

## Deuterium Isotope Effects on the Interaction between Hyperbranched Polyethylene Imine and an Anionic Surfactant

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Solvent isotope effects on the interaction between the hyperbranched cationic polyelectrolyte, polyethylene imine (PEI), and the anionic surfactant sodium dodecyl sulfate (SDS) were investigated using potentiometric titration and electrophoretic mobility measurements. In the basic pH range, a significantly higher fraction of the amine groups was found to be protonated when the PEI was dissolved in D<sub>2</sub>O compared to H<sub>2</sub>O at the same pH/pD. The difference in polymer charge in the two solvents decreases gradually with decreasing pH, and it completely diminishes at around pH = 4. Electrophoretic mobility measurements of PEI/SDS complexes at different pH values correlated very well with these observations. At pH/pD  $\approx$  9 a much higher mobility of the PEI/SDS complexes was found in D<sub>2</sub>O than in H<sub>2</sub>O at low surfactant concentrations, and the charge neutralization point shifted to a considerably larger surfactant concentration in heavy water. These results can be explained by the significantly higher charge density of the PEI in D<sub>2</sub>O compared to H<sub>2</sub>O. However, at the natural pH/pD as well as at pH = 4 and pD = 4 conditions the PEI molecules have roughly equal charge densities, which result in very similar charged characteristics (mobilities) of the PEI/SDS complexes as well as the same charge neutralization SDS concentration. It can be concluded that extreme care must be taken in the general analysis of those experiments in which weak polyelectrolyte/surfactant aggregates are investigated in heavy water, and then these observations are correlated with structures of the same system in water.

### Introduction

The necessity of using heavy water (D<sub>2</sub>O) for small-angle neutron scattering, NMR, and other experimental techniques has been very well documented.<sup>1–6</sup> In many cases it is assumed that the systems studied in D<sub>2</sub>O maintain the same properties that they have in H<sub>2</sub>O. However, as is well known, the physicochemical properties of water and heavy water differ to a certain extent. D<sub>2</sub>O is more structured (i.e., it forms stronger hydrogen bonds than H<sub>2</sub>O), which results in a higher density, viscosity, boiling point, and lower solubility of inorganic salts.<sup>7</sup>

Kinetic isotope effects in organic reactions are very well known and have been studied extensively.<sup>8</sup> The use of deuterium has been reported to have an effect in biological systems, as well as in superconductivity.<sup>7</sup>

Isotopic effects have also been observed on the formation of surfactant self-assembly aggregates,<sup>9</sup> where the substitution of H<sub>2</sub>O for D<sub>2</sub>O in *n*-nonyl- $\beta$ -D-glucoside (C<sub>9</sub>G<sub>1</sub>) solutions induced an increment in the micellar size. The difference between the solvents was diminished by increasing the temperature, and it was attributed to the shorter length of the O–D bond compared to the O–H bond making the surfactant headgroup size smaller in D<sub>2</sub>O as compared to H<sub>2</sub>O, allowing more surfactant molecules to pack in the micelles.

A similar explanation was suggested for the large deuterium isotope effects reported to affect the phase diagram of mixtures

of two alkylglucoside surfactants (C<sub>9</sub>G<sub>1</sub> and *n*-decyl- $\beta$ -D-glucoside, C<sub>10</sub>G<sub>1</sub>).<sup>10</sup> For this system, considerable shifts in both critical points and phase boundaries were detected when H<sub>2</sub>O was replaced by D<sub>2</sub>O.

In another study, when investigating the synergistic effects in mixtures of SDS and C<sub>10</sub>G<sub>1</sub>, using small-angle neutron and light scattering,<sup>11</sup> a very large solvent isotopic effect was detected. In these mixtures in which the nonionic surfactant was present in excess ([SDS/C<sub>10</sub>G<sub>1</sub>] = 1:3) and at moderate electrolyte concentrations (NaCl = 0.1 M), the micelles formed in water were a factor of 5 smaller than the micelles formed in the corresponding samples in heavy water. The reason for this difference in micellar size was attributed to a lower solubility of the C<sub>10</sub>G<sub>1</sub> in D<sub>2</sub>O compared to that in H<sub>2</sub>O, which enhanced the incorporation of the nonionic surfactants to the micelles even at very low surfactant concentrations.

Solvent isotopic effects are not exclusive of surfactant systems. Polymeric systems can also be affected by isotopic changes in the solvent. In a study on poly(ethylene oxide)–water systems, the deuteration of both the solvent and the polymer backbone was varied.<sup>12</sup> Small-angle neutron scattering measurements showed more clustering on deuterated PEO (dPEO) samples in D<sub>2</sub>O compared to the same sample in H<sub>2</sub>O. The clusters were suggested to be due to H bonds formed through the CH<sub>2</sub>CH<sub>2</sub>O $\cdots$ HOH $\cdots$ OCH<sub>2</sub>CH<sub>2</sub> interactions. These results thus indicated a stronger deuterium bonding compared to hydrogen bonding. Moreover, deuteration of the polymer backbone resulted in stronger hydrophobic interactions compared to the protonated polymers. This observation was based on the fact that the strongest cluster formation was obtained

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for dPEO/D<sub>2</sub>O, then for hPEO/D<sub>2</sub>O, then for dPEO/H<sub>2</sub>O, and finally for hPEO/H<sub>2</sub>O.

Solvent isotope effects have also been detected in the swelling kinetics and volume phase transition of polymer hydrogels of poly(*N*-isopropylacrylamide) (PNIPAM) and polyacrylamide (PAAM).<sup>13</sup> The slower swelling kinetics of these hydrogels in D<sub>2</sub>O compared to that in H<sub>2</sub>O was attributed to the higher viscosity of the former solvent. In the same report, the phase separation temperature of PAAM and poly(acrylic acid) (PAA) complexes in H<sub>2</sub>O and D<sub>2</sub>O solutions was studied. The complexes prepared in D<sub>2</sub>O presented a phase separation temperature of about 16 K higher than that in H<sub>2</sub>O. In this case, the deuterium isotope effect on the polymer–solvent interactions was considered to be small compared to the isotope effect on the polymer–polymer interactions.

There are other polymeric systems that are very sensitive to solvent composition; among them we can find the weakly charged polyelectrolytes such as polyethylene imine (PEI) and polyvinylamine. In the case of PEI, a lot of effort has been made in order to understand the unusual characteristics of the system in aqueous solution, at different electrolyte concentrations, pH values, and in the presence of surfactants, as well as the effects these parameters may have on the PEI structure.<sup>14–18</sup>

The interactions between PEI and SDS have been investigated recently in an aqueous medium by Meszaros et al.<sup>17</sup> at the high pH range. In this study the authors observed three characteristic concentration ranges. With increasing SDS concentration, the DS<sup>−</sup> ions first bind to the protonated amine groups of the PEI molecules. Because of the binding of the surfactant anions, the PEI–DS<sup>−</sup> complex becomes more hydrophobic and the excess positive charge density decreases. This results in shrinkage of the complex (concentration range A). The collapse of the molecules leads to increasing dispersion forces acting between them. When these attractive forces become large enough, the PEI/SDS system coagulates because the electric potential of the particles is too small to stabilize the dispersion (concentration range B). The surfactant ions bound to the protonated groups in the surface layer give the particle surface a hydrophobic character. A further increase in the surfactant concentration results in adsorption of SDS by hydrophobic interactions in a second binding process (concentration range C), causing charge reversal of the PEI/SDS complex. At low ionic strength, the excess negative charge of the particles may result in an electrostatically stabilized colloid dispersion of PEI/SDS nanoparticles. In this concentration range, the polymer/surfactant nanoparticles can be coagulated via the addition of supporting electrolytes. Small-angle neutron scattering and static light scattering measurements (in D<sub>2</sub>O) have also been employed in order to characterize the structures formed under these conditions and determine how the structures evolve with increasing SDS concentrations at different pH values.<sup>14</sup>

Surprisingly, very little attention has been given to the effect that an isotopic change of the solvent may have on PEI. This study incorporated the use of potentiometric titration and electrophoretic mobility measurements in order to investigate the effects that an isotope solvent change has on PEI molecules and its association with an oppositely charged surfactant.

## Experimental Section

**Materials.** HCl, carbonate free NaOH, NaCl, DCl, and NaOD were obtained from Sigma-Aldrich. The concentration of the HCl stock solution was specified by the supplier, but it was checked via standard titration of HCl with KHCO<sub>3</sub> in the presence of a methyl-orange indicator.<sup>19</sup> The concentration of

DCl was determined by an argentometric method.<sup>19</sup> The concentration of NaOH (NaOD) was determined from its potentiometric titration with HCl (DCl) in water (heavy water), respectively. The aqueous solutions were prepared from double distilled water, whereas the solutions in heavy water were prepared from D<sub>2</sub>O having 99.96% isotopic purity and provided by Sigma-Aldrich. The solutions were prepared and stored under N<sub>2</sub> atmosphere in a glovebag provided by Sigma-Aldrich.

Polyethylene imine ( $M_w = 3.3 \times 10^6$  g/mol as determined by static light scattering) was provided by BASF as an aqueous solution of 50 wt %. Sodium dodecyl sulfate, SDS, (99.9%) was obtained from Sigma-Aldrich and used as received.

**pH Measurements.** The charge density of PEI was determined via its potentiometric titration with NaOH (NaOD) and HCl (DCl), respectively. All of the titrations were performed under N<sub>2</sub> atmosphere. The concentration of PEI ( $c_{\text{PEI}} = 500$  mg/dm<sup>3</sup>), the concentration of the inert electrolyte (0.1 M NaCl), and the temperature ( $T = 25$  °C) were kept constant during the titration. The pH was measured by means of a combined pH-glass electrode and a research pH-meter (Radelkis). The inner reference solution was 1 M KCl in water for the measurements in aqueous medium, whereas a 1 M KCl in D<sub>2</sub>O was used for the solutions made from heavy water. In both cases, the electrodes were conditioned in the same solutions for 24 h.

The measured EMF (electromotive force) values of the galvanic cell are given as

$$\text{EMF} = \epsilon_o + \frac{kT}{e} \ln(\gamma_{\text{H}^+} c_{\text{H}^+}) - \epsilon_{\text{Ag/AgCl}} - \epsilon_{\text{diff}} \text{ (in water) and}$$

$$\text{EMF} = \epsilon_o + \frac{kT}{e} \ln(\gamma_{\text{D}^+} c_{\text{D}^+}) - \epsilon_{\text{Ag/AgCl}} - \epsilon_{\text{diff}} \text{ (in heavy water)}$$

where  $\epsilon_o$  is the standard electrode potential of the glass electrode,  $\epsilon_{\text{Ag/AgCl}}$  is the electrode potential of the Ag/AgCl reference electrode, and  $\epsilon_{\text{diff}}$  is the diffusion potential of the ions in the liquid junction;  $k$  denotes the Boltzman constant,  $T$  is the absolute temperature, and  $e$  is the elementary charge.  $\gamma_{\text{H}^+}$  and  $c_{\text{H}^+}$  as well as  $\gamma_{\text{D}^+}$  and  $c_{\text{D}^+}$  denote the activity coefficient and the concentration of hydrogen ions and deuterium ions, respectively.

In the case of ordinary electrolyte solutions, the diffusion potential in the liquid junction is negligible ( $\epsilon_{\text{diff}} \approx 0$ ) assuming that the salt bridge contains similar mobility cations and anions. However, if macroions are present in the solution (e.g., polyelectrolyte molecules) then the diffusion potential is anomalous<sup>20</sup> and its value could be as high as 100 mV. The anomalous diffusion potential diminishes in the presence of a sufficient amount of inert electrolyte, and for this reason the pH measurements were carried out in the presence of 0.1 M NaCl.

The EMF of the glass electrode, measured in an aqueous solution, is usually converted to a pH value by means of suitable buffer solutions with accurately known hydrogen ion activity. The EMF values measured in heavy water could be converted to pD values by means of a similar calibrating procedure assuming the usage of buffer solutions prepared in D<sub>2</sub>O from deuterated acids (bases). Because appropriate buffer solutions in heavy water are not straightforward to obtain, the pH meters are usually calibrated in aqueous buffer solutions and used immediately after this calibration for the measurements in heavy water.

In these cases, an empirical formula has been used for a long time, based on the classical work of Mikkelsen et al. and Glasoe

et al.<sup>21,22</sup> to convert the pH meter reading ( $\text{pH}^*$ ) into a pD value

$$\text{pD} = \text{pH}^* + 0.44 \quad (1)$$

Krezel et al. have investigated very recently the relationship of pK values of various acids and bases in heavy and light water, respectively.<sup>23</sup> In their paper, the following expression was derived in 0.1 M  $\text{KNO}_3$ .

$$\text{pH} = 0.9291\text{pH}^* + 0.421 \quad (2)$$

(or equivalently  $R_{\text{pH}} = 0.9291R_{\text{pH}^*} + 0.421$  using the notation of Krezel et al.<sup>23</sup>), where  $R_{\text{pH}^*}$  ( $\text{pH}^*$ ) is the pH meter reading of an acid/base solution in  $\text{D}_2\text{O}$  with a  $\text{H}_2\text{O}$  calibrated pH-meter,  $R_{\text{pH}}$  is the pH meter reading of the same amount of acid/base in water. Using the ionic products of water and heavy water, we can give the theoretical relationship between the pH and pD of the same amount of an acid/base in  $\text{D}_2\text{O}$  and in  $\text{H}_2\text{O}$ , respectively, (eq 4 in ref 23) in 0.1 M  $\text{KNO}_3$  as

$$\text{pD} = 1.076\text{pH} \quad (3)$$

The combination of eqs 2 and 3 gives  $\text{pD} = 0.9997\text{pH}^* + 0.452$ , which is in very good agreement with the classical formula of  $\text{pD} = \text{pH}^* + 0.44$ .

To avoid any ambiguity, the EMF versus pD and the EMF versus pH functions of the electrode were determined experimentally with the potentiometric titration of the medium (0.1 M NaCl in  $\text{D}_2\text{O}$  or  $\text{H}_2\text{O}$ ) with a strong monovalent acid ( $\text{DCl}/\text{HCl}$ ) as well as with a base ( $\text{NaOD}/\text{NaOH}$ ) of precisely known concentration. Then the  $\text{H}^+$  or  $\text{D}^+$  activity of the polyelectrolyte solutions were determined from the measured EMF values by means of these calibration curves. It should be noted that this calibration method proved to be in excellent agreement with the standard calibration method of pH meters using aqueous buffer solutions and eq 1.

#### Determination of $\text{H}^+$ and $\text{OH}^-$ Concentration from the Measured Activities.

To determine the concentration of the polymer bound  $\text{H}^+$  ( $\text{D}^+$ ) or  $\text{OH}^-$  ( $\text{OD}^-$ ) ions, we had to convert the electrochemically measured activities to the volume averaged (equilibrium) concentration of the investigated ions, which means that their activity coefficients should be known

$$a_{\text{H}^+} = \gamma_{\text{H}^+} [\text{H}^+] \quad (4)$$

where  $[\text{H}^+]$  denotes the equilibrium concentration of  $\text{H}^+$  ions and  $\gamma_{\text{H}^+}$  is its activity coefficient. In the case of dilute solutions of simple 1:1 electrolytes, the activity coefficient can be determined by means of the Debye–Huckel theory. However, in the presence of macroions (polyelectrolytes) the distribution of small ions is influenced strongly in the solution. The ions having a charge similar to that of the macroions are expelled from their close vicinity, whereas the ions having an opposite charge are accumulated around them. It can be shown<sup>24,25</sup> that in the presence of macroions the volume averaged concentration of the small ions (e.g.,  $\text{H}^+$ ) can be expressed in the following form (by neglecting the small ion interactions)

$$[\text{H}^+] = a_{\text{H}^+} \langle e^y \rangle \quad (5)$$

where  $y = e\phi/kT$  is the dimensionless electric potential and the brackets denote volume averaging. The factor  $\langle e^y \rangle$  takes into account that in the presence of macroions (polyelectrolytes) the distribution of small ions is not uniform in the solution. This factor can become important in low ionic strength solutions where it must be determined from independent measure-

ments.<sup>24,25</sup> In our case, the measurements were done in the presence of a large amount of inert electrolyte (0.1 M NaCl); thus, because of the high ionic strength the  $\langle e^y \rangle$  factor becomes practically equal to one.

**Electrophoretic Mobility Measurements.** A Zetasizer 2000 from Malvern Instruments, England was used to measure the electrophoretic mobility of the PEI/SDS aggregates at different pH values in different solvents, by means of a standard zeta potential capillary cell. The calibration of the instrument was done using Malvern Zeta Potential Transfer Standard (code MRK403-02) prior to use. All of the measurements were performed at  $T = 25^\circ\text{C}$ .

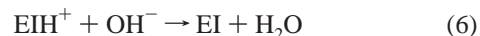
Stock solutions of PEI at 1%, both in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$ , were prepared 12 h before the measurements and diluted to the desired concentration prior to the experiments. No salt was added. The water used for these measurements was obtained from a Millipore RiOs-8 and Milli-Q PLUS 185 purification system and finally filtered through a 0.2- $\mu\text{m}$  Millipak filter.

For these measurements, the samples were studied at three different pH/pD values. The “natural pH” (i.e., the pH/pD of the samples was not adjusted), where the pH/pD values of the pure PEI samples (0.05%) were 10.1 and 10.9, respectively, was chosen as one of the sample conditions. The samples were also analyzed at pH/pD just below 9 and at pH/pD = 4. The pH/pD of the polymer stock solutions was adjusted using HCl or DCl depending on the solvent. When investigating the samples dissolved in  $\text{D}_2\text{O}$ , 5 mL of that solvent was injected into the instrument prior to injecting each sample.

## Results and Discussion

The most important characteristic of a polyelectrolyte is its charge density. In the case of weak polyelectrolytes, the polymer molecules acquire their charge by the protonation/dissociation of their basic and acidic groups. It is well known from the literature that weak acids and bases show a considerable isotopic effect. Thus, it can be expected that the charge density and consequently the physical properties of the weak polyelectrolyte solutions will be influenced by the solvent ( $\text{H}_2\text{O}$  or  $\text{D}_2\text{O}$ ). Poly(ethylene imine) contains primary, secondary, and tertiary amine groups, whose protonation is affected strongly by the pH of the solution.<sup>17</sup> As a consequence, it is an ideal candidate for investigating the effect of the solvent ( $\text{H}_2\text{O}$  or  $\text{D}_2\text{O}$ ) on the characteristics of its aqueous solutions.

**Characterization of the Charge Density of Poly(ethylene Imine) in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$ .** To characterize the effect of isotopic substitution on the protonation equilibrium of PEI, the fraction of the protonated/deuterated amine groups,  $x_{\text{EIH}^+}/x_{\text{EID}^+}$  as a function of the pH/pD value in water and heavy water was determined, respectively. The charge of the PEI at and above the natural pH/pD values of the solutions was determined by titration with NaOH/NaOD



where EI and  $\text{EIH}^+$  denote the unprotonated and the protonated amine groups of PEI, respectively. The concentration of the  $\text{OH}^-$  ions neutralized by the polymer ( $\text{B}_{\text{OH}^-}(\text{pH})$ ), at a given pH, can be calculated as

$$\text{B}_{\text{OH}^-}(\text{pH}) = c_{\text{OH}^-} - ([\text{OH}^-] - [\text{OH}^-]_0) \quad (7)$$

where  $c_{\text{OH}^-}$  and  $[\text{OH}^-]$  are the total added concentration and the equilibrium concentration of the  $\text{OH}^-$  ions, respectively, and  $[\text{OH}^-]_0$  is the concentration of  $\text{OH}^-$  ions at the natural pH



of PEI. The charge density of the PEI at its natural pH ( $[EIH^+]_{pH_0}$ ) can be determined by measuring the amount of  $OH^-$  ions required to completely neutralize the PEI. Practically, this means the determination of the limiting value of the concentration of the  $OH^-$  ions neutralized by the polymer ( $B_{OH^-}(pH \rightarrow \infty)$ ). This limiting value was reached when the pH/pD exceeded 11/12.

The fraction of the protonated amine groups above the natural pH/pD value can be expressed as

$$x_{EIH^+} = \frac{[EIH^+]}{[EI]_{tot}} = \frac{[EIH^+]_{pH_0} - B_{OH^-}(pH)}{[EI]_{tot}} \quad (8)$$

where  $[EI]_{tot}$  is the total concentration of amine groups and  $[EIH^+]$  is the equilibrium concentration of the protonated amine groups of the PEI.

The PEI charge below the natural pH/pD was determined by titration with HCl/DCI



The fraction of the protonated amine groups below the natural pH/pD value can be given as

$$x_{EIH^+} = \frac{[EIH^+]_{pH_0} + B_{H^+}(pH)}{[EI]_{tot}} \quad (10)$$

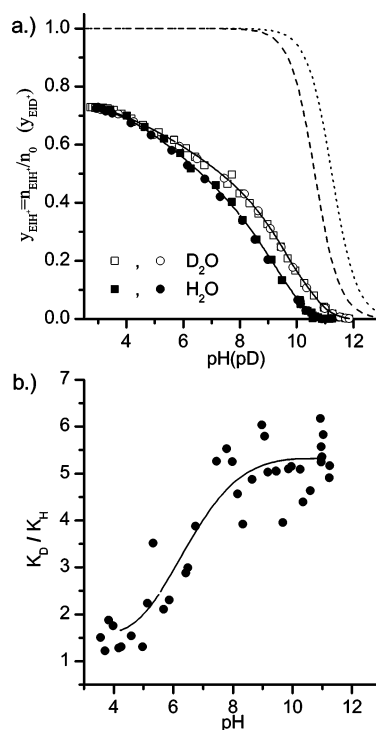
where  $B_{H^+}(pH)$  is the concentration of "bound"  $H^+$  ions taking part in the protonation of PEI

$$B_{H^+}(pH) = c_{H^+} - ([H^+] - [H^+]_0) - ([OH^-]_0 - [OH^-]) \quad (11)$$

where  $c_{H^+}$  and  $[H^+]$  are the total added concentration and the equilibrium concentration of the  $H^+$  ions, respectively, and  $[H^+]_0$  is the concentration of  $H^+$  ions at the natural pH of PEI. The last term of the above equation takes into account that between pH = 7 and the natural pH of PEI a considerable portion of the added  $H^+$  ions takes part in the neutralization of the  $OH^-$  ions present in the PEI solution.

The fraction of protonated amine groups in the PEI solution is plotted in Figure 1a as a function of pH/pD. As a reference, the fraction of protonated methylamine in  $H_2O$  and  $D_2O$  is also plotted in the figure using  $pK_{b,H}(\text{MeNH}_2) = 3.36$  and  $K_{b,D}/K_{b,H} = 3.26$  in the calculation (where  $K_{b,H}$  and  $K_{b,D}$  denote the base dissociation constants of methylamine in  $H_2O$  and  $D_2O$ , respectively). The figure clearly demonstrates the existence of an isotope effect in the protonation equilibrium of PEI. At a given  $H^+/D^+$  activity, the PEI has a larger charge density in  $D_2O$  than in  $H_2O$ , which is in accordance with the general observation that acids ( $EIH^+$  in this case) are less acidic in  $D_2O$  than in water.<sup>22,23,25</sup> This effect is most pronounced from pH (pD)  $\approx 7$  to the natural pH of the PEI solution but diminishes both at high and low pH values. It is easy to show theoretically that with increasing pH, the ratio of  $x_{EID^+}$  and  $x_{EIH^+}$  tends to a constant value, which for  $\text{MeNH}_2$  should equal  $K_{b,D}/K_{b,H}$ . However, as the pH increases the PEI molecules become deprotonated, and the theoretically predicted difference of the polymer charge becomes unobservable experimentally.

At low pH values, the difference between the fractions of the charged amine groups in the two solvents also diminishes. Theoretically, it can be predicted that in the case of a monovalent weak base the ratio of  $x_{EID^+}$  and  $x_{EIH^+}$  tends to 1 as the pH



**Figure 1.** (a) The fraction of charged (protonated or deuterated) amine groups of PEI in normal and heavy water as a function of the pH/pD (the circles and squares denote parallel measurements). The lines are guides for the eyes. The dashed and the dotted lines indicate the fraction of protonated (deuterated) methylamine in  $H_2O$  and  $D_2O$ , respectively. (b) The ratio of the dissociation constants calculated in  $H_2O$  and  $D_2O$  determined by eq 12.

decreases because of the fact that all of the amine groups become protonated. However, as it is indicated in Figure 1a, the charge difference of the PEI diminishes in the two solvents as the fraction of the charged groups tends to 0.75 instead of 1. This observation can be interpreted in terms of the PEI structure. It is well established that the hyperbranched PEI contains primary, secondary, and tertiary amine groups in the ratio of 1:2:1.<sup>14,16,17</sup> Because the least basic amine groups are the tertiary ones, their protonation can be expected at the end. Furthermore, if the primary and secondary amine groups are protonated in the PEI, then the remaining tertiary amine groups have three charged neighbors in the  $\gamma$  position exerting considerable negative inductive effects on the nitrogen of the tertiary amine groups, thus substantially reducing their basic character.

A similar interpretation was given for the results of early titration studies in aqueous solutions of PEI at different electrolyte concentrations.<sup>16</sup> In this study, the least basic nature of the tertiary amine groups was confirmed, for which the  $pK_{b,H} = 4.1$  (at 25 °C), whereas for primary and secondary amine groups the  $pK_{b,H}$  values are 3.25 and 3.19, respectively.

To determine the characteristic pH range of protonation for the tertiary amine groups, a  $c_{PEI} = 2\%$  solution was also titrated with a 1 M HCl solution. However, further protonation of the PEI could not be observed up to  $pH \approx 0.2$ , which is consistent with previous experimental results.<sup>16</sup>

For the characterization of the magnitude of an equilibrium isotope effect for a weak base, the ratio of the intrinsic base dissociation constants measured in  $D_2O$  and  $H_2O$  can be used (for a more detailed description of the dissociation behavior of polyelectrolytes, see, e.g., ref 26). It is straightforward to show that the ratio of the equilibrium constants can be determined from the shift of the titration curves at equal protonation/deuteration degree of the PEI in the two solvents ( $x_{EIH^+} = x_{EID^+}$ )-

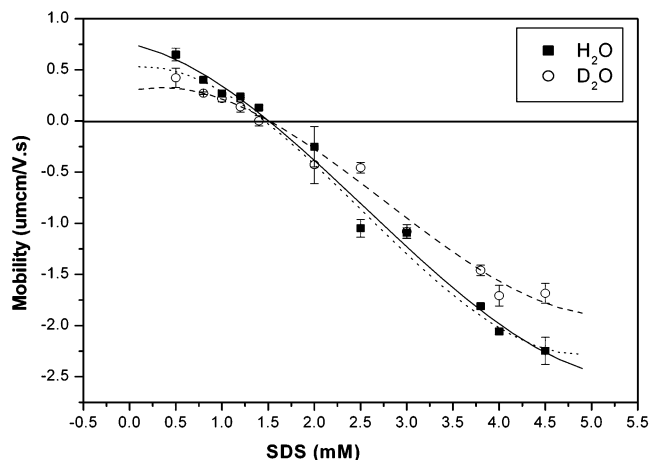
The ratio of the equilibrium constants calculated from the

$$(\text{pD} - \text{pH})_{x_{\text{EID}}^+} = x_{\text{EIH}^+} = \log(K_{\text{D}}/K_{\text{H}}) \quad (12)$$

experimental titration curves is plotted in Figure 1b. As indicated by the figure, a large constant isotope effect ( $K_{\text{b,D}}/K_{\text{b,H}} \approx 5$ ) can be observed if the pH exceeds  $\sim 7$ . However, below  $\text{pH} = 7$  the ratio of the equilibrium constants decreases significantly. This observation can be interpreted in terms of the change in the electronic structure of the polyelectrolyte molecules. Simple statistical physical models derived for small molecules indicate that the equilibrium isotope effect is determined primarily by the difference of zero-point vibration energies between the hydrogen and deuterium substituted forms of the molecule.<sup>27</sup> The models predict that the larger the force constant of the vibrations, in which isotope substituted bonds are involved, the larger the observed isotope effect. Because usually the stronger the bond involved in the vibration the larger the force constant, it can be expected that the decrease of the isotope effect reflects the weakening of the bond involved in the isotope substitution. Assuming that this conclusion holds for macromolecules, the observed pH dependence of the isotope effect can be interpreted in terms of the properties of the  $\text{NH}^+$  bonds. As indicated in Figure 1b, above  $\text{pH} \approx 7$  the magnitude of the isotope effect is not affected considerably by the pH. In this pH range, as has been mentioned, less than 50% of the amine groups are protonated, which means that their basic character (the strength of the  $\text{N}-\text{H}^+$  bond) is only influenced slightly by the rest of the molecule. That is not the case at low pH, where the weakening of the basic character of the amine groups (the weakening of the  $\text{N}-\text{H}^+$  bond) due to the negative inductive effects on the nitrogen of the tertiary amine groups is reflected by the decrease of the isotope effect.

**Polyethylene Imine/SDS Complex Formation in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$ .** Because several physicochemical properties of the polyelectrolytes are determined by their overall charge, the effect of isotope substitution on the protonation equilibrium of PEI can have a pronounced effect on its properties. To illustrate this effect, we investigated the interaction between PEI and the anionic surfactant SDS in both solvents at three different pH/pD values.

It should be noted that the electrophoretic mobility measurements were performed without added salt (because of the disturbing effect of the supporting electrolyte on the stability of the PEI/SDS complexes as well as the diminishing mobility values at high ionic strengths), whereas the potentiometric titrations of PEI were done in 0.1 M NaCl. The charge density of the PEI is affected by the ionic strength of the solution.<sup>16,17</sup> In general, it can be expected that the larger the ionic strength, the larger the charge density of the PEI. However, the effect of ionic strength diminishes at both high and low pH values, while at intermediate pH values the effect of ionic strength may be considerable. Nevertheless, the relevant parameter is not the absolute charge density of the PEI but its relative charge in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  compared to each other. If the PEI has similar charge densities in the two solvents at a given pH and ionic strength, then it can be expected that a change of the ionic strength shifts the polymer charge densities in a similar manner. This means that the change of the mentioned charge ratio in the two solvents initiated by the change of the ionic strength is a second order (small) effect. (It should be noted that the expression “similar charge density” means here that the difference in the charge densities is small compared to the amount of unprotonated amine groups. This assumption is fulfilled in the entire pH range, see Figure 1.) On the basis of this reasoning,



**Figure 2.** Electrophoretic mobility of PEI/SDS aggregates as a function of SDS concentration, in two different solvents, as indicated on the legend on the right-hand side of the figure. The measurements were done at the natural pH of the samples, and the PEI concentration was 0.05%. The points shown are average values of five measurements, whereas the solid and dashed lines are guides for the eyes. The dotted line illustrates the viscosity corrected mobility data obtained for the samples in  $\text{D}_2\text{O}$ .

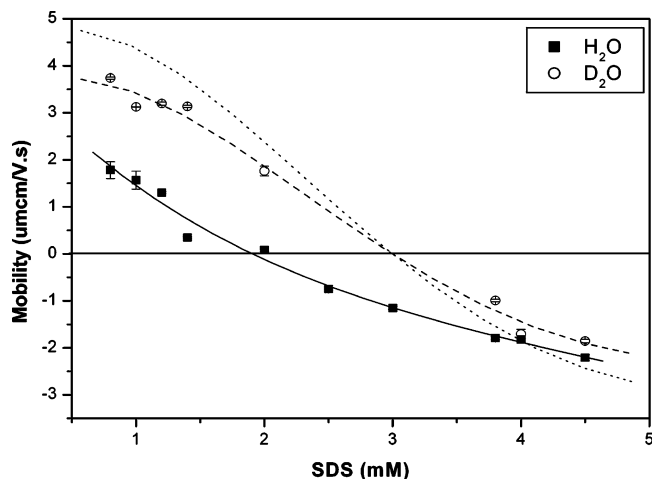
it is straightforward to assume that similar trends in the relative charge of PEI in the two solvents may be predicted with and without the presence of NaCl.

**Samples at Natural pH.** The PEI/SDS solutions were clear in both solvents at all of the surfactant concentrations studied. First, the pH values of pure polyelectrolyte samples were measured. In the case of the samples prepared in water, the pH of the PEI solution at a concentration of 1% was found to be 11.0. When this solution was diluted to 0.05%, the pH value decreased to 10.1. However, the pD of the 1% polymer solution prepared in  $\text{D}_2\text{O}$  was 11.8. When it was diluted to a polymer concentration of 0.05%, the pD value decreased to 10.9. These data indicate that the PEI samples prepared in  $\text{D}_2\text{O}$  have pD values around 0.8–0.9 units higher than the pH values of the polymer solutions prepared in water; this demonstrates that the PEI is a stronger base in  $\text{D}_2\text{O}$  than in  $\text{H}_2\text{O}$ , which is in agreement with our titration data.

The electrophoretic mobility data for PEI/SDS samples in both water and heavy water are presented in Figure 2 at their natural pH/pD. Clearly, the mobility values of the PEI molecules in the two solvents (without SDS) are very similar. Because the charge of the PEI increases with decreasing solution pH/pD<sup>14,17,28</sup> the observed roughly equal electrophoretic mobility values of the PEI at pD = 10.9 in  $\text{D}_2\text{O}$  and at pH = 10.1 in  $\text{H}_2\text{O}$  seem to indicate that at the high pH range the charge density of PEI molecules is higher in  $\text{D}_2\text{O}$  than in  $\text{H}_2\text{O}$  at the same hydrogen/deuterium ion activity. However, when the data in Figure 1 is considered, it is evident that the charge density of PEI in  $\text{D}_2\text{O}$  at pD = 10.9 is low and very similar to the charge density of PEI in  $\text{H}_2\text{O}$  at pH = 10.1.

The two mobility curves are also similar to each other in the presence of SDS. The most important feature is that the PEI/SDS complex exhibits zero mobility (charge neutralization) at the same SDS concentration (around 1.5 mM) in both solvents. It is also clearly seen that the mobility of the complexes is slightly smaller in  $\text{D}_2\text{O}$  than in water. The higher the mobility (the larger the charge density of the polymer/surfactant complex), the more significant the difference in the mobility becomes.

The observed smaller electrophoretic mobility values in  $\text{D}_2\text{O}$  can be attributable to the approximately 20% larger viscosity

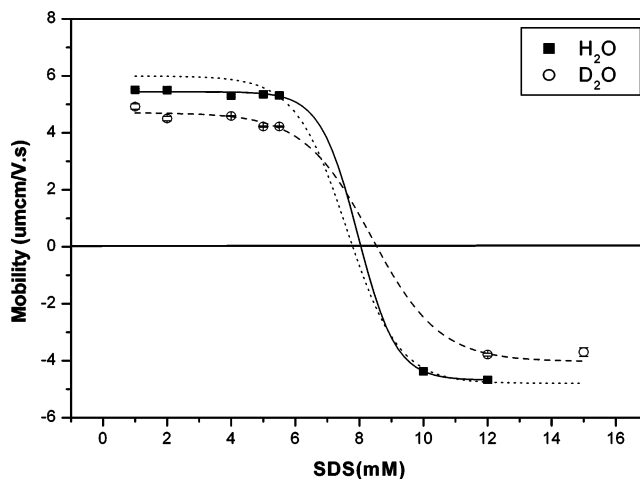


**Figure 3.** Electrophoretic mobility of PEI/SDS aggregates as a function of SDS concentration in two different solvents, as indicated on the legend on the right-hand side of the figure. The measurements were done at pH = 8.8 and pD = 8.5. The PEI concentration was 0.05%. No data are shown for samples in which precipitate had formed. The points shown are average values of five measurements, whereas the solid and dashed lines are guides for the eyes. The dotted line illustrates the viscosity corrected mobility data obtained for the samples in D<sub>2</sub>O.

value of heavy water compared to that of H<sub>2</sub>O.<sup>29</sup> In fact, if we assume that the structure of the complexes formed is the same in the two solvents, the effect of the viscosity can be accounted for. The dotted line in Figure 2 illustrates the viscosity corrected mobility data obtained for the samples in D<sub>2</sub>O. Clearly, the differences between the samples prepared in H<sub>2</sub>O and D<sub>2</sub>O are minimal. These results correlate well with the results of the titrations suggesting that PEI molecules have approximately equal, low charge density at the natural pH/pD. Assuming that the surfactant binding is dependent primarily on the PEI charge density in both solvents, similar charge density of the PEI/SDS nanocomplexes can be expected as a function of the surfactant concentration in both H<sub>2</sub>O and D<sub>2</sub>O.

**Samples at pH Just Below pH/pD = 9.** The pH/pD of the PEI solutions (1%) were adjusted to 9.7 in both H<sub>2</sub>O and D<sub>2</sub>O. When these solutions were diluted to a polyelectrolyte concentration of 0.05%, the pH/pD decreased to pH = 8.8 (in H<sub>2</sub>O) and to pD = 8.5 (in D<sub>2</sub>O), respectively. The electrophoretic mobility data of the PEI/SDS solutions at 0.05% PEI concentration at pH = 8.8 and pD = 8.5 initial conditions are plotted in Figure 3. Clearly, the difference in viscosity cannot explain the difference between the samples in the two solvents. Moreover, if the viscosity difference is taken into account (dotted line in Figure 3), then the difference in mobility values between the two samples is enhanced.

The differences between the samples containing PEI and SDS prepared in D<sub>2</sub>O and H<sub>2</sub>O, respectively, were evident to the naked eye. The PEI samples prepared in water started to become turbid in the presence of 1.2 mM SDS. The samples were turbid around the charge neutralization point and became clear again at 3.8 mM of SDS. In the case of the samples prepared in D<sub>2</sub>O, the samples were just as turbid as those in H<sub>2</sub>O, at 1.2 mM SDS. However, unlike the samples in water, the turbidity persisted even at the highest surfactant concentration studied (4.5 mM). Furthermore, there was a precipitate present in the D<sub>2</sub>O samples containing 2.5 and 3.0 mM SDS. This observation is consistent with a higher charge density of the PEI in D<sub>2</sub>O at this pH/pD condition because it has been shown previously that the high turbidity region increases in width for a polyelectrolyte with a higher charge density.<sup>30</sup>



**Figure 4.** Electrophoretic mobility of PEI/SDS aggregates as a function of SDS concentration in two different solvents, as indicated in the legend on the right-hand side of the figure. The measurements were done at pH/pD = 4.0. The PEI concentration was 0.05%. No measurements are reported for samples in which precipitate had formed. The points shown are average values of five measurements, whereas the solid and dashed lines are guides for the eyes. The dotted line illustrates the viscosity corrected mobility data obtained for the samples in D<sub>2</sub>O.

In dilute SDS solutions, the electrophoretic mobility values for samples prepared in D<sub>2</sub>O were twice as large as for those prepared in water (see Figure 3) and a significant shift in the charge neutralization point toward a higher surfactant concentration (3.1 mM SDS) was observed in heavy water compared to the value (2 mM) observed in H<sub>2</sub>O. These findings are also well correlated with the fact that PEI has a significantly higher charge in D<sub>2</sub>O than in H<sub>2</sub>O at this pH/pD (Figure 1). In this surfactant concentration range, the compensation of the PEI charge occurs; therefore, the larger the initial charge density of the PEI, the larger amount of surfactant is needed to neutralize the PEI/SDS complex.

Above the charge neutralization concentration measured in water (2 mM SDS), the difference in the electrophoretic mobility values of the samples, prepared in the two solvents, decreases gradually. Above 4 mM SDS, similar mobility values were detected, with slightly lower values in D<sub>2</sub>O, which is due to the difference in solvent viscosity.

**Samples at pH/pD = 4.** The electrophoretic mobility data of the PEI/SDS samples, in which the pH/pD of the 0.05% PEI solution was adjusted to pH = 4 (in H<sub>2</sub>O) and pD = 4 (in D<sub>2</sub>O), are shown in Figure 4. In this case the samples became turbid at a SDS concentration of 4 mM in both cases, and they did not become clear even at the highest surfactant concentration studied (15 mM). Phase separation (i.e., a precipitate in equilibrium with a clear solution) was evident at 8 mM of SDS for the samples in both solvents, which is an unmistakable indication that the PEI/SDS aggregates are close to uncharged at this surfactant concentration. There are no large differences between the mobility values of the samples prepared in D<sub>2</sub>O and H<sub>2</sub>O, respectively, but a slightly smaller magnitude of the mobility data is observed in D<sub>2</sub>O in the whole surfactant concentration range. This can be explained fully by the difference in viscosity between the two solvents (dotted line in the figure).

The data suggest similar and high charge densities of the PEI molecules at this pH/pD range, which is in very good agreement with the results of the potentiometric titrations (Figure 1). The electrophoretic mobility data of the PEI/SDS complexes also demonstrate that the charge density of the PEI/SDS nanocom-

plexes is very similar in the two solvents at the whole surfactant concentration range.

## Conclusions

Depending on the pH, the nature of the monomer, and so forth, the charge density of weak polyelectrolytes can be significantly different in normal and heavy water. This should be kept in mind when correlating the neutron scattering data of weak polyelectrolytes and/or polyelectrolyte/surfactant solutions, measured in heavy water, with the structures of the same system in an aqueous medium. In this study, it was observed that PEI has a higher charge density in heavy water than in water at high pH values. This difference in the charge density was especially large in the pH range  $7 < \text{pH} < 11$ .

To demonstrate the solvent isotope effect on the properties of a weak polyelectrolyte, we investigated the interaction of PEI with SDS in three characteristic pH/pD ranges (at the natural pH of PEI, at pH/pD  $\approx 9$ , and pH/pD  $\approx 4$ ).

Although the natural pD of the PEI solutions in D<sub>2</sub>O were higher than their natural pH in H<sub>2</sub>O, the PEI molecules were found to have a similar low charge in the two solvents. Further, the PEI molecules were similar but highly charged at pH = pD = 4. Mixtures of PEI and SDS at the natural pH/pD value as well as at the pH/pD = 4 of the polyelectrolyte solution revealed identical electrophoretic mobility values after correcting for differences in solvent viscosity, and the charge neutralization point was found at the same surfactant concentration. This finding can be rationalized by the similar charge densities of the PEI at both pH/pD conditions.

However, when the pH/pD of the PEI samples was adjusted to  $\sim 9$ , the mobility of the PEI samples prepared in D<sub>2</sub>O was considerably higher than that of the samples prepared in H<sub>2</sub>O. The charge neutralization point was also found to be at a significantly larger surfactant concentration in heavy water than in water. These results are rationalized by the considerably larger charge density of the PEI molecules in D<sub>2</sub>O compared to that in H<sub>2</sub>O at these pH/pD conditions.

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