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Association of κ-Carrageenan Induced by Cs^+ Ions in Iodide Aqueous Solution: A Light Scattering Study

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ABSTRACT: Light scattering (LS) investigations have additionally confirmed the intramolecular nature of the fundamental ordered conformation (single helix) of κ-carrageenan in aqueous 0.1 M NaI. The partial replacement of sodium with cesium ions induces a (thermoreversible) intermolecular association of the polysaccharide in the ordered conformation. The association-inducing effect can be observed already at the lowest investigated concentration of the cation. We found no significant change of the thermodynamic properties related to an alleged composition threshold value in the range of the cesium equivalent fraction, X_{Cs}, from 0.12 to 0.40. The extent and the rate of the association process strongly increase with the concentration of cesium. Equilibrium values for the association constant could be obtained except for the case of $X_{Cs} = 0.40$, where the process is too massive to allow long-time LS investigation. In the latter condition, some additional stiffening of the chain was noticed.

Introduction

Carrageenans are a family of linear sulfated polysaccharides extracted from red algae. Three main species can be identified, namely, κ-, ι-, and λ-carrageenan, though other types have been recognized. The present study is restricted to κ-carrageenan, the primary structure of which ideally consists of a regularly alternating α-(1−3)-D-galactose-4-sulfate and β-(1−4)-3,6-*anhydro*-D-galactose (Figure 1). One of the most peculiar features of κ-carrageenan is related to its ability to form thermoreversible, ionotropic gels, in particular with alkaline metal counterions of high atomic number.[1]

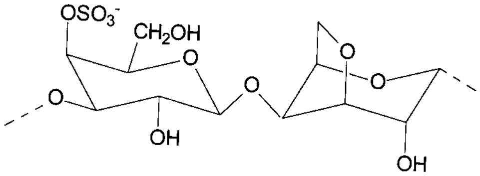

Figure 1. Ideal repeating unit of κ-carrageenan.

Despite its widespread and massive application, mainly by the food industry as a thickening and stabilizing agent, fundamental aspects concerning the mechanism of gelation have been a controversial matter until very recently,[2−12] and further investigation is still needed for a full comprehension of the process. On the other hand, new interesting features have been recently reported, such as the observation of a chiral nematic phase in aqueous κ-carrageenan under specific experimental conditions.[13−17]

The molecular requirements for gel formation have now been safely assessed, demonstrating that a single-stranded ordered helix is the conformational prerequisite for any further level of intermolecular organization.[2−6,10−12,18−22] An intermolecular association of helical stretches follows this stage, ultimately giving rise to the three-dimensional gel network. The same interchain association can occur on the microscopic scale without the development of macroscopic gel behavior, e.g., at high dilution and/or in the presence of suitable cosolutes. The type and concentration of ions in solution mediate the whole process: both co-ions and counterions show a specific effect on the conformational and associative behavior of aqueous κ-carrageenan.[6] Some cations, like K^+ and Cs^+, strongly promote the association of the helices, while others, like Li^+ and Na^+, are much less effective.[1,10,11,22] On the other hand, co-ions can also strongly influence the solution behavior of κ-carrageenan.[21,23] Iodide, in particular, is known to play a key role in establishing a wider range of conditions where intramolecular conformational ordering of the polymer develops without intermolecular association.[2,3,5,6,10,11] It should be noted, however, that association unavoidably occurs upon increasing iodide concentration.[24] The specific action of both cations and anions is balanced depending on the overall salt composition. Thus, for example, in aqueous 0.1 M NaCl, at room temperature, a low-ordered loose-helix[25] is expected, while an ordered single helix is stabilized by NaI under the same conditions of salt concentration and temperature. Intermolecular association, however, can be promoted by replacing Na^+ with gelling cations in iodide solutions. Hence, it is possible to control the conformational−associative properties of κ-carrageenan by appropriate modifications of the ionic environment.

A better characterization of the molecular properties of κ-carrageenan in aqueous solution is crucial in order to contribute to the elaboration of a self-consistent physical−chemical model for the formation of the gel and of the liquid-crystalline phases. Such a need arises from the controversial results reported in the literature. The discrepancy between experimental data found by different authors can be traced back to the complex

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solution behavior of the biopolymer, which is extremely sensitive to experimental conditions and procedures. This issue, already addressed by several authors[2,3,24,26] with reference to light scattering measurements, must actually be regarded as the origin of the long debate on fundamental physicochemical features of κ-carrageenan solutions.

This study is devoted to the investigation, performed by WALLS (wide-angle laser light scattering), of the κ-carrageenan solution behavior under different salt conditions. The effect of a gradual change from the ordered (helical), nonassociated state (0.1 M NaI) to increasingly association-inducing conditions (NaI/CsI mixtures, at constant 0.1 M ionic strength and increasing cesium fraction) has been studied, with particular interest for the time dependence of the associative process.

Referring to the above-mentioned liquid-crystalline behavior of κ-carrageenan, it should be acknowledged that mesophases can only be produced under specific experimental conditions. In particular, they develop at a relatively high polymer concentration ($C_p \geq 50$ g L^{-1}), i.e., well beyond the limits of the light scattering technique. Nevertheless, a thorough description of the polyelectrolyte behavior as reflected by the ion dependence of the conformational−associative properties of κ-carrageenan is strongly needed. Light scattering can provide fundamental information in the dilute regime ($C_p \leq$ ca. 1 g L^{-1}), which can then be extended to higher concentrations by means of other experimental techniques (such as small-angle neutron and X-ray scattering).

Experimental Section

Materials. κ-Carrageenan was supplied by Sigma−Aldrich (type III, from *Eucheuma cottonii*, lot 73H0840). It was dissolved in Milli-Q water by stirring and heating to 70 °C and subsequently precipitated by dropwise addition to 2-propanol (analytical grade, from BDH Laboratory Supplies) under vigorous stirring. The dissolution−precipitation procedure was then repeated two more times. The final product was dialyzed three times against NaCl (0.2 M), then dialyzed against water until the test for the presence of chloride ($AgNO_3$) was negative, and finally freeze-dried.

To avoid an acid hydrolysis of the polymer, the dialysis was performed at 4 °C under slightly alkaline conditions (pH ∼ 8) by addition of 0.1 M NaOH after every change of the outer solution. Furthermore, to avoid bacterial degradation a few drops of $CHCl_3$ were added.

NaCl, NaI, and CsI employed in the course of the experimental work were analytical-grade products, respectively from BDH Laboratory Supplies, Acros Organics, and Avocado Research Chemicals. Ultrapure water (Milli-Q reagent-grade water systems, Millipore) was used throughout the whole purification procedure and experimental work.

Methods. Light scattering measurements were performed on an Amtec MM 1000 instrument (equipped with a Spectra-Physics Stabilite, model 124B, He−Ne laser operating at 632.8 nm), as previously reported.[3,5] The data were treated as described in the following section, taking the refractive index increment equal to 0.150 mL/g for all the salt compositions[3] (0.1 M NaCl, 0.1 M NaI, and NaI/CsI mixtures with total ionic strength 0.1 M and Cs^+ equivalent fraction up to 0.4). Particular attention was given to the preparation of the κ-carrageenan solutions, to avoid aggregation phenomena that can actually impair the reliability of the experimental results.[2,3,24,26] The proper amount (≤0.1 g) of the lyophilized polymer was dissolved into 50 mL of water by stirring for about 15 min at about 55 °C. The stock solution was left under stirring at room temperature overnight and then diluted in order to obtain the proper final concentrations. A suitable volume of salt solutions (NaCl 1 M, NaI 1 M, and CsI 0.5 M) was slowly added to the half-filled flasks (i.e., at half dilution), under stirring at 65 °C, to reach the desired final ionic composition. By use of a peristaltic pump in a closed circuit, the solutions were filtered several times through a Millipore filter (pore size 0.22 μm) directly in the cells to obtain dust-free solutions. The WALLS measurements were first performed on each solution immediately after filtration; afterward, the solutions were kept at room temperature in the light scattering cells, to repeat the experiments at different times so as to investigate the time evolution of the systems. Given the time required for each measurement (about 2 h, including the instrument alignment test and the filtration of the solutions) it is not possible to define a time coordinate for each set of measurements in a rigorous way. It was then chosen to set $t = 0$ h for the measurements performed immediately after the preparation of the samples, while the beginning (lowest concentration) of the following measurements was taken into account.

Elaboration of WALLS Data: (A) Concentration Dependence. The elaboration of the concentration dependence of the light scattering data was performed in the framework of the Elias open association model (OAM), which has already been demonstrated to give the best description of the experimental data for κ-carrageenan under associating conditions.[5,18,26] As a more detailed theoretical discussion of the model can be found in the original treatment by Elias[27] as well as in the quoted previous work, only the fundamental features and equations will be reported here.

The basic assumption of the OAM is that the association process occurs through a series of thermodynamically equivalent steps, leading from the fundamental nonassociated form, the "unimer", to dimers, trimers, tetramers, etc. Each step is described by an equilibrium constant, which is assumed to be independent of the extent of association: $K_1 = K_2 = K_3 ... = K$.

The (apparent) weight-average molar mass of the associated forms, $(\bar{M}_w)_{assn,}$ can thus be defined, according to the model, as

$$(\bar{M}_w)_{assn} = \sqrt{(\bar{M}_w)_0^{\,2} + 4000K(PI)(\bar{M}_w)_0 C_p} \tag{1}$$

where $(\bar{M}_w)_0$ is the weight-average molar mass of the nonassociated species, PI is the polydispersity index, and C_p is the polymer concentration.

The reduced scattering intensity, extrapolated to zero angle, is given by

$$\frac{KC_p}{R_\theta}\Big|_{\theta=0°} = [(\bar{M}_w)_0^{\,2} + 4000K(PI)(\bar{M}_w)_0 C_p]^{-1/2} + 2A_2C_p \tag{2}$$

where A_2 is the second virial coefficient.

Equation 2 as a two-parameter equation (namely K and A_2) was used to fit the experimental data. $(\bar{M}_w)_0$ was obtained under nonassociating conditions. For PI the value 2.27 was taken from gel permeation chromatography-low angle laser light scattering (GPC−LALLS) results for a similar sample.[28] Previous work from our laboratory[24,26] has indicated that A_2 can safely be assumed to be constant during the C_p-induced association, both of κ- and of ι-carrageenan. This finding was presently confirmed in a few sample cases. A_2 was then treated as a constant for each set of polymer concentrations at a given value of X_{Cs}.

(B) Angular Dependence. The Zimm plots of the polymer aqueous solutions, under nonassociating conditions (0.1 M NaCl and 0.1 M NaI), are linear with respect to the concentration dependence. The angular dependence always shows some curvature, more pronounced at the highest concentrations (Figure 2). The Berry approach[29] (a variant of the usual Zimm treatment[30]) was tested, but no significant reduction of the curvature was found. On the other hand, under strongly associating conditions (i.e., high Cs^+ concentration) a very pronounced distortion of the Zimm plots appears (Figure 3), in both the concentration and the angular dependence. In a

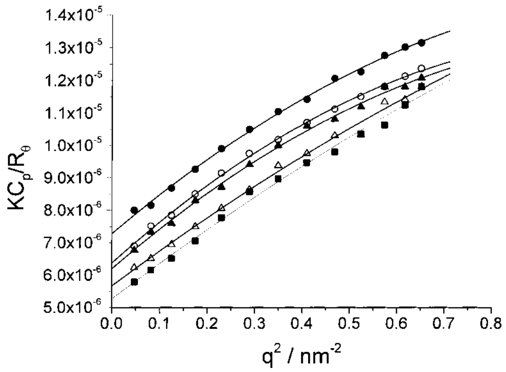

Figure 2. Dependence of the reduced scattering intensity on the square of the scattering vector for κ-carrageenan in 0.1 M NaI at 25 °C in the concentration range 0.168−0.730 g L^{-1}.

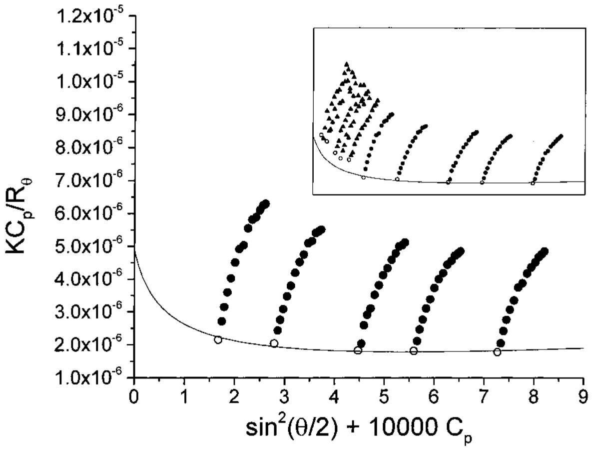

Figure 3. Zimm plot obtained for κ-carrageenan in aqueous 0.1 M iodide solution at $X_{Cs} = 0.40$ at 25 °C. The line represents the fitting of the values extrapolated to $\theta = 0$, according to the standard Elias method. The insert contains also the set of data obtained in the very low concentration range ($C_p \leq 0.120$ g L^{-1}). The scale of the inset is the same as the main plot.

few initial cases separate extrapolations to zero angle and to zero concentration were performed, just to get a preliminary indication of the molecular parameters. Thereafter, simultaneous treatment of both concentration and angular data was performed in all cases. The data were treated according to the well-known equation

$$\frac{KC_p}{R_\theta} = \{[(\bar{M}_w)_0^{\,2} + 4000K(PI)(\bar{M}_w)_0C_p]^{-1/2} + 2A_2C_p\}[(P(\theta))]^{-1} \quad (3)$$

where $P(\theta)$ is the scattering form factor. At first, both a linear and a quadratic dependence of $P(\theta)$ were used in all cases. The fitting was generally poor except for the conditions of little or no cesium present. Distortions of the plots of the reduced scattering intensity may have different origins. Macromolecular association, intra- and interparticle interference, change of shape and dimensions of the scattering entities, and nonlinear effects in the higher order terms of the dependence of $P(\theta)$ on the (square of the) scattering vector, q^2, are among the many possible causes. A purely phenomenological approach was then followed, using various expressions for a polynomial dependence of $P(\theta)$ on q^2 that was not limited to the first term. In addition, the various coefficients of the polynomial q^2 expansion were set as dependent on C_p as well, in turn with a polynomial dependence. In fact, $P(\theta)$ was treated as $P(\theta, C_p)$. This choice was based on separate trials with the different functions, performed on different sets of experimental data. Eventually, out of the several possible combinations of complex polynomial expressions, eight functions were selected. In all cases they gave very similar (although never identical) fitting; those fits were always significantly better than those given by a simple linear or quadratic dependence of $P(\theta)$ on q^2 only. In all cases the first term of the complex polynomial expansion of $P(\theta, C_p)$ (for $C_p \rightarrow 0$) contained the value of the z-average of the radius of gyration of the isolated chain, $(R_g)_z$. This was the only parameter that was eventually retained from the angular dependence. Eight sets of values of the LS parameters $[(\bar{M}_w)_0, A_2, (R_g)_z,$ and $K]$ were then obtained for each system: each final value was then the average of those. The new numerical approach described here turned out to be very suitable for the elaboration of both linear and highly distorted plots. The experimental results have been shown to be consistent with the usual Zimm/Elias treatment but with the additional advantage of allowing a unified treatment of the whole set of experimental data. Standard deviation values of all parameters in Table 1 refer to the error in the parametric fitting of the Zimm plot data.

(C) Persistence Length. The persistence length (L_p) was calculated from the Doty−Benoit equation:

$$(R_g)_z^{\,2} - L_p^{\,2}\left\{\left(N_z\frac{b}{3L_p}\right) - 1 + \left(\frac{2L_p}{N_wb}\right)\left[1 - \left(\frac{L_p}{N_nb}\right)\right]\right\} = 0 \quad (4)$$

where N_n, N_w, and N_z are, respectively, the number-average, weight-average, and z-average degrees of polymerization and b is the monomeric projection length. A molar mass distribution M_z:M_w:M_n = 3.98:2.27:1.00 was taken from GPC−LALLS measurement previously performed on a similar sample.[28] b values of 1.03 and 0.82 nm were taken for the disordered and for the helical conformation, respectively.[31] Values are given with the probable error based on the estimated standard deviation of the radius of gyration values.

Results and Discussion

Determination of the Fundamental Parameters of the κ-Carrageenan Sample. The first task at hand was to get a reliable value of the M_w of the isolated chain of the κ-carrageenan sample under allegedly nonassociating conditions, i.e., in 0.1 M NaCl and in 0.1 M NaI. These salt compositions correspond to the isolated, conformationally disordered chain and to the isolated helical state (for at least 90% of ordering), respectively, as indicated by several authors[2,3,6] on the basis of optical rotation and molar mass measurements. The stability of the system in NaI was tested over a period of 5 h, after which no change of the experimental parameters was observed beyond the experimental error. The results of the independent evaluation of M_w from the three sets of experiments turned out to be in good statistical agreement. They fall certainly within the limits of the light scattering accuracy, which is usually assumed to be about 10% (see Table 1a, variable M_w). An average value of M_w (M_w = 216 000 g mol^{-1}) was then estimated from these measurements.

This result is in complete agreement with all previously reported data obtained under reproducible preparation conditions (see ref 2 and references therein). Were it necessary, it further confirms the intramolecular nature of the disorder-to-order conformational transition of κ-carrageenan, first suggested by Smidsrød et al. more than 20 years ago.[21]

The data were then treated again with a fixed value for M_w in order to get the other LS parameters (see Table 1a, fixed M_w). For the case of NaI, reference will hereafter be made to the average of the two sets of results.

As for the second virial coefficient (A_2), the values in NaI are remarkably lower with respect to those in NaCl. These results are very close to those previously reported

Table 1. Zimm Plot Fitting Molecular Parameters (±SD) Obtained for κ-Carrageenan under Different Salt Conditions (CsI/(CsI + NaI) Equivalent Ratios, X_{Cs}) at 25 °C[a]

(a) $X_{Cs} = 0$

	0.1 M NaCl	0.1 M NaI, $\Delta t = 0$	0.1 M NaI, $\Delta t = 5$ h	NaI avg
Variable M_w				
$M_w \times 10^{-3}$, g mol^{-1}	227 ± 3	211 ± 5	211 ± 3	
$A_2 \times 10^3$, mol cm^3 g^{-2}	3.93 ± 0.09	1.78 ± 0.17	1.74 ± 0.10	
$(R_g)_z$, nm	75.3 ± 5.2	83.2 ± 6.6	88.7 ± 4.8	
L_p, nm	21.4 ± 3.2	39.1 ± 7.5	45.5 ± 6.2	
Fixed M_w				
$M_w \times 10^{-3}$, g mol^{-1}	*216*	*216*	*216*	*216*
$A_2 \times 10^3$, mol cm^3 g^{-2}	3.72 ± 0.05	1.88 ± 0.07	1.85 ± 0.08	1.87 ± 0.08
$(R_g)_z$, nm	69.0 ± 2.8	86.1 ± 3.4	91.7 ± 3.4	88.9 ± 4.0
L_p, nm	15.7 ± 1.3	33.0 ± 2.9	38.0 ± 3.2	35.6 ± 3.6

(b) $X_{Cs} = 0.12$

	$\Delta t = 0$ h	$\Delta t = 24$ h
Fixed M_w		
$(M_w)_0 \times 10^{-3}$, g mol^{-1}	*223*	
$K \times 10^{-3}$	114 ± 12	
$A_2 \times 10^3$, mol cm^3 g^{-2}	2.46 ± 0.11	
$(R_g)_{z,0}$, nm	90.9 ± 4.0	
L_p, nm	37.3 ± 3.7	
Fixed M_w, R_g		
$(M_w)_0 \times 10^{-3}$, g mol^{-1}		*223*
$(R_g)_{z,0}$, nm		*90.9*
$K \times 10^{-3}$		124 ± 16
$A_2 \times 10^3$, mol cm^3 g^{-2}		2.34 ± 0.16

(c) $X_{Cs} = 0.20$

	$\Delta t = 0$ h	$\Delta t = 18$ h	$\Delta t = 23$ h
Fixed M_w			
$(M_w)_0 \times 10^{-3}$, g mol^{-1}	*228*		
$K \times 10^{-3}$	116 ± 12		
$A_2 \times 10^3$, mol cm^3 g^{-2}	2.29 ± 0.08		
$(R_g)_{z,0}$, nm	93.5 ± 5.9		
L_p, nm	39.7 ± 5.8		
Fixed M_w, R_g			
$(M_w)_0 \times 10^{-3}$, g mol^{-1}		*228*	*228*
$(R_g)_{z,0}$, nm		*93.5*	*93.5*
$K \times 10^{-3}$		166 ± 9	151 ± 21
$A_2 \times 10^3$, mol cm^3 g^{-2}		2.25 ± 0.44	2.09 ± 0.44

(d) $X_{Cs} = 0.28$

	$\Delta t = 0$ h	$\Delta t = 24$ h	$\Delta t = 45$ h	$\Delta t = 70$ h	after heating
Fixed M_w					
$(M_w)_0 \times 10^{-3}$, g mol^{-1}	*233*				
$(R_g)_{z,0}$, nm	86.1 ± 5.0				
$K \times 10^{-3}$	115 ± 10				
$A_2 \times 10^3$, mol cm^3 g^{-2}	1.28 ± 0.04				
L_p, nm	32.9 ± 4.3				
Fixed M_w, R_g					
$(M_w)_0 \times 10^{-3}$, g mol^{-1}		*233*	*233*	*233*	*233*
$(R_g)_{z,0}$, nm		*86.1*	*86.1*	*86.1*	*86.1*
$K \times 10^{-3}$		260 ± 28	514 ± 70	737 ± 159	39.7 ± 22.0
$A_2 \times 10^3$, mol cm^3 g^{-2}		0.26 ± 0.04	0.29 ± 0.05	0.30 ± 0.04	2.03 ± 0.20

(e) $X_{Cs} = 0.40$

	$\Delta t = 0$ h	$\Delta t = 20$ h
Variable M_w, R_g		
$(M_w)_0 \times 10^{-3}$, g mol^{-1}	240 ± 15	
$(R_g)_{z,0}$, nm	111 ± 9	
$K \times 10^{-3}$	441 ± 42	
$A_2 \times 10^3$, mol cm^3 g^{-2}	0.42 ± 0.02	
L_p, nm	59.8 ± 12.3	
Fixed M_w, R_g		
$(M_w)_0 \times 10^{-3}$, g mol^{-1}		*240*
$(R_g)_{z,0}$, nm		*111*
$K \times 10^{-3}$		$(4.18 \pm 0.46) \times 10^3$
$A_2 \times 10^3$, mol cm^3 g^{-2}		−0.12 ± 0.05

[a] Parameters used as fixed values in the fitting are given in italic type.

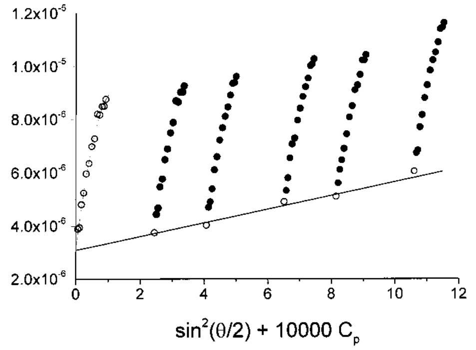

Figure 4. Zimm plot of the reduced scattering intensity of κ-carrageenan in aqueous 0.1 M iodide solution at $X_{Cs} = 0.20$ at 25 °C. The points extrapolated to zero angle are fitted according to a linear dependence of the reduced scattering intensity on C_p.

under the same or very similar conditions.[2,3,11,12] They indicate that iodide is a poorer solvent for the ordered polymer conformation than chloride is for the disordered one. This fits the current model for the basic transition, which is envisaged to take place from a disordered wormlike conformation to a semirigid ordered one.[2,3] The latter is stabilized by a cooperative network of interresidue hydrogen bonds.[20,32] A substantial development of (intramolecular) polymer−polymer interactions ensues, which overcompensates the expected higher value of A_2 for a (quasi)rodlike chain with respect to a more coiled one.[33] Another source[34] of negative contribution to A_2 stems from the associative tendency of κ-carrageenan, which is manifested even in NaI, at suitable concentrations.[24]

Also, the values of the radius of gyration [$(R_g)_z$] are thoroughly in line with our previous light scattering findings: the ratio of the values of $(R_g)_z$ for iodide and chloride is presently 1.29, as compared[3] to 1.30. $(R_g)_z$ is larger for the helix than for the disordered form, in agreement with obvious expectations, as well as with viscometric evidence.[3,6,10] Correspondingly, the value of the persistence length, L_p, in iodide is about twice as large as that in NaCl (see Table 1a, fixed M_w).

Evidence for the Association. The experimental work was then focused on the characterization of κ-carrageenan solutions under increasingly association-inducing conditions. In particular, different mixed NaI/CsI aqueous solutions were used as a solvent, keeping the total ionic strength constant (= 0.1 M) and varying the CsI equivalent fraction (X_{Cs}) in the range 0−0.4. The experimental data at different values of X_{Cs} were treated according to the most common procedures to get both the values of the reduced scattering intensity at zero angle and those at zero concentration. In particular, a linear fitting function was used for the former dependence. A representative example is given by the data of Figure 4 for the case $X_{Cs} = 0.20$. Values of M_w, A_2, and $(R_g)_z$ were obtained in this way for the other cases at $t = 0$: they are plotted in Figure 5, panels a, b, and c, respectively. They all manifest a clear dependence on X_{Cs}, which can be univocally interpreted as an evidence for intermolecular association that is established from the introduction of the very first Cs^+ ions into the system. Consequently, the parameters of Figure 5 should be more correctly looked upon as merely apparent values. In fact, for an associating system, different values of the parameters are obtained depending upon the range of polymer concentration that is being used for the linear extrapolation. The pronounced curvature of the Zimm plot of the scattering data obtained at $X_{Cs} = 0.40$ (Figure 3) gives clear supporting evidence for the association. An attempt to study the latter system at lower concentrations was made by performing a new series of experiments in a very low concentration range, as shown in the inset in Figure 3. The two data sets could not be merged into a single one, given the difficulty in achieving accurate light scattering values at very low concentration. Consequently, the Elias fitting has been applied only to the values at the higher concentration. Still, the picture of a remarkable intermolecular association that is normally regarded as the origin of the strong upturn of the plot at very low concentrations is neatly confirmed. In addition, the results of Figure 3 warn against an insufficient attention paid to the very low concentration regime and the misuse of deceivingly linear extrapolation procedures.

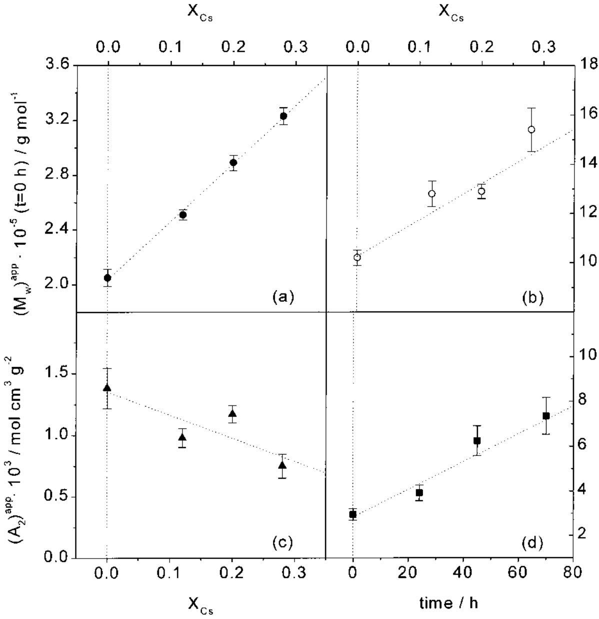

Figure 5. Experimental light scattering parameters obtained for κ-carrageenan (at 25 °C) by means of a standard linear extrapolation procedure. (a−c) Values calculated immediately after the preparation of the samples ($t = 0$ h) as a function of X_{Cs}. (d) Values calculated for $X_{Cs} = 0.28$ at different times.

The possible time dependence of aggregation was investigated by performing different measurements on the same samples at different times. The values of $(M_w)^{apparent}$ for the case of $X_{Cs} = 0.28$ are reported in Figure 5d. Also with time, the apparent relative molar mass appears to increase, suggesting that the association process is not very rapid (at least in those conditions). The effect of time should then not be overlooked in drawing conclusions on the process. On the other hand, the time scale is so long that for the duration of a typical LS experiment (i.e., about 2 h) the system can be considered as amenable to treatment by an equilibrium formalism, namely, the open association model by Elias.[27] The calculated association constants should more properly be called time-dependent association ratios, $K(t)$. Nevertheless, for the above argument, they will be treated operationally as constants, K, that progressively increase toward the true equilibrium value at $t \rightarrow \infty$, K_{eq}.

Evaluation of the Molar Mass of the Polyelectrolyte in Mixed Counterion Conditions. In the treatment of Elias, the molar mass of the fundamental isolated chain needs to be known. Although the weight-average degree of polymerization, DP_w, of the sample can be calculated from the determined value of M_w and that of the molar mass of the repeating unit in the sodium form, the latter is not known a priori in NaI/CsI mixed systems. The most obvious hypothesis is that a Na^+/Cs^+ exchange on the sulfate group takes place, so that the counterion distribution on the polymer chain reflects the salt composition in solution. In this case, the molar mass of the repeating unit, $(M)_0$, is given by

$$(M)_0 = (408X_{Na} + 518X_{Cs}) \tag{5}$$

where X_{Na} and X_{Cs} are, respectively, the equivalent fractions of sodium and cesium and 408 and 518 are the corresponding molar mass values of the repeating units of the two pure forms.

The validity of this assumption was tested at the highest Cs^+ concentration ($X_{Cs} = 0.40$). The resulting polymer molar mass was 240 000, whereas the expected one, according to eq 5 and the DP_w value obtained for the pure Na^+ form, was 239 000. It corresponds to a DP_w of 531, very close to the mean value (529) obtained in NaCl and NaI solutions. Given the excellent agreement between the values, it was decided to fix the average value of DP_w to 530 for all the other investigated compositions.

Determination of the Macromolecular and Association Parameters in the Presence of Cs^+. The OAM was applied to all mixed counterion systems (X_{Cs} = 0.12, 0.20, 0.28, and 0.40). Calculations were first made at $t = 0$, in each case fixing the value of $(M_w)_0$ according to eq 5, mostly to determine $(R_g)_z$ (but also K and A_2). The rationale for this is the following. Due to lack of information on the geometrical architecture of the interpolymeric assemblies and the complexity of the corresponding form factor(s), no attempt was made to calculate $(R_g)_z$ for the associated forms at finite concentrations. In fact, the value of $(R_g)_z$ coming out from the fitting pertains to the condition of infinite dilution, being therefore that of the isolated fundamental ordered chain, $(R_g)_{z,0}$. Still, one cannot completely rule out that the numerical value of $(R_g)_{z,0}$ may be biased, at least partly, by the trend of the values of the reduced scattering intensity at finite concentration, i.e., in the presence of association. In fact, a slight but nonnegligible trend to increase with increasing time (that is, increasing association) was noticed. It was therefore decided that the most reliable value of $(R_g)_{z,0}$ should be that obtained for $t = 0$. A comparative analysis of the results will show that our hypothesis is amply justified (vide infra). The value of $(R_g)_{z,0}$ so calculated for the isolated chain was then used as a fixed parameter [together with the proper fixed value of $(M_w)_0$] to eventually get the values of K and A_2 for all X_{Cs} values. All the values of the parameters are reported in Table 1b–e. As a general statement, one could say that the results are internally consistent, with comparatively low values of the standard deviation that enable one to make safe quantitative comparisons. The results have been collectively reported as a function of X_{Cs} in Figure 6, using instead of K the corresponding value of $\Delta G°$.

The time-zero results show that, in the very first lapse of time after preparation of solution, a constant substantial extent of intermolecular association takes place over the whole X_{Cs} range 0.12–0.28, as shown by a constant value of the apparent association constant of 1.0×10^5.

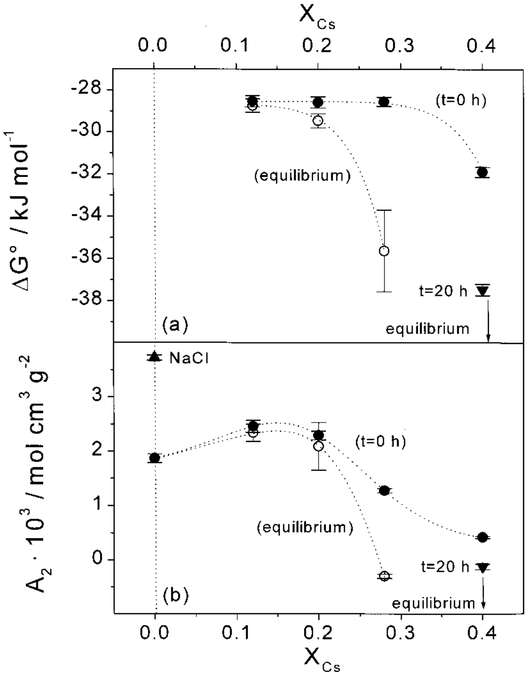

Figure 6. X_{Cs} dependence for κ-carrageenan (at 25 °C) of the free energy of association (a) and the second virial coefficient (b) at $t = 0$ h (●) and at equilibrium (○). The triangles at XCs = 0.40 represent experimental values at t = 20 h. The lines have been drawn to guide the eye.

The second virial coefficient shows a downward curvature with a maximum at about $X_{Cs} = 0.12$ (Figure 6b). This apparently anomalous behavior can be easily reconciled with the interplay of two opposing effects. The first one is related to the initial mixing of Cs^+ and Na^+ counterions in the condensation volume around the iodide-stabilized helix. This fact certainly brings about a perturbation of any quasi-regular arrangement of the condensed Na^+ counterions in the pure sodium form of the polyelectrolyte. The accompanying increase in entropy will then disfavor polymer–polymer intersegmental interactions and produce a positive effect on A_2. However, as soon as the equivalent fraction of Cs^+ increases, the probability of forming cooperative stretches of helix in the pure Cs^+ form also increases. This brings about a negative entropic contribution, likely to be accompanied by the development of even more favorable polymer–polymer interactions, stemming from the peculiar specificity of Cs^+ for the ordered κ-carrageenan chain. This latter negative effect should be triggered by a threshold value for the formation of the cooperative segment, thereby manifesting itself with a delay on the X_{Cs} scale. However, it is also expected to be quantitatively larger than the former one, altogether producing a maximum of A_2 as a function of X_{Cs} but then very rapidly negative A_2 values. In fact, Cs^+ ions can condense on specific sites on the isolated iodide-stabilized helix but still preserve their translational freedom within the volume immediately surrounding the chain: this was indicated by an elegant ^{133}Cs NMR experiment (H. Grasdalen, private communication of unpublished results). A few Cs^+ ions ($X_{Cs} \ll 0.1$) were added to a κ-carrageenan solution in aqueous tetramethylammonium iodide 0.15 M at 25 °C: a chemical shift of the cesium signal was observed, very similar to the one reported[22] for ordered κ-carrageenan in the gel form (obtained in the absence of iodide). However, at variance

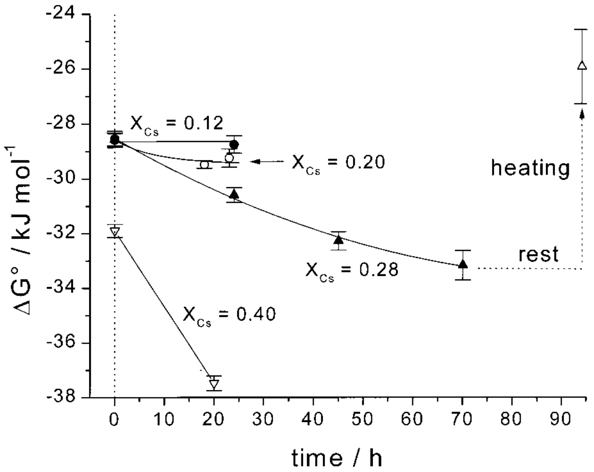

Figure 7. Time dependence of the free energy of association of κ-carrageenan (at 25 °C) at different values of X_{Cs}. The data at $X_{Cs} = 0.28$ have been fitted by an exponential decay in order to estimate the equilibrium conditions. In the other cases, the lines have been drawn to guide the eye.

with the latter case, no line broadening was observed, thus indicating that in the conditions of existence of the isolated iodide-stabilized single helix the condensed Cs^+ ions are not trapped in rigid structures such as those proposed for the interchain junction in the gel.

The quantitative analysis of the scattering data for $X_{Cs} = 0.40$ shows an increase of the apparent association constant by a factor about 4 with respect to the measurement at $X_{Cs} = 0.28$. This result is in partial agreement with the findings reported by Wittgren et al.[7] from field-flow fractionation-multiangle light scattering (FFF-MALS) and by Viebke et al.[8] from differential scanning calorimetric (DSC) data, both reporting a threshold value at $X_{Cs} = 0.4$ for aggregation of κ-carrageenan. However, in the latter paper the authors state that no aggregation occurs at X_{Cs} lower than 0.4, while, on the contrary, an associative behavior at low X_{Cs} (0.12−0.28) turns out clearly from our results. This issue finds even stronger evidence from the time evolution of the associative process, which is discussed in the following paragraph.

Time Effects. The aggregation of κ-carrageenan has already been described[19,24] as a continuous open process; that is, the apparent molar mass increases continuously with time, possibly without reaching an equilibrium even at long times. The recent work by Meunier et al.[19] has been devoted to a better characterization of this behavior. However, that study was limited to about 14 h, and decreasing temperature in the presence of K^+ induced the association. Thus no observation at longer times, with Cs^+ as the association-inducing ion, has been reported yet.

The stability of the samples at $X_{Cs} = 0.12$ was tested 24 h after the first measurement, but no significant increase of the association constant could be detected beyond the experimental error (Table 1b). The average of the two $\Delta G°$ values was taken as the equilibrium value, $(\Delta G°)_{eq}$ (Figures 6 and 7).

The same procedure was applied at $X_{Cs} = 0.20$, with repeated measurements at 18 and 23 h. In this case (Table 1c) the association constant results are slightly higher at $t = 18$ h, while no further change occurs at $t = 23$ h. The average of the two latter $\Delta G°$ values was taken as the equilibrium value, $(\Delta G°)_{eq}$, for $X_{Cs} = 0.20$ (Figures 6 and 7).

Very different behavior was observed at $X_{Cs} = 0.28$, as reported in Table 1d and Figure 7. In this case a continuous, linear increase of the association constant can be observed at different times up to 70 h, while the second virial coefficient falls to a negative value already at $t = 24$ h and remains constant thereafter. At $t = (70 + 24)$ h the solutions at different C_p for $X_{Cs} = 0.28$ were heated for 1 h at 60 °C, then immediately subjected to a light scattering measurement at 25 °C. The resulting association constant is even lower than the one at $t = 0$ h, clearly pointing to a thermally induced dissolution of the polymer aggregates. The data at $X_{Cs} = 0.28$ show that aggregation is still proceeding after 70 h, even though the upward curvature of the plot indicates that the system is slowly approaching an equilibrium state. In a purely phenomenological approach, we fitted the $\Delta G°(t)$ data at $X_{Cs} = 0.28$ using the general equation for the exponential decay of the first order. The curve in Figure 7 corresponds to that fitting. The calculated equilibrium free-energy value, $(\Delta G°)_{eq}$, is as low as -35.6 ± 1.9 kJ mol^{-1} (Figure 6), and the curve is predicted to reach 99% of its asymptotic value for $t \cong 300$ h.

A very pronounced time-dependent association was found at $X_{Cs} = 0.40$ (Table 1e, Figure 7). Owing to the extreme curvature of the Zimm plots (see Figure 3), in this case it was not possible to perform measurements at times longer than 20 h. The analysis of the data, however, reveals a 10-fold increase of the apparent association constant within this period. It is worth mentioning that a visual inspection during the second measurement ($t = 20$ h) revealed that the scattering was not uniform, showing a peculiar sparkling of the laser beam. This is most likely due to a colloidal-type phase separation, with a polymer-enriched suspended phase on a semimacroscopic scale. In any event and despite the lack of data at times longer than 20 h, it turns out clearly that at $X_{Cs} = 0.40$ aggregation is much faster and more extensive than at $X_{Cs} = 0.28$.

The results reported above can be summarized by stating that, under the given experimental conditions, the Cs^+- induced aggregation process of κ-carrageenan develops continuously, albeit showing quantitative differences depending on the salt composition. At $X_{Cs} = 0.12$, in fact, no time dependence can be observed, even though the extent of association, given by the apparent association constant, is already noticeable. At $X_{Cs} = 0.20$ a small time dependence is shown, but an equilibrium is seemingly reached relatively fast within 18 h, as no further change of the experimental parameters is detectable thereafter. At $X_{Cs} = 0.28$, on the contrary, a marked time dependence can be observed. At $X_{Cs} = 0.40$, however, the aggregation process is notably faster and more extended than at $X_{Cs} = 0.28$. This could possibly confirm the existence[7,8] of a sort of threshold value at $X_{Cs} = 0.40$. A preliminary hypothesis might involve the formation, at a certain minimum cesium fraction, of a cooperative sequence of condensed Cs^+ ions on the κ-carrageenan chains, which could strongly promote the interchain association process. However, further data are necessary to fully describe the kinetic and thermodynamic behavior of the system in the X_{Cs} range 0.28−0.40, as well as at higher cesium content.

Chain Dimensions and Stiffness. The values of the radius of gyration of κ-carrageenan as a function of X_{Cs} are reported in Figure 8a. The corresponding values of

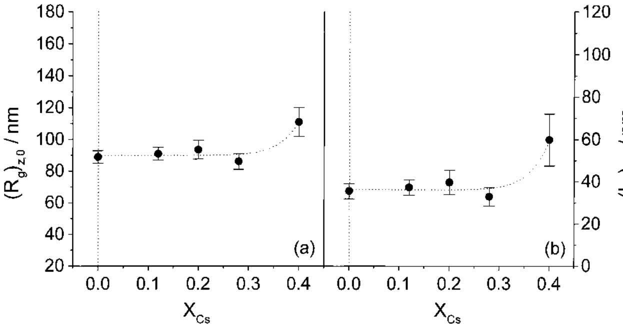

Figure 8. Dependence on X_{Cs} of the dimensional parameters $(R_g)_{z,0}$ (a) and $(L_p)_{z,0}$ (b) for *κ*-carrageenan (at 25 °C).

the persistence length, L_p, are given in Figure 8b. $(R_g)_{z,0}$ and L_p are practically constant within the experimental error in range of salt composition from 0 to 0.28. The latter finding shows that the chain dimensions and stiffness are practically invariant of upon the initial progressive exchange of Na^+ with Cs^+. In addition, they strongly suggest that the choice made about $(R_g)_{z,0}$ is a very reasonable one.

For $X_{Cs} = 0.40$ an increase of the values both of $(R_g)_{z,0}$ and of L_p is noticed. Given the very high tendency of the polysaccharide toward association under those circumstances, one cannot exclude that the numerical value of $(R_g)_{z,0}$ may be affected by the strong nonlinearity of both the angular and the concentration dependence of KC_p/R_θ. Therefore, the values of $(R_g)_{z,0}$ and L_p should be considered as upper bounds of the true values. Nevertheless, there is no reason to believe that such an error is significantly larger than the standard deviation reported in Table 1. Then, the helix at high content of cesium is somewhat more expanded and more rigid than the corresponding helix in the pure (or prevailing) sodium form. This could well stem from a better accommodation of the little-solvated Cs^+ ions around the chain, in clusters of similar counterions associated with a stretch of chain corresponding to the cooperative unit. It is not difficult then to conceive how prone such an arrangement will be to finding its natural counterpart on another (facing) chain, thereby giving rise to the elementary junction unit for the ensuing massive association process. Finally, it should be pointed out that the value of L_p obtained for our system in iodide for $X_{Cs} = 0.40$ (i.e., 60 nm) is very close to the lower limit recently suggested by Borgström et al.[35]

Conclusions

The main conclusions of the paper are the following: (i) It has been confirmed that the attainment of an ordered form of *κ*-carrageenan is possible without any increase in the molar mass of the polymer. (ii) Such a single helix is considerably more expanded [$(R_g)_z$] and more rigid (L_p) than the disordered form. (iii) The addition of Cs^+ ions brings about an increase of the molar mass of *κ*-carrageenan, which can be traced back to two contributions. As to the isolated chain, an increase of $(\bar{M}_w)_0$ is due to the exchange of Na^+ with Cs^+ counterions. This process reflects the ionic composition of the solution. For finite values of the polymer concentration, a thermally reversible association immediately takes place. Under all investigated conditions LS experiments could be successfully performed on the associating system. (iv) The association process requires an amount of time to reach a true equilibrium, which is proportional to X_{Cs}. It has been possible to calculate the equilibrium value of the association constant up to a value of the equivalent ionic fraction of cesium of 0.28. The corresponding values of $\Delta G°$ show a dramatic tendency to decrease upon increasing X_{Cs}. (v) The dimensions and the stiffness of the isolated chain seem to increase significantly only when X_{Cs} reaches the value of 0.40.

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References and Notes

(1) Rees, D. A.; Morris, E. R.; Robinson, G. *J. Mol. Biol.* **1980**, *138*, 349−362.
(2) Bongaerts, K.; Reynaers, H.; Zanetti, F.; Paoletti, S. *Macromolecules* **1999**, *32*, 675−682.
(3) Slootmaekers, D.; De Jonghe, C.; Reynaers, H.; Varkevisser, F. A.; Bloys van Treslong, C. J. *Int. J. Biol. Macromol.* **1988**, *10*, 160−168.
(4) Ueda, K.; Itoh, M.; Matsuzaki, Y.; Ochiai, H.; Imamura, A. *Macromolecules* **1998**, *31*, 675−680.
(5) Bongaerts, K. Ph.D. Thesis, Katholieke Universiteit Leuven, Heverlee, Belgium, 1999.
(6) Ciancia, M.; Milas, M.; Rinaudo, M. *Int. J. Biol. Macromol.* **1997**, *20*, 35−41.
(7) Wittgren, B.; Borgström, J.; Piculell, L.; Wahlund, K. G. *Biopolymers* **1998**, *45*, 85−96.
(8) Viebke, C.; Borgström, J.; Carlsson, I.; Piculell, L.; Williams, P. *Macromolecules* **1998**, *31*, 1833−1841.
(9) Hjerde, T.; Smidsrød, O.; Christensen, B. E. *Biopolymers* **1998**, *49*, 71−80.
(10) Smidsrød, O.; Grasdalen, H. *Hydrobiologia* **1984**, *116*, 178−186.
(11) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 1842−1845.
(12) Meunier, V.; Nicolai, T.; Durand, D. *Int. J. Biol. Macromol.* **2001**, *28*, 157−165.
(13) Borgström, J.; Quist, P.-O.; Piculell, L. *Macromolecules* **1996**, *29*, 5926−5933.
(14) Borgström, J.; Egermayer, M.; Sparrman, T.; Quist, P.-O.; Piculell, L. *Langmuir* **1998**, *14*, 4935−4944.
(15) Zani, V. Graduation Thesis, University of Genova, Italy, 1998.
(16) Piculell, L.; Sparrman, T.; Ramzi, M.; Borgström, J.; Quist, P.-O. *Macromolecules* **1998**, *31*, 5152−5154.
(17) Ramzi, M.; Borgström, J.; Piculell, L. *Macromolecules* **1999**, *32*, 2250−2255.
(18) Denef, B. Ph.D. Thesis, Katholieke Universiteit Leuven, Heverlee, Belgium, 1997.
(19) Meunier, V.; Nicolai, T.; Durand, D. *Macromolecules* **2000**, *33*, 2497−2504.
(20) Ueda, K.; Imamura, A.; Brady, J. W. *J. Phys. Chem.* **1998**, *102*, 2749−2758.
(21) Smidsrød, O.; Andresen, I.-L.; Grasdalen, H.; Larsen, B.; Painter, T. *Carbohydr. Res.* **1980**, *80*, c11−c16.
(22) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 229−231.
(23) Norton, I. T.; Morris, E. R.; Rees, D. A. *Carbohydr. Res.* **1984**, *134*, 89−101.
(24) Bongaerts, K.; Reynaers, H.; Zanetti, F.; Paoletti, S. *Macromolecules* **1999**, *32*, 683−689.
(25) Rochas, C.; Brûlet, A.; Guenet, J. M. *Macromolecules* **1994**, *27*, 3830−3835.
(26) Bongaerts, K.; Paoletti, S.; Denef, B.; Vanneste, K.; Cuppo, F.; Reynaers, H. *Macromolecules* **2000**, *33*, 8709−8719.
(27) Elias, H. G. In *Light Scattering from Polymer Solutions*; Huglin, M. B., Ed.; Academic Press: London, 1972; Chapter 9, pp 397−457.
(28) Slootmaekers, D.; van Dijk, J. A. P. P.; Varkevisser, F. A.; Bloys van Treslong, C. J.; Reynaers, H. *Biophys. Chem.* **1991**, *41*, 51−59.
(29) Ioan, C. E.; Aberle, T.; Burchard, W. *Macromolecules* **2001**, *34*, 326−336.

(30) Kratochvíl, P. *Classical Light Scattering From Polymer Solutions*; Elsevier: Amsterdam, 1987.
(31) Paoletti, S.; Smidsrød, O.; Grasdalen, H. *Biopolymers* **1984**, *23*, 1771−1794.
(32) Bosco, M.; Picotti, F.; Radoicovich, A.; Rizzo, R. *Biopolymers* **2000**, *53*, 272−280.
(33) Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: New York, 1961; p 210.
(34) Richards E. G. *An Introduction to Physical Properties of Large Molecules in Solution*; Cambridge University Press: Cambridge, U.K., 1980; pp 61−62.
(35) Borgström, J.; Egermayer, M.; Sparrman, T.; Quist, P. O. *Langmuir* **1998**, *14*, 4935−4944.

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