Polyelectrolytes, polyelectrolyte complexes, counterion condensation and ligand binding

Die elektrostatische Wechselwirkung dominiert die Adsorption von Polyelektrolyten an Oberflächen, die Bildung von Komplexen und die Bindung von Liganden an Makromoleküle. Die effektive Ladung von Polyelektrolyten wird durch die nominelle Ladungsdichte und die Gegenionenkondensation, die vom umgebenden Medium abhängt, bestimmt. Elektrophorese-NMR zusammen mit Diffusions-NMR erlaubt es, die effektive Ladung direkt zu messen. Die spektroskopische Auflösung in beiden Experimenten ermöglicht es, einzelne Spezies im Gemisch oder Komplex zu separieren. Aus der effektiven Ladung, die am Polyelektrolyt gemessen wird, wird der Effekt der Gegenionenkondensation quantifiziert. Liegen organische Gegenionen vor, werden diese simultan charakterisiert und der Anteil kondensierter Gegenionen berechnet. Daraus und aus der effektiven Ladung wird die Zahl der Wiederholeinheiten und damit die Molmasse von Polyelektrolyten bestimmt. Das gleiche Vorgehen erlaubt es, den gebundenen Anteil von Liganden zu bestimmen oder die Dynamik in Polyelektrolytkomplexen zu erfassen. In den Dispersionen liegen freie Polyelektrolyte, Polyelektrolytkomplexe und Polyelektrolytnanopartikel vor. Über den hydrodynamischen Radius ist die Größe von Polyelektrolyten und Polyelektrolytkomplexen zugänglich.

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Since the charge of the polyelectrolytes is one of the driving forces for adsorption and binding, knowledge of the effective charge of polyelectrolytes in solution is important. The effective charge is determined by the nominal charge density on the polymer, the degree of dissociation and the fraction of condensed counterions [1]. From a point of view of electrostatics counterions may be replaced by multivalent counterions, which are oppositely charged polyelectrolytes.

The fraction of condensed counterions is determined by the energy required for a counterion to move away from the polyelectrolyte escaping the electrostatic field of the polyelectrolyte charges, which is lowered by counterions compensating a fraction of the charges on the polymer. Condensed counterions move with the macromolecule. On longer time scales there may be exchange between free and condensed counterions [2]. Poly(4-styrene-sulfonate), PSS and poly(diallyldimethylammonium chloride), PDADMAC have been choosen as examples. Both are strong polyelectrolytes, which are completely dissociated in aqueous solution at the pH ranges of interest. The remaining variable is the fraction of condensed counterions that has a significant influence on the number of charges available depending on the solvent properties [3].

For the determination of the effective charge pulsed field gradient NMR has been applied, which has been proven to be a versatile tool. In the stimulated echo pulsed-field-gradient NMR experiment the coefficient of the self diffusion of the molecule in solution has been determined from the signal attenuation as a function of the magnetic field gradient applied [4, 5]. From diffusion coefficient the coefficient of the velocity-proportional hydrodynamic friction has been determined according to Einstein's formula [6]. On the other hand from the diffusion coefficient the hydrodynamic radius has

been calculated following Stokes-Einstein's formula [7, 8], which gives a measure of the space occupied by the molecule in the solution [9, 10]. In the case of polymer usually a wide distribution of molecular weights and therefore of hydrodynamic sizes is found. In that case a numeric inversion of the Laplace transform has been applied to calculate the diffusion-ordered (DOSY) spectrum correlating diffusion coefficient and chemical shift [11]. Thus it has been possible to identify the diffusion coefficients of different species in the solution.

When a DC electric field has been applied during the PFG NMR experiment, charged molecules move coherently along the field lines [12, 13, 14]. This coherent motion results in a phase modulation of the NMR signal as a function of either the strength of the magnetic field gradient or the electric field strength. If only the electric field strength has been varied while keeping all other parameters constant, effects of relaxation and the superimposed diffusion of the molecules do not influence the signal. Thus even slow coherent motion resulting in displacements comparable to those resulting from diffusion have been resolved [15, 16], which is important for the possible resolution in the experiment.

Dedicated two-dimensional data processing according to [15] based on [17] permitted the generation of two-dimensional electrophoresis NMR spectra correlating the chemical shift, identifying the moving species, with its electrophoretic mobility. This model-free approach has the advantage that no assumption on the number of components is required. An example of a two-dimensional electrophoresis NMR spectrum is shown in Fig. 1.
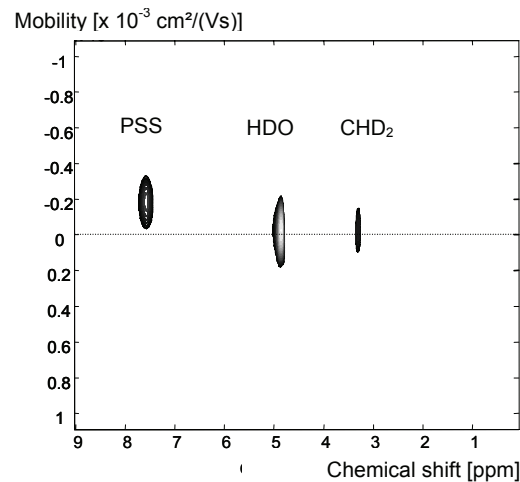

Fig. 1:
Two-dimensional electrophoresis NMR spectrum of PSS in a mixture of water and methanol

From the force balance between the force that the electric field exerts on the effective number of charges on the molecule and the hydrodynamic friction, the effective number of charges is calculated according to equation (1) from the electrophoretic mobility μ_{el}, the diffusion coefficient D, the sample temperature T and natural constants.

$$z = \frac{\mu_{el} \cdot k_B \cdot T}{e \cdot D} \qquad (1)$$

This is the number of charges on the polyelectrolyte that interact with other molecules and charged surfaces. This number is significantly smaller than the nominal number. Fig. 2 depicts the effective charge of PSS as a function of the ionic strength of the aqueous solution. In the solution of PSS with a molecular weight of 77 kg·mol^{-1} corresponding to 377 repeat units only 20 % of the
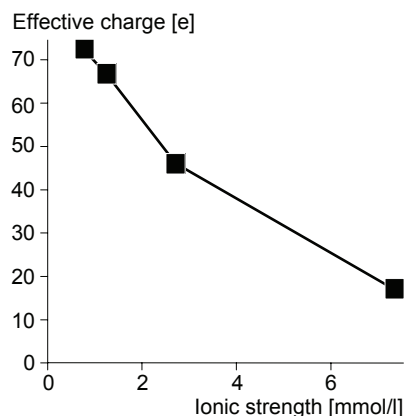
nominal charge is found. This fraction decreases further upon the addition of salt to the solution. Increasing the charge density along the polymer chain would not at all enhance the electrostatic interaction of this polyelectrolyte with other charged objects. A similar effect is found for proteins, where the effective charge is significantly smaller than the nominal charge as it would be expected from the degree of dissociation of the various functional groups on the macromolecule [18]

When the relative permittivity of the solution has been decreased, the effective charge of the polyelectrolyte decreased accordingly as has been demonstrated for mixtures of water and methanol [19]. The hydrodynamic size of polyelectrolytes strongly depends on the effective charge, since the repelling force between the charges brings the polyelectrolyte into a more extended conformation [20]. Accordingly the hydrodynamic radius decreases with decreasing relative permittivity.

The counterions may be replaced by multivalent counterions, by oppositely charged polyelectrolytes. In that case the oppositely charged polyelectrolyte does not only compensate the charges and thus the repelling forces, attractive forces between the oppositely charged polyelectrolytes, are introduced as shown in Fig. 3. The hydrodynamic size has been reduced when NaCl has been added. When the same ionic strength has been adjusted by the addition of PSS, the size distribution has become significantly broader. However, the mean of the distribution shifts towards significantly smaller sizes. This may be explained by the reduction of repelling forces and the subsequent formation of denser complexes.
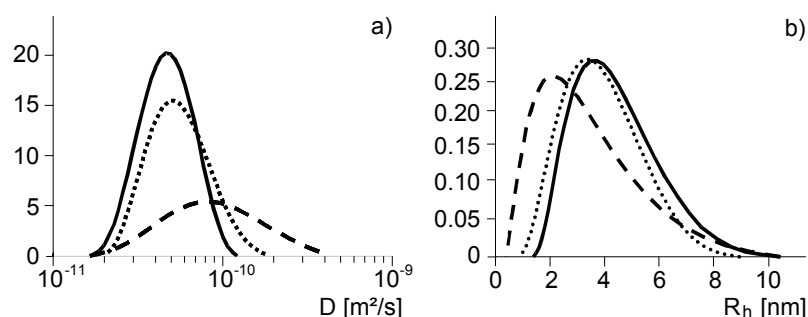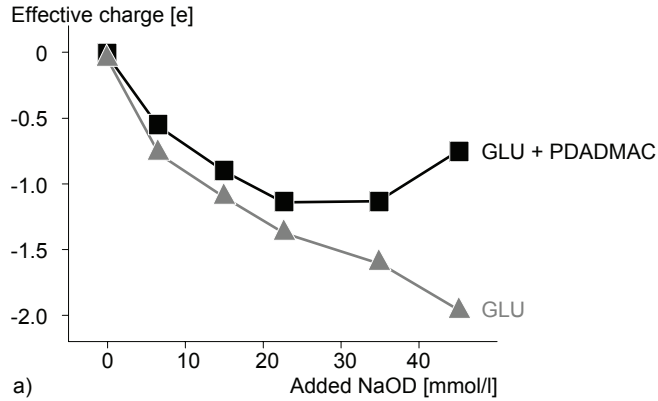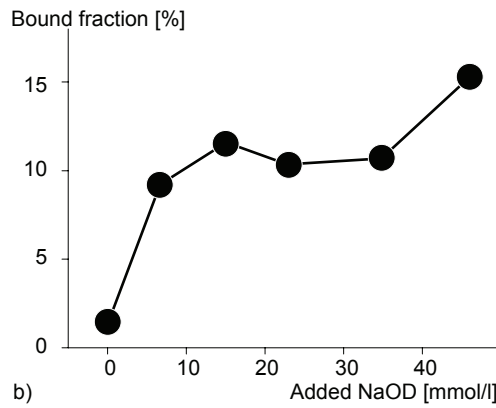
To investigate binding of small molecules to polyelectrolytes the example of glutamic acid and PDADMAC has been investigated. The effective charge of free glutamic acid and glutamic acid in contact with PDADMAC has been investigated as depicted in Fig. 4.

It has been shown that the charge of glutamic acid increases nearly linearly with increasing pH from zero at low pH to -2 at high pH. Simultaneously the hydrodynamic size increases since the solvation shell becomes larger, because of the increasing charge more water is bound.

Effective charge [e]

a)

Bound fraction [%]

b)

Fig. 4:
Effective charge of glutamic acid (GLU) (a) At low pH glutamic acid appears uncharged, yielding two negative charges at high pH, when both acid groups are dissociated. In contact with the polycation the effective charge in the time average is lower. The fraction of glutamic acid bound to PDADMAC (b) is derived from the PFG NMR.

For the investigation of the polyelectrolyte behaviour in the absence of conformational degrees of freedom, rigid-rod poly(aramide) polyelectrolytes have been synthesized. Opposed to other rigid-rod polyelectrolytes these do not require flexible side chains. For the present study the poly(aramides) have the additional advantage, that, as a result of the synthetic route, pyridinium counterions are present, which are identified in the NMR spectra as shown in Fig. 5. Comparing the diffusion coefficient observed for the free pyridinium ion in pyridinium chloride D_f and that of pyridinium as a counterion of poly(aramide) D_{obs}, the fraction of bound pyridinium ions has been calculated, since the time average between bound and free states is observed on the time scale of the PFG-NMR experiment according to equation (2). The diffusion coefficient of the pyridinium bound to the poly(aramide) D_b is that of the poly(aramide), since the pyridinium does not change the hydrodynamic size of the polymer.

$$D_{obs} = (1 - x_b)D_f + x_b D_b \qquad (2)$$

The nominal charge has been derived from the effective charge and the fraction of bound counterions according to equation (3)

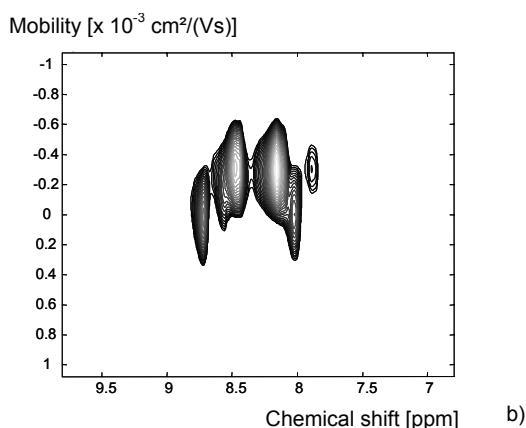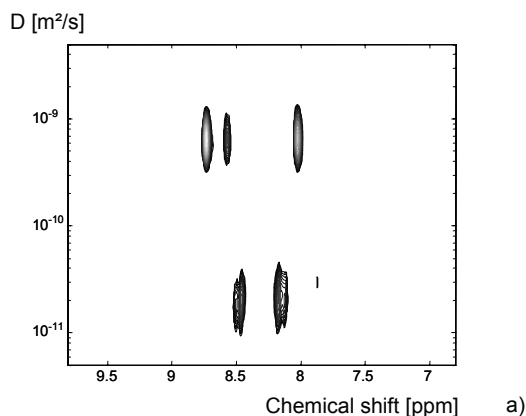$$z_{nom} = \frac{z_{eff}}{x_f} \qquad (3)$$

D [m²/s]

Chemical shift [ppm]   a)

Mobility [x 10^{-3} cm²/(Vs)]

Chemical shift [ppm]   b)

Fig. 5:
Diffusion-ordered spectrum (a) and two-dimensional electrophoresis NMR spectrum (b) of poly(aramide) and pyridinium counterions

With an effective charge of -35 elementary charges of the poly(aramide) and a fraction of 35 % of bound counterions the nominal charge has been calculated to 50. With two charges per repeat units a molecular weight of 14 kg·mol^{-1} has been calculated [21], which is in close agreement to the result from light scattering. This route is an alternative to the molecular weight determination for polyelectrolytes, where other methods like chromatography fail due to the specific interaction of the charged molecules with the columns. It is not limited to rod-like polyelectrolytes.

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