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^{23}Na NMR in Aqueous Solutions of Sodium Polyuronates. Counterion Binding and Conformational Conditions

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ABSTRACT: ^{23}Na NMR line widths and longitudinal relaxation were studied for aqueous solutions of sodium pectate and sodium alginates of different sequential structure as a function of polyanion concentration, degree of neutralization, and temperature at two distinct frequencies. The line width of Na^+ in 0.128 M sodium acetate increased linearly with the concentration of added tetramethylammonium alginate and narrowed with increasing temperature, indicating fast exchange of Na^+ between a "free" and a "polymer-bound" state, with a rate of exchange exceeding the quadrupolar relaxation in the latter state. The frequency-dependent line-broadening effects of polygalacturonate and polyguluronate were much larger than that of polymannuronate. This was ascribed to a shorter correlation time, a smaller quadrupolar coupling constant, and a lower degree of counterion binding in solutions of polymannuronate. In dilute solutions of the polyuronates, the correlation time of bound Na^+ was sufficiently long to cause biexponential decay of magnetization and hence non-Lorentzian lines. The biexponential character of transversal relaxation increased with decreasing polymer concentration in salt-free solutions, suggesting that it is correlated with the flexibility of the polymer chain and/or the translational diffusion of Na^+ ions from one polyanion to another. Electrostatic theories (Poisson-Boltzmann) account satisfactorily for the counterion binding, but a small degree of specific site binding of Na^+ to polyguluronate and polygalacturonate cannot be ruled out. No evidence in support of a recently reported pH-induced intramolecular conformational transition in polygalacturonate was obtained.

Introduction

Alginate occurs in the cell wall and intracellular spaces of brown-algal thalli.[1] It is a linear copolymer of 1,4-linked D-mannuronate (ManA) and L-guluronate (GulA) arranged in a blockwise fashion.[2] Pectic substances are present as cell wall constituents in higher plants and consist predominantly of linearly 1,4-linked D-galacturonate (GalA) and its methyl ester. Typical samples of pectin also contain small amounts of neutral sugars.[3] These polycarboxylates show selective interactions with divalent cations, which are especially important for their biofunctional properties.[4] Previous studies have, therefore, mostly dealt with this aspect.[5-9]

However, the properties of these polyelectrolytes in solution are also sensitive to monovalent ionic environments.[10] Recently, Seale et al.[11] studied the interaction of alginate with univalent cations in solution by circular dichroism and rheological measurements. Among the alkali-metal ions, Na^+ was found to be exceptional. It was suggested that in the presence of excess salt specific site binding of Na^+ ions occurs between adjacent guluronate residues, leading to cooperative association of polyguluronate sequences similar to that brought about by Ca^{2+} ions.[8]

Since the pioneering work of the NMR group in Lund on quadrupolar relaxation in solutions of synthetic and biological polyelectrolytes,[12,13] this method has become a most valuable tool for studying polyelectrolyte systems. Besides providing a direct probe of the ion-binding phenomenon at the molecular level, the NMR of ionic nuclei may also serve as an indirect probe of the conformational and dynamic behavior of macromolecules.[14]

Recently, Gustavsson et al. demonstrated the utility of ^{23}Na NMR in studies of Na^+ binding to poly(acrylic acid) and poly(methacrylic[15] acid) as well as to mucopolysaccharides.[14] Additional evidence for the reported pH-induced conformational transition in poly(methacrylic acid)[16-19] was provided by an increased Na relaxation, with markedly different T_1 and T_2 values, and non-Lorentzian NMR lines occurring for the degrees of neutralization between 0.1 and 0.3. With poly(acrylic acid) this behavior was absent, thus fully supporting the potentiometric and spectroscopic results, which indicated that no conformational transition occurred. A pH-induced conformational transition has also been reported for polygalacturonate on the basis of calorimetric, potentiometric, viscosimetric, and chiroptical measurements.[20]

Table I
Composition, Intrinsic Viscosities, and Molecular Weights of Polyuronate Samples

sample	content, % ManA	content, % GulA	content, % GalA	intrinsic viscosity $[\eta]^a$	mol wt
polygalacturonate			>95		$\bar{M}_n \approx 20000$
polymannuronate	98	2		12.0	$\bar{M}_w \approx 670000^b$
polyguluronate	10	90		2.1	$\bar{M}_w \approx 100000^b$

[a] 100 $cm^3\ g^{-1}$ in 0.1 M NaCl. [b] Estimated from an experimental relationship between $[\eta]$ and $\bar{M}_w$[26].

In the present work, we report ^{23}Na NMR studies of alginates and pectate under various experimental conditions, mostly in the absence of salt. The data were analyzed along the lines published by Gustavsson[15] and Gunnarsson,[21] with the widely used single correlation time model for quadrupolar relaxation, and also by the newly developed, more general theory by Halle et al.[22]

An attempt was made to determine whether (1) specific site binding of Na^+ occurs in aqueous sodium alginate solutions and (2) intramolecular conformational transitions take place in aqueous polyuronate solutions.

Experimental Section

(1) Materials. Sodium polygalacturonate was obtained by neutralizing a commercial pectic acid (Fluka A/G) with NaOH. Alginates were isolated from seaweed samples.[23,24] The polymannuronate was a fractionated alginate from fruiting bodies of *Ascophyllum nodosum*. The polyguluronate was synthesized by the soil bacterium *Azotobacter vinelandii*.[25] The molecular weights were estimated from osmometric and viscosity measurements.[26,27] To remove excess ions, solutions of the materials were dialyzed exhaustively against distilled water for several hours and then freeze-dried and analyzed by ^{1}H NMR.[28] The degree of esterification of the pectate was <2%, as determined from ^{13}C NMR.[29] No signals from neutral sugars were detected. Tetramethylammonium (TMA) salts of the polymers were prepared from the sodium forms by ion exchange.

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Some characteristics of the main samples are reported in Table I, and hereafter they are denoted by the idealized structures they approach.

(2) Methods. The degree of neutralization, α, is defined as the stoichiometric ratio of added HCl to charged groups on a fully neutralized polyelectrolyte, for which $\alpha = 1$.

Samples for NMR were dissolved in D_2O in 10-mm NMR tubes. ^{23}Na NMR spectra were recorded with JEOL JNM-FX100 (26.4 MHz) and Bruker WM-400 (105.6 MHz) FT-NMR spectrometers using 8000 data points and spectral widths of 5 kHz (26.4 MHz) or 10 kHz (105.6 MHz). Pulse angle and repetition time were varied to optimize the quality of the spectra. Spin-lattice relaxation times T_1 were measured at 105.6 MHz by the inversion recovery method.[30] The relation $1/T_2 = \pi\Delta\nu_{1/2}$ was applied to obtain the transversal relaxation time (for Lorentzian lines). The excess values of the line widths and relaxation rates were obtained by subtracting values for a reference solution of 0.05 M sodium acetate in D_2O. At 105.6 MHz the sample temperature was 293 K and at 26.4 MHz it was 297 K unless otherwise stated. Values measured at the latter conditions were converted to 293 K when the frequency dependence was compared (by utilizing the measured line width temperature dependence). The samples were heated to $\approx$353 K just before measurement to avoid aggregation effects.

^{13}C NMR relaxation times, T_1, and the nuclear Overhauser enhancement were measured on a JEOL JNM-FX100 spectrometer at 25 MHz, as reported previously.[31]

Theory

Poisson–Boltzmann Calculation. A polyelectrolyte in solution will attract ions of opposite charge and repel ions of the same charge. When the polyelectrolyte is considered as a cylinder of radius a with a uniform surface charge density,

$$\sigma = \frac{e}{2\pi a l} \tag{1}$$

where e is the unit charge and l is the projection of the intercharge distance onto the cylinder axis; the electrostatic potential, ψ, and the resulting distribution of ions in a medium of relative permittivity ϵ_r will follow the cylindrical Poisson–Boltzmann equation (in SI units)[32]

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\psi(r)}{dr}\right) = -\frac{e}{\epsilon_0\epsilon_r}\sum_i z_i c_{i0} \exp\left(\frac{-z_i e\psi(r)}{kT}\right) \tag{2}$$

Here r is the distance from the cylinder axis, z_i is the valence of ion i, ϵ_0 is the permittivity of a vacuum, and c_{i0} is the concentration of species i at $\psi = 0$. Although the charge density is not very high in alginates, the cylindrical model is justified by the relatively high degree of mechanical rigidity in the polymer chain, which results in a very extended coil.[33] In order to apply eq 2, the polyelectrolyte is assumed to be placed at the center of a cylindrical cell of radius R.[32] The dimensions of this cell containing the counterions of one polyion are chosen so as to give the correct overall concentration of polymer, c_m molar (on a monomer basis), according to

$$R = (\pi l c_m N \times 1000)^{-1/2} \tag{3}$$

where N is Avogadro's number. In an assembly of parallel cells the potential must have a minimum midway between the axes, thus providing the first boundary condition.

$$\left.\frac{d\psi}{dr}\right|_{r=R} = 0 \tag{4}$$

The second boundary condition follows from Gauss' law.

$$\left.\frac{d\psi}{dr}\right|_{r=a} = \frac{-\sigma}{\epsilon_r\epsilon_0} \tag{5}$$

In the absence of salt the general solution of eq 2 valid for the polyuronates is[22,32]

$$\psi(r) = \frac{2kT}{ze}\ln\left(\frac{r}{R}\left(\cos\left(s\ln\frac{R}{r}\right) + \frac{1}{s}\sin\left(s\ln\frac{R}{r}\right)\right)\right) \tag{6}$$

where the dimensionless parameter s is the solution to the transcendental equation

$$\xi = \frac{e^2}{4\pi kTl\epsilon_0\epsilon_r} = \frac{1+s^2}{1+s\cot(s\ln(R/a))} \tag{7}$$

ξ is a dimensionless charge parameter which, according to Manning's theory,[34] states that a fraction of $1 - (|z|\xi)^{-1}$ counterions associates or condenses onto the polyion to reduce its charge to a critical value given by $\xi_{crit} = 1/|z|$. The amount of condensed ions per charged group on the polymer is, according to Manning's model, independent of concentration, excess salt, and the radial dimension of the polyelectrolyte.

In the Poisson–Boltzmann model the radial concentration profile of univalent counterions is given by

$$n(r) = n_0\exp\left(\frac{-e\psi}{kT}\right) \tag{8}$$

and the fraction of ions unspecifically bound to the polyelectrolyte, p_b, can be defined by

$$p_b = \frac{\int_a^{a+\Delta}\exp(-e\psi/kT)r\,dr}{\int_a^R\exp(-e\psi/kT)r\,dr} \tag{9}$$

where Δ is the thickness of the region where the ions are considered "bound" to the polymer. The distance Δ is usually chosen approximately as the diameter of a hydrated counterion because the NMR effects are of short range.[22] In this work Δ is set to 0.5 nm. When eq 6 is substituted into eq 9, the integration gives[22]

$$p_b = 1 - \frac{1}{\xi}\left(1 - \frac{1 - s\tan\left(s\ln\frac{R}{(a+\Delta)}\right)}{1+(1/s)\tan\left(s\ln\frac{R}{(a+\Delta)}\right)}\right) \tag{10}$$

The behavior of p_b as a function of the polymer concentration does not depend on Δ. The magnitude of p_b is not especially sensitive to the value of Δ in our concentration interval. According to eq 10, the value of $\partial p_v/\partial\Delta|_{\Delta=0.5\text{nm}}$ for polyguluronate is 0.19 nm^{-1} at a concentration of 0.01% and 0.33 nm^{-1} at 1.0%.

^{23}Na NMR Relaxation. In polyelectrolyte solutions the quadrupolar relaxation of Na is completely dominated by the interaction of its quadrupole moment with the electric field gradients brought about by the polymer.[35] A two-state model is justified by the overwhelming contribution to the relaxation effect from the counterions close to the polyelectrolyte surface, where their local concentrations easily reach molar values. The resulting asymmetric charge distribution between the species together with the perturbation of the hydration symmetry, caused by the polyion field, is the main source of the field gradients.[22] Since these effects are of short range, we regard the counterions affected only if they are within a small distance, Δ, from the surface of the polyelectrolyte. Generally, the relaxation of spin 3/2 nuclei is biexponential and gives rise to two NMR line components having different widths and slightly different absorption frequencies (dynamic shift).[36] In the NMR spectra under consideration here, no asymmetry in the line shape was observed, and accordingly it could be satisfactorily fitted by the superposition of two Lorentzian lines with the same resonance frequency. Under condition of fast exchange and upon the assumption that the relaxation is dominated by isotropic motions, the broad component accounting for 60% of the total intensity has a transversal relaxation rate given by[37]

$$\frac{1}{T_{2f}} = (1 - p_b)\frac{1}{T_{20}} + p_b\frac{\pi^2}{5}\chi^2\tau_c\left(1 + \frac{1}{1 + \omega_0{}^2\tau_c{}^2}\right) \qquad (11)$$

The slower relaxing, narrow-line component accounting for the remaining intensity relaxes according to

$$\frac{1}{T_{2s}} = (1 - p_b)\frac{1}{T_{20}} + p_b\frac{\pi^2}{5}\chi^2\tau_c\left(\frac{1}{1 + \omega_0{}^2\tau_c{}^2} + \frac{1}{1 + 4\omega_0{}^2\tau_c{}^2}\right) \qquad (12)$$

Here χ denotes the quadrupolar coupling constant, this being proportional to the magnitude of the field gradient sensed by the counterion nuclei, ω_0 is the angular NMR frequency, p_b is the fraction of bound ions, and τ_c is the correlation time for the motion modulating the relaxation of these ions. T_{20} is the relaxation time for free ions. The resulting NMR line shape will depend on the value of the product $\omega_0\tau_c$. In cases where $\omega_0\tau_c > 0.25$, it is possible to deconvolute (separate) the two line components, and τ_c can be obtained from the ratio of their widths. If $\omega_0\tau_c$ becomes smaller, the relaxation approaches an exponential behavior with T_2 and T_1 values given by the approximate relations[38]

$$\frac{1}{T_2} = (1 - p_b)\frac{1}{T_{20}} + p_b\frac{1}{5}\pi^2\chi^2\tau_c\left(0.6 + \frac{1}{1 + \omega_0{}^2\tau_c{}^2} + \frac{0.4}{1 + 4\omega_0{}^2\tau_c{}^2}\right) \qquad (13)$$

$$\frac{1}{T_1} = (1 - p_b)\frac{1}{T_{10}} + p_b\frac{2}{5}\pi^2\chi^2\tau_c\left(\frac{0.2}{1 + \omega_0{}^2\tau_c{}^2} + \frac{0.8}{1 + 4\omega_0{}^2\tau_c{}^2}\right) \qquad (14)$$

Under conditions where there is still a difference between T_1 and T_2, it is possible to evaluate τ_c from the ratio T_2/T_1. A third way to determine τ_c is to utilize the frequency dependence.

Studies of ^{23}Na NMR in many dilute polyelectrolyte solutions have usually demonstrated a marked increase in the biexponential character of the relaxation upon dilution.[21,39] The only reasonable explanation for these observations has previously been that the increase in the correlation time, τ_c, is governed by a structural expansion of the polymer chain in dilute solutions.[39]

Recently, Halle et al.[22] presented a theory for quadrupolar spin relaxation of counterions that quantitatively accounted for the observed behavior, independently of any conformational changes. The counterion diffusion within a cell, as defined above, is insufficient to average out the quadrupolar interaction completely. This has been realized both from the quadrupole splittings observed in macroscopically anisotopic systems[40] and from theoretical treatment.[41] The only way to accomplish this for large, straight molecules (slow tumbling) is by counterion diffusion from one cell to another. The resulting modulation of the residual electric field gradient gives rise to the slow correlation time, τ_{eff}. The difference $\Delta\nu_{1/2f} - \Delta\nu_{1/2s} = \Delta(\Delta\nu_{1/2})$ was shown to take the simple form[22]

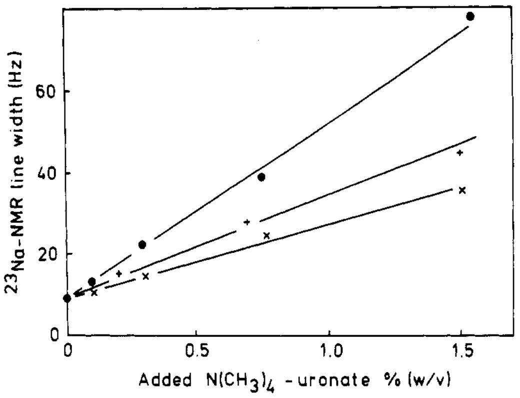

Figure 1. Linear increase of the ^{23}Na NMR line widths of 0.128 M NaOAc in D_2O produced by addition of TMA alginates at 26.4 MHz. The temperature was 297 K and the pD 7. (●): Alginate of content $F_G = 0.5$ and $F_{GG} = 0.3$. (×): Polymannuronate of low molecular weight ($\bar{M}_n \approx 25\,000$). (+): Polymannuronate of high molecular weight ($\bar{M}_w \approx 670\,000$).

$$\Delta(\Delta\nu_{1/2}) = (\pi/20)(p_b\chi)^2\tau_{\mathrm{eff}} \qquad (15)$$

Here $\tau_{\mathrm{eff}} = 2\tau_{\mathrm{cell}}$, where τ_{cell} is the mean residence time for a counterion in the cell. A fairly long analytical expression for τ_{cell} in salt-free solutions is given by eq A7 in ref 22. In case of curved polyions, having a finite persistence length, L_p, a possibility of modulation of the residual electric field gradient also exists through axial counterion diffusion along the polyion. The effective correlation time is then given by[22]

$$\frac{1}{\tau_{\mathrm{eff}}} = \frac{1}{2\tau_{\mathrm{cell}}} + \frac{1}{2(\tau_{\mathrm{cell}}\tau_{\mathrm{axi}})^{1/2}} \qquad (16)$$

where

$$\tau_{\mathrm{axi}} = \frac{L_p{}^2}{2D} \qquad (17)$$

D is the free counterion self-diffusion coefficient. An estimate of the persistence length can be obtained by using the relationship between the unperturbed mean-square end-to-end distance, $\langle r^2\rangle_0$, the contour length, L_c, and the persistence length, L_p, for a Porod–Kratky wormlike chain given by Flory:[42]

$$\langle r^2\rangle_0 = 2L_cL_p\left(1 - \frac{L_p}{L_c}\left(1 - \exp\left(\frac{L_c}{L_p}\right)\right)\right) \qquad (18)$$

Results and Discussion

In Figure 1, the ^{23}Na NMR line width of 0.128 M sodium acetate in D_2O solution is plotted as a function of added TMA polyuronates. The linear dependence is in agreement with rapid exchange of Na$^+$ between a "free" state in bulk water and a "bound" state associated with the polymers, where the quadrupolar relaxation is very fast. Provided the nature of the bound state is independent of polymer concentration, the effect is described by[12]

$$\Delta\nu_{1/2,\mathrm{obsd}} = (1 - p_b)\Delta\nu_{1/2,\mathrm{F}} + p_b\Delta\nu_{1/2,\mathrm{B}} \qquad (19)$$

Here $\Delta\nu_{1/2,\mathrm{F}}$ and $\Delta\nu_{1/2,\mathrm{B}}$ are the line widths characterizing the free, F, and the bound, B, states, respectively. $\Delta\nu_{1/2,\mathrm{obsd}}$ is the observed, averaged line width, and p_b is the fraction of "bound" Na$^+$ ions. The fact that the line width narrowed with increasing temperature (Figure 2) indicated that the exchange of Na$^+$ occurs faster than the relaxation in the bound state.

The relatively small line-broadening effect of polymannuronate compared to that of polyguluronate and poly-

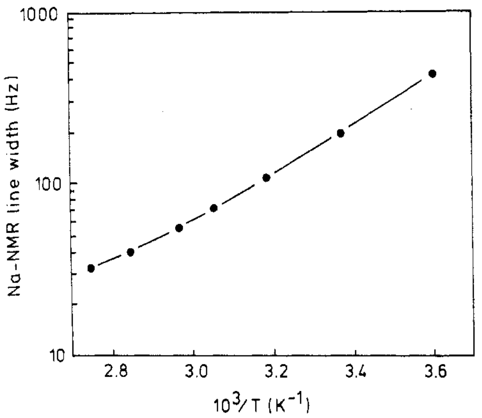

Figure 2. Temperature dependence of the ^{23}Na NMR line width of 3% (w/v) sodium alginate ($F_G \approx 0.9$, $\bar{M}_w \approx 100000$) in D_2O solution, pD 7, at 26.4 MHz.

Table II
^{23}Na NMR Line Widths (Hz) $\Delta\nu_{1/2}$, $\Delta\nu_{1/8}$, $\Delta\nu_{1/2s}$, and $\Delta\nu_{1/2f}$ and Estimated Correlation Times τ_c (ns) of Bound Na Ions in Salt-Free Solutions of Sodium Polyguluronate and Sodium Polymannuronate at Different Polymer Concentrations c (%(w/v)) at 293 K

c	$\Delta\nu_{1/2}$	$\Delta\nu_{1/8}$	$\Delta\nu_{1/2s}$[a]	$\Delta\nu_{1/2f}$[a]	τ_c[b]	B[c]	τ_c[d]
			Sodium Polyguluronate				
1.0	198[e]						
	126[f]	369	93	195	1.4	1.62	1.6
0.5	175.6[e]						
	121[f]	320	121	121		1.49	1.3
0.25	163[e]						
	117[f]	305	117	117		1.43	1.2
0.1	146.3[e]						
	105[f]	318	76	174	1.6	1.43	1.2
0.05	138[e]						
	84[f]	320	61	227	2.5	1.73	1.9
0.01	117[e]						
	64.8[f]	322	50	289	4.0	1.95	3.8
			Sodium Polymannuronate				
1.0	55.3[e]						
	46[f]	123	40.2	51.5	0.6	1.26	0.8
0.5	49[e]						
	42.4[f]	114	35.6	50	0.9	1.20	0.7
0.25	47.7[e]						
	38.4[f]	102	36	40.3		1.33	0.95
0.1	42.6[e]						
	37[f]	100	30.8	44.3	1.0	1.20	0.7
0.01	38[e]						
	31.5[f]	100	22.6	59.3	2.4	1.27	0.85

[a] Line widths of narrow (slow) and broad (fast) components, respectively, in the decomposed non-Lorentzian NMR lines. [b] Determined by comparison of $\Delta\nu_{1/2s}$ and $\Delta\nu_{1/2f}$. [c] $B = \Delta\nu_{1/2\text{exptl}}(26.4\ \text{MHz})/\Delta\nu_{1/2\text{exptl}}(105.6\ \text{MHz})$. [d] Determined by the frequency dependence of $\Delta\nu_{1/2}$. [e] 26.4 MHz. [f] 105.6 MHz.

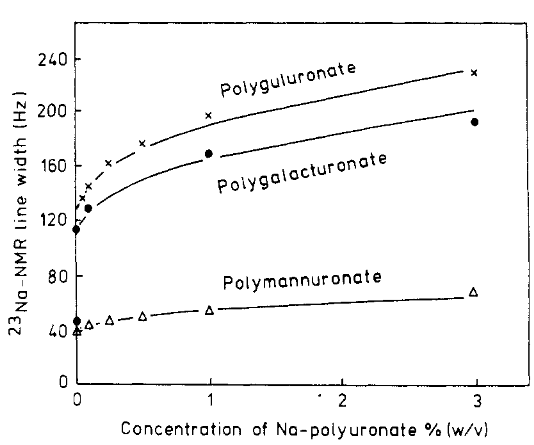

Figure 3. ^{23}Na NMR line widths at different sodium polyuronate concentrations in salt-free D_2O solutions at 297 K, pD 7, and 26.4 MHz. The points represent measured values. The curves were calculated by using the p_b values from Table V, $\Delta\nu_{1/2F} = 10$ Hz, and $\Delta\nu_{1/2B} = 636$ Hz for polyguluronate, 540 Hz for polygalacturonate, and 193 Hz for polymannuronate.

Table III
^{23}Na NMR Relaxation Rates, $1/T_1$, and $1/T_2 = \pi\Delta\nu_{1/2}$, and Correlation Times, τ_c, Evaluated from the Ratio T_2/T_1 at 105.6 MHz, in Salt-Free Neutral D_2O Solutions of 1% (w/v) Sodium Polyuronates at 293 K

sample	$1/T_1$, s^{-1}	$1/T_2$,[a] s^{-1}	T_2/T_1	τ_c, ns
polygalacturonate	260	407	0.64	0.9
polyguluronate	229	397	0.58	1.1
polymannuronate	96	145	0.67	0.8
sodium acetate, 50 mM	23.6[b]	33.3[b]		

[a] Since the transversal relaxation rates evaluated from the half-height of non-Lorentzian lines are underestimated compared to those obtained by proper pulse methods,[15] these $1/T_2$ rates represent minimum values. [b] Values representative for "free" Na^+ ions.

galacturonate (Figure 1) is reminiscent of their widely explored, different interaction with divalent cations.[8,9] It is well-known that both polyguluronate and polygalacturonate offer binding sites with a strong affinity for cations within a certain range of ionic radii, among which Ca^{2+} is one of the most important. Since Na^+ has much the same ionic radius as Ca^{2+}, it could well be that a specific site binding occurred also for Na^+, as has, in fact, recently been suggested.[11] However, as shown below, specific site binding does not seem to play an important role, if any, in explaining the features of the ^{23} Na NMR data in salt-free solutions.

In Figure 3 are plotted the results from measurements (Table II) at 26.4 MHz of the full ^{23}Na line width at half height on dilution of the polyuronates, without adding salt, at pD 7 (i.e., at full ionization). As will be shown below, the line-broadening effect displays a polymer-concentration dependence that is typical for delocalized ion binding to polyelectrolytes, as described by the Poisson–Boltzmann equation,[43,44] an approach based purely on electrostatic effects.

The curves in Figure 3 show the fits of eq 19 to the line-width data. Values of p_b were taken from Table V and the measured value of $\Delta\nu_{1/2,F}$ was 10 Hz. Only the $\Delta\nu_{1,2,B}$ values were adjusted in computing the three theoretical curves, which fit the experimental line widths quite well. The disagreement at the lowest concentration of polygalacturonate, corresponding to 5×10^{-5} M (monomolar), is probably due to a contamination by Na^+ from the glassware used. The only parameter varying with the polymer concentration in this calculation is the fraction of bound ions, p_b, as calculated by use of eq 10. Its value decreases only slightly and retains an appreciable value even at the lowest polymer concentrations, in contrast to the expectations from a specific site binding model governed by the mass-action law. At a concentration of 4.0% (w/v) p_b was found to be 0.39 for polyguluronate, in agreement with the constant value predicted by Manning's limiting law, $p_b = 1 - \xi^{-1}$. The difference between polyguluronate and polygalacturonate, which have a similar structure as seen by the Na^+ ions, is mainly due to the differences in molecular weight, this being larger for polyguluronate. Reducing its molecular weight to $\bar{M}_n \approx 9000$, decreased the line width by ≈20%.

At 26.4 MHz the NMR spectra showed no noticeable deviation from single Lorentzian peaks. However, the extreme narrowing condition ($\omega_0\tau_c \ll 1$) is not fulfilled, since the transversal relaxation is frequency-dependent, and significantly different T_1 and T_2 values were measured

Table IV

^{13}C NMR Longitudinal Relaxation Rates, T_1, and Nuclear Overhauser Enhancement Factors (nOe Values) of Sodium Polyguluronate and Sodium Polymannuronate Measured at 25 MHz in Salt-Free D_2O Solutions at Different Temperatures[a]

sample	temp, K	$10^3/T$, K^{-1}	T_1, s	τ_c, ns	nOe	τ_c, ns
polyguluronate	293	3.41	0.086	0.6		
	323	3.10	0.13	0.4		
	363	2.75	0.24	0.2	2.6	0.9
polymannuronate	323	3.10	0.17	0.3		
	363	2.75	0.27	0.2	2.7	0.8

[a] Corresponding carbon correlation times, τ_c, are evaluated by standard methods.[47] The given values are averaged over all carbons. $c \approx$ 8% (w/v), $\bar{M}_n \approx 8000$.

at the highest frequency applied (Table III). At this frequency, a clearly non-Lorentzian character in the NMR absorption also appeared for the high molecular weight polyguluronate sample at concentrations below 0.25% (w/v). A similar tendency, although much smaller, was observed in the case of polymannuronate. Similar effects have been reported for poly(acrylic acid) and poly(methacrylic acid) of high molecular weight.[21] This dilution dependence is mainly caused by slow polyion–counterion interaction dynamics, leading to an increase in the effective correlation time, τ_c, for motions modulating the relaxation of bound ions.

In an attempt to deconvolute the experimental line shapes, the method of Delville et al.,[45] based on measured widths at one-half, $\Delta\nu_{1/2}$, and at one-eighth of the height, $\Delta\nu_{1/8}$, was applied. This is illustrated in Figure 4, showing the ^{23}Na NMR spectrum of a typical polyguluronate solution. Mathematical treatment in accordance with NMR theory for spin 3/2 nuclei leads to the widths $\Delta\nu_{1/2\mathrm{s}}$ and $\Delta\nu_{1/2\mathrm{f}}$ of the narrow and broad component, respectively. The results obtained at 105.6 MHz for polyguluronate and polymannuronate listed in Table II show a more pronounced concentration dependence for the former sample. At 0.5 and 0.25% (w/v) a Lorentzian line is displayed, characterized by $\Delta\nu_{1/2\mathrm{f}}/\Delta\nu_{1/2\mathrm{s}} = 1$. At 0.01%, this ratio has increased almost sixfold.

The dynamic information evaluated under the assumption that the single correlation time model applies is given in Tables II and III in terms of τ_c values. The sixth column in Table II shows values of τ_c as calculated by applications of eq 11 and 12 to $\Delta\nu_{1/2\mathrm{s}}$ and $\Delta\nu_{1/2\mathrm{f}}$ at 105.6 MHz. As can be seen from the last column in Table II, essentially similar values of τ_c were deduced from the frequency dependence of $\Delta\nu_{1/2}$ by use of eq 13, including a significant increase at low concentrations of polyguluronate. The τ_c values obtained from eq 13 and 14 by comparing the nonequality of the measured T_1 and T_2 values at 105.6 MHz and a concentration of 1% (w/v), shown in Table III, are also practically similar.

Although it may be ambiguous to assign a particular molecular motion to the observed τ_c values, a few possible mechanisms are worth considering. The rate of exchange of Na$^+$ between a "free" and a "bound" state could well be correlated to τ_c values in the nanosecond range. The overall tumbling of the coiled polymer should definitely be too slow to affect the correlation time. The increase in τ_c values upon dilution more probably indicates that it is dominated by some local motion in the chains and that the contribution from the fast two-site exchange of Na$^+$ is of minor importance. The segmental flexibility is slowed down by dilution, due to the Coulombic expansion of charged flexible chains at low ionic strength. This is supported both by viscosity measurements[27] and dielectric studies of sodium alginate in salt-free, aqueous solutions.[46] The reported dielectric measurements indicated that the rigidity of the chains started to increase at a similar concentration, as judged by ^{23}Na NMR. In addition, the effect was found to be markedly dependent upon the molecular weight, being more pronounced with higher $\overline{\mathrm{DP}}_n$ at corresponding concentrations. Furthermore, the measurements of ^{13}C NMR relaxation and nuclear Overhauser enhancement (nOe) give convenient access to segmental or local motions in the polymer chain described by the correlation time of carbon atoms in the rigid structure of the uronate residues. Tables II, III, and IV show that the τ_c values are roughly the same for carbon and bound sodium at relatively high concentrations of polymer. As can be seen from Table IV, the T_1 and nOe values of the carbons cannot be rationalized by a single correlation time. The larger τ_c value obtained from nOe is typical of chainlike molecules in the random coil conformation in which the internal chain dynamics involve a broad spectrum of local motions attributable to a certain distribution of correlation times.[47] τ_c values related to possible migration of counterions along a curved polyelectrolyte will also depend upon the dilution due to a concomitant increase in the persistence length caused by expansion of the chains.

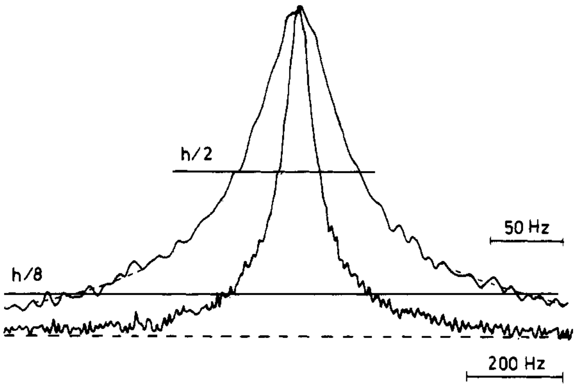

Figure 4. ^{23}Na NMR spectrum of a 0.05% (w/v) sodium polyguluronate solution in D_2O at 293 K and pD 7 measured at 105.6 MHz at two different expansions. The number of scans was 13 366, the pulse width 35 μs (corresponding to a 90° pulse), repetition time 150 ms, and the frequency range 5000 Hz. The levels for measurements of line widths, $\Delta\nu_{1/2}$ at one-half and $\Delta\nu_{1/8}$ at one-eighth of the total height, are indicated.

Once the Na relaxation in terms of the correlation time has been obtained, its value can be used in eq 11–14 to calculate the quantity $p_b\chi^2$ for different concentrations. The NMR lines were nearly Lorentzian for polymannuronate and for polyguluronate at concentrations of 0.5 and 0.25% (w/v), respectively, and eq 13 was employed. The $p_b\chi^2$ values are shown in Table V and Figure 5, where the vertical bars indicate the limits due to the extreme p_b values, 0 and 1. At polyguluronate concentrations below 0.25% and in excess of 0.5, eq 11 and 12 were used together with the widths of the corresponding lines in Table II. When the average value for the correlation time in Table V was used, slightly different values were obtained from eq 11 and 12, as indicated by the vertical bars in Figure 5. From Table V it can be inferred that the evaluated $p_b\chi^2$

Table V
Correlation Time, τ_c, for Bound Na^+ Ions, Fraction of Bound Na^+ Ions, p_b, and Values of the Product $p_b\chi^2$, in Salt-Free Neutral D_2O Solutions of Sodium Polyuronates at Different Polymer Concentrations at 293 K

c, % (w/v)	τ_c,[a] ns	$p_b\chi^2 \times 10^{-10}$, Hz^2 (obsd)	p_b[b]	$p_b\chi^2 \times 10^{-10}$, Hz^2 (calcd)[c]	χ, kHz
		Polyguluronate			
1.0	1.5	13.7	0.29	13.4	700
0.5	1.3	11.0	0.26	12.4	
0.25	1.2	11.1	0.24	11.7	
0.1	1.4	10.9	0.22	10.9	
0.05	2.2	10.3	0.21	10.4	
0.01	3.9	10.0	0.19	9.5	
		Polymannuronate			
1.0	0.8	5.1	0.26	5.0	440
0.5	0.8	4.6	0.22	4.5	
0.25	0.9	3.9	0.20	4.1	
0.1	0.9	3.7	0.17	3.8	
0.01	1.6	2.3	0.14	3.1	

[a] Average values from Table II. [b] p_b was calculated from eq 7 and 10. Parameter values: $l = 0.52$ nm,[52] $a = 0.45$ nm for polymannuronate; $l = 0.435$ nm,[52] $a = 0.5$ nm for polyguluronate and polygalacturonate. $\Delta = 0.5$ nm, $T = 297$ K, $\epsilon_r = 78.3$. We used 216 as the weight of the monomer unit including one water molecule. [c] Calculated by using the p_b values and a constant χ value corresponding to a least-squares fit given in the last column.

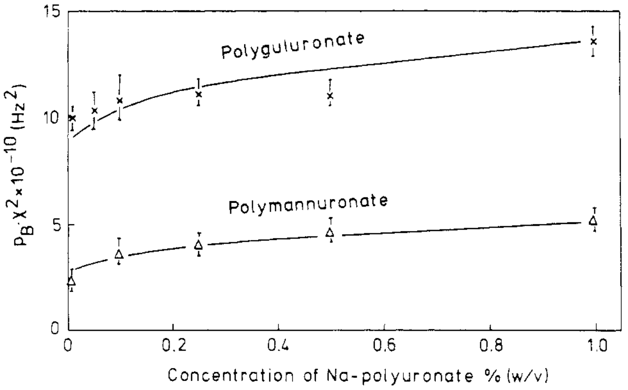

Figure 5. $p_b\chi^2$, i.e., the product of the fraction of bound Na^+ and the squared quadrupolar coupling constant, as a function of sodium polyuronate concentration. The points represent values evaluated from measured ^{23}Na NMR line widths and relaxation times, and the drawn lines indicate predicted values by using theoretical p_b values from the Poisson–Boltzmann cell model and χ values giving a best least-squares fit.

values of alginates resemble the concentration dependence of p_b given by the Poisson–Boltzmann model. Hence, it is reasonable to assume constant values for the quadrupolar coupling constants, χ. These were found to be 700 kHz and 440 kHz for polyguluronate and polymannuronate, respectively, from a least-squares fit of the observed $p_b\chi^2$ values by utilizing the theoretical p_b values in Table V. The fit is remarkably good for polymannuronate (Figure 5). The scattering among the values shown for polyguluronate may be due to small irregularities in either p_b or χ or both. At high concentrations this could be caused by a tendency to aggregation, which was indicated by slightly larger line widths when measured after long storage of the solution. A small increase in the correlation time of Na^+ and significantly non-Lorentzian absorption at 1% may also be ascribed to aggregational phenomena, which, however, certainly are not of any significance at lower concentrations. This assumption was further checked by applying samples purified by ultracentrifugation to get rid of possible aggregates and impurities. No significant alteration in the ^{23}Na NMR line from the apparently clear supernatant of 0.25% and 0.1% (w/v) sodium polyguluronate and sodium polymannuronate was observed after centrifugation at 140000*g* for 2 h.

The line widths and the quadrupolar couplings reported here are larger than have hitherto been reported for purely electrostatic binding to polyions bearing carboxyl groups, namely 100–200 kHz. The χ values are still smaller than those expected for real site binding, including dehydration of the counterions. For sodium heparinate a χ value of 1.2 MHz has been reported and attributed to Na binding to anionic sites.[48] However, since the local concentration of counterions close to the polyanion is fairly high, about 0.6–0.7 M in salt-free solution of 1% (w/v) sodium polyguluronate, a small degree of specific site binding to adjacent guluronate residues cannot be ruled out. This would also result in enhanced relaxation, i.e., larger χ value.

As far as the quadrupolar coupling constants are concerned, the relatively high value also observed for polymannuronate, for which site binding of Na^+ is very unlikely, provides additional support that they can be accounted for by structural features of the polymer chains.

Hence, interpretation of the present data by using a single correlation time model for Na quadrupolar relaxation suggests a delocalized binding mechanism, as described by the Poisson–Boltzmann cell model.

Consideration must also be given to the theory recently presented by Halle et al.[22] by which the dilution dependence of the relaxation behavior is quantitively explained by assuming that it is controlled by the diffusion of counterions between uncorrelated macromolecule segments. This is an isotropic process, and in dilute solutions it is relatively slow. From eq A7 in ref 22 values of τ_{cell} are calculated. The time scale of this process is shown in Table VI. Application of eq 15 to the difference between the deconvoluted line widths, $\Delta\nu_{1/2f} - \Delta\nu_{1/2s}$, using the calculated p_b values in Table V and a value of $\tau_{eff} = 2\tau_{cell}$, leads to the χ values shown in the eighth column in Table VI. The quadrupolar coupling constants deduced are smaller than those in Table V. According to the theory, they represent a minimum residual value that cannot be averaged out by rapid local motions in the perturbed region close to the polyion.

To test how the χ value might be affected by possible modulation through axial counterion diffusion along the polyions, numerical values of the polyuronate persistence length, L_p, shown in Table VI, have been evaluated by using eq 18. Root mean square end-to-end distances were extrapolated from the reported molecular weight and ionic strength dependence of $\langle r^2\rangle^{1/2}$ for polymannuronate and guluronate-enriched alginate.[26] Unperturbed values, $\langle r^2\rangle_0^{1/2}$, were obtained by taking the ratio $\langle r^2\rangle^{1/2}/\langle r^2\rangle_0^{1/2} = 1.2$ from the data in ref 27. This ratio, indicating the chain expansion caused by the excluded-volume effect, is very small for alginate since the molecules are highly ex-

Table VI
Residual ^{23}Na Quadrupolar Coupling Constants, χ, and Magnitude of Physical Properties Used in Estimating These Constants in Dilute Polyuronate Salt-Free Solutions

c_m, mM	$\langle r^2\rangle_0^{1/2}$, nm	L_p,[a] nm	τ_{axi},[b] ns	τ_{cell},[b] ns	τ_{eff},[b] ns	$\Delta(\Delta\nu_{1/2})$, Hz	χ,[c] kHz	χ,[d] kHz
				Polyguluronate				
4.6	80	16	128	175	161	98	192	283
2.3	83	17	145	375	287	166	182	294
0.46	97	24	288	2097	1134	238	102	212
				Polymannuronate				
11.5	180	9	41	43	42	4.3	90	129
4.6	200	12	72	118	104	13.5	110	165
0.46	240	17	145	1380	676	36.7	67	135

[a] L_p was calculated from eq 18. [b] τ_{axi}, τ_{cell}, and τ_{eff} were calculated from eq 17, eq A7 in ref 22, and eq 16, respectively. The self-diffusion coefficient of Na$^+$ ions $D = 1 \times 10^{-9}$ m^2 s^{-1}. [c] Obtained from eq 15 by using $\tau_{eff} = 2\tau_{cell}$. [d] Obtained from eq 15 by using τ_{eff} in the table.

tended due to hindered rotation.[33] In Table VI we present the resulting values of the correlation times, τ_{axi} and τ_{eff}, and, in the last column, the corresponding χ values. The reduction of τ_{eff}, brought about by axial diffusion, leads to higher χ values, up to 100% increase at the lowest polyion concentrations. The magnitude of χ has a low sensitivity to L_p. Hence, regardless of the uncertain L_p values, it may be concluded that the residual ^{23}Na quadrupolar coupling constants in sodium polymannuronate, estimated by using the theory of Halle et al.,[22] fall within a range that is typical for χ values previously obtained from Na NMR quadrupolar line splittings in macroscopically anisotropic systems, such as oriented fibers[40] and lyotropic liquid crystals,[49,50] while the χ values in sodium polyguluronate are slightly higher. However, there exists a great variety among the values found in the different systems, ranging from 35 to 135 kHz. Therefore, it is not possible at present to settle whether the difference between the polyuronates is due to their different chain structures or is caused by a small degree of specific site binding to polyguluronate.

In an attempt to explore and quantify the difference in Na relaxing behavior of alginates further, the ^{23}Na NMR line width was measured for a series of alginates having different composition and sequential structure. As shown in Figure 6, the magnitude of the excess line width increased progressively with the fraction of guluronate residues, F_G. The observed relationship is certainly due both to dynamic and structural properties of the polymer chain. Conformational-energy calculations of chain flexibility[51] predict a higher characteristic ratio (i.e., a more stiff and expanded coil) for polyguluronate than for polymannuronate and, most interestingly, with heterotypic sequences being more flexible than either. The general tendency which emerges from the sparing data in Figure 6 is that for samples containing about equal amounts of both residues, $F_G \approx 0.5$, the sequence in terms of the fractional content of contiguous guluronate residues, F_{GG}, seems to play a significant role in broadening of ^{23}Na NMR line. Apart from structural features, this may be caused either by site binding, which should be promoted by the increase in effective binding sites constituted by adjacent guluronate residues[5], or by a sequence dependent chain flexibility.[51] However, the fact that the F_{GG} values seem to play a minor role at F_G values higher or lower than 0.5 favors the latter explanation.

Of particular interest in relation to secondary structure are the data presented in Figure 7, which show the influence of the degree of neutralization on the line width of Na$^+$ in solutions of sodium polymannuronate and sodium polygalacturonate, the latter polyion at two different concentrations. At the lowest concentration a weak association of counterions and a concomitant, small degree of line broadening starts already at a very low value of α.

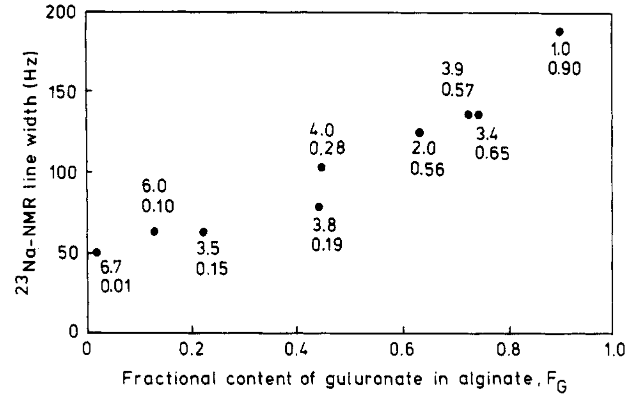

Figure 6. Variation of ^{23}Na excess NMR line width with the fractional content of guluronate residues, F_G, in 1% (w/v) solutions of alginate at 26.4 MHz. Temperature was 297 K and pD 7. The upper figure parameters indicate the molecular weight, $\bar{M}_w \times 10^{-5}$, and the lower figure parameters indicate the fraction of contiguous guluronate residues, F_{GG}, in the respective samples.

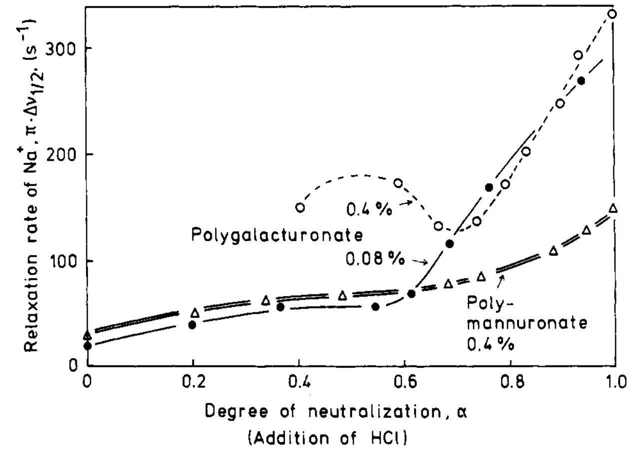

Figure 7. Transversal ^{23}Na NMR relaxation rates, $1/T_2 = \pi\Delta\nu_{1/2}$, for sodium polymannuronate solutions and for sodium polygalacturonate solutions at two concentrations as a function of the degree of neutralization, α, at 297 K and 26.4 MHz.

Around $\alpha = 0.61$ and 0.72 for polygalacturonate and polymannuronate, respectively, where the linear charge density exceeds the critical value predicted by Manning's limiting law, a rapid increase in the line width occurs, indicating an appreciable binding of counterions. This result is typical for polyelectrolyte solutions in which the secondary structure of the polymer is retained upon neutralization. However, at a higher concentration of polygalacturonate, a quite different result was obtained. The ^{23}Na NMR line widths exhibit a marked maximum around a degree of neutralization ≈ 0.5. Such behavior can be accounted for by processes affecting τ_c and/or a change in the nature or amount of counterion binding. Due to the very low solubility of polyuronic acids, it is reasonable to assume that it is primarily governed by aggregation, which is expected to affect both τ_c (reduced local mobility) and $p_b\chi^2$ (higher local charge density and increased field gradients). Actually, it could be seen by eye that the concentrated solution of polygalacturonate became turbid in this lower α region, whereas the polymannuronate solution was apparently clear.

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Registry No. Sodium pectate, 9049-37-0; sodium alginate, 9005-38-3; sodium polygalacturonate, 28087-73-2; sodium polyguluronate, 28964-87-6; sodium polymannuronate, 28961-37-7.

References and Notes

(1) Percival, E.; McDowell, R. H. *Chemistry and Enzymology of Marine Algal Polysaccharides*; Academic: London, 1967.
(2) Haug, A.; Larsen, B.; Smidsrød, O. *Acta Chem. Scand.* **1967**, *21*, 691.
(3) Aspinall, G. O. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 333.
(4) Painter, T. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic: New York, 1983; Vol. 2, pp 195–285.
(5) Smidsrød, O.; Haug, A.; Whittington, S. G. *Acta Chem. Scand.* **1972**, *26*, 2563.
(6) Morris, E. R.; Rees, D. A.; Thom, D.; Boyd, J. *Carbohydr. Res.* **1978**, *66*, 145.
(7) Morris, E. R.; Powell, D. A.; Gidley, M. J.; Rees, D. A. *J. Mol. Biol.* **1982**, *155*, 507.
(8) Smidsrød, O. *Faraday Discuss. Chem. Soc.* **1974**, *57*, 263.
(9) Smidsrød, O.; Grasdalen, H. *Hydrobiologia* **1984**, *116*/*117*, 19.
(10) Dingsøyr, E.; Smidsrød, O. *Br. Polym. J.* **1977**, *9*, 56.
(11) Seale, R.; Morris, E. R.; Rees, D. A. *Carbohydr. Res.* **1982**, *110*, 101.
(12) Forsên, S.; Lindman, B. In *Methods of Biochemical Analysis*; Laszlo, P., Ed.; Academic: New York, 1983; Vol. 1, pp 193–245.
(13) Lindman, B. In *NMR of Newly Accessible Nuclei*; Laszlo, P., Ed.; Academic: 1983; Vol. 1, pp 193–245.
(14) Gustavsson, H.; Siegel, G.; Lindman, B.; Fransson, L.-Å. *Biochim. Biophys. Acta* **1981**, *677*, 23.
(15) Gustavsson, H.; Lindman, B.; Bull, T. *J. Am. Chem. Soc.* **1978**, *100*, 4655.
(16) Mandel, M.; Leyte, J. C.; Stadhouder, M. G. *J. Phys. Chem.* **1967**, *71*, 603.
(17) Crescenzi, V. In *Polyelectrolytes*; Sêlêgny,, E., Ed.; D. Reidel: Dordrecht, 1974; p 115.
(18) Liquori, A. M.; Barone, G.; Crescenzi, V.; Quadrifoglio F.; Vitagliano, V. *J. Macromol. Chem.* **1966**, *1*, 291.
(19) Barone, G.; Crescenzi, V.; Quadrifoglio F.; Vitagliano, V. *Ric. Sci.* **1966**, *36*, 447; **1966**, *36*, 503.
(20) Cesaro, A.; Ciana, A.; Delben, F.; Manzini, G.; Paoletti, S. *Biopolymers* **1982**, *21*, 431.
(21) Gunnarsson, G.; Gustavsson, H. *J. Chem. Soc., Faraday Trans. 1*, **1982**, *78*, 2901.
(22) Halle, B.; Wennerström, H.; Piculell, L. *J. Phys. Chem.* **1984**, *88*, 2482.
(23) Haug, A.; Larsen, B.; Smidsrød, O. *Acta Chem. Scand.* **1966**, *20*, 183.
(24) Haug, A.; Larsen, B. *Acta Chem. Scand.* **1962**, *16*, 1908.
(25) Skjåk-Bræk, G. In *Application of Hydrocolloids in Foods: Progress in Food and Nutrition Science*; Phillips, G. O., Ed.; Pergamon: Oxford, 1983; pp 523–528.
(26) Mackie, W.; Nog, R.; Sellen, D. B. *Biopolymers* **1980**, *19*, 1839.
(27) Smidsrød, O. *Carbohydr. Res.* **1970**, *13*, 359.
(28) Grasdalen, H. *Carbohydr. Res.* **1983**, *118*, 255.
(29) Rinaudo, M.; Ravanat, G.; Vincedon, M. *Makromol. Chem.* **1980**, *181*, 1059.
(30) Vold, R. L.; Waugh, J. S.; Klein, M. P.; Phelps, D. E. *J. Chem. Phys.* **1968**, *48*, 3831.
(31) Grasdalen, H.; Larsen, B.; Smidsrød, O. *Carbohydr. Res.* **1981**, *89*, 179.
(32) Fuoss, R.; Katchalsky, A.; Lifson, S. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 579.
(33) Smidsrød, O.; Glover, R. M.; Whittington, S. G. *Carbohydr. Res.* **1973**, *27*, 107.
(34) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924.
(35) Forsén, S.; Lindman, B. *Chem. Br.* **1978**, *14*, 29.
(36) Werbelow, L. G.; Marshall, A. G. *J. Magn. Res.* **1981**, *43*, 443.
(37) Bull, T. E. *J. Magn. Res.* **1972**, *8*, 344.
(38) Gustavsson, H.; Siegel, G.; Lindman, B.; Fransson, L.-Å. *Biochim. Biophys. Acta* **1976**, *437*, 106.
(39) Levij, M.; de Bleijser, J.; Leyte, J. C. *Biochem. Biophys. Res. Comm.* **1981**, *87*, 183; *Ibid.* **1982**, *87*, 34.
(40) Edges, H. T.; Rupprecht, A.; Berendsen, H. J. C. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 790.
(41) Wennerström, H.; Lindblom, G.; Lindman, B. *Chem. Scr.* **1974**, *6*, 97.
(42) Flory, P. J. In *Statistical Mechanics of Chain Molecules*; Wiley-Interscience: New York, 1969; pp 401–403.
(43) Gouy, G. *J. Phys.* **1901**, *9*, 457.
(44) Chapman, D. L. *Philos. Mag.* **1913**, *25*, 475.
(45) Delville, A.; Detellier, C.; Laszlo, P. *J. Magn. Res.* **1979**, *34*, 301.
(46) Allgên, L.-G.; Roswall, S. *J. Polym. Sci.* **1957**, *33*, 635.
(47) Bovey, F. A. In *Stereodynamics of Molecular Systems*; Ramaswany, H. Sarma, Ed.; Pergamon: Elmsford, NY, 1979; pp 53–73.
(48) Herwats, L.; Laszlo, P.; Genard, P. *Nouv. J. Chim.* **1977**, *1*, 173.
(49) Lindblom, G.; Lindman, B.; Tiddy, G. J. *J. Am. Chem. Soc.* **1978**, *100*, 2299.
(50) Leigh, I. D.; McDonald, M. P.; Wood, R. M.; Tiddy, G. J.; Trevethan, R. E. *J. Chem. Soc., Faraday Trans. 1* **1981**, *77*, 2867.
(51) Whittington, S. G. *Biopolymers* **1971**, *10*, 1481, 1617.
(52) Atkins, E. D. T.; Nieduszynski, I. A.; Mackie, W.; Parker, K. D.; Smolko, E. E. *Biopolymers* **1973**, *12*, 1865.