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The Complex Formation between Pb^{2+}, Ca^{2+} and Some Pentoses

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The complex formation between Pb^{2+}, Ca^{2+} and arabinose, ribose, and xylose has been studied by emf measurements. Results obtained at different ionic strengths have been compared with those from NMR measurements. It is concluded that the more detailed information obtained from NMR spectra at high concentrations is also applicable at low concentrations. The emf measurements have also confirmed that the presence of an *ax-eq-ax* or a *cis-cis-cis* sequence of hydroxyl groups is necessary for a significant complex formation to occur.

Weak interactions have long been known to exist between carbohydrates or cyclitols and metal ions in neutral and acid aqueous solution.[1] Quantitative studies of these interactions have been made by Angyal *et al.*[2,3] using ^1H NMR spectroscopy and stability constants have been determined for a large number of carbohydrate complexes especially with the alkaline earth metal ions.

For the complex formation to occur to a significant extent, three vicinal hydroxyl groups either in an *ax-eq-ax* sequence on a six-membered ring or in a *cis-cis* sequence on a five-membered ring must be present. The conclusions reached from ^1H NMR spectroscopy about the nature of the binding site have been confirmed by Andrasko and Forsén [4] from measurement of the spin-lattice relaxation time of ^{23}Na in aqueous carbohydrate and inositol solutions. Practical use of the complex formation has been made in preparative work.[5]

The NMR measurements have to be carried out with comparatively high concentrations of metal ion and carbohydrate (0.5 – 2 mol/dm^3). In the present investigation the complex formation has been studied at low concentrations with the emf technique. By carrying out measurements at different ionic strengths it would then be possible to establish if values of stability constants determined at high concentrations are applicable at low concentrations. As models in these studies Pb^{2+}-pentose interactions have been used. Some measurements with Ca^{2+} employing the competition technique have also been included in this investigation.

Emf methods have been little used previously. Angyal and Hickman [6] have employed a divalent ion-sensitive electrode to investigate the complex formation between Ca^{2+}, Sr^{2+}, and Ba^{2+} and *epi*- and *cis*-inositol.

EXPERIMENTAL

Method. The interaction between Pb^{2+} and the carbohydrate was studied at 25.0(1) °C by measurements of the cells

Pb(Hg),Glass	B Pb^{2+} H H^+ S pentose A ClO_4^- $(A-H-2B)$ Na^+	Ref

The reference electrode was

A $NaClO_4$	0.01 M Ag^+ $(A-0.01$ M) Na^+ A ClO_4^-	Ag,AgCl

The emfs of the cells may be written

$$E_{Pb} = E_{Pb}^\circ - 29.58 \text{ (mV) lg } (b/\text{M}) - 29.58 \text{ (mV) lg } (y_{Pb^{2+}}) - E_j \quad \text{(I)}$$

$$E_g = E_g^\circ - 59.16 \text{ (mV) lg } (h/\text{M}) - 59.16 \text{ (mV) lg } (y_{H^+}) - E_j \quad \text{(II)}$$

Capital letters denote total concentrations whereas small letters are used for the corresponding free concentrations. The activity

coefficient, y, refers to activity scales where $y=1$ in the pure ionic medium. The test solutions were prepared by the titration technique and in an experiment B and H were kept constant usually at 1×10^{-3} and 5×10^{-3} M, respectively. S was varied from 0 to 0.09 M. The influence of the sodium perchlorate used as salt background was followed by making measurements at the $(Na)ClO_4$ concentrations 0.025, 0.050, 0.100, 0.250, 0.500, and 1.000 M. Some experiments were also made in 0.1 M $(C_2H_5)_4NClO_4$ in order to study the effect of a qualitative change in the medium.

In the experiments with Ca^{2+}, S was kept constant at 0.1 M and the total concentration of Ca^{2+}, C, was varied from 0 to 0.15 M. Measurements were performed in 1 M $(Na)ClO_4$ only.

Chemicals. Sodium, calcium, lead and silver perchlorates, and perchloric acid solutions were prepared and analysed as described in Refs. 7 and 8.

Tetraethylammonium perchlorate was prepared by neutralizing a hot 20 % water solution of tetraethylammonium hydroxide (Kebo, *purum*) with perchloric acid. The product was recrystallized twice from methanol. The crystals were dried and stored over silica gel in a desiccator.

L(+)-Arabinose (Merck, für die Mikrobiologie), D(−)-ribose, and D(+)-xylose (both Merck, für biochemische Zwecke) were purified by recrystallization after treatment with activated charcoal. Aqueous ethanol was used for recrystallization of arabinose and xylose and aqueous 1-propanol for ribose. The crystals were dried and stored over silica gel in a vacuum desiccator. The purification of the chemicals was repeated until the emf measurements on the products from two consecutive recrystallizations gave the same result. The analytical hydrogen ion concentrations in solutions of the pentoses were found from potentiometric titrations with dilute perchloric acid. Gran extrapolations were used for the determination of the equivalence point.

Lead amalgam was prepared by dissolving lead metal (Merck, silberfrei) in mercury (Kebo, *puriss*, twice distilled). The amalgam (0.1 % Pb) was stored under 0.1 M $HClO_4$ in a N_2 atmosphere. Ribose was deuterated for the NMR measurements by dissolving it in D_2O (99.7 %, Ciba-Geigy) and evaporating the solvent under vacuum. This was repeated twice. $CaCl_2$ and $Pb(NO_3)_2$ were dried at 200 °C for 20 h. $Pb(ClO_4)_2$ was obtained from the stock solution in H_2O. The water was driven off under an infrared lamp and the evaporation was repeated twice with D_2O.

Apparatus. The titrations were carried out with an automatic titrator. The salt bridge, electrodes, and other experimental details were essentially the same as in Ref. 8. All potentials were measured to ± 0.01 mV. Since the changes in the measured potentials are small particular care was taken to exclude oxygen from the measuring system. All solutions were thus continuously flushed with oxygen-free argon. All plastic tubing from the buret was mantled and nitrogen passed between the inner and outer tube in order to prevent oxygen from diffusing through the walls of the inner tubing.

The NMR spectra were recorded on a JEOL JNM-FX100 spectrometer at 24 °C. Chemical shifts were measured from sodium 3-(trimethylsilyl)propionate as internal standard.

DATA TREATMENT, RESULTS AND DISCUSSION

Lead complexes. The data from the main series of measurements in which Pb^{2+} complexes were studied will be treated first. H^+ does not interact with the pentoses, *i.e.* $h=H$. Then

$$\Delta E_g = E_g(s=S) - E_g(s=0) = E_j(s=0 - E_j(s=S) - 59.16 \lg (y_{H^+}) \quad (1)$$

will be a measure of the changes in the liquid junction potential and the activity coefficient for H^+ upon addition of the pentose. The solution with $s=0$ is chosen as the standard state for each perchlorate concentration. In the same way ΔE_{Pb} can be calculated. $\lg(y_{Pb^{2+}})$ will then include any specific interaction between Pb^{2+} and the carbohydrate.

ΔE_g and ΔE_{Pb} were found to be independent of B (1×10^{-3} M $\leq B \leq 5 \times 10^{-3}$ M) and H (2×10^{-3} M $\leq H \leq 10 \times 10^{-3}$ M). Furthermore the same values were obtained whether s was increased or decreased during a titration, indicating that any interactions occurring are reversible.

In Fig. 1 the results in 1 M $(Na)ClO_4$ are shown. These are typical for all concentrations of the ionic media except 0.025 M. ΔE_g decreases and ΔE_{Pb} increases to a good approximation linearly with the carbohydrate concentration. The slopes of these lines, which are virtually independent of A, are presented in Table 1.

If complex formation occurs E_{Pb} must increase with S. This is found to be the case but only to a small extent except for the D-ribose system. When stability constants are calculated from data obtained in a constant ionic medium it is generally assumed that the activity factors are constant. E_g would then be a measure of the change in the liquid junc-

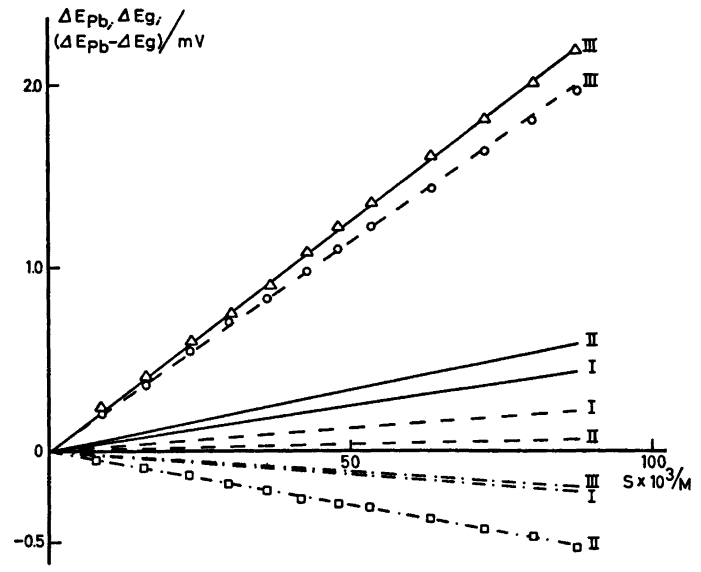

Fig. 1. The changes in the potential of the glass electrode (ΔE_g — · · —), and the lead amalgam electrode (ΔE_{Pb} - - -) and $\Delta E = \Delta E_{Pb} - \Delta E_g$ (———) as a function of the concentration of the pentose, S, in 1 M (Na) ClO_4. The lines for L-arabinose, D-xylose, and D-ribose are identified by the symbols I, II, and III, respectively. For clarity the experimental points have in general been left out.

Table 1. Summary of the results from the emf measurements on the Pb^{2+} complexation.

A	L-Arabinose			D-Xylose			D-Ribose		
M	$d\Delta E_g/dS$	$d\Delta E_{Pb}/dS$	β	$d\Delta E_g/dS$	$d\Delta E_{Pb}/dS$	β	$d\Delta E_g/dS$	$d\Delta E_{Pb}/dS$	β
	mV M^{-1}	mV M^{-1}	M^{-1}	mV M^{-1}	mV M^{-1}	M^{-1}	mV M^{-1}	mV M^{-1}	M^{-1}
1.000	−2.5	2.4	0.38	−6.0	0.6	0.51	−2.3	22.6	1.94
0.500	−1.5	3.5	0.39	−4.2	0.0	0.34	−1.7	22.4	1.88
0.250	−1.9	3.4	0.41	−4.8	0.1	0.38	−2.0	21.3	1.81
0.100	−1.9	2.9	0.37	−4.7	0.5	0.40	−2.9	21.7	1.91
0.050	−1.2	4.2	0.42	−4.8	0.8	0.44	−2.7	21.7	1.90
0.025	[a]	[a]	0.41	[a]	[a]	0.40	[a]	[a]	1.88
0.100 [b]	−1.5	4.0	0.43	−5.9	−0.6	0.43	−2.6	22.6	1.96

[a] In 0.025 M $NaClO_4$ E_g and E_{Pb} did not vary linearly with S. The difference $\Delta E_{Pb} - \Delta E_g$ was, however, a linear function of S. [b] in 0.1 M $(C_3H_5)_4NClO_4$.

tion. Since E_j has the same value in (I) and (II) combination of the expressions for ΔE_g and ΔE_{Pb} yields

$$\Delta E_{Pb} = \Delta E_g + 29.58 \lg (B/b) + 29.58 \lg (y_{H^+}^2/y_{Pb^{2+}}) \quad (2)$$

In solution the pentose is present in a number of forms which will be denoted $S_a, S_b, \cdots S_N$ and assumed to form the 1:1 complexes $PbS_a, PbS_b, \cdots PbS_N$. Charges have been omitted for convenience. Between the various species the following equilibria exist

$$\begin{array}{ll} S_a \rightleftharpoons S_b & k_{ab} = [S_b]\,[S_a]^{-1} \\ \vdots & \vdots \\ S_a \rightleftharpoons S_N & k_{aN} = [S_N]\,[S_a]^{-1} \end{array} \quad (3)$$

$$\begin{array}{ll} Pb + S_a \rightleftharpoons PbS_a & \beta_a = [PbS_a] b^{-1} [S_a]^{-1} \\ Pb + S_N \rightleftharpoons PbS_N & \beta_N = [PbS_N] b^{-1} [S_N]^{-1} \end{array} \quad (4)$$

Activity factors have been left out since they may be expected largely to cancel and for uncharged species to be almost constant.

The total concentrations can be written

$$S = \sum[S_n] + \sum[PbS_n] \quad (5a)$$

$$B = b + \sum[PbS_n] \quad (5b)$$

Combination of eqns. (3), (4), and (5) yields

$$B = b[1 + \sum(\beta_n k_{an}[S_a])]; \; k_{aa} = 1 \quad (6)$$

and from eqns. (5a), (5b), and (3)

$$[S_a] = (S - B + b)\,(\sum k_{an})^{-1} \approx S(\sum k_{an})^{-1} \quad (7)$$

where the approximate expression is valid when the complex formation is weak. Inserting eqns. (6) and (7) in (2) and differentiating the resulting expression in the case of weak complex formation yields

$$d(\Delta E_{Pb})/dS - d(\Delta E_g)/dS = \Phi \approx 29.58(\ln 10)^{-1}(\sum \beta_n k_{an})(\sum k_{an})^{-1} + 29.58\, d[\lg(y_{H^+}^2/y_{Pb^{2+}})]/dS \quad (8)$$

The experimental results are thus in accord with eqn. (8) if the activity factors are constant or $\lg(y_{H^+}^2/y_{Pb^{2+}})$ varies linearly with S. Emf measurements evidently cannot be used to find the stability constants of the complexes formed by the individual forms of the carbohydrate. Instead a "mixed" constant, $\beta = \sum \beta_n k_{an} / \sum k_{an}$, is obtained. The values of this constant calculated from eqn. (8) for the three carbohydrates, neglecting the last term, are given in Table 1. The constant for D-ribose is five times larger than the constants for L-arabinose and D-xylose, which are small and have the same value within the limits of the experimental error. There is no significant variation of the constants with the concentration of $(Na)ClO_4$. This is a common result for complex formation with neutral ligands.[9] It also indicates a negligible interaction between Na^+ and the carbohydrates. This was confirmed by measurements in 0.1 M $(C_2H_5)_4NClO_4$ which led to the same β-values as the measurements in $NaClO_4$.

Calcium complexes. When treating the data from the competition measurements it will be found convenient to let the symbols carry an index $[d_1,d_2]$ in order to indicate the compositions of the equilibrium solutions in cell (I) and (II). The first position in the index refers to the absence ($d_1 = 0$) or presence ($d_1 = 1$) of Ca^{2+}. In the same way $d_2 = 0$ and $d_2 = 1$ denote the absence or presence of pentose.

With no pentose present the changes of the emf's of cell (I) and (II) upon addition of Ca^{2+} can be written

$$\Delta E_g[1,0] = E_g[1,0] - E_g[0,0] = \Delta E_j[1,0] - 59.15 \lg y_{H^+}[1,0] \quad (9)$$

$$\Delta E_{Pb}[0,0] = E_{Pb}[1,0] - E_{Pb}[0,0] = \Delta E_j[1,0] - 29.58 \lg y_{Pb^{2+}}[1,0] \quad (10)$$

where $\Delta E_j[1,0] = E_j[0,0] - E_j[1,0]$, and the solution with $c = 0$ has been chosen as the standard state. The ratio $y_{H^+}^2[1,0]/y_{Pb^{2+}}[1,0]$ can be found from

$$\Delta E[1,0] = \Delta E_{Pb}[1,0] - \Delta E_g[1,0] = 29.58 \lg (y_{H^+}^2[1,0]/y_{Pb^{2+}}[1,0]) \quad (11)$$

In the presence of pentose the corresponding changes on addition of Ca^{2+} are

$$\Delta E_g[1,1] = \Delta E_j[1,1] - 59.15 \lg y_{H^+}[1,1] \quad (12)$$

$$\Delta E_{Pb}[1,1] = \Delta E_j[1,1] + 29.58 \lg (b[0,1]/b[1,1]) - 29.58 \lg y_{Pb^{2+}}[1,1] \quad (13)$$

and

$$\Delta E[1,1] = 29.58 \lg (b[0,1]/b[1,1]) + 29.58 \lg (y_{H^+}^2[1,1]/y_{Pb^{2+}}[1,1]) \quad (14)$$

On the assumption that the quotients of the activity coefficients in Equations (11) and (14) are equal, one obtains

$$\Delta E = \Delta E[1,1] - \Delta E(1,0] = 29.58 \lg (b[0,1]/b[1,1]) \quad (15)$$

When only 1:1 complexes are formed the expressions for the mass balances and equilibria yield the following equation for calculating b.

$$b^3(\beta_1\beta_2 - \beta_1^2) + b^2(\beta_1\beta_2 S - \beta_1^2 S - \beta_1 + \beta_2 + \beta_1^2 B - 2\beta_1\beta_2 B - \beta_1\beta_2 C) + b(-\beta_1\beta_2 BS + \beta_1 B - 2\beta_2 B + \beta_1\beta_2 B^2 + \beta_1\beta_2 BC) + \beta_2 B^2 = 0 \quad (16)$$

The stability constants $\beta_1 = \beta_{Pb}$ and $\beta_2 = \beta_{Ca}$ are mixed constants as previously defined.

The value of β_{Ca} can be found by minimizing the error function $X = \sum(\Delta E_{exp} - \Delta E_{calc})^2$. ΔE_{calc} is obtained from the right-hand side of eqn. (15) with $b[0,1]$ and $b[1,1]$ calculated from eqn. (16). Since ΔE_{exp} may be beset by a systematic error from the measurements of the reference potentials $E_g[0,0]$, $E_g[1,0]$, $E_{Pb}[0,0]$,

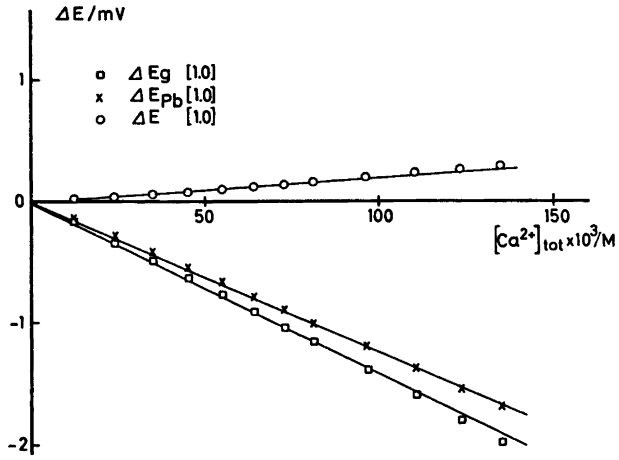

Fig. 2. The changes in the potential of the glass electrode (ΔE_g) and the lead amalgam electrode (ΔE_{Pb}) upon exchange of Na^+ against Ca^{2+} in 1 M (Na) ClO_4. $\Delta E = \Delta E_{Pb} - \Delta E_g$.

and $E_{Pb}[1,0]$ a parameter, ε, has been added to ΔE_{calc} in order to allow for this situation.

Fig. 2 presents results from the titrations with $S = 0$. $\Delta E_g[1,0]$ and $\Delta E_{Pb}[1,0]$ vary linearly with c. The slopes of the lines are not very different. The change in $y_{H^+}{}^2[1,0]/y_{Pb^{2+}}[1,0]$ is therefore small, when Na^+ in the background electrolyte is exchanged for Ca^{2+}.

The results in the presence of a pentose are shown in Fig. 3. ΔE for L-arabinose or D-xylose is very small. This is the expected result, since β_{Pb} is small (≈ 0.5 M^{-1}). ΔE for D-ribose is large enough to permit a calculation of β_{Ca}. The minimum in X was found by systematic variations of β_{Ca} and ε. From 4 titrations the mean value and the standard deviation of β_{Ca} was found to be 1.6(3) M^{-1}. The ε-values were 0.02 mV or less.

Discussion and comparison with NMR data. The results of the present measurements agree qualitatively with those obtained from other kinds of measurements. D-Ribose has an

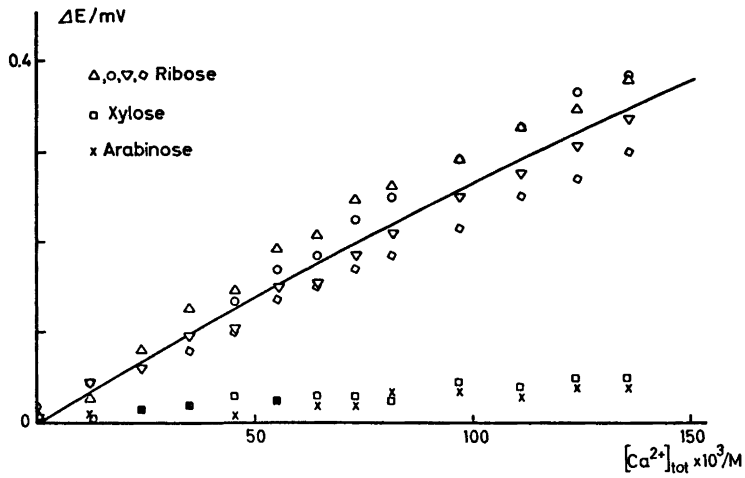

Fig. 3. ΔE defined by Equation (15) as a function of $[Ca^{2+}]$. The drawn curve represents calculated ΔE values for $\beta_{Ca} = 1.6$ M^{-1}.

ax-eq-ax sequence of hydroxyl groups in the α-pyranose form and in the 1*C* conformation of the β-pyranose form. A *cis-cis* sequence is present in the α-furanose form. L-Arabinose and D-xylose exist almost exclusively in pyranose forms which lack the *ax-eq-ax* sequence and the stability constants for these carbohydrates are expected to be small as observed.

Angyal [2] reports that no substantial change in the NMR spectrum of D-arabinose was observed on addition of calcium chloride. If it is assumed that arabinose and xylose do not form complexes the last term in eqn. (8) can be estimated from the Φ-values for these carbohydrates. Φ is independent of the $NaClO_4$ concentration and its mean value is 5 ± 1 mV M^{-1} for both compounds. Substituting this figure in eqn. (8) the stability constant for ribose is found to drop from 1.9 to 1.5 M^{-1}. Calculated as a percentage the difference is large between the two values of β_{Pb} and reflects the difficulties in estimating stability constants in weakly interacting systems.[10,11] The last value might be too small, since it is unlikely that the interaction would be wholly dependent on the presence of one hydroxyl group to complete an *ax-eq-ax* sequence.

In order to obtain more information on this point some experiments were also done with simple alcohols. It was then found that addition of monools and diols with nonadjacent OH-groups resulted in negative values of Φ. Hence these alcohols decrease $y_{H^+}^2/y_{Pb^{2+}}$.

Diols with vicinal OH-groups and glycerol on the other hand yielded positive values of Φ, the value for glycerol being the greatest and about the same as that for arabinose and xylose. There is thus qualitative agreement between the results of these experiments and the expected order of the strength of the interaction between the metal ion and the alcohols.

Angyal [2] has determined the stability constants for the complexes between Ca^{2+} and the various forms of D-ribose from NMR spectra. From his results the mixed constant β_{Ca} can be calculated to be 2.0 M^{-1}, which compares quite well with our figure. The mixed constant defined in eqn. (8) was computed from

$$\beta = \sum \beta_n k_{an} / \sum k_{an} = \sum \beta_n [S_n] / \sum [S_n] = \sum \beta_n p_n / 100 \quad (9)$$

Table 2. Chemical shifts and coupling constants for anomeric protons of ribose, and the equilibrium compositions of ribose solutions and the equilibrium constants calculated on the basis of these compositions.

	Pyranose α	Pyranose β	Furanose α	Furanose β
0.5 M ribose				
δ	4.86	4.93	5.4	5.25
J/Hz	2.2	6.3	–	1.3
%	21.6	57.6	6.6	14.2
0.5 M ribose + 1.5 M $CaCl_2$				
δ	4.99	5.13	5.50	5.31
J/Hz	2.2	3.9	4.0	–
%	43.4	41.7	10.9	3.9
β_n/M^{-1}	5.5	1.4	4.4	[a]
0.5 M ribose + 1.0 M $Pb(ClO_4)_2$				
δ	5.02	5.12	5.48	5.28
J/Hz	1.7	4.2	4.3	–
%	43.7	43.2	7.9	5.2
β_n/M^{-1}	6.7	1.5	3.4	[a]
0.5 M ribose + 1.0 M $Pb(NO_3)_2$				
δ	5.0	5.07	5.47	5.29
J/Hz	1.7	4.6	–	–
%	34.1	52.2	7.5	6.3
β_n/M^{-1}	3.5	1.4	2.2	[a]

[a] Assumed not to form complexes.

where p_n is the percentage of the n'th form of the pentose. No constants are available for the Pb^{2+} complexes and these were therefore determined from NMR spectra as outlined in Ref. 2. The results are entered in Table 2. The data for the pure ribose solution and the constants for the calcium complexes agree well with those reported in Ref. 2. The value of the mixed constant is 2.5 M^{-1} with $Pb(ClO_4)_2$ and 1.7 M^{-1} with $Pb(NO_3)_2$ and thus about the same as found by the emf method. The smaller value found in nitrate medium probably reflects the fact that Pb^{2+} is complexed by NO_3^-.[12] Complexation – in this case by acetate ions – is probably the reason why the constant for the Pb^{2+}-D-allose complex is considerably smaller than for the corresponding alkaline earth metal complexes.[3]

The results of the present investigation suggest that stability constants for carbohydrate complexes determined at high concentrations are also applicable at low concentrations,

but the small values of the constants make it unlikely that metal ion-carbohydrate interactions are of much importance in, for instance, biological systems.

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