

Experimental Evidence of Counterion Affinity in Alginates: The Case of Nongelling Ion Mg^{2+}

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The present contribution aims at testing experimentally the theoretical model previously devised (Donati, I.; Cesàro, A.; Paoletti, S.; *Biomacromolecules* **2006**, 7, 281–287) for the description of the interaction between alginate and nongelling Mg^{2+} ions. The model, based on an extension of the counterion condensation theory, introduces a contribution of free energy of affinity, $\Delta G^{\text{aff},0}$, which depends on the monomer composition of the polyuronate. In the present work, three different alginates separately mimicking the mannuronan (polyM), the guluronan (polyG), and the polyalternating (polyMG) components of alginate, together with a natural alginate isolated from *Laminaria hyperborea* (*L. hyperborea*), were examined. They were treated with Mg^{2+} ions, and relative variations in scattered light intensity, isothermal calorimetry (ΔH^{mix}), specific viscosity, and ^{23}Na NMR longitudinal relaxation rates were monitored with respect to samples at the same ionic strength but containing only Na^+ ions. The fraction of condensed magnesium counterions was found to be strongly dependent on alginate composition, increasing along the series mannuronan < polyalternating \sim *L. hyperborea* < guluronan, thus in good agreement with the theoretical predictions.

Introduction

Long-range electrostatic interactions profoundly affect the structure, solubility, and stability of polyelectrolytes. The concentration and valence of the small ions in solution determine the overall conformation of a polyelectrolyte and, under certain circumstances, can induce its large scale aggregation (e.g., hydrogel formation). Conformation of DNA was shown to strongly depend both on the counterion species and on its valence, e.g., including supercoiling around globular octamers of histone proteins.^{1–3} As to the effect of small ions, a marked ion selectivity was reported for DNA;⁴ the competition between Na^+ and Mg^{2+} ions for the polynucleotide was experimentally demonstrated and theoretically tackled by means of a Poisson–Boltzmann approach.⁵ Recently, sequence-specific binding of monovalent counterions in the minor groove of AT tract was demonstrated by several authors.^{6–8}

Alginate is a collective term for a family of algal polysaccharides composed of 1–4 linked β -D-mannuronic (M) acid and α -L-guluronic (G) acid arranged in a blockwise pattern with homopolymeric regions of G residues (G-blocks, guluronan—or polyG—sequences) and M residues (M-blocks, mannuronan—or polyM—sequences) interspersed by regions of strictly alternating sequence (MG-blocks, polyalternating—or polyMG—sequences). This polysaccharide is very well-known for its selectivity toward different divalent cations. For example, Ca^{2+} (as well as Ba^{2+}) induces instantaneous hydrogel formation, whereas Mg^{2+} does not. The strong and very fast interaction between alginate and the former ions has been largely exploited for biotechnological applications. In fact, encapsulation of several different cells types and tissues, among which Langerhans islets represent the most well-known example, has been exploited in research and

preclinical studies.^{9–12} A marked compositional dependence of the ion binding of alginate has been reported since the late 1970s, as it was recognized that G-blocks do possess structural features allowing the strong coordination of calcium ions.^{13–15} More recently, this overall picture has been extended owing to the availability of compositionally homogeneous alginates prepared by an enzymatic approach.^{16,17}

With respect to the great amount of work performed on Ca^{2+} (and to a lower extent on Ba^{2+}), relatively little attention has been devoted to the behavior of this polysaccharide in the presence of Mg^{2+} . Although a limited amount of experimental data on Mg^{2+} –alginate are available, the lack of strong polymer–ion interactions and chain–chain associations have been unquestionably demonstrated.¹⁸ Therefore, despite the less appealing industrial and biotechnological application of such system, Mg^{2+} ion can represent the best candidate to test the applicability of the counterion condensation (CC) theory, as originally developed by Manning.¹⁹ The latter approach, although derived in the limit of zero salt concentration, has proved to describe fairly well the experimental behavior of biopolyelectrolytes^{20–23} and in particular of ionic polysaccharides.^{24–27} The case of counterion systems of mixed valence has been consistently tackled²⁸ in the framework of the so-called “chemical model” of the CC theory developed by Manning himself.^{22,29} Recently, extensions of Manning theory which take into account finite salt concentration have been proposed.^{30,31}

Some of us have previously dealt with the description of the interaction between Mg^{2+} ions and alginates of different compositions on the basis of heat-of-mixing (microcalorimetric) data and by means of an extension of the CC theory to include a specific affinity between the divalent ion and the polyelectrolyte(s).³² However, it is important to stress that such affinity does not imply an inner-sphere “bonding” of the ion; on the contrary, the condensed counterions are still considered as free to move within the condensation volume (“territorial binding”).

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TABLE 1: Chemical Composition and Intrinsic Viscosity of Mannuronan (PolyM), Polyalternating (PolyMG), Guluronan (PolyG), and Alginate from *L. hyperborea*^a

sample	F_G	F_M	F_{GG}	$F_{GM,MG}$	F_{MM}	$[\eta]$ (dL/g) ^b
mannuronan	0	1	0	0	1	9.25 ± 0.03
polyalternating	0.45	0.55	0	0.45	0.1	6.95 ± 0.02
guluronan	0.84	0.16	0.77	0.07	0.09	9.86 ± 0.02
<i>L. hyperborea</i>	0.65	0.35	0.53	0.12	0.23	6.41 ± 0.02

^a F_G denotes the fraction of alginate consisting of guluronic acid. F_{GG} indicates the fraction of alginate consisting of guluronic acid in blocks of dimers, whereas F_{MM} indicates the fraction of alginate consisting of mannuronic diads. $F_{GM,MG}$ indicates the fraction of alginate consisting of mixed sequences of guluronic and mannuronic acid. ^b Solvent: NaCl 0.1M, 20 °C.

In fact, Mg^{2+} ions can be described as diffusely bound counterions, in contrast with the strongly site-bound ones (e.g., Ca^{2+}).^{33,34}

This work aims at presenting novel experimental data which, interpreted by means of the theoretical model based on the free energy of counterion affinity for magnesium ion, provide more information on the role played by the free energy of affinity for magnesium and its dependence on alginate composition. In particular, results collected from viscometry and ²³Na NMR relaxation measurements have been compared with theoretical predictions calculated by means of the above-reported adaptation of the CC theory.³²

Materials and Methods

Sodium alginate isolated from *Laminaria hyperborea* stipe (*L. hyperborea*) was provided by FMC Biopolymer (Norway). Mannuronan (polyM, fraction of guluronic residues, F_G , <0.001) was isolated from an epimerase-negative mutant (AlgG⁻) of *Pseudomonas fluorescens*.³⁵ Guluronan (polyG) and polyalternating (polyMG) have been prepared from mannuronan using the epimerases AlgE6 and AlgE4, respectively, following the conditions reported elsewhere.³⁶ The main compositional features of the polymers used in the present study are summarized in Table 1, and their structures are reported in Scheme 1. NaClO₄, Mg(ClO₄)₂ were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Light Scattering. A Perkin-Elmer LS50B spectrofluorimeter was used to record the intensity of the light ($\lambda = 500$ nm) scattered at 90° by a 3.5×10^{-3} monomol/L ($= C_p \equiv [\text{polym}]_{\text{rep,unit}}$) solution of alginate from *L. hyperborea* in aqueous 0.05 M NaClO₄ upon irradiation with an incident light at 500 nm. Ten intensity values corrected for background were averaged for each data point. A constant temperature of 25 °C was used throughout the experiments. The Z-average size for alginate prior and after treatment with Mg^{2+} ions was evaluated by using a Zetasizer Nano ZS system equipped with a 4 mW He–Ne laser (633 nm) (Malvern Instruments, Inc., Southborough, MA).

Viscosity Measurements. Reduced capillary viscosity was measured at 25 °C by means of a Schott-Geräte AVS/G automatic measuring apparatus and an Ubbelohde type viscometer upon addition of Mg(ClO₄)₂ at different values of the $[Mg^{2+}]/C_p$ ($R_{Mg^{2+}}$) ratio. A 3.5×10^{-3} monomol/L polymer solution (C_p) in 0.05 M NaClO₄ was used. For comparison, the variations of the specific viscosity were recorded upon dilution and with a solution of NaClO₄ at a concentration adjusted to match the values of the ionic strength corresponding to the additions of Mg^{2+} .

Calorimetry. Calorimetric experiments were performed using a LKB 10700-2 batch type microcalorimeter. The ΔH^{mix} was determined upon mixing a solution of alginate from *L. hyperborea* with a solution Mg^{2+} ions to get a specific $[Mg^{2+}]/C_p$ molar ratio ($R_{Mg^{2+}}$). All experiments were performed with a final polymer concentration equal to 3.5×10^{-3} monomol/L (C_p) and in the presence of 0.05 M NaClO₄. The results have been corrected taking into account the enthalpy of dilution both of polymers and of salts.

Circular Dichroism Spectroscopy. Circular dichroism spectra of the sodium forms of the polymers of Table 1 were recorded in 0.05 M NaClO₄ ($C_p = 3.5 \times 10^{-3}$ monomol/L, pH = 7) with a JASCO J-700 spectropolarimeter in a quartz cell of 1 cm optical path. The following setup was used throughout: bandwidth, 1 nm; time constant, 2 s; scan rate, 20 nm/min. Four spectra, corrected for the background, were averaged for each sample. The spectra were recorded at different $[Mg^{2+}]/C_p$ ($R_{Mg^{2+}}$) molar ratios prior and after the addition of Mg(ClO₄)₂.

NMR Spectroscopy. ²³Na NMR measurements were performed at 25 °C on a JEOL 270 NMR spectrometer (6.34 T) operating at 270 MHz for proton and at 71.38 MHz for sodium. The R_1 relaxation rate of sodium were measured by means of the inversion recovery sequence using 19 different τ intervals. The relaxation rate of ²³Na in the absence of polymers (R_{1f}) was determined in aqueous 0.05 M NaClO₄ ($R_{1f} = 16.44$ s⁻¹). All experiments were performed at 3.5×10^{-3} monomol/L (C_p) polymer concentration and in the presence of 0.05 M NaClO₄. The ¹H NMR spectra were recorded at 90 °C with a Bruker DPX 400 spectrometer (9.4 T) operating at 400 MHz for proton and samples were prepared as described by Grasdalen et al.³⁷ The chemical shifts are expressed in ppm downfield from the signal for 3-(trimethylsilyl)propanesulfonate. The integration of the ¹H NMR signals allowed us to determine the composition of the different alginate samples reported in Table 1.

Theory

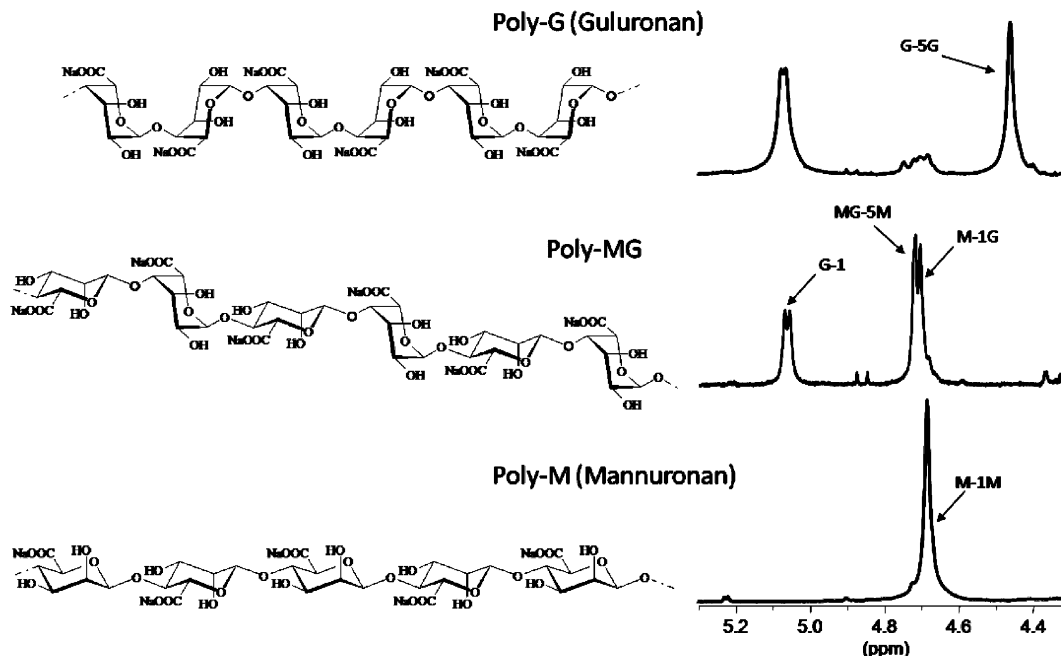
Before recalling the main features of the treatment of affinity reported in ref 32 it may be useful to briefly summarize the underlying theoretical foundations, namely, the so-called “chemical model” of counterion condensation, extended to include ions of different valence.²⁸ The “chemical model” was developed by Manning^{22,29} to extend his theory¹⁹ to “allow calculation of the charge fraction of polyelectrolytes as a function of the ionic strength”.²² It provided a sum of terms contributing to the total (molar) free energy of a polyelectrolyte, $\overline{\Delta G}^{\text{ion}}$:

$$\overline{\Delta G}^{\text{ion}} = \overline{\Delta G}^{\text{el}} + \overline{\Delta G}^{\text{mix}} \quad (1)$$

where $\overline{\Delta G}^{\text{mix}}$ is the free energy of mixing of mobile species and $\overline{\Delta G}^{\text{el}}$ is the electrostatic free energy that can be expressed as

$$\overline{\Delta G}^{\text{el}} = -RT(q^{\text{eff}})^2 \xi^{\text{str}} \ln(1 - e^{-\kappa b^{\text{str}}}) \quad (2)$$

q^{eff} is the effective charge on the polyelectrolyte, ξ^{str} is the dimensionless linear charge density parameter: it is the ratio of the Bjerrum length, l_B (l_B for water at 298.15 K is 7.135 Å), over b^{str} (Å), the average value of the distance between the projections of the fixed charges onto the axis of the polyelectrolyte: $\xi^{\text{str}} = l_B/b^{\text{str}}$; κ is the Debye screening parameter. The key feature of the CC theory is the assertion that thermodynamic instability will occur for $\xi > \xi_{\text{crit}}$, with $\xi_{\text{crit}} = |z|^{-1}$, and z is the valence of the counterions. The system will regain its thermo-

SCHEME 1: Schematic Structure and ^1H NMR Spectra (Anomeric Region) of PolyG (Guluronan), PolyMG (Polyalternating), and PolyM (Mannuronan)^a


^a M-1M and M-1G represent the anomeric proton of an M residue neighboring another M residue or a G residue, respectively. MG-5M refers to the H-5 proton of the central G residue in a MGM triad. G-1 refers to the anomeric proton of G residues and G-5G refers to the H-5 proton of G residues in G-blocks.

dynamic stability if a fraction r of counterions will be permanently associated (*condensed*) with the polyion, as the result of the following equilibrium condition:¹⁹

$$z(1 - zr)\xi = 1 \quad (3)$$

leading to the well-known general expression:

$$r = \frac{1}{z} \left(1 - \frac{1}{z\xi} \right) \quad (4)$$

which attains the forms $r = 1 - (1/\xi)$ for $z = 1$, and $r = (1/2)[1 - (1/(2\xi))]$ for $z = 2$, respectively. In parallel, the *effective* value of ξ , ξ^{eff} , will be

$$\xi^{\text{eff}} = \xi(1 - zr) = \frac{1}{z} \quad (5)$$

The effective charge on the polyion will then also be

$$q^{\text{eff}} = q_0(1 - zr) \quad (6)$$

and eq 2 will read

$$\overline{\Delta G}^{\text{el}} = -RTq_0^2(1 - zr)^2\xi^{\text{str}} \ln(1 - e^{-\kappa b^{\text{str}}}) \quad (7)$$

Within the Debye–Hückel approximation, $\overline{\Delta G}^{\text{el}}$ accounts for the electrostatic free energy of formation of the polyelectrolyte array. The volume (per polyelectrolyte charge) available for translation to associated counterions is V_p ; therefore, two phases will form and coexist, with volumes $V_p C_p$ and $V - V_p C_p$,

respectively, where C_p is the (equivalent) concentration of the polyelectrolyte charges and V is the total volume of the solution. $\overline{\Delta G}^{\text{mix}}$ corresponds to the sum of the energies of mixing of the condensed counterions, the noncondensed ones (“free”), the similions, and the solvent molecules, namely:

$$\overline{\Delta G}^{\text{mix}} = \overline{\Delta G}^{\text{cond}} + \overline{\Delta G}^{\text{free}} + \overline{\Delta G}^{\text{simil}} + \overline{\Delta G}^{\text{solv}} \quad (8)$$

Those terms are based on entropy of mixing only,^{28,38} and therefore so far no specific affinity is considered.

The treatment of Paoletti et al.²⁸ extended the “chemical model” to a polyelectrolyte system containing various amounts of counterions of different valence (most typically, mono- and divalent counterions, with index value i and j , respectively). In brief, it introduced two equilibrium parameters, r and x_i , where r is now the fraction of *all* condensed counterions (of all valences) and x_i is the mole fraction of ions of type i in r (it obviously holds: $x_i + x_j = 1$).

In the presence of a mixture of two ions, e.g., i and j , the total fraction of condensed counterions, r , can be written as follows:

$$r = r_i + r_j = r(x_i + x_j) \quad (9)$$

The equilibrium values of r and x_i can be determined following the procedure of separate minimization of (polyelectrolytic) free energy with respect to both r and either x_i or x_j , respectively. Their equilibrium values depend on ξ^{str} , on C_p , and on the ratios, R_i and R_j , of the total concentration in solution of ions of valence i and j , respectively, over C_p itself.

Throughout this paper the ionic species i will be supposed to be the monovalent sodium counterions: $x_{\text{Na}^+} = r_{\text{Na}^+}/r$, being the species j of any valence. For this case, the treatment of ref

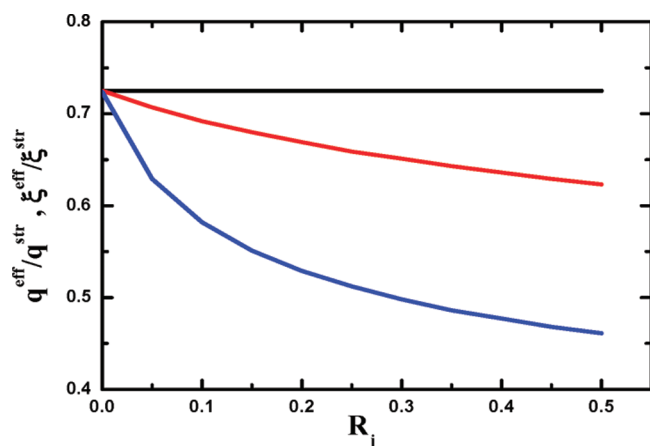


Figure 1. Dependence on R_j of the ratio q^{eff}/q_0 (and identically ξ^{eff}/ξ_0) for counterions of valence $z_j = 1$ (black), $z_j = 2$ (red), and $z_j = 3$ (blue). Polymer concentration and supporting salt are as reported in the caption to Figure 2. The terms q^{eff} and ξ^{eff} are calculated according to the model presented in the Theory section by using a ξ of 1.38 (i.e., ξ of mannuronan).

28 shows that the effective charge density on the polyelectrolyte, ξ^{eff} , varies according to²⁸

$$\xi^{\text{eff}} = \frac{1}{(z_j - x_{\text{Na}^+}(z_j - 1))} \quad (10)$$

Equation 10 attains the familiar values of 0.5 or 1 (as per eq 5) when only divalent ($z_j = 2$) or monovalent (Na^+) ions, respectively, are present.¹⁹ Figure 1 shows that when z_j is unity (i.e., when a mixture of two monovalent counterions is considered) there is, obviously, no variation in q^{eff} and ξ^{eff} . However, upon increasing z_j to 2, a decrease of the effective charge and charge density on the polyelectrolyte is detected, which is even more marked when a trivalent ion is considered. As to the free energy, eq 8 will become

$$\overline{\Delta G}_{ij}^{\text{mix}} = \sum_{n=i,j} (\overline{\Delta G}_n^{\text{cond}} + \overline{\Delta G}_n^{\text{free}}) + \overline{\Delta G}^{\text{simil}} + \overline{\Delta G}^{\text{solv}} \quad (11)$$

On the basis of the above extension,²⁸ ref 32 further introduced a *specific affinity* between a divalent ion and the polyelectrolyte:

$$\overline{\Delta G}^{\text{ion}} = \overline{\Delta G}^{\text{el}} + \overline{\Delta G}_{ij}^{\text{mix}} + \overline{\Delta G}_j^{\text{aff}} \quad (12)$$

To simplify treatment, a “reference” system of interaction of the polyion with counterion i (hereafter, Na^+) had to be chosen, with respect to which to evaluate the “excess” free energy of affinity, $\overline{\Delta G}^{\text{aff},0}$ of species j . Formally, it corresponds to

$$\overline{\Delta G}_j^{\text{aff}} = r(1 - x_{\text{Na}^+})\overline{\Delta G}_j^{\text{aff},0} \quad (13)$$

The experimental (microcalorimetric) data of heat-of-mixing sodium ions with mannuronan (see Figure 1 of ref 32) perfectly followed the theoretical predictions with no interaction in excess to the purely polyelectrolytic ones, i.e., Na^+ as a counterion displayed no affinity for the polymer itself. (Rigorously speaking, such an absence of deviations from the theoretical behavior

could simply stem from perfect equalization of attractive and repulsive interactions, much like in the case of θ conditions for polymer solutions, still without affecting the general conclusions). Accordingly, in the following treatment the data obtained with sodium have been used as the “baseline” with respect to which detect and define Mg^{2+} affinity, corresponding to: $\overline{\Delta G}_i^{\text{aff},0} = \overline{\Delta G}_{\text{Na}^+}^{\text{aff},0} = 0$ and $\overline{\Delta G}_j^{\text{aff},0} = \overline{\Delta G}_{\text{Mg}^{2+}}^{\text{aff},0} = \overline{\Delta G}^{\text{aff},0}$.³² Besides extending the formal approach of ref 28 to include affinity, ref 32 focused on the experimental determination of the enthalpy of mixing alginates of different composition with a Mg^{2+} salt. The deviations of the calorimetric data from the theoretical “no-affinity”²⁸ behavior of the heat-of-mixing divalent counterions with monovalent counterions (and a polyelectrolyte) were interpreted in terms of the two relevant thermodynamic parameters $\overline{\Delta G}^{\text{aff},0}$ and the corresponding enthalpy of affinity, $\overline{\Delta H}^{\text{aff},0}$. Both $\overline{\Delta G}^{\text{aff},0}$ and $\overline{\Delta H}^{\text{aff},0}$ resulted to be strongly dependent on alginate composition. They are supposed to trigger the condensation of Mg^{2+} counterions without violating the fundamental assumptions of the CC theory about the charge fraction of the polyelectrolyte. This short-range affinity simply affects the distribution of the divalent ions between the bulk solution and the condensation volume, V_p . Thus, the affinity induces a variation—with respect to the ideal condensation fractions, determined only by the entropy of mixing^{28,38}—of both the *total* fraction of condensed counterions, r ($r = r_{\text{Mg}^{2+}} + r_{\text{Na}^+}$), and $r_{\text{Mg}^{2+}}$, the *fraction of divalent* (Mg^{2+}) counterions condensed on the polyelectrolyte.

Results and Discussion

Alginates characterized by different diadic compositions display different enthalpy variations upon mixing with the nongelling Mg^{2+} ion.³² This experimental evidence can be accounted for by the theoretical model based on the CC theory which implies a free energy of affinity, $\overline{\Delta G}^{\text{aff},0}$ (assumed independent of the state of occupation of the neighboring sites), of the divalent counterion toward the polyelectrolyte. The presence of this contribution, however, does not imply per se the presence of any long-lasting “bonding” between the ion and the polyelectrolyte to the level of charge annihilation,³³ and therefore this model is suitable for the description of the Mg^{2+} –alginate system.

The relative intensity of the scattered light at 90° upon addition of Mg^{2+} was measured to rule out any possible magnesium-mediated chain association. The results have been reported in Figure 2a for alginate from *L. hyperborea*. The substantial invariance of the relative scattering intensity ratio, $I_{R_j}^{90}/I_{R_j=0}^{90}$, upon addition of the divalent cation points to the absence of polysaccharide chain association induced by the divalent ion and, hence, of strong “bonding” ion–polysaccharide interactions. The modification of the CD spectra of alginate from *L. hyperborea* (Figure 2b) upon addition of the Mg^{2+} ion is practically negligible and equal to the one displayed by the polymer with Na^+ ions at the same ionic strength value (same results are given by the other systems, data not shown). Since strong “bonding” interactions induce notable modifications of the electronic environment at the chromophore on the polyelectrolyte (the carboxylic moiety in the present case),³⁴ the absence of any significant change in the CD spectra leads to conclude that the addition of the divalent cation Mg^{2+} does not imply such a strong interaction. Nevertheless, as shown in Figure 3 and in line with what previously reported, the addition of magnesium ions to the polyelectrolyte solution produces a more positive enthalpy of mixing than that predicted by the CC theory in the absence of specific interactions.³² Experimental micro-

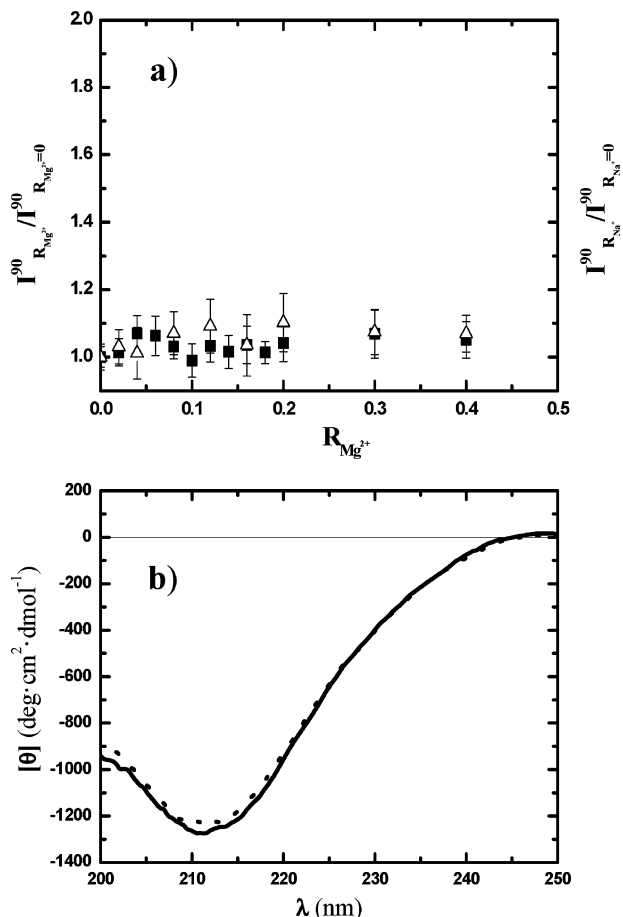


Figure 2. (a) Dependence on $R_{\text{Mg}^{2+}}$ of the intensity of scattered light at 90° for a solution of alginate from *L. hyperborea* treated with (■) $\text{Mg}(\text{ClO}_4)_2$ (left y-scale). Treatment with (Δ) NaClO_4 at the same total ionic strength has been reported for comparison (right y-scale). (b) Circular dichroism spectra of the solution of alginate from *L. hyperborea* prior to (—) and after (····) the addition of $\text{Mg}(\text{ClO}_4)_2$ to an $R_{\text{Mg}^{2+}} = 0.4$. Polymer concentration, $C_p = 3.5 \times 10^{-3}$ monomer/L; supporting salt, NaClO_4 0.05 M.

calorimetry results for the different alginate samples can be rationalized by using a negative free energy of affinity, $\overline{\Delta G}^{\text{aff},0}$, the magnitude of which decreases along the series polyG > *L. hyperborea* > polyMG > polyM. The biparametric analysis of ref 32 allowed to state that $\overline{\Delta G}^{\text{aff},0}$ is dominated by the entropy of affinity $\overline{\Delta S}^{\text{aff},0}$ rather than by $\overline{\Delta H}^{\text{aff},0}$ (Table 2).³²

Here we extend the experimental observations resorting to the combination of two methods particularly well-suited to respond, on one side, to overall macromolecular properties (viscosity) and, on the other side, to short-range interactions (²³Na NMR longitudinal relaxation rate).

The specific viscosity of a polyelectrolyte is strongly affected by the number and distribution of the fixed charges, as well as by their interaction with the mobile counterions. It can then provide a direct evidence of the condensation of counterions of generic charge z_j on a polyelectrolyte.

In order to assess any effect on chain dimensions brought about by the condensation of Mg^{2+} counterions on the different alginates of Table 1, we have measured the dependence of the specific viscosity of the same polysaccharide solutions on $R_{\text{Mg}^{2+}}$ in the presence of a constant concentration of monovalent supporting salt (Figure 4a–d). To separate the effects due to specific interactions of the divalent ion from the purely

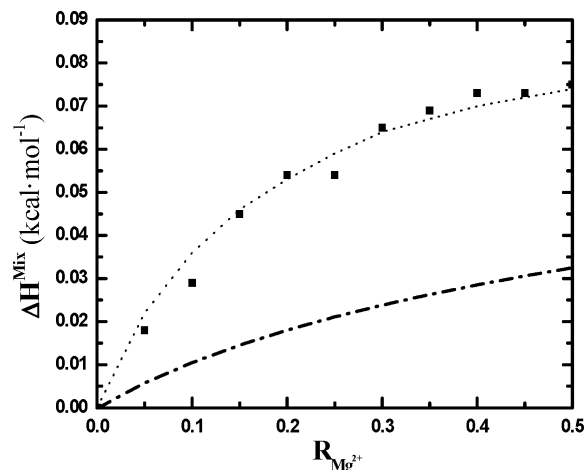


Figure 3. (■) Experimental enthalpy of mixing for a solution of alginate from *L. hyperborea* treated with Mg^{2+} ions. The dotted curve represents the best fit of the experimental data achieved with the theoretical approach presented in ref 32. The dash-dot curve represents the theoretical enthalpy of mixing with Mg^{2+} ions assuming no affinity interaction.

TABLE 2: Structural Parameters of Polyuronates and Thermodynamic Parameters, Experimentally Determined by Calorimetry, of Their Affinity for Magnesium Ions

sample	b^{str} (Å) ^a	ξ^b	$\overline{\Delta G}^{\text{aff},0}$ (kcal·mol ⁻¹) ^c	$\overline{\Delta H}^{\text{aff},0}$ (cal·mol ⁻¹) ^c	$-\overline{T\Delta S}^{\text{aff},0}$ (kcal·mol ⁻¹) ^c
mannuronan ^d	5.17	1.38	-0.30	734	-1.03
polyalternating ^d	4.80	1.49	-1.18	-23.7	-1.16
<i>L. hyperborea</i> ^c	4.66	1.53	-1.78	22.1	-1.80
guluronan ^d	4.44	1.61	-3.55	41.4	-3.59

^a b^{str} is the average value of the distance between the projections of the fixed charges onto the axis of the polyelectrolyte, and it is calculated on the basis of the composition of each polymer using the values 4.35 and 5.17 Å for guluronate and mannuronate residues, respectively (ref 28). ^b ξ is the ratio of the Bjerrum length— l_B —and b^{str} . l_B for water at 298.15 K is 7.135 Å. ^c Intrinsic total thermodynamic parameters calculated per mole of Mg^{2+} from the minimization procedure. The error associated with the calculation of the intrinsic free energy of affinity term was estimated to be approximately 10%. The error associated with the intrinsic entropy of affinity was found to range from 15% (polyalternating) to 8% (mannuronan). Experimental conditions: aqueous NaClO_4 0.05 M, 25 °C. ^d Data from ref 32. ^e Present work.

electrostatic ones, the specific viscosity of the same polymer solutions has been measured in conditions providing the same dilution and the same value of the ionic strength (as NaClO_4) for each $R_{\text{Mg}^{2+}}$ value. In fact, the addition of the divalent ion varies the total ionic strength of the solution and this brings about a decrease of the intramolecular electrostatic repulsion, a decrease of chain dimensions, and finally a decrease of the reduced specific viscosity.³⁹

As shown in Figure 4, the specific viscosity of all polyuronates is more reduced by the addition of magnesium perchlorate than by the addition of sodium perchlorate at the same ionic strength. This is traced back to the presence, in the polyelectrolyte condensation volume, of divalent counterions which, by significantly decreasing the effective charge density and hence the intrachain electrostatic repulsions, lead to a decrease of the polyuronate hydrodynamic volume.

Further support, although preliminary, to this interpretation has come from the analysis of the z -average dimensions of the alginate chains by means of a Malvern Nanosizer instrument. In the case of native alginate from *L. hyperborea*, a z -average

radius of gyration of 55 ± 4 nm was measured for the polyelectrolyte chain. Upon addition of Mg^{2+} ions to $R_{\text{Mg}^{2+}} = 0.16$, a slight contraction of the z -average dimension to 51 ± 3 nm was noted. This effect was even more evident when the amount of added divalent counterions reached the value of $R_{\text{Mg}^{2+}} = 0.24$; in this case the z -average dimension was 41 ± 4 nm. Albeit limited to the case of *L. hyperborea* alginate, these results provide a confirmatory evidence of a reduction of the polyuronate dimensions upon addition of the divalent counterion, thus supporting the interpretation of the decrease of the specific viscosity of the polyelectrolyte solutions as arising from a reduction of the effective charge.

The key feature of the model developed in the previous paper is represented by the $\overline{\Delta G}^{\text{aff},0}$,³² which stems from the affinity of the specific polyuronate for the divalent ion. As a consequence, the more negative is $\overline{\Delta G}^{\text{aff},0}$ the higher is the fraction of divalent magnesium ions in the condensation volume, $r_{\text{Mg}^{2+}}$ (eq 14)

$$r_{\text{Mg}^{2+}} = r_{\text{Mg}^{2+}}(\overline{\Delta G}^{\text{aff},0}) \quad (14)$$

The direct outcome of the divalent ion condensation is the reduction of the effective charge on the polyelectrolyte, q^{eff} , which, in turn, depends on the free energy of affinity, on the structural value of the linear charge density, ξ^{str} , and on the number (and charge) of the condensed counterions (eq 15):

$$q^{\text{eff}} = q^{\text{eff}}(r_{\text{Na}^+}, r_{\text{Mg}^{2+}}) = q^{\text{eff}}(\overline{\Delta G}^{\text{aff},0}, \xi^{\text{str}}, R_{\text{Mg}^{2+}}) \quad (15)$$

where $\overline{\Delta G}^{\text{aff},0}$ and ξ^{str} are characteristics of the polyelectrolyte considered and $R_{\text{Mg}^{2+}}$ is an independent variable.

To assess the relative importance of the different factors, we calculated the dependence of $q_{R_{\text{Mg}^{2+}}=0}^{\text{eff}}/q_{R_{\text{Mg}^{2+}}}^{\text{eff}}$ on $R_{\text{Mg}^{2+}}$ for the different alginates, at first considering only the difference in charge density, ξ^{str} . It was chosen to use for all polymers equal values of $\overline{\Delta G}^{\text{aff},0}$, to equalize any possible effect of affinity interactions, while using the accepted ξ^{str} values of the different alginates, which increase along the series polyM < polyMG < polyG. ξ^{str} obviously affects the fraction of condensed divalent counterions and, in turn, the effective charge on the polyelectrolyte. Even for the largest possible difference in ξ^{str} (see Table 2) and for the highest considered value of $R_{\text{Mg}^{2+}}$ (i.e., 0.26), the difference in $q_{R_{\text{Mg}^{2+}}=0}^{\text{eff}}/q_{R_{\text{Mg}^{2+}}}^{\text{eff}}$ between mannuronan and guluronan is very small and does not significantly depend on the value of $\overline{\Delta G}^{\text{aff},0}$, since it ranges from 1.6% (when $\overline{\Delta G}^{\text{aff},0} = 0$) to 2.6% (when $\overline{\Delta G}^{\text{aff},0}$ is fixed for all polymers at -3.55 kcal·mol⁻¹ calculated for polyG; data not shown). Such tiny difference rules out the possibility that any strong polymer selectivity for Mg^{2+} ions stems from differences in ξ^{str} .

At variance, Figure 5a shows that the ratio $q_{R_{\text{Mg}^{2+}}=0}^{\text{eff}}/q_{R_{\text{Mg}^{2+}}}^{\text{eff}}$ largely differs among the various polyuronates over the whole range of $R_{\text{Mg}^{2+}}$ when it is calculated considering, in addition to the corresponding ξ^{str} values, the $\overline{\Delta G}^{\text{aff},0}$ values reported in Table 2. In particular, the more negative the free energy of affinity of

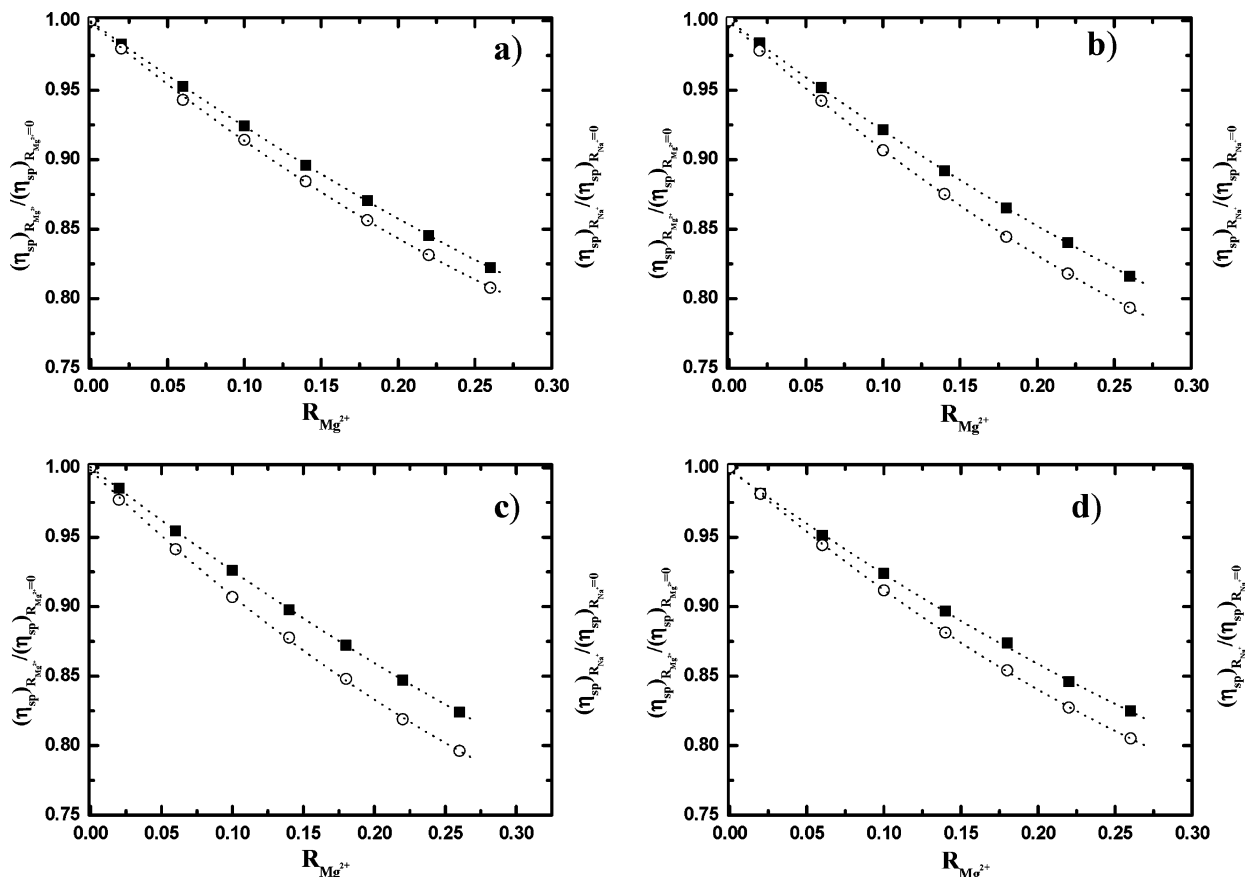


Figure 4. Dependence on $R_{\text{Mg}^{2+}}$ of the relative decrease of the specific viscosity of (a) mannuronan, (b) polyalternating, (c) guluronan, and (d) alginate from *L. hyperborea* upon addition of (O) $\text{Mg}(\text{ClO}_4)_2$ (left y-scale). Addition of (■) NaClO_4 at the same total ionic strength has been reported for comparison (right y-scale). Polymer concentration and supporting salt are as reported in the caption to Figure 2.

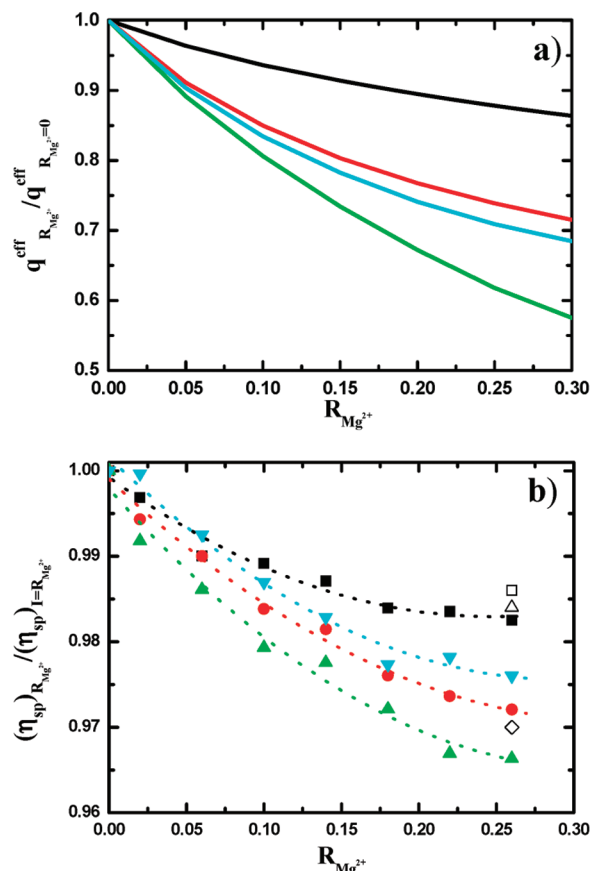


Figure 5. (a) Dependence on $R_{Mg^{2+}}$ of the relative effective charge ratio ($q_{R_{Mg^{2+}}}^{eff}/q_{R_{Mg^{2+}}=0}^{eff}$) for mannuronan (black), polyalternating (red), alginate from *L. hyperborea* (cyan), and guluronan (green) assuming the values of $\Delta G^{aff,0}$ reported in Table 2 for each sample. Polymer concentration and conditions are as in the caption to Figure 2. (b) Dependence on $R_{Mg^{2+}}$ of the relative specific viscosity ratio ($(\eta_{sp})_{R_{Mg^{2+}}}/(\eta_{sp})_{I=R_{Mg^{2+}}}$) of mannuronan (black), polyalternating (red), alginate from *L. hyperborea* (cyan), and guluronan (green). Lines are drawn to guide the eye. In panel b, the value of the relative specific viscosity ratio calculated for mannuronan (□) and guluronan (△) assuming $\Delta G^{aff,0} = -0.30$ kcal·mol⁻¹ and for guluronan (◇) assuming -3.55 kcal·mol⁻¹ is also reported (details in the Supporting Information).

the polyelectrolyte for the divalent counterion, the higher the reduction of its effective charge. At $R_{Mg^{2+}} = 0.26$, the largest difference of $q_{R_{Mg^{2+}}}^{eff}/q_{R_{Mg^{2+}}=0}^{eff}$ reaches almost 40%. As already pointed out, the effect of a marked reduction of $q_{R_{Mg^{2+}}}^{eff}/q_{R_{Mg^{2+}}=0}^{eff}$ should be reflected by a reduction of the polymer hydrodynamic volume. This prediction is clearly fulfilled when one considers the experimental dependence on $R_{Mg^{2+}}$ of the ratio between the specific viscosity of the polymer with Mg^{2+} and the corresponding one determined at the same ionic strength ($I = R_{Mg^{2+}}$) but with monovalent Na^+ counterions only $(\eta_{sp})_{R_{Mg^{2+}}}/(\eta_{sp})_{I=R_{Mg^{2+}}}$ (Figure 5b). Being intrinsically corrected for both dilution and ionic screening, such ratio should depend exclusively on the conformational effects produced by the effective polymer charge (q^{eff}) deriving from different amounts of condensed divalent ions, $r_{Mg^{2+}}$. It should be noted that the variation of such relative specific viscosity increases along the sequence polyM < polyMG \sim *L. hyperborea* < polyG, thus closely paralleling the trend theoretically calculated for $q_{R_{Mg^{2+}}}^{eff}/q_{R_{Mg^{2+}}=0}^{eff}$ (Figure 5a) using the relevant free energy of affinity of values of Table 2.

As a confirmatory evidence, a semiempirical calculation of the theoretical value of the relative specific viscosity at $R_{Mg^{2+}} = 0.26$ has been performed for mannuronan and guluronan

employing both their respective ξ^{str} parameters and their values of the free energy of affinity (see Table 2). Those values were implemented into a functional trend of the specific viscosity on effective charge density of the companion polyuronate poly(galacturonate)⁴⁰ (for details, see the Supporting Information). The results (reported in Figure 5b as an open square and an open diamond, respectively) agree fairly well with the corresponding experimental $(\eta_{sp})_{R_{Mg^{2+}}}/(\eta_{sp})_{I=R_{Mg^{2+}}}$ results. On the contrary, the same calculation gives for $(\eta_{sp})_{R_{Mg^{2+}}}/(\eta_{sp})_{I=R_{Mg^{2+}}}$ of guluronan a value much closer to that of mannuronan (open triangle in Figure 5b) if $\Delta G^{aff,0}$ is taken equal for both polymers ($\Delta G^{aff,0} = -0.30$ kcal·mol⁻¹), thus restricting the difference between the two polyuronates to the charge density only.

It is then safe to state that the marked variation of $(\eta_{sp})_{R_{Mg^{2+}}}/(\eta_{sp})_{I=R_{Mg^{2+}}}$ with $R_{Mg^{2+}}$ reported in Figure 5b is due almost completely to the much higher magnitude of the free energy of affinity for magnesium of the other alginates with respect to mannuronan. The negative $\Delta G^{aff,0}$ terms increase the amount of condensed Mg^{2+} counterions, which reduces the polyelectrolyte charge density, ξ^{eff} , and the chain expansion and finally produce a significant viscosity decrease.

The condensation of Mg^{2+} on alginate implies the displacement of sodium ions from the condensation volume, with a reduction of the fraction of condensed Na^+ ions which can be written as $\Delta r_{Na^+} (= r_{Na^+R_{Mg^{2+}}} - r_{Na^+R_{Mg^{2+}}=0})$, the dependence of Δr_{Na^+} on $R_{Mg^{2+}}$ being determined by the value of the free energy of affinity, $\Delta G^{aff,0}$ (Figure 6a). ²³Na NMR relaxation measurements were performed in order to get evidence of sodium displacement. The spin quantum number of the nucleus of sodium is $3/2$, and its magnetic relaxation is dominated by the quadrupolar mechanism, which results from the interaction between the nuclear quadrupole moment and the fluctuating electric field gradients at the nucleus. The latter ones, in the case of monoatomic ions, are of intermolecular origin. Although outside the extreme narrowing conditions the relaxation of ²³Na is biexponential,^{41–43} in the present case the spin–lattice relaxation curve is fitted very well using an averaged relaxation time value (T_1). According to the “two-state, all-or-none” model, the sodium ions exchange between a free (X_f) and a bound (X_b) situation and, when the exchange between the sites is fast on the NMR time scale, eq 16 holds:^{4,44–46}

$$R_{1obs} = R_{1f}X_f + R_{1b}X_b = R_{1f}(1 - X_b) + R_{1b}X_b \quad (16)$$

where $R_{1obs} (= 1/T_1)$ is the experimentally observed relaxation rate and R_{1f} and R_{1b} are the relaxation rates of “free” and “bound” sodium ions, respectively. The former represents the sodium ions in a free state (i.e., in the bulk) where the electric field gradient experienced by the nucleus is supposed not to be affected by the presence of the polyelectrolyte. At variance, the bound sodium ions, i.e., those associated with the polyanion, are characterized by different intrinsic relaxation rates, R_{1b} , larger than R_{1f} . As pointed out by Nordenskiöld et al.,⁴⁶ the bound sodium ions are subjected to a different electric field gradient as compared to free ions, defined as those in a (polyelectrolyte-free) dilute aqueous solution of a sodium salt.⁴⁷ Hence, the bound ions in the NMR, i.e., those the electric field gradient of which is perturbed due to the presence of the polyelectrolyte, do not necessarily correspond to the amount of condensed counterions calculated by using the counterion condensation theory or by integrating the Poisson–Boltzmann counterion distribution function. Anyway these ions are closely arranged in the proximity of the polyelectrolyte and the latter theories

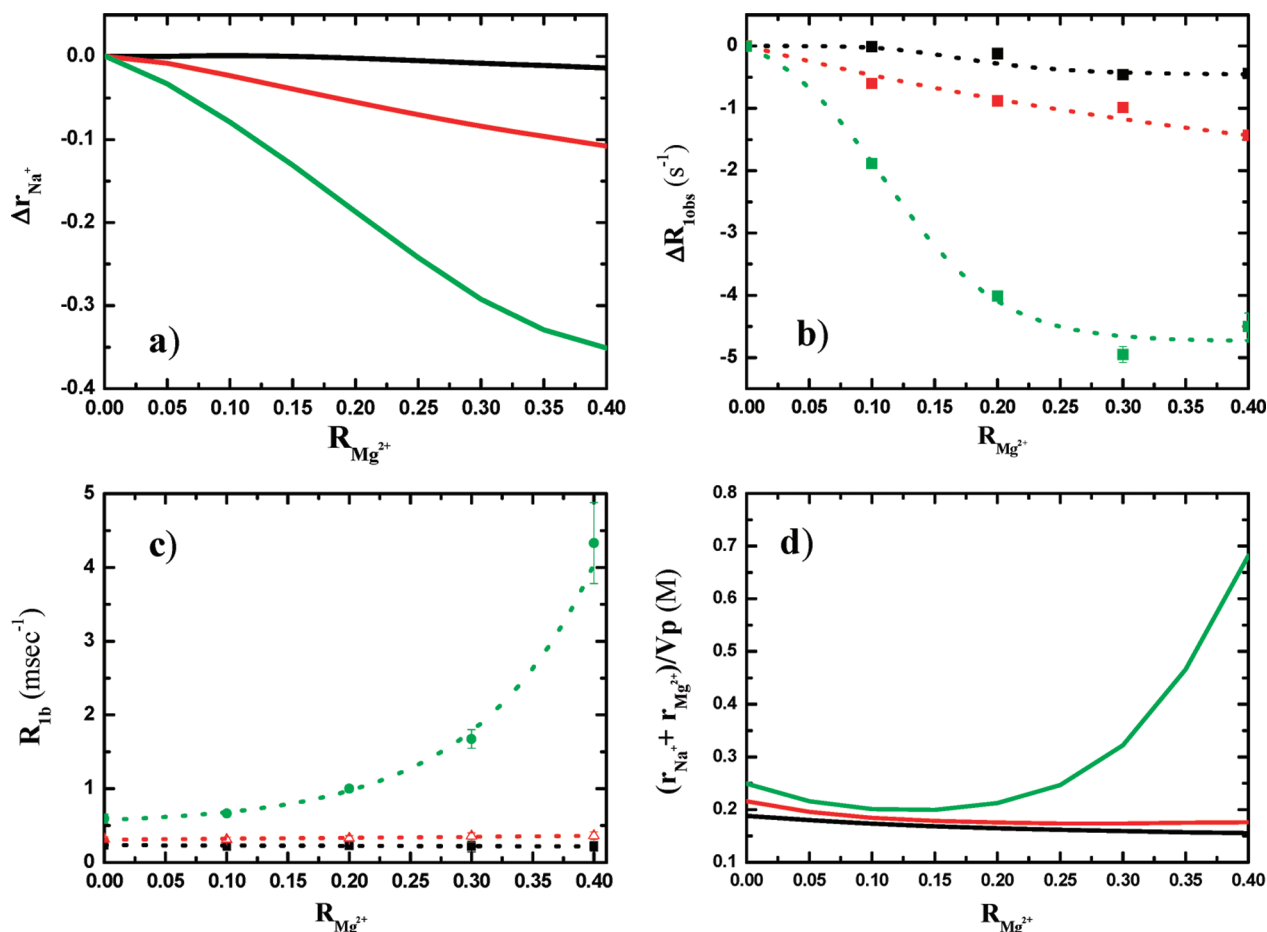


Figure 6. Dependence on $R_{Mg^{2+}}$ of (a) Δr_{Na^+} , (b) ΔR_{1obs} , (c) R_{1b} , and (d) the local concentration (in V_p) of all condensed counterions (Na^+ and Mg^{2+}) around the different polyuronates for mannuronan (black), polyalternating (red), and guluronan (green). Polymer concentration and conditions are as reported in the caption to Figure 2. In panels b and c the curves are drawn to guide the eye.

do provide a good approximation of X_b . The addition to the polyelectrolyte of competing divalent cations, such as Mg^{2+} , should bring about the displacement of condensed sodium ions that is expected to cause a decrease of R_{1obs} because of the reduction of X_b . It follows (eq 17)

$$\Delta R_{1obs} = R_{1obs, R_{Mg^{2+}}} - R_{1obs, R_{Mg^{2+}}=0} \propto \Delta r_{Na^+} \quad (17)$$

Considering the specific case of alginate samples treated with Mg^{2+} , the above considerations about the modification of the effective charge, q^{eff} , lead to predict that ΔR_{1obs} is strongly affected by the free energy of affinity.

The behavior theoretically predicted by the model (Figure 6a) parallels nicely the ^{23}Na NMR ΔR_{1obs} results (Figure 6b). This result confirms the validity of the hypothesis of a free energy of affinity of alginate for the nongelling Mg^{2+} ion, which specifically depends on the polysaccharide composition and triggers the Mg^{2+}/Na^+ competition. In particular, it can be noticed that the amount of sodium ions released from the condensation volume of the polyelectrolyte decreases along the series polyG > polyMG > polyM.

The calculation of the relaxation rate of the bound Na^+ ions, R_{1b} , has been attempted by means of eq 16, using for R_{1f} the value of R_1 determined in a dilute solution of $NaClO_4$ and calculating X_b from the value of r_{Na^+} .³² The results have been reported in Figure 6c (dotted curves). It can be noticed that both polyM and polyMG display a slight decrease of R_{1b} upon

increasing the amount of added divalent ion. The opposite tendency is shown for guluronan, with a significant increase of the calculated relaxation rate of the bound sodium ions.

Although the full interpretation of such effect requires further work, some preliminary considerations can nevertheless be drawn:

- First of all, experiments show that, even in the absence of added magnesium ions, sodium ions do experience different electric field gradients in the presence of the three polyuronates at the same concentration, resulting in different values of the R_{1obs} and R_{1b} .⁴⁸ In fact, R_{1obs} of Na^+ for guluronan, polyalternating, and mannuronan (in $NaClO_4$ 0.05 M without added Mg^{2+}) is 31.2, 23.0, and 20.4 s^{-1} , respectively, and the corresponding estimated values of R_{1b} are 594, 317, and 233 s^{-1} , respectively. These results are in agreement with those reported previously by Grasdalen et al. on alginates of different composition obtained from fractionation processes.⁴⁸ In the present work, it should be stressed that the polyuronate samples have been obtained by means of an enzymatic approach and are thus characterized by a higher compositional purity.
- One further consideration can arise from the analysis of theoretical results obtained from the model. It is interesting to note that the affinity-driven condensation of the counterions accounts for a notable decrease of the condensation volume, V_p .^{25,32} This effect markedly in-

creases with the increase of $|\Delta G^{\text{aff},0}|$ of the polyuronates for the divalent ion (see Figure 2 of the Supporting Information). As a result, the local concentration of all ions (i.e., both Na^+ and Mg^{2+}) will be increased, and much more so in the case of polyG (see Figure 6d). The straightforward consequence will be to strongly affect ion (de)hydration, counterion/counterion correlation increasing the electric field gradient. The strong similarity of the trends of Figure 6, parts c and d, seems to corroborate such qualitative interpretation.

- (iii) Over and above, the condensation of the divalent cations has a marked impact on the hydration of both the polysaccharide and of the condensed counterions, which, in turn, can be reflected in a variation of the relaxation rate of the residual Na^+ in the condensation volume.
- (iv) The NMR quadrupolar relaxation of sodium ions bound to a polyelectrolyte is determined both by the magnitude and by the fluctuations of the electric field gradients at the sodium nucleus. Considering that the overall hydrodynamic volume of the polyelectrolytes varies upon addition of Mg^{2+} ions, changes in the dynamics of bound Na^+ ions may occur.

Conclusions

The system composed of alginate and Mg^{2+} ions represents an ideal candidate to be described by means of the CC theory for the weak specific interactions which take place between a multivalent counterion and a polyelectrolyte. The theoretical model, originally devised to account for the experimental variations of the enthalpy of mixing,³² and implying an “affinity” of alginate for the nongelling Mg^{2+} ion strongly dependent on the composition of the polyuronate, has proven successful in rationalizing the present experimental trends of viscometry and ^{23}Na NMR relaxation. Albeit indirectly, the viscosity showed that the increasing fraction of condensed Mg^{2+} ions along the series mannuronan < polyalternating $\sim L$. *hyperborea* < guluronan determined a decrease of the polyelectrolyte unscreened fixed charges, at the root of the decrease of the observed chain dimensions. The same trend was found looking, via ^{23}Na NMR relaxation, at the displacement of sodium ions from the condensation volume of the polyelectrolyte upon addition of the nongelling magnesium ion. Both results point to confirm the importance of the free energy of affinity, $\Delta G^{\text{aff},0}$, of the polyuronate for Mg^{2+} ions, which results strongly dependent on the frequency of the different sequences in alginate.

Overall, the combination of the theoretical model, self-consistently derived from the CC theory, with experimental results allows to shed light over the thermodynamic features of affinity-driven interactions in ionic polysaccharides and looks promising for interpreting the thermodynamic parameters of such systems in a generalized way.

On the contrary the development of a more complex theoretical model is required in order to gain deeper knowledge over the more challenging alginate– Ca^{2+} system, where also bonding interactions between the polyelectrolyte and the counterions play a role. Work is in progress along this line.

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Supporting Information Available: Additional discussion of viscosity and condensation volume. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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