Light Scattering Investigation of ι-Carrageenan Aqueous Solutions. Concentration Dependence of Association

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Received June 7, 2000

ABSTRACT: The conformational transition of ι-carrageenan in different salt solutions under nongelling conditions has been studied by light scattering (WALLS). The focus is on the thermodynamic interactions and the concentration dependence of the reduced scattering intensity at zero angle. An upward curvature of (KC_p/R_0) in conditions of conformational ordering has been interpreted as due to a concentration-dependent reversible chain association. The open association model of Elias has been applied to analyze the data. In conditions of conformational ordering a decrease of polymer concentration, C_p, is accompanied by a decrease of $(\bar{M}_w)_{assoc}$. These results have been confirmed by using low-angle light scattering (LALLS). In the lowest range of C_p, the decreasing values of $(\bar{M}_w)_{assoc}$ are already very close to that of the single-stranded disordered conformation. In the same range, the values of the specific optical activity remain constant. This is a confirmatory evidence that the fundamental ordered conformation of ι-carrageenan is a single helix.

Introduction

Carrageenans are sulfated polysaccharides extracted from different species of marine red algae. The ability to induce gelation of aqueous solutions is one of the most useful properties of both κ- and ι-carrageenan. The idealized alternating structure of the latter copolymers is reported in Figure 1. The molecular basis of gelation has been identified with a thermoreversible, ion-induced, disorder-to-order conformational transition, followed by intermolecular association and network formation.

Whether the fundamental ordered state is a single-stranded helix or a double-stranded helix has been a matter of debate for quite some time.[1−8] In principle, molar mass (MW) determination is expected to be the method of choice to solve the problem. However, the exceptionally high tendency of both κ- and ι-carrageenan to give rise to extensive chain association was often underestimated. This feature has severely impaired the possibility of using most of the published data to obtain reliable structural information.

The first light-scattering data of ι-carrageenan, involving a discussion of the conformational change, were reported by Jones et al.[9] in 1973. Those authors performed a selective cleavage of the ι-carrageenan chain to give short segments, which did not gel, but which still exhibited the change in optical rotation commonly attributed to a coil-to-helix transition. The molar mass of those segments was determined in 0.10 M NaCl at different temperatures, and a doubling of both the weight-average molar mass ($\bar{M}_w$) and the number-average molar mass ($\bar{M}_n$) upon cooling was found. It is worth to underline that the authors observed an upward bending of the concentration dependence of the reduced scattering intensity, (KC_p/R), which, in later studies, was attributed to a concentration dependent association.

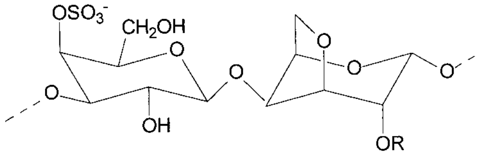

Figure 1. Repeating unit of κ- (R = H) and ι- (R = SO_3^-) carrageenan.

Morris et al.[2] carried out a similar light-scattering study on ι-carrageenan segments in the presence of potassium ions (KCl, 0.15 M). Upon cooling, substantially higher $\bar{M}_w$ values were observed, which were considered to be a direct evidence for aggregation of ι-carrageenan double-helices in the presence of K^+ ions. Light scattering measurements on native ι-carrageenan under allegedly nonaggregating conditions (Na^+ salt form) showed a 10-fold increase of $\bar{M}_w$ upon increasing the salt content. This observation was interpreted in terms of chain−chain association into limited cluster domains through double-helical junction zones.

Smidsrød and co-workers in the same year published results leading to the opposite conclusion.[4,5] The $\bar{M}_n$ values of three different samples of ι-carrageenan segments in aqueous 0.30 M LiI did not change on passing from disordering conditions (60 °C) to order-inducing conditions (30 °C), both states being revealed by specific optical activity data, [α]. At both temperatures, [α] did not change upon dilution of the polysaccharide solutions from 15% down to 0.025%. Those findings are in contrast with what is expected for a concentration-dependent association of single-stranded disordered chains, as in the case of double-helix formation.

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[‡] This paper is dedicated to Prof. M. Mandel.

10.1021/ma000996y CCC: $19.00

Another light-scattering study on segments of ι-carrageenan was presented by Norton et al.[10] Three salt forms of segmented ι-carrageenan (K^+, Na^+, and $(CH_3)_4N^+$, respectively) were studied over the range of 1:1 added salt from 0.10 to 0.50 M, at two different temperatures. An increase of the refractive index increment, d*n*/d*c*, was reported on changing temperature from 0.124 mL/g at 25 °C to 0.147 mL/g at 69 °C. A doubling of $\bar{M}_w$ was observed when $(CH_3)_4NCl$ was the added salt, whereas 2.5- and a 12-fold increases were reported in NaCl and KCl aqueous solutions, respectively. These observations were again considered as evidence for the formation of double helices followed by subsequent aggregation. However, experimental data for angles below 50° were rejected from the Zimm extrapolation, and the very dilute polymer concentration range was not explored. Both choices may well lead to a substantial overestimation of the values of $\bar{M}_w$ obtained by extrapolation to zero-angle and infinite dilution.

Austen et al.[11] reported low-angle laser light scattering (LALLS) results of ι-carrageenan segments in 0.20 M $(CH_3)_4NBr$ and observed an approximate doubling of the molar mass on cooling the carrageenan solution. Ter Meer[12] investigated the structural aspects of the thermoreversible gelation of ι-carrageenan in 0.10 M KCl and 0.25 M $(CH_3)_4NCl$, respectively. The author used both native ι-carrageenan as well as its segments. The native sample was in fact a mixture of ι- and κ-carrageenan. From light-scattering measurements, the gelling ι-carrageenan segments showed a strong tendency for aggregation even at high temperatures. It was impossible to determine their molar mass. However, from the extrapolation of the data obtained at the highest scattering angles, the temperature dependence of the molar mass could be estimated. A 2−3-fold decrease of the molar mass with increasing temperature was observed. On the other hand, light-scattering measurements of the κ/ι mixture exhibited a minimum in the concentration dependence of the inverse apparent molar mass in 0.25 M $(CH_3)_4NCl$. According to Ter Meer, this had to be ascribed to an associative interaction (“binding”) between two chemically different molecules in which one component shows a much smaller tendency to self-association—or even not at all (namely the κ-component).

Recently the solution behavior of both κ- and ι-carrageenan samples was reinvestigated by light-scattering measurements.[13] The authors interpreted the experimental data in terms of a disorder-to-order transition. They admitted that under specific conditions an intramolecular conformation transition could be seen.[4,5] However, they claimed that a doubling of the molar mass could be calculated from a linear extrapolation of the scattering data as a function of polymer concentration, under the given experimental conditions. The latter result was interpreted as an evidence for the formation of an ordered double helix. Still, the authors did admit that sometimes an upward curvature of the reduced scattering intensity at zero angle, KC_p/R_0, had been observed also in their experiments.

The influence of acid hydrolysis of ι-carrageenan has been studied in both ordered and disordered states by Hjerde et al.[14] in the presence of a high concentration of supporting electrolyte (total 0.30 M: 0.20 M LiI and 0.10 M HCl). The partially hydrolyzed ι-carrageenan, on passing from the ordered conformation to the disordered one, showed at least a 2-fold decrease in $\bar{M}_w$ leading those authors to conclude that the ordered form of ι-carrageenan is predominantly a double-stranded helix.

In another study[15] the same research group analyzed high and low MW fractions of ι-carrageenan by size-exclusion chromatography (SEC) combined with LALLS over a temperature range in which the conformational transitions occurred. Also in this case the concentration of the supporting 1:1 electrolyte was quite high (i.e. 0.20 M LiI or 0.20 M LiCl). For ι-carrageenan the authors reported an increase of $\bar{M}_w$ that was attributed to a strong tendency for aggregation of ordered chains.

An extensive analysis of the association properties and of the values of MW of κ-carrageenan across the transition has been recently reported in this journal.[7,8] Following those lines of investigation, it was decided to study the similar behavior of ι-carrageenan.

Wide-angle laser light scattering (WALLS) has been used in the present study to explore the influence of both ionic strength, *I*, and temperature, *T*, on $\bar{M}_w$ and on the polymer−solvent interactions in the very dilute polymer concentration regime. In a number of significant cases, low-angle laser light scattering (LALLS) has been additionally used to confirm the behavior of the scattering values extrapolated to zero angle from the (KC_p/R) data collected in WALLS experiments. Great attention has been paid also to the importance of a careful and accurate sample preparation and of the description of all the experimental conditions when doing light-scattering measurements in polysaccharide studies. It is well-known that the preparation procedure strongly affects the values of the molecular parameters.[7,8,16,17]

Under all experimental conditions, which encompass the conformational transition to the ordered form of native ι-carrageenan, a concentration-dependent upward bending of (KC_p/R_0) is clearly observed, which is typical for a concentration dependent association. A minimum in (KC_p/R_0) as a function of C_p was already reported in the light-scattering study on the dilute and semidilute solutions of several polymers. For example, in the case of poly(γ-benzyl-L-glutamate) (PBLG) in 1,2-dichloroethane, Wissenburg et al.[18] attributed this behavior to an aggregation effect of the semiflexible PBLG chains. Upon increasing the temperature from 25 to 50 °C, the upward curvature became less pronounced, which has been ascribed to a universal behavior of the PBLG chains in various solvents (*N*,*N*-dimethylformamide; 1,2-dichloroethane).

For the sake of clarity, the interpretation of the experimental data in the present paper is restricted to the most fundamental molecular parameter, i.e., $\bar{M}_w$. In fact, it is felt that a sound assessment of this fundamental property is a mandatory prerequisite for any further analysis, like that of the angular dependence of the scattering function or the study of dynamic light scattering. To achieve this goal, a nontrivial and self-consistent method is needed to reveal the value of $\bar{M}_w$ extrapolated to infinite dilution in conditions of conformational ordering. The observed upward bending of (KC_p/R_0) has been interpreted in terms of the open association model of Elias.[19]

One of the main goals of this work is therefore to verify experimentally if the occurrence of the ordered state of ι-carrageenan, at *low* polymer concentration, in NaCl, NaI, and LiCl, is unequivocally accompanied by a chain dimerization or if it is not. In this way it will be possible to ascertain whether an intermolecular double-stranded helix can be the *fundamental* ordered conformation of ι-carrageenan.

Materials and Methods

ι-Carrageenan samples were from Sigma Chemicals Co. (Bornem, Belgium), Type V, No. C4014 Lot 27 F0373 from *Eucheuma spinosum*. The samples were transformed into their sodium or lithium salts, respectively, following a general procedure which had already proved to be safe and reliable for avoiding undesired aggregation phenomena, for both ι- and κ-carrageenan.[7,8,16,17] The purification procedure which was followed produced samples of high compositional purity. Combined NMR and IR analyses showed that contaminating κ-type sequences amounted to 8% at most.[16] The polydispersity of the sample was checked with gel permeation chromatography GPC-LALLS, and the polydispersity index was found to be 2.0.[16] All chemicals used were analytical grade, and ultrapure water was used throughout.

Light-scattering measurements (both WALLS and LALLS) were performed as previously described.[16,20] To remove dust, the solutions were slowly filtered through Millipore filters (pore size of 0.22 μm).

Results and Discussion

Sample Preparation and Handling. Polysaccharide solutions are particularly difficult to handle due to their high viscosity and their tendency to form entanglements, aggregates and other forms of interchain association.[21] Moreover, it is common knowledge that the method for preparing polysaccharide solutions is very critical: the way in which the solutions are prepared, the filtration procedure, and the thermal treatment as well as the cleaning procedure of the sample cells may strongly affect the ultimate values of the obtained molecular parameters.[7,8,22]

Since the light-scattering method by its own nature is very sensitive to the quality of the solutions, the presence of small amounts of aggregates can strongly influence the calculated values of $\bar{M}_w$. Therefore, it is necessary to conform to "safe" protocols and to report all the experimental details concerning the sample preparation in order to guarantee the reproducibility of the measurements and to allow for comparison with data obtained by others.

In previous studies[7,8,17,23] κ-carrageenan solutions were prepared starting from either an aqueous κ-carrageenan solution of known concentration or from a weighted amount of lyophilized κ-carrageenan sample dissolved in pure water. In both cases, the proper amount of salt was added afterward to obtain the desired molarity of the final solutions. Light scattering measurements revealed poor reproducibility[16] and/or a higher MW when freeze-dried carrageenans were dissolved directly into an aqueous salt solution.[13,24] Similar observations have been reported by Lecoutier et al.[25] for light-scattering data obtained on lyophilized xanthan.

At first, light-scattering measurements were carried out starting from given aqueous salt solutions to which a known amount of lyophilized ι-carrageenan in water was added under vigorous stirring. A dilution series was obtained by addition of increasing amounts of the 1:1 electrolyte solutions. Concentrations of ι-carrageenan used for the experiments ranged between 0.2 and 1.8 g$\cdot$L^{-1} and the (final) simple salt concentrations varied from 0.02 to 0.10 M. All solutions underwent light-scattering measurements immediately after preparation, starting with the lowest polymer concentration.

Table 1. Weight-Average Molar Mass Values of ι-Carrageenan in Different Salt Solutions As Obtained from WALLS Experiments at 25 °C[a]

	$\bar{M}_w \times 10^{-3}$ for salt types		
C_s (M)	NaCl	NaI	LiCl
0.02	400	206	278
0.04	212	262	289
0.05	547	312	250
0.07	681	323	300
0.10	1865	571	1302

[a] Data obtained immediately after sample preparation.

From the initial light-scattering measurements unusual Zimm plots were obtained. The $\bar{M}_w$ values of ι-carrageenan obtained from the linear extrapolation to $C_p = 0$ of the initial light-scattering data are summarized in Table 1. A general tendency to an increase of $\bar{M}_w$ as a function of increasing salt concentration is apparent. All the experimental observations point to an irreversible association/aggregation effect as manifested by irregular Zimm plots and by the presence of time-dependent effects.[16,20] Filter clogging was frequently observed. It can be concluded that such a method of preparation gives nonreproducible and then unreliable molar mass values. Moreover, the importance of these results becomes fully manifested only if a large number of experiments are performed, since one single occasional experiment can give apparently reliable data.

Because most of the initial attempts failed to yield reproducible results, further attention has been paid to the sample preparation of ι-carrageenan solutions. To an aqueous carrageenan solution of known C_p, a fixed amount of a salt solution was slowly added while stirring. After filling up the volumetric flask with pure water to the graduation mark to get the desired polymer and salt concentrations, the solutions were stored for 24 h at room temperature. The concentrations of ι-carrageenan used for the light-scattering experiments were reduced to the range between 0.02 and 0.50 g$\cdot$L^{-1}. All solutions were filtered immediately after preparation. The reason the solutions were stored for 24 h before measuring became clear from the following experiments. The scattered light intensity was measured as a function of time from freshly prepared Na$-\iota$-carrageenan solutions (0.37 g$\cdot$L^{-1}) in aqueous 0.08 M NaCl and in NaI, respectively, as reported earlier in ref 20. A rapid increase of the light-scattering intensity was observed during the first 2 h after the filtration, followed by a slower increase, up to constant values after approximately 5 h. The observed effect was less pronounced in similar experiments, carried out in 0.03 M salt solutions and at lower carrageenan concentration (0.09 g$\cdot$L^{-1}). Such findings point to a nonequilibrium state of ι-carrageenan solutions at high salt concentrations immediately after preparation. Also the perturbing effect of shear during the filtration step should probably be taken into serious account. Another point of interest is the fact that in NaI the effect was less pronounced, which illustrates the influence of the anion in the process.

The influence of time after sample preparation on the molecular parameters is additionally demonstrated in Figure 2. A linear behavior of (KC_p/R_0) with a positive slope is observed when measured immediately after sample preparation. If the solution is stored for some time at ambient temperature, a concentration depend-

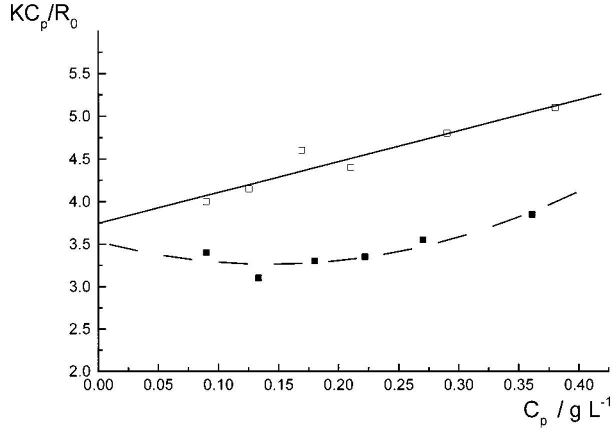

Figure 2. Concentration dependence of the reduced scattered intensity at zero angle of ι-carrageenan in 0.08 M NaCl: (□) measured immediately after sample preparation and filtration; (■) measured after 24 h. The curves have been drawn to guide the eye.

ent upward bending of the zero angle light scattering is observed. The data points of the latter nonlinear curve keep constant and well reproducible for several hours, suggesting that thermodynamic equilibrium has been reached. All following experiments were performed on solutions at thermodynamic equilibrium.

The "Open Association Model" of Elias. To obtain KC_p/R_0 from WALLS data, the reduced scattered intensity values, KC_p/R_θ, have to be fitted with a function of $\sin^2(\theta/2)$. The intercepts give then the values of KC_p/R_0. For all cases, a polynomial function of the second order, i.e., $F(\sin^2(\theta/2)) = b_0 + b_1 \sin^2(\theta/2) + b_2 (\sin^2(\theta/2))^2$, where $F(\sin^2(\theta/2)) = KC_p/R_\theta$ and $b_0 = KC_p/R_0$, has given almost the same value of χ^2 as the linear one. However the b_2 values have always been very low, and therefore negligible. Thus, preference has been given throughout to the linear extrapolation procedure to obtain KC_p/R_0. A detailed discussion of this point is reported in the following paper.[26]

At higher salt concentrations and lower temperatures the concentration dependence of KC_p/R_0 shows a minimum. The trend of the KC_p/R_0 vs C_p plots is markedly curved in a very asymmetric way, as one expects for associating systems. Hence, it is not surprising to find that the familiar classical light-scattering equation for nonassociation systems, eq 1, gives a very poor fit of the data:

$$\frac{KC_p}{R_0} = \frac{1}{(\bar{M}_w)_0} + 2A_2C_p \tag{1}$$

Here all the symbols have their usual meaning, and $(\bar{M}_w)_0$ stands for the weight-average molar mass of the fundamental molecular form undergoing association.

In Figure 3a an example of the linear fitting is shown for Na$^+$–ι-carrageenan in 0.08 M NaCl at 25 °C. A similar behavior is also shown by all the other measurements, both in the WALLS and in the LALLS modes. It can be qualitatively assessed by inspection of Figure 3a and more quantitatively from the large values of χ^2 given in Table 2, that the linear fitting is not at all adequate, erroneously overestimating the values of $(\bar{M}_w)_0$ for ι-carrageenan in associating conditions.

Some improvement is obtained by using a virial expansion including a third term:

$$\frac{KC_p}{R_0} = \frac{1}{(\bar{M}_w)_0} + 2A_2C_p + 3A_3{C_p}^2 \tag{2}$$

The curve obtained by quadratic fit is reported in Figure 3b; the lower value of χ^2 (see Table 2) points to a better fit. The negative value of the second virial coefficient, A_2, is strongly suggestive of an association process. Still, the intrinsic symmetry of eq 2 does not seem to provide the best shape for fitting the experimental data. Empirical hyperbolic functions of the type of eqs 3 and 4 appear to be more adequate as shown by their fitting curves given in Figure 3, parts c and d, respectively.

$$\frac{KC_p}{R_0} = \frac{1}{(\bar{M}_w)_0 + \alpha C_p} + 2A_2C_p \tag{3}$$

$$\frac{KC_p}{R_0} = \frac{1}{[{(\bar{M}_w)_0}^2 + \beta C_p]^{1/2}} + 2A_2C_p \tag{4}$$

The progressive improvement of the fitting on passing form the classical light-scattering equation at zero angle to eqs 3 and 4, as indicated by a decrease of χ^2, is accompanied by a decrease in $(\bar{M}_w)_0$.

The general equation for light scattering of associating systems in solution can be defined as follows:

$$\frac{KC_p}{R_0} = {(\bar{M}_w)_{assoc}}^{-1} + 2A_{2,0}(C_p)C_p \tag{5}$$

Here $(\bar{M}_w)_{assoc}$ is the apparent weight-average molar mass of the associating system (which is a function of C_p) and $A_{2,0}(C_p)$ the second virial coefficient of the associating system, which in principle can be a function of $(\bar{M}_w)_{assoc}$, and therefore of C_p.

Associating systems can be analyzed by various physical models. The one which by general consensus seems to give the best results is the so-called "open association" model, as described by Elias.[19] In this model, a continuous distribution of the associated forms of the fundamental nonassociated form—the "unimer"—into dimers, trimers, tetramers, ..., is assumed. Each step is described by an equilibrium constant, K_1, K_2, K_3, K_4, ... = K_{app}, assuming that all steps of the open association are thermodynamically equivalent.

Since any intermolecular association process is always accompanied by a change of particle mass, it can be studied by means of the concentration dependence of $(\bar{M}_w)_{assoc}$. The observed initial decrease of KC_p/R_0 with increasing C_p is due to an increase of the degree of association. For higher values of C_p the curve will increase again due to the prevailing effect of the second virial coefficient. The expression for $(\bar{M}_w)_{assoc}$ according to Elias is given by eq 6. Here $(\bar{M}_w)_0/(\bar{M}_n)_0$ is the

$$(\bar{M}_w)_{assoc} = \sqrt{{(\bar{M}_w)_0}^2 + 4000K_{app}\frac{(\bar{M}_w)_0}{(\bar{M}_n)_0}(\bar{M}_w)_0C_p} \tag{6}$$

polydispersity of the polymer in nonassociating conditions, PI, and K_{app} is the "apparent" association constant. In the present work the latter value will be treated as a phenomenological thermodynamic parameter, the absolute value of which and the dependence of which on temperature, ionic strength and other

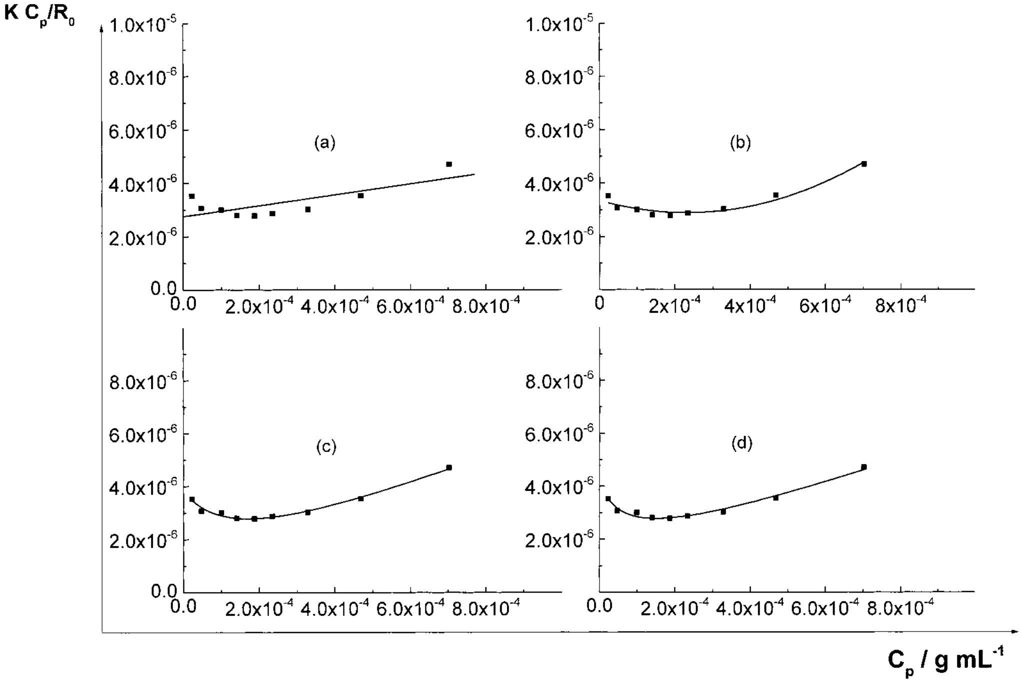

Figure 3. KC_p/R_0 plots of Na$^+$–ι-carrageenan in 0.08 M NaCl at 25 °C. Lines represent the following: (a) linear fitting (eq 1); (b) quadratic fitting (eq 2); (c and d) hyperbolic fitting based on eqs 3 and 4, respectively, of the experimental data points.

Table 2. Weight-Average Relative Molar Mass Values and the Second Virial Coefficients for Na$^+$–ι-Carrageenan in 0.08 M at 25 °C, Obtained by Using Different Fitting Equations

	$\bar{M}_w \times 10^{-3}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$\chi^2 \times 10^{14}$
eq 1	362 ± 30	1.0 ± 0.4	19.1
eq 2	298 ± 11	−2.0 ± 0.5	2.6
eq 3	260 ± 8	2.8 ± 0.1	0.7
eq 4	240 ± 14	2.6 ± 0.1	1.0

physical-chemical parameters will not be interpreted on the basis of any particular model.

By substitution of $(\bar{M}_w)_{\text{assoc}}$ into eq 5, one gets

$$\frac{KC_p}{R_0} = \frac{1}{\{(\bar{M}_w)_0^{\,2} + 4000K_{\text{app}}\text{PI}(\bar{M}_w)_0C_p\}^{1/2}} + 2A_2(C_p)C_p \tag{7}$$

It is immediately evident that the analytical form of eq 7 is the same as that of eq 4. Although eq 7 is a three-parameter equation (namely of $(\bar{M}_w)_0$, K_{app}, and $A_2(C_p)$), the value of $(\bar{M}_w)_0$ has to be consistently the same for all cases, if the same polymer sample is used both in nonassociating and in associating conditions. Therefore, when $(\bar{M}_w)_0$ is fixed to the value of the nonassociating form, eq 7 can be simplified to an equation with two parameters, i.e. K_{app} and $A_2(C_p)$.

Determination of $(\bar{M}_w)_0$. Having ascertained that interchain association is an intrinsic feature of the solution behavior of ι-carrageenan, it is very important to obtain a sound estimate of $(\bar{M}_w)_0$. The series of experiments at constant T (T = 25 °C) and variable salt concentration (C_s), for both NaCl and NaI and from WALLS and LALLS, are considered at first. The hyperbolic-type of curve fitting has been previously demonstrated to be the best one. However, to avoid introducing even the slightest form of bias into the data treatment it was chosen on purpose to use the quadratic-type of fitting of the scattering data for C_s values equal or larger than 0.06 M, where major departure from linearity appears to be evident. For low C_s values (i.e. <0.06 M) a linear extrapolation has been used for the same reasons. In this way, a series of $\bar{M}_w$ values as a function of C_s have been obtained, which can be undoubtedly considered as *upper bounds* with respect to the real value of $(\bar{M}_w)_0$. A plot of the results of such calculations is reported in Figure 4b, both for NaCl and NaI and for WALLS and LALLS. The points are somewhat scattered and a statistical analysis reveals that they can be well described by a linear correlation as a function of C_s (i.e. $\bar{M}_w(C_s) = \bar{M}_w(C_s = 0) + a_1C_s$), with a positive slope, a_1 = 0.54). This is a further indication that an ionic strength-induced association is indeed present, and that the value of $\bar{M}_w(C_s = 0)$ = 203 000 ± 18 000 can be taken as an upper bound for $(\bar{M}_w)_0$.

In Figure 4a, the result of the application of the same procedure to the data obtained as a function of temperature in 0.08 M NaCl is reported. Again, some solutions underwent also LALLS analysis. As it can be seen, also in this case $\bar{M}_w(T)$ decreases on increasing temperature. If the values of the two lowest temperature measurements are left out, the series can be fitted with a polynomial function leveling off at 200 000 as found in the salt-concentration dependent series. By comparing the two sets of independent data, it was concluded that a value of 200 000 for $(\bar{M}_w)_0$ of the Na$^+$–ι-carrageenan form can be very safely assumed for the further calculations.

In the case of the Li$^+$ salt form, the same procedure was followed for the data obtained as a function of the molar concentration of LiCl (results not reported). Again a linear correlation between $\bar{M}_w(C_s)$ and C_s is obtained, the positive slope being a_1 = 0.53, also in this case indicating the presence of interchain association. The value of the intercept, $\bar{M}_w(C_s = 0)$ = 197 000 ± 31 000, can be taken as the upper bound of $(\bar{M}_w)_0$, the true value of the relative molar mass of the nonassociated species. $(\bar{M}_w)_0$ was taken as 200 000 for the Na$^+$ salt form, with

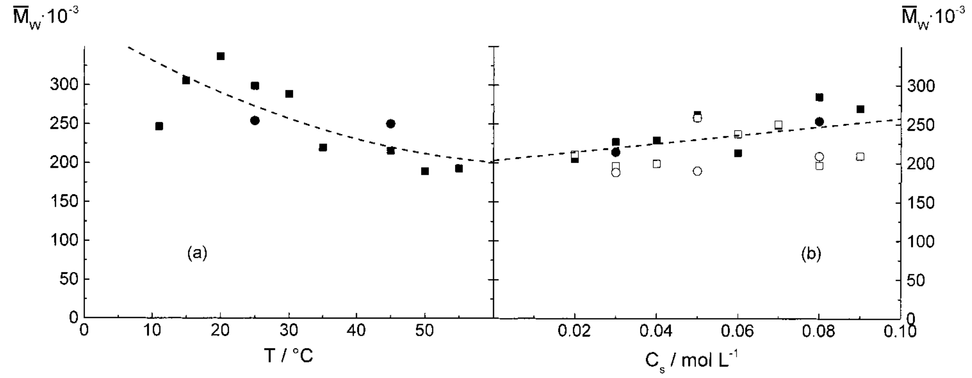

Figure 4. $\bar{M}_w$ of Na$^+$–ι-carrageenan: (a) as a function of temperature in 0.08 M NaCl (full squares, WALLS; full circles, LALLS); (b) as a function of the concentration of salt (C_s) at 25 °C in water. Key: full symbols, NaCl (squares, WALLS; circles, LALLS); open symbols, NaI (squares, WALLS; circles, LALLS).

Table 3. Dependence of the Second Virial Coefficient on Polymer Concentration for ι-Carrageenan in Aqueous NaCl Solutions: Comparison between Different Fitting Procedures

C_s (M)	dependence	$K_{app} \times 10^{-5}$	$A_{2,0} \times 10^3$ (mL·mol·g^{-2})	$B_2 \times 10^3$	$\chi^2 \times 10^{14}$
0.08	const, eq 8	6.24 ± 0.68	2.95 ±0.20		1.42
	linear, eq 9	6.89 ± 2.37	3.58 ± 2.03	0.24 ± 0.72	1.83
	log, eq 10	7.09 ± 3.11	4.02 ± 3.64	1.84 ± 5.98	1.83
0.09	const, eq 8	4.89 ± 0.24	2.36 ±0.90		0.26
	linear, eq 9	4.43 ± 0.69	1.75 ± 0.89	−0.30 ± 0.47	0.30
	log, eq 10	4.34 ± 0.86	1.43 ± 1.44	−1.88 ± 3.06	0.31

some logical priority in view of the larger number of experimental data points available for that case. The value of $(\bar{M}_w)_0 = 187\,000$ was then consistently obtained for Li$^+$–ι-carrageenan, taking into account the difference in atomic weight between Na$^+$ and the Li$^+$ ions (two ions per repeating unit). This value lies well within the experimental error, and it will therefore be used in the following calculations. The values of $(\bar{M}_w)_0$ for the two salt forms correspond to a weight-average degree of polymerization, $\langle\Pi\rangle_w$, of 391.

Before proceeding further, it should be clearly stressed that *neither* the choice of a particular association model (i.e. eq 7) *nor* the choice of $(\bar{M}_w)_0$ implies any bias toward either of the two models of carrageenan conformation. At infinite dilution, mass-action law prescribes that both associated single helices, associated double helices, and double helices themselves dissociate into isolated chains. Only the combination of $\bar{M}_w$ *and* conformation-sensitive properties (like, e.g., optical activity) can resolve the conformational problem.

Concentration Dependence of A_2. Interchain association can be accompanied by changes in the dimensions of the macromolecular solute, and an effect on the values of the second virial coefficient cannot be excluded a priori. In the absence of any previous information for ι-carrageenan, different mathematical models have been used to investigate whether there is any dependence of A_2 on $(\bar{M}_w)_{assoc}$ and hence on C_p. Three different cases are analyzed and compared. In the first case, no concentration dependence on the second virial coefficient is assumed:

$$A_2(C_p) = A_{2,0} = A_2 \tag{8}$$

A linear dependence was considered as a second possibility:

$$A_2(C_p) = A_{2,0} - B_2(1 - ((\bar{M}_w)_{assoc}/(\bar{M}_w)_0)) \tag{9}$$

In the third case the logarithmic dependence was used (resembling the results of the study of κ-carrageenan samples of different MW, in the disordered conformation[27]), having

$$A_2(C_p) = A_{2,0} - B_2 \log((\bar{M}_w)_{assoc}/(\bar{M}_w)_0) \tag{10}$$

K_{app}, $A_{2,0}$, and B_2 values have been calculated for the data of ι-carrageenan both in 0.08 M NaCl and in 0.09 M NaCl, each time using eq 7 and one of the three different concentration dependence forms of A_2. The values of $(\bar{M}_w)_0$ and of PI were always fixed at 200 000 and 2, respectively. The results are given in Table 3. It can be seen that there is no clear-cut dependence of A_2 on $(\bar{M}_w)_{assoc}$ in the investigated C_p regime. In fact, in the cases of the application of eqs 9 and 10, both the errors on $A_{2,0}$ and the χ^2 values do not indicate any significant improvement of the fitting. It was therefore decided not to introduce any specific correction for the second virial coefficient dependence on $(\bar{M}_w)_{assoc}$, (and hence on C_p) but rather to use a constant value of A_2 throughout.

Analysis of the Results Obtained under Different Experimental Conditions. a. Data as a Function of the Concentration of the Added Salt. An extensive WALLS analysis was carried out of the conformational and association behavior of ι-carrageenan in aqueous salt solutions at different molarity values, covering the region where the coil-to-helix conformation takes place as indicated by optical activity measurements.[28] The salts used were NaCl, NaI, and LiCl. The investigated C_p range varied from 1×10^{-5} to 9×10^{-4} g·mL^{-1} and the 1:1 salt concentrations were between 0.02 and 0.09 M. The series in LiCl was repeated twice, after a long time interval (i.e., 3 years), to check reproducibility. The specific optical activity, [α], of ι-carrageenan had already been shown to be independent of C_p, over a wide range of C_p, for various values of conformational order and for all salts considered.[20] [α] was used to assess the extent of conformational transformation. At 25 °C, [α] indicated that the system

Table 4. Thermodynamic Parameters of ι-Carrageenan in NaCl at 25 °C Obtained from WALLS and LALLS Data under Thermodynamic Equilibrium Conditions

C_s (M)	$K_{app} \times 10^{-5}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$\chi^2 \times 10^{14}$	$(c^*)_0$ (g·L^{-1})
0.02	(≤0.01)	9.15 ± 0.13	3.27	0.55
0.03	(≤0.01)	7.15 ± 0.21	4.92	0.70
0.03[a]	1.07 ± 0.26[a]	9.22 ± 0.37[a]	2.70[a]	0.54[a]
0.04	0.67 ± 0.32	8.11 ± 0.70	3.20	0.62
0.05	3.22 ± 0.74	7.19 ± 0.41	3.87	0.70
0.05[a]	3.96 ± 0.60[a]	6.43 ± 0.33[a]	4.27[a]	0.78[a]
0.06	4.48 ± 0.10	4.23 ± 0.50	5.16	1.2
0.07	7.37 ± 0.80	4.58 ±0.19	1.38	1.1
0.08	6.24 ± 0.68	2.95 ± 0.20	1.47	1.7
0.08[a]	7.46 ± 0.65[a]	2.38 ± 0.07[a]	2.64[a]	2.1[a]
0.09	5.34 ± 0.18	2.43 ± 0.70	0.14	2.1

[a] LALLS data.

Table 5. Thermodynamic Parameters of ι-Carrageenan in NaI at 25 °C[a]

C_s (M)	$K_{app} \times 10^{-5}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$\chi^2 \times 10^{14}$	$(c^*)_0$ (g·L^{-1})
0.02	(≤0.01)	9.14 ± 0.16	3.23	0.55
0.03	(≤0.01)	8.77 ± 2.03	4.67	0.57
0.03[b]	(≤0.01)[b]	8.78 ± 0.11[b]	2.97[b]	0.57[b]
0.04	(≤0.01)	7.04 ± 0.29	11.17	0.71
0.05	2.59 ± 0.13	5.37 ± 0.10	0.24	0.93
0.05[b]	(≤0.01)[b]	4.90 ± 0.15[b]	4.56[b]	1.0[b]
0.06	1.74 ± 0.33	3.54 ± 0.36	2.30	1.4
0.07	2.41 ± 0.19	3.53 ± 0.15	0.47	1.4
0.08	1.75 ± 0.34	2.70 ± 0.37	2.44	1.9
0.08[b]	2.61 ± 0.27[b]	2.35 ± 0.20[b]	2.16[b]	2.1[b]
0.09	2.29 ± 0.27	2.36 ± 0.23	1.18	2.1

[a] Except as noted, WALLS data obtained under thermodynamic equilibrium conditions. [b] LALLS data obtained under thermodynamic equilibrium conditions.

is in a disordered conformation for molarities of the 1:1 supporting electrolyte at least up to 0.02 M, whereas the fully ordered conformation is not attained below about 0.08 M and certainly is obtained at and beyond 0.09 M. This was irrespective of the identity of the cation or of the anion. The light-scattering experiments at 25 °C were therefore performed within such salt molarity extremes.

The conformational transition of ι-carrageenan can be obtained also by variation of temperature. [α] indicated that the transition extremes for solutions containing [NaCl] = 0.08 M are at 25 (ordered form) and 40 °C (disordered form).[29] At that fixed value of ionic strength light-scattering experiments were carried out in the temperature range from 11 to 55 °C. For each type and concentration of simple electrolyte, a constant value of A_2 has been used independent of C_p, according to the conclusions of the preceding paragraph. The fitting of (KC_p/R_0) as a function of C_p using a combination of eqs 7 and 8 was excellent for all cases, as can be seen from the χ^2 values and from the errors on the fitting parameters. The results obtained at 25 °C are reported in Tables 4−6 for NaCl, NaI, and LiCl, respectively. A number of solutions was also investigated by LALLS, giving results which fully confirm those obtained by WALLS (e.g., see Figure 3 of ref 20). It is possible to conclude that the choice of the model was sound and that the presence of a polymer concentration dependent association shown by the previous findings[16,20,29] is demonstrated as a peculiar feature of ι-carrageenan in a very wide range of physical−chemical conditions. In general, the association is more extensive in conditions producing a higher conformational order, thus confirming a well-known correlation already found both for κ-carrageenan and for ι-carrageenan.[1−11]

Good character of the solvent and association-inducing ability interplay in a complex manner. Both features specifically depend on the chemical nature of counterions and co-ions. Analysis of A_2 reveals that aqueous LiCl is the best solvent for ι-carrageenan, showing the highest values of A_2 for each value of the ionic strength, I (see Figure 5). Oppositely, aqueous NaI produces the lowest values of A_2 at all salt concentrations. The relative role of the counterion and of the coion in determining the values of A_2 is worth to be analyzed. The average ratio of the values of A_2 for NaCl over the corresponding values of A_2 for NaI, for all values of C_s, is 1.1 ± 0.2, pointing to a closely similar behavior of aqueous solutions of the two co-ions with Na^+ as the counterion. On the contrary, the corresponding average ratio of A_2 for LiCl over A_2 of NaCl is 1.6 ± 0.3, showing a neatly better solvent character of aqueous Li^+ ions over Na^+ ions, with chloride as the co-ion. Anyway, for all the systems investigated the trend of A_2 with the ionic strength is parallel to the transition from the disordered conformation to the ordered one. This is expected for the latter system in which the intramolecular polymer−polymer interactions (intersegmental) are much stronger than in the disordered state. The same behavior was found for the salt-induced transition of κ-carrageenan.[17] Over and above this, the increase of I contributes to shield the repulsive polyelectrolytic interactions, and it then further brings about a decrease of the favorable polymer−solvent interactions.

The thermodynamic overlap concentration, $(c^*)_0$, can be easily calculated for the isolated, nonassociated ι-carrageenan chain using the equation[30]

$$(c^*)_0 = \frac{1}{A^2(\bar{M}_w)_0} \qquad (11)$$

where $(\bar{M}_w)_0$ is equal to 200 000 and 187 000 for the Na^+ salt form and for the Li^+ salt form of ι-carrageenan, respectively. As expected, the values of $(c^*)_0$ for NaCl and NaI are very similar, whereas those of LiCl are always the lowest ones for any given value of C_s. Quite interestingly, for all salts the range of $(c^*)_0$ values is from about 0.3 to about 3 g·L^{-1}, indicating a large chain expansion as a general feature of this polysaccharide, the Li^+ salt form being the most expanded one. This observation, albeit quite unquestionable, will certainly further benefit from the quantitative support provided by the values of the z-average radius of gyration obtained from the analysis of the angular dependence of the scattering data, which is the subject of the following paper.[26]

The analysis of the association behavior can be conveniently separated into two parts, the former one related to C_s values corresponding to almost 50% ordering or more (up to 100%), the latter one being confined to the lowest values of C_s (i.e. $C_s < 0.05$ M). In the former range of ionic strength values solutions of NaI show the lowest values of K_{app}. All that demonstrates once more the “solubilizing” properties of aqueous iodide solutions for carrageenans in the ordered conformation (at least for C_s values up to about 0.10 M), as already shown for the first time by Smidsrød and coworkers about 20 years ago.[4,5] On the average, both NaCl and LiCl solutions show K_{app} values which are slightly more than twice as large as those of NaI, the

Table 6. Thermodynamic Parameters of ι-Carrageenan in LiCl at 25 °C[a]

	first series			second series			"χ^2" average		
C_s (M)	$K_{app} \times 10^{-5}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$\chi^2 \times 10^{14}$	$K_{app} \times 10^{-5}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$\chi^2 \times 10^{14}$	$K_{app} \times 10^{-5}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$(c^*)_0$ (g·L^{-1})
0.02	4.78 ± 0.2	15.69 ± 0.035	2.807	2.43 ± 1.07	12.05 ± 0.05	54.85	4.7 ± 0.2	15.51 ± 0.04	0.34
0.03	2.75 ± 0.47	13.54 ± 0.041	2.662	(≤0.01)	7.60 ± 0.24	5.14	2.8 ± 0.5[b]	11.5 ± 0.1	0.43
0.04	4.06 ± 0.68	12.47 ± 0.04	3.279	(≤0.01)	6.57 ± 0.49	21.92	4.1 ± 0.7[b]	11.7 ± 0.1	0.43
0.05	3.24 ± 0.46	9.85 ± 0.34	2.058	2.05 ± 0.51	9.86 ± 0.27	16.25	3.1 ± 0.5	9.9 ± 0.3	0.54
0.06				1.63 ± 0.44	8.74 ± 0.29	15.14	1.6 ± 0.4[b]	8.7 ± 0.3[b]	0.61
0.07	4.96 ± 0.43	5.93 ± 0.20	0.951	5.23 ± 0.94	4.11 ± 0.17	7.54	5.0 ± 0.5	5.7 ± 0.2	0.94
0.08	7.75 ± 1.37	4.84 ± 0.38	4.286	4.62 ± 0.87	3.77 ± 0.20	10.11	6.8 ±1.2	4.5 ± 0.3	1.2
0.09				6.61 ± 0.86	4.77 ± 0.12	5.53	6.6 ± 0.9[b]	4.8 ± 0.1[b]	1.1

[a] WALLS data obtained under conditions of thermodynamic equilibrium. [b] From a single value.

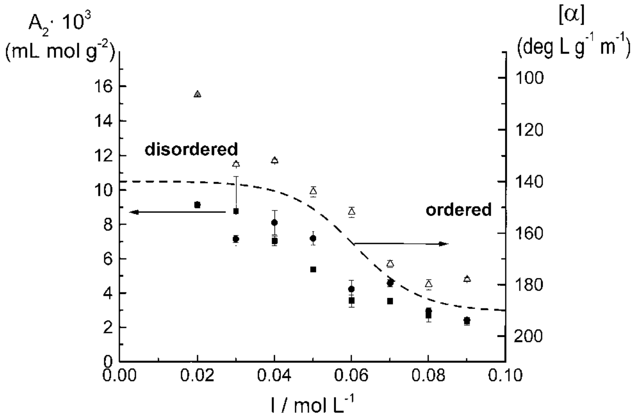

Figure 5. A_2 of ι-carrageenan as a function of the ionic strength, *I*, in different aqueous NaCl (solid circle), NaI (solid square), and LiCl (open upward triangle) salt solutions (lhs scale). The dashed line (data taken from ref 28, Figure 7a) follows the transition from the *ordered* conformation to the *disordered* one, as indicated by optical activity measurements (rhs scale).

latter salt taken as a reference. Statistical analysis reveals that it is not possible to ascertain whether in this range of C_s there is any significant difference between the set of K_{app} values of NaCl and those of LiCl. The association behavior in the range of low C_s values is clearly correlated with, and reasonably determined by, the critical overlap concentration, $(c^*)_0$, and hence by A_2. In fact Li^+−ι-carrageenan in LiCl, being the most expanded salt form, has the highest probability of chain encounter. This behavior produces a higher "effective" concentration, which in turn gives K_{app} values which are of the same order of magnitude as those shown at high C_s. There is no contradiction between a low fraction of ordered conformation, typical for low values of C_s, and a nonnegligible value of K_{app}. In fact, even a few ordered repeating units on an otherwise mostly disordered chain could be enough to produce the junction of highly expanded chains, and then association. On the other side, the smaller expansion of the Na^+ form of ι-carrageenan seems to produce barely detectable association for $C_s < 0.05$ M.

The whole of the thermodynamic analysis of the scattering data at $\theta \rightarrow 0°$ for ι-carrageenan points to the following issues:

(i) Aqueous solutions containing I^- ions are confirmed as a "good solvent" also for the ordered form of ι-carrageenan as was already known for that of κ-carrageenan.

(ii) Li^+ counterions are shown to induce a chain expansion significantly larger than that produced by Na^+ ones. This very likely stems from an unfavorable specific Li^+ counterion/polyion interaction, possibly due to the clashes of the "hard" hydration sheath of the sulfated ester group with the very large hydration shell of Li^+ ions. Longer distances of closest approach would produce less effective polymer charge screening, higher repulsion and larger chain expansion. This point would greatly benefit from polyelectrolyte analysis including the effect of specific counterion−polymer interaction.[31]

(iii) The thermodynamic critical overlap concentration values, $(c^*)_0$, are very low for all ι-carrageenan systems. This possibility has been given too little attention so far in the analysis of the solution properties of this polymer. Such a demonstrated behavior can profoundly affect not only equilibrium experiments, but also nonequilibrium ones, like, e.g., SEC or rheological ones.

Table 7. Thermodynamic Parameters of ι-Carrageenan in 0.08 M NaCl as a Function of Temperature[a]

T (°C)	$K_{app} \times 10^{-5}$	$A_2 \times 10^3$ (mL·mol·g^{-2})	$\chi^2 \times 10^{14}$	$(c^*)_0$ (g·L^{-1})
11	3.30 ± 0.54	2.96 ± 0.20	6.20	1.7
15	11.23 ± 0.87	2.04 ± 0.01	1.14	2.5
20	13.52 ± 2.77	2.44 ± 0.28	7.43	2.0
25	10.02 ± 0.77	2.70 ± 0.09	1.90	1.9
30	2.27 ± 0.43	4.21 ± 0.23	0.66	1.2
35	1.84 ± 0.12	4.62 ± 0.08	0.79	1.1
45	0.86 ± 0.10	7.27 ± 0.13	1.18	0.69
50	0.61 ± 0.17	7.07 ± 0.29	4.12	0.71
55	1.09 ± 0.12	6.54 ± 0.12	1.22	0.76

[a] WALLS data obtained under thermodynamic equilibrium conditions.

b. Data as a Function of Temperature. As far as the experiments carried out in 0.08 M NaCl as a function of *T* are concerned, the features of the scattering curves at zero angle are the same as those of the curves obtained at constant temperature for different salts. Therefore, it was fully justified to use the same approach also for those data. The results of the fitting procedure are reported in Table 7. At and below 25 °C, 100% ordering is indicated by independent optical activity data.[16] In that range, the association is the highest, as shown by the values of K_{app}; at higher temperatures, a decrease of the fraction of the ordered conformation is perfectly paralleled by a decrease of K_{app}. The increase of the values of A_2 on increasing temperature stems from the transition from the ordered to the disordered conformation, much like in the case of the conformational change brought about by a decrease of C_s (See Figure 5) and follows the transition from the *ordered* conformation to the *disordered* one, as indicated by optical activity measurements (see Figure 6).

A very interesting result to be underlined is that at a temperature as high as 55 °C, at which the values of the optical activity is very close (albeit not equal) to that of the fully disordered conformation, still the value of

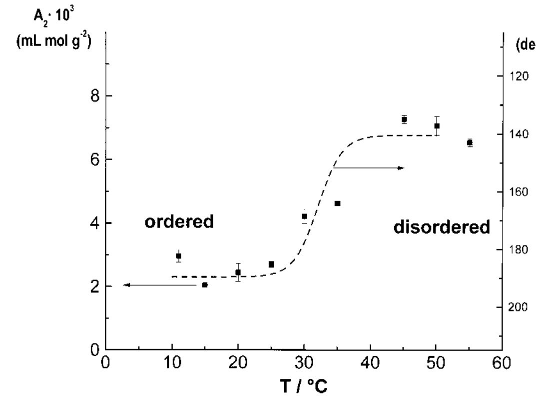

Figure 6. A_2 as a function of T for ι-carrageenan in 0.08 M NaCl (lhs scale). The dashed line (data taken from ref 29, Figure 14) follows the transition from the *ordered* conformation to the *disordered* one, as indicated by optical activity measurements (rhs scale).

K_{app} is as high as about 10^5, which corresponds to a substantial degree of association. Clearly, the latter must derive from small, but not nil, portions of remaining ordered tracts.

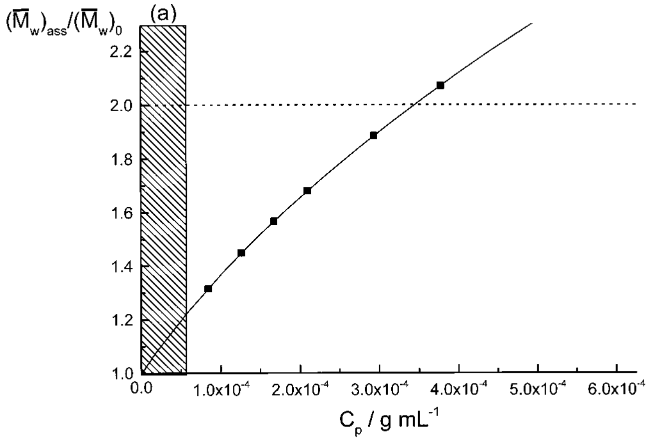

Figure 7. Dependence of the ratio $(\bar{M}_w)_{assoc}/(\bar{M}_w)_0$ on C_p for ι-carrageenan in aqueous 0.08 M NaI, at 25 °C. The points correspond to the values of C_p used in the LS experiments. The unshaded area at the rhs of region (a) corresponds to the range of C_p in which [α] data[27] show that the polymer is in its fully ordered conformation.

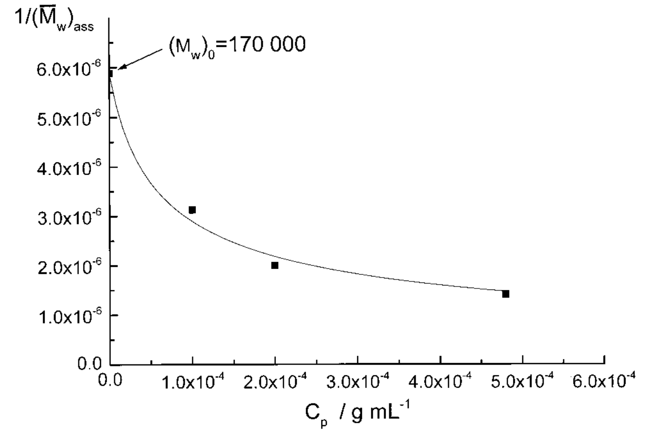

Figure 8. Dependence on C_p of the values of $1/(\bar{M}_w)_{assoc}$ of Li^+ ι-carrageenan in 0.20 M LiCl at 35 °C (data taken from Figure 8 of ref 15).

Conclusions

The data presented in this paper are probably the most extensive light-scattering investigation of ι-carrageenan in aqueous solution reported so far, including the study of the effect of ionic strength, salt-specificity, and temperature. The WALLS results have been confirmed by significant sample cases investigated by LALLS. They all lead to the convergent evidence that ι-carrageenan, under a wide range of physical and chemical conditions, shows a remarkable tendency to give rise to intermolecular association. The latter process is absolutely nonnegligible even at very low values of C_s and at high values of T. This fact should be taken into due account when using such conditions as an alleged state in which conformationally disordered chains are expected to be in the isolated single-stranded form, especially so for values of C_p corresponding to a semidilute regime.

The most important conclusion, however, deals with the very sound finding that in *all* conformational conditions the $\bar{M}_w$ of the fundamental unassociated form, $(\bar{M}_w)_0$, is the same. This has been found to be true both for partial (C_s < 0.08 M) and for total conformational ordering ($C_s \geq$ 0.08 M). It necessarily means that the partially or fully ordered conformations of ι-carrageenan, both in the Na^+ and in the Li^+ salt forms, are single-stranded.[16,20] As a hypothesis, the idea was put forth[13] that the previously observed curvature in the reduced scattering intensity curves[13,16,20]—which has been amply confirmed by the present data—was due to a dissociation from an *ordered dimer* to single-stranded *disordered "coils"*. Recently published data of specific optical activity, [α], were carefully taken in conditions of high ionic strength (both in 0.08 M NaCl and in 0.08 M NaI, at 25 °C) over a very wide range of C_p.[28] They indicate that the completely ordered conformation persists down to a value of C_p ($C_p = 6.12 \times 10^{-5}$ g mL^{-1}), which fully encompasses to the minimum of the KC_p/R_0 plots. All [α] data fall within the range in which the association/dissociation process takes place. By use of eq 6 and of the K_{app} values from Tables 4 and 5 (average values of WALLS and LALLS data), one finds that, for the quoted lowest value of C_p, the ratio $(\bar{M}_w)_{assoc}(C_p)/(\bar{M}_w)_0$ is as low as 1.62 and 1.24 for NaCl and for NaI, respectively. The graphical evidence for the latter case is reported in Figure 7. It shows that the values of $(\bar{M}_w)_{assoc}$ for all polymer concentrations (except the highest one) for which complete ordering is indicated by [α] data fall *below* that corresponding to a double-stranded structure. Therefore, it can be concluded that the single chains that are progressively liberated upon polymer dilution (at 25 °C and 0.08 M NaI) are still in their fully ordered conformation. This finding could appear at variance with some statements of ref 15, which suggest the onset of disordering upon dilution, albeit only a *partial* disordering. Unfortunately, the [α] data on which they are based cannot be taken into account for two reasons. First, the [α] values of Figure 11 of ref 15 are numerically in error by about 2 orders of magnitude, as can be easily verified by comparison with well-established published data,[28,33,34] including recent ones[14] from the same laboratory. Second the error in the absolute reading of α (in deg) at low temperature for the two different curves obtained for the same value of C_p (C_p = 0.48 mg mL^{-1}) reported in Figures 8[15] and 11,[15] respectively, is *larger* than the *total absolute* reading of α for the concentration for which an alleged slight decrease of [α] is claimed (C_p = 0.05 mg mL^{-1}).

The experimental MW results of the quoted recent SEC−LALLS study[15] of ι-carrageenan in highly associating conditions (0.20 M LiCl), namely those of Figure 8 of Reference 15, fully agree with the findings of this work. The $(\bar{M}_w)_{assoc}$ data obtained at different polymer concentrations at 35 °C can be conveniently reported as a $1/(\bar{M}_w)_{assoc}$ vs C_p plot (see Figure 8). Such a plot shows a definite upward curvature, as expected for a highly associating system. It can be nicely fitted by use of eq 6 taking the known values of $(\bar{M}_w)_0$ and PI.[15] The fitting parameter, K_{app}, is $(8.9 \pm 0.8) \times 10^5$, in very good agreement with the data of Table 6 of this work, in particular if account is made for the much higher value of the salt concentration. The results were obtained in a C_p range very similar to that used in previous work[16,20] and in the present one, in contrast with the statement of the authors.[15] Moreover, Table 2 of ref 15 reports a monotonic increase of $\bar{M}_w$ upon decreasing temperature for two samples of ι-carrageenan, having values of $(\bar{M}_w)_0$ equal to 65 000 and 170 000, respectively. Such a continuous trend was also reported recently for κ-carrageenan both by ref 15 and by Ueda et al.[32] It was recently demonstrated[8] that such a behavior is the result of a continuous association process brought about by lowering of temperature and/or by increase of the ionic strength. It can be easily predicted that a further progress in the change of either of the two variables would give rise to relative changes of the apparent $\bar{M}_w$ much larger than two, as found for κ-carrageenan.[32] One should never forget that the $\bar{M}_w$ values obtained from SEC−LALLS measurements at finite concentration are truly "apparent" values. Only extrapolation to $C_p \rightarrow 0$, as performed in Figure 8, allows one to determine the true $(\bar{M}_w)_0$ value. Thus, the experimental results of ref 15 do not allow to conclude that a double-stranded structure is the fundamental ordered conformation of carrageenans, in contrast with the conclusions of previous reports.[1,13] Therefore, it is rewarding to notice the convergence of our present conclusions with the experimental findings of ref 15. They both demonstrate that comparatively high values both of C_p and of C_s, at low T, bring about a massive association of the chains of the Li^+ form of ι-carrageenan.[16] At the same time they must univocally be interpreted as confirming that the $\bar{M}_w$ of the fundamental ordered form giving rise to a progressive association is the same as that of the single-stranded disordered conformation (see Figure 8): the basic step in conformational ordering is an intramolecular process.[20]

According to the conceptually simplest model, such a fundamental ordered conformation is a single helix. The existence of the single-helical conformation was indicated almost 20 years ago,[4−6] and since then it has been repeatedly confirmed both for ι- and for κ-carrageenan.[7,16,17,20,23,32,34−37] Moreover, it has to be stressed that many of the interpretations of light-scattering data of ι-carrageenan leading to the opposite conclusion (i.e. that the ordered conformation of the polysaccharide in solution could exist only in a double-stranded conformation) can be now easily explained by the insufficient attention paid toward the solution preparation methods and the much too high values of C_p and C_s used. The intrinsic difficulty of a correct determination of $\bar{M}_w$ of gelling polysaccharides should not be underestimated.

The following paper deals with the study of the angular dependence of the scattered light, to gain further insight into the average chain dimensions of ι-carrageenan under the presently described conditions.[26]

Acknowledgment. K.V., K.B., B.D., and F.C. are indebted to KULeuven and/or IWT for their research grants and to the University of Trieste for the hospitality at the Department of BBCM. K.V. is grateful to the University of Leiden for the experimental facilities supporting the LALLS measurements. Travel grants were obtained from FWO-Vlaanderen and Vlaamse Leergangen Leuven. Part of the work is sponsored by an FWO research grant, by KULeuven, the University of Trieste and INTAS. The scientific support of the POLY-biòs Research Center, Trieste, Italy, is gratefully acknowledged.

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MA000996Y