Helix-Coil Transitions of Ionic Polysaccharides Analyzed within the Poisson-Boltzmann Cell Model. 1. Effects of Polyion Concentration and Counterion Valency

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ABSTRACT: The effects of polyelectrolyte concentration and counterion valency (monovalent, divalent, and mixtures of these) on the coil-helix transition of κ- and ι-carrageenan (two moderately charged polysaccharides) are investigated experimentally and theoretically. In the theoretical model presented, the electrostatic contribution to the propagation constant of the transition is calculated from the full Poisson-Boltzmann equation in the cylindrical cell model. In this model all solute concentrations (including that of the polyion) are finite, and the effects of polyion-polyion interactions—which are found to be important—are thus treated implicitly. It is experimentally found that in the presence of divalent counterions, the helix conformation may be either stabilized or destabilized by the addition of a 1:1 electrolyte, depending on the salt composition of the solution and on the charge densities of the helix and coil conformations. These effects are explained by the theoretical model, which gives a qualitatively correct and semiquantitatively accurate description of the experimental data presented, as well as giving insight into other documented effects of increasing ionic strength on the coil-helix transition of polyelectrolytes.

I. Introduction

The helix-coil transition of polymers in solution is an important phenomenon, well-known in systems like polypeptides, nucleic acids, and, as in this study, polysaccharides. The cooperative nature of the transition as well as its dependence on the degree of polymerization has been well understood for 3 decades through the theoretical work of Zimm and Bragg[1] and later refinements. The current understanding of the additional complications arising from Coulomb interactions in the case of a charged polyelectrolyte undergoing the transition is, however, less satisfactory, and this latter problem is the subject of this article.

To illustrate the effects of electrostatic interactions on the coil-helix transition of polyelectrolytes we here present new experimental data on the transitions of κ- and ι-carrageenan and their dependence on the counterion valency in mixed (1:1 and 2:1) electrolyte solutions as well as their dependence on the carrageenan concentration. (The dependence of the transition on other variables, such as salt concentration and solvent, will be analyzed in future publications in this series.) ι- and κ-carrageenan[2] (Figure 1) are two moderately charged sulfated galactans, well-known for their ability to form aqueous gels at low concentrations. Since the coil-to-helix transition, where the helix conformation is favored by high salt concentration and low temperature, is the first step in the gelation of carrageenans,[2] it is of interest to understand the electrostatic contribution to the conformational transition in these systems. This is, in fact, the problem which originally inspired this investigation. However, we wish to stress that the experimental results reported here—which, in part, are rather counterintuitive—should be of relevance to the understanding of coil-helix transitions in polyelectrolytes in general.

To account for our experimental observations theoretically we need an electrostatic model, and we will here employ the so-called Poisson-Boltzmann cell model.[3] This model has previously proven quite successful in the thermodynamic analysis of aqueous surfactant systems (see ref 4 and references cited therein), and it has very recently been used in the analysis of counterion self-diffusion data in solutions of ι-carrageenan in its two conformations.[5] However, with the exception of a very recent work by Jamnik and Bratko,[6] this electrostatic model seems not to have been used previously in the thermodynamic analysis of the coil-helix transition of ionic polymers. (Jamnik and Bratko use the cell model in a thermodynamical analysis of the salt concentration dependence of the transition midpoint temperature for κ-carrageenan. This work and its conclusion will be discussed more fully in a future publication in our series, where we adress the same problem.) In the Poisson-Boltzmann cell model, as applied to the present case, the electrostatic contribution to the polyelectrolyte chemical potential is calculated from the full Poisson-Boltzmann equation with the polyelectrolyte (coil or helix conformation) modeled as an infinitely long charged rod centered in a cylindrical cell, the radius of which is given by the concentrations of the helix and coil molecules. By the use of a cell with finite radius, effects of polyion-polyion interactions at finite concentrations—which may have important consequences, as will emerge below—are thus treated implicitly. To our knowledge, these interactions have not been considered in previous theoretical attempts to rationalize electrostatic effects on the coil-helix transition of polyelectrolytes. Furthermore, the theoretical models used previously have, in almost all cases (exceptions are the above cited work by Jamnik and Bratko[6] and an early study by Nagasawa and Muroga[7]), been based on approximations to the Poisson-Boltzmann equation, such as the linearized Poisson-Boltzmann equation[8] or Manning's limiting laws,[9] the latter approach being the one most frequently taken in the analysis of experimental data. While these approaches have shed light on general effects such as that of increasing salt concentration on the coil-helix transition temperature, they will nevertheless prove insufficient to account for the experimental results obtained in the present study. In contrast, it will be shown that the Poisson-Boltzmann cell model gives a qualitatively (and semiquantitatively) correct description of all experimental results obtained in this work.

The paper is organized as follows. After a brief section on materials and methods (section II), we give an account of our experimental studies (section III). Then we present our theoretical model in some detail (section IV), relating the thermodynamic expressions to the parameters of the conventional helix-coil transition theory (the Zimm-Bragg theory[1]). In the subsequent section (V) we present model calculations for the systems studied, which we discuss and compare with our experimental results as well as with

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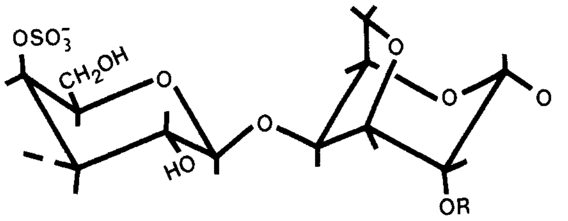

Figure 1. Repeating disaccharide structures of κ- (R = H) and ι- (R = SO_3^-) carrageenan.

previously developed theory. Finally, a summary of the main conclusions emerging from our work is given in section VI.

II. Experimental Section

Materials. Commercial samples of ι- (from Eucheuma spinosa, Lot. No. 124F-0605) and κ- (from Eucheuma cottonii, Lot No. 124-0604) carrageenan were obtained from Sigma Chemical Co. Segments of enhanced structural regularity were prepared as described by Bryce et al.[10] The carrageenan segments were dialyzed against millipore-filtered water and freeze-dried. Pure ion forms (sodium or calcium) were obtained by ion exchange at elevated temperature. The efficiency of our ion-exchange procedure was controlled by atomic absorption spectroscopy. The calcium content of a sodium ι-carrageenan sample obtained in this way was found to be below the detection limit (<5 ppm of the dry weight of the sample). Sample concentrations are given as moles (disaccharide) per decimeter cubed (M), assuming ideal disaccharide molecular weights for the freeze-dried segments. Gel permeation chromatography (on uncalibrated columns) showed that the molecular weight distributions of both carrageenan segment preparations were quite broad.

Methods. The carrageenan coil–helix transition was monitored[2] by optical rotation measurements at 436 nm. The temperature was controlled by the circulation of thermostatically regulated water through the jacketed cell. Most measurements were performed with a 10-cm path length cell in a Jasco DIP-360 polarimeter. The onset of helix formation was measured as a function of the temperature (cooling runs) and the salt composition was varied until the onset of helix formation occurred at the desired temperature (cf. section III).

III. Experimental Results

The effects of added salt on the coil–helix transition and the gelation of carrageenans have been the subject of much research in the past, and a much-discussed issue in this context is the occurrence of cation specificity in these transitions, in addition to the effects of purely electrostatic origin which are the subject of the present paper. It has recently been demonstrated that pure ι-carrageenan displays no marked cation specificity;[11] however, systematic studies on single cation forms of κ-carrageenan (see, e.g., ref 12) have established that the helix–coil transition of this polysaccharide is very sensitive to the presence of certain monovalent cations. On the other hand, literature data[12] also clearly show that a range of monovalent cations, including sodium (the monovalent cation used in the present study), have closely similar effects on the transition. Similarly, a group of divalent cations (including calcium as used here) have been shown[12] to be virtually equivalent with respect to their effect on the κ-carrageenan conformational transition. These results we take as evidence that in these systems Coulomb interactions alone are responsible for the observed salt effects; consequently, there should be no specific binding of cations influencing the experimental results presented here.

In the present study, we wish to focus our attention on two consequences of Coulomb interactions on the coil–helix transition of polyelectrolytes, i.e., that the transition will be dependent on the valency of the counterion as well as on the concentration of the polyelectrolyte. In the experimental investigations, we have chosen to characterize our samples by the onset rather than the midpoint of the coil-to-helix transition (from a theoretical point of view, this choice is completely arbitrary; cf. below), for the following reasons. (i) The experimental determination of the onset of helix formation can be made with good accuracy without the requirement that the entire transition to 100% helix be measured. (ii) In polydisperse samples, like ours (cf. section II above), the onset of helix formation reflects properties of the high molecular weight end of the distribution, whereas the transition midpoint bears no particular significance. (iii) It is generally believed that the onset of helix formation in the carrageenans reflects the coil–helix equilibrium *in solution*, also under conditions when the helices, once formed, will form aggregates.[13] The last point is of relevance only for κ-carrageenan where a temperature hysteresis in the order–disorder transition is often seen, indicating the formation of larger aggregates subsequent to helix formation. ι-Carrageenan on the other hand gives perfectly reversible transitions[11] which suggests that there is no aggregate formation.[13]

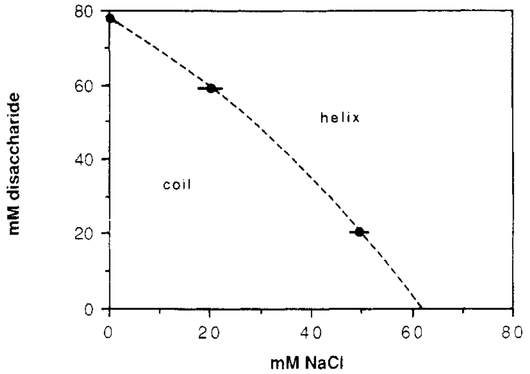

Figure 2. Experimental conformational stability diagram for Na ι-carrageenan in NaCl solutions. Points indicate compositions (carrageenan and salt concentration) of solutions in which the onset of helix formation commences at 32.5 °C.

Furthermore, we have chosen to vary the component concentrations in each experiment in such a manner that the onset of helix formation occurs at the same temperature, rather than (which is more common) observing the result of, say, added 1:1 electrolyte on the transition temperature. The advantage we find in restricting ourselves to isothermal experiments is that such data enable us to compare our experimental results with the predictions of the theory presented below without requiring any adjustable parameters. (The variation of the carrageenan transition onset temperature with the concentration of added electrolyte will be treated in a subsequent publication.)

Polyelectrolyte Concentration Dependence. Figure 2 shows the results of an experiment determining the concentration of added sodium chloride required to initiate helix formation, at constant temperature (32.5 °C), in solutions with different contents of sodium ι-carrageenan. As is well-known, the ι-carrageenan helix conformation may be obtained in salt-free solutions of sufficiently high concentration,[14] whereas more dilute solutions require the addition of salt for the onset of helix formation.[15] At a high concentration of added salt (0.4 M NaCl), the onset of helix formation was found to be independent of the polyelectrolyte concentration in the range investigated (20–78 mM disaccharide), which thus ensures that the polyelectrolyte concentration dependence at low salt concentration (Figure 2) depends on electrostatic interactions only.

Counterion Valency Dependence. Although extensive experimental investigations of the coil–helix transition

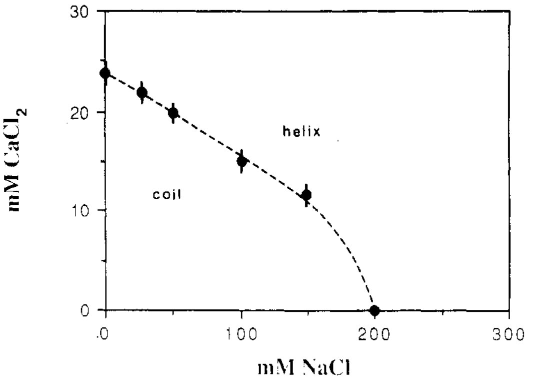

Figure 3. Experimental conformational stability diagram for κ-carrageenan (5 mM) in mixed electrolyte solutions. Points indicate salt concentrations (NaCl and $CaCl_2$) of solutions in which the onset of helix formation occurs at 18 °C.

of different single cation forms of the carrageenans have been reported in the literature (see, e.g., ref 12), systematic studies on systems containing mixtures of cations of different valencies seem not to have been published previously. Figure 3 shows the result of such a study in the form of a conformational stability diagram of κ-carrageenan in mixed salt (sodium or calcium chloride) solutions. (In this experiment, as in the experiment reported below, the carrageenan concentration was kept constant at a low enough value so that the counterion contribution to the ionic activities was negligible.) The experimental points indicate the calcium concentration required to initiate the κ-carrageenan coil-to-helix transition at a constant temperature (18 °C) as a function of the sodium ion concentration in the solution. The points are seen to fall on a monotonic curve connecting the pure calcium and the pure sodium axes, a result which intuitively seems very reasonable: The higher the sodium chloride content of the sample, the less calcium chloride is required to initiate the electrostatically disfavored helix conformation.

All the more surprising is the result of a similar investigation on ι-carrageenan, which carries twice as many charged groups as κ-carrageenan (cf. Figure 1), the result of which is shown in Figure 4. (The higher temperature, 72.5 °C, of the ι-carrageenan transition reflects the curious fact that the more charged ι-carrageenan helix is actually *more stable* than the κ-carrageenan helix under similar conditions of ionic strength.) In this system, the calcium concentration required to initiate helix formation at different concentrations of sodium salt is seen to go through a pronounced *maximum* for samples containing about 100 mM sodium chloride. This means that the initial addition of sodium chloride to a calcium chloride solution of ι-carrageenan actually *destabilizes* the helix conformation, so that more calcium has to be added to restore the original equilibrium. This anomalous behavior persists up to quite high salt concentrations; only when a large excess of sodium chloride has been added does the system revert to the normal behavior displayed by κ-carrageenan (Figure 3). While we believe that this is the first time that the behavior displayed in Figure 4 is reported for carrageenans, it is of interest to note that previous studies, concerned with the conformational equilibria of polynucleotides, have shown similar anomalous effects when 1:1 electrolyte is added to a system containing a sufficient amount of divalent counterions.[16-18] To account for the above observations—the dependence of the transition on the ι-carrageenan concentration at low salt, the anomalous behavior of ι-carrageenan in salt mixtures, and the differences, in the latter respect, between ι- and κ-carrageenan—we will make use of an electrostatic model, as introduced in the next section.

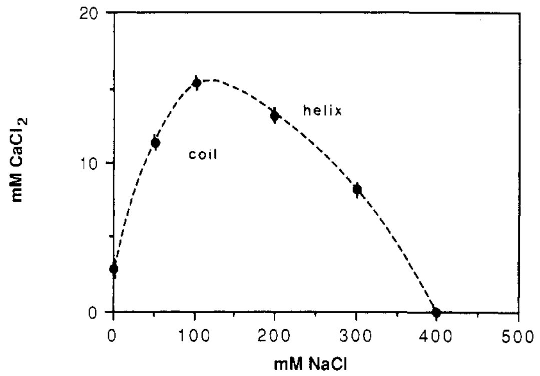

Figure 4. Experimental conformational stability diagram for ι-carrageenan (2.9 mM) in mixed electrolyte solutions. Points indicate salt concentrations (NaCl and $CaCl_2$) of solutions in which the onset of helix formation occurs at 72.5 °C.

IV. Theoretical Model

General Considerations. In the Zimm–Bragg model[1] of the polymer coil–helix transition, the polymer is assumed to consist of repeating units such that each unit may exist in either a helix or a coil conformation. The free energy of helix formation is described by two parameters: an initiation constant, σ, and a propagation constant, s, where ln (σs) corresponds to the free energy of forming the first helical unit and ln (s) corresponds to the free energy of transforming subsequent units from the coil to the helical state. For the case of an ionic polymer, the propagation parameter may, formally, be divided into two parts, representing electrostatic and nonelectrostatic contributions respectively:

$$kT \ln (s) = m(\Delta\mu_{\mathrm{el}} + \Delta\mu_{\mathrm{nonel}}) \qquad (1)$$

where m is the number of charged groups per repeating unit and $\Delta\mu_{\mathrm{el}}$ and $\Delta\mu_{\mathrm{nonel}}$ represent (the negative of) the electrostatic and the nonelectrostatic contributions (expressed per unit polyion charge) to the free energy difference associated with the addition of another unit to the growing helix. The nonelectrostatic contribution, $\Delta\mu_{\mathrm{nonel}}$, is a function of temperature, but will be assumed to be independent of the concentration of added salt in the following.

The cooperativity of the transition is governed by σ (a low value of σ leads to a high cooperativity), the value of which may be obtained from the width of the conformational transition (under conditions when electrostatic interpolyion interactions are not important; cf. below) measured as a function of, for instance, the temperature or the ionic strength. As the initiation constant is generally believed to depend mainly on chain conformational factors associated with the helix initiation,[1,19] we will here assume σ to be independent on the amount or type of added electrolyte. In the following, we will thus assume that the effects of electrostatic interactions on the coil–helix equilibrium are restricted to their influence on $\Delta\mu_{\mathrm{el}}$ as defined in eq 1.

Electrostatic Model. Before attempting to evaluate $\Delta\mu_{\mathrm{el}}$ we note that since electrostatic interactions are of long range, the electrostatic contribution to the free energy difference associated with the transformation of a coil unit to a helic unit must, in general, vary with the number of helical units preceding the unit under consideration, as well as with the number of coil units succeeding it. Only when the regions of both conformations are sufficiently long (compared to the Debye length of the system) will the free energy difference approach a constant value. In our model we will assume that this condition is met, which is equivalent to postulating a sufficiently cooperative transition. For the purpose of evaluating the electrostatic interactions, we may then treat both the helical and the coil regions as sections of infinite length. (Although the validity of this assumption cannot be quantitatively assessed for the particular systems of this study—unfortunately, no attempts to determine σ for carrageenans seem to have appeared in the literature—it should be noted that in the experiments reported here, the longest Debye length is (at 3 mM $CaCl_2$) 28 Å, which would correspond to about six to seven repeating disaccharide units. As a comparison the cooperative length for polypeptides is of the order of 100 repeating units.)

The electrostatic free energy associated with each conformation, coil and helix, may then be calculated from the Poisson–Boltzmann equation in a cylindrical cell model.[3,20] The cell model consists of an infinite cylindrical cell, containing water and mobile ions, where the polyion is represented as a charged rod of uniform surface charge density centered in the cell. The model polyion is thus characterized by two parameters; a, the radius of the rod, and l, its length per unit surface charge. The cell radius, b, is determined by the polyion concentration and, for systems containing both coils and helices, by the conditions expressed in eq 11, 13, and 14 below. In cylindrical symmetry, the Poisson–Boltzmann equation reads

$$\frac{1}{r}\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}\phi}{\mathrm{d}r}\right) = -\frac{e}{\epsilon_r\epsilon_0}\sum c_{i0}z_i \exp(-e\phi z_i/kT), \qquad a < r \le b \quad (2)$$

to be solved with the boundary conditions $\mathrm{d}\phi/\mathrm{d}r(b) = 0$ and $\mathrm{d}\phi/\mathrm{d}r(a) = -\sigma/\epsilon_0\epsilon_r$. Here $\phi(r)$ is the electrostatic potential at a distance r from the center of the cell, c_{i0} is the concentration of mobile ions of charge z_i at $r = b$, σ is the surface charge density of the charged rod, and the other symbols carry their usual meanings (a complete list of all symbols used is given in Appendix 2). By convention, the electrostatic potential is taken to be zero at the cell boundary; $\phi(b) = 0$. From the solution of the Poisson–Boltzmann equation the electrostatic free energy of the cell, G_{el}, may be evaluated[20] as

$$G_{el} = E_{el} - TS_{mix} \quad (3)$$

(neglecting volume changes), where

$$E_{el} = (\epsilon_r\epsilon_0/2)\int(\nabla\phi)^2\,\mathrm{d}V \quad (4)$$

and

$$TS_{mix} = -N_AkT\sum_i\int c_i[\ln(c_i/c_{solvent}) - 1]\,\mathrm{d}V \quad (5)$$

and where c_i and $c_{solvent}$ designate the concentrations of mobile ionic species i and solvent (water) molecules, respectively. In these expressions, E_{el} is the electrostatic energy and S_{mix} the entropy of mixing, where the nonuniform distribution of mobile ions has been considered (this expression reduces to the ideal entropy of mixing in the absence of electrostatic interactions). The chemical potentials are then obtained by differentiating the free energy in the usual way, leading to the following expressions[4]

$$\mu_{solvent} = -kT\sum c_{i0}/c_{solvent} \quad (6)$$

$$\mu_i = kT\ln(c_{i0}/c_{solvent}) \quad (7)$$

(single ion potential of mobile ionic species i), and

$$\mu_{el,pi} = -e\phi(a) - E_{el} + kTV_{solvent}N_A\sum(c_{i,av} - c_{i0}) \quad (8)$$

Here $\mu_{el,pi}$ is the electrostatic contribution to the polyion chemical potential expressed per unit polyion charge, $\phi(a)$ is the electrostatic potential at the polymer surface, $V_{solvent}$ is the volume of solvent (per unit polyion charge) in the cell, and $c_{i,av}$ is the cell average concentration of mobile ions of charge z_i. The polyelectrolyte chemical potential per unit charge is then given by

$$\mu_{el,pe} = \mu_{el,pi} + \mu_i^+/z_i^+ \quad (9)$$

where μ_i^+ is the chemical potential of the counterion. We may now obtain an expression for the desired electrostatic contribution to the chemical potential difference between the coil and the helix conformations, defined as

$$\Delta\mu_{el} \equiv \mu_{el,pe}(\text{coil}) - \mu_{el,pe}(\text{helix}) \quad (10)$$

At equilibrium between the coil and the helix conformations, this expression may be simplified. The equilibrium condition specifies that the chemical potentials of all mobile species are the same for coil and helix cells, or, equivalently (cf. eq 6 and 7)

$$c_{i0}(\text{coil}) = c_{i0}(\text{helix}) \quad (11)$$

(It should be noted that in obtaining eq 7, the convention $\phi(b) = 0$ was used; however, only at equilibrium may we assign the *same* boundary electrostatic potential to both the coil and the helix cells.) The counterion contributions to the polyelectrolyte chemical potential difference therefore cancel and $\Delta\mu_{el}$ simplifies to

$$\Delta\mu_{el} = \mu_{el,pi}(\text{coil}) - \mu_{el,pi}(\text{helix}) \quad (12)$$

For the polyion this difference in the electrostatic chemical potential between the coil and the helix conformations will be finite also at equilibrium ($\Delta\mu_{el} \neq 0$). However, the total chemical potential difference between the helix and the coil conformations must, of course, be zero at equilibrium; $\Delta\mu_{el}$ is then exactly balanced by $\Delta\mu_{nonel}$ and by the entropy of mixing helix and coil units as given by, e.g., the Zimm–Bragg theory.

Apart from the equilibrium condition, two conservation conditions, i.e., that the average salt concentration and the total amount of solvent should correspond to the overall composition of the solution, have to be satisfied:

$$c_{i,av}(\text{coil})(1 - p) + c_{i,av}(\text{helix})p = \langle c_i\rangle \quad (13)$$

$$V_{solvent}(\text{coil})(1 - p) + V_{solvent}(\text{helix})p = \langle V_{solvent}\rangle \quad (14)$$

Here p represents the fraction of units in the helix conformations and bracketed quantities designate global averages. With reference to eq 13 it is important to realize that under conditions where the polyion significantly affects the ionic *activities* (which, by thermodynamic necessity, are constant throughout an equilibrium system), the cell-averaged ion *concentrations* $c_{i,av}$(coil) and $c_{i,av}$(helix) will differ, both deviating from the global average $\langle c_i\rangle$. The equilibrium condition (eq 11) merely states that the ionic concentrations are equal at the cell borders. The average concentration within each cell is obtained by an integration over the cell volume and, since the charge densities are different for the two conformations, the concentration profiles and consequently also their averages will generally be different in the two cells.

At sufficiently high concentrations of added salt, however, c_{i0} will approach $\langle c_i\rangle$ and the above conservative conditions may be neglected. The system may then be regarded as dilute from the point of view of electrostatic polyion–polyion interactions, as these interactions will become independent of the polyion concentration. (In the case of ι-carrageenan, the polyion interactions may be regarded as dilute at carrageenan concentrations lower than 20 mM disaccharide when the salt concentration exceeds 0.2 M; cf. Figure 5.) A salt-free solution, on the

Table I
Carrageenan Model Parameters

	coil		helix	
carrageenan type	l, Å	a, Å	l, Å	a, Å
κ	10	3.3	4.1	5.1
ι	5	3.5	2.2	5.3

other hand, is always concentrated from an electrostatic point of view.

The Model Applied to Carrageenans. The model is now complete (the iterative procedure for the calculation of $\Delta\mu_{el}$ is described in Appendix 1), and to perform the desired calculations we need only specify the parameters l and a of the two polymer conformations. The parameter values used for κ- and ι-carrageenan throughout this work are listed in Table I. The lengths per unit charge have been obtained from fully extended molecular models for the coil conformation and from X-ray diffraction data[21,22] on oriented fibers for the helix conformation. Assigning values to the rod radii is less straightforward; here we have taken the rod volume per unit charge to be equal to the corresponding experimental partial molar volume[23] which is assumed to be the same for both conformations (the small volume difference (about 1%) indicated by the results of Gekko et al.[24] has been neglected). To approximate the average electrostatic radius with what could be called an average geometric radius seems to work quite well for carrageenans and there appears to be no systematic way of changing the polymer radius in order to obtain a better fit with the experimental data. The approximation probably works well also for other polyelectrolytes undergoning conformational transitions if both conformations are reasonably compact (as is the case for carrageenans); it is likely to be less satisfactory for describing the conformational equilibria for polyelectrolytes like xanthan[25] where the charges are situated on flexible side chains.

Up to this point we have neglected the fact that if the helix formation is a bimolecular reaction the transition will, in principle, also be dependent on the polymer concentration through the concentration dependence of the initiation step.[26] For a highly cooperative transition involving high molecular weight polymers, this effect should, however, be weak.[26] Whether carrageenan forms a double helix[10,15] or a single helix[27] is a matter of some controversy in the literature. However, in the present investigation we will not need to consider the *stoichiometry* of the coil-helix transition since the polymer concentration is either kept constant or varied over a narrow range (20–80 mM disaccharide), within which we have found the transition to be independent of the polymer concentration at a high salt concentration, where the polyelectrolyte counterions do not contribute significantly to the ionic activities. On the other hand, it should be clear that our model is quite sensitive to the *shape* of the coil and helix conformations as manifested in the parameters l and a; in particular, the linear charge densities of the two conformations will have a great influence on the model behavior as investigated here.

V. Model Predictions and Discussion

In this section, we will present and discuss some predictions of the model outlined in the previous section as applied to the systems under study here, i.e., κ- and ι-carrageenan. A primary task is of course to explain the experimental data presented in section III, but we will also illustrate some general features of the electrostatic contribution to the chemical potential difference, $\Delta\mu_{el}$, calculated as described above. We will also make some brief comparisons between the predictions of our model and earlier theoretical results by Manning and co-workers.[9,28]

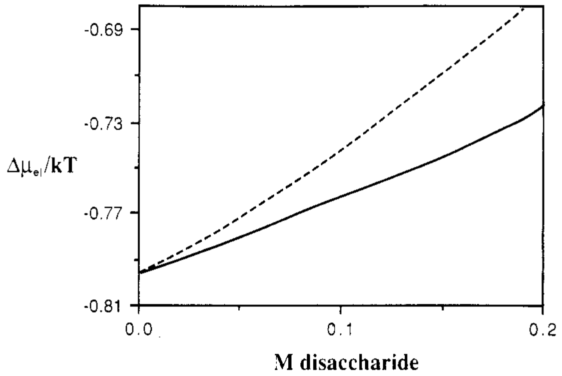

Figure 5. Calculated (see text) variation of $\Delta\mu_{el}/kT$ for ι-carrageenan with the polyion concentration in the presence of 0.2 M NaCl and at 20 °C. The full line represents 100% helix conformation and the dotted line 100% coil conformation.

Effects of Finite Polyelectrolyte Concentration. Figure 5 shows how the calculated $\Delta\mu_{el}$ of ι-carrageenan varies with the concentration of the polyelectrolyte at constant concentration (0.2 M) of added 1:1 electrolyte. Two curves are shown, corresponding to systems containing 100% coil and 100% helix, respectively. These $\Delta\mu_{el}$ values refer to the processes of forming one infinitely long helix in an infinite system of coils (at a finite polyion concentration), or vice versa. In either of these limiting cases, $\mu_{el,pi}$ of the dominant conformation, as well as the chemical potentials of all mobile components of the system, may be calculated as if the system contained only this conformation. All properties of the conformation at infinite dilution (cell radius, cell average concentrations and, through eq 8, $\mu_{el,pi}$) are then completely determined by the equilibrium condition as manifested in eq 11 (cf. also Appendix 1).

Figure 5 shows that when the electrostatic interactions are not dilute, $\Delta\mu_{el}$ will be a function not only of the salt composition and the polyelectrolyte concentration but also of the helical content of the system through the conditions in eq 11 and, for finite concentrations of both conformations, eq 13 and 14. The effect of this will be to make $\Delta\mu_{el}$ more unfavorable for helix formation as the helical content increases. This dependence on the helical content arises because the counterion activities decrease on helix formation due to the higher charge density of the helix. The effect is significant in salt-free systems but will rapidly disappear when salt is added, causing the transition not only to shift to higher temperatures but also to sharpen at higher ionic strengths when the polyion–polyion interactions are reduced. It is interesting that exactly this effect has been noted for the case of DNA;[16,18] however, the explanation offered here has, to our knowledge, not been proposed previously. Experimental studies in our laboratory have confirmed this effect of ionic strength on the width of the helix–coil transition also for carrageenans (although the effect is not so readily apparent in these systems as in the case of the much more highly charged DNA) and these results will be discussed in detail in a future paper in this series. Here we only wish to point to this phenomenon as an effect which is entirely due to *inter*polyion interactions. Thus, they can only be captured by a theory which, like the one used here, assumes a finite polyion concentration (the two curves of Figure 5 are seen to coincide at infinite dilution of the polyion, when excess salt is present).

Polyelectrolyte Concentration Dependence. In Figure 6, we compare the experimental data of Figure 2 with the predictions of the theoretical model. The theo-

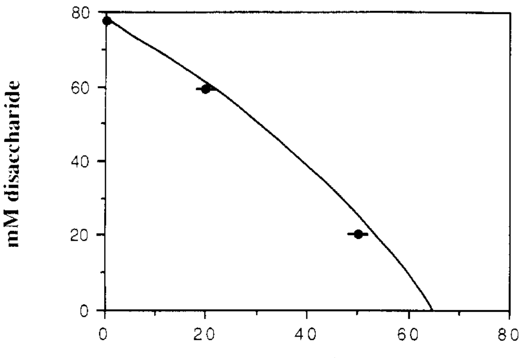

Figure 6. Comparison between calculated (solid line; see text) and experimental conformational stability diagrams for ι-carrageenan in NaCl solutions. Conditions as given in Figure 2.

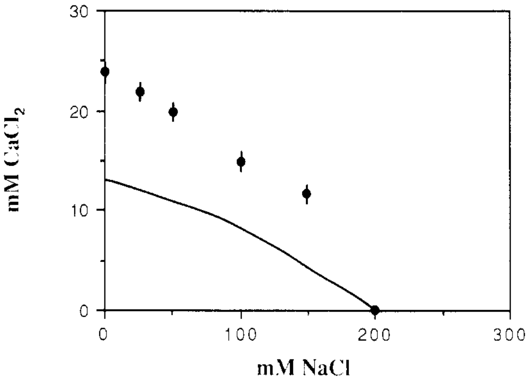

Figure 7. Comparison between calculated (solid line; see text) and experimental conformational stability diagrams for κ-carrageenan in mixed salt solutions. Conditions as given in Figure 3.

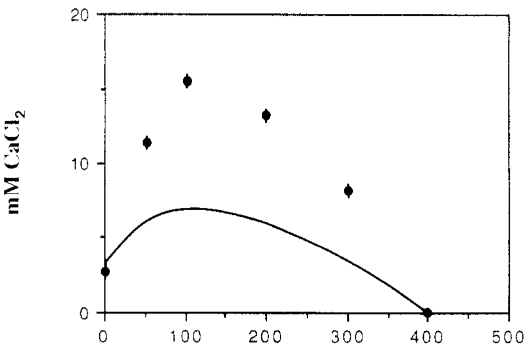

Figure 8. Comparison between calculated (solid line; see text) and experimental conformational stability diagrams for ι-carrageenan in mixed salt solutions. Conditions as given in Figure 4.

retical curve was obtained in the following way. First the value of $\Delta\mu_{el}$ at a polyelectrolyte concentration of 78.4 mM disaccharide and with no added salt was calculated. This was chosen as our (arbitrary) reference point, and we have drawn the theoretical curve through the experimental value at this point in Figure 6. With the assumptions of our model, i.e., that $\Delta\mu_{nonel}$ is only dependent on temperature (cf. above) it then follows (since the propagation term is an equilibrium constant for the formation of helical units[1,19] and therefore has to be constant *at a constant helical content*) that all other points of the theoretical curve are given by the condition that $\Delta\mu_{el}/kT$ be kept constant. The calculations were performed assuming 0% helix content, as this corresponds to the conditions of the experimental data. (Repeated calculations at 1% helix content gave slightly higher salt concentrations (0.5–1 mM) for the systems containing added salt, in agreement with the conclusions of the previous paragraph.) From Figure 6 it is clear that the theory is very good at predicting the amount of added NaCl required to yield the onset of helix formation in progressively more dilute solutions of ι-carrageenan.

Counterion Valency Dependence. We will now turn to the dependence of the coil–helix transition on the counterion valency and test our theoretical model on the experimental data given in Figures 3 and 4. The theoretical curves were obtained as described in the previous paragraph, using the pure monovalent salt forms as reference points. Figure 7 shows the result of κ-carrageenan, and it is seen that the monotonic dependence on the concentration of 1:1 electrolyte is reproduced by theory. Quantitatively, our model is off by a factor of nearly 2 for the pure divalent salt form; however, the order-of-magnitude difference between the concentrations of monovalent and divalent cations required to initiate helix formation is correctly predicted. (A better agreement between theory and experiment was obtained when the system containing only divalent ions was chosen as a reference system; the calculated curve was then found to fall only slightly above four of the remaining five experimental points, joining the NaCl-axis at 276 mM.)

Theoretical and experimental results for the more highly charged ι-carrageenan are compared in Figure 8, and it is gratifying to note that also in this case the correct qualitative behavior is predicted by our model; i.e., the model predicts a maximum in the concentration of divalent cations required for an onset of helix formation as the concentration of monovalent cations is increased. The reason for this behavior can now be understood, in terms of our model, if we examine the concentration profile of the ions in the cell. The counterion concentration is given by $c^{2+}(r) = c_0^{2+} \exp(-2e\phi(r)/kT)$ for divalent ions and by $c^{+}(r) = c_0^{+} \exp(-e\phi(r)/kT)$ for monovalent ions. If the magnitude of ϕ is sufficiently large, it is obvious that $c^{2+} > c^{+}$ even for a low bulk fraction of divalent ions. As a result, the electrostatic potential profile away from the polyion surface and the chemical potential of the polyelectrolyte will be determined mainly by the divalent ions. This is the same as to say that the sum on the right-hand side of eq 2 may be approximated with a sum where contributions from the monovalent counterions are excluded. This approximation becomes better as the magnitude of the electrostatic potential increases. For a helix in equilibrium with a coil, c_{i0} is the same for both conformations, but ϕ(helix) will always be more negative than ϕ(coil) (except at the cell boundary) due to the higher charge density of the helix. In the limit where the electrostatic potential profile of the helix cell is determined mainly by divalent counterions, an increase in the concentration of monovalent ions will therefore result in a *change* in the chemical potential of the coil conformation which is larger than that of the helix conformation, which is contrary to the normal behavior in the absence of divalent ions. (The added salt will, of course, change the potential of both conformations in the same direction, but the equilibrium is governed by the *difference* in chemical potential between the two states.)

As more 1:1 electrolyte is added, the electrostatic potentials will eventually be reduced (and, also, the c_0^{+}/c_0^{2+} ratio will have increased) to a point where the above approximation no longer applies. Then the normal behavior, where the helix is stabilized by the addition of salt, is regained. The locus of the crossover point separating the two types of behavior will depend on the difference in charge density between the two conformations and, also, on the ratio c_0^{+}/c_0^{2+}. If the potentials are low enough for the exponentials to be linearized, the anomalous effect will

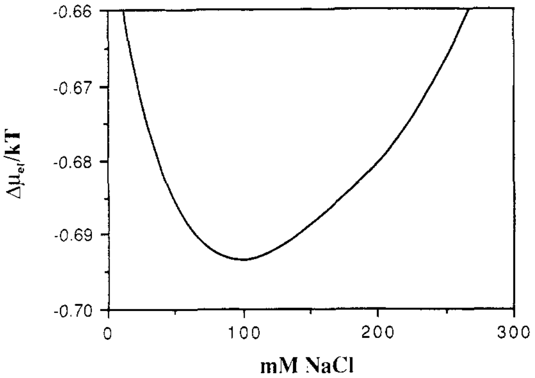

Figure 9. Calculated (see text) variation in $\Delta\mu_{el}/kT$ for ι-carrageenan (infinitely diluted) with the concentration of NaCl in the presence of 6 mM $CaCl_2$ and at 70 °C.

disappear regardless of the value of c_0^+/c_0^{2+}. (If, in addition, the polyelectrolyte concentration is low, the electrostatic free energy will be a single-valued function of the ionic strength.) Thus, the nonmonotonic behavior in the electrostatics stems entirely from the nonlinearity of the exponential function. The above discussion also explains why a monotonic curve was obtained for κ-carrageenan. κ-Carrageenan has half the charge density of ι-carrageenan and hence the potentials are too low (under conditions where the helix conformation is stable) for the maximum in the concentration of divalent ions to appear.

For illustrative purposes, a few cases are shown where $\Delta\mu_{el}$ has been calculated as a function of the concentration of 1:1 electrolyte in systems containing a constant concentration of 2:1 salt. Figure 9 shows results of such calculations for ι-carrageenan in the presence of 6 mM calcium chloride; here a minimum in $\Delta\mu_{el}$, corresponding to a minimum in the helix stability, is seen at a sodium chloride concentration of 100 mM. A further increase in the sodium chloride concentration results in an increase in $\Delta\mu_{el}$, as the electrostatic potentials have now been sufficiently reduced so that the added monovalent ions can stabilize the helix as they would have done in the absence of divalent ions. (The relationship between the theoretical curves of Figures 8 and 9, which are merely two different ways of illustrating the same physical phenomenon, should be appreciated at this stage: Whereas Figure 8 is an isothermal conformational stability diagram, giving the conditions under which $\Delta\mu_{el}$ is constant, Figure 9 shows the *variation* in $\Delta\mu_{el}$ with added monovalent salt in systems the salt compositions of which correspond to a horizontal line at $[CaCl_2]$ = 6 mM in Figure 8. The curve in Figure 9 is thus closely related to the theoretically predicted variation in the transition onset temperature, in ι-carrageenan samples initially containing 6 mM $CaCl_2$, with successive additions of NaCl.)

Figure 10 shows results of two sets of similar calculations for κ-carrageenan at two different concentrations of 2:1 electrolyte. At the higher concentration of 2:1 electrolyte (corresponding to 13 mM $CaCl_2$; Figure 10a), $\Delta\mu_{el}$ increases monotonically as monovalent salt is added (the helix is always stabilized) which is consistent with the conformational stability diagram shown in Figure 7. However, when the concentration of divalent ions is reduced to 2 mM (Figure 10b), the magnitudes of the electrostatic potentials increase and the minimum in the helix stability appears again. Unfortunately, this behavior cannot be verified experimentally since an onset of helix formation in κ-carrageenan at such low salt concentrations does not occur above the freezing point of water. Similarily, the minimum in the helix stability for ι-carrageenan can be suppressed by increasing the concentration of 2:1 electrolyte, but this would shift the temperature of the onset of helix formation above the boiling point of water.

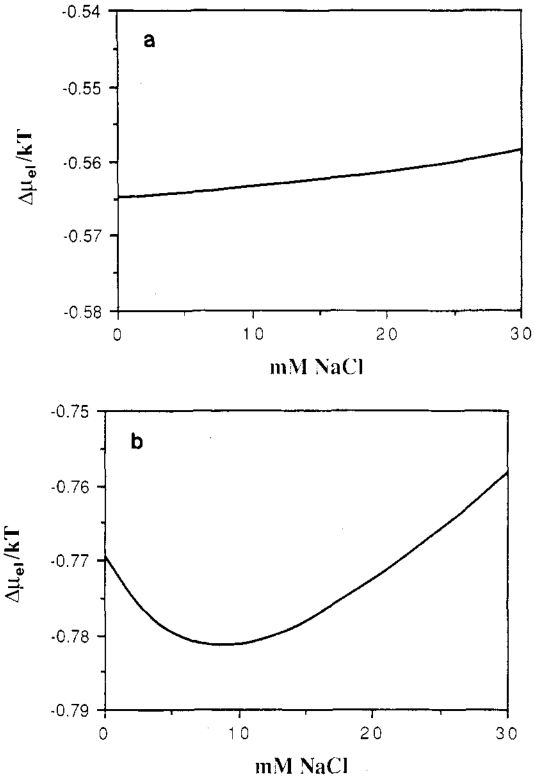

Figure 10. Calculated (see text) variation in $\Delta\mu_{el}/kT$ for κ-carrageenan (infinitely diluted) at 18 °C with the concentration of NaCl in the presence of (a) 13 mM $CaCl_2$ and (b) 2 mM $CaCl_2$.

Previously, on the basis of the counterion condensation concept,[9] de Marky and Manning[28] have derived theoretical expressions which make a priori predictions of the variation of $\Delta\mu_{el}$ as 1:1 electrolyte is added to a polyelectrolyte solution containing a constant amount of divalent counterions. In its title and in all numerical examples their paper refers to the case of DNA—the work undoubtedly having been inspired by experimental results of the kind reported in ref 16–18 (some of which are also correctly predicted by the theory[9])—but as the theoretical approach taken was rather general, we think it is appropriate for us to cite those results which would seem relevant for the systems studied here, making comparisons with experiment and with the predictions of the Poisson–Boltzmann cell model. de Marky and Manning restricted themselves to the case $c_0^+ \gg c_0^{2+}$ (in our notation) distinguishing six different cases characterized by the magnitude of the equivalent ratio of divalent cations to negative polyion charges as compared to the linear charge density parameters, ξ_{coil} and ξ_{helix}, of the coil and helix conformations. For the case when the divalent ions are in excess of the polyion charges, which is appropriate here, the theory predicts (eq 55 of ref 28):

$$\Delta\mu_{el}/kT = C - (1/8)(1/\xi_{coil} - 1/\xi_{helix}) \ln c_0^+ + (1/4)(1/\xi_{coil} - 1/\xi_{helix}) \ln c_{pi} \quad (15)$$

where C is a constant, the value which depends on the temperature and on the concentration of divalent ions. If the polyion concentration is also taken to be constant, the expression reduces to

$$\Delta\mu_{el}/kT = \text{constant} - (1/8)(1/\xi_{coil} - 1/\xi_{helix}) \ln c_0^+ \quad (16)$$

As $\xi_{helix} > \xi_{coil}$ for carrageenans (as for DNA), it is seen that eq 16 predicts a monotonic decrease in the helix stability as monovalent counterions are added. This behavior has, as noted above, also been verified by experiment in the case of DNA.[18] For aqueous solutions of the less charged carrageenans, however, the situation is evidently more complex, as may be inferred both from the experimental results presented here (Figures 3 and 4) and from the theoretical results based on the Poisson–Boltzmann cell model (Figures 9 and 10). In particular, the experimentally observed conformational stability diagrams only display a behavior consistent with the predictions of eq 16 (i.e., a decrease in the helix stability with addition of monovalent counterions) in the case of ι-carrageenan, and then only at sufficiently low concentrations of 1:1 electrolyte (cf. the left part of Figure 4). de Marky and Manning[28] clearly stated that their theoretical approach is correct only for solutions sufficiently dilute in all ionic species, and the results of our study would then suggest that this criterion is not generally met for carrageenans under experimentally relevant conditions (i.e., when they undergo a coil–helix transition in aqueous solution).

VI. Summary and Concluding Remarks

In this work, we have presented new experimental data illustrating the dependence of the coil–helix transition of two moderately charged polysaccharides, κ- and ι-carrageenan, on the polyion concentration as well as on the counterion valency. To account for our observations, we have used the Poisson–Boltzmann cell model, with model polyelectrolyte parameters given by the linear charge densities and the partial molar volumes of the real polyelectrolyte chains. With no free parameters the model succeeds very well in giving a qualitatively and semiquantitatively correct description of the experimental results. As a corollary, it was found that the model provides an explanation for effects of added salt on the sharpness of the coil–helix transition, such as has previously been observed in the case of DNA.

The experimental results of this study as well as our theoretical analysis show that the conformation with the highest charge density (the helix) may, in the presence of an intermediate concentration of divalent counterions, be destabilized on addition of monovalent ions up to a certain concentration, after which it is stabilized by further addition of monovalent ions. This is a general electrostatic effect which is not limited to helix–coil transitions or cylindrical symmetry; however, the explanation of this anomalous behavior offered here has, to our knowledge, not been presented previously. A probably related phenomenon lies behind the observation that the internal collapse of a DNA molecule requires higher concentrations of di-, tri-, and tetravalent counterions to occur as the concentration of monovalent salt is increased;[29,30] however, in this case the reversed behavior at higher concentrations of monovalent salt has not been observed. This would require rather high salt concentrations, due to the high charge density of DNA, at which intermolecular aggregation might cause problems.

The fact that the anomalous effect of added monovalent counterions should disappear at suffiently high concentrations of divalent ions seems not to have been recognized earlier. However, it is probably difficult to experimentally verify this prediction for systems like DNA or ι-carrageenan since the required concentrations of divalent ions would shift the transitions above the boiling point of water. Conversely, the prediction that a minimum in the helix stability of κ-carrageenan appears when the concentration of divalent ions is sufficiently low (cf. Figure 10b) also defies experimental verification, since a transition under these conditions would require temperatures below the freezing point of water.

Finally, we should point out that, to our knowledge, this is the first time that the well-known effects of counterion valency on the carrageenan coil–helix transition have been accounted for theoretically. Furthermore, the success of our model lends support to the following assumptions regarding the carrageenan helix made in deriving our results, namely, that (i) the conformation of the carrageenan helix is the same in the presence of NaCl as in the presence of $CaCl_2$ and that (ii) its solution dimensions are close to those of the solid state as deduced from X-ray diffraction data. With reference to the ongoing discussion regarding the multiplicity of the carrageenan helix, we point out that our model will not give results in agreement with experiment if it is assumed that the lengths per unit charge of the helix conformations are twice as large as those given in Table I. A carrageenan single-helix conformation, in solution, corresponding to one strand of the proposed solid-state double helix[21,22] (such a conformation has been proposed in the literature[31]) does therefore not receive support from our study.

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Appendix 1

The basic difficulty in calculating $\Delta\mu_{\rm el}$ is to obtain, at a given helical content, the ionic activities (c_{i0}) of the system as well as the cell radii ($b_{\rm c}$ and $b_{\rm h}$) of the two conformations. Once these interdependent quantities have been obtained the calculations are straightforward (cf. eq 2–12). Schematically, the calculations may be performed as follows. Starting with the special case $p = 0$, the cell radius of the coil conformation then follows directly from the polymer concentration. The Poisson–Boltzmann equation (eq 2) is then solved as usual for the coil cell by an iteration in the ionic activities to yield the correct average salt concentration ($c_{i,\rm av}(\text{coil}) = \langle c_i \rangle$, since $p = 0$) of the system. From the c_{i0} values thus obtained, the cell radius of the helix conformation at the onset of helix formation (i.e., at $p = 0$) may now be calculated by solving the Poisson–Boltzmann equation through an iteration in the cell radius, keeping the c_{i0} values constant (see eq 11). The electrostatic chemical potentials of both conformations can now be calculated. An analogous procedure for $p = 1$ gives (apart from $\Delta\mu_{\rm el}(p{=}1)$) the range within which the cell radii vary over the entire helix–coil transition, since

$$b_{\rm c}(p{=}0) \geq b_{\rm c}(p) \geq b_{\rm c}(p{=}1) \qquad \text{(A1)}$$

and

$$b_{\rm h}(p{=}0) \leq b_{\rm h}(p) \leq b_{\rm h}(p{=}1) \qquad \text{(A2)}$$

The calculation of $\Delta\mu_{\rm el}$ at any value of p ($0 \leq p \leq 1$) is now possible through the following iterative scheme: (1) select a value of $b_{\rm c}$ within the limits of eq A1; (2) guess values of $c_{i,\rm av}(\text{coil})$; (3) calculate c_{i0}; (4) given c_{i0}, calculate $b_{\rm h}$ and $c_{i,\rm av}(\text{helix})$; (5) given $b_{\rm h}$, $b_{\rm c}$, and the total polymer concentration, calculate p through eq 14; (6) given p, $c_{i,\rm av}(\text{coil})$, and $c_{i,\rm av}(\text{helix})$, calculate $\langle c_i \rangle$ (see eq 11). If this value does not correspond to the actual concentration in the system return to point 2 and adjust $c_{i,\rm av}(\text{coil})$, for instance using regula falsi. When the process has converged, calculate $\Delta\mu_{\rm el}$ from $b_{\rm h}$, $b_{\rm c}$, and c_{i0}.

One additional comment regarding point 2 may be appropriate: If the system contains n different mobile ionic species it is only necessary to solve for $n - 1$ values of $c_{i,\rm av}(\text{coil})$, since the remaining one is taken care of by the electroneutrality of the cell (the electroneutrality is guaranted by the boundary condition $\mathrm{d}\phi/\mathrm{d}r(b) = 0$ in eq

2). A mixture of 2:1 and 1:1 electrolytes thus requires a two-dimensional iteration.

Appendix 2. Symbols

a	polyion radius
b	cell radius
c_i	concentration of mobile ionic species i
c_{i0}	concentration of mobile ionic species i at the cell boundary
$c_{i,\mathrm{av}}$	cell average concentration of mobile ionic species i
e	the unit charge
E_{el}	electrostatic interaction energy (per unit polyion charge) of the cell
G_{el}	electrostatic free energy (per unit polyion charge) of the cell
k	Boltzmann's constant
l	projected charge separation of the polyion
m	number of charges per repeating unit
N_{A}	Avogadro's number
p	fraction of repeating units in the helix conformation
r	radial coordinate in the cell
S_{mix}	entropy of mixing
s	propagation parameter in the Zimm–Bragg theory for helix–coil transitions
T	absolute temperature
T_{m}	helix–coil transition midpoint temperature
V_{solvent}	volume of the solvent (per unit polyion charge) in the cell
z_i	charge number of ion i
ϵ_0	dielectric permittivity of vacuum
ϵ_r	dielectric constant
μ_i	chemical potential of component i
$\Delta\mu_{\mathrm{el}}$	electrostatic chemical potential difference between the coil and the helical states
$\Delta\mu_{\mathrm{nonel}}$	nonelectrostatic chemical potential difference between the coil and the helical states
ξ	reduced linear charge density ($\equiv e^2/(4\pi\epsilon_0\epsilon_r lkT)$) in Manning's polyelectrolyte theory
σ	initiation parameter in the Zimm–Bragg theory for helix–coil transitions; polyion surface charge density
ϕ	electrostatic potential

Registry No. κ-Carrageenan, 11114-20-8; ι-carrageenan, 9062-07-1.

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