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Cooperative Interactions of Unlike Macromolecules 2: NMR and Theoretical Study of Electrostatic Binding of Sodium Poly(styrenesulfonate)s to Copolymers with Variably Distributed Cationic Groups

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Cooperative electrostatic binding of sodium poly(styrenesulfonate)s (PSS) of varying chain length to poly[diallyl(dimethyl)ammonium chloride-*co*-acrylamide] (DADMAC-AA) copolymers with varying charge density were investigated using ^{1}H, ^{23}Na, and ^{35}Cl NMR spectra and relaxations and ^{1}H pulsed-gradient stimulated echo (PGSE) self-diffusion experiments. First, DADMAC-AA copolymers were shown to exhibit increasing chain rigidity and tighter counterion binding both with increasing charge density and progressive dilution by D_2O. These effects can be alleviated by increasing the ion strength of the solution and are thus mainly due to the polyelectrolyte effect. Second, *p*-toluenesulfonate as a model of a lone PSS unit was shown to bind to DADMAC-AA to a less-than-statistical degree α indicating thus a positive single-group increment of binding Gibbs energy for PSS, $\Delta G^{(1)} > 0$. Third, PSS of various chain lengths were found to bind to DADMAC-AA to a larger-than-statistical (mostly almost quantitative) α, exhibiting thus an intramolecular cooperativity of the second (or true) kind. In the cases of lower cooperativity (short PSS and DADMAC-AA with low charge density), $\alpha \approx 0.6$ and is lowered to 0.35 when increasing the ion strength to 1 M NaCl. For large cooperativity (longer PSS and high-charge-density DADMAC-AA), α is somewhat decreased (to $\alpha \approx 0.9$) at low ion strength but increases to almost 1.0 in 1 M NaCl. Mobility studies of the AA sequences in the products obtained at low ion strength, using transverse relaxations and PGSE measurements, indicate the existence of uncoupled regions (rather than single groups), hence non-equilibrium coupling due to the *parking problem*. As increased ion screening by an added electrolyte as well as prolonged storage of the sample increases α to almost 1.0, this behavior can be diagnosed as a *pseudo*-irreversible or kinetic effect.

1. Introduction

Cooperative binding has been recognized as a crucial factor in the spontaneous self-assembly of macromolecules into more or less ordered aggregates[1−4] and formal aspects of cooperativity have been studied accordingly.[5−11] However, detailed understanding of structural and other factors of cooperativity is lacking in most cases. Among the interactions, which can be operative in macromolecular binding, the electrostatic (ionic) interaction appears to be the simplest due to its relative insensitivity to structural details and relatively long-range effect. A recent study[12] of cooperative ionic coupling of linear polyphosphates to a block copolymer bearing densely distributed trimethylammonium cations showed a rather complex interplay of various factors, however. The most important result is that the stabilization energy decreases with increased coupling degree and has to be considered a counter-cooperative factor, whereas entropy and to a lower degree kinetic and phase factors appear to be the driving force. To clarify this question further, we turn in this study to polycations with variably distributed cationic groups, namely, the statistical copolymers of diallyl(dimethyl)ammonium chloride with acrylamide. These copolymers prepared by a feeded radical polymerization[13] were shown[14] to interact readily with poly(styrenesulfonate)s, although their structure can be shown to preclude direct contact of the majority of the complementary ionic groups. To get a deeper insight into the structure and internal mobility of the resulting coupling products, multinuclear NMR has been chosen as the chief method of investigation.

2. Experimental Section

2.1. Materials. The statistical copolymers of diallyl(dimethyl)ammonium chloride (DADMAC) with acrylamide (AA) named, according to the molar percentage of DADMAC, COP8 to COP73, were prepared by a feeded radical copolymerization of the comonomers.[13]

Sodium poly(styrenesulfonate) (PSSNa) was purchased from Polymer Standard Service, Mainz, Germany ($P_n = 1640$) and from Polymer Source, Inc., Lajoie, Canada ($P_n = 66$ and 240). PSS samples being of high purity and low polydispersity were used as purchased.

2.2. Preparation of Solutions of Polyelectrolytes and Aggregates. Both types of polyelectrolytes were dissolved to 1% w/w in 99.6% D_2O. For diluted solutions or coupling experiments, the solutions were diluted 50 times with D_2O or NaCl/D_2O solutions (0.02 to 2 mol NaCl/L). In coupling experiments, the equivalent amount of the PSSNa solution was added slowly (in 2−5 min) to a vigorously magnetically stirred COPX solution.

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2.3. NMR Measurements. ^1H, ^{23}Na, and ^{35}Cl NMR spectra and relaxations were measured with an upgraded Bruker Avance DPX300 spectrometer (^1H resonance frequency 300.13 MHz), using a multinuclear broad-band probehead for ^{23}Na and ^{35}Cl, or inverse z-gradient multinuclear probehead for ^1H spectra and PGSE experiments in extremely diluted solutions. In ^1H spectra, residual H_2O signal had to be suppressed using the Watergate pulse sequence[19,20] attached to all other sequences used. All methods of zero, single, and multiple quantum coherence relaxation measurement and evaluation were described in the previous study.[12] PGSE experiments were done using the Stejskal−Tanner sequence[21] with Δ delays constant in one experiment ($\Delta = 30$, 40, 50, 60, and 70 ms) and variable z-field gradient (5 ms pulses of 12.5 to 50 G cm^{-1}). Pseudo-solid-echo decays were measured using the Cohen−Addad−Schmidt sequence.[18]

3. Results and Discussion

3.1. Structure and Mobility of the DADMAC-AA Copolymers. The copolymers used (named COPX throughout the text, X being the molar percentage of DADMAC) were extensively characterized elsewhere,[13] including ^{13}C NMR spectra. As the study of their coupling with sodium poly(styrenesulfonate) (PSSNa) in the 1:1 ion ratio required extreme dilution in D_2O, ^1H, ^{23}Na, and exceptionally ^{35}Cl NMR spectra were attainable on sensitivity grounds. Figure 1 shows ^1H NMR spectra of the copolymers at 1.0% w/w in D_2O, and Figure 2 shows those of 0.02% w/w solutions in D_2O and in 0.2 M NaCl/D_2O. The signals were assigned using 2D COSY-DQF, TOCSY, and NOESY techniques, and their numbers correspond to Scheme 1 except 1*, which belongs to equivalent methyl groups in the *trans*-bonded DADMAC group. Signal 6 (CH_2 of the AA groups) is split into two to three broad signals in proportions varying with the copolymer composition as well as conditions such as concentration and ionic strength. As all the copolymers were prepared by the same polymerization mechanism, this splitting reflects chemical and conformational microstructure rather than stereoregularity of the AA sequences. NOESY spectra of 1% solutions of COP8, COP47, and COP73 strongly indicate that the downfield part of signal 6 belongs to AA-CH_2 groups attached or near to the DADMAC groups.

The very apparent feature of these spectra is the change of both shape and intensity of individual signals with changing DADMAC content, dilution, and ion strength of the solution. The broadening of signals, which is the cause of most shape as well as intensity changes, is due to residual static dipolar interactions if substantial motional hindrances are present. Such dipolar broadening $\delta\omega$ has been found[15] to be approximately

$$\delta\omega \approx {\omega_D}^2\tau_c/[1 + \kappa^2{\omega_D}^{-2}{\tau_R}^{-2}] \quad (1)$$

where ${\omega_D}^2$ is the dipolar broadening in the absence of any motion, τ_c and τ_R are the respective correlation times of the local and collective isotropic motions, and κ is an empirical constant of the system (usually between 60 and 150). The dependence of $\delta\omega$ on τ_c and τ_R is sufficiently steep in the vicinity of $\omega_D\tau \approx 1$ to lead to almost *all or nothing* detection of the signal in high resolution (in contrast to solid-state) NMR. The hydrodynamic radius R_H of the copolymers was established[13] to be 37.1−70.7 nm. Taking the well-known Debye relation as an approximation for the rotational correlation time,

$$\tau_R = \pi {R_H}^3\eta/kT \quad (2)$$

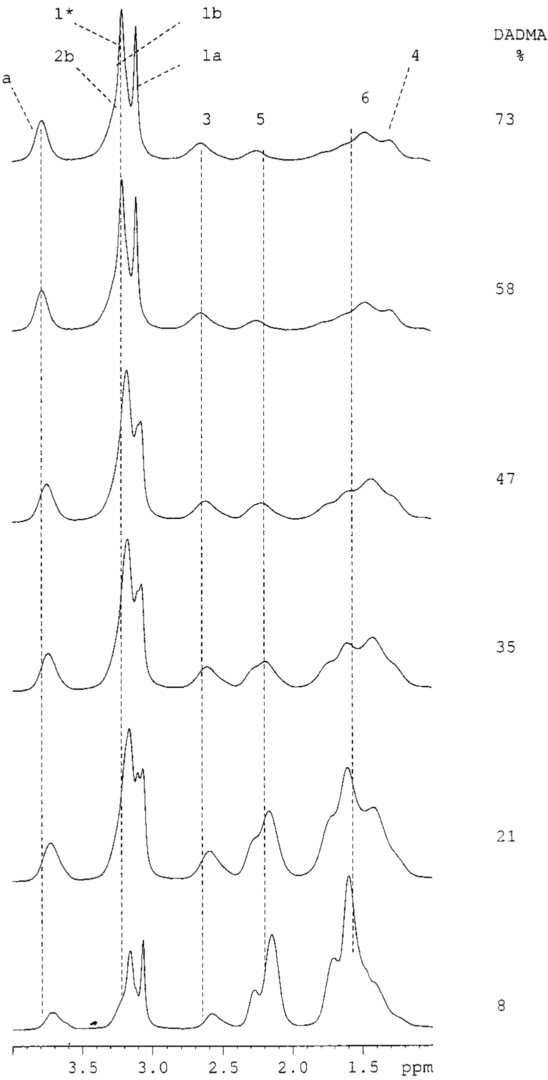


Figure 1. 300.13 MHz ^1H NMR spectra of COPXX copolymers in 1% D_2O solution at 300 K (intensities adjusted).

(where η is the viscosity of the system, k is the Boltzmann constant, and T is temperature) we have to conclude that the residual static dipolar interactions ω_{Di} of the nuclei, which do not contribute to the detectable signal at 300 K, must exceed 3 kHz. This points to a substantial local or semilocal immobilization of the molecule in their vicinity. The local as well as collective motion can be slowed or hindered by (i) specific polyelectrolyte effects, (ii) inter- and intramolecular association, and (iii) local conformation constraints due to stiff or bulky groups. Among (i), the relative chain stiffening due to increased charge and/or dilution and ion strength decrease has been observed earlier.[16] Intensity changes due to (ii) (extreme broadening of a part of the signal) are generally known (see, for example, ref 17). Local constraints usually need collaboration of either (i) or (ii) for their influence on the signal intensity. In the present case, all factors (i) to (iii) are present. Figure 3 shows the dependence of T_2 (leading terms in a generally multiexponential decay) corresponding to the methyl signals of the ionic groups, neutral monomer units (CH_2) as well as Cl^- counterions. This behavior shows an increased polyelectrolyte nature of the copolymer with increased DADMAC content, corroborated by increased conformational restraints due to stiff cation-bearing groups.

In ^{35}Cl spectra, the increase in quadrupolar relaxation rate (or the decrease in the corresponding relaxation time) indicates[16]

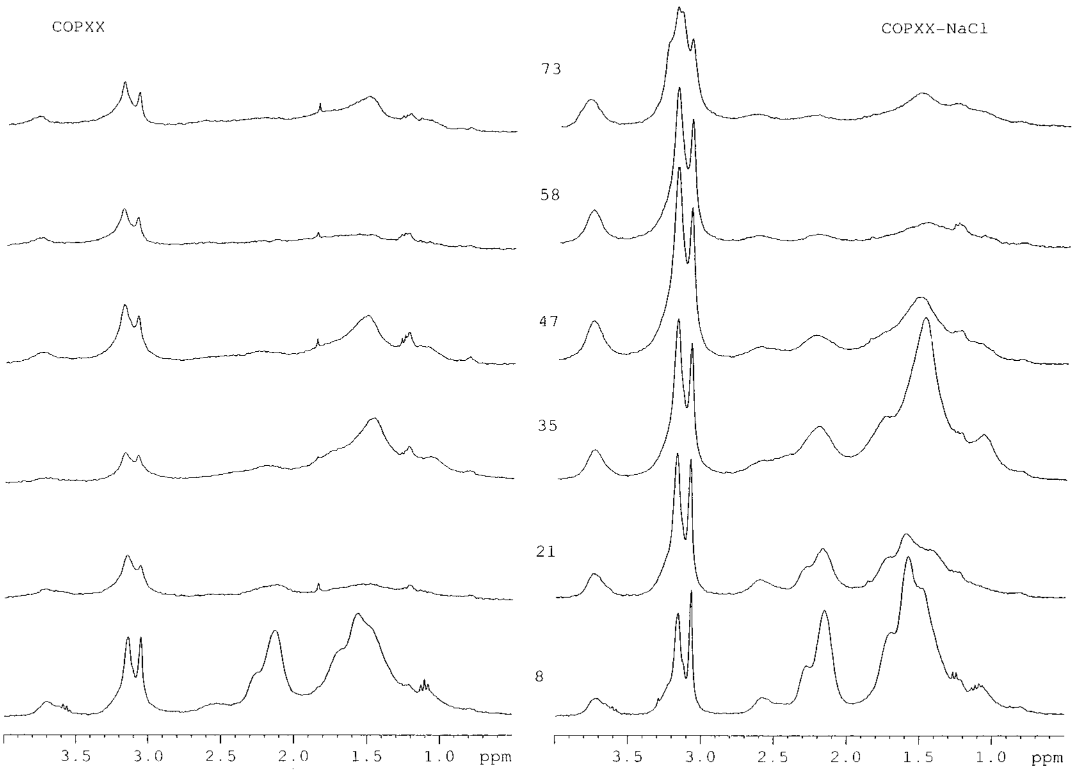


Figure 2. ^{1}H NMR spectra of COPXX copolymers in 0.02% D_2O and 0.2 M NaCl/D_2O (genuine intensities.)

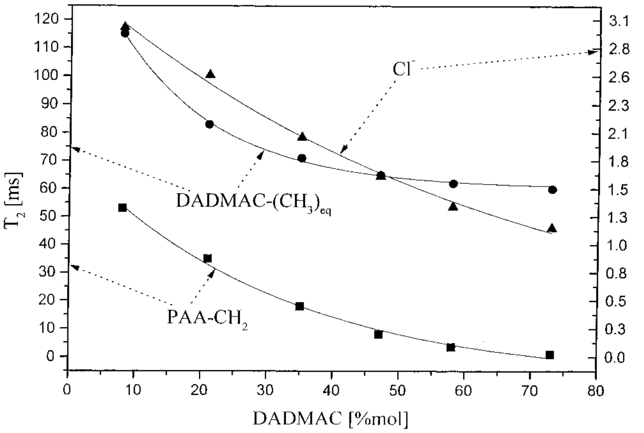


Figure 3. Transverse relaxation times T_2 of the ^{1}H (CH_3 and CH_2) and ^{35}Cl resonances in 1% D_2O solutions of COPX at 300 K.

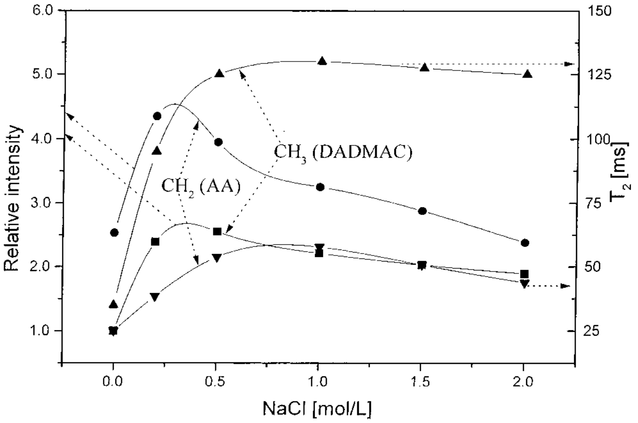
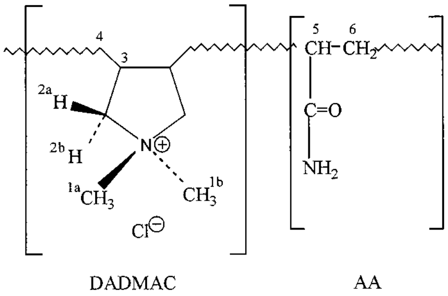


Figure 4. Relative intensities and T_2 values of CH_3 and CH_2 ^{1}H NMR signals in 0.02% solutions of COP47 in different NaCl/D_2O solutions at 300 K.

SCHEME 1



tighter binding of the counterions to a higher charged (and thus progressively stiffer) polymer. With increasing amounts of DADMAC, the charge distance decreases below the Bjerrum length leading to counterion condensation and thus tighter average binding of Cl^- ions. According to the theory of Halle and Piculell,[22,23] the main effect in counterion transverse relaxation is brought about by the ion diffusion between polyions molecules rather than by their residence in the vicinity of the polyion. The relaxation rate (or its inverse, the relaxation time) depends on the probability of counterion diffusion, its correlation time, the quadrupolar interaction, and an ordering parameter. Its dependence on the average ion distribution is thus less straightforward but still existing.

In ^{1}H spectra, the observed decrease in T_2 should be due to increasing residual static dipolar interactions as documented by pseudo-solid-echo (PSE) experiments,[18] which give T_2 values generally larger than ordinary CPMG sequences. Because most of the dipolar-coupled nuclei do not contribute to the detected signal, the PSE decays cannot be analyzed unambiguously. Unfortunately, MAS NMR spectra, which could reveal them, are out of question on sensitivity grounds. However, charge screening by an increased ionic strength of the solution gradually increases their mobility and thus visibility of their resonance to high-resolution NMR. As an example of this effect, Figure 4 shows the dependence of intensity and corresponding T_2 values

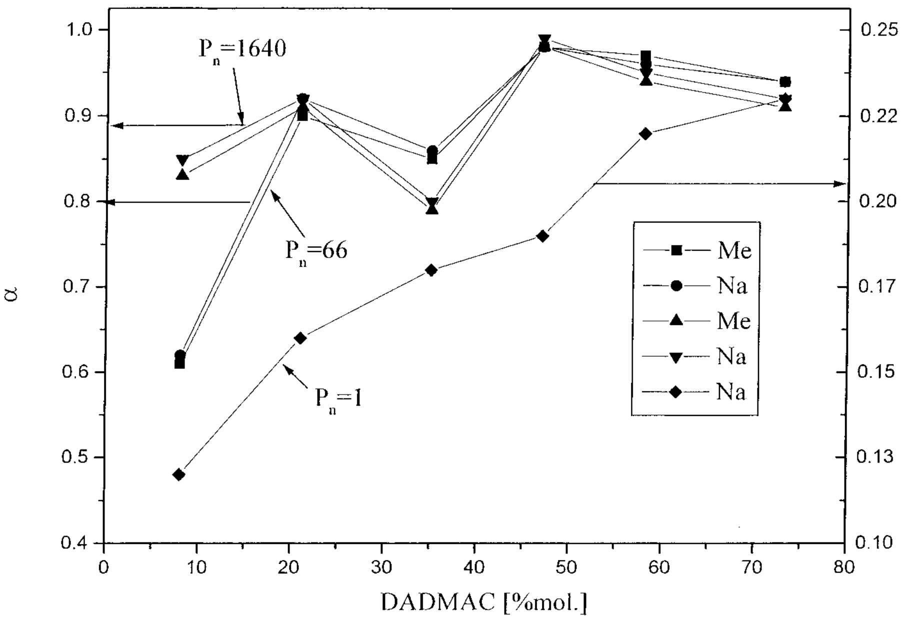


Figure 5. Coupling degrees α achieved in mixing the 0.02% D_2O solutions of COPX copolymers with equivalent amounts of PSSNa (P_n = 66, 1640) or PTSNa (P_n = 1) in D_2O determined from CH_3 signal intensity (Me) and ^{23}Na relaxation (Na).

of signals 1* and 5 in a 0.02% solution of COP47 in NaCl/D_2O solution on the electrolyte concentration. Increasing signal intensities as well as T_2 values with the increasing ionic strength point a gradual mobilization of the corresponding groups. The apparent delays in the increase in T_2 values (leading terms in biexponential fitting) as compared to the corresponding signal intensities must be due to the groups just liberated from relative immobilization and thus increasing the minority of fast-relaxing but observable nuclei. The slight decrease of both intensity and T_2 of signal 5 (and, to a lower degree, signal 1*) at higher NaCl concentrations points to partial association of PAA-rich segments of the chain (salting-out of PAA); the same phenomenon can be observed at elevated temperatures, at which PAA partly loses its hydrophilic nature. The behavior of the other copolymers is similar, only less dramatic.

In general, we can conclude that the copolymers under study (i) exhibit increasing polyelectrolyte behavior as well as chain rigidity with increasing DADMAC content, in connection with it and (ii) exhibit relatively lose chain flexibility and decreased ionization with extreme dilution, but (iii) their flexibility can be restored by a moderately increased ionic strength of the solution (0.5 to 1.0 M NaCl).

3.2. Coupling of the Polyions with Low-Molecular-Weight Analogues. In contrast to high molecular weight poly(styrenesulfonate)s (PSSNa, see below), sodium *p*-toluenesulfonate (PTSNa) added in an equivalent amount to 1.0 or 0.02% solution of COPX (X = 8−73) has no visible effect on their ^{1}H NMR spectra. Also the signals of PTS (aromatic protons) have unchanged chemical shifts, the only difference being 8−16% rel. decrease (almost in the limits of experimental error) of the corresponding T_2 values. The degree of PTSNa coupling to COPX was established from ^{23}Na T_1 relaxation time, using the fast-exchange relation[12] $\alpha = [R_{1(\mathrm{PTS})} - R_1]/[R_{1(\mathrm{PTS})} - R_{1(\mathrm{NaCl})}]$, where R_1, $R_{1(\mathrm{NaCl})}$, and $R_{1(\mathrm{PTS})}$ are the respective longitudinal relaxation times of the actual sample, NaCl and PTS, at the equivalent concentration. The obtained α values (0.12−0.23) are shown in Figure 5 together with those of high molecular weight PSSNa. Conversely, the coupling of tetramethylammonium bromide (equivalent amount) with PSSNa (P_n = 66, 1640) was investigated by the same method. The coupling degree calculated from an analogous relation $\alpha = [R_{1(\mathrm{PSS})} - R_1]/[R_{1(\mathrm{PSS})} - R_{1(\mathrm{NaBr})}]$, was about 0.32, irrespectively of the polymerization degree of PSS. As both values do not reach even the statistical value 0.5, we can conclude that the single-coupling change of the Gibbs energy $\Delta G^{(1)} > 0$, i.e., the cooperativity of coupling, found below for the high molecular weight reactants, is not given by sheer summation of $\Delta G^{(1)}$ but is of a second, or true, kind (for the discussion of this term, see Appendix).

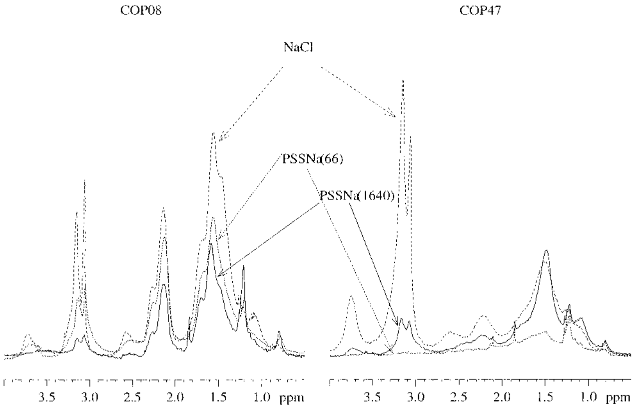


Figure 6. ^{1}H NMR spectra of the parent copolymers COP8 and COP47 in 0.2 M NaCl/D_2O and their complexes with two kinds of PSSNa (P_n = 66, 1640) in D_2O.

3.3. Coupling of the Copolymers with Poly(styrene sulfonates). In a recent study,[14] ready coupling of PSSNa (P_n = 1640) with COPX (X = 8−73) has been observed, and most compact structures of the aggregates for equimolar mixing of the complementary ions could be deduced from the viscosity of their solutions. Following these results, we have studied the equi-ionic systems at 0.02% w/w of the reactants in D_2O. As the dilution is rather extreme, considering the width of the signals, only ^{1}H and ^{23}Na spectra could be measured, using sensitive probeheads and accumulating 800 or more scans. In ^{1}H spectra, even the residual signal of H_2O was too intensive to be effectively eliminated by mere presaturation, and the Watergate pulse sequence[19] with pulsed z-field gradients had to be used.

Figure 6 shows an example of the ^{1}H NMR spectra of the coupling products of COP8 and COP47 with two kinds of

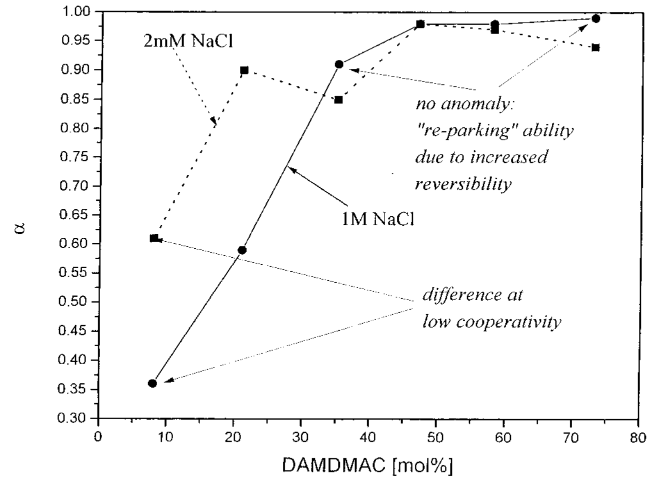


Figure 7. Coupling degrees α achieved in mixing the 0.02% NaCl/D_2O solutions of COPX copolymers with equivalent amounts of PSSNa (P_n = 66).

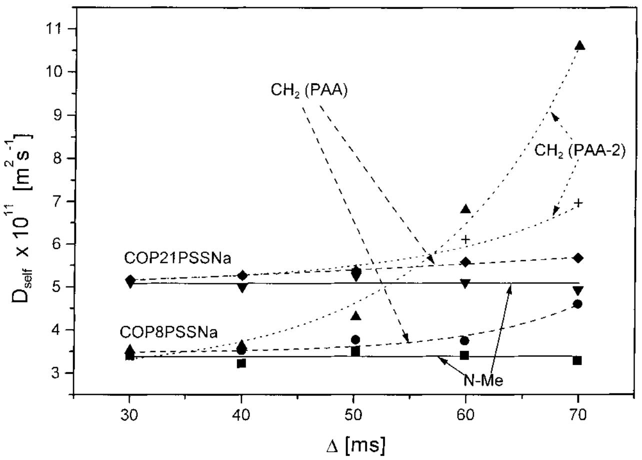


Figure 8. Apparent self-diffusion coefficients obtained by PGSE (monoexponential fitting) for the DADMAC methyl groups (N-Me) and topologically near (CH_2(PAA)) and distant (CH_2(PAA-2)) AA groups in COP8PSSNa and COP21PSSNa at different diffusion delays Δ

PSSNa differing in polymerization degree, as compared with those of the original copolymers (measured at the same concentration in 0.2 M NaCl to reveal full intensities of the signals). As seen, coupling leads to profound change both of intensities and shape of the signals. Again, these changes are due to relative immobilization of the corresponding groups and consequent increased residual static spin interactions. The loss of DADMAC methyl signal (1a,b and 1*) is directly correlated with the coupling degree α as proved by comparison with the results of ^{23}Na relaxation in the same systems. The relaxation method was analogous to that used above, only relaxation in the corresponding PSSNa was compared to that of NaCl and the actual system. Each of the methods used for the determination of α have their drawbacks, but relative agreement between them within 8% rel., documented in Figure 5 for all the copolymers and two different PSSNa, gives a relative certainty that the obtained α values are realistic. As seen in Figure 5, the values of α generally increase with increasing DADMAC content (with relative anomalies for COP35 and COP73) as well as with increasing polymerization degree of PSS. As explained below and in the Appendix, this is a clear sign of true cooperative coupling. The anomalies can be due to three main reasons: (i) local constraints in the structure (self-association, stiff folding), (ii) irreversible coupling in a configuration not optimal for energy minimization (the so-called *parking problem*), and (iii) chemical inhomogeneity of the copolymer. The first kind (and possibly the third one) appears to be operative in COP35 which itself behaves somewhat anomalously in solution (see above). The second kind can be important in the copolymers with higher charge density (COP73), where the coupling is stronger. According to earlier results,[24] highly aggregated frozen structures (far from thermodynamic equilibrium) are formed in pure water, supporting thus the importance of the *parking problem.* This agrees with the less anomalous coupling of COP73 with the much shorter PSS, where the coupling probably is less tight. Both hypotheses are nicely documented in Figure 7, where the coupling of short PSSNa (P_n = 66) is shown in two media: (i) 2 mM NaCl, already freeing a substantial part of the rotational hindrances in the copolymers, and (ii) 1 M NaCl, affecting (by charge screening and equilibrium) the coupling degree and probably the kinetics of coupling, too. Whereas in case (i) the anomalies are only slightly removed and the course of α is virtually unchanged, in (ii) there is a much lower α in the cases where charge density is low and cooperativity is weak (COP08, COP21), but all remaining anomalies are removed including the increase of α almost to 1.0 for COP73. Thus, a partial reversibility of coupling appears to be favorable in the case of strong cooperativity.

3.4. Local Mobility in the Coupling Products. The investigation of local mobility in the coupling products is hampered by the fact that substantial parts of the signals are not detected due to extreme broadening. However, in some cases there is a substantial part of the signal visible and can thus be put to analysis. We have done PGSE self-diffusion measurements of the 1:1 products of PSSNa (P_n = 65) with COP8 and COP21 using the diffusion delay Δ constant in one experiment but varied between 30 and 70 ms in the experiment series. The decays were analyzed as monoexponential despite minor deviations in some curves. The apparent self-diffusion coefficients obtained in these experiments for *N*-methyl groups of DADMAC units (referred to as N-Me), CH_2 groups of AA units near to DADMAC [referred to as CH_2(PAA)] and those of AA units topologically more distanced from DADMAC groups [CH_2(PAA-2)] are given in Figure 8. While the apparent value of D_{self} remains approximately constant for N-Me or changes only slightly for CH_2(PAA), it increases steeply for CH_2(PAA-2) in the case of COP8PSSNa. At the same time, transverse relaxation of both types of CH_2 is polyexponential, with a leading term characterized by $T_{2(1)} = 42$ ms and a slight tail with $T_{2(2)} = 106$ ms. Accordingly, the intensity loss during Δ at zero gradient for both CH_2(PAA) and CH_2(PAA-2) sharply increases with increasing Δ, indicating thus that only a fraction of the particles contribute to the apparent value of D_{self} at higher Δ. As the relative diffusion length $\Delta\lambda_d$ for Δ = 70 ms and the apparent D_{self} of CH_2(PAA-2) relative to N-Me is about 1.3×10^{-6} m, i.e., about 30 times the hydrodynamic radius, there must be some particles richer in acrylamide that are smaller and thus more mobile. This indicates a chemical heterogeneity of COP8PSSNa, which could be partly expected due to its lower coupling degree α but probably also brought about by the parent copolymer. As seen in Figure 8, this effect is much less apparent in COP21PSSNa.

There is a possibility to turn the complicating influence of chemical heterogeneity to an advantage. As faster transverse relaxation (lower T_2) of the CH_2 groups indicate their lower local mobility, we can conclude from the dependences in Figure 8 that products with lower relative content of DADMAC groups

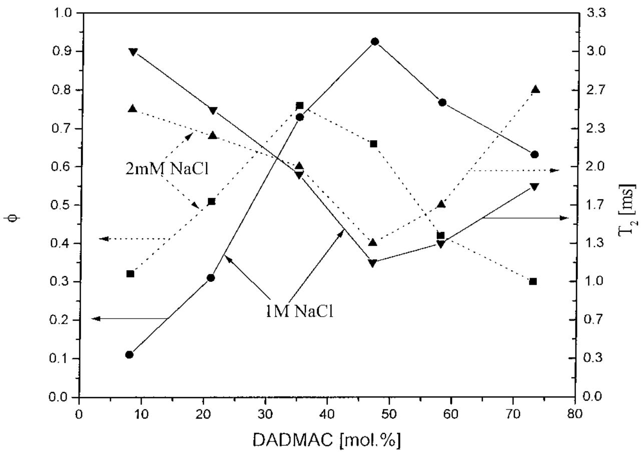


Figure 9. Intensity fractions ϕ of the extremely broadened (invisible) and the time constants T_2 of the fast component in transverse relaxation of the PAA resonance in the complexes of COPXX with PSSNa prepared in two indicated media.

exhibit not only larger collective (translational) but also larger local (conformational) mobility. As this effect can be mostly observed with AA groups topologically distant to DADMAC, the structure of the polycation apparently does not collapse uniformly during ion coupling: the existence of domains with less crowded folding of AA sequences could be surmised.

Because of lower mobility and faster relaxation, no such interesting observation was possible with the other copolymers (even in the reported cases, the number of scans per one point needed in the PGSE measurement grows up to 3000 for the longest Δ). However, the larger α and lower probability of chemical heterogeneity in the respective parent polycations suggest that the heterogeneity effect should be even weaker than in COP21PSSNa.

The only criteria of mobility in coupling products with higher DADMAC content could be the intensity loss (i.e., the part of immobilized groups) and the transverse relaxation rate of the remaining visible resonance. In the latter case, the fast component in the biexponential fitting should be decisive as it probably reflects the transit to completely collapsed part of the structure. Figure 9 shows both these quantities for the acrylamide CH_2 groups in the coupling products obtained in the already mentioned two media, viz. 2 mM and 1 M NaCl. As seen in the figure, the largest part of the collapsed and immobilized structure is obtained for the medium-distributed ionic groups, and also faster relaxation (lower mobility) of the visible signals is obtained here. For the copolymers with highest ion density better alignment (and more effective immobilization) is obtained in a medium with a higher ionic strength. These results parallel those of coupling degrees.

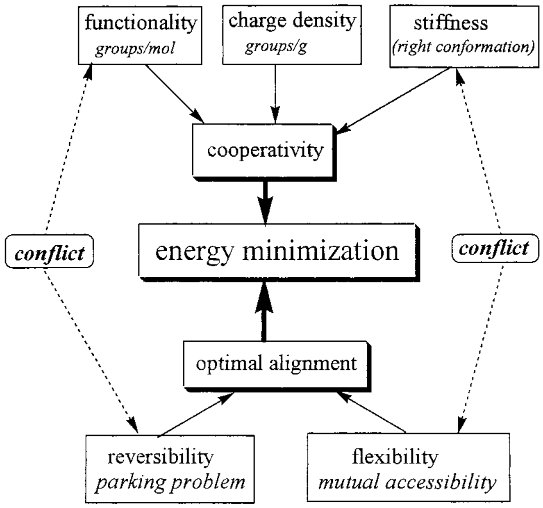
The decrease of local mobility of the neutral groups with increasing coupling degree and density of the coupled groups is quite logical. Nevertheless, the slight relative increase in mobility for the highest DADMAC disturbs this picture. One possible explanation is based on the collective mobility of the aggregate. Equation 2 shows that a two times smaller R_H leads to an eight times smaller τ_R, which in turn could be critical for the resolution of residual static broadening $\delta\omega$ in eq 1, providing that $\delta\omega$ is in the most probable interval 3−5 kHz. In other words, the apparent higher mobility of neutral AA groups in COP73 in principle could reflect the higher collective rather than local mobility. However, the estimation of the dimensions of the mobile aggregates is a complicated problem. As found earlier,[24,25] already in nonstoichiometric systems the level of aggregation and the hydrodynamic radii are very high. In the presence of salt at low mixing ratios, highly swollen particles with a low level of aggregation were formed with COP47, which collapsed at a mixing ratio 0.5 to compact and highly aggregated ones.[23] Subsequent addition of salt to complexes between Cop47 and PSS leads at first to a disintegration into small primary particles.[24] In addition to these phenomena, a strong tendency to secondary aggregation and flocculation at a mixing ratio near 1.0 was observed. Thus, the behavior of the systems is rather complex. Viscosity measurements[14] revealed that COP73 has an exceptional low structural density of the flocs, what may favor the mobility of the primary complex particles. On the other hand, the tendency of the copolymers with higher DADMAC contents to a somewhat lower coupling degree can also indicate less optimal alignment, which could be sensitively detected by the local mobility of AA groups in the product. The reason may consist in the mismatching of the charge distances of the components, which can be better compensated by more flexible chains.

3.5. General Discussion of the Observed Cooperativity of Coupling. Despite the limited scope of the systems studied so far (sodium polyphosphates of different lengths with a densely charged poly[2-(trimethylammonio)ethyl methacrylate chloride]-*block*-poly[*N*-(2-hydroxypropyl)methacrylamide][12] and sodium poly(styrenesulfonate)s of different lengths with variously charge-diluted DADMAC-AA copolymers in this study), some tentative generalizations about the cooperativity of coupling can be made.

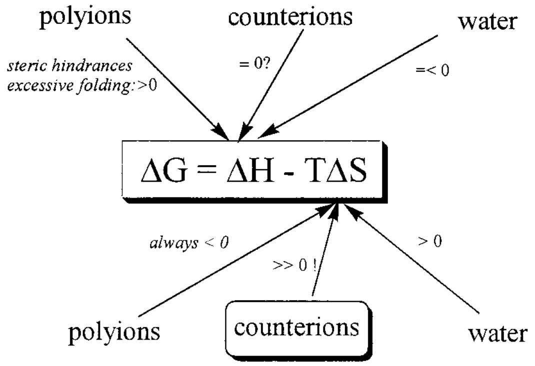
Thermodynamic Effects. We have found in both cases that intramolecular cooperation is of the second, or *true* kind (see Appendix), i.e., the Gibbs energy change of a single coupling $\Delta G^{(1)} > 0$. The coupling degree α generally increases with longer (up to a critical length above which it remains constant) and/or more densely charged polyions except the cases where the intensive and irreversible coupling leads to nonoptimal alignment of the complementary polyions, leaving out some of the ionic groups. This is the so-called *parking problem* (i.e., one misplaced molecule or group sequence precluding successful coupling of the others), which is generally more acute in the case of longer and more readily coupling molecules. Misplacement of polyions can be alleviated by sufficient ion screening (i.e., increasing the reversibility of early couplings). The coupling can be hampered by steric hindrances (due to conformations, folding or intra- or intermolecular association). Despite the impossibility of real contact of all complementary ionic groups, their near-as-possible approach is thus clearly important. Hence, the energy or rather enthalpy, although not being the decisive term in ΔG, has to be minimized. Its factors, recognized in both our studies, are qualitatively summarized in Scheme 2.

However, as discussed in some detail in Appendix, ΔH cannot be the driving force of electrostatic coupling in our cases nor the source of its cooperative effect simply because it is zero at best (actually in our cases, the coupling must be slightly endothermic). As ΔG is decisive, the driving force must be in the entropy term, $T\Delta S$. As illustrated in Scheme 3 and discussed in Appendix, the increment of ΔS brought about by the polyions must be negative. Thus the entropy increase of the small counterions liberated by coupling and of the molecules of water liberated by the increased hydrophobicity of the coupling product is the true driving force of the coupling. This as such has been generally known for many years. However, on the grounds of our studies it can be added that the *cooperativity* of coupling must be due to a *larger* entropy change per one

SCHEME 2



SCHEME 3



counterion for a *longer* or *more densely charged* polyion: as shown here and elsewhere[12,16] by relaxation studies, the counterions are more tightly bound and thus restricted in their position in these cases due to the polyelectrolyte effect.

However decisive for coupling and its cooperativity, the entropy term is always in balance with enthalpy as illustrated in Scheme 3. Thus, details of structure and mobility of the polyions precluding an effective alignment of the polyions can upset the balance and take over the cooperative or counter-cooperative role.

Kinetic Effects. In all systems studied by either of us, the electrostatic coupling produced more hydrophobic structures usually aggregated into some kind of micro- or nanophase. Hence, the coupling has to be considered to be irreversible (at least in some stage), and kinetic effects thus play an important role in the process. However, there seems to be no kinetic effect in our observations contrary to those already listed as the thermodynamic ones. In particular, the restricted ability of the polyion to align optimally to its partner, which can be the source of the *parking problem,* can be largely a kinetic effect.

Coarse-Grain Thermodynamic (Phase) Effects. The just mentioned aggregation of the products into nanoparticles based on (i) optimal electrostatic interaction (the scrambled-egg arrangement) and (ii) hydrophobicity of the coupled part of the structure not only increases the importance of the kinetic effects but brings about additional thermodynamic effects. Among them, perhaps the most important is an additional entropy gained by reducing the specific surface of the product and thus the area where the motion of water molecules is restricted. The potentially serious importance of this effect is difficult to evaluate and in particular relate to the polyion structure.

In conclusion, we probably recognized the main thermodynamic, kinetic, and phase effects influencing the cooperativity of electrostatic coupling of polyions. At the same time, we have to admit that much more research has to be done for their quantitative evaluation, in particular in relation to the structure of the polyions.

4. Conclusions

In this study, cooperative electrostatic binding of sodium poly(styrene sulfonate)s (PSS) of varying chain length to poly[diallyl(dimethyl)ammonium chloride-*co*-acrylamide] (DADMAC-AA) copolymers with varying charge density was demonstrated.

First, DADMAC-AA copolymers were shown to exhibit increasing chain rigidity and tighter counterion binding both with increasing charge density and progressive dilution by D_2O. These effects can be alleviated by increasing the ion strength of the solution and are thus mainly due to the polyelectrolyte effect. DADMAC-AA copolymers with higher charge density thus restrict the positions of their counterions to a larger degree offering thus larger entropy gain when releasing them into solution after coupling with PSS.

Second, *p*-toluenesulfonate as a model of a lone PSS unit was shown to bind to DADMAC-AA to a less-than-statistical degree α. The same was found for tetramethylammonium chloride as a rather approximate model of a cationic group in the DADMAC-AA copolymer. These results thus indicate a positive single-group increment of binding Gibbs energy, $\Delta G^{(1)} > 0$, for both PSS and DADMAC-AA.

Third, PSS of various chain lengths were found to bind to DADMAC-AA to a larger-than-statistical (mostly almost quantitative) α, exhibiting thus an intramolecular cooperativity of the second (or true) kind. In the cases of lower cooperativity (short PSS and DADMAC-AA with low charge density), $\alpha \approx 0.6$ and is lowered to 0.35 when increasing the ion strength to 1 M NaCl. For large cooperativity (longer PSS and high-charge-density DADMAC-AA), α is somewhat decreased (to $\alpha \approx 0.9$) at low ion strength but increases to almost 1.0 in 1 M NaCl. Mobility studies of the AA sequences in the products obtained at low ion strength, using transverse relaxations and PGSE measurements, indicate the existence of uncoupled regions (rather than single groups), hence nonequilibrium coupling due to the *parking problem*. As increased ion screening by an added electrolyte as well as prolonged storage of the sample increases α to almost 1.0, this behavior can be diagnosed as a *pseudo*-irreversible or kinetic effect.

As $\Delta G^{(1)}$ for both polyions is clearly positive, the sum of higher-order contributions to Gibbs energy change $\Sigma\Delta G^{(n)}$ must be large and negative, i.e., the cooperativity in the studied case must be of the real (or higher-order) kind (see Appendix). Our results indicate that this is due to $\Sigma\Delta S^{(n)} > 0$, the entropy gain being achieved mainly by the released small counterions. This has been surmised for many years. Our finding that this effect increases with increased ion density and, for shorter chains, with increased functionality (or length) of the polyions, i.e., with denser and more restricted counterion distribution, gives additional evidence to this hypothesis.

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Appendix: Simplified Thermodynamic Model of the Cooperative Coupling

Cooperativity of interactions can be either (i) intermolecular (numerous molecules interacting with the matrix) or (ii) intramolecular (numerous groups of the same molecule interacting with the matrix). Let stand ΔG for the Gibbs energy (equilibrium) or activation free energy (kinetics) of the process and restrict the discussion to the type (ii), i.e., that of n groups of the same molecule coupling with the matrix. ΔG can be partitioned into contributions of group singles Δg_i (i.e., the hypothetical contribution of the isolated coupling of the ith group), doubles Δg_{ij} (i.e., the *additional* contribution brought about by the fact that *both* ith and jth groups coupled), triples Δg_{ijk}, etc. Having n groups in the same molecule interacting with the matrix, we thus have (assuming that the groups need not follow in a row):

$$\Delta G = \sum_{i=1}^{n} \Delta g_i + \sum_{i=1}^{n-1}\sum_{j=i+1}^{n} \Delta g_{ij} + \sum_{i=1}^{n-2}\sum_{j=i+1}^{n-1}\sum_{k=j+1}^{n} \Delta g_{ijk} + ... \qquad \text{(A1)}$$

providing that we allow for not properly ordered n-tuples. Some of the terms in eq A1 (e.g., for $j \gg i+1$) can be very small. For macromolecules without gross mismatch of their complementary ions and thus coupling in a ladder-like manner, it is thus perhaps more natural to count only properly ordered n-tuples, viz.

$$\Delta G = \sum_{i=1}^{n} \Delta g_i + \sum_{i=1}^{n-1} \Delta g_{i,i+1} + \sum_{i=1}^{n-2} \Delta g_{i,i+1,i+2} + ... = \Delta G^{(1)} + \Delta G^{(2)} + \Delta G^{(3)} + ... \qquad \text{(A1a)}$$

We can have *first-order* (or apparent) cooperation if $\Delta G = \Delta G^{(1)}$ and for most of the groups $\Delta g_i < 0$ so that $\Delta G << \Delta G^{(1)}/n$, i.e., the coupling of a collection of groups is much more effective than that of any of the individual groups even though there is no mutual assistance between them. In the *higher-order* (or real) cooperation,

$$\sum_{j=2}^{n} \Delta G^{(j)} < 0$$

so that $\Delta G < \Delta G^{(1)}$.

Using $\Delta G^{(j)} = \Delta H^{(j)} - T\Delta S^{(j)}$, we have to consider both the enthalpy and the entropy j-order contributions. For the direct enthalpy term for electrostatic coupling, we have to consider the spatial probability densities $\varphi_i(\mathbf{r})$, $\psi_j(\mathbf{r})$ of the ith anion and jth cation on the polymers, respectively, and the respective spatial densities $\rho(\mathbf{r})$, $\sigma(\mathbf{r})$ of countercations and counteranions. Their respective j-order changes are $\Delta\varphi_i(\mathbf{r})^{(j)}$, $\Delta\psi_j(\mathbf{r})_j^{(j)}$, $\Delta\rho(\mathbf{r})^{(j)}$, $\Delta\sigma(\mathbf{r})^{(j)}$. In principle, $\Delta H^{(j)}$ must have all the contributions $\kappa\int q^2 d\mathbf{r}_i d\mathbf{r}_j\, \Delta\mu^{(j)}(\mathbf{r}_i)\Delta\nu^{(j)}(\mathbf{r}_j)/\epsilon_{ef}|\mathbf{r}_i - \mathbf{r}_j|$, where μ and ν stand for φ, ψ, ρ, σ and $\kappa = +1$ or -1 for attractive or repulsive interactions, respectively. In complexes with a gross mismatch of complementary ions, most distances between them $R_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$ could be rather large even if optimized in a *scrambled egg* manner. Even then, vanishing contribution $\Delta H^{(j)}$ does not necessarily follow considering the $1/R_{ij}$ long-range nature of the interaction and, in particular, the value of the effective permittivity ϵ_{ef}, which can easily be 10 times lower than that of water. Our results indicate that $\Delta H^{(j)}$, which depends on the nature of the ionic groups as well as their ability to align optimally, is an important contribution.

Turning now to entropy, let V_A, V_C be the respective excluded volumes of the polyanion and polycation present as N macromolecules each at the start. Thus, $W_{AT} = [V - N(V_A + V_C)]/V_A \approx V/V_A$ is the number of positions accessible to the polyanion in the total volume V. W_{AT} is roughly proportional to the probability of A in the translation degree of freedom. For the coupling product, we can write in the same manner $W_{AC,T} = [V - (N - 1)(V_A + V_C) - V_{AC}]/V_{AC} \approx V/V_{AC}$. Thus, the corresponding translational entropy change $\Delta s_T = k[\ln W_{AC,T} - \ln W_A - \ln W_C] \approx k\ln[V_{AC}/(V_A + V_B)]$. It is easy to see that Δs_T must be always negative and, by analogous reasoning, so must be the rotational increment Δs_C. We thus can assume in general for the direct coupling contribution $\Delta s_d(j) \leq 0$. This gives a special importance to the contribution from the release of side products $\Delta s_s(j)$. In particular for electrostatic interaction, we clearly must have $\Delta s_s(j) > 0$ to accomplish coupling at all ($\Delta s_s(1) > 0$) and to observe real cooperativity ($\Delta s_s(j) > 0$, $j > 1$). Using as above the symbols $\rho^{(j)}$ and $\sigma^{(j)}$ for the corresponding probability densities at the point $\mathbf{r}$ of space and in the jth step, we can write

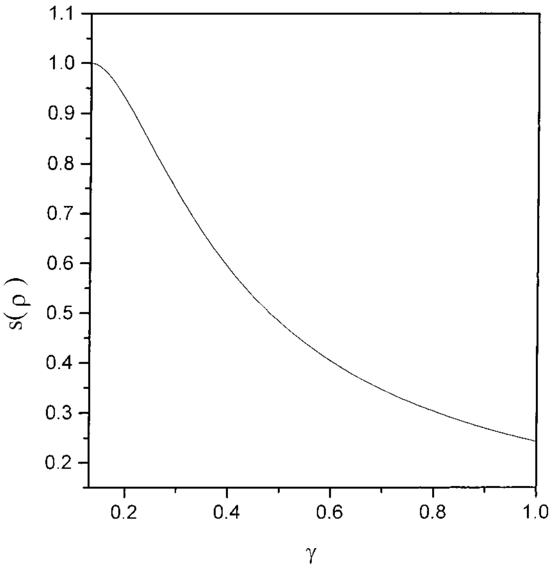

Figure 10. Simulated dependence of $s(\rho)$ on the parameter γ [$s(\rho)$ normalized to 1.0 for $\gamma = 0$].

$$\Delta s_s^{(j)} = k\int_V [\rho^{(j-1)} \ln(\rho^{(j-1)}) - \rho^{(j)}\ln(\rho^{(j)})]\mathrm{d}\mathbf{r} + k\int_V [\sigma^{(j-1)}\ln(\sigma^{(j-1)}) - \sigma^{(j)}\ln(\sigma^{(j)})]\mathrm{d}\mathbf{r} \qquad \text{(A2)}$$

As we intend to show by model calculations in another study, not only $\Delta s_s^{(1)} > 0$ for *any* electrostatic coupling but also $\Delta s_s(j) > 0$ for $j = 2, ..., k$, where k depends on the strength of the polyelectrolyte effect, i.e., on the dependence of ρ or σ on n. Incidentally, this already can be deduced from the recently published solutions[27−29] of the Poisson−Boltzmann equation. As shown by Deshkovski, Obukhov and Rubinstein[29] for a rodlike polyion in a dilute solution (exact solution, achieved in cylindrical symmetry), $\rho(r) \propto r^{-2\gamma}$, where γ can adopt different values for three different phases: $\gamma = 1$ for saturated counterion condensation (strongly charged polyelectrolyte), $\gamma = \gamma_R$ (net charge density of the cylinder) and $\gamma = \gamma_0$ (net charge density of the polyion, for weakly charged polyelectrolytes). It is easy to see that the entropy $s(\rho)$ must be much lower for strongly charged polyion. Figure 10 illustrates the steep dependence of normalized entropy $s(\rho)$ on γ. The entropy change due to liberation of small counterions (and, perhaps to a smaller degree, that due to the liberation of hydrating water molecules) is thus the driving force not only of coupling as such but of its cooperativity as well.

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