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# Light scattering study of polyelectrolyte polysaccharides - the carrageenans

## 1. Introduction

Polyelectrolytes are long-chain molecules carrying a large number of ionisable groups. For every charged group on the macromolecule, there is, somewhere in the vicinity, a small mobile counter-ion of opposite charge. A polyelectrolyte solution may, and generally does, also contain other low-molar mass electrolytes, which ionise to give small ions. The small ions that carry a charge of the same sign as the 'macro'-ion are called co-ions [1,2].

Biopolymeric polyelectrolytes, in contrast to synthetic polyelectrolytes, are a substantial part of the components of living cells. Such polyelectrolytes may exist in solution either in a linear open conformation, as do many nucleic acids for example, or in a tertiary structure, as do proteins. Apart from these two well-known groups of charged biopolymers, there is another very important class of naturally occurring polyelectrolytes, namely acidic polysaccharides, such as cellulose sulphate, hyaluronan, carrageenans, alginates, xanthan, etc. [2].

The physical-chemical polyelectrolyte behaviour has been the subject of many investigations [3]. Their properties strongly differ from simple electrolytes, and a great many theoretical and experimental studies have been performed in order to understand their behaviour in solutions, as they play such an important role in nature. The present work focuses on the associating behaviour of a polyelectrolytic polysaccharide: more specifically, iota- ( $\iota$ -) carrageenan has been investigated by means of light scattering studies to elucidate its conformation-induced network-forming abilities.

Apart from the relevance of any fundamental study of the molecularity of biopolymeric polyelectrolytes, the potentials of biopolymeric engineering, as clarified in Figure 1 stress the importance of studies of the physical-chemical

### Abstract

The results of light scattering investigations on the conformational/associative properties of  $\iota$ -carrageenan in aqueous solution are presented. The effect of different salts (NaCl, NaI, LiCl) has been investigated. The experimental data has been treated according to the Open Association Model by Elias. The results fit into the current model for the intermolecular association (i.e. the lateral junction of single-helical stretches) of the polysaccharide. The importance of preparative procedures in order to obtain reproducible light scattering data from aqueous solutions of ( $\kappa$ - and  $\iota$ -) carrageenan solutions is discussed.

**Key words:** polyelectrolytes, carrageenans, light scattering, association

behaviour of polysaccharide polyelectrolytes in solution [4]. The present work intends to contribute to the better understanding of gelling polysaccharides. Both in biological and artificial systems, a gel appears to be a most common state for polysaccharides. The polymer chains form a three-dimensional interconnected network immobilising a large quantity of solvent (water) with or without other water-soluble components. Apart from their biological function, polysaccharide gels have widespread commercial uses in the food, cosmetic, pharmaceutical and medical industries [5].

### 1.1. Applications

Low costs and high quality are commercially important. Phycocolloids such as agar, carrageenans and alginates are especially good at fulfilling these conditions. Therefore they are widespread in the food industry, as they are in addition non-toxic and have unique rheological properties [6]. Their viscosity, gel strength and stability in aqueous mixtures, solutions and emulsions make them common additives in 'modern' food such as ice-cream, pudding, sauces, dressings, canned food, syrups, jams, artificially whipped toppings, etc. The special ability of carrageenans to interact with and stabilise milk proteins means they are widely used in dairy products. Because they are well tolerated by the human organism, they can be safely used in skin applications. Therefore they are also employed in the cosmetic and pharmaceutical industry, where they are used as emulsifiers, stabilisers and thickeners in creams, lotions, dentifrice, shampoos, soaps, etc.

Their softening and refreshing properties, due to the evaporation of the water they incorporate, are also valued properties [4]. The mechanism of gel formation related to the behaviour of the phycocolloids upon heating and cooling, pH changes and the addition of monovalent and divalent cations, differentiate their applications in various products.

### 1.2. Carrageenans

Carrageenans are sulphated linear polysaccharides extracted from red algae of the class *Rhodophyceae* [7]. Carrageenans have many different functions in seaweed. For instance, they play a role as a cation-exchange barrier and as a sea-water reservoir to prevent desiccation. Moreover, they also supply the mechanical protection necessary for cells to withstand the force of the waves.

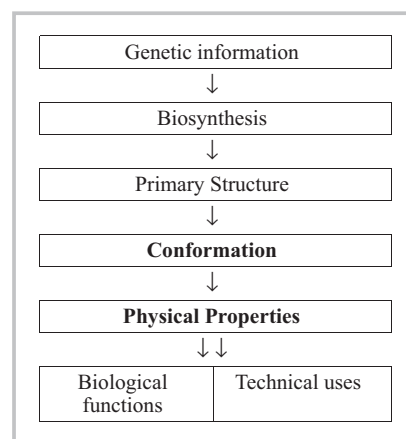


Figure 1. The localisation of the importance of conformational and physical studies in biopolymer engineering.

On the basis of their chemical composition, the carrageenans can be differentiated into three major groups, kappa- ( $\kappa$ -) carrageenan, iota- ( $\iota$ -) carrageenan and lambda- ( $\lambda$ -) carrageenan. Present and former studies have been mainly restricted to  $\kappa$ - and  $\iota$ -carrageenan, which differ only in their sulphate content:  $\kappa$ -carrageenan is formed of alternating  $\beta$ -(1-3)-D-galactose 4-sulphate and  $\alpha$ -(1-4) 3,6-anhydro-D-galactose units, (see Figure 2a), and  $\iota$ -carrageenan of alternating  $\beta$ -(1-3)-D-galactose 4-sulphate and  $\alpha$ -(1-4)-3,6-anhydro-D-galactose-2-sulphate units (see Figure 2b).

One of their basic properties is the ability to induce thickening or gelation of solutions. The most characteristic features are related to their ability to form ionotropic gels, in particular in the presence of alkaline metal counter-ions ( $K^+$ ,  $Rb^+$ ,  $Cs^+$ ). This process appears to be thermoreversible with or without hysteresis, and has been the object of numerous studies [8-10]. Detailed molecular interpretation of the gelation mechanism has, however, been controversial for a long time.

It is now universally accepted that in the first step of the gelation mechanism of carrageenans at moderate polymer concentration, a thermoreversible, ion-induced conformational transition takes place from a disordered (coil) to an ordered (helix) form. This is followed by aggregation and network formation when the polymer concentration and/or

the ionic strength are increased and/or the temperature is decreased. The disagreement between the research teams involved is at the level of the nature of the disorder-to-order transition and the degree of helix formation. Several investigators favour a coaxial double helix as the fundamental ordered conformation [11-15]: upon reducing the temperature or increasing the salt concentration, double-helical stretches will be formed in a thermoreversible process. Afterwards, these double helices will pair and form the physical junctions responsible for the gelling behaviour of carrageenans (see Figure 3). Other groups of researchers present evidence of a single helix as the fundamental ordered state of the carrageenans prior to association and gel formation. In the latter context, it is accepted that single-helical regions along the polymer chain associate into larger stretches upon increasing salt concentration and/or polymer concentration and/or decreasing temperature without levelling of at a special integral number, such as two for a double and three for a triple helix [16-22] (see Figure 4).

It is well-known that carrageenan, especially  $\iota$ -carrageenan, easily undergoes association even in low salt concentrations and at moderate temperatures. Therefore the light scattering data has been analysed within the frame of the Open Association Model of Elias, which has already been shown in the past to be the best model available with

respect to the type of association, *i.e.* open or closed [23,24].

A comprehensive study of the literature on  $\kappa$ -carrageenan clarifies the two models and the ongoing debate. However, the recent findings of three different groups strongly support the single helical strand as the candidate for association into larger segments. Moreover, it was shown that the Open Association Model of Elias could be applied to describe the association process. In order to probe whether the latter approach has more general validity, a research project was started on the polyelectrolyte behaviour of iota- ( $\iota$ -) carrageenan in saline solutions.

## 2. Light scattering study of $\iota$ -carrageenan in saline solutions

Wide-angle laser light scattering is used to explore the influence of both ionic strength and temperature on the weight-average molar mass, on the polymer-solvent interactions, and on the chain dimensions of  $\iota$ -carrageenan in diluted salt solutions. For more information on the static light scattering technique, the reader is referred to classical textbooks, where the basic principles are explained. It is well known that careful and accurate sample preparation plays a crucial role in performing reproducible and reliable light scattering measurements [23,25]. Particularly in the case of

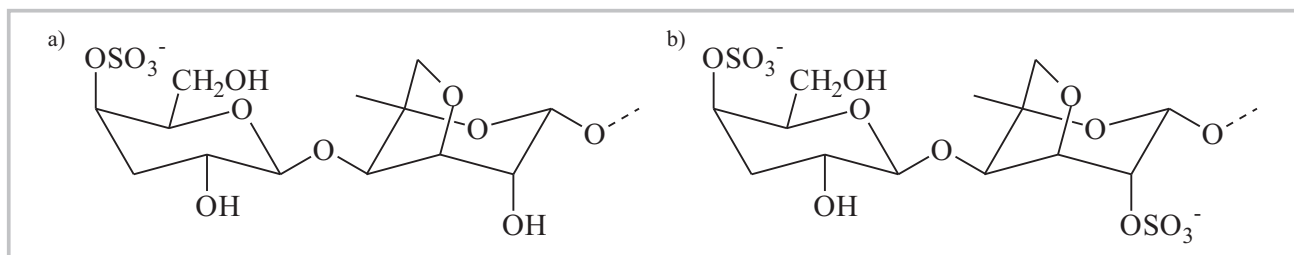


Figure 2. The chemical structure of a)  $\kappa$ -carrageenan and b)  $\iota$ -carrageenan co-polymeric repeating unit.

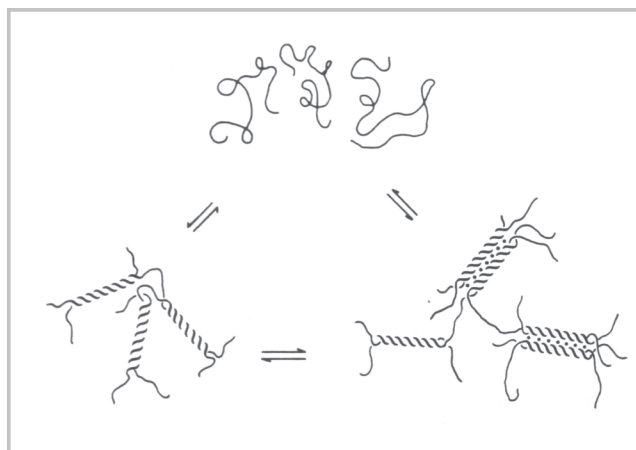


Figure 3. Gelation model for carrageenans according to Rees [5]

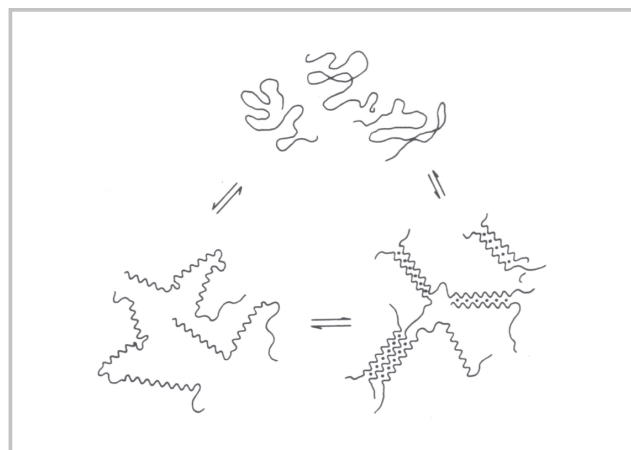


Figure 4. Gelation model for carrageenans according to Smidsrød [17,18]

$\iota$ -carrageenan, it has been found that (depending on the preparation method) there is a time dependence on the intensity measurements, which strongly affects the molecular parameters obtained from light scattering [23]. Hence, an exact description of the experimental conditions of the light scattering measurements on polysaccharides is necessary. The present light scattering study on native  $\iota$ -carrageenan samples shows that under all experimental conditions, *i.e.* during the conformational transition from the disordered to the ordered form, intermolecular association is induced as indicated by an increase of  $\bar{M}_w$ . At sufficiently high salt concentrations and depending on the nature of the added salt, a concentration-dependent upward bending in the curve of the inverse of the reduced Rayleigh ratio at zero-angle is clearly observed. This behaviour is thermoreversible. The upward bending of the zero-angle light scattering data is especially striking in chloride-containing solutions, whereas the presence of iodide ions seems to partially impede the association. At low polymer concentrations, both in NaCl, NaI and LiCl, the occurrence of the ordered state in  $\iota$ -carrageenan is not accompanied by chain dimerisation. Hence the possibility that the fundamental ordered conformation of  $\iota$ -carrageenan is a double stranded helix can *a priori* be ruled out.

A minimum in the zero-angle scattering intensity as a function of the polymer concentration was already reported in the light scattering study on the dilute and semi-dilute solutions of, *e.g.*, poly( $\gamma$ -benzyl-L-glutamate), PBLG, in 1,2-dichloroethane by Wissenburg *et al* [26]. This behaviour was attributed to an aggregation effect of the semi-flexible PBLG chains. Upon increasing the temperature from 25°C to 50°C, the upward curvature became less pronounced. This has been ascribed to the universal behaviour of the PBLG chains in various solvents (DMF, 1,2-dichloroethane).

Here we focus on interpreting the associate behaviour of the experimental light scattering data at zero-angle in the frame of the Open Association Model of Elias [27]. Particular attention is paid to the most fundamental molecular parameter, *i.e.* the weight-average molar mass,  $\bar{M}_w$ , because 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.

The study of macromolecular association was performed by analysing the

concentration dependence of the scattering at zero angle (extrapolated WALLS data). Hence the second part of this work is restricted to interpretation of the angular dependence of the data. In order to achieve this goal, a non-trivial and self-consistent method is developed in detail, and the obtained knowledge on the weight average molar mass and the second virial coefficient under associating conditions is used to calculate the radius of gyration and the persistence length of  $\iota$ -carrageenan.

## 2.1. Concentration dependence of association phenomena

### 2.1.1. 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 [28]. 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, the thermal treatment and the procedure of cleaning the sample cells may strongly affect the ultimate values of the molecular parameters obtained [29,30]. Since the light scattering method is, by its own nature, very sensitive to the quality of the solutions, the presence of small amounts of dust or aggregates can strongly influence the calculated values of the weight average molar mass. Therefore it is necessary to achieve '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.

When  $\kappa$ -carrageenan solutions [31] are prepared, starting from either an aqueous  $\kappa$ -carrageenan solution of known concentration or from a weighed amount of lyophilised  $\kappa$ -carrageenan sample dissolved in pure water, reliable light scattering data are obtained. In both cases the correct amount of salt is then added to obtain the desired molarity of the final solutions. Light scattering measurements reveal poor reproducibility and a higher molar mass when freeze-dried  $\kappa$ -carrageenan is dissolved directly into an aqueous salt solution [15].

If light scattering measurements are carried out starting from given salt solutions to which an aqueous  $\iota$ -carrageenan solution of known concentration is added, and a dilution series is obtained by addition of increasing amounts of the

salt solutions, unusual Zimm-plots are obtained [23]. There appears to be a general tendency towards an increase in the molar mass as a function of increasing salt concentration, and all the experimental observations point to an irreversible association-aggregation effect as manifested by unusual Zimm-plots and by the presence of time-effects [23,32]. Filter clogging was frequently observed, and the 'routine' laboratory experiments result in non-reproducible molar mass values. Moreover, the importance of these results is fully manifested only if a large number of experiments are performed, since one single occasional experiment can yield apparently reliable data.

When starting from an aqueous carrageenan solution of known concentration, to which a fixed amount of a salt is slowly added while stirring and filling up the volumetric flask with pure water to the graduation mark in order to obtain the desired polymer and salt concentrations, good results are obtained only if the solutions are stored for 24 hours at room temperature before measurement. The reason why the solutions are stored for 24 hours before measuring becomes clear as a rapid increase of the light scattering intensity is observed during the first two hours after the filtration, followed by a slower increase, up to constant values after approximately 5 hours. Such findings point to a non-equilibrium state of the  $\iota$ -carrageenan solutions at higher salt concentrations immediately after preparation. The perturbing effect of the shear during the filtration step must certainly be taken seriously into account. Another point of interest is the fact that in the presence of NaI the effect is less pronounced, which illustrates the influence of the anion in the process.

Therefore experiments are always performed on  $\iota$ -carrageenan solutions in thermodynamic equilibrium, *i.e.* 24 hours after following this 'safe' preparation protocol.

### 2.1.2. Application of the Open Association Model of Elias

To obtain the reduced scattered intensity at zero angles,  $KC_p/R_0$ , from wide-angle laser light scattering data, the reduced scattered intensity values,  $KC_p/R_0$ , are fitted with a function of  $\sin^2(\theta/2)$ .  $K$  is the optical constant,  $R_0$  the Rayleigh ratio at angle zero and  $C_p$  the polymer concentration. The intercepts then give the values of  $KC_p/R_0$ . For all cases, a polynomial function of the second order, *i.e.*  $F(\sin^2(\theta/2)) = B_0 + B_1\sin^2(\theta/2) + B_2(\sin^2(\theta/2))^2$ , yields almost the same results as the linear one, and the  $B_2$  values



are always very low, and therefore negligible. So, in order to obtain  $KC_p/R_0$ , preference is given to the linear extrapolation procedure.

At higher salt concentrations and lower temperatures, chain association is observed: the light-scattering concentration dependence passes through a minimum. The trend of the  $KC_p/R_0$  versus  $C_p$  plots is markedly curved in a very asymmetric way, and much more so than one expects for associating systems. Hence the familiar classical light scattering equation for non-associating systems gives a very poor fit for these systems, as is clearly evidenced from the results in reference [24].

Some improvement is obtained by using a virial expansion including a third term: Eq 1.

Here  $A_2$  and  $A_3$  are respectively the second and third virial coefficient, and  $(\bar{M}_w)_0$  stands for the weight-average molar mass of the fundamental molecular form undergoing association. The curve obtained by this quadratic fit has a lower value of  $\chi^2$ , which points to a better fit. A negative value of the second virial coefficient,  $A_2$ , is expected for an associating system. Nevertheless, the intrinsic symmetry of Equation 1 does not seem to provide the best shape for fitting the experimental data. Empirical hyperbolic functions of the type of Equations 2 and 3 appear to be more adequate, as shown by their fitting curves in reference [24]. Eq 2., Eq 3.

where

$\alpha$  and  $\beta$  are empirical fitting constants. The progressive improvement of the fittings on passing from the classical light scattering equation at zero angle to Equation 2 and Equation 3, as indicated by a decrease in  $\chi^2$ , is accompanied by a decrease  $(\bar{M}_w)_0$ . It is anyway evident that a linear function for the treatment of the zero-angle intensity data erroneously overestimates the value of  $(\bar{M}_w)_0$  for  $\iota$ -carrageenan in associating conditions.

The general equation for light scattering of associating systems in solution is mostly written as follows: Eq 4.

where

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

Associating systems can be analysed 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 [27]. In this model, a continuous distribution of the associated forms of the basic non-associated form, the 'unimer', into dimers, trimers, tetramers etc., is assumed. Each step is described by an equilibrium constant,  $K_1$ ,  $K_2$ ,  $K_3$ ,  $K_4$ , ..., and all steps of the open association are assumed to be thermodynamically equivalent, i.e. they all have identical equilibrium constants,  $K$ .

Since an intermolecular association process is always accompanied by a change of particle mass, association can be studied by means of the concentration dependence of  $(\bar{M}_w)_{app}$ . Elias [27] defines  $(\bar{M}_w)_{app}$  as the molar mass calculated from experimental data at finite concentrations using an equation valid for infinite dilution only. The initial decrease observed of  $(\bar{M}_w)_{app}^{-1}$  with increasing  $C_p$  is due to an increase in the degree of association. For higher values of  $C_p$ , the curve increases again to the prevailing effect of the second virial coefficient. The expression for the apparent weight-average molar mass according to Elias is given by Equation 5.

Here  $(\bar{M}_w)_0/(\bar{M}_n)_0$  is the weight-average molar mass over the number-average molar mass of the non-associating system, or, in other words, the polydispersity of the system, and  $K_{app}$  is the apparent association constant. In the present work, it was conservatively decided to consider it as an 'apparent' association constant, in as much as it will be treated as a phenomenological parameter of thermodynamic nature whose absolute value

Equation 1-6

$$\frac{KC_p}{R_0} = \frac{1}{(\bar{M}_w)_0} + 2A_2C_p + 3A_3C_p^2 \quad (1)$$

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

$$\frac{KC_p}{R_0} = \frac{1}{\left[ (\bar{M}_w)_0^2 + \beta C_p \right]^{1/2}} + 2A_2C_p \quad (3)$$

$$\frac{KC_p}{R_0} = (\bar{M}_w)_{app}^{-1} + 2A_{2,0}(C_p)C_p \quad (4)$$

$$(\bar{M}_w)_{app} = \sqrt{(\bar{M}_w)_0 + 4000K_{app} \frac{(\bar{M}_w)_0}{(\bar{M}_n)_0} (\bar{M}_w)_0 C_p} \quad (5)$$

$$\frac{KC_p}{R_0} = \frac{1}{\left[ (\bar{M}_w)_0^2 + 4000K_{app} P.I. (\bar{M}_w)_0 C_p \right]^{1/2}} + 2A_2(C_p)C_p \quad (6)$$

and whose dependence on temperature, ionic strength and other physical-chemical parameters will not be calculated *a priori* on the basis of any particular model. By substitution of  $(\bar{M}_w)_{app}$  into Equation 4, one obtains: Eq 6.

The analytical form of Equation 6 is the same as that of Equation 3. Although Equation 6 is a three-parameter equation, namely of  $(\bar{M}_w)_0$ ,  $K_{app}$  and  $A_2(C_p)$  when the same polymer sample is used in both non-associating and associating conditions, the value of  $(\bar{M}_w)_0$  must remain consistently the same for all cases. Therefore, when  $(\bar{M}_w)_0$  is fixed to the value of the non-associating form, Equation 6 can then be simplified to a two-parameter equation, i.e. of  $K_{app}$  and  $A_2(C_p)$ .

### 2.1.3. Determination of $(\bar{M}_w)_0$

As it has been clearly pointed out in the previous paragraphs that  $\iota$ -carrageenan undergoes interchain association, it is very important to obtain a sound estimate of  $(\bar{M}_w)_0$ . The series of experiments performed with  $\text{Na}^+$  as a counter-ion at constant temperature ( $T=25^\circ\text{C}$ ) and variable salt concentrations,  $C_s$ , are considered first. To introduce even the slightest form of bias into the data treatment, a quadratic-type of fitting function is chosen for fitting the scattered data for  $C_s$  values larger than 0.06 M. At this concentration the major deviation from linearity begins, even if the hyperbolic-types have been demonstrated to be much better. In all other cases a linear extrapolation is used. The series of  $\bar{M}_w$  values as a function of  $C_s$  obtained in this way can be best described by a linear correlation with  $C_s$ , i.e.

$\bar{M}_{w(C_s)} = \bar{M}_{w(C_s=0)} + a_1 C_s$ , with a positive slope,  $a_1=0.54$ ; this is a further indication that a concentration-dependent association indeed occurs. (See Figure 5.) The value of  $\bar{M}_{w(C_s=0)} = 203\,000 \pm 18\,000$  is a reasonable estimate for the upper boundary of  $(\bar{M}_w)_0$  in  $\text{Na}^+$ -containing solutions.

The results of applying the same procedure to the data obtained, as well as the weight-average molar mass of  $\text{Na}^+$ - $\iota$ -carrageenan in  $\text{NaCl}$  0.08 M as a function of temperature are also reported in Figure 5. In this case, a quadratic fit is used for temperature values lower than  $35^\circ\text{C}$ . The linear correlation, as can be seen in the salt concentration series, is absent. Instead, if the values of the two lowest temperature measurements are left out, the series can be fitted nicely with a polynomial fit resulting levelling off at the same value of 200 000 as found in the salt-concentration dependent series.

By comparing the two sets of independent data, it can be concluded that a value of 200 000 for  $(\bar{M}_w)_0$  of the  $\text{Na}^+$ - $\iota$ -carrageenan form can be very safely assumed for subsequent calculations.

For the  $\text{Li}^+$ -salt, similar results are obtained: a linear correlation is found with the value of the intercept  $\bar{M}_{w(C_s=0)}=197\,000 \pm 31\,000$  and the slope  $a_1=0.53$ . Having taken  $(\bar{M}_w)_0=200\,000$  for the  $\text{Na}^+$ -salt form, with some logical priority and by straightforward application of stoichiometric calculations to take into account the difference in atomic mass between the (two/repeating unit)  $\text{Na}^+$ - and the  $\text{Li}^+$ -ions, one obtains for  $\text{Li}^+$ - $\iota$ -carrageenan  $(\bar{M}_w)_0=187\,000$ . This value lies well within the experimental error of the above-cited upper bound of

197 000, and it will therefore be used in the following calculations.

#### 2.1.4. Concentration dependence of $A_2$

Interchain association might in principle be accompanied by changes in chain dimensions, and an effect on the values of the second virial coefficient cannot *a priori* be excluded. Three different cases are analysed and compared. In the first case, no concentration dependence on the second virial coefficient is taken into account for  $\iota$ -carrageenan, *i.e.*:

$$A_2(C_p) = A_2 \quad (7)$$

A linear dependence is considered as a second possibility: Eq. 8.

Equation 8,9

$$A_2(C_p) = A_{2,0} - B_2 \left( 1 - \left( \bar{M}_{w,app} / (\bar{M}_w)_0 \right) \right) \quad (8)$$

$$A_2(C_p) = A_{2,0} - B_2 \log \left( \bar{M}_{w,app} / (\bar{M}_w)_0 \right) \quad (9)$$

In the third case, the logarithmic dependence as found for  $\kappa$ -carrageenan [36] is written as: Eq. 9.

$K_{app}$ ,  $A_2$ , or  $A_{2,0}$  and  $B_2$ , are calculated for the data of  $\iota$ -carrageenan in 0.08M  $\text{NaCl}$  and for those in 0.09M  $\text{NaCl}$  both at  $25^\circ\text{C}$  with Equation 6, using the three different concentration dependency forms of  $A_2$ . The molar mass is always fixed at 200 000 and the polydispersity is 2. The results are given in Table 1.

It can be seen that there is no clear-cut dependence of  $A_2$  on  $(\bar{M}_w)_{app}$  in the investigated  $C_p$  regime. In fact, in the cases where a correction is used, the errors and the chi-square factors\*  $\chi^2$  become larger, indicating that the fitting

Table 1. Dependence of the second virial coefficient on polymer concentration for  $\iota$ -carrageenan in aqueous  $\text{NaCl}$  solutions: comparison between different concentration dependency forms in the Open Association Model of Elias.

$C_s$ (M)	Dependence	$K_{app} \cdot 10^5$	$A_{2,0} \cdot 10^3$ , $\text{mL} \cdot \text{mol}^{-2}$	$B_2 \cdot 10^3$ , $\text{mL} \cdot \text{mol}^{-2}$	$\chi^2 \cdot 10^{14}$
0.08	Constant, Equation 7	$6.24 \pm 0.68$	$2.95 \pm 0.20$	--	1.416
	Linear, Equation 8	$6.89 \pm 2.37$	$3.58 \pm 2.03$	$0.24 \pm 0.72$	1.830
	Logarithmic, Equation 9	$7.09 \pm 3.11$	$4.02 \pm 3.64$	$1.84 \pm 5.98$	1.832
0.09	Constant, Equation 7	$4.89 \pm 0.24$	$2.36 \pm 0.90$	--	0.264
	Linear, Equation 8	$4.43 \pm 0.69$	$1.75 \pm 0.89$	$-0.30 \pm 0.47$	0.305
	Logarithmic, Equation 9	$4.34 \pm 0.86$	$1.43 \pm 1.44$	$-1.88 \pm 3.06$	0.311

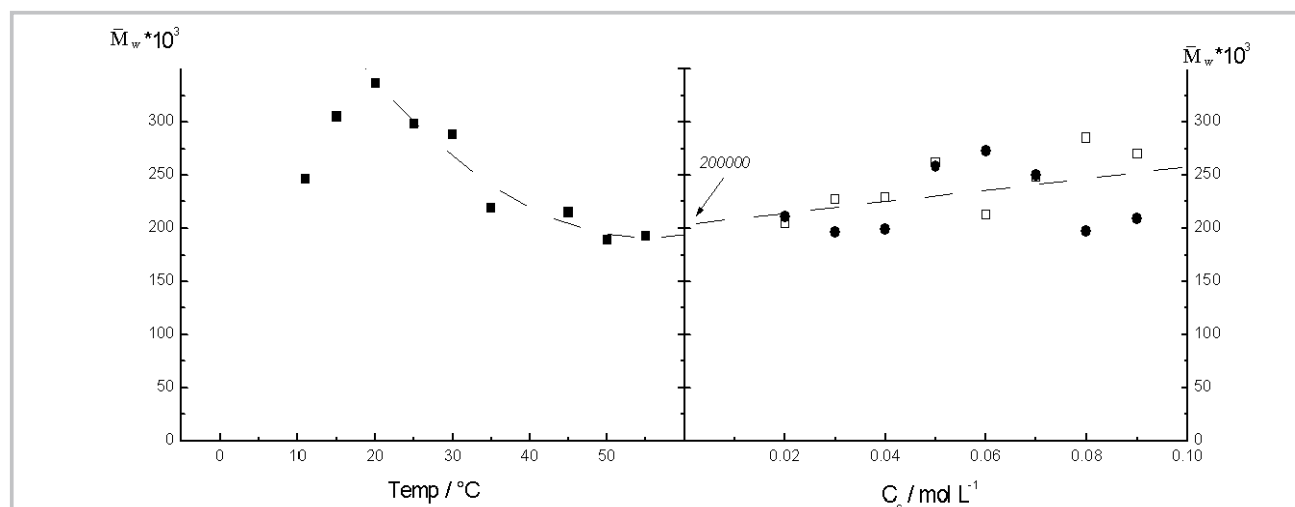


Figure 5. The weight-average molar mass of  $\text{Na}^+$ - $\iota$ -carrageenan in 0.08 M  $\text{NaCl}$  as a function of the temperature (solid squares), and as a function of  $\text{NaCl}$ , (open squares) and  $\text{NaI}$  (solid circles), concentration at  $25^\circ$ .

\* The standard way of defining the best fit is to choose parameters so that the sum of the squares of the deviations of the theoretical curve(s) from the experimental points for a range of independent variables,  $\chi^2$ , is at its minimum.

is less good. There is no need to introduce any specific correction for the second virial coefficient dependence on  $(\bar{M}_w)_{app}$ , and hence on  $C_p$ , without affecting the reliability of the derived parameters.

## 2.2. Angular dependence and conformational properties

### 2.2.1. Theoretical approach

The tedium involved in collecting multi-angle data and the often hazardous extrapolation in the case of non-linear Zimm-plots, as is the case for associating systems, are probably the root cause of the scarce data sets which can be retraced in the current literature. This is especially true for ι-carrageenan as originally shown by Vanneste [23]. It is, however, worthwhile and rewarding to consider the information content of the form factor,  $P_\theta$ , as it enables the extraction of the z-average root mean square radius of gyration,  $(\langle Rg^2 \rangle_z)^{1/2}$ , an important parameter related to the average shape of the macromolecules in solutions as given by Equation 11,

where:

$\lambda$  is the wavelength of light and  $\theta$  the scattering angle.

Whether or not the second virial coefficient is affected by an angular dependence contribution has long been and still is a matter of debate. Different equations have been proposed to introduce

$P_\theta$  in the angular independent light scattering equation. The two most simple and widely used forms are: Eq. 12, 13

Equation 12 represents the classical approach, whereas in Equation 13 the intermolecular effects are related to thermodynamic properties. In between these two extreme cases, several other cases have been proposed and discussed [10,33,34]. It is known from this literature data that intermolecular interactions more readily affect  $A_2$  than the molecular dimensions as reflected by  $\langle Rg^2 \rangle_z$ . The theoretically derived equations, however, are rather complex and difficult to use in practice [10].

For the present purpose, the angular dependent light scattering data has been initially fitted to derive the radius of gyration with several empirical functions. From preliminary calculations, it is found that when no thermodynamic interactions are accounted for in the angular dependence of the light scattering data, inconsistencies arise in fitting wide-angle laser light scattering data in the coil-to-helix transition region by using Equation 12. There is considerable scatter in the values of the resulting radii of gyration, and strongly deviating results appear under the experimental conditions at which the conformational transition takes place.  $P_\theta$ , measured as a function of scattering angle and polymer concentration, can also be said to depend on both variables, as stated in Equation 13. Nevertheless, in treating the light scattering data of macromolecular solutions

showing some degree of association, the straightforward use of the latter Equation 12 also leads to incorrect values of  $\langle Rg_0^2 \rangle_z$  in the coil-to-helix transition. Hence when analysing the angular scattering data in the coil-to-helix transition region, it seems that one has to account in some way for thermodynamic interactions. A best set of fitted results is obtained using the following empirical function, essentially based on the principles of the Open Association Model of Elias [27]. Eq. 14.

with  $(\bar{M}_w)_0$  being the molar mass of the non-associating species, *i.e.* 200 000, and  $\bar{M}_{w,app}$  the apparent molar mass,  $T_1$  and  $T_2$  are thermodynamic parameters related to intermolecular interactions. The parameter  $a_1$  in the power of the ratio of the apparent molar mass over the molar mass of the non-associating system accounts for the molar mass dependence of the radius of gyration for associating systems.

Hydrodynamic and conformational parameters of similar macromolecules, differing only in their molar mass, are often related to  $M^a$  [35]. The value of 'a' depends on the conformational characteristics of the polymer. The slope of a double logarithmic plot of the radius of gyration versus the weight average molar mass gives for  $a_1$  the theoretical value 1 for rod-like molecules, 0.6 for flexible coils in good solvents and 0.33 for spheres [1]. Due to the high number of parameters in Equation 14, it was preferred not to use  $a_1$  as a completely independent parameter, but rather to fix it. In fact, it was found that the effect of  $a_1$  on the fitting and, in particular, on the value of  $Rg_0$ , varied between the following extremes. For low extent of association (*e.g.* at low I) there was no effect of  $a_1$  on the quality of the fitting, as monitored by the sum of the squares of the residuals,  $\chi^2$ , and on  $Rg_0$ . For high values of association, a slight dependence of  $\chi^2$  and of  $Rg_0$  on  $a_1$  was noticed. In particular,  $Rg_0$  slightly decreased upon increasing  $a_1$  from 0.33 to 0.85. However, the accompanying decrease of  $\chi^2$  was small enough to justify the assumption of a constant value of  $a_1$  for all values of I. In the absence of additional independent information, the simplest theoretical choice was obviously that of the sphere. In addition, the latter model seems to be the most plausible one for the intermolecular associate, highly branched and likely to be star-shaped because of the high electrostatic repulsion.

Another important parameter in the characterisation of a polymer is the persistence length  $L_p$ , which characterises

Equation 11-15

$$\lim_{\theta \rightarrow 0} P_\theta^{-1} = 1 + \frac{16\pi^2}{3\lambda^2} \langle Rg^2 \rangle_z \sin^2(\theta/2) \quad (11)$$

$$\frac{KC_p}{R_0} = \frac{1}{\bar{M}_w P_\theta} + 2A_2 C_p \quad (12)$$

$$\frac{KC_p}{R_0} = \frac{1}{P_\theta} \left( \frac{1}{\bar{M}_w} + 2A_2 C_p \right) \quad (13)$$

$$\frac{KC_p}{R_\theta} = \left( \frac{1}{\bar{M}_{w,app}} + 2A_2 C_p \left( 1 - T_1 \sin(\theta/2) - T_2 \sin^2(\theta/2) \right) \right) \left( 1 + \frac{16\pi^2}{3\lambda^2} \langle Rg_0^2 \rangle_z \left( \frac{\bar{M}_{w,app}}{(\bar{M}_w)_0} \right)^{2a_1} \sin^2(\theta/2) \right) \quad (14)$$

$$\langle Rg_0^2 \rangle_z = L_p^2 \left( \frac{\bar{N}_z b}{3L_p} - 1 + \frac{2L_p}{\bar{N}_w b} \left( 1 - \frac{L_p}{\bar{N}_n b} \right) \right) \quad (15)$$

the flexibility of the chain. The persistence length is defined as the integral of the average projections of chain elements of the infinitely long chain on its initial direction. From the radius of gyration, the persistence length of a wormlike chain can be calculated using the equation of Benoit & Doty [36] as modified for poly-disperse samples [37] (see Equation 15).

Here  $\bar{N}_z$ ,  $\bar{N}_w$  and  $\bar{N}_n$  are the average number of chain elements, with the index indicating the appropriate kind of average and  $b$  is the monomeric projection length. Again assuming a Schulz-Zimm distribution (as indicated by previous analyses of the sample) [23], the persistence length  $L_p$  can be calculated. For  $b$ ,

the following position was used:  $b(x)=b_c(x_c)+b_h(1-x_c)$ , where  $b_c$  and  $b_h$  were 1.09 nm and 0.867 nm, and the indexes  $c$  and  $h$  refer to 'coil' and 'helix' respectively.  $x_c$  is the molar fraction of segments in the disordered ('coil') conformation, as given by optical activity measurements.

### 2.2.2. Analyses of the conformational parameters in different experimental conditions

Wide-angle laser light scattering experiments are performed in NaCl, NaI and LiCl solutions at different salt concentrations, from 0.02 M to 0.10 M. In this concentration range, a conformational

transition takes place in all studied salts from a disordered (coil) to an ordered (helix) conformation, as indicated by optical activity measurements. The series in LiCl has been repeated after a long time interval to test the reproducibility of the measurements [10,24]. The angular dependence of  $\iota$ -carrageenan solutions is also studied using a constant molarity of salt, 0.08M NaCl, as a function of temperature.

In Table 2 and Table 3,  $(\langle Rg_0^2 \rangle_z)^{1/2}$  is given for NaCl and NaI, and for LiCl respectively as calculated from Equation 14 using a Levenberg-Marquardt optimisation in the non-linear least squares fit.  $\bar{M}_w$ ,  $K_{app}$  and  $A_2$  are used as constants, obtained as described in part 2.1 of this work. The error on the z-average mean square root radius of gyration of the non-associated form is always rather small. The values of  $T_1$  are in most cases between 0 and 1, and  $T_2$  oscillates around 0 (data not shown here).

In all four cases there is a clear distinction between the radii of gyration above 0.05M and below 0.05M, as can be seen from the plots of the data in Figure 6 and Figure 7, where the radius of gyration is plotted against the inverse square root of the ionic strength.

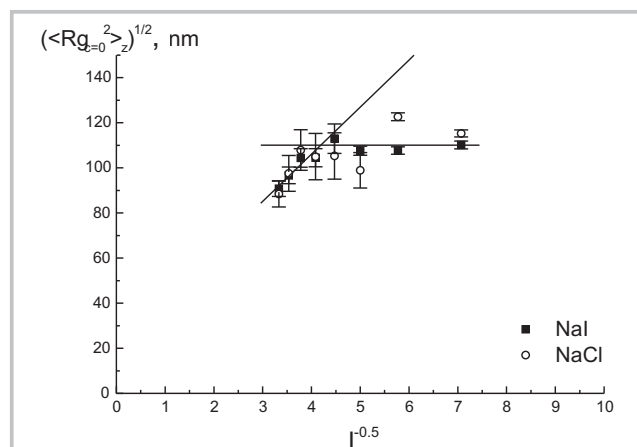
**Table 2.** The radius of gyration of  $\iota$ -carrageenan as calculated from Equation 14 in NaCl and NaI salt solutions at different molarities at 25°C.

$C_s$ (M)	$(\langle Rg_0^2 \rangle_z)^{1/2}$ , nm	
	NaCl	NaI
0.02	115.30 ± 1.57	110.20 ± 1.74
0.03	122.70 ± 1.72	107.70 ± 1.63
0.04	98.93 ± 7.83	107.60 ± 2.02
0.05	105.30 ± 10.28	113.00 ± 6.53
0.06	105.00 ± 10.30	104.50 ± 4.02
0.07	107.90 ± 9.00	104.40 ± 4.11
0.08	97.57 ± 7.95	96.69 ± 3.67
0.09	88.44 ± 5.74	90.80 ± 3.44

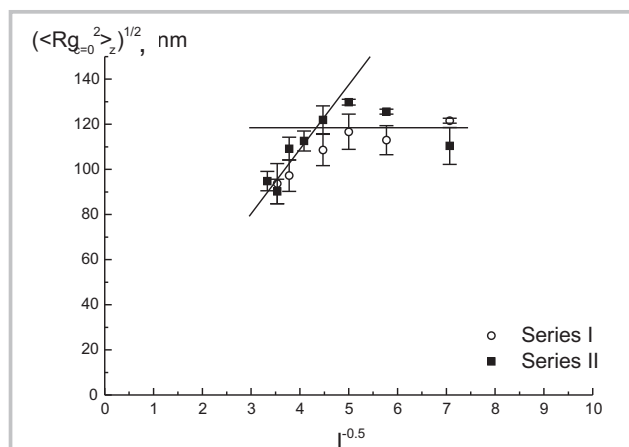
**Table 3.** The radius of gyration of  $\iota$ -carrageenan as calculated from Equation 14 in LiCl salt solutions (Series I and Series II) at different molarities at 25°C.

$C_s$ (M)	$(\langle Rg_0^2 \rangle_z)^{1/2}$ , nm	
	Series I	Series II
0.02	121.60 ± 1.10	110.40 ± 8.19
0.03	113.00 ± 6.44	125.60 ± 1.09
0.04	116.70 ± 7.79	129.80 ± 1.26
0.05	108.60 ± 6.92	122.00 ± 6.17
0.06	--	112.60 ± 4.40
0.07	97.25 ± 6.99	109.20 ± 5.04
0.08	93.70 ± 8.89	90.25 ± 5.43
0.09	--	94.83 ± 4.28

This phenomenon is no pure coincidence. In the behaviour of the second virial coefficient and the apparent association constant, as demonstrated and explained before, similar conclusions were found. Therefore it was decided to fit the two regions separately. In particular the analysis of the data in NaCl and NaI solution indicates that there is no statistical reason for not making the simple assumption that, in the range of  $C_s$  from 0.02M to 0.05M, a single average value of  $Rg_0$  holds for both salts and all concentrations. The high ionic strength regime, *i.e.* above



**Figure 6.** The radius of gyration for  $\iota$ -carrageenan in NaCl and NaI solutions as obtained from Equation 14; the solid lines represent the linear fit in the high ionic strength range and the mean value in the low ionic strength range.



**Figure 7.** The radius of gyration for  $\iota$ -carrageenan in LiCl solutions as obtained from Equation 14; the solid lines represent the linear fit in the high ionic strength range and the mean value in the low ionic strength range.



0.05M, where the dependence of the radius of gyration on the ionic strength is large, is fitted with a linear function. The fitted values of the radius of gyration are reported in Table 4 and Table 5 for Na<sup>+</sup> and Li<sup>+</sup> respectively as the counter-ion. The plots of the fitted values of R<sub>g0</sub> as a function of I are reported in Figure 8. The use of I on the abscissa allows for a clearer identification of the two regions of the ionic strength – and hence of the conformational transition – in which the behaviour differs. From Figure 8 one can note that, beyond about 50% of conformational change, the average chain dimensions are the same for the sodium and the lithium forms, whereas for lower ionic strength values the Li<sup>+</sup>-form is more expanded than the Na<sup>+</sup>-form. This is in perfect agreement with the results found for the second virial coefficient and the apparent association constant in the previous part of this chapter.

From the radius of gyration obtained after fitting, the persistence length of the unassociated chain is calculated using the Doty-Benoit equation modified for polydisperse systems, *i.e.* Equation 15. The results are also reported in Table 4 and Table 5, and a plot is given in Figure 9. The trend of the values of the persistence length as a function of the ionic strength is similar to that of the radius of gyration. More or less similar values for the two counter-ions, Na<sup>+</sup> and Li<sup>+</sup>, are found in the high salt ranges, whereas a marked difference is noted in the lower salt region of the first part of the conformational transition. In the latter range the values of the persistence length for the Li<sup>+</sup>-form are very high (L<sub>p</sub> > 100 nm), almost reaching the values for the per-

sistence length of a rod with the same contour-length as the disordered Li<sup>+</sup>- $\iota$ -carrageenan conformation. This means that the calculated values of L<sub>p</sub> are at the limit of validity of the Doty-Benoit equation, and that a concept of a 'loose helix' is much more valid for describing the conformation of  $\iota$ -carrageenan in the disordered form and in the first stages of its transition to the regular helical conformation. The overall behaviour as a function of increasing ionic strength can be qualitatively explained in this way: at first the 'loose helix' is very extended due to the low screening effect of the ionic atmosphere. During the conformational transition one can envisage two opposite phenomena: a progressive stiffening of the backbone, which should lead to a more expanded conformation, and, on the other hand, a progressive shielding of the polyelectrolyte charges due to the increase in ionic strength. The calculated behaviour of the persistence length indicates that the first phenomenon prevails in the first part of the conformational transition, whereas the second predominates beyond 50% of transformation. This explains the pseudo-biphasic behaviour of the persistence length as a function of the ionic strength. For mere comparison purposes, in Figure 10 the dependence on I<sup>-0.5</sup> of the persistence length for I values corresponding to conformational ordering  $\geq$  about 50% have been reported. In Figure 10 a line was drawn through those points to the intercept at infinite ionic strength (L<sub>p∞</sub>) calculated for the helical conformation of the accompanying molecule  $\kappa$ -carrageenan (26 nm) [31]. This operation is only aimed at indicating that the calculated values of L<sub>p</sub> for the ordered

conformation of  $\iota$ -carrageenan are compatible with that of the L<sub>p∞</sub> value, thereby giving some confidence to the calculation procedure used. Finally, it should be mentioned that we attempted to calculate L<sub>p</sub> values for the ordered conformation of  $\iota$ -carrageenan assuming a double-helical conformation. The only conformation of this type compatible with the experimental observation that the disorder-to-order transition is an intramolecular one, is that of a perfectly matched hairpin-like double-helix. However, in all cases it was not even possible to calculate L<sub>p</sub> values of R<sub>g0</sub>. This seems to be a clear-cut evidence that the only ordered conformation of  $\iota$ -carrageenan which is compatible with the experimental data is that of a single-stranded helix.

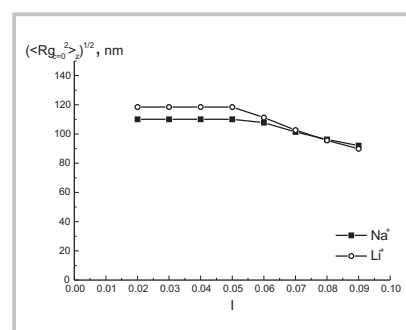


Figure 8. The fitted values of the radius of gyration for  $\iota$ -carrageenan in both Na<sup>+</sup> and Li<sup>+</sup>-solutions.

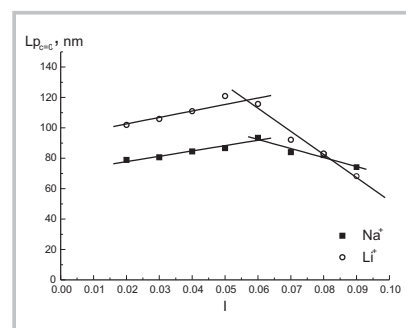


Figure 9. The persistence length for  $\iota$ -carrageenan in both Na<sup>+</sup> and Li<sup>+</sup>-solutions.

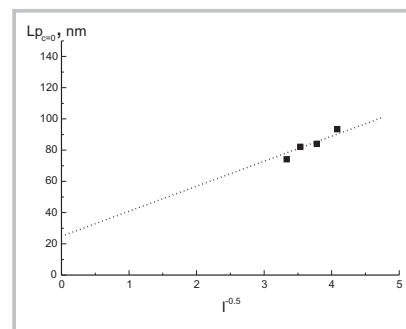


Figure 10. The persistence length of  $\iota$ -carrageenan as a function of the inverse of the ionic strength for the high ionic strength regime in Na<sup>+</sup>. The dotted line represents the extrapolation to L<sub>p∞</sub> as calculated for the helical conformation of the accompanying molecule  $\kappa$ -carrageenan.

Table 4. The fitted radius of gyration and calculated persistence length for  $\iota$ -carrageenan in Na<sup>+</sup>-solutions of the two co-ions, Cl<sup>-</sup> and I<sup>-</sup> at 25°C.

C <sub>s</sub> (M)	(<Rg <sub>0</sub> <sup>2</sup> > <sub>z</sub> ) <sup>1/2</sup> , nm	x <sub>coil</sub>	L <sub>p</sub> , nm
0.02	110.09	1.00	78.90
0.03	110.09	0.95	80.62
0.04	110.09	0.90	84.47
0.05	110.09	0.85	86.67
0.06	107.76	0.55	93.42
0.07	101.43	0.35	84.05
0.08	96.33	0.10	85.13
0.09	92.10	0.00	74.21

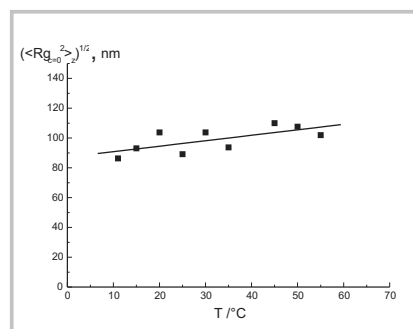
Table 5. The fitted radius of gyration and calculated persistence length for  $\iota$ -carrageenan in Li<sup>+</sup>-solutions at 25°C.

C <sub>s</sub> (M)	(<Rg <sub>0</sub> <sup>2</sup> > <sub>z</sub> ) <sup>1/2</sup> , nm	x <sub>coil</sub>	L <sub>p</sub> , nm
0.02	118.46	1.00	101.98
0.03	118.46	0.95	105.87
0.04	118.46	0.90	111.02
0.05	118.46	0.85	120.96
0.06	111.29	0.56	115.74
0.07	102.64	0.30	92.16
0.08	95.67	0.05	83.15
0.09	89.90	0.00	68.26



**Table 6.** The radius of gyration of  $\iota$ -carrageenan in 0.08 M NaCl solution at different temperatures.

T, °C	$(\langle Rg_0^2 \rangle_z)^{1/2}$ , Calc, nm	$(\langle Rg_0^2 \rangle_z)^{1/2}$ , Fit, nm	$\chi_{coil}$	Lp, nm
11	86.34 ± 4.10	91.20	0.00	71.65
15	93.11 ± 3.20	95.67	0.00	75.93
20	103.78 ± 6.05	94.50	0.10	76.10
25	89.12 ± 5.21	96.33	0.14	79.67
30	103.77 ± 7.38	98.17	0.69	64.08
35	93.70 ± 2.16	100.00	0.90	62.32
45	109.97 ± 1.74	103.37	1.00	65.82
50	107.64 ± 1.96	105.51	1.00	69.68
55	101.96 ± 2.43	107.37	1.00	73.19

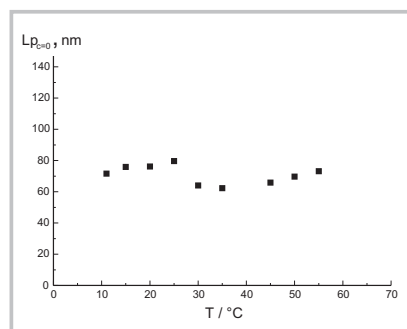


**Figure 11.** The radius of gyration as a function of the temperature in 0.08M NaCl solutions; the line is a guide to the eye.

For the dependence of the radius of gyration against the temperature in 0.08 M NaCl solution, similar results are found. When the radius of gyration is calculated in the same way as for the ionic strength dependence, one finds a more or less continuous increase with temperature (see Figure 11). The results of  $\langle Rg_0^2 \rangle_z^{1/2}$  are presented in Table 6. After fitting the calculated radius of gyration against the temperature with a linear fitting function, new, smoothed, radii of gyration can be calculated, which are also given in Table 6. From these results, the persistence length is calculated using the Doty-Benoit equation. During the conformational transition a break in the linearity of the persistence length is clearly observed, indicating the presence of two opposing mechanisms. The results are plotted in Figure 12.

### 2.3. Conclusions

$\iota$ -Carrageenan solutions are studied by wide-angle laser light scattering as a function of ionic strength in NaCl, NaI and LiCl solutions at constant temperature and as a function of temperature in 0.08 M NaCl in order to explore the influence of these factors on coil dimensions, helical conformation and polymer-solvent interactions. The radius of gyration is obtained by fitting the light scattering data with an empirical function, accounting for some thermodynamic interaction in the particle-scatter-



**Figure 12.** The persistence length as calculated by the equation of Doty-Benoit for  $\iota$ -carrageenan in 0.08M NaCl solution at different temperatures.

ing function. The function permits us to calculate the radius of gyration using the Levenberg-Marquardt optimisation method when the weight-average molar mass in non-associating conditions, the apparent association constant and the second virial coefficient are known.

The radius of gyration depends on the conformation of the  $\iota$ -carrageenan chains, and a break is observed in the linear dependence of  $(\langle Rg^2 \rangle_z)^{1/2}$  on  $I^{-0.5}$  when changing from a coil to a helix. A similar phenomenon is noticed in the intrinsic viscosity measurements during the conformational transition. □

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