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STRUCTURAL STUDY OF THE SOLUTIONS OF ACIDIC POLYSACCHARIDES. I. STUDY OF THE SOLUTIONS OF ACIDIC POLYSACCHARIDES BY MEASURING THE ACTIVITY COEFFICIENTS OF COUNTERIONS

V. P. YURYEV, I. G. PLASHCHINA, E. E. BRAUDO & V. B. TOLSTOGUZOV

A. N. Nesmeyanov Institute of Organoelement Compounds, USSR Academy of Sciences, Moscow, USSR

ABSTRACT

The activity coefficient of sodium ions in the presence of acidic polysaccharides (alginates, pectins and κ-carrageenan) has been measured potentiometrically. The activity determined in limit-dilute solutions normally exceeded the values calculated from the Manning theory. The difference ($\Delta\gamma_{Na^+}$) *was regarded as a measure of the chain flexibility. The change in* $\Delta\gamma_{Na^+}$ *when a polysaccharide is transferred from water to* 8 M *urea solution gave information about the contribution of hydrogen bonds to the stabilisation of the polysaccharide conformation in aqueous solutions. The function* n *is independent of polymer concentration at high concentration.* $n = 1 - (\gamma_{Na^+}^{exp})/(\gamma_{Na^+}^{NaCl})$ *where* $\gamma_{Na^+}^{exp}$ *is the experimentally determined value of the activity coefficient of the counterions and* $\gamma_{Na^+}^{NaCl}$ *is the activity coefficient of sodium ions in a NaCl solution of the same equivalent concentration as the polymer solution.*

This suggests that the polysaccharide solution has a microheterogeneous structure. The results of the viscosity measurements suggest that there is agreement between equilibrium thermodynamic and rheological evaluation criteria for the structure of polymer solutions.

INTRODUCTION

The activity coefficient of counterions in polyelectrolyte solutions depends on the chemical structure of the polyelectrolyte, its conformation, the nature of the polymer–polymer interaction and also on the structure of the solution.

This paper attempts a study of the structure of the solutions of sodium salts of some acidic polysaccharides (alginates of various chemical structures, pectins of

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various degrees of esterification and κ-carrageenan) by potentiometric measurement of the activity of sodium ions.

MATERIALS AND METHODS

Since the activity coefficient of counterions in acidic polysaccharide solutions depends strongly on the structural features of the polysaccharide and the presence of admixed ions, the following information must be available to interpret the experimental data reliably:

1. The structure of the polysaccharide (i.e. the mannuronate : guluronate ratio and block distribution in alginates; the content and composition of the nonuronide components in pectins and the ratio of the anhydrogalactose groups to sulphate groups in κ-carrageenan).
2. The content of admixed ions and/or the ash content of the acidic form of the polysaccharide.
3. The conductivity of the solvent (water).

The study was made on samples of sodium alginates (A1 and A2) manufactured by British Drug Houses (England) and Fluka (Switzerland) (A3), and also on κ-carrageenan (K1) manufactured by Sigma (USA). The methods of polysaccharide purification and analysis have been described by Yuryev *et al.* (1979), Berth *et al.* (1981) and Grinberg *et al.* (1980).

Pectic acid (P1) manufactured by Sigma (USA) and citrus pectin (P3) manufactured by Koch-Light (England) were also investigated. The preparations were purified by reprecipitation with acid isopropanol as described by Plashchina *et al.* (1978). The preparation P2 was obtained by saponification of P3 at pH 12 and 25°C, and the preparation P4 by methylation of P3 with diazomethane at −20°C as described by Vollmert (1950). The uronide content and degree of esterification were determined colorimetrically as described by Fili-pov & Vlasyeva (1973) and Fili-pov & Kuzminov (1971), respectively. The results of the characterisation of the polysaccharides are shown in Table 1a–c. The acidic forms of the alginates and pectins after purification contained no ash.

The conductivity of the water used for the preparation of the acidic polysaccharide solutions was 1×10^{-6} ohm^{-1} m^{-1}.

The polymer concentration is expressed as the number of g-equivalents of ionogenic groups per kg of solvent.

The sodium ion activity was determined with a sodium ion analyser OP-263 (Radelkis, Hungary) fitted with a micro-capillary sodium-selective electrode. The device was calibrated with standard solutions of NaCl before and after each activity measurement, the activity of the sodium ions (a_{Na^+}) being calculated by the equation

TABLE 1a–c
Characterisation of Polysaccharides

Table 1a

Alginate sample	*Content of blocks in the chain, %*[a]			*Content of mannuronic acid residues, %*		*Intrinsic viscosity, $cm^3 g^{-1}$, in 0·19 M NaCl + 0·01 M NaF at 20°C*[b]
	MG	*MM*	*GG*	*Calculated from block composition*	*Determined experimentally*	
A1	50	30	20	55·0	56·8	970
A2	37	33	30	51·5	52·6	555
A3	20	33	47	43·0	46·0	695

Table 1b

Pectinate sample	*Percentage galacturonic acid (moisture-free basis)*	*Degree of esterification, %*	*Intrinsic viscosity, $cm^3 g^{-1}$, in 0·09 M NaCl + 0·01 M NaF at 25°C*[b]
P1	88	0	–
P2	88[c]	15	197
P3	88[c]	58	294
P4	88[c]	78	295

Table 1c

κ-Carrageenan sample	*Galactose : 3,6-anyhydro-galactose : SO_4^{2+} ratio*	*Content of ions, %*		
		Na^+	*K^+*	*Ca^{2+}*
K1	1:0·95:1·02	5·82	0·136	–

[a] M = mannuronic acid residue, G = guluronic acid residue.
[b] Mean shear rate = 700–1200 s^{-1}.
[c] The content of neutral sugars in the pectins under study was as follows: rhamnose 3–7%, arabinose 4–6%, xylose less than 1% (Berth *et al.*, 1981).

$$a_{Na^+} = c_{Na^+} \frac{(\gamma\pm)^2_{NaCl}}{(\gamma\pm)_{KCl}} \qquad (1)$$

Implicit in this equation is the MacInnes assumption that $\gamma_{K^+} = \gamma_{Cl^-}$ in KCl.

The values of $(\gamma\pm)_{NaCl}$ and $(\gamma\pm)_{KCl}$, relating to the same concentrations of the two salts, were taken from the literature. The concentrations of the standard solutions covered the range of activities measured.

It is known that the adsorption of acidic polysaccharides can affect potentiometric studies carried out with glass electrodes (Streltsova *et al.*, 1975). In this case, however, the operating parameters of the device were kept constant during measurements.

The activity of the sodium ions was measured at 298·2 ± 0·1 K (25·0°C). The relative error in the measurement of the activity coefficient was 3%.

The viscosity of the sodium alginate solutions was measured by means of a rotational viscosimeter (VMV-03, manufactured by the Special Design Bureau of the A. V. Topchiyev Institute of Petrochemical Synthesis, USSR Academy of Sciences). The measurements were made in the shear rate range 0·5–7·0 s^{-1} and the results obtained were extrapolated to zero shear rate. However, the viscosity of all the solutions under study was practically independent of shear rate.

RESULTS AND DISCUSSION

The dependence of the activity coefficients of the sodium ions ($\gamma_{Na^+}^{exp}$) on the concentration of polysaccharide in aqueous solutions is shown in Figs 1 and 2. The curves are similar to those obtained in studies of aqueous solutions of sodium alginate (Podlas & Ander, 1970) and κ-carrageenan (Podlas & Ander, 1969). A comparison of our

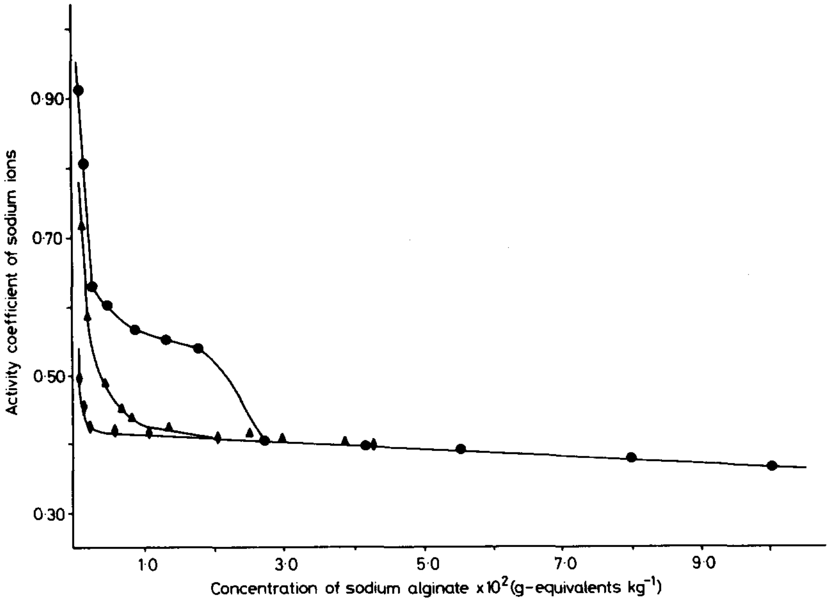

Fig. 1. Dependence of the activity coefficient of counterions (Na^+) on the sodium alginate concentration: –●– A1; –▲– A2; –◆– A3.

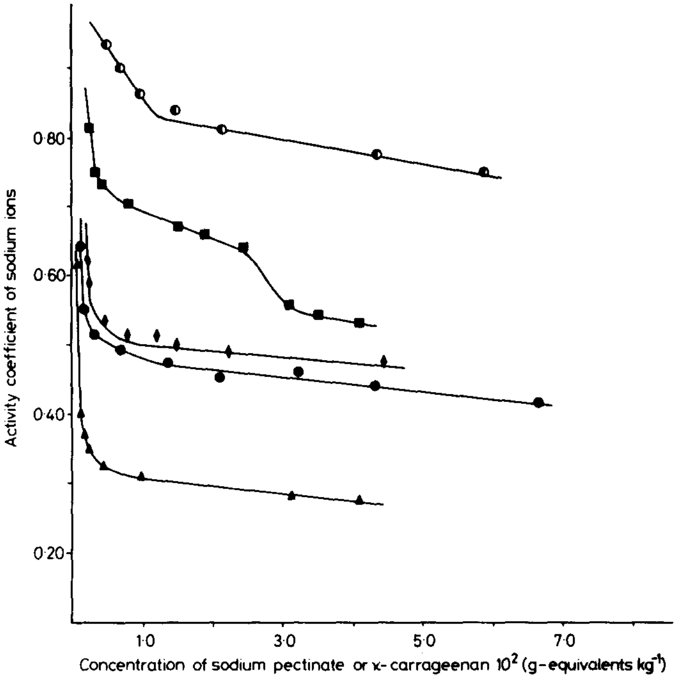

Fig. 2. Dependence of the activity coefficient of counterions (Na^+) on the pectin and κ-carrageenan concentration: –●– P1; –♦– P2; –■– P3; –▲– P4; –◐– K1.

values of $\gamma_{Na^+}^{exp}$ with the literature data can be made for κ-carrageenan. The values shown in Fig. 2 are significantly higher than those obtained by Podlas & Ander (1969), Pass *et al.* (1977) and Rochas & Rinaudo (1980). The reason for the differences is still obscure.

The polysaccharide concentrations studied covered the region of the so-called limit-dilute solutions for which the contribution of the polymer-polymer interaction to the thermodynamic functions of the counterions is negligible, and also the region of more concentrated solutions where the behaviour of the counterions depends on this interaction. The characteristics of these two concentration regions will be considered separately below.

Limit-dilute Solutions

The activity coefficients of counterions in the aqueous solutions of polysaccharides: The methods by which the activity coefficients of counterions in dilute solutions of linear polyelectrolytes are calculated generally involve approximation of the counterion-polyion interaction by the interaction of a counterion with a cylindrical segment not interacting with the other macromolecules and segments. The applicability of such a model is restricted to a definite region of intermediate polyelectrolyte concentrations (Manning, 1969, 1974). In extremely dilute solutions the interaction of the counterions with the functional groups belonging to different segments takes place, and above a certain concentration there is a contribution to the colligative properties of a solution from the polymer-polymer interaction.

Because of the rapid change in the activity coefficient with polyelectrolyte concentration in the low-concentration region (Figs 1 and 2), it is necessary to carefully select a polymer concentration (c) at which to interpret the data theoretically. In our work we have taken the concentration (c^*) to correspond to the point of departure from the linear dependence of the activity coefficient on concentration. The values of c^* are given in Table 2. The departure from linearity was assumed to be due to the appearance of the polymer-polymer interaction. Experimental support for such a view is given below.

The activity coefficient of the sodium ions in the limit-dilute solutions was calculated from equations proposed by Manning (1969):

$$\gamma_{\mathrm{Na}^+}^{\mathrm{theor}} = \frac{1}{\xi}\exp(-0{\cdot}5); \qquad \xi > 1 \tag{2a}$$

$$\gamma_{\mathrm{Na}^+}^{\mathrm{theor}} = \exp(-0{\cdot}5\,\xi); \qquad \xi < 1 \tag{2b}$$

where $\xi = q^2/DKTb$ is the charge density parameter in which q is the electron charge, D, the dielectric constant and b, the projection of the distance between the adjacent charged groups on to the macromolecule axis. For water at 298·2 K, $D = 78{\cdot}5$ and $\xi = 7{\cdot}135\,b^{-1}$ if b is expressed in Ångströms. For 8 M urea at the same temperature $D = 97$ (Wyman, 1933) and $\xi = 5{\cdot}779\,b^{-1}$.

It is obvious that the model of a stiff cylindrical macroion is best suited to the macromolecules whose segments have a regular helical conformation (Rees, 1973). The possibility of using Manning's equations to describe the colligative properties of helical macromolecules in salt-free solutions was established by Skolnik (1979).

The parameter b for polyelectrolytes with a helical conformation can be calculated by the equation

$$b = \frac{h}{n\alpha} \tag{3}$$

where h is the helix pitch, n is the order of the screw symmetry and α is the degree of ionisation. For sodium pectate and pectins with a degree of esterification $\leqslant 70\%$, $h = 13{\cdot}1$ Å and $n = 3$ (Palmer & Hartzog, 1945). For a single chain of κ-carrageenan $h = 24{\cdot}6$ Å and $n = 3$ (Anderson *et al.*, 1969), but taking into account the double stranded macromolecule structure, the value of the parameter b calculated from the above equation should be halved.

A more complex situation exists with sodium alginate whose macromolecules consist of blocks of polymannuronate (MM) and polyguluronate (GG) residues and regions where the two sugars alternate (MG) (Haug *et al.*, 1966). The mean value of

TABLE 2

Experimental and Theoretical Activity Coefficients of Sodium Ions in Limit-dilute Salt-free Polyelectrolyte Solutions

Polyelectrolyte index	b (Å)	*Water*					*8 M Urea*					$\Delta(\Delta\gamma_{Na^+})$
		ξ	*Concentration of polyelectrolyte* × 10^2 *(g-equivalents kg^{-1})*	$\gamma_{Na^+}^{theor}$	$\gamma_{Na^+}^{exp}$	$\Delta\gamma'_{Na^+}$	ξ	*Concentration of polyelectrolyte* × 10^2 *(g-equivalents kg^{-1})*	$\gamma_{Na^+}^{theor}$	$\gamma_{Na^+}^{exp}$	$\Delta\gamma''_{Na^+}$	
A1	4·74	1·505	0·25	0·40	0·65	0·25	1·219	0·35	0·50	0·70	0·20	−0·05
A2	4·71	1·515	0·20	0·40	0·57	0·17	1·227	0·53	0·50	0·85	0·35	0·18
A3	4·66	1·531	0·05	0·40	0·51	0·11	1·240	0·10	0·49	0·76	0·27	0·16
P1	4·37	1·633	0·12	0·37	0·58	0·21	1·322	0·14	0·46	0·84	0·38	0·17
P2[a]	5·14 4·37	1·388 1·633	0·17	0·44 0·37	0·59	0·15 0·22	1·124 1·322	0·19	0·54 0·46	0·84	0·30 0·38	0·15 0·16
P3[a]	10·4 4·37	0·686 1·633	0·30	0·71 0·37	0·73	0·02 0·36	0·555 1·322	1·65	0·76 0·46	0·97	0·21 0·51	0·19 0·15
P4[a]	19·9 4·37	0·359 1·633	0·05	0·84 0·37	0·38	−0·45 0·01	0·291 1·322	0·26	0·86 0·46	0·64	−0·22 0·18	0·24 0·15
K1	4·1	1·740	1·05	0·35	0·85	0·50	1·409	0·16	0·43	1·25	0·82	0·32

[a] The upper number refers to pectin with a statistical distribution of methoxyl groups, the lower number refers to a block-wise distribution.

b for sodium alginate macromolecules may be obtained from the formula

$$\bar{b} = \sum_i \varphi_i b_i \tag{4}$$

where φ_i is the content of blocks of a given type in the macromolecule, and b_i is the projection of the end-to-end vector of the block on to the 'axis' of the macromolecule.

For sodium polymannuronate $h = 15{\cdot}1$ Å and $n = 3$ (Mackie, 1971; Atkins, 1977) and $b_{MM} = 5{\cdot}03$ Å (pH $\gg$ pK) (Haug, 1961). For the polymannuronates of various metals $h = 8{\cdot}7$ Å, $n = 2$ (Mackie, 1971) and $b = 4{\cdot}35$ Å (pH $\gg$ pK) (Haug, 1961). The structure of the MG blocks being unknown, it was assumed in the calculation of the parameter b for sodium alginate that $b_{MG} = \frac{1}{2}(b_{MM} + b_{GG})$. Hence

$$b = \varphi_M b_{MM} + \varphi_G b_{GG} \tag{5}$$

where φ_M and φ_G are the general contents of the residues of mannuronic and guluronic acid, respectively, in the alginate macromolecule.

In the calculation of the parameter b for pectins a knowledge of the distribution pattern of the ester groups and hence the charged groups in the molecule is essential. The distribution pattern depends both on the structure of the initial pectin sample (P3) and the methylation conditions (Kohn & Furuda, 1969). Two limiting distribution patterns are possible, viz., statistical and block-wise. In the first case, at pH $\gg$ pK (Katchalsky *et al.*, 1954; Rinaudo & Milas, 1974), $b = h/(1 - E \times 10^{-2})n$ where E is the percentage degree of esterification. In the second case $b = 4{\cdot}37$ Å, irrespective of the degree of esterification E. As the charge distribution pattern of samples under study in unknown, the calculations were made with the values of b corresponding both to statistical and block-wise distributions.

The calculated results are shown in Table 2 together with the values of $\gamma_{Na^+}^{exp}$ for the limit-dilute solutions. In the case of pectins (samples P2–P4) the upper value corresponds to calculated data for the statistical charge distribution, and the lower one for the block-wise distribution.

It can be seen from Table 2 that normally $\gamma_{Na^+}^{exp} > \gamma_{Na^+}^{theor}$. The differences between these values ($\Delta\gamma'_{Na^+}$) can be regarded as a measure of the departure of the properties of the real molecule from the behaviour of the model underlying Manning's theory. It can be assumed that the value of $\Delta\gamma_{Na^+}$ characterises the degree to which the segment structure of the macromolecules of the polysaccharides under study deviates from the rigid cylinder model. Hence $\Delta\gamma'_{Na^+}$ is a measure of chain flexibility. (A similar approach was used by Katchalsky *et al.* (1966). When calculating the osmotic coefficient, they included deviations of the real polyelectrolyte structure from the completely extended chain by introducing an effective value for the parameter b.)

Deviations from a local cylindrical structure can be due, first, to the distortion of the regular chemical structure of the chain (Rees & Welsh, 1977) and, second, to the conformational fluctuations of those parts of the chain that have a regular structure, i.e. intramolecular mobility. In the case of alginates (A1-A3) and pectins (P2-P4) the values of $\Delta\gamma'_{Na^+}$ include also a possible error associated with the selection of the parameter *b*.

Figure 3 shows the dependence of $\Delta\gamma'_{Na^+}$ on the content of the MG blocks in the sodium alginate macromolecule φ_{MG}. It can be seen here that $\Delta\gamma'_{Na^+}$ depends linearly on φ_{MG} becoming zero at $\varphi_{MG} = 0$. (The extrapolation to zero of $\Delta\gamma'_{Na^+}$ at $\varphi_{MG} = 0$ is evidence for the absence of systematic errors in the measurements of $\gamma^{exp}_{Na^+}$.)

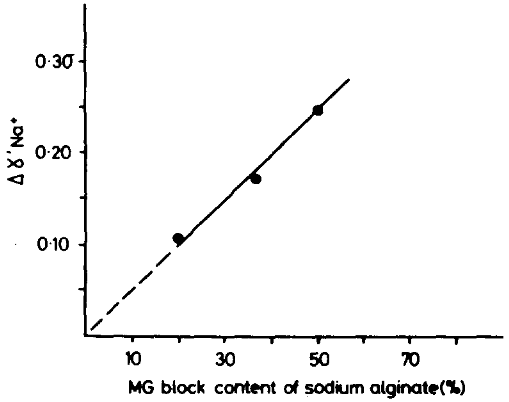

Fig. 3. Dependence of $\Delta\gamma'_{Na^+}$ on the content of the MG blocks in sodium alginate.

This means that possible deviations from the local cylindrical structure in the sodium alginate macromolecule are completely controlled by the presence of the MG blocks. This result is consistent with existing conceptions of the role of MG blocks as hinges responsible for the flexibility of the alginate macromolecule (Whittington, 1971). A hypothetical alginate molecule free of MG blocks would correspond to a cylindrical macroion model and thus have a limiting high stiffness. The studies of alginate solutions therefore support the assumption that $\Delta\gamma'_{Na^+}$ is controlled by deviations of the local macromolecule structure from a rigid cylinder model.

The values of $\Delta\gamma'_{Na^+}$ for pectins can be far more readily interpreted based on the assumption of a block-wise charge distribution rather than a statistical one. By adopting the former distribution pattern, we obtain the largest deviation from the local cylindrical structure (the largest flexibility) for P3 having the least regular chemical structure ($E = 58\%$). The methylation of this sample to sample P4 is accompanied by such a large increase in stiffness that the macromolecular structure can be approximated to a set of stiff cylindrical segments ($\Delta\gamma'_{Na^+} \approx 0$). The data obtained is consistent with the results of viscosimetric studies (Smidsrød & Haug, 1971). These authors, report that the pectin macromolecule chain flexibility increases somewhat as *E* increases from 0 to 58% but sharply falls as *E* increases further to 78% and then to 89%. Thus highly esterified pectins have a higher chain stiffness than those with a low degree of esterification. The chain stiffness passes through a minimum in the region $E = 50$–60%. If the charge distribution is statistical then the effect of degree of esterification on chain stiffness discussed above may still be valid if additional nonelectrostatic binding of the sodium ions to the ester groups of pectin macromolecules occurs. This will make a negative contribution to $\Delta\gamma'_{Na^+}$, which will increase with the degree of esterification. The possibility of alkali metal ions being bound due to chelation involving ester and carboxyl groups has been shown by Strauss (1974). It should be noted that the data obtained on the effect of the degree of pectin esterification on the activity coefficients of sodium ions conflicts with the results reported by Rinaudo & Milas (1974). According to these authors, the activity coefficients of sodium ions show a monotonic decrease as the degree of esterification increases from 55 to 90%.

Attention should also be drawn to the relatively high value of $\Delta\gamma'_{Na^+}$ for sodium pectate despite the fact that the content of the nonuronide residues, some of which act as defects or 'kinks' in the regular molecular backbone (Rees & Welsh, 1977), is lower than the content of MG blocks in the alginates A2 and A3 which have a smaller value of $\Delta\gamma'_{Na^+}$.

The largest deviation from the theoretical value of $\gamma_{Na^+}^{theor}$ is observed for κ-carrageenan. The high value of $\Delta\gamma'_{Na^+}$ is probably due to the fact that the real conformation of the sodium salt of κ-carrageenan differs radically from the two-stranded helix conformation used as the basis for the calculation of $\gamma_{Na^+}^{theor}$. It is suggested by Anderson *et al.* (1969) that all salts of κ-carrageenan with univalent cations have the same conformation. On the other hand Grinberg *et al.* (1980) have reported that the sodium salt of κ-carrageenan in the absence of low molecular weight electrolytes does not show any conformational changes as the solution is being cooled down from 70 to 30°C and, probably, has an unordered conformation at room temperature. According to Vreeman *et al.* (1980) the sodium salt of κ-carrageenan in 0·1 M NaCl and 0·005 M EDTA at 20°C has a random coil conformation. (Some authors (Rochas & Rinaudo, 1980; Ooi *et al.*, 1980) compare the values of γ^{exp} to γ^{theor}, the latter being calculated for the random coil conformation of the polyelectrolyte using Manning's theory. We cannot accept the applicability of the Manning theory to random coil polyelectrolytes.)

Within the same series of polysaccharides the macromolecule flexibility parameter $\Delta\gamma'_{Na^+}$ increases with the polymer concentration c^* (Table 2) in accord with the applicability criteria of Manning's theory. The dependence of c^* on the chain flexibility parameter shows that the increase in γ'_{Na^+} as $c \rightarrow 0$ is due to changes in polyion interaction with counterions associated with the decrease in ionic strength of the solution and is not caused by an increase in the contribution to the activity of admixed ions present in the solvent (water).

Comparison of counterion activity coefficients in water and 8 M urea solution: The helix conformations of the acidic polysaccharides under study are probably stabilised by intra- and interchain hydrogen bonds. Thus, as reported by Atkins *et al.* (1973), each unit of the polymannuronic acid chain contains three hydrogen bonds. The conformation of polyguluronic acid is possibly stabilised by an intramolecular hydrogen bond O(2)H . . . O(6)D and intermolecular hydrogen bonds are formed with the participation of water molecules (Atkins *et al.*, 1973). The intermolecular forces are assumed to contribute to the pectic acid structure (Sathyanarayana & Rao, 1973).

To find out to what extent hydrogen bonds affect the stiffness of acidic polysaccharide molecules, consideration was given to how the value of $\gamma_{\mathrm{Na}^+}^{\mathrm{exp}}$ deviated from that predicted for a cylindrical model as a polyelectrolyte is transferred from water to an 8 M urea solution. This deviation was expressed as the quantity (see Table 2, p. 145 and I of 'Notes Added in Proof', p. 156)

$$\Delta(\Delta\gamma_{\mathrm{Na}^+}) = \Delta\gamma'_{\mathrm{Na}^+} - \Delta\gamma''_{\mathrm{Na}^+}$$

The ratio obtained experimentally for the activity coefficient of the sodium ions for solutions of NaCl in 8 M urea to solutions in water, is equal to the ratio between the dielectric constants of the two solvents. This means that urea concentrated to 8 M produces no specific effect on the activity of the sodium ions or the instrument reading. In other words, the observed variations of $\Delta\gamma_{\mathrm{Na}^+}$ as polysaccharides are transferred from water to 8 M urea are associated with a changing contribution of the polyion to the colligative properties of the solution.

The increase in the activity coefficient of the counterions is in the order: the model macroion for the helix conformation < the real polyelectrolyte in aqueous solution (the skeletal stiffness + contribution of the electrostatic interactions + contribution of the hydrogen bonds) < the real polyelectrolyte in 8 M urea (skeletal stiffness + contribution of the electrostatic interactions) and is governed by increases in the mean distance between the charged groups when passing from the helical conformation to the less ordered states.

It can be seen from Table 2 that the largest contribution of hydrogen bonds to the macromolecule stiffness occurs for κ-carrageenan despite its probable 'random coil' conformation. For sodium alginate with a 50% content of MG blocks (A1) the hydrogen bonds do not contribute to the macromolecule stiffness whereas in the samples A2 and A3 (a 37% and a 20% content of the MG blocks, respectively) this contribution is the same despite the different chain stiffnesses. The different nature of the effect of hydrogen bonds on the conformation of sample A1, compared with A2 and A3, may suggest a cooperative stabilisation mechanism of the alginate helix by hydrogen bonds. This problem, however, should be studied in more detail. It is probable that the values of $\Delta(\Delta\gamma_{\mathrm{Na}^+})$ reflect differences not only in the block composition of the samples but in the distribution of the individual blocks along the chain.

In 8 M urea the chain flexibility characterised by the parameter $\Delta\gamma''_{\mathrm{Na}^+}$ is higher for the samples A2 and A3 than for A1, despite a higher content of MG blocks in the latter. This also points to a substantial contribution of the hydrogen bonds to the stabilisation of the ordered structure of the homopolymer alginate macromolecule fragments.

Hydrogen bonds contribute equally to the stiffness of the alginate macromolecules (A1 and A3) and pectins. For the latter the degree of esterification has practically no affect on $\Delta(\Delta\gamma_{Na^+})$.

As distinct from aqueous solutions, no correlation of $\Delta\gamma''_{Na^+}$ with c^* is observed for polysaccharide solutions in 8 M urea.

Concentrated Solutions

Figures 4 and 5 show the dependence of the activity coefficient of the sodium ions on the polyelectrolyte concentration in salt-free solutions. The function

$$n = \left(1 - \frac{\gamma^{\mathrm{exp}}_{\mathrm{Na}^+}}{\gamma^{\mathrm{NaCl}}_{\mathrm{Na}^+}}\right)$$

where $\gamma^{\mathrm{NaCl}}_{\mathrm{Na}^+}$ is the activity coefficient of the sodium ions in the NaCl solution of the same equivalent concentration as the solution under study, characterises the contribution of the polymeric nature of the polyelectrolyte to the activity coefficients of the counterions. It is readily apparent that the function n becomes zero when $\gamma^{\mathrm{exp}}_{\mathrm{Na}^+} = \gamma^{\mathrm{NaCl}}_{\mathrm{Na}^+}$ (in the absence of a polymer effect) and assumes a maximum value equal to unity at $\gamma^{\mathrm{exp}}_{\mathrm{Na}^+} = 0$.

As can be seen from Figs 4 and 5 for the aqueous solutions the function n increases rapidly at low polymer concentrations to reach a constant value. The change in the nature of the concentration dependence of this function corresponds to the transition from a dilute to a concentrated solution.

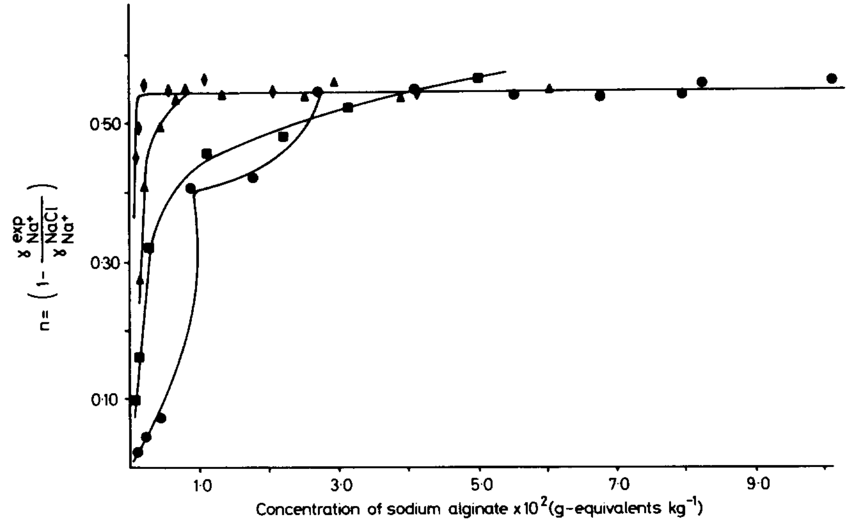

Fig. 4. Dependence of the function n on the sodium alginate concentration: —●— A1; —▲— A2; —◆— A3; —■— A1 in 8 M urea solution.

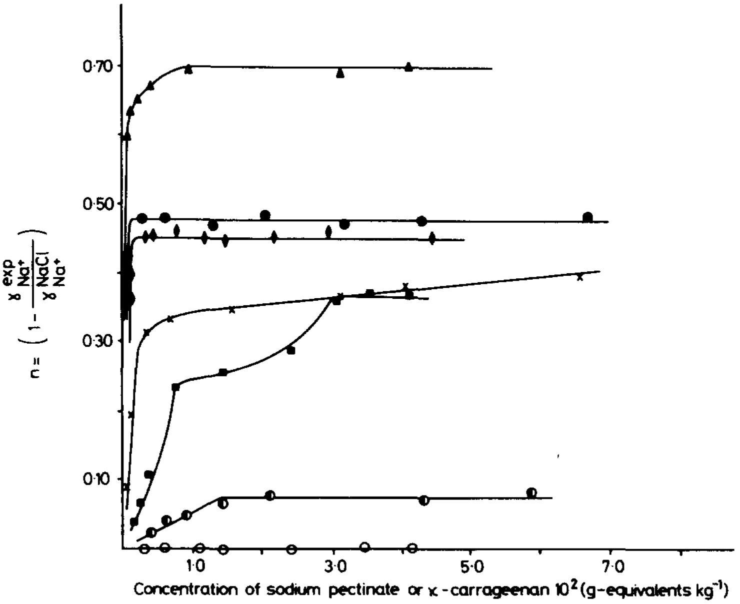

Fig. 5. Dependence of the function *n* on the pectin and κ-carrageenan concentration: —●— P1; —♦— P2; —■— P3; —▲— P4; —◐— K1; —×— P1 in 8 M urea solution; —○— K1 in 8 M urea solution.

Using the function *n* it will be possible to define the chemical counterion potential in a salt-free solution as follows:

$$\mu_2 = (\mu_2^0)' + \mathrm{RT} \ln X_2' \qquad (6)$$

$$(\mu_2^0)' = \mu_2^0 + \mathrm{RT} \ln \gamma_{\mathrm{Na}^+}^{\mathrm{NaCl}} \qquad (6a)$$

$$X_2' = (1 - n) X_2 \qquad (6b)$$

where X_2 is the molar counterion fraction.

The parameter X_2' defines formally the fraction of 'free' counterions, i.e. such ions whose activity coefficient is equal to that of the sodium ions in a NaCl solution of the same equivalent concentration, the activity coefficient of the 'bound' counterions being taken as zero. 'The standard state' at each point is found by taking into account the activity of the sodium ions in the NaCl solution of the corresponding concentration.

When *n* = constant, eqn (6) describes the chemical potential of the counterions in a pseudo-ideal polyelectrolyte solution with a reduced standard state, as shown above.

The value of the function *n* in concentrated solutions is the same for all the sodium alginate samples under study and is approximately the same for pectic acid. However, this function depends strongly on the degree of pectin esterification in that the polymeric contribution to the activity coefficient of the counterions increases with the chain stiffness. In the case of a block-wise charge distribution, n depends linearly on $\Delta\gamma'_{Na^+}$. It is possible that the function n is governed partially by the non-electrostatic counterion-macroion interactions which may occur at high degrees of pectin esterification.

In the case of κ-carrageenan the polymeric contribution to the activity of the counterions is far smaller than for alginates and pectins; this is probably due to the relatively low charge density of κ-carrageenan in the non-ordered conformation.

The constancy of n implies that the immediate environment of the counterions is independent of the polymer concentration in solution. This may imply that the structure of the concentrated solutions of acidic polysaccharides in water is microheterogeneous. The addition of a polymer to such a solution increases the number of structural elements (associates) without any change in their microstructure, particularly the concentration of a species in a structural element. The idea of a microheterogeneous structure of the solutions of the acidic polysaccharides under study suggests that water is a poor solvent for these biopolymers.

The results of the light-scattering studies of solutions prepared from pectins with degrees of esterification in the range of 5–100% also suggest extensive macromolecular association at polymer concentrations greater than 10^{-3} g-equivalents kg^{-1}, the solution being characterised by the negative values of the second virial coefficient (Berth *et al.*, 1981).

The concentration dependence of γ_{Na^+} and hence n, for the acidic polysaccharides studied, is different from the sodium salts of flexible-chain polymers (Katchalsky *et al.*, 1966; Chu & Marinsky, 1967; Ise & Okubo, 1965, 1966, 1967, 1968; Asai *et al.*, 1969; Boyd, 1974; Dolar, 1974; Gekko & Noguchi, 1975). For these species the osmotic coefficient (ϕ_p) is either independent of the polymer concentration or increases with it. In both cases the function n increases with the polymer concentration. (See **II** of 'Notes Added in Proof', p. 156.)

A similar type of dependence of the function n on concentration is observed for solutions of the alginate A1 and the pectate P1 in 8 M urea (Figs 4 and 5). This may suggest a more homogeneous structure in solutions of these polysaccharides in 8 M urea as compared with their aqueous solutions. In the cases of κ-carrageenan solutions in 8 M urea no polymeric contribution to the activity of the counterions is observed ($n = 0$).

The low solvent affinity of a polymer is expected to favour the formation of a fluctuating entanglement network in solution. This is reflected, particularly, by a sharply increasing dependence of the solution viscosity on polymer concentration (Vinogradov & Malkin, 1977). The concentration plot for the viscosity of the aqueous sodium alginate solution (A1) (Fig. 6) covers three concentration regions. At $c < 0{\cdot}2 \times 10^{-2}$ g-equivalents kg^{-1} η is linearly related to c, which is typical of dilute solutions. The maximum concentration, still corresponding to the region of dilute solutions, is close to the concentration c^*. This provides support for the criterion by which c^* has been selected in the present work.

In the region of concentrated solutions $\eta \propto c^{2{\cdot}3}$. The value of the critical concentra-

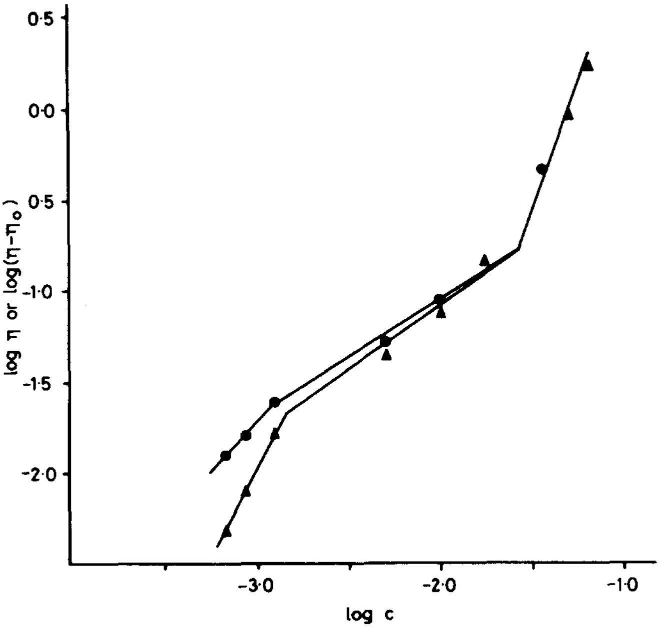

Fig. 6. Dependence of the initial viscosity of the sodium alginate solution (A1) on the polyelectrolyte concentration: —●— $\eta = f(c)$; —▲— $(\eta - \eta_0) = f(c)$.

tion at which one can observe the appearance of the fluctuating entanglement network ($2{\cdot}37 \times 10^{-2}$ g-equivalents kg^{-1}) coincides virtually with the value of c^{**} at which the function n starts to reach a constant value (Fig. 4). Here, as in the case of the dilute solutions, there is agreement between the equilibrium thermodynamic and rheological evaluation criteria for the solution structure.

In the intermediate concentration region ($0{\cdot}2 \times 10^{-2}$ g-equivalents kg^{-1} $< c <$ $2{\cdot}37 \times 10^{-2}$ g-equivalents kg^{-1}) the relationship $\eta \propto c^{0{\cdot}7}$ is approximately true. The diminishing contribution of the alginate macromolecules to the solution viscosity as compared with the dilute solution is likely to be due to the compression of the macromolecular coils as θ-conditions are approached with increasing polymer concentration (Rafikov *et al.*, 1978). A further concentration increase leads to coil overlap which is accompanied by a rapid increase in viscosity.

The intermediate region on the curve describing the dependence of viscosity on alginate concentration (Fig. 6) is matched with a section in the concentration dependence plot for the function n, where the latter changes nonlinearly (Fig. 4). For the alginates with a higher chain stiffness the intermediate region between the dilute and concentrated solutions is less well defined. A similar picture is observed for pectins (Fig. 5). A clear-cut intermediate region between the dilute and concentrated regions is observed for the pectin P3 only, which probably has the largest chain flexibility.

For polysaccharides in the same series the polymer concentration at which the transition to concentrated solution behaviour is observed (c^{**}) generally decreases, as in the case of c^*, with increasing chain stiffness. Thus for alginates c^{**} decreases as the content of the MG blocks becomes smaller (Fig. 4). In the pectin series the dependence of c^{**} on the chain stiffness holds for the samples P1-P3 only.

Thus dependence of the activity of the counterion on the concentration of acidic polysaccharide in solution reflects variations in the type of polymer-polymer interaction. The similar behaviour of salt-free solutions of poly(S-carboxy-methyl-L-cysteine) is explained by Ooi *et al.* (1980) in terms of the stacking of polypeptide pleated sheets (β-structure), i.e. also by polymer-polymer interactions. The thermodynamic properties of concentrated aqueous solutions of acidic polysaccharides are consistent with the current views regarding their microheterogeneous structure.

CONCLUSIONS

1. Correlation of the experimentally found activity coefficients of the sodium ions in the solutions of acidic polysaccharides with the values calculated from the Manning theory enable an estimate of the relative polysaccharide chain stiffness to be made.
2. The dependence of the activity coefficient of the counterions on the acidic polysaccharide concentration in solution reflects variations in the type of polymer-polymer interaction.
3. The thermodynamic properties of the concentrated aqueous solutions of acidic polysaccharides are consistent with current views regarding their microheterogeneous structure.

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REFERENCES

Anderson, N. S., Campbell, J. W., Harding, M. M., Rees, D. A. & Samuel, W. B. (1969). *J. Mol. Biol.* 45, 85.

Asai, K., Fakuya, K. & Ise, N. (1969). *J. Phys. Chem.* 73, 4071.

Atkins, E. D. T., Nieduszynski, J. A., Mackie, W., Parker, K. D. & Smolko, E. E. (1973). *Biopolym.* 12, 1865, 1879.

Atkins, E. D. T. (1977). *Pure Appl. Chem.* 49, 1135.

Berth, G., Anger, H., Plashchina, I. G., Braudo, E. E. & Tolstoguzov, V. B. (1981). Submitted to *Carbohydr. Polymers.*

Boyd, G. E. (1974). In *Polyelectrolytes*, ed. E. Sélégny. Dordrecht-Boston, Reidel Publ. Co., p. 135.
Chu, P. & Marinsky, J. A. (1967). *J. Phys. Chem.* **71**, 4352.
Dolar, D. (1974). In *Polyelectrolytes*, ed. E. Sélégny. Dordrecht-Boston, Reidel Publ. Co., p. 97.
Fili-pov, M. P. & Kuzminov, V. I. (1971). *Zh. Analit. Khim.* **26**, 143.
Fili-pov, M. P. & Vlasyeva, T. V. (1973). *Prikl. Biokhim. i Mikrobiol.* **9**, 134.
Gekko, K. & Noguchi, H. (1975). *Biopolym.* **14**, 2555.
Grinberg, N. V., Plashchina, I. G., Braudo, E. E. & Tolstoguzov, V. B. (1980). *Colloid. and Polymer. Sci.* **258**, 1098.
Haug, A. (1961). *Acta Chem. Scand.* **15**, 950.
Haug, A., Larsen, B. & Smidsrød, O. (1966). *ibid.* **20**, 183.
Ise, N. & Okubo, T. (1965). *J. Phys. Chem.* **69**, 4102.
Ise, N. & Okubo, T. (1966). *ibid.* **70**, 1930.
Ise, N. & Okubo, T. (1967). *ibid.* **71**, 1287.
Ise, N. & Okubo, T. (1968). *ibid.* **72**, 1361, 1366, 1370.
Katchalsky, A., Shavit, N. & Eisenberg, H. (1954). *J. Polym. Sci.* **13**, 69.
Katchalsky, A., Alexandrowicz, Z. & Kedem, O. (1966). In *Chemical physics of ionic solutions*, eds. B. E. Conway and R. G. Barrads. New York, Wiley, p. 295.
Kohn, R. & Furuda, J. (1969). *Coll. Czech. Chem. Commun.* **34**, 641.
Mackie, W. (1971). *Biochem. J.* **125**, 89.
Mackie, W., Noy, R. & Sellen, D. B. (1980). *Biopolym.* **19**, 1839.
Manning, G. S. (1969). *J. Chem. Phys.* **51**, 924.
Manning, G. S. (1974). In *Polyelectrolytes*, ed. E. Sélégny. Dordrecht-Boston, Reidel Publ. Co., p. 9.
Ooi, K., Maeda, H. & Ikeda, Sh. (1980). *Biopolym.* **19**, 1743.
Palmer, K. J. & Hartzog, B. M. (1945). *J. Amer. Chem. Soc.* **67**, 2122.
Pass, G., Phillips, G. O. & Wedlock, D. J. (1977). *Macromol.* **10**, 197.
Plashchina, I. G., Braudo, E. E. & Tolstogusov, V. B. (1978). *Carbohydr. Res.* **60**, 1.
Podlas, T. J. & Ander, P. (1969). *Macromol.* **2**, 432.
Podlas, T. J. & Ander, P. (1970). *ibid.* **3**, 154.
Rafikov, S. R., Budtov, V. P. & Manakov, Yu. B. (1978). *Introduction to the physico-chemistry of polymer solutions*. Moscow, Nauka, p. 186. (In Russian.)
Rees, D. A. (1973). In *M.T.P. international review of science. Organic chemistry, series one, carbohydrates*, Vol. 7. London, Butterworths, p. 251.
Rees, D. A. & Welsh, E. J. (1977). *Ang. Chem. Intern. Ed. Engl.* **16**, 214.
Rinaudo, M. & Milas, M. (1974). *J. Polym. Sci., Polym. Chem. Ed.* **12**, 2073.
Rinaudo, M., Kariman, A. & Milas, M. (1979). *Biopolym.* **18**, 1673.
Rochas, C. & Rinaudo, M. (1980). *Biopolym.* **19**, 1675.
Sathyanarayana, B. K. & Rao, V. S. R. (1973). *Curr. Sci.* **42**, 775.
Skolnik, J. (1979). *Macromol.* **12**, 515.
Smidsrød, O. & Haug, A. (1971). *Biopolym.* **10**, 1213.
Strauss, U. P. (1974). In *Polyelectrolytes*, ed. E. Sélégny, Dordrecht-Boston, Reidel Publ. Co., p. 79.
Streltsova, Z. A., Braudo, E. E. & Tolstoguzov, V. B. (1975). *Bioorganich. Khim.* **1**, 267.
Vinogradov, G. V. & Malkin, A. Ya. (1977). *Polymer rheology.* Moscow, Khimiya, p. 210. (In Russian.)
Vollmert, B. (1950). *Macromol. Chem.* **5**, 101.
Vreeman, H. J., Snoeren, T. H. M. & Payens, T. A. J. (1980). *Biopolym.* **19**, 1357.
Whittington, S. G. (1971). *ibid.* **10**, 1481.
Wyman, J. R. (1933). *J. Amer. Chem. Soc.* **55**, 4146.
Yuryev, V. P., Grinberg, N. V., Braudo, E. E. & Tolstoguzov, V. B. (1979). *Starch/Stärke.* **31**, 121.

NOTES ADDED IN PROOF

I. It is to be pointed out that a possible absolute error resulting from an inaccurate selection of the parameter b in the calculation of $\gamma_{\mathrm{Na}^+}^{\mathrm{theor}}$ decreases, on passing from $\Delta\gamma'_{\mathrm{Na}^+}$ to $\Delta(\Delta\gamma_{\mathrm{Na}^+})$, by $D_{\mathrm{i}}/(D_{\mathrm{j}} - D_{\mathrm{i}}) = 4{\cdot}2$ times (i = water, j = 8 M urea solution). If the effective value of the parameter b differs from the real value (b_0) by σ, then at $\xi > 1$ (see eqn (2a)) $\Delta\gamma_{\mathrm{Na}^+} = \gamma_{\mathrm{Na}^+}^{\mathrm{exp}} - cD(b_0 + \sigma)$. For the i-th and j-th solvents $\Delta(\Delta\gamma_{\mathrm{Na}^+}) = (\gamma_{\mathrm{Na}^+}^{\mathrm{exp}})_{\mathrm{i}} - (\gamma_{\mathrm{Na}^+}^{\mathrm{exp}})_{\mathrm{j}} - c(D_{\mathrm{i}} - D_{\mathrm{j}}) . (b_0 + \sigma)$. Consequently, the ratio between the absolute errors in the determination of $(\Delta\gamma_{\mathrm{Na}^+})_{\mathrm{i}}$ and $(\Delta\gamma_{\mathrm{Na}^+})$ is $D_{\mathrm{i}}/(D_{\mathrm{j}} - D_{\mathrm{i}})$.

II. According to Manning (1969) and Sholnik (1979), in salt-free solutions $\phi_{\mathrm{p}} = 1 + \log \gamma_1^{\mathrm{p}}$ at $\xi < 1$ and $\phi_{\mathrm{p}} = 1/2\ \mathrm{e}^{-1/2} \gamma_1^{\mathrm{p}}$ at $\xi > 1$ (where γ_1^{p} is the coefficient of the counterion activity).