Response to Comment on "The Helix-Coil Transition of an Ionic Polysaccharide Probed by Counterion Self-Diffusion Measurements" by G. S. Manning

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Manning's comment[1] on our recent paper in *Macromolecules*[2] contains elements of a general critique of the so-called Poisson-Boltzmann cell model (PBCM) as well as a suggestion that the main conclusion of our paper (the rejection of an extended single-strand helical conformation of ι-carrageenan) might be model dependent and should possibly be reevaluated. I will here attempt to briefly respond to the criticism on both these levels.

Manning has one fundamental objection to the application of the PBCM to isotropic polyelectrolyte solutions: He states that "the cell model requires the polyions to be aligned". This is an unfortunate, but common, misinterpretation of the approximations inherent in the cell model. Obviously, the objective of the model is not to give an accurate geometrical description of a polyelectrolyte solution. Rather, the model is used as a simple, approximate way to find the relevant boundary conditions at any finite concentration of the polyion. As such, it neglects effects due to the distributions in the relative orientation and in the separation of neighboring polyions in an isotropic and homogeneous system. The validity of these approximations is certainly open to scrutiny, and, for dispersions of spherical particles, the accuracy of the cell model has been tested with regard to its predictions of thermodynamic properties[3] (of charged systems) and of the self-diffusion of small molecules.[4] However, the fact that it is *necessary*, in general, to consider effects of the polyion concentration justifies the attempt to introduce such effects, albeit in an approximate manner, via the cell model. Studies of polyion conformational equilibria[5,6] and of the spin relaxation[7] of counterions in polyelectrolyte solutions clearly illustrate this point and, also, that effects of the polyion concentration may indeed be handled in a satisfactory way by the PBCM. It should, furthermore, be noted that, in the limit of infinite dilution, the PBCM is very similar to the counterion condensation model (CCM) and shows the same condensation behavior.

As to the question[1] of the break in the counterion diffusion coefficient at the condensation point, such a break is indeed predicted[8] by the PBCM in the infinite dilution limit. The difference between the CCM and the PBCM in this respect is only quantitative, not qualitative. Experimentally, the situation is not clear, however. In the work[9] cited[1] by Manning the experimental diffusion coefficients obtained on the low charge side of the condensation point were independent of the charge density of the polyion, and in the case of NaPA/HPA,[9,10] a *discontinuity* in the diffusion coefficient was observed at the condensation point. Both these observations disagree with the predictions of the PBCM *or* the CCM. On the other hand, neither a break nor a discontinuity at the condensation point was observed in other studies[11-13] of the polyacrylate system.

The cell model as such provides a description of the static properties of the system. Further problems are connected specifically with the use of the model[8,14] to predict the self-diffusion of small ions in a polyelectrolyte solution. Here the PBCM is only expected to give an accurate description of the *radial* diffusion within the cell, since it only captures the dominating features of the radial variation in the electrostatic potential outside a polyion. The *axial* variation of the electrostatic potential profile (along the polyion), which is due to the discreteness of the polyion charges and to counterion correlations, is not at all taken into account. The (finite) reduction in the axial rate of diffusion of the counterions, which is indicatd by experiments[15] and simulations,[16,17] is therefore not predicted by the model.

Because of the assumptions and limitations of the model, we should not expect the PBCM to make accurate predictions of the *absolute* values of counterion diffusion quotients, D/D_0, in real polyelectrolyte solutions. Comparisons[13,14] between experiments and model predictions clearly illustrate this point. However, it is also found[13] that the model predictions of *changes* in D/D_0 following a change in polyion charge density are quantitatively much more reliable, and it is on the basis of this observation (on which we elaborate more in ref 2) that we find that our experimental data do not support the single-strand helix conformation for ι-carrageenan: The predicted *difference* in D/D_0 between the coil and the single-strand helix is much smaller than the difference found experimentally.

Manning[1] challenges this conclusion. He first argues that our experimental observation of a concentration independence in the counterion diffusion coefficient is at variance with the predictions of the mode we use, whereas the same observation could, on the other hand, indicate that the "conditions of the applicability of the limiting law" (the CCM) are satisfied. To understand the possible origin of the observed concentration invariance, we must recall the general features[9,10,13,18-21] of the concentration dependence of counterion diffusion coefficients in polyelectrolyte solutions. At high concentrations ($>10^{-2}$ M), a concentration dependence in agreement with the prediction[2,13] of the PBCM is to be seen, i.e., a decrease in the counterion diffusion coefficient on dilution. At sufficiently low concentrations, however, the counterion diffusion coefficient dramatically *increases* on dilution. It has been suggested[20,21] that the latter effect, which is not predicted by *any* theory that treats infinitely long polyions, has its origin in the finite length of real polyions. In agreement with this interpretation, it is found[20] that the increase in the diffusivity of the counterion on dilution appears at increasingly lower concentrations as the molecular weight of the polyion increases.

The result of the two competing concentration effects is that there exists, in any real polyion solution (including ι-carrageenan in the coil state[18]), a concentration interval where the variation of the counterion diffusion coefficient with the concentration of the polyelectrolyte is very weak. Moreover, both the concentration *range* where this concentration independence (or weak dependence) occurs and the *value* of the observed counterion diffusion coefficient in this range depend on the molecular weight of the polyion.[20] Therefore, in view of the pronounced concentration dependence observed on both sides of the minimum,[9,10,13,18-21] an observed concentration independence as such may not be taken as an indication of infinite dilution behavior, as Manning[1] suggests. As regards the particular ι-carrageenan solutions discussed here, it may be noted that, thermodynamically, the limiting behavior has *not* been reached, since the coil-helix transition temperature differs[2] for the two concentrations studied. This concentration dependence is purely an electrostatic effect.[5]

Lastly, Manning[1] points out that the experimental value[2]

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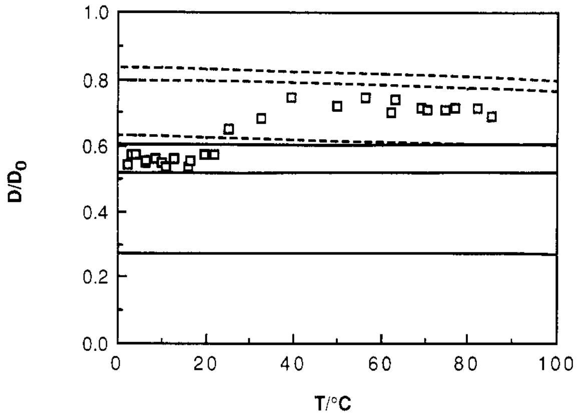

Figure 1. Diffusion quotients of the tetramethylammonium counterion in a 55 mM solution of ι-carrageenan. Boxes indicate experimental results (from ref 2), and lines, theoretical predictions for (from top to bottom) the coil, the single-strand helix, and the double-strand helix conformations according to the CCM (from ref 1; solid lines) and the PBCM (from ref 2; dashed lines).

of the counterion diffusion quotient for the ι-carrageenan helix is in good agreement with the CCM prediction for the single-strand helix, whereas it deviates very much from the prediction for the double-strand helix. "On this basis", he concludes, "the authors' data can be interpreted as suggesting the presence of a single-stranded helix." Fundamental arguments speaking against the latter helical conformation have been presented elsewhere;[6] the issue to be discussed here is, however, whether the results from the CCM may cast doubts on our interpretation of counterion self-diffusion data. To facilitate this discussion, I have in Figure 1 reproduced the experimental data of ref 2, together with the theoretical predictions[1,2] by the CCM and the PBCM for the coil, the single-strand helix, and the double-strand helix conformations, respectively. The PBCM curves correspond to calculations[2] with an unreduced axial diffusion rate and without the addition of a contact radius of the counterion to the radii of the model polyions. (If a contact radius of the ion is introduced or if a constant reduction in the axial diffusion is assumed, all the PBCM curves in Figure 1 will be shifted, by equal amounts, upward or downward, respectively.[2])

If it is unrealistic to expect that the PBCM should give an accurate a priori prediction of the counterion diffusion quotient in an arbitarily chosen polyelectrolyte solution, the same certainly holds true for the CCM. Fundamentally, the latter model must be regarded as more approximate than the former, since the CCM only considers the limiting behavior of the system, where the properties depend solely on the linear charge density of the polyion. Consequently, the observed large variations in D/D_0 with the counterion,[13,21] the pendant ionic group,[19] and the backbone[9] of the polyion are not predicted by the CCM. In the high charge limit, which is relevant here, the general pattern that emerges from comparisons[9,10,14,18,19,22] with experiments on a large number of polyelectrolytes is that the CCM overestimates the effect of the polyions on D/D_0 of monovalent counterions, except for vinylic carboxylate polyions with alkali-metal counterions. Therefore, it must be suspected that the agreement between the experimental helix data and the CCM predictions for the single-strand helix in Figure 1 is purely coincidental. This conclusion is strongly supported by the clear discrepancy between the experimental data for the *coil* conformation and the corresponding CCM prediction (Figure 1; cf. also ref 18). Manning,[1] however, is not disturbed by this discrepancy: He finds that the difference between experiment and theory in Figure 1 amounts to 15% and concludes that the theoretical value for the coil "bears comparison" with the measured value. However, since the effect that the theory predicts is really the relative *reduction* in the counterion diffusion coefficient in a polyelectrolyte solution (i.e., the quantity $1 - D/D_0$), Manning's estimate of the discrepancy between theory and experiment is misleading: The reduction predicted by the CCM for the coil conformation is actually 39% larger than that observed experimentally. (Trivially, the relative error in the theoretical estimate of D/D_0 should become smaller as D approaches D_0.)

My interpretation of Figure 1 is as follows. The CCM predictions of D/D_0 are considerably *lower* than the corresponding experimental values, indicating that the CCM assumptions (the infinite dilution behavior and the vanishing diffusion for condensed counterions) are inappropriate for the investigated solution of ι-carrageenan with the bulky tetramethylammonium counterion. Qualitatively, this kind of discrepancy between CCM predictions and experiment fits well with previous results involving ι-carrageenan[18] or the tetramethylammonium counterion.[13] Still, the CCM predicts (as does the PBCM) a *difference* in D/D_0 between the coil and the single-strand helix conformations that is considerably smaller than that obtained experimentally. This small difference is simply a consequence of the *very small difference in shape*[2] between the coil and the extended single-stand helix. I therefore find that the CCM predictions, although they generally exaggerate the reduction of the counterion diffusion in the present system, quite clearly speak against the interpretation of the data of Figure 1 in terms of a conformational transition from a coil to a single-strand helical conformation.

To summarize the main conclusions of this note: The essential difference between the PBCM and the CCM is that systems at finite concentrations are considered in the former model. Experimentally, the dependence of various properties (including the counterion diffusion coefficient) on the concentration of the polyelectrolyte is verified. The derivation of the counterion diffusion quotient within the PBCM or the CCM requires further assumptions regarding the axial diffusion, and the cases most commonly considered ("free axial diffusion of all ions" or "no diffusion of condensed ions") are both incorrect, in general. As a consequence of these and other assumptions, neither the PBCM nor the CCM predictions of diffusion quotients are quantitatively reliable. On the other hand, the predicted differences in the diffusion quotients for polyelectrolytes differing in charge density are more reliable in both models. From this point of view, it is clear that both the PBCM and the CCM analyses speak against the single-strand helical conformation of ι-carrageenan.

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