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Dilute solution properties of κ-carrageenan polysaccharides: effect of potassium and calcium ions on chain conformation

M.T. Nickerson[a], A.T. Paulson[a,*], F.R. Hallett[b]

[a]Department of Food Science and Technology, Dalhousie University, Halifax, NS, Canada B3J 2X4
[b]Department of Physics, University of Guelph, Guelph, ON, Canada N1G 2W1

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Abstract

The effect of added ions (0.5 mM K^+ or 0.25 mM Ca^{2+}) and temperature (25 or 60 °C) on the solution properties of κ-carrageenan (0.2%, w/w) were investigated using static light scattering, circular dichroism and viscometry. At 60 °C (coil conformation), added salts caused a decrease in the radius of gyration (R_g), as chains became less spatially extended. As temperatures declined (60–25 °C), κ-carrageenan underwent a temperature-induced transition whereby R_g decreased by ~18% with or without added K^+, and 2.7% with added Ca^{2+}. Temperature dependence of the molecular ellipticity $[\theta]$ at 620 nm, described by the van't Hoff relationship, estimated enthalpic changes for the unraveling of the ordered conformation of 2.3–4.1 kJ/mole depending on the ionic conditions. At 25 °C (ordered conformation), R_g decreased and $[\theta]$ increased with added salts. No significant effect of ionic strength on intrinsic viscosity was found.

Keywords: κ-Carrageenan; Coil; Helix; Light scattering; Circular dichroism; Viscosity

1. Introduction

Kappa-carrageenan is a sulphated polysaccharide extracted from red seaweed (Rhodophyceae), used primarily as a gelling agent in a wide range of food applications (Millane, Chandrasekaran, Arnott, & Dea, 1988). Structurally, it consists of alternating (1→3)-α-D-galactose-4-sulphate and (1→4)-β-3,6-anhydro-D-galactose residues joined in a linear chain. The gelation mechanism of κ-carrageenan polysaccharides is thought to involve a two-step process, a disordered–ordered transition and then the aggregation of these ordered structures into an infinite network. However, the exact nature of the ordered conformation is unclear. At higher temperatures or low ionic strengths, the disordered (coil) conformation is favored due to the high conformational entropy arising from the rotation and fluctuation about the glycosidic bonds along the κ-carrageenan backbone (Morris, Rees, Thom, & Welsh, 1977; Rees, 1973). As temperatures decline or ionic strength increases, conformational entropy decreases allowing chains to overcome thermal motion and adopt an ordered structure.

The most widely accepted model of the ordered conformation was proposed by Anderson, Campbell, Harding, Rees, and Samuel (1969) based on X-ray diffraction data of κ-carrageenan fibers to be a parallel double helix having a pitch of 2.5 nm with chains offset by a 28° rotation and shifted by 0.1 nm from the half-pitch position. However, unlike ι-carrageenan, which gives good diffraction patterns, κ-carrageenan patterns are diffuse in nature, as fibers are unable to reorient further into microcrystallites (Piculell, 1995). Millane et al. (1988) reported that an antiparallel double helix model could also explain the X-ray diffraction patterns. These authors also excluded other proposed models of the ordered conformation, such as single helices, dimers, and mixtures of single helices and iota-like double helices.

In solution, the nature of the ordered conformation has been debated based on conflicting evidence supporting the double helix model or an intramolecular 'dimer' model (i.e. chains undergo a hairpin turn or go through cyclization to

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* Corresponding author. Fax: +1-902-420-0219.
E-mail address: allan.paulson@dal.ca (A.T. Paulson).

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orient into either parallel or anti-parallel intramolecular ‘dimers’ (Smidsrød & Grasdalen, 1982)), even though the latter is not supported by X-ray diffraction patterns. This debate is fueled by the inherent differences in the carrageenan molecule itself and by differences in sample preparation. Millane et al. (1988); Ueda, Itoh, Matsuzaki, Ochiai, and Imamura (1998) reported the disordered–ordered transition should be accompanied by a doubling of the molecular weight, according to the double helix model. Hjerde, Smidsrød, and Christensen (1999); Viebke and Williams (2000) reported this phenomenon to occur for κ-carrageenan using size-exclusion chromatography-multi-angle laser light scattering and field-flow fractionation, respectively. These findings, along with the X-ray diffraction patterns found by Anderson et al. (1969) are thought to support the double helix model. In contrast, the formation of an ordered conformation under specific salt conditions (0.15 M TMA-l and TMA-Cl) was not accompanied by an increase in molecular weight, even though intrinsic viscosity increased (Smidsrød & Grasdalen, 1982, 1984), nor was a concentration dependence of the coil–helix transition found (polymer concentration: 0.1–3.0% (w/v); 0.15 M TMA-Cl and TMA-I) (Grasdalen & Smidsrød, 1981). As such, Smidsrød & Grasdalen proposed that κ-carrageenan associated to form an intramolecular ‘dimer’. Bongaerts, Reynaers, Zanetti, and Paoletti (1999) reported similar molar masses between the two conformations by light scattering. Presently, there does not appear to be conclusive evidence favoring either model.

In the present study, the behavior of the coil and ordered conformations in dilute solution (i.e. below the critical overlap concentration) of commercial κ-carrageenan was investigated as a function of temperature and added salt by light scattering, circular dichroism (CD) and viscometry. A greater understanding of the behavior of commercial products will lead to better controlled-use by the food industry. Many previous studies have purified or modified the commercial form to investigate mechanisms of interactions, however, this may not give a true representation of chain behavior in industrial applications.

Conventionally, the disordered–ordered transition of κ-carrageenan has been studied by optical rotation to investigate conformational changes over the entire polymer. CD offers a means for studying spatial changes around specific reactive groups, in response to changes in temperature, pH or added ions. In terms of gelation, these groups represent the sites for ionic interactions and facilitate junction zone formation and aggregation. However, problems arise when attempting to study κ-carrageenan using CD since sulphate groups have no transition within the wavelength range of commercial instruments (190–1000 nm) (Johnson, 1987). Rees, Williamson, Frangou, and Morris (1982) added cationic dyes, such as methylene blue or acridine orange to fragmented and modified ι-carrageenan to study the coil to helix transition. These dyes were found to associate more with the helix structure rather than the coil to induce optical activity within the visible spectrum. This activity originates from coupling effects between dye molecules, a consequence of a greater linear charge density in the helical structure (Michon, Konate, Cuvelier, & Launay, 2002). Rees et al. (1982) suggested that the tendency for dye molecules to stack along the chain backbone might adversely affect the true transition, limiting their use as ‘conformational’ probes. Nevertheless in the present study, CD offers insight into the influence of added salts on the conformation of commercial κ-carrageenan near the sulphated group. Advancements in vacuum-ultraviolet CD instruments (∼155–200 nm) should lead to better characterization of the carrageenan molecule, without the use of cationic dyes (Balcerski, Pysh, Chen, & Yang, 1975).

2. Materials and methods

2.1. Materials

Kappa-carrageenan powder (Genugel carrageenan, type CHP-2F, lot #93756, 1999) (CP Kelco, San Diego, CA) consisted of 77.47% (w/w) polymer, 14.35% (w/w) potassium, 7.3% (w/w) water, and minute amounts of calcium (0.12%, w/w), sodium (0.64%, w/w), magnesium (0.12%, w/w) (atomic adsorption), and phosphorous (0.002%, w/w) (colorimetric analysis). Kappa-carrageenan powder was used without further purification. Salt stock solutions were prepared in distilled de-ionized water (DDW) using $CaCl_2 \cdot 2H_20$ and KCl.

2.2. Static light scattering

A 0.2% (w/w) solution was prepared by dispersing κ-carrageenan powder in Milli-Q-Plus™ water at room temperature with a magnetic stirrer. Dispersions were heated to 100 °C and allowed to boil vigorously for 10 min, with continuous stirring. Potassium (0.5 mM) or Ca^{2+} (0.25 mM) ions were added from a stock solution once boiling had initiated. Evaporative losses were corrected for by the addition of Milli-Q-Plus™ water. Solutions were filtered through a 0.45 μm filter (GN-6, 25 mm Metricel membrane filter, ID #63068, Pall Corporation, Michigan, USA) directly into the sample cell. All concentrations were below the critical overlap concentration, as determined by an inflection point in a plot of specific viscosity vs. polymer concentration on logarithmic axes (Nickerson, 2003). Nickerson (2003a,b) reported the overlap concentration to be 0.291% (w/w) in κ-carrageenan solutions without any added salt, 0.227% (w/w) with added K^+ (0.5 mM) and 0.273% (w/w) with added Ca^{2+} (0.25 mM).

Static light scattering (SLS) measurements were performed using a Malvern 4700 Photon correlator spectrometer (Malvern Instruments Inc., Malvern, GBR) equipped with a Siemens Helium–Neon laser ($\lambda = 632.8$ nm). A water bath served to maintain temperature. The angular dependence of the incident scatter (I_s) was measured over an angular range of 20–150° at 5° intervals, at both 25 and 60 °C. Two angular scans were performed on each of duplicate samples. Data at wider angles ($>90°$) were negligibly above the solvent and, therefore, were eliminated. The scattering angle was related to a wave vector, q, defined by

$$q = (4n\pi/\lambda)\sin(\theta/2) \qquad (1)$$

where n is the refractive index of the medium and θ is the angle at which I_s was detected (Hallett, 1996; Higgins & Benoit, 1996).The refractive index of water at 25 and 60 °C was 1.332 and 1.327 mg/ml, respectively (Weast, 1975). Measurements were made on a 0.2% (w/w) κ-carrageenan solution in the absence and presence of added 0.5 mM K^+ or 0.25 mM Ca^{2+}. Changes to chain conformation were described by the radius of gyration (R_g) determined using a Guinier plot ($\ln I_s(q)$ vs. q^2), where R_g was derived from the slope ($R_g^2/3$) of the linear portion of the curve and describes the spatial extensibility of the chains in solution. Scattering intensity data were first normalized to a reference $I_s(90°)$ to account for changes in scattering volume at different angles:

$$I_s(q) = I_s(q)(\sin\theta/I_s(90^\circ)). \qquad (2)$$

A second normalization accounted for changes in the refractive index of the sample with the addition of ions, which affects the magnitude of I_s in the Guinier plots by reducing the contrast between solvent and polysaccharides. Linear regression of I_s (after the first normalization) in a Guinier plot was extrapolated to zero (not shown), and then normalized to an arbitrarily selected value of 100 (or $e^{4.6}$).

2.3. Circular dichroism

CD measurements were performed with a Jasco J-810 spectropolarimeter (Jasco Corporation, Tokyo, JPN) using a flat-faced quartz sample cell (path length = 1 mm). Solutions were prepared as described in Section 2.2, except DDW was used. Kappa-carrageenan lacks a transition within the wavelength range of commercial instruments, therefore, methylene blue (0.98 mg/l) was added during preparation in order to form a κ-carrageenan–dye complex to give a visible adsorption band (Dea, McKinnon, & Rees, 1972). Samples were cooled to room temperature prior to measurements.

Spectral scans were performed first over a wavelength range of 450–700 nm at a rate of 20 nm/min and temperature of 25 °C. Data were collected every 0.2 nm for a total of 1250 points during each spectral scan. Temperatures were then ramped up to 60 °C by 1 C°/min at a constant wavelength (620 nm). A second spectral scan followed the thermal scan at 60 °C. Duplicate spectral scans were performed on each duplicate at 25 and 60 °C, whereas during heating, only one scan was performed on each duplicate. Temperature was controlled using a circulating water bath. Thermal scans were not performed during cooling due to instrument limitations. A mean molecular ellipticity $[\theta]$ (deg cm^2 decimol^{-1}) was determined by

$$[\theta] = \theta_p/(10 n_r C_p l) \qquad (3)$$

where θ_p is the polymer ellipticity (mdeg) (i.e. solution ellipticity minus the solvent-dye), n_r is the average number of repeating disaccharide (κ-carrageenan) units per chain, C_p is the molar polymer concentration (M) and l is the path length (cm). Molar concentrations are expressed in relation to the repeating disaccharide unit, assuming the chains are homogenous for the κ-form.

2.4. Capillary viscometry

An automatic Viscomat capillary viscometer (Pfeuffer GmbH, Kitzingen, DEU) was used to measure the viscosity of solutions at 25 °C. Solutions were measured in duplicate and prepared as described in Section 2.2 except that DDW was used and samples were unfiltered. Kappa-carrageenan solutions were prepared over a concentration range of 0.03–0.15 g/dl (or %, w/w). Intrinsic viscosity was determined for solutions in the absence and presence of added Ca^{2+} (0.075–0.25 mM) or K^+ (0.2–0.5 mM) using a polynomial function $\eta_{sp} = [\eta]c + a_2c^2 + a_3c^3$, where c is the polymer concentration. A general linear model was applied in a backward-stepwise process to determine the significance of equation constants and $[\eta]$ using Systat software (SPSS Inc., Ver. 10, 2000, Chicago, IL).

2.5. Statistical analysis

SLS (R_g) and viscometry ($[\eta]$) data were analyzed using Systat software by an individual degree of freedom orthogonal contrast analysis performed using the general linear model (Li, 1964) The effects of added salts (K^+ and Ca^{2+}) vs. no added salts, K^+ vs. Ca^{2+}, temperature (25 vs. 60 °C) and associated interactions (i.e. (1) no added salts vs. added salts × temperature and (2) K^+ vs. Ca^{2+} × temperature) were contrasted for the R_g data. A similar analysis was performed on $[\eta]$, contrasting only the effects of salt (i.e. no added salts vs. added salts and K^+ vs. Ca^{2+}). An analysis of co-variance was performed to determine differences between slopes of $[\theta]$-temperature data applied to the van't Hoff relationship ($\ln[\theta]$ vs. inverse temperature) for κ-carrageenan solutions in the absence and presence of added 0.5 mM K^+ or 0.25 mM Ca^{2+}, during heating at 620 nm.

3. Results and discussion

3.1. Static light scattering

A Guinier plot of SLS data showed a linear reduction in $\ln I_s(q)$ with increasing q^2 for all treatments (Fig. 1). Mean

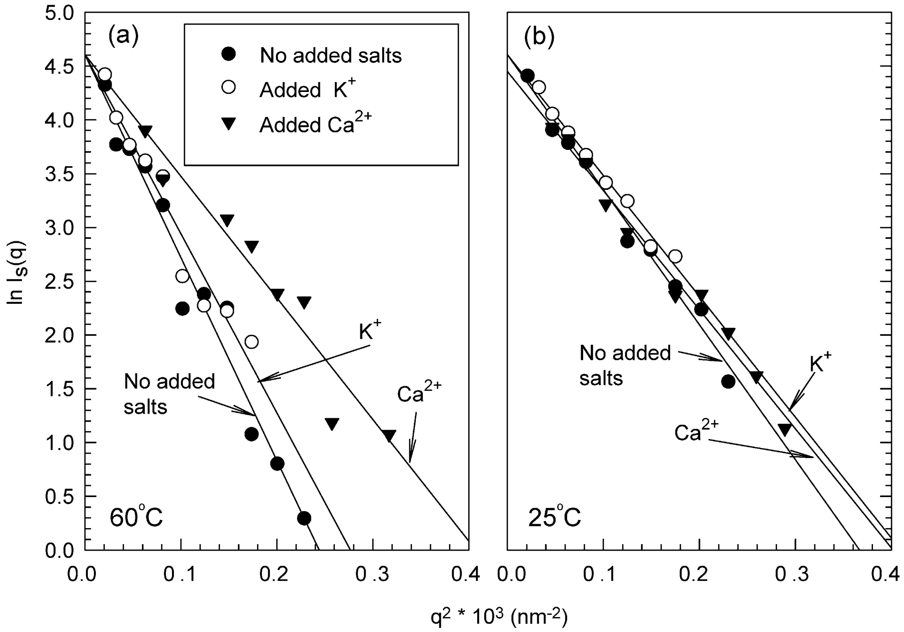

Fig. 1. Guinier approximations for the disordered and ordered conformation of κ-carrageenan (0.2%, w/w) in the absence and presence of added K^+ (0.5 mM) or Ca^{2+} (0.25 mM), at 60 °C (a) and 25 °C (b), respectively.

R_g values are shown in Fig. 2 for all solutions. An individual degree of freedom (orthogonal) contrast analysis found the interactions of the main effects contrasts (i.e. (1) no added salts vs. added salts × temperature, and (2) K^+ vs. Ca^{2+} × temperature) to be significant ($p < 0.001$). Overall, the effect of temperature was significant ($p < 0.001$), however, the effects of the main contrasts were different at each temperature, where R_g was substantially higher at 60 than 25 °C for K^+ and with no added salt systems, but not with added Ca^{2+}.

At 60 °C, the addition of salts caused chains to become less spatially extended (i.e. lower R_g) relative to solutions without any added salts, proposed to be due to a salt-induced transition, a consequence of the high residual K^+ content (14.35%, w/w) in the commercial powder. It is proposed that the addition of K^+ induced a more ordered conformation through intramolecular associations caused by a hairpin turn (anti-parallel arrangement) or through cyclization (parallel arrangement) (Piculell, 1995). Intermolecular interactions might also result in a more ordered conformation, although it is hypothesized to be a secondary effect since intermolecular interactions would most likely result in an increase in R_g. All solutions followed Newtonian flow behavior (Nickerson, 2003).

The addition of counterions above a critical salt concentration (i.e. an ionic strength above which any additional ions induce a conformational change) causes a shift from polymer–solvent to polymer–polymer interactions (Lahaye, 2001). te Nijenhuis (1997) proposed that K^+ ions associate through intramolecular interactions by forming an ionic bond with a sulphate group of one galactose residue, and then an electrostatic attractive force with the anhydro-O-3, six-ring of the adjacent galactose residue. At higher polymer concentrations, similar attractions are thought to occur with neighboring chains. Excessive salts screen electrostatic repulsive forces to promote polymer–polymer interactions (Piculell, 1995). The salt-induced disorder–order transition of κ-carrageenan is well documented in the literature (Mischenko, Denef, Koch, & Reynaers, 1996; Vanneste, Slootmaekers, & Reynaers, 1996). Smidsrød and Grasdalen (1984) reported a salt-induced transition for a 0.2% (w/v) κ-carrageenan solution by optical rotation (except at 20 °C, using 0–0.15 M TMA-I, 0–0.15 M LiI and 0–0.6 M TMA-Cl).

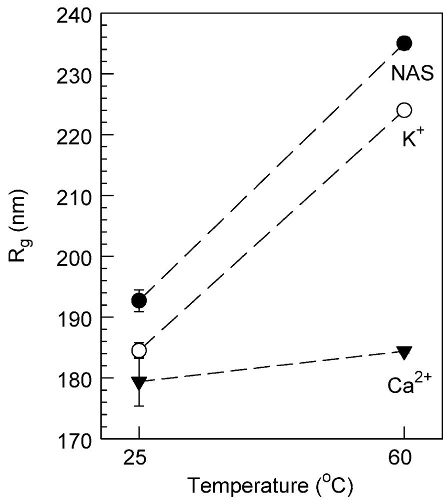

Fig. 2. The radius of gyration (R_g) as a function of salt content (no added salts (NAS), added K^+ (0.5 mM) or added Ca^{2+} (0.025 mM)) and temperature for a 0.2% (w/w) κ-carrageenan solution ($n=2$). Error bars represent one standard deviation.

With the addition of Ca^{2+}, R_g decreased substantially relative to the solutions without any added salts or with added K^+. Similar to solutions with added K^+, chains are proposed to undergo a hairpin turn or go through cyclization to form a more ordered structure, although intermolecular interactions will still occur. In the present study, the exact mechanism of Ca^{2+} interaction is unknown, although two possibilities are suggested that lead to a stable ordered conformation. First, it is proposed that Ca^{2+} screens long-range Coulombic interactions to enhance polymer–polymer interactions rather than polymer–solvent. Secondly, it is proposed that Ca^{2+} ions might cross-link two sulphated groups similar to the iota-form: (1) positioned on galactose residues on opposite sides of parallel or anti-parallel aligned segments, a consequence of cyclization or hairpin turns or lateral associations of neighboring chains, and/or (2) positioned on neighboring residues along the backbone. It is proposed that ordered structures are further stabilized by hydrogen bonding.

As temperatures declined (60–25 °C), R_g decreased by 18.0% for solutions without any added salts, 17.6 and 2.7% with added K^+ and Ca^{2+}, respectively (Fig. 2). At lower temperatures, thermal motion in the solution decreases to allow intramolecular attractive forces and co-operative (i.e. short-range local forces that function cooperatively to achieve long-range stability) interactions over the chain to increase (Rees, 1973). This results in a decrease in the conformational entropy associated with the chain, favoring a more ordered conformation (Morris et al., 1977; Rees, 1973), and will enhance associations between neighboring chains, if nearby. Although the effect of temperature on R_g was similar for solutions without any added salts and those with added K^+, it is hypothesized that, overall, there is a greater number of ordered domains present in the latter because of the proposed salt-induced transition at 60 °C. The substantial decrease in R_g at 60 °C for samples with Ca^{2+} relative to those with added K^+ or without any added salts, as well as by the lack of temperature dependence of R_g between 60 and 25 °C, is consistent with the presence of Ca^{2+}-salt bridges. Different solution properties of κ-carrageenan chains with added Ca^{2+} than without (i.e. no added salts or added K^+) suggest a different polymer-ion mechanism might be occurring, and possibly a different ordered conformation may be present.

At 25 °C, the reduction in R_g with added salts is proposed to be a consequence of charge screening, which reduces electrostatic repulsion to promote more polymer–polymer interactions. As a result, it is proposed that the ordered conformation is more tightly associated than structures found at 60 °C. In the present study, R_g values determined for the ordered conformation ranged between ~180 and 193 nm, depending on the salt content.

3.2. Circular dichroism

The CD spectrum of κ-carrageenan–dye complexes at 25 and 60 °C is shown in Fig. 3. The magnitude of the optical activity is related to the coupling effects of bound and stacked methylene blue molecules associated with the κ-carrageenan backbone. Increased optical activity occurs through long-range interactions between dye molecules that are closer than 0.35–0.77 nm apart (Michon et al., 2002). Sulphate groups in the coil conformation were estimated to be separated by 1.03 nm based on X-ray diffraction of

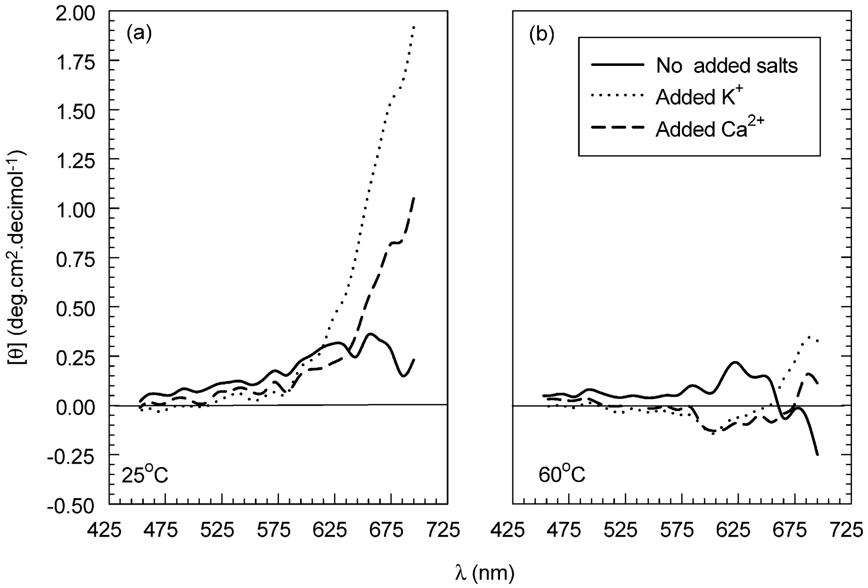

Fig. 3. Mean molecular ellipticity [θ] as a function of wavelength for the disordered and ordered conformation of a κ-carrageenan (0.2%, w/w): methylene blue (0.98 mg/l) solution in the absence and presence of added K^+ (0.5 mM) or Ca^{2+} (0.25 mM), at 25 °C (a) and 60 °C (b), respectively. Smooth lines represent the moving average of every 50 data points.

the double helix structure (Rochas & Rinaudo, 1980; Takemasa, Chiba, & Date, 2002). This makes long-range interactions between dye molecules unlikely in the coil state; consequently, the optical activity within the CD spectrum should be close to the background spectrum (i.e. water-dye). In contrast, spacing between sulphated groups in the double helix structure is estimated to be 0.43 nm (Rochas & Rinaudo, 1980), allowing for coupling between dye molecules to occur (Michon et al., 2002).

The magnitude of [θ] was highest at similar wavelengths at both 25 and 60 °C, occurring at ~620–650 and 680–690 nm for solutions in the absence and presence of added salts (independent of valency), respectively. Methylene blue solutions alone (i.e. no polymers present) have been found by Michon et al. (2002) and by Rees et al. (1982) to have a maximum absorbency near 610 and 660 nm. The shift to longer wavelengths in the presence of κ-carrageenan and added salts is hypothesized to be due to: (a) ion disruption of dye–polymer complexes through competing electrostatic attraction for the sulphate groups or electrostatic repulsion of free dye molecules, and/or (b) counterion induced conformational change of the polysaccharide that affected dye stacking tendencies, and/or (c) counterion induced conformational change of the dye molecule, which affects coupling interactions required for absorbance. Further binding studies are required to elucidate these competing or independently functioning mechanisms.

The CD spectrum of κ-carrageenan–dye complexes at 25 °C is shown in Fig. 3a. The highest magnitude of [θ] was approximately eight and four times greater for solutions with added K^+ and Ca^{2+}, respectively, than solutions with no added salts. The large rise of [θ] with K^+ is hypothesized to be the result of enhanced dye coupling, as the linear charge density becomes greater as chains are more spatially 'compacted' (i.e. lower R_g). A similar phenomenon is proposed to occur with Ca^{2+}, although ionic bridging and charge screening by the Ca^{2+} may disrupt the amount of dye coupling resulting in less of a rise in [θ].

As temperatures were raised (25→60 °C), the ordered–disordered transition was accompanied by a decrease in [θ] for all ionic conditions (Fig. 3). Rees et al. (1982) reported a decrease in the magnitude of the CD signal as temperatures were raised from 30 to 90 °C for ι-carrageenan–methylene blue complexes, which they attributed to dye binding to the helix structure, but not the coil. The van't Hoff relationship was used to describe the temperature dependence of this transition at temperatures between 25 and 60 °C, and a constant wavelength (620 nm) during heating

$$d(\ln[\theta])/d(1/T) = \Delta H/R \tag{4}$$

where T is temperature (K), R is the gas constant and ΔH is the enthalpy change associated with the unraveling of the ordered state to the disordered conformation. A linear increase in ln [θ] with $1/T$ (i.e. a decrease in [θ] with increasing temperature) was found for all treatments (Fig. 4). Enthalpic changes were derived using the slope ($\Delta H = \text{slope} \times R$) determined by linear regression. Kappa-carrageenan solutions in the absence of added ions had an enthalpy for the unraveling of the ordered conformation of 2.3 ± 0.2 kJ/mole, whereas the addition of K^+ and Ca^{2+} produced ΔH values of 4.1 ± 0.2 and 3.9 ± 0.2 kJ/mole, respectively. Analysis of covariance revealed that the treatment slopes were significantly different ($p < 0.001$, $n = 528$). Norton, Goodall, Morris, and Rees (1983a)

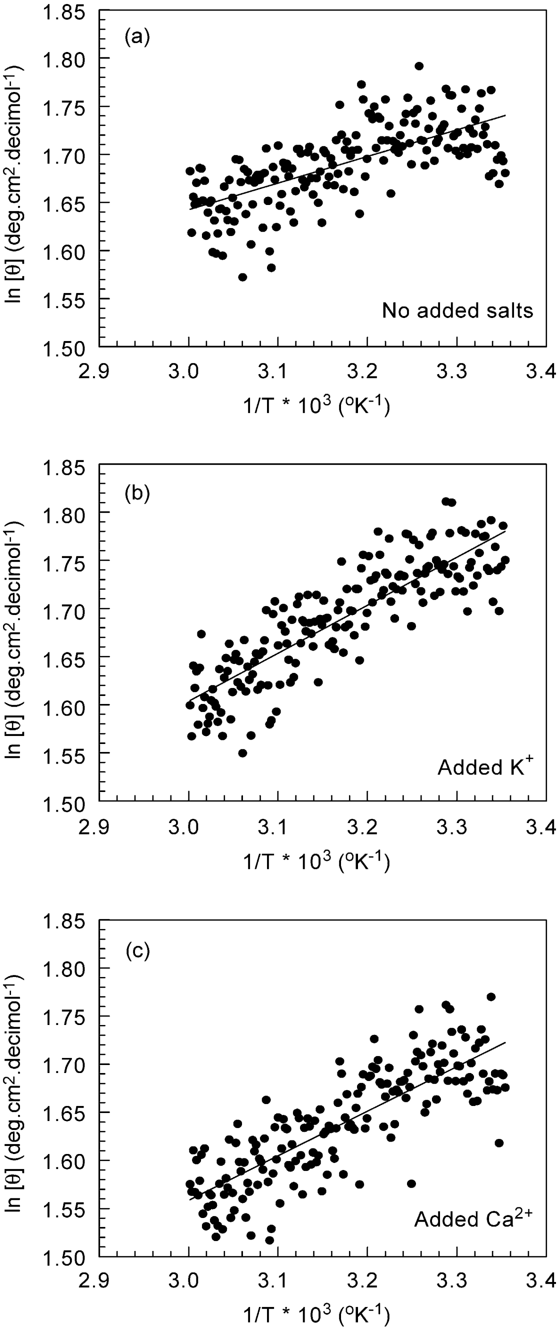

Fig. 4. van't Hoff analysis of the temperature dependence of [θ] at 620 nm during heating for a 0.2% (w/w) κ-carrageenan: methylene blue (0.98 mg/l) solution in the absence (a) and presence of added K^+ (0.5 mM) (b) or Ca^{2+} (0.25 mM) (c).

determined enthalpies during heating, using differential scanning calorimetry of the K^+-form κ-carrageenan, of 7.3–8.8 and 8.1–10 kJ/mole for different polymer concentrations (0.047–0.118 M) at 0.1 and 0.25 M K^+, respectively. In addition, the same authors reported enthalpy values for ordered ι-carrageenan unraveling ranging between 4.2 and 5.5 kJ/mole, depending on the polymer concentration, ionic strength, and carrageenan-form (Me_4N^+ or K^+). Norton, Goodall, Morris, and Rees (1983b) also found enthalpy values to range between 5 and 10 kJ/mole for κ-carrageenan solutions during heating with 0.5 and 0.1 M KCl, respectively. Caution should be taken when interpreting enthalpic data in the present study since dye binding may influence conformational changes.

At 60 °C, $[\theta]$ was reduced substantially relative to solutions at 25 °C (Fig. 3b). The small amount of optical activity suggests that the complete unraveling of the ordered domain into the coil conformation was not achieved. Rees et al. (1982) reported that dye–helix complexes were more stable than helices alone, finding a similar phenomenon with ι-carrageenan–dye complexes after heating. At 60 °C, the magnitude of $[\theta]$ increased with added K^+ relative to the solution without any added salts, whereas the magnitude remained the same with added Ca^{2+}.

3.3. Viscometry

Intrinsic viscosity was determined to be 41.2 dl/g for κ-carrageenan in the absence of added cations, whereas in the presence of added K^+ (0.5 mM) and Ca^{2+} (0.25 mM), $[\eta]$ equaled 38.0 and 39.6 dl/g, respectively. An individual degree of freedom contrast analysis found no significant differences between solutions with and without added salts (i.e. independent of ion-type), or between solutions with added K^+ or Ca^{2+} ($p > 0.05$). Harding, Day, Dhami, and Lowe (1997) obtained an intrinsic viscosity of 63 dl/g for Na^+-κ-carrageenan at 25 °C. No trend was found with $[\eta]$ as a function of ionic strength, possibly because of the high residual ion concentration in the carrageenan powder (Fig. 5). Attempts to determine the $[\eta]$ by the Huggin's equation failed because polymer concentrations were prepared by serial dilution, which increases error in the η_{sp}/c term (Kozicki & Kuang, 1996). Launay, Doublier, and Cuvelier (1986) reported the Huggin's equation is only valid at $\eta_{sp} < 0.7$. In the present study, η_{sp} ranged between 1 and 7.

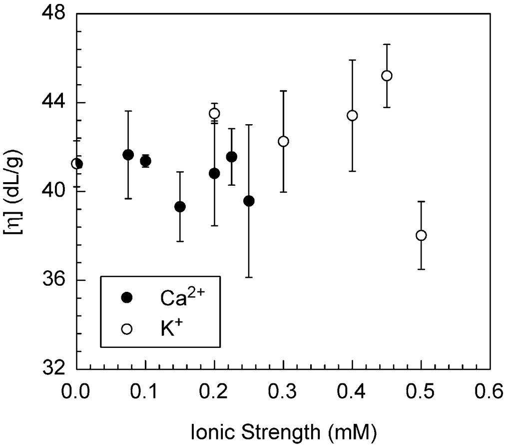

Fig. 5. Intrinsic viscosity ($[\eta]$) of κ-carrageenan solutions as a function of K^+ and Ca^{2+} concentration ($n=2$). Error bars represent one standard deviation.

The upper limit of dilute solution behavior is typically considered to occur at the critical overlap concentration (C^*), where significant chain overlap leads to substantial changes to viscosity (Harding, 1998; Jampen, Britt, & Tung, 2000; Morris, Culter, Ross-Murphy, Rees, & Price, 1981). In dilute solution, κ-carrageenan samples were found by Nickerson (2003) to display Newtonian behavior until entanglement becomes sufficient to induce non-Newtonian flow above C^*. This concentration has been determined previously using a rolling ball viscometer (Jampen et al.) and by steady shear rheometry (Linemann & Kruger, 1997; Morris et al., 1981; Nickerson, Paulson, & Speers, 2003; Nickerson, 2003) for various polysaccharides. However, the onset of chain entanglement occurs at lower concentrations, described by a Berry number ($c[\eta]$) of unity (Kasaai, Charlet, & Arul, 2000). Specific viscosity, as measured by capillary viscometry, increases linearly with polymer concentration at $c[\eta]$ values <1. Above the onset of chain overlap, entanglement becomes increasingly more significant with polymer concentration until reaching a $c[\eta]$ of 10 above which a substantial rise in viscosity occurs with increasing concentration. Launay et al. (1986) deemed the first weaker onset of chain entanglement C^*, and the second substantial change in solution properties, C^{**}.

In the present study, the mean $[\eta]$ for all solutions was found to be 41.5 dl/g by averaging all data in Fig. 5. Using the critical overlap concentrations for these same samples, determined by Nickerson (2003), $c[\eta]$ was estimated to be 12.1 for solutions without any added salts, 9.4 with added K^+, and 11.3 with added Ca^{2+}. These estimates coincide with a substantial rise in viscosity with increasing concentration (Nickerson, 2003a,b). In the present study, concentrations used had $c[\eta]$ ranging between 1.2 and 8.3. According to Kasaai et al. (2000) our samples would be considered semi-dilute rather than dilute. Although the literature is unclear on where exactly dilute solution behavior ends, its determination depends on instrument sensitivities needed to discern chain overlap to varying degrees. Although in the present study chain overlap does occur, it is proposed that intramolecular interactions are the primary mechanism of chain ordering for two reasons. Firstly, R_g values decreased as temperatures were lowered from 60 to 25 °C, whereas if intermolecular interactions were more substantial, R_g values would be expected to increase. Secondly, solutions were Newtonian as measured by steady shear rheometry (Nickerson, 2003). For similar reasons, the degree of aggregation is proposed to be limited in the present study, although a few aggregates might form.

4. Conclusion

Kappa-carrageenan is strongly influenced by the types of ions found in solution. Since the polymer concentrations were below the overlap concentration, chains are believed to undergo a conformational transition from a coil to a more ordered structure through primarily intramolecular interactions, although intermolecular interactions might occur. The exact nature of this state is still being debated, and was not elucidated by this investigation. However, based on the present findings it appears that solvent conditions may dictate the ordered conformation of the commercial powder. It is proposed that in the presence of added Ca^{2+}, cross-bridges develop between sulphate groups to form an 'intra' or intermolecular dimer, based on the absence of a thermally induced transition from light scattering, and distinctly different optical activity from solutions with added K^+. In contrast, the ordered conformation without any added salts and with added K^+ could develop either into a double helix or a dimer, stabilized by electrostatic attractive forces involving the K^+ ion and by hydrogen bonding. This is evident by a large temperature-induced transition during light scattering and differences in optical activity from solutions with added Ca^{2+}. Although studying the commercial form is complicated by the high residual salt content within the powder, it behaves similarly to the modified or purified forms previously studied in the literature.

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