Cooperative Interactions of Unlike Macromolecules: NMR Study of Ionic Coupling of Poly[2-(trimethylammonio)ethyl Methacrylate Chloride]-*block*-Poly(*N*-(2-hydroxypropyl) Methacrylamide) Polycation with Oligophosphates in D_2O

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Cooperative effects in the interactions of poly[2-(trimethylammonio)ethyl methacrylate chloride]-*block*-poly(*N*-(2-hydroxypropyl) methacrylamide) polycation with oligophosphates (phosphate glasses) leading to micelle-like complexes were studied using NMR. ^{1}H, ^{13}C, ^{14}N, ^{23}Na, ^{31}P,^{35}Cl, and ^{79}Br single- and double-quantum high-resolution and MAS NMR at 7.2 and 12 T of the complexes were studied at concentrations 0.1 and 0.02 mol/L of the ionic groups in D_2O at 300−350 K. ^{1}H, ^{13}C, and ^{31}P longitudinal and transverse relaxations and ^{23}Na, ^{35}Cl, and ^{79}Br T_1^1, T_1^3, and T_2^3 time evolutions were studied to reveal the ionization states, local and semilocal mobilities, and ion coupling conversions in the products of the same polycation with polyphosphates of P_n = 15, 25, 35, 45, 65, and 75. Pulsed gradient spin−echo (PGSE) was used to measure self-diffusion coefficients and thus the size of both reactants and products. The results show that the ionic coupling is almost complete with polyphosphates of P_n up to 35 but decreases steeply with higher values of P_n. In all cases, the products are aggregates of ionic complexes, but their size increases with higher P_n of the polyphosphate. The cooperativity in the products of self-assembling is discussed in terms of kinetic, thermodynamic (entropy), and microphase stabilization.

Introduction

Cooperative effects in the interaction of multiple analogous groups, sometimes combined with a complex influence of the medium and other effects, are at the core of most processes of spontaneous self-assembling of macromolecules into various kinds of supramolecular complexes.[1−3] They are thus of interest, both practical and theoretical, in a wide range of macromolecular science from molecular biology to synthetic polymers. To state that a cooperative effect must operate in the formation of a given macromolecular complex, considering its stability and the strength of the individual interactions, is usually quite easy. However, it is often quite difficult to discern various kinds of interaction taking part in the complex, let alone describe it in a quantitative way.[4−6]

If the interactions are hydrogen bonds, dipolar and dispersion forces, the main difficulties are in (i) their steep dependence on distance, (ii) their directionality and thus strong dependence on the attainable conformations, and (ii) their relative weakness relative to steric constraints. Due to this, the self-assembling of macromolecules based on these interactions is often limited to exclusive molecular partners, which is one of the basic conditions of the existence of living matter but makes any generalization extremely difficult. This makes the electrostatic interaction of oppositely charged polyions the obvious candidate for a basic study, considering its independence of direction as well as only a linear decrease in interaction energy with distance. Clearly, even here, there are complicating factors making this type of interaction an object worth an experimental study rather than a mere exercise in calculus.

There is another strong motive for an intensive study of the interaction of ionomers, namely, the use of polycations (as homopolymers or blockcopolymers) as protecting agents and synthetic vectors for delivery of genes or DNA plasmids designed for use in in vivo gene therapy.[7,8] The interaction of poly[2-(trimethylammonio)ethyl methacrylate chloride] ((PTMAEM)Cl) or block copolymer poly[2-(trimethylammonio)ethyl methacrylate chloride]−poly(*N*-(2-hydroxypropyl) methacrylamide) ((PTMAEM)Cl−PHPMA) with DNA has been studied mainly by means of light scattering.[9] These studies have shown a ready coupling of the polycation with DNA accompanied by a collapse of the DNA tertiary structure. However, no structural information on the complexes or deep understanding of the interaction could be given on the grounds of these methods. Clearly, the main interaction here should be the electrostatic coupling of the trimethylammonium cations with the phosphate anions of DNA. But changes in the DNA shape as well as spatial considerations indicate a possible role of hydrophobic interactions as well.

In this NMR study, we chose sodium polyphosphates (phosphate glasses) partly as a (very poor) model of the repeating phosphate groups in DNA, partly for their own sake, as well-defined polyanions of a relatively narrow distribution of polymerization degrees. As the polycation counterpart, we use (PTMAEM)Cl−PHPMA, an A−B type block copolymer (DO166) because it allows complete saturation of its polycation part by the polyanion without precipitation of the product, which would hamper NMR observation and complicate the kinetics and equilibrium of ion coupling.

The system under study offers an exceptional variety of NMR-active nuclei, namely, ^{1}H, ^{13}C, ^{14}N, ^{15}N, ^{17}O,^{23}Na, ^{31}P, ^{35}Cl, and ^{79}Br. We utilize them all except ^{15}N and ^{17}O, both of them being beyond sensitivity of our spectrometer at the working concentration 0.1 or 0.02 M of the phosphate or trimethylammonium units.

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Experimental Part

Chemicals and Sample Preparation. Poly[2-(trimethyammonio)ethyl methacrylate]-*block*-poly[*N*-(2-hydroxypropyl)-methacrylamide] (DO166) was prepared by coupling of semitelechelic poly[*N*-(2-hydroxypropyl)methacrylamide] terminated in reactive *N*-hydroxysuccinimidyl ester group (PHPMA-COOSu) with semitelechelic poly[2-(trimethyammonio)ethyl methacrylate chloride] terminated in primary amino group ((PTMAEM)Cl−NH_2) in dimethyl sulfoxide.[9] An excess of PHPMA-COOSu was used (the COOSu:NH_2 ratio was 1.2:1). Briefly, (PHPMA-COOSu) was prepared by a radical precipitation polymerization in acetone carried out in the presence of a chain-transfer agent 3-sulfanylpropionic acid followed by esterification with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide.[9] Polymer terminated in a carboxylic group was purified by double precipitation from methanol into a mixture of acetone−diethyl ether followed by fractionation on Sephadex LH-60 in methanol. Polymer succinimide ester was purified by double precipitation. ((PTMAEM)Cl−NH_2) was prepared by a radical solution chain-transfer polymerization in methanol in the presence of cysteamine hydrochloride.[9] The polymer was purified by precipitation and dialysis (molecular weight cutoff of the dialyzing tubing was 3500) and isolated by freeze-drying. Both polymers were characterized by size exclusion chromatography (FPLC Pharmacia) using a Superose 12 column and 0.05 M Tris buffer (pH 8.1). The weight average and number average of the molecular weights were calculated using calibration with PHPMA standards. M_w and M_n of the PHPMA block were 14 400 and 8400 g mol^{-1}, respectively; M_w and M_n of the (PTMAEM)Cl block were 10 600 or 7500 g mol^{-1}.

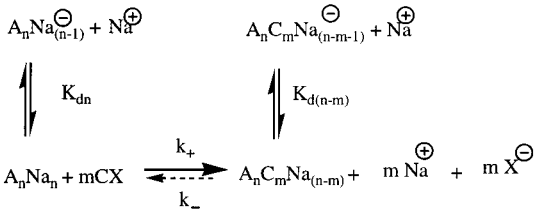
The final block copolymer was isolated by precipitation into acetone. Excess PHPMA was removed by extraction with dimethylformamide.

Sodium polyphosphates (phosphate glasses, PG*X*) of the given P_n were purchased from Aldrich and, after their purities were checked by ^{31}P NMR, were used in their commercial grade. Complexes of DO166 with PG*X* were prepared by equimolar (chloride to phosphate) mixing of their 0.1 or 0.02 mol/L solutions in D_2O at 297 K, adding dropwise the PG solution to a vigorously mixed solution of DO166 during 5 min. The obtained mixtures were transferred into a NMR tube or MAS spacer and measured immediately.

NMR Measurements. ^{1}H, ^{13}C, ^{14}N, ^{23}Na, ^{31}P,^{35}Cl, and ^{79}Br single- and double-quantum high-resolution NMR spectra at 7.2 T were measured with a Bruker Advance DPX 300 spectrometer, those at 12 T with the Bruker 500 MHz spectrometer equipped with the MAS probehead (Faculty of Mathematics and Physics, Charles University, Prague). For ^{31}P, the $\pi/2$ pulse, the preacquisition delay and the acquisition time were 13.2 μs, 5.5 μs, and 1.348 s at 7.2 T and 3.7 μs, 4.5 μs, and 0.348 s at 12 T, respectively. A high-resolution ZrO_2 rotor with a HR spacer and KEL-F caps was used for the MAS of liquid samples; the spinning frequency was 5 and 10 kHz. The pulse sequences used for the spectra acquisition and relaxation measurements were published elsewhere.[10,18,26] PGSE measurements were done on a Z-gradient broadband inverse probehead using two 3 ms gradient pulses with variable gradient strength (15−95% of the maximum 49.7 G/cm) symmetrically placed before and after a refocusing electromagnetic π pulse.[27] Due to the limited gradient field intensity, maximum diffusing delays (up to 0.16 s) permitted by transverse relaxation were used.

Quantum mechanical calculations were done in the MNDO, ab initio SCF 3-21G or 6-31G, and density functional theory (DFT) formats using the GAUSSIAN[28] program. The geometries of the molecules were fully optimized using the gradient optimization routine in the program, and all the stationary states were verified by inspecting their Hessian. In the case of MNDO calculations of larger complexes, smaller models of similar structure and geometry were always calculated using MNDO and ab initio SCF 6-31G and DFT formats. Quite analogous optimum structures and energy trends were obtained on different levels of calculation so that no artifacts are introduced by the semiempirical method. All calculations were performed in C_1 symmetry.

SCHEME 1



Theory

This part explains and partly develops the physical basis of the determination of the efficiency of ion coupling, the ionization state, and local or semilocal mobility by NMR.

In most cases, the ^{31}P signals of the phosphate groups coupled with a trimethyammonium ion can be discerned from that of the free ones and their relaxation behavior gives some information about the coupled sites. Sometimes, the signal of the coupled groups has broad wings reaching into the second one and the signal intensity can be impaired by various factors. The degree of ion coupling then has to be checked by an independent method. In the present case, the relaxation rate of the counterions partially or totally freed into solution offer such possibility. In addition, the relaxation behavior of these ions bound permanently or transiently to the sites, which avoided coupling, can offer additional information about the coupling product.

We consider a general reaction shown in Scheme 1. For simplicity, the polycation salt is represented by its individual monomer units CX and the number m is related to the conversion α ($\alpha = m/n$ for an equimolar mixture of both components). Here and in the whole subsequent text, molarity is expressed for monomer, i.e., trimethylammonium cation (C) or phosphate anion (A) units, without respect to the polymerization degree of the reacting components. The ion dissociation in A_nNa_n naturally offers a number of equilibria characterized by different dissociation constants. In a polyionomer, however, the corresponding different ionization states are in a fast exchange,[10] so that it is reasonable to consider a mean dissociation constant K_d related to a single monomer unit of the given polymeric species. It has to be born in mind, however, that the value of K_d depends on the ionization state of the surrounding units, in particular on the length of a sodium polyphosphate sequence. Hence the symbol K_{dn}.

Na atoms in the $A_nC_mNa_{(n-m)}$ ($m \in \langle 0,n \rangle$) are in labile ionic bonds, i.e., in fast exchange with the free Na^+ ions in solution. In principle, we thus could determine conversion α by measuring ^{23}Na (or ^{35}Cl) longitudinal relaxation rate R_{1ex}. As shown by Bull,[11] the longitudinal relaxation decay of a $I = {}^3/_2$ nucleus can be expressed by the function

$$I_z(t) = I_z(0)[(1/5)\exp(-r_1 t) + (4/5)\exp(-r_2 t)] \qquad (1)$$

where

$$r_j = \chi_Q \frac{\tau_c}{1 + (j\omega\tau_c)^2} \tag{2}$$

with $j = 1, 2$. In eq 2, the quadrupole coupling constant $\chi_Q = (1/10)(1 + \eta^2/3)(e^2qQ/h)^2$, where η is the asymmetry factor, eq the gradient of electrostatic field, eQ the quadrupole moment of the nucleus, h the reduced Planck constant, ω the resonance frequency, and τ_c the correlation time of the main motion active in the relaxation. As shown by Bull,[11] the relaxation decay under exchange of the nucleus between two or more sites adopts a form of at least four exponentials, and some approximation is the usual approach. However, under fast exchange and the extreme narrowing condition fulfilled well (fast motion, Lorentzian line shape, $\omega\tau_c < 1$) at the more populated site, the relaxation decay collapses into a single exponential and one can write[11]

$$I_z(t) = I_z(0) \exp(-R_{1ex}t) \tag{3}$$

where

$$R_{1ex} = R_{1F} + \frac{0.2\varphi}{1 - \varphi} \sum_{j=1}^{2} \frac{j^2}{r_{B_j}^{-1} + \tau_B} \approx R_{1F} + \frac{0.2\varphi}{1 - \varphi} \sum_{j=1}^{2} j^2 r_{B_j} \tag{4}$$

Here φ is the molar fraction of the bound nucleus, i.e., $(1 - \varphi) = \alpha$ in this case, and τ_B is the mean residence time at the bound site (if $\tau_B \ll 1/r_{B_j}$, the second approximation in eq 4 is acceptable). If eqs 3 and 4 are good approximations, the conversion α can be obtained from R_{1ex} providing there are known the following quantities: (i) the relaxation rate R_{1F} of the free ions, (ii) the relaxation rates r_{B_j} of the ions in the $A_nC_mNa_{(n-m)}$ product, and (iii) the dissociation constant $K_{d(n-m)}$. From these quantities, only R_{1F} can be known from an independent measurement. However, $K_{d(n-m)}$ will probably depend on both n and m due to the polyelectrolyte effect, and also r_{B_j} could be dependent on m due to different motional behavior of the corresponding products. Nevertheless, both $K_{d(n-m)}$ and r_{B_j} can be obtained by the following procedure. The original solution with the concentration c of the product is subsequently diluted with p (p=0, 1, ..., 0.6) equivalent volumes of NaCl with the same concentration so that the total ion strength of the system remains constant and the necessary correction on Na^+ ion concentration[12] can be avoided. It is easy to show that for a given p,

$$\varphi = \frac{\beta - [\beta^2 - 4(p + 1)(1 - \alpha)c^2]^{1/2}}{2(p + 1)c} \tag{5}$$

where

$$\beta = c(p + 2 - \alpha) + K_{d(n-m)}(p + 1) \tag{5a}$$

If α does not change with addition of the electrolyte (a condition that could be checked in an independent way, e.g., from ^{31}P in our case) and the value of R_{1F} is known from an independent measurement, the values of $K_{d(n-m)}$, r_{B_j}, and α can be fitted to the series of measured $R_{1ex}(p)$ using eqs 3−5. $R_{1ex}(p)$ is strongly dependent on all these quantities, so that at least six points should be measured for the fitting. This method (called method A in the subsequent text) can be used almost universally for well-hydrated monovalent cations such as $^7Li^+$ or $^{23}Na^+$, providing that the basic assumptions, in particular the extreme narrowing, are fulfilled to a reasonable degree. Also, the model used is based on a two-site exchange, which again is an approximation. Possible improvements using the Manning model or Poison−Boltzmann distribution are being developed.

In the case of "structure breaking" anions such as $^{35}Cl^-$ or $^{79}Br^-$, the relaxation can be complicated by their partial adsorption to hydrophobic groups and asymmetric hydration.[12] In such case, eq 4 holds no longer and has to be replaced, as a viable approximation, by

$$R_{1ex} = \varphi R_{1B} + (1 - \varphi)[(1 - \psi)R_{1F} + \psi R_{1FA}] \tag{6}$$

where $\psi = \xi c/(1 + p)$, ξ being a parameter characterizing the number of ion adsorption sites and the probability to adsorb to them in the given system, and R_{1FA} is the mean relaxation rate of the uncoupled but adsorbed ions, $R_{1FA} > R_{1F}$. Equation 6 has the uncomfortable property of introducing two more parameters ξ and R_{1FA} into the model, which decreases the reliability of the fitting.

Under the conditions of this study, longitudinal relaxation of both ^{23}Na and ^{35}Cl has monoexponential shape. To reveal the dynamics of the system, transverse relaxation has to be used. Under fast exchange between the free and bound ions and at the same level of approximation as above, we can write (in a slight extension of the approximation made by Bull[11])

$$I_{xy}(t) = I_{xy}(0)\left[\frac{3}{5}\exp(-s_0t) + \frac{2}{5}\exp(-s_1t)\right] \tag{7}$$

where

$$s_j = R_{1F} + \frac{\varphi\, r_{B_j}}{(1 - \varphi)(r_{B_j}t_B + 1)} \tag{8}$$

providing that the relative shift of the signal between the exchanging sites is vanishing. In eq 8, we reasonably assume the extreme narrowing for the free ions; i.e., $R_{1F} = R_{2F}$. The meaning of r_{B_j} is the same as in eq 2 on the condition that only one type of motion contributes to relaxation of the bound ions. This is known[10,13−16] to be a problem. Apparently, we can consider neither the free ions in their cloud around a polyion to be completely independent nor the bound ions to be tightly attached to the oppositely charged group and thus copying its motions. Even assuming the existence of the ionization state extremes as a first approximation, the relaxation-relevant motion of the bound ions can be a superposition of that given by the local dynamics and that connected with the whole molecular or supramolecular system. In such a case, a pragmatic approach has to be chosen. An analogy of the Lipari−Szabo model-free analysis[17,18] in the present case is the modification of eq 2,

$$r_j = \chi_Q\left[\frac{\tau_M S}{1 + (j\omega\tau_M)^2} + \frac{\tau_e(1 - S)}{1 + (j\omega\tau_e)^2}\right] \tag{9}$$

where τ_M and τ_e are the correlation times of the collective and local motions, respectively, and S is the generalized order parameter expressing here the coupling of the motion of the bound ion to that of the whole molecular system. In cases where τ_M and τ_c can be discerned by relaxation analysis, S is the measure of the stiffness of the binding site and its molecular surroundings. If such neat analysis is impossible, eq 2 has to be used instead of eq 9. It can be shown by simulation that the apparent correlation time τ_c invariably grows with growing S and can thus be considered at least a semiquantitative measure of the local or semilocal mobility.

An alternative way to examine the mobility of the bound $I = {}^3/_2$ ions is to follow the time evolution of their multipolar states using double- or triple-quantum filtered NMR.[19−21] Only the tensors $T_1{}^3$ and $T_2{}^3$ ($T_i{}^j$ meaning the tensor of the jth rank and ith order) can be realistically expected even if $S > 0$, but the system is not spatially oriented. Neglecting the dipolar contribution to relaxation,[20] the time development of $T_1{}^3$ and $T_2{}^3$ ($f_{31}{}^{(1)}$ and $f_{32}{}^{(2)}$, respectively) can be expressed

$$f_{31}{}^{(1)} = (\sqrt{6}/5)[\exp(-R_s t) - \exp(-R_f t)] \tag{10}$$

$$f_{32}{}^{(2)} = \exp(-R_f t) \tag{11}$$

with $R_s = r_1 + r_2$ and $R_f = r_0 + r_1$ with r_j being defined in eq 2. For $q = R_f/R_s$,

$$\tau_c = \{(5q - 9) + [(5q - 9)^2 + 32(q - 1)]^{1/2}\}^{1/2}/(\sqrt{8}\omega_0) \tag{12}$$

The values of τ_c obtained from eq 12 correspond to the motionally hindered bound state and can thus be used as an independent indicator of a nonstatistical binding.

For the $I = {}^1/_2$ nuclei such as ^{31}P in our case, subject to two main periodical motions as above, the longitudinal (R_1) and transverse (R_2) relaxation rates for a mixed dipolar (by a like spin) and chemical shift anisotropy (CSA) mechanism are[19] (in the absence of residual static interactions)

$$R_1 = \zeta_d \delta_d [J(\omega_0) + 4J(2\omega_0)] + \zeta_a \delta_a J(\omega_0) \tag{13}$$

$$R_2 = \frac{1}{2}\zeta_d \delta_d [3J(0) + 5J(\omega_0) + 2J(2\omega_0)] + \frac{1}{6}\zeta_a \delta_a [4J(0) + 3J(\omega_0)] \tag{14}$$

where ζ_d and ζ_a are the statistical weights of the dipolar and CSA mechanisms, respectively. $\delta_d = 3\gamma^4\hbar^2/(10r^6)$, where γ is the gyromagnetic ratio and r is the distance between relevant magnetic moments, $\delta_a = (2/15)(\gamma B_0 \Delta\sigma)^2$, B_0 is the intensity of the static magnetic field, and the magnetic shielding anisotropy $\Delta\sigma$ is the difference between the parallel and perpendicular components of the diagonalized magnetic shielding tensor. The reduced spectral densities can be approximated[17,18] by the relation

$$J(j\omega) = \frac{S^2\tau_M}{1 + (j\omega\tau_M)^2} + \frac{(1 - S^2)\tau_c}{1 + (j\omega\tau_c)^2} \tag{15}$$

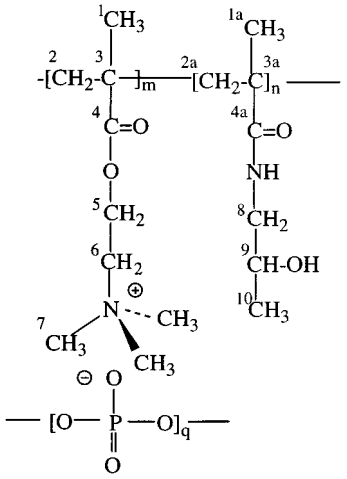
Residual static (dipolar or CSA) interactions due to hindered internal motion in the molecular or supramolecular system can contribute to the transverse relaxation by the factor $R^S{}_{2i}$ (the subscript i implying that there could be different sources of residual broadening),

$$R^S{}_{2i} = \delta\omega_i \tau_c{}^2[(\exp(-t/\tau_c) + t/\tau_c - 1] \tag{16}$$

where $\delta\omega_i$ is the residual static broadening, which can be partly or completely removed either by magic angle spinning (MAS) of the sample or isotropic tumbling of the molecular system. The empirical approximation to such narrowing has been found[18] to be

$$\delta\mathbf{w}_i = \frac{\mathbf{\Delta}_i}{1 + \exp\{\zeta[(2\pi\tau_M\Delta_i)^{-1} - 1]\}} \tag{17}$$

CHART 1



where $\Delta_I = S^2\delta\omega_0$ with $\delta\omega_0$ being the residual static broadening in the absence of MAS and/or fast collective tumbling. The observed relaxation rate is then

$$R_{2ex} = R_2 + \sum_i R_{2i}{}^S \tag{18}$$

The second factor in eq 15 can cause nonexponentiality of the transverse magnetization decay, although the usual effect in the absence of motional constraints is simply an increase in R_{2ex}. Residual static interactions influencing transverse relaxation consequently influence the signal shape, giving rise to signal broadening or a super-Lorentzian signal. As it can be shown by simulation,[18−20] there are two main cases in which a "super-Lorentzian" (i.e., a signal with unusually broad, sometimes slightly asymmetric wings) occurs. (i) The motion of the nucleus is spatially restricted and the signal is a *convolution* of two unequal Lorentz−Gaussian signals; in such a case, the transverse relaxation decay is nonexponential in the sense that its trail is a decreasing *faster*. (ii) There exist two or more types of analogous nuclei hindered in motion to a different degree so that their signals are differently broadened (possibly also by residual static interactions), and the resulting signal is a *superposition* of two or more Lorentz−Gaussians; in such a case, the transverse relaxation decay is polyexponential in the sense that its trail is decreasing *slower* than expected. In the course of relaxation decay, the shape of the signal does not alter significantly in case i, whereas it is progressively narrowed in case ii.

Results

Structure and Ionization of the Reactants. The polycation carrying copolymer, namely, poly(2-(trimethylammonio)ethyl methacrylate)-*block*-poly(*N*-(2-hydroxypropyl) methacrylamide) shown in the upper part of Chart 1 and referred to as DO166 in the text was used in the chloride form. Its ionic block ((PTMAEM)Cl) had the molecular weight averages M_w = 10 600, M_n = 7500, and those of the neutral hydrophilic block (PHPMA) were M_w = 14 400 and M_n = 8400, according to GPC analysis. Thus the m and n values in Chart 1 were 36 and 59, respectively. According to ^{1}H NMR, m/n = 0.615 which corresponds within 5% relative to GPC analysis. Parts a and b of Figure 1 show the ^{1}H and ^{13}C DEPT spectra (proton bearing carbons) of 0.1 M DO166 in D_2O (the concentration being expressed in moles of the (TMAEM)Cl unit per liter) at 300 K. The assignment of the signals corresponds to Chart 1 and was

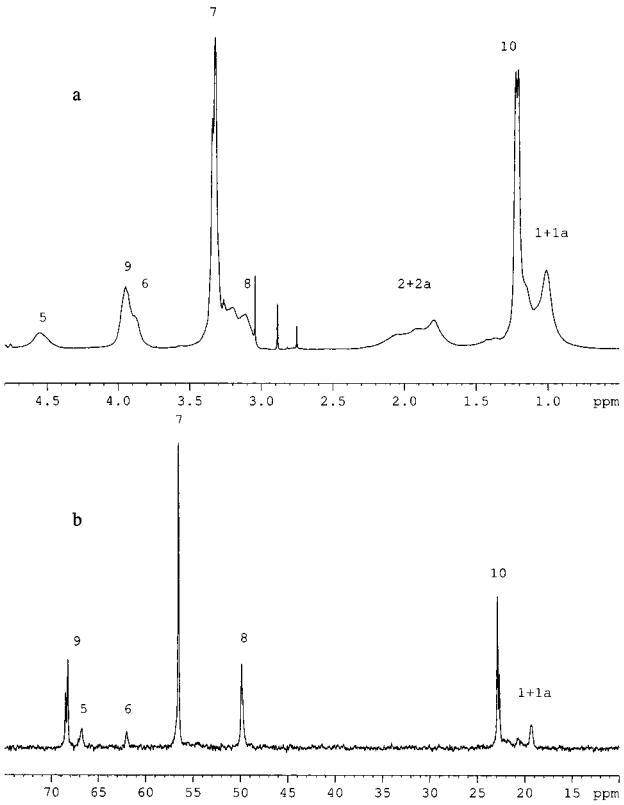


Figure 1. DEPT45 NMR spectra of the parent poly(2-(trimethylammonio)ethyl methacrylate)-*block*-poly(*N*-(2-hydroxypropyl) methacrylamide) chloride (DO166) in D_2O (0.1 M, 300 K): 300.13 MHz ^1H (a) and 75.45 MHz ^{13}C (b).

TABLE 1: ^1H and ^{13}C Relaxation Times[a] (s) of the Individual Signals in DO166 and DO166−PG25 (0.1 M in D_2O) at 300 K

	DO166			DO166−PG2 5		
	^1H		^{13}C	^1H		^{13}C
signal	T_1	T_2	T_1	T_1	T_2	T_1
1a	0.30	0.04	0.06	0.27	0.04	0.03
2a	0.30	0.04		0.28	0.04	
3a			1.62			1.60
5	0.18	0.08	0.19			
6	0.22	0.09	0.17			
7	0.32	0.16	0.23	0.27	0.06	0.20
8	0.42	0.28	0.18	0.40	0.22	0.16
9	0.69	0.22	0.29	0.61	0.20	0.28
10	0.46	0.16	0.42	0.45	0.15	0.40

[a] Estimated error ±5% rel.

made by combination of COSY, NOESY, and HSQC spectra. The splitting of the signal 7 into two uneven peaks in the ^1H spectrum (not paralleled in ^{13}C NMR) suggests two different states of the (TMAEM)Cl groups. Their relative intensities do not correspond to the ionization equilibrium (see below) and probably reflect different preferred conformations of the polyion chain; accordingly, the splitting disappears under coupling of the (TMAEM)Cl units with the polyphosphate. The T_1 and T_2 relaxation times of the main ^1H resonances given in Table 1 reflect the usual dynamics of a hydrated polymer chain except for the rather low values in the case of the signal 7 where fast rotation of the methyl groups could be expected. The plausible explanation is the quadrupole coupling to the ^{14}N nucleus. In sharp contrast to the tetramethylammonium analogue, ^{14}N as well as ^{35}Cl nuclei in the (TMAEM)Cl unit have broad NMR signals, the respective half-widths being 110 and 600 Hz with

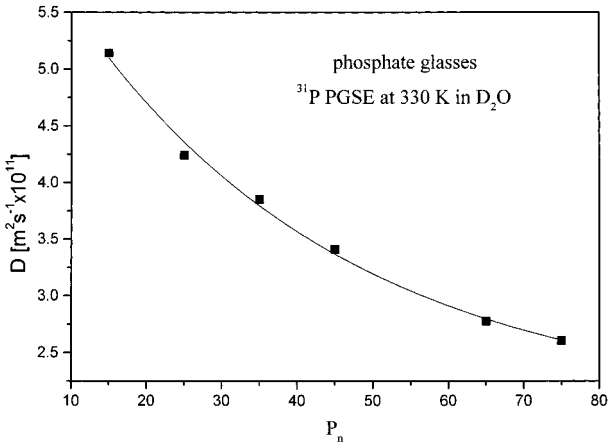


Figure 2. Dependence of the self-diffusion coefficient of oligophosphates (PG15 to PG75) on their P_n as measured by the ^{31}P NMR PGSE method (0.1 M in D_2O, 300 K).

the corresponding T_1 values 3.2×10^{-3} and 1.29×10^{-3} s (measured independently by the inversion−recovery method). These values suggest substantial electric field gradient at these two nuclei and distorted symmetry of their surroundings, in other words, a rather low ionization degree of the (TMAEM)Cl unit. Although the relaxation method of K_d estimation outlined above works much better with Na^+ ions, we also used ^{35}Cl NMR to obtain an approximate value of K_d for the $TMAEM^+Cl^-$ ion dissociation equilibrium. Because of the difficulties with fast relaxing nuclei and anomalies in the relaxation of "structure breaking" quadrupolar ions,[12] we used the concentration 0.02 M and the values of p added NaCl equivalents from 1 to 7. Since the R_1/p dependence was anomalous even at this concentration, we repeated the same row of relaxation experiments (i) with the poly($TMAEM^+Cl^-$) homopolymer and (ii) with the nonionic PHPMA progressively diluted by the 0.02 N solution of NaCl. Both types of experiments showed chloride ion adsorption to the organic molecules. After the influence of this effect was removed, the ^{35}Cl relaxation values obtained by fitting were as follows: $R_{1F} = 30.3\ s^{-1}$, $R_{1B} = 1709.5\ s^{-1}$, and $K_d = 0.033$ mol/L. Because of the complicating effect of ion adsorption, these values have to be considered rather crude estimations. According to the K_d value, more than half (56.7%) of the $TMAEM^+Cl^-$ units exist as ion pairs at the 0.1 M concentration, the Cl^- ions being in a dynamic exchange between solvent separated and contact states.

The sodium polyphosphates (phosphate glasses) were used in their commercial form and are referred to in the following text as PG*X*, *X* denoting the average number of the phosphorus atoms in the chain (the actual values of *X* being 5, 15, 25, 35, 45, 65, and 75). Their ^{31}P NMR spectra exhibit one strong signal of the inner-chain phosphate units near −22 ppm (to the signal of sodium metaphosphate) and several minor signals corresponding to the terminal groups (see below, in the figures comparing them to those of the coupling products). After prolonged storage in D_2O solution, in particular at elevated temperature, signals in the range from +1 to −0.5 ppm of hydrolytic products (monophosphates) appear with growing intensity. At the typical experimental temperature of 300 K, the solutions were sufficiently stable to allow even prolonged measurements without any distortion.

Figure 2 shows the dependence of the self-diffusion coefficient of the polyphosphate, measured by ^{31}P PGSE, on its average chain length. The shape of this dependence suggests a rather extended chain, which is not surprising in view of the

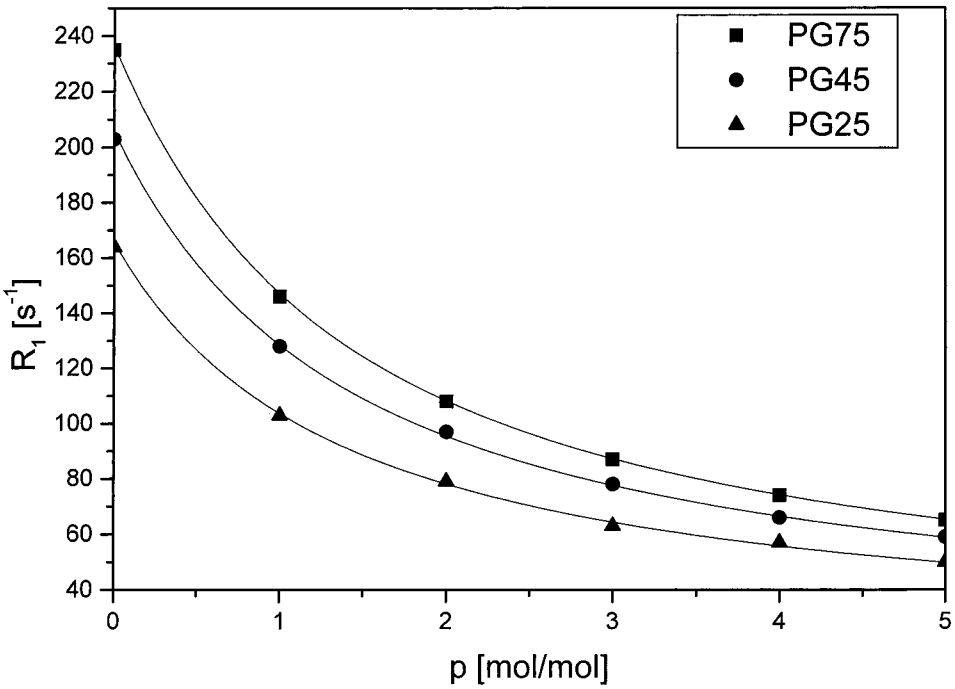


Figure 3. Dependence of the ^{23}Na longitudinal relaxation rate in the 0.1 M D_2O solution of three different polyphosphates on the added *p* equivalents of 0.1 N NaCl in D_2O at 300 K.

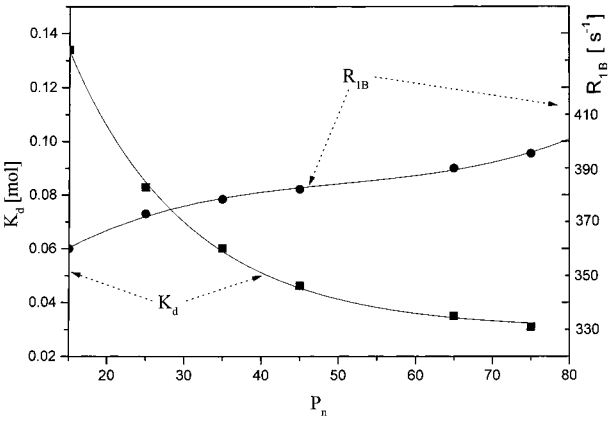


Figure 4. Mean constant of Na^+ ion dissociation K_d and longitudinal relaxation of the bound ^{23}Na nuclei R_{1B} in dependence on P_n of the polyphosphates (0.1 M in D_2O, 300 K).

structure. The T_1 values of the prevailing ^{31}P NMR signal almost do not depend on the chain length (varying in the range from 1.22 to 1.18 s). In contrast to longitudinal relaxation, the transverse decay curves are at least biexponential with a varying relative weight of a faster component (T_2 in the range from 0.31 to 0.33 s) and a slower one (from 0.73 to 0.88 s). Somewhat surprisingly, the largest weight of the faster component is in the shortest polyphosphates (starting with 15 atoms in the chain). This could suggest their relatively larger internal stiffness given by a larger relative charge.

To investigate both the degree of ionization and the properties of the Na-bound state, we measured the ^{23}Na longitudinal relaxation under dilution of the polyphosphates with 0.1 N NaCl. Three examples of the fittings of the obtained relaxation rates using method A are given in Figure 3. The obtained values of K_d and R_{1B} are shown in Figure 4. As seen, K_d depends rather steeply on *n*, in particular in shorter polyphosphates. Na^+ ions are from 33.4 to 58.2% in the bound state. Their relaxation rate R_{1B} does not change much with *n*, indicating thus a rather flexible nature and swift motion of the binding sites. The

TABLE 2: T_1 Relaxation Times[a] (s) of the Reaction Components and the Mixture in the Interaction of (TMN)Br with PG75 (0.05 M, 300 K)

	^{1}H	^{14}N	^{23}Na	^{31}P	^{79}Br
NaBr			0.069		0.000$53
(TMN)Br	8.07	18.89			0.000$44
PG75			0.0060	1.26	
(TMN)Br−PG75	5.14	9.22	0.0066	1.09	0.000$45

[a] Estimated error ±5% rel.

monoexponential shape of the transverse decay for the bound ions points to the same conclusion.

We can thus conclude that both reacting partners DO166 and PG*X* are only partly ionized at the experimental concentration so that ionization and/or ion exchange is an integral part of the course of their coupling.

Interaction of a Monomeric Cation with a Polyanion. To evaluate the degree of cooperation in the interaction of two complementary polyions, we studied first the interaction of tetramethylammonium bromide ((TMN)Br) with the polyphosphates, in particular with the longest one (PG75) where the interaction could be expected to be most pronounced. The concentration of both (TMN)Br and the phosphate units of PG75 in the mixture were 0.05 M in D_2O. The temperature of measurement was 300 K. For all the nuclei, i.e., ^{1}H, ^{14}N, ^{23}Na, ^{31}P, and ^{79}Br, the corresponding spectra consisted of one line (except ^{31}P NMR where minor signals of the end groups of the polyphosphate can be detected). Due to the tetrahedral symmetry in the $(CH_3)_4N^+$ ion, the quadrupole coupling of ^{14}N is weak and the NMR signal is very narrow. Under mixing of (TMN)-Br with PG75, very small relative shifts of ^{14}N (0.5 Hz) and ^{31}P (0.13 ppm) were observed. There was a change in longitudinal relaxation rate, however. The corresponding T_1 relaxation times are given in Table 2. As expected, the nucleus most sensitive to bonding is ^{14}N and, due to cross-relaxation, ^{1}H in the TMN^+ cation. There is also a change in the relaxation of both ^{23}Na and ^{79}Br, due to their changed ionization states. The highest T_1 value of ^{14}N calculated for the bound state of

TMN^+, if only the changed field gradient and no distortion of symmetry is taken into account, is 1.35 s. On the basis of this, the fraction of bound TMN^+ is about 0.08. On the basis of ^{23}Na and ^{79}Br relaxation, it is 0.09 and 0.08, respectively. Hence the mean fraction is around 0.08, while 0.5 or even a higher value could be expected under free ion exchange or under preferential bonding of TMN^+ to the polyphosphate, respectively. The plausible explanation of this discrepancy is the lower electrostatic energy (higher stabilization) achieved by the polyphosphate when binding Na^+ ions with a much smaller ion radius compared to TMN^+. On the other hand, the TMN^+ ion, in particular in its bound state, is evidently less tightly hydrated than Na^+, making thus the TMN−phosphate units relatively hydrophobic. Although highly improbable considering the found fraction of primary couplings, a row of several coupled units would produce a hydrophobic microphase which could shift the equilibrium of coupling. From the absence of such effect, a provisional hypothesis could be made that electrostatic rather than hydrophobic interaction is the primary factor of the polyion coupling described below, though the latter could be important in its second stage.

Coupling of the Polycation with the Polyphosphates. The procedure of mixing the reaction components was always done in the following way. To a vigorously mixed solution of DO166 at the given concentration (from 0.1 to 0.02 M) was added very slowly (in about 5 min) an equivalent amount of the equally concentrated solution of the given polyphosphate at ambient temperature (usually 296 K). At all concentrations and all polyphosphate polymerization degrees, a slight opalescence appeared in an early stage of mixing, indicating the forming of supramolecular particles. At higher concentrations and higher polymerization degrees of the polyphosphate, this opalescence deepened into slight turbidity, which did not change even after several months if the samples were stored at normal temperature.

Figure 5 shows examples of the ^1H and ^{13}C NMR spectra of the products; Figures 6 and 7 give the corresponding ^{35}Cl, ^{14}N, and ^{31}P NMR spectra. Except for ^{31}P, there is almost no change in the chemical shift of the NMR signals of the parent compounds after their coupling. Some changes in signal shape are quite dramatic, however. Both ^1H and ^{13}C signals 5 and 6 are entirely missing due to their broadening beyond detection. Somewhat surprisingly, even the methyl signals 7 are broadened and decreased in absolute intensity. ^{35}Cl signals are much narrowed as expected for mostly free Cl^- ions freed by exchange; however, with the increasing polymerization degree in the polyphosphate, they gradually broaden. ^{14}N signals are perceptibly narrower than in the parent DO166 but much weaker in absolute intensity so that they must correspond to residual, presumably uncoupled, groups, whereas those of the coupled ones are broadened beyond detection. ^{23}Na signals (not reproduced) in DO166−PG15 are somewhat broadened and non-Lorentzian but in the coupling products of the higher polyphosphates take the course analogous to those of ^{35}Cl. Since the reactants were mixed in an equimolar ratio of (TMAEM)Cl and phosphate groups, there remains a perceptible influence of terminal P−O−Na groups, which remain untouched and evidently influence the signal shape. It has to be born in mind that both ^{23}Na and ^{35}Cl signals reflect a fast exchange between the corresponding bound and free ions. ^{31}P spectra are the only ones reflecting the coupling between (TMAEM)Cl and phosphate groups by a relative shift of the main signal as well as its broadening and decrease in absolute intensity. The effect is most pronounced at the polyphosphate length comparable to that of the (PTMAEM)Cl block. With higher polyphosphates, it has a more complex behavior discussed below.

The changes just described are comprehensible if analyzed from two points of view: (i) structure and mobility of the coupled products and (ii) achieved degree of coupling. Table 1 shows an example of T_1 and T_2 values for the observable ^1H signals and ^{13}C T_1 values (T_2 of this nucleus being unreliable) measured for one coupling product in comparison with those of the parent polycation copolymer. According to these results, the dynamics of the PHPMA blocks is not perceptibly influenced by the coupling reaction, in sharp contrast to that of the ionic (PTMAEM)Cl block. In the latter case, groups 5 and 6 appear to be immobilized and even methyl groups 7 are clearly hindered in motion. (Comparison of the respective changes of T_1 and T_2 shows that the broadening of signal 7 is not given simply by long-range interaction with the fast relaxing ^{14}N nucleus.) Simulation of a trimeric sequence (Figure 8) of the coupled product by MNDO (checked for possible artifacts on a smaller model by ab initio SCF 6-31G and DFT quantum mechanical method), shown for the lowest energy minimum in Figure 9, indicates perceptible crowding in the coupling area. In consequence, the rotational barrier of the methyl and in particular the whole trimethylammonium group is increased. The local mobility can be increased by thermal activation, as illustrated by Figure 9 where the ^1H spectra of DO166−PG65 measured at temperatures from 300 to 340 K are shown. The narrowing of the relevant signals is observed in the order 7 > 6 > 5. However, even at 340 K there remains a substantial broadening of these signals, compared to DO166, indicating thus that the observed narrowing is not given by thermal dissociation of the coupling product. This is also fully evidenced by the corresponding ^{31}P, ^{23}Na, and ^{35}Cl spectra. The change of spectra due to heating is almost fully reversible except that an abrupt change takes place after several heating cycles. As discussed below, this change in behavior is due to hydrolysis of the polyphosphate part below the critical level.

The apparent feature of the ^1H and ^{13}C spectra of the coupling products containing different polyphosphates is an increasing broadening of signal 7 with increasing phosphate chain length up to 65 (at $P_n = 75$ there is a slight relative decrease, which will be analyzed below). The same trend is copied by the ^{31}P spectra except that a maximum broadening and intensity loss is achieved at $P_n = 45$. At higher P_n, the signal adopts a partly "super-Lorentzian" shape; i.e., it behaves like a convolution of at least two Lorentzians. For DO166−PG65, the corresponding line widths $\Delta\nu_{1/2}$ at 7.2 T are 11.4 and 55.8 Hz, whereas at 12.0 T they increase to 30.2 and 156.2 Hz, which is almost exactly in proportion to B_0^2, indicating thus that shielding anisotropy is the main mechanism of the corresponding transverse relaxation. However, with the hard pulse and fast acquisition intended for solid-state NMR, used at 12 T, the $\Delta\nu_{1/2}$ values of the ^{31}P components increase to 130 and 652 Hz, respectively, and the increase in absolute intensity is about 1.4. Under MAS at the same B_0, there is a further increase in absolute intensity and, in particular, perceptible narrowing of the signal. The $\Delta\nu_{1/2}$ values (and relative weights) of the two deconvoluted Lorentzian components are 87 (0.312) and 417 (0.678) Hz at 5 kHz MAS and 111 (0.642) and 382 (0.358) at 10 kHz MAS (note the inverted ratio of the narrow and broad components). Analogously, the ^1H signal 7 is narrowed and increased by MAS. And vice versa, in analogy to ^1H spectra, ^{31}P signal narrowing similar to that obtained by MAS can be achieved by heating the sample up to 340 K.

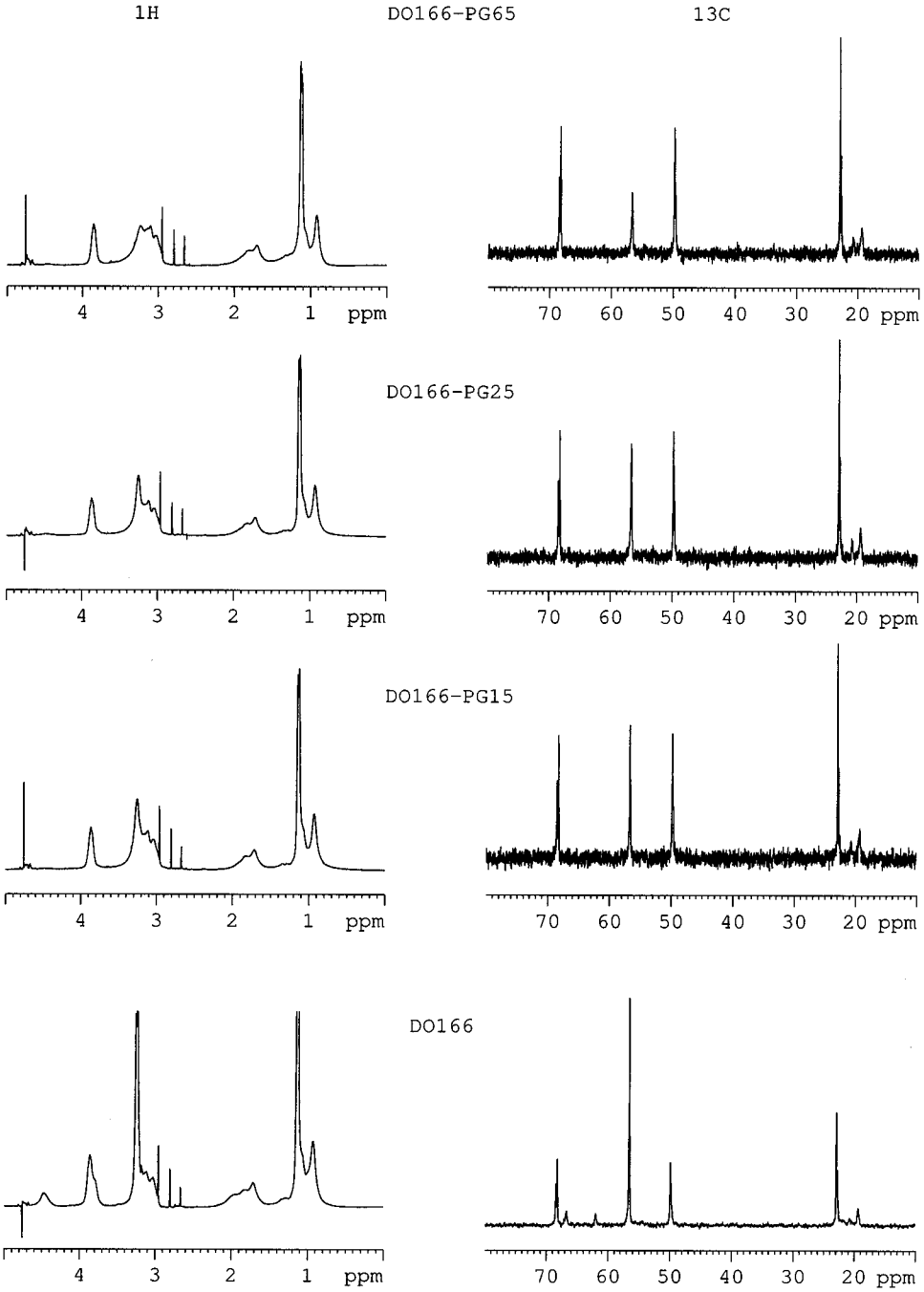


Figure 5. ^{1}H NMR and ^{13}C NMR DEPT45 spectra of three different coupling products of DO166 with the indicated polyphosphates compared with the corresponding spectra of DO166.

The only partial effectiveness of MAS in narrowing the ^{31}P signal has two main reasons: (i) the molecular complex tumbles with a frequency comparable to that of MAS,[18,21] and (ii) the width of the signal is due, partly at least, to a distribution of chemical shifts of superimposed signals. This can be seen when collecting ^{31}P spectra after 1, 2, 3, and 4 ms of transverse relaxation filtering. From the changing pattern and progressive narrowing of the remaining signals, it can be deduced that the coupled phosphate groups are in a state of different mobility. Almost no such effect could be directly detected in the coupling products of the polyphosphates with P_n up to 35. The analysis of relaxation behavior offers some deeper if complex insight into this matter. In contrast to ^{31}P longitudinal relaxation, which is apparently monoexponential, transverse relaxation is at least biexponential, with a varying weight of the fast and slow components. Table 3 shows the T_1 and T_{2i} values with the corresponding normalized weights w_i satisfying the relation

$$I_{xy}(t) = I_{xy}(0)\sum_i w_i \exp(-t/T_{2i}) \qquad (19)$$

for the parent polyphosphates, Table 4 contains analogous values for the corresponding coupling products. As explained in Theory, the multiexponentiality of transverse decay together with the signal shape development during it points to a superposition of signals, i.e., to the existence of phosphate groups with different mobility. The dependence of relaxation behavior on P_n of the polyphosphate is obviously complex in both the parent compounds and the coupling products. It is clear, however, that the fraction of groups with higher mobility increases with the

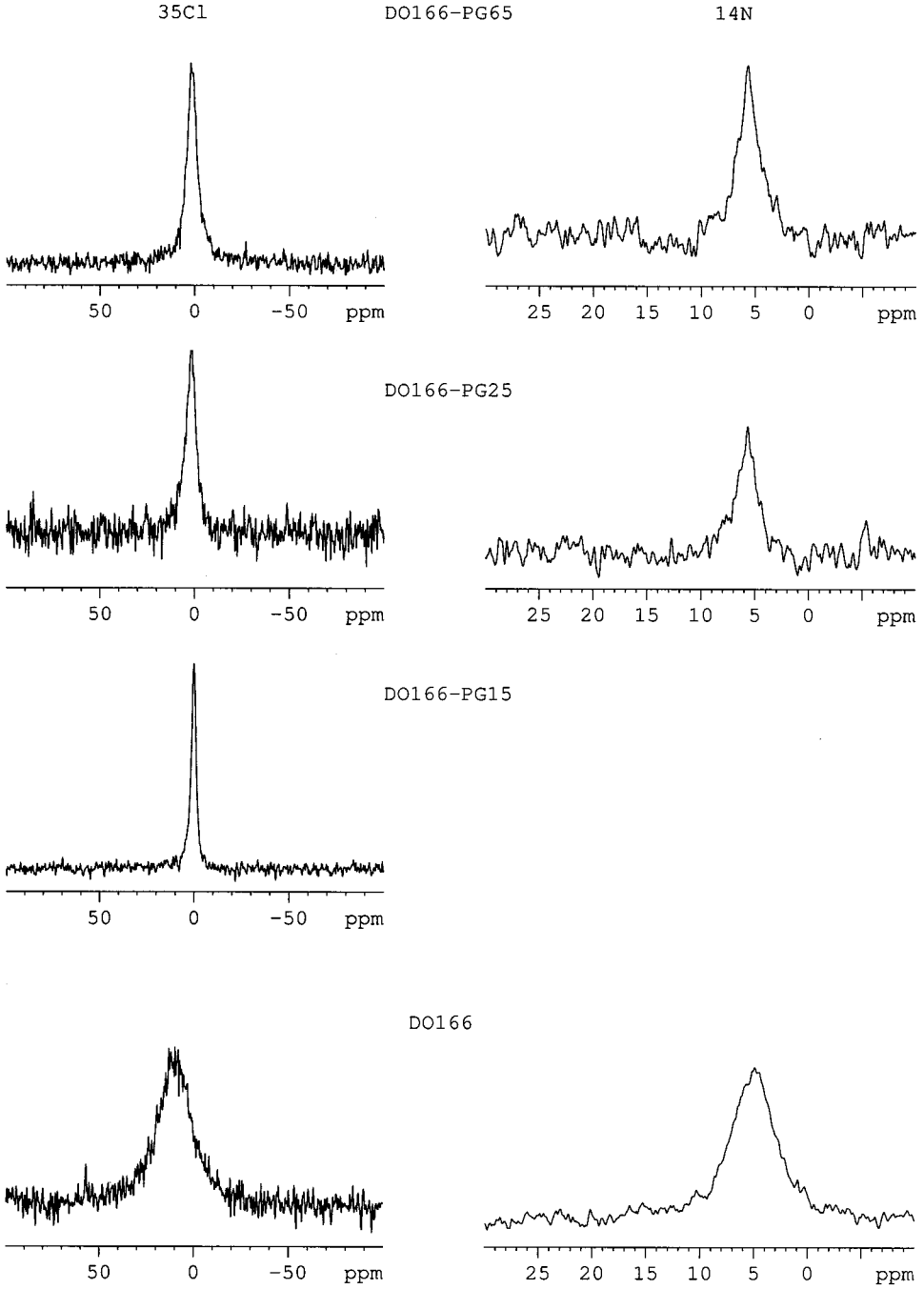


Figure 6. ^{35}Cl and ^{14}N NMR spectra of three different coupling products of DO166 with the indicated polyphosphates compared with the corresponding spectra of DO166.

chain length in the parent polyphosphates. In the coupling products, the mobility of all groups is relatively decreased, some of them to a much higher degree. Starting with $P_n = 45$, an increasing part of phosphate groups with distinctly higher mobility (compared to the rest of phosphate groups in the product) appears. These can be shown to be uncoupled by the evidence of (i) dimensions of the product, (ii) conversion of coupling obtained by three different methods, and (iii) the course of ^{23}Na single and multiple quantum relaxation.

(i) Figure 10 shows ^{1}H NMR PGSE decays for DO166 in 0.1 N NaCl and the coupling products DO166−PG15, DO166−PG45, and DO166−PG75. Due to the small gradient field intensity attainable with our inverse probehead (up to 50 G/cm) and fast transverse relaxation of the nuclei in the coupled groups of the product, only the proton signal of group 10 belonging to the neutral part of DO166 was suitable for PGSE measurements. Self-diffusion coefficients D_{sd} calculated from the shown intensity-gradient dependences are included in Table 5. The mean diffusion length λ_d achieved in the diffusion interval τ_d is $\lambda_d \approx (\tau_d D)^{1/2}$, D being the translation diffusion coefficient. Taking the lowest value of D_{sd} in Table 5 and $\tau_d = 0.16$ s, we arrive at $\lambda_d \approx 980$ nm, which is definitely larger than the diameter of a molecule or even any stable supermolecular aggregate of the coupling product. Hence, the value of D_{sd} can be considered a measure of the diffusing species size as a whole and we can conclude that e.g. the coupling product of DO166 with PG75 is substantially larger than that with PG25.

(ii) Parallel to the increasing size of the complex there is a change of coupling conversion α. The values shown in Table 5 were obtained from (a) the relative intensities of the corre-

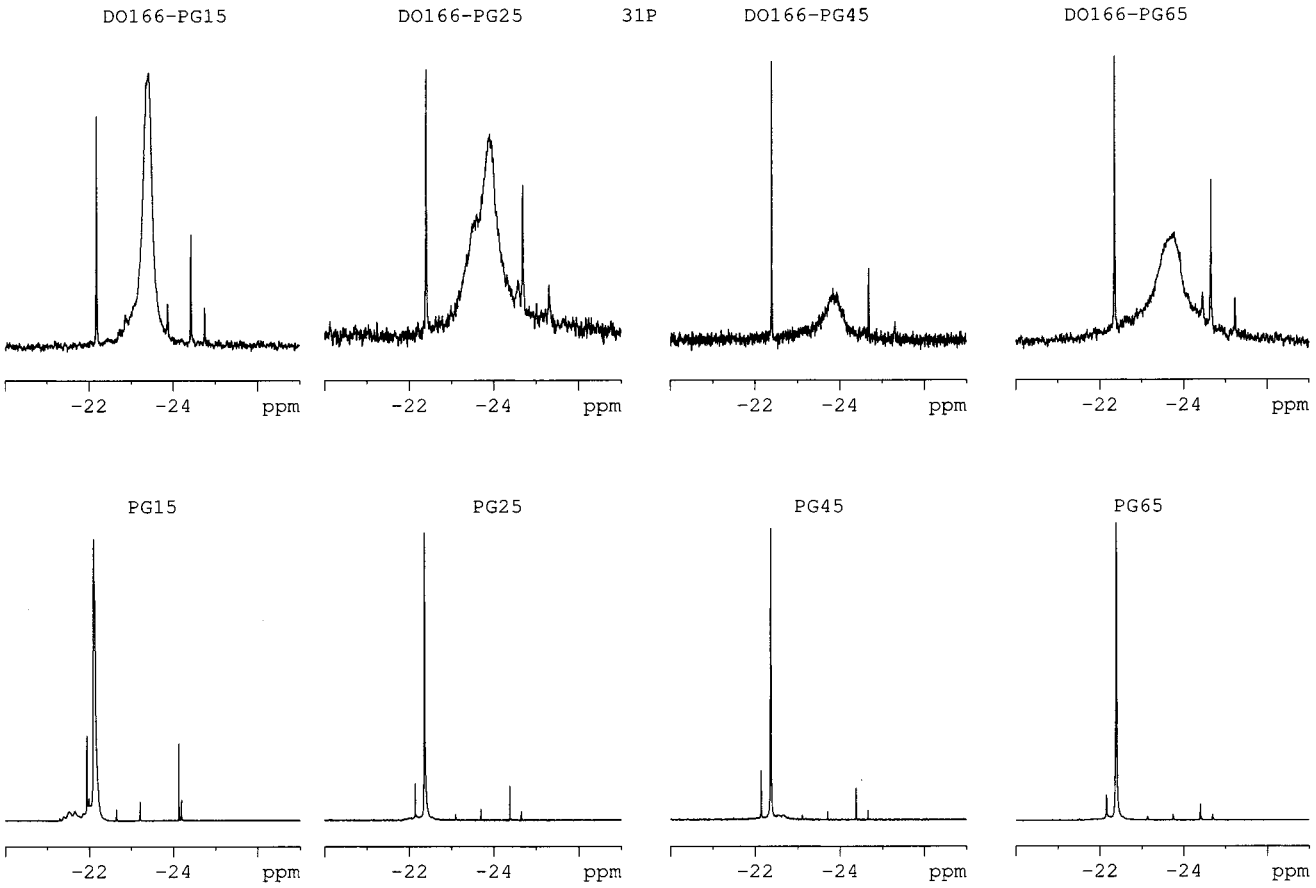


Figure 7. ^{31}P NMR spectra of the indicated polyphosphates and their coupling products with DO166.

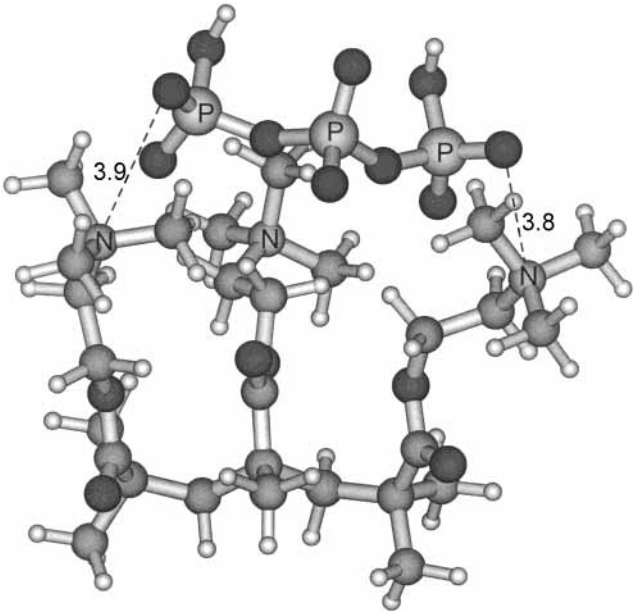


Figure 8. MNDO simulated optimum structure of a trimeric unit of a (TMAEM)Cl−polyphosphate complex.

sponding signals in ^{31}P NMR spectra; (b) ^{23}Na longitudinal relaxation (method A in Theory), corrected for terminal O−Na groups in the case of DO166−PG12 and DO166−PG25; and (c) ^{35}Cl longitudinal relaxation (method B). There is some scatter in the values of α determined by the three independent methods, but the tendency is the same in all of them. The breaking points in the dependence of α as well as D_{sd} on P_n of the polyphosphate

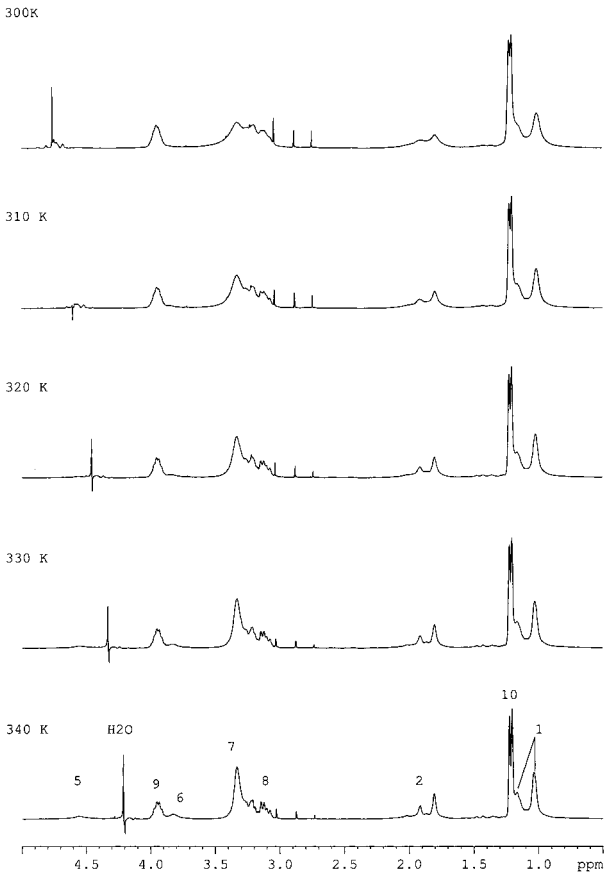


Figure 9. Temperature dependence of the ^{1}H NMR spectrum of the coupling product DO166−PG65 (0.1 M in D_2O).

TABLE 3: ^{31}P Longitudinal T_1 (s) and Transverse T_2 (s) Relaxation Times[a] and Corresponding Normalized Weights w_i for the Polyphosphates of the Given P_n (0.1 M, 300 K)

P_n	T_1	w_1	T_{21}	w_2	T_{22}
15	1.229	0.81	0.313	0.19	0.895
25	1.187	0.68	0.320	0.32	0.862
35	1.183	0.66	0.320	0.34	0.848
45	1.182	0.64	0.322	0.36	0.803
65	1.179	0.60	0.326	0.40	0.782
75	1.168	0.58	0.328	0.42	0.727

[a] Estimated error ±5% rel.

TABLE 4: ^{31}P Longitudinal T_1 (s) and Transverse T_2 (ms) Relaxation Times[a] and Corresponding Normalized Weights w_i for the Coupling Products of DO166 with the Polyphosphate of the Given P_n (0.1 M, 300 K)

P_n	T_1	w_1	T_{21}	w_2	T_{22}	w_3	T_{23}
15	0.616	0.72	7.2	0.28	34.5		
25	0.376	0.68	5.9	0.32	28.9		
35	0.276	0.66	5.6	0.34	22.3		
45	0.252	0.58	4.1	0.32	18.6	0.10	39.2
65	0.323	0.55	3.0	0.28	14.4	0.17	52.3
75	0.382	0.36	2.9	0.24	16.2	0.40	69.8

[a] Estimated error ±5% rel.

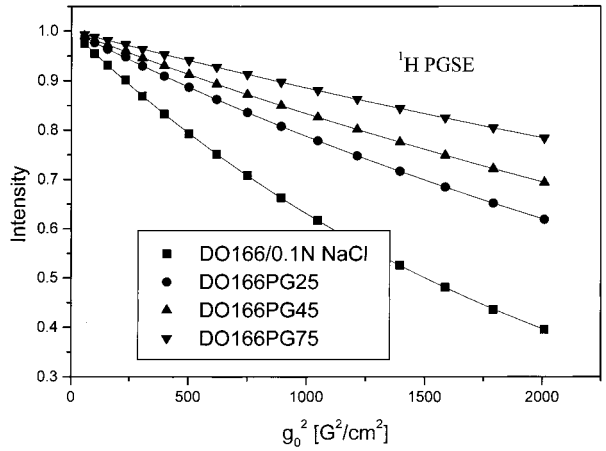


Figure 10. ^{1}H PGSE decay curves of D_2O solutions of 1 M DO166 in 0.1 N NaCl and the indicated coupling products (300 K).

TABLE 5: Self-Diffusion Coefficients[a] D_{sd} (m^2 s^{-1} × 10^{-11}) and the Corresponding Coupling Conversions[b] α Determined by ^{31}P NMR (α_P), ^{23}Na T_1 (α_{Na}), and ^{35}Cl T_1(α_{Cl})

product	D_{sd}	α_P	α_{Na}	α_{Cl}
DO166−PG15	0.69	0.95	0.93	0.94
DO166−PG25	0.63	0.95	0.94	0.94
DO166−PG35	0.61	0.92	0.93	0.92
DO166−PG45	0.48	0.90	0.89	0.89
DO166−PG65	0.42	0.87	0.86	0.87
DO166−PG75	0.32	0.79	0.77	0.76

[a] Estimated error ±5% rel. [b] Estimated error ±3% rel.

is near $P_n = 36$ and 72, i.e., near the P_n of the (PTMAEM)Cl block and its double.

(iii) As expected due to the high conversion of polyion coupling, the transverse relaxation of both ^{23}Na and ^{35}Cl is monoexponential and no double quantum filtered (DQF) signals are detected for the products of polyphosphates up to PG35. Hence, both Na^+ and Cl^- ions are almost completely freed into the surrounding water, and no detectable exchange of them with the few bound sites takes place. From DO166−PG45 to DO166−PG75, the biexponentiality of transverse (or single quantum coherence, T_1^1) ^{23}Na relaxation is increasingly apparent

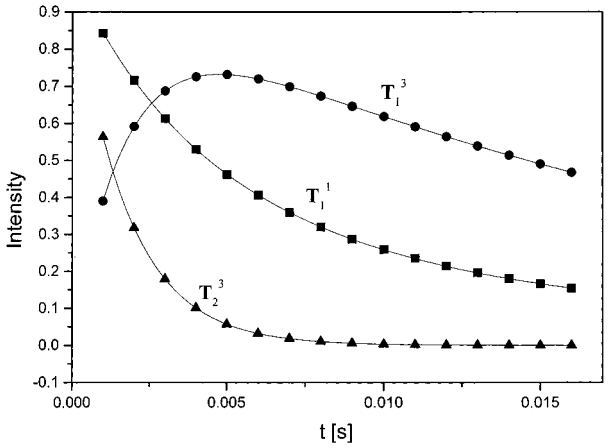


Figure 11. ^{23}Na NMR time decays of T_1^1, T_1^3, and T_2^3 coherences.

and DQF signals can be detected, in particular with DO166−PG75. In contrast to it, ^{35}Cl SQC relaxation remains monoexponential for all products. Since the ion liberation must be symmetric, the difference in relaxation behavior of these ions must be due to either their respective mobility in the bound state or Larmor frequency (or both). The Larmor frequencies of ^{35}Cl and ^{23}Na in these experiments were 29.4×10^6 and 79.39×10^6 Hz, respectively. The critical value of the main correlation time τ_c below which $r_0 \approx r_1$ (see Theory, eq 2) for the bound state is about 0.7×10^{-10} and 2×10^{-10} s, respectively. Figure 11 shows the evolution of T_1^1, T_1^3, and T_2^3 coherences of ^{23}Na in DO166−PG75. The values of τ_c obtained from T_1^1 (eqs 7 and 8, after correcting the data for R_{1F}) and T_1^3 and T_2^3 (eq 12) are 3.4×10^{-9} and 7.3×10^{-9} s, respectively. The difference could be due to experimental and evaluation error but also to the fact that we detect preferentially free or near-free ions in transverse relaxation, whereas in DQF there is a strongest response from a tightly bound state. However, both values are more than 3 orders of magnitude higher than expected for usual diffusion. From this, one can conclude that a fraction of Na ions must be bound to a relatively stiff polyanion sequence, whereas the corresponding Cl ions are bound to relatively less hindered, possibly lone uncoupled (TMAEM)Cl groups.

In agreement with this finding, a relative narrowing of signal 7 in ^{1}H and ^{13}C NMR spectra can be observed in the polyphosphates with P_n progressing from 45 to 75. This phenomenon is superficially complementary to the opposite regression from 35 to 15, but the reason is different: as shown in Theory, the signals are narrowed by (i) larger local mobility (45−75) as well as (ii) faster collective tumbling of the complex (35−15). The larger local mobility of some (TMAEM)Cl groups in DO166−PG75 is almost certainly due to imperfect but multicentric coupling of several DO166 and PG75 molecules into one complex. This is illustrated in Figure 12, comparing ^{1}H spectra of DO166−PG75 obtained by mixing the components at concentrations 0.1 and 0.02 M, respectively. The five times lower concentration evidently reduces the probability of bridging between the coupling products.

The products reported so far were all measured immediately after mixing. No detectable differences in their behavior were observed in the span of several days needed for their reported measurements. However, after a month of storage there was observed a very strong broadening of signal 7 in ^{1}H (and ^{13}C) spectra. The ^{31}P signal is broadened, too, but no significant change in the size of the complex has been observed by PGSE.

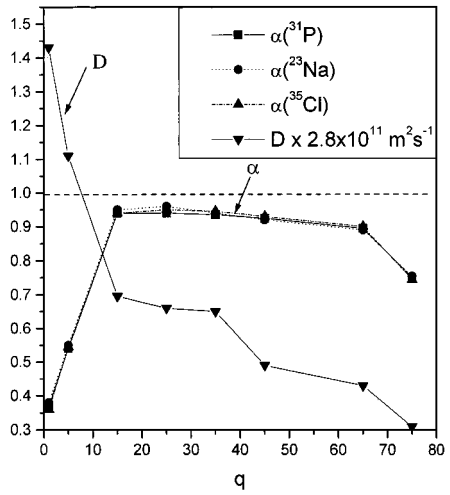


Figure 12. Coupling degrees α established by three independent methods and self-diffusion coefficients of the products of DO166 with phosphates of different chain lengths (equimolar ratio of anions and cations).

Hence, the products change very slowly to a more stable form different in the alignment of phosphate groups.

Quite complementary behavior of the complexes can be observed under heating. As already said above, the recovering and narrowing of the signals under gradual heating to 350 K is apparently reversible. However, after two or three heating cycles, there is an abrupt change in behavior: signals 5 and 6 in ^{1}H and ^{13}C NMR spectra are recovered, and signal 7 is narrowed (though not to the degree observed in free DO166). The ^{31}P broad signal corresponding to the complex disappears completely, transferring its intensity partly to that of the free polyphosphate and partly to that of the monophosphate product of hydrolysis. In the case of DO166−PG65, the relative intensities of the ^{31}P signals correspond to 27% mol of the hydrolyzed phosphate. Even under random cleavage, P_n of the remaining polyphosphate chain should be almost 12; if its hydrolyzed end groups were not fully involved in complexation, the effective P_n would be about 10. Since the behavior of the system after hydrolysis is quite similar to that of DO166 with phosphates having P_n in the range from 1 to 7, a quite sharp lower limit of the polyphosphate P_n for a cooperative interaction is indicated.

Discussion

Brief Summary of the NMR Results. The main results are illustrated in Figure 12: the degree of ion coupling α (established by three independent methods) steeply increases with increasing length of the polyphosphate up to $n \approx 20$, goes through a plateau and slightly decreases for $n > 55$. At the same time, the size of the product increases (self-diffusion coefficient decreases), in particular at the start of the dependence. When analyzing the statistics of the remaining bound Na^+ and Cl^-counterions using various relaxation modes of the corresponding nuclei, one can deduce that they tend to form longer sequences (in particular if $n > 55$). Thus the cooperativity of coupling is demonstrated in two different ways: (i) the coupling needs a longer sequence of both complementary ions to be effective, and (ii) the ions of both reactants are coupled and tend to be coupled in rather unbroken sequences.

Conditions of the Cooperative Coupling and Its Expression in Our System. The interactions can be cooperative in the kinetic or thermodynamic sense. In nonequilibrium systems, these two senses differ and the corresponding effects can be caused by different factors. The slow change of our systems in time indicates that they are not formed in an equilibrium state. At normal temperature, their evolution leads to more stable complexes of the same kind, however. Hence the kinds of cooperativity in forming and maintaining our complexes are not conflicting.

Given the nature of the coupled macromolecules, their primary interaction must be Coulombic (in other words, electrostatic or ionic). The cooperativity of such forces is comparatively well understood for an interaction of a polyion with small counterions and is based on the slow ($1/r$) decrease of electrostatic energy and spatial restrictions in one kind of the complementary charges. In our case there are complementary restrictions in both ion types given by their skeletal bonds and allowed conformations.

As shown above, the probability of the first binding of a (TMAEM)Cl cation to a polyphosphate is less than statistical due to its bulkiness. The same, only more so, must be true for the next few neighboring groups, due to crowding. This holds if only potential energy is taken into account. In the same time, however, the probability of encounter of the complementary ionic groups behaves in a quite different manner.

Let the probability of the first encounter of a *j*th TMAEMA group in the polycation with a complementary phosphate group in the time interval from t to $(t + \Delta t)$ be $p_1(t)\Delta t$. Then the probability of the complementary encounter of an n-tuple of the respective matching groups will be

$$p_n^{+}(t + n\Delta t) = p_1(t)\Delta t^n \prod_{i=j-1}^{1} (1 - \psi_i) \prod_{k=j+1}^{n} (1 - \psi_k) \quad (20)$$

where the function ψ_i expressing the conformation freedom of the given group (the superscripts denoting the cation or anion and $\psi_I < 1$) rapidly converges to some small value, $\psi_i \ll 1$, with increasing $|i - j|$. The probability that the groups $i = 1, ..., j$ will attach to one molecule of the complementary polyion and the groups $k = j + 1, ..., n$ will do so to another one then is

$$p_{j,n-j}^{+}(t + \Delta\tau) = \pi_1(\tau)^2 \Delta\tau^{\nu} \psi_{j+1} \prod_{i=1}^{j} (1 - \psi_i) \prod_{k=2}^{n-j-1} (1 - \psi_k) \quad (21)$$

Since $p_1(t)$ is concentration dependent, $p_{j,n-j}^{+}$ will be lower than p_n^{+} for lower concentrations, which is what we observe. At the given concentration, the ratio of these two quantities is given by the descent of ψ_i with growing i. Thus from the purely statistical point of view, the ability of one polycation to bind exclusively to a complementary and matching polyanion is one kind of a measure of cooperativity. The simple binding we observe at least in the range from PG15 to PG45 thus indicates a fairly strong cooperative effect. However, there is a problem of matching even here, which we shall discuss below.

Once formed, the complex must have a low probability to be cleaved again from a purely statistical point of view. The probability of the jth group being knocked out of its binding position by a small couterion is proportional to ψ_j and that of the whole complex to be cleaved to $\prod_{i=1}^{n} \psi_i$, which is always a small number. In other words, the cleavage of the *j*th bond is being counterbalanced by maintaining the surrounding bonds along the chain unimpaired. If some other *k*th bond were cleaved in the next time interval, the *j*th site very probably snaps back into binding. This effect could be called a *kinetic* stabilization

of the complex. However improbable the cleavage of the complex from this point of view, it would finally happen if its thermodynamic balance were unfavorable.

The electrostatic stabilization energy of the *j*th couple of bound cation and anion groups in the complex is

$$\epsilon_j = \frac{\epsilon^2}{\chi_{\mathrm{ef}}} \sum_{i=1}^{n} \int_V [\sigma_i^+(r_{ji})\, \sigma_j^-(r_{ji}) + \sigma_i^-(r_{ji})\, \sigma_j^+(r_{ji})] r_{ji}^{-1}\, \mathrm{d}r_{ji} \quad (22)$$

where e is the elementary charge, χ_{ef} is the effective permittivity, σ^+ and σ^- are the radial distributions of charge relative to the center of the opposite charge, r_{ji} is the respective distance, and the integral is taken in some realistic limits. Equation 22 could be augmented to include quantum effects or reduced to point charges; i.e.,

$$\epsilon_j = \frac{e^2}{\chi_{\mathrm{ef}}} \sum_{i=1}^{n} r_{ij}^{-1}$$

The energy of an *n*-tuple of coupled ions is then

$$E_n = E_{na} + E_{nc} - \sum_{i=1}^{n} \epsilon_i \quad (23)$$

where E_{na} and E_{nc} are the cumulative electrostatic repulsion energies of the *n*-anion and *n*-cation, respectively, and the total electrostatic energy is $E_{\mathrm{el}} = E_n + E_{\mathrm{ion}}$, where the last term designates the mean Coulombic energy of the liberated counterions in their random positions. Simulations indicate that E_{el} in our case for any n is somewhat higher than the corresponding sum of electrostatic energies of the parent polyion salts.

However, the change of entropy ΔS is another—probably the most important factor—in the buildup of the free energy ΔG determining the thermodynamic stability. The respective entropy changes for both polyions as such are negative, their conformations being relatively frozen in a ladderlike structure of the complex. But there is surely a larger *positive* entropy change due to the small counterions and hydrating water molecules being freed from the binding sites of both polyions into random positions in the surrounding medium. Hence we suggest that the *thermodynamic* stabilization of the complex is an *entropy* effect. Unfortunately, the hydrolytic instability of the polyphosphate chain does not allow checking this conjecture, but we plan to study it on other systems.

Finally, there is an additional effect stabilizing the complex, which could be called *microphase separation.* Both by visual observation and by PGSE, we see that the products are substantially larger than it could be surmised for simple coupling products. We are planning to study this phenomenon by SANS but are rather sure even now that the ammonium−phosphate parts of the complexes, being relatively hydrophobic, aggregate into the core of a micelle-like system. This takes them out of reach of the surrounding small ions and shifts the coupling equilibrium like ordinary precipitation of insoluble salts does.

The only problem in the just given explanation is the observation that polyphosphates with smaller $\mathbf{\Pi}_\nu$ from 15 to 35 offer almost quantitative coupling, whereas the longer ones, in particular those with $P_n = 65$ and larger are less effective. P_n of the polycation being 36, no combination of P_n 15, 25, or 35 can match it exactly, so that more molecules of complementary polyions must be involved in the complex formation. In addition, there is a $1/(15 \times 36) = 1.85 \times 10^{-3}$ relative probability that, e.g., the first cation group of DO166 will encounter the first

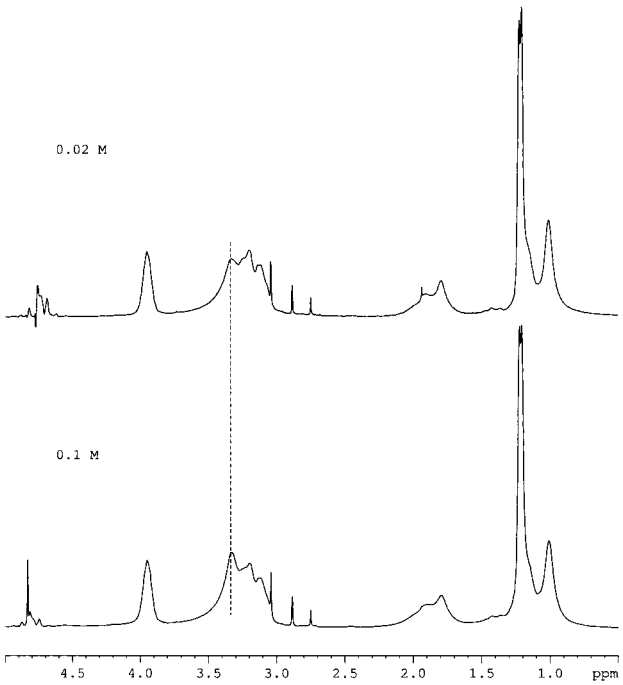


Figure 13. ^{1}H NMR spectra of DO166−PG75 prepared at the concentrations 0.1 and 0.02 M of the parent compounds (300 K).

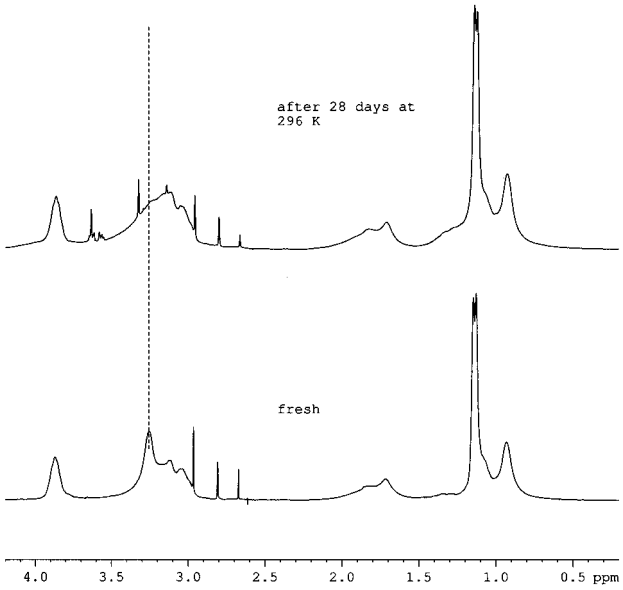


Figure 14. ^{1}H NMR spectra of DO166−PG25 immediately after mixing of the components and after 28 days at 296 K (measured at 300 K).

group of PG15 so that the coupling groups will align from both starts of the chain. Hence, either the coupled polyphosphate has to travel in a kind of *caterpillar* motion into its optimal position or—as suggested above—more molecules of polyphosphate and polycation have to take part in the final buildup of the complex, probably in the aggregation stage. The second explanation could be preferred on the grounds of ^{31}P NMR spectra where no fast exchange between the bound and free phosphorus states can be observed. However, slower motions exceeding in their characteristic period the NMR time window could be important. Figure 13 shows an example of the coupling degree being substantially increased by decreasing the concentration of the reactants. Figure 14 gives another example of the coupling degree increase with time. Also MNDO simulation suggests that the coupling of

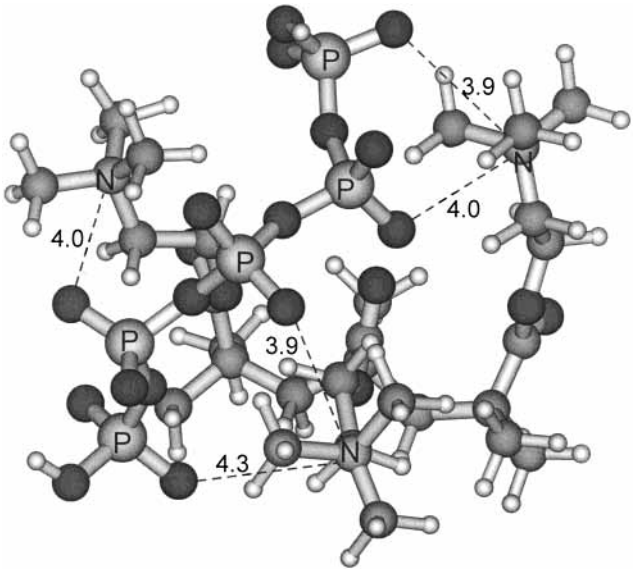


Figure 15. MNDO simulated optimum structure of a product of model (TMAEM)Cl trimer with a longer polyphosphate chain.

(TMAEM)Cl cations with phosphate anions need not proceed in an ordered manner but can leave out one anion, making thus the motion along the polyphosphate chain easier. This is illustrated in Figure 15. The reason the longer polyphosphates ($P_n = 65-75$) do not match so easily probably is in (i) the faster aggregation of the coupling products and (ii) the lower flexibility or approachability of the polyphosphate chain compared to the dangling (TMAEM)Cl groups. Despite entropy change being the main driving force of the coupling, the ability of the polyions to align optimally in the coupling product and thus lower the enthalpy change evidently is important too.

Conclusions

Using multinuclear multiquantum NMR spectroscopy and relaxation analysis in combination with quantum chemical calculations, we demonstrate the cooperative effect in electrostatic coupling of sodium polyphosphates with the trimethylammonium chloride groups of the block copolymer. In addition to the entropy change driven by the liberation of small counterions, optimal alignment of the reactants, ensured either by their structure or experimental conditions and leading to a lowering of the product's enthalpy, has been shown to be an important factor. Another factor, which could not be proved in a direct way, appears to be the microphase separation of the product leading to a slight irreversibility of the coupling.

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