

## CALCIUM AND STRONTIUM ION ACTIVITY IN SOLUTIONS OF THE CORRESPONDING PECTINATES AND ITS DEPENDENCE ON THEIR DEGREE OF ESTERIFICATION

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Single-ion activity coefficients ( $\gamma$ ) of counterions  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  have been determined in solutions of lower calcium and strontium oligogalacturonates and in solutions of the corresponding pectinates, using the metal-indicator method. Dependence of these activity coefficients on the degree of esterification of the pectinates ( $E$  5 to 90%) and on the concentration of the solutions has been studied. For a given degree of esterification, the free carboxyl groups in pectinates have the same affinity for both counterions  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$ . In the region of low degree of esterification  $E$ , the function  $\gamma_{\text{Me}^{2+}} = f(E)$ , as well as the dependence of the ionisation parameter on the charging parameter of the macromolecule, exhibits an anomalous course characterized by low values of the activity coefficients  $\gamma_{\text{Me}^{2+}}$ . These anomalously low values of  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  in solutions of pectinates are in all probability due to an intermolecular bonding of the  $\text{Ca}^{2+}$  or  $\text{Sr}^{2+}$  ions to carboxyl groups of two or more macromolecules in small soluble aggregates.

The selectivity of cation exchange in pectin-based natural ion exchangers depends considerably on the degree of esterification of their carboxyl groups with methanol. The pectin selectivity toward calcium ions in the exchange reaction  $\text{Ca}^{2+}/\text{K}^{+}$  increases with a decreasing degree of esterification of pectin<sup>1,2</sup> (*i.e.* with increasing linear electric charge density). Pectin and its sodium salt proved to be effective as prophylactics in poisoning with heavy metals. Good results were also obtained on application of these compounds to removal of radioactive strontium from the gastro-intestinal tract (for a review of the literature see *e.g.* ref.<sup>3</sup>). From this point of view a more detailed study of the binding of  $\text{Sr}^{2+}$  and  $\text{Ca}^{2+}$  to pectin appeared desirable.

In one of our previous papers<sup>4</sup>, devoted to the binding of cations to polyuronides, we have determined the activity of  $\text{Ca}^{2+}$  ions in solutions of calcium pectinates and its dependence on their degree of esterification ( $E$ ). In the course of our work the need of a further study of this problem has arisen. It was desirable to determine also the activity of  $\text{Sr}^{2+}$  ions in solutions of strontium pectinates and particularly to study the binding of calcium and strontium to the pectinates of low degree of esterification ( $E < 43\%$ ), using an improved technique. In this region of low degree of esterification, the preparation of calcium and strontium pectinate solutions is accompanied with partial coagulation of the corresponding pectinates and therefore the binding

of calcium and strontium to pectin can be affected, besides the purely electrostatic forces, by other factors.

Our present paper deals with the dependence of the interaction of  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  ions with free carboxyl groups of pectin (expressed as single-ion activity coefficients,  $\gamma_{\text{Me}^{2+}}$ ) on the esterification degree of pectin. The activity of  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  ions was determined spectrophotometrically by the metal-indicator method, using tetramethylmurexide as metallochromic indicator. The measurements were done in clear dilute solutions of the corresponding pectinates prepared by special procedures.

## EXPERIMENTAL

### Material

Tetramethylmurexide was the same preparation as used in the previous experiments<sup>4,5</sup>. The solutions of hydroxides had the following concentration: 0.1M-NaOH (carbonate-free), 0.1M-Sr(OH)<sub>2</sub>, and clear saturated calcium hydroxide solution (c. 0.042M). The starting pectin samples were commercial preparations of Danish provenience. They were purified by washing with acidified and neutral ethanol, as was described previously<sup>4</sup>. D-Galacturonic acid (monohydrate) was a "puriss" Fluka (Switzerland) sample. Sodium salts of di-, tri- and tetragalacturonic acids were chromatographically pure samples, which were prepared and characterized as described previously<sup>6</sup>. The other chemicals were of analytical purity. The redistilled water used in the experiments had a conductivity  $2 \cdot 10^{-6} \Omega^{-1} \text{cm}^{-1}$ .

### Preparation of Solutions of Calcium and Strontium Oligogalacturonates and Pectinates

The solutions of calcium and strontium oligogalacturonates were prepared by exact neutralisation of oligogalacturonic acids with the corresponding hydroxide<sup>6</sup>.

Samples of pectinic acids of various degree of esterification were prepared by partial alkaline deesterification of highly esterified pectin, which in turn was synthesized by treatment of pectin with methanolic sulphuric acid at low temperature<sup>4</sup>.

The samples of pectinic acids of low degree of esterification (insoluble or only partially soluble in water) were first solubilized by neutralisation with 0.1M-NaOH. The solutions containing 5 to 7 mequiv.  $[-\text{COONa}] \cdot \text{l}^{-1}$  were centrifuged at 13000g and passed through a column of Dowex 50WX2 ( $\text{H}^+$ ), affording thus clear solutions of pectinic acids. Samples of pectinic acids of higher degree of esterification were dissolved directly in water and centrifuged.

Solutions of the corresponding pectinates were then prepared by a slow neutralisation of dilute pectinic acid solutions (concentration about 5 mequiv.  $[-\text{COOH}] \cdot \text{l}^{-1}$ ) with calcium or strontium hydroxide. The neutralisation was carried out exactly to the equivalence point and was followed potentiometrically. The neutralisation of pectinic acids of a degree of esterification  $E \leq 43\%$  was accompanied by partial coagulation of calcium or strontium pectinate; the extent of the coagulation increased with decreasing  $E$  (*vide infra*). The gel was removed by centrifugation at 13000g, and in the resulting completely clear solutions of calcium or strontium pectinates the activity  $a_{\text{Me}^{2+}}$  was determined either directly or upon dilution to the concentration 3.00 mequiv.  $[-\text{COOMe}_{0.5}^{\text{I}}] \cdot \text{l}^{-1}$ .

### Analytical Methods

The content of free carboxyl groups in pectin, the esterification degree of pectin and the polyuronide content in dry material were determined alkalimetrically by potentiometric titration<sup>4,7</sup>.

Concentration of the groups  $[-\text{COOMe}_{0.5}^{\text{II}}]$  in solutions of well soluble calcium and strontium pectinates was calculated from the consumption of the corresponding hydroxide, necessary for the neutralisation of the pectinic acid.

In the case of partially soluble pectinates, the concentration of the groups  $[-\text{COOMe}_{0.5}^{\text{II}}]$  in the supernatant was determined using two procedures: 1) calculation from the total concentration of carboxyl groups in solution (determined by the carbazole method<sup>8</sup>) and from the degree of esterification of the corresponding pectinate, 2) determination of the concentration  $[\text{Me}^{2+}]$  by chelatometric titration with Chelaton IV using Eriochrome black T in the presence of  $\text{Mg}^{2+}$  ions, the end point being indicated photometrically (interference filter Zeiss Jena, IF 650 nm). The analytical methods were checked, using very dilute solutions of known concentration; both procedures afforded identical results within the limit of the experimental error<sup>9</sup>. The degree of esterification of calcium and strontium pectinates in the supernatant and in the gel phase was determined by the method of precipitation of insoluble copper pectates and pectinates<sup>10,11</sup>. The molecular weight of the pectinates was determined viscometrically<sup>12</sup>. Potentiometric titrations were carried out using a Radelkis OP-205 potentiometer (Hungary) with a Radiometer glass electrode (Denmark).

The mean degree of substitution ( $\overline{DS}$ ) with free carboxyl groups ( $-\text{COOH}$ ) in saccharide units of the linear pectinate molecule was calculated from the degree of esterification of the pectinate ( $E$ ) using the relationship  $\overline{DS} = 1 - E/100$  (this calculation neglects a very small number of L-rhamnose units which can be present in the linear pectin molecule).

The charging parameter,  $\lambda$ , of calcium pectate (CaP) and of the calcium polymannuronate (CaM) was calculated for the temperature 25°C,  $\epsilon = 78.54$ ,  $e_0 = 4.803 \cdot 10^{-10}$  esu,  $k = 1.3805 \cdot 10^{-16}$  erg. deg<sup>-1</sup> (ref.<sup>13</sup>),  $b_{(\text{CaP})} = 4.35 \text{ \AA}$  (ref.<sup>14,15</sup>),  $b_{(\text{CaM})} = 5.0 \text{ \AA}$  (ref.<sup>16-18</sup>).

### Determination of $\text{Ca}^{2+}$ and $\text{Sr}^{2+}$ Ion Activity

Activity of  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  ions was determined spectrophotometrically by the metal-indicator method, using tetramethylmurexide as metallochromic indicator<sup>4,19</sup>. Unless otherwise stated, the measurements were carried out in clear calcium or strontium pectinate solutions of concentration 3.00 mequiv.  $[-\text{COOMe}_{0.5}^{\text{II}}] \cdot 1^{-1}$ , corresponding to 1.5 mmol  $[\text{Me}^{2+}] \cdot 1^{-1}$ . For blank samples, pure pectinate solutions were used. The activity of calcium ions was determined by absorbance measurements at 490 nm and 530 nm, that of the strontium ions<sup>19</sup> at 500 nm and 550 nm. Pure solutions of  $\text{CaCl}_2$  or  $\text{SrCl}_2$  were used for calibration, with no further electrolyte added. The absorbances were determined at 23–25°C on a compensating Hilger-UVISPEC spectrophotometer.

## RESULTS AND DISCUSSION

The analyses of pectin samples of various degree of esterification ( $E$ ) are summarised in Table I. The samples without specified polyuronide content contained at least 90% of this compound in dry material (based on the composition of the starting material). Besides the polyuronide, the pectin macromolecule contained about 10% of neutral saccharides (for details see ref.<sup>1</sup>). The samples contained a very small

amount of ash, which by no means influenced the determination of activity of  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  ions (sulphate ash  $< 0.5\%$ ).

*Comparison of the Affinity of Free Carboxyl Groups of Pectinates toward  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  Ions*

The interaction of the counterions  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  with free carboxyl groups in pectinates is assessed on the basis of their single-ion activity coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$ . The activity of  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  was determined in completely clear solutions of calcium or strontium pectinates at concentrations, corresponding to  $1.500 \text{ mmol } [\text{Me}^{2+}] \cdot \text{l}^{-1}$ , except for sample 1. In this case the concentration of the solutions was a little lower ( $1.1$  to  $1.5 \text{ mmol } [\text{Me}^{2+}] \cdot \text{l}^{-1}$ ), due to greater partial coagulation of calcium and strontium pectinates.

The results of the measurements are summarised in Table II. The single-ion activity coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  in the samples of the same degree of esterification  $E$  are very similar in the whole range of  $E$  values ( $5$  to  $90\%$ ).

The basic structural unit of the pectin macromolecule is D-galacturonic acid. We studied therefore the activity of  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  ions also in solutions of calcium and strontium salts of lower oligogalacturonic acids of polymerisation degree  $DP$  1 to 4 (Table III). As a result of cumulation of carboxyl groups in the oligomer molecule, the activity  $a_{\text{Me}^{2+}}$ , as well as the activity coefficient  $\gamma_{\text{Me}^{2+}}$ , decreases with the increasing  $DP$ , the decrease being analogous in both the calcium and strontium oligogalacturonate series. The differences between the activities  $a_{\text{Ca}^{2+}}$  and  $a_{\text{Sr}^{2+}}$  and between the activity coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  in the corresponding samples are near the experimental errors.

The affinity of the free carboxyl groups in solutions of pectinates with a given degree of esterification toward both counterions  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  is thus practically identical. The results are in good accord with our previous work<sup>3</sup>, studying selectivity of exchange of  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$  in pectates in the gel form. The pectate (fully deesterified pectin) exhibited only very small differences in the affinity for these cations ( $\text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+}$ ). The same result was obtained by Haug and Smidsrød<sup>20</sup>.

*Dependence of the Activity Coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  on the Degree of Esterification of Pectinates*

In one of our previous papers<sup>4</sup> we studied also the dependence of the activity coefficient  $\gamma_{\text{Ca}^{2+}}$  on the degree of esterification ( $E$ ) of calcium pectinate. In the region of low values of  $E$ , the activity  $a_{\text{Ca}^{2+}}$  was determined only on a small number of samples. At that time, we did not know any procedure suitable for preparing solutions of calcium pectate or calcium pectinate of a very low degree of esterifica-

TABLE I  
Analyses of the Pectin Samples

Sample	Degree of esterification $E, \%$	Poly-galacturonide content %	$[\eta]$ 100 ml/g	Molecular weight $\overline{M}_\eta$
1	4.6	90.4	0.70	18 000
2	14.8	89.2	0.86	21 000
3	19.2	90.6	2.34	44 000
4	22.8	—	1.72	35 000
5	35.4	—	2.14	41 000
6	36.1	—	2.60	48 000
7	37.8	—	2.67	49 000
8	41.7	—	3.00	53 000
9	43.8	—	3.08	54 000
10	54.5	90.7	2.09	41 000
11	54.8	86.7	3.76	63 000
12	69.7	—	2.96	53 000
13	89.7	90.6	4.41	71 000

TABLE II  
Dependence of the Single-Ion Activity Coefficients,  $\gamma_{Ca^{2+}}$  and  $\gamma_{Sr^{2+}}$ , in Solutions of Calcium and Strontium Pectinates on their Degree of Esterification ( $E$ )

Sample	$E$ %	$\overline{DS}$	$\lambda$	$\gamma_{Ca^{2+}}$	$\gamma_{Sr^{2+}}$
1	4.6	0.954	1.57	$0.073 \pm 0.005$	$0.080 \pm 0.006$
2	14.8	0.852	1.40	$0.085 \pm 0.005$	$0.089 \pm 0.005$
3	19.2	0.808	1.33	$0.095 \pm 0.005$	$0.090 \pm 0.005$
4	22.8	0.772	1.27	$0.101 \pm 0.008$	$0.088 \pm 0.005$
5	35.4	0.646	1.06	$0.162 \pm 0.005$	$0.151 \pm 0.005$
6	36.1	0.639	1.05	$0.198 \pm 0.007$	—
7	37.8	0.622	1.02	$0.220 \pm 0.004$	—
8	41.7	0.583	0.96	$0.265 \pm 0.003$	—
9	43.8	0.562	0.92	$0.285 \pm 0.009$	—
10	54.5	0.455	0.75	—	$0.352 \pm 0.002$
11	54.8	0.452	0.74	$0.360 \pm 0.003$	$0.347 \pm 0.001$
12	69.7	0.303	0.50	$0.466 \pm 0.003$	$0.467 \pm 0.002$
13	89.7	0.103	0.17	$0.604 \pm 0.001$	$0.617 \pm 0.001$

tion. These compounds were generally assumed to be insoluble or only sparingly soluble. The activity  $a_{\text{Ca}^{2+}}$  in a sample of  $E$  5% was therefore determined only in a gel suspension. The found value of  $a_{\text{Ca}^{2+}}$  which represented the activity of  $\text{Ca}^{2+}$  in the solution, was incorrectly ascribed to the ions, bonded in the gel phase of calcium pectinate. Later we have demonstrated<sup>21</sup> that completely clear dilute solutions of calcium pectate can be prepared by a suitable application of ion-exchangers. For this reason we repeated the determination of activity coefficients  $\gamma_{\text{Ca}^{2+}}$  in calcium pectinate solutions, studying in detail particularly the change of  $\gamma_{\text{Ca}^{2+}}$  in the region  $E < 45\%$ .

The dependence of the activity coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  on  $E$  in the solutions of the corresponding pectinates is depicted in Fig. 1, together with the activity coefficients  $\gamma_{\text{Sr}^{2+}}$  and  $\gamma_{\text{Ca}^{2+}}$  determined in the solutions of monomers (*i.e.* strontium D-galacturonate and calcium D-galacturonate). All the activity coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  lie practically on the same curve.

In pectin of a high degree of esterification (*e.g.*  $E \sim 95\%$ ) the free carboxyl groups are far from each other and therefore, in the first approximation, they can be regarded as isolated. As shown by the extrapolated curve in this region (Fig. 1), the affinity of such isolated free carboxyl groups toward the  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  ions is very similar to that of the free carboxyl groups in monomeric D-galacturonate. The activity coefficient  $\gamma_{\text{Me}^{2+}}$  decreases with the decreasing  $E$ , *i.e.* with the increasing linear electric charge density of the macromolecule: this behaviour is in full accord with the present knowledge of the binding of cations to polyelectrolytes<sup>22,23</sup>.

In the region of  $E \sim 40$  to  $35\%$ , the curve deviates unexpectedly from its anticipated course and the activity coefficient  $\gamma_{\text{Me}^{2+}}$  suddenly drops. In the region of  $E \sim 20$  to  $0\%$ , the value of  $\gamma_{\text{Me}^{2+}}$  changes already very little. Although the values of  $\gamma_{\text{Ca}^{2+}}$ , determined in our previous study<sup>4</sup>, lie very near the recently found curve (Fig. 1), this phenomenon escaped our attention because in the previous work only a small number of samples was measured. The anomalous course of the curve  $\gamma_{\text{Me}^{2+}} = f(E)$  in the region of low  $E$  values will be explained below.

#### *Dependence of the Activity Coefficient $\gamma_{\text{Ca}^{2+}}$ on the Concentration of Calcium Pectinate Solutions*

In addition to the characteristic of the free carboxyl groups in pectinates of various degree of esterification, we studied the dependence of  $\gamma_{\text{Ca}^{2+}}$  on the concentration ( $c$ ) of calcium pectinate solutions. The results of these measurements are given in Fig. 2. The solutions were prepared by diluting the corresponding calcium pectinate solution of the highest studied concentration. The activity coefficient  $\gamma_{\text{Ca}^{2+}}$ , determined in calcium D-galacturonate solution (curve 2), varies with concentration in the same manner as theoretical values of  $\gamma_{\text{Ca}^{2+}}$  calculated for a simple calcium salt (curve 1) *ref.*<sup>24</sup>. Similar situation was found also for a highly esterified pectin ( $E = 89.7\%$ ,

TABLE III

Single-Ion Activities,  $a_{Me^{2+}}$ , and Single-Ion Activity Coefficients,  $\gamma_{Me^{2+}}$  in Solutions of Calcium and Strontium Oligogalacturonates

D-Galacturonate	$a_{Ca^{2+}} \cdot 10^3$ <sup>a</sup>	$a_{Sr^{2+}} \cdot 10^3$	$\gamma_{Ca^{2+}}$ <sup>a</sup>	$\gamma_{Sr^{2+}}$
Mono-	$1.095 \pm 0.010$	$1.119 \pm 0.010$	0.730	0.746
Di-	$0.972 \pm 0.008$	$0.994 \pm 0.020$	0.648	0.663
Tri-	$0.834 \pm 0.005$	$0.849 \pm 0.007$	0.556	0.566
Tetra-	$0.737 \pm 0.005$	$0.737 \pm 0.017$	0.491	0.491

<sup>a</sup> Previously published results<sup>6</sup>.

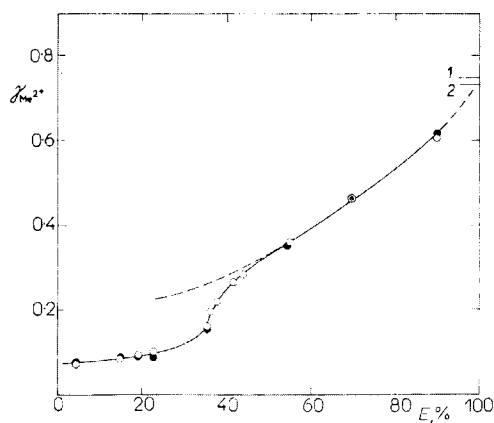


FIG. 1

Dependence of the Single-Ion Activity Coefficient,  $\gamma_{Me^{2+}}$ , in Solutions of Calcium and Strontium Pectinates on Their Degree of Esterification  $E$

○  $\gamma_{Ca^{2+}}$ , ●  $\gamma_{Sr^{2+}}$ , 1 strontium D-galacturonate (monomer), 2 calcium D-galacturonate (monomer).

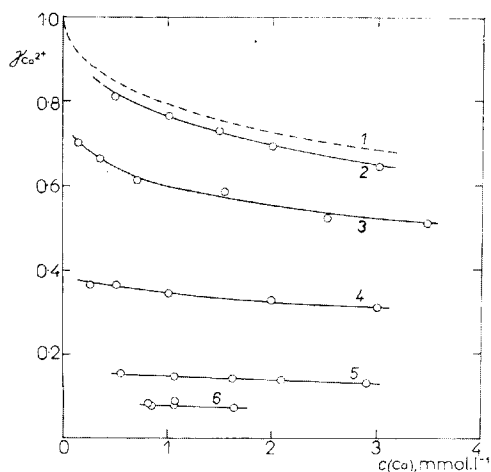


FIG. 2

Dependence of the Single-Ion Activity Coefficient,  $\gamma_{Ca^{2+}}$ , on the Concentration of Calcium Pectinate with Various Degrees of Esterification  $E$

$c$  (Ca) Total calcium concentration in solutions of calcium pectinates ( $\text{mmol} \cdot \text{l}^{-1}$ ), 1 theoretical values of  $\gamma_{Ca^{2+}}$  calculated according to Debye and Hückel for a simple strong electrolyte<sup>24</sup>, 2 calcium D-galacturonate (monomer), 3-6 calcium pectinates of the respective esterification degree  $E = 89.7\%$ ,  $54.8\%$ ,  $35.4\%$ , and  $4.6\%$ .

curve 3) in which free carboxyl groups are far apart from each other. The lower the degree of esterification of the pectinates (*i.e.* the higher the linear electric charge density of the macromolecule) (curves 4–6) the smaller the change of  $\gamma_{\text{Ca}^{2+}}$  with the concentration of the solution; this is a behaviour characteristic for polyelectrolytes.

The activity coefficient of counterions is only slightly affected by the concentration of the solutions of polyelectrolytes with a high charge density of macromolecules. For example, in sodium heparinate solutions the value of  $\gamma_{\text{Na}^+}$  is almost constant in a wide range of concentrations; the same was observed for sodium polyacrylate solutions and for other polyelectrolytes of a high linear charge density<sup>25,26</sup>. Nagasawa and collaborators<sup>22</sup> found a small decrease of  $\gamma_{\text{Na}^+}$  values with decreasing concentration of solutions of sodium salts of various polyacids. Kozak and Dolar<sup>27</sup> have shown that the osmotic coefficient of counterions  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  in solutions of corresponding polystyrenesulphonates is almost independent of concentration. On the other hand, a small increase of  $\gamma_{\text{Na}^+}$  values in the region of the lowest concentrations<sup>26</sup> was found for polyelectrolytes with a lower charge density.

The course of the curves  $\gamma_{\text{Ca}^{2+}} = f(c)$ , depicted in Fig. 2, together with the previous results (Table II, Fig. 1), shows that the cation binding ability of the pectinates changes in a wide range, depending on the linear charge density of the macromolecules. In this respect, pectinates with high degree of esterification are similar to simple electrolytes, whereas pectinates with low degree of esterification behave as typical polyelectrolytes.

#### *Dependence of the Ionisation Parameter of Calcium Pectinate on the Charging Parameter*

The ionisation parameter  $i_m$  expresses the effective degree of ionisation of a monomeric unit of a polyelectrolyte:

$$i_m = \alpha \gamma_{\text{Me}} \cdot \overline{DS}, \quad (1)$$

where  $\alpha$  is degree of ionisation of macromolecules (*i.e.* the ratio of the number of ionised groups to the total number of ionizable groups of the polyelectrolyte),  $\gamma_{\text{Me}}$  is the single-ion activity coefficient of the counterions and  $\overline{DS}$  is the mean degree of substitution of the monomeric units of the linear macromolecule with an ionisable group). The parameter  $i_m$  may also be described as the effective charge of the monomeric unit<sup>33</sup>.

As follows from the quantitative evaluation of the interaction of counterions with the molecule of the polyelectrolyte according to the rodlike model of Lifson and Katchalsky<sup>23,28</sup>, the charging parameter  $\lambda$  is an important structural characteristic of a polyelectrolyte solution:

$$\lambda = e_0^2 / ekTb, \quad (2)$$



where  $e_0$  is the elementary charge,  $\epsilon$  is dielectric constant of the solvent,  $k$  is Boltzmann constant,  $T$  is absolute temperature and  $b$  is the repeating distance between the neighbouring ionised groups in the direction of the main axis of the linear polyelectrolyte macromolecule.

For a solution of calcium pectinate as a salt of a polyacid  $\alpha = 1$ ,

$$i_m = \gamma_{Ca^{2+}} \cdot \overline{DS}. \quad (3)$$

The basic skeleton of the pectin molecule is formed by polygalacturonic acid. As a result of the diaxial glycosidic bond  $\alpha(1 \rightarrow 4)$  between the D-galacturonic acid units with C 1 configuration, the molecule of pectin behaves as a linear, considerably rigid molecule. We have discussed in detail the structure of the pectin molecule earlier<sup>29</sup>. For such linear macromolecule of calcium pectinate, the value of  $b$  can be calculated in a first approximation from the relation:

$$b = b_0 / \overline{DS}, \quad (4)$$

where  $b_0$  is the distance between the neighbouring carboxyl groups in the molecule of calcium pectate ( $\overline{DS} = 1$ ). The value  $b_0 = 4.35 \text{ \AA}$  was determined by X-ray analysis of pectic acid, sodium pectate and of the oriented calcium pectate gel<sup>14,15</sup>. For calcium pectate the value of  $\lambda$  is 1.64. A certain deviation of the actual distance  $b$  from the value calculated by the above mentioned procedure can be expected for compounds with a very low degree of substitution (e.g.  $\overline{DS} = 0.1$ ) where the partial flexibility of the linear macromolecule already begins to operate.

Numerous studies of the binding of cations to polyelectrolytes have shown that in the region of very low charge of the macromolecule the value  $i_m$  increases initially

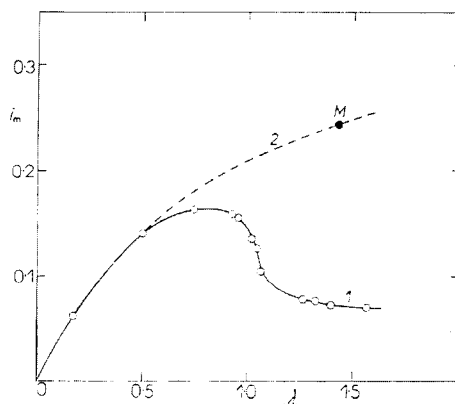


FIG. 3

Dependence of the Ionisation Parameter,  $i_m$ , in Solutions of Calcium Pectinates on their Charging Parameter,  $\lambda$

1 Experimental data, 2 theoretical curve,  $M$   $i_m$  in calcium polymannuronate solution.

almost linearly with the charging parameter  $\lambda$ , then the curve  $i_m = f(\lambda)$  is bent towards the abscissa, and finally, in the region of high values,  $i_m$  attains a constant value (e.g.<sup>23,30</sup>).

The dependence of  $i_m$  on  $\lambda$  in calcium pectinate solutions is shown in Fig. 3 (curve 1); the theoretical course, under assumption of purely electrostatic interactions of  $\text{Ca}^{2+}$  ions with carboxyl groups of the pectinate ( $-\text{COO}^-$ ), is represented by curve 2 (Fig. 3).

The theoretical curve 2 was constructed according to the study of interactions of the ions  $\text{Me}^{2+}$  with carboxymethylcellulose<sup>30</sup>, using the activity coefficient  $\gamma_{\text{Ca}^{2+}}$  determined in a dilute solution of calcium polymannuronate<sup>21</sup>. For both these systems a purely electrostatic interaction is assumed. Under condition of such an interaction, the activity coefficient  $\gamma_{\text{Ca}^{2+}}$  in solutions of various calcium polyuronates is independent of their structure and depends only on the distance between the neighbouring ionised carboxyl groups ( $b$ ). The activity coefficient  $\gamma_{\text{Ca}^{2+}}$  in a solution of calcium polymannuronate should therefore be identical with the value  $\gamma_{\text{Ca}^{2+}}$  in a solution of pectinate of the same linear charge density of the macromolecule (i.e. with the same value of  $b$ ). In a calcium polymannuronate solution,  $\gamma_{\text{Ca}^{2+}} = 0.281$  (ref.<sup>21</sup>),  $b = 5.0 \text{ \AA}$  (ref.<sup>16-18</sup>),  $\lambda = 1.43$ . The value  $b = 5.0 \text{ \AA}$  corresponds to the molecule of a pectinate with degree of substitution  $\overline{DS} = 0.87$ . The ionisation parameter  $i_m$  is then:  $0.281 \cdot 0.870 = 0.244$  (curve 2, point  $M$ ).

The curve 1 on Fig. 3 which depicts the function  $i_m = f(\lambda)$  has an anomalous course. In a relatively narrow range of  $\lambda$  values, the  $i_m$  values decrease substantially relatively to the anticipated values; this phenomenon witnesses of a very strong binding of  $\text{Ca}^{2+}$  ions to the pectinate carboxyl groups.

#### *Anomalous Course of the Curves $\gamma_{\text{Me}^{2+}} = f(E)$ and $i_m = f(\lambda)$*

The alkaline deesterification of pectin, used in the preparation of samples, is accompanied by some cleavage of the glycosidic bonds in the macromolecule; even though the extent of cleavage is small, this leads already to a large drop in the molecular weight of pectin. The average molecular weights,  $\overline{M}_n$ , of the pectin samples (Table I) differ therefore considerably and they are in the range 18000 to 71000. We have proved in our previous paper<sup>21</sup> that for solutions of calcium polymannuronates and polyguluronates with the degree of polymerisation higher than 30 ( $\overline{M}_n > 5300$ ), the interaction of  $\text{Ca}^{2+}$  ions with carboxyl groups of polyuronates does not depend on the length of the macromolecular chain. The same conclusion was reached in the study of binding of  $\text{Ca}^{2+}$  to oligo- and polygalacturonates<sup>31</sup>. Therefore, the varying molecular weight of the studied pectin samples has no effect on the activity of the counterions  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  and it hence cannot cause the anomalous course of the above mentioned curves.

The anomalous drop of the  $\gamma_{\text{Me}^{2+}}$  values was exhibited by all the pectinate solutions in which the calcium or strontium pectinate already partially coagulated during the neutralisation. The neutralisation of pectinic acids of a degree of esterification  $E > 43\%$  with calcium or strontium hydroxide led to completely clear solutions. On the other hand, in samples with  $E \leq 43\%$  the insoluble pectinates partially coagulated, the extent of coagulation increasing with the decreasing degree of esterification and with increasing starting concentration of the pectinic acid. Thus, for example, in the solutions of calcium pectinates with  $E$  40, 35, 20 and 5%, at the total concentration 5 mequiv.  $[-\text{COOCa}_{0.5}^{\text{II}}] \cdot \text{l}^{-1}$ , the extent of the coagulation of calcium pectinate was 2, 5, 20 and 30%, respectively.

As a result of partial coagulation of calcium and strontium pectinates, the anomalous course of the curves  $\gamma_{\text{Me}^{2+}} = f(E)$  and  $i_m = f(\lambda)$  could be due to a fractional coagulation according to the different degree of esterification of the individual macromolecules. The samples with a lower degree of esterification were prepared by the alkaline deesterification of a highly esterified pectin. Heri and collaborators<sup>32</sup> have demonstrated that this deesterification procedure leads to highly homogeneous preparations, concerning the degree of esterification. In all these samples we determined the degree of esterification of the pectinate in the clear supernatant as well as in the coagulate. The degree of esterification of pectinate both in solution and in the coagulate was the same within the experimental error of the analytical method ( $\Delta E$  1 to 2%). The neutralisation of pectinic acid with hydroxide was therefore not accompanied with fractionation of calcium and strontium pectinate according to their degree of esterification; this fact represents also a proof of a considerable homogeneity of the studied samples.

We have found anomalously low values of  $\gamma_{\text{Ca}^{2+}}$  also in clear solutions of calcium polyguluronates ( $DP > 18$ ) in all cases when during their preparation the polyguluronate<sup>21</sup> already partially coagulated.

The activity coefficient  $\gamma_{\text{Me}^{2+}}$  was determined in the clear supernatant after removal of the coagulate at 13000g ( $\gamma_{\text{Me}^{2+}}$  was calculated, taking the polyuronate concentration in the supernatant). On the basis of the above mentioned findings we therefore assume that the clear solutions of calcium or strontium pectinates with a low degree of esterification are not molecularly dispersed but they contain small soluble aggregates of macromolecules. The anomalously low activity coefficients  $\gamma_{\text{Ca}^{2+}}$  and  $\gamma_{\text{Sr}^{2+}}$  are in all probability caused by an intermolecular bonding of the ions  $\text{Me}^{2+}$  to carboxyl groups of two or more macromolecules.

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