Macromol. Symp. ***190***, *65–74 (2002)*

Light Scattering Investigation of Association Phenomena in Saline Carrageenan Solutions

*Fabio Cuppo and Harry Reynaers**

Laboratory of Macromolecular Structural Chemistry, Department of Chemistry, Catholic University of Leuven, Celestijnenlaan 200F, B-3001 Heverlee, Belgium

SUMMARY: Intermolecular association of ι- and κ-carrageenan in aqueous solution has been studied by wide-angle laser light scattering as a function of salt composition and temperature. For ι-carragenan, the effect of three different salts (LiCl, NaCl, NaI) on the associative properties was investigated. As to κ-carrageenan, the association-inducing effect of Cs^+ ions was studied, with particular interest in the time dependence of the aggregation process.

Introduction

Carrageenans are a family of sulfated polysaccharides extracted from marine red algae. Three main species can be identified, namely ι-, κ- and λ-carrageenan. This study is restricted to ι- and κ-carrageenan, the primary structure of which is shown in Fig. 1. Despite their widespread application, especially in food industry as thickening and stabilizing agents, the mechanism of gelation has been a matter of discussion until very recently, and further investigation is still needed for a full comprehension of the process[1-4]. The controversial data so far reported in literature must be related to the extreme sensitivity of these polymers to preparative and experimental conditions, as already pointed out in previous work[1-4].

Compositional (polymer concentration, nature and concentration of added salts, etc.) as well as physical parameters (temperature) play a key role in the gelling behavior of κ- and ι-carrageenan[1-5]. The mechanism of gelation has currently been shown to consist of a two-step process[1-4]: first, a conformational transition from the disordered to an ordered (single-helix) state takes place; then, interchain association between helical stretches gives rise to the three-dimensional gel network. However, it is worth noting that interchain association may occur on the microscopic scale without the development of a macroscopic gel behavior, *e.g.*, at high dilution and/or in the presence of suitable co-solutes.

CCC 1022-1360/00/$ 17,50+.50/0

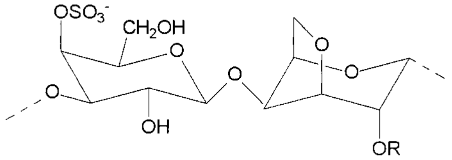

Figure 1. Repeating unit of κ-carrageenan (R = H) and ι-carrageenan (R = SO_3^-).

Carrageenans are known to modulate their conformational-associative behavior depending on the specific nature (and concentration) of ionic species in solution[1-5]. Whereas older studies suggested a different behavior of ι- and κ-carrageenan in this respect[6], more recent work has led to opposite conclusions, i.e., only a small difference is observed between the two forms[7,8]. In general, it can be stated that the alkali metal ions with higher atomic number (K^+, Rb^+, Cs^+) are more effective in inducing gelation than the lighter ones (Na^+, Li^+). ι-Carrageenan, however, shows a higher tendency to interchain association, which is reflected by a lower ion specificity in comparison with κ-carrageenan.

The present research focuses on solution properties of ι-carrageenan and κ-carrageenan by exploring the influence of different physicochemical parameters, such as the nature of added salts, ionic strength, temperature and polysaccharide concentration.

A light scattering approach (wide-angle laser light scattering, WALLS) has been employed, inasmuch as this method offers the remarkable advantage of allowing the study of essentially unperturbed systems in comparison with other experimental techniques (osmometry, viscosimetry, chromatographic techniques, etc.).

Preliminary results are reported on the associative behavior of κ-carrageenan in aqueous NaI/CsI mixed systems. This aspect has recently found increasing interest due to the observation of a nematic phase of κ-carrageenan[9] under those salt conditions (although at higher polymer concentrations).

Materials and Methods

ι- and κ-carrageenan were supplied by Sigma-Aldrich (ι-carrageenan type V, from *Eucheuma spinosa*, lot no. 27F0373; κ-carrageenan type III, from *Eucheuma cottonii*, lot no. 73H0840). The commercial samples were purified following a general procedure which had already proved to be safe and reliable avoiding undesired aggregation phenomena[1-3,10]. The purified ι-carrageenan sample was subjected to combined NMR and IR analyses. It turned out that the contaminating κ-type fraction amounted to 8 % at most. The polydispersity of the sample was checked by gel permeation chromatography (GPC-LALLS) the found polydispersity index being 2.0. All chemicals used were of analytical grade; ultrapure water (Milli-Q Reagent Grade Water Systems, Millipore) was used throughout.

Light scattering measurements were performed as previously described[2,11]; the polymer solutions were prepared according to the procedure discussed in the following paragraph.

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. As a consequence, the method for preparation of polysaccharide solutions is very critical, and can possibly affect the reliability of the experimental results when proper procedures are not employed.

When ι- or κ-carrageenan solutions are prepared starting from an aqueous carrageenan solution of a known concentration, to which suitable volumes of salt solutions are carefully added (under stirring at sufficiently high temperature), reliable light scattering data are obtained. On the contrary, light scattering measurements show poor reproducibility and, in particular, a higher molar mass when the freeze-dried polymer is dissolved directly in an aqueous salt solution. Similar observations are reported by Lecoutier *et al.* for light scattering data on lyophilized xanthan[12].

In the present case, light scattering measurements were carried out first starting from aqueous salt solutions to which a known amount of lyophilized ι- or κ-carrageenan was added under vigorous stirring. A dilution series was then obtained by addition of increasing amounts of the 1:1 electrolyte solutions. All solutions underwent light-scattering measurements immediately after preparation, starting with the lowest polymer concentration.

From the initial light scattering measurements unusual Zimm plots were obtained. In particular, a general tendency to an increase in the resulting molar mass as a function of increasing salt concentration appeared. All the experimental observations, including time-dependent effects, pointed to the occurrence of intermolecular association. It was then concluded that the preparation procedure was inappropriate. It should be noted furthermore that only a comparison of repeated experiments could lead to this conclusion, as a single occasional experiment might give apparently reliable data.

After several trials with different samples and under different conditions (polymer and salt composition, curing times, etc.), the following procedure turned out to give the best results: an aqueous (ι- or κ-) carrageenan solution of a known concentration was diluted in order to obtain solutions of the desired final concentrations. A suitable volume of salt solutions was slowly added to the half-filled flasks (*i.e.* at half dilution), under stirring at 65 °C, so as to reach the proper final ionic composition. Using a peristaltic pump in a close circuit, the solutions were filtered several times through a Millipore filter (pore size 0.22 μm) directly in the light scattering cells to obtain dust-free solutions. In all cases, the polymer concentration was lower than 0.50 g L^{-1}. In the case of ι-carrageenan, the solutions were stored at room temperature for 24 h before the measurements since it was found that, under the investigated conditions, an equilibrium state could be reached within that period. Hence, the light scattering data on ι-carrageenan can be safely regarded as equilibrium data. On the other hand, it turned out from several experiments that the equilibration time for κ-carrageenan can often be much longer than 24 h under aggregating conditions (while no time dependence immediately after the preparation of the samples is observable under non-aggregating conditions). It was then chosen to start the measurements on the κ-carrageenan samples immediately after their preparation, studying the time dependence of association under different experimental conditions and performing consecutive experiments on the same samples at different times.

Analysis of Scattering Data: The Open Association Model of Elias

The processing of the light scattering data was performed in the framework of the Elias open association model (OAM), which has been demonstrated to give the best description of the experimental data for ι- and κ-carrageenan under associating conditions[2-4]. As a more detailed theoretical discussion of the model can be found in the original treatment by Elias[13] as well as in the quoted previous work, only the fundamental features and equations will be reported here.

The basic assumption of the open association model is that the association process occurs through a series of thermodynamically equivalent steps, leading from the fundamental non-associated 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: $K_1 = K_2 = K_3 \ldots = K_{\text{assoc}}$.

The weight-average molar mass of the associating polymer system, $(\overline{M}_w)_{\text{assoc}}$, can thus be defined, according to the model, as

$$(\overline{M}_{\mathrm{w}})_{\mathrm{assoc}} = \sqrt{(\overline{M}_{\mathrm{w}})_0^2 + 4000\cdot K_{\mathrm{assoc}}\cdot PI\cdot(\overline{M}_{\mathrm{w}})_0\cdot C_{\mathrm{p}}} \qquad (1)$$

where $(\overline{M}_{\mathrm{w}})_0$ is the weight-average molar mass of the non-associated species, PI is the polydispersity index and C_{p} is the polymer concentration.

The reduced scattering intensity, extrapolated to zero angle, is given by the following equation:

$$\left.\frac{K\cdot C_{\mathrm{p}}}{R_\theta}\right|_{\theta=0^\circ} = \frac{1}{\left\{(\overline{M}_{\mathrm{w}})_0^2 + 4000\cdot K_{\mathrm{assoc}}\cdot \mathrm{PI}\cdot(\overline{M}_{\mathrm{w}})_0\cdot C_{\mathrm{p}}\right\}^{1/2}} + 2\cdot A_2\cdot C_{\mathrm{p}} \qquad (2)$$

where A_2 is the second virial coefficient.

Equation (2) as a two-parameter equation (K_{assoc} and A_2) was used to fit the experimental data. $(\overline{M}_{\mathrm{w}})_0$ was obtained under non-associating conditions. The value of PI for the ι-carrageenan sample was determined as described above (see Materials and Methods), while for κ-carrageenan the value 2.27 was taken from GPC-LALLS results previously obtained for a similar sample[14].

Results and discussion

ι-Carrageenan

The conformational-associative behavior of ι-carrageenan has been studied in a wide range of salt and temperature conditions. In particular, the effect of three different salts, namely NaI, NaCl and LiCl, has been investigated as a function of the ionic strength (and temperature), in order to span the region of conformational transition from the disordered to the ordered (single-)helical state. In fact, from optical activity measurements[15] it follows that at 25 °C, independently of the type of the 1:1 supporting electrolyte in solution, the biopolymer is in a fully disordered state at ionic strength equal or lower than 0.02 M, while, on the contrary, a fully ordered state is always reached at ionic strength equal or higher than 0.09 M. The experimental light scattering parameters under these two extreme conditions are given in Table 1.

Table 1. Light scattering parameters for ι-carrageenan under different salt conditions at 25 °C.

	0.02 M LiCl	0.02 M NaCl	0.02 M NaI	0.09 M LiCl	0.09 M NaCl	0.09 M NaI
$K_{\mathrm{assoc}}\cdot 10^{-5}$	≤0.01 ± 0.00	≤0.01 ± 0.00	≤0.01 ± 0.00	6.6 ± 0.9	5.34 ± 0.18	2.29 ± 0.27
$A_2\cdot 10^3$ mol cm^3 g^{-2}	15.51 ± 0.04	9.15 ± 0.13	9.14 ± 0.16	4.8 ± 0.1	2.43 ± 0.70	2.36 ± 0.23

The associative behavior of ι-carrageenan, under the given experimental conditions, seems to vary between the limits of LiCl and NaI, where the highest and the lowest degree of association were respectively observed for all the investigated ionic strengths (0.02÷0.09 M); NaCl represents an intermediate state between the two limits. Hence, it can be concluded that Li^+ is more effective than Na^+ in promoting aggregation, and Cl^- more than I^-. The effect of the ionic strength reflects the progressive conformational ordering of macromolecules upon increasing salt concentration, since interchain association requires the transition to the helical state as a preliminary stage[1-3,5,11].

As to the second virial coefficient, a decrease in the order LiCl > NaCl > NaI was observed over the whole range of ionic strength. This result, at first glance, appears to be in contradiction with the association-inducing properties of the three salts. It must be noted, however, that the physicochemical meaning of the second virial coefficient cannot be regarded in an oversimplified way, especially in a rather complex system such as a polyelectrolyte in aqueous salt solution undergoing intermolecular association. Several effects should be taken into account in such cases, like chain conformation and stiffness, excluded-volume effects, specific interactions between the polymer and ions, etc. Proper interpretation of the experimental data in terms of molecular parameters would then require a deeper theoretical approach, which is beyond the purpose of this work.

Some examples of Zimm plots, showing typical effects of intermolecular association on the angular and concentration dependence of the light scattering data, are given in Figs 2, 3 and 4.

Figure 2 shows how the NaCl salt concentration affects the Zimm plots of ι-carrageenan (treated according to the Elias method, as described above) at constant temperature, 25 °C.

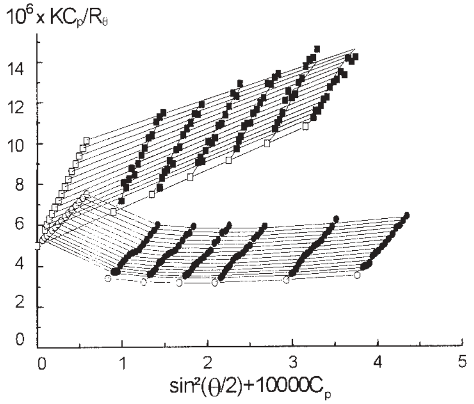

Figure 2. Zimm plots of ι-carrageenan in 0.02 M NaCl (squares) and in 0.09 M NaCl (circles). (The solid symbols represent the experimental data and the open symbols the extrapolated values.)

In 0.09 M NaCl, an upward curvature can be observed; the scattered intensities are higher than in 0.02 M NaCl, where the curvature is absent (*i.e.*, little or no association occurs). From the slope of the values extrapolated to zero angle, it is evident that the second virial coefficient is larger in 0.02 M than in 0.09 M NaCl (Table 1). It can also be seen, from the slope of the angular dependence extrapolated to $C_p = 0$, that the radius of gyration is larger in 0.02 M than in 0.09 M NaCl (115.3 ± 1.6 nm and 88.4 ± 5.7 nm, respectively).

Figure 3 shows a similar Zimm plot of ι-carrageenan in 0.07 M NaI at 25 °C. Here the association effect is also clearly visible.

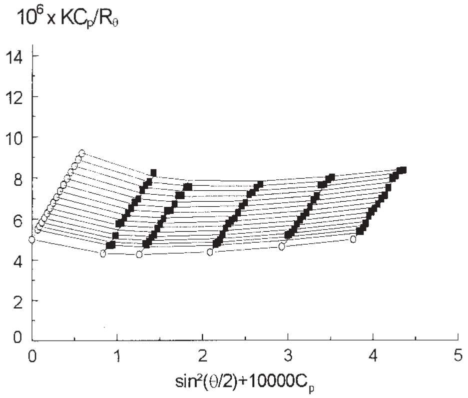

Figure 3. Zimm plot of ι-carrageenan in 0.07 M NaI. (The solid squares are the experimental data and the open circles the extrapolated values.)

In Fig. 4, the influence of temperature on the associative behavior of ι-carrageenan is clearly manifested: in 0.08 M NaCl, an upward curvature of the Zimm plots, especially in the low-concentration region, appears at 11 °C.

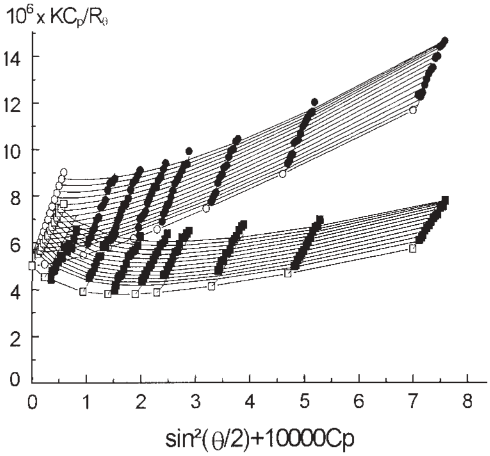

Figure 4. Zimm plots of ι-carrageenan in 0.08M NaCl at 11 °C (squares) and at 55 °C (circles). (The solid symbols represent the experimental data and the open symbols the extrapolated values.)

The scattered intensities clearly decrease upon raising temperature to 55 °C; from the slope at zero polymer concentration, it can be seen that the radius of gyration increases with increasing temperature. At 55 °C, a certain curvature is still present in the Zimm plot, pointing to a small but persistent association effect.

κ-Carrageenan

The experimental work currently carried out in our laboratory deals with the associative behavior of κ-carrageenan as a function of salt concentration and temperature. In particular, the effect of a gradual change from non-associating (NaI 0.1 M) to increasingly associating conditions (NaI/CsI mixtures, at constant 0.1 M ionic strength and increasing the CsI mole fraction, X_{Cs}) is being studied. Some preliminary results will be shown hereinafter.

The first characterization of the κ-carrageenan sample under non-associating conditions confirmed the fundamental features already reported in previous work performed in our laboratory[1-2,11] (Table 2).

Table 2. Light scattering parameters for κ-carrageenan under non-associating conditions at 25 °C.

	0.1 M NaCl	0.1 M NaI $\Delta t = 0$	0.1 M NaI $\Delta t = 5h$
$M_w \cdot 10^{-3}$ / g mol^{-1}	227 ± 4	211 ± 5	211 ± 3
$A_2 \cdot 10^3$ / mol cm^3 g^{-2}	3.72 ± 0.05	1.88 ± 0.07	1.85 ± 0.08

In particular, the conformational transition between a disordered state (NaCl 0.1 M) and a helical state (NaI 0.1 M), already demonstrated by several authors on the basis of optical rotation and molar mass measurements[1,5,11], is reflected by the experimental light scattering data. No change of the weight-average molar mass is observable between the two conformational states beyond the experimental error. This finding provides further evidence of the presence of a single-helical conformation as the fundamental ordered state in aqueous solution[1-4]. The thermodynamic stability of the system in NaI was tested over a period of 5 h, after which no significant change of the experimental parameters was detected. As to the second virial coefficient, its values in NaI are remarkably lower in comparison with those in NaCl. This result is very close to those previously reported under same or very similar conditions[1,11]. They indicate that iodide is a poorer solvent for the ordered polymer conformation than chloride is for the disordered one.

The occurrence of intermolecular association becomes evident when a part of the Na^+ ions is replaced by Cs^+ ions. In Table 3 and in Fig. 5, the results at $X_{Cs} = 0.28$ are shown.

Table 3. Light scattering parameters obtained for κ-carrageenan in mixed 0.1 M CsI/NaI solution at CsI mole fraction $X_{Cs} = 0.28$, at 25 °C.

	$\Delta t = 0$ h	$\Delta t = 24$ h	$\Delta t = 45$ h	$\Delta t = 70$ h	After heating
$K_{assoc} \cdot 10^{-3}$	115 ± 10	260 ± 28	514 ± 70	737 ± 159	39.7 ± 22.0
$A_2 \cdot 10^3$ / mol cm^3 g^{-2}	1.28 ± 0.04	-0.26 ± 0.04	-0.29 ± 0.05	-0.30 ± 0.04	2.03 ± 0.20

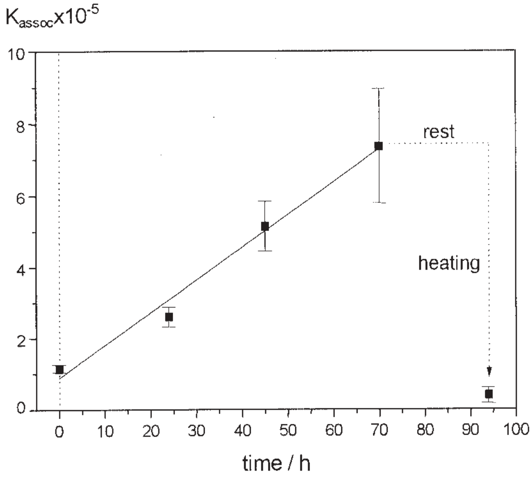

Figure 5. Time evolution of the "apparent" association constant for κ-carrageenan at X_{Cs} = 0.28, at 25 °C and after heating for 1 h at 60 °C. The solid line is a linear fit of the experimental data.

A continuous increase in the "apparent" association constant (which is actually a function of time, slowly approaching a true equilibrium value at $t \rightarrow \infty$) can be observed; this clearly indicates that the system is still far from reaching equilibrium state after 70 h. Meanwhile, the second virial coefficient falls to a negative value already at t = 24 h and remains constant thereafter.

At t = 94 h (*i.e.*, 24 h after the last experiment), the solutions at different C_p for $X_{Cs} = 0.28$ were heated for 1 h at 60 °C and then immediately subjected to light scattering measurement at 25 °C. The resulting association constant is even lower than that at t = 0 h, clearly pointing to a thermally induced dissolution of polymer aggregates.

Even though at a preliminary stage, the results reported above clearly demonstrate that (thermoreversible) intermolecular association of κ-carrageenan under the investigated experimental conditions occurs below the "threshold value" of $X_{Cs} = 0.4$ recently suggested by Viebke *et al.* from DSC measurements[16]. This finding, anyway, deserves further study and analysis, which will be fully reported in a forthcoming paper.

Conclusions

The associative behavior of ι-carrageenan, at 25 °C, is strongly enhanced by increasing the ionic strength of the supporting salt (LiCl, NaCl, NaI) in the range 0.02÷0.09 M. However, the ability to induce association follows the order LiCl > NaCl > NaI.

From preliminary experimental results, it turns out that κ-carrageenan undergoes remarkable intermolecular association in NaI/CsI mixed systems at 0.1 M ionic strength and the CsI mole fraction, X_{Cs}, equal to 0.28. The associative process, under the investigated conditions, is rather slow, as the system is still far from reaching equilibrium after 70 h. This result clearly indicates that association occurs at cesium concentration lower than the threshold value (X_{Cs} = 0.4) recently suggested by other authors.

Measurements performed under non-associating conditions (NaCl or NaI, 0.1 M) show no increase in the molar mass of κ-carrageenan upon conformational ordering. This finding provides further evidence of the monomolecular nature (single helix) of the conformational transition.

References

1. K. Bongaerts, H. Reynaers, F. Zanetti, S. Paoletti, *Macromolecules* **1999**, 32, 675.
2. K. Bongaerts, Ph.D. Thesis, Katholieke Universiteit Leuven, Heverlee, Belgium, 1999.
3. B. Denef, Ph.D. Thesis, Katholieke Universiteit Leuven, Heverlee, Belgium, 1997.
4. K. Bongaerts, S. Paoletti, B. Denef, K. Vanneste, F. Cuppo, H. Reynaers, *Macromolecules* **2000**, 33, 8709.
5. M. Ciancia, M. Milas, M. Rinaudo, *Int. J. Biol. Macromol.* **1997**, 20, 35.
6. D.A. Rees, E.R. Morris, D. Thom, J.K. Madden, in: *The Polysaccharides*, G. O. Aspinall, Ed., Academic Press, New York 1982.
7. L. Piculell, C. Håkansson, S. Nilsson, *Int. J. Biol. Macromol.* **1987**, 9, 297.
8. M.J. Ridout, S. Garza, G.J. Brownsey, V.J. Morris, *Int. J. Biol. Macromol.* **1996**, 18, 5.
9. J. Borgström, P.-O. Quist, L. Piculell, *Macromolecules* **1996**, 29, 5926.
10. K. Bongaerts, H. Reynaers, F. Zanetti, S. Paoletti, *Macromolecules* **1999**, 32, 683.
11. D. Slootmaekers, C. De Jonghe, H. Reynaers, F.A. Varkevisser, C.J. Bloys van Treslong, *Int. J. Biol. Macromol.* **1988**, 10, 160.
12. J. Lecoutier, G. Chauveteau, G. Muller, *Int. J. Biol. Macromol.* **1986**, 8, 306.
13. H.G. Elias, in: *Light Scattering from Polymer Solutions*, M. B. Huglin, Ed., Academic Press, London 1972, Chap. 9, p.397.
14. D. Slootmaekers, J.A.P.P. van Dijk, F.A. Varkevisser, C.J. Bloys van Treslong, H. Reynaers, *Biophys. Chem.* **1991**, 41, 51.
15. B. Denef, A. Gamini, F. Delben, S. Paoletti, H. Reynaers, K. Vanneste, *Biopolymers* **1998**, 45, 105.
16. C. Viebke, J. Borgström, I. Carlsson, L. Piculell, P. Williams, *Macromolecules* **1998**, 31, 1833.