

Scaling Equations for a Biopolymer in Salt Solution

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The effect of the simultaneous presence of monovalent and divalent cations on the thermodynamics of polyelectrolyte solutions is an incompletely solved problem. In physiological conditions, combinations of these ions affect structure formation in biopolymer systems. Dynamic light scattering measurements of the collective diffusion coefficient D and the osmotic compressibility of semidilute hyaluronan solutions containing different ratios of sodium and calcium ions are compared with simple polyelectrolyte models. Scaling relationships are proposed in terms of polymer concentration and ionic strength J of the added salt. Differences in the effects of sodium and calcium ions are found to be expressed only through J .

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Polyelectrolytes are polymers with ionizable groups. In spite of their wide range of commercial applications and the interest that they continue to attract in fields ranging from biology to polymer physics, the complexity of their interactions still eludes a complete theoretical description [1,2]. Their almost universal presence in biological processes in concert with various monovalent or multivalent ions makes it important to understand their general behavior, particularly in physiological or near-physiological salt conditions. Self-assembly and structure formation of charged biopolymers (DNA, proteins, etc.), for example, are mediated by the charge density of the polymer, as well as by the concentration and valence of the surrounding ions. In many polyelectrolyte solutions increasing the concentration of multivalent ions above a threshold value induces phase separation or irreversible structural changes [3–8].

Few systematic investigations into well-defined biopolymers or synthetic polyelectrolytes have been reported over an extended range of salt conditions, and none involving solutions with mixed valence ions. Existing theories have little to say about the effects of multivalent ions, as they have focused on solutions containing only monovalent ions [9,10]. In highly charged systems with multivalent ions, the mean field Debye-Hückel and Poisson-Boltzmann models break down. More advanced physical models contain numerous assumptions and parameters, the experimental interpretation of which is not always straightforward. Large-scale computer simulation in principle offers a promising approach to investigate polyelectrolyte molecules in solution, but the complexity of the phase behavior of these systems, with their many length scales, and the large number of components in the case of added salt, make these studies both technically challenging and time-consuming, even with the most recent tools [11–13].

In their pioneering work Flory and Osterheld investigated the effect of monovalent and divalent cations on the

average linear expansion of polyacrylic acid chains in dilute aqueous solution [14]. In particular, they found that at fixed degrees of neutralization the chain expansion factor increased linearly with $1/J$, where J is the ionic strength of the solution.

In this Letter systematic dynamic light scattering (DLS) observations of the effect of ions on the dynamics of semidilute solutions of a high molecular weight biopolymer, hyaluronan (HA), are reported and compared with the predictions of current theoretical models. HA, the sodium salt of hyaluronic acid, which is one of the most abundant biopolymers in the body, is involved in the control of tissue hydration, in the rheology of synovial fluid, as well as in the self-assembly of proteoglycans in cartilage [15]. The collective diffusion coefficient D and the dynamic intensity were measured over a wide concentration range both of polymer and physiologically relevant ions (sodium and calcium). The measurements, using an ALV 5022F goniometer with a HeNe laser working at 6328 Å, were made on aqueous solutions of HA ($M_w = 1.2 \times 10^6$) at 50 and 100 mM NaCl and also in 100 mM NaCl with varying amounts of CaCl₂ (20, 50, 100, and 200 mM CaCl₂). The temperature was 25.0 ± 0.1 °C and the pH of the samples was 6.5, at which HA is completely dissociated.

According to standard theory [16], the thermodynamic properties of neutral polymer solutions are defined at the molecular level by a correlation length ξ such that

$$D = k_B T / 6\pi\eta\xi, \quad (1)$$

η being the viscosity of the solvent. Deviations of η from its value in the bulk solvent due to the finite polymer volume fraction are small in the semidilute concentration regime studied here [17,18]. D , which characterizes the mutual (collective) diffusion between the solvent and the segments of the polymer chain, depends on the concentration c_p of polymer in the solution. In neutral polymer

solutions under excluded volume conditions ξ varies as $c_p^{-3/4}$.

To describe the behavior of ξ in polyelectrolyte solutions with added salt, Odijk [9] employed the Debye-Hückel screening length $1/\kappa$, leading to the scaling expression

$$1/\xi \propto c_p^{3/4} \kappa^{3/4} \propto c_p^{3/4} J^{-3/8}, \quad (2)$$

where J is the ionic strength due to the added salt. With slightly different assumptions, Dobrynin and Rubinstein propose an analogous expression [10],

$$1/\xi \propto c_p^{1/2} (1 + 2c_s/c_p)^{-1/4}, \quad (3)$$

in which c_s is the concentration of added salt, assumed to be composed of monovalent ions. At high salt content, $2c_s/c_p \gg 1$ and Eq. (3) reduces to

$$1/\xi \propto c_p^{3/4} J^{-1/4}, \quad (4)$$

where we have replaced $2c_s$ in Eq. (3) by J to account for ions of higher valency. Experimentally, the difference between Eqs. (2) and (4) is difficult to distinguish. DLS measurements by various authors on polyelectrolyte solutions containing sodium chloride are in general accord with these scaling predictions [19–23].

For the osmotic pressure Π , however, the pressure exerted by the ions must be added to that due to the polymer,

i.e., $\Pi_{\text{pol}}/k_B T \propto \xi^{-3}$. This yields for the total osmotic pressure [10]

$$\Pi/k_B T \propto (c_p^2 + J^2)^{1/2} - J + \xi^{-3}, \quad (5)$$

where k_B is Boltzmann's constant and T is the absolute temperature. Experimentally, it is observed by DLS that the intensity correlation functions from semidilute polyelectrolyte solutions display two distinct components, a fast (f) and a slow (s) relaxation [19,21–23]. The slow mode is generally ascribed to the motion of multichain aggregates in the solution, while the fast relaxation is attributed to the gel mode, as in neutral polymers. The slow mode may be represented either by a sum of exponentials or, over the observed time window, by an expression of the form $\exp[-(\Gamma_s t)^m]$, where Γ_s is the relaxation rate of the slow process. In the present system it was found, to an excellent approximation, that $m = 2/3$.

In semidilute polymer solutions and gels, however, the slow mode is practically osmotically inactive. It is the fast mode with its much smaller intensity (Fig. 1 inset) that defines the osmotic modulus $c_p \partial \Pi / \partial c_p$ of the system [24]. Analysis of the intensity correlation function $g^{(2)}(t)$ allows both the relative amplitude a and relaxation rate $\Gamma_f = Dq^2$ of this collective diffusion component to be determined for different scattering angles θ , q being the transfer wave vector. The Rayleigh ratio of the dynamically scattered light is then $R_\theta = a \langle I(t) \rangle$, where $\langle I(t) \rangle$ is the total scattered intensity averaged over the experimental measuring time. As both D and R_θ were found to be

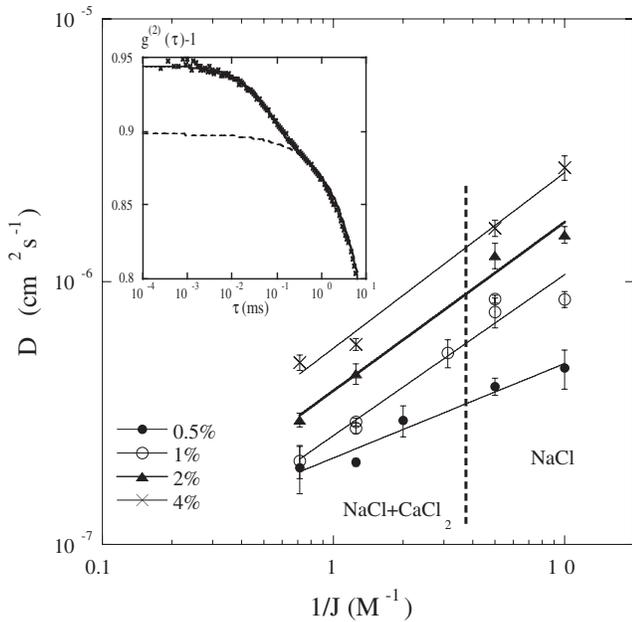


FIG. 1. Dependence of D (fast mode) upon $1/J$ in HA solutions at 25 °C for four different polymer concentrations c_p . Dotted line separates results from samples with and without CaCl_2 . Inset: Enlarged view of intensity correlation function $g^{(2)}(t) - 1$ ($c_p = 26.7 \text{ mM}$, $c_s = 100 \text{ mM NaCl}$, $\theta = 120^\circ$) showing fast (continuous curve) and slow (dashed curve) components.

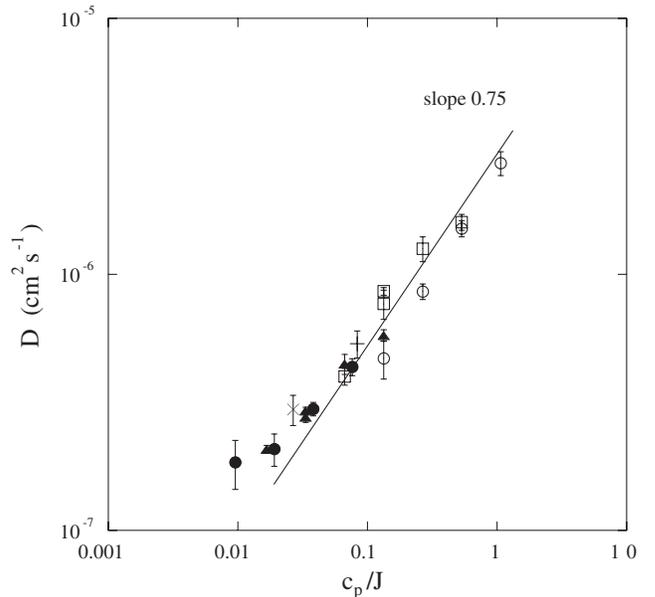


FIG. 2. Dependence of D on c_p/J . Slope of straight line $3/4$. (Open circles) 50 mM NaCl; (open squares) 100 mM NaCl; (+) 100 mM NaCl and 20 mM CaCl_2 ; (\times) 100 mM NaCl and 50 mM CaCl_2 ; (filled triangles) 100 mM NaCl and 100 mM CaCl_2 ; (filled circles) 100 mM NaCl and 200 mM CaCl_2 .

independent of θ , averages were taken over the four angles of observation, 60° , 90° , 120° , and 150° . R_θ is related to the osmotic pressure Π by

$$R_\theta = \frac{Kk_B T c_p}{\partial \Pi / \partial c_p}, \quad (6)$$

where K is the optical contrast factor for the polymer-solvent pair.

The experimentally observed dependence of D , expressed as a function of $1/J$, is shown in Fig. 1 for different values of c_p . For each c_p , D exhibits power law dependence on $1/J$, the exponent of which increases with increasing c_p . On introduction of divalent ions (left of vertical dotted line), the power law remains invariant. This implies that the interactions are dominated mainly by electrostatic effects, rather than by specific interactions between counterions and polymer backbone. This in turn means that the divalent ions appear to exert their influence entirely through the ionic strength.

Figure 2 displays the same values of D as a function of the reduced variable c_p/J . To a reasonable approximation, the points fall on the same straight line of slope 0.75. Such a strong dependence on J suggests that both Eqs. (2) and (4) underestimate the effect of multivalent ions. The overall slope of Fig. 2, however, confirms that the concentration dependence $D \propto c_p^{3/4}$ resembles that of neutral polymers in a good solvent. These findings also indicate that semirigid polyelectrolyte solutions in the presence of ions with mixed valence can be described in terms of the same variables, c_p and J , as suggested by Flory and Osterheld. The curvature observed at the lower end of Fig. 2, corre-

sponding to the lowest polymer concentration at the highest ionic strength, mimics the approach to the dilute concentration regime, where the diffusion coefficient becomes independent of c_p [16]. As will be seen later, however, this behavior is the result of changes in the persistence length of the polymer.

The intensity of the dynamically scattered light R_θ can be estimated on taking the derivative $\partial \Pi / \partial c_p$ in Eq. (5), by setting

$$\xi^{-3} = \alpha c_p^{9/4} J^{-3/4}, \quad (7)$$

where α is a constant. This yields

$$R_\theta = bJ / \{ [1 + (c_p/J)^2]^{-1/2} + \frac{9}{4}\alpha(c_p J)^{1/4} \}, \quad (8)$$

where $b \equiv K$ represents a normalization constant. On plotting R_θ as a function of the right-hand side of Eq. (8), however, linear behavior is recovered only when α is large. It follows that the square root term in the denominator of Eq. (8) is very small and, consequently, that the ionic contribution of the osmotic pressure in the light scattering intensity is negligible. Figure 3 shows the behavior of R_θ in the limit of large α . The slope in the resulting power law representation

$$R_\theta \propto J^{3/4} c_p^{-1/4} \quad (9)$$

of the least squares fit through the data is 0.96 ± 0.04 , thus verifying the validity of Eq. (9). Although the measurement of R_θ is intrinsically noisier than that of D , this representation is acceptable for all the data, including for the samples with high divalent salt content. By comparison, when the same data are plotted in the formalism

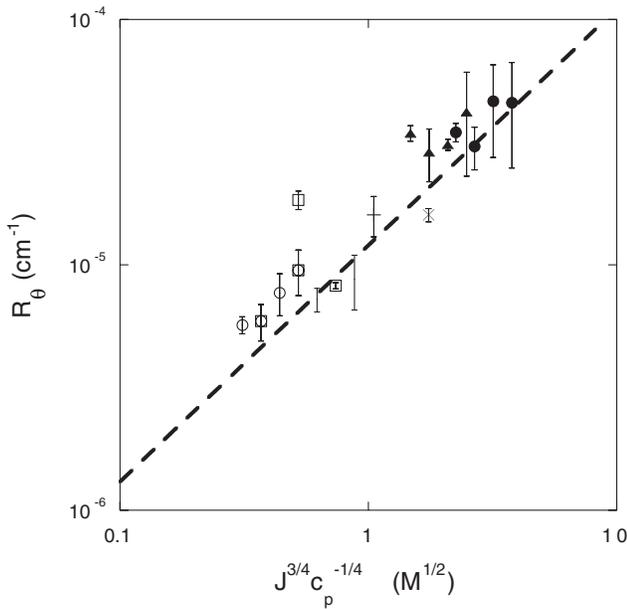


FIG. 3. Variation of DLS intensity R_θ of the collective (fast) mode with $J^{3/4}/c_p^{1/4}$. Dashed line is least squares straight line fit through data. Symbols as in Fig. 2.

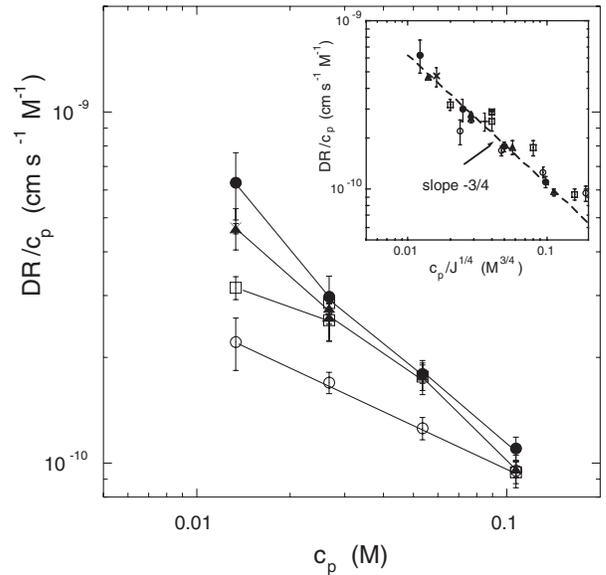


FIG. 4. The product $DR_\theta/c_p = Kk_B T/f$. In this plot, the least squares fit of Fig. 3 is linearized to $R_\theta = 1.20 \times 10^{-5} J^{3/4}/c_p^{1/4} \text{ cm}^{-1}$. Lines are guides for the eye. Inset: Same data as a function of $c_p/J^{1/4}$. Symbols as in Fig. 2.

derived from Eq. (2), namely $R_\theta \propto J^{9/8} c_p^{-1/4}$, the fit is substantially poorer and a linear relationship is not obtained. The present intensity measurements thus tend to support Eq. (4), in which, however, the osmotic contribution from the ions is small.

In contrast to the purely thermodynamic quantity R_θ , the diffusion coefficient D contains information of both osmotic and hydrodynamic nature.

$$D = (\partial\Pi/\partial c_p)/f, \quad (10)$$

where f is the friction coefficient describing the hydrodynamic interaction between the polymer chains and the solvent. It follows from Eq. (6) that the quantity

$$DR_\theta/c_p = Kk_B T/f \quad (11)$$

is independent of the osmotic pressure and depends only on the local structure of the polymer chain in its surrounding liquid. In the present experiment, variations in the solvent viscosity η , whether due to finite polymer [18] or salt [25] concentration, together amount to no more than 15%. Any change recorded in f in excess of this therefore signals a modification of the effective size of the chain subunits.

In Fig. 4 the variation of DR_θ/c_p is plotted as a function of c_p . In this figure, to minimize scatter, R_θ has been replaced by the right-hand side of Eq. (9). The results show that in this system $1/f$ depends not only on c_p but also on J . With increasing salt concentration the dependence of $1/f$ on c_p becomes more pronounced: at low polymer concentrations the segment mobility increases strongly with increasing J , while at high c_p the effect is attenuated, even tending to disappear. Owing to the intrinsic rigidity of the chain, a simple scaling relationship is not in general expected for the persistence length [26]. The inset of Fig. 4 shows, however, that the same data, when plotted as a function of $c_p/J^{1/4}$, can be approximated by a master curve with slope $-3/4$. The most marked increase in $1/f$ (i.e., decrease in persistence length) with increasing ionic strength J occurs at low polymer concentration. This result, which is consistent with the findings of Flory and Osterheld [14], is not addressed explicitly in current theoretical models. More strikingly, no specific effect attributable to the divalent calcium ions is observed, except in their contribution to J . This finding is remarkable in view of the many experimental observations on polyelectrolyte systems (e.g., polyacrylic acid, DNA) in which phase separation occurs as the concentration of divalent ions increases [27].

In summary, in semidilute polyelectrolyte solutions in the presence of monovalent and divalent counterions, the dynamic light scattering intensity R_θ varies as $c_p^{-1/4} J^{3/4}$, where J is the ionic strength. At the polymer concentrations c_p investigated here, no direct contribution to the osmotic modulus from the ions is detected. By contrast, the collective diffusion coefficient D can be described by a

simple scaling relationship similar to that of neutral polymer solutions, using the normalized variable c_p/J . The observed dependence of D is not of purely thermodynamic origin in the usual sense, but is also the result of changes in the persistence length of the polymer chains due to electrostatic screening by the dissolved ions. A corollary of these observations is that the effects of monovalent and multivalent ions, which were believed to be qualitatively different, are fully described by the ionic strength.

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- [1] F. Oosawa, *Polyelectrolytes* (Marcel Dekker, New York, 1971).
 - [2] P. Dubin, J. Bock, R. Davis, D.N. Schulz, and C. Thies, *Macromolecular Complexes in Chemistry and Biology* (Springer, New York, 1994).
 - [3] S. Lifson and A. Katchalsky, *J. Polym. Sci.* **13**, 43 (1954).
 - [4] G. S. Manning, *J. Chem. Phys.* **51**, 934 (1969).
 - [5] Y. Zhang, J. F. Douglas, B. D. Ermi, and E. Amis, *J. Chem. Phys.* **114**, 3299 (2001).
 - [6] M. Muthukumar, *J. Chem. Phys.* **120**, 9343 (2004).
 - [7] Z. Ou and M. Muthukumar, *J. Chem. Phys.* **124**, 154902 (2006).
 - [8] V. A. Bloomfield, *Curr. Opin. Struct. Biol.* **6**, 334 (1996).
 - [9] T. Odijk, *Macromolecules* **12**, 688 (1979).
 - [10] A. V. Dobrynin and M. Rubinstein, *Prog. Polym. Sci.* **30**, 1049 (2005).
 - [11] M. J. Stevens, *Biophys. J.* **80**, 130 (2001).
 - [12] C. N. Patra and A. Yethiraj, *J. Phys. Chem. B* **103**, 6080 (1999).
 - [13] P. Y. Hsiao, *J. Chem. Phys.* **124**, 044904 (2006).
 - [14] P. J. Flory and J. E. Osterheld, *J. Phys. Chem.* **58**, 653 (1954).
 - [15] S. Hokputsa, K. Jumel, C. Alexander, and S. E. Harding, *Carbohydr. Polym.* **52**, 111 (2003).
 - [16] P. G. de Gennes, *Scaling Concepts in Polymer Physics* (Cornell University, Ithaca, 1979).
 - [17] H. Vink, *J. Chem. Soc., Faraday Trans. 1* **81**, 1725 (1985).
 - [18] A. L. Kholodenko and J. F. Douglas, *Phys. Rev. E* **51**, 1081 (1995).
 - [19] R. L. Cleland, *Macromolecules* **15**, 382 (1982).
 - [20] R. S. Koene, T. Nicolai, and M. Mandel, *Macromolecules* **16**, 220 (1983).
 - [21] S. Förster, M. Schmidt, and M. Antonietti, *Polymer* **31**, 781 (1990).
 - [22] M. Sedlak and E. J. Amis, *J. Chem. Phys.* **96**, 817 (1992).
 - [23] J. J. Tanahatloe and M. E. Kuil, *J. Phys. Chem. B* **101**, 9233 (1997).
 - [24] F. Horkay, W. Burchard, E. Geissler, and A. M. Hecht, *Macromolecules* **26**, 1296 (1993).
 - [25] I. M. Abdulgatov and N. D. Azizov, *Fluid Phase Equilib.* **240**, 204 (2006).
 - [26] A. V. Dobrynin, *Macromolecules* **39**, 9519 (2006).
 - [27] F. Horkay, A. M. Hecht, and E. Geissler *J. Polym. Sci., Part B: Polym. Phys.* **44**, 3679 (2006).