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BINDING OF DIVALENT CATIONS TO OLIGOMERIC FRAGMENTS OF PECTIN

RUDOLF KOHN
Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, 842 38 Bratislava (Czechoslovakia)

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ABSTRACT

Activities of counterions M^{2+} (M = Ca, Cd, Cu, Pb, Sr, and Zn) determined in dilute solutions of the corresponding metal oligo-D-galactosyluronates of degree of polymerization 1 to 9, have been interpreted. The metallochromic-indicator method (tetramethylmurexide) and ion-specific electrodes were applied to activity measurements. The interaction of these cations with carboxyl groups was evaluated on the basis of activity coefficients ($\gamma_{M^{2+}}$), degree of association (β), and the multiple-equilibria theory. It was proved that Ca^{2+}, Sr^{2+}, and Zn^{2+} ions are bound to these oligouronates with intramolecular electrostatic bonds, whereas Cd^{2+}, Cu^{2+}, and Pb^{2+} form complexes. The results obtained with model derivatives of D-galactopyranuronic acid bring deeper insight into the formation of chelates of Cu^{2+} and Pb^{2+} ions with mono- and oligo-D-galactosyluronates.

INTRODUCTION

The complexity of the process of elimination of toxic cations from the human body by means of pectin (see for example, refs. 1–6) prompted us to study systematically the binding of some essential and some toxic cations to oligo-(D-galactosyluronic acid)s having various degrees of polymerization[7–13], to metabolites of pectin (galactaric acid and 2,5-furandicarboxylic acid), and to model substances[14], as well as to plant tissues isolated from some nutritionally important vegetables and apples[2,15]. A further aim of our study was to elucidate the complicated rules governing cation binding to polymeric glycuronans. Solution of these problems dictated proceeding from simple substances to complex ones, *i.e.* from monomeric glycuronic acids, through oligouronic acids, to macromolecular glycuronans.

Binding of various divalent cations to monomeric D-galactopyranuronic acid had been studied by several authors. However, the binding of these cations to their oligomers had not been investigated. Therefore, we systematically studied the binding of several cations (Ca^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+}), to oligo-(D-galactosyluronic acid)s with degree of polymerization (d.p : *m*) 1 to 9.

The present paper, a collection of the results of our earlier[7–14] studies, offers a deeper insight into this topic. The aim of this work was to derive general laws for cation binding to acidic plant polysaccharides, as natural polyelectrolytes, from the results achieved in individual experiments on compounds of low molecular weight.

EXPERIMENTAL

Sodium oligo-D-galactosyluronates were prepared, and characterized, by the methods described earlier[7,12], *i.e.*, by partial, acid hydrolysis of pectic acid, rechromatography, and desalting of the hydrolysis products on Sephadex G-25 (Fine) and G-10 columns. Chromatographic separation of higher oligomers (m = 7 to 9) was repeated 3 to 4 times, selecting a narrow region of fractions. Sodium oligo-D-galactosyluronates were chemically pure according to the ratio of terminal reducing groups to carboxyl groups (CHO)/(CO_2H) for the lower oligomers[7], and according to chromatographic movements of the higher ones on paper sheets and plates of silica gel, respectively [fitting the linear relationship $\log(1/R_F - 1) = f(m)$].

Sodium oligo-D-galactosyluronates were converted into the corresponding oligouronic acids by percolation of their aqueous solutions (c_{CO_2Na} = 4–5 mmol.L^{-1}) through a column of Dowex-50W X2 (H^+) resin. Solutions of calcium and strontium oligo-D-galactosyluronate were respectively obtained by direct neutralization of these oligouronic acids with calcium or strontium hydroxide. Solutions of other (Cd, Cu, Pb, and Zn) oligouronates could not be prepared in the same way. For them, solutions of an oligouronic acid were first neutralized with potassium hydroxide, and then an equivalent amount of the respective metal nitrate was added, and the ionic strength was adjusted with potassium nitrate. The systems in which precipitation of oligouronates occurred were centrifuged at 20,000g

The activity of the counterions M^{2+} was determined in solutions of metal oligo-D-galactosyluronates. The activity of Ca^{2+}, Sr^{2+} (refs. 16 and 17), and Zn^{2+} (refs. 13 and 18) ions was tested by means of the metallochromic-indicator method (tetramethylmurexide: TMM), whereas the activity of Cd^{2+}, Cu^{2+}, and Pb^{2+} ions was determined by use of ion-specific electrodes[11,12] [Crytur (Czechoslovakia), Model 82-17 (Pb), 48-17 (Cd), 29-17 (Cu)] and Orion (U.S.A.), Model 94-82A (Pb) at 25.0°. (Application of both the metallochromic indicator TMM and the Orion ion-specific electrode for the determination of the Ca^{2+} activity in solutions of calcium oligo-D-galactosyluronates[19] and acidic polysaccharides[20] led to identical results; for details, see ref. 20.) The activities $a_{Ca^{2+}}$ and $a_{Sr^{2+}}$ were measured in solutions of the respective oligo-D-galactosyluronates, without addition of further electrolyte, at the starting ionic strength of the solution, I_0 = 4.5 mmol.L^{-1}. Determinations of activities of the other cations were performed at the starting ionic strength, I_0 = 0.01 mol.L^{-1} (additional electrolyte, KNO_3). When calculated I_0 values, the concentration of the oligomer was expressed as the molar concentration of uronic acid units in the solution tested. (Molal and molar activity coefficients $\gamma_{M^{2+}}$ are identical for these dilute solutions[16].) With water-soluble oligouronates, the activity $a_{M^{2+}}$ was measured at the concentration of 3.00 mmol L^{-1} ($CO_2M_{0.5}$) in most cases, with partially soluble oligo-D-galactosyluronates in supernatant liquors, after centrifugation of suspensions. (Binding of cations: Ca^{2+}, m = 1–7, refs. 7, 9, and 10; Sr^{2+}, m = 1–4, ref. 8; Zn^{2+}, m = 1–7, ref. 13; Cd^{2+}, m = 1–9, ref. 12; Cu^{2+}, m = 1–5, ref. 12; and Pb^{2+}, m = 1–5, ref. 11).

The interaction of divalent cations with carboxyl groups of oligo-D-galactosyluronates was evaluated according to the activity coefficients of counterions ($\gamma_{M^{2+}}$) and the degree of association (β) of M^{2+} with oligouronates, respectively.

$$\beta = (c_{M^{2+}} - [M^{2+}]_f)/c_{M^{2+}}, \qquad (1)$$

where $c_{M^{2+}}$ is the starting concentration of M^{2+} ions, added in amounts equivalent to the carboxyl groups, and $[M^{2+}]_f$ is the concentration of free cations in the equilibrium solution or in the suspension. Values of $[M^{2+}]_f$ were calculated from the measured activities, $a_{M^{2+}}$, using tabulated[21] theoretical values of single-ion activity-coefficients $\gamma^0_{M^{2+}}$ (Debye–Hückel theory), corresponding to the ionic strength, I, of the equilibrium solution. The ionic strength (I_0) of the starting solution undergoes a change due to the binding of counterions to carboxyl groups, this being reflected in some decrease in its value. The ionic strength (I) of the equilibrium solution was calculated by the iterative procedure described[11], starting with $a_{M^{2+}}$ and I_0 values.

RESULTS AND DISCUSSION

Binding of divalent cations to oligo-(D-galactosyluronic acid)s. Solubility of oligouronates. — Cadmium, calcium, strontium, and zinc oligo-D-galactosyl-

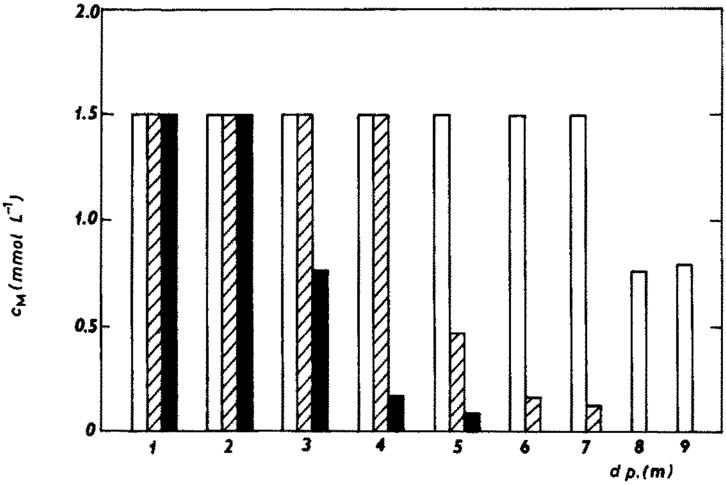

Fig 1. Solubility of cadmium, copper(II), and lead oligo-D-galactosyluronates. [Starting concentration $c_{CO_2M_{0.5}}$ = 3.00 mmol L^{-1}; i e , c_M = 1.50 mmol L^{-1}; m = d.p. of oligomers. Key: □, Cd, ▨, Cu, and ■, Pb.]

TABLE I

BINDING OF DIVALENT CATIONS TO MONOMERIC D-GALACTOPYRANURONIC ACID

M^{2+}	$c_{CO_2M_{0.5}}$ (mmol L^{-1})	I_0 (mol L^{-1})	$\gamma^0_{M^{2+}}$	$\gamma_{M^{2+}}$	$\gamma_{M^{2+}}/\gamma^0_{M^{2+}}$
Ca^{2+}	3.00	0.0045	0.759	0.730 ±0.007	0.96
Sr^{2+}	3.00	0.0045	0.754	0.746 ±0.007	0.99
Zn^{2+}	2.00	0.01	0.675	0.649 ±0.000	0.96
Cd^{2+}	3.00	0.01	0.670	0.579 ±0.022	0.86
Cu^{2+}	3.00	0.01	0.675	0.432 ±0.007	0.64
Pb^{2+}	3.00	0.01	0.665	0.387 ±0.008	0.58

uronates having a d.p. of $m \leq 7$ were soluble in water under the experimental conditions. With calcium[9] and cadmium[12] oligo-D-galactosyluronates of higher d.p. ($m > 10$ and ≥ 8, respectively), partial precipitation occurred. A similar phenomenon could be expected with strontium and zinc oligo-D-galactosyluronates of higher d.p. Copper and lead oligo-D-galactosyluronates are even less soluble. Precipitation of copper oligo-D-galactosyluronates occurs at $m \geq 5$, and that of lead oligo-D-galactosyluronates at $m \geq 3$. Water solubility of these substances is presented in Fig. 1 (the starting concentrations of oligo-D-galactosyluronates: $c_{CO_2M_{0.5}} = 3.00$ mmol.L^{-1}, *i.e.* $c_M = 1.50$ mmol.L^{-1}). The solubility of metal oligo-D-galactosyluronates decreases in the order (Ca, Sr, Zn) > Cd > Cu > Pb

Activity coefficients of counterions ($\gamma_{M^{2+}}$). — The binding of divalent cations to D-galactopyranuronic acid and its oligomers is first evaluated on the basis of activity coefficients of counterions ($\gamma_{M^{2+}}$). Only the values of $\gamma_{M^{2+}}$ corresponding to soluble oligomers have been interpreted.

The activity coefficients of divalent cations interacting with monomeric D-galactopyranuronic acid are reported in Table I. $\gamma_{M^{2+}}$ is the activity coefficient determined experimentally, $\gamma^0_{M^{2+}}$ is its theoretical value corresponding to a solution of the respective strong electrolyte of the same ionic strength, calculated according to Debye and Hückel[21], and I_0 is the starting ionic strength of the solution. Because not all activity determinations were performed at the same ionic strength, the ratio of activity coefficients $\gamma_{M^{2+}}/\gamma^0_{M^{2+}}$ was used as the measure for the interaction of M^{2+} ions with CO_2^- groups.

On the basis of the results presented in Table I, the cations tested can be devided into two groups.

The first group of cations, *i.e.*, Ca^{2+}, Sr^{2+}, and Zn^{2+}, interact only slightly with D-galactopyranuronic acid. The activity coefficients $\gamma_{M^{2+}}$ are very similar to the $\gamma^0_{M^{2+}}$ values corresponding to a completely dissociated salt of a strong electrolyte ($\gamma_{M^{2+}}/\gamma^0_{M^{2+}} = 0.96$ to 0.99). The results are in good agreement with the very small values of stability constants found by Buddecke and Drzeniek[22] for Ca D-galactopyranuronate and Ca D-glucopyranuronate. According to Makridou *et al.*[23], Ca^{2+}

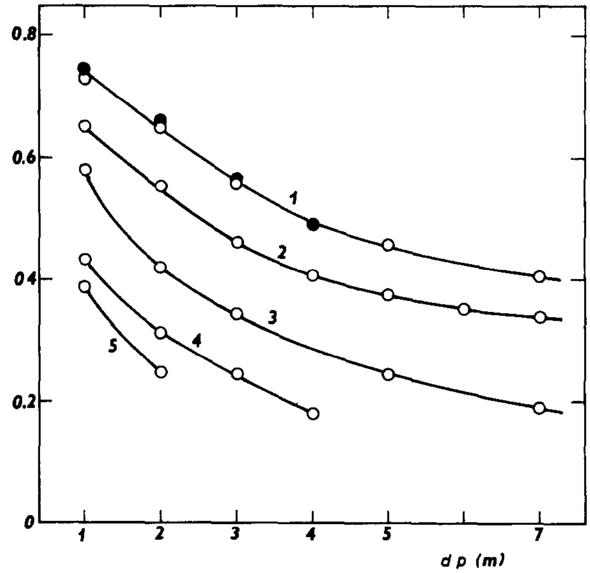

Fig 2 Dependence of single-ion activity-coefficient of counterions, $\gamma_{M^{2+}}$, on the d.p. (m) of oligo-D-galactosyluronates. [Key $c_{CO_2M_{0.5}}$ and I_0, see Table I. 1, ○ Ca^{2+}, ● Sr^{2+}, 2, Zn^{2+}, 3, Cd^{2+}, 4, Cu^{2+}; and 5, Pb^{2+}]

ions do not form any complexes with these uronic acids in neutral and acid media; complex-formation appears only in strongly alkaline solution (pH >10). Gould and Rankin[24] determined the stability constants of the same Ca uronates by using various experimental techniques (experimental conditions not given in their short communication). The values obtained were low as well; however, they depended on the structure of the uronic acids. The somewhat higher stability of the Ca D-galactopyranuronate complex, than that of the Ca D-glucopyranuronate was ascribed to the axial position of the OH group on C-4 of D-galactopyranuronic acid as against the equatorial position in D-glucopyranuronic acid.

The second group includes Cd^{2+}, Cu^{2+}, and Pb^{2+} cations, which are bound substantially more strongly to D-galactopyranuronic acid, in the order: $Cd^{2+} < Cu^{2+} < Pb^{2+}$ (see Table I). The results are in accord with the findings of Makridou and co-workers[23], who, by a protometric method, studied the interaction of several M^{2+} cations with D-galactopyranuronic and D-glucopyranuronic acid.

With increasing d.p. (m) of oligo-D-galactosyluronates, the activity coefficient of counterions $\gamma_{M^{2+}}$, decreases continuously with all cations tested (see Fig. 2). Such a finding was to be expected, considering purely electrostatic interactions of counterions with oligouronates. Curve 2, corresponding to zinc oligo-D-galactosyluronates, is shifted to lower $\gamma_{M^{2+}}$ values compared to the binding of Ca^{2+} and Sr^{2+} ions to the same oligomers (curve 1). The reason for this phenomenon is the higher ionic strength of solutions ($I_0 = 0.01$ mol.L^{-1}) used in determination of the activity of Zn^{2+} ions.

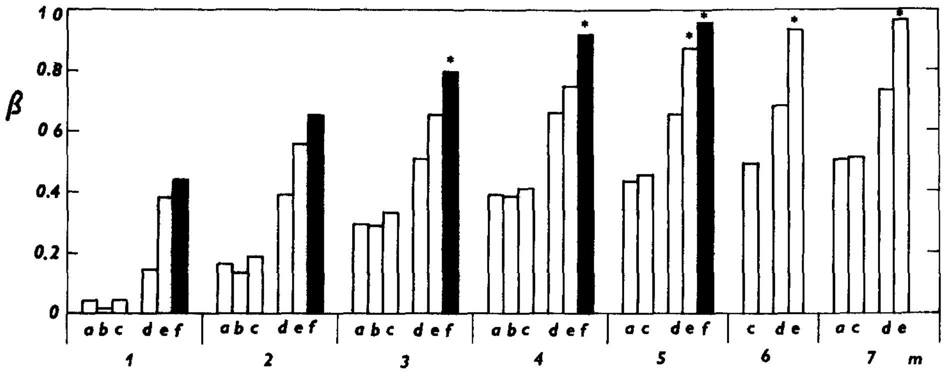

Fig. 3 Degree of association, β, of divalent cations with oligo-D-galactosyluronates of various values of d p (m) [Key: asterisk denotes system with partial precipitation of oligomers, electrostatic bond, a, Ca; b, Sr, c, Zn, formation of chelates, d, Cd, e, Cu, and f, Pb]

Degree of association (β). — The interaction of M^{2+} cations with carboxyl groups of oligo-D-galactosyluronates was also evaluated by means of the degree of association, β. The association degree β (see Eq. *1*) conveys the portion of bound cations of their total, initial amount in the solution, which is equivalent to CO_2^- groups of oligomers. The concentration of bound cations represents the sum of cations contained in the insoluble part of the oligo-D-galactosyluronate and in the soluble, undissociated part of this oligomer.

The results in Fig. 3 demonstrate that, with increasing d.p. of oligo-D-galactosyluronates, the portion of bound cations increases regularly. The association degree, β, of lead and copper(II) cations approaches asymptotically the value $\beta = 1$, already at a relatively low d.p. Columns marked with an asterisk stand for systems with a partial phase-separation of oligo-D-galactosyluronates.

It is evident that low-molecular-weight fragments of pectin also exhibit different affinities towards divalent cations, increasing in the order $Ca^{2+} \sim Sr^{2+} \sim Zn^{2+} < Cd^{2+} < Cu^{2+} < Pb^{2+}$.

In the first group of cations (Ca^{2+}, Sr^{2+}, Zn^{2+}), the differences in binding of the individual cations to oligomers are very small, irrespective of different electronic structure of their atoms. The amount of Ca^{2+}, Sr^{2+}, and Zn^{2+} cations bound to monomeric D-galactopyranuronate is negligible (close to experimental errors). On the basis of ^{1}H- and ^{13}C-n.m.r. spectra and osmotic coefficients of Ca^{2+} ions, determined in solutions of Ca oligo-D-galactosiduronates (m = 1–5), Rinaudo and Ravanat[19] concluded that calcium ions are bound to carboxyl groups of these substances purely by electrostatic interactions. This conclusion is supported by interpretation of activity coefficients $\gamma_{M^{2+}}$ (M = Ca, Zn), determined on solutions of various oligo- and poly-uronates and some derivatives of pectic acid[10,13], as well as by the findings that Ca^{2+}, Sr^{2+}, and Zn^{2+} ions are bound to nearly the same extent, to oligo-D-galactosyluronates (see Fig. 3).

The cations of the second group (Cd^{2+}, Cu^{2+}, and Pb^{2+}) are bound to carboxyl groups of oligo-D-galactosyluronates to substantially greater extents. Lead cations are bound to the highest extent (nearly quantitatively to the pentamer). We presumed that, besides the carboxyl groups, other functional groups of the uronic acid units could also be involved in the cation binding in this group (formation of chelates). Therefore, the following experiments were carried out.

Formation of complexes of lead and copper(II) cations with D*-galactopyranuronic acid and oligo-(*D*-galactosyluronic acid)s.* — Divalent-metal cations are bound stoichiometrically by polymeric D-galacturonan, as well as by pectin having different degree of esterification (d.e.), *i.e.*, one M^{2+} cation is bound by two CO_2^- groups, irrespective of the kind of bond, intra- or inter-molecular. We have proved exact stoichiometry in the binding of Ca^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Pb^{2+}, and Sr^{2+} cations to potassium pectate cross-linked by epichlorohydrin[25] and of Ca^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} cations[1,13,26–28] by pectin (d.e. 0–90%). Analytical determination of the d.e. of pectin by precipitation of insoluble copper pectate and pectinates[28,29] is based on a stoichiometric binding of Cu^{2+} ions with the free (unesterified) carboxyl groups of pectin.

Conjectures as to the mode of binding of M^{2+} ions to monomeric D-galactopyranuronic acid have not so far been unified. In the preceding it was shown that Ca^{2+}, Sr^{2+}, and Zn^{2+} ions are only slightly bound to D-galactopyranuronic acid ($\beta = 0.01$–0.04). Lead and copper(II) are much more strongly bound to this uronic acid and very firmly to penta- and hepta-(D-galactosyluronic acid)s ($\beta = 0.97$), practically the same as to a polymeric pectate chain[11,12]. The mode of cation binding to low-molecular-weight fragments of pectin (d.p. 1–4) remains an open question, because, for different experimental conditions[23,24], different stoichiometries were postulated.

The formation of complexes of divalent cations with oligo- and poly-uronic acids can be well studied by using the theory of multiple equilibria[30], according to which

$$r^{-1} = (nK[M^{2+}]_f)^{-1} + n^{-1}, \tag{2}$$

where r is the number of M^{2+} cations bound to the polyacid molecule or to the repeating segment of the macromolecule having n binding sites, $[M^{2+}]_f$ is the concentration of free M^{2+} ions in the equilibrium solution, and K is the stability constant of the respective complex.

We have demonstrated earlier that interaction of Ca^{2+}, Cd^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+} cations with carboxyl groups of pectins having different d.e. values proceeds exactly in accordance with the theory of multiple equilibria[1,13,26,27], with formation of a stoichiometric bond. On the other hand, interaction of Cu^{2+} ions with 2,3-dicarboxy derivatives of starch and amylose leads to formation of more than one type of complex, depending on the ratio of interacting components; the function $r^{-1} = f([Cu^{2+}]_f^{-1})$ is no longer linear, but convex (for details, see ref. 31).

The activity $a_{Cu^{2+}}$ was determined in solutions of potassium mono-, di-, and

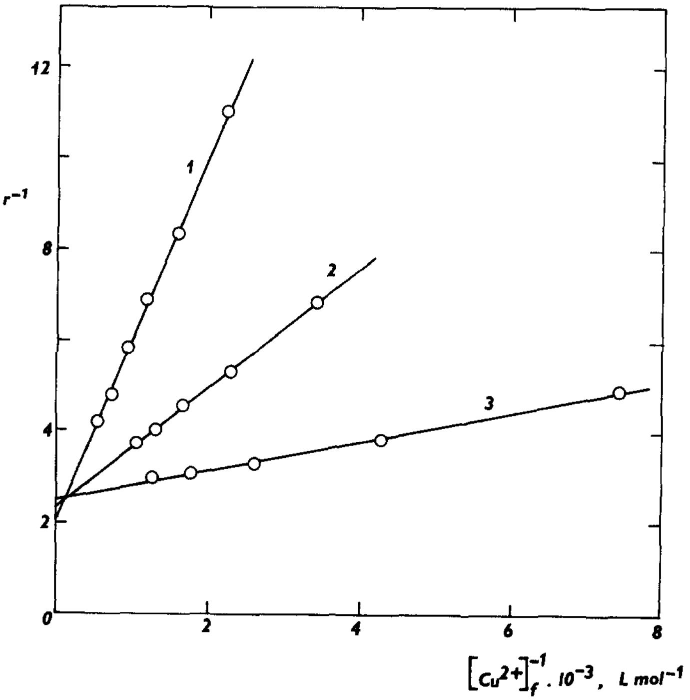

Fig 4 Binding of Cu^{2+} ions to oligo-D-galactosyluronates, theory of multiple equilibria [Key r, the number of Cu^{2+} ions bound to one uronic acid unit, $[Cu^{2+}]_f$, the concentration of free Cu^{2+} ions in the equilibrium solution; 1, 2, and 3, mono-, di-, and tetra-D-galactosyluronates.]

tetra-D-galactosyluronates[12] containing various amounts of copper(II) nitrate [3.00 mmol.L^{-1} (CO_2K); 0.45–1.95 mmol.L^{-1} (Cu^{2+}); I_0 = 0.02 mol.L^{-1}; additional electrolyte, KNO_3]. The concentration of free copper(II) ions in equilibrium solutions $[Cu^{2+}]_f$ was determined by making assumptions described in the Experimental section, the r value was enumerated, and the results were evaluated according to Eq. 2. One D-galactopyranuronic acid units was chosen as the ligand unit.

The results are illustrated in Fig. 4. The linear course of the function $r^{-1} = f([Cu^{2+}]_f^{-1})$, with minimal scattering of experimental data, suggests that only one type of complex is formed. The theoretical value of n (*viz.*, 1.00) corresponds to the positively charged complex $(CuA)^+$, and the value of n'_0 = 0.50, to the stoichiometric bond of 1 Cu^{2+} with 2 CO_2^- groups. The intercepts on the ordinate ($r^{-1} = n^{-1}$), obtained by extrapolation of the experimental data, were r^{-1} = 2.20, 2.36, and 2.47 for copper mono-, di-, and tetra-D-galactosyluronate, respectively. The numbers of binding sites for cations on one uronic acid unit are then n = 0.454, 0.424, and 0.405, respectively These values, close to the theoretical value of n'_0 = 0.50, suggest that Cu^{2+} ions are also bound stoichiometrically on monomeric D-galactopyranuronic acid and its oligomers; formation of the $(CuA)^+$ complex, presumed by Makridou *et al.*[23], was not evident. Line slopes ($1/nK$) reflect the dependence of the stability constant K of Cu complexes on the d.p. of oligomers, in accordance with β values presented in Fig. 3. We ascribe the small difference between the experimental values of n (0.454–0.405) and the theoretical one (0.50) to lower pH (5.25–5.7) of equilibrium solutions, brought about by hydrolysis of cupric salts. Dissociation of carboxyl groups is partially lessened at lower pH. Consequently, the proportion of CO_2^- groups entering the interactions is somewhat lower, *i.e.*, 81 to 90% of their starting concentration (for details, see ref. 12). The binding of Cu^{2+} ions to polymeric D-galacturonan is also stoichiometric at lower pH (5.2–5.5).

On the basis of these results, we analogously presume that Pb^{2+} and Cd^{2+} ions are also bound stoichiometrically to mono- and oligo-D-galactosyluronates, in accordance with their stoichiometric bond to D-galacturonan, proved earlier[1,27].

In general, the multiple-equilibria theory does not help in identifying the kind of bond, *i.e.*, whether it is electrostatic or chelate. In the case of complex-formation of Cu^{2+} ions with glycuronic acid anions, several types of interaction can be considered[32]: (*a*) monodentate interaction of the cation with the CO_2^- group as the sole binding site[23], (*b*) bidentate interaction with CO_2^- and OH-4 or the oxygen atom of the pyran ring as binding sites, and (*c*) tridentate interaction with CO_2^-, OH-4, and the ring-oxygen atom; see ref. 33. On the basis of microcalorimetric studies of Cu^{2+} ion binding to D-galactopyranuronate, Aruga[32] came to the conclusion that, besides the carboxyl group, the ring-oxygen atom also participates in formation of the positively-charged, $(CuA)^+$ complex by means of an outer-sphere, electrostatic bond.

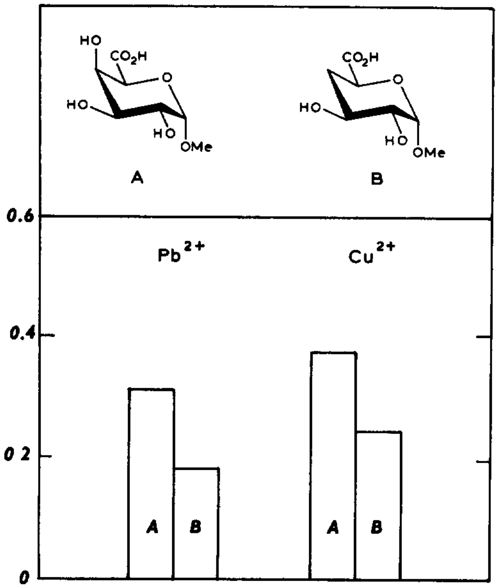

Fig. 5 Binding of Pb^{2+} and Cu^{2+} ions to model derivatives of D-galactopyranuronic acid. [Key β, degree of association, A, methyl α-D-galactopyranosiduronic acid, B, methyl 4-deoxy-α-D-*xylo*-hexopyranosiduronic acid]

Paoletti and co-workers[34] showed that the strong binding of Cu^{2+} to CO_2^- groups of a microbial "alginate" brings about loss of a considerable amount of water from the hydrate shells of both kinds of ion. A fully hydrated gel of Cu D-galacturonan displays formation of carboxylate inner-sphere complexes, as evidenced by e.s.r. and i.r. spectra[35].

In order to assess the role of different oxygen atoms in the binding of lead and copper(II) ions to D-galactopyranuronic acid, we investigated[14] the interactions of these cations with potassium salts of methyl α-D-galactopyranosiduronic acid (*A*), and methyl α-4-deoxy-D-*xylo*-hexopyranosiduronic acid (*B*) [3.00 $mmol.L^{-1}$ (CO_2K); 1.50 $mmol.L^{-1}$ (M^{2+}); $I_0 = 0.01$ $mol.L^{-1}$; additional electrolyte, KNO_3]. The results are summarized in Fig. 5. The binding of Pb^{2+} and Cu^{2+} ions to the substances under study was evaluated according to the association degree β. Lead and cupric salts of D-galactopyranuronic acid and its derivatives *A* and *B* are soluble in water.

Elimination of OH-4 of D-galactopyranuronic acid causes a significant decrease in association both of Pb^{2+} and Cu^{2+} ions with this uronic acid (see Fig. 5, columns *A* and *B*). The results prove unambiguously that the oxygen atom of this hydroxyl group is included in complexes of Pb and Cu monomeric D-galactopyranuronates, respectively.

On the basis of the results just mentioned, it may be assumed that, besides the ring-oxygen atom[32], the bridge-oxygen atom will participate in formation of the Pb and Cu complexes of the polymeric D-galacturonan chain. The chelate formation proved in the case of monomeric D-galactopyranuronic acid with lead and copper(II) ions, the weak dependence of the stability constant *K* of lead pectinates on the linear charge-density of pectin[1], and the strong binding of Cu^{2+} ions to the "isolated", very distant, free carboxyl groups in a highly esterified pectin, may all be good reasons to believe that Pb^{2+} and Cu^{2+} ions can probably form intramolecular chelates with the D-galacturonan macromolecules, in addition to the well known, intermolecular chelates. This conclusion can also be supported by the effect of ammonia on Cu pectate and Cu pectinate gels (unpublished results). A very dilute, aqueous solution of ammonia (0.1%) readily converts these gels into sols. However, Cu^{2+} ions bound to insoluble pectate cross-linked by epichlorohydrin cannot be displaced on percolation with a dilute solution of ammonia; the Cu^{2+} ions remain bound to carboxyl groups. It is evident that the intramolecular is stronger than the intermolecular chelate.

Stoichiometric, intermolecular binding of divalent cations to D-galacturonan and L-guluronan gels can be explained by interaction of the M^{2+} ion with two carboxyl groups belonging to two different macromolecular chains that are in close proximity to this cation. Besides, the oxygen atoms of hydroxyl groups, the ring-oxygen atoms, and the bridge-oxygen atoms of the component sugar units can participate in this binding process by way of their free-electron pairs, according to the well known "egg box" model[36,37] (see also, refs. 38 and 39). Intramolecular binding of Pb^{2+} and Cu^{2+} ions to oligo-D-galactosyluronates and D-galacturonan by chelate formation requires close contact of the M^{2+} ion with at least one CO_2^- group. Direct contact with the other, neighboring carboxyl group is not possible, due to the twofold screw symmetry of the M(II) salts of D-galacturonan[40]. Carboxyl groups of the vicinal uronic acid units are situated on opposite sides of the sugar rings.

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