Hydrodynamic Size and Charge of Polyelectrolyte Complexes[†]

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Polyelectrolyte complexes have a wide range of applications for surface modification and flocculation and sorption of organic molecules from solutions. As an example, complexes between poly(diallyl dimethyl ammonium chloride) and poly(styrene sulfonate) have been investigated by diffusion and electrophoresis NMR. The formation of primary or soluble complexes is monitored. The hydrodynamic size is characterized by the hydrodynamic radius, calculated from the diffusion coefficient determined by pulsed field gradient NMR. In the combination with electrophoresis NMR, the effective charge of the molecules and complexes is determined. The hydrodynamic size of the primary complex is smaller than that of the pure polyelectrolyte of the larger molecular weight, in the present case poly(styrene sulfonate), in solution, since charges are compensated by the oppositely charged polyelectrolyte and hence the repelling forces diminish. The effective charge of the complexes is drastically reduced.

Introduction

Electrostatic interaction plays an important role in the formation of complexes from molecules in aqueous solution and the binding of ligands to proteins.[1] Complexes of oppositely charged polyelectrolytes may act as a model system for such studies. However, most synthetic polyelectrolytes have additional conformational degrees of freedom, mostly missing for proteins. Polyelectrolytes in solution adopt a much more extended conformation than uncharged polymers in a good solvent. If the ionic strength of the solution is increased, this repelling force becomes weaker, this effect is even stronger for bivalent salts at the same ionic strength.[2] In addition, polyelectrolyte complexes and polyelectrolyte complex nanoparticles offer a much wider range of application conditions than the pure polyelectrolytes; they are more tolerant against changes in pH and ionic strength for flocculation[3] and sorption.[4] Polyelectrolyte complex nanoparticles are formed from primary or soluble polyelectrolyte complexes.[5,6] These primary complexes are the subject of the present study.

The hydrodynamic size is conveniently probed by diffusion measurements using pulsed field gradient (PFG) NMR.[7] Compared to dynamic light scattering PFG NMR has distinct advantages: it is applicable to small molecules and in salt-free solution as well. The detection of NMR spectra in each experiments permits the data analysis for each species, that is resolved by its respective chemical shift in a mixture separately.[8] There is a sufficiently large range of conditions in which both light scattering and NMR are applicable as well permitting an effective comparison.[9] From the diffusion coefficient the hydrodynamic radius R_h is calculated by the Stokes–Einstein equation given in eq 1:[10] with k_B Boltzmanns constant, η the

$$D = \frac{k_B T}{6\pi\eta R_h} \tag{1}$$

solvent viscosity, and T the sample temperature.

The effective charge on small molecules, polymers or complexes is derived from a combination of diffusion and electrophoresis NMR. In the electrophoresis NMR experiment, a constant electrophoretic drift velocity is the result of the force balance between the force of the electric field on all the charges on the molecule and the hydrodynamic friction, which is derived from the diffusion coefficient using Einsteins formula.[11] The electrophoretic mobility is measured in the electrophoresis NMR experiment, where the NMR experiment again offers the possibility to assign the species that is moving especially in mixtures.[12,13,14] From the electrophoretic mobility and the diffusion coefficient, the effective charge of the molecule or complex is calculated assuming a steady state resulting from the force balance between the force of electric field acting on the effective charges and the hydrodynamic friction according to eq 2: with D being the diffusion coefficient, μ the electro-

$$Z = \frac{k_B T \mu}{e D} \tag{2}$$

phoretic mobility, k_B Boltzmanns constant, and T the sample temperature.

On the time scale of the PFG-NMR experiment of tens of milliseconds, in many situations, only a population-weighted average between free and bound states is observed, which has to be taken into account.

Experimental

Poly(diallyl dimethyl ammonium chloride) (PDADMAC) with a molecular weight of 5 kg/mol, has been provided by W. Jaeger, Golm. Synthesis and characterization are specified elsewhere.[15,16] Poly(styrene sulfonate) (PSS, for GPC, M_w = 77 kg/mol) has been purchased from Fluka. Both chemicals have been dried under vacuum and used without further treatment. Samples were prepared from stock solutions in D_2O (99.95%, Deutero GmbH). Deuterated water has been used to minimize the residual proton signals of the solvent and thus to improve the measurements of low polyelectrolyte concentration. Different monomer ratios of polyanion to polycation (n^-/n^+) have been

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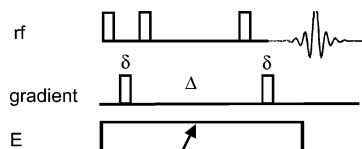
Figure 1. Schematic of the stimulated echo pulsed field gradient NMR experiment illustrating the radio frequency pulses and acquisition, the magnetic-field-gradient pulses of duration δ and separation Δ and in the case of the electrophoresis NMR experiment the DC electric field.

adjusted by dropwise addition of corresponding amounts of PDADMAC stock solution to the stirred PSS stock solution. The concentration of PSS in all samples investigated has been 5 mmol/L (monomer).

Pulsed field gradient (PFG) NMR experiments with stimulated echo pulse sequences as depicted in Figure 1 have been used to measure both diffusion coefficient and electrophoretic mobility. The effect on PFG NMR signals caused by different motion and their evaluation are described in detail elsewhere.[17,18,]

All experiments have been conducted on a Bruker Avance 300 NMR spectrometer operating at a Larmor frequency of 300 MHz for protons. The spectrometer is equipped with a microimaging accessory generating magnetic field gradients of up to 1 T/m. An in-house built electrophorsis NMR probe with a U-shaped sample tube has been applied.[19] The U-shaped design provides, that the electric-field induced motion is colinear with the static magnetic field and thus the magnetic field induced by the motion of charged molecules is perpendicular to the static field and can thus be neglected. The platinum electrodes are placed on top of the experimental volume and gas bubbles originating from electrolysis do not disturb the experiment. One of the three gradient channels of the gradient controller of the spectrometer is connected to a custom-made DC amplifier [(Rohrer GmbH Mess- and Systemtechnik, München, Germany)] providing the electric field synchronized with the NMR experiment.

The electrophoresis NMR experiments were performed incrementing the electric field in 16 steps between -120 and $+120$ V/cm. The gradient strength and time constants have been adapted to the diffusion behavior of the sample. The diffusion time Δ was in the range between 25 and 45 ms and was kept shorter then in the diffusion experiments to minimize Joule heating. The gradient pulse duration δ varied from 2 to 6 ms and the applied magnetic field gradient from 0.4 to 0.6 T/m between the several experiments.

For samples exhibiting narrow lines the resulting phase modulation can be evaluated slice by slice, which yields a linear relationship between the phase acquired by the NMR signal and the electric field applied, which proofs, that the electrophoretic mobility is constant. Alternative data processing is based on the States method for phase-sensitive two-dimensional NMR spectra[20] and requires acquisition with positive and negative electric field, resulting in a two-dimensional spectrum correlating electrophoretic mobility and chemical shift for each species.[18]

In the diffusion experiments the gradient was incremented linearly in 32 steps up to the maximum of 1 T/m. The gradient pulse duration varied from 4 to 5 ms and the diffusion time Δ varied in the range from 45 to 100 ms. The data evaluation was performed by fitting the Stejskal-Tanner-equation[21,22] to the integral of the proton signals as a function of the square of the gradient strength. For evaluation the aromatic protons of PSS in the region at 7.6 ppm and the methyl protons of PDADMAC at 3.2 ppm have been consulted. All experiments have been performed at 20 °C.
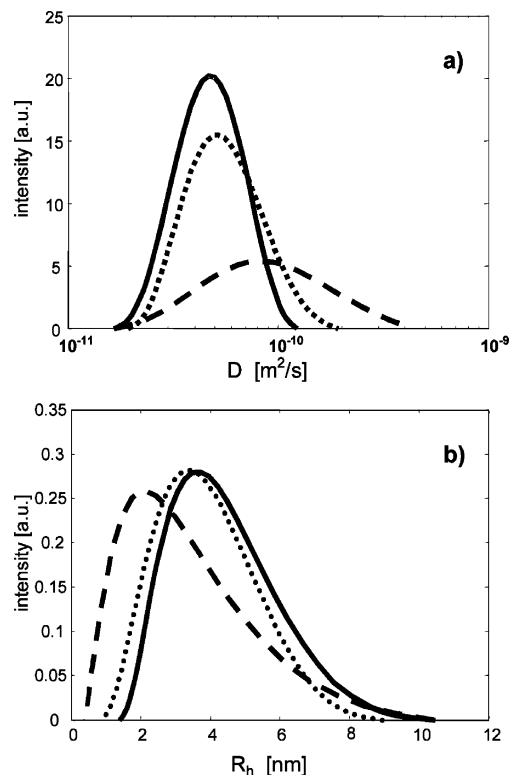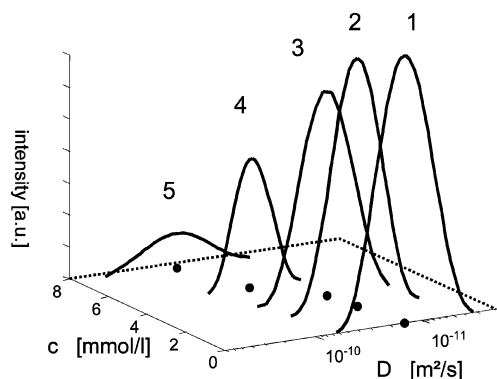

Figure 2. Distribution of diffusion coefficient (a) and hydrodynamic radii (b) of polycation PDAMAC for different anions. Solid line, pure PDADMAC; dotted line, PDAMAC with addition of monovalent salt, NaCl; dashed line, with addition of a PSS. Molar ratio of the charges to PDADMAC (monomers) n^-/n^+ in both cases is 0.66.

Data processing has been performed in MATLAB and MatNMR,[23] for the evaluation of data exhibiting a distribution of diffusion coefficients like in the diffusion of polymers with a polydisperse molecular weight a numerical inversion of the Laplace transform has been applied[24]

Results and Discussion

The conformational degree of freedom has been probed via the influence of the ionic strength on the hydrodynamic size of the polyelectrolytes. Because of the distribution of the molecular weight of the polyelectrolytes, a distribution of diffusion coefficients has been determined by inverse Laplace transfrom of the Stejskal-Tanner plot, that subsequently has been converted into a distribution of hydrodynamic radii following Einsteins equation[11] as shown in Figure 2. For a comparison to the formation of a polyelectrolyte complex, the same ionic strength as in the polyelectrolyte complex has been adjusted by a monovalent salt NaCl. The shape of the distribution of hydrodynamic sizes does not change; it remains a log-normal distribution, as expected from the natural molecular weight distribution. It is, however, shifted to smaller sizes. If, on the other hand, the same ionic strength has been adjusted by the addition of the appropriate amount of PSS to the solution, a different shape of the distribution is observed. The maximum size compares to that of the pristine PDADMAC, the minimum and correspondingly the mean of the distribution is shifted toward significantly smaller sizes.

Since PSS is available with narrow molecular weight distribution, in the following a constant concentration of PSS has been investigated under increasing concentration of PDADMAC. As depicted in Figure 3, the distribution of the diffusion coefficients changes its shape only at a high load of PDAD-

Figure 3. Distribution of diffusion coefficients of polyelectrolyte complexes as function of variable content of polycation PDADMAC (M_w = 5kg/mol). Basic solution is 5mmol/l (monomer) PSS (M_w = 77kg/mol). The numbers refer to (1) pure PSS, (2) addition of 2 mmol/l (monomer) PDAMAC, ratio n^-/n^+ =2.5, (3) ratio n^-/n^+ =1.5, (4) pure PDADMAC, c = 5 mmol/L (monomer), and (5) ratio n^-/n^+ = 0.66.
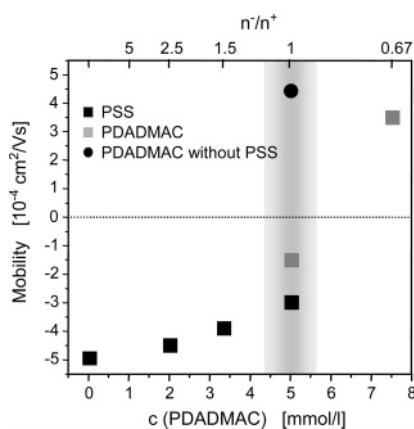
Figure 4. The electrophoretic mobility of the complexes as measured by electrophoresis NMR.

MAC. It shifts toward higher diffusion coefficients upon the addition of the polycation. Any particles formed that leading to turbidity of the solution do not contribute to the NMR signal in the range of diffusion coefficients accessible here. Therefore, only the soluble complexes have been studied.

Due to restrictions in the local mobility in the complex, the transverse relaxation time T_2 becomes significantly shorter and the signals become more difficult to detect. Most of the signals obtained are from methyl groups or dangling chain ends.

In Figure 4 the electrophoretic mobility, that has been detected for the mobile part of the complexes is shown. With increasing concentration of PDAMAC the size of the complexes, that are investigated is decreasing and therefore the hydrodynamic friction. As seen in the electrophoretic mobility is decreasing as well, clearly indicating, that the charge on the complex is becoming smaller. The shaded area indicates the range of charge compensation, where only weak signals are detected. In the range of excess of PDADMAC, only the signals of PDADMAC have been evaluated since the signal from PSS has been too weak. The charge of the complex is significantly smaller than that of the pure polyelectrolytes and follows a clear trend of the polyelectrolyte concentrations.

Conclusions

Primary complexes of poly(diallyl dimethyl ammonium chloride) and poly(styrene sulfonate) have been investigated by pulsed-field gradient and electrophoresis NMR. The hydrodynamic radii have been determined from the diffusion coefficients

using Einsteins equation. While polyelectrolytes in pure solution adopt a rather extended conformation, they become increasingly compact upon the addition of the oppositely charged polyelectrolyte. Because of the large number of charges on the polyelectrolyte, the effect is much more pronounced than by the addition of low-molecular-weight salt. The hydrodynamic radius of the primary polyelectrolyte complexes is significantly smaller than that of the uncomplexed poly(styrene sufonate), proofing, that the polyelectrolytes in the complex adopt a significantly more compact conformation than the free polyelectrolytes in solution. This is due to the compensation of charges by the oppositely charged polyelectrolyte and thus a reduction of the repelling force along the polymer chain. To study the effect of electrostatic interaction on the formation of the polyelectrolyte complexes the effective charge, that is the charge acting in an external electric field has been determined by electrophresis NMR. The magnitude of the effective charge of any complex is smaller than that of the uncomplexed polyelectrolytes as expected since the complex formation is understood to be driven by electrostatic interaction. There is a smooth transition between the initial anionic complexes to the cationic complexes. However a significant negative charge is observed at the nominal charge compensation.

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