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# Charge on Poly(ethylene imine): Comparing Electrophoretic NMR Measurements and pH Titrations

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## Introduction

The presence of charged or ionizable groups on a polymer offers the opportunity to control the behavior of the polymer—solubility, tendency to adsorb at surfaces—by changes in external variables such as pH or ionic strength. This character is harnessed in many diverse practical applications, such as wastewater remediation,[1] a flocculant of negatively charged fibers,[2] an additive for production of ink-jet paper,[3] and in drug delivery.[4] Accordingly, the overall behavior is determined by the chemical nature of the polymer, its molecular weight, and effective charge, the last quantity determined by the degree of counterion dissociation/binding.

The effective charge of a polyelectrolyte in solution, at least when strongly ionized, is often significantly lower than the number of ionizable groups due to charge neutralization as a result of condensation of the counterions onto the polymer chain, the so-called Manning counterion condensation.[5] Consequently, there is poor agreement between the charge density calculated from pH titration curves and other experimental approaches such as osmotic pressure,[6] electrophoretic mobility,[7] and dielectric measurements.[8] This general discrepancy arises due to the fact that not all techniques can distinguish the counterions that are “free” in solution from those bound to the polymer.

Further, there are few experimental techniques that can probe the charge on a polymer or surfactant micelle in complex, multicomponent mixtures due to the fact that the detection approach (light scattering, osmotic pressure, dielectric spectroscopy) cannot distinguish the various components. Essentially, a technique is required that is chemically selective, and NMR is the most obvious candidate. In an electrophoretic NMR (eNMR) experiment, an applied electric field generates a coherent motion in the sample, with the charged molecules acquiring a flow dependent on their charge. This flow is quantified by encoding a spatial and temporal dependence to the NMR signal.

eNMR is still a relatively novel technique even though proof of concept was shown many years ago.[9−12] Recently, eNMR has been applied to a number of model colloidal systems, specifically polymers (poly(styrenesulfonate), PSS;[13,14] poly(diallyldimethylammonium chloride), PDADMAC;[15] surfactant micelles (the single surfactant tetramethylammonium and tetrabutylammonium dodecyl sulfates TMADS and TBADS, respectively[16]), and binary mixtures of sodium dodecyl sulfate SDS/tetra(ethylene oxide) dodecyl ether, $C_{12}E_4$,[17] and SDS/dodecyl malonobis(*N*-methylglucamide), DBNMG[18,19]) and polymer/surfactant complexes (poly(ethylene oxide), PEO/SDS).[20] eNMR studies of the mixed surfactant and PEO/SDS systems are particularly insightful as the interactions between the nonionic and ionic species manifest themselves as an “effective charge” on the nonionic species. Similarly, the two components in each of the TMADS, TBADS[16] (surfactant “tails” and headgroup counterions), and the PDADMAC/perfluorinated succinic acid[21] systems show a charge reversal dependent on the molar composition. Drawbacks of the NMR aspects of eNMR include sample size/form/type amplifications of the already inherent insensitivity of NMR compared to other spectroscopic techniques. The electrophoretic element of eNMR introduces further complications; there is an upper limit of the sample conductivity whose value is determined by the maximum power output of the electric field generator, coupled with disturbing factors such as Joule heating of the sample, causing convective effects and various related measurement artifacts.[22]

Poly(ethylene imine) (PEI) is a cationic polymer exhibiting one of the highest charge densities when fully protonated in aqueous solution.[23] Intrinsic viscosity[24] and small-angle neutron scattering of aqueous solutions[25] studies of branched PEI show that the polymer does not significantly change conformation over the pH range $2 < \mathrm{pH} < 11$ due to the highly branched globular structure. Therefore, branched PEI is a suitable choice to probe the electrophoretic mobility of the polymer as a function of pH.

## Experimental Section

**Materials.** Two samples of poly(ethylene imine), PEI, with molecular weights $M_w = 2000$ and $25\,000$ g mol$^{-1}$ were purchased from Aldrich. While the $M_w = 25\,000$ g mol$^{-1}$ sample was used as received, the water was removed from the as-supplied $M_w = 2000$ g mol$^{-1}$ solution under reduced pressure. These polymers comprise primary, secondary, and tertiary amines in a ratio of 1:2:1. Low molecular weight PEI samples have been used to minimize the interpretation problems arising from the unavoidable polydispersity.

All PEI solutions were prepared from stock solutions made up with $D_2O$. Samples at a concentration of 5 wt % were prepared by mass (with appropriate corrections for the density of $D_2O$) on a 1 g scale. Sample conductivities were measured at the experimental temperature (20 ± 0.1 °C) using a microprobe (Radiometer Meter Laboratory CDM210 conductivity meter) calibrated with 1 M KCl.

**Electrophoretic NMR.** The eNMR measurements were conducted on a Bruker AMX300 NMR spectrometer using a custom-designed glass U-tube of 2 mm outside diameter coated with polyacrylamide to reduce electroosmosis.[26] The arrangement is held securely in the center of the active volume of a 10 mm diffusion probe (Cryomagnet Systems, Indianapolis, IN). A constant current pulsed electric field generator designed by S. Woodward (University of North Carolina, Chapel Hill, NC) was used to deliver the current to two blackened platinum wires extending just below the liquid surfaces.

The displacement induced by the electric field, typically 20 $\mu$m, was quantified using the spatial resolution of a simple form of NMR imaging, based on the attenuation of the spin echo caused by the molecules experiencing different “read” and “write” field gradient pulses. Whereas the random diffusional motion of the polymer result in an exponentially damped

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attenuation, the coherent motion induced by the electric field results in a phase shift (the first factor) of the spin echo. For a U-tube geometry, the phase shift of the two sample sections are opposite and equal. Effectively, the added result of the signals from the two U-tube sections is instead a cosinusoidal amplitude variation and the phase information (containing information on the direction of transport relative to the magnetic field) is lost:

$$I(G,\delta,\Delta,\nu) = I_0 \cos(\gamma G \delta \nu \Delta) \exp\left[-\gamma^2 \delta^2 G^2 \left(\Delta - \frac{\delta}{3}\right) D_s\right] \quad (1)$$

where $\gamma$ is the magnetogyric ratio. $G$, $\delta$, and $\Delta$ correspond respectively to the magnitude, duration, and separation of the field gradient pulses, $D_s$ is the self-diffusion coefficient, and $\nu$ is the velocity of the charged entity in the electric field. Accordingly, $I$ is the integral of the relaxation-weighted Fourier transformed peak intensity in the absence ($I_0$) and presence ($I(G,\delta,\Delta,\nu)$) of flow and field gradients. The exponential term accounts for the attenuation due to diffusion. To maximize the electric field induced displacements with manageable pulsed electric fields, a diffusion time ($\Delta$) of 0.5 s was used, which necessitated a stimulated echo sequence with $\tau = 10$ ms. The attenuation of the signal due to relaxation was therefore kept to a minimum and was approximately 30%. Typically, gradient pulses were 1 ms in duration ($\delta$) with intensity ($G$) 0.44 T/m while the electric field pulses were of the order of 250−300 ms duration with intensity increasing from 0 to 2 mA (335 V).

**PGSE-NMR.** Measurements were conducted on a Bruker AMX360 NMR spectrometer using a stimulated echo sequence as described elsewhere.[27] The self-diffusion coefficient is extracted by fitting the measured peak integrals to eq 2.

$$A(\delta,G,\Delta) = A_0 \exp[-(kD_s)^\beta] \quad (2)$$

where $k = -\gamma^2 G^2\{[30\Delta(\delta + \sigma)^2 - (10\delta^3 + 30\sigma\delta^2 + 35\sigma^2\delta + 14\sigma^3)]/30\}$, $\sigma$ is the ramp time of the trapezoidal field gradient pulse, and $\beta$ is a phenomenological parameter that which quantifies the distribution of coefficients associated with polydispersity.

## Results and Discussion

The standard method for determining the charge on a polymer as a function of pH is to titrate the polymer using acid or base and record the pH.[28] Unlike small molecules, the ability to (further) protonate a particular group on a polyelectrolyte depends on its current degree of protonation, α; i.e., the effective protonation constant, log $K_i$, of the polymer is different from the protonation of the completely unionized polymer, log $K_i^0$, and indeed depends on pH.[29,30]

Here, we calculate α from the measured pH via

$$\alpha = \frac{[\mathrm{PEI}]_{\mathrm{total}}}{[\mathrm{PEI{-}H^+}]} \qquad [\mathrm{H^+}]_{\mathrm{total}} - [\mathrm{H^+}]_{\mathrm{free}} = [\mathrm{PEI{-}H^+}] \quad (3)$$

where $[\mathrm{PEI}]_{\mathrm{total}}$ and $[\mathrm{H^+}]_{\mathrm{total}}$ are the total concentrations of PEI and added acid, and $[\mathrm{H^+}]_{\mathrm{free}}$ is the concentration of acid determined by the pH measurement.

The degree of protonation is plotted in Figure 1 as a function of pH for the two samples of PEI used in this study. This behavior is in excellent agreement with that presented by, for example, Mészáros et al.[31] (PEI is effectively uncharged at pH > 10.5, 30% charged at pH = 7, and ~65% charged at pH = 4) and Suh et al.[32]

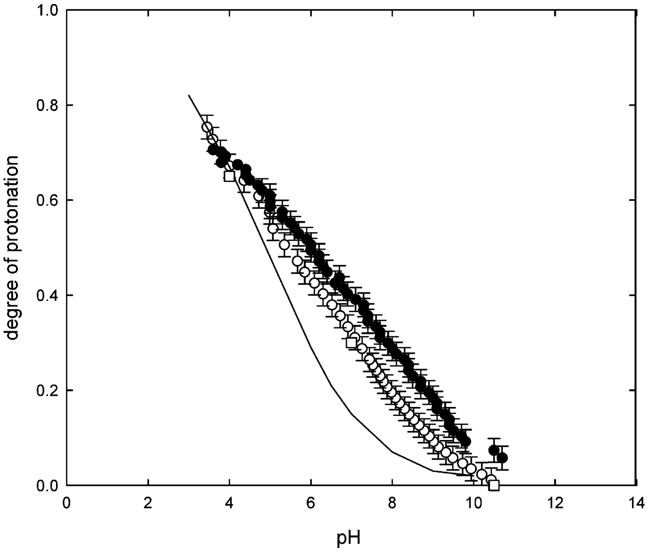

**Figure 1.** Effective charge on the PEI as a function of pH for branched BPEIs of molecular weight 2500 g mol$^{-1}$ (open circles) and 2000 g mol$^{-1}$ (filled circles) calculated from the pH titration data. The solid line is the empirical analysis of pH titration data following the method of Suh et al.[32] and the data of Mészáros et al.[31]

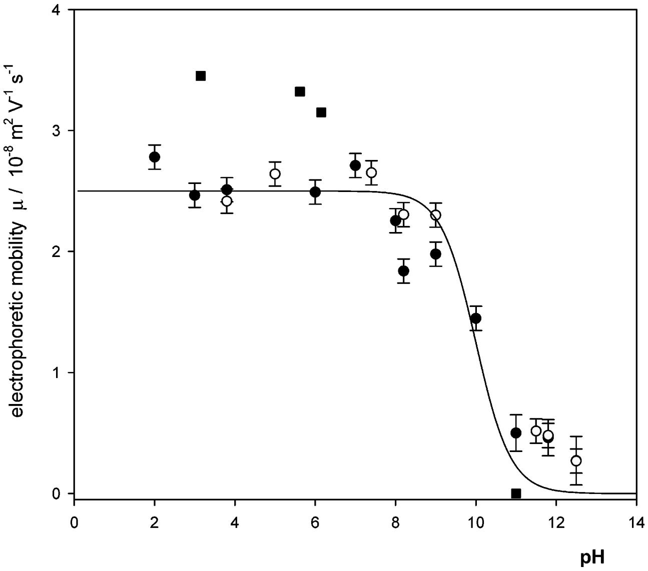

**Figure 2.** Electrophoretic mobility of two branched PEI samples as a function of pH: closed symbols, $M_w = 2000$ g/mol; open symbols, $M_w = 25\,000$ g/mol. Shown for comparison is the predicted charge (line) based on p$K_a$ ~ 10. The solid squares are data taken from ref 21, for PEI of $M_w = 7200$ g mol$^{-1}$ in 0.1 N NaCl.

Figure 2 presents the measured electrophoretic mobilities of the two PEIs as a function of pH. A roughly sigmoidal shape is observed with the electrophoretic mobility increasing dramatically over the range 8 < pH < 10, as the pH is lowered from pH = 11. For pH < 8, the electrophoretic mobility is largely constant. The closed symbols correspond to the measured conductivity data for PEI $M_w = 2000$ g mol$^{-1}$. Also presented are the electrophoretic mobilities of PEI $M_w = 7200$ g mol$^{-1}$ taken from the light scattering data of Lindquist and Stratton;[28] the agreement is reassuringly good given the nature of the two techniques. For the electrophoretic NMR, measurements at pH > 11 are not possible as the electroosmosis suppressive polyacrylamide coating of the U-tube dissolves in highly basic media.

While undergoing the coherent flow associated with the charge on the polymer, the friction opposing the motion of the polymer is the same as that opposing the normal diffusion of the polymer. Thus, the effective charge on the polymer, $z$, may be calculated knowing

$$\mu = \frac{zeD_{\mathrm{s}}}{k_{\mathrm{B}}T} \tag{4}$$

where $\mu$ is the electrophoretic mobility, $e$ is the charge of an electron, $k_{\mathrm{B}}$ is the Boltzmann constant, $T$ is temperature, and $D_{\mathrm{s}}$ is the self-diffusion coefficient. The charge on the polymer scales linearly with the number of ionizable groups and therefore, to a first approximation, molecular weight; $z(\mathrm{pH}) = \alpha(\mathrm{pH})(M_{\mathrm{w}}/\mathrm{RMM})$, where RMM is the molar mass of the monomer unit. Consider the low pH range, i.e. pH $\approx$ 5, the self-diffusion coefficient scales approximately linearly with molecular weight, viz. $D_{\mathrm{s}}$ (PEI $M_{\mathrm{w}}$ = 2000 g mol$^{-1}$) = $1.6 \times 10^{-11}$ m$^2$ s$^{-1}$, whereas $D_{\mathrm{s}}$ (PEI $M_{\mathrm{w}}$ = 25 000 g mol$^{-1}$) = $2.6 \times 10^{-12}$ m$^2$ s$^{-1}$. Hence, from this simple comparison $z \propto M_{\mathrm{w}}^{+1}$ and $D_{\mathrm{s}} \propto M_{\mathrm{w}}^{-1}$ so the product $zD_{\mathrm{s}}$ ($\cong \mu$) scales as $M_{\mathrm{w}}^{0}$, as shown in Figure 1.

As a simple comparison, the eNMR and PGSE-NMR studies on PSS[13] show that for a polymer of molecular weight 77 000 g mol$^{-1}$ the electrophoretic mobility of the polymer increases somewhat on addition of salt but then decreases to a value slightly smaller than the no-salt case. The self-diffusion coefficient simply increases—because the hydrodynamic radius decreases—over the same ionic strength region. Accordingly, the charge on the polymer goes from ~36$e$ to ~20$e$ at the higher ionic strength considered, 0.04 mol L$^{-1}$. The measured electrophoretic mobility of the PSS,[13] $\mu = 2.2 \times 10^{-8}$ m$^2$ V$^1$ s$^{-1}$, calculated from eq 4 corresponds to an effective charge of 45$e$, in excellent agreement with the charge (40$e$) calculated using the electrophoretic friction constant derived from Manning's model.[32] Interestingly, this is approximately 15% of the total number ionizable groups, based on the molecular weight. Further, this simple picture highlights the rather complex interplay between the molecular weight dependencies of the electrophoretic mobility and the self-diffusion coefficient (or friction factor)—the charge on a homopolymer polyelectrolyte at a given degree of protonation will always scale linearly with molecular weight; the self-diffusion coefficient will reflect the friction vs molecular weight scaling behavior, the latter being sensitive to the solution conformation (and inter alia the degree of charging).

In Figure 3, the (fractional) charge on the polymer calculated from the pH titration is compared with the normalized fractional charge calculated from the electrophoretic NMR results. For the latter, the electrophoretic mobility at each pH has been normalized by the average of the mobilities for each polymer over the range pH < 6, i.e., over that region of pH where the electrophoretic mobility is constant at its maximum value. Clearly, there is a significant difference between the magnitudes of the degree of protonation extracted from the two approaches—a similar observation was made by Lindquist and Stratton.[28] One possible reason for this could be the effects of ionic strength—the polymer concentration was constant in the electrophoretic NMR study compared to the pH titration, where the polymer is significantly diluted. Accordingly, the ionic strength will be different between the two cases and, significantly so, over the intermediate pH range. It is known that the degree of protonation increases significantly with ionic strength.[15,31] Alternatively, if one uses Manning's model of counterion condensation to calculate an electrophoretic friction factor, strictly only valid for linear polymers[5,33] and hence the charge on the polymer, the degree of protonation calculated from the electrophoretic mobility measurements is further reduced by a factor of approximately 2−3, and the agreement between the two approaches improves. An analogous conclusion was reached in the PSS study,[13] but further work is required to understand the differences between the conclusions reached by these two approaches.

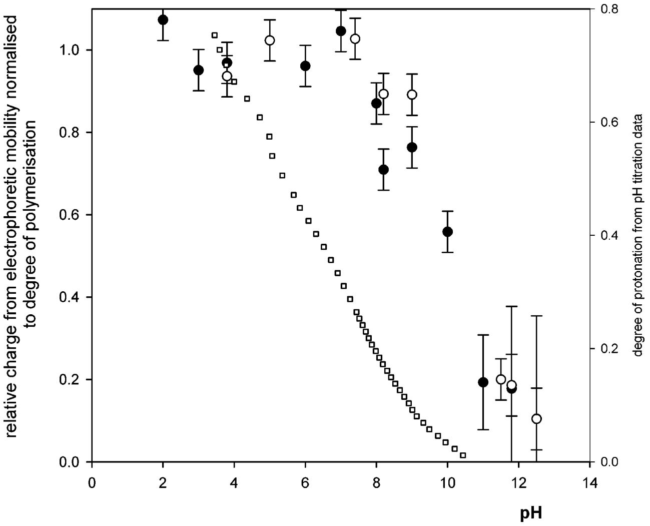

**Figure 3.** Charge from electrophoretic mobility measurements normalized to the molecular weight for the two samples of branched PEI $M_{\mathrm{w}}$ = 2000 g/mol (closed symbols) and $M_{\mathrm{w}}$ = 25 000 g/mol (open symbols) as a function of pH (left-hand axis). The open squares are the comparable parameter calculated from the $M_{\mathrm{w}}$ = 2000 g mol$^{-1}$ pH titration data presented in Figure 1 (right-hand axis).

## Conclusions

The electrophoretic mobility of two samples of PEI has been measured by electrophoretic NMR. The effective charge on the polymer controlled via the solution pH can easily be quantified and is shown to be independent of molecular weight due to the opposite molecular weight dependencies of the charge (~$M^{+1}$ and the friction opposing the flow of the polymer ~$M^{-1}$). This approach is probably only valid for small branched polymers as with increasing molecular weight the distribution of polymer segments will become more Gaussian, and the linear scaling of friction with molecular weight will break down. The experiment further confirms the potential of electrophoretic NMR to the study of charging phenomena in complex multicomponent systems.

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