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Conformational Transition in Aqueous Solution of Poly(L-glutamic acid). A Low-Frequency Electrical Conductivity Study

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The low-frequency electrical conductivity of poly(L-glutamic acid) in aqueous solutions has been measured during the whole conformational transition from α-helix to random coil, induced by change of pH. The results are compared with the conductivity deduced on the basis of the Manning condensation theory for cylindrical polyelectrolytes and with the Poisson–Boltzmann spherical cell model assuming a simple additive relationship of the polymer and small ion contribution. The influence of the molecular weight is discussed.

Introduction

The helix to random coil transitions of natural polymers in aqueous solutions has been for three decades a subject of extensive theoretical and experimental studies, since this transition should play an important role in the structure and functions of proteins.[1,2]

Poly(L-glutamic acid) [PGA] has often been used as a model system[3,4] for this class of compounds to obtain an insight into their conformation and the dynamical behavior in solution. In aqueous solution, PGA exhibits a helix–coil transition[5,6] with varying pH values, from values lower than 4, where the polymer is completely in α-helix conformation, to values greater than 8 when the polymer is randomly coiled. In the transition region, chain segments partially α-helix and partially random coil coexist.

During the past decade, a number of experimental data derived from a variety of physicochemical methods have been collected and considerable progress has been achieved in the knowledge of the conformation of polypeptides in solution.[7] Nevertheless, some questions regarding the electrostatic interactions which enhance the stability of the α-helix conformation and the different contributions of the polymer–solvent and solvent–solvent interactions still remain not completely resolved.

The purpose of the present work is to study the conductometric behavior of aqueous solutions of poly(L-glutamic acid) at three different degrees of polymerization, during the conformational helix to random coil transition induced by change of pH. Low-frequency electrical conductivity measurements are particularly convenient for exploration in a wide range of experimental conditions of the influence of counterions on the structure of charged polypeptide chains, where repulsive interactions between charged groups and steric interactions coexist, determining the conformation of the polymer backbone.

The electrical conductivity investigations reflect the dynamic properties of the charged polymer–counterion systems and is expected to give useful information on the electrostatic interactions between polyion and small ions, in the charge-induced helix–coil transition. On the other hand, the phenomenology of the transport

properties, such as the electrical conductivity, continues to attract a broad interest for experimental and theoretical investigations owing to the potentiality in the understanding these complex nonequilibrium phenomena.

The conductivity measurements have been analyzed considering two different models. The first one is based on the counterion condensation theory derived by Manning for rodlike polyions,[8,9] and the second takes advantage of the fact that different thermodynamic and transport properties of the polyelectrolyte solutions can be described with the aid of the Poisson–Boltzmann equation[10–12] that, on the other hand, is not confined to polyions of cylindrical geometry. In fact, although locally rodlike, flexible polymers, such as high molecular weight polyions, occupy a roughly spherical volume in solution. Consequently we have analyzed the conductivity data within the Poisson–Boltzmann spherical cell model, considering the contributions to the conductivity derived from the polyion domain and from the small ions in solution by means of a "hybrid" potential approximately valid both inside and far from the polyion domain.

While the bulk of our experimental results supports the validity of the Poisson–Boltzmann cell model of a polyelectrolyte solution, for polyions of higher molecular weight, this model, owing to the difficulty in defining the extension of the polymer domain, proves to be insufficient to account for the results obtained in the present study. On the other hand, when deviation from the cylindrical geometry occurs, the Manning theory for a range of degrees of ionization larger than about 0.7 yields too large fractions of condensed counterions and the calculated conductivity become smaller than that observed experimentally.

Experimental Section

(a) Material. Three samples of sodium poly(L-glutamate) salt (NaPG) used in this work were purchased from Sigma Chemical Co. The molecular weights obtained from viscosity methods given by the manufacturer were 13.3×10^3, 32.0×10^3, and 74.0×10^3. The polydispersity ratio (the ratio of weight to number

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(8) Manning, G. S. *J. Phys. Chem.* **1969**, *51*, 924, 3249.
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(10) Anderson, C. F.; Record Jr., M. T. *Annu. Rev. Phys. Chem.* **1982**, *33*, 191.
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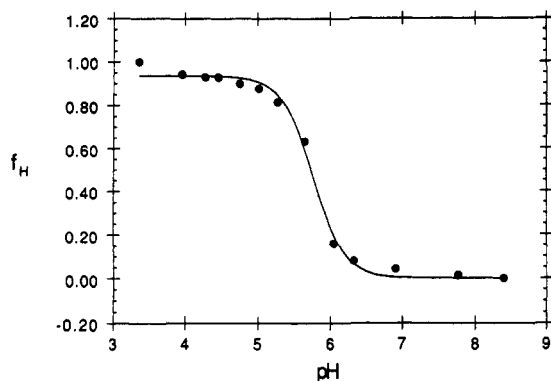(12) Jonsson, B.; Wennerstrom, H. *J. Phys. Chem.* **1987**, *91*, 338.

Figure 1. Fraction f_H of the helical content as a function of pH deduced from the molar ellipticity measured at 222 nm.

average molecular weight) ranged from 1.13 to 1.22 for the samples investigated.

In order to remove the Na^+ ions, the initial NaPG solutions were passed throughout a column containing a severalfold excess of strong cation-exchange resin (Amberlite IR-120, Merck). The acid form thus obtained is then neutralized with a titrated NaOH solution. We shall designate the three molecular weight samples as PGA-1, PGA-2, and PGA-3, respectively. Double distilled, deionized water with an electrical conductivity of less than 1×10^{-6} mho/cm was used throughout the preparation of the PGA solutions. The polymer concentration in solution (expressed as the concentration of the carboxylic acid groups) was determined by potentiometric titration as that of NaOH at the end point of the titration, and for all the samples investigated, the concentration was confined in the range of semidilute regime (about 0.5–0.8 mg/mL).

(b) Circular Dichroism. The conformational transition from the helix to random coil was monitored by circular dichroism measurements recording the molar ellipticity, $[v]$ (deg cm²/dmol) at 222 nm for each pH value with a J-600 CD spectropolarimeter (Japan Spectroscopy Co.). The fraction f_H (Figure 1) of the helical content was evaluated using the expression

$$f_H = \frac{[v]_{222} - [v]_{222}^C}{[v]_{222}^H - [v]_{222}^C}$$

where $[v]^C$ and $[v]^H$ are the molar ellipticity for the all-coil and all-helix conformations, respectively.

(c) Potentiometric Titration. Potentiometric titrations were carried out with a Radiometer Model PHN82 calibrated with standard buffers (pH = 4.01 and pH = 7.00). Different amounts of PGA in acid form, from 0.4 to 0.8 mg/mL depending on the different run, was titrated with 0.1 M NaOH. The degree of ionization α was calculated from the titrant volume in the usual manner.

(d) Conductometric Measurements. Measurements of the electrical conductivity were carried out at the temperature of 20 °C by means of a impedance analyzer Hewlett-Packard Model 4193A in the frequency range from 0.5 to 100 MHz. The conductivity cell consists of a short section of a cylindrical waveguide excited far beyond its TM_{01} cutoff frequency, thus utilizing in the measurements only the stray field of the coaxial line–waveguide transition. The cell constants were determined from measurements of standard liquids of known conductivity and dielectric constant, according to the procedure suggested by Bottomley.[13] The overall accuracy of the experimental setup was confined within 0.5% over the whole frequency range. The conductivity is independent of frequency, within the experimental uncertainties, up to about 10 MHz. At higher frequencies, the typical dependence described by a power law

$$\sigma = \sigma_0 + A\omega^s$$

is observed. In the present work, we report only the low-frequency

conductivity values, whereas the dependence on frequency and particularly the behavior of the exponent s during the helix-to-coil transition, will be discussed in a future paper.

Theoretical Background

(a) The Electrical Conductivity of a Polyelectrolyte Solution in the Light of the Manning Theory. When the Debye length is lower than the average distance between charged groups on the polyion chain, high molecular weight polyions behave locally as rod-like polyions and the counterion condensation theory[8,9] in the cylindrical cell model holds.

The theory of counterion condensation in the neighboring of a charged polyion has been developed and reviewed clearly by Manning. The basic formulation is that the polyion is characterized by the charge-density parameter ξ defined as

$$\xi = e^2/\epsilon K_B T b \qquad (1)$$

where e is the electronic charge, b the average spacing between charged groups on the polyion chain, $K_B T$ the thermal energy, and ϵ the dielectric constant of the aqueous phase. In an environment containing counterions of valence z_c, when ξ exceeds the critical value $\xi_c = 1/|z_p z_c|$, where z_p is the valence of the polyion charged groups, a fraction f

$$f = 1 - \frac{1}{|z_0 z_p|\xi} \qquad (2)$$

of counterions condenses on the polyion to reduce its effective charge to the constant value $(z_p z_c \xi)^{-1}$. Moreover, in the Manning theory, the number of counterions condensed on the polyion per unit charge is independent of concentration, radial dimension of the polyelectrolyte, and excess salt. This charge reduction means a reduction of the electrophoretic mobility of the polyion and, as a consequence, a modification of the electrical conductivity of the whole solution.

The electrical conductivity σ of a dilute charged polyion solution can be written as

$$\sigma = \sum_i C_i \lambda_i \qquad (3)$$

where C_i and λ_i are the concentration and the equivalent conductivities of the ith ion in the solution. In the present case, the conductivity σ (in mho/cm) of the polymer solution (without added salt) was expressed as

$$\sigma = 10^{-3}(C_{H^+}\lambda_{H^+} + C_{OH^-}\lambda_{OH^-} + (1 - f)(C_{Na^+}\lambda_{Na^+} + \alpha C_p \lambda_p)) \qquad (4)$$

where C_p, C_{Na^+}, C_{H^+}, and C_{OH^-} are the concentrations (expressed in monomol/L) of the polyion, Na^+, H^+, and OH^- ions, respectively, λ_p, λ_{H^+}, λ_{OH^-}, and λ_{Na^+} are the equivalent conductivities (expressed in mho cm² mol⁻¹) of the polyion and small ions, α is the degree of polyion ionization, and f is the degree of counterion condensation. The equivalent conductivity λ_p of the polyion can be derived from the Manning theory. According to this theory,[14] the electrophoretic mobility and hence the equivalent polyion conductivity has been derived taking into account the charge–solvent effect, the asymmetry field, the interactions between units of the same polymer and a simple unit and the pure solvent. Two different cases must be distinguished according to the value of the charge parameter ξ, which controls the counterion condensation.

For $\xi > 1$ the polyion equivalent conductivity λ_p is given by

$$\lambda_p = \frac{\epsilon K_B T |\ln K_D b|}{3\pi\eta e|z_c|}\left(\frac{\delta}{\beta}\right)$$

where η is the viscosity of the solvent and $1/K_D$ the Debye screening length defined by

$$K_D^2 = \frac{4\pi e^2 z^2}{\epsilon K_B T}\sum_i C_i$$

(13) Bottomley, P. A. *J. Phys. E: Sci. Instrum.* **1978**, *11*, 413.

(14) Manning, G. S. *J. Phys. Chem.* **1981**, *85*, 1506.

The full expressions for the correction terms δ and β taking into account the asymmetry field are given in eqs 37 and 38 of ref 14. For monovalent charged groups and monovalent ions, these quantities reduce to

$$\delta = 1$$

$$\beta = 1 + \frac{1}{6}\frac{|\ln K_D b|}{3\pi\eta}\frac{\epsilon K_B T}{e}\left(\frac{1}{u^+} + \frac{1}{u^-}\right)$$

where u^+ and u^- are the mobilities of the cations and anions, respectively.

For $\xi < 1$, the equivalent conductivity λ_p can be written as

$$\lambda_p = e / \left[\frac{3\pi\eta b}{|\ln K_D b|} + \frac{1}{6}\xi e\left(\frac{1}{u^+} + \frac{1}{u^-}\right)\right]$$

Equation 4 furnishes the calculated electrical conductivity σ when the degree of ionization α is obtained from the potentiometric titration and the concentrations and the equivalent conductivities of the ion species present in the solution are properly considered.

In order to compare the observed values with the prediction of the counterion condensation theory, the average distance b between the charged group on the polyion chain is required. This quantity, that for a weak polyacid, depends on the degree of ionization α, has been determined by making an appropriate average of the distance d of the ionized groups in the helix- and coiled configuration, according to

$$b = f_H\left(\frac{d}{\alpha}\right)_{\text{helix}} + (1 - f_H)\left(\frac{d}{\alpha}\right)_{\text{coil}}$$

by treating the chain as a succession of aligned helix and coiled sections and assuming that the presence of each section does not affect the overall distribution of different helix and coiled segments along the polymer chain. In the above equation, it is assumed that α is the same for coil and helix configuration.[12]

A somewhat different approach can be attempted, consisting of a different evaluation of the degree of ionization α. In fact, this parameter for the helical and coiled portion of the polymer may be also deduced from the theoretical titration curve derived on the basis of the Poisson–Boltzmann (PB) equation in the cylindrical cell model. The PB equation, which represents the mathematical basis of the counterion condensation in polyelectrolyte solution without added salt,[15] is commonly used by assuming the polyelectrolyte to have a rodlike shape with a uniform surface charge distribution. In this manner, for any actual degree of neutralization, the effective degree of ionization of the helical and coiled chains in the same solution may be determined.

Each polyion is modeled as an impermeable cylinder of radius a and length $L = Nd$, with N the degree of polymerization and d the length of the monomeric unit projected onto the polyion axis, and the polyelectrolyte solution is represented as an ensemble of cylindrical cells whose radius R is given by the polymer concentration C_p (expressed in monomol per unit volume) according to

$$R = \left(\frac{1}{\pi d C_p N_a} + a\right)^{1/2}$$

where N_A is the Avogadro number.

The PB equation, expressed in terms of the reduced potential $\Phi = -e\varphi/K_B T$ and reduced distance $u = r/a$ is given by

$$\frac{d^2\Phi}{du^2} + \frac{1}{u}\frac{d\Phi}{du} = 4\pi a^2 L_B \sum_i z_i n_i(R) \exp(z_i\Phi) \tag{5}$$

where L_B is the Bjerrum length ($L_B = e^2/\epsilon K_B T$) and $n_i(R)$ are the concentrations of small ions at the cell boundary (the other symbols have their usual meanings).

For given values of the characteristic parameters of the polyion, eq 5 is solved by iterative procedure under the boundary conditions

$$\left(\frac{d\Phi}{du}\right)_{u=R/a} = 0$$

$$\left(\frac{d\Phi}{du}\right)_{u=1} = -2\alpha z_p \xi$$

and the further condition[16]

$$\alpha = \frac{C_{H^+}\exp[\Phi(a)]}{K_0 + C_{H^+}\exp[\Phi(a)]} \tag{6}$$

which defines the degree of ionization α through the law of mass action, where K_0 is the intrinsic dissociation constant and C_{H^+} the ion activity of H^+ ion. In this way, the calculated conductivity values are obtained by combining the Manning theory with the theoretical titration curve deduced from the PB equation.

(b) The Electrical Conductivity within the Poisson–Boltzmann Spherical Cell Model. Flexible polyion chains, especially for high molecular weight, occupy a roughly spherical volume in solution. In this case, the PB spherical cell model might be more appropriate. In the spherical cell model, in the center of each cell whose radius is given by

$$R = \left(\frac{3}{4\pi N_A N_p}\right)^{1/3}$$

where N_p is the number of polyion per unit volume, a spherical domain of radius R_p is occupied by the polymer in a random coil state. The Poisson–Boltzmann equation for spherical symmetry is

$$\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{d\Psi}{dr}\right) = -\frac{4\pi ez}{\epsilon}\sum_j\left(n_j(R)\exp\left(-\frac{z_i e\Psi}{k_B T}\right)\right) + \frac{4\pi ez}{\epsilon}n_0 \tag{7}$$

with $n_0 = 3\alpha N/4\pi R_p^3$ for $0 < r < R_p$ and $n_0 = 0$ for $R_p < r < R$.

In eq 7, n_0 is the number (density) of ionized groups on the polymer chain per unit volume whereas $n_j(R)$ ($j = 1, 2$) are, as usually, the concentrations of the positive and negative ions at the cell boundary, when the potential Ψ is set equal to zero. The other symbols carry their usual meanings.

In this case the degree of ionization α, in analogy to eq 6, can be related to the potential Ψ with the aid of the law mass action, according to

$$\alpha = \frac{C_{H^+}\exp\left(-\frac{ze\bar{\Psi}}{k_B T}\right)}{K_0 + C_{H^+}\exp\left(-\frac{ze\bar{\Psi}}{k_B T}\right)}$$

where $\bar{\Psi}$ is assumed to be the average potential over the whole polymer domain

$$\bar{\Psi} = \frac{3}{R_p^3}\int_0^{R_p}\Psi(r)r^2\,dr$$

The effective charge of each polymer domain is calculated integrating the total charge density from zero to the radius R_p of the polymer domain

$$zen^* = ze\int_0^{R_p}4\pi r^2\,dr\,[n_0 + \sum_j n_j(R)e^{-[z_j e\Psi/k_B T]}]$$

The overall conductivity of the solution is thus obtained by adding the contribution due to the N_p polymer domains per unit volume, each bearing the effective charge zen^*, according to the expression deduced by Henry[17] for rigid spherical particle in a medium of viscosity η

$$\sigma_{\text{polymer}} = ezn^*N_p\frac{\epsilon}{4\pi\eta}\Psi(R_p)$$

(15) LeBret, M.; Zimm, B. H. *Biopolymers* **1984**, *23*, 287.

(16) Nilsson, S.; Zhang, W. *Macromolecules* **1990**, *23*, 5234.

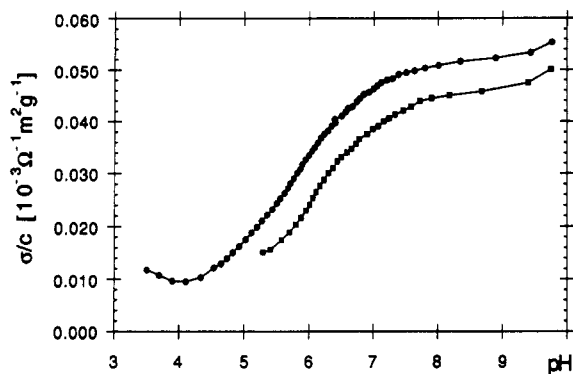(17) Henry, D. C. *Proc. R. Soc. London* **1931**, *A133*, 106.

Figure 2. Conductivity σ normalized to the polymer concentration c (expressed in g/L) as a function of pH at the temperature of 20 °C: (●) PGA-1 (molecular weight 13.3 × 10³; c = 0.77 g/L); (■) PGA-2 (molecular weight 32.0 × 10³; c = 0.48 g/L).

and that due to the ions in the remaining volume of the spherical cell according to the expression

$$\sigma_{ion} = 10^{-3}[C_{H^+}\lambda_{H^+} + C_{OH^-}\lambda_{OH^-} + C_{Na^+}\lambda_{Na^+}]$$

The effective calculation of the conductivity is based on the knowledge of the radius R_p which defines the volume occupied by the polyion in solution. The conformational properties of the polyion chain are determined by the total persistence length L_T due to the intrinsic persistence length L_p and the electrostatic persistence length L_e

$$L_T = L_p + L_e = [C_\infty + 1]\frac{d}{2} + \frac{L_B}{4K_D^2d^2}$$

where C_∞ is the characteristic ratio of the polymer and d is the distance between two units. The characteristic ratio can be estimated from the Stockmayer–Fixman equation,[18] which relates the intrinsic viscosity [η] to the weight-averaged molecular weight through appropriate hydrodynamic constants and values for PGA polymers have been recently reported by Oka et al.[19] The mean-square end-to-end distance may be calculated using the expression

$$\langle L^2 \rangle = 2L_TL_c - 2L_T^2(1 - \exp(-L_c/L_T))$$

where L_c is the contour length, and if the polyion in the random coil state is represented within the freely jointed chain model, the mean maximum cross sectional dimension, to a first approximation, is given by

$$\bar{s} \cong 0.7\langle L^2 \rangle^{1/2}$$

This quantity is assumed to be a measure of the diameter $2R_p$ of the volume occupied by the polymer in solution.

Results and Discussion

Figures 2 and 3 show the electrical conductivity at the frequency of 0.5 MHz and temperature of 20 °C as a function of pH for polymer solutions of three different molecular weights, and at slightly different polymer concentrations. To compare the results relative to solutions with different polymer concentration, and in order to reduce all the data to a common basis, the conductivity σ has been normalized to the polymer concentration c expressed in grams per liter.

The parameters needed to model the polyion, the length d per repeating unit and the radius a, have been chosen as the standard values in the helical and coiled conformation.[16,20,21] The average distance between projection of COOH groups on the polyion axis is assumed to be 1.5 Å for helical conformation and 3.4 Å for fully
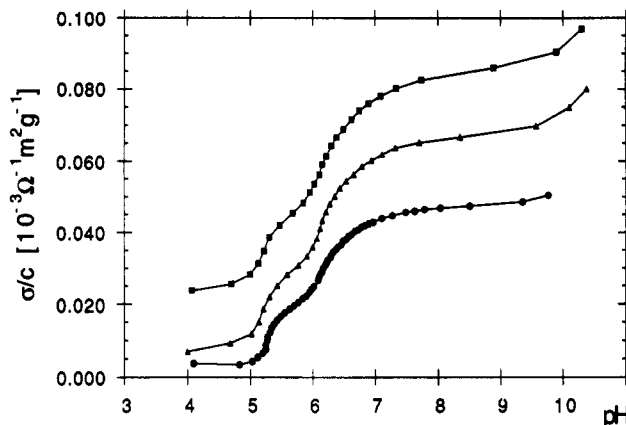

Figure 3. Conductivity σ of PGA-3 (molecular weight 73 × 10³) normalized to the polymer concentration c (expressed in g/L) as a function of pH at the temperature of 20 °C: (●) no added salt; polymer concentration c = 0.75 g/L; (▲) 10⁻⁴ M NaCl electrolyte solution; polymer concentration c = 0.66 g/L; (■) 10⁻³ M NaCl electrolyte solution; polymer concentration c = 0.59 g/L.
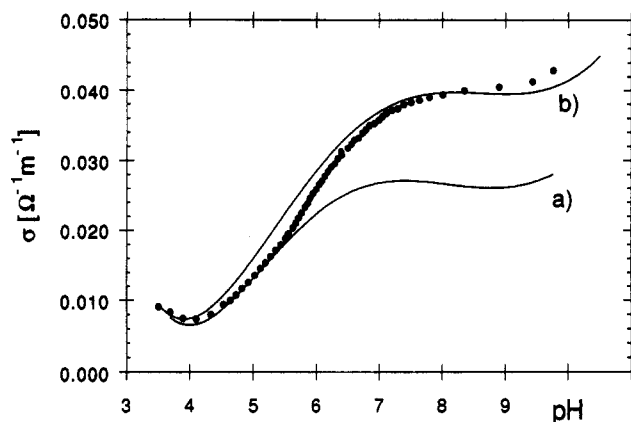

Figure 4. Conductivity σ of PGA-1 (molecular weight 13.3 × 10³; C_p = 6.0 × 10⁻³ monomol/L) as a function of pH at the temperature of 20 °C. The full lines represent the calculated values according to (curve a) Manning theory, eq 4; (curve b) PB spherical cell model.
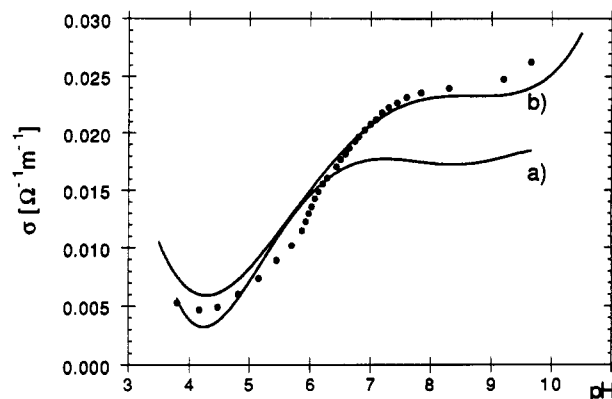

Figure 5. Conductivity σ of PGA-2 (molecular weight 32.0 × 10³; C_p = 3.75 × 10⁻³ monomol/L) as a function of pH at the temperature of 20 °C. The full lines represent the calculated values according to (curve a) Manning theory, eq 4; (curve b) PB spherical cell model.

extended coils. The radii of 14 and 11 Å for all-helix and all-coil conformational states respectively have been chosen.[16] The same intrinsic dissociation constant for the helix and coil conformation has been assumed and equal to pK_0 = 4.375.

Maxfield et al.[22] have pointed out that the dissociation constant pK_0 might be significantly different for the helix and coil con-

(18) Stokmayer, W. H.; Fixman, M. *J. Polym. Sci.* **1963**, *C1*, 137.
(19) Oka, M.; Hayashi, T.; Nakajima, A. *Polym. J.* **1985**, *17*, 621.
(20) Paoletti, S.; Cesaro, A. *C. Arce Semper, J. C. Benegas—Biophys. Chem.* **1989**, *34*, 301.
(21) Hiltner, W. A.; Hopfinger, A. J.; Walton, A. G. *J. Am. Chem. Soc.* **1972**, *94*, 4324.

(22) Maxfield, F. R.; Alter, J. E.; Taylor, G. T.; Scheraga, H. A. *Macromolecules* **1975**, *8*, 479.
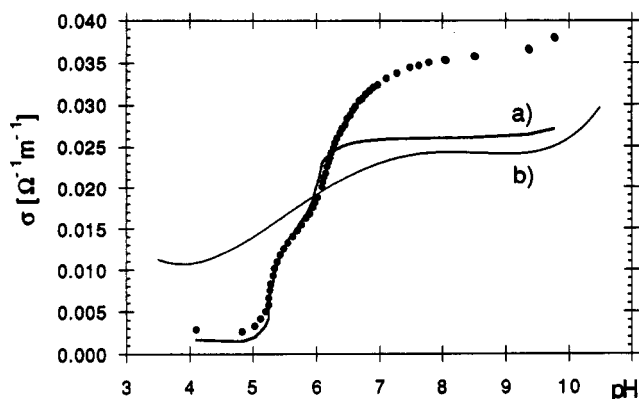
Figure 6. Conductivity σ of PGA-3 (molecular weight 74 × 10³; C_p = 5.8 × 10⁻³ monomol/L) as a function of pH at the temperature of 20 °C. The full lines represent the calculated values according to (curve a) Manning theory, eq 4; (curve b) PB spherical cell model.
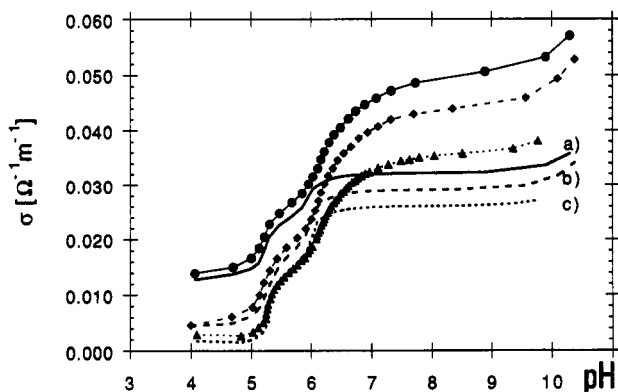

Figure 8. Fraction f of condensed counterions in PGA-2 solution (molecular weight 32 × 10³) deduced from eq 4 compared with the fraction $f = (1 - 1/\xi)$ of the Manning theory: (curve a) calculated for fully helical conformation (d = 1.5 Å); (curve b) calculated from the measured helical content according to eq 9; (curve c) calculated for fully coil conformation (d = 3.4 Å).

Figure 7. Conductivity σ of PGA-3 (molecular weight 74 × 10³) as a function of pH for three different NaCl electrolyte concentration at the temperature of 20 °C: (●) 10⁻³ M NaCl; (♦) 10⁻⁴ M NaCl; (▲) no added salt. The curves a, b, and c refer to the calculated values according to the Manning theory (eq 4) in the order from high to low ionic strength.

Figure 9. Electrical conductivity of PGA-1 (molecular weight 13.3 × 10³) as a function of pH, at the temperature of 20 °C. Calculated values: (—) degree of ionization derived from the experimental titration curve; (---) degree of ionization derived from the cylindrical PB equation with different values of the intrinsic dissociation constant for helix (pK_0 = 3.4) and coil (pK_0 = 4.2) conformation; (———) degree of ionization derived from the cylindrical PB equation with the same intrinsic dissociation constant (pK_0 = 4.375) for both helix and coil conformation.

formation up to 0.13 units. The effects of this difference on the theoretical titration curve calculated on the basis of the PB equation and hence on the calculated conductivity have been considered and briefly discussed (see below).

We will now analyze our results in terms of the Manning theory above described and in terms of the PB – spherical cell model. The results are shown in Figures 4–6, where the conductivity calculated from eq 4 on the basis of the Manning condensation theory (curve a) and that calculated from eqs 8 and 9 on the basis of the spherical model (curve b) is compared to the measured values in the whole pH range, when the conformational transition takes place, for the three polymers of different molecular weights in aqueous solution.

As far as the Manning theory is concerned, these results are in reasonable agreement with the experiment for all the polyions studied in the low pH value range up to pH = 6.5 to which corresponds a degree of ionization of about α = 0.7 and a charge density parameter of about ξ = 1.45. These results provide support to the validity of the Manning theory for low to moderate value of the charge density parameter and furthermore furnish evidence for the absence of condensation (as predicted) for a polyion with a charge parameter lower than the critical value ξ_c = 1. As the charge parameter ξ increases, deviations from the observed values become more serious, suggesting that the Manning theory predicts too large a fraction of the condensed counterions.

To find whether the discrepancy at high value of degree of ionization can be attributed to polyion–polyion electrostatic interactions we have measured the electrical conductivity of PGA-3 in NaCl electrolyte solution of different molarities (10⁻⁴ and 10⁻³ mol/L). In this way, the Debye length is varied from 30 to 10 nm with a consequent reduction of the electrostatic interactions and the system can be considered as dilute. As can be seen in
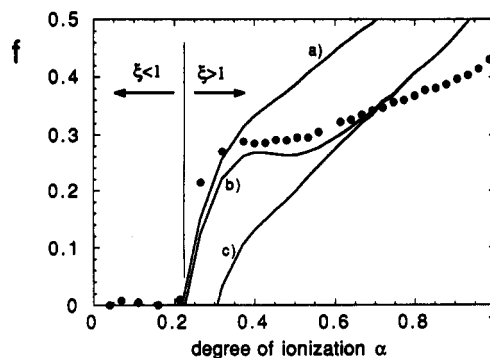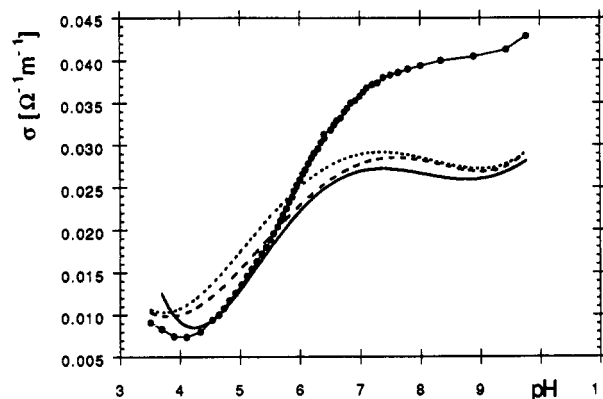
Figure 7, a substantial similar behavior emerges, indicating that the observed deviations cannot be attributed to effects induced by ionic strength. Nevertheless, it is interesting that the fraction of condensed counterions f reflects the helix–coil transition clearly, as shown in Figure 8 for PGA-2 solution, where its peculiar behavior below and above the critical value of the parameter ξ_c = 1 is evident. Furthermore, f follows the complete conformational transition, in good agreement with the Manning theory, up to α = 0.7, where, as noted above, a gradual increasing deviation is observed.

Finally, Figure 9 shows the influence on the calculated conductivity values of the degree of ionization derived from the cylindrical PB equation with different choice of the values for the dissociation constant pK_0 for the all-coil and all-helix conformation. The effect is quite small, indicating a substantial agreement of the theoretical titration curve with that observed on the basis of the effective titration curve.

Recently, Zhang et al.[23] have pointed out that the value a = 14 Å for α-helical PGA used to fit the titration data is roughly twice the value a = 6.5 Å expected for the α-helix. When this value is inserted in the analysis of the conductivity data employing eqs 5 and 6, the results do not appreciably deviate from those shown in Figures 4–6, indicating that this parameter does not influence significantly the expected values.

As noted above, the calculated values of the electrical conductivity show a disagreement with the observed ones for all the

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samples investigated at a high degree of ionization. These discrepancies are too large to be accounted for by approximations involved in the Manning theory, but it seems reasonable to attribute them to the flexibility of the polymer chain when the transition to the coil conformation takes place. In this theory, the condensed counterions do not participate to the electrical conduction. A further possibility is that these counterions might possess a surface conductivity along the polyion chain, thus giving rise to a contribution to the whole conductivity. An evaluation of this contribution would require the knowledge of the surface mobility and the extension of the chain segment where this process takes place.

The results of the PB spherical cell model are shown in Figures 4–6 (curve b). When this model is applied to the polymer in solution, the value of the polymer domain radius must be assigned unambiguously. In the present case, R_p is derived from the characteristic ratio obtained from viscosity measurements. However, it is found that the calculated conductivity results are not so sensitive to the choice of the polymer radius. As can be seen, good agreement has been obtained in the whole range of pH values for PGA-1 and PGA-2 and more pronounced dis-

crepancies occur only for the polyion of highest molecular weight (PGA-3). For a high molecular weight polymer, the application of this rough approximation is more difficult and requires a more sophisticated theoretical treatment. It must be noted that the crossover from the linear to the spherical model is governed by the Debye length K_D in the polymer domain in comparison to the length R_p. As K_D decreases, the electrostatic interactions are strongly reduced and a fraction of counterions in the polymer domain behave preferably as free ions. As the molecular weight is increased, at an intermediate degree of ionization (pH = 6.5), the ratio K_D/R_p decreases from 0.25 to 0.14, indicating that counterions inside the polymer domain should contribute to the polymer conductivity.

While both the approaches used give a remarkably good description of the experimental data at least under moderate value of the degree of ionization, they will prove insufficient to account for the whole experimental results involving high molecular weight polymer where the situation is evidently more complex.

Emf and Ultrasonic Relaxation Measurements of Premicellar and Micellar Aggregation in the Drug Promethazine Hydrochloride

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A membrane electrode selective to the cation of the drug promethazine hydrochloride has been constructed and used in various electrochemical measurements on aqueous solutions of the drug in the presence of different but constant amounts of sodium chloride ranging from 10^{-3} to 2×10^{-1} mol dm^{-3}. At low concentrations of the drug, the emf is directly proportional to log (drug concentration) and displays the theoretical Nernstian behavior for the majority of solutions. The drug is micellar, showing a critical micellization concentration (cmc) characterized by a distinct break in the emf/concentration plots. At concentrations immediately below the cmc, the emf data display nonideal behavior which has been interpreted in terms of the formation of premicellar aggregates. In the micellar region, at a low salt concentration, the monomer drug concentration decreases with increasing overall drug concentration. As the salt concentration is increased, this decrease in monomer concentration becomes less pronounced and eventually levels off when the salt concentration is 2×10^{-1} mol dm^{-3}. In addition, the nonideal behavior corresponding to the formation of premicellar aggregation also becomes less pronounced as the salt concentration is increased, until at 2×10^{-1} mol dm^{-3} premicellar aggregation is effectively eliminated. By measurement of the emf of the drug electrode relative to a double-junction reference electrode and also by investigation of the variation of the cmc of the drug with salt concentration, the degree of micellar dissociation has been evaluated. Ultrasonic relaxation measurements have also been carried out in micellar solutions of the drug containing 10^{-3} and 2×10^{-1} mol dm^{-3} sodium chloride. The relaxation is attributed to the perturbation of the monomer/micelle exchange process. The concentration dependences of the relaxation times and amplitudes have been analyzed to give kinetic and thermodynamic parameters associated with the micellar processes.

Introduction

The aggregation behavior of phenothiazine drugs has been extensively studied using a variety of techniques.[1] A recent calorimetric study[2] focused on aqueous solutions of the drug promethazine hydrochloride (PHCl) in the presence of sodium chloride. It was shown that the drug exhibited a critical micellar concentration (cmc) and in addition at concentrations immediately below this cmc the calorimetric data showed nonideal behavior which was interpreted in terms of premicellar association involving the formation of the small aggregates, dimers, trimers, and possibly tetramers. In the interpretation of the calorimetric data leading to the above conclusions, various association models were tested.

The object of this exercise was to see which model best fitted the experimetal data. In practice this trial and error type of approach, which by necessity has to be used when dealing with macroscopic measurements such as calorimetric[2] and colligative[1,3] data, can sometimes lead to a situation in which more than one type of association model fits the experimental data. A less ambiguous type of equilibrium approach to investigate aggregation behavior of this kind would result if direct measurements of the monomer concentration of drug in the presence of aggregates could be achieved. As a result of developments in drug selective electrodes,[4] we have shown that devices of this kind can easily be constructed and used in a routine fashion to measure the monomer concen-

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