

## BINDING OF LEAD CATIONS TO OLIGOGALACTURONIC ACIDS

Rudolf KOHN

*Institute of Chemistry,  
Slovak Academy of Sciences, 842 38 Bratislava*

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The activity of  $Pb^{2+}$  ions in solutions of lead oligogalacturonates of polymerization degree  $DP$  1 to 5 (starting concentration of the investigated solutions  $3.00 \text{ mmol } (COOPb_{0.5})^{-1}$ , the ionic strength of the solution  $I = 0.01 \text{ mol l}^{-1}$ , electrolyte  $KNO_3$ , was determined by lead specific ion electrodes. The activity  $a_{Pb^{2+}}$  is independent on the kind of anion in very diluted solutions of lead perchlorate and nitrate. Ions of lead become bound quite firmly even to the monomeric D-galacturonic acid (association degree of  $Pb^{2+}$  ions to carboxyl groups of uronic acid  $\beta = 0.43$ ). The increase of polymerization degree is associated with a strong decrease of the lead oligogalacturonate solubilities on one hand, and increase of the bond strength of the  $Pb^{2+}$  ions to these oligomers on the other. The association degree of  $Pb^{2+}$  ions to carboxyl groups of the pentamer ( $\beta = 0.96$ ) almost reaches the value found for the polymeric chain of pectate molecule (D-galacturonan); here, the stoichiometric binding of  $Pb^{2+}$  to two carboxyl groups takes place with a great deal of probability. Lead has been found to bind highly preferentially to oligogalacturonates even in the presence of calcium ions. Results presented in this paper help to clear the mechanism of elimination of lead from the human body through pectin and its oligomeric fragments.

Pectin has proved to be an efficient prophylactic substance and remedy for people working with lead and its salts. This has been evidenced not only by many results obtained with laboratory animals, but also by the clinical experience with people suffering from lead poisoning. A survey of articles dealing with this problem was embodied in our preceding papers concerning the mechanism of lead binding to pectin<sup>1</sup> and plant tissues of selected sorts of vegetables and fruits<sup>2</sup>. Elimination of lead proceeds predominantly *via* gastrointestinal tract. Lead cations are firmly bound to carboxyl groups of pectin in the small intestine only at a neutral or weakly alkaline medium, whereas their binding ability at a low pH of the gastric juice is very low. Lead cations bound to pectin are transported through the digestive tract without being resorbed in the small intestine and are eliminated to a great extent by excrements.

Pectin has been found an effective remedy not only against poisoning of the gastrointestinal tract, but also against intoxications of respiratory organs by aerosols of lead<sup>3</sup> and mercury vapour<sup>4</sup>. A peroral application of pectin resulted, in this case, in an enhanced secretion of cations of toxic metals through urine<sup>3,4</sup>. Similarly, it has

been shown<sup>5</sup> that application of pectin for medical treatment of people permanently working with lead resulted in an increased excretion of lead in urine, what can be associated with the elimination of lead previously deposited in the body. These findings indicate that a considerably complex mechanism of lead eliminations was involved, which has not been cleared as yet. Cleavage of the pectin molecule into low-molecular fragments prevalently occurs in the large intestine due to enzyme systems of the microbial intestinal flora. It has been suggested<sup>5,6</sup> that the excretion of lead in urine can considerably be associated with the binding of lead cations to D-galacturonic acid (the monomeric structure unit of pectin molecule), which can be resorbed to some extent, from the intestinal tract<sup>7,8</sup>. Formation of complexes of  $Pb^{2+}$ ,  $Cu^{2+}$ ,  $Cd^{2+}$  and further cations with monomeric D-galacturonic and D-glucuronic acids has already been proved<sup>9</sup>. This paper deals with the investigation of binding of lead ions to low-molecular, exactly defined oligomeric fragments of pectin (oligogalacturonic acids of polymerization degree  $DP$  1 to 5) in order to throw more light to this complex problem.

## EXPERIMENTAL

Oligogalacturonic acids were prepared from a purified commercial pectin preparation Genu Pectin (Medium Rapid Set, Københavns Pektinfabrik, Denmark). Oligogalacturonic acids of polymerization degree 2 to 5 were obtained and characterized by methods described earlier<sup>10</sup>, by a partial acid hydrolysis of pectic acid, repeated chromatography and desalting on Sephadex G 25 and G 10 columns. Uronic acids in effluents were detected by UV spectrometry at 205 nm in contrast to the original procedure. Oligogalacturonic acids were chemically pure and showed a linear dependence of the function  $\log(1/R_f - 1) = f(DP)$  on paper chromatography on Whatman No 1 paper and on silica gel plates (Silufol, Kavalier, Czechoslovakia). Oligogalacturonic acids were stored as sodium salts at  $+5^\circ C$ .

D-Galacturonic acid puriss. was a preparation of Fluka (Switzerland). Potassium pectate was obtained by an alkaline deesterification of the same preparation of pectin in suspension in a 60% ethanol<sup>11</sup> and was characterized by methods reported earlier<sup>12</sup>. The pectate contained 87% D-galacturonan in dry substance; its esterification degree  $E = 0\%$ , the mean molecular weight determined viscometrically  $\bar{M}_r = 26\ 000$ . Chemicals were of *p.a.* grade; a standard solution of lead perchlorate (Orion Research Inc., Cambridge, USA) and redistilled, freshly reboiled and cooled, carbon dioxide-free water were used. The 0.05M-KOH was carbonate-free. Digital potentiometer Radiometer, type PHM 64, glass electrode Radiometer, type 222B, and the reference saturated calomel electrode, type K 401, were used for pH measurement and potentiometric titrations.

*Preparation of lead oligogalacturonate solution for the determination of  $Pb^{2+}$  ion activity.* A solution of sodium oligogalacturonate (concentration  $5-7\text{ mmol (COONa)}l^{-1}$ ) was percolated through a Dowex 50W ( $H^+$ ) column. The potassium oligogalacturonate solution was obtained by neutralization of the effluent with a 0.05M-KOH solution to  $pH \sim 7.2$ . Solution of lead oligogalacturonate was prepared by mixing together the equivalent amounts of potassium oligogalacturonate solution with 0.01M- $Pb(NO_3)_2$  and an addition of 0.1M- $KNO_3$  so as the resulting concentration of the lead oligogalacturonate solution was  $3.00\text{ mmol (COOPb}_{0.5})l^{-1}$  at an ionic strength  $I = 0.01\text{ mol}l^{-1}$ . Should a partial precipitation of the lead salt take place

during preparation of the solution, the suspension is centrifuged at 20 000*g* for 15 min and the clear supernatant used for the determination of the activity of  $\text{Pb}^{2+}$  ions; the total lead concentration was estimated concurrently. A like procedure was employed when preparing the lead pectate solution. The total lead concentration in standards 0.01M- $\text{Pb}(\text{ClO}_4)_2$  and 0.01M- $\text{Pb}(\text{NO}_3)_2$  solutions and supernatants was determined chelatometrically using a spectrophotometric indication of the point of equivalence. Employed were 0.01M- or 0.002M-Complexon IV and  $\text{MgCl}_2$  solutions, and an interference IF 650 nm filter (Zeiss, Jena); indication with Eriochrome Black T.

The activity of  $\text{Pb}^{2+}$  counterions bound to carboxyl groups of oligogalacturonic acids and pectic acid was estimated using the lead specific ion electrodes Orion Research (USA), model 94-82A and Crytur (Monokrystal, Turnov, Czechoslovakia), type 82-17. Saturated calomel electrode with a double salt bridge (the outer cell filled with a 10%  $\text{KNO}_3$  solution) Radiometer (Copenhagen, Denmark), type K 711 was employed as a reference electrode. The  $\text{Pb}^{2+}$  ion activity was determined in solutions of lead oligogalacturonates at  $25.0 \pm 0.1^\circ\text{C}$ . Used were values of single-ion activity coefficients  $\gamma_{\text{Pb}^{2+}}$ , calculated on the basis of the theory of strong electrolytes according to Debye and Hückel, as already published<sup>13,14</sup>. Calibration curves  $\text{mV} = f(\text{pPb})$  were determined for each measurement series. Potential of the indication electrode was read after equilibration under constant stirring, after 10 min.

*Calculation of the corrected concentration of free  $\text{Pb}^{2+}$  ions in equilibrium solutions of lead mono- and oligogalacturonates.* First of all the approximate concentration of free lead ions in an equilibrium solution  $[\text{Pb}^{2+}]$  was calculated using values of experimentally determined  $a_{\text{Pb}^{2+}}$  activity and activity coefficient  $\gamma_{\text{Pb}^{2+}}$  corresponding to the ionic strength of the starting solution ( $I = 0.01 \text{ mol l}^{-1}$ ,  $\gamma_{\text{Pb}^{2+}} = 0.665$ ). This value served for calculating the corrected ionic strength of the solution taking the presumed mechanism of binding of lead ions in mind; i.e. the  $\text{Pb}^{2+}$  bond in form of a positive charged complex of  $(\text{PbA})^+$  type, or in form of a complex of  $(\text{PbA}_2)$  type, where  $\text{A}^-$  is the anion of D-galacturonic acid, or the uronic acid unit in the oligomer. The activity coefficient  $\gamma_{\text{Pb}^{2+}}$  corresponding to the corrected ionic strength of the solution was estimated using tabulated values<sup>14</sup> and the corrected concentration of free  $\text{Pb}^{2+}$  ions in the equilibrium solution was calculated. This iterative procedure was 2 to 3 times repeated till constant  $I$  and  $[\text{Pb}^{2+}]$  values were achieved.

The association degree  $\beta$  of lead ions with carboxyl groups of oligogalacturonates is defined by expression:

$$\beta = (c_{\text{Pb}^{2+}} - [\text{Pb}^{2+}]) / c_{\text{Pb}^{2+}},$$

where  $c_{\text{Pb}^{2+}}$  is the starting concentration of lead ions ( $c_{\text{Pb}^{2+}} = 1.500 \text{ mmol l}^{-1}$ ) and  $[\text{Pb}^{2+}]$  is the concentration of free  $\text{Pb}^{2+}$  ions in an equilibrium solution, or in the supernatant.

*Determination of the amount of electrolyte diffused from the reference calomel electrode into the distilled water.* The potassium nitrate solution (10%) in the outer cell of the reference saturated calomel electrode was replaced by a potassium chloride solution of the same molar concentration. The reference electrode was immersed in redistilled water (5 ml) and after a 15 min stirring the amount of  $\text{Cl}^-$  diffusing into water was determined by means of a potentiometric argentometric titration with 0.01M- $\text{AgNO}_3$  (silver electrode, salt bridge - 10%  $\text{KNO}_3$  solution).

## RESULTS AND DISCUSSION

*Verification of the method of  $\text{Pb}^{2+}$  activity determination.* Interaction of  $\text{Pb}^{2+}$  ions with carboxyl groups of oligouronic acids can be well observed at a low ionic strength  $I$ . Nevertheless, a little change in concentration of the secondary electrolyte

in such solutions considerably influences the activity coefficient of counterions  $\gamma_{\text{Pb}^{2+}}$ . Therefore, we investigated the diffusion of the electrolyte from the outer cell of the reference electrode into the solution under study at the same conditions at which the activity  $a_{\text{Pb}^{2+}}$  was determined. Aiming to make the analytical determination of concentration easier, potassium nitrate was replaced by the chloride of the same molar concentration in these orientating experiments. As found, roughly  $2.3 \pm 0.3$  micromol of the electrolyte diffused from the salt bridge into redistilled water (5 ml) within 15 min; this corresponded to its final concentration  $0.46 \text{ mmol l}^{-1}$ .

To eliminate the unfavourable influence of diffusion of the electrolyte from the salt bridge on the change of the activity coefficient  $\gamma_{\text{Pb}^{2+}}$ , activities of  $\text{Pb}^{2+}$  ions were measured in solutions of lead oligogalacturonates of the starting ionic strength  $I = 0.01 \text{ mol l}^{-1}$  (electrolyte  $\text{KNO}_3$ ). Diffusion of the electrolyte from the salt bridge can cause a change of the activity coefficient of lead ions in such solutions  $\Delta\gamma_{\text{Pb}^{2+}} \leq -0.005$ , what is in limits of the  $a_{\text{Pb}^{2+}}$  determination accuracy by a specific ion electrode.

Standard solutions of lead salt are recommended to prepare from lead perchlorate, and sodium perchlorate for adjustment the ionic strength. As already evidenced<sup>12,15</sup> with the application of the metallochromic indicator method for determination of activity of calcium ions, the estimated single-ion activity coefficients  $\gamma_{\text{Ca}^{2+}}$  in  $\text{CaCl}_2$  solutions, in which the ionic strength of solution was adjusted by addition of  $\text{KCl}$  or  $\text{KNO}_3$  ( $I = 0.01$  to  $0.15 \text{ mol l}^{-1}$ ) were in full agreement with those calculated on the basis of the theory of strong electrolytes according to Debye and Hückel (the proof of validity of MacInnes' convention for the secondary electrolyte  $\text{KCl}$  or  $\text{KNO}_3$ ). This was also the reason to use  $\text{KNO}_3$  as an additional electrolyte for determination of  $a_{\text{Pb}^{2+}}$ . (Potassium chloride is not suitable for a low solubility of  $\text{PbCl}_2$  in water). Therefore, the potential of each of the indication electrodes was alternatively checked in solutions of lead perchlorate and nitrate of various concentration.

Potentials of the tested electrode determined in solutions of both kinds of the lead salt (Fig. 1) lay exactly on the line expressing the function  $\text{EMF} = f(\text{pPb})$  irrespective of the kind of the lead salt anion ( $\text{ClO}_4^-$ , or  $\text{NO}_3^-$ ). The value of the Nernst factor is  $29.6 \text{ mV}$  at  $25^\circ\text{C}$ . A linear dependence of the above-mentioned function with the line slope ( $\Delta\text{mV}/\Delta\text{pPb}$ ) lying predominantly in the  $-29$  to  $-31 \text{ mV}$  interval was always found when determining the calibration curves. Well reproducible results with a mean error of one activity  $a_{\text{Pb}^{2+}}$  determination roughly  $\pm 3\%$  ( $\pm 10\%$  reported for the Orion Research electrode<sup>16</sup>) was found under optimum experimental conditions (precise temperating of solutions, stirring with a constant velocity, sufficient equilibration of the potential after 10 min). Both electrodes (Orion, USA, and Crytur, Czechoslovakia) afforded the same results of  $a_{\text{Pb}^{2+}}$  activity determination.

A precipitation of the lead salt was observed during preparation of lead oligogalacturonate solutions of polymerization degree  $DP \geq 3$ . After centrifugation of the suspension the total concentration of lead in the supernatant was determined, by a chelatometric titration, in addition to the  $a_{Pb^{2+}}$  estimation. Spectrophotometric indication of the point of equivalence enabled to determine with a sufficient accuracy even a very small concentration of lead in solution when using 2 mM-titrating agents. The lowest concentration of lead encountered in experiments (approximately  $1 \cdot 10^{-4}$  mol  $l^{-1}$ ) was determined with a  $\pm 2\%$  error.

*Binding of lead ions to oligogalacturonic acids ( $K^+$  form).* The activity of  $Pb^{2+}$  counterions was determined in solutions of lead oligogalacturonates of polymerization degree  $DP$  1 to 5 by means of lead specific ion electrodes. The  $a_{Pb^{2+}}$  activities were measured in solutions of lead oligogalacturonates of a 3.00 mmol ( $COOPb_{0.5}$ ),  $l^{-1}$  concentration at a starting ionic strength of the solution  $I = 0.01$  mol  $l^{-1}$  (electrolyte  $KNO_3$ ), or in supernatants after removal of the precipitated lead uronate.

Activity of counterions ( $a_M$ ), or their activity coefficient  $\gamma_M$  is a significant criterion for the interaction between cations and anionic groups of the polyelectrolyte in diluted solutions of a polyacid. The activity coefficient  $\gamma_M$  does not virtually depend, at a sufficiently high linear charge density of the macromolecule, on the concentration of the polyelectrolyte solution<sup>17</sup>. On the other hand, the activity coefficient of counter-

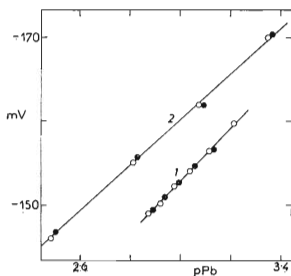


FIG. 1

Potential of lead specific ion electrodes in solutions of lead perchlorate and nitrate. Electrodes: 1 Orion, 2 Crytur,  $\circ$   $Pb(ClO_4)_2$ ,  $\bullet$   $Pb(NO_3)_2$

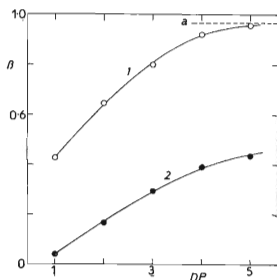


FIG. 2

Binding of lead and calcium ions to oligogalacturonic acids ( $K^+$  form).  $\beta$  Association degree of  $Pb^{2+}$ , or  $Ca^{2+}$  ions,  $DP$  polymerization degree of oligogalacturonic acids, 1 binding of lead ions, 2 binding of calcium ions,  $a$  association degree  $\beta$  of lead ions bound to pectate ( $E$  0%)

rions  $\gamma_M$  is with monomeric acid or lower oligomers, a function of concentration of the solution. (The  $\gamma_M$  values can be here utilized as criteria for binding of cations only when all solutions of oligomers under investigation are of the same concentration). Solubility of lead oligogalacturonates in solutions considerably decreased with the increase of polymerization degree  $DP$  (Table I). The interaction of  $Pb^{2+}$  ions with carboxyl groups of the oligomer is judged, therefore, according to the association degree of lead ions  $\beta$ , which gives the ratio of bound lead (in the precipitate of oligogalacturonate and in the soluble portion of the oligomer) and the total starting concentration of lead ions ( $c_{Pb^{2+}} = 1.500 \text{ mmol l}^{-1}$ ). Due to an interaction of  $Pb^{2+}$  ions with  $(COO^-)$  groups a change in the starting ionic strength  $I$  takes place in an equilibrium solution according to the type of interaction considered. Concentration of free lead in an equilibrium solution  $[Pb^{2+}]$  was, therefore, calculated employing the corresponding corrected value of ionic strength  $I$ .

The measured values are summarized in Table I. Starting with the trimer a precipitation of the lead oligogalacturonates occurred. The total lead concentration  $c_{Pb}$  in equilibrium solutions gradually decreases and reaches only 6% of the starting concentration with the pentamer. A greater scattering of  $a_{Pb^{2+}}$  values, found in lead oligogalacturonate solutions ( $DP$  3 to 5), is associated with a greater variation of the total concentration of lead ( $c_{Pb}$ ) in supernatants after centrifugation of the suspension. The association degree  $\beta$  of lead ions with carboxyl groups of oligomers strongly increases with their polymerization degree  $DP$ . Ions of lead become bound in a considerable measure even to the monomeric fragment of D-galacturonan, namely 43% of the starting lead concentration. Binding of  $Pb^{2+}$  ions to the pentamer ( $\beta = 0.96$ ) is virtually consistent with that to the polymeric chain of pectate (D-galacturonan),

TABLE I

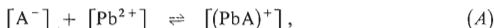
Binding of  $Pb^{2+}$  ions to oligogalacturonic acids ( $K^+$  form). Starting concentration of  $(COOPb_{0.5}) = 3.00 \text{ mmol l}^{-1}$

$DP$	$c_{Pb}$ $\text{mmol l}^{-1}$	$a_{Pb^{2+}} \cdot 10^3$	$\beta$
1	1.500	$0.581 \pm 0.012$	0.43
2	1.500	$0.370 \pm 0.016$	0.65
3	$0.768 \pm 0.039$	$0.214 \pm 0.017$	0.80
4	$0.175 \pm 0.026$	$0.086 \pm 0.007$	0.92
5	$0.093 \pm 0.004$	$0.046 \pm 0.002$	0.96
Pb-pectate	0.112 0.073	0.048 0.027	0.96 0.97

where the association degree  $\beta$  was found to be 0.96 to 0.97 and the total lead concentration in supernatant after centrifugation of the suspension approximately the same as that in the solution of pentamer.

The association degree  $\beta$  of calcium ions for those oligomers is also presented (Fig. 2) for comparison of the selectivity of exchange and binding of ions to potassium galacturonates. The  $\beta$  values were calculated from activities  $a_{Ca^{2+}}$  determined in solutions of calcium oligogalacturonates of the same concentration, as reported earlier<sup>10,18</sup>; this calculation was also based on the corrected ionic strength of the equilibrium solution. As evident, lead will be bound to oligogalacturonates highly preferentially even in the presence of calcium ions.

Makridou and coworkers<sup>9</sup> employed a method of protometric titration to investigate the interaction of  $Pb^{2+}$  ions with carboxyl groups of monomeric D-galacturonic acid. These authors presume the formation of a  $(PbA)^+$  complex according to the following equation



where  $A^-$  stands for the anion of D-galacturonic acid. The stability constant of the complex  $\log K = 2.00$  ( $I = 1 \text{ mol l}^{-1}$ , secondary electrolyte  $NaClO_4$ ). Providing the equation (A) holds, we obtained, basing upon results of our measurements, value  $\log K = 2.51$  for the same constant of stability. This a little higher value is primarily associated with the considerably lower ionic strength of systems investigated in this paper ( $I = 0.01 \text{ mol l}^{-1}$ ), when compared with that ( $I = 1 \text{ mol l}^{-1}$ ) employed by the already mentioned authors<sup>9</sup>.

We evidenced, when studying the binding of lead ions to polymeric D-galacturonan and pectin of various esterification degree of carboxyl groups by methanol<sup>1</sup>, that lead ions become bound to carboxyl groups of these polymers stoichiometrically in contrast to equation (A), *i.e.* one  $Pb^{2+}$  ion is bound to two  $(COO^-)$  groups according to reaction mechanism:



The ligand unit ( $L^{2-}$ ) with one binding site is a segment of the macromolecule comprising just two free (not esterified) carboxyl groups. This reaction mechanism exactly obeys the theory of multiple equilibria<sup>1</sup>. The quantity of lead ions bound to pentamer ( $\beta = 0.96$ ) is virtually identical with that bound to polymeric D-galacturonan chain ( $\beta = 0.96$  to  $0.97$ , Table I). We assume, therefore, that the binding of  $Pb^{2+}$  ions to pentamer proceeds with a great probability according to the reaction mechanism (B). Different conceptions considering the binding of  $Pb^{2+}$  ions to monomeric D-galacturonic acid on one hand and to oligogalacturonic acids of higher polymerization degree ( $DP \geq 5$ ) on the other suggest that elucidation of the mode of binding of  $Pb^{2+}$  ions to those substances would require more experimental data.

The high association degree  $\beta$  of lead ions with carboxyl groups of monomeric D-galacturonic acid and with low-molecular fragments of pectic acid shows that these compounds can play an important role in elimination of lead from the human body in the sense of the afore-mentioned conceptions.

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