Biophysical Chemistry 113 (2005) 201–208

Biophysical Chemistry

www.elsevier.com/locate/bpc

Unperturbed dimensions of Carrageenans in different salt solutions

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Received 8 July 2004; received in revised form 10 September 2004; accepted 10 September 2004

Abstract

Commercial samples of κ-, ι-, and λ-carrageenans were studied by means of Size Exclusion Chromatography with dual detection, i.e. employing a Refractive Index (concentration sensitive) and Multiangle Light Scattering (size sensitive) detectors. The eluent was water containing 0.1 M concentration of different ionic salts, namely LiCl, NaCl, KCl and NaI, with the exception of κ-carrageenan that aggregates in presence of KCl. Molecular weight distributions and averages, coefficients of the scaling law of molecular dimensions and unperturbed dimensions were thus obtained from a single polydisperse sample of each polymer. Measurements were performed at 25 °C and all the systems were above theta conditions with values of the q exponent of the scaling law ranging from 0.51 to 0.59. Extrapolation to unperturbed conditions provides values of the characteristic ratio $C_N=56\pm1$ and 40 ± 5 respectively for λ- and ι-carrageenans regardless of the ionic salt employed. However, κ-carrageenan gives $C_N=31$, 35 and 59, respectively, in presence of LiCl, NaCl and NaI, which clearly indicates that this polymer behaves on a different way in presence of NaI than with the other two salts. A tentative explanation of this difference is provided.

Keywords: Unperturbed dimensions; SEC-MALS; Carrageenan

1. Introduction

Carrageenans are a family of polysaccharides extracted from several species of marine red algae. The basic structure of carrageenan is a linear polysaccharide made up of a repeating disaccharide sequence of β-D-galactopyranose, linked 1,3 and α-D galactopyranose linked through positions 1,4. The three main species, κ-, ι-, and λ-carrageenan, differ in the number of sulphate groups per repeating unit, as shown in Scheme 1. Moreover, κ- and ι-carrageenans contain 3,6-anhydro bridges in the α-D galactopyranose residue that play an important role in their gelling properties. The anhydro bridge forces the sugar to flip from a 4-C-1 conformation to a 1-C-4 conformation and can then form cross-linked networks and gels. λ-Carrageenan has both its sugar residues in a 4-C-1 conformation and does not form gels [1].

The mechanism of gelation in κ- and ι-carrageenans has been investigated by many authors [2–7]; it implies a coil-helix conformational transition followed by aggregation of the helices in a network. However, the formation and nature of the resulting gel is still a matter of study and discussion. The process is very complex and depends not only on the chemical structure of carrageenan and temperature, but also on the nature of salts present in the system that can either prevent or promote the gelation. Although there is agreement in the coil-helix step that precedes the gelation, there is no consensus on whether κ-carrageenan forms double or single helix or if the multiple helix mechanism involves the aggregation of fully developed or incomplete helices [8–11].

Incorporation of a Multiangle Light Scattering detector to a traditional SEC equipment allows the determination of molecular weight M_i and radius of gyration, $\langle s^2\rangle_i$, for each individual slice i across the whole chromatogram of a polydisperse sample. Thus, a single measurement performed on a widely polydisperse sample paramount to many separated measurements carried out on monodisperse samples of different molecular weights. Consequently, the

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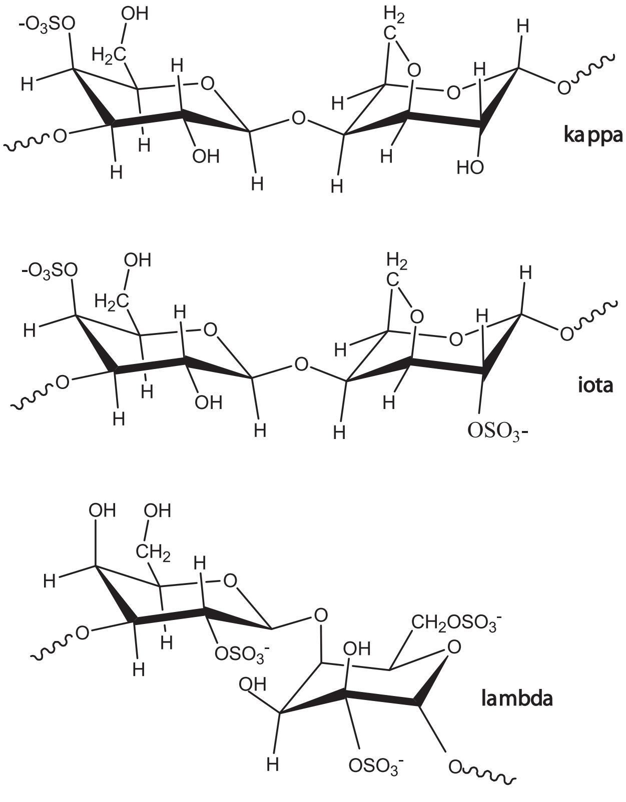
doi:10.1016/j.bpc.2004.09.005



Scheme 1.

SEC-MALS results allow the analysis of the variation of the radius of gyration with molecular weight which is customarily written as a scaling law [12,13], $\langle s^2 \rangle^{1/2} = QM^q$, whose coefficients provide valuable information about thermodynamical quality of the solvent and about the size and shape of the polymer. Moreover, the variation of the ratio $\langle s^2 \rangle / M$ with M allows the extrapolation to unperturbed conditions and the evaluation of unperturbed dimensions which, again depend on the shape of the polymer. Finally, the variation of the light scattering form factor $P(\theta)$ with the scattering vector h provides further information on the overall shape of the polymer chains.

We have employed SEC-MALS measurements for the analysis of several polymer solutions [14–17], including some polysaccharides. As a continuation of that work, we present here experimental measurements obtained from water solutions of κ-, ι-, and λ-carrageenans in presence of different ionic salts, namely LiCl, NaCl, KCl and NaI. Three of these salts contain the same anion, i.e. Cl^- while the effect of their cations on the solution behavior of carrageenans is quite different. Thus, it has been well established that the ability of the polymer to form gels from the salty solution depends on the nature of the cation and increases in the order Li^+, Na^+, K^+. The fourth salt that we have employed, NaI, contains the iodide anion which has been proven to stabilise the helical structures of κ-carrageenan in solution through a specific site binding mechanism that prevents association of helical segments and network formation [3,5,18,19]. Thus, even if anions usually produce rather minor effects on the gelation process, the pair iodide/κ-carrageenan is quite different since the formation of helical segments is controlled by the same factors as in other systems (i.e. temperature, polymer concentration, ionic strength, nature of the cation, etc.), but the iodide binding to the helix conformation of κ-carrageenan gives rise to a stabilisation of this structure. Consequently, the choice of salts employed in this work allows studying both coiled (in presence of LiCl or NaCl) and helical (in presence of NaI) structures of κ-carrageenan [3,5,18,19].

In our opinion, critical interpretation of SEC-MALS results could provide a better understanding of the solution properties of these polymers, including the conformational transitions that take place prior to the gelation process.

2. Experimental

2.1. Materials

The polymers ι-, κ- and λ-carrageenan were commercial samples (Aldrich). The salts used were NaCl, LiCl, NaI and KCl (Scharlau). The water (Milli-Q, Millipore) and the aqueous 0.1 M salt solutions used as eluents in SEC were filtered through a 0.2 μm cellulose membrane (Millipore) and degassed.

2.2. Measurements

SEC measurements were carried out using an equipment consisted of a Waters Associates model 510 pump with a 0.1-μm on-line filter (Millipore), a U6K injector (Waters) and two different detectors: an Optilab Interferometric Refractometer (RI) operating at 632.8 nm and a Dawn DSP-F multiangle light scattering (MALS) photometer, equipped with a He–Ne laser (λ=632.8 nm), both from Wyatt Technology. The chromatographic columns used, two Ultrahydrogel Linear 6–13 μm (Waters) were kept in an oven at 25 °C. The flow rate was 0.5 ml/min. Repeated injections were made for each sample at different concentrations (<5 mg/ml) to ensure the reproducibility of the samples.

The MALS photometer was calibrated with spectrometric grade toluene (Scharlau). The normalization of the detectors in the different aqueous solutions was performed with low molecular weight, standard samples of poly (ethylene oxide) and dextran of low molecular weight. The software used, ASTRA 4.73 from Wyatt Technology, allowed on-line collection of data of molecular mass and radius of gyration, as well as calculation of the distributions and averages.

Several values of differential refractive index increment dn/dc are found in the literature of carrageenans [8,20–22]. We used values ranging from 0.13 to 0.14 ml/g following the data of Viebke et al. [8] and used one of the options of the Astra software which allows to verify the dn/dc values from data of injected mass and calibration constant of the interferometric refractometer. Thus, we could check the reproducibility of data.

2.3. Theoretical background

The basic light scattering equation [23] is:

$$\frac{Kc}{\Delta R_\theta}\left[1+\frac{16\pi^2}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\langle s^2\rangle_z-\ldots\right]\left[\frac{1}{M_\mathrm{w}}+2A_2c+\ldots\right] \tag{1}$$

where ΔR is the excess Rayleigh ratio, c is the concentration, λ is the wavelength of the incident light in the medium, θ is the scattering angle and K is the optical constant given by:

$$K=\frac{4\pi^2 n_1^2}{\lambda_0^4 N_\mathrm{A}}\left(\frac{\mathrm{d}n}{\mathrm{d}c}\right)^2 \tag{2}$$

where λ_0 is the vacuum wavelength, n_1 is the solvent refractive index, N_A is Avogadro's number, and dn/dc is the refractive index increment.

The first parentheses on Eq. (1) contains the terms of the particle form factor that takes into account the interferences among light scattered at different angles for large molecules, while the second parentheses include the non ideal terms arising from the polymer–polymer interactions. Since the concentrations employed in SEC-MALS are very small, and the solutions are further diluted while passing through the columns, Eq. (1) could be approximated by [24]:

$$\frac{Kc}{\Delta R_\theta}=\left[1+\frac{16\pi^2}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\langle s^2\rangle_z-\ldots\right]\left[\frac{1}{M_\mathrm{w}}\right] \tag{3}$$

The RI detector allows to measure the concentration of the polymer and the MALS detector measures simultaneously the excess Rayleigh ratio at different θ angles for each slice i of the chromatogram, which is assumed to be monodisperse, both in composition and molecular mass [24]. Hence, a plot of $Kc/\Delta R\theta$ versus $\sin^2(\theta/2)$ affords to calculate the molecular mass M_i and the mean square radius of gyration $\langle s^2\rangle_i$ for each slice across the chromatogram. These data can be used to obtain the calibration curves (M or $\langle s^2\rangle$ versus elution volume), the molecular weight distribution (MWD) and the scaling law of the polymer.

3. Results and discussion

3.1. Averaged values

The averaged values of molecular weight and radii of gyration are presented in Table 1. The first differences among the solution behavior of the three polymer are apparent in this table. For κ-carrageenan the values obtained for the molecular weight and radius of gyration in NaI are roughly twice those obtained in NaCl and LiCl. This polymer was not soluble in KCl. For ι-carrageenan different values of both the molecular weight and radii of gyration are obtained in the different eluents as can be seen in Table 1. And last but not least, λ-carrageenan yields very similar molecular weights and radii of gyration in the four eluents.

As it has been proved the values of the radii of gyration do not depend on the differential refractive index increments [24]. Thus, the easiness to draw a parallelism between the values of molecular weight and radii of gyration for the three polymers, as can be seen in Table 1, dispels any doubt about the influence of any possible errors in the refractive index increments on the molecular weights obtained.

3.2. Chromatograms

Figs. 1 and 2 show one of the MALS signals (at 90°) and the RI signal respectively, for κ, ι and λ carrageenan in the different salt solutions used as eluents. The light scattering signals are proportional to the product of molecular weight and concentration, so they have a different shape than the RI signals that are proportional to concentration. The most significant fact is that the peak for the 0.1 M NaI solution of κ-carrageenan is noticeably displaced towards lower elution volumes (i.e. higher molecular weights) than those obtained in NaCl and LiCl solutions of the same polymer.

The calibration curves of molecular weight versus elution volume, for the three polymers, are also presented in Fig. 1 whereas the corresponding logarithmic plot of the root mean squared radii of gyration versus elution volume are depicted in Fig. 2.

The molecular weight calibration curves obtained for κ-carrageenan (Fig. 1κ) are linear, as expected for good chromatographic separation, and very similar in the three eluents, although in the NaI solution significantly higher molecular weights are obtained, in good agreement with both, the averaged molecular weight obtained in this solvent (see Table 1) and the displacement of the corresponding

Table 1
Polydispersities, averaged molecular weights and root mean square radii of carrageenans in different salt solutions

Eluent: Aqueous 0.1 M of	κ-Carrageenan			ι-Carrageenan			λ-Carrageenan		
	$M_\mathrm{w}/M_\mathrm{n}$	10^6 M_w (g/mol)	$\langle s^2\rangle^{1/2}$ (nm)	$M_\mathrm{w}/M_\mathrm{n}$	10^6 M_w (g/mol)	$\langle s^2\rangle^{1/2}$ (nm)	$M_\mathrm{w}/M_\mathrm{n}$	10^6 M_w (g/mol)	$\langle s^2\rangle^{1/2}$ (nm)
LiCl	1.47	0.432	66	1.62	1.01	106	1.16	0.92	88
NaCl	1.55	0.410	67	1.53	2.07	144	1.50	1.12	111
KCl		–	–	1.40	3.04	179	1.53	1.21	109
NaI	2.09	1.06	137	1.27	1.74	132	1.35	1.17	108

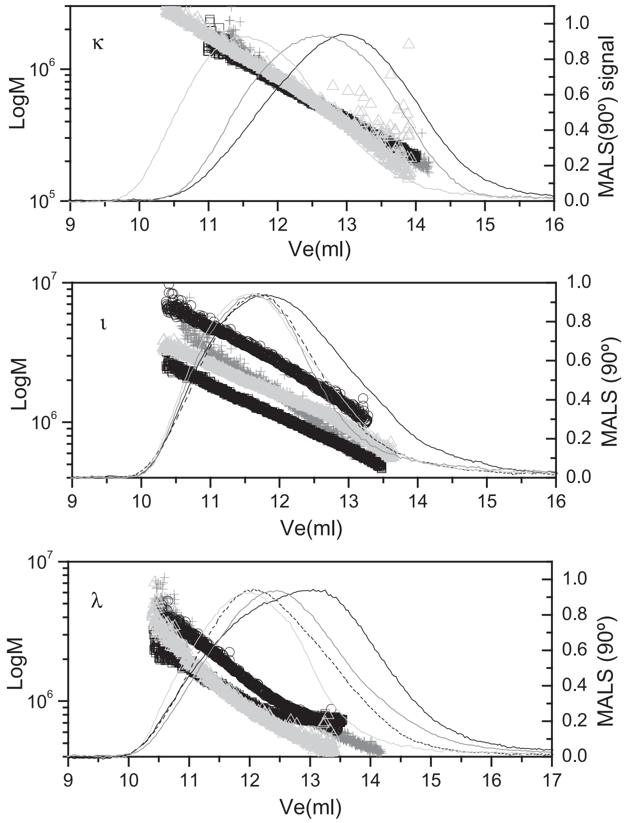


Fig. 1. Molecular weight versus elution volume for κ-, ι- and λ-carrageenan in different salt solutions: LiCl (black square), NaCl (dark grey cross), NaI (light grey triangle) and KCl (black circle). Also depicted the 90° MALS signal versus elution volume for κ-, ι- and λ-carrageenan in LiCl as solid black line, NaCl as dark grey line, NaI as light grey line and KCl as dotted black line.

MALS and RI peaks towards lower elution volumes noted above. The radius of gyration calibration curves of κ-carrageenan (Fig. 2κ) in NaCl and LiCl aqueous solutions are similar whereas the one obtained in the NaI solution is different, not only appears a zone with much higher radii of gyration, in a manner similar to the molecular weight behavior, but the whole curve is displaced towards higher radii for the same elution volumes.

On the contrary, the calibration curves of molecular weight and radius of gyration versus volume obtained for ι-carrageenan, in the various solutions are very different. The dependence of the curves on the cation Li^+, Na^+ and K^+ is evident in Figs. 1ι and 2ι, with a marked displacement to higher molecular weights and radii of gyration for the same elution volume when the size of the cation increases. It is worth to note that, in spite of these differences, the chromatographic peaks start at the same elution volume in the four solvents. The calibration curves obtained for NaI, depicted also in Figs. 1ι and 2ι appear close to the one for NaCl as indicating that the effect of the anion is less significant than that of the cation. These findings are in god agreement with the role of cations in the processes associated with coil - helix transition and gelation of carrageenans. K^+ is much more effective than Li^+ or Na^+ in promoting helix formation and then gelation [2,8,18]. Thus, the intermolecular aggregation of the chains, as a previous step to gelation, is favored in the order K^+>Na^+>Li^+. The interactions that are responsible of this effect might be general electrostatic effects which increase the cation concentration around the negatively charged polymeric chains [3].

The calibration curves of molecular weight and radius of gyration versus volume obtained for λ-carrageenan, in the various solutions are depicted in Figs. 1λ and 2λ. The chromatographic peaks start at the same elution volume in the four solvents and the dependence of the curves on the cation is less evident than for ι-carrageenan. Similar curves are obtained in NaCl and KCl solutions and only the calibration curves for the polymer in the LiCl are a little different and they show a slight displacement to lower molecular weights and radii of gyration. The calibration curves obtained in the NaI eluent appear in the same range of molecular weights and radii of gyration.

3.3. Scaling laws

The scaling laws relating the size (radius of gyration) and the molecular weight $\langle s^2\rangle^{1/2}=QM^q$ provide information about shape and size of the polymer and the thermodynamic quality of the solvent [12,13]. Thus, for random coil polymers at theta conditions, q=0.5 and the unperturbed

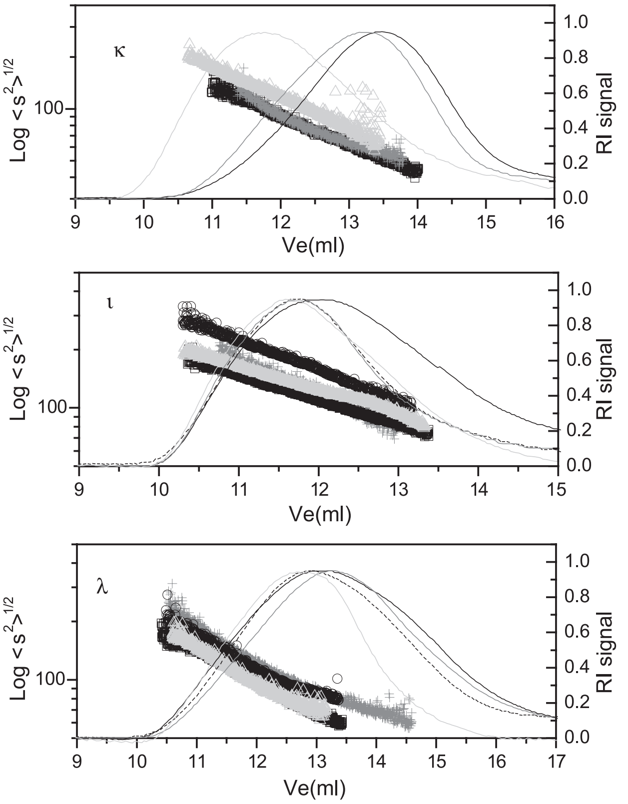


Fig. 2. Radius of gyration and refractive index signal versus elution volume for κ-, ι- and λ-carrageenan. RI signal versus elution volume for κ-, ι- and λ-carrageenan. See legend for Fig. 1.

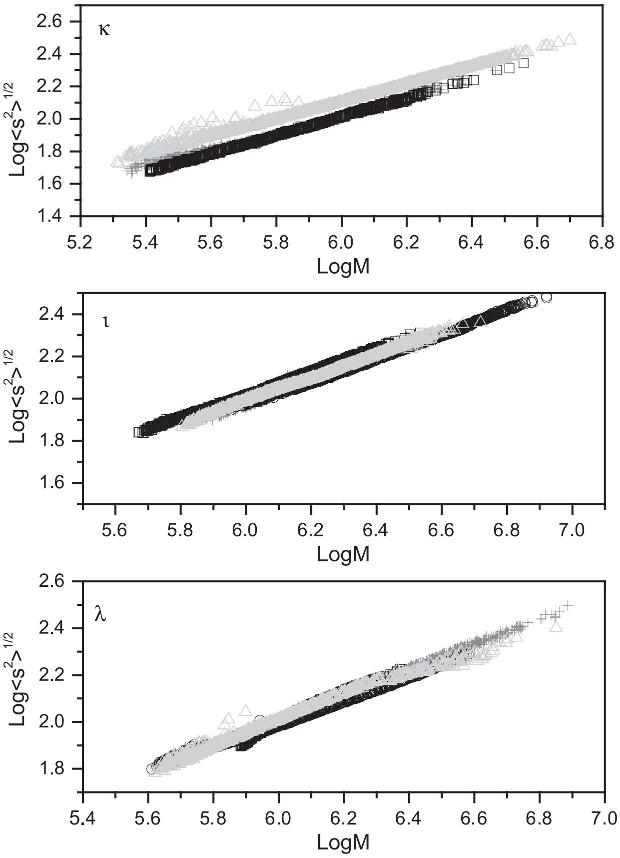


Fig. 3. Scaling laws for κ-, ι- and λ-carrageenan. See legend for Fig. 1.

radius of gyration $\langle s^2\rangle_0$ scales with M in the random walk manner. The exponent q increases with the quality of the solvent and reaches the value 0.6 for very good solvents. Below the theta conditions, the chains shrink avoiding the solvent molecules, q decreases and, in the limit, the polymer assumes a globular shape for which q=1/3. For rigid polymers q increases up to the value for a rigid rod (q=1).

The scaling laws for the three carrageenans are presented in Fig. 3, and the values of the q and Q parameters are collected in Table 2. The plots for κ-carrageenan (Fig. 3κ), are linear and the polymer is above theta conditions in the three eluents, as the values of q>0.5 (Table 2) point out. Our values are in good agreement with the literature results [4,8,25] obtained by using a few fractionated samples of κ-carrageenan in solvents that promote either the coil (NaCl) or the helix (NaI) conformations. Moreover, the fact that we obtain the same value of q=0.53 in NaCl and NaI, in good agreement with the just cited literature results, can be explained, since the q parameter is an universal exponent and the value 0.53 only indicates that the polymer chain behaves as a random coil in a solvent above θ conditions. However, as explained below, the unperturbed dimensions will provide more information about the different behavior of the polymer in the presence of Cl^- or I^-, encountered in the chromatographic peaks, and calibration curves. The value of q=0.57 obtained in the LiCl solution indicates that the presence of Li^+ instead of Na^+ improves the thermodynamic quality of the solvent.

The scaling laws for the ι-carrageenan are presented in Fig. 3ι. The scaling laws are very similar regardless of the cation, Li^+, Na^+ or K^+ employed, on spite of the results obtained for the calibration curves (Figs. 1ι and 2ι). This lack of dependence of the scaling law on the type of cation is in good agreement with the SAXS studies of gelation [26] of the polymer. The presence of I^- diminish the quality of the solvent but, still the polymer behaves as a random coil over θ conditions in all the eluents.

The results obtained for λ-carrageenan are shown in Fig. 3λ. It is interesting to notice that the value of q decreases as the size of the cation increases. Thus, the LiCl solution is a very good solvent, while the KCl solution is close to unperturbed conditions (q=0.51). The parameters for NaI are almost identical to those of NaCl which again indicates the negligible effect of the anion of the salt in the behaviour of the polymer. It should be pointed out that the change in the thermodynamical quality of the solvent observed from LiCl to KCl solutions is not accompanied by any intermolecular aggregation, as it could be ascertained by observation of Figs. 1λ and 2λ and the results collected in Table 1.

3.4. Particle scattering factor

The analysis of light scattering data can also be studied on the basis of the scattering function $P(\theta)$ defined as the ratio of the excess Rayleigh ratio at scattering angles θ and 0. The reciprocal scattering function, $P(\theta)^{-1}$, can be expanded in terms of the scattering vector h as [23]

$$\begin{aligned} P(\theta)^{-1} &= 1 + \frac{h^2\langle s^2\rangle}{3} - \ldots \\ &= 1 + \frac{16\pi^2}{3\lambda^2}\langle s^2\rangle \sin^2\left(\frac{\theta}{2}\right) - \ldots \end{aligned} \qquad (4)$$

Table 2
Scaling parameters and experimental unperturbed dimensions according to Fixman extrapolation

Eluent: Aqueous 0.1 M of	κ-Carrageenan			ι-Carrageenan			λ-Carrageenan		
	Q	q	$\langle s^2\rangle_0/M$ nm^2 mol g^{-1}	Q	q	$\langle s^2\rangle_0/M$ nm^2 mol g^{-1}	Q	q	$\langle s^2\rangle_0/M$ nm^2 mol g^{-1}
LiCl	0.039	0.57	0.0072	0.045	0.56	0.0086	0.031	0.59	0.0090
NaCl	0.066	0.53	0.0080	0.044	0.56	0.0080	0.064	0.54	0.0090
KCl				0.040	0.56	0.0066	0.084	0.51	0.0087
NaI	0.089	0.53	0.0136	0.044	0.53	0.0073	0.052	0.55	0.0087

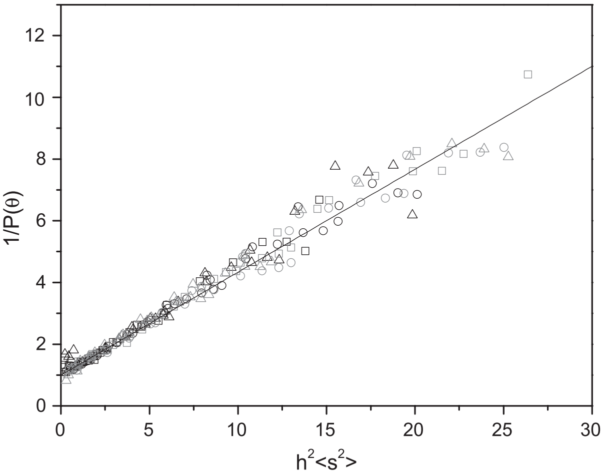


Fig. 4. $P(\theta)^{-1}$ as a function of $h^2\langle s^2\rangle$ for different slices of κ (square), ι (circle) and λ (triangle), in LiCl (black) and NaI (grey). See Table 3.

Fig. 4 shows the experimental values of $P(\theta)^{-1}$ against $\sin^2(\theta/2)$ for three different slices of each carrageenan in two different solvents NaCl and NaI at different elution volumes. The values of molecular weight, the root mean square radius of gyration and the elution volume, obtained for these slices are listed in Table 3. The fitting according to Eq. (4) (solid line) as a function of $h^2\langle s^2\rangle$ is also depicted in Fig. 4 and suggests that the conformations of the polymer chains are random coil in all the solvents in agreement with the scaling laws obtained.

3.5. Unperturbed dimensions

The results obtained from the scaling laws of carrageenans in the different solvents are not very distinctive and therefore, they do not afford to ascertain the disparities in the solution behavior observed in the averages, peaks and calibration curves that were indicated above. However, much more conclusive data can be obtained by comparison of the results of the unperturbed dimensions calculated for these chains.

The relationship between the dimensions of a polymer in a given solvent and the dimensions at unperturbed (i.e. theta) conditions is given by [12]

$$\langle s^2\rangle = \alpha\langle s^2\rangle_0 \tag{5}$$

where the expansion factor, α, depends on the quality of the solvent and the molecular weight of the polymer chain. The expansion factor becomes unity in two circumstances: First at theta conditions when the dimension of the chain reaches the unperturbed value, and second at the limit of such a low molecular weight that the interaction between segments of the same chain is not possible. The dependence of the expansion factor on molecular weight M can be used to extrapolate results obtained in perturbed condition to theta conditions. However, the dependence is complex and several theoretical and empirical expressions can be found in the literature. For polymers in good solvents, a widely used expression is the Fixman [27] extrapolation, defined in Eq. (6), which provides $\langle s^2\rangle_0/M$ as the intercept:

$$\frac{\langle s^2\rangle}{M} = \frac{\langle s^2\rangle_0}{M} + 0.0286\ \beta g(\alpha)\frac{M}{\langle s^2\rangle^{1/2}} \tag{6}$$

Since the samples of carrageenans used are polydisperse, they afford broad sets of values of radii of gyration versus molecular weight (scaling laws) that can be used to obtain unperturbed dimensions with very good results [28–30]. Fig. 5 shows the experimental values of $\langle s^2\rangle/M$ versus $M^{1/2}$ for the three polymers. The positive slopes of the extrapolations are a straight consequence of the over theta conditions of the systems, i.e. $\alpha>1$. Thus, the ratio $\langle s^2\rangle/M$ will increase with increasing M for all the samples in all the eluents although in the case of λ-carrageenan in KCl solution, the slope of this extrapolation is close to zero since the polymer is close to theta condition (see Fig. 4λ and Table 2). Extrapolations of the linear parts of the curves as shown in Fig. 5 provide the values of $\langle s^2\rangle_0/M$ collected in Table 2.

The length of a polymeric chain is customarily represented by the characteristic ratio C_N, defined as the ratio of the unperturbed dimensions of the real chain to the dimensions of a freely jointed chain containing the same number of skeletal bonds N:

$$C_N = \frac{\langle r^2\rangle_0}{Nl^2} = \frac{6M_0}{nl^2}\frac{\langle s^2\rangle_0}{M} \tag{7}$$

where M_0 is the molecular weight of the repeating unit, and $\langle r^2\rangle_0$ the unperturbed value of the mean square end to end distance, which for flexible chains can be obtained as $\langle r^2\rangle_0=6\langle s^2\rangle_0$. The values of C_N computed according to Eq.

Table 3
Elution volume, molecular weight and radius of gyration for different slices of the SEC chromatogram of carrageenans

Slice		κ			ι			λ		
		V_e (ml)	$10^{-6}\ M_w$ (g mol^{-1})	$\langle s^2\rangle^{1/2}$ (nm)	V_e (ml)	$10^{-6}\ M_w$ (g mol^{-1})	$\langle s^2\rangle^{1/2}$ (nm)	V_e (ml)	$10^{-6}\ M_w$ (g mol^{-1})	$\langle s^2\rangle^{1/2}$ (nm)
LiCl	1	10.9	1.83	150	10.4	2.56	176	10.4	2.53	175
	2	11.8	0.88	95	10.8	2.04	155	11.1	1.60	138
	3	13.1	0.39	60	11.8	1.27	118	12.0	0.94	97
NaI	1	10.2	4.02	275	10.4	3.51	196	10.6	3.60	197
	2	10.8	2.06	187	11.7	1.82	134	11.2	1.64	135
	3	11.5	1.22	142	11.8	2.83	173	11.8	1.15	113

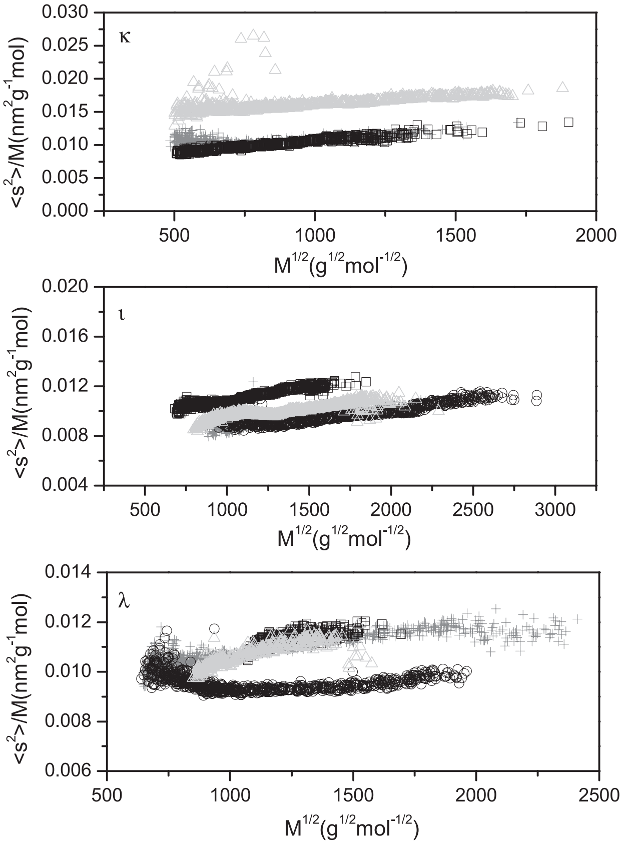


Fig. 5. Extrapolation to unperturbed condition for κ-, ι- and λ-carrageenan. See legend for Fig. 1.

(7) are shown in Table 4. Although the definition of C_N is quite straightforward, comparison of the results reported by different authors could be risky because different approaches might be used while defining the units of the freely jointed chain. Thus, we represent each repeat unit of the real chain by two freely jointed bonds linking consecutive skeletal oxygen atoms. The lengths of these bonds are 0.48 nm for the 1,3 substituted ring and 0.55 nm in the case of 1,4 substitutions. Consequently the term Nl^2 appearing on Eq. (7) amounts to 0.533 M/M_0. However, Le Questel et al. [31], who report theoretical values of C_N=3.0, 3.5 and 4.9, respectively, for κ, ι and λ carrageenans represent each repeat unit by a single bond connecting the first skeletal oxygen atom of consecutive repeat units. The length of these bonds is ca. 1 nm and therefore their values should be roughly doubled before comparing them with ours. At any rate, our experimental results are noticeably larger than the theoretical ones of Le Questel et al. [31].

According to the results summarized in Table 4, λ-carrageenan extrapolates to the same value of unperturbed dimensions, C_N=56±1 regardless of the ionic salt employed in the eluent. In the case of ι-carrageenan the dispersion of values is larger although does not suggest any correlation with the kind of salt employed. Therefore we take C_N=40±5 for ι-carrageenan which is noticeably smaller than the result obtained in the case of λ. Finally, the case of κ-carrageenan is quite different from the other two since the results obtained with LiCl and NaCl extrapolate to about the same value of C_N=33±2 which is smaller that the values obtained for λ and ι. However, in presence of NaI, the unperturbed dimensions of κ-carrageenan, C_N=59, are roughly twice than in the other two salts, although the value of q, and therefore the thermodynamical quality of the solvent, is the same for NaCl and NaI. And yet, the extrapolation to unperturbed conditions is quite good (see Fig. 5) which indicates that the polymeric chains behave as random coils, as the value of q=0.53 suggests. A tentative explanation of this different behavior of κ-carrageenan in presence of NaI as compared with the other two salts could be as follows: As it was indicated above, iodide ion favors the formation of helical sequences on the chain of κ-carrageenan [3,18,19]. However, the helical sequences do not expand over all the polymeric chain, since in that case, $\langle s^2 \rangle$ would scale with M^2 and this behavior is not observed in our experimental results. Instead, the chain might be formed by relatively short helical segments separated by a few units in different conformations that would act as flexible joints. The chain would then behave as a random coil with helical segments instead of single sugar residues as rigid units and therefore produce much larger molecular dimensions than in the case of the other two salts when the rigid units are the sugar residues.

4. Conclusions

Analysis of a single polydisperse commercial sample of κ-, ι- and λ-carrageenans by SEC with double detection employing water with different ionic salts as eluent allows the determinations of molecular weight distributions and averages, molecular dimensions and their scaling laws and unperturbed dimensions. At 25 °C, all the systems are above theta conditions, with values of the q parameter of the scaling law ranging from 0.51 to 0.59 and the polymeric chains behave as random coils. Extrapolation to unperturbed conditions provides values of the characteristics ratios C_N which are roughly independent of the ionic salt added in the case of ι- and λ-carrageenans, i.e. C_N=40±5 and 56±1, respectively, for ι and λ. However, in the case of κ-carrageenan, the value of C_N=59 obtained in presence of NaI is roughly twice the result of C_N=33±2 measured in presence of LiCl or NaCl salts. This effect could be

Table 4
Experimental values of the characteristic ratio of the unperturbed dimension $C_N=\langle r^2 \rangle_0/Nl^2$ computed according to Eq. (7)

Eluent: Aqueous 0.1 M of:	κ-Carrageenan	ι-Carrageenan	λ-Carrageenan
LiCl	31	45	57
NaCl	35	42	57
KCl	–	34	55
NaI	59	38	55

explained by assuming than in presence of NaI the polymeric chain contains relatively short helical segments linked by a few units in different conformations that act as flexible joints. Consequently the chain behaves as a random coil whose units are helical segments rather than the much shorted sugar residues that would be the rigid segments in the other cases.

Acknowledgements

The financial support provided by the DGICYT through project BQU2001-1158 and the grant PFPU (G.M.).

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