Separation of Polyelectrolyte Chain Dynamics and Dynamics of Counterion Attachment by EPR Spectroscopy

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Summary: Rotational dynamics and local enrichment of counterions close to polyelectrolyte chains were studied by EPR spectroscopy in solvents of different viscosity. The results confirm previous findings (D. Hinderberger, G. Jeschke, and H. W. Spiess, Macromolecules 2002, 35, 9698) that electrostatic attachment of counterions to the chains is dynamic with lifetimes of contact ion pairs shorter than 1 ns. While in low-viscosity solvents linewidths for a dianionic nitroxide probe and their dependence on polyelectrolyte concentration are dominated by the gradient of local concentration in the vicinity of the chain, they are more strongly influenced by changes in rotational dynamics in a glycerol/water mixture. The slowdown of dynamics at higher viscosity strongly depends on polyelectrolyte concentration, suggesting that the lifetime of the attached state increases. The linewidths of trianionic triarylmethyl probes and of the center line of the nitroxide probes are dominated by local counterion enrichment both at low and high viscosity. Comparison of these linewidths and of the extent to which the lineshapes are non-Lorentzian indicates build-up of larger concentration gradients at higher viscosity.

Keywords: chain dynamics; counterion condensation; ESR/EPR; nanoheterogeneity; polyelectrolytes

Introduction

Polyelectrolytes – polymers with a large number of charged groups – play an important role in fields of scientific research as diverse as molecular biology and nanotechnology.[1,2] Furthermore, there is a wide variety of commercial applications already for polyelectrolyte materials in e.g. cosmetics, fuel cells, and food and oil industry.

Despite this widespread interest in and large amount of studies on polyelectrolytes, there are fundamental interactions whose influence on the polyelectrolyte structure has not yet been understood satisfactorily. Open questions include i) the role of conformation-dependent electrostatic interactions between charged repeat units of the polyelectrolyte on the one hand and between the polyelectrolyte and counterions on the other hand; ii) the role of especially *intra*molecular hydrophobic interactions, and iii) the role of specific solvation. It is commonly acknowledged that some of the very interesting properties of polyelectrolytes in

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solution and at interfaces are a consequence of these interactions. Within the last decade theoretical investigations of the aforementioned interactions, driven by increasing computing power and sophisticated algorithms, have been carried out and led to predictions of polyelectrolyte conformations in solution that could not yet be verified experimentally.[3]

Many characterization methods can either only probe macroscopic properties (e.g. conductivity) or require some long-range order in the systems, such as the well-established scattering techniques (light-, x-ray-, and neutron-scattering). Magnetic resonance methods, such as electron paramagnetic resonance (EPR) spectroscopy,[4] being local, highly sensitive and highly selective, have potential to deliver valuable information on polyelectrolyte materials, which is otherwise inaccessible.

EPR spectroscopy in combination with spin labeling has been used for three decades to elucidate structure and dynamics of larger molecules, in particular of biomolecules (e.g. DNA, membrane proteins), but also in polymer science.[5,6] In spin labeling, the paramagnetic probe molecule is covalently attached to the molecule of interest, while in spin probing weak non-bonding interactions, such as hydrophobic interactions between polymers and spin-labeled surfactants are used to direct the label to the site of interest.[6] In polyelectrolyte-counterion systems spin probing can be based on the electrostatic interaction. In such experiments changes in the EPR spectra induced by changes in polyelectrolyte concentration are directly related to the interaction of the spin-carrying counterions with the polyelectrolyte chain.

Using divalent ionic spin probes together with continuous-wave (CW) EPR and Fourier-Transform (FT) EPR at room temperature we have recently shown that di- and trivalent probe ions are condensed to cationic polyelectrolyte chains via *dynamic electrostatic attachment*.[7] This term is used for counterion attachment in order to emphasize that a dynamic equilibrium between closely attached (site bound, according to Manning),[8] non-specifically attached (territorially bound) and detached (free) counterions is attained. Our previous work showed that exchange between site-bound and territorially bound spin-carrying counterions proceeds on a sub-nanosecond timescale.[7]

In our recent paper we moreover found that the effect of solvent in these systems is more complex than just that of a dielectric continuum between charges. Changing relative permittivity from $\varepsilon \approx 50$ (ethanol/water 1:1) to $\varepsilon \approx 140$ (N-methylpropionamide (NMPA)/water 2.3:1) did not result in a significant change of dynamic electrostatic attachment and local concentrations of probe ions, while the results for both these solvents differed strongly from the results for pure water. We attributed this to the interaction of the hydrophobic parts of the

organic solvent molecules (in organic solvent/water mixtures) with the polyelectrolyte backbone, which leads to a screening of intra-polyelectrolyte hydrophobic interactions and thus prevents conformational changes that take place in pure water.

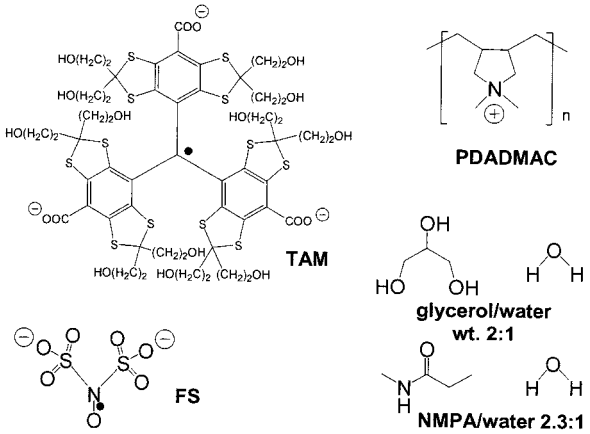
In this earlier study we did not discuss the effect of polyelectrolyte chain dynamics on the spectra. In our qualitative discussions we assumed that the polyelectrolyte molecules are fixed on the timescale of counterion dynamics. In this work we assess how the dynamics of counterion condensation is influenced by polyelectrolyte motion.

Our study is organized as follows. In the first part we describe new experimental results for a more viscous solvent mixture of water with glycerol and compare them with our earlier results.[7] In the second part, we extend our previous discussion of the effects taking particular care of the separation of spectral changes caused by a slowdown of rotational diffusion of the counterions due to temporary electrostatic attachment to the polyelectrolyte chain from those caused by more frequent counterion collisions due to increased concentration close to the polyelectrolyte chain. Finally, we consider how the two processes are influenced by polyelectrolyte chain dynamics.

Materials and Methods

As spin probes (Scheme 1) we used Fremy's salt dianion (Potassium nitrosodisulfonate), FS, with an unknown grade of purity (ICN Biomedicals) and triarylmethyl trianion, TAM (sodium salt, gift from Nycomed Innovations AB, Sweden). As cationic polyelectrolyte we chose poly(diallyldimethylammonium chloride), PDADMAC, with an M_W of 240 000, (Polysciences, Inc.). All chemicals were used as received. As solvent systems we chose deionized Milli-Q-water (permittivity: $\varepsilon_r = 80$ at 293 K), 66 wt.-% glycerol/34 wt.-% water (made from 87 wt.-% glycerol/water mixture, Fluka, density $\rho \approx 1.17$ g/ml, approximate $\varepsilon_r = 57$ at 293 K) and 70 vol.-% N-methylpropionamide (Aldrich Chem. Co.)/30 vol.-% water (NMPA/H_2O, $\rho = 0.95$ g/ml, approximate $\varepsilon_r = 140$ at 293 K). Small amounts of KOH were added to adjust the solution to pH > 8. Spin probe concentration was fixed at 0.5 mM (FS) or 0.4 mM (TAM), and polyelectrolyte concentration was varied from 4 mM (in monomeric units) to 140 mM. Data are presented as a function of the ratio R of spin probes to polymer repeat units, so that no assumption on the actual number of repeat units per chain, and thus on M_n, had to be made. EPR spectra at X-band (~9.7 GHz) were measured on a Bruker ELEXSYS 580 spectrometer using an AquaX inlet and a rectangular cavity (4103TM, Q-values typically ~3000). The temperature during all measurements was set to 293 K.

Data analysis is based on three facts about the spectral changes observed on addition of polyelectrolyte that were established in our previous work.[7] First, there is line broadening for both the TAM and FS spin probes that affects all lines in the spectra to roughly the same extent and can be traced back to Heisenberg spin exchange broadening due to more frequent collisions of the counterion in regions close to the polyelectrolyte chain, where local concentration is enhanced. Second, this broadening is heterogeneous in the sense that a gradient of local concentration – from high spin probe concentration close the polyelectrolyte chains to approximately bulk concentration – leads to a distribution of relaxation times and thus to a non-Lorentzian lineshape. Third, for the FS spin probe additional broadening is observed that affects the high-field line to the largest and the center line to the smallest extent and can be traced back to a slowdown of rotational diffusion.



TAM = triarylmethyl trianion; FS = Fremy's salt dianion; PDADMAC = poly(diallyldimethylammonium chloride); NMPA = N-methylpropionamide.

Scheme 1. Molecular structures of charged spin probes, polyelectrolyte and solvents used in this study.

The EPR spectra of both spin probes throughout most of the polyelectrolyte concentration range can be fitted by a homewritten program which assumes that relaxation can be described by a stretched exponential decay.[9] The time-domain signal for a single line with resonance offset ω_i, characteristic transverse relaxation time T_2, and stretch factor x is then given by

$$V(t) = \sum_{k=1}^{n} \exp\left(-\frac{t}{T_{2,k}}\right)^{x} \exp(i\omega_k t), \qquad (1)$$

where n = 1 for TAM and n =3 for FS spin probes with k = 1 corresponding to the low-field line and k = 3 corresponding to the high-field line. The CW spectrum is obtained by Fourier-transformation of the sum signal $V(t)$, and subsequent pseudomodulation[10] with the same modulation amplitude as used in the experiments. This simulated CW EPR spectrum is fit to the experimental spectrum, which provides a quantification of the non-Lorentzian lines with a minimum number of fit parameters and without making any assumptions on the physical process leading to relaxation. It provides good fits of the data for all compositions of the system in low-viscosity solvents but is restricted to $R < 0.1$ in the glycerol/water mixture. Above this value we do not consider the simulated data reliable any more, as the deviation of the simulated spectra from the experimental data becomes significant.

All values reported for T_2 in this work are average values of the Williams-Watts distribution of T_2 [11]

$$\langle T_2 \rangle = \frac{1}{x} \cdot T_2 \cdot \Gamma\left(\frac{1}{x}\right), \tag{2}$$

where the brackets denote the average value and $\Gamma(1/x)$ is the gamma function.

A characteristic EPR linewidth $<\Delta B>$ of the non-Lorentzian lines can be computed by $\langle \Delta B_k \rangle$ = [0.412 (G/MHz) $*\langle T_{2,k} \rangle^{-1}$].[12] This averaged linewidth is a quantitative measure of the electron spin-electron spin interaction: $<\Delta B> \propto \langle T_2 \rangle^{-1} \propto T_{2,intrinsic}^{-1} + T_{e\text{-}e}^{-1}$, which is in turn related to local concentration of the spin-carrying counterions. Here, $T_{2,intrinsic}^{-1}$ denotes the intrinsic relaxation rate at infinite dilution and $T_{e\text{-}e}^{-1}$ denotes the concentration-dependent rate due to intermolecular electron spin interaction. The intrinsic rate differs for the three lines (k = 1, 2, 3) for nitroxides, and these differences are related to the rotational correlation time (see below). We assume broadening to be mainly due to Heisenberg spin exchange, but in solutions of high viscosity an additional effect due to residual magnetic dipolar interaction cannot be excluded.[13,14] As broadening due to both mechanisms is related to local concentrations our semi-quantitative conclusions do not depend on the detailed broadening mechanism.

For the FS spin probe, a characteristic value $\langle \tau_c \rangle$ for the rotational correlation time was computed from the so-called "B-parameter": $B = (<\Delta B(k=3)> - <\Delta B(k=1)>)/2$ as introduced by Goldman et al.[15, 16] Note that our $\langle \tau_c \rangle$ is not the strictly defined average of the distribution of rotational correlation times, which cannot be extracted by this simple data analysis procedure. This slightly modified data analysis compared to Ref. [7] does not lead to

qualitatively different results but provides quantitative average values that are better characteristics of the distributions of T_2 and τ_c.

Results

Dynamic Electrostatic Attachment of Spin Probes

As reported earlier,[7] we have found that addition of oppositely charged polyelectrolyte to solutions of multivalent spin probes leads to a marked increase in the peak-to-peak linewidths and changes the lineshapes. Typical spectra for the probe FS in glycerol/water with and without added PDADMAC are displayed in Figure 1. Qualitatively, the lineshape changes on adding polyelectrolyte resemble those ones observed before in other solvent mixtures.[7]

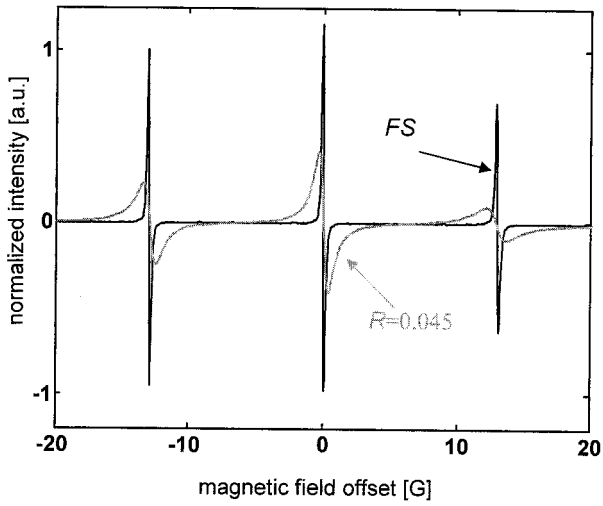


Figure 1. X-band (9.79 GHz) EPR spectra in 66 wt.-% glycerol/34 wt.-% water at two different spin probe/DADMAC ratios *R*. Black line: *no polyelectrolyte, only FS*; grey line: $R = 0.045$. Spectra were normalized to equal double integral.

However, the effects are much larger in glycerol/water than in low-viscosity solvents such as water and NMPA/water. By measurements of neutral and like charged nitroxide spin probes together with polyelectrolyte it has been verified that these changes are directly related to electrostatic interactions and are not pure viscosity effects.[7]

Fremy's Salt and PDADMAC in Glycerol/Water and NMPA/Water

In Figure 2 parameters $<T_{2,k}>$ and stretch factor x are shown for FS/PDADMAC in the low-viscosity solvent NMPA/water and the higher-viscosity solvent glycerol/water. Stretch factors x according to Equation (1) are a measure of the width of the distribution of T_2-relaxation times. With increasing R-value stretch factors deviate more and more strongly from their values in the absence of polyelectrolytes, thus the distribution of relaxation times becomes more heterogeneous with higher R. The trend for the stretch factors as a function of R is almost identical in both solvent systems, just the absolute values are shifted by approximately 0.05.

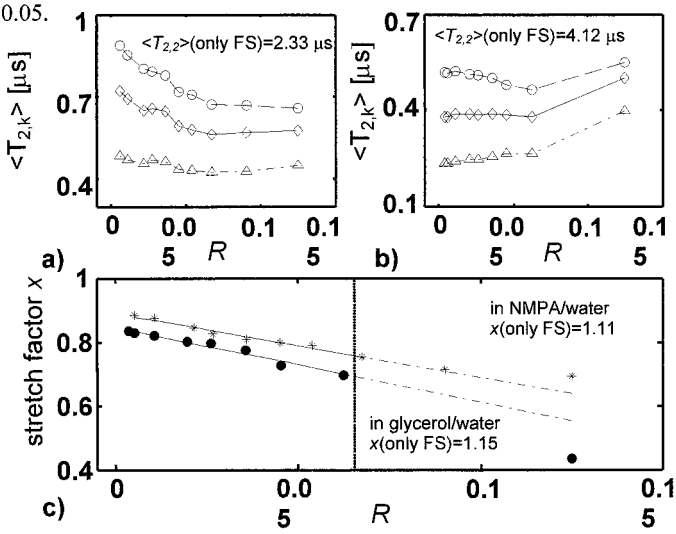


Figure 2. Relaxation time averages $\langle T_{2,k} \rangle$ and stretch factors x (see Equation (1) and (2)) for spin probe FS as function of FS/PDADMAC ratio R in two different solvents (EPR frequency: 9.79 GHz). Parameter uncertainties are within marker size.
a) In 70 vol.-% NMPA/30 vol.-% water (based on data from Ref. 5).
b) In 66 wt.-%.glycerol/34 wt.-% water. Diamonds/solid lines: low-field manifolds ($k = 1$); circles/dashed lines: center-field manifolds ($k = 2$); triangles/dash-dot lines: high-field manifolds ($k = 3$). Values for the center-field line of pure FS are also given, lines are meant as guide to the eye.
c) Stretch factors x as characterization of relaxation time distribution (see Equation (1)). Straight solid lines are linear fits including data points up to $R = 0.067$ (marked by vertical dotted line; extrapolation of the linear fits: dash-dot lines). Linear fits: in NMPA/water: $x = 0.889 - 2.019R$; in glycerol/water: $x = 0.847 - 2.353R$.

The averaged relaxation times $<T_{2,k}>$ of the fits to the EPR spectral line manifolds in glycerol/water are significantly lower than their respective values in NMPA/water. Plots of the $\langle T_{2,k} \rangle$ for both solvent systems show that the relaxation times – unlike the stretch factors –

do not show the same trends. Firstly, in glycerol/water, the values for $R = 0.125$ deviate from the trends of the relaxation times in the range $R = 0 \rightarrow 0.067$. Secondly, in this range the low-field line relaxation time is almost unaltered, whereas the center-field relaxation times decrease and the high-field relaxation times increase. In the same R-range, relaxation times of all three lines in NMPA/water steadily decrease and reach a plateau for $R > 0.067$.

Data for FS/PDADMAC in glycerol/water at $R = 0.125$ clearly deviate from the described trends. Using Equation (1), one could not fully fit the FS EPR spectrum at R=0.125 in glycerol/water (spectrum not shown) or at even higher R. A certain amount of the spectral intensity in these spectra is distributed over a large spectral width and is not accounted for in the fit. Thus, the extracted values represent relaxation-, distribution- and dynamic data of only a fraction ($R = 0.25$: 50%, $R = 0.125$: 85%) spin probes and cannot be compared to the data in the R-range up to 0.067.

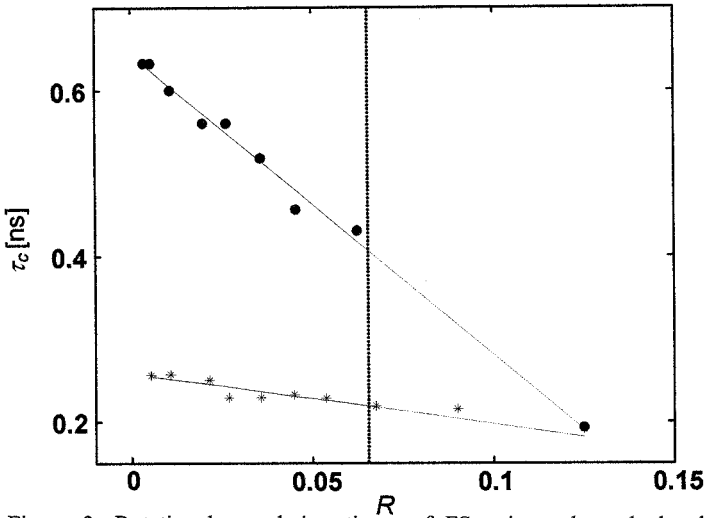


Figure 3. Rotational correlation times of FS spin probe calculated from the linewidth differences of high-field and low-field line manifolds (see text) as function of FS/PDADMAC ratio R in different solvents. Filled circles: in glycerol/water (τ_c(only FS) = 0.025 ns); asterisks: in NMPA/water (τ_c(only FS): 0.011 ns). Solid lines are linear fits including data points up to $R = 0.067$ (marked by vertical dotted line; extrapolation of linear fits: dash-dot lines). Linear fits: in glycerol/water: τ_c=0.644 ns – 3.630 ns*R; in NMPA/water: τ_c=0.258 ns – 0.616 ns*R.

Despite the obvious differences, FS/PDADMAC spectra in both solvents have a certain feature in common, namely that the differences between the average relaxation times of the high-field line ($k = 3$) and the low-field line ($k = 1$) become smaller with larger R-values. This corresponds to on average faster rotational motion of the counterion probe with decreasing

polyelectrolyte content of the solution. In Figure 3 rotational correlation times $\langle \tau_c \rangle$ for FS in both solvent systems are plotted as a function of R. The decrease in $\langle \tau_c \rangle$ with increasing R is much larger in glycerol/water ($\langle \tau_c \rangle$ decreases by ~66%) as compared to NMPA/water ($\langle \tau_c \rangle$ decreases by ~20%). The slowdown of rotational diffusion with increasing polyelectrolyte content is linear in R in both solvents.

TAM and PDADMAC in Glycerol/Water and Water

CW EPR spectra of the spin probe TAM consist of only one single line because of lack of hyperfine interaction of the electron spin with magnetic nuclei.[17] Typical spectra for the TAM probe with different concentrations of PDADMAC in glycerol/water are presented in Figure 4a. Clearly broadening in the EPR spectra increases with increasing R. Averaged relaxation times $<T_2>$ and stretch factors x that were gained from fitting spectra to Equarion (1) are dis-

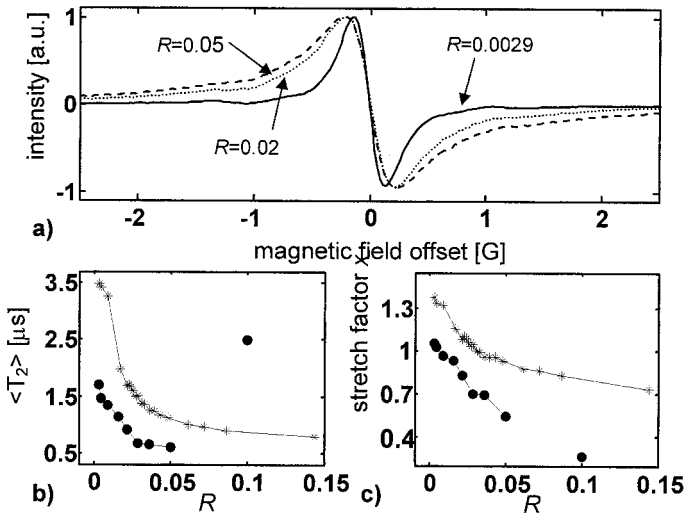


Figure 4. Representative EPR spectra, averaged relaxation times $<T_2>$, and stretch factors x (see Equation (1) and (2)) for spin probe TAM as function of TAM/PDADMAC ratio R in two different solvents (EPR frequency: 9.79 GHz). Parameter uncertainties are within marker size.

a) Three EPR spectra of TAM in glycerol/water at different R values; solid line: R = 0.0029; dotted line: R = 0.02; dashed line: R = 0.05; spectra are normalized to equal amplitude.

b) Averaged relaxation times $<T_2>$; filled circles: in glycerol/water ($<T_2>$(only FS) = 3.49µs); asterisk: in water ($<T_2>$(only FS) = 4.65µs).

c) Stretch factors x as characterization of relaxation time distribution (see Equation (1); filled circles: in glycerol/water (x(only FS) = 1.31); asterisks: in water (x(only FS) = 1.48). Lines are meant as guide to the eye.

played in Figure 4b,c. The trends not only for $<T_2>$, but also for the stretch factors x are similar in both solvents, water and glycerol/water, in the range $0< R \leq 0.05$. The only difference is that both quantities are shifted to lower values for glycerol/water.

As was the case with FS, the value for $<T_2>$ in glycerol/water at the smallest polyelectrolyte concentration (here R=0.1) deviates from the trend at lower concentration. This can again be attributed to our model not being able to completely fit the experimental data in glycerol/water at such R-values.

Discussion

General consequences of the observed spectral changes for a model of counterion distribution and dynamics

As the EPR transition frequency of the TAM spin probe at X-band frequencies (~9.7 GHz) does not significantly depend on orientation, the strong line broadening on addition of polyelectrolyte can only be due to a change in the environment of the probe, but not due to changes in dynamics. The observed strong changes with maximum broadening at low polyelectrolyte concentration are easily rationalized by enrichment of this counterion probe close to the polyelectrolyte chain, resulting in more frequent collisions and thus in Heisenberg exchange broadening. Any alternative explanations such as for instance effects of different local oxygen concentration could hardly explain changes of the observed magnitude. The deviation from a Lorentzian lineshape then proves a heterogeneous distribution of the TAM probes, i.e. a concentration gradient.

The same effect is apparent in the spectra of the FS spin probe, but in this case the spectrum is in addition sensitive to rotational diffusion. The presence of polyelectrolyte causes a moderate slowdown of this motion, with apparent rotational correlation times increasing with increasing polyelectrolyte concentration and never exceeding 1 ns.

These findings exclude the most simple model of counterion condensation in which one fraction of the counterions forms long-lived (lifetime $\geq$ 1 ns), static *contact ion pairs* with ionic groups of the polyelectrolyte while the remainder freely diffuses in solution. In such a case, the condensed counterions would contribute a spectral component analogous to spin labels attached to a polymer chain (restricted sidegroup motion, no isotropic averaging on the EPR timescale), while the freely diffusing probes would contribute a narrow component with the same τ_c as observed in the absence of polyelectrolyte. Such bimodal spectra are clearly not what we observe. In contrast, isotropic averaging (i.e. almost unhindered rotation about

all three molecular axes) for the vast majority of the spin-carrying counterions on a sub-nanosecond timescale is evidence for only temporary electrostatic attachment to the chains in solution. This is a finding already indicated in our previous work,[7] but reinforced now by the observation that even at higher solvent viscosity and thus both slower polyelectrolyte chain dynamics and slower translational diffusion of the counterions there is no indication for bimodal spectra.

Note that contact ion pairs consisting of two ions with different charge have been extensively discussed before in the context of solutions of low molecular weight electrolytes (see, e.g., Ref. [18]) and that for ion pairs formed after covalent bond breaking the dynamics of dissociation and reencounter have been studied by picosecond absorption spectroscopy.[19] Results of the latter study show that the lifetime of ion pairs consisting of a diphenylmethyl cation and a chloride anion in acetonitrile is on the order of 150 ps. A model of dynamic electrostatic attachment in which at any given time there exists a fraction of contact ion pairs with such a short (sub-nanosecond) lifetime could be reconciled both with Manning theory,[8,21] and with our present results. From the experiments and analysis undertaken in this paper it is not possible to determine the molecular structure of the short lived contact ion pairs between FS spin probes and PDADMAC monomeric units. Results of experiments on shock-frozen solutions, which allow for such characterization, will be published elsewhere.[20]

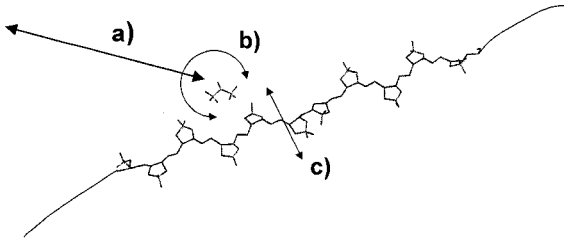


Figure 5. Sketch of the domain of an FS spin probe electrostatically attached to a PDADMAC chain. This is meant as a snapshot of the state when the spin probe is very close to the chain. Three dynamic processes are important:
a) attachment – detachment dynamics;
b) rotational dynamics of FS spin probe;
c) motion of PDADMAC polyelectrolyte chains.
A fully stretched polymer conformation is not implied.

The CW EPR spectra discussed here depend on local concentration and rotational correlation time of the spin-carrying counterions. A qualitative or semi-quantitative discussion of their

dependence on R should thus consider the counterion concentration gradient in the vicinity of the chains and the three dynamic processes indicated in Figure 5:

a) attachment/detachment of counterion probes due to translational diffusion,

b) rotational diffusion of freely diffusing spin probes, and

c) reorientation of attached counterion probes due to polyelectrolyte chain dynamics.

While full quantification of such a model is beyond the scope of this work, we shall try to identify which of the processes dominate spectral lineshapes in which regimes of solvent viscosity and polyelectrolyte concentration.

Data Interpretation: FS and PDADMAC

It has been investigated whether the influence of solvent composition on polyelectrolyte chain conformation induces changes in the dynamic electrostatic attachment of probe ions to the polyelectrolyte.[7] In solvent mixtures such as 70 vol.-% NMPA/30 vol.-% water, which has a relative permittivity higher than pure water ($\varepsilon_r \approx 140$ at 293 K, pure water: $\varepsilon_r \approx 80$ at 293 K), or 50 vol.-% ethanol/50 vol.-% water, which has a relative permittivity lower than pure water ($\varepsilon_r \approx 50$ at 293 K), analysis of the EPR lineshapes remarkably gave very similar results, while the results in pure water deviated strongly from those of the two aforementioned systems. This indicated that relative permittivity is not the most important solvent property in our system.[7] Consistent with our data, we concluded that only in water a polyelectrolyte chain conformational transition (as a second-order type transition) takes place from an extended conformation to a more globular conformation. We attributed the absence of such a counterion-induced chain collapse in the solvent mixtures of water and organic solvent with both higher and lower permittivity to screening of intramolecular hydrophobic interactions between repeat units of the polyelectrolyte chains by the organic solvent, which preferentially solvates the polyelectrolyte backbone.

Against this background we now compare the low-viscosity system in NMPA/water and the higher viscosity system in glycerol/water (Figure 2).

It is obvious that for NMPA/water (Figure 2a) $<T_{2,k}>$ decreases for all k with increasing R and reaches a plateau value for $R > 0.067$. This can be explained as follows: Assuming that over the long run all divalent probe ions partake in the dynamic attachment to the polyelectrolyte,[7] the average amount of charged PDADMAC repeat units per probe ions decreases with increasing R. Thus both the average local concentration and the heterogeneity of environments (i.e. the concentration gradient from the polyelectrolyte chains towards the free solution) increase with increasing R. The increase in local concentration is accompanied

by spin-exchange coupling starting to dominate line broadening.[16] Increased heterogeneity is exhibited in an increasing deviation of the stretch factor x from unity, the distribution of relaxation times becomes broader, the stretch factor deviates more strongly from unity (asterisks in Figure 2c).

In contrast, the acceleration of rotational diffusion with increasing R (asterisks in Figure 3) causes a narrowing of the lines which is most pronounced for the high-field line and least pronounced for the center line. Indeed, the decrease of $\langle \tau_c \rangle$ is expected for a dynamic equilibrium between the slowly reorienting attached state and the fast reorienting detached state, as larger R and thus lower polyelectrolyte concentration correspond to a smaller fraction of time spent in the attached state. The monotonic decrease of all the $<T_{2,k}>$ with increasing R shows that in NMPA/water Heisenberg exchange broadening dominates over dynamic broadening for all lines.

The situation is slightly different for the system in glycerol/water (Figure 2b). Trends similar to those in NMPA/water are observed only for the stretch factor x and the averaged relaxation time $<T_{2,2}>$ of the center-field line, which is the line least affected by changes in the rotational correlation time.[16] The decrease in relaxation time in the range $0 < R \leq 0.067$ for this line still reflects increased spin-exchange coupling and local concentration of spin probes, the same behavior as found for NMPA/water as solvent. The plateau observed at very low R values with the decrease starting only after the third data point can be attributed to the fact that at our lowest R values polyelectrolyte concentration is so high that we are in an *overlap regime* with respect to the polyelectrolyte chains. Local counterion probe concentration approaches the bulk concentration in this regime.

The trend for the stretch factor x (filled circles in Figure 2c) also indicates similar behavior in glycerol/water as in NMPA/water. Center-field relaxation times $<T_{2,2}>$ and stretch factors x thus indicate that PDADMAC chain conformation in glycerol/water is similar to that in NMPA/water and that no conformational change takes place in the studied range of concentrations.

Exchange broadening is expected to depend on viscosity, as the frequency of molecular collisions depends on the diffusion coefficient. For free diffusion and assuming that the Stoke-Einstein relation holds, the exchange frequency and thus also the linewidth is proportional to the inverse viscosity, so that $<T_{2,2}>$ should be proportional to the viscosity.[13] However, what we observe is a shorter $<T_{2,2}>$ (i.e. larger linewidth) in the glycerol/water mixture (higher viscosity) than in the NMPA/water mixture (lower viscosity) as well as a larger deviation of the stretch factor x from unity. In particular the latter finding indicates that

slower diffusion in the more viscous solvent causes build-up of a larger concentration gradient, so that local counterion concentration in the immediate vicinity of the polyelectrolyte chain would also be larger.[22] The collision frequency is proportional to local concentration, and the concentration increase may thus compensate or even overcompensate the decrease due to slowing down of translational diffusion. Note however, that part of the stronger broadening of the center line is due to slower rotational diffusion.[15, 23, 24]

The trend for the low-field ($<T_{2,1}>$) and high-field $<T_{2,3}>$ averaged relaxation times in glycerol/water clearly differs from the one in NMPA/water. In the glycerol/water mixture these linewidths are thus dominated by slow rotational diffusion. Consequently this solvent mixture is better suited for characterizing dynamic attachment, while the NMPA/water (or ethanol/water) mixture is better suited for characterizing the concentration gradient.

The larger sensitivity of the glycerol/water mixture to dynamics rather than concentration gradients is manifest in the plot of $\langle \tau_c \rangle$ (Figure 3). In the R-range from 0 to 0.067 we obtain good fits for a linear dependence of $\langle \tau_c \rangle$ on R for both solvent systems but the slope of the decay for glycerol/water is sixfold as compared to the one for NMPA/water. It is also apparent that the slowdown of rotational diffusion in glycerol/water mixtures with respect to NMPA/water is much more dramatic at small R values than in the absence of the polyelectrolyte (slowdown by only a factor of 2.3) or in the presence of only a small concentration of polyelectrolyte. Therefore, this slowdown cannot be attributed to the viscosity effect on rotational diffusion that is described by the Stokes-Einstein relation or a modified version of this theory.[23, 24] Indeed, a stronger dependence of $\langle \tau_c \rangle$ on solvent viscosity is expected for dynamically attached counterions than for freely diffusing counterions for two reasons. First, slower translational diffusion may increase the lifetime of the attached state. Second, influence of the viscosity is much more dramatic for the polyelectrolyte molecule with its large radius of gyration than for a small probe. Residual motion in the attached state is thus dramatically reduced.

TAM and PDADMAC

The TAM spin probe is trivalent, much larger, and tristar-shaped with a large separation of the ionic groups (~1.1 nm) , so that direct comparison between data with TAM and FS as spin probe is not possible. On the other hand, linewidths are determined exclusively by exchange broadening for this probe, which increases reliability of conclusions on concentration gradients.

In fact, the results for this probe (Figure 4b,c) confirm our conclusions on exchange broadening for the FS probe. The trends for averaged relaxation times and stretch factors are similar in both solvents, water and glycerol/water. As in the case of FS, the decrease of $<T_2>$ and the larger deviation of the stretch factor x from unity point to build-up of a larger concentration gradient in the higher-viscosity solvent compared to the lower-viscosity solvent. However, both effects are more dramatic for the larger TAM probe and slowdown of rotational diffusion cannot explain the strong decrease in $<T_2>$ in this case. For TAM, the data thus prove rather than only indicate that the effect of an increase in local concentration in the vicinity of the polyelectrolyte on the exchange frequency overcompensates the effect of a decrease in the translational diffusion rate.

There is a sharp transition of $<T_2>$ from a steep decrease to a plateau when increasing R above ≈ 0.03. This may correspond to a transition from a strong *overlap regime* that is dominated by TAM molecules building physical crosslinks between PDADMAC chains ($R < 0.03$) to a regime in which TAM molecules interact with single polyelectrolyte chains and force the chains to form globular structures: each TAM by effectively screening three charges of the polymer and bending the chain in one domain, or by crosslinking remote domains of one single chain. This may lead to an approach to a maximum local concentration of TAM counterions that can accommodated by the chain. A further decrease of the polyelectrolyte concentration might then lead to the appearance of a fraction of counterions that interacts more weakly with only the surface of the crosslinked PDADMAC globules and thus gives rise to a spectral component with a smaller linewidth as seen at $R = 0.1$.

Conclusions

The viscosity dependence of the EPR spectra of counterion probes in polyelectrolyte solutions of varying concentration confirms our model of counterion condensation as a fast dynamic process with lifetimes of the contact ion pairs being shorter than 1 ns. While EPR lineshapes of nitroxide counterion probes in low-viscosity solvents and solvent mixtures are dominated by exchange broadening due to increased local concentration in the vicinity of the polyelectrolyte chain, they are more strongly influenced by slowdown of rotational diffusion in the high-viscosity solvent glycerol/water. The dramatic effect of an increase in polyelectrolyte concentration on the rotational correlation time can be explained by a longer lifetime of the attached state due to slower translational diffusion combined with much slower dynamics in the attached state due to the slowdown of polymer chain dynamics. Analysis of the viscosity dependence of the exchange broadening for both the FS and TAM spin probes

indicates that slowdown of translational diffusion in more viscous solvents causes the build-up of a larger counterion concentration gradient in the vicinity of the chain.

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