BINDING OF ZINC CATIONS TO PECTIN AND ITS OLIGOMERIC FRAGMENTS

Anna MALOVÍKOVÁ and Rudolf KOHN

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

Received November 18th, 1982

The activity of Zn^{2+} ions bound to carboxyl groups of pectin of esterification degree E 0 to 90% and to oligomeric potassium galacturonates of polymerization degree 1 to 7 was estimated by the metallochromic indicator (tetramethylmurexide) method. The zinc ions are stoichiometrically bound to pectin, one Zn^{2+} cation to two free carboxyl groups. The stability constant K of zinc pectinates strongly decreases with the increasing esterification degree in a relationship close to a linear logarithmic function, $\log K = f(E)$ similarly, as with the binding of Ca^{2+} ions to pectin. Pectin reveals a little higher selectivity to Zn^{2+} ions when considering this couple of cations. The zinc ions are bound to the monomeric D-galacturonic acid to a very low extent in contrast to some toxic cations as *e.g.* Pb^{2+}, Cu^{2+}, and Cd^{2+}. A continuous increase of the degree of association of Zn^{2+} ions with carboxyl groups of oligomers takes place with the increasing polymerization degree. The validity of the increment additivity of terminal and inner uronic acid units to the final activity of zinc counterions bound to these oligomers was proved. The results are discussed from the standpoint of application of pectin as an active component of prophylactic diets against poisoning with cations of toxic metals with respect to the excretion of Zn^{2+} ions from the human body. Findings concerning the interaction of Zn^{2+} ions with pectin in dependence on its esterification degree (E) constitute a theoretical basis for a potential application of pectin as a useful ligand of Zn^{2+} ions in medical treatment of zinc deficiency in the human body.

Zinc belongs to a group of elements which are inevitable in a trace amount for the living organism, but toxic when in surplus. The principal role of zinc in the metabolism of man probably lies in its effect on the activity of enzymes. The clinical response to the zinc deficiency is first of all manifested by both the growth and sexual maturity retardation, deterioration of taste and visual acuity, mental lethargy and a worse healing of wounds. A detailed information on this matter offers the review article[1]. The zinc content in the body is controlled by the gastrointestinal tract, which has the physiological ability for absorption, but also for excretion of this cation. Application of zinc is dependent to a great extent on the composition of food and on the amount of zinc-specific binding substances excreted by pancreas into the intestine.

Several clinical studies dealing with the binding of zinc cations to dietary fibres, an important component of our every-day food, have recently been published. These compounds, mainly polysaccharides (cellulose, hemicelluloses, pectin, plant gums,

mucilage) and lignin considerably influence the digestion processes[2]. The majority of papers[3-6] dealt, in addition to zinc, with binding of further essential cations (Fe^{3+}, Ca^{2+}, Cu^{2+}, Mg^{2+}) to individual components of dietary fibres. The observed level drop of the investigated elements in the organism due to their bonding to plant fibres was generally not significant. As reported by Camire and Glydesdale[7], who investigated the individual fibre components (pectin, cellulose, lignin) and wheat bran, pectin and lignin reveal an extraordinary high binding capacity towards Zn^{2+}, Ca^{2+} and Mg^{2+}; capacity of their binding is pH-dependent. The laws of binding of zinc to pectin with a precisely defined physicochemical properties have so far not been investigated. The presented paper, which is a continuation of our preceding reports on the binding of lead[8,9], cadmium[10,11] and copper ions[11] to carboxyl groups of pectin and its oligomeric fragments, concerns the binding of zinc cations to these substances.

EXPERIMENTAL

Material and Instruments

The pectin samples of various esterification degree of carboxyl groups with methanol were prepared from the purified citrus pectin (Genu Pectin, Medium Rapid Set, Type A, Københavns Pektinfabrik, Denmark) by a controlled alkaline deesterification[12]. The highly esterified pectin (sample 6) was obtained from the purified pectin preparation by esterification with methanol in acid medium (1M-H_2SO_4) at 3°C for 3 weeks. Potassium pectate was prepared by an alkaline deesterification of pectin in suspension using potassium hydroxide in 60% ethanol (v/v). Samples were analyzed (the esterification degree E, the polyuronide content, the limit viscosity number $[\eta]$ and relative molecular mass $\bar{M}_r$) by methods already described[12]. The equation by Owens and coworkers[13] was employed for transformation of the $[\eta]$ value to mean relative molecular mass.

Sodium oligogalacturonates of polymerization degree $m = 2$ to 7 and tetramethylmurexide were prepared and characterized in our Laboratory; they were chemically pure and already used in the preceding study[11]. D-Galacturonic acid (monohydrate) *puriss.* was the commercial preparation of Fluka (Switzerland). All chemicals were of *p.a.* grade, the 0·05M-KOH was carbonate free; the specific conductivity of redistilled water was less than $2 \cdot 10^{-4}$ S m^{-1}.

Employed were: the compensation spectrophotometer UVISPEC-Hilger and digital pH-meter Radelkis, type OP-208, glass electrode Radiometer, type 222 B, and saturated calomel electrode Radiometer, type K 401.

Analytical Methods

The metallochromic indicator (tetramethylmurexide) method[12,14], was applied for investigation of the interaction of Zn^{2+} cations with carboxyl groups of pectin and its oligomeric fragments: this method has already been employed for Ca^{2+} and Sr^{2+} activity determination in solutions of the above-mentioned substances (*e.g.* ref.[15]).

Solutions of oligogalacturonic acids were prepared from the starting sodium oligogalacturonates by ion exchange; the corresponding potassium salts were obtained by neutralization with potassium hydroxide. Graded amounts of the $Zn(NO_3)_2$ solution were added to solutions

of potassium pectinates of various esterification degree E and equivalent amounts to solutions of potassium oligogalacturonates. The ionic strength I was adjusted by addition of KNO_3. Concentration of free Zn^{2+} ions and activity $a_{Zn^{2+}}$ were estimated in these pectinate and oligogalacturonate solutions of the respective starting concentration of carboxyl groups 3·00 and 2·00 mmol l^{-1} at the ionic strength of the starting solution $I_0 = 0{\cdot}02$ and $0{\cdot}01$ mol l^{-1}, respectively; the concentration of tetramethylmurexide was $4 \cdot 10^{-5}$ mol l^{-1}. Tabulated values of the single-ion activity coefficients $\gamma_{Zn^{2+}}$, calculated by Kielland[16] on the basis of the theory of strong electrolyte solutions were employed for $a_{Zn^{2+}}$ activity calculations; considered were the corrected values of ionic strength I corresponding to equilibrium solutions (*cf.* ref.[9]).

Absorbances A of the investigated solutions and calibration solutions ($Zn(NO_3)_2$, KNO_3) were determined at two wavelengths $\lambda = 455$ and 520 nm corresponding to the maximum of absorption of the Zn-tetramethylmurexide complex and the metallochromic indicator itself in a 1 cm-cell. Concentration of free $[Zn^{2+}]$ ions in equilibrium solutions was determined using the calibration curve $\varphi = f([Zn^{2+}])$; $\varphi = A_{455}/A_{520}$.

The binding of Zn^{2+} ions to potassium oligogalacturonates was considered on the basis of the degree of association β:

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

where $c_{Zn^{2+}}$ is the starting total concentration of zinc ions in the investigated oligogalacturonate solutions, and $[Zn^{2+}]$ is the concentration of free zinc cations in equilibrium solution, or in the supernatant.

The interaction of Zn^{2+} ions with pectin of various esterification degree E was evaluated by means of stability constant K of zinc pectate and pectinates. These were calculated using equation (*2*), as follows from the multiple equilibria theory[17]:

$$r^{-1} = (nK[Zn^{2+}])^{-1} + n^{-1}, \qquad (2)$$

where r is the number of Zn^{2+} ions bound to the iterative macromolecule segment with n binding sites, $[Zn^{2+}]$ is the concentration of free Zn^{2+} ions in the equilibrium solution. Extrapolation of function (2), $r^{-1} = f([Zn^{2+}]^{-1})$ to value $[Zn^{2+}]^{-1}$ limiting to zero serves for determination of the section at ordinate, for which $r^{-1} = n^{-1}$; thus the number of binding sites n was calculated. A segment of macromolecule comprising just two free (unesterified) carboxyl groups, which binds one Zn^{2+} cation providing the stoichiometric bond, were chosen for the ligand unit.

The total starting concentration of Zn^{2+} ions needed for calculation of the degree of association β and stability constant K was corrected with respect to the amount of Zn^{2+} ions bound to metallochromic indicator β'. The degree of association of Zn^{2+} with the metallochromic indicator β' was calculated according to equation:

$$\beta' = \frac{A - A_{(TMM)^-}}{A_{(TMMZn)^+} - A_{(TMM)^-}}, \qquad (3)$$

where A is absorbance of the solution of zinc salt under investigation, $A_{(TMM)^-}$ and $A_{(TMMZn)^+}$ are absorbances of the tetramethylmurexide solution itself and of its complex with zinc ions at $\lambda = 455$ nm. The $A_{(TMMZn)^+}$ was determined in the solution containing $4 \cdot 10^{-5}$ mol (TMM) . l^{-1} and 0·25 mol $Zn(NO_3)_2$ l^{-1}.

Concentration of free carboxyl groups in starting solutions of potassium pectate and pectinates was determined by the method of precipitation of the insoluble copper pectate and pectinates[18,19]. Concentration of Zn^{2+} ions in the reference $Zn(NO_3)_2$ solution was estimated chelatometrically (Complexon IV, 0·01 mol l^{-1}, indicator Eriochrome Black T, ammonium buffer solution) employing a spectrophotometric indication of the point of equivalence (interference filter Zeiss, Jena, IF 650 nm). All measurements are corrected against blank.

DISCUSSION AND RESULTS

Binding of Zinc Cations to Pectin of Various Esterification Degree

The interaction of Zn^{2+} ions with carboxyl groups of pectin in relation to its esterification degree E of carboxyl groups by methanol (E 0 to 90·3%) was studied by the spectrophotometric method using tetramethylmurexide as metallochromic indicator. The characteristic data of pectin samples are listed in Table I. The $\bar{M}_r$ values evidence that the relative molecular mass of the samples investigated is great enough so that the interaction of Zn^{2+} ions with carboxyl groups is no more function of the macromolecule chain length, but only function of its linear charge density.

Metallochromic indicator murexide, or its tetramethyl derivative give coloured complexes with a series of bivalent cations as Ca^{2+}, Sr^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, *etc.*[20]. Therefore, it is useful for determination of the activity of these cations in solutions of low-molecular and macromolecular natural substances. This method has proved suitable for determination of Ca^{2+} and Sr^{2+} ion activities in solutions of the corresponding oligo- and polyuronates[21-15]. Its was also useful for examination of binding of zinc ions to acid polysaccharides (dextran sulfate)[22]. The application of tetra-

TABLE I

Characteristic data of pectin samples and the corresponding stability constants K of zinc pectate and pectinates (I_0 = 0·02 mol l^{-1})

Sample	E %	Content of polyuronide[a] %	$[\eta]$ $m^3\ kg^{-1}$	$\bar{M}_r$	log K
1	0·0	88·2	0·125	28 000	4·56
2	25·0	87·3	0·208	40 000	3·96
3	38·6	91·3	0·297	53 000	3·82
4	47·8	89·8	0·305	54 000	3·41
5	67·4	86·0	0·505	78 000	3·20
6	90·3	85·7	0·261	48 000	2·70

[a] D-Galacturonan partially esterified with methanol.

methylmurexide in comparison with murexide employed in the original method is advantageous since the colour of its complex with the afore-mentioned cations does not depend on the pH of the solution in the pH 4·7–8·1 interval. The complex of tetramethylmurexide with zinc cations gives an absorption spectrum having the absorbance maximum at 455 nm. The molar absorption coefficient ε, estimated for this complex $\varepsilon_{(TMMZn)+} = 26\,500$, is in good agreement with the value determined earlier[20] for the complex of murexide with zinc ions $\varepsilon_{(MZn)+} = 27\,400$.

Correction to the amount of cations bound to the metallochromic indicator has to be taken into consideration when applying the indicator method. Therefore, we determined the dependence of the degree of association of Zn^{2+} ions with tetramethylmurexide (β') upon the concentration of free Zn^{2+} ions in the solution (Fig. 1, $c_{(TMM)} = 4 \cdot 10^{-5}$ mol l^{-1}). The β' values were calculated employing equation (*5*). The colour of the indicator solution is a function of β'. As shown in the drawing, determination of the concentration of free Zn^{2+} ions is quite precise in solutions containing $1 \cdot 10^{-4} - 10 \cdot 10^{-4}$ mol of free Zn^{2+} ions in one liter. The experimentally determined function $\beta' = f([Zn^{2+}])$ in the $\beta' = 0{\cdot}29 - 0{\cdot}81$ interval served for calculation of the stability constant of the tetramethylmurexide–zinc complex, $\log K = 3{\cdot}66 \pm 0{\cdot}01$ ($I \leq 3$ mmol l^{-1}). The $\log K$ value for the murexide-zinc complex was found to be 3·0–3·1 ($I = 0{\cdot}1$ mol l^{-1}, pH ~6), ref.[20,23]. The stability constant estimated in this paper is somewhat higher when compared with that of murexide–zinc complex, due mainly to the different values of ionic strength of the solutions under study.

Concentrations of free Zn^{2+} ions in solutions of zinc pectate and pectinates were determined at a 3·00 mmol (COOK) l^{-1} starting concentration and various amounts of zinc salt added. The starting ionic strength of the solutions investigated was adjusted with KNO_3 to $I_0 = 0{\cdot}02$ mol l^{-1}. A gel formation of the most of pectin samples took place during addition of the zinc salt. Therefore, concentration of free Zn^{2+} ions was determined in supernatants after centrifugation of the suspension (15 000*g* for 15 min). The total starting concentration of zinc in solutions was corrected by the amount of Zn^{2+} ions bound to tetramethylmurexide calculated using the function $\beta' = f([Zn^{2+}])$.

The binding of zinc cations to carboxyl groups of pectin was evaluated employing equation (*2*), following from the multiple equilibria theory. Application of this equation allowed to determine the stability constant K of zinc pectate and pectinates, as well as the number of binding sites n at the ligand unit (segment). Fig. 2 shows the function $r^{-1} = f([Zn^{2+}]^{-1})$ for samples of pectin of various esterification degree E. The linear course of this function let us conclude that interaction of Zn^{2+} ions with carboxyl groups of pectin is governed by the multiple equilibria theory. A little greater scattering of the values measured is caused by a gel formation of zinc pectinates.

The macromolecule segment comprising two free carboxyl groups was chosen

for the ligand unit. This unit has one binding site ($n_0 = 1$) providing a stoichiometric bond of Zn^{2+} cation to two carboxyl groups. The experimentally determined n values of various pectin samples ($n = 1{\cdot}00 \pm 0{\cdot}15$) proved this presumption. The stoichiometric binding of Zn^{2+} ions was also evidenced with an insoluble, 1-chloro-2,3-epoxypropane-crosslinked potassium pectate, where a simple ion-exchange method could be used. Kolawole and coworkers[24] investigated the binding of Zn^{2+} ions to polymethacrylate by potentiometric and conductometric titrations and dialysis. These authors employed the multiple equilibria theory for evaluation of the binding of cations, as well; they concluded that zinc forms two distinct complexes with polymethacrylate. Zinc is stoichiometrically bound to polymethacrylate, *i.e.* one Zn^{2+} ion per two carboxyl groups, at lower and medium quantities of the zinc salt added; at higher ones the zinc cation forms a complex with one carboxyl group only. The stoichiometric bond prevails and is more stable. The linear course of function (*2*) (Fig. 2), concerning the binding of Zn^{2+} to carboxyl groups of pectin, evidences the formation of exclusively one complex with a stoichiometric bond similarly, as with binding of other bivalent cations, *e.g.* Pb^{2+} (ref.[8]) and Cd^{2+} (ref.[10]) to pectin of various esterification degree E.

Stability constants of zinc pectinates are listed in Table I; their dependence upon the esterification degree of pectin E shows curve *1* in Fig. 3. The same dependence for the binding of physiologically important Ca^{2+} ions to pectin at the same ionic strength $I = 0{\cdot}02$ mol l^{-1} (curve 2) is presented just for comparison[25]. The stability

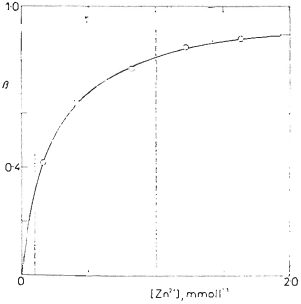

FIG. 1

Dependence of the association degree β' of Zn^{2+} ions with tetramethylmurexide on the concentration of Zn^{2+} ions in solution. [TMM] = $4 \cdot 10^{-5}$ mol l^{-1}

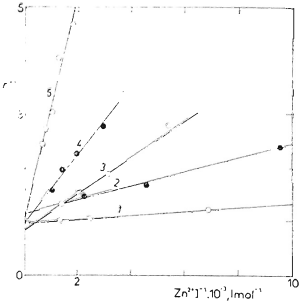

FIG. 2

Binding of Zn^{2+} ions to potassium pectinates. Function $r^{-1} = f([Zn^{2+}]^{-1})$. *1–5* Esterification degree of pectin E 0, 25·0, 47·8, 67·4, 90·3%; $I_0 = 0{\cdot}02$ mol l^{-1}

constant of zinc pectinates strongly depends on the esterification degree of pectin. The binding of Zn^{2+} ions to carboxyl groups of pectin gets markedly weaker with the increasing esterification degree E, *i.e.* with extending the mean distance of neighbouring free carboxyl groups. This dependence is close to a linear logarithmic function $\log K = f(E)$ similarly, as with binding of Ca^{2+} ions to pectin. The Zn^{2+} ions are, to some extent, preferentially bound to pectin when compared with binding of Ca^{2+} ions, this being documented by the difference between stability constants K of zinc and calcium pectinates.

Binding of Zinc Cations to Potassium Oligogalacturonates

The same technique was employed when studying the interaction of Zn^{2+} ions with carboxyl groups of potassium oligogalacturonates (degree of polymerization $m = 1-7$) at 2·00 mmol $(COOZn_{0.5})\,l^{-1}$ concentration. The starting ionic strength of these solutions I_0 was 0·01 mol l^{-1}, analyses were 3 to 4 times repeated. The total starting concentration of zinc in solution ($c_0 = 1{\cdot}00$ mmol $(Zn)\,l^{-1}$) was corrected by the amount of Zn^{2+} ions bound to tetramethylmurexide according to function $\beta' = f([Zn^{2+}])$ shown in Fig. 1. In contrast to preparation of zinc pectinates no gelation took place when solutions of zinc oligogalacturonates were prepared.

The binding of Zn^{2+} ions to those oligomeric fragments of pectin was characterized both by the association degree β of Zn^{2+} ions with carboxyl groups of oligogalacturonates and the activity coefficient $\gamma_{Zn^{2+}}$ of zinc counterions in solutions of zinc oligogalacturonates. The corrected value of ionic strength I, corresponding to an equilibrium solution, was employed for calculation of activities $a_{Zn^{2+}}$ and activity coefficients $\gamma_{Zn^{2+}}$. The $\gamma_{Zn^{2+}}$ values can also be the criterion for interaction of the respective cations with oligogalacturonates, since all solutions investigated had the same concentration. The results are summarized in Table II and Fig. 4 (c_{Zn} is the corrected total zinc concentration in an equilibrium solution). Curve *1* expresses the dependence of association degree β of Zn^{2+} ions with oligogalacturonates upon their polymerization degree m. Values β for calcium oligogalacturonates, calculated from the data already published[26] (curve 2) are given for comparison purposes. The Zn^{2+} ions are bound to oligomeric fragments of pectin similarly as Ca^{2+} ions in contrast to Cd^{2+} and expecially Cu^{2+} and Pb^{2+} ions, which are bound to oligogalacturonates to a far greater extent and form precipitates at a relatively low polymerization degree of oligomers (Cd^{2+} at $m \geq 8$, Cu^{2+} at $m \geq 5$, Pb^{2+}at $m \geq 3$). The degree of association β continuously increases with the increasing polymerization degree m. Binding of Zn^{2+} ions to monomeric D-galacturonate is very weak, $\beta = 0{\cdot}041$. Likewise, the activity coefficient $\gamma_{Zn^{2+}}$, as determined in solution of monogalacturonate ($\gamma_{Zn^{2+}} = 0{\cdot}649$) is close to the calculated value $\gamma_{Zn^{2+}} = 0{\cdot}675$ corresponding to the solution of a strong electrolyte of zinc salt of the same ionic strength ($I = 0{\cdot}01$ mol l^{-1}).

Additivity Rule of Activities of Zn^{2+} *Counterions Bound to Oligogalacturonates*

The validity of the additivity of chiroptic activity increments of inner and terminal uronic acid units in the D-galacturonan molecule to its total optical activity was

TABLE II

Binding of Zn^{2+} ions to potassium oligogalacturonates; $(COOZn_{0.5}) = 2.00$ mmol l^{-1}; $I_0 = 0.01$ mol l^{-1}

Polymerization degree m	c_{Zn} mmol l^{-1}	$[Zn^{2+}]$ mmol l^{-1}	I mol l^{-1}	$\gamma_{Zn^{2+}}$	β
1	0·968	0·928	0·0098	0·649 ± 0·000	0·041
2	0·969	0·786	0·0094	0·553 ± 0·002	0·189
3	0·971	0·649	0·0089	0·459 ± 0·008	0·332
4	0·972	0·572	0·0087	0·406 ± 0·003	0·411
5	0·972	0·528	0·0086	0·375 ± 0·012	0·457
6	0·973	0·494	0·0085	0·351 ± 0·005	0·492
7	0·973	0·473	0·0084	0·337	0·514

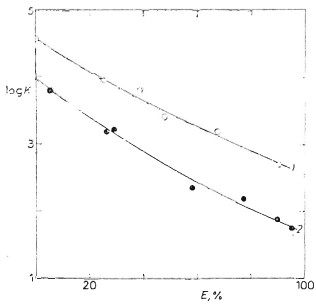

FIG. 3

Dependence of the stability constant K of pectinates on their esterification degree E. *1* Zn-pectinates, *2* Ca-pectinates

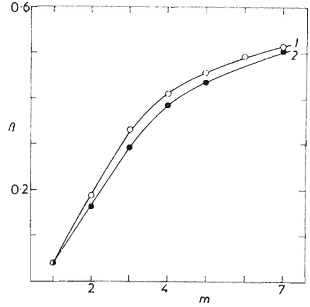

FIG. 4

Association degree β of Zn^{2+} ions with potassium oligogalacturonates. $[COOZn_{0.5}] = 2.00$ mmol l^{-1}; $I_0 = 0.01$ mol l^{-1}. m Polymerization degree of oligomers; *1* Zn-oligogalacturonates, *2* Ca-oligogalacturonates

proved on the basis of the circular dichroism, studied in dilute solutions of sodium oligogalacturonates and sodium D-galacturonan. This proves the chromophoric carboxyl groups of inner galacturonic acid units of the linear macromolecules to be mutually equally oriented in space. This phenomenon is based on a remarkable rigidity of the D-galacturonan molecule due to its $\alpha(1\rightarrow4)$ glycosidic diaxial bonds. (The principle of additivity of optical rotation in polysaccharide solutions was first considered by Freudenberg[28]).

We have proved the validity of the additivity rule also for Ca^{2+} counterion activities in solutions of calcium oligogalacturonates and oligoguluronates[26]. The final activity $a_{Ca^{2+}}$ can be expressed by the sum of activities of Ca^{2+} ions bound to both terminal uronic acid units and inner units in the macromolecule chain. Considering the respective activities, following equation can be deduced for activity coefficient $\gamma_{Ca^{2+}}$:

$$\gamma_m = \frac{\gamma_r}{m} + \frac{\gamma_{nr}}{m} + \frac{(m-2)}{m}\gamma_i \qquad (4a)$$

$$\frac{\gamma_r + \gamma_{nr}}{2} = \gamma_2 , \qquad (4b)$$

where γ_m stands for the resulting activity coefficient of Ca^{2+} counterions in solutions of calcium oligo- and polyuronates of polymerization degree m; γ_r, γ_{nr} and γ_i are the activity coefficients of Ca^{2+} ions bound to reducing and nonreducing terminal uronic acid units and inner units in the macromolecule chain (γ_2 corresponds to the dimer). Rearrangement of equation (*4*) gives the expression

$$\gamma_m = \gamma_2 - (\gamma_2 - \gamma_i)\left(\frac{m-2}{m}\right), \qquad (5)$$

which is a linear function $\gamma_m = f[(m-2)/m]$ for $m \geq 2$ (Freudenberg equation).

The additivity rule relating to the counterion activities in solutions of polyelectrolytes, first reported in one of our previous papers[26], has no general validity. It can be applied only for electrostatic binding of cations to an polyeletrolyte of a considerably rigid linear macromolecule (molecular-disperse solutions of D-galacturonan, L-guluronan). This rule does not hold any more *e.g.* in solutions of calcium D-mannuronan, which has markedly more flexible chain due to glycosidic diequatorial $\beta(1\rightarrow4)$ bonds[26].

A very close course of curves $\beta = f(m)$ describing the binding of Zn^{2+} and Ca^{2+} ions to oligogalacturonates (Fig. 4) provides evidence for an electrostatic bond of Zn^{2+} ions to carboxyl groups of these oligomers. The validity of the principle of additivity of counterion activities was therefore verified by interpretation of $\gamma_{Zn^{2+}}$ values determined in solution of zinc oligogalacturonates of polymerization degree

$m = 1-7$ (Table II). The linear course of the function $\gamma_{Zn^{2+}} = f[(m-2)/m]$ with a minimal scattering of values measured (Fig. 5) is a further proof for validity of the additivity rule concerning activities of counterions (Zn^{2+}, Ca^{2+}) bound to individual structural units of uronic acid in oligogalacturonates.

The value of the activity coefficient $\gamma_{Zn^{2+}} = 0{\cdot}252$, corresponding to binding of Zn^{2+} ions to polymeric D-galacturonan was determined by extrapolation of the function $\gamma_{Zn^{2+}} = f[(m-2)/m]$ to m limiting to ∞; the correlation coefficient, estimated by the least squares method was $-0{\cdot}9994$. The corresponding value of $\gamma_{Ca^{2+}}$, reflecting the binding of Ca^{2+} ions to D-galactunonan, $\gamma_{Ca^{2+}} = 0{\cdot}295$, has already been reported[26]. These activity coefficients ($\gamma_{Zn^{2+}}$, $\gamma_{Ca^{2+}}$) refer to an intramolecular electrostatic bond of Zn^{2+} and Ca^{2+} cations to D-galacturonan in molecular-disperse solutions. On the other hand, an intermolecular chelate bond of Ca^{2+} ions (by analogy also of Zn^{2+} ions) to uronan is manifested by anomalously low activity coefficients $\gamma_{Ca^{2+}}$ (details were reported *e.g.* in[29]). The difference in activity coefficients $\gamma_{Zn^{2+}} = 0{\cdot}252$ and $\gamma_{Ca^{2+}} = 0{\cdot}295$, indicating a little firmer binding of Zn^{2+} ions to carboxyl groups of D-galacturonan, is in accordance with the stability constants determined in zinc pectate ($\log K = 4{\cdot}56$) and calcium pectate solutions ($\log K = 3{\cdot}97$, Fig. 3).

The binding of Zn^{2+} ions to pectin and its oligomeric fragments was purposedly investigated at a relatively low ionic strength $I = 0{\cdot}02$ or $0{\cdot}01$ mol l^{-1} aiming to attain the highest accuracy of analyses. The binding of bivalent cations to pectin is influenced by the presence of a secondary electrolyte in solution due to the ion exchange. The amount of cations bound (M^{2+}) drops with the raising ionic strength of the solution. So, *e.g.* the value of the stability constant of calcium pectate (E 0%) at $I = 0{\cdot}02$ mol l^{-1} is $\log K = 3{\cdot}96$, at $I = 0{\cdot}15$ mol l^{-1}, corresponding to ionic

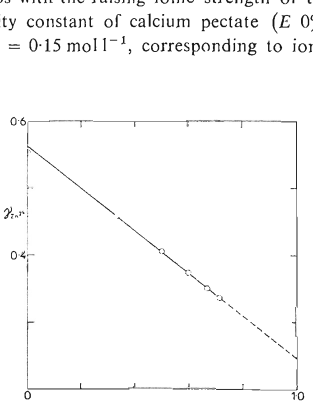

FIG. 5

Binding of Zn^{2+} ions to potassium oligogalacturonates. Function $\gamma_{Zn^{2+}} = f[(m-2)/m]$; $[COOZn_{0{\cdot}5}] = 2{\cdot}00$ mmol l^{-1}; $I_0 = 0{\cdot}01$ mol l^{-1}

strength of physiological saline, $\log K = 3{\cdot}05$ (ref.[25]). Such a behavior can be anticipated also with binding of Zn^{2+} ions to pectin.

Pectin present in vegetables and fruit is a component of our every-day food; it is a question, to what extent will its consumption influence the excretion of zinc from the human body. Pectin in vegetables has a medium degree of esterification, roughly *E* 50%, pectin in fruit is mostly highly esterified *E* 75–85%. As we have shown, the stability constant *K* of zinc pectinates fairly decreases with the increasing esterification degree (Fig. 3). The stability constant of a fully deesterified pectin (*E* 0%) $\log K = 4{\cdot}56$, whereas those of pectin with a medium and high esterification degree *E* 50% and *E* 85% equal 3·45 and 2·83, respectively. If we further consider, besides these values, the drop of stability constant *K* at a higher ionic strength of solution corresponding to that in the gastrointestinal tract, following conclusions can be made: the consumption of fruit will surely not influence the excretion of zinc. A normal consumption of vegetables is unlikely to cause a substantial excretion of zinc from the human body. A prophylactic diet against poisoning with toxic metal cations including a greater amount of vegetables (*e.g.* cabbage, carrot) as an effective component can be manifested by a little greater excretion of zinc when compared with consumption of a normal food. The diet can be, however modified (if even necessary) by food components richer in zinc. A short-time application of sodium pectate (*E* 0%) as a remedy against an acute intoxication with heavy-metal cations should be considered from the viewpoint of a concurrent binding of zinc to pectate. Sodium pectate (*E* 0%), showing not only the highest content of free carboxyl groups (the highest binding capacity), but also the greatest affinity towards Zn^{2+} ions, should be applied against intoxication with greater amounts of zinc. The binding of Zn^{2+} to oligomeric fragments of pectin is virtually the same as that of Ca^{2+} ions.

Our thanks are due to Mr A. Fekete for experimental assistance.

REFERENCES

1. Sandstead H. H. in the book: *Disorders of Mineral Metabolism*, Vol. I, p. 93. Academic Press, New York 1981.
2. Kay R. M., Strasberg S. M.: Clin. Invest. Med. *1*, 9 (1978).
3. Drews L. M., Kies C., Fox H. M.: Amer. J. Clin. Nutr. *32*, 1893 (1979).
4. Kelsay J. L., Jacob R. A., Prather E. S.: Amer. J. Clin. Nutr. *32*, 2307 (1979).
5. Mercurio K. C., Behm P. A.: J. Food Sci. *46*, 1462 (1981).
6. Lei K. Y., Davis M. W., Fang M. M., Young L. C.: Nutr. Reports Internat. *22*, 459 (1980).
7. Camire A. L., Glydesdale F. M.: J. Food Sci. *46*, 548 (1981).
8. Maloviková A., Kohn R.: This Journal *44*, 2915 (1979).
9. Kohn R.: This Journal *47*, 3424 (1982).
10. Maloviková A., Kohn R.: This Journal *47*, 702 (1982).
11. Kohn R., Heinrichová K., Maloviková A.: This Journal *48*, 1922 (1983).
12. Kohn R., Furda I.: This Journal *32*, 1925 (1967).
13. Owens H. S., Lotzkar H., Schultz T. H., Maclay W. D.: J. Amer. Chem. Soc. *68*, 1628 (1946).

14. Kohn R.: Chem. Zvesti *28*, 625 (1974).
15. Kohn R., Luknár O.: This Journal *40*, 959 (1975).
16. Conway B. E.: *Electrochemical Data*, p. 102, Elsevier, New York 1952.
17. Klotz I. M. in the book: *The Proteins* (H. Neurath, K. Bailey, Eds), Vol. I, Part B, p. 748. Academic Press, New York 1953.
18. Tibenský V., Rosik J., Zitko V.: Nahrung *7*, 321 (1963).
19. Kohn R., Tibenský V.: Chem. Zvesti *19*, 98 (1965).
20. Schwarzenbach G., Gysling H.: Helv. Chim. Acta *32*, 1314 (1949).
21. Kohn R.: Carbohyd. Res. *20*, 351 (1971).
22. Mattai J., Kwak J. C. T.: Biophys. Chem. *14*, 55 (1981).
23. Geier G.: Helv. Chim. Acta *50*, 1879 (1967).
24. Kolawole E. G., Olayemi J. Y.: Macromolecules *14*, 1050 (1981).
25. Kohn R., Furda I.: This Journal *32*, 4470 (1967).
26. Kohn R., Malovíková A.: This Journal *46*, 1701 (1981).
27. Bystrický S., Kohn R., Sticzay T.: This Journal *44*, 167 (1979).
28. Freudenberg K., Kuhn W., Dürr W., Bolz F., Steinbrunn G.: Ber. Deut. Chem. Ges. *63*, 1510 (1930).
29. Kohn R.: Pure Appl. Chem. *42*, (3), 371 (1975).

Translated by Z. Votický.