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Separation of polyelectrolytes of variable compositions by free-zone capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) of a series of random copolymers of the ionic monomer, sodium 2-acrylamido-2-methylpropanesulfonate (AMPS), and the nonionic monomer, acrylamide (AAm), was carried out. The absolute value of the electrophoretic mobility μ_E increases as expected with AMPS content. However, μ_E clearly shows a discontinuity when the reduced polymer linear charge density, ξ, becomes unity. This phenomenon is a confirmation of Manning's counterion condensation theory. It is concluded that free-zone CE can be used to separate and characterize charged copolymers below $\xi=1$.

Keywords: Polymers; Acrylamide; Acrylamidomethylpropanesulfonate; Polyelectrolytes

1. Introduction

The application of capillary electrophoresis (CE) to the study of polyelectrolytes is relatively new but shows considerable promise. Grossman and Soane [1] studied the effect of the electric field across the free zone capillary on the electrophoretic mobility, μ_E, and related this effect to orientation of the rod-shaped polyion under high field strength. Although it is generally understood that the electrophoretic mobility of polyelectrolytes is independent of MW, Poli and Schure [2] were able to separate sulfonated polystyrene over a wide range of molecular weight three times faster than with the conventional SEC method, by using CE with a carrier solution containing hydroxyethyl cellulose. Schmitt et al. [3] reported the use of free-zone CE to separate cationic hydroxytriazine from anionic humic polyelectrolytes. Wang and Li [4] were able to achieve baseline separation of two polyanions, polyphosphates and polycarboxylates, via CE with indirect UV detection. These research studies indicate the potential of CE for separating and analyzing polyelectrolytes in aqueous solution.

Encouraged by the high efficiency and rapid analysis that free-zone CE offers, we set out to investigate the potential of free-zone CE for analysis of the linear charge densities of strong polyelectrolytes. Our initial goal was to determine the extent of sulfonation of sodium polystyrenesulfonate (NaPSS) standards from different suppliers. The failure of CE to display any significant difference in the electrophoretic mobility of different NaPSS standards underlined the need to study the dependence of the electrophoretic mobility on the linear charge density of strong polyelectrolytes. To do so,

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we used a series of random copolymers of a charged monomer, 2-acrylamido-2-methylpropanesulfonate (AMPS), and a uncharged monomer, acrylamide (AAm). The results we report here not only help to explain the absence of any difference in the mobility of different sulfonated polystyrene standards, but also provide a direct and clear observation of counterion condensation on polyelectrolytes.

Polyelectrolytes in aqueous solution are typically modeled as charged polyion chains tightly surrounded by an atmosphere of counterions [5–9]. Different theoretical models may vary with regard to the spatial distribution of the counterions; nevertheless, the concept of counterion condensation [5] on polyelectrolytes of linear charge densities above certain critical values has been supported by numerous experimental results, such as pH titration [10,11], solution refractive index measurements [12], conductivity measurements [13–15], tracer counterion diffusion measurements [16], counterion activity measurements [17], ultrasonic absorption measurements [18], ^{23}Na NMR [19], fluorescence quenching [20], small-angle X-ray scattering [21], electrophoretic light scattering [22], isotachophoresis [15,23], and gel electrophoresis [24]. The counterion condensation theory developed by Manning [5] states that, if the dimensionless linear charge density (ξ) of the polyion covalent structure exceeds the reciprocal of the valence of the counterions ($|Z|^{-1}$), there will be a layer of condensed counterions along the contour length of the polyion which will effectively reduce ξ to $|Z|^{-1}$. Here, the dimensionless linear charge density, ξ, is defined in S.I. units as

$$\xi = \frac{e^2}{4\pi D \epsilon_0 k_B T b} \quad (1)$$

where e is the elementary charge of a proton (C), D is the dimensionless dielectric constant of the solvent, k_B is the Boltzmann constant (J/K), ϵ_0 is the permittivity of vacuum (F/m), T is the temperature (K), and b is the average charge spacing along the polyion chain (m). Although various experimental observations confirm the existence of counterion condensation, electrophoresis seems to be the most direct and most convenient method to investigate the phenomenon and evaluate the corresponding theories. However, it is necessary to point out various factors that complicated the interpretation of some previously reported electrophoresis results. With electrophoretic light scattering (ELS), Klein and Ware [22] observed a discontinuity in electrophoretic mobility of 6,6-ionene at $\xi = 1$. However, they varied ξ by changing the dielectric constant of the solvent. This approach, which is correct only if the solvent is a continuous dielectric medium with no specific solvation effects as assumed in Manning theory, changes the environment of the counterions in a way not accounted for in their analysis. Shaaban et al. [15] adjusted the solution pH of weakly ionized polyacrylic acid to vary the linear charge density, but this approach raises the problem of local fluctuations in the microscopic degree of ionization. These difficulties are overcome by using random copolymers of the strongly ionized monomer, AMPS, and the uncharged monomer, AAm, which make it possible to vary ξ without changing the solvent.

2. Experimental

Random copolymers (MW≈50 000) of 2-acrylamido-2-methylpropanesulfonate (AMPS) and acrylamide (AAm) with AMPS mole fraction ranging from 10 to 100% were synthesized by polymerizing mixtures of AMPS and AAm at appropriate mole ratio in dimethylformaldehyde (DMF) at 60°C for 6–12 h with 0.5% azobisisobutyronitrile (AIBN) as initiator and 20 ppm EDTA as complexing reagent to remove heavy metal ions. The synthesized polymers were precipitated twice in acetone, dialyzed for 24 h, and freeze-dried. The average charge spacing (b) for vinyl polymers carrying a charge on each monomeric unit is 2.55 Å, corresponding to a linear charge density of $\xi = 2.8$ at room temperature [20]. The linear charge densities of the copolymers were therefore obtained by multiplying the linear charge density of the homopolymer, poly(AMPS), by the mole fraction of AMPS monomer in the copolymers. Samples for CE were prepared by dissolving copolymers at 1.0 mg/ml and the neutral marker (mesityl oxide) at 0.05% (w/w) in 0.05 M phosphate and 0.05 M borate buffer, respectively, with the ionic strength of each buffer maintained at 0.05 M with 0.05% neutral marker (mesityl oxide) added. Although it is common practice to use a deactivated

capillary to prevent the adsorption of macromolecules on capillary surfaces, the bare-fused-silica capillary is appropriate for strong negatively charged polyanions which will not be adsorbed on negatively charged surfaces. Therefore, CE was carried out on a Beckman P/ACE 5500 instrument using a 50 μm I.D. uncoated capillary, 47 cm in total length and an effective length (from the injection end to the detection window) of 40 cm. The voltage applied across the capillary was 30 kV, and the temperature was maintained at 25.0±0.1°C with fluorocarbon coolant. Sample injection was hydrodynamic with UV detection at 200 nm. Duplicate runs of each sample showed that the electrophoretic mobility, μ_E, calculated by subtracting the electroosmotic flow (EOF) of the run buffer from the sample's apparent electrophoretic mobility, was reproducible to within 3%.

3. Results and discussion

Fig. 1 clearly shows well-defined breaks in the slope of the best-fit lines at $\xi=0.96$ and $\xi=0.97$ for data obtained in phosphate buffer and borate buffer, respectively. This is quite close to the condition for the onset of counterion condensation at $\xi=1.0$ predicted by Manning's theory for systems with monovalent counterions. Counterion condensation explains very well the marked decrease in the effect of polymer composition on the electrophoretic mobility of the copolymers, characterized by the slope of the best-fit lines (going from $\xi<1$ to $\xi>1$, $d\mu_E/d\xi$ changes from 2.1 to 0.18 in phosphate buffer and from 1.9 to 0.18 in borate buffer). Free-zone CE is therefore effective in separating polyelectrolytes based on linear charge density for $\xi<1$, but due to counterion condensation, it can not distinguish different polymers with $\xi>1$. The same conclusion was also drawn by Whitlock and Wheeler [23] based on their electrophoretic mobility data obtained for copolymers of AMPS and 2-hydroxyethylmethacrylate (HEMA) via isotachophoresis. The mobilities we measured in the two buffers differ by an amount larger than experimental error. The reason for this difference is still unknown.

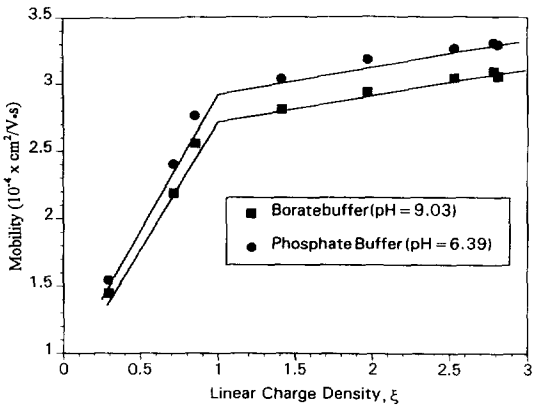

Fig. 1. Electrophoretic mobility of a series of copolymers of poly(AMPS/AAm) with different linear charge densities.

Concurrent with our work, Hoagland et al. [25] carried out a similar but independent study to determine whether charge distribution could be ignored in the separation of the highly charged synthetic polymers by gel electrophoresis, and observed a clear break point in the slope of $\mu_E(\xi)$ centered around $\xi=1.0$. Like Hoagland et al., we also find non-zero values for μ_E in the limit of $\xi\rightarrow 0$, namely, $0.9\cdot10^{-4}$ cm^2/V s at either pH=9.03 or pH=6.39, close to the extrapolated $\mu_E(0)\approx 1.0\cdot10^{-4}$ cm^2/V s in Ref. [25]. Considering that the mobility varies inversely with the square root of ionic strength, our mobility values are in good semi-quantitative agreement with those of Hoagland et al. However, for $\xi>1$, our data show a more prominent dependence of μ_E upon ξ (by approximately a factor of two) than that found by Hoagland et al. [25], i.e., a larger discrepancy from the constant value of μ_E at $\xi>1$ predicted by counterion condensation theory.

We believe that the behavior of copolymers of strongly ionized and uncharged monomers, such as the copolymer between AAm and AMPS, can be studied in a more straightforward way than carboxylic acid polymers. First, these polymers show no pH dependence of ξ, making it possible to work at any pH. In addition, the charged sites on the copolymer used in this experiment are fixed and can not fluctuate spatially, nor can they change as a result of electrostatic induction effects on the pK of acrylic acid. Furthermore, with this copolymer we avoid the adsorption of unionized acrylic acid units onto the negatively charged wall of the bare fused-silica capillary, as reported in Ref. [25].

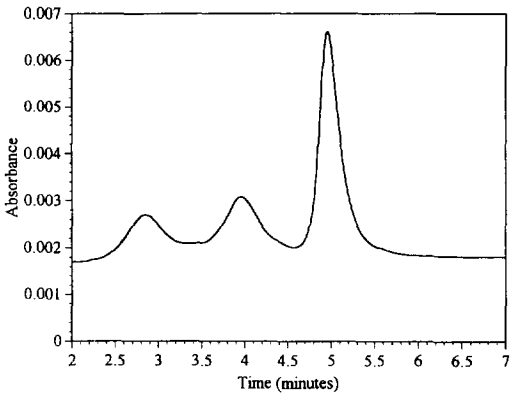

Fig. 2. Separation of a mixture of poly(AMPS/AAm) copolymers of three major compositions in elution order: 90% AAm, 75% AAm, and 50% AAm.

The forgoing results suggest that CE can be a useful tool in analyzing polymers of low ξ. Not only can CE be used to separate these polyelectrolytes based on their difference in linear charge density, but also to characterize the composition distribution of copolymers made up of charged and uncharged monomers such as poly(AMPS/AAm). Fig. 2 demonstrates the separation and composition distributions of poly(AMPS/AAm) consisting of three major mole compositions, 90% AAm, 75% AAm and 50% AAm. Since the linear charge densities of these copolymers are low, three components in the mixture are well resolved by CE. The width of the peaks may also reveal the extent of polydispersity in terms of monomer compositions. However, because of counterion condensation, CE cannot distinguish one polymer of high ξ from another, nor can it characterize composition distributions of copolymers such as poly(AMPS/AAm) of high charge density.

Acknowledgments

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