

Preparation of Water-soluble Polyuronic Acids and their Calcium Salts, and the Determination of Calcium Ion Activity in Relation to the Degree of Polymerization

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If solutions of sodium polyuronates with a low concentration are filtered through a cation-exchanger in H^+ -form, the precipitation of polyuronic acids does not occur (except for a partial precipitation occurring with polyguluronic acids of intermediate DP). Soluble calcium oligo- and polyuronates were prepared from such solutions, or suspensions, by potentiometric titration with calcium hydroxide. Solutions of the polyuronic acids and their calcium salts are stable only at low ionic strengths.

The Ca^{2+} -activities were determined in solutions of calcium oligo- and polyguluronates (DP 1–81), calcium oligo- and polymannuronates (DP 1–4000), calcium pectate, and calcium polymethacrylates. These measurements were performed by means of the metal-indicator method, using tetramethylmurexide as an auxiliary ligand. The activity coefficient $\gamma_{Ca^{2+}}$ in solutions of calcium monouronates is close to that of a corresponding solution of calcium chloride. With the increasing degree of polymerization of oligo- and polyuronates the activity coefficients $\gamma_{Ca^{2+}}$ decrease. They reach roughly constant values for calcium polyguluronates and calcium polymannuronates with DP higher than 30. The $\gamma_{Ca^{2+}}$ values of calcium polyguluronate and calcium pectate solutions are very close and several times lower than $\gamma_{Ca^{2+}}$ of the calcium polymannuronate. The results are discussed on the basis of linear charge densities of the polyuronates.

It is generally known that alginic acids and alginates are natural ion-exchangers. During the last ten years many experimental data concerning this subject have accumulated.^{1,2} For many mono- and divalent cations the selectivity of the ion-exchange is closely related to the uronic acid composition of the alginates.^{1,3–6} In most cases, the higher the content of L-guluronic acid units in the alginate, the higher the selectivity of exchange of these cations.

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The polyuronides and their low molecular fragments are of a special interest in human medicine as prophylactic substances and remedies against intoxication by radioactive strontium (see, *e.g.*, Refs. 7–9; survey of papers ^{7,10}). From this point of view, the selectivity of the exchange of Ca^{2+} – Sr^{2+} appears as the most important. In systems containing calcium and strontium salts of natural polyanions and synthetic derivatives of polysaccharides with anionic groups ($-\text{COOH}$, $-\text{SO}_3\text{H}$), strontium is bound selectively only to alginates and their low molecular fragments which are rich in L-galuronic acid units.^{4,1,6,11} Similar results were obtained in biological tests studying the effect of polyuronates and different polyanions on the inhibition of strontium absorption.^{12,13} The only exception was observed by using sodium pectate and pectin *in vivo*, which also gave a reduction of strontium absorption.^{14–19}

To be able to understand the high selectivity of alginates in ion-exchange reactions we need further data on the interaction of cations with carboxyl groups of the two characteristic polyuronates (polyguluronates and polymannuronates) present in alginate dependent upon the degree of polymerization, starting from the monomeric uronic acid up to the polyuronates. Until now, such measurements were performed only with the lower calcium oligogalacturonates ²⁰ (degree of polymerization DP 1 up to 4). In solutions of these oligogalacturonates the activity of Ca^{2+} was determined by means of the metal-indicator method.

In the present paper the same experimental technique was used for elucidating the effect of the degree of polymerization of oligo- and polyguluronates (DP 1–81) and oligo- and polymannuronates (DP 1–4000) on the interaction of Ca^{2+} with their carboxyl groups. Since the system (simultaneously with the exchange of some cations on polyuronates) changes from the *sol*- to the *gel*-state, the study of ion-binding becomes difficult. A special effort was therefore made to prepare the calcium salts of these oligo- and polyuronic acids in a soluble form, so that the activity measurements with both polyuronates could be realized under the same experimental conditions *i.e.* in solution.

EXPERIMENTAL

Materials. The sodium alginates I and II, with a high content of L-galuronic and D-mannuronic acid, respectively, were prepared as described elsewhere.⁵ In the following, the terms “*polyguluronate*” and “*polymannuronate*” are used for these preparations. The alginate fraction used as starting material in the preparation of oligo- and polyguluronides was prepared as described previously (preparation C).³³ Polymannuronic acid was prepared from fruiting bodies of *Ascophyllum nodosum*.³⁴ The pectic acid was prepared by an alkaline de-esterification of a commercial preparation of apple pectin as published earlier.⁵ The analytical composition of these polyuronides is given in Table 1.

The sodium polymethacrylates I and II were preparations by the Research Institute of Synthetic Varnishes and Resins in Pardubice (Czechoslovakia) and the Central Research Institute of Sugar Industry in Kiev (USSR), respectively. Sample I was purified by dialysis against deionized water; both samples were desalted by means of the ion-exchange technique. The corresponding polymethacrylic acids were pure substances with a degree of polymerization $\overline{\text{DP}}_\eta = 390$ (I) and 3200 (II), respectively.

Tetramethylmurexide was prepared from caffeine *via* alloxanthine and characterized as described previously.²¹ The 0.05 N potassium hydroxide was carbonate-free. The aqueous calcium hydroxide was a clear, saturated solution, having a concentration of

Table 1. Composition of polyuronic acids.

Sample	Source	Uronic acid composition %		D-GalUA	Degree of polymerization	
		L-GulUA	D-MannUA		\overline{DP}_n	\overline{DP}_η
Alginic acid I, "polyguluronic acid"	<i>Laminaria hyperborea</i> stipes	89.5	10.5	—	81	—
Alginic acid II, "polymannuronic acid"	<i>Fucus vesiculosus</i> receptacles	8	92	—	—	4000
Pectic acid	Apples	—	—	90.6	—	91

\overline{DP}_η = average degree of polymerization determined viscometrically.

about 0.042 N. All other chemical reagents were of analytical grade; the distilled water had a conductivity of about 2.5×10^{-6} ohm⁻¹ cm⁻¹.

Methods. In the following, the low molecular weight fragments of polyuronides with a number degree of polymerization $\overline{DP}_n \leq 10$ are defined as oligomers, fragments with $\overline{DP}_n > 10$ as polymers.

The total concentration of carboxyl groups was determined by potentiometric titration with calcium hydroxide to the equivalent point (pH ca. 7.5). In systems of calcium oligo- and polyguluronates (DP 16–81), which are only partly soluble, the concentration of calcium was determined separately in the supernatant and in the gel-phase after centrifugation. The two fractions were acidified with hydrochloric acid to an approximately 0.1 N solution, and the precipitate filtered off on Schleicher-Schüll filter, "blue ribbon" No. 589³, previously washed with 0.1 N HCl. Calcium was then quantitatively washed out with 0.1 N HCl and determined in the filtrate with a Perkin-Elmer Atomic Absorption Spectrophotometer. With calcium pectate, the concentration of calcium in the supernatant and gel was determined indirectly by precipitation of the insoluble copper pectate.^{22,23}

The amount of uronate units was estimated by the phenol-sulphuric acid method,²⁴ applying the factors already determined for mannuronic and guluronic acids.²⁵ The number average degree of polymerization (\overline{DP}_n) of oligo- and polyuronates up to $\overline{DP}_n = 81$ was determined by using the Nelson method.²⁶ The degree of polymerization of the high-molecular sodium polymannuronate,^{27,28} sodium pectate,²⁹ and samples of sodium polymethacrylate³⁰ was determined viscometrically. The uronic acid composition was determined according to Haug and Larsen.³¹ The content of carbon dioxide in distilled water was estimated by potentiometric titration.³²

Determination of calcium ion activity. The solutions were prepared with great care to achieve solutions containing the smallest possible amount of carbon dioxide absorbed from the air and of potassium chloride diffused from the salt-bridge during the potentiometric titration. Freshly boiled and cooled distilled water was used containing less than 1×10^{-5} mol CO₂/l. A special saltbridge with 0.1 N KCl was applied. The resulting solution included about 1×10^{-5} mol KCl/l.

The activity of Ca²⁺ in solutions of calcium oligo- and polyuronates was determined by means of the metal-indicator method using tetramethylmurexide as an auxiliary ligand. The principle and the detailed description of the method were published earlier.^{21,20} The measurements were performed in solutions containing 1.20 up to 3.00 mequiv. of [–COOCa_{1/2}] and 4×10^{-5} mol of tetramethylmurexide per liter. The absorbances were determined at 490 and 530 nm in a Hitachi Perkin-Elmer model 139 spectrophotometer, using solutions of calcium oligo- and polyuronates as reference.

Preparation of oligouronides and of low molecular weight polyuronides. Details concerning acid hydrolysis of the appropriate alginate fractions to provide a suitable starting material for the preparation of oligomers, and polymers of low DP, have been published previously.^{33,35,34}

To prepare the lower polyuronides the reaction was stopped when DP_n reached approximately 20 (time of hydrolysis about 100 min). To prepare the oligouronides with DP 2–4 the reaction was stopped when DP_n was approximately 3, thus requiring about 6 h of hydrolysis. The solution was then cooled to room temperature, neutralized with 0.1 N NaOH and evaporated to 5 ml.

The fractionation. A column (22 × 1500 mm) of Sephadex G-50 was used to prepare polydisperse polyuronide fractions with DP higher than 8. The appropriate hydrolysate was washed on the column and eluted with 0.1 M Na_2SO_4 solution. The elution rate was approximately 12 ml/h and fractions of 2 ml were collected. The tubes were analyzed for uronide and reducing end-groups and pooled to provide fractions with the desired DP . The number of tubes in each fraction was always between 10 and 20.

The separation of the monodisperse fractions of the oligouronides (DP 2–4) was carried out on column (19 × 1750 mm) of Bio-Gel P-4 equilibrated with 0.1 M Na_2SO_4 . The elution was performed with the same solvent at a rate of 7 ml/h and fractions of 2 ml were collected. The elution curve obtained for the separation of the lower oligoguluronides is shown in Fig. 1. It has been previously demonstrated³⁵ that from this column the lower oligouronides are eluted as monodisperse entities.

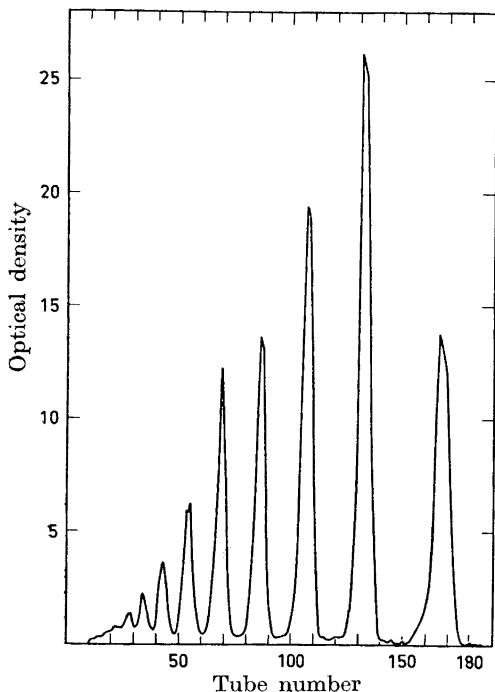


Fig. 1. Separation of oligoguluronides on a column of Bio-Gel P 4.

Desalting of oligo- and polyuronide fractions. The fractions were evaporated in vacuum until the solution was saturated with respect to Na_2SO_4 . They were desalted using a column of Sephadex G-10 (14 × 1700 mm). Each fraction was analysed for uronide content, and its conductivity was determined. The first appearance of salt in the eluate was indicated by an increase in the ratio of conductivity to the uronate content. The desalting process was repeated twice, and the fractions were isolated in form of white powders by freeze-drying.

An accidental contamination of starting samples of the polymannuronate (DP 4000), polyguluronate (DP 81), pectate, and of polymethacrylates with salts was eliminated by the filtration of very dilute solutions of these samples on columns of Dowex 50 W × 4

(H⁺ form) and Dowex 1 × 8 (OH⁻ form). Solutions of polyacids completely free of salts were obtained. With the solutions of sodium polyguluronate (DP 81) and potassium pectate (DP 91) a partial absorption occurred on the anion-exchange resin.

Preparation of solutions of oligo- and polyuronic acids and of their calcium salts. The sodium oligo- and polyuronates were converted to the corresponding acids by means of Dowex 50 W × 4 in H⁺ form. The starting solutions contained about 1.4–1.6 mg of sample in 1 ml. All samples of oligo- and polymannuronates filtered on the cation-exchanger yielded clear solutions of acids. Guluronates with DP from 10 to 28, on the other hand, gave a partial coagulation of the polyguluronic acid on the resin. The filtrate was a suspension containing the polyguluronic acid partly in a soluble and partly in an insoluble form. The polyguluronate with DP 81 yielded a solution with a hardly detectable opalescence.

The insoluble pectic acid was previously converted to its soluble potassium salt by potentiometric titration with potassium hydroxide. The solution was centrifuged at 13 000 *g* and then filtered through the cation-exchange resin; a clear solution of pectic acid was obtained.

The solutions, or suspensions, of oligo- and polyuronic acids contained 4–7 mequiv. of [–COOH]/l. They were neutralized with a clear solution of calcium hydroxide by potentiometric titration to the equivalence point (pH 7.2–7.5). Fractions of oligo- and polymannuronic acids gave clear solutions of their corresponding calcium salts. During the titration of pectic acid and polyguluronic acids with DP higher than 12 a partial coagulation of the corresponding calcium salt occurred. The insoluble part was removed by centrifugation at 13 000 *g*. A clear supernatant containing calcium polyuronates in a soluble form was obtained.

The solution of the high molecular calcium polymannuronate (DP 4000) was prepared in two different ways: (1) using the ion-exchange technique, (2) using the following method: To the solution of sodium polymannuronate two volumes of ethanol, acidified with hydrochloric acid, were added. The precipitated polymannuronic acid was carefully washed with acidified 60 % ethanol, then with neutral 60 and 96 % ethanol, and dried at room temperature. This insoluble polymannuronic acid was slowly neutralized with a clear solution of calcium hydroxide to the equivalence point. With both methods a clear solution of the calcium polymannuronate was obtained.

The solution of sodium polymethacrylate was converted to the polymethacrylic acid by the ion-exchange technique, and then neutralized with calcium hydroxide to pH 8.0–8.1. At this pH the degree of neutralization was about 0.98.

No coagulation occurred during these processes.

The clear solutions of calcium oligo- and polyuronates were properly diluted with distilled water and then used for measurements of calcium ion activities.

RESULTS

Preparation of solutions of polyuronic acids and of calcium polyuronates. It is well known, that the addition of an excess of a strong acid to a solution of sodium alginate or pectate causes precipitation of the corresponding polyuronic acid. Only low molecular fragments of polyuronic acids have so far been prepared in a soluble form, e.g. fragments of pectic acid (DP 1–4).^{36,37} Similarly, the addition of calcium chloride leads to precipitation of calcium alginates and calcium pectate, respectively. The precipitation of insoluble calcium pectate is the basis of the method of Carré and Haynes,³⁸ for many years used as a standard method in the analysis of pectic substances.

In the present study, it was necessary to eliminate the influence of the gel-formation on the interaction of Ca²⁺ with carboxyl groups of the polyuronides under investigation. By using the ion-exchange technique it was possible to prepare diluted solutions of polyuronic acids and of their calcium salts, which up to now were held to be insoluble or soluble only to a low extent.

If solutions of sodium (potassium) polyuronates with a low concentration are filtered using a cation-exchanger in H^+ -form, the precipitation of polyuronic acids does not occur, probably due to the progressive exchange of Na^+ (K^+) and H^+ -ions. Using this treatment solutions of the polymannuronic acid (DP 4000), polyguluronic acid (DP 81), and pectic acid (DP 91) were prepared. The composition of polyuronates used is described in Table 1. The solutions of polyuronic acids contained 4–6 mequiv. uronic acid per liter. Atomic absorption spectrometry failed to show the presence of sodium, potassium, calcium, or magnesium ions.

The solutions containing polymannuronic acid or pectic acid were completely clear; those of polyguluronic acid had a hardly detectable opalescence. Addition of an excess of hydrochloric acid to these solutions gave an immediate precipitation of the acids.

All samples of sodium oligo- and polymannuronates (DP 1–4000) yielded clear solutions of the corresponding acids. The same also applies to the lower oligomers (DP 1–4) of guluronic acid. With samples of $DP > 10$ the precipitation of polyguluronic acids occurred directly on the cation-exchange resin; the tendency of the polyguluronic acids to precipitate at first increases, reaches a maximum at DP about 16 and then decreases. The sample of DP 28 gave a clear solution which had a low turbidity only after some hours. Since the low molecular fragments of polyguluronic acid were prepared only in small amounts, it was not possible to follow quantitatively the precipitation. It should be pointed out that this process is to a large extent dependent upon the concentration of the starting solution.

Calcium oligo- and polyuronates. The solutions of calcium oligo- and polyuronates were prepared by potentiometric titration of solutions, or suspensions, of the corresponding acids with calcium hydroxide.

The titration curves were in both cases sufficiently steep in the neighbourhood of the equivalence point to allow the neutralisation to be carried out with an error of less than 0.5 %.

Polymethacrylic acid, as well as all oligo- and polymannuronic acids, yielded clear solutions of the corresponding calcium salts. The same also applies to oligoguluronic acids with $DP \leq 12$, including the insoluble acid fractions of DP 9.5 and 12.3, which became soluble during the titration with calcium hydroxide. Polyguluronic acids of higher DP and pectic acid were partially precipitated during the neutralisation. The distribution of material between the two fractions for the individual samples are given in Table 2. The amount of gel separated by centrifugation was weighed for each sample and the figures are recorded in Table 2. To render these figures comparable they were recalculated to the amount of gel corresponding to 2 mequiv. of polyuronates in the gel.

The three experiments with pectic acid were performed to investigate the influence of the rate of addition during the neutralisation with calcium hydroxide. The first sample (1) was neutralised very slowly by a continuous addition of $Ca(OH)_2$ (40 min), the second (2) by the standard titration technique, and the third (3) by a very rapid addition of about 90 % of the quantity required for neutralisation and subsequent titration to the end point.

Table 2. Content of calcium polyuronate in the soluble and insoluble phase. All figures correspond to 1 liter starting solution.

Polyuronic acid	DP	[-COOCa _{1/2}] mequiv.			% precipitated	gel % (w/w)	gel _{corr.} ^a % (w/w)	
		total	super-natant	gel				
Polyguluronic acid	16.3	4.03	3.31	0.72	17.9	< 0.2	< 0.6	
		3.95	3.12	0.83	21.0			
		3.95	3.11	0.84	21.3			
	18.4	4.23	1.72	2.51	59.3	< 0.2	< 0.2	
		4.58	2.15	2.43	53.1			
		2.62	1.57	1.05	40.1			
	28.4	4.00	1.62	2.38	59.5	1.8	1.5	
		4.20	2.04	2.16	51.4	2.0	1.8	
		4.46	2.40	2.06	46.2	1.5	1.5	
	81	3.20	1.54	1.66	51.9	4.9	5.9	
		3.20	1.56	1.64	51.3	5.1	6.2	
		2.16	1.52	0.64	29.6	1.64	5.1	
2.16		1.44	0.72	33.3	1.77	4.9		
Pectic acid 1)	91	4.02	2.04	1.98	49.3	6.2	6.2	
		2)	4.02	1.88	2.14	53.2	11.1	10.4
		3)	4.02	1.62	2.40	59.7	14.4	12.0
			4.71	2.04	2.67	56.7		

1) 2) 3) See text.

^a Values corrected to the amount of gel corresponding to 2 mequiv. of polyuronates in the gel.

All activity measurements with mono-, oligo-, and polymannuronates were carried out in solutions containing 1.500 mmol Ca/l. The results are presented in Table 3 and Fig. 2. The calcium activity ($a_{Ca^{2+}}$) in the solution

Table 3. Activity of Ca²⁺ and activity coefficient $\gamma_{Ca^{2+}}$ in solutions of Ca mono-, oligo- and polymannuronates. [Ca]=1.500 mmol/l.

Sample	DP	$a_{Ca^{2+}}$ mmol/l	$\gamma_{Ca^{2+}}$
1 ^a	1	1.05 ± 0.01	0.70 ± 0.01
2	2	1.02 ± 0.01	0.68 ± 0.01
3	3	0.91 ± 0.01	0.61 ± 0.01
4	4	0.90 ± 0.01	0.60 ± 0.01
5	11.5	0.613 ± 0.004	0.409 ± 0.003
6	16.0	0.577 ± 0.007	0.385 ± 0.005
7	16.3	0.582 ± 0.008	0.388 ± 0.005
8	20	0.485 ± 0.005	0.323 ± 0.003
9	25	0.496 ± 0.003	0.331 ± 0.002
10	48	0.441 ± 0.010	0.294 ± 0.006
11	4000	0.421 ± 0.003	0.281 ± 0.002

^a Published previously.⁵

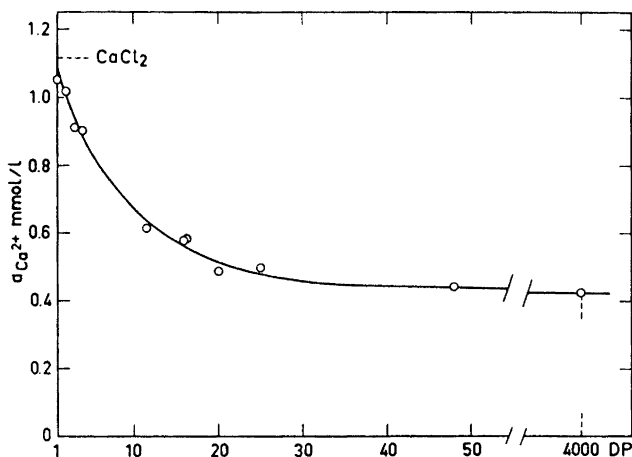


Fig. 2. Activity of Ca^{2+} in solutions of calcium oligo- and polymannuronates dependent on the degree of polymerization (DP). ----- $CaCl_2$. Activity of Ca^{2+} in solution of 1.500 mmol $CaCl_2/l$.

of the monomeric calcium mannuronate is close to the $a_{Ca^{2+}}$ -value in a solution of calcium chloride with the same concentration ($CaCl_2$; $a_{Ca^{2+}} = 1.12$ mmol/l; $\gamma_{Ca^{2+}} = 0.75$). With an increase in degree of polymerization the calcium activity decreases until it becomes practically independent of the chain length at a DP > 30. (The activity coefficient $\gamma_{Ca^{2+}}$ changes in the same manner.) The Ca^{2+} -activity measurements with mono- and oligoguluronates, and with polyguluronates of DP < 16, were carried out in solutions containing 1.500 mmol Ca/l. At DP \geq 16 a partial precipitation of calcium polyguluronates occurred. With polyguluronates of DP \geq 16 the Ca^{2+} -activity measurements were performed in the clear supernatant, separated by centrifugation. (The concentration of the calcium polyguluronate in the supernatant, illustrating the solubility of this substance, is presented in Table 2.) Owing to the restricted solubility of calcium polyguluronates, it was not possible to perform all measurements at the same concentration. The results of Ca^{2+} -activity for different samples may, therefore, be compared only on the basis of $\gamma_{Ca^{2+}}$ -values (Table 4).

To allow a direct comparison, the activity coefficients obtained for calcium guluronates and mannuronates are presented graphically in Fig. 3. With an increasing degree of polymerization, the activity coefficients for the calcium guluronates decrease faster than those of the corresponding calcium mannuronates, particularly in the DP-region between 18 and 28. Above DP 30, the activity coefficients are virtually independent of the chain length and considerably lower for the calcium polyguluronates (0.083) than for calcium polymannuronates (0.281).

The calcium activity, and the corresponding activity coefficients, obtained with the samples of calcium pectate and calcium polymethacrylate are given in Table 5 with the previously published²⁰ figures for calcium oligo-galact-

Table 4. Activity of Ca^{2+} and activity coefficient $\gamma_{\text{Ca}^{2+}}$ in solutions of Ca mono-, oligo- and polyguluronates.

Sample	DP	[Ca] mmol/l	$\alpha_{\text{Ca}^{2+}}$ mmol/l	$\gamma_{\text{Ca}^{2+}}$
1	1 ^a	1.500	1.11 ± 0.01	0.74 ± 0.01
2	2	1.500	0.957 ± 0.002	0.638 ± 0.002
3	3	1.500	0.772 ± 0.004	0.515 ± 0.003
4	4	1.500	0.688 ± 0.014	0.459 ± 0.010
5	9.5	1.500	0.560 ± 0.005	0.373 ± 0.003
6	12.5	1.500	0.509 ± 0.001	0.339 ± 0.001
7	16.3	1.183	0.408	0.345
		1.182	0.398	0.337
		1.233	0.405	0.328
8	18.4	0.880	0.263	0.299
		0.704	0.222	0.315
		0.611	0.179	0.293
		0.705	0.198	0.281
9	28.4	0.606	0.066	0.109
		0.732	0.086	0.117
		0.804	0.069	0.086
10	81	0.720	0.064	0.089
		0.720	0.067	0.093
		0.729	0.072	0.099
		0.722	0.049	0.068
		0.683	0.045	0.066

^a Published previously.⁵

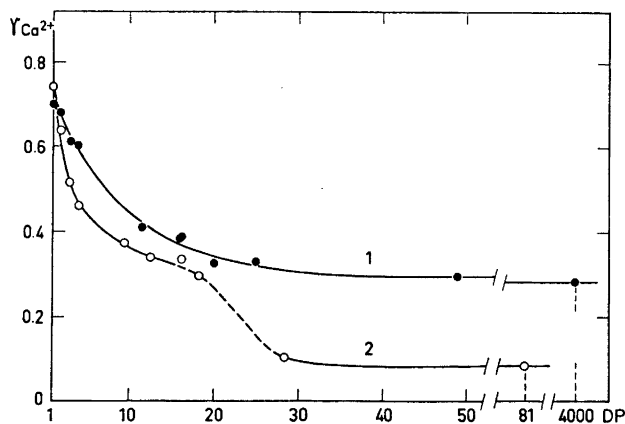


Fig. 3. Activity coefficient $\gamma_{\text{Ca}^{2+}}$ in solutions of calcium oligo- and polymannuronates and calcium oligo- and polyguluronates dependent on the degree of polymerization (DP). 1.... Calcium oligo- and polymannuronates. 2.... Calcium oligo- and polyguluronates.

Table 5. Activity of Ca^{2+} and activity coefficient $\gamma_{\text{Ca}^{2+}}$ in solutions of calcium oligogalacturonates, pectate, and polymethacrylates.

Sample	DP	[Ca] mmol/l	$a_{\text{Ca}^{2+}}$ mmol/l	$\gamma_{\text{Ca}^{2+}}$
Calcium oligogalacturonate ^a	1	1.500	1.095 ± 0.010	0.730
	2	1.500	0.972 ± 0.008	0.648
	3	1.500	0.834 ± 0.005	0.556
	4	1.500	0.737 ± 0.005	0.491
Calcium pectate	91	0.69	0.046	0.067
		0.61	0.046	0.075
		0.603	0.037	0.061
		0.699	0.039	0.056
		0.758	0.041	0.054
Calcium polymethacrylate	390	1.500	0.112 ± 0.006	0.075
	3200	1.500	0.099 ± 0.003	0.066

^a Published previously.²⁰

uronates included for comparison. Calcium pectate and polymethacrylate both have low activity coefficients, in the same range as those of calcium polyguluronates.

DISCUSSION

The most direct way to investigate the interaction between polyanion and counterions in polyelectrolyte solution is to study the activity of the counterions. The determination of Ca^{2+} -activities obviously requires a soluble calcium salt of the polyanion in question. Our observation that soluble polyuronic acids may be prepared by the ion-exchange technique and that these acids in turn gave soluble calcium salts by careful neutralisation with calcium hydroxide rendered this method open for use with oligo- and polyuronides, which hitherto have been considered to be insoluble as their calcium salts. Within the concentration range tested, oligo- and polymannuronates, and the polymethacrylates, all gave soluble calcium salts. The polyguluronates, and pectates, however, gave a partial precipitation during the conversion process. The amount of polyuronate precipitated depended both on the DP and the rate of neutralisation (Table 2). Owing to this limited solubility it was necessary to work at a lower concentration with guluronates and pectates, and only activity coefficients should be compared.

It is generally accepted that the concentration of the polyelectrolyte influences the activity coefficient of the counterion only to a very limited extent. The activity coefficient of sodium ions (γ_{Na^+}) in sodium heparinate solutions was nearly independent of the concentration within a rather wide range of concentrations.⁴⁵ The same also applies to sodium polyacrylate and other polyelectrolytes with a high linear charge density.⁴⁶ Nagasawa *et al.*³⁹

observed a slow decrease in γ_{Na^+} in solutions of sodium salts of some polyacids with decreasing concentration. On the basis of these findings it is reasonable to assume that the difference in concentration among the samples is not going to influence the value of the activity coefficient.

For calcium mannuronates there is a steady decrease in the calcium activity, and in the activity coefficient, with increasing DP up to a chain length of about 30 (Table 3, Fig. 2). Calcium guluronates, on the other hand, show a much more complicated dependence on molecular weight by giving a pronounced drop in the activity coefficient in the DP-region between 18 and 28. All oligomeric and polymeric fractions of DP higher than 4 are to a certain extent polydisperse as they are prepared as cut-outs from a molecular-weight distribution obtained by gel-permeation chromatography. Due to the fact that a partial precipitation occurs during neutralisation with calcium hydroxide, it is easy to suspect that the shape of the curve may have been influenced by a molecular weight fractionation taking place during the precipitation. For the sample with $\overline{\text{DP}}=18$, we demonstrated, however, that the $\overline{\text{DP}}$ was the same, within the experimental error, for both the soluble and the insoluble fraction. This rules out polydispersity as being responsible for the sharp decrease in the activity coefficient, and we can at present offer no explanation for this particular DP-dependence occurring with the polyguluronides.

The activity coefficients $\gamma_{\text{Ca}^{2+}}$ of the calcium salts of polyacids under investigation are collected in Table 6. In the solution of calcium polymann-

Table 6. Activity coefficient $\gamma_{\text{Ca}^{2+}}$ in solutions of calcium salts of some polyacids.

Polyacid	DP	Number of measurements	[Ca] mmol/l	$\gamma_{\text{Ca}^{2+}}$	Y Å
Polymannuronic	4000	3	1.50	0.281 ± 0.002	5.18
Polyguluronic	81	5	0.68–0.73	0.083 ± 0.007	4.36
Pectic	91	5	0.60–0.76	0.063 ± 0.004	4.35
Polymethacrylic	390	2	1.50	0.075 ± 0.004	2.5
	3200	2	1.50	0.066 ± 0.002	

Y = distance between two adjacent carboxyl groups in a perpendicular projection on the main axis of the macromolecule.

uronate, a relatively high activity coefficient ($\gamma_{\text{Ca}^{2+}} 0.281$) was found, indicating a weaker interaction of Ca^{2+} with carboxyl groups. The polymannuronate exerts only a low selectivity for Ca^{2+} in the ion-exchange reaction of $\text{Ca}^{2+} - \text{K}^+$,⁵ thus it is not surprising that this $\gamma_{\text{Ca}^{2+}}$ -value can be compared with the osmotically active fraction of monovalent counterions in solutions of sodium and potassium alginates, $\Phi_p 0.30 - 0.40$.⁴⁴ Compared to calcium polymannuronate, the activity coefficients $\gamma_{\text{Ca}^{2+}}$ in solutions of calcium polyguluronate and calcium pectate are several times lower ($\gamma_{\text{Ca}^{2+}} 0.083$ and 0.063 , respec-

tively). These findings are in agreement with the ion-exchange properties of these polyuronates. The polyguluronate and pectate exert a high selectivity for Ca^{2+} in ion-exchange reaction of $\text{Ca}^{2+} - \text{K}^+$.⁵ It should also be mentioned that the same tendency persists for the lower oligomers (DP 2–4) with very similar $\gamma_{\text{Ca}^{2+}}$ -values for guluronates and galacturonates and consistently higher values for the mannuronates.

The interaction between polyanion and counterions is influenced by the linear charge density of the macromolecule. The linear charge density is expressed by the distance between the perpendicular projection of adjacent charged groups on the main axis of the macromolecule. The higher the linear charge density, the stronger the interaction of counterions with anionic groups and the lower the activity coefficient of the counterions (see, *e. g.*, Ref. 39). Such a dependence of activity coefficient $\gamma_{\text{Ca}^{2+}}$ on the linear charge density was well documented for solutions of calcium pectinates with different degrees of esterification of carboxyl groups with methanol from 25 to 95 %.²¹

Table 6 shows the mean values of activity coefficients $\gamma_{\text{Ca}^{2+}}$ of calcium polyuronates and calcium polymethacrylate with the repeating distance of the corresponding macromolecules (Table 6), taken from X-ray data in the literature.^{40–43}

The conformation of sugar rings is supposed to be *C1* for polygalacturonic,^{41–43,48} and polymannuronic acids⁴⁰ and *1C* for polyguluronic acid.⁴⁰ It should be pointed out, however, that in the case of the two latter polyuronides, this assumption is based on X-ray data of the polyacids only. The activity coefficients $\gamma_{\text{Ca}^{2+}}$ of calcium polyguluronate and calcium pectate are clearly lower than expected on the basis of the linear charge densities, compared to the calcium polymethacrylate. This demonstrates that the linear charge density is not the only factor controlling the interaction between polyanion and counterions.

Katchalsky and co-workers⁴⁴ found that the osmotically active fraction Φ_p of calcium ions in oriented calcium alginate gels only reached 1 % of the total calcium concentration in the gel. Compared with the results presented here for polyuronates in solution, this indicates that calcium ions are bound much stronger in a gel than when the polyanion molecules are in solution. It is supposed that calcium ions in an alginate gel form bridges between the alginate chain molecules,⁴⁴ and the results therefore indicate that calcium ions bound in this way have a lower activity coefficient than calcium ions bound to isolated alginate molecules. According to Smidsrød and Haug,¹ the high selectivity of guluronic acid units of the alginate macromolecule in alkaline earth ion exchange reactions appears to be dependent upon the existence of the alginate in the insoluble, gel state. This will be discussed in more detail in a forthcoming publication.

The low activity coefficients $\gamma_{\text{Ca}^{2+}}$ found in solutions of calcium polyguluronate and pectate can be caused by three factors having an additive character: (1) The higher linear charge density of macromolecules of these polyuronates in comparison with that of calcium polymannuronate, (2) The intermolecular binding of Ca^{2+} with carboxyl groups of different chains in small soluble aggregates. (An intramolecular interaction of Ca^{2+} with distant carboxyl groups of the same macromolecule forming “loops” can also be considered, in

the sense used in findings of Cooper and Wassermann⁴⁷) (3) The distance between the carboxyl group and hydroxyl group, or groups, participating in the binding process.

Further work is in progress, and a more detailed discussion of these points will be published later.

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