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A Differential Scanning Calorimetry Study of κ-Carrageenan in the NaCl/NaI/CsI/CsCl Systems and Analysis by Poisson−Boltzmann Calculations

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ABSTRACT: The dependence on salt composition of the coil−helix−aggregate transition of κ-carrageenan has been studied experimentally and theoretically. The transition enthalpy on cooling has been measured by differential scanning calorimetry (DSC). The salt composition was varied systematically using mixtures of the four salts NaCl, NaI, CsI, and CsCl, which includes both aggregating and nonaggregating conditions. One anion or cation have been exchanged for another, keeping the total salt concentration at 0.1 M. An electrostatic model, based on the nonlinear Poisson−Boltzmann equation solved in cylindrical symmetry, was used to describe the specific binding of cesium and iodide ions to the κ-carrageenan helix. This modeling resulted in a semiquantitative description of the variation of the ion binding and the charge density in the different salt mixtures. It was found that the model can reproduce the transition enthalpies in the nonaggregated systems very well while in the aggregated systems the model predictions deviates markedly from the experimental results. This deviation, interpreted as an aggregation enthalpy, varies with the extent of thermal hysteresis. The hysteresis occurs only when the charge density is lower than the charge density for the bare κ-carrageenan helix (without bound ions). Another interesting observation is that the width of the DSC peak obtained on heating increases drastically when the aggregation occurs. Finally, the uniaxial gel strength was found to depend linearly on the extent of the hysteresis.

I. Introduction

κ-Carrageenan is a linear sulfated polysaccharide, which may be extracted from red seaweeds.[1,2] The backbone is based on a repeating disaccharide sequence of β-D-galactopyranose residues linked through the 1, 3 position and α-galactopyranose residues linked through the 1, 4 position (Figure 1). κ-Carrageenan is well-known for its gel forming properties and is used extensively in the food industry as a gelling agent[3].

The gelation mechanism of κ-carrageenan is still poorly understood. However it is agreed that gel formation involves a conformational transition of the carrageenan molecules according to the following scheme: coil−helix−gel. This scheme provides various mechanisms for gel formation. It is not specific for κ-carrageenan, but is commonly seen for helix forming systems, including synthetic polymers.[2] Gelation can occur on the helical level, where branching and association occurs through incomplete double helix formation. Another possibility is on the superhelical level, where the network is built up by aggregation of fully developed helixes. A more complex model, where the network formation is a combination of the two models above, is also a possibility. The question on which level the network formation takes place is complicated by the fact that helix formation and gel formation occur simultaneously. It has been shown that certain salts containing iodide prevent κ-carrageenan gel formation but stabilize the double helix.[4] This is in contrast to certain alkali metal ions, such as cesium, potassium, and rubidium[5] that promote both helix and gel formation. The origin of the above ion specificity has been attributed to binding of the ions to the κ-carrageenan double helix conformation[6−8] but not to the coil.

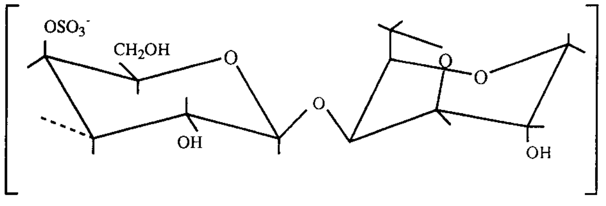

Figure 1. Ideal repeating disaccharide unit for κ-carrageenan.

The finding by Smidsrød and Grasdalen of a good solvent for κ-carrageenan helixes indicates that it is possible to alter the solvent conditions for κ-carrageenan by altering the salt composition. This strategy has been used in a number of recent studies and new important results regarding the behavior of κ-carrageenan has been found.[9−12] It was shown that solutions of κ-carrageenan helixes can be gelled isothermally and reversibly by dialysis against gel inducing salts, such as KCl or CsCl.[10] This result indicates that gel formation occurs at the superhelical level. Further in another study[11] on dilute solutions of degraded κ-carrageenan molecules in mixtures of sodium iodide and cesium iodide, cryo-TEM micrographs showed the formation of microfibers when a certain amount of cesium ions was present. The occurrence of the microfibers correlated well with the onset of thermal hysteresis in the conformational transition and an increase in specific viscosity. A rheological study on intact κ-carrageenan chains at higher concentration under the same salt conditions[12] correlated well with the findings in dilute solution.

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The aim of this paper is to distinguish and estimate the enthalpy contribution from the specific ion-binding, the nonelectrostatic interactions, and the formation of aggregates in the κ-carrageenan transition enthalpy, which can be done by systematically utilizing the abovementioned ion specificity. Earlier calorimetric studies on κ-carrageenan[13−19] have been performed under widely different ionic strengths and for some different types of salt. However the ensemble of experimental evidence is not sufficient to separate the different contributions to the transition enthalpy and the reported enthalpies show a very large variation. Our approach is to exchange one ion (cation or anion) at a time, keeping the total salt concentration constant at 0.1 M. We can cover both aggregating and nonaggregating conditions by using the following salt systems: NaCl → NaI → CsI → CsCl → NaCl. The binding of specific cations and anions to the κ-carrageenan double helix has previously been modeled by the Poisson−Boltzmann equation in cylindrical symmetry[20,21] and we have used the same model to calculate the degree of ion binding in the abovementioned salt mixtures. From the modeling and the experimental data, it is possible to estimate the contribution from the aggregation to the transition enthalpy and thereby get a clearer picture of the interplay between ion binding and aggregation in the κ-carrageenan−salt system.

II. Experimental Section

Materials. κ-carrageenan (from *Euchema cottonii*) was a gift from Sanofi Bio-Industries (France). The sample was converted to the pure sodium form by pouring a hot carrageenan solution through an ion-exchange column at elevated temperature. The carrageenan solution was collected and freeze-dried. The weight average molecular weight was estimated to be ~350 000 by light scattering.[22]

Methods. Preparation of samples. The desired salt compositions were prepared by mixing the two different salts and adding distilled water. Freeze-dried κ-carrageenan (sodium form) was dissolved in the appropriate electrolyte solution by heating to at least 80 °C for about 30 min to obtain a true solution. A 0.5% w/w solution of sodium κ-carrageenan contains approximately 12 mM Na^+. The contribution from the counterions has been neglected in the presentation of experimental data (but not in the Poisson−Boltzmann analysis), and only the salt content of the solvent is quoted.

Differential Scanning Calorimetry Measurements. The calorimetric data were collected using a SETARAM (France) C 80 calorimeter, and the data processing was performed using a HP 86. An accurate weight of solution was transferred into the DSC cell. An exact equivalent weight of pure solvent was also weighed into the reference cell. After the sample and solvent cell had been introduced into the calorimeter they were initially heated to 80 °C and allowed to equilibrate for 30 min. A program cycle was followed in which the sample was cooled to 5−30 °C (depending on the sample) at 0.3 °C/min, maintained at this low temperature for 30 min, followed by heating back to 80 °C. We chose the above scan rate to compare transition temperatures and thermal hysteresis with a previous optical rotation study of κ-carrageenan in NaI/CsI mixtures.[11] A few samples were also analyzed at a scan rate of 0.5 °C/min, which gave the same result as the lower scan rate.

Texture Analyzer. A Stevens-LFRA texture analyzer was used to collect the uniaxial gel strength data using a 10 mm probe at a speed of 0.2 mm/s. The samples were prepared as described above and then left standing at room temperature to cool. No difference between samples standing from 2 to 24 h could be observed using this technique. The maximum pressure before the gel broke was defined as $F_{\max}$.

III. Theoretical Model

The effects of ions on the conformation and association of macromolecules can be classified into three categories: [23] (1) general electrostatic effects (if the macromolecule is charged), 2) general ion-specific effects that do not depend on the particular macromolecular association/conformation (also called "lyotropic" or "Hofmeister" effects), and (3) site binding, which depends on the detailed structure of the macromolecule.

The general electrostatic effects, which are for example responsible for the enrichment of counterions outside any charged surface, can often be described by the Poisson−Boltzmann differential equation.[24]

The lyotropic effects are observed for example when precipitating proteins (salting-in and salting-out) and are well documented experimentally for many macromolecular association and conformational equilibria. The effect is stronger for the anions and one can order them in series with increasing ability to salting-in (to increase solubility of for example a protein): $SO_4^{2-} < F^- < Cl^- < Br^- < NO_3^- < I^- < SCN^-$. The effect does not yet have any satisfactory theoretical explanation, although many have investigated the subject.[25,26] A plausible explanation is that the lyotropic effect is due to an enrichment or depletion of the ions at the macromolecule−solution interface, which gives an interfacial energy contribution to the conformational/association equilibria. The ions at the right-hand side of the series (I^- and SCN^-) would therefore favor the conformation (or aggregate) with the largest surface exposed toward the solvent.

Site binding, finally, is specific to the particular macromolecule. Well-known examples include Ca^{2+} ions binding to alginate and to the protein calbindin. The specificity of the site-binding could be different for different macromolecules, but it can involve a size selective step, since the ions often have to fit into some sort of cavity.

κ-Carrageenan is negatively charged and undergoes conformational and association equilibria. It has specific binding of both anions and cations to the helix conformation, and we thus have to take all the abovementioned effects into account when evaluating the DSC results.

In this study we have used Na^+, Cl^-, Cs^+, and I^- to vary the ionic atomsphere around the κ-carrageenan. We have varied the concentration of all these ions in a systematic way by using mixed salts but at a constant ionic strength (0.1 M). Both Cs^+ and I^- are ions that bind to the helix conformation. Previous studies have shown that Cs^+ and I^- bind to separate sites and thus that we have noncompetitive binding. The specific binding has been demonstrated[6,18,21] by NMR measurements, and it affects the coil-to-helix transition by increasing the transition temperature (stabilizing the helix). However the specific mechanism and nature of the binding site is not clear.[21] For I^- we have to consider also the lyotropic effect but apparently the site binding of iodide to the helix is stronger than the lyotropic effect, since it stabilizes the (more compact) helix conformation relative to the coil. Na^+ and Cl^- are "inert" ions and they experience mainly the general electrostatic effect.

To describe the general electrostatic effect we have solved the Poisson−Boltzmann equation in cylindrical symmetry[27]

$$\frac{1}{r}\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}\phi}{\mathrm{d}r}\right) = -\frac{eN_A}{\epsilon_0\epsilon_r}c_o(\exp(-e\phi/k_BT) - \exp(e\phi/k_BT)), \qquad a < r \le b \quad (1)$$

where ϕ is the electrostatic potential, r is the radial distance from the center of the cylinder, e is the protonic charge, N_A is Avogadros number, $\epsilon_0\epsilon_r$ is the dielectric constant of water, k_BT is the Boltzmann temperature, and a is the radius of the κ-carrageenan helix (approximated as a cylinder of radius of 5.1 Å). We have applied excess amount of salt condition, i.e., the limit $b \rightarrow \infty$, so c_o is the salt concentration in the bulk where $\phi = 0$. The boundary conditions are given by the electrostatic field in the bulk ($d\phi/dr(b) = 0$) and at the polymer surface ($d\phi/dr(a) = -\sigma/\epsilon_0\epsilon_r$, where σ is the surface charge density). These conditions corresponds to infinite dilution of κ-carrageenan in a 0.1 M salt solution. The relative error in $\phi(a)$ due to finite integration was less than 10^{-6}.

The specific binding of Cs^+ and I^- to the κ-carrageenan helix has been modeled by introducing binding sites on the cylinder surface.[6,17] Associated with each site is an equilibrium binding constant which determines the degree of occupancy of the site from the law of mass action (eq 2) where $c_{m,0}$ is the concentration of

$$f_m = \frac{K_{m,0}\exp(-ze\phi(a)/k_BT)c_{m,0}}{1 + K_{m,0}\exp(-ze\phi(a)/k_BT)c_{m,0}} \qquad (2)$$

ion m in the bulk, $K_{m,0}$ is the corresponding binding constant, and z is the charge of the ion (+1 or −1). The surface charge of the cylinder is affected by the fraction of occupied sites according to eq 3, where σ_0 is the

$$\sigma = \sigma_0\left(1 - \frac{f_{Cs}}{n} + \frac{f_I}{n}\right) \qquad (3)$$

surface charge density in the absence of binding (−0.122 C/m^2), corresponding to an area per charge of 131 Å^2. f_{Cs} and f_I are the fraction of occupied sites, and n is the number of polymer charges per binding site.

One can divide the calorimetric enthalpy of the coil−helix−aggregate transition into the following contributions:

$$\Delta H_{cal} = \Delta H_{helix} + \Delta H_{agg} \qquad (4a)$$

$$\Delta H_{helix} = \Delta H_{non-el} + \Delta H_{el} + \Delta H_{bind} \qquad (4b)$$

where ΔH_{non-el} includes all nonelectrostatic contributions to the helix formation (for example hydrogen binding), ΔH_{el} represents the enthalpy cost in creating the more highly charged double helix from the less charged coil conformation, ΔH_{bind} is the contribution from the binding of the specific ions, and ΔH_{agg} is the enthalpy from the formation of superhelical aggregates. In principle ΔH_{agg} may include the same type of terms as ΔH_{helix}, but since the nature of the aggregate is poorly known, we cannot model these contributions in detail. Therefore ΔH_{agg} will not be included in the theoretical enthalpy calculations but estimated as the difference between the calculated ΔH_{helix} and the observed ΔH_{cal}.

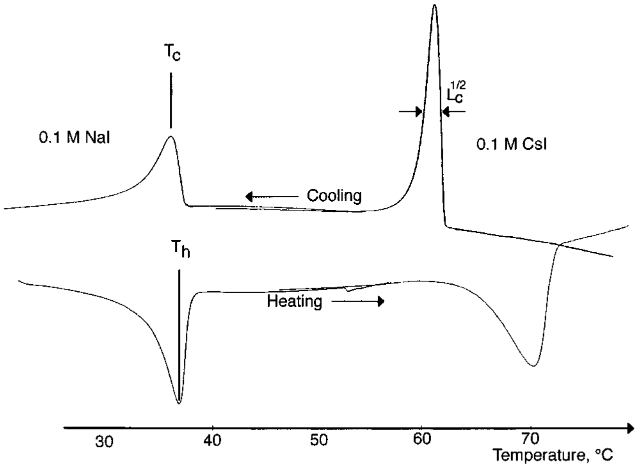

Figure 2. Two typical DSC thermograms for κ-carrageenan. The cooling and heating curves for both $X_{Cs} = 0$ (0.1 M NaI) and $X_{Cs} = 1$ (0.1 M CsI) are displayed.

The fraction of occupied sites was calculated for all the salt compositions investigated by solving the Poisson−Boltzmann equation (equation 1) in a self-consistent way to satisfy eqs 2 and 3. The fraction of occupied sites was converted into ΔH_{bind} following the formula $\Delta H_{bind} = [f_{Cs}\Delta H_{Cs^+}/n] + [f_I\Delta H_{I^-}/n]$. To calculate ΔH_{helix} we also need ΔH_{non-el} and ΔH_{el}. ΔH_{el} is neglected since it for the κ-carrageenan system is on the order of 0.1−0.4 kJ/mol[6] and the ΔH_{non-el} is unknown and has to be obtained from experimental data.

IV. Experimental Results

The experiments were designed to study the enthalpy change of the coil−helix−aggregate transition, in respect to ion binding and aggregation, over four different environments, namely, NaI → CsI, CsI → CsCl, CsCl → NaCl and NaCl → NaI. Thus, they contain two specific binding ions[2](cesium and iodide) and two inert ones. The chosen environments make it possible to vary one ion at time and keep the total ion concentration constant at 0.1 M. The salt composition is described by the following molar fractions

$$X_{Cl} = [NaCl]/\{[NaCl]+[NaI]\}; \quad NaCl \rightarrow NaI$$

$$X_{Cs} = [CsI]/\{[CsI]+[NaI]\}; \quad NaI \rightarrow CsI$$

$$X_I = [CsI]/\{[CsI]+[CsCl]\}; \quad CsI \rightarrow CsCl$$

$$X_{Na} = [NaCl]/\{[NaCl]+[CsCl]\}; \quad CsCl \rightarrow NaCl$$

which were all varied between 0 and 1. The above definitions give four equalities: $X_{Cs} = 1 = X_I$ (0.1 M CsI); $X_{Cs} = 0 = X_{Cl}$ (0.1 M NaI); $X_{Na} = 1 = X_{Cl}$ (0.1 M NaCl); $X_{Na} = 0 = X_I$ (0.1 M CsCl).

Typical DSC graphs for two different solutions are displayed in Figure 2, and both the cooling and heating curves for each solution are included. T_c, as indicated in the figure, is the temperature at the peak of the cooling curve (exotherm); T_h is the temperature at the peak of the heating curve (endotherm) and $\Delta T = T_h - T_c$ is the hysteresis. In all electrolyte compositions investigated here, T_h is equal to or higher than T_c. A difference lower than 1 deg can probably be attributed to thermal lag between the cooling and heating cycle. However no correction for thermal lag has been done. As can be seen there is some difficulty in drawing a

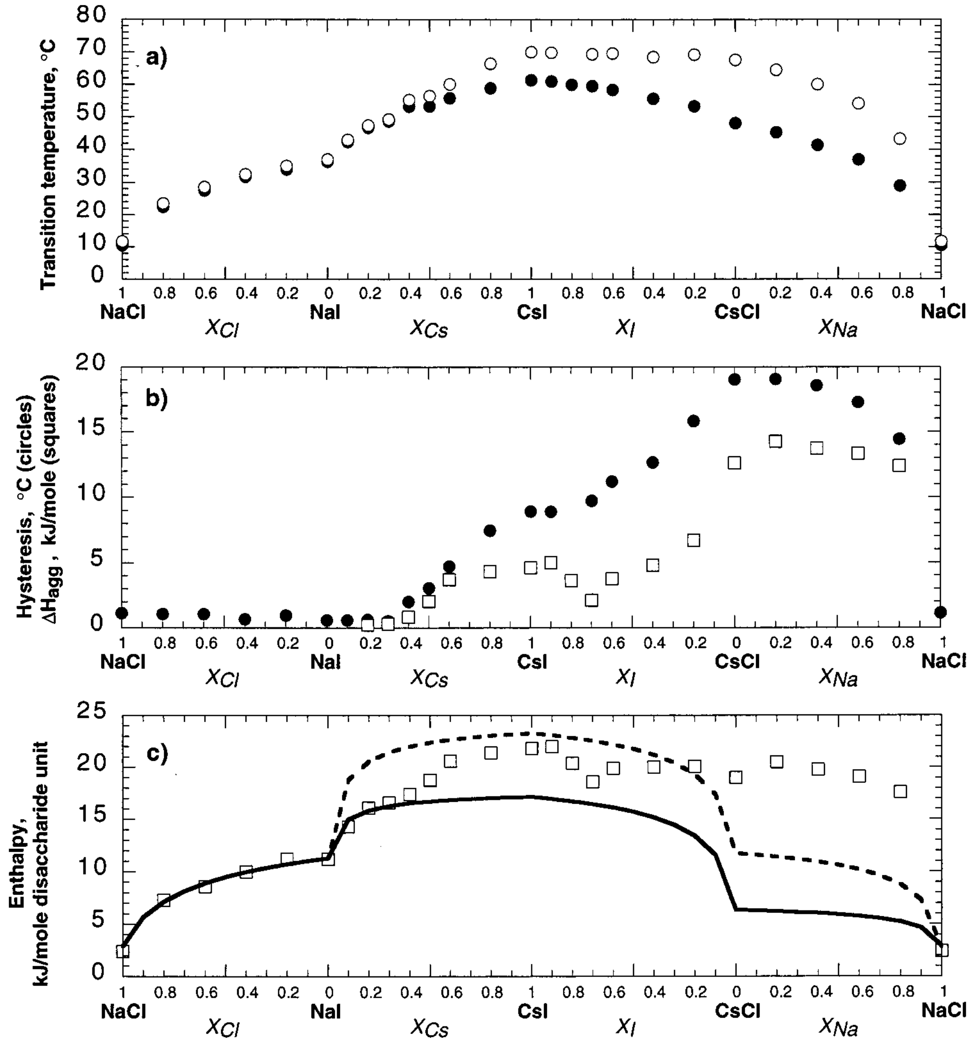

Figure 3. Experimental data for 0.5% w/w κ-carrageenan in mixed system of NaCl/NaI, NaI/CsI, CsI/CsCl and CsCl/NaCl: (a) Conformational transition temperatures T_c (●) and T_h (○); (b) hysteresis (●) and ΔH_{agg} (□); (c) experimental transition enthalpies (□) together with the calculated ΔH_{helix} (lines). The fully drawn line corresponds to parameter set 1 ($\Delta H_{Cs^+} = 22.4$, $\Delta H_{I^-} = 26.0$ and $\Delta H_{non-el} = 2.9$ kJ/mol) and the dashed line to set 2 ($\Delta H_{Cs^+} = 22.4$, $\Delta H_{I^-} = 26.0$ and $\Delta H_{non-el} = 2.9$ kJ/mol).

baseline to calculate the enthalpy, and the calculated value could vary within ~5%. However the peaks were evaluated as closely as possible to each other.

The different temperature features that can be observed in this system as a function of the salt composition are displayed in Figure 3a (see also Table 1). Starting in 0.1 M NaCl, where the transition temperature is ~12 °C, we observe a gradual increase in T_c as the chloride ions are replaced by iodide ions up to 37 °C in 0.1 M NaI. T_c continues to increases in the NaI → CsI system to ~62 °C (0.1 M CsI). This is followed by a gradual decrease on going from CsI → CsCl, and T_c reaches about 48 °C at 0.1 M CsCl. The gradual decrease continues on going from CsCl → NaCl until about $X_{Na} = 0.8$ after which a sharper decrease is observed and T_c returns to ~12 °C in 0.1 M NaCl. A similar trend is seen for the melting temperature, T_h, except that on going from CsI → CsCl the melting temperature is more or less constant.

The hysteresis, represented by the circles in Figure 3b, occurs first at $X_{Cs} \approx 0.4$ in the salt mixture NaI → CsI (no hysteresis in the system, NaCl → NaI), increases up to $\Delta T \sim 8°$ in 0.1 M CsI and is then more or less constant on going from CsI → CsCl until $X_I \sim 0.7$. A second increase is then observed, from ~10 to 20°, at 0.1 M CsCl. A gradual decrease occurs on replacement of cesium with sodium ions (CsCl → NaCl) until about $X_{Na} = 0.8$ (from ~20 to ~14 °C); thereafter a much sharper decrease down to 0 °C at 0.1 M NaCl is seen.

The corresponding enthalpy data from the cooling curve (represented as kJ/mol disaccharide unit) as a function of molar ratios are displayed in Figure 3c. The enthalpies were calculated using a molecular weight of 408 for the repeating disaccharide unit, which corresponds to the sodium form of κ-carrageenan. A slight difference between the cooling and heating enthalpies was observed under aggregating conditions. The reason for this is unknown; however, it might be due to slow (time-dependent) processes. When going from NaCl → NaI, a gradual increase in transition enthalpy is seen from 2.5 to ~12 kJ/mol of disaccharide unit. Entering the NaI → CsI system, an initially steep increase is observed that levels off at 16 kJ/mol. Above $X_{Cs} = 0.4$ there is a second increase, and an enthalpy of 22 kJ is attained in 0.1 M CsI. On replacement of the iodide by chloride ions (CsI → CsCl) a slight decrease is observed. A slight decrease is also seen in the system CsCl → NaCl to about $X_{Na} = 0.8$, followed by a sharp drop to about 2.5 kJ/mol of disaccharide unit in 0.1 M NaCl.

All the above experiments have been done at a κ-carrageenan concentration of 0.5% w/w. To check the concentration dependence of the transition enthalpy,

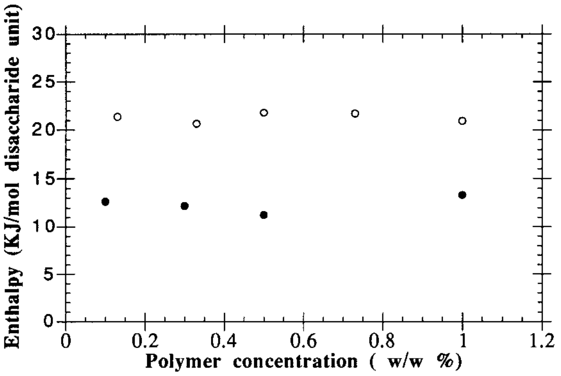

Figure 4. Enthalpy (kJ/mol disaccharide unit) as a function of κ-carrageenan concentration for 0.1 M NaI (●) and 0.1 M CsI (○).

Table 1. Parameters Obtained from DSC and Uniaxial Gel Strength Measurements

salt composition		ΔH, kJ/mol	ΔT, °C	$L^{1/2}_h$, °C	$L^{1/2}_c$, °C	F_{max}	T_c, °C	T_h, °C
X_{Cl}	1	2.4	1.15	2.95	3.08		10.5	11.7
	0.8	7.3	1.08	2.75	3.30		22.4	23.5
	0.6	8.6	1.08	2.75	3.08		27.5	28.6
	0.4	10.0	0.70	2.34	2.63		31.8	32.5
	0.2	11.2	0.99	2.42	3.08		34.1	35.1
X_{Cs}	0	11.2	0.60	2.41	2.63		36.5	37.1
	0.1	14.3	0.60	2.19	2.63		42.5	43.1
	0.2	16.1	0.64	2.19	2.41		46.8	47.5
	0.3	16.6	0.54	2.19	2.41		48.8	49.3
	0.4	17.4	2.02	4.16	1.97		53.3	55.3
	0.5	18.4	3.07	4.16	1.97		53.4	56.5
	0.6	20.6	4.72	4.09	2.04	22	55.8	60.1
	0.8	21.4	7.47	4.09	1.75	37	58.9	66.4
X_I	1	21.8	8.93	4.23	1.61	44	61.4	70.0
	0.9	22.0	8.92	4.16	1.64		61.0	69.8
	0.8	20.4	9.00	4.16	1.59	60	60.0	
	0.75	19.2	9.31	4.38	1.75		59.5	69.3
	0.7	18.6	9.73	4.38	1.75		58.4	69.6
	0.6	19.9	11.23	4.38	1.54	66	55.7	68.4
	0.4	20.0	12.68	4.60	1.54	86	53.4	69.2
	0.2	20.1	15.84	4.16	1.46	125	48.2	67.6
X_{Na}	0	19.0	19.34	3.94	1.31	151	45.4	64.5
	0.2	20.5	19.08	4.84	1.53	149	41.5	60.1
	0.4	19.8	18.58	5.05	1.75	96	37.0	54.2
	0.6	19.1	17.28	6.06	2.02	72	28.9	43.3
	0.8	17.6	14.44	6.42	2.75		10.5	11.7

two salts were selected (0.1 M NaI and 0.1 M CsI) corresponding to the nonaggregated and aggregated state. Figure 4 clearly demonstrates that the enthalpy in both salts is independent of the κ-carrageenan concentration, in the range of 0.1%–1% w/w.

The width of the transition, measured at the half of the peak maximum for the heating ($L^{1/2}_h$) and cooling ($L^{1/2}_c$) curves, are shown in Figure 5together with the ratio $L^{1/2}_h/L^{1/2}_c$. From NaCl to $X_{Cs} \approx 0.4$ the widths of the cooling and heating peaks decrease slowly; however, the ratio does not change and is close to 1. After $X_{Cs} \approx 0.4$ an abrupt increase in the width of the heating peak is clearly observed while the width of the cooling peak continues to decrease gradually. The ratio increases sharply from 1 to 2 at this point and continues to increase up to 3 in 0.1 M CsCl. In the CsCl → NaCl system a steep increase is seen for both peak widths, but interestingly the ratio stays constant (around 3).

A uniaxial gel strength measurement was performed on the gelled samples. In Figure 6the gel strength, in arbitrary units, as a function of ΔT is displayed. The data points are an average of at least two measurements. A good correlation between gel strength and the extent of hysteresis is observed for all the systems (The NaCl → NaI system does not gel at 0.5% w/w κ-carrageenan concentration). It is seen that the two points from the NaI → CsI system fall on the same curve as the five points from the CsI → CsCl system. However, the two points from the CsCl → NaCl deviate from this curve but show the same trend.

V. Model Parameters and Model Predictions

The model (described under section III) contains many parameters, and the most important of them, for example the binding constants, are known from the litterature (see below). The individual ion binding enthalpies (ΔH_{Cs^+} and ΔH_{I^-}) are unknown, as well as ΔH_{non-el}, and were used as fitting parameters. The relative change in ion binding in the different salt mixtures, rather than the absolute values of ion binding degree, is the primary result of the model calculations. We verified (as described in detail below) that our particular choise of parameters gave qualitatively the same final results, i.e., the predicted transition enthalpy (ΔH_{helix}) in the different salt mixtures, over some other, equally probable, parameter sets

The absolute value of the binding constants, $K_{0,m}$, were taken from previous work[7,21] where they were obtained by a fit to the isothermal coil–helix stability diagrams. The density of binding sites is not known, and in the earlier work, it was found that the assumption of one or two binding sites per polymer charge gave an equally good fit to experimental data. In this work we have used $n = 2$ (two polymer charges per site) and the corresponding values for $K_{0,m}$, 760 and 5 M^{-1} for the iodide and cesium binding equilibria, respectively. The set of parameters for $n = 1$ ($K_{0,Cs^+} = 1.86$ M^{-1} and $K_{0,I^-} = 330$ M^{-1}) was tried, for comparison, but no qualitative difference was found in the resulting charge

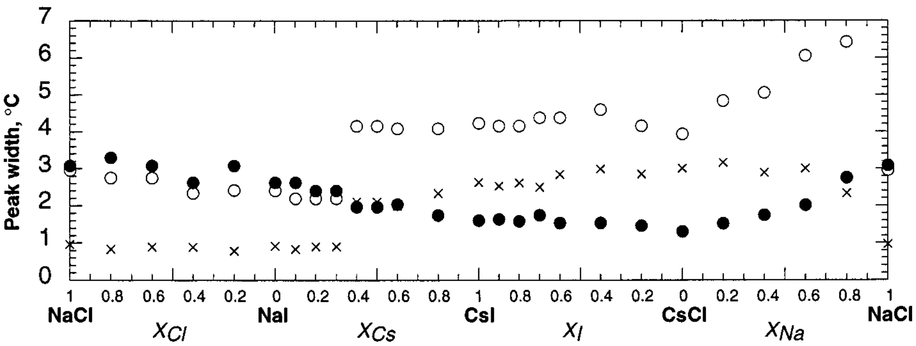

Figure 5. Width of the cooling($L^{1/2}_c$, ●) and heating curves ($L^{1/2}_h$, ○) at half of the peak maximum and the ratio $L^{1/2}_h/L^{1/2}_c$ (×), in the mixed system of NaCl/NaI, NaI/CsI, CsI/CsCl, and CsCl/NaCl.

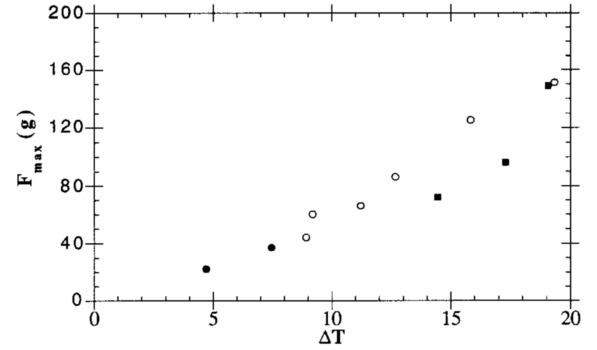

Figure 6. Uniaxial gel strength as a function of hysteresis (ΔT). See Table 1 for data points: (●) X_{Cs}; (○) X_I; (■) X_{Na}; (□) X_{Cl}.

density and, more importantly, the calculated enthalpy (ΔH_{helix}) was practically unchanged. The individual ion binding enthalpies depended however on the choise of $n = 1$ or 2, and the extracted values were between 3 and 8 kJ/mol and 17−25 kJ/mol for ΔH_{Cs^+} and ΔH_{I^-}, respectively. The temperature for all calculations was set to 290 K since the binding constants were determined at this temperature. The sensitivity to the absolute values of the binding constants was tested by a variation of ±10% in both K_{Cs} and K_I. This was found to affect the fraction of bound ions slightly, the effect being stronger for iodide (±5%) than cesium (±2%), but it did not change ΔH_{helix} and gave only a minor variation in the individual ion binding enthalpies (ΔH_{Cs^+} and ΔH_{I^-}).

The results from the Poisson−Boltzmann model calculations regarding ion binding of cesium and iodide ions are displayed in Figure 7. The data are presented as the fraction of occupied sites ($f = 1$ corresponds to 100% binding) for the various salt mixtures.

For both the cesium and the iodide, the fraction of occupied sites roughly follows the bulk concentration of the ion. As the ion concentration increases, the cesium binding increases much more rapidly than the binding of iodide whose sites are occupied more gradually. The maximum binding for the cesium ion is also higher than for the iodide. These differences are due to the electrostatic enrichment of counterions on the helix surface while the coions are depleted. Figure 7 also clearly demonstrates that there is a coupling between the binding of the two ions; for example, when the cesium ions are first introduced (NaI → CsI), the binding of the iodide ions also increases. A similar effect is observed on the cesium ions when the iodide ions are removed (CsI → CsCl). This synergistic effect is caused by the opposite charge of the ions and their ability to change the charge density. The resulting charge density of the κ-carrageenan double helix is displayed in Figure 8 as a function of the salt composition. In this figure the charge densities for κ-carrageenan, ι-carrageenan, and furcellaran double helixes have been included.

As described in section III (Theoretical Model), the ion binding data can be used to calculate ΔH_{helix}. First we have to find suitable values for the individual ion binding enthalpies, ΔH_{Cs^+}, ΔH_{I^-}, and we also need to know ΔH_{non-el}. These parameters are not known, but we can use the experimental data to extract them. In Figure 3c the calculated transition enthalpies are presented as a function of salt composition for two different sets of parameters. The fully drawn line (set 1: $\Delta H_{Cs^+} = 8.7$ kJ/mol, $\Delta H_{I^-} = 26.0$ kJ/mol, and $\Delta H_{non-el} = 2.9$ kJ/mol) represents a least-squares fit to those enthalpy points where the transition is free from hysteresis (from $X_{Cl} = 0.80$ to $X_{Cs} = 0.3$), which are the only points where we expect our model to be valid. The point obtained in 0.1 M NaCl ($X_{Cl} = 1$) was not used in the fit since this value (2.5 kJ/mol) probably is to low due to experimental limitations; the low-temperature tail of the transition (below 5 °C) could not be collected.

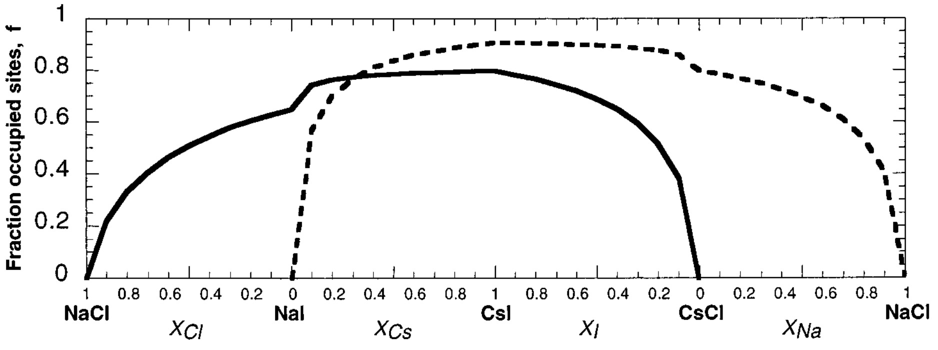

Figure 7. Calculated number of occupied sites for cesium ions (dashed line) and iodide ions (full line) as a function of NaCl/NaI, NaI/CsI, CsI/CsCl, and CsCl/NaCl mixtures.

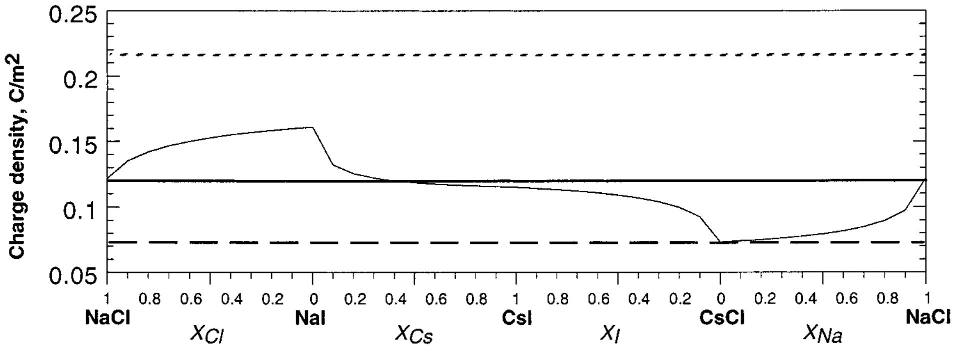

Figure 8. Calculated charge density (C/m^2) as a function of salt composition (wide line). The lines correspond to ι-carrageenan (--), κ-carrageenan (—), and furcellaran (— —).

The dotted line (set 2: $\Delta H_{Cs^+} = 22.4$ kJ/mol, $\Delta H_{I^-} = 26.0$ kJ/mol, and $\Delta H_{non-el} = 2.0$ kJ/mol) was calculated with the previously estimated enthalpy[7] for the binding of cesium ions. In Figure 3b, the difference between ΔH_{cal} and ΔH_{helix}, calculated from set 1, is plotted as a function of salt composition, together with the hysteresis. This difference represents our estimate of ΔH_{agg} and it follows the same trend as the hysteresis.

VI. Discussion

Transition Enthalpies. The transition enthalpy has contributions from the following processes: the coil−helix transition, ion binding to the double helix (both iodide and cesium), and the association of double helixes. To distinguish the different processes we start with the simple salts, i.e., 0.1 M of NaCl, NaI, CsI, and CsCl. The sodium chloride salt gave the lowest transition enthalpy. This is expected, due to the fact that only the coil−helix transition contributes to the enthalpy. The value ~2.5 kJ/mol of disaccharide unit is comparable to recently obtained data under similar conditions;[19] however, it is higher than previously published data of[13] 0.4 kJ/mol of disaccharide unit. The transition enthalpy for 0.1 M NaI is ~11 kJ/mol of disaccharide unit. This is a combination of the coil−helix transition and the binding of iodide ions. The value is comparable with previously obtained data in 0.15 M tetramethylammonium iodide (10.4 kJ/mol)[16] and sodium iodide (11 kJ/mol).[19] In 0.1 M CsI and 0.1 M CsCl the transition enthalpies are similar (22 and 20 kJ/mol of disaccharide unit, respectively) and in both salts we have contribution from all of the above-mentioned processes. The value in CsCl correlates reasonably well with previously obtained data in CsCl[18] which gave ~18 kJ/mol of disaccharide unit for a 0.04% w/w κ-carrageenan solution and a value of ~23 kJ/mol of disaccharide unit for a 0.06% w/w κ-carrageenan solution.[17] Other studies on the comparable potassium chloride salt have given values between 9,[30,15,17] 17,[31] and ~21 kJ/mol of disaccharide unit.[17] Further a study on a 0.1 M potassium nitrate solution (1% w/w κ-carrageenan concentration) gave a value of 18 kJ/mol per disaccharide unit.[32]

The NaCl → NaI system shows a smooth increase in transition enthalpy when chloride ions are replaced by iodide ions (Figure 3c). The increase levels off at approximately $X_{Cl} = 0.8$. This agrees with data in a previous study where no large difference was seen between a sample containing 100 mM NaI and 200 mM NaI.[19] In the NaI → CsI system the transition enthalpy also increases, but the increase occurs in two separate "steps". The initial increase reflects only the binding of cesium to the double helix since no aggregation (hysteresis) is present, and it is correlated to the increase in f_{Cs} and f_I, calculated from our model (Figure 7). This increase seems to level off just below $X_{Cs} = 0.4$ (16kJ/mol disaccharide unit) but is followed by a second increase above $X_{Cs} = 0.4$. The second increase is not predicted by our model, but it coincides with the onset of hysteresis, which indicates that the aggregation contributes to the transition enthalpy. After this increase, the enthalpy levels off once more at ~22 kJ/mol of disaccharide unit around $X_{Cs} = 0.8$ and is more less constant until 0.1 M CsI. In the CsI → CsCl system a slight decrease is observed from 22 kJ/mol to just below 20 kJ/mol. The transition enthalpy is derived from the cesium binding (more or less constant), the iodide binding (decreasing), and the aggregation (increasing). The decrease in iodide binding, which reaches zero at 0.1 M CsCl, should lower the transition enthalpy. However, the increase in aggregation (hysteresis) apparently compensates for most of the loss and only a slight decrease in transition enthalpy is observed. The trend continues for most of the next regime (CsCl → NaCl), where the transition enthalpy gradually decreases to a molar ratio of $X_{Na} = 0.8$. No iodide ions are present and the hysteresis (and the charge density) is more or less constant, with only a slight decrease of the hysteresis (increase in charge density) observed. There is an abrupt decrease in transition enthalpy between $X_{Na} = 0.8-1$ (from ~17 to ~2.5 kJ). This corresponds to the difference in transition enthalpy between the coil−helix transition containing no specific ions and no aggregation with one containing a small proportion of binding cesium ions and the accompanying aggregation.

The constant transition enthalpy (Figure 4) in 0.1 M sodium iodide over a polymer concentration range from 0.1 to 1% w/w is expected. In the 0.1 M sodium iodide system the contributions to the transition enthalpy originates from the coil−helix transition and the binding of iodide ions to the κ-carrageenan double helix. Both of these processes should depend linearly on the number of disaccharide units present, if the iodide ions are in excess. Further the transition temperature does not vary significantly (slight increase due to counterions, data not shown) over the concentration range. In the 0.1 M cesium iodide system we have a further contribution to the transition enthalpy from the aggregation process. The hysteresis does not change over the range (~8−9 °C, data not shown), and the transition enthalpy is constant, implying that the aggregation contribution to the transition enthalpy is also proportional to the number of disaccharide units.

In this context it is also interesting to compare previous estimates of the transition enthalpies for κ-carrageenan:[7] 12.7 kJ/mol for ΔH_{non-el} and 22.4 kJ/mol for $\Delta H_{bind(Cs^+)}$, with the experimental values from parameter set 1 obtained in this study: 2.9 kJ/mol (ΔH_{non-el}) and 8.7 kJ/mol ($\Delta H_{bind(Cs^+)}$). Clearly the former calculations made using the Poisson−Boltzmann cell model[27,7] overestimates the enthalpies but considering that the Poisson−Boltzmann model is only semiquantitative in the best cases, the results are acceptable. The nonelectrostatic contribution, ΔH_{non-el}, was estimated from the salt induced shift in the conformational transition temperature, based on the data in ref 5 for Na^+ ions. Upon inspecting the original data we note that the temperature range is rather narrow (4−25 °C) which could cause some uncertainty in the estimated ΔH_{non-el}. ΔH_{agg} values vary in the different salts investigated, and ΔH_{agg} can sometimes represent more than 50% of ΔH_{cal}. A value for ΔH_{agg} of 4 kJ/mol, similar to the lowest ΔH_{agg} obtained here, has been reported by previous investigators as the difference between free (nongelling) and aggregated (gelling) helixes.[32,15]

In conclusion, the transition enthalpies in the nonaggregated systems are fully explained by the ion binding to the double helix, including also the small step occurring around 0.1 M NaI. For the aggregated systems, the observed transition enthalpy is more or less independent of salt composition but our model calculations predict a decreasing ΔH_{helix} when we move to the right in Figure 3c. To obtain a constant total transition enthalpy, the decrease in ΔH_{helix} has to be compensated for by an increase in ΔH_{agg}. We find that ΔH_{agg} increases from approximately 5 kJ/mol, when we have iodide present, up to around 13 kJ/mol for the CsCl → NaCl system.

Transition Temperatures. A first observation is that all curves vary smoothly for the four systems, indicating that there is no change of the primary conformation[1] with the salt compositions.

In the NaCl → NaI system no hysteresis is present. The iodide stabilizing effect is seen as an increase in T_c (and T_h), correlated with an increase in the number of occupied iodide sites which leads to a higher charge density of the helix. The transition is just the normal reversible coil−helix transition of κ-carrageenan. In the NaI → CsI system an increase in T_c is observed between $X_{\text{Cs}} = 0$ to 1 (from 36° to 62 °C). This increase can be attributed to the well-known stabilizing effect, through ion binding, of the cesium ions to the κ-carrageenan double helix and correlates well with the increase in the number of occupied cesium sites. The coil−helix transition temperature at 0.1 M CsI (~62 °C) is the highest temperature for all the measured salt mixtures, and this maximum in helix stability correlates with the maximum of ion binding of both cesium and iodide ions as seen from the model calculations. A similar increase in T_h is observed with $X_{\text{Cs}} \sim 0.4$, where T_h starts to deviate from T_c ($T_h > T_c$). At and above this ratio the occurrence of aggregates has been demonstrated by a wide range of techniques: optical rotation, viscosity, cryo-TEM, rheology, and FFF-MALS.[11,12,28] The onset of hysteresis is therefore clearly a result of aggregation between double helixes which results in a stabilization of the helix state.[29,14] It is known that iodide ions promote helix formation but prevent aggregation, and from the above data, it is seen that aggregation (hysteresis) does not occur until a certain amount of gel promoting cesium ions are present. The ratio ($X_{\text{Cs}} \sim 0.4$) is also the approximate crossover between the iodide and cesium ion binding degree as seen in the model calculation. The charge density of the double helix above this point is lower than for κ-carrageenan helix in an environment containing no binding ions (e.g. 0.1 M NaCl) while in 0.1 M NaI is ~75% of the higher charged ι-carrageenan double helix (compared with ~55% in 0.1 M NaCl). However as the number of cesium ions increases, the charge density decreases and becomes closer to that of the lower charged furcellaran polymer. It is well-known that ι-carrageenan does not form any aggregates (no hysteresis has been observed) and that furcellaran is highly aggregated.[2]

Considering the CsI → CsCl system, a decrease in T_c is expected due to the exchange of iodide to chloride ions. Such a decrease is also clearly observed going from $T_c \sim 62$ °C at 0.1 M CsI to $T_c \sim 48$ °C at 0.1 M CsCl. This decrease correlates well with the decrease in the number of occupied iodide sites. However, T_h is more or less constant and only a very small decrease from about 70 °C at 0.1 M CsI to approximately 68 °C at 0.1 M CsCl is observed. The difference in behavior between T_c and T_h is clearly observed in the hysteresis which reaches ~20 °C at 0.1 M CsCl. This is the maximum hysteresis observed in all four systems and correlates to the maximum difference in ion binding between cesium and iodide and consequently the lowest charge density in all four systems.

The behavior in the next system, CsCl → NaCl, is explained by the stabilizing effect on the κ-carrageenan double helixes by the cesium ions. A similar decrease in both T_c and T_h is observed when cesium ions are exchanged for sodium and correlates well with the decreasing binding degree of cesium ions. The similar behavior of T_c and T_h is also reflected in the hysteresis which is more or less constant from $X_{\text{Na}} = 0-0.6$, followed by a slight decrease between 0.6 and 0.8, and it finally abruptly disappears as X_{Na} approaches 1. The observed trend for the charge density is a slight increase (more or less constant) between $X_{\text{Na}} = 0$ and 0.6; thereafter, a sharper increase is observed.

The transition temperatures were also measured by optical rotation (data not shown) and it was found that upon lowering the total ionic strength (0.03 M) the onset of hysteresis was shifted to lower cesium contents (at $X_{\text{Cs}} = 0.3$ instead of $X_{\text{Cs}} = 0.4$ in 0.1 M salt). When the model calculations were repeated at the lower ionic strength we found that the charge density was lowered close to the hysteresis onset, compared to the charge density in 0.1 M salt. This supports the idea that the occurrence of hysteresis (and aggregation) is indeed controlled by the charge density of the helix. When the charge density decreases below a certain value, the aggregation can occur.

Transition Width. The parameter varied in the above experiments is the salt composition; thus, polydispersity and polymer concentration have been kept constant. This suggests that any change in transition sharpness should be due to a change in the equilibrium between the coil and the helix state. The equilibrium constant consists of the coil concentration, helix concentration, and an association factor to form the first helical unit between two coil strands.[33] A change in the sharpness of the cooling curve should reflect a change in the helix concentration or the association factor. The association factor contains mainly the entropic loss in free energy when two chains associate and should be independent of the electrolyte condition. Under nonaggregating conditions, the width of the cooling curve is the same as the width of the heating curve, but a slight decrease in peak width is seen with an increasing amount of specific ions. This is probably a result of the higher stability of the double helix when specific ions are present. An abrupt increase of peak width of the heating curve is clearly observed when we enter the aggregated systems. This observation can be a kinetic effect, for example if the melting of the double helix inside an aggregate is much slower than the melting of a free helix. On the other hand, if it is an equilibrium effect, it would mean that the melting of the aggregates is less cooperative than the melting of free helixes. The width of the cooling peak in the aggregating systems is lower than the cooling peak of the nonaggregated systems. This could be explained by a higher stability of the helix, as above, but this is not compatible with the decrease of T_c in the CsI → CsCl interval. More probable is that the sharper transition upon cooling is a result of that the helixes aggregate as soon as they are formed and thereby drive the coil−helix transition faster to helical state.

Gel Strength. The uniaxial gel strength measurements show a good correlation between the maximum gel strength and the extent of hysteresis. All measurements have been done on 0.5% w/w κ-carrageenan samples, and no measurements were done on the weak gels that form under nonaggregating conditions at higher polymer concentrations.[12] However there seems to be a difference between gels containing both cesium and iodide compared to the gels containing only cesium. This indicates that the gel strength does not depend only on the extent of hysteresis but also that the ions present might also play a part.

VII. Concluding Remarks

A semiquantitative description of the experimental transition enthalpies under nonaggregated conditions was obtained by the electrostatic model, where the specific ion binding was included. Thermal hysteresis, a sign of aggregate formation, occurred in the experiments when the calculated charge density (with bound ions) was lower than the "bare" charge density (in the absence of binding ions). However, the model calculations are not able to reproduce the transition enthalpies for those systems where aggregation occur and a contribution from the aggregation process to the total transition enthalpy is therefore implied.

From these DSC results, and the belonging ion binding calculations, we can obtain some indications on the nature of the superhelical aggregates but much remains to be understood. An interesting point is that the stability of the aggregates (interpreted as T_h, the melting temperature of the aggregates) is controlled by the concentration of cesium only. The aggregation enthalpy, ΔH_{agg}, varies from 5 to 13 kJ/mol and contributes with more than 50% of the total transition enthalpy in some salt mixtures. This large variation in aggregation enthalpy can mean that the structure of the aggregates depend on the salts present. For example, the strength and size of the aggregates could vary with the salt composition. As we observe a higher turbidity for the systems with the higher hysteresis, there is definitely some structural difference between samples with low and high hysteresis. It is very interesting to note that the increase in aggregation enthalpy follows the increase in hysteresis (Figure 3b).

The structure (nature) of the aggregates is poorly known. In cryo-TEM micrographs[11] they appear as very stiff long rods consisting of an unknown number of double helixes. The cryo-TEM study was done on dilute polymer solutions in the NaI/CsI system. No growth of the rods could be observed when the cesium content increased, but the rods aggregated into larger bundles with increasing cesium content.

The above-mentioned observations suggest that a term describing the association has to include a primary term describing the association of double helixes and a second term describing the aggregation of these aggregates. However, available information on the structure and ion binding properties is still not sufficient to describe such a term and include it in the model calculations.

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