[42] is given in section 2.5. The crude glucuronoside could not be crystallised, but the hygroscopic gum obtained was freeze dried and stored over phosphorus pentoxide. The galacturonoside was recrystallised from alcohol. (m.p.162-164° ; reported[43] 165° after sintering at 126°C).

The extraction of D-Mannurone and D-Gulurone, the lactones of mannuronic and guluronic acids, from alginic acid was attempted. The work is described in section 2.5.

2 . 2 Studies on Formation and Hydrolysis of Lactones

The equilibrium which can exist in aqueous solution between a uronic acid and its lactone has already been discussed (Chapter 1). Since these lactones form in acid solution and are hydrolysed to the anion of the parent acid by alkali, it is essential to determine rates of formation and hydrolysis of these species to allow accurate measurement of concentrations of ligand in each form during potentiometric titrations.

The most satisfactory solution for use in potentiometric titration would be one in which the lactone had been fully hydrolysed. Therefore, an investigation was carried out to measure the rate of hydrolysis of the lactone relative to the rate of degradation of the acid in alkaline solution, and the rate of relactonisation when the solution was made acid.

Results

Two methods were used to investigate the rate of

uronolactone hydrolysis in mildly alkaline solutions. In the first of these, the change in ratio of the concentration of parent acid and anion to the total concentration, including lactone, is determined by titrimetric measurement as a function of the time under the test conditions. (The total concentration is determined by dissolving a weighed sample of ligand in the preparation of the test solution while the actual concentration of titratable acid present is determined by the method described in Section 2.5).

When the pH of a stock solution of D-galacturonic acid is adjusted to pH 8-9 with potassium hydroxide solution, no change occurs in the apparent purity of the ligand over a period of two hours (Fig. 2.1). If lactonised species were present, these would be hydrolysed and the concentration of acid would increase. For this acid no such change occurs in keeping with the fact that it does not normally form a lactone (see Section 1.2). On the other hand, methyl α-D-glucuronoside, under similar conditions, shows the apparent increase in concentration over the initial period, as the lactone is hydrolysed to the anion of the acid, after which the concentration remains unchanged[44] (Fig. 2.2).

When the purity of a sample of D-glucuronic acid stored at pH5 was examined as a function of time, a slight decrease was observed over a period of 20 hours, indicating the gradual formation of the lactone (Fig. 2.3).

A summary of the change with time in the concentration of acid as measured by pH titration for these tests is

Rate of Hydrolysis of Lactone (pH 9) - Galacturonic Acid

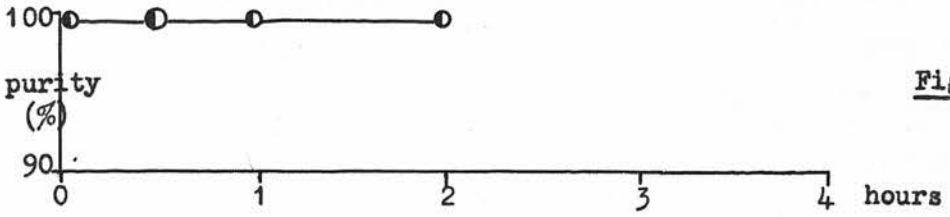


Fig. 2.1

Rate of Hydrolysis of Lactone (pH 9) - Methyl α-D-Glucuronoside

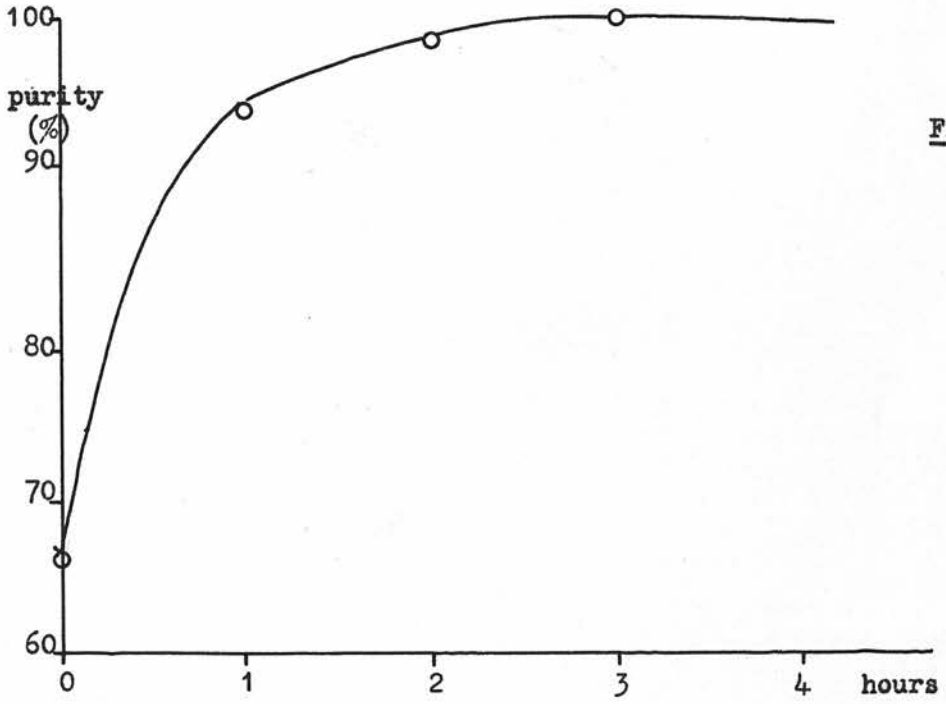


Fig. 2.2

Rate of Formation of Lactone (pH 5) - Glucuronic Acid

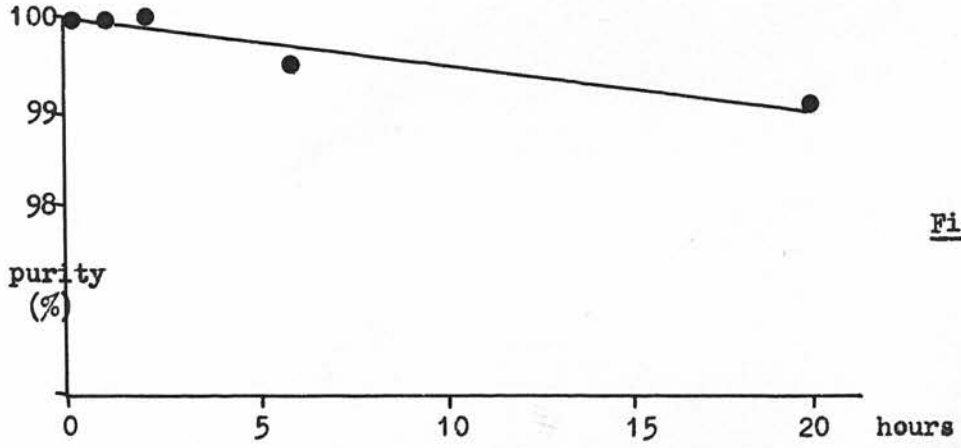


Fig. 2.3

given in Table 2.1.

Table 2.1 - Change in Ligand Purity with Time under Acid and Alkaline Conditions

Time (hrs.)	Gal. acid pH 8-9	Methyl-α-D-glucoronoside pH 8-9	Gluc. acid pH 5
0	100.0%	65.6%	100.0%
½	99.8%	-	-
1	99.9%	93.7%	100.0%
2	99.8%	98.7%	100.0%
3	-	99.8%	-
6	-	-	99.5%
20	-	-	99.0%

The second method involves a study of the hydrolysis of a partially lactonised solution of D-glucuronic acid to glucuronate in mildly alkaline solution (pH 9) by polarimetry (see Section 5.5 for experimental method). Since the lactone has a different specific rotation from that of the acid, it is possible to measure the rate of hydrolysis of the lactone by following the change with time in rotation of a solution of the ligand which has attained anomeric equilibrium. The results from this show that the rate of change of rotation, which is rapid immediately after the addition of alkali, slackens with time until after about 45 minutes there is little further change (see Fig. 2.4). This again corresponds

Rate of hydrolysis of Lactone (pH 9)- Glucuronic Acid

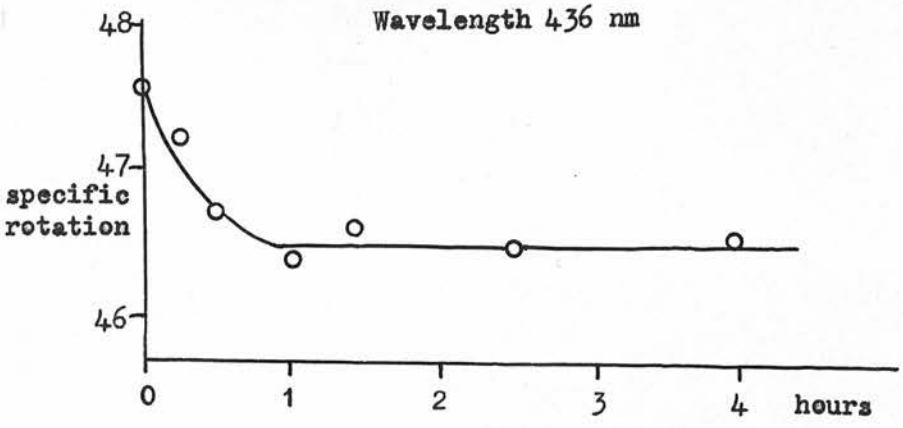


Fig. 2.4

Action of Aqueous Alkali (pH 9) - Glucuronic Acid

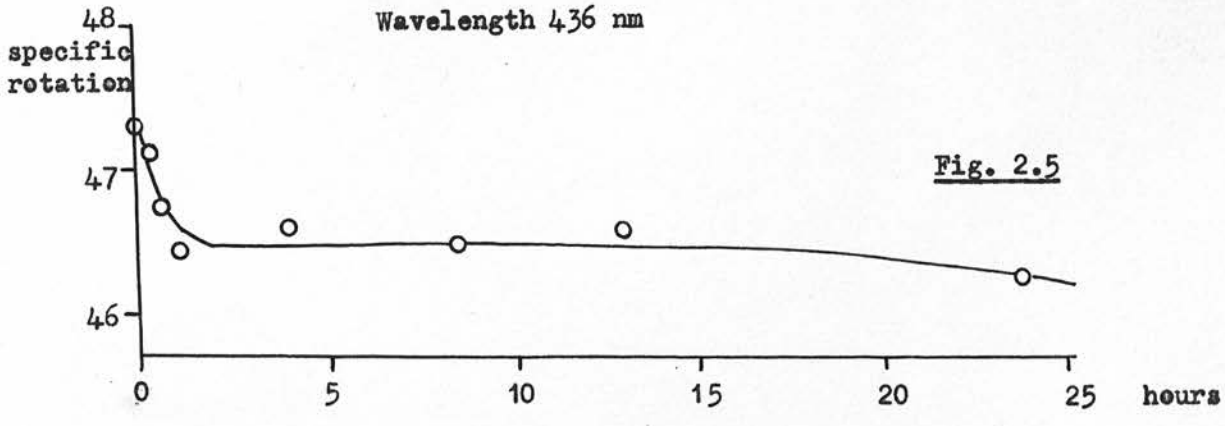


Fig. 2.5

to hydrolysis of the lactone to the anion of the parent acid. (Changes in optical rotation observed on the addition of alkali (see below - section 2.3) will include a contribution due to any change in the position of the anomeric equilibrium due to the pH change and do not therefore represent unambiguous evidence of lactone hydrolysis).

The degradative effect of alkali on uronic acids has been discussed (Chapter 1). It is therefore essential to show that no such degradation takes place during the hydrolysis of lactones under mildly alkaline conditions. Two methods were employed to determine the rate at which these changes occur in the ligands due to the presence of dilute aqueous alkali.

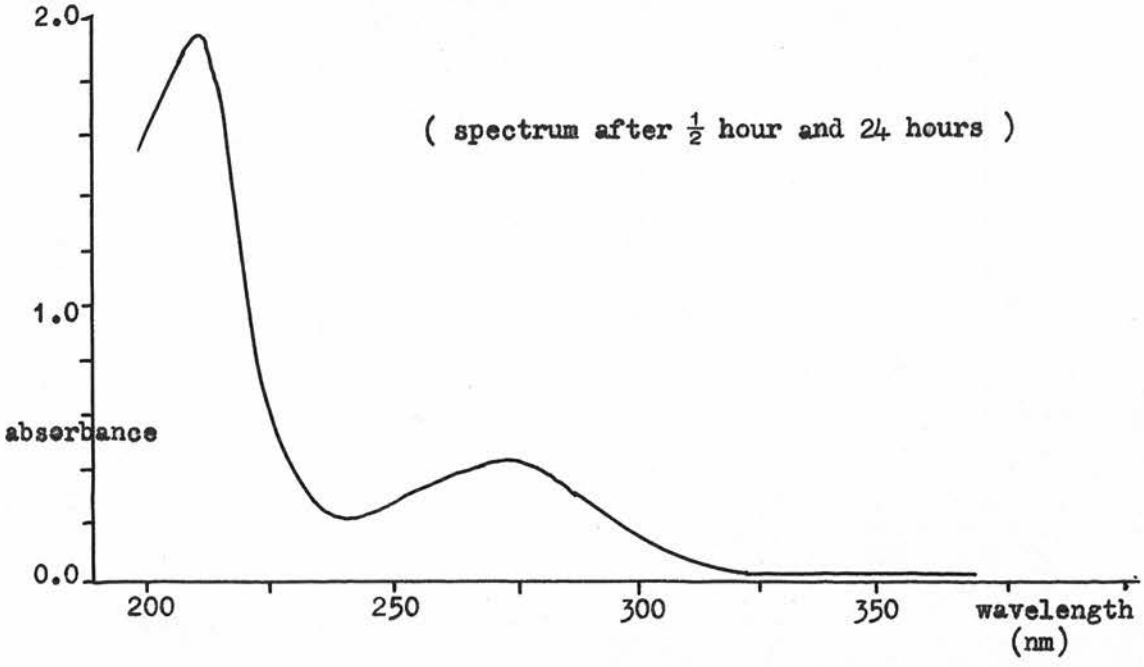
The first method was the measurement of a specific rotation of the ligand solution at pH9 over a prolonged period. The specific rotation based on the total concentration of ligand which has reached mutarotation equilibrium and which contains a proportion of lactonised species decreases rapidly initially until a steady value is attained after about 45 minutes corresponding to the hydrolysis of all lactone to the parent acid (Fig. 2.5). The rotation remains constant for about 24 hours indicating that no further reaction was taking place, since it is likely that any species produced by the degradation would have a different rotation and would therefore cause a change in the measured specific rotation.

The second method of determining the effect of dilute aqueous alkali on the ligands was u.v. spectroscopy. If alkali causes degradation, the u.v. spectrum of the ligand would change due to the presence of species containing double bonds (see Section 1.2). Such changes have been reported and examples of these include the appearance of additional intense absorption bands at 301 nm and 290 nm in the spectrum of 2,5-dimethyl Δ^4-1--glucosaccharo-3,6-lactone 1-methyl ester in alkaline solution[45] and the shift in absorption of mannosaccharo-dilactone from 229 nm to 263 nm on moving from acid to alkaline solution[46].

The spectra obtained with both D-glucuronic acid and methyl α-D-glucuronoside showed no change on storing in aqueous alkali (pH 9) for 24 hours (Figs. 2.6A and 2.6B). The results from this together with the polarimetric method show that the only action of dilute aqueous alkali over a period of 24 hours is the hydrolysis of lactone and that any degradation resulting from the action of alkali over this period can be ignored.

Thus, solutions virtually free of lactone or degradation products may be prepared by maintaining ligand solutions at pH 8-9 for $1\frac{1}{2}$ hours, and solutions for use in potentiometric titrations were treated in this way.

Action of Aqueous Alkali (pH 9) - Glucuronic Acid



Action of Aqueous Alkali (pH 9) - Methyl α-D-Glucuronoside

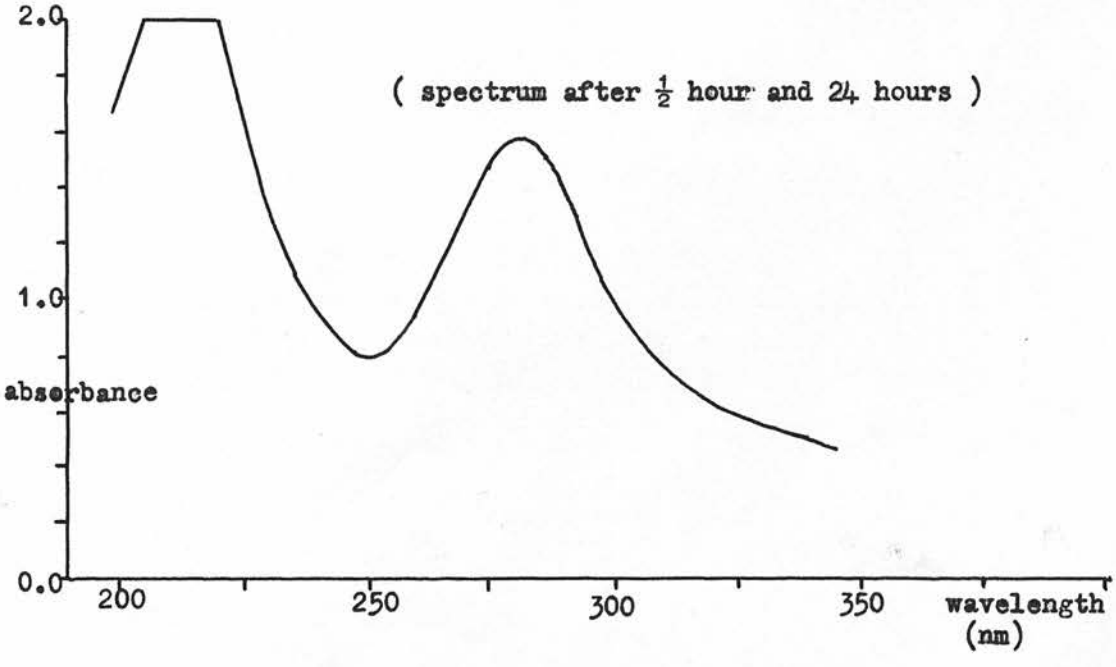


Fig. 2.6

2 . 3 Studies of Mutarotation

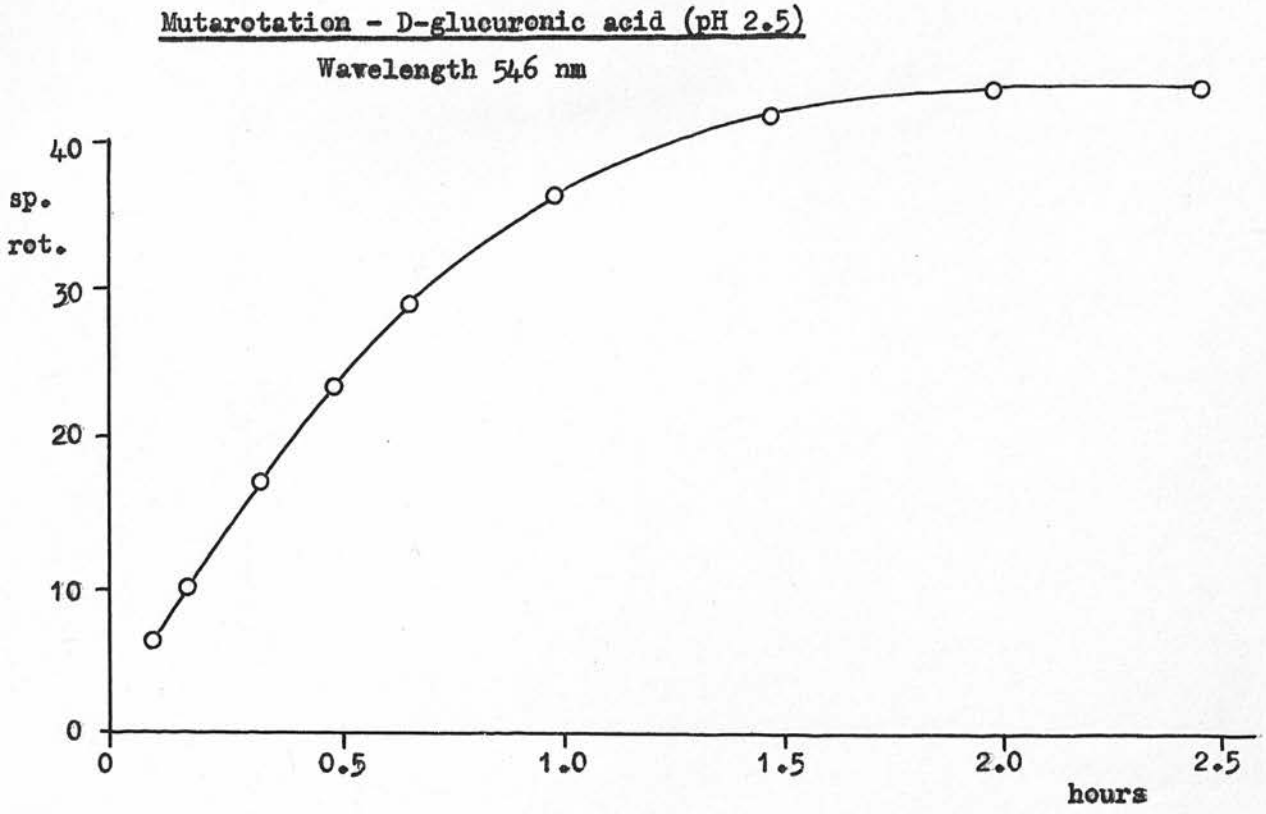
The gradual change in the anomeric composition of a sugar in aqueous solution and the effect this mutarotation has on the specific rotation of the sugar has been discussed (Chap. 1). Since a part of the parent study involves the measurement of equilibrium specific rotation values, it is necessary to have a quantitative measure of the rate at which anomeric equilibrium is attained. The mutarotation of D-glucuronic acid and D-galacturonic acid at different pH values was studied and the time taken to reach equilibrium recorded.

A weighed sample of the ligand together with sufficient sodium carbonate to give the desired pH was dissolved in water and the solution was immediately transferred to a polarimeter cell fitted with a water jacket filled by means of a pump from a water bath equipped with a thermostat. The specific rotation for the ligand was determined and plotted as a function of time from the preparation of the solution. As was previously discussed (Chap. 1), the graph representing D-glucuronic acid illustrates a simple mutarotation whereas that of the D-galacturonic acid is of a complex nature involving not only the equilibrium between the α- and β-anomers but also apparently that of furanose forms. Since the C(1) position in the uronosides is blocked eliminating any anomerisation, no mutarotation is observed. The mutarotation curves for D-glucuronic acid

and D-galacturonic acid at various pH values are illustrated in Fig. 2.7 and 2.8 respectively.

Mutarotation is accelerated by all substances capable of donating or accepting protons[47,48]. In sugar solutions, the principal catalysts of this type are the undissociated water molecules, hydrogen ions and hydroxyl ions although additional species which affect the rate of the reaction in the case of sugar acids are the undissociated acid and the dissociated salt form. From the results above, it is observed that the time taken for the neutralised solutions to reach anomeric equilibrium is less than that for the solutions of ligand at low pH. Thus at pH 7-8, mutarotation is essentially complete in 1 hour, while at pH3 the time required to reach equilibrium is 2-2$\frac{1}{2}$ hours. It has been reported[49] that the more rapid mutarotation of the salts appears to have been caused by the catalytic effect of the glucuronate or galacturonate ion. In the present case, the combined catalytic effect of the undissociated acid, hydrogen ions and salt must be less than that of the salt and undissociated water.

In order to ascertain whether the specific rotation of the ligands in acid and alkaline solutions is different, stock solutions of each ligand were prepared (approx. 0.3 mol/l) and these were allowed to stand for about 3 hours before use to ensure complete mutarotation. A series of solutions containing aliquots of the stock


Mutarotation - D-glucuronic acid (pH 2.5)
Wavelength 546 nm
sp.
rot.
40
30
20
10
0
0
0.5
1.0
1.5
2.0
2.5
hours

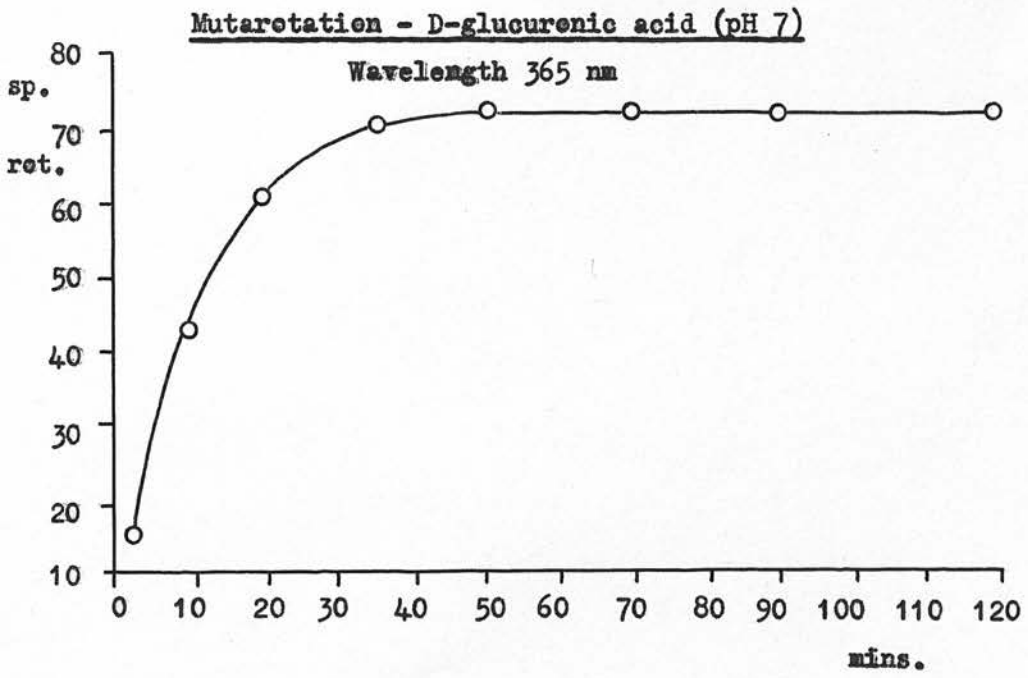

Mutarotation - D-glucuronic acid (pH 7)
Wavelength 365 nm
sp.
rot.
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
mins.

Fig. 2.7

Mutarotation - D-Galacturonic Acid (pH 7)

Wavelength 365 nm

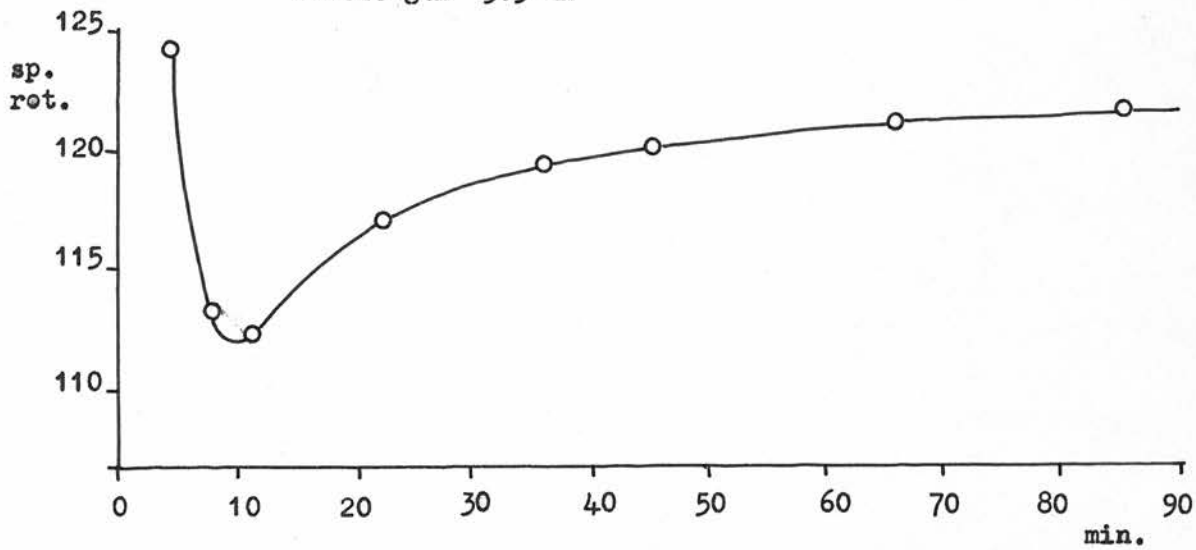


Fig. 2.8

Change in Specific Rotation with pH - D-Glucuronic Acid

Wavelength 365 nm

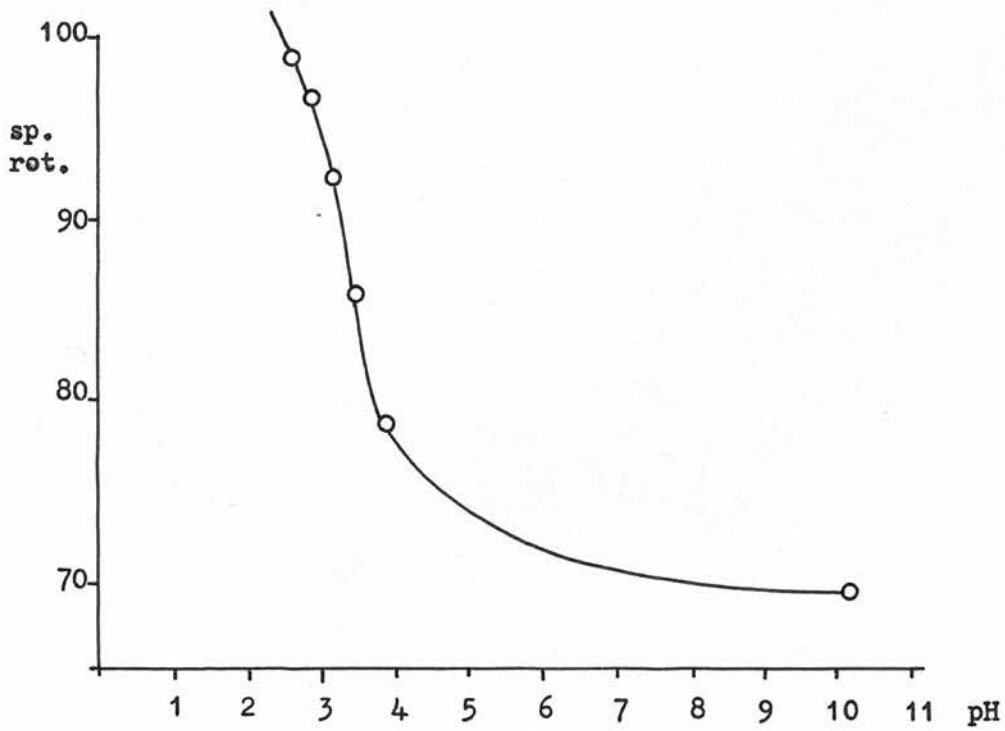


Fig. 2.9

solutions for each ligand were prepared and varying volumes of 0.1 mol/l potassium hydroxide solution or nitric acid were added to each to give a range of pH values. The specific rotation based on the total ligand concentration was determined for each solution and graphs showing specific rotation as a function of pH could be plotted. This graph, which is shown in Fig. 2.9 for D-glucuronic acid, is similar to the typical titration curve for the acid. For the remaining ligands, values were obtained for the specific rotations of the protonated and dissociated forms of each acid and these are tabulated in Table 2.2.

Table 2.2 Change in Specific Rotation with pH

Ligand	Wavelength	Protonated form (pH 2)	Dissociated form (pH 7)
D-gluc.	365 nm	98.4	72.3
Me α-D-gluc.	546	121.7	110.1
D-gal.	365	163.2	124.2
Me β-D-gal.	546	-44.1	-57.5

The specific rotation of the ligand in acid and alkaline solutions is different and although the rotation of the protonated acid and its salt may not be identical, it is likely that part of the difference is due to a shift in the position of the anomeric equilibrium.

2 . 4 Dissociation Constants

The method most commonly used for the determination of acid dissociation constants is based on following the changes in the pH of a solution of an acid as it is titrated with standard alkali. The pH is commonly followed by a potentiometric setup with a glass electrode sensitive to hydrogen ions (see section 4.4). As is explained in section 1.3, the equilibrium constant which may most conveniently be determined is a mixed constant, involving the activity of hydrogen ions, and the analytical concentrations of other species. All of the materials studied here function as monobasic acids, and their equilibria may be summarised thus:

$$HL \rightleftharpoons H^+ + L^-$$

$$k' = \frac{(H^+)[L^-]}{[HL]} = k_t \cdot f_{HL}/f_- = k_c \cdot f_+ \qquad 2.1$$

where $\underline{k}'$, $\underline{k}_t$ and $\underline{k}_c$ are the mixed, thermodynamic, and concentration constants, and the other terms are defined in section 1.3.

In the course of a titration of a solution of a known concentration of acid in a solution containing a known amount of potassium chloride or nitrate with standard potassium hydroxide, two useful relationships are:

$$L_t = [HL] + [L^-] \qquad 2.2$$

and

$$[K^+] + [H^+] = [L^-] + [OH^-] + [Cl^-] \qquad 2.3$$

The first of these simply defines the total concentration of acid in all forms, L_t, while the second expresses the electroneutrality of the solution. In the second

equation, $[K^+]$ represents potassium ions from two sources, the potassium chloride, and the potassium hydroxide added. Calling this last concentration OH_a,

$$[K^+] = [Cl^-] + OH_a \qquad 2.4$$

substituting this into equation 2.3 and rearranging gives:

$$[L^-] = OH_a + [H^+] - [OH^-] \qquad 2.5$$

Generally either or both of the terms involving hydrogen and hydroxide ions will be insignificant compared with the others, but if they are required, they may be calculated from the pH of the solution, providing that some appropriate activity coefficient may be applied:

$$[H^+] = 10^{-pH}/f_{\pm} \quad ; \quad [OH^-] = 10^{-pOH}/f_{\pm}$$

where pOH = pKw - pH and pKw = $\log[(H^+)(OH^-)]$ = 13.996 (25°)

This correction will generally be small, so that an approximate value for the activity coefficient will be satisfactory.

Hence, using equations 2.5 and 2.2, values for $[L^-]$ and [HL] may be determined. It is convenient to express these in terms independent of total ligand concentration, and this may be done using an auxiliary variable g, the degree of neutralisation, defined:

$g = [L^-]/L_t$.

Taking logarithms in equation 2.1 and substituting gives:

$$\log k' = \log (H^+) + \log(g) - \log (1-g)$$

or: $$pk' = pH - \log(g) + \log (1-g) \qquad 2.6$$

A complication which proved to be significant here is the formation of associated species between the cation of the base and the anion of the ligand. If a species KL is formed, its concentration must be considered, and equations 2.2 and 2.4 become:

$$L_t = [HL] + [L^-] + [KL] \qquad 2.7$$

and $$[K^+] = [Cl^-] + OH_a - [KL] \qquad 2.8$$

The stability constant of the potassium complexes is generally low, and its determination is described in Chapter 3.

Calculations and Results:

Stock solutions were prepared by dissolving a known weight of the acids. Before use, the pH of an aliquot of the solution was adjusted by means of potassium hydroxide solution to pH 8-9 and the solution allowed to stand under a nitrogen atmosphere for $1\frac{1}{2}$ hours to allow hydrolysis of the lactones present.

A known volume of this solution (25 ml.), of acid concentration generally between 0.004 mol/l and 0.02 mol/l, and of ionic strength kept effectively constant at 0.1 by suitable additions of 1 mol/l potassium chloride solution, was introduced to the titration cell. Nitrogen was bubbled through the solution which was allowed to stand for about fifteen minutes to attain thermal equilibrium. Titration with standard (either 1 mol/l or 0.1 mol/l) hydrochloric or nitric acid solution was then effected as quickly as possible, values of pH being recorded after the addition of aliquots of acid. Nitrogen was bubbled

through the solution after each addition, and, during the recording of pH values, it was passed over the surface of the solution. The purity of the ligand solution was calculated from the end-point of the back titration of excess alkali. (Details of electrodes and potentiometric equipment are described in Section 4.4). A typical titration curve is shown in fig. 2.10.

A computer program (Ionconst) was written to calculate the pK by means of the following steps (see Appendix).

1. Calculation of a value of pK' from each individual pair of (pH, g) results, using eqn. 2.6.
2. A weighted least squares treatment of the values of pK', eliminating any values outwith predetermined probability limits. The weight for each value was taken as g when $0 < g < 0.5$ and as (1 - g) when $0.5 \leq g < 1$.

   A final average pK' value and overall standard deviation was obtained.
3. A recalculation of pK' making allowance for the formation of potassium-acid complex (see section 3.1) followed by a further weighted least squares treatment to obtain a corrected value for pK'.

In any particular case, titration curves were generally obtained in duplicate for at least three different ligand concentrations. The best value of pK' was taken as the weighted average of all the individual values (see above). The values obtained where $g \simeq 0.5$

were given greatest weight in all cases since the error in calculating the concentrations of the protonated and deprotonated forms was at a minimum.

Typical sets of (pH,g) points for the ionisation of D-glucuronic and galacturonic acids at 25°C are shown in figures 2.10, 2.11. Typical tabular (pH,g) and pK' data for all solutions can be seen in section 2.6.

The values obtained for pK' for each acid at an ionic strength of 0.1 were:

D-glucuronic acid	3.19
D-galacturonic acid	3.49
Methyl α-D-glucuronoside	3.16
Methyl β-D-galacturonoside	3.48

[These values include correction for potassium complex formation. The values for the stability constants determined for potassium glucuronate and potassium galacturonate (section 3.1) were used in the calculations for the glucuronoside and galacturonoside respectively].

Discussion

In the following series of carboxylic acids in which the number of hydroxyl substituent is increased, the acidity of the acid also increases.

Table 2.3 - Variation in Acidity with Degree of Hydroxyl Substitution

Acid	Acetic Acid	Lactic Acid	Glucuronic Acid
pKa	4.76	3.86	3.19
no. of hydroxyl substituents	0	1	4

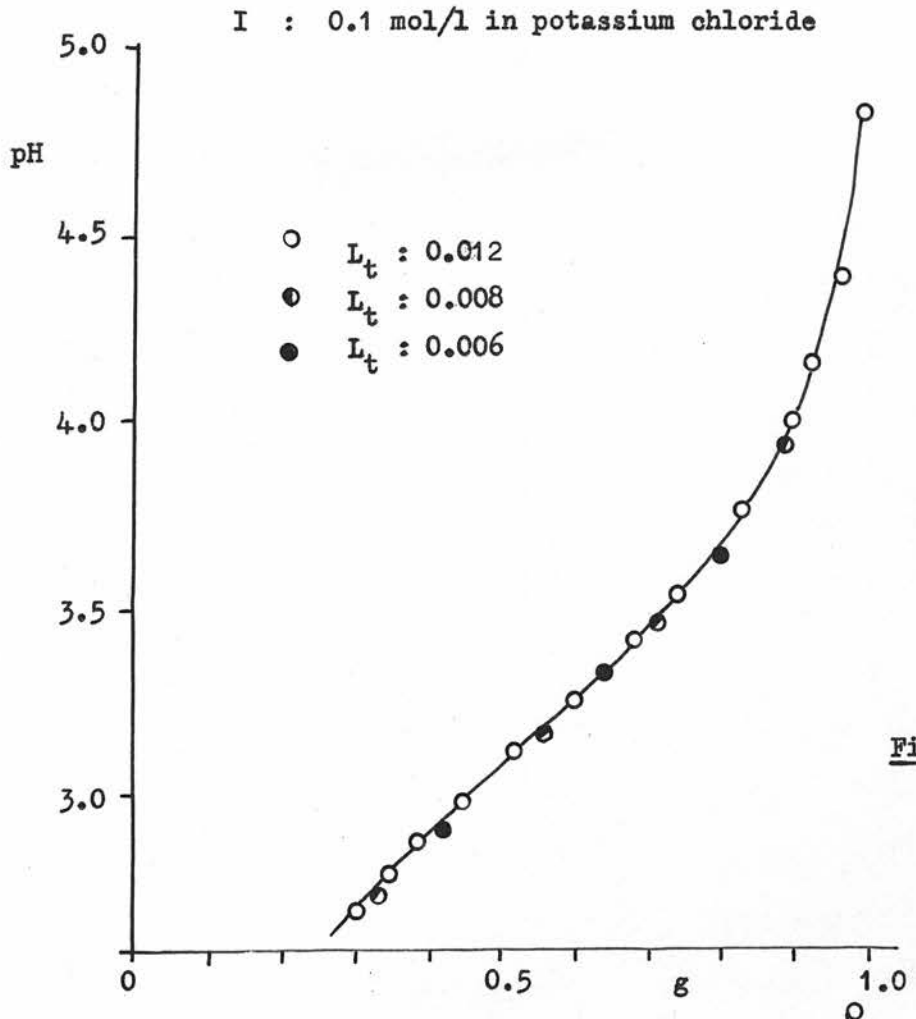


Fig. 2.10

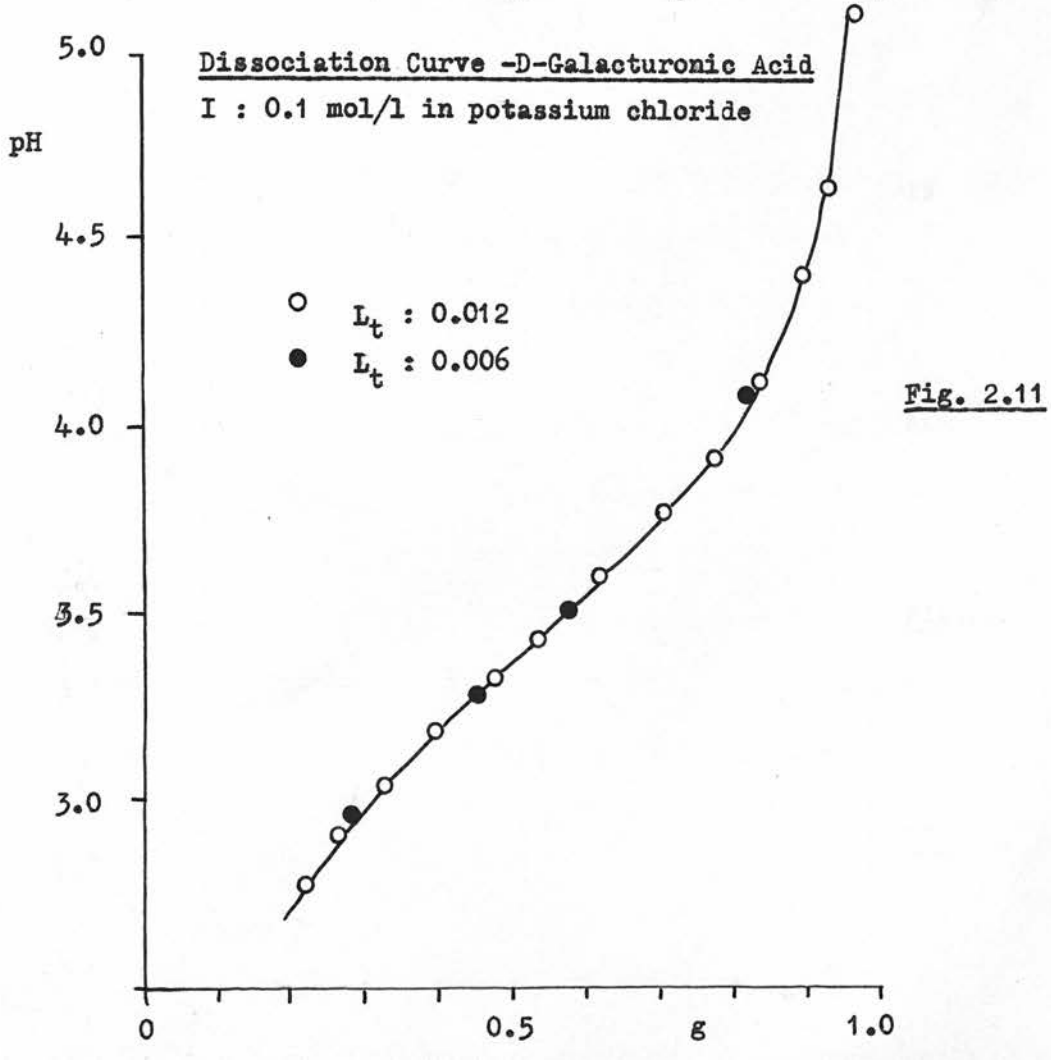


Fig. 2.11

The explanation for this behaviour is the greater delocalisation of charge possible in the acid with the greatest number of hydroxyl substituents causing increased stability for the anion formed. It is therefore to be expected that the uronic acids are moderately strong acids.

However, within the uronic acid series (within the series, each member has the same number of hydroxyl substituents), there is considerable variation in acid strength, e.g. D-glucuronic acid - pK_a 3.12[50]

D-guluronic acid - pK_a 3.65[50]

Two possible reasons for this variation in acidity have been postulated[51]. The first involves hydrogen bonding between hydroxyl and carboxyl groups giving increased stability to the anion form and therefore increased acidity while the second explains differences in terms of conformational differences. In this case, it is reported[52] that in the cyclohexane series axial carboxyl groups are less acidic than their equatorial counterparts. In the uronic acid series in the C-1 conformation, the less acidic have axial carboxyl groups whereas in the stronger acids the carboxyl groups are equatorial[51]. It has been concluded[51] that the two effects combine to give rise to the observed differences in pK_a as well as other physical properties. However, since the acids in the present work all have equatorial carboxyl groups in the C-1 conformation, it is likely that the differences in pK can only be explained in terms of hydrogen bonding.

The results obtained were 3.11 and 3.12 respectively for the pK_a of the acid at an ionic strength of 0.1 (the former in a sodium chloride medium and the latter in potassium chloride). The apparent discrepancy between these values and that quoted in the present work is in fact that neither determination makes any correction for the concentration of potassium or sodium complex present. Although the stability constants for the potassium uronates are small (see Section 3.1), the concentration of potassium ions in the solution is high, coming mainly from the background electrolyte, and this implies that the equilibrium concentration of the potassium complex will be sufficiently large that correction must be made in the free ligand concentration to allow for it. In fact, if the present value were quoted for pK_a of glucuronic acid at 25°C and at an ionic strength of 0.1 but taking no account of the potassium complex correction, the value would be 3.11 which corresponds with the other determinations above.

There is one further reported determination of the pK_a for D-glucuronic acid[53]. The value in that case was 3.33 but the conditions were not specified making comparison with the above results impossible.

The only reported value for D-galacturonic acid is that of Haug[50]. As in the case above, the value of 3.42 is at an ionic strength of 0.1 but takes no account of the sodium complex correction. This value is also in good agreement with the present determination without correction.

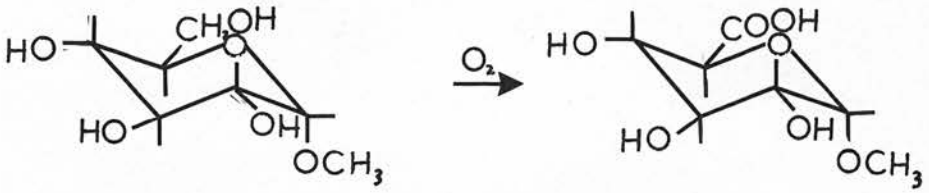
There are no reported determinations of the ionisation constants for methyl uronosides in the literature.

However, it is interesting to compare the value obtained in the present study for methyl α-D-glucuronoside with those for the parent acid, D-glucuronic acid. It has been reported[54] that the α-anomer of a sugar is a slightly weaker acid than the β-anomer. Since the pK_a determined for D-glucuronic acid must be an overall ionisation constant comprising both anomeric ionisation constants, it would be expected that pK_a for the α-uronoside would be slightly greater than that of the uronic acid being a slightly weaker acid. It is for similar reasons that the two anomeric species present in D-glucuronic acid give rise to different stability constants with metal ions (see Section 5.7). However, the present dissociation constant results show little difference between those for the uronoside and those for the parent acid.

2 . 5 Experimental

Preparations:

(a) Methyl α-D-glucuronoside Methyl β-D-galacturonoside



Methyl α-D-glucoside Methyl α-D-glucuronoside

Methyl α-D-glucuronoside and methyl β-D-galacturonoside were prepared from methyl α-D-glucoside and methyl β-D-galactoside respectively using Marsh's[42] catalytic oxidation method. The first stage was the preparation of the platinum catalyst, by the hydrogenation of Adam's Catalyst (platinum dioxide). Gaseous oxygen was then passed through an aqueous solution of the methyl glycoside containing the catalyst in suspension. The solution was maintained at a temperature of 60-65°C and was well stirred throughout to keep the catalyst in suspension.

During the preparation, it was necessary to maintain the solution at pH7 or slightly above to prevent the formation of lactones. The pH was adjusted by the addition of 1 mol/l sodium bicarbonate solution to neutralise the acid as it formed.

The course of the reaction was followed by electrophoresis. Samples were withdrawn at regular time

intervals to detect the point at which the maximum amount of product had been formed. This method of control was possible since the product was acidic whereas the starting material was neutral. An extra spot appeared on the electrophoretogram (fig. 2.12) and the intensity of this spot increased with time. The reaction time at which a maximum yield of product was obtained, shown by the greatest intensity of the product spot on the electrophoretogram, was taken as $9\frac{1}{2}$ hours.

The resultant solution, after filtration to remove the catalyst, was eluted through a column of DEAE--Sephadex in the formate form with a solution of increasing concentration of formic acid. The eluate was collected in 50 ml. aliquots in tubes which were tested by means of the phenol-sulphuric acid test for sugar. A graph of optical density against the number of the tube was drawn (fig. 2.13) and the tubes under the main peak were combined. The product was tested by electrophoresis using D-glucuronic acid as a standard and the combined solution was evaporated to a gum using a rotary evaporator. The products were then purified as described in section 2.1.

(b) Mannurone

Alginic acid (100 g.) was treated with 80% v/v sulphuric acid (1 l.) at room temperature for 5 hours. The solution was diluted with water to 17.5 l. and heated at 90°C for 5 hours. The acid was neutralised with

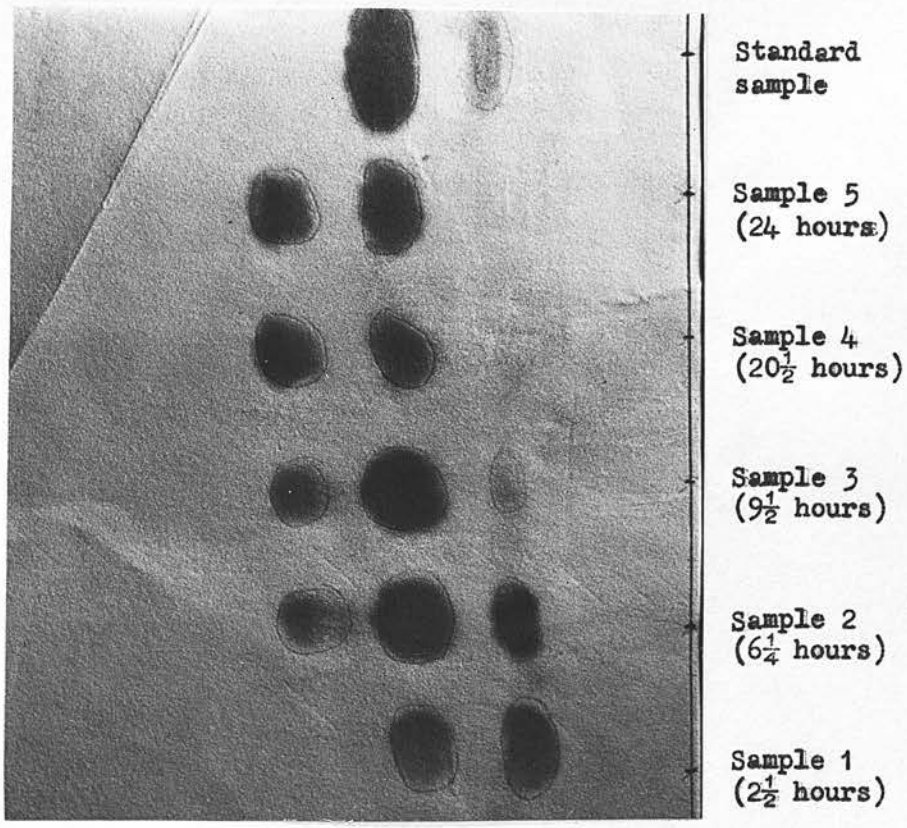


Fig. 2.12 - Electrophoretogram - Rate of formation of oxidation products - Methyl α-D-glucoside

Separation of Oxidation Products of Methyl- β -D-Galactoside on a DEAE - Sephadex Column

(Analysis by phenol-sulphuric acid test)

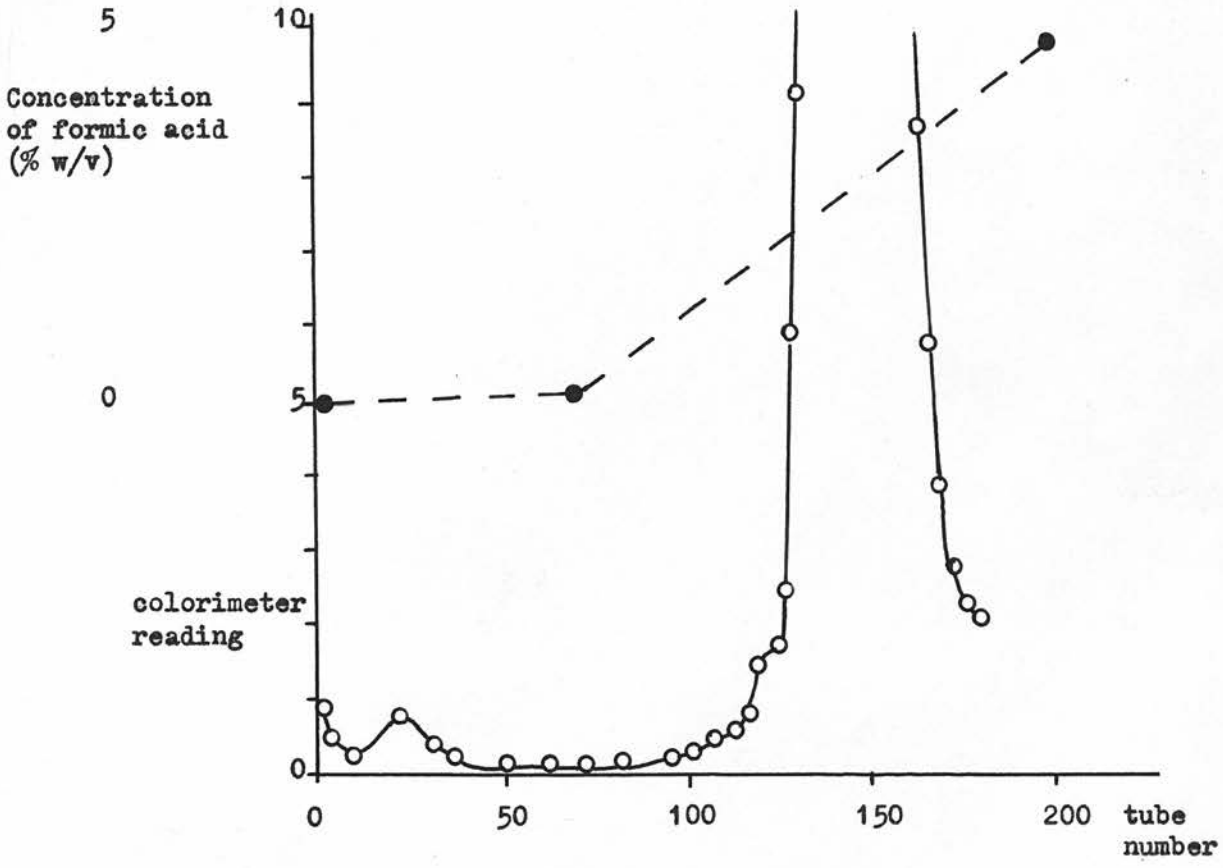


Fig. 2.13

calcium carbonate and the precipitate removed by filtration and washed. The combined filtrate and washings were adjusted to pH 2.5 and were concentrated in a cyclone evaporator to 1 litre. Calcium sulphate was removed by filtration and the solution was further concentrated to 500 ml. Calcium sulphate was removed and the filtrate was adjusted to pH 2.8 with calcium carbonate when further sulphate was collected. After treatment with decolourising charcoal, water (20 ml.) was added followed by glacial acetic acid (120 ml.). The solution was heated, filtered and set aside to cool when needle-like crystals formed.

The crystals were found to have no readily detectable melting point and were shown to be magnesium mannuronate. It is likely that the magnesium was introduced into the system as an impurity in the calcium carbonate. The mannuronate salt was passed through a cation exchange column in the acid form to remove the magnesiums ions. The solution was concentrated on a rotary evaporator and seeded with crystals of mannurone but no crystals could be induced from the solution which contained both mannuronic and guluronic acids.

Electrophoresis

Electrophoresis was carried out in pyridine-acetic acid buffer (0.1M) at pH6. Spotting of the paper was by the method of Trevelyan, Procter and Harrison[55], which involved the passage of the dried strip through a reagent solution prepared by diluting 0.1 ml. of saturated

aqueous silver nitrate solution to 20 ml. with acetone and adding water dropwise, with shaking, until the silver nitrate, which separated on addition of acetone, had redissolved. The dry paper was sprayed with 0.5 mol/l sodium hydroxide solution in ethanol. Brown silver oxide which was immediately produced, formed black spots of silver in presence of reducing sugars. Excess silver oxide was dissolved by immersing the strip in 6 mol/l ammonium hydroxide solution and then washing with running water. The electrophoretogram was then dried in an oven.

DEAE-Sephadex Column

Diethylamino ethyl-Sephadex - A25 was initially swollen in water and the fines removed by decantation. The resin was washed alternately with 0.5 mol/l hydrochloric acid and 0.5 mol/l sodium hydroxide before generating in the formate form by washing with 0.5 mol/l formic acid. The resin was packed in a column plugged with glass wool and was equilibrated with 0.005 mol/l formic acid solution. During elution of the column, a flow rate of 60 ml. per hour was maintained. Separation of product was effected by eluting the column with a constant concentration gradient (0-5% w/v) of formic acid.

Phenol-sulphuric acid test[56]

1 ml. of carbohydrate solution was placed in a test tube and 1 ml. of aqueous phenol (5% w/v) was added. 5 mls. of concentrated 'AnalaR' sulphuric acid was added

from a fast delivery pipette and the solution was mixed and cooled. The optical density of the solution was measured using an EEL colorimeter with a filter to give a wavelength of 495 nm.

Evaporations

These were carried out at reduced pressure using a rotary evaporator with the temperature at or below 40°C.

Estimation of purity of ligand

A measure of the purity of the ligand was obtained for each titration from the end point as determined by pH meter. Titrations of uronic acids with alkali, and of excess alkali with nitric acid were both used. There is a rapid change in pH near the endpoint, the maximum rate occurring about pH 7.5. Purity of a weighed sample can thus be directly determined by titration with alkali, and change in purity estimated by successive additions of nitric acid and alkali to the titrated solution.

Potentiometric equipment and electrodes

These are described in section 4.4.

2 . 6 Tabular Results - Acid Dissociation

This section comprises typical data obtained from pH titrations to determine dissociation constants for D-glucuronic acid, D-galacturonic acid, methyl α-D--glucuronoside and methyl β-D-galacturonoside. The data were calculated using computer program IONCONST (see Appendix) and include correction for the presence of complexes formed between the ligands and potassium ions.

A. D-glucuronic acid

Ionic strength: 0.1 mol/l in potassium chloride.
Concentration of hydrochloric acid solution: 0.1001 mol/l.

	Vol. acid solution (ml).	pH	g	pK	Acceptance*
L_t: 0.0120	0.140	5.962	0.995	3.749	0
	0.200	4.844	0.976	3.319	0
	0.299	4.405	0.945	3.253	0
	0.400	4.169	0.913	3.227	0
	0.499	4.012	0.882	3.216	1
	0.600	3.890	0.851	3.211	1
	0.701	3.784	0.821	3.202	1
	0.798	3.702	0.791	3.201	1
	1.000	3.552	0.732	3.197	1
	1.101	3.488	0.702	3.193	1
	1.200	3.430	0.674	3.192	1
	1.300	3.375	0.646	3.192	1
	1.401	3.319	0.618	3.187	1
	1.500	3.269	0.591	3.186	1

Vol. acid solution (ml).	pH	g	pK	Acceptance *
1.599	3.222	0.565	3.185	1
1.700	3.172	0.539	3.180	1
1.800	3.128	0.514	3.180	1
1.900	3.082	0.490	3.176	1
2.000	3.039	0.466	3.173	1
2.100	2.998	0.443	3.172	1
2.200	2.956	0.422	3.168	1
2.301	2.917	0.401	3.167	1
2.401	2.880	0.380	3.167	1
2.500	2.842	0.362	3.163	1
2.602	2.805	0.343	3.161	1
2.701	2.768	0.328	3.154	1
2.800	2.734	0.312	3.152	1
2.900	2.700	0.297	3.147	0
3.000	2.668	0.283	3.145	0
L_t: 0.0080				
0.140	5.279	0.996	2.992	0
0.200	4.591	0.968	3.189	1
0.300	4.192	0.923	3.193	1
0.400	3.977	0.878	3.198	1
0.500	3.820	0.834	3.197	1
0.600	3.697	0.791	3.197	1
0.700	3.592	0.749	3.195	1
0.800	3.503	0.708	3.197	1
0.899	3.421	0.668	3.195	1
1.000	3.347	0.628	3.197	1

Vol. acid solution (ml.)	pH	g	pK	Acceptance*
1.100	3.277	0.591	3.195	1
1.200	3.211	0.555	3.193	1
1.300	3.148	0.521	3.189	1
1.400	3.090	0.488	3.188	1
1.501	3.032	0.457	3.183	1
1.600	2.979	0.428	3.181	1
1.700	2.927	0.401	3.177	1
1.800	2.877	0.377	3.171	1
1.899	2.830	0.354	3.166	1
2.000	2.783	0.334	3.158	1

Average value for the acid dissociation constant of

D-glucuronic acid: 3.19

(Standard deviation: 0.015)

Number of determinations averaged: 241.

B. <u>D-galacturonic acid</u>

Ionic strength: 0.1 mol/l in potassium chloride.

Concentration of hydrochloric acid solution: 0.1001 mol/l.

Vol. acid solution (ml.)	pH	g	pK	Acceptance*
L_t: 0.01199				
0.160	5.715	0.988	3.939	0
0.200	5.086	0.975	3.637	0
0.300	4.627	0.942	3.556	0
0.400	4.389	0.910	3.527	0
0.500	4.225	0.878	3.511	1
0.600	4.104	0.845	3.508	1
0.700	3.997	0.814	3.498	1
0.799	3.906	0.782	3.492	1

Vol. acid solution (ml.)	pH	g	pK	Acceptance*
0.900	3.829	0.750	3.492	1
1.000	3.752	0.719	3.483	1
1.100	3.689	0.688	3.485	1
1.201	3.626	0.657	3.483	1
1.300	3.570	0.627	3.483	1
1.400	3.512	0.598	3.479	1
1.560	3.428	0.551	3.478	1
1.599	3.408	0.539	3.478	1
1.640	3.387	0.528	3.477	1
1.699	3.358	0.511	3.477	1
1.799	3.308	0.483	3.475	1
1.900	3.256	0.456	3.470	1
2.000	3.208	0.430	3.467	1
2.099	3.161	0.405	3.465	1
2.200	3.113	0.380	3.461	1
2.300	3.070	0.356	3.463	1
2.400	3.024	0.334	3.459	1
2.500	2.978	0.313	3.454	1
2.600	2.934	0.293	3.451	1
2.701	2.890	0.275	3.446	0
2.800	2.849	0.257	3.443	0
2.900	2.806	0.242	3.435	0
3.000	2.765	0.228	3.427	0
L_t: 0.00605				
0.180	5.102	0.974	3.671	0
0.220	4.710	0.950	3.581	0
0.300	4.352	0.901	3.540	0

Vol. acid solution (ml.)	pH	g	pK	Acceptance *
0.400	4.092	0.841	3.515	1
0.501	3.913	0.781	3.505	1
0.599	3.781	0.724	3.506	1
0.700	3.660	0.668	3.500	1
0.800	3.557	0.614	3.500	1
0.860	3.499	0.582	3.499	1
0.920	3.444	0.551	3.498	1
0.979	3.391	0.522	3.496	1
1.040	3.340	0.492	3.496	1
1.100	3.289	0.465	3.492	1
1.200	3.211	0.421	3.491	1
1.301	3.132	0.382	3.483	1
1.400	3.063	0.345	3.482	1
1.499	2.995	0.314	3.476	1
1.601	2.928	0.285	3.467	1

Average value for the acid dissociation constant of D-galacturonic acid: 3.49

(Standard deviation: 0.015)

Number of determinations averaged: 166

C. Methyl α-D-glucuronoside

Ionic strength: 0.1 mol/l in potassium nitrate

Concentration of nitric acid solution: 1.000 mol/l.

Vol. acid solution (ml.)	pH	g	pK	Acceptance *
L_t: 0.0235				
0.020	6.026	0.986	4.224	0
0.040	4.727	0.954	3.473	0

Vol. acid solution (ml.)	pH	g	pK	Acceptance*
0.080	4.152	0.890	3.306	0
0.120	3.869	0.826	3.251	1
0.160	3.674	0.764	3.223	1
0.200	3.517	0.703	3.202	1
0.240	3.381	0.644	3.184	1
0.280	3.260	0.586	3.169	1
0.320	3.148	0.531	3.154	1
0.360	3.042	0.478	3.140	1
0.400	2.941	0.429	3.125	1
0.440	2.845	0.384	3.111	1
0.480	2.751	0.343	3.093	1
0.520	2.661	0.308	3.073	1
0.560	2.573	0.279	3.045	1

Average value for the acid dissociation constant of methyl α-D-glucuronoside: 3.16

(Standard deviation : 0.057)

Number of determinations averaged: 94

D. Methyl β-D-galacturonoside

Ionic strength: 0.1 mol/l in potassium nitrate

Concentration of nitric acid solution: 1.0035 mol/l.

	Vol. acid solution (ml.)	pH	g	pK	Acceptance*
L_t: 0.00385	0.015	4.739	0.940	3.691	0
	0.020	4.364	0.894	3.584	1
	0.025	4.148	0.849	3.544	1
	0.030	3.984	0.805	3.512	1

Vol. acid solution (ml.)	pH	g	pK	Acceptance*
0.035	3.856	0.763	3.492	1
0.0402	3.744	0.721	3.477	1
0.045	3.654	0.683	3.465	1
0.050	3.571	0.645	3.456	1
0.055	3.499	0.607	3.454	1
0.060	3.430	0.572	3.448	1
0.065	3.366	0.538	3.443	1
0.070	3.307	0.506	3.441	1
0.075	3.198	0.447	3.434	1
0.080	3.146	0.422	3.428	1
0.085	3.146	0.422	3.428	1
0.090	3.098	0.397	3.424	1
0.095	3.054	0.373	3.424	1

Average value for the dissociation constant of methyl β-D-galacturonoside: 3.48

(Standard deviation: 0.057)

Number of determinations averaged: 91

[* The Acceptance column signifies whether the value was included in the final average by computer program IONCONST (see Appendix)].

CHAPTER 3

STABILITIES OF URONATE COMPLEXES: CATION RESPONSIVE ELECTRODE STUDIES

Stabilities of Uronate Complexes: Cation Responsive Electrode Studies.

Although the majority of complex stability constants in aqueous solution have been determined by measuring changes in the pH of the solution due to complexing using a conventional pH electrode[57] (see Chap. 4), this method must be used in the pH range within which significant concentrations of protonated and dissociated ligand occur, permitting the greatest possible changes in the pH on complexing. When the ligand is a strong or moderately strong acid and the metal complexes have low stability (a reported attempt at determining stability constants for complexes of this type concluded that no complexing took place[58]), the change in solution acidity on complexing is extremely small, reducing the accuracy of measurement and hence the potential accuracy of the stability constant determination.

It is therefore preferable, in order to improve the accuracy of determination, to adopt a method in which the concentration of complex, free ligand or free metal, can be measured directly. Two such methods have been adopted in which this principle is used. In this chapter, the concentration of metal ion remaining uncomplexed is determined directly using cation responsive electrodes. The second method (Chap. 5) involves the measurement of ligand in complexed and uncomplexed forms using a polarimetric determination, a method applicable only to studies involving optically active ligands.

The complexes between various bivalent and univalent cations and uronic acids have been studied and stability constants determined. A potassium-responsive glass electrode was used in the study of complexing between potassium ions and D-glucuronic acid and D-galacturonic acid and liquid ion-exchange membrane electrodes were used in the study of bivalent ion complexes with various uronic acids and uronosides.

3 . 1 Potassium Complexes

An equilibrium, similar to that in the dissociation of the acids (Chap. 2), exists between potassium ions and the dissociated acid in which the associated species is a potassium-acid complex.

$$K^+ + L^- \rightleftharpoons KL$$

The equilibrium constant, here known as a formation or stability constant, is given by:-

$$K = \frac{(KL)}{(K^+)\ (L^-)}$$

where KL is the potassium-acid complex and L^- is the dissociated acid.

The method employed to determine the concentration of the species present in the solution must be extremely sensitive, as the complexes formed between potassium ions and ligands are of relatively low stability. Since a method which makes direct measurement of one of the species is likely to reduce the sources of error, an electrode sensitive to potassium ions was used.

The method[59] adopted was one of potential comparison which involved the adjustment of the alkali metal concentration of a solution containing a known concentration of complexing ligand by the incremental addition of a concentrated solution of alkali metal ions until the potential of the complex-containing solution became exactly equal to that of a solution containing only a known concentration of alkali metal ions.
All solutions used in this set of determinations of the

formation constant were prepared in 0.1 mol/l. 2-amino-2-(hydroxymethyl)-1,3-propanediol ('Tris') buffer. This fulfilled three functions; namely, to ensure that the ligand was fully in the dissociated form in order to eliminate other equilibria, to maintain the concentration of hydrogen ions at a sufficiently low level that no electrode interference was observed, and to maintain a constant strength of background electrolyte. The choice of buffer solution was made because 'Tris' effectively does not complex with ligands of the type in present study and does not affect the electrode response. Under these conditions, the concentrations as well as the activities of the potassium ions in the two solutions could be considered to be equal. Then, from a knowledge of the potassium ion concentration in the complex--containing solution and the total concentration of potassium ions present, the concentration of complex was calculated. From this and total concentration of ligand, the concentration of free dissociated ligand was calculated enabling a value for the stability constant to be determined.

The reference electrodes used throughout were of the silver/silver chloride type. These are more reproducible than other types since they effectively eliminate errors due to liquid junction fluctuation (see section 4.4).

Results

The standard solution was prepared by diluting a known volume of a standard potassium chloride solution

(prepared in 'Tris' buffer as described in section 4.4), with 'Tris' buffer to a total volume of 30 mls. This solution, which was allowed to attain thermal equilibrium by immersion in a water bath, was used as defining the potential recorded by the electrodes at a known potassium activity. Since the ionic strength was known and was effectively identical to that of the test solution, comparison not only of potassium activity between the solutions when the same potential was recorded but also of potassium concentration was possible.

A known weight of ligand was dissolved in 'Tris' buffer to give a stock ligand solution. An aliquot of this solution was diluted to 30 mls. with 'Tris' buffer to give the test solution which was brought to thermal equilibrium in the water bath.

The incremental procedure described above was used, giving the results tabulated in Table 3.1.

Discussion

The small stability constant for the potassium glucuronate complex is a measure of the weak association between the species. It is to be expected that any association involving potassium ions would be relatively weak since the metal has no suitable orbitals to permit bonding. This leaves ion pairing of the oppositely charged species as the sole binding force.

The results obtained for this associated species are of similar order to those obtained by Rechnitz and Zamochnick[59] for sodium malate. In that case also, the association can only be maintained by ion pair formation

since sodium is unable to form covalent bonds to ligands to stabilise the resulting complex.

However, although the association is extremely weak and the concentration of the complexed species present in any solution will be small, allowance must be made for the presence of the complex in all calculations of equilibrium constants involving uronic acids, when substantial concentrations of potassium ions are present.

The effect of alkali metal ions on the measured dissociation constants for uronic acids has been mentioned in Chapter 2, and it will be seen in the next section that the effect is even more marked for the determination of stability constants of the complexes of divalent metals, as they are only about 5-15 times larger than the constants determined here.

TABLE 3.1 Stability Constant Data for Potassium Uronate Complexes

	Potassium Galacturonate.[44]			
Determination No.	1	2	3	4
Standard Solution				
Volume of Potassium soln. (ml)	10	10	10	10
Concentration of Potassium ions present (mol/l)	0.001665	0.001665	0.001665	0.001665
Ionic Strength	0.102	0.102	0.102	0.102
Test Solution (at balance point)				
Volume of Potassium soln (ml)	0.115	0.100	0.098	0.099
Total concentration of Potassium ions present (mol/l)	0.001678	0.001676	0.001676	0.001675
Ionic Strength	0.103	0.103	0.103	0.103

Test Solution (at balance point)	1	2	3	4
Concentration of Potassium Uronate complex (mol/l)	0.000013	0.000011	0.000011	0.000011
Total ligand Concentration (mol/l)	0.001668	0.001668	0.001659	0.001660
Concentration of free ligand (mol/l)	0.001655	0.001657	0.001648	0.001649
Concentration Stability constant of potassium uronate	4.7	4.0	4.0	4.1

	Potassium Glucuronate					
Determination No.	1	2	3	4	5	6
Standard Solution						
Volume of Potassium soln. (ml)	5	5	5	2	2	2
Concentration of Potassium ions present (mol/l	0.008345	0.008345	0.008345	0.003339	0.003339	0.003339
Ionic Strength	0.108	0.108	0.108	0.103	0.103	0.103
Test Solution (at balance point)						
Volume of Potassium soln. (ml)	0.3424	0.3410	0.3419	0.1352	0.1350	0.1350
Total concentration of Potassium ions present (mol/l)	0.008463	0.008429	0.008451	0.003363	0.003359	0.003359
Ionic Strength	0.117	0.117	0.116	0.107	0.107	0.107
Concentration of Potassium uronate complex (mol/l)	0.000118	0.000084	0.000106	0.000024	0.000020	0.000020

Test Solution (at balance point	1	2	3	4	5	6
Total Ligand concentration(mol/l)	0.008584	0.008366	0.008233	0.003379	0.003373	0.003374
Concentration of free ligand (mol/l)	0.008468	0.008282	0.008127	0.003355	0.003353	0.003354
Concentration Stability Constant of Potassium Uronate	1.7	1.2	1.6	2.1	1.8	1.8

Average value of the concentration stability constants:-

Potassium D-glucoronate - 1.7

Potassium D-galacturonate - 4.2

3 . 2 Bivalent Cation Responsive Electrodes

Since the majority of complexes which are of importance in the present investigation are weak complexes formed between uronic acids and bivalent ions, it is advantageous to adopt a direct-measurement method similar to that for potassium complexes (section 3.1). It is claimed[60] that a recently developed type of bivalent-ion electrode based on a liquid-liquid membrane principle gives a theoretical electrode response for a number of ions over a wide range of activities with good ion selectivity over univalent or other bivalent ions. Two electrodes of this type were tested for response against different bivalent cations and for selectivity against these cations in the presence of interfering ions before being used in the determination of metal complex stability constants.

The two types of electrode which were employed were obtained from Orion Research Inc. - Model 92-20 Calcium Ion Activity Electrode and Model 92-32 Water Hardness Electrode. The electrodes detect bivalent cations by means of the potential caused by the difference between the metal ion activity in the test solution and that of the internal filling solution being developed across a thin layer of a water immiscible liquid ion exchanger held in place by a porous membrane disc. This is similar to the detection of hydrogen ions using a conventional glass pH electrode, where the potential is developed across a thin glass membrane, having ion-exchange properties.

The internal structure of the electrode is shown in Fig. 3.1 in which it is compared with that of a glass electrode. It contains an aqueous filling solution which contains fixed levels of calcium and chloride ions. The calcium ions provide a stable potential between the inside surface of the porous membrane, which is saturated with ion exchanger, and the internal filling solution. The chloride ions in the reference electrode filling solution provide a constant potential between the filling solution and the internal reference electrode, which is of the silver/silver chloride type (as described in section 4.4). Changes in potential which are measured are therefore due only to changes in potential across the membrane which correspond to changes in the test sample bivalent cation activity. The potentials are measured relative to an external reference electrode of either silver/silver chloride, or calomel with liquid junction.

Calibration of Electrode

All electrodes respond to a particular ion in a manner given by the expression:

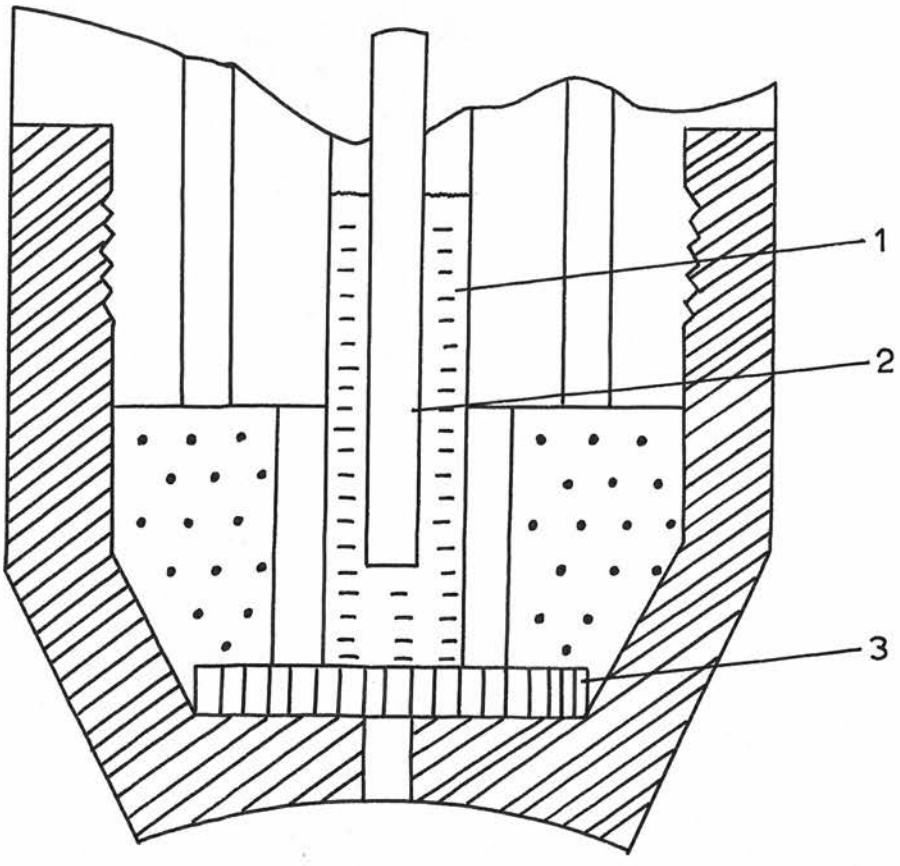
$$E = E_o + \frac{RT}{zF} \ln (M^{z+}) \quad \ldots\ldots\ldots \quad (3.1)$$

where E is the measured potential from the electrode set.

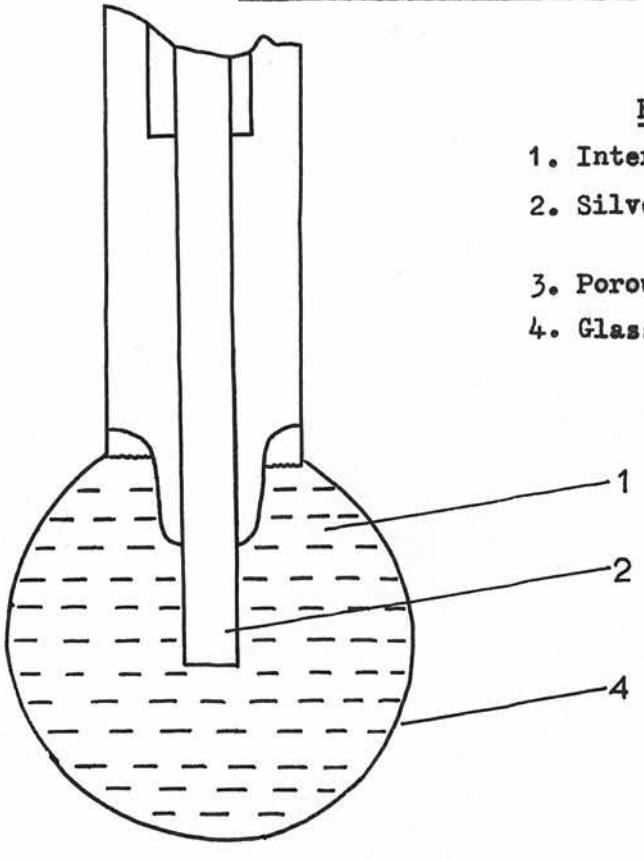
E_o is the potential which would be recorded for the solute in its standard state and is characteristic of the ion responsive electrode and the reference electrode,

R and F are constants, ($R = 8.317$ J $mol^{-1} k^{-1}$,

$F = 9.652 \times 10^4$ V)



Liquid Membrane Electrode



Glass pH Electrode

KEY

1. Internal aqueous filling solution
2. Silver/silver chloride reference electrode
3. Porous membrane
4. Glass membrane

Fig. 3.1

T is the absolute temperature

z is the charge on the ion

and (M^{z+}) is the activity of the ion.

If a reference electrode whose response is independent of ionic concentrations in the test solution is used, it follows that a linear relationship should exist between measured potential and the logarithm of the activity of the ion to which the electrode is responsive.

The slope of such a graph is given by $\frac{(2.303\ RT)}{zF}$ and the intercept by E_o. For bivalent ions at 298K, the theoretical slope is 29.58 mV for each tenfold change in the ion activity and this is used as a measure of the electrode response to that ion.

When cation concentration, and not activity, is to be measured, it is necessary to convert equation 3.1 into the following form:-

$$E = E_o + \frac{RT}{zF} \ln [M^{z+}] .f \qquad \ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

where f is a suitable activity coefficient for the cation M^{z+}.

A graph of electrode response against the logarithm of the cation concentration is a curve which at low ionic strengths approximates to the straight line of slope $\frac{(2.303\ RT)}{(zF)}$ above, but which becomes increasingly divergent with increasing ionic strength on account of the decreasing value for the activity coefficient.

Graphs were plotted for several metal ions using the water hardness electrode and for calcium ions using the calcium responsive electrode. In addition, a graph

of electrode response against calcium concentration in the presence of a constant ionic strength background electrolyte was plotted.

Results

Both electrodes were regularly calibrated for response against the cations used in the present study by immersion in a solution of the nitrate salt of the cation at 298.0 ± 0.1K followed by the addition of successive aliquots of a more concentrated salt solution. The electrode potential after each addition was recorded and graphs were plotted of these measurements against the logarithm of the cation concentration in the solution. The curves obtained were similar for a particular combination of electrode and cation but the absolute magnitude of the potential varied with factors such as the age of the electrode, the depth of immersion of the electrode and the stirring speed of the solution (see also ref. 61). Typical curves are shown in Fig. 3.2. Similar curves are shown in Fig. 3.3 in which there is a constant ionic strength due to the presence of a background electrolyte.

The departure from the theoretical linear relationship between the electrode response and the logarithm of the cation activity was used as a measure of the response of the electrode to that ion. Clearly it is necessary to select a suitable activity coefficient and this choice was discussed in Chapter 1. Typical graphs of electrode potential against the logarithm of the cation activity

Table 3.2 Calibration Data - Calcium

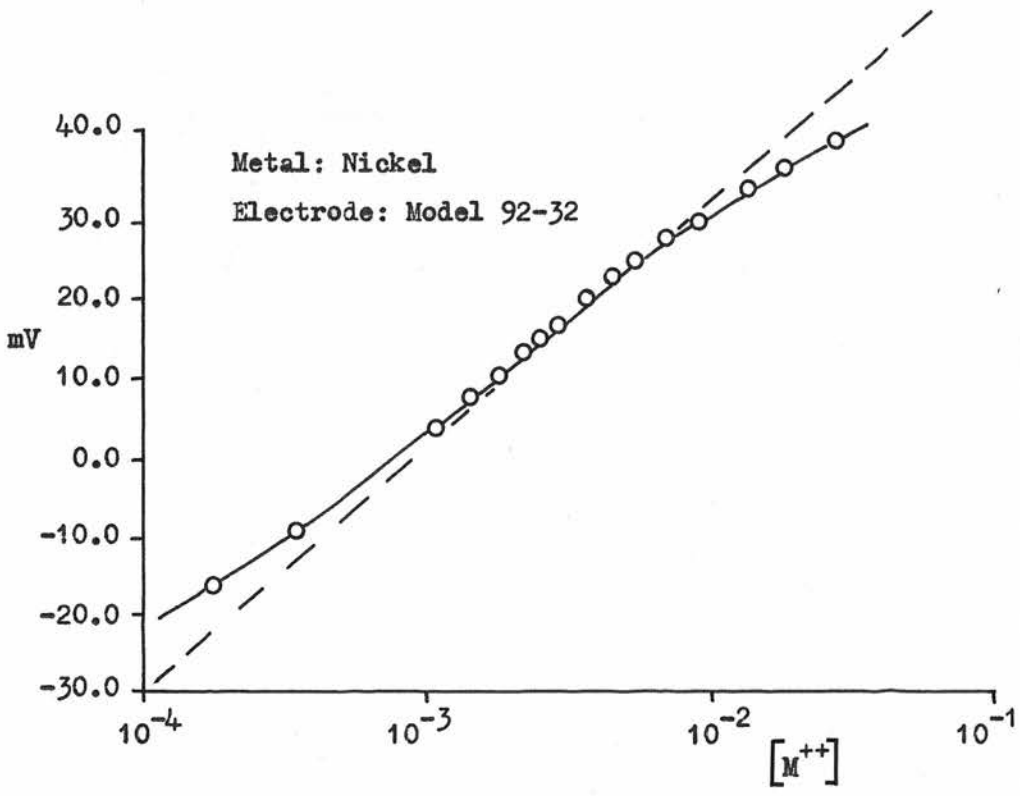
Electrode : Model 92.20 (Orion Research Inc.)

pH : 7 Temperature : 25.0 ± 0.1°C

Initial volume: 50.0 mls.

Concentration of calcium nitrate solution : 1.0721 mol/l.

Vol. added	$[Ca^{++}]$	Potential (mV)
0.005	1.072×10^{-4}	-22.8
0.010	2.144×10^{-4}	-18.6
0.020	4.287×10^{-4}	-12.25
0.040	8.570×10^{-4}	-4.75
0.060	1.285×10^{-3}	0.2
0.080	1.713×10^{-3}	3.4
0.100	2.140×10^{-3}	5.5
0.120	2.567×10^{-3}	7.3
0.140	2.994×10^{-3}	8.9
0.170	3.633×10^{-3}	11.0
0.200	4.271×10^{-3}	12.6
0.250	5.334×10^{-3}	14.9
0.300	6.394×10^{-3}	16.8
0.400	8.509×10^{-3}	19.65
0.500	1.061×10^{-2}	21.8



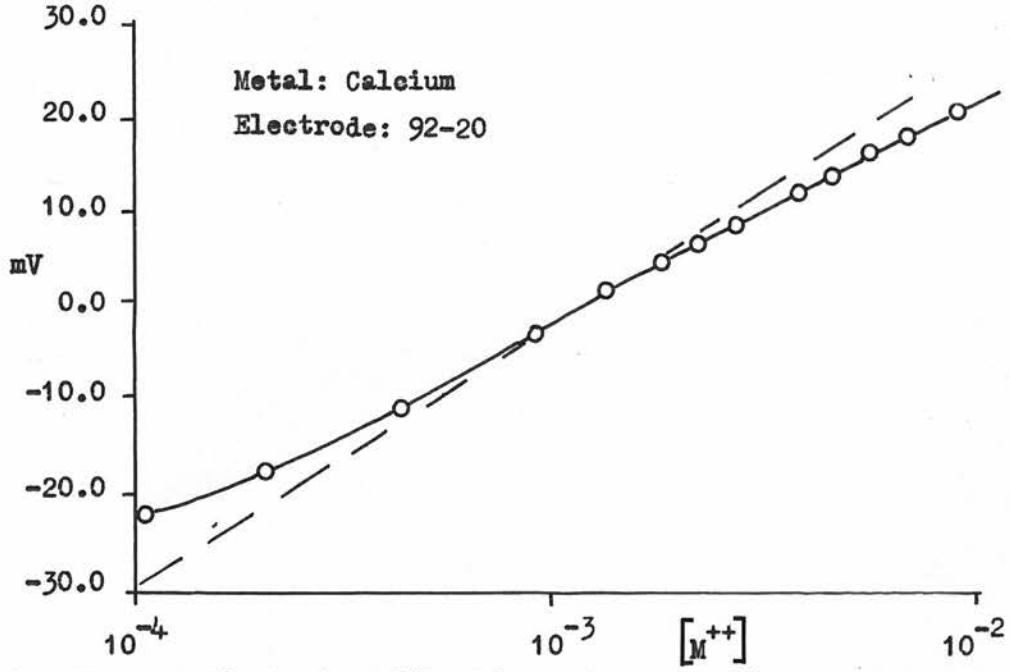
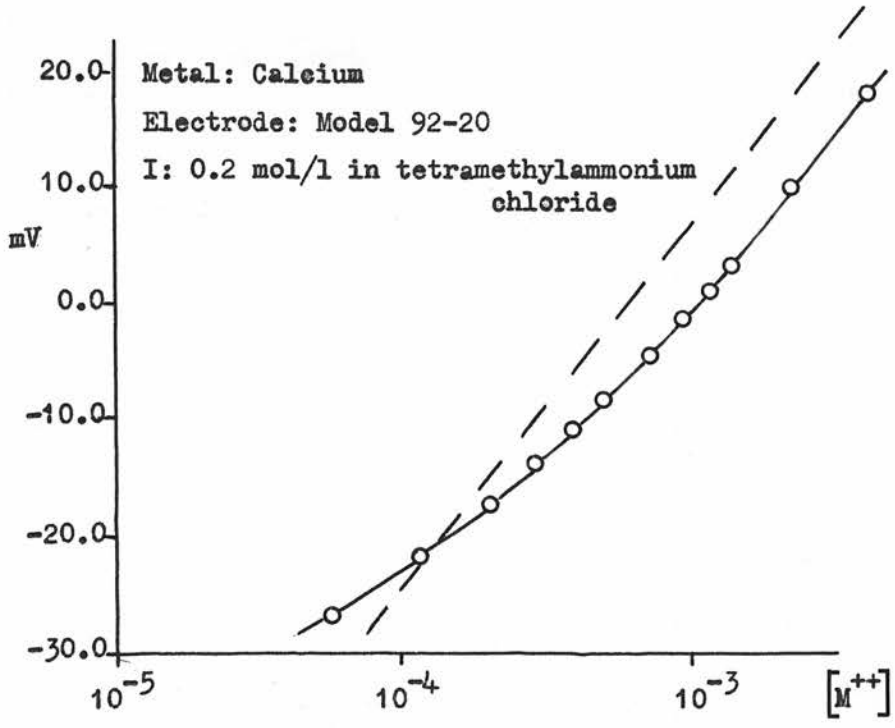


Fig. 3.2 - Electrode Calibration - Concentration



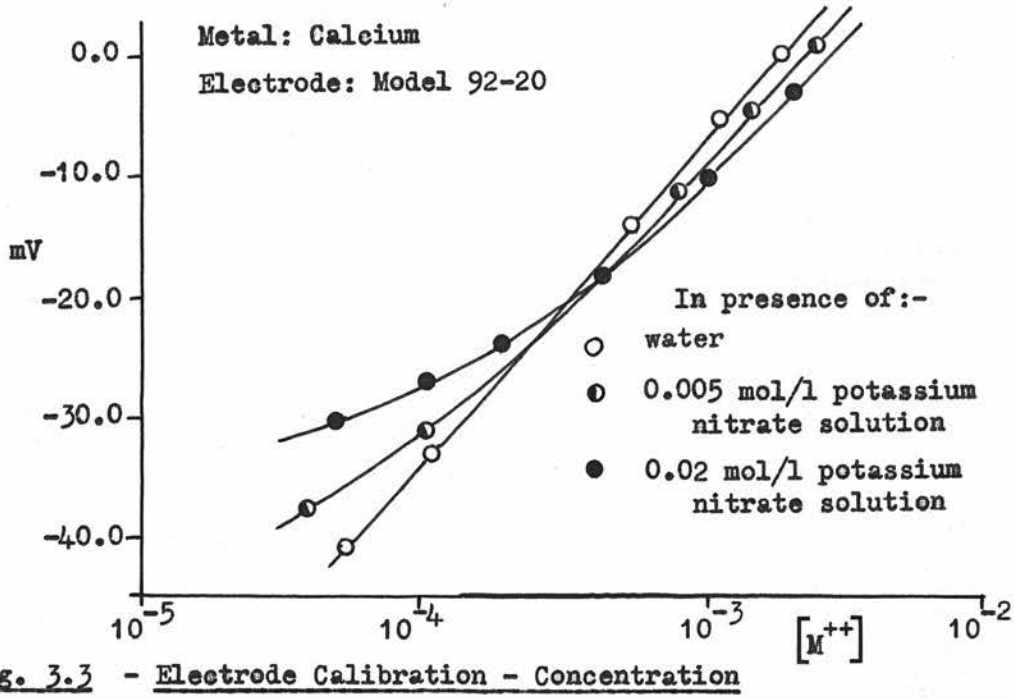


Fig. 3.3 - Electrode Calibration - Concentration
(in presence of background electrolytes)

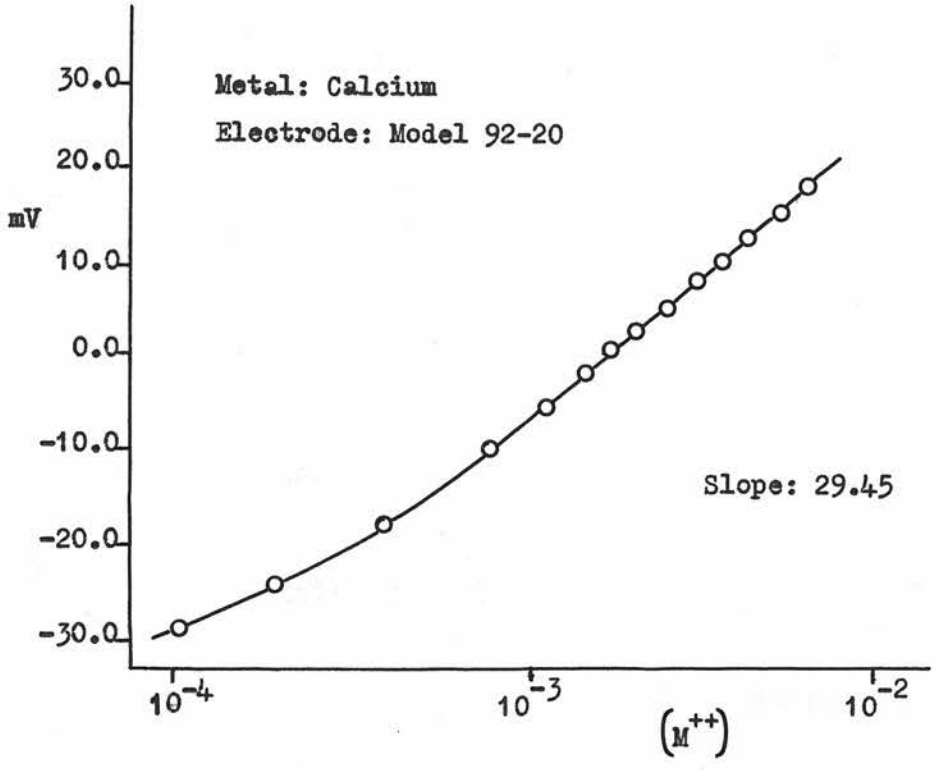


Fig. 3.4 Electrode Calibration - Activity

(calculated from the cation concentration and the relevant activity coefficient) are shown in Fig. 3.4. From these the actual slope was compared with the theoretical slope of 29.58 mV for each tenfold change in ion activity. The results showed each electrode responds to the cations under investigation in a manner similar to that expressed in equation 3.1. Results from typical calibration tests are tabulated in Table 3.2.

Discussion

Calibration data with electrodes of this type have been reported with several cations but the majority of data were collected for Model 92-20 as a test of the response to calcium ions. The results from some of these are summarised in Table 3.3 for comparison together with the choice of activity coefficient and the range over which linearity of response was obtained.

Although some variations in the slope are observed in Table 3.3, all determinations give linear response of the electrode to calcium ions in the range $1.0 \leq pCa \leq 4.0$. This confirms that, in this activity range, this electrode responds satisfactorily to calcium ions.

Selectivity:

The response of liquid ion exchange membrane electrodes to bivalent cations according to expression 3.1, however, applies not only to calcium or magnesium ions but also to any other individual bivalent cations. The absolute magnitude of the electrode potential measured at a fixed activity of various different ions

TABLE 3.3 Comparison of Calibration Data for Electrode Model 92-20

	Orion Research[60] Instruction Manual = 92-20	Ross[62]	Rechnitz and Lin[61]	Shatkay[63]	Present Work
Slope ($mV/(M^{++})$)	29.58	29.3	29.58	26.5	29.45
Range of Linearity	$10^{-4} \leq (Ca^{++}) \leq 3 \times 10^{-1}$	$10^{-4} \leq (Ca^{++}) \leq 1$	$10^{-4} \leq (Ca^{++}) \leq 10^{-1}$	$5 \times 10^{-5} \leq [Ca^{++}] \leq 1$	$10^{-3} \leq (Ca^{++}) \leq 10^{-1}$
Activity Coefficient	unspecified	Mean activity Coefficient for $CaCl_2$	unspecified	single ion	single ion

would differ and this would reflect the different values for the E_o term but changes in the potential, or the slope of the calibration line, with changing activity for any cation would be similar.

If two or more bivalent ions are present in the same solution, the electrode response is given by:-

$$E = E_o + 2.303 \frac{RT}{2F} \log \quad (M_1^{++}) + k_2(M_2^{++}) + k_3(M_3^{++}) + \ldots \quad (3.3)$$

where k_2, k_3, etc. are selectivity constants for the species M_2, M_3, etc, compared with M_1 taken as standard. The electrode responds more or less strongly to different bivalent cations and this is expressed in the value for the selectivity constant.

On the other hand, if the solution contains a mixture of bivalent and univalent cations, the electrode response is given by:-

$$E = E_o + 2.303 \frac{RT}{2F} \log (M^{++}) + 2.303 \frac{RT}{F} \log k_N (N^+) \quad \ldots\ (3.4)$$

or

$$E = E_o + 2.303 \frac{RT}{2F} \log (M^{++}) + k'_N(N^+)^2 \quad \ldots\ (3.5)$$

which includes the electrode response to the univalent ion. The relative response is expressed in the selectivity constant, k'_N.

The effect of any foreign cation should be to change the electrode response in a positive direction by an amount which will be related to the concentration of that ion and to its selectivity constant. Detectable changes in the potential due to the presence of univalent ions should only be observed if the concentration of the univalent cation is relatively high as the activity appears as a squared term in equn. 3.5.

An alternative relationship has been proposed to express the electrode response in presence of foreign ions, particularly in concentrated solutions[64].
In this case, the selectivity considered was that of the electrode for calcium ions in the presence of sodium ions. The electrode response is given by:-

$$E = E_o + \frac{RT}{2F} \ln m_2 (m_1 + 2m_2)^2 f_{21}^3 + K m_1^2 (m_1 + 2m_2)^2 f_{12}^4$$

where m_1 is the molarity of the sodium chloride solution, m_2 is the molarity of the calcium chloride solution, f_{12} and f_{21} are the mean activity coefficients for sodium chloride and calcium chloride in the mixed electrolyte respectively and K is the selectivity constant. It was not necessary to use an expression of this type in the present work since expressions such as 3.3 and 3.5 adequately describe electrode response in solutions used.

An explanation of increased selectivity constant in the presence of high concentrations of sodium chloride is also proposed[64]. If a species such as CaClR (where R is the organic resin supported by the membrane) is transported across the organic phase instead of CaR_2 at high chloride concentrations, this would cause the electrode to become permeable to chloride ions and destroy the theoretical response to calcium ions. Furthermore, the species CaClR would be expected to be more readily interchangeable with NaR causing the selectivity of the electrode for calcium ions over sodium ions to decrease (and the selectivity constant to increase) since this selectivity depends on the preferential formation of the calcium complex CaR_2.

Results

(a) Response in the presence of hydrogen ions

The electrode potential measured in solutions containing a fixed concentration of copper ions but at different pH levels was plotted against the pH of the solution. This was carried out using the Orion Model 92-32 Water Hardness Electrode.

The results, shown in Fig. 3.5, show that the permitted level of hydrogen ions varied with different bivalent ion concentrations. Thus, at a copper ion concentration of about 1×10^{-2} mol/l, solution acidities of pH 3.5 could be tolerated without significant interference while at a copper concentration of 1×10^{-3} mol/l, the hydrogen ion concentration could not exceed 10^{-4} mol/l without interference.

Since similar values for the selectivity constant of this electrode for hydrogen ions in the presence of other bivalent cations have been reported (see Table 3.5), it was concluded that all determinations should be carried out in the range pH 6-9 to avoid interference by hydrogen ions.

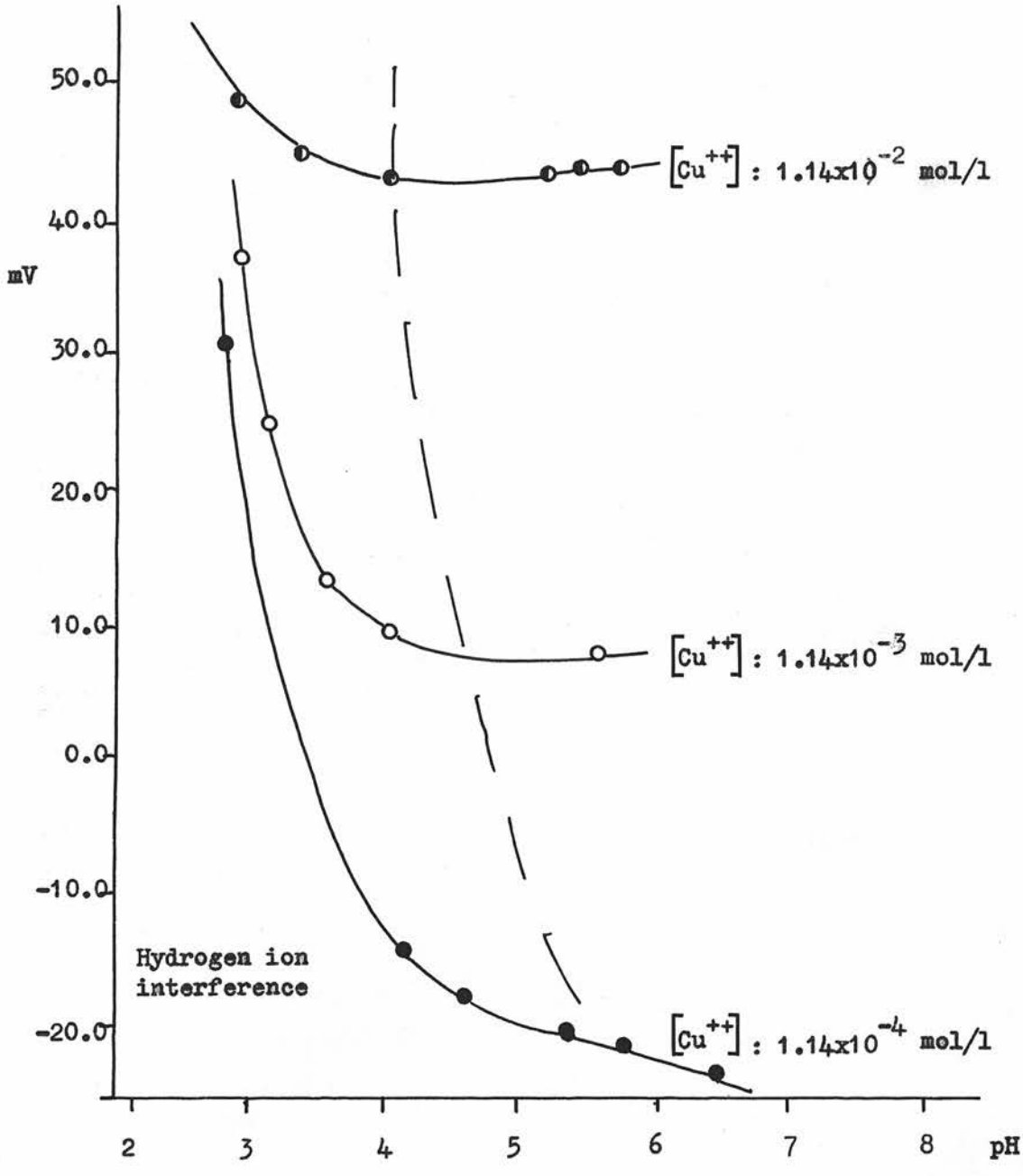
(b) Response of Water Hardness Electrode to Potassium Ions in Nickel Nitrate Solutions

The electrode response to changes in the concentration of potassium ions in a solution containing a constant amount of nickel nitrate solution was determined.

The observed electrode potential was used to obtain the apparent nickel ion activity, $(Ni^{2+})_{app.}$ from a cation

Fig. 3.5 Effect of pH on response of the liquid membrane electrode

Metal: Copper
Electrode: Model 92-32



activity calibration curve and this, in conjunction with the actual nickel ion activity (Ni^{2+}), and the potassium ion activity, (K^+), (calculated from the cation concentrations and the single-ion activity coefficient) was used in the calculation of a selectivity constant, k_{K-N}. Since from equations 3.1 and 3.5:-

$$(Ni^{2+})_{app.} = (Ni^{2+}) + k_{K-Ni}(K^+)^2$$

a value can be determined for the selectivity constant:-

$$k_{K-Ni} = \frac{(Ni^{2+})_{app.} - (Ni^{2+})}{(K^+)^2}$$

Some typical electrode potential results for nickel ions in the presence of potassium ions are listed in Table 3.4. The average selectivity constant with this electrode to potassium ions in nickel solutions is 0.080.

(c) Response of Calcium Ion Selective Electrode to Potassium Ions in Calcium Ion Solutions

An alternative method of considering the selectivity of the electrode was used with the calcium ion selective electrode (Model 92-20). Potassium nitrate solution of similar concentration to that of the potassium uronate solution to be used in the stability constant determination was added by syringe to a calcium nitrate solution and the electrode response recorded (see Section 4.4 for method). This curve was considered as a standard correction curve for the metal complex studies using similar conditions.
The change in electrode response due to the addition of a known volume of potassium nitrate solution was subtracted from the change in electrode response due to the addition

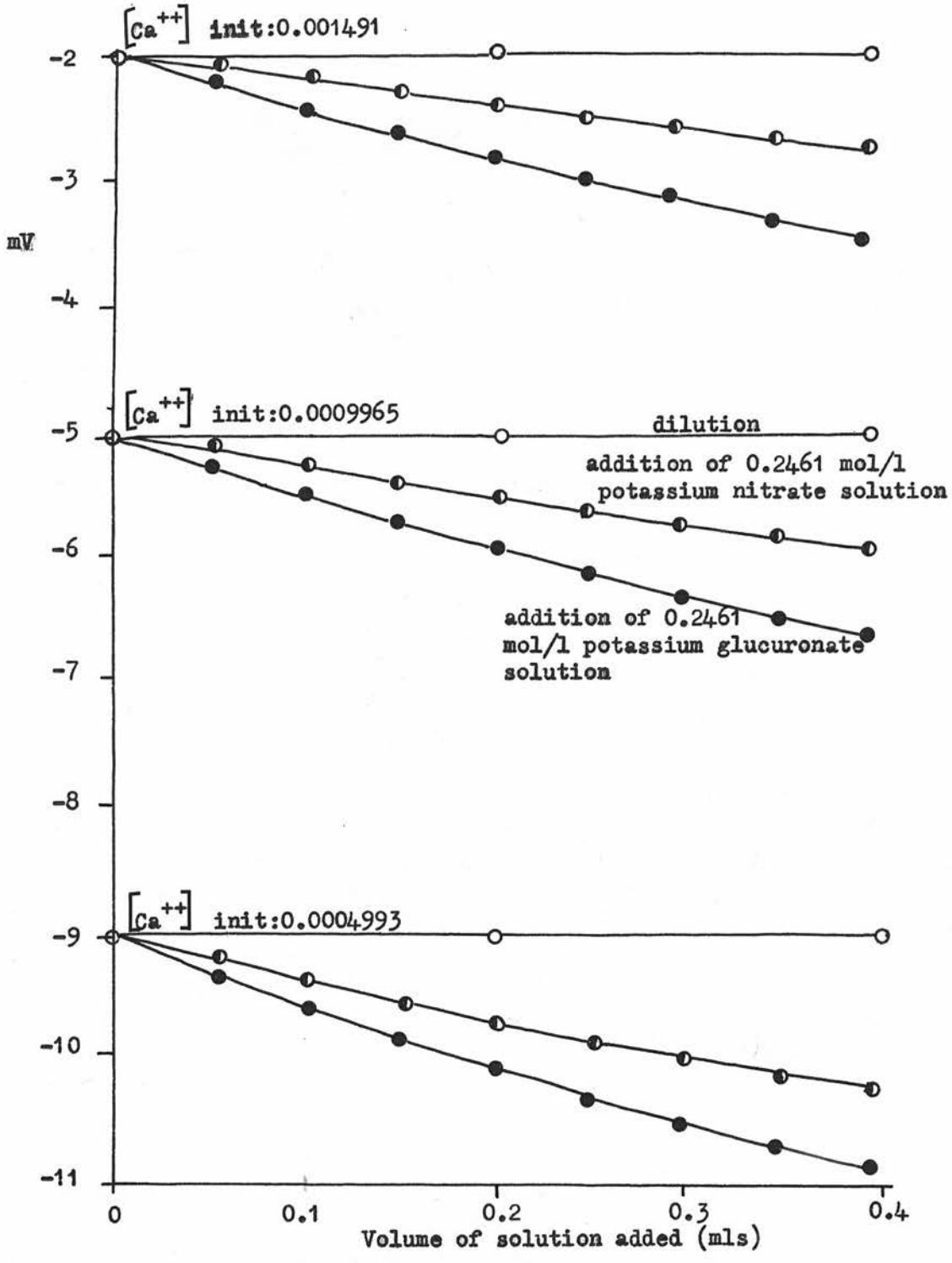


Fig. 3.6 Addition of different solutions to calcium nitrate solution

of an identical volume of potassium uronate solution and the difference was taken as the change in potential due to complex formation.

In fig. 3.6 typical curves are shown illustrating the change in potential due to the addition of water, potassium nitrate solution and potassium uronate solution to a solution of calcium nitrate.

A value for the selectivity constant, k_{K-Ca}, has been calculated as in Section (b) above and the value was 0.015.

(d) Response of Calcium Ion Selective Electrode to Tetramethyl Ammonium Ions in Calcium Ion Solutions

The response of the calcium ion selective electrode (Model 92-20) in the presence of tetramethyl ammonium ions was determined by an identical method to that in Section (b). The selectivity constant, k_{T-Ca} was 0.024.

Some typical electrode potential results are listed in Table 3.4.

Some reported values for selectivity constants are given in Table 3.5.

Table 3.4 Selectivity Constant Data

Cation: Nickel Foreign ion: Potassium

Electrode: Model 92-32.

$[Ni^{++}]$	$[K^{+}]$	Electrode Potential(mV)	k_{K-Ni}
0.00275	-	14.1	-
0.00269	0.0196	-3.3	0.084
0.00264	0.0385	-4.9	0.090
0.00259	0.0564	-6.8	0.073
0.00254	0.0737	-6.4	0.094
0.00250	0.0905	-5.7	0.094
0.00245	0.1069	-5.2	0.086
0.00241	0.1227	-4.65	0.081
0.00275	-	14.2	-
0.00269	0.0196	-3.2	(0.46)
0.00264	0.0384	-5.1	(0.18)
0.00259	0.0563	-6.95	0.069
0.00254	0.0739	-6.5	0.092
0.00250	0.0907	5.95	0.091
0.00247	0.1067	-5.35	0.086
0.00241	0.1125	-4.65	0.082

Average value for k_{K-Ni} : 0.080

Table 3.4 (contd.)

Cation: Calcium Foreign ion: Tetramethylammonium

Electrode: Model 92-20

$[Ca^{++}]$	$[T^{+}]$	k_{T-Ca}
0.0001207	-	0.070
0.0001201	0.00856	0.011
0.0001199	0.01281	0.011
0.0001196	0.01705	0.011
0.0001193	0.02126	0.012
0.0001188	0.02964	0.010
0.0001180	0.04206	0.007
0.0002412	-	-
0.0002401	0.00856	0.023
0.0002396	0.01281	0.034
0.0002391	0.01704	0.031
0.0002385	0.02125	0.025
0.0002375	0.02962	0.019
0.0002359	0.04204	0.015
0.0004818	-	-
0.0004797	0.00855	0.017
0.0004786	0.01279	0.038
0.0004776	0.01702	0.043
0.0004765	0.02123	0.027
0.0004745	0.02959	0.023
0.0004714	0.04199	0.015

Average value for k_{T-Ca} : 0.024

Table 3.5 Selectivity Constants for Divalent Cation Responsive Electrode

Electrode	Cation	Foreign Ion	Selectivity Constant	Ref.
Model 92.20	Calcium	Hydrogen	10^{5}	62
	Calcium	Sodium	10^{-4}	62
	Calcium	Potassium	10^{-4}	62
	Calcium	Ammonium	10^{-4}	62
	Calcium	Magnesium	0.014	62
	Calcium	Barium	0.010	62
Model 92.32	Calcium	Magnesium	1.0	60
	Calcium	Barium	0.94	60

3 . 3 Metal Complex Studies

Having determined the sensitivity and selectivity of the electrodes, it is possible to employ the electrodes to determine change in cation activity due to complex formation on addition of ligand solution. Values can be calculated from these measurements for the stability constant of the complexes formed between the cation and ligand.

Initial studies were based on a method which involved transfer of the electrode between solutions. The first method comprised transferring the electrode through a range of solutions each containing the same metal concentration but each with a different ligand concentration. The results were related to a calibration curve for the cation (see Section 3.1) to give the free metal concentration in each solution allowing a determination of the stability constant from each.

Results from this method (see below) were not satisfactory because the electrode showed fluctuations in potential resulting in the potential recorded being irreproducible. To improve upon this, a method similar to that used in the determination of potassium complexes (Section 3.1) was adopted. In this way, measurements of divalent ion activity in ligand-containing solutions were always measured relative to a calibration point. However, there were two major drawbacks to this method - fluctuations in the potential on transfer of the electrode were too great to permit measurements to be made to a

sufficient accuracy and it was only possible to obtain single point determinations for the stability constant.

A determination of the suitability of a transfer method was carried out in which a divalent ion electrode was transferred through a series of solutions containing pure divalent ion solutions at a range of concentrations. The results, shown in Table 3.6, show that reproducibility is poor and that readings can only be obtained to an accuracy of about $\pm$ 0.1 - 0.15 mV. As will be shown in later results (see below), this error is relatively large when compared with the changes observed as a result of complexing. Furthermore, the reproducibility achieved in mixed solutions of ligand and metal ions is poorer.
It was therefore decided to develop a method which avoided the need for electrode transfer.

A further consideration was that the reproducibility of the electrodes in quaternary ammonium salt solutions was poor. As a result, subsequent determinations were carried out in the absence of such cations.

Such a method, involving no transfer of the divalent ion electrode is to add aliquots of ligand solution to a pure solution of the divalent ion and to record the change in potential. This method showed the advantage over the transfer methods of lesser electrode potential drift.
It also allowed a single point calibration of the electrode response for every titration from the potential measured in the starting solution which comprised pure metal ion solution.

Table 3.6. Reproducibility of Water Hardness Electrode - Transfer Method

Bivalent ion electrode: Orion Model 92-32

Reference electrode : Ag/AgCl type

Test 1

Solution	1	2	3	4	5
Calcium chloride concentration (mol/l)	5×10^{-4}	1×10^{-3}	2×10^{-3}	4×10^{-3}	8×10^{-3}
Solution	1	2	3	4	5
Potential (mV)	-159.9	-135.7	-110.7	-87.2	-63.8
	-156.4	-133.8	-110.2	-87.2	-
	-	-133.7	-109.7	-86.0	-63.0
	-	-	-109.7	-	-

Test 2 All solutions were prepared in an ionic medium of 0.1 mol/l tetramethylammonium chloride.

Solution	1	2	3	4
Calcium nitrate concentration (mol/l)	2.5×10^{-4}	5×10^{-4}	1×10^{-3}	2×10^{-3}
Solution	1	2	3	4
Potential	-45.75	-38.20	-30.50	-22.90
	-46.00	-38.25	-30.35	-
	-	-38.35	-30.60	-22.95
	-	-	-30.35	-

A slight modification of the method was required on account of the acidic nature of the ligands. Since the selectivity of the electrode in the presence of hydrogen ions was poor, alkali was added to neutralise the acid bringing the pH of the test solution into the range pH 6-9 (the optimum range for the electrode). The initial titrations with ligand involved separate addition of potassium hydroxide solution but this was modified and solutions of the potassium salt of the ligand were titrated (see Section 4.4).

The ligand titration technique required a calibration curve for the cation used in the relevant concentration range and a correction factor for the influence of potassium ions on this curve. With this method, reasonable reproducibility can be achieved provided external conditions are kept constant. Care was taken to avoid changes in electrode response due to fluctuations in any of these conditions.

The stability constants for the complexes between D-glucuronic acid and calcium and nickel, and D-galacturonic acid, methyl α-D-glucuronoside and methyl β-D-galacturonoside and calcium were determined by this method.

Method of Calculation

The primary equilibrium involved is similar to that in Section 3.1:-

$$M^{++} + L^{-} \rightleftharpoons ML^{+}$$

and the thermodynamic stability constant is given by:-

$$K_t = \frac{(ML^+)}{(M^{++})(L^-)} = \frac{[ML^+].f_{ML^+}}{(M^{++}).[L^-].f_{L^-}} \quad \ldots\ldots\ldots\ldots \quad (3.6)$$

The activity coefficients for the singly-charged species, ML^+ and L^-, are to a reasonable approximation identical which allows equn. 3.6 to be simplified to:-

$$K_t = \frac{[ML^+]}{(M^{++}).[L^-]} \qquad \ldots\ldots\ldots\ldots \quad (3.7)$$

Calibration of the electrode response to divalent ions has already been discussed (Section 3.2) in which the metal ion concentration was converted into the activity by means of a single ion activity coefficient.
The electrode potential recorded on the addition of each aliquot of ligand solution after correction for potassium ion interference was related to this calibration curve giving a measure of the divalent ion activity.

In calculating the concentration of complexed species present, the cation activity was converted to concentration using the single ion activity coefficient. The resulting concentration was subtracted from the total cation concentration to give the concentration of complex in the solution, $[ML^+]$. The concentration of free ligand, $[L^-]$, was calculated by subtraction of the complex concentration, $[ML^+]$, from the total ligand concentration.
The thermodynamic stability constant, K_t, was then determined from equation 3.7. The concentration stability constant, K_c is given by:-

$$K_c = \frac{[ML^+]}{[M^{++}][L^-]} = \frac{[ML^+].f_{M^{++}}}{(M^{++}).[L^-]} \qquad \ldots\ldots\ldots\ldots\ldots \quad (3.8)$$

This can obviously be calculated from the thermodynamic constant using the single ion activity coefficient for the divalent cation:-

$$K_c = K_t \cdot f_{M^{++}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.9)$$

Calculation of the stability constants (see below) was by means of a computer program DIVCAT (see Appendix) in which a modification was made to the above method to allow correction for the presence of potassium complex. As in the calculation of dissociation constants (Chap.2), some small concentration of the complex between the ligand and potassium was present in the solution and correction must be made to obtain the true value for the free ligand concentration.

An alternative method of calculating stability constants from similar methods was reported by Rechnitz and Lin[61]. The electrode response is normally expressed as:-

$$E = E_o + 0.0295 \log \left[(Ca^{++}) + \sum_{o}^{i} K_i \, (M_i^{+})^{1/2} \right]$$

In the presence of a complexing ligand, L, this may be written as:-

$$E = E_o + 0.0295 \log \left[\frac{[Ca^{++}] \cdot f_{Ca^{++}}}{1 + \sum_{o}^{i} \beta_j [L_j]} + \sum_{o}^{i} K_i \, (f_i [M_i])^{1/2} \right]$$

where β_j and $[L_j]$ are the overall formation constants and the ligand concentrations of the complexing ligand.

This method is based on the assumption that there is only one ligand species present and that it complexes only with calcium ion not the interfering ion.

The assumption that the foreign ion, in this case potassium, does not complex with ligand could not be made in the present study since it has already been shown

(Section 3.1) that potassium forms complexes with the ligands of detectable strength. It was therefore necessary to introduce a correction for the presence of potassium complexes in the calculation. The concentration stability constant for the potassium complex (Section 3.1) was used in calculating the concentration of potassium complex:-

$$[KL] = (K_t \times [L^-] \times KK') \,/\, (1 \times KK' . [L^-]) \quad \ldots\ldots\ldots (3.10)$$

where KK' is the concentration stability constant for the potassium complex which is related to the value determined in Section 3.1 by the expression:-

$$KK' = KK_{(0.1)} \times \frac{f_{\pm}^2}{f_{\pm(I = 0.1)}^2} \quad \ldots\ldots\ldots (3.11)$$

where $f_{\pm}$ and $f_{\pm(I = 0.1)}$ are the mean activity coefficient of uni-univalent electrolytes at the ionic strength of the test solution and at an ionic strength of 0.1 respectively.

With this correction, equation 3.8 becomes:-

$$K_c = \frac{[ML^+]}{[M^{++}]\left[L_t - [ML^+] - [KL]\right]} \quad \ldots\ldots\ldots (3.12)$$

Results

The method described above involving the incremental addition of neutralised ligand solution was used giving the results listed in Table 3.7. The complexes formed between calcium ions and D-glucuronic acid, D-galacturonic acid, methyl α-D-glucuronoside and methyl β-D-galacturonoside were studied using the calcium ion selective electrode while that between nickel ions and D-glucuronic acid was studied using the water hardness electrode. Typical sets of data obtained are tabulated in Section 3.4.

Table 3.7 Thermodynamic Stability Constants for Some Metal Uronate Complexes Using a Divalent Ion Responsive Electrode

Complex	Thermodynamic Stability Constant
Calcium glucuronate	32
Calcium galacturonate	64
Calcium methyl α-D-glucuronosidate	40
Calcium methyl β-D-galacturonosidate	48
Nickel glucuronate	58

Discussion

The principal recommendation for adopting the use of a divalent ion sensitive electrode for the determination of stability constants was that this would permit direct measurement of one of the required ion activities allowing improved accuracy of determination. However, with this equipment, the absolute accuracy of any individual reading is at best about 0.5 mV but may be as poor as 1 mV. With this latter limit, the overall accuracy in the determination of the metal ion activity is only 5-7% in the range of ion activities used (approximately 10^{-3} mol/l). This accuracy of measurement is insufficient to permit the expected improvement over the results obtained by indirect measurement such as pH measurements (Chap. 4).

Satisfactory results have been obtained using electrodes of this type in the study of complexes of divalent ions of greater stability. For example, Rechnitz and Lin[61] achieved reasonable reproducibility in determining stability constants of calcium complexes with constants in the range $1\text{-}3 \times 10^{4}$. In this case, changes in calcium ion activity with complexing was large relative to fluctuations in potential due to the electrode set.

3 . 4 Tabular Results - Metal Complexes

This section includes some typical data obtained from measurements with the calcium responsive liquid membrane electrode to determine stability constants. The data tabulated were calculated using computer program DIVCAT (see Appendix).

Average values for the complexes between calcium ions and D-glucuronic acid, D-galacturonic acid, methyl α-D--glucuronoside and methyl β-D-galacturonoside, and between nickel ions and D-glucuronic acid are tabulated in Table 3.7.

A. Calcium glucuronate

Ionic strength: variable.

Electrode Potential (mV)	(M^{++}) $(x10^{-3})$	$[ML^{+}]$ $(x10^{-4})$	$[L^{-}]$ $(x10^{-3})$	K_t
-0.256	1.095	0.11	0.423	24.7
-0.627	1.063	0.37	0.829	41.7
-0.878	1.043	0.48	1.247	36.8
-1.140	1.022	0.61	1.661	35.8
-1.351	1.005	0.68	2.079	32.6
-1.573	0.988	0.77	2.492	31.4
-1.745	0.975	0.81	2.909	28.6
-1.916	0.962	0.85	3.323	26.7
-0.335	0.765	0.10	0.425	31.4
-0.701	0.743	0.24	0.844	37.7
-1.004	0.726	0.33	1.265	37.5
-1.307	0.709	0.42	1.683	35.6
-1.521	0.697	0.46	2.105	31.2
-1.737	0.685	0.50	2.524	28.8

Electrode Potential (mV)	(M^{++}) $(x10^{-3})$	$[ML^{+}]$ $(x10^{-4})$	$[L^{-}]$ $(x10^{-3})$	K_t
-1.953	0.674	0.54	2.941	27.4
-2.130	0.665	0.56	3.358	25.2
-2.357	0.653	0.62	3.769	25.4
-2.535	0.644	0.65	4.182	24.2

Average value for the thermodynamic stability constant for calcium glucuronate: 32.4

Number of results included in averate: 119.

B. Calcium galacturonate

Electrode Potential (mV)	(M^{++}) $(x10^{-3})$	$[ML^{+}]$ $(x10^{-4})$	$[L^{-}]$ $(x10^{-3})$	K_t
-0.188	0.773	0.10	0.130	97.8
-0.355	0.763	0.18	0.261	88.8
-0.488	0.755	0.23	0.395	76.8
-0.656	0.746	0.31	0.526	79.0
-0.830	0.736	0.40	0.656	82.0
-0.955	0.728	0.44	0.789	77.0
-1.038	0.724	0.46	0.926	68.3
-1.123	0.719	0.47	1.062	62.1
-1.258	0.711	0.53	1.193	62.5
-1.343	0.707	0.55	1.328	58.5
-1.478	0.699	0.61	1.460	59.4
-1.562	0.695	0.63	1.594	56.5
-1.698	0.688	0.68	1.724	57.7
-1.737	0.685	0.67	1.862	52.5
-1.826	0.681	0.69	1.995	51.1

Electrode Potential (mV)	(M^{++}) $(x10^{-3})$	$[ML^{+}]$ $(x10^{-4})$	$[L^{-}]$ $(x10^{-3})$	K_t
-1.915	0.676	0.72	2.128	50.0
-2.004	0.671	0.74	2.261	49.1
-2.095	0.667	0.98	1.695	86.8
-0.303	1.394	0.34	0.244	99.8
-0.390	1.384	0.40	0.376	77.5
-0.492	1.373	0.49	0.505	70.6
-0.618	1.360	0.62	0.630	72.2
-0.696	1.352	0.67	0.763	64.8
-0.829	1.338	0.81	0.887	68.0
-0.911	1.329	0.86	1.018	63.8
-1.046	1.315	1.00	1.141	66.9
-1.085	1.311	0.99	1.279	59.2
-1.174	1.302	1.06	1.408	57.9
-1.263	1.293	1.13	1.537	56.8
-1.402	1.279	1.28	1.658	60.2
-1.441	1.275	1.27	1.795	55.4
-1.530	1.267	1.34	1.923	54.9
-1.570	1.263	1.33	2.059	51.1

Average value for the thermodynamic stability for calcium galacturonate: 64.4

Number of results included in average: 272.

CHAPTER 4

STABILITIES OF URONATE COMPLEXES: pH ELECTRODE STUDIES

Chapter 4. Stabilities of Uronate Complexes: pH Electrode Studies

Of the many available methods for determining the stability constants of metal complexes of organic ligands, the most widely used involves the measurement of the pH of the test solution to allow calculation of the free ligand concentration (see Section 1.4). Early determinations by this method employed the determination of pH by colorimetry or by using a hydrogen gas electrode[65] which is subject to many practical difficulties. Subsequent developments in glass electrode technology and in potentiometry have however improved the speed and accuracy of pH measurement to the point where this type of 'pL' method is one of the simplest and quickest for stability constant measurements in cases where protons and metal cations compete for the ligand.

4 . 1 Divalent Cation Complexes

The equilibrium which exists between bivalent cations and dissociated uronic acids in which the associated species is a metal-ligand complex has already been discussed (Section 3.3)

$$M^{++} + L^{-} \rightleftharpoons ML^{+}$$

where M^{++} is a divalent cation and ML^{+} is the associated species. The concentration formation constant of the complex is then given by:-

$$K_f = \frac{[ML^{+}]}{[M^{++}][L^{-}]}$$

If both this equilibrium and the acid dissociation equilibrium (section 2.4) are considered to occur simultaneously, it can be seen that the result of introducing metal ions into a solution of an acidic ligand is to dissociate the acid to a greater extent. This increases the acidity of the solution and it is this increase in acidity of a ligand solution in the presence of metal ions over a corresponding solution containing no metal ion which gives a measure of the degree of association.

Since the determinations are to be carried out at a constant ionic strength, a third equilibrium must be considered since the potassium ions in the background electrolyte also form an associated species with the ligands (cf Section 2.4 and see Section 3.1). The effect of this association is to cause a further small increase in the acidity. However, since a stability constant can

be determined for this complex, correction can be made for this increase in acidity allowing calculation of a true stability constant for the complex between the ligand and the other metal ion.

Treatment of Experimental Data

As in the determination of dissociation constants for the ligands, the data obtained are measurements of pH of a solution of the ligand on addition of increasing volumes of a standard potassium hydroxide solution. The difference is that the present titrations were carried out in the presence of metal ions.

The data obtained from these titrations were corresponding pairs of pH measurements and volumes of hydroxide solution added. In addition, the total concentrations of ligand, metal ions, potassium ions, the dissociation constant of the ligand and the stability constant for the potassium complex of the ligand are known.

The relationships which must be considered in calculating the stability constant data are:-

$$[K^+] + [H^+] + 2[M^{2+}] + [ML^+] = [OH^-] + [L^-] + [NO_3^-] \quad \ldots\ldots (4.1)$$

$$L_t = [L^-] + [HL] + [ML^+] + [KL] \quad \ldots\ldots\ldots (4.2)$$

$$M_t = [M^{2+}] + [ML^+] \quad \ldots\ldots\ldots (4.3)$$

$$K_t = [K^+] + [KL] \quad \ldots\ldots\ldots (4.4)$$

The first of these expresses the electroneutrality of the solution while the remaining equations are the definitions of the total concentration of ligand, bivalent metal cations and potassium ions respectively.

The data obtained from the titrations, namely pH of the solution and volume of hydroxide solution added, are

converted to the concentration of hydrogen ions, $[H^+]$, and the concentration of hydroxide added, OH_a, respectively:-

$$[H^+] = \text{antilog } (-pH) \,/\, f_{H^+} \qquad \ldots\ldots\ldots (4.5)$$

$$\text{and } OH_a = (VB \times MB) \,/\, (V + VB) \qquad \ldots\ldots\ldots (4.6)$$

where VB is the volume of hydroxide solution of molarity MB added, V is the initial volume of the solution, and f_{H^+} is the activity coefficient for the hydrogen ion.

By rearrangement of equation (4.1) (cf derivation of equation 2.5) the concentration of ligand which remains undissociated can be expressed as:-

$$[HL] = L_t - OH_a - [H^+] + [OH^-]$$

and, since the solutions are predominantly acidic, the correction term for free hydroxyl ions can be omitted:-

$$[HL] = L_t - OH_a - [H^+] \qquad \ldots\ldots\ldots (4.7)$$

The concentration of acid which has dissociated can then be calculated from the concentration dissociation constant for the acid at this ionic strength:-

$$[L^-] = ([HL] \,.\, K_a) \,/\, (H^+) \qquad \ldots\ldots\ldots (4.8)$$

where K_a is the mixed dissociation constant.

The correction term for the formation of the complex between the ligand and potassium must be introduced:-

$$[KL] = (K_t \times [L^-] \times KK) \,/\, (1 + KK.\,[L^-]) \qquad \ldots\ldots\ldots (4.9)$$

where KK is the concentration stability constant for the associated species formed between potassium ions and the ligand.

The concentration of the complex formed between the ligand and the bivalent cation can be calculated as:-

$$[ML^+] = L_t - [HL] - [L^-] - [KL]$$

or, by substitution from equation 4.7:-

$$[ML^+] = OH_a + [H^+] - [L^-] - [KL] \quad \ldots\ldots\ldots\ldots (4.10)$$

and the concentration of free cation as:-

$$[M^{2+}] = M_t - [ML^+] \quad \ldots\ldots\ldots\ldots (4.11)$$

The concentration stability constant for the divalent cation complex with the acid can then be calculated as:-

$$K_f = \frac{[ML^+]}{[M^{++}][L^-]} \quad \ldots\ldots\ldots\ldots (4.12)$$

Values for this constant can be calculated for each pair of readings for pH and volume of hydroxide solution added and an average stability constant for the complex determined.

A computer program for carrying out this calculation and averaging the results is described in the Appendix.

4 . 2 Results

The values determined for the stability constants are true concentration constants because all the parameters in the calculation are expressed in terms of concentration. To allow the calculation of the free ligand concentration, it is necessary to convert the hydrogen activity measured into concentration terms by means of an activity coefficient. The values used for this were those calculated from the Debye-Huckel expression in Section 1.3. The concentration dissociation constant is suitable for use in this calculation directly because the stability constant determinations were carried out at the same ionic strength.

The calculation of values for the stability constants was carried out using a computer program (see Appendix) in which the purity of the ligand solution was allowed to vary to obtain the optimum correlation between the obtained results giving the minimum least squares deviation. Typical results for the determinations of the stability constants for the complexes between calcium and magnesium and D-glucuronic acid are tabulated in Section 4.5.

The average values for the concentration stability constants for several complexes, determined at a temperature of 25°C and at an ionic strength of 0.1 mol/l, are given in Table 4.1.

Table 4.1 Stability Constants of Some Uronate Complexes - pL Method

Ligand	Metal	Concentration Stability Constant (25°C: ionic strength 0.1)			
Glucuronic Acid	Calcium	23	x: 96%		
	Cobalt	27	x: 98%		
	Copper	118	x: 97%		
	Nickel	29	x: 95%		
	Magnesium	28	x: 97%		
Galacturonic Acid	Calcium	52	x: 89%	y: 0.22×10^{-3};	cxy: 0.967
	Cobalt	59	x: 91%	y: 0.20×10^{-3};	cxy: 0.977

(For the glucuronate complexes, the value of y was constrained to be zero).

The values of x (the purity of ligand), y (the concentration of strong base or acid introduced in either ligand or metal solution) and cxy (the correlation factor between x and y) refer to the computer program used in the calculation (see Appendix).

4 . 3 Discussion

The 'pL' method of determining stability constants using a pH electrode is the most widely used when the cation and hydrogen ions compete for the ligand. This will be satisfactory when the ligand is a weak acid and relatively large changes in pH are observed on complexing due to the displacement of hydrogen ions. In the present study, the ligands, uronic acids, are moderately strong acids and changes in the pH will be small.

The maximum accuracy which can be attained in the measurement of pH using a standard glass pH electrode with a standard calomel electrode as reference electrode is about 0.005 pH units and the reproducibility may be as low as 0.01 pH units. This last error of 0.01 pH units would account for the change in concentration stability constant of (say) calcium glucuronate from 20 to 25 (i.e. about 25%) when the solution is at pH3. Clearly this method shows insufficient sensitivity for the accurate quantitative study of complexes of the type considered and, although a large number of determinations were made for each complex, the standard deviation is relatively large.

4 . 4 Experimental

The preparation of the ligands used has been dealt with in Chapter 2. Estimation of the purity of the ligand was carried out during each titration from the end-point of the acid-base titration measured by pH meter. The potassium nitrate solution was made up by dissolving the calculated amount of 'Analar' material in boiled-out water.

The solutions for use with the bivalent cation responsive electrodes were prepared by dissolving a known weight of the ligand in boiled-out water and adjusting the pH to pH7 using standard potassium hydroxide solution. The concentration of the ligand was calculated from the concentration of alkali required to prepare the solution.

Standard potassium hydroxide, hydrochloric acid and nitric acid solutions were prepared from B.D.H. ampoules. The alkali was standardised by titration against weighed samples of 'Analar' potassium hydrogen phthalate, previously dried for an hour at 115°C and the acids were standardised by titration against the standard alkali. The end-points were detected by the use of phenolphthalein as indicator.

The metal solutions were prepared by dissolving the corresponding metal nitrate in boiled-out water. The solution was standardised by passage through a cation exchange resin column, previously washed with acid followed by water. The acid eluant was titrated against standard potassium hydroxide solution.

All solutions which were used in the study of the potassium complexes were prepared using Tris-buffer as solvent. 'Analar' 2-amino-2-(hydroxymethyl)propane-1,3-diol was weighed out and dissolved in the required volume of boiled-out distilled water which contained the theoretical concentration of hydrochloric acid necessary to half-neutralise it.

Estimation of purity of ligand

A measure of the purity of the ligand was obtained for each titration from the end point as determined by pH meter. With alkali as titrant, the end point was assumed to be the point at which the ligand was completely neutralised.

Preparation and use of Buffer Solutions

Buffers used as standards in the potentiometric study were potassium hydrogen phthalate (0.05 mol/l) and a solution containing a mixture of potassium dihydrogen phosphate (0.025 mol/l) and disodium hydrogen phosphate (0.025 mol/l) which gave solutions of pH 4.005 and 6.86 respectively at 25°C[66]. The solutions were prepared by weighing out the calculated amounts of 'Analar' material, dried at 115°C, and dissolving in the appropriate volume of freshly boiled-out distilled water. Buffer solutions were stored in hard glass bottles and were discarded after about one month.

The response of the glass electrodes used was checked periodically at 25°C using the two buffer solutions by setting the pH in the first and measuring the pH of the

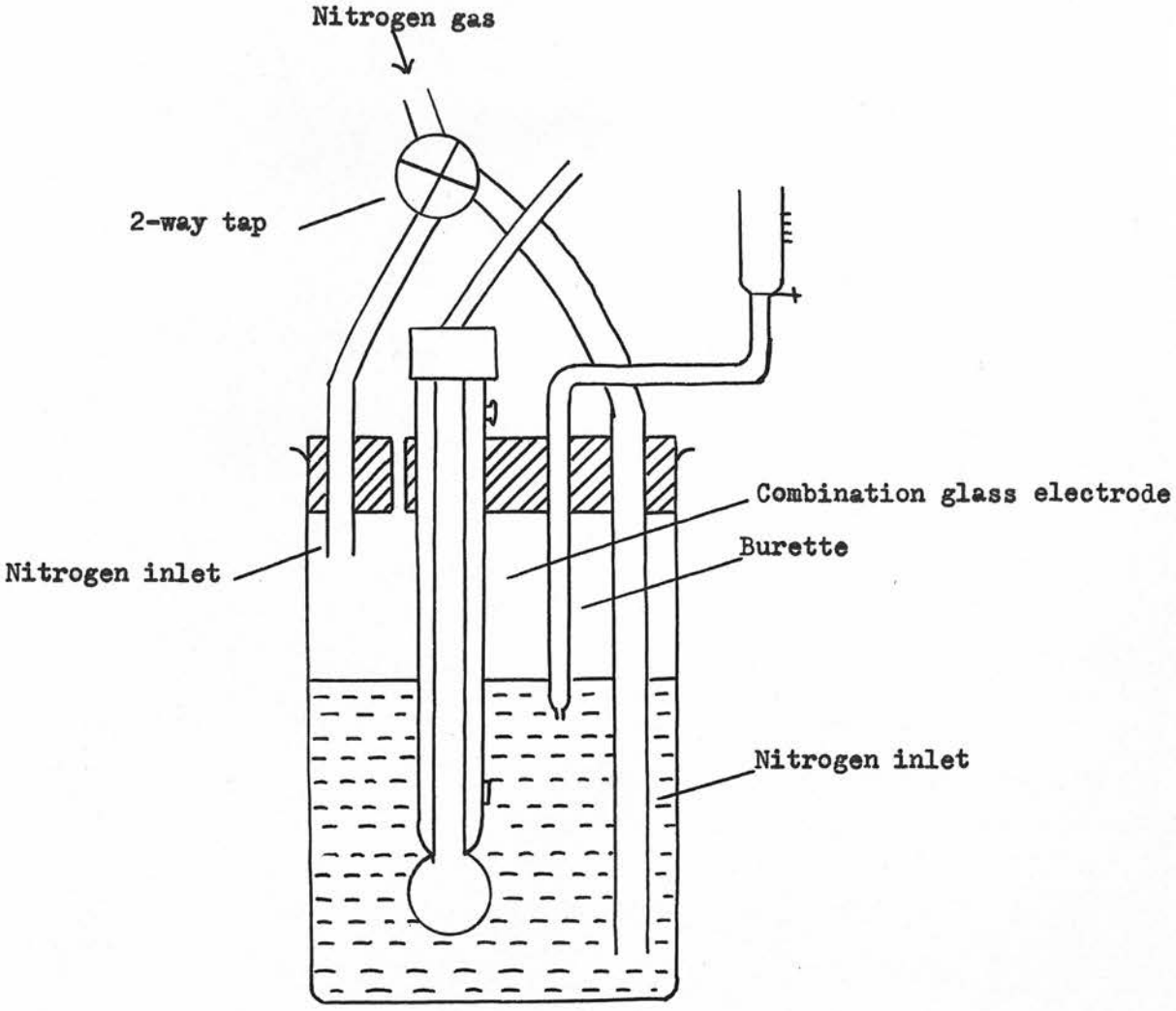
second. It was found to be satisfactory to make all pH measurements relative to the potassium hydrogen phthalate buffer which was used as a primary standard buffer and whose pH value was that of the specification in British Standard 1647 (1950). The use of this buffer as the standard for all titrations sometimes necessitated a more extended time lapse after transfer of the electrodes in cases where the pH being measured was far removed from 4.005, so that equilibrium might be established. Nevertheless, the standardisation rarely varied by more than ± 0.01 pH units during a titration and this was taken as the limit of accuracy.

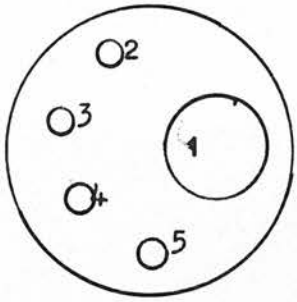
Thermostats and cells

All the work in this section was carried out at 25°C using a thermostatically controlled copper water bath of 200 litres volume. The temperature regulator was of the toluene mercury type and this controlled the temperature to an accuracy of at least ± 0.1°C.

The nitrogen gas used for stirring was bubbled through water at the same temperature as the solution through (or over) which it was to pass, so that it might be saturated with water vapour at that temperature.

Three main types of titration cell were used. The first consisted basically of 100 ml. tall form beaker sealed with a rubber bung with holes for the gas inlets and outlet, and the electrodes. (This cell is shown diagrammatically in fig. 4.1). This cell was immersed in the water bath during titrations to ensure accurate temperature control. The second type was similar and is

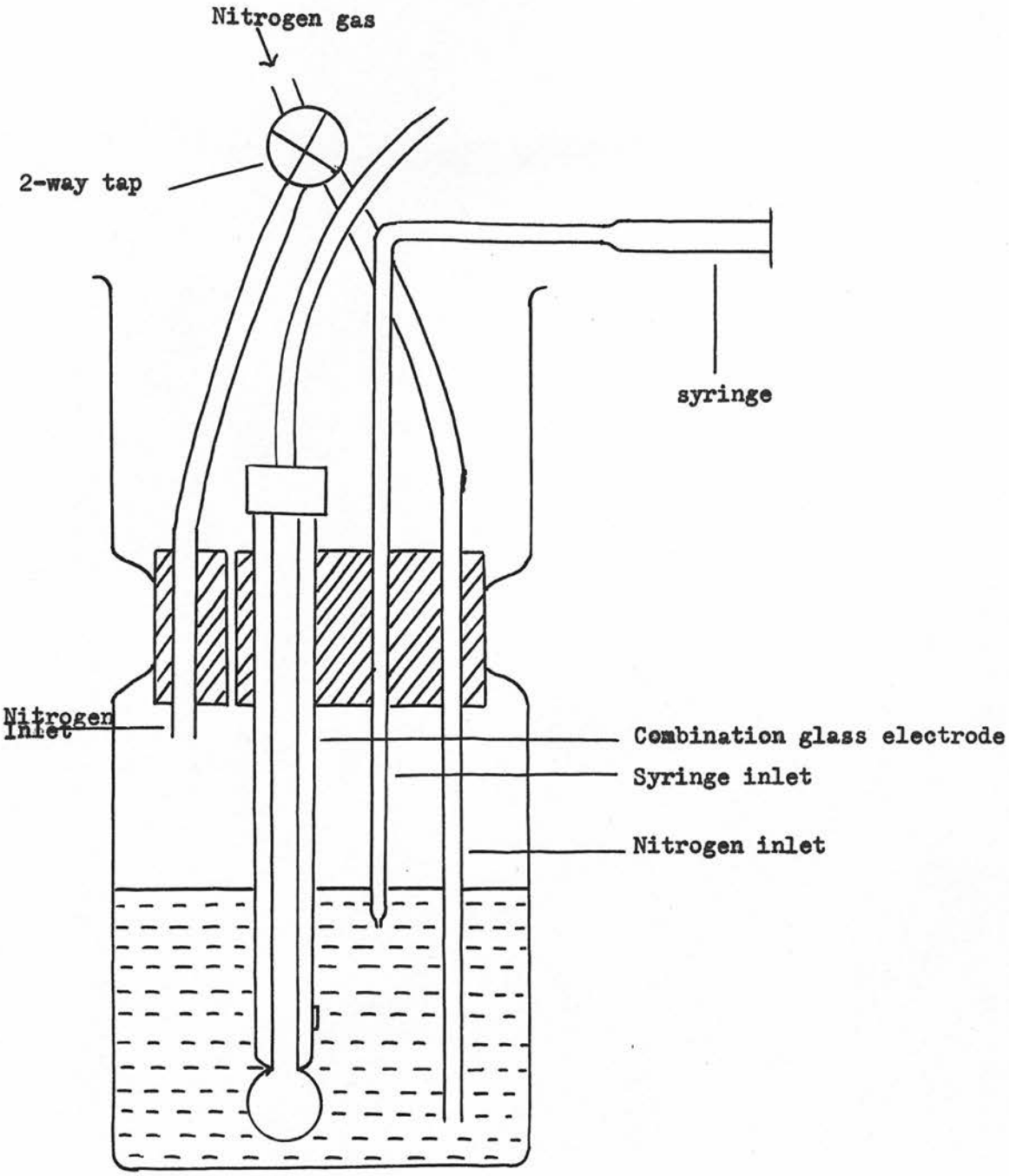


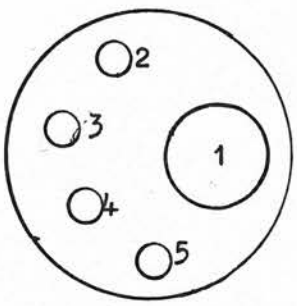


KEY:-

1. Combination electrode
2. Burette
3. Nitrogen inlet (for bubbling through solution)
4. Nitrogen inlet (for passing over solution)
5. Nitrogen outlet

Fig. 4.1 Titration Cell A

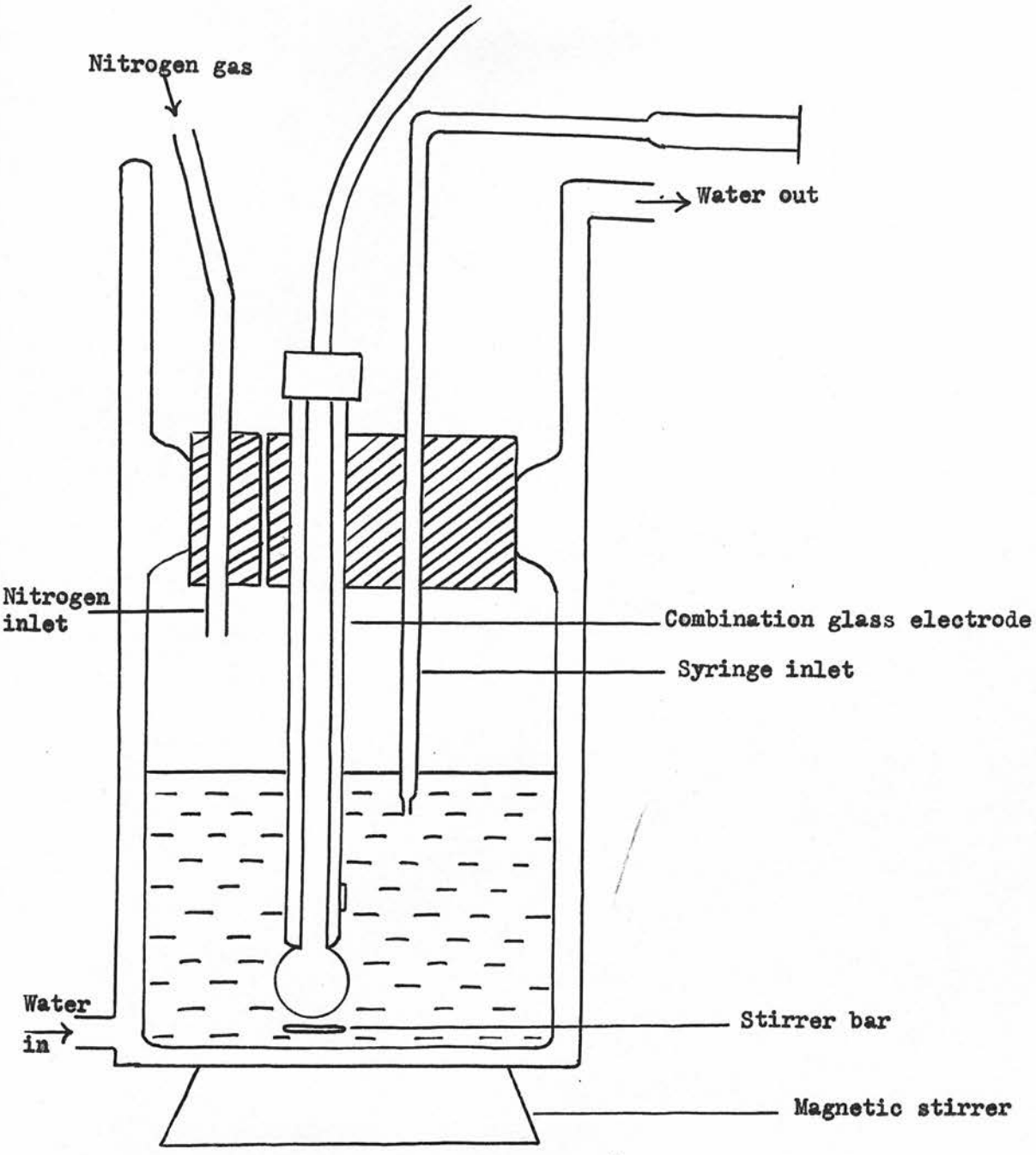


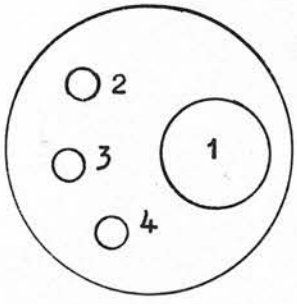


KEY:-

1. Combination electrode
2. Syringe inlet
3. Nitrogen inlet (for bubbling through solution)
4. Nitrogen inlet (for passing over solution)
5. Nitrogen outlet

Fig. 4.2 Titration Cell B





KEY:-

1. Combination electrode
2. Syringe Inlet
3. Nitrogen inlet
4. Nitrogen outlet

Fig. 4.3 Titration Cell C

shown in Fig. 4.2. The third type of cell used differed in that it had a water jacket through which water from the thermostat bath was pumped, and that stirring of the solution was effected by a magnetic stirrer bar which was operated by standing the cell on a magnetic stirrer during a titration (see fig. 4.3 for details of the cell).

pH Meter and Electrometer

pH measurements were made on a Beckman Research pH meter calibrated to 0.002 pH unit, readable to 0.0005 pH units and reproducible to 0.001 pH unit for any solution. The leads from the meter were all adequately shielded to eliminate the effect of stray electromagnetic fields.

In the study of potassium complexes, the potentials were measured and recorded using a set-up consisting of a vernier potentiometer, an electrometer (E.I.L. Vibron Electrometer model 33B-2) and a flat bed recorder to assist in measuring the potential. The potentiometer was standardised using a calibrated Weston Normal Cell (Cambridge Instruments Ltd) with a stated e.m.f. of 1.01861 absolute volts at 20°C. All leads were shielded and the shields were connected to a common earth.

With these precautions, the readings of the meters were found to be completely stable and free from interference from any possible source of stray field.

Electrodes

For all the pH titrations, the electrodes used were either Beckman or Activion glass electrodes, of the type suitable for work at temperatures in the range 10-90°C, with

combined calomel reference electrode. The glass electrodes were prepared for use in the normal way by soaking for about 24 hours in 0.1 mol/l hydrochloric acid followed by thorough washing in distilled water. When transferred from one solution to another, the glass electrode was washed quickly with distilled water, excessive liquid was removed from the electrode by a tissue without touching the glass and was allowed up to 20 minutes to equilibrate in the new solution. When not in use, the electrodes were left standing in distilled water.

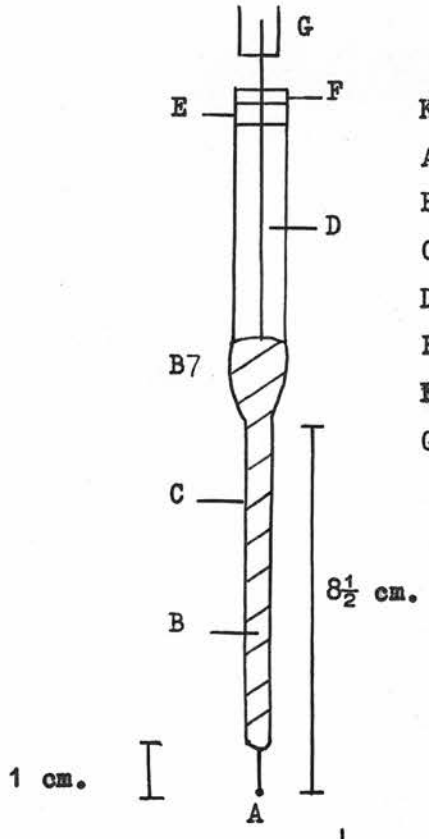
The electrode used for determination of potassium ion activity was E.I.L. type GKN 33 which was prepared for use and stored in distilled water. The reference electrode used with this type of electrode was of the silver silver chloride type.

Silver silver chloride electrodes

These were of the electrolytic type and were prepared in accordance with the method of Brown[67] and Ives and Janz[68]. The electrodes were mounted on an extended B7 soda glass cone (fig. 4.4). The electrode base was made from 0.02'' diameter platinum wire fused into the soda glass support. The tip of the platinum wire was fused to avoid sharp edges which may cause uneven deposition of silver. The tube was thoroughly dried before clean mercury was poured into it.

The electrodes were prepared in batches of eight. The platinum bases were cleaned by anodising in concentrated nitric acid using a platinum cathode and a

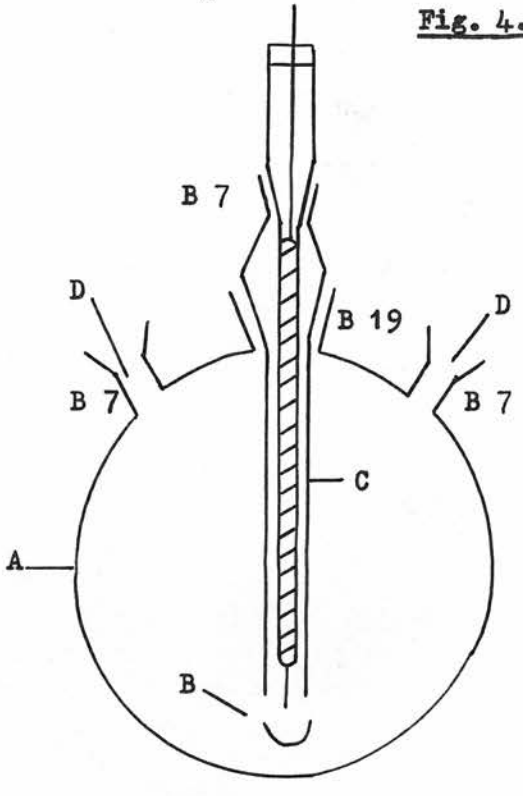
Fig. 4.4 Silver, silver chloride Electrode construction



KEY:-

A Platinum wire base
B Purified mercury
C Extended B7 soft glass cone
D Thick copper wire
E Split polythene tube support
F Araldite seal
G Terminal connector

Fig. 4.5 Electrolysis Apparatus



KEY:-

A 500ml. flask
B Silver cyanide trap
C Central electrode
D Silver, silver chloride electrode sites (8)

current of $1\frac{1}{2}$ mA. This was followed by prolonged washing in conductivity water. (The electroplating apparatus is shown in fig. 4.5). After anodising and washing, the electrodes were silver plated. This was accomplished using a solution of recrystallised potassium silver cyanide (10 g/l). The central platinum electrode was separated from the other electrodes in order to prevent silver cyanide formed at the anode during the electrolysis from mixing with the bulk of the solution. The concentration of cyanide in this solution was reduced to a minimum by the addition of a solution of 'Analar' silver nitrate until the precipitated silver cyanide remained undissolved on shaking. The silver plating was carried out at a current density of 0.4 mA cm^{-2} (about 0.5 mA for the eight electrodes) for about 7 hours. In the initial stage of the plating, a current of 0.8 mA was used for 30 min to ensure that a large number of silver nuclei were formed and that consequently the silver layer would adhere well to the platinum surface. The silver layer was snowy white.

The plated electrodes were washed for a few hours with conductivity water, left overnight in 'Analar' ammonia and then washed for 2 days in conductivity water. The electrodes were then made the anode in a cell containing bromide free 0.1 mol/l hydrochloric acid and electrolysis was carried out with a current of 0.5 mA for 45 mins. This was sufficient to convert about 10% of the silver to silver chloride, this proportion having been found by previous workers to give the most reproducible

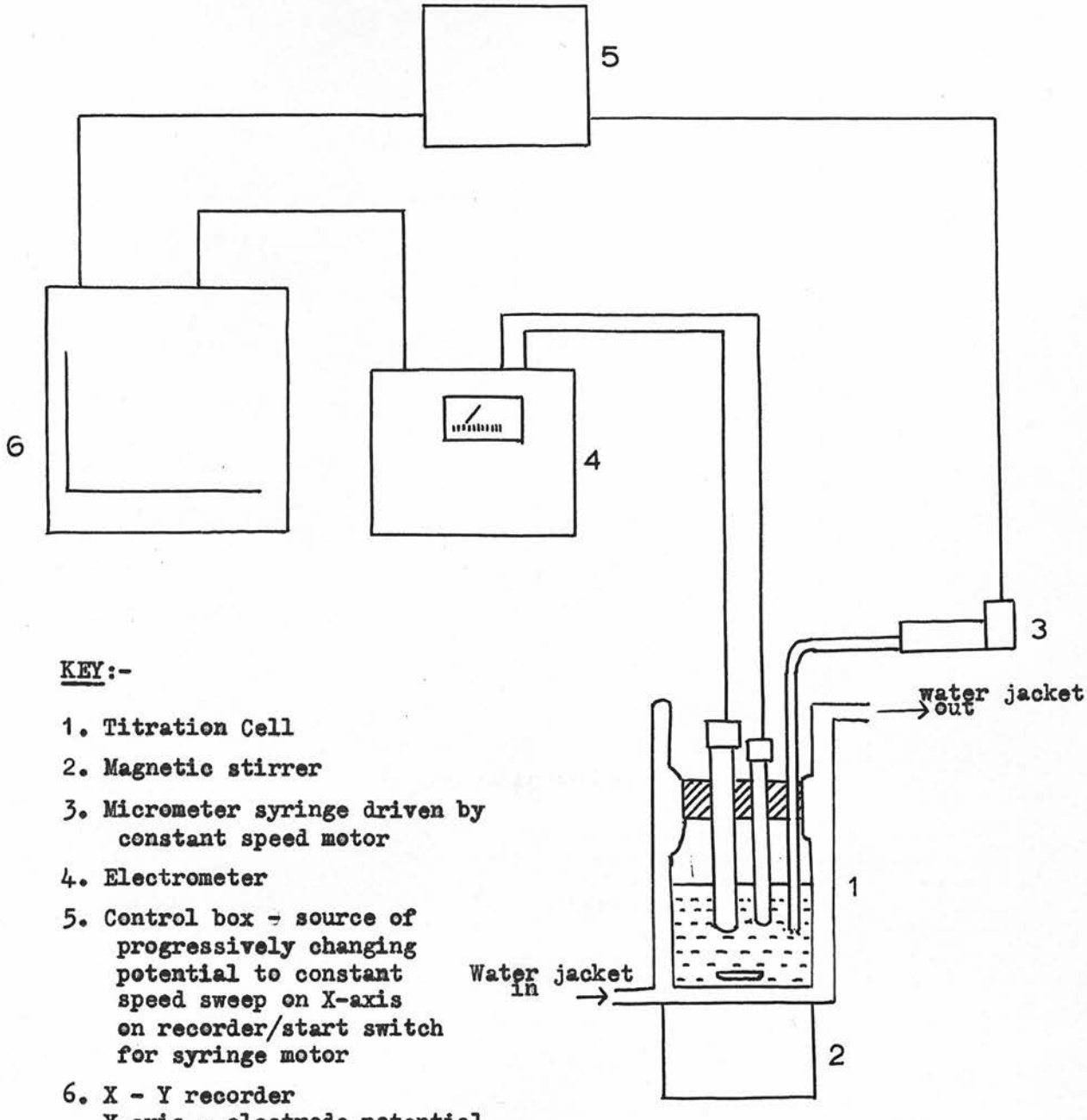
results. The electrodes at this stage were brownish--purple. They were well washed with water and then stored under a nitrogen atmosphere in conductivity water in the dark. They were always stored at 25°C. At least 3 days were required before reliable bias potentials were obtained. A Vibron Electrometer was used for the intercomparison, the electrolyte was a very dilute sodium chloride solution. In general, 6 out of each batch of 8 electrodes agreed to within 0.02 mV. Interconnecting the electrodes made no improvement in the bias potentials.

Method of Titration - pH titrations

Standard potassium hydroxide solution was added to the titration cell either by micro-burette or by micrometer syringe. Using the former method, the alkali solution was 0.1 mol/l and with the latter, 1 mol/l. The solution was mixed during the addition by bubbling nitrogen through the solution. The nitrogen flow was then diverted to pass over the solution and, after a settling period of 2 minutes, a reading of the solution pH was obtained.

Method of Titration - Bivalent Cation Responsive Electrode

The electrode was allowed to equilibrate in a solution of the metal nitrate until a steady potential was obtained. Neutralised ligand solution was added by micrometer syringe at a constant rate with changes in the electrode potential being constantly recorded on an automatic recorder. The micrometer syringe was driven by



KEY:-

1. Titration Cell
2. Magnetic stirrer
3. Micrometer syringe driven by constant speed motor
4. Electrometer
5. Control box - source of progressively changing potential to constant speed sweep on X-axis on recorder/start switch for syringe motor
6. X - Y recorder
   Y-axis - electrode potential
   X-axis - time scale (from control box)

Fig. 4.6 **Potentiometric titration apparatus - divalent cation responsive electrode**

a motor to allow addition of ligand solution at a constant rate. A diagrammatic sketch of the set-up is given in Fig. 4.6.

4 . 5 Tabular Results - Metal Complexes

This section includes some typical data obtained from pH titration to determine stability constants. Although data are tabulated only for some titrations in the presence of D-glucuronic acid and calcium nitrate and magnesium nitrate solution, data were obtained for the complexes between calcium, cobalt, copper, nickel and magnesium ions with D-glucuronic acid and calcium and cobalt ions with D-galacturonic acid (see Table 4.1 and Section 4.2). The results were calculated using computer program (see Appendix B).

A. Calcium glucuronate

Ionic strength: 0.1 mol/l in potassium nitrate.

OH_a ($x10^{-3}$)	$[H^+]$ ($x10^{-3}$)	$[L^-]$ ($x10^{-2}$)	$[ML^+]$ ($x10^{-3}$)	$[M^{++}]$ ($x10^{-2}$)	K_f
L_t: 0.0066					
M_t: 0.0080					
0.000	2.238	0.158	0.428	0.757	35.9
0.499	1.910	0.177	0.376	0.762	27.9
0.999	1.633	0.195	0.390	0.760	26.4
1.498	1.380	0.215	0.405	0.758	24.8
1.996	1.153	0.236	0.425	0.756	23.8
2.494	0.948	0.260	0.438	0.754	22.3
2.991	0.769	0.285	0.471	0.751	22.0
3.488	0.609	0.311	0.504	0.747	21.7

OH_a ($x10^{-3}$)	$[H^+]$ ($x10^{-3}$)	$[L^-]$ ($x10^{-2}$)	$[ML^+]$ ($x10^{-3}$)	$[M^{++}]$ ($x10^{-2}$)	K_f
3.984	0.469	0.338	0.544	0.742	21.7
4.480	0.344	0.367	0.576	0.739	21.3
4.975	0.234	0.394	0.648	0.731	22.5

Calculated ligand purity: 96.1%

L_t: 0.0068

M_t: 0.0222

0.000	2.483	0.144	0.916	2.133	29.9
0.500	2.157	0.158	0.931	2.131	27.6
0.999	1.857	0.174	0.953	2.127	25.7
1.498	1.581	0.193	0.976	2.124	23.9
1.996	1.327	0.213	0.993	2.121	22.0
2.494	1.106	0.234	1.043	2.115	21.1
2.991	0.908	0.256	1.094	2.109	20.3
3.488	0.726	0.282	1.121	2.105	18.9
3.984	0.523	0.305	1.208	2.095	18.9
4.480	0.435	0.329	1.298	2.085	18.9
4.975	0.315	0.351	1.437	2.070	19.8
5.470	0.208	0.370	1.605	2.052	21.1
5.964	0.113	0.371	1.996	2.012	26.8

Calculated ligand purity: 97.8%

B. Magnesium glucuronate

Ionic strength: 0.1 mol/l in potassium nitrate

OH_a ($\times 10^{-3}$)	$[H^+]$ ($\times 10^{-3}$)	$[L^-]$ ($\times 10^{-2}$)	$[ML^+]$ ($\times 10^{-3}$)	$[M^{++}]$ ($\times 10^{-2}$)	K_f
L_t: 0.0066					
M_t: 0.0042					
0.000	2.243	0.161	0.333	0.385	53.9
0.500	1.927	0.179	0.302	0.388	43.5
0.999	1.644	0.198	0.287	0.389	37.3
1.498	1.390	0.219	0.283	0.389	33.2
1.996	1.159	0.242	0.269	0.390	28.4
2.494	0.957	0.266	0.278	0.389	26.8
2.991	0.778	0.292	0.290	0.388	25.6
3.488	0.618	0.320	0.291	0.387	23.5
3.984	0.480	0.347	0.326	0.384	24.5
4.480	0.357	0.374	0.369	0.374	26.0
4.975	0.251	0.398	0.472	0.369	32.2
5.470	0.157	0.413	0.692	0.347	48.4
Calculated ligand purity: 97.9%					
L_t: 0.0066					
M_t: 0.0084					
0.000	2.264	0.160	0.421	0.794	33.1
0.500	1.927	0.181	0.345	0.801	23.8
0.999	1.659	0.198	0.380	0.797	24.1
1.498	1.409	0.218	0.400	0.795	23.3
1.996	1.180	0.240	0.412	0.793	21.7
2.494	0.973	0.265	0.411	0.793	19.6

OH_a ($\times 10^{-3}$)	$[H^+]$ ($\times 10^{-3}$)	$[L^-]$ ($\times 10^{-2}$)	$[ML^+]$ ($\times 10^{-3}$)	$[M^{++}]$ ($\times 10^{-2}$)	K_f
2.991	0.791	0.291	0.423	0.791	18.4
3.488	0.634	0.316	0.472	0.786	19.0
3.984	0.493	0.343	0.512	0.781	19.1
4.480	0.369	0.370	0.565	0.776	19.7
4.975	0.260	0.396	0.655	0.766	21.6
5.470	0.165	0.411	0.873	0.744	28.6

Calculated ligand purity: 97.5%

CHAPTER 5

STABILITIES OF URONATE COMPLEXES: N.M.R. AND POLARIMETRIC STUDIES

Chapter 5 Stabilities of Uronate Complexes: N.M.R. and Polarimetric Studies

The use of spectroscopy to determine the stability constants of metal complexes of certain classes of ligand, has been mentioned in Chapter 1. In fact, determination of this type using u.v.[69], n.m.r.[70] and polarimetric[71,72,73] methods have previously been reported.

In order that a polarimetric method can be used in stability constant determinations it is necessary that the ligand species exhibits different optical activities in the free and complexed forms. When this condition is fulfilled, the degree of activity can be measured for a series of solutions in which the ratio of the total concentration of metal to that of ligand is varied, allowing the stability constant for the complex to be calculated.

5 . 1 Preliminary Studies - Polarimetry Method

Before this method can be applied to stability constant determinations, it is necessary to select suitable values for the wavelength of light to be used, and for the pH of the test solutions. The choice of wavelength was limited by the polarimeter which could operate only at five fixed wavelengths, 589 nm with a sodium lamp and 578, 546, 436 and 365 nm with a mercury lamp. It was also necessary that the wavelength was selected so that none of the species present have absorption bands at that point in the spectrum. Since, of the species present, only the metal ions would be

expected to show absorption in this region, the visible and ultra violet spectra of each of the metal ions to be studied was recorded both with and without the presence of ligand solution (figs. 5.1 and 5.2). From these spectra, suitable wavelengths were selected for each metal ion and these are tabulated in Table 5.1 (the value used is underlined).

Table 5.1 Polarimetry - Suitable Wavelengths for Metal Uronate Studies

Cation	Suitable Wavelengths (nm)
Ca^{++}	365, 436, 546, 578, 589
Co^{++}	365
Cu^{++}	365, 436, 546
Ni^{++}	546, 578

A constraint on the choice of pH is the limited solubility of some metal hydroxides and the high stability of many hydroxo complexes such as those of copper, with the result that some complexes can only be studied at relatively acid pH values. The use of acidic solutions has the disadvantage that additional ligand species - protonated ligand and lactone - are present.
Since complexing occurs between the metal ion and the dissociated ligand, it is preferable to use neutralised ligand species. As a result, complexes of calcium with various uronic acid ligands can be studied at pH 7 whereas those of the transition metal ions are studied at pH 4.2.

Fig. 5.1 Visible Spectra - Metal Nitrates

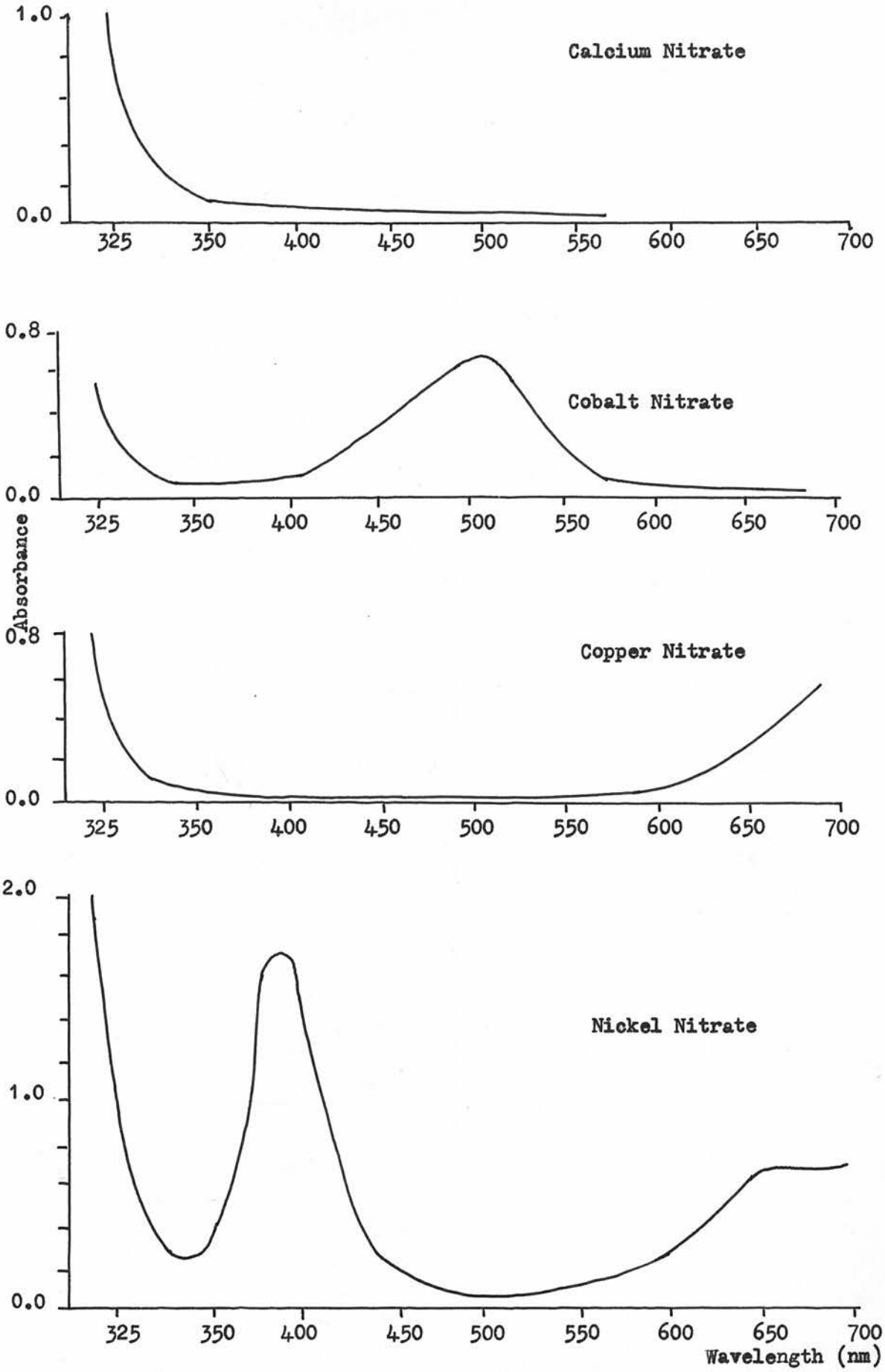
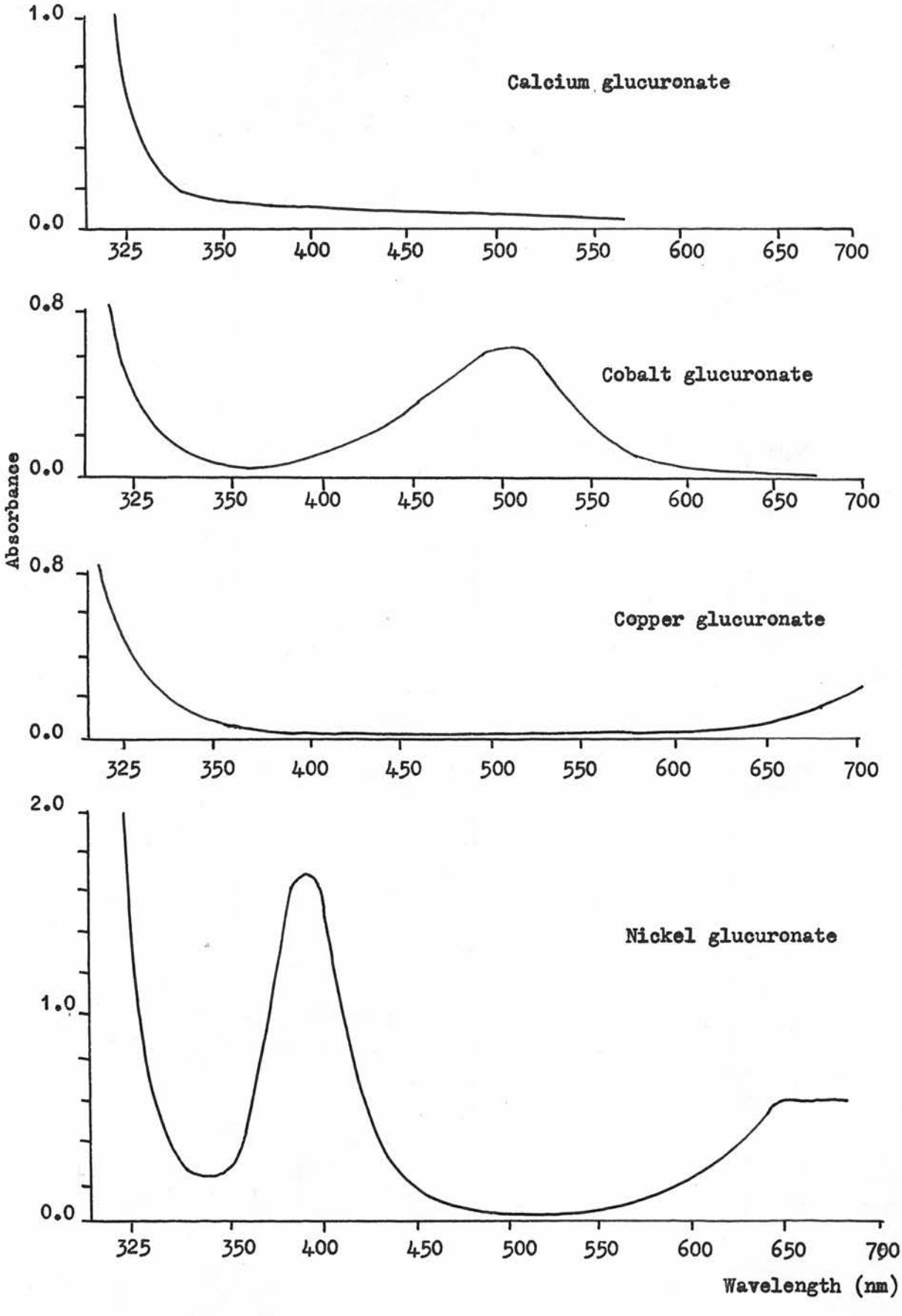


Fig. 5.2 Visible Spectra - Metal Uronates



5 . 2 Changes in Rotation on Metal Addition to Uronate Solutions

The specific rotation of neutralised and partly neutralised uronic acid solutions is observed to change with increasing concentrations of some metal nitrates in the solution. The value continues to change as the metal ion concentration is increased until a limiting value of the rotation is approached. Since it was confirmed that the metal nitrate solutions did not contribute to the rotation and also allowed light to be fully transmitted at the wavelengths selected, the changes observed are attributable only to complex formation and the limiting value to the case in which the ligand is entirely complexed by metal ions. From the measurement of rotation at points intermediate between the two extreme cases, it is possible to determine the fractions of the ligand in complexed and uncomplexed forms and, since the total concentrations of both ligand and metal ions are known, to calculate values for the stability of the 1:1 complex from each measurement.

From a preliminary investigation, it was observed that, although uronic acid solutions at pH 4.2 and pH 7 showed changes in rotation on addition of metal ions, no such change in rotation occurred using ligand solutions adjusted to pH 2 suggesting that only the dissociated form of the acid is capable of complexation. The result of this observation is that all uronate complex studies by the polarimetric method require the pH to be sufficiently high

for the ligand to be at least partly dissociated. However, as already discussed above, the pH must not be high enough to cause precipitation of the hydrolysed metal ions as metal hydroxides.

Study of Uronate Complexes of Calcium at pH 7

Two series of solutions were prepared each containing a fixed volume of a stock ligand solution (0.02 mol/l) - D-glucuronic acid in the first case and D-galacturonic acid in the second. The pH was adjusted to 7 and the solution was allowed to reach mutarotation equilibrium; then various volumes of a solution of calcium nitrate (0.5 mol/l) were added. The optical rotation of each solution was recorded and was observed to increase with increasing metal ion concentration until a maximum value was approached (Figs. 5.3 and 5.4 and Tables 5.2 and 5.3).

The equilibria which must be considered are identical to those in Chapters 3 and 4, viz:-

$$Ca^{++} + L^{-} \rightleftharpoons CaL^{+} \quad \ldots\ldots\ldots\ldots \quad (5.1)$$

and

$$K^{+} + L^{-} \rightleftharpoons KL \quad \ldots\ldots\ldots\ldots \quad (5.2)$$

where L^{-} signifies the ligand anion.
Measurement of the specific rotation, based on total ligand present, allows the calculation of the ratio of complexed to uncomplexed ligand and hence the determination of the stability constant in the following manner.
(Since the concentration of calcium ion in the solution is now generally in considerable excess over that of the potassium ion, the concentration of potassium complex will be considerably lower than that of the calcium complex and will not be included in the calculation).

TABLE 5.2 Stability Constant for Calcium Glucuronate - polarimetry

a_1 : 72.3 a_2 : 88.3

Wavelength : 365 nm.

$[\alpha]$	n_{ML}	M_t	L_t	$[CaL^+]$	$[Ca^{++}]$	$[L^-]$	K_c	K_t
77.45	0.322	0.0521	0.0183	0.0059	0.0462	0.0124	10.3	27.0
78.61	0.394	0.0764	0.0179	0.0071	0.0693	0.0109	9.4	27.0
79.62	0.458	0.0996	0.0175	0.0080	0.0915	0.0095	9.3	29.1
80.65	0.522	0.1216	0.0171	0.0089	0.1127	0.0082	9.7	31.2
81.33	0.565	0.1428	0.0168	0.0095	0.1334	0.0073	8.0	26.5
83.03	0.672	0.1825	0.0161	0.0108	0.1717	0.0053	12.0	-
84.57	0.769	0.2190	0.0154	0.0119	0.2072	0.0036	16.1	-

Average value of thermodynamic constant for calcium glucuronate: 28.2

TABLE 5.3 Stability Constant for Calcium Galacturonate - polarimetry

a_1 : 124.2 a_2 : 143.3

Wavelength : 365 nm

$[\alpha]$	n_{ML}	M_t	L_t	$[CaL^+]$	$[Ca^{++}]$	$[L^-]$	K_c	K_t
130.6	0.333	0.0230	0.0258	0.0086	0.0143	0.0171	35.2	74.9
133.5	0.487	0.0416	0.0234	0.0114	0.0302	0.0120	31.4	76.6
135.7	0.602	0.0701	0.0197	0.0583	0.0583	0.0075	25.9	70.0
136.1	0.623	0.1151	0.0255	0.0159	0.0992	0.0096	16.7	51.4
138.6	0.754	0.1599	0.0243	0.0183	0.1416	0.0060	21.6	69.7
140.5	0.853	0.2084	0.0231	0.0197	0.1887	0.0034	30.8	-
141.3	0.895	0.2429	0.0222	0.0199	0.2221	0.0023	38.5	-

Average value of thermodynamic constant for calcium galacturonate: 72.8

Fig. 5.3 Polarimetric Studies - Calcium Glucuronate (pH 7)
Wavelength 365 nm

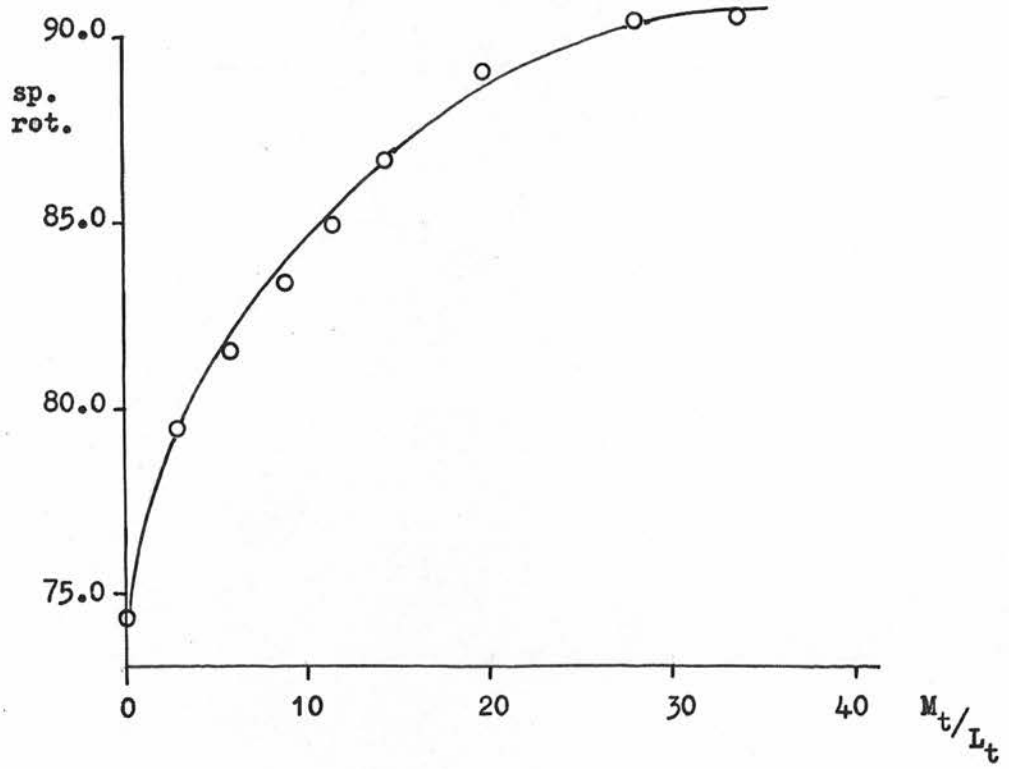


Fig. 5.4 Polarimetric Studies - Calcium Galacturonate (pH 7)
Wavelength 365 nm

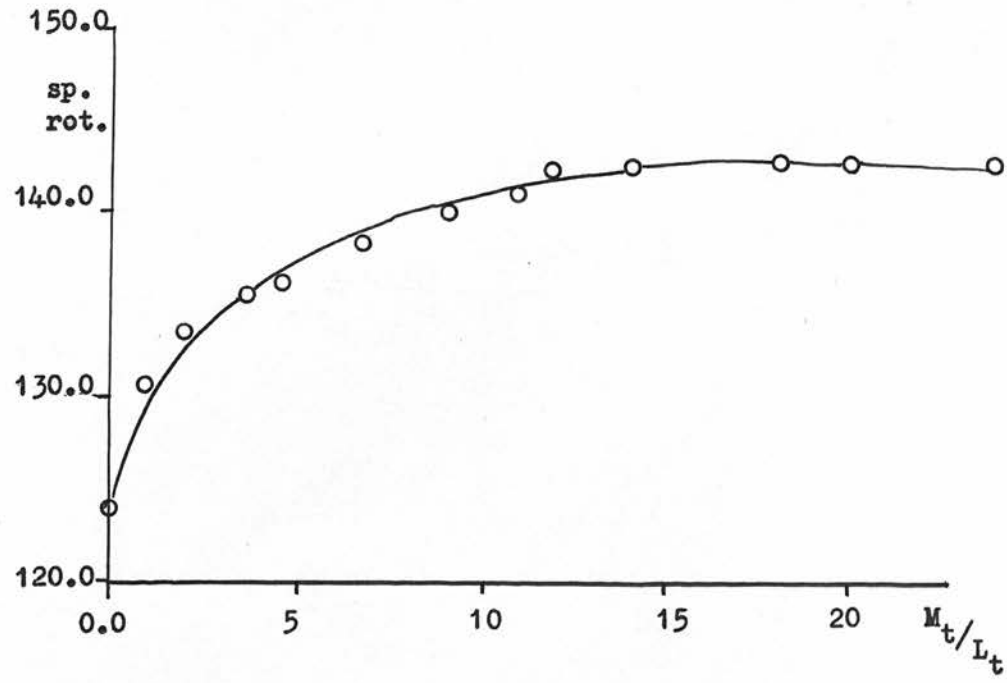
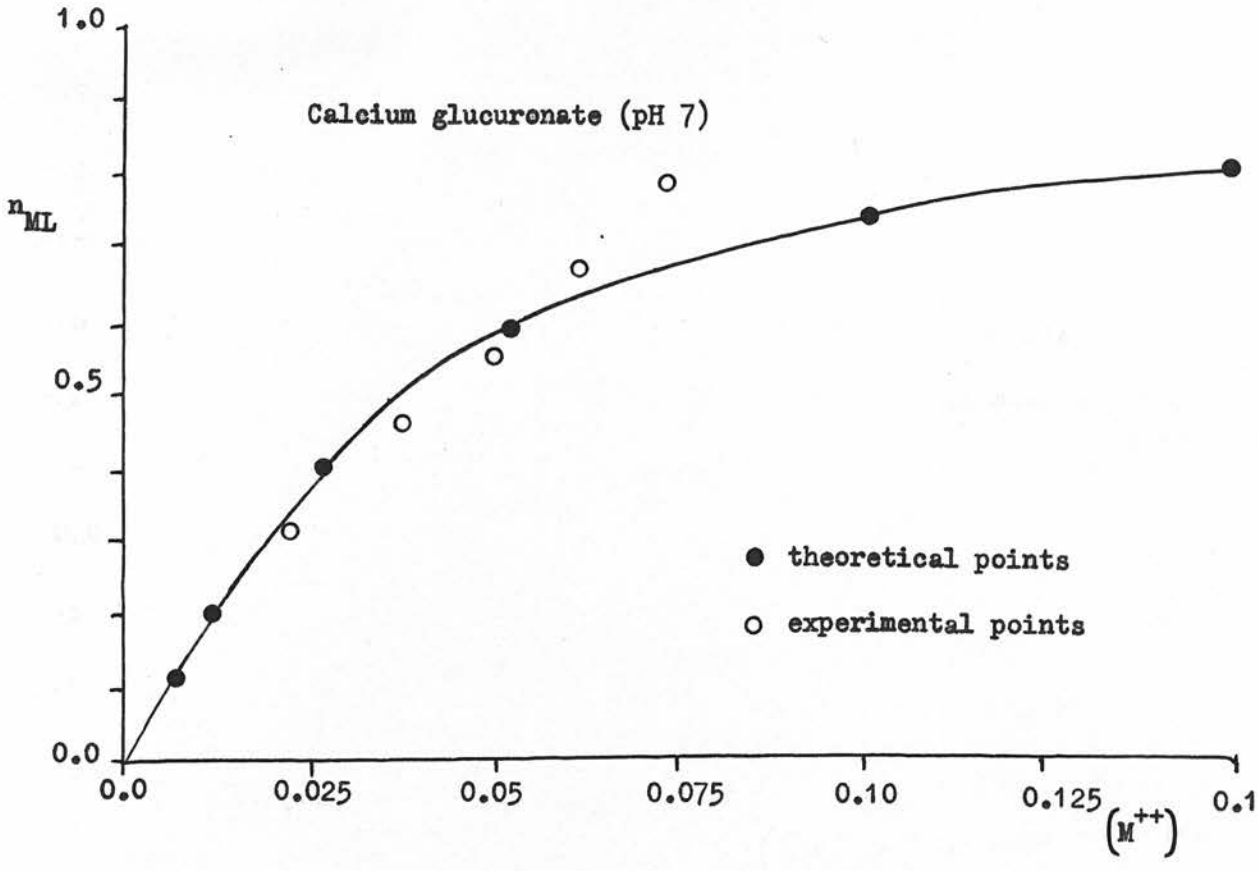
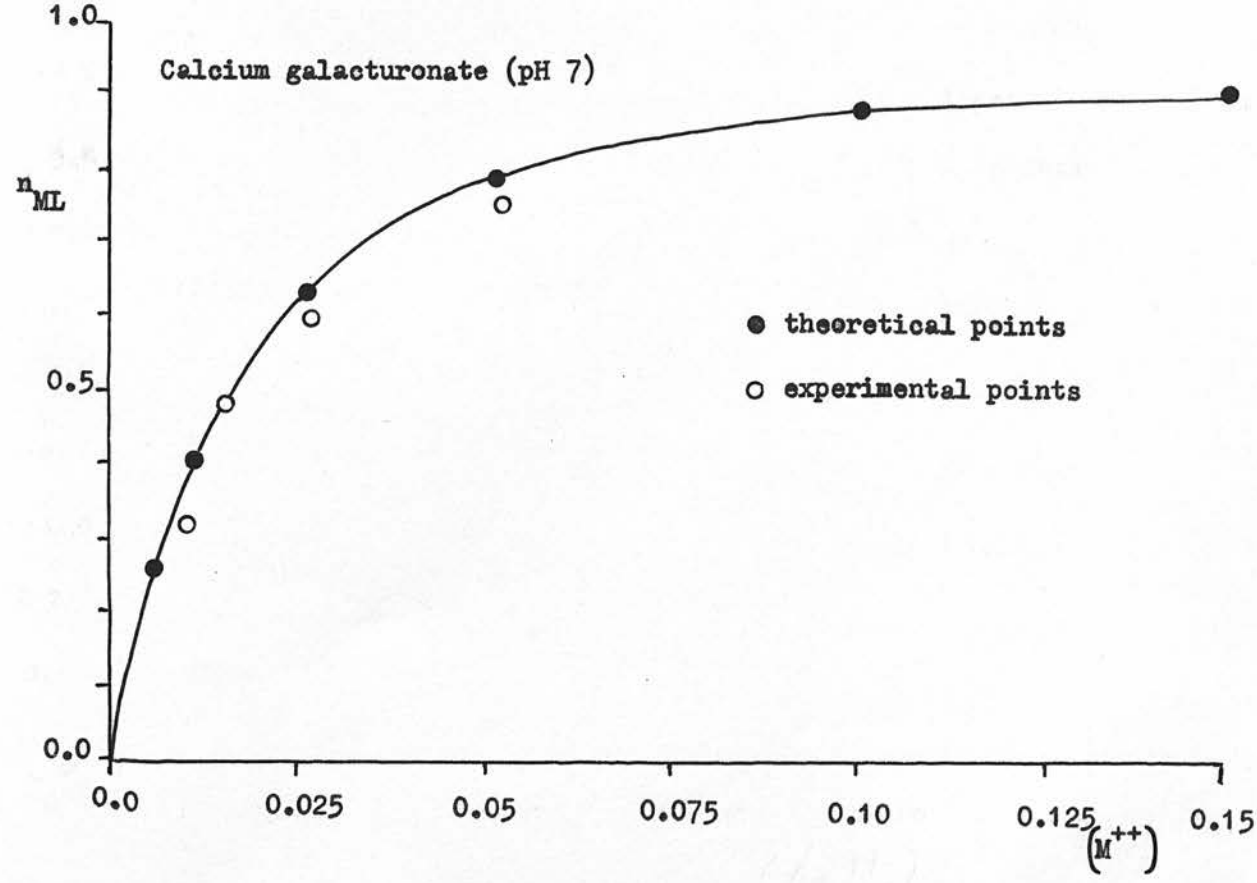


Fig. 5.5 Polarimetric Studies - Theroetical Curve Fitting





The mole fraction of ligand in the complexed form is related to the measured values of optical rotation by the expression:-

$$n_{ML} = \frac{a - a_1}{a_2 - a_1} \quad \ldots\ldots\ldots\ldots (5.3)$$

where a is the observed specific rotation,

a_1 is the specific rotation of the uncomplexed species (i.e. the initial value before addition of metal solution)

and a_2 is the specific rotation of the complexed species (i.e. the limiting value for the specific rotation).

The concentration of complexed ligand is then given by:-

$$[ML^+] = n_{ML} \cdot L_t \quad \ldots\ldots\ldots\ldots (5.4)$$

and of the uncomplexed ligand by:-

$$[L^-] = (1 - n_{ML}) \cdot L_t \quad \ldots\ldots\ldots\ldots (5.5)$$

where L_t is the total concentration of ligand present.

The concentration of uncomplexed cation can also be readily determined by subtraction of the concentration of complexed species from the total metal concentration. Thus the concentration of all species taking part in complex formation have been determined and values for the concentration stability constant can be calculated from the expression:-

$$K_c = \frac{[ML^+]}{[M^{++}][L^-]} = \frac{n_{ML} \cdot L_t}{(L_t - n_{ML}.L_t)(M_t - n_{ML}.L_t)} \ldots (5.6)$$

This value, which can be calculated from each pair of values for metal ion concentration and the corresponding specific rotation, can be converted into the thermodynamic

stability constant to allow comparison of the different values. The conversion to the thermodynamic constant is by means of activity coefficient correction (Chap. 1) but this can be simplified by employing the assumption that the activity coefficients for the univalent species are approximately equal (see Chap. 1) and can therefore be ignored. The expression for the thermodynamic constant is:-

$$K_t = \frac{[ML^+]}{(M^{++})\,[L^-]} = K_c \cdot \frac{1}{f_{M^{++}}} \quad \ldots\ldots\ldots (5.7)$$

An alternative method of expressing the results is to compare graphs of the mole fraction of complex against the metal activity in the solution for the experimental results with those obtained using calculated figures. These theoretical curves are obtained by assuming various values for the thermodynamic stability constant for each complex. From these the ratio of complex to uncomplexed ligand can be calculated for a series of cation activities using equn. 5.7 and this ratio in combination with a value for the total concentration of ligand allows calculation of values for the mole fraction of complex, n_{ML}, corresponding to any value of cation solution activity. Comparison of the experimental curve for any complex with a series of theoretical curves calculated for different thermodynamic stability constants to find the most readily superimposed theoretical curve allows an average thermodynamic stability constant to be estimated from the experimental points (Fig. 5.5).

The activity coefficients used in this work are those of Harned and Owen[74] since, at the ionic strength used,

the single ion activity coefficient used in previous calculations (Chaps. 3 and 4) is not satisfactory. At this ionic strength, a modified form of the Debye-Huckel equation (see Section 1.3) must be used since the limiting form applies only to very dilute solutions although it can be used with reasonable precision at finite but low ionic strengths.

The average values for the overall thermodynamic stability constants for calcium glucuronate and calcium galacturonate, excluding any correction for the formation of any potassium complexes, were taken as 30 and 73 respectively.

Study of uronate complexes at pH 4.2

One of the principal restrictions on an investigation of complexes by this method at pH 7 is in the choice of cations. Most metal hydroxides are of limited solubility and, since the metal is in great excess in the solution and would therefore be largely uncomplexed, precipitation would occur. To overcome this, a more acidic solution of pH is necessary. The limitation in lowering the pH of the test solution is, as discussed above, that complexing only takes place in the presence of the dissociated form of the ligand. Therefore, the pH of the solution must be maintained at a sufficiently high level to allow some degree of dissociation.

It was decided to study the stability of some complexes of D-glucuronic acid, notably transition metal complexes, using this method at a solution pH value of 4.2. At this pH, the ligand is about 90% dissociated while most

TABLE 5.4 Change in Rotation with Increasing Metal Ion Concentration - Nickel Nitrate (pH 4.2)

a_1 : 31.75 a_2 : 37.75

Wavelength : 546 nm.

$[\alpha]$	n_{ML}	L_t	M_t	$[ML^+]$	$[M^{++}]$	$[L^-]$	Vol.hydroxide soln.added(ml.)	K_c
33.77	0.337	0.0162	0.0239	0.00547	0.0184	0.01077	3.63	27.5
35.16	0.568	0.0153	0.0563	0.00870	0.0476	0.00661	3.63	27.7
35.33	0.597	0.0150	0.0663	0.00896	0.0574	0.00606	3.64	25.8
35.79	0.673	0.0148	0.0760	0.00993	0.0660	0.00482	3.62	31.2
36.12	0.728	0.0145	0.0852	0.01054	0.0747	0.00393	3.64	35.9
36.54	0.798	0.0142	0.0943	0.01136	0.0829	0.00287	3.60	47.7
36.69	0.802	0.0140	0.1031	0.01124	0.0919	0.00278	3.64	44.0
36.81	0.843	0.0137	0.1110	0.01156	0.0994	0.00215	3.67	54.1
37.28	0.878	0.0135	0.1192	0.01186	0.1073	0.00164	3.64	67.3
37.48	0.922	0.0131	0.1345	0.01203	0.1225	0.00102	3.63	96.1

TABLE 5.5 - Change in Rotation with Increasing Metal Ion Concentration - Cobalt Nitrate (pH 4.2)

a_1 : 76 63 a_2 : 85.09

wavelength: 365 nm

$[\alpha]$	n_{ML}	L_t	M_t	$[ML^+]$	$[M^{++}]$	$[L^-]$	Vol.hydroxide soln.added (ml)	K_c
79.53	0.343	0.0163	0.0226	0.00560	0.0110	0.01074	3.47	30.8
80.09	0.409	0.0159	0.0330	0.00652	0.0265	0.00942	3.59	26.1
80.48	0.455	0.0157	0.0433	0.00713	0.0361	0.00854	3.52	23.1
81.56	0.583	0.0529	0.0529	0.00894	0.0440	0.00640	3.58	31.8
82.06	0.642	0.0150	0.0622	0.00964	0.0526	0.00538	3.62	34.1
82.56	0.701	0.0147	0.0710	0.01034	0.0607	0.00441	3.63	38.6
82.69	0.716	0.0145	0.0799	0.01037	0.0696	0.00411	3.63	36.3
83.01	0.754	0.0142	0.0884	0.01074	0.0777	0.00350	3.60	39.5
83.44	0.805	0.0140	0.0964	0.01125	0.0852	0.00273	3.62	48.4
83.82	0.850	0.0138	0.1044	0.01169	0.0928	0.00207	3.57	61.0
83.71	0.837	0.0135	0.1121	0.01133	0.1007	0.00221	3.58	50.9
84.37	0.915	0.0131	0.1262	0.01195	0.1142	0.00111	3.63	94.2

TABLE 5.6 - Change in Rotation with Increasing Metal Ion Concentration
Calcium Nitrate (pH 4.2)

a_1 : 76.67 a_2 : 84.05

Wavelength: 365 nm

$[\alpha]$	n_{ML}	L_t	M_t	$[ML^+]$	$[M^{++}]$	$[L^-]$	Vol.hydroxide soln.required (ml)	K_c
77.69	0.138	0.0163	0.0302	0.00225	0.0279	0.01153	3.53	7.0
78.50	0.248	0.0156	0.0579	0.00388	0.0540	0.00965	3.57	7.4
78.83	0.293	0.0154	0.0707	0.00449	0.0662	0.00890	3.57	7.6
79.42	0.373	0.0150	0.0835	0.00561	0.0779	0.00774	3.59	9.3
79.91	0.439	0.0148	0.0956	0.00648	0.0891	0.00679	3.61	10.7
80.35	0.499	0.0145	0.1072	0.00722	0.0999	0.00596	3.62	12.1
81.09	0.599	0.0140	0.1293	0.00837	0.1209	0.00460	3.63	15.1
82.86	0.839	0.0126	0.1867	0.01058	0.1762	0.00167	3.69	36.0

TABLE 5.7 - Change in Rotation with Increasing Metal Ion Concentration

Copper Nitrate (pH 4.2)

a_1 : 76.70 a_2 : 123.0

wavelength : 365 nm

$[\alpha]$	n_{ML}	L_t	M_t	$[ML^+]$	$[M^{++}]$	$[L^-]$	Vol.hydroxide soln.added (ml)	K_c
99.92	0.502	0.0162	0.0181	0.00813	0.00997	0.00807	3.64	100.9
105.96	0.632	0.0156	0.0343	0.00986	0.02444	0.00574	3.70	70.3
109.00	0.698	0.0150	0.0499	0.01047	0.03943	0.00463	3.75	57.3
112.48	0.773	0.0144	0.0641	0.01113	0.05297	0.00327	3.78	64.2
115.19	0.831	0.0139	0.0773	0.01155	0.06575	0.00235	3.77	74.8
116.71	0.864	0.0137	0.0895	0.01184	0.07766	0.00186	3.82	82.0
117.52	0.882	0.0130	0.1011	0.01147	0.08963	0.00153	3.83	83.6
118.84	0.910	0.0122	0.1219	0.01110	0.11080	0.00110	3.86	91.1
120.86	0.954	0.0115	0.1402	0.01096	0.12924	0.00054	3.90	157.1

Fig. 5.6 Polarimetric Studies - Nickel glucuronate (pH 4.2)

Wavelength 546 nm

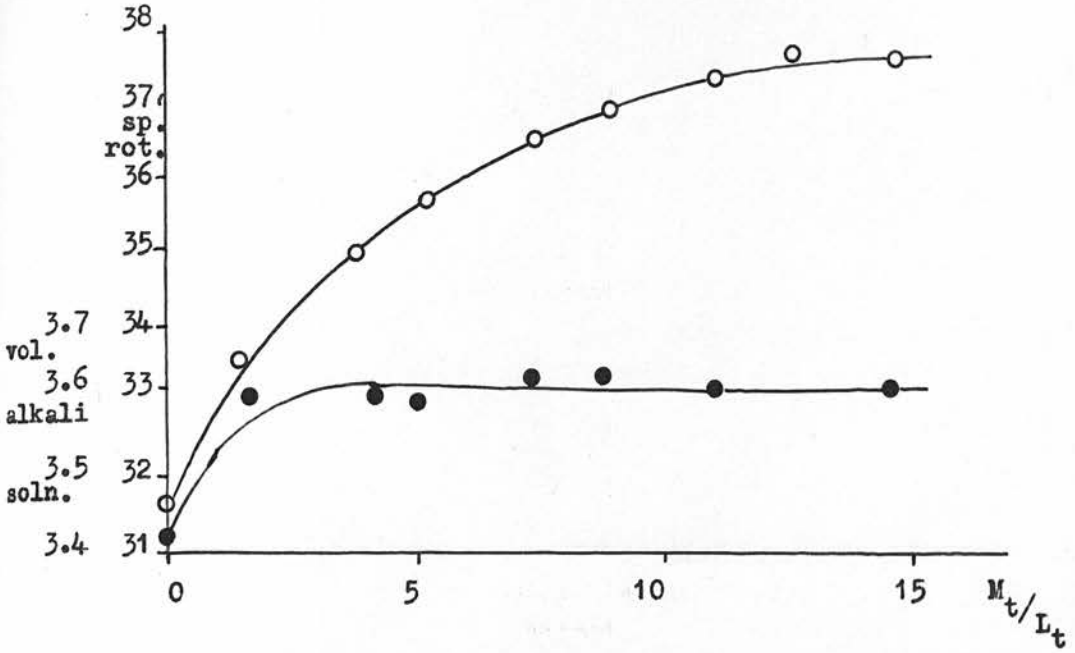


Fig. 5.7 Polarimetric Studies - Cobalt glucuronate (pH 4.2)

Wavelength 365 nm

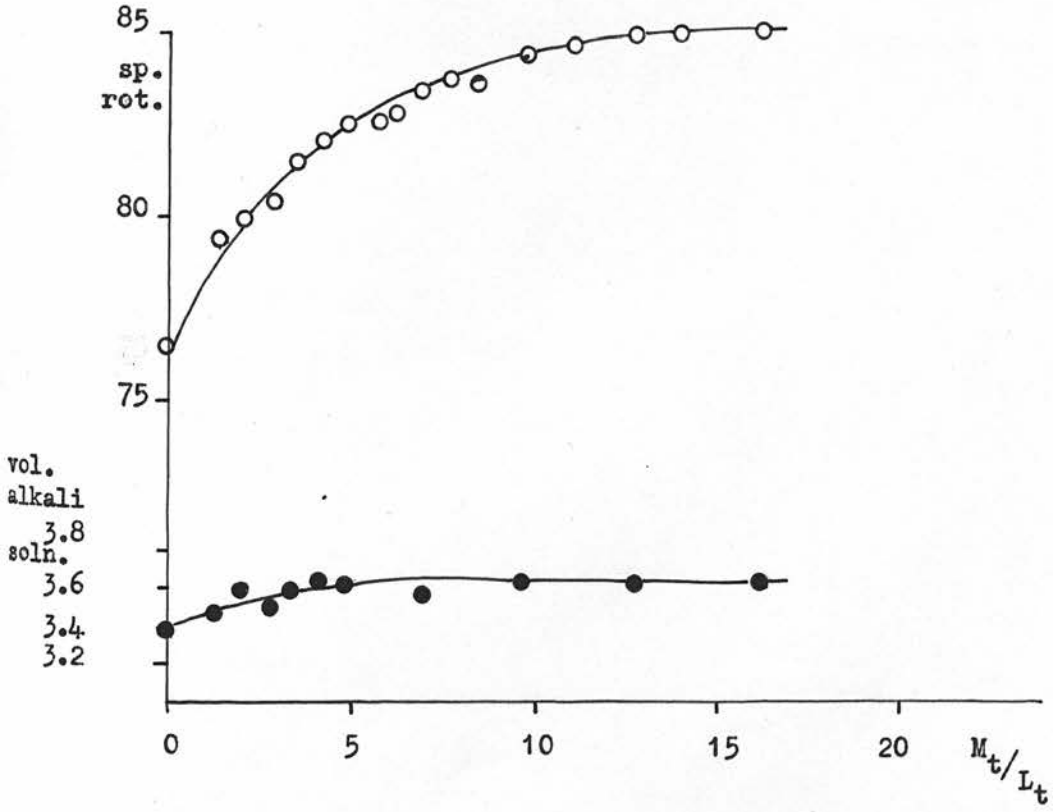


Fig. 5.8 Polarimetric Studies - Calcium glucuronate (pH 4.2)
Wavelength 365 nm

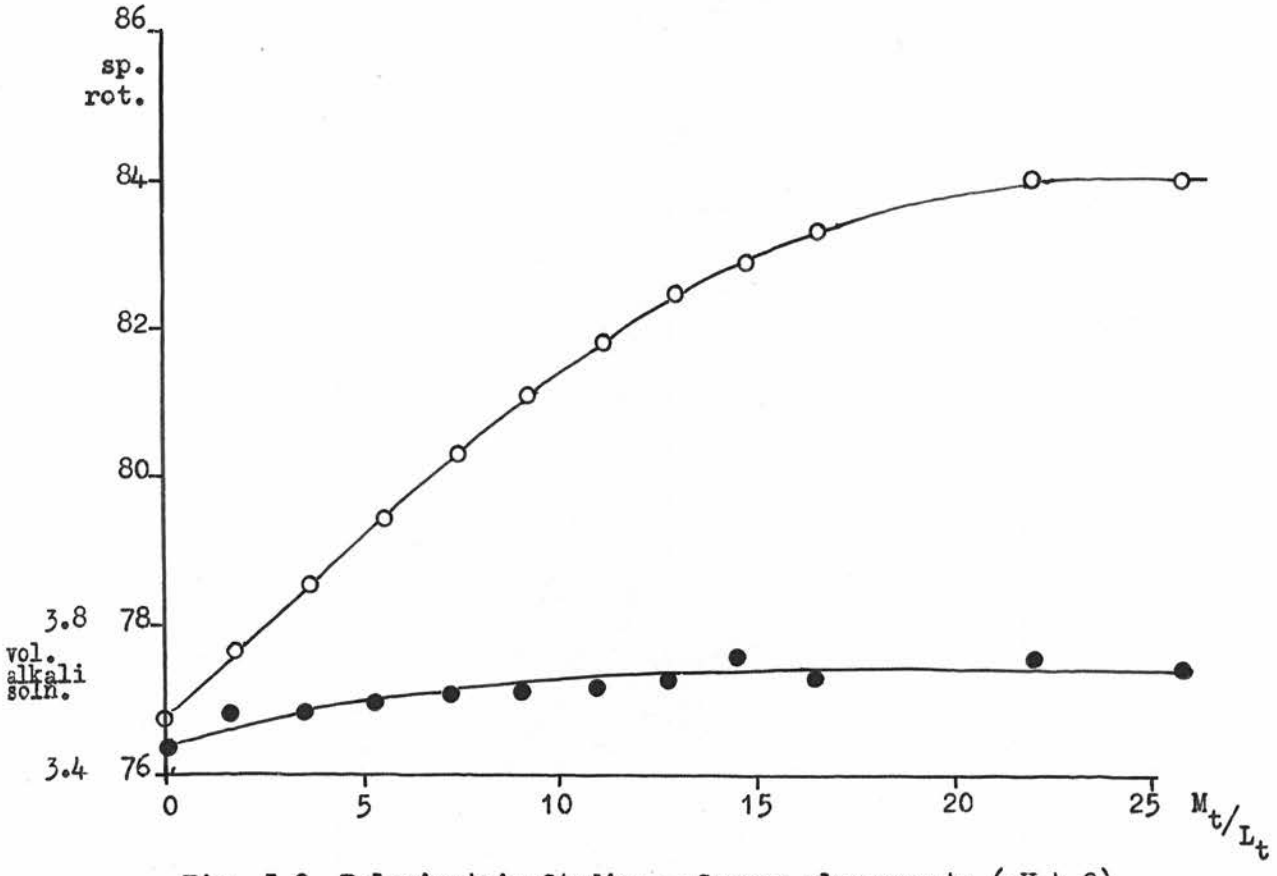
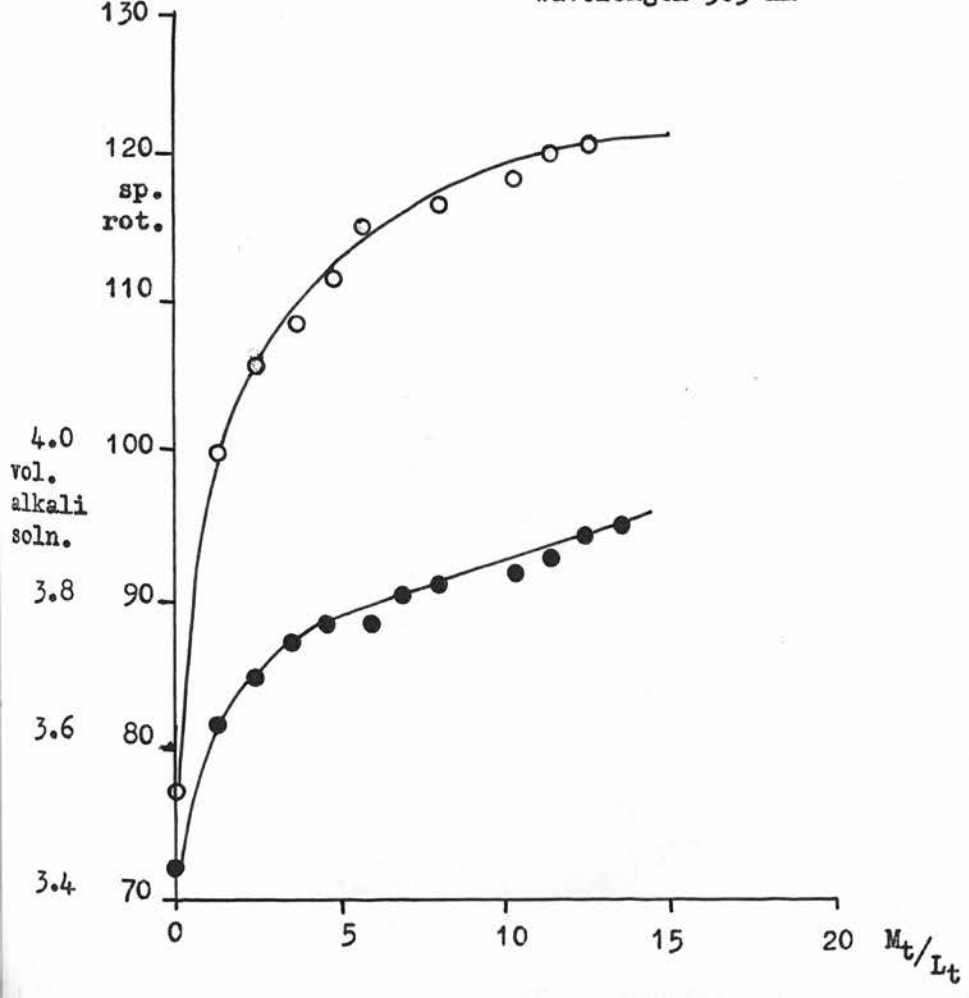


Fig. 5.9 Polarimetric Studies - Copper glucuronate (pH 4.2)
Wavelength 365 nm



Values for the stability constants for the complexes with nickel, cobalt and calcium were calculated by a method similar to that used for the complexes at pH 7 (see above). The principal difference was that the mole fraction of complex, which was obtained from equation 5.3, was multiplied by the concentration of dissociated ligand, not the total concentration of ligand as above, to give the concentration of complex. (This takes account of the incomplete dissociation of the ligand at this pH). The concentration of dissociated ligand was obtained from the total concentration of ligand, solution pH and the pK_a of the ligand as determined in Chapter 3.

The results obtained from these complexes could also be expressed by a comparison method with theoretical values similar to that employed for the complexes at pH 7. Such a comparison, however, showed poor agreement which could be due to a number of factors. The solutions under test contained three ligand species - protonated, dissociated and complexed forms - each of which, in addition to showing a different degree of optical rotation, could exist as different mixtures of anomeric forms (see Chap. 1). A further source of possible error is in the unknown concentration of lactone which might be present. This could not occur in the determinations at pH 7 since such species are hydrolysed to the anion of the acid.

Study of Calcium Complexes of Uronosides at pH 7

An attempt to determine the stabilities of the calcium complexes of methyl α-D-glucuronoside and methyl β-D--galacturonoside at pH 7 was carried out in an identical

manner to that above. For these ligands, however, no change in rotation was observed with increasing calcium concentration (Tables 5.8 and 5.9). The reason for this surprising observation cannot be that the complexes do not form since it has already been found (Chap. 3) that the complexes of the methyl uronosides are of comparable stability to those of the corresponding uronic acids. Since, in solutions of the uronosides only one anomeric form is present, whereas both anomeric forms co-exist in equilibrium in solutions of the uronic acids, the change in rotation observed on complexing with uronic acids may be caused by a shift in the anomeric equilibrium due to the different relative stabilities of the complexes formed by each anomer. No such change could take place during uronoside complex formation and this method is inapplicable for complexes of ligands of this type. In order to test this hypothesis, the nuclear magnetic resonance spectra of uronate solutions were studied.

Table 5.8 Rotation of Methyl α-D-glucuronoside in presence of Nickel Nitrate Solutions

Wavelength: 546 nm.

$[\alpha]$	M_t/L_t
109.75	0
110.8	15

Table 5.9 Rotation of Methyl β-D-galacturonoside in presence of Nickel Nitrate Solutions

Wavelength: 546 nm

$[\alpha]$	M_t/L_t
-57.5	0
-57.5	15
-57.7	40

5 . 3 N.M.R. Studies

The relative concentrations of the two anomers in a sugar solution can be directly measured by N.M.R. spectroscopy[75]. The position in the sugar ring of the anomeric proton on C(1) is unique, as C(1) is attached to two electron-withdrawing oxygen atoms. In addition, the protons on C(1) from each of the anomeric forms resonate at different applied field resulting in the direct identification of the doublet due to each anomer. The axially oriented hydrogen (on the β-anomer) is more shielded than the equatorially oriented hydrogen [H^{α}] and resonates at higher applied field[76]. This method of determining the relative concentrations of the anomeric forms can be used to study the shift in equilibrium which may occur when the ligands form complexes with metal ions.

N.M.R. spectra of sodium D-glucuronate and sodium D-galacturonate (each acid neutralised with sodium carbonate solution) both in the presence of calcium ions and in the absence of calcium ions were recorded. (Details of the preparation of solutions and methods of measurement are contained in Section 5.5). The data collected from these spectra were the chemical shifts of the anomeric protons due to the α- and β-anomers and the relative intensities of these absorptions as determined by the integrals. The results are tabulated in Table 5.10.

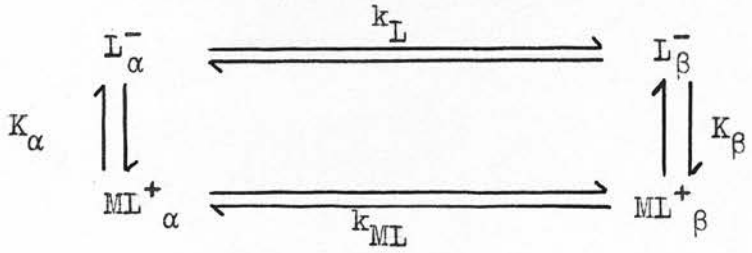
Table 5.10 Changes in Anomeric Equilibrium due to Complexing

Ligand	Chemical Shift		% in each anomeric form.	
	α-anomer	β-anomer	α-anomer	β-anomer
Sodium D-glucuronate (0.3 mol/l)				
(a) in deuterium oxide solution	4.77	5.37	41.8	58.2
(b) in a solution of calcium nitrate (approx. 2 mol/l) in deuterium oxide	4.73	5.40	49.8	50.2
Sodium D-galacturonate (0.3 mol/l)				
(a) in deuterium oxide solution	4.72	5.43	37.5	62.5
(b) in a solution of calcium nitrate, (approx. 2 mol/l) deuterium oxide	4.51	5.39	47.8	52.2

The results from this study clearly show that a shift in anomeric equilibrium occurs during complexing. This suggests that the complexes between the α-anomer of the acids with calcium ions are more stable than those of the β-anomer, the shift being towards the α-anomer.

5 . 4 Anomeric Constants

It has already been noted that, although the molecular rotation of the uronic acids changes on the addition of calcium ions, that of the methyl uronosides is not affected. Since it has been established (Chap. 3) that their calcium complexes are of comparable strength to those of the free uronates, the shift in rotation in this latter case must be due to a shift in the equilibrium between the α- and β-forms of the anion. The implication of this is that the stability of the complex formed between the α-form of the anion and calcium is different to that between the β-form and calcium. In order to calculate the stability constants for the complexes of both forms, K_α and K_β it is necessary to evaluate constants k_L and k_{ML}:-



where $k_L = \frac{[L_\alpha^-]}{[L_\beta^-]}$ and $k_{ML} = \frac{[ML_\alpha^+]}{[ML_\beta^+]}$

and K_α and K_β are the stability constants for the α- and β-forms of the anion respectively.

The values of k_L and k_{ML} for the ligands were estimated from the relative sizes of the ^1H n.m.r. signals of the anomeric protons in a deuterium oxide solution (Table 5.11). The results are tabulated in Table 5.11.

Table 5.11 Changes in Ratio of Anomeric forms on Complexing

Ligand	k_L	k_{ML}
Glucuronate	0.72	0.94
Galacturonate	0.66	0.96

Using these constants, it is possible to convert the overall thermodynamic stability constants, K', for each ligand into true stability constants for complexes of each of the two anomeric forms of the ligand. The overall thermodynamic stability constant is defined as:-

$$K' = \frac{([ML^+_\alpha] + [ML^+_\beta])}{(M^{++})\,([L^-_\alpha] + [L^-_\beta])}$$

where the subscripts represent the two modifications of the ligand, and it is assumed that activity coefficients for the singly charged species effectively cancel one another. The stability constants for the anomeric forms are defined as:-

$$K_\alpha = \frac{[ML^+_\alpha]}{(M^{++})[L^-_\alpha]} \quad \text{and} \quad K_\beta = \frac{[ML^+_\beta]}{(M^{++})\,[L^-_\beta]}$$

These constants can also be defined in terms of k_L and k_{ML} as:-

$$K_\alpha = K' \cdot \frac{k_{ML}\,(1 + k_L)}{k_L\,(1 + k_{ML})} \quad \text{and} \quad K_\beta = K' \cdot \frac{(1 + k_L)}{(1 + k_{ML})}$$

By substitution of the values for k_L and k_{ML}, values for the stability constants of the complexes of the anomeric forms of D-glucuronic acid and D-galacturonic acid can be predicted. These are compared with the measured values

for the methyl glycuronosides (Chap. 3) in Table 5.12.

Table 5.12 Stability Constants for the Calcium Complexes of the Anomeric Forms of Uronic Acids

Ligand	Measured values (see Chap. 3)	Calculated values
Glucuronate	32	-
Galacturonate	64	-
α-Methyl glucuronosidate	40	37
β-Methyl glucuronosidate	-	28
α-Methyl galacturonosidate	-	79
β-Methyl galacturonosidate	48	54

5 . 5 Experimental

Preparation of Ligand Solutions

For the polarimetric studies, stock ligand solutions were prepared by dissolving weighed amounts of the ligand (prepared as described in Chap. 2) in distilled water. These solutions were allowed to stand for at least 2 hours before use to ensure complete mutarotation had occurred. Ligand solutions, which were stored in sealed glass bottles in a refrigerated room, were replaced regularly (2-3 weeks) to minimise the rise of bacterial contamination.

To prepare the ligand solutions in deuterium oxide for use in the n.m.r. studies, it was necessary to deuterate the hydroxyl substituents. This was achieved by dissolving a weighed amount of the ligand in deuterium oxide allowing an equilibrium to take place in which some of the hydroxyl groups were deuterated. The deuterium oxide - water mixture was removed using a vacuum desiccator before the process was repeated twice. The resulting solid was dissolved in a known volume of deuterium oxide and, for the studies involving neutralised solutions, the theoretical amount of 'Analar' sodium carbonate was added to give the sodium salt of the ligand.

Preparation of Metal Ion Solutions

Weighed amounts of 'Analar' metal nitrates were dissolved in distilled water in the preparation of stock metal ion solutions.

These solutions were standardised by passage through a cation exchange column in the acid form and titrating the

liberated acid with standard alkali.

The calcium solutions for use in the n.m.r. studies were prepared by adding a weighed amount of 'Analar' calcium nitrate to the deuterated ligand solution described above.

Method of Measurement - Polarimetry

The solution whose optical rotation was to be measured was added to a polarimeter cell. This cell was fitted with a water jacket through which water at a given temperature could be pumped.

The polarimeter cell was placed in a Perkin-Elmer 142B Polarimeter set at the required wavelength. The value quoted for the optical rotation is the average of at least five separate determinations.

Method of Measurement - N.M.R.

N.M.R. charts were recorded on a Varian HA-100 N.M.R. Spectrometer. The sample in a tube was placed in the instrument and maintained at a standard temperature of 28°C.

The signal was locked on the chemical shift attributable to tetra-methyl silane (TMS) and, for some charts, benzene was also used as an external integral standard.

CHAPTER 6

CONCLUSION

Chapter 6 Conclusion

In this study, a number of experimental methods were used to determine the degree of association between uronic acids and some metal ions in aqueous solutions. This provides both a comparison of the effectiveness of the different methods and a comparison of the complexing power of species under varied experimental conditions. Consequently, a more reliable estimate of the weak degree of complexing between some uronic acids and metal ions is possible than would have been had a single method been employed.

Uronic acids exist in aqueous solution as an equilibrium mixture of forms - the principal equilibrium being that between the α- and β- anomers (see Chap. 1). Throughout most of the determinations, it was assumed that the complexing takes place between the equilibrium mixture of these anomers (considered as one species) and the metal ion. Stability constants for the complexes of each of the anomeric species can, however, be measured using certain types of experimental methods, especially polarimetry and proton magnetic resonance.

6 . 1 Uronate Complexes

The occurrence in biological systems of alkali and alkaline earth metal ions has already been described (Section 1.1). The importance of these ions in such systems lies in the power with which each complexes with certain organic compounds and it is for this reason that

stability constants have been determined for the complexes formed between some of these cations and certain uronic acids. In addition, the complexing of these ligands with certain transition metal ions plays a lesser but still important role and stability constants of this type of complex have also been determined.

Three types of methods have been adopted in the determination of stability constants of these complexes - pL method, pM method and polarimetric. Since each of these is carried out under different experimental conditions, subject to different systematic errors, a more reliable value for the stability constant of the complex can be obtained by comparing values from all methods. The different conditions under which the complexes have been studied are summarised in Table 6.1.

The relative precision of the stability constant determinations by the various methods is different. The error involved in determining a concentration will, in general, be large when that concentration is the difference of two concentrations of similar magnitude and will be least when the concentration to be determined is similar to the analytical concentration of the species. This principle can be applied to the stability constants calculated from the different methods. In the case of the potentiometric titrations, the principal sources of error lie in the measurement of electrode response and in the values of solutions added whereas, in the polarimetric studies, the errors lie in the rotation measured and the volumes of solution. These errors are transmitted into the calculated value in the following manner.

The error in the measured electrode response is transmitted into each term of the expression for the

TABLE 6.1 Comparison of the Methods of Study

Method	Concentration Variation within a single run for Total Ligand	Concentration Variation within a single run for Total Metal	pH	Ionic Strength	Comments
1 Glass Electrode (pL)	Constant	Constant	Variable (pH3-8 approx)	Constant (0.1 mol/l)	Metal ion concentration is similar or in excess of ligand
2 Liquid Membrane Electrode (pM)	Variable	Constant	Constant (in range: pH6-8)	Variable (in range: 0.004-0.012)	Ligand is normally in excess
3 Polarimetric	Constant	Variable	Constant (at various pH values)	Variable (in range: 0.05-0.3)	Excess metal ion over ligand.

stability constant for the pL method (Chap. 4). In this case, the hydrogen ion concentration, which is directly related to the measured value of pH, appears directly in the derivation of the free ligand concentration (see equation 4.8) and of the protonated ligand concentration (equation 4.7). It will therefore also affect the derivation of the concentration of complexed species (equation 4.10) as this includes the concentration of ligand in the different forms. The concentration of free metal ion in the solution will only be affected indirectly and the error in this term will be relatively small since the concentration of complexed species was always much lower than the total metal concentration. The greatest error by this method occurs in the concentration of complex since the sum of concentrations of ligand in all other forms is of similar magnitude to the total ligand concentration. The value for the free ligand concentration will show least precision at the beginning of the titration when the concentration of hydrogen ion is of similar to that of the total ligand, and at the end of the titration when the concentration of base added is similar to the total ligand concentration. This latter error is due to the second reading error - that of the volume of base added.

The sources of error in determinations by the pM method (Chap. 3) are similar. In this case, a direct measure of the free metal ion activity is possible and this can, by means of a suitable activity coefficient, be converted into the concentration of free metal ion.

It is likely that this will cause a degree of error since no exact determination of activity coefficients can be made. A further error will occur in this concentration caused by the electrode potential reading error. The combination of these errors is introduced into the derivation of the concentration of complex where the accuracy will be low since the difference in concentration of the free metal ion and total metal ion is small. The precision in the determination of free ligand concentration is high since it is little different from the concentration of total ligand in the solution. For this method, the chief source of error again lies in the concentration of complexed species but the precision will improve as the titration progresses since the difference between the total metal ion and observed metal ion becomes greater.

The determination of stability constants by polarimetry employs direct measurement of the ratio of complexed to uncomplexed ligand. This method therefore eliminates small differences in concentration from the calculation. However, any error in the specific rotation is transferred directly into the concentration stability constant (this term also includes the ratio of complexed to uncomplexed ligand). The main source of error, particularly in the glucuronate determinations, arises from the uncertainty in the limiting value for the rotation in the presence of excess metal.

The stability constants determined for a range of uronate complexes by different methods are tabulated in

TABLE 6.2 Thermodynamic Stability Constants for Some Metal Uronates

Ligands	Cations									
	H^{+}*	Ca^{++}			Co^{++}	Cu^{++}	Mg^{++}	Ni^{++}		K^{+}
	A	A	B	C	A	A	A	A	B	B
D-glucuronic acid	3.19	48	32	30	56	236	58	60	58	2
D-galacturonic acid	3.49	115	64	73	124	-	-	-	-	4
Methyl α-D-glucuronoside	3.16	-	40	a	-	-	-	-	-	-
Methyl β-D-galacturonoside	3.48	-	48	a	-	-	-	-	-	-

Method A - pH titration

Method B - cation responsive electrode titration

Method C - polarimetry

a - not applicable

* expressed as pK_a - a 'mixed' constant

Table 6.2 together with an estimate of the thermodynamic constant for each complex. These values can then be compared with values for other similar types of complexes.

In general, complexes formed between transition metal ions and ligands are considerably more stable than similar alkaline earth metal complexes. This is due to the transition metal ion having suitable vacant electronic orbitals for bonding which are not present in the alkaline earth metal ion. However, in the present work, the stability of the complexes formed by D-glucuronic acid with calcium ions and with nickel ions show little difference. By comparison, the corresponding complexes with lactic acid show a marked stability difference, that for the calcium complex being 11.7[77] (ionic strength 0.2) while that for the nickel complex is 164[78] (ionic strength unknown). It is to be expected that the increased number of substituent hydroxyl groups with D-glucuronic acid as ligand would have increased the degree of chelation with the transition metal ions thereby causing greater stability. This would then follow the pattern of change in complex stability between acetic acid and lactic acid (see Table 6.3).

Table 6.3 Changes in Complex Stability with Increasing Hydroxyl Substitution - Calcium and Nickel

Ligand		Acetic Acid	Lactic Acid	D-glucuronic Acid
K_t	Calcium Complex	15[77]	60[77]	35
	Nickel Complex	13[79]	164[78]	60
Number of hydroxyl substituents		0	1	4

However, in the present series, the difference in stability of the complexes of alkaline earth metal ions and transition metal ions with uronic acids is small. This deviation from the expected pattern is explicable in terms of the spatial configuration of the ions involved in complex formation. Unlike the lactate anion, which will be relatively free to allow some distortion of the molecular shape, the glucuronate ion is a comparatively rigid molecule with limited degrees of freedom. The hydroxyl groups, which will be slightly charged on account of delocalisation of charge from the carboxylate anion, will be fixed in the glucuronate whereas that in the lactate will be more able to orientate to take part in bonding. From this, chelation may occur in the formation of complexes of lactic acid with cations having suitable vacant electronic orbitals, such as transition metals. This is less likely in the case of the uronic acids where the bonding will consist largely of strong ion-pair formation between the anion in which the charge is partly delocalised and the metal ions. In such a case, the stability of complexes with cations of the same charge would be similar.

The complex between D-glucuronic acid and copper ions is apparently of much greater stability. It is, however, possible that this value is erroneous to some extent on account of the formation of hydrolysed forms of copper. Hydrolysed copper species can form at relatively low pH values and would result in the liberation of hydrogen ions reducing the pH of the solution. Since the main method

employed in the study of this complex was a glass electrode 'pL' Method (see Chapter 4) which used changes in the pH as a measure of the degree of complexing, this could lead to false values of the stability constant. For example, at pH4 approximately 1% of the total concentration of copper ions will be in the form of a singly-charged monohydrate. In addition, solid copper hydroxide precipitates at pH5 when the copper concentration is approximately 0.02 mol/l.

The weaker acidity of D-galacturonic acid relative to D-glucuronic acid was discussed in Chapter 2 largely in terms of the greater delocalisation of charge giving rise to a less stable anion. This less stable anion will complex more strongly with cations giving greater stability of the complexes which is clearly observed in Table 6.2.

The alkaline earth metal complexes with ligands with different degrees of hydroxyl substitution show a marked increase in stability with degree of substitution (Table 6.4).

Table 6.4 Change in Complex Stability with Increasing Hydroxyl Substitution - Calcium and Magnesium

Ligand	Acetic Acid	Lactic Acid	D-Glucuronic Acid	D-galacturonic Acid
K_t Calcium Complex	15[77]	60[77]	35	80
K_t Magnesium Complex	15[77]	50[77]	55	-
Number of Hydroxide substituents	0	1	4	4

Cannan and Kibrick[77] concluded that the effect of α-hydroxyl groups in hydroxy acids is greater on the

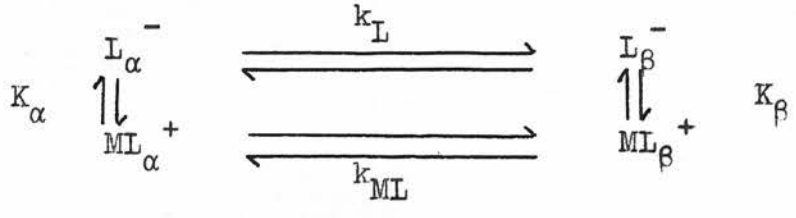
complex stability than that of any other. Hydroxyl groups in other positions can cause a slight enhancement of the stability in alkaline earth metal complexes but can cause a slight depression of the constant with some cations including magnesium. The above results in Table 6.4 for the complexes of calcium are in agreement with this but those for the magnesium complexes show no depression of the complex stability with increasing hydroxyl substitution.

6 . 2 Anomeric Constants

The values for stability constants of uronate complexes which have been reported (Section 6.1) are for the main part those for a mixture of two complexes. Since a uronic acid exists in solution as an equilibrium mixture of two anomeric forms, the complexes of the acids which have been considered must in fact consist of mixtures of the corresponding complexes of each of the anomeric forms.

In order to allow a further understanding of the relative stability of each of these complexes, an attempt was made to determine the relative amounts of each anomer in both complexed and uncomplexed ligand solutions. The most readily available method for such a study is n.m.r. spectroscopy and, since the contributions due to each anomer are clearly distinguishable, direct measurement of the relative concentrations is possible.

The method of study described in Section 5.4 is based on the assumption that the equilibrium occurring in solution can be simply described as:-

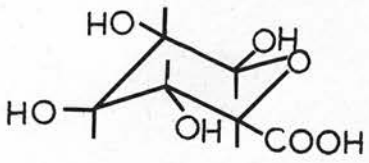


with the equilibrium constants being K_α, K_β, k_L and k_{ML}.

The values obtained for k_{ML} from ^{1}Hnmr data may not be completely correct since it is unlikely that the limiting case in which all the ligand has been complexed will be reached since this would require an extremely high

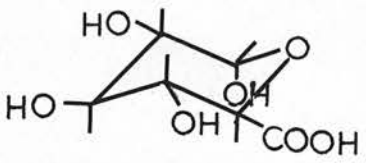
concentration of metal ion. However, the relative magnitude of this error will be small since the limiting value can be approximated.

If the stereochemistry of the four forms of D-glucuronic and D-galacturonic acids is considered, the differences lie in the orientation of one or two hydroxyl groups (fig. 6.1).



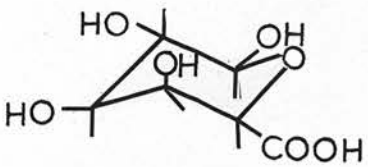
β-D-Glucuronic acid

I



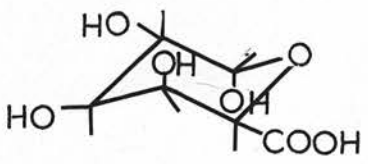
α-D-Glucuronic acid

II



β-D-Galacturonic acid

III



α-D-Galacturonic acid

IV

Figure 6.1 Stereochemistry of Some Uronic Acids

The increased stability of the complexes of the α-anomer relative to the β-anomers lies in part in the physical position of the hydroxyl on C(1). In the β-anomer, it is in a position on the opposite side of the ring from the carboxyl group reducing the degree of complexing possible if C(1) is one of the positions which is involved in bond formation.

A similar explanation would summarise the increased stability of calcium D-galacturonate complexes over the corresponding calcium D-glucuronate complexes. In this case, the difference lies in the orientation of the hydroxyl group on C(4). Another factor in the enhancement of stability is mentioned in the next section.

The increased stability demonstrated in the series of complexes:-

β-D-glucuronate < α-D-glucuronate < β-D-galacturonate < α-D-glacturonate

(I) (II) (III) (IV)

is probably related to the varying degree of acidity of the different hydroxyl groups in the ring. It has been reported that, for D-glucose the anomeric hydroxyl group in its acidic function is slightly stronger than for any other hydroxyl group[54]. It was also reported that β-D-glucose is a slightly stronger acid than its alpha anomer[54]. If this was applied to the uronic acid series, the alpha form, being the slightly weaker acid, might be expected to complex more strongly with cations than the

beta form.

The stability constants which were calculated for the complexes of the α- and β-anomers compared favourably with those values which were obtained for complexes of the uronosides in which there is no anomeric equilibrium (Table 5.12).

6 . 3 Crystal Structure of Some Uronate Salts

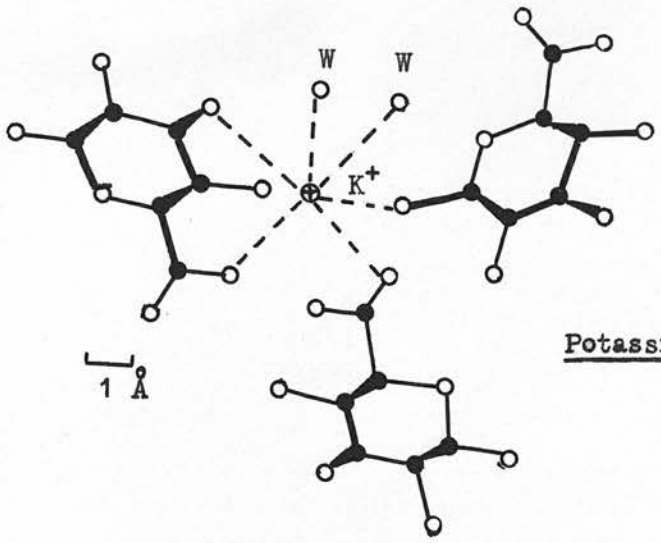
A very limited amount of work has been done on the crystal structures of the salts of uronic acids, but two structures which have been determined give an interesting comparison with the results reported here.

The structure of potassium β-D-glucuronate which was determined by Gurr[80] (Figure 6.3a) shows the environment of the potassium ion, which has a very distorted octahedral coordination. The asymmetric unit of the crystal contains one cation, one anion, and two water molecules, both of which are co-ordinated at distances of 2.79 and 2.84Å. The rest of the coordination is made up by O(6) and O(3) of one molecule, at 2.80 and 2.81Å, O(7) of a second molecule, at 2.85Å, and O(4) of a third at 2.80Å.

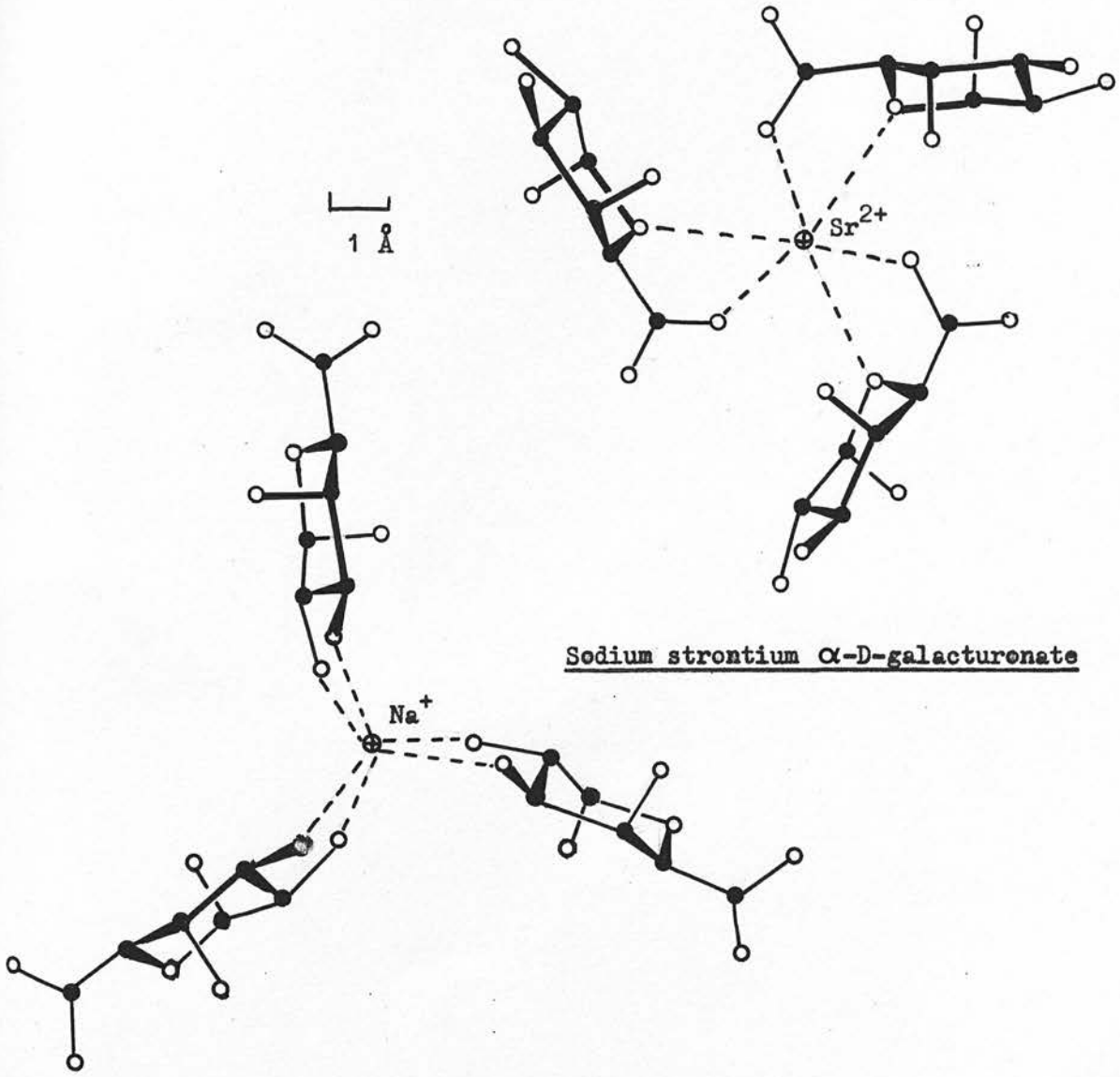
The structure of sodium strontium α-D-galacturonate hexahydrate has been recently determined[81]. In this double salt, both metal ions lie on crystallographic three-fold axes. The coordination about strontium is nine-fold, similar to that of the calcium ions in apatite[82]. The three coordinating atoms for each asymmetric unit are: O(5) at 2.87Å, and O(6) at 2.52Å from one residue, and a water molecule (directly above O(6) in the figure) also at 2.52Å. The sodium ions are coordinated by a slightly distorted octahedron consisting of three each of O(2) at 2.37Å and O(3) at 2.53Å. The environments of these ions are shown in projection down the three-fold axes in figure 6.3b and c.

The differences in interatomic distances are approximately those which would be expected, taking the

ionic radii of potassium, sodium, and strontium to be 1.33, 0.95, and 1.13Å respectively[83]. In both cases water molecules are involved in coordination, but the extent to which they do so is less in the galacturonate salt, although the relative numbers of water molecules per anion is the same (2:1). The much neater packing of the galacturonate ions, and the avoidance of the two axial oxygen atoms for coordination is particularly notable. These atoms, which are strongly hydrogen bonded in this structure, are the ones involved in the inter--residue link in polygalacturonic acid. This could well correspond to more effective solvation of galacturonate in solution with consequent enhancement of complex stability.



Potassium β-D-glucuronate



Sodium strontium α-D-galacturonate

Fig. 6.3 Crystal structure of some uronate salts

6 . 4 Conclusions

The stability constants of the uronate complexes studies have been found to be relatively small. As a result, the methods which can be used in a quantitative determination of these constants must be of extremely high accuracy. The three types of methods which were employed in this study have each been shown in varying degrees to be insufficiently sensitive to obtain fully accurate values but, by averaging values determined by the different approaches, a reasonably accurate determination was possible.

The principal objective of this investigation was to evaluate the stability of some complexes of a type which can exist in primary plant cell walls. As a preparatory stage in the study of the complexes of the polymeric material in the cell walls, the ligands used were pure uronic acids. Some stability constants for this type of ligand with both typical cations found in the plant and some transition metal ions were studied. In addition, the relative stability of the anomeric complexes with calcium ions was estimated.

The results from the investigation will allow a greater understanding of the type of complexes formed between uronic acids and some cations and will facilitate the evaluation of stability data from oligo- and poly--uronic acids.

APPENDIX

Appendix Computer Programs

Computer programs used in the calculation of dissociation constants and stability constants were written in Atlas Autocode for the KDF-9 computer; and later in FORTRAN IV for the IBM 360 50, chiefly by the author. The three main programs referred to in the text of this thesis are briefly described below:

(1) IONCONST

Purpose To calculate an average value of the dissociation constant for a monobasic acid from the experimental pH titration data, discarding any spurious or unreliable results and making correction for any complexed species formed between the acid anion and cations present in the solution.

Method Individual values of the dissociation constant are obtained by application of equation 2.6 to each pair of values of volume of titrant and pH. Correction is made for the removal of a fraction of the free ligand ion by complexation with the cation in the background electrolyte from the stability constant for this complex.

From the individual values, weighted average pK and overall standard deviation are found by a least squares procedure. This value is refined by the repeated application of the least squares procedure excluding all individual values outwith $2\frac{1}{2}$ times the standard deviation (i.e. the 95% probability limit) until no further values are discarded. The weighting factor used in averaging

values is g when $0 < g < 0.5$ and (1-g) when $0.5 < g < 1$.

Input Number of sets of results, molarity of titrant alkali, molarity of titrant acid, the molecular weight of the ligand, the ionic product of water at the appropriate temperature, molarity of the background electrolyte and the stability constant for the complex formed between the ligand and the cation in the background electrolyte (once only); the percentage purity of the ligand solution, molarity of the stock ligand solution and the volume added to the test solution, the volume of alkali added to the test solution, the total volume of the test solution, and the volume of background electrolyte solution added (for each set); volume of titrant alkali added and recorded pH (for each addition of alkali within a set).

Output (The output presents the following data both with and without correction for complexing between the ligand anion and the cation in the background electrolyte). Tabulated values of the volume of titrant acid, the corresponding volume of titrant alkali un-neutralised, pH, g, and pK, together with an indication of whether the particular value of pK was discarded at any stage in the successive least squares procedure, and, if so, at what stage this occurred. The weighted average value of the dissociation constant after each least squares cycle, along with its standard deviation, is also shown.

(2) Calculation of complex stability constants - pH titration.

Purpose To calculate an average value of the stability

constant for complexes formed between the anions of monobasic acids and metal cations making correction for any other metal complex simultaneously formed in solution by the ligand and a second metal cation.

Method Individual values of the concentration stability constant are obtained by application of equations 4.5-4.12 to each pair of (OH_a, pH) results. This calculation makes allowance for the presence of the complexed species formed between the ligand and the background electrolyte cation.

The standard deviation in the arithmetic average value of the stability constant is minimised by a repetitive least squares procedure allowing the purity of the ligand solution and the concentration of strong acid or base to be used as variables.

The shifts required in the purity of the ligand and concentration of strong acid or base present (introduced either in the ligand or metal solution) were calculated from simultaneous equations derived from the expression:-

$$\Delta K = \frac{\delta K}{\delta x} \cdot \Delta x + \frac{\delta K}{\delta y} \cdot \Delta y$$

where K is the stability constant

and x, y are the variables.

After each cycle the shift calculated in each variable was applied and the calculation repeated to give the most consistent value of the stability constant.

This refining procedure was carried out four times.

Input Molarity of titrant alkali, molarity of metal ion solution and the volume added, molarity of ligand

solution and the volume added, total volume of test solution, ionic product of water at the appropriate temperature, the concentration dissociation constant of the ligand at the appropriate ionic strength, molarity of background electrolyte solution and the volume added, the concentration stability constant for the complex between the ligand and the background electrolyte cation at the appropriate ionic strength, and the number of results in the data set (for each set); the pH values recorded after each addition of a fixed aliquot of titrant alkali.

Output Tabulated values for each determination of OH_a, together with the concentrations of hydrogen ions, protonated ligand, ligand complex with background electrolyte cation, free ligand anion, metal complex and free metal cation, and a value for the concentration stability constant.

For each set of data, there are tabulated values of the ligand purity, the required change in this purity and an average value for the concentration stability constant.

(3) DIVCAT - Calculation of complex stability constant - bivalent ion responsive electrode titration.

Purpose as for program 2.

Method Individual values for both the concentration and thermodynamic stability constants are obtained by the application of equations to each pair of values for the volume of ligand solution and recorded electrode potential. This calculation makes allowance for the presence of the complexed species formed between the ligand and the cation used in the neutralisation of the ligand.

The recorded electrode potential, after correction for the contribution due to the secondary cation, is converted to the free metal ion activity by interpolation from a standard calibration curve for that system. This value, after conversion to the metal ion concentration by the application of an appropriate activity coefficient, is used in conjunction with calculated concentrations of the other species present in the solution in the calculation of the concentration stability constant. This latter value is then converted to a thermodynamic stability constant by applying the appropriate activity coefficients.

An overall average value for the thermodynamic stability constant from a number of sets of data is calculated together with the standard deviation.

Input A table of values to allow calculation of the required correction in the recorded electrode potential for each concentration of secondary cation (once for each initial divalent cation concentration); an activity calibration curve for the cation under test (once for each initial divalent cation concentration); molarity of ligand solution, molarity of metal ion solution and the volume added to the test solution, total volume of test solution, and the stability constant of the complex formed between the ligand and the secondary cation (for each set); pairs of values for the recorded electrode potential and the volume of ligand solution added.

Output Tabulated values for the corrected electrode potential, the bivalent cation activity, the concentrations of free metal ion, complexed species and free ligand ion, the concentration stability constant and the thermodynamic constant.

In addition, an average thermodynamic constant is printed for each set of data and an overall thermodynamic constant, together with standard deviation, for several sets of data representing results for one complexed species.

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Calcium Complexes of Uronic Acid Monomers

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Reprinted from

Chemical Communications 1970

The Chemical Society, Burlington House, London W1V OBN

Calcium Complexes of Uronic Acid Monomers

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Summary Stability constants are reported for calcium complexes with anomeric forms of two uronic acids, and complex stability is related to structure and biological function.

THE interaction of alkaline-earth cations with acidic polysaccharides is considered to be of major significance in cellular structure of both land plants and algae,[1] and also in cation regulation in mammalian bone formation.[2] Kohn *et al.*,[3] in a study of the cation exchange properties of alginates, report, however, that no detectable complexing occurs between calcium ions and uronic acid monomers. We have investigated the interaction between calcium(II) and D-glucuronate and D-galacturonate ions, and have measured stability constants which, although small, vary significantly with the structure of the acid. We have not detected complexes of higher order than 1:1.

Free alduronic acids in aqueous solution rapidly establish equilibrium between α- and β-pyranose forms, so a stability constant measured for their complexes with a bivalent ion will be essentially of the form:

$$K' = \frac{[ML_\alpha^+] + [ML_\beta^+]}{[M^{2+}]\{[L_\alpha^-] + [L_\beta^-]\}}$$

where the subscripts represent the two modifications of the ligand, and it is assumed that activity coefficients for the singly charged species effectively cancel one another. Stability constants were also measured for the calcium complexes of α-D-methylglucuronoside and β-D-methylgalacturonoside, substances which cannot undergo mutarotation.

Stability constants were estimated at various ionic strengths by three methods. In the first, the fall in calcium ion activity on the addition of a potassium uronate solution to a solution of calcium nitrate was followed potentiometrically using an Orion liquid ion exchange membrane electrode (No. 9220). The second was the standard pH method, using a glass electrode. The third method was polarimetric, making use of the difference in molecular rotation between potassium uronate alone and in the presence of a large excess of calcium ions, to determine the degree of complexing at intermediate molar ratios. In the latter two cases, single-ion activity coefficients were applied to the calculated free metal concentrations. The results are summarised in Table 1.

The molecular rotation of the methyl glycosides is not affected by the presence of calcium ions. Since their calcium complexes are of a similar strength to those of the free uronates, the shift in rotation in the latter case must be

due to a shift in the equilibrium between the α- and β-forms of the anion. Equilibrium constants of the form:

$$k_L = \frac{[L_\alpha^-]}{[L_\beta^-]} \qquad k_{ML} = \frac{[ML_\alpha^+]}{[ML_\beta^+]}$$

were evaluated. Those for glucuronate were estimated from the relative sizes of the ^1H n.m.r. signals of the anomeric protons in a deuterium oxide solution, and those for galacturonate were calculated from the data of Isbell and Frush.[4] Using these constants, it is possible to convert values of K' into true stability constants for complexes of the two anomeric forms of the ligand. These are given in Table 2, and may be compared with those for the glycoside complexes in Table 1.

TABLE 1. *Measured stability constants for calcium complexes* (K′ *or* K; M^{-1})

Ligand	Method 1	Method 2	Method 3
Glucuronate	32	33	30
Galacturonate	64	72	73
α-Methylglucuronosidate ..	40	(a)	(b)
β-Methylgalacturonosidate ..	56	(a)	(b)

(a) Not measured; (b) method not applicable.

The greater strength of the galacturonate complexes can be related to the hydroxyl group on C(4), which is axial in galacturonate and equatorial in glucuronate. A smaller enhancement of the stability may be associated with the axial hydroxyl group at C(1) in the α-anomers.[5] These axial groups allow for greater interaction with a cation, and cause galacturonic acid to be a significantly weaker acid than glucuronic.[5]

TABLE 2. *Constants from mutarotation data*

Ligand	k_L	k_{ML}	K_α	K_β
Glucuronate	0·72	0·94	37M^{-1}	28M^{-1}
Galacturonate	0·27	0·44	101M^{-1}	61M^{-1}

The results also suggest structure-function relationships for α-linked polygalacturonic acid in the tissues of higher plants. Similarly, the β-linked poly-L-guluronic acid in brown seaweeds has been shown to have the ^1C conformation with three axial oxygen atoms,[6] while the D-mannuronic acid residues also present have only one. It is significant that an enzymic conversion of mannuronic into guluronic acid residues has been reported, in which the presence of calcium ions is required.[7] The considerably stronger binding of calcium by the guluronate groups is indicated, as the process would otherwise be thermodynamically unfavourable.[8]

We thank the Science Research Council for an equipment grant, and Dr. D. A. Rees for considerable stimulating discussion.

(*Received, March 17th*, 1970; *Com.* 376.)

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