Biomacromolecules **2006,** *7,* 3229−3238

Effect of Polycation Length on Its Complexation with DNA and with Poly(oxyethylene-*block*-sodium methacrylate)

Toni Andersson,[†] Susanna Holappa,[‡] Vladimir Aseyev,*,[†] and Heikki Tenhu[†]

Laboratory of Polymer Chemistry, University of Helsinki, PB 55, FIN-00014, Finland, and Laboratory of Forest Products Chemistry, Helsinki University of Technology, PL 6300, 02015, Finland

Received May 19, 2006; Revised Manuscript Received September 5, 2006

Polyelectrolyte complexes of a synthetic polycation with either a genomic DNA or a synthetic poly(oxyethylene-*block*-sodium methacrylate), POE-*b*-PMANa, have been studied in aqueous solutions as a function of cation:anion ratio, the degree of polymerization of the polycation, the ionic strength, and temperature using dynamic light scattering and turbidity measurements. The polycation was a copolymer of methacryl oxyethyl trimethylammonium chloride and poly(oxyethylene) monomethyl ether monomethacrylate with 4−5 oxyethylene repeating units, PMOTAC-*g*-POE. The molar masses of the polycations in a homological series were 0.3, 0.9, and 2.1 × 10^6 g/ mol. The amount of comonomers with poly(oxyethylene) tails in the copolymers was 15 mol %. The molar mass of the POE-*b*-PMANa was 75000 g/mol and that of the POE-block was 5000 g/mol. The molar mass of the polycation was shown to have a dramatic effect on the stability and size of the complexes formed by either of the polyanions. An increase in the polycation molar mass shifts the cloud point toward the lower polycation content in the complexes, and a macro phase separation occurs in the solutions with the cation to anion molar ratios much below than 1:1. Increasing the ionic strength has a similar effect. Further addition of salt to turbid and phase-separated solutions results in dissociation of the complexes, and the polyions dissolve as individual macromolecules. The effect of POE on the stability of polyelectrolyte complexes is discussed as well.

Introduction

Oppositely charged polyelectrolytes with high charge densities can form highly aggregated, compact, and nearly spherical polyelectrolyte complexes, PECs, in aqueous solution. Complex formation owes to the Coulomb attraction of the polyions and the entropy gain that take place when the low molar mass counterions are released by the polyions to the outer solution.[1−3] Uncomplexed ionic groups of the polymers provide the colloidal stability of PECs. Typically, PECs are not soluble in the stoichiometric mixtures, i.e. when the cation to anion molar ratio of the monovalent ionic groups in the polymers is 1:1. As the ratio approaches unity, interpolymer binding leads to the effective neutralization of the polyions and the complex of two hydrophilic polymers becomes hydrophobic; secondary aggregation takes place, followed by the macro phase separation.

PECs have widely been investigated for the last few decades. Various studies have been done on water-soluble polyelectrolyte complexes,[4,5] as well as on the PEC systems showing phase-separation behavior.[6,7] It has been demonstrated that the stoichiometric complexes can be stabilized as aqueous colloids by utilizing double-hydrophilic block copolymers.[5,8−9] The steric stabilization, provided by neutral segments, enables the formation of the equimolar PEC particles, which are stable in a much broader pH and ionic strength range in comparison to the PECs formed by homopolymers.

Poly(oxyethylene), POE, has often been used in solubilizing electroneutral polymers in aqueous solvents. Thus grafting of the neutral thermosensitive poly(*N*-isopropylacrylamide), PNIPAAM, with POE has been shown to prevent the precipitation of the PNIPAAM above its LCST.[10] POE can also be used to stabilize the stoichiometric polyelectrolyte complexes; PECs formed by polyelectrolytes with hydrophilic neutral grafts or blocks are more stable, and the phase separation can be prevented even at 1:1 anion-to-cation ratio.[8,11] Nonionic POE grafts or blocks affect the conformation and solution behavior of polyelectrolytes and their complexes, significantly modify physicochemical properties of the PECs, and thus bring them new applications.

The ionic strength, temperature, the polymer concentration, and even the rate of mixing of the components affect the formation and the stability of the PECs.[3,12−16] Due to the electrostatic nature of the interactions, the solution ionic strength plays a decisive role in the formation of the PECs and in their properties. The presence of salt enables the rearrangement of the ionic bonds as well as the exchange processes and shifts the reaction closer to the thermodynamic equilibrium.[17] The degree of interpolymer association can also be controlled with the concentration of the added salt.[3] A small amount of added salt decreases the particle size, compared to that of the PECs formed in pure water. Increasing the salt concentration decreases the colloidal stability of the PECs and induces the secondary aggregation, which eventually leads to precipitation if temperature is kept constant. One may expect that the precipitate of the complexes formed by weak polyelectrolytes can be redissolved again by a further increase in the ionic strength. At a certain ionic strength, the amount of low molar mass counterions is sufficient to screen the Coulomb attraction of the polyions and make the entropy gain due to the release of the counterions negligible. In this case PECs are not formed, and a polymer solution consists of individual macromolecules. Such an effect of the ionic strength is schematically represented in Figure 1 and will be experimentally supported and discussed more in

---

* To whom correspondence should be addressed. Ph.: +358-9-19150335, Fax: +358-9-19150330, E-mail: Vladimir.Aseyev@helsinki.fi.

[†] University of Helsinki.

[‡] Helsinki University of Technology.

10.1021/bm0604911 CCC: $33.50

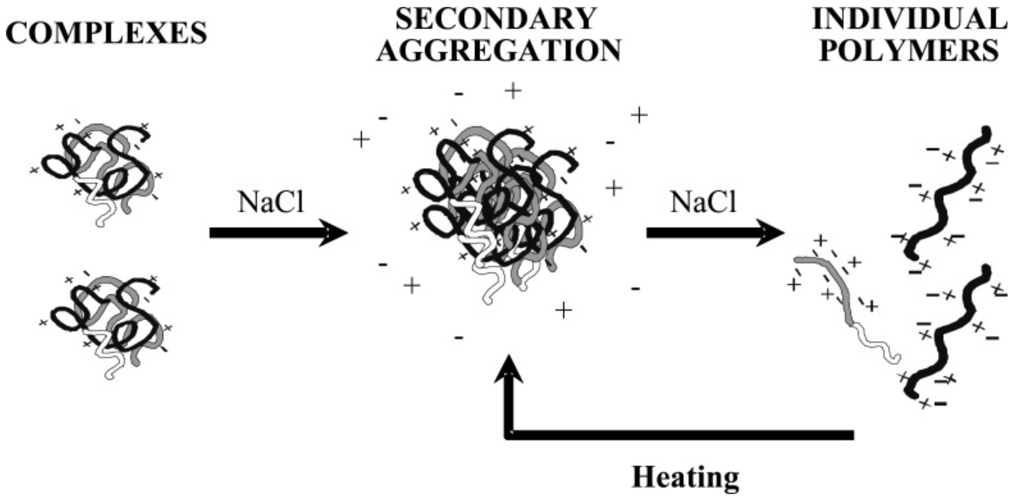

Figure 1. A schematic presentation of the polyelectrolyte complex formation as a function of the ionic strength and temperature.

detail later in the text. PECs formed by strong polyelectrolytes are not likely to be redissolvable.

Polyelectrolyte complexes of cationic poly(methacryloyl oxyethyl trimethylammonium chloride), PMOTAC, and anionic poly(oxyethylene-*block*-sodium methacrylate), POE-*b*-PMANa, with two different lengths of the PMANa block have been investigated by dynamic and static light scattering, viscosimetry, asymmetrical flow field-flow fractionation, and fluorescence spectroscopy.[18−20] POE blocks of POE-*b*-PMANa provide the colloidal stability of the polyelectrolyte complexes, including the stoichiometric PECs. Increasing the length of the anionic block increases the particle size, due to the smaller fraction of the solubilizing POE in the block copolymer. A larger number of POEs are needed also for the stabilization of the PECs in solutions of higher salt concentration, and thus bigger particles are formed. Chain exchange processes between the stoichiometric complexes have been shown to take place via two mechanisms, insertion and expulsion of the single chains, and merging and splitting of the interpolyelectrolyte complexes. With an excess of either the cationic or anionic component, charged nonstoichiometric complexes are formed. The mechanism of the chain insertion, as well as the structure of the PECs at different degrees of overcharging has been evaluated.

The complexation of PMOTAC and its poly(oxyethylene)-grafted analogue, PMOTAC-*g*-POE, with two different polyanions has been investigated.[12,18,21] Polyanions were POE-*b*-PMANa and a polydisperse sodium salt of salmon DNA. Special attention was paid to the effect of the ionic strength and also temperature on the formation of PECs. The effect of poly(oxyethylene), POE, on the stability and shape of the PECs formed by two totally different polyanions has been demonstrated. It has been shown that POE grafts increase the solubility and stability of the complex particles and prevent the phase separation even with 1:1 cation to anion molar ratios.

The complex formation of DNA with various polycations is of growing interest owing to the potential use of these complexes in gene therapy as stimuli responsive carriers for DNA segments[22] and, for example, in biosensors.[23−26] Due to this potential, it is important to understand the effect of various parameters such as the ionic strength, temperature, and the degree of polymerization on the size, shape, and stability of the complexes. As a logical continuation of the previous research,[12,21] this work focuses on the effect of the molar mass of the polycation on the physicochemical properties of the interpolyelectrolyte complexes. Three samples of cationic PMOTAC-*g*-POE of different molar masses were complexed with two polyanions. In the first series of experiments, the polyanion was a polydisperse genomic DNA with a much higher average molar mass (host) than that of the polycations (guest). In the second experimental series, a short synthetic polyanion, POE-*b*-PMANa, was used as a guest, whereas the polycations were hosts with chains longer than those of the polyanion. Particles of PECs were investigated in aqueous dispersions, which the cation:anion ratio, the ionic strengths, and temperature was varied. As will be shown, the molar mass of the polycation has a remarkable effect on the size and stability of the complexes formed by either of the polyanions.

Experimental Section

Materials. For the synthesis of the polycation, an aqueous solution (75-wt %) of methacryl oxyethyl trimethylammonium chloride, MOTAC, was purchased from Aldrich and used as received. Poly(oxyethylene) monomethyl ether monomethacrylate with 4−5 oxyethylene repeating units, POE-methacrylate (Polysciences Inc.), ammonium peroxydisulfate APS (Merck), and *N*,*N*,*N*,*N*-tetramethylethylenediamine 99.5%+ TEMED (Aldrich) were also used with no further purification. The phosphate buffer used in polymerization was Titrisol pH 7.00 (Merck). Salmon DNA (sodium salt) was purchased from Sigma ($M_w = 10.4 \times 10^6$ g/mol). For the synthesis of the polyanion, 4,4′-azobis(4-cyanopentanoic acid), ACPA, was purchased from Fluka and dried under vacuum for 48 h. Tris(methylsilyl)methacrylate, TMSMA (Aldrich), was distilled in a vacuum. 3-Mercaptopropionic acid, 3-MPA (Fluka), was used as received. α-Amino-*ω*-methoxy-PEG, $M_w = 5000$ g/mol (Shearwater Polymers, Inc.), was dried under vacuum at 50 °C overnight. *N*-Hydroxysuccinimide, HoSu, (Fluka) and 1,3-dicyclohexylcarbodiimide, DCC (Fluka), were used as received. Dioxane, acetone, and acetonitrile were dried on molecular sieves. DNA was dialyzed for 3 days in water. After dialysis, it was dried under vacuum in a freeze-drier.

Polymer Syntheses. *PEO-b-PMANa Copolymer*. In the synthesis of the anionic PEO-*b*-PMANa copolymer, TMSMA (10 mass-%) was first polymerized using ACPA as an initiator and 3-MPA as a chain transfer agent. The molar ratios, monomer:initiator:chain transfer agent in the TMSMA polymerization were 100:0.25:0.3. Reaction was carried out in dioxane at 60 °C under nitrogen for 18 h. The solvent was evaporated, and the product was dried overnight under vacuum at room temperature. The polymer was purified by precipitation from acetone to acetonitrile and then filtered and dried under vacuum overnight. As the second step of the synthesis, the carboxylic acid end groups were activated by an excess amount of *N*-hydroxysuccinimide. This was carried out in dioxane in the presence of DCC for 2 days. The reaction mixture was precipitated in acetonitrile and the product was dried under

Table 1. The Polycations: Graft-Copolymers of Methacryl Oxyethyl Trimethylammonium Chloride and Poly(oxyethylene) Monomethyl Ether Monomethacrylate with 4−5 Oxyethylene Repeating Units, PMOTAC-*g*-POE

polycation	M_w (g/mol)	M_w/M_n	amount of POE grafts
PMOTAC-*g*-POE1	0.3×10^6	2.7	15 mol-%
PMOTAC-*g*-POE2	0.9×10^6	2.6	14 mol-%
PMOTAC-*g*-POE3	2.1×10^6	3.6	15 mol-%

vacuum. In the final step, the activated PTMSMA and the amino-terminated PEO were attached to each other in a condensation reaction. The reaction was carried out in dioxane under nitrogen at room temperature in the dark for two weeks. The product was precipitated in acetonitrile. All the reaction steps were done under dry conditions in order to prevent the hydrolysis of the protecting trimethylsilyl group. The protecting silyl groups were removed by washing the product first with 0.10 M NaOH and subsequently with water. PEO-*b*-PMANa had $M_w = 75000$ and $M_w/M_n = 1.6$ according to SEC.

Copolymer PMOTAC-g-POE. Copolymerization of MOTAC with POE-methacrylate was conducted at −4 °C to prevent the gelation of the solution. The aqueous phosphate buffer was used as a solvent, and a redox pair APS and TEMED was used as an initiator (0.3−0.5 mass-% of total monomer charge). Decreasing the amount of initiator dramatically increased the molar mass of the polymer. The polymerization was started as a homopolymerization of MOTAC. After 1 h, POE-methacrylate (15 mol-% of the monomer charge) was added, and the reaction was continued for 45−80 min. It was noticed that increasing the polymerization time increases the molar mass of the polycation as well as the yield mainly due to the chain transfer reactions. The polymer was precipitated into acetone. Prior to the precipititation, NaCl was added to the solution to block the cations in the polymer chain and thus facilitate the precipitation. Evaporation of water was not used in the present case because of the possible chain transfer reactions. As is often the case, POE acts as a chain transfer agent in the radical polymerization thus leading to branching and gelation of the product especially at elevated temperatures. Other details of the synthesis have been reported elsewhere.[12,18,21]

The molar masses, polydispersities and POE-contents of the studied polycations are presented in Table 1. The amount of POE-grafts on the polycations was kept constant (14−15 mol-%). The molar mass of the POE-graft in polycations was 200 g/mol, i.e. 4−5 ethylene oxide repeating units. POE-*b*-PMANa had $M_w = 75000$ g/mol and $M_w/M_n = 1.6$. The molar mass of the POE-block in the polymer was 5000 g/mol. The molar mass of the polymers were measured by size exclusion chromatography (Waters) using POE standards.

Preparation of Polymer Complexes. DNA, POE-*b*-PMANa, and PMOTAC-*g*-POE were first dissolved separately in aqueous 0.02 M NaCl. The distributions of hydrodynamic radius, R_h, of uncomplexed DNA, the POE-*b*-PMANa, and the PMOTAC-*g*-POE have been presented in our previous publications.[12,21] All the size distributions of the polymers before the complex formation are broad and do not vary much with the temperature or the ionic strength above 0.02 M NaCl.

To prepare stable dispersions of PMOTAC-*g*-POE/DNA complexes, PMOTAC-*g*-POE solutions with concentration 0.5 mg/mL were added to 0.2 mg/ mL DNA solutions in four mixing ratios. The average value of the molar mass of four deoxyribonucleotide units in DNA has been used to calculate the molar ratios of cationic to anionic repeating units of the polymers in the interpolymer complexes. The molar ratios of cationic to anionic groups of the studied polyions were 0.30, 0.45, 0.60, and 0.9. Polycation solutions were added dropwise with a syringe to DNA solutions upon mixing to avoid the macroscopic phase separation. In each solution the final DNA concentration was kept constant 0.17 mg/mL whereas the polycation concentration was varied. The DNA concentration was set after the addition of the polycation by adding an appropriate amount of 0.02 M NaCl solution so that the total volume was always the same.

Aqueous 0.02 M NaCl dispersions of the PMOTAC-*g*-POE/DNA complexes were clear at the low mixing ratios and became turbid upon increasing the polycation content. Thus, in the case of the PMOTAC-*g*-POE1/DNA, the dispersions clouded at the 0.6 cation:anion ratio and the complexes partly precipitated above the mixing ratio of 0.9. For comparison, the stoichiometric PMOTAC-*g*-POE1/POE-*b*-PMANa complexes were stable under the similar solution conditions. The dispersions of the complexes formed between DNA, and the polycations of the higher molar masses turned cloudy at the lower mixing ratios. Thus, in the case of the PMOTAC-*g*-POE3/DNA (the polycation #3 of the highest molar mass studied, see Table 1), the dispersions became turbid at as low mixing ratio as 0.3.

To prepare the complexes of polycations with POE-*b*-PMANa, the solution of polyanion was added dropwise to solutions of polycations with a syringe. Only stoichiometric complexes of synthetic POE-*b*-PMANa with PMOTAC-*g*-POE were studied as a function of the molar mass of the polycation, the ionic strength, and temperature. Solutions of 0.5 and 1.0 mg/mL polyion concentrations were prepared in aqueous 0.02 M NaCl. Polyanion and polycation solutions with equal polymer concentrations were mixed together in predetermined ratios to obtain cation-to-anion ratio of 1:1, and the overall polymer mass concentration of the complexes solutions was the same as for the solutions. The complexes of two largest polycations (PMOTAC-*g*-POE2 and PMOTAC-*g*-POE3) formed flakelike precipitates instantly after mixing when the polymer concentration was 1.0 mg/mL. However, precipitation was minimal for the complexes with polymer concentration 0.5 mg/mL, which allowed the light scattering studies. DLS measurements were performed on the supernatant. The precipitated fraction dissolved as individual polymers above 0.39 M NaCl even in the case of polymer concentration 1.0 mg/mL.

After mixing the components, the dispersions of both types of complexes were shaken for 2 h at room temperature. The dispersions were stored in refrigerator and then kept before the actual measurement for 24 h at the temperature of interest to reach equilibrium. After stabilization, the ionic strength was adjusted by salt addition to reach the final concentrations in the range of 0.02−0.54 M NaCl. After adding the salt, dispersions were stabilized in the refrigerator at least 24 h and the turbidity measurements were started at 5 °C.

Instrumentation. *Turbidity measurements*. Turbidity was measured to study the complex formation and phase separation behavior in solutions with the high ionic strengths as a function of temperature. Turbidity measurements were conducted using a Shimadzu 160 1PC UV−Vis spectrophotometer at the wavelength of 500 nm (the studied polymers do not specifically absorb light of the wavelengths above 300 nm). Measurements were performed on the PMOTAC-*g*-POE/POE-*b*-PMANa complexes within the temperature range 5−50°C. The concentration of NaCl was varied in the range 0.33 M − 0.54 M. Two polymer concentrations were studied: 0.5 mg/mL and 1.0 mg/mL. A macroscopic flakelike precipitation occurred in the dispersions with the total polymer concentration of 1.0 mg/mL and disturbed the measurements even when the ionic strength was kept low (0.02 M NaCl). Though the dispersions with polymer concentration of 0.5 mg/mL turned turbid upon heating as well, these dispersions were homogeneous and colloidally stable. Temperature was elevated with a step of 5°C. At each temperature step, the solutions were kept for 15 min to reach the equilibrium prior to the turbidity measurement.

***^{1}H NMR and ^{13}C NMR*.** NMR spectra were measured to determine the chemical compositions of the polymers after purification, with a Varian Gemini 2000. D_2O was used as a solvent. The polymer concentration was 40.00 mg/mL. The amounts of poly(oxyethylene) in the POE-grafted polycations were determined from quantitative ^{13}C NMR spectra. The ratios of the components were calculated using the ratio of poly(oxyethylene) (68 ppm) and $N(CH_3)_3$ (53 ppm) signal areas. All other ^{13}C NMR signals of the polymers overlap.

Size Exclusion Chromatography. The molar masses of the synthesized polymers and DNA were determined by size exclusion chromatography (SEC) using Waters 717 Plus autosampler and Waters 2410 refractive index detector. Poly(ethylene oxide) standards (Polymer Laboratories) were used, and 0.8 M aqueous $NaNO_3$ was used as an eluent. In each case the M_w/M_n was rather high, and the chromatograms were monomodal.

Light Scattering. Dynamic light scattering, DLS, was conducted using a Brookhaven Instruments BI-200SM goniometer and BI-9000AT digital correlator. A Lexel 85 argon laser operating at 514.5 nm wavelength and 15−50 mW power was the light source. The effect of the ionic strength, temperature, and the molar mass of polymers on the macromolecular conformation and the polymer complex formation were studied for the solutions having the polymer concentration of 0.5 and 1.0 mg/mL in the case of PMOTAC-*g*-POE/POE-*b*-PMANa complexes and 0.17 mg/mL in the case of PMOTAC-*g*-POE/DNA complexes. NaCl concentration varied from 0.02 to 0.54 M. In DLS measurements the temperature stabilization time was the same as in the turbidity measurements, i.e., 15 min with the heating step of 5 °C. Temperature range was 10°C to 50°C. Numerical data presented herein are not absolute and only represent qualitative changes in the dispersions. Thus, correlation functions of scattered light intensity, $G_2(t)$, for analysis of the distributions of the hydrodynamic size were collected at scattering angle 90°. Distributions of the hydrodynamic sizes of PMOTAC-*g*-POE/DNA complexes were investigated at scattering angles ranging between 30° and 150° as in our earlier studies.[21]

Results and Discussion

DNA(host) with PMOTAC-*g*-POE(guest). The effect of the molar mass of the polycation on the formation of the PMOTAC-*g*-POE/DNA complexes was investigated on the dispersions of 0.30, 0.45, 0.60 and 0.90 cation:anion ratios and DNA concentration of 0.17 mg/mL. PMOTAC-*g*-POE/DNA particles were studied in aqueous 0.02 M NaCl solution at 20 °C. Distributions of the hydrodynamic radius were obtained at 90° scattering angle and analyzed as a function of mixing ratio and M_w of the polycation. Figures 2 and 3 present the distributions of R_h of the complexes. PMOTAC-*g*-POE1 ($M_w = 0.3 \times 10^6$ g/mol) forms soluble complexes with DNA over the whole measured range of mixing ratios (0.3−0.9) in a 0.02 M aqueous NaCl solution (Figure 2). The size distributions are bimodal consisting of two narrow peaks. In fact, such bimodal distributions may also be a sign of an actual broad monomodal distribution owing to the well-known ill-posed character of the inverse Laplace transform, which is the basis of the CONTIN algorithm. However, the corresponding autocorrelation function of scattered electric field $g_1(t)$ represented as $\ln(|G_2(t)|^{1/2})$ vs t has two obvious linear regions, meaning that the size distributions are bimodal indeed and both peaks are narrow. Moreover, broad distributions are not typical for PECs.[11,27] The angular dependence of these two peaks has been investigated in detail in the earlier study.[21] At the scattering angles below 75°, only one narrow peak representing the PEC particles was detected, which allows for estimation of the radius of gyration R_g.

Evidently, the peak corresponding to the larger particles represents the polyelectrolyte complexes. Mean R_h of PEC particles is approximately constant upon increasing the polycation content (Figure 2). The other peak corresponding to the smaller scatterers has much lower relative intensity. Position and relative intensity of the low intensity peak does not significantly vary as a function of the polycation content increasing from 0.3 to 0.9 cation:anion ratio. However, since the total time average intensity of light scattered by the PEC dispersions increases upon increasing the polycation content, the absolute intensity value that corresponds to the lower intensity peak increases as well. Taking also into account the stronger scattering from the dense PEC particles, one may suggest that the origin of the low intensity peak owes to the presence of the uncomplexed polycation chains. Uncomplexed DNA chains may actually exist at low cation:anion ratios and gradually disappear upon addition of polycation in the mixture. However, DNA has a broad size distribution and, therefore, can hardly result in a narrow peak. One of the possible reasons for the existence of the polycation chains not bound to DNA at 0.9 cation:anion ratio could be the difference in the degree of ionization of polyions, i.e., the effective charge of the polyelectrolytes. In this aspect, special attention has also been paid to the effect of the POE grafts and blocks on the formation of PECs. POE not only increases the solubility of PEC particles in water, but may form inter- and intramolecular complexes with polycations via cationic binding[12,21] and also with weak polyanions, as recently demonstrated for POE-*b*-PMAA.[19]

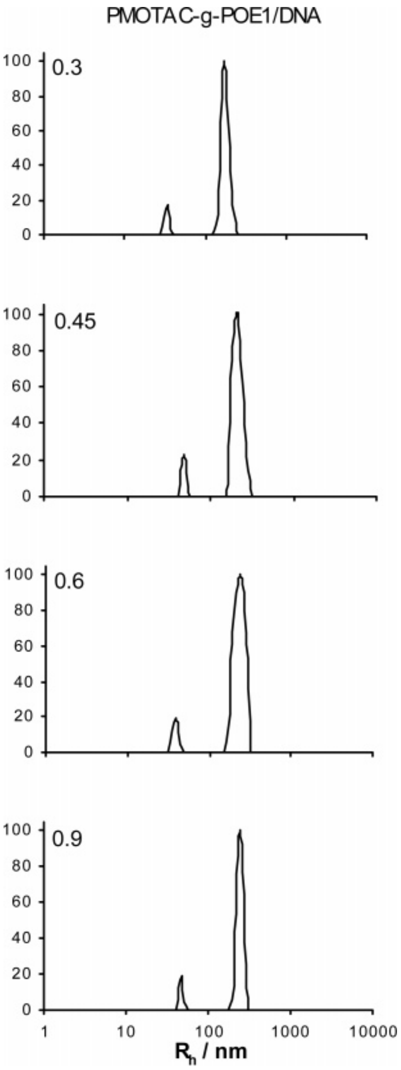

Figure 2. The effect of mixing ratio on the size distribution of PMOTAC-*g*-POE1/ DNA complex in 0.02 M aqueous NaCl solution. The polycation concentration increases from top to bottom (cation: anion ratios of 0.3, 0.45, 0.6, and 0.9)

The stabilizing effect of POE is especially pronounced for the shorter PMOTAC-*g*-POE1 in solutions of 0.02 M NaCl, when the difference in the chain length of PMOTAC-*g*-POE and DNA is maximal. R_g/R_h values of PMOTAC-*g*-POE1/DNA particles were typical for a hard (not a flowing-through) sphere, close to 0.77.[21] The R_h of PMOTAC-*g*-POE1/DNA complexes was 230 nm, and the corresponding peak in the size distribution was narrow even with a mixing ratio of 0.9. At a 0.9 ratio, the dispersion turned only slightly turbid without any obvious

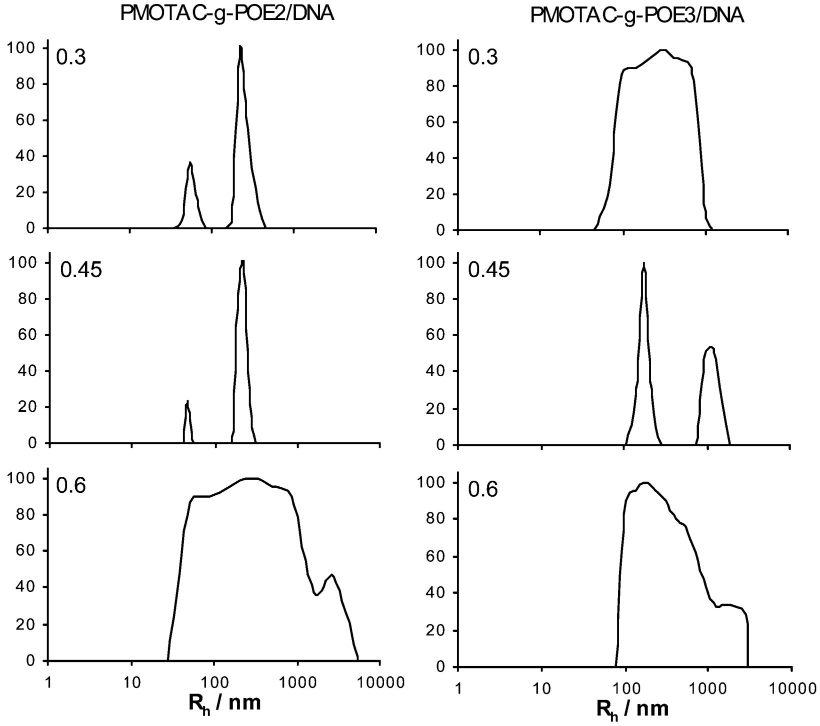

Figure 3. The effect of mixing ratio on the size distributions of PMOTAC-*g*-POE2/ DNA (on the left) and PMOTAC-*g*-POE3/DNA (on the right) complexes in 0.02 M aqueous NaCl solution. The polycation concentration increases from top to bottom (cation:anion ratios of 0.3, 0.45, 0.6)

macroscopic precipitation. Solutions with a 1:1 mixing ratio are turbid, and the PMOTAC-*g*-POE1/DNA complexes partly precipitate.

As can be seen from comparison of Figures 2 and 3, the molar mass of the polycation together with the mixing ratio has a dramatic effect on the formation of PECs. In the case of PMOTAC-*g*-POE2/DNA in 0.02 M NaCl solutions below mixing ratio 0.45, PEC particles with narrow size distribution were formed, see Figure 3. The size distributions were bimodal also in this case, similar to those of PMOTAC-*g*-POE1/DNA. A strong tendency toward phase separation was observed upon increasing the polycation concentration above mixing ratios of 0.45. A broad size distribution obtained from supernatant together with the low intensity of scattered light from the PECs of a 0.6 mixing ratio and higher indicate that the complexes phase separate almost completely. A similar process of aggregation followed by precipitation was observed in the case of the largest polycation PMOTAC-*g*-POE3. However, PMOTAC-*g*-POE3/DNA particles were even larger and less stable under the same conditions, see Figure 3. Precipitation of PMOTAC-*g*-POE3/DNA complexes took place in the dispersions with the mixing ratios lower than those for the shorter polycations. Thus, the PMOTAC-*g*-POE3/DNA dispersion with a mixing ratio of 0.3 was slightly turbid, whereas the formation of the large secondary aggregates of $R_h = 1000$ nm was observed in the dispersions with the mixing ratio of 0.45. In the latter case, the intensity of light scattered from the supernatant was low and a partial macroscopic precipitation was visually observed. In the supernatants of DNA and two largest polycations with the 0.9 mixing ratio, the dynamic light scattering measurements were not possible due to weak scattering.

It can be concluded that in 0.02 M NaCl solutions, the PEC particles formed between DNA and short PMOTAC-*g*-POE1 are much more stable in respect to the increasing polycation content in the complexes than the PECs formed between DNA and the two other polycations of the higher molar mass. Longer chains of PMOTAC-*g*-POE form larger PECs. Particles of PMOTAC-*g*-POE2 or PMOTAC-*g*-POE3 with DNA form secondary aggregates and precipitate from the dispersions of the mixing ratios lower than that of PMOTAC-*g*-POE1/DNA.

POE-*b*-PMANa(guest) with PMOTAC-*g*-POE(host). The complex formation between a short chain POE-*b*-PMANa as a guest molecule and a relatively long cationic PMOTAC-*g*-POE as a host molecule was studied to establish a possible effect of the M_w of the polycation. Additional POE blocks of the polyanion improve the stability of the PECs in comparison to complexes with DNA and allow for investigation of PMOTAC-*g*-POE/ POE-*b*-PMANa complexes with a mixing ratio 1:1 and polymer concentrations of 0.5 mg/mL and 1.0 mg/mL.

In the Figure 4, the effect of the ionic strength on the PMOTAC-*g*-POE1/POE-*b*-PMANa complexes is presented. The total polymer concentration was 0.5 mg/mL. One can notice that the polyelectrolytes form complexes with extremely narrow size distribution at the ionic strengths below and equal 0.1 M NaCl. Similar to the dispersions of PECs formed by PMOTAC-*g*-POE and DNA at 0.02 M NaCl, the low intensity peak represents polycations not bound to POE-*b*-PMANa. Owing to the lower degree of ionization of anionic groups of the PMANa blocks in comparison to the polycation, all POE-*b*-PMANa chains are incorporated in the PEC particles, resulting in the overcharging of the PECs, whereas some cationic chains remain free in solution. This is evident for the NaCl concentrations below 0.02 M. In solutions of a higher ionic strength, the difference in ionization of the polyions effectively disappears owing to the Debye−Hückel screening by low molar mass counterions. Interpolymer complexes become more hydrophobic and larger upon increasing the ionic strength, and above 0.1 M NaCl the low intensity peak is no longer detected.

Strictly speaking, the term overcharging does not necessarily mean the existence of an actual surface charge of PECs, but rather shows that a larger relative number of anionic groups in PEC particles is needed to compensate the charges of the polycations. In the present work, the polyanions were either in

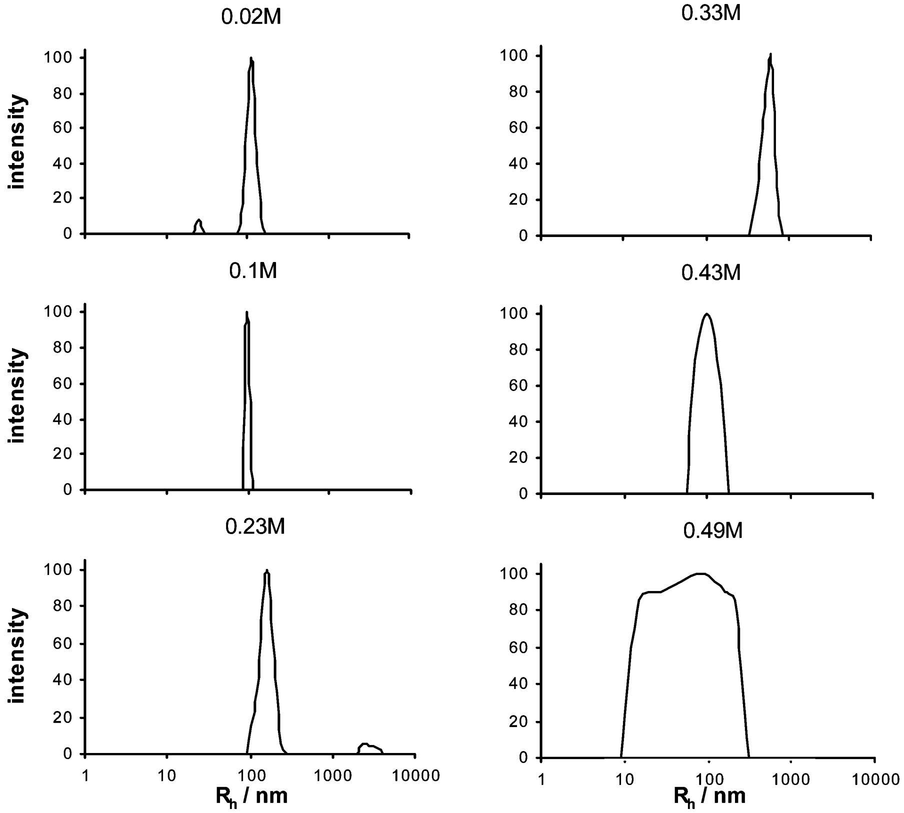

Figure 4. The effect of ionic strength on the size distribution of 1:1 complex of PMOTAC-*g*-POE1 and POE-*b*-PMANa. The total polymer concentration is 0.5 mg/mL.

excess in respect to the polycation or at a stoichiometric ratio. Despite of that, the polycation (PMOTAC-*g*-POE1 below 0.02 M NaCl) existed as individual chains even at a cation-to-anion ratio close to stoichiometric. PMOTAC-*g*-POE/DNA complexes can be overcharged by the longer host (DNA), which agrees with the guest−host theory of polyelectrolyte complexes.[17,28−30] In contrast, the PMOTAC-*g*-POE/POE-*b*-PMANa complexes reveal overcharging by the guest component in the presence of free cationic hosts. This underlines once again the specific role of POE chains in stabilizing the intermolecular interactions.

In 0.23 M aqueous NaCl solutions PMOTAC-*g*-POE1/POE-*b*-PMANa complexes form larger secondary aggregates observed in bimodal size distribution as a peak at higher R_h values from the individual PECs, see Figure 4. At 0.23 M the relative intensity of the peak for the larger particles is still low. Upon further increase of the ionic strength, the complexes become more hydrophobic due to the screening of the free ionic groups in the complex particles. The relative intensity of the peak corresponding to the secondary aggregates increases and swamps the scattering from the smaller PECs. The dispersion becomes turbid, but macroscopic precipitation is minimal. The dispersion turns clear again upon increasing the ionic strength. Large secondary aggregates break at 0.43 M NaCl, see Figure 4. The intensity of the scattered light is still 5-fold compared to the intensities of pure polymer solutions of the components, and the observed size distribution is narrow compared to those for pure individual polymers in aqueous NaCl.[12] This indicates that complexes still exist at 0.43 M NaCl. Upon a further increase in the ionic strength, the complexes disintegrate and polymers dissolve as individual chains. At 0.49 M NaCl the intensity decreases dramatically to a value comparable with the PMOTAC-*g*-POE solution without added polyanion, and the observed size distribution is as broad as those for pure polymer solutions.

In the case of two larger polycations, PMOTAC-*g*-POE2 and PMOTAC-*g*-POE3, the size distributions of the complexes were also narrow at 0.02 M NaCl. The corresponding mean peak value of R_h of about 100 nm was equal to the value of PMOTAC-*g*-POE1/POE-*b*-PMANa complexes and was smaller than that of PMOTAC-*g*-POE1/DNA. The complexes of the polycations of higher M_w with POE-*b*-PMANa form secondary aggregates at ionic strengths lower than that for the PMOTAC-*g*-POE1/POE-*b*-PMANa complexes. In a way, this behavior is similar to what has been observed earlier for complexes with DNA. However, it must be emphasized that this time PMOTAC-*g*-POE plays the role of a host molecule, and the effect of the molar mass on the PEC formation is expected to be opposite, i.e., the stability of the complexes should increase with increasing difference in the lengths of host and guest molecules.[17,28−30] The secondary aggregation in the PEC dispersions of the longer PMOTAC-*g*-POEs appears already at 0.1 M NaCl in contrast to those by the smaller PMOTAC-*g*-POE1. For PMOTAC-*g*-POE2 and PMOTAC-*g*-POE3, in the range of 0.23−0.43 M NaCl, the peak corresponding to the secondary aggregates dominates and a partial macroscopic phase separation is observed. Starting from 0.49 M NaCl, aggregates break apart and the precipitated fraction dissolves as individual polymer chains as was observed in the case of the complexes formed by PMOTAC-*g*-POE1.

A possible influence of the total polymer concentration on the formation of larger PEC particles and secondary aggregates was verified on the dispersions with a higher total polymer concentration of 1.0 mg/mL. These complexes were also studied as a function of the ionic strength. Polymer concentration appeared to have no effect on the dimension of PECs formed by PMOTAC-*g*-POE1. The process of the aggregation of the PECs as a function of increasing the ionic strength was similar to described above, and no significant macroscopic phase

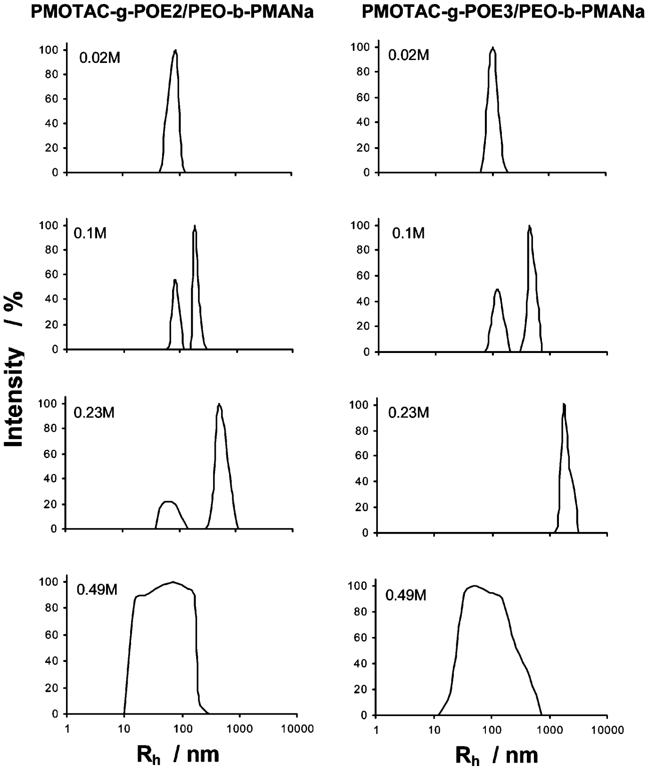

Figure 5. The effect of the ionic strength on the size distribution of the 1:1 complexs of PMOTAC-*g*-POE2/POE-*b*-PMANa and PMOTAC-*g*-POE3/POE-*b*-PMANa. The total polymer concentration is 0.5 mg/mL.

separation was observed. With two larger polycations, the increase in the polymer concentration from 0.5 mg/mL to 1.0 mg/mL had considerable effects. PMOTAC-*g*-POE2 complexes with POE-*b*-PMANa formed secondary aggregates already at 0.02 M NaCl. Above 0.23 M NaCl, a strong tendency to a macroscopic phase separation was observed for both larger polycations. For precipitated solutions, the scattered light intensity approached the value of the pure solvent indicating nearly complete macroscopic phase separation. In the case of PMOTAC-*g*-POE3 the precipitation was significant even with the lowest studied the ionic strength of 0.02 M NaCl.

Effect of Temperature on PMOTAC-*g*-POE/POE-*b*-PMANa Complexes. As has been demonstrated above, all PEC particles segregate and dissolve as individual macromolecules if the ionic strength is high enough. On the other hand, once broken complexes are expected to form again upon heating due to the increasing mobility of low molar mass counterions, which results in the weakening of the effective screening of the polyions.[12] In the present study, the effect of polycation size on the critical temperature of re-formation of the PECs was studied as a function of salt concentration. The total polymer concentration was 0.5 mg/mL and 1.0 mg/mL. Re-formation of the complexes was followed by the strong aggregation and precipitation upon heating. The temperature at which the PECs form again and the dispersion becomes cloudy were shown to depend on the ionic strength, the polymer concentration, and on the amount of hydrophilic nonionic POE grafts.

Before mixing, the anionic POE-*b*-PMANa and the cationic PMOTAC-*g*-POE polymer solutions were clear in the whole range of temperature (5−50 °C) and salt concentration (0.02−0.54 M). However, solubility of the PMOTAC-*g*-POE/ POE-*b*-PMANa complexes is strongly affected by temperature and salt. In Figure 6, the turbidity results are presented for the PECs formed by the polycations with different molar masses in the dispersions with the ionic strengths of 0.41 M, 0.43 M, and 0.49 M NaCl. Turbidity increases with increasing temperature. The same effect can be seen upon decreasing the ionic strength or increasing the molar mass of the polycation at constant temperature. One can see from Figure 6 that the dispersions of two larger polycations mixed with POE-*b*-PMANa turn turbid at lower temperatures than that for the shortest PMOTAC-*g*-POE1. Figure 7 represents the phase diagram obtained for the dispersions of PECs formed by polycations of different M_w. The dependence of the cloud point temperature on the molar mass of the polycations is obvious.

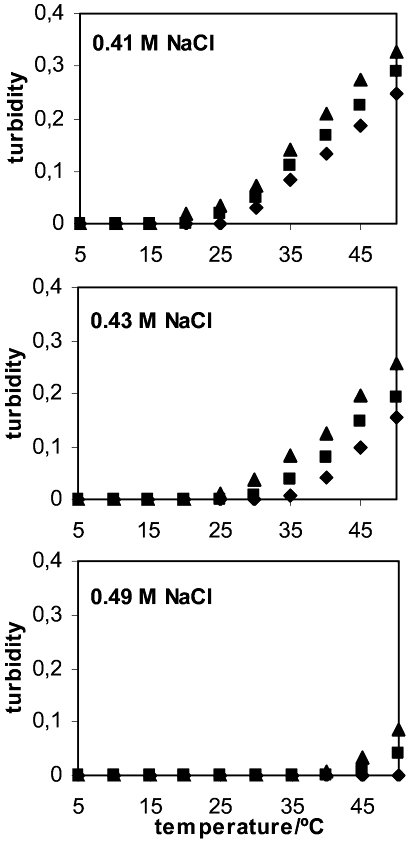

Figure 6. Turbidity results of 1:1 complexes of PMOTAC-*g*-POE1 (◆), PMOTAC-*g*-POE2 (■), and PMOTAC-*g*-POE3 (▲) with POE-*b*-PMANa measured with a total polymer concentration 0.5 mg/mL in aqueous NaCl solutions: from top to bottom 0.41, 0.43, and 0.49 M.

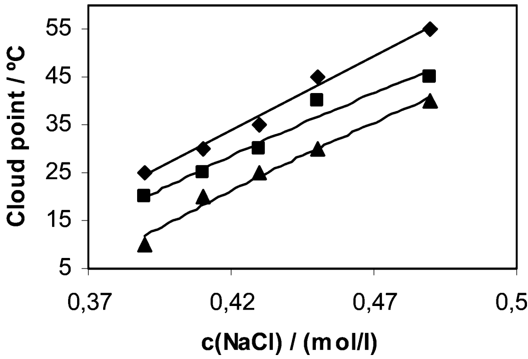

Figure 7. Phase diagram of 1:1 complexes of PMOTAC-*g*-POE1 (◆), PMOTAC-*g*-POE2 (■), and PMOTAC-*g*-POE3 (▲) with POE-*b*-PMANa measured with a total polymer concentration 0.5 mg/mL in aqueous NaCl solutions.

In respect to the PMOTAC homopolymer, POE grafts of PMOTAC-*g*-POE enhance the stability of PECs in the saline dispersions and increase the cloud point temperature. Lower NaCl concentration is needed to break the secondary aggregates

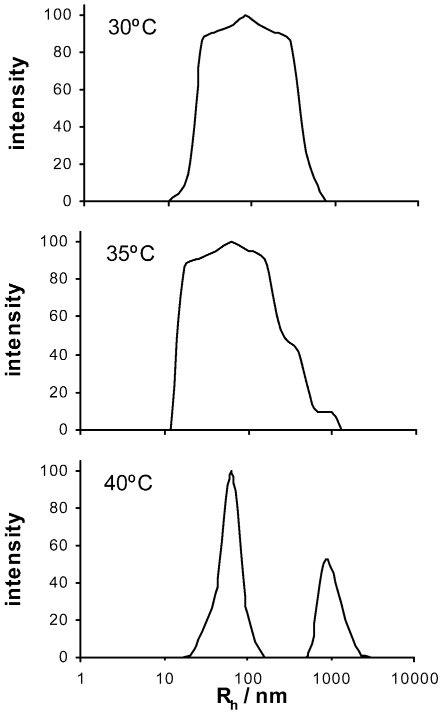

Figure 8. Effect of the temperature on the size distribution of 1:1 complexes of PMOTAC-*g*-POE3 and POE-*b*-PMANa measured with DLS in 0.49 M aqueous NaCl solution. The total polymer concentration is 0.5 mg/mL.

formed by the PMOTAC-*g*-POE/POE-*b*-PMANa complexes at a fixed temperature compared to the complexes formed by PMOTAC homopolymer. However, the complexes break and dissolve as individual polymers at approximately the same ionic strength and temperature. The temperature interval, in which PECs are formed again upon heating, is much broader for POE-grafted PMOTAC than that in the case of PMOTAC homopolymer of the same molar mass. In the case of PMOTAC-*g*-POE, the cloud point is observed far above the real complexation temperature, i.e., completely soluble PECs are formed.[12]

Re-formation of the PECs upon heating was studied using light scattering in aqueous 0.49 M NaCl. Within a temperature range 5−50 °C, the dispersions of the PMOTAC-*g*-POE1 with POE-*b*-PMANa were completely transparent. DLS revealed that the size distributions in aqueous saline solutions below 20 °C are monomodal with a maximum at 50 nm. Mean value of the hydrodynamic size distributions, R_{h} , is similar to those of the individual polymers under the same solution conditions, and the size distributions are broad. This suggests that complexes do not form under these conditions. Upon heating the solutions above 25 °C, complexes form again, and the size distribution turns bimodal with the maximum of the peak corresponding to the PECs at 200 nm. PECs of PMOTAC-*g*-POE1/POE-*b*-PMANa can also be observed with the higher polymer concentration of 1.0 mg/mL. However, further heating causes clouding and formation of the secondary aggregates of PECs. R_h increases dramatically up to 600−800 nm at 55 °C.

The results for either of the larger polycations were similar to each other even for the dispersions with the smaller polymer concentration 0.5 mg/mL, where the aggregation is expected to be less than for the PMOTAC-*g*-POE1/POE-*b*-PMANa with 1.0 mg/mL concentration. Figure 8 represents the effect of temperature on PMOTAC-*g*-POE3/POE-*b*-PMANa dispersions in 0.49 M NaCl with the total polymer concentration of 0.5 mg/mL. In the cases of PMOTAC-*g*-POE2 and PMOTAC-*g*-POE3 mixed with POE-*b*-PMANa, re-formation of the PECs and the secondary aggregation upon heating was not observed. Below the cloud point temperature the size distributions were as broad as those for pure polymer solutions. However, when the solutions became turbid, the large PECs were immediately detected. Within that temperature region, for the two longer polycations a monotonic increase in the absolute scattering intensity was observed. In comparison to the results obtained for the shorter polycation, the increasing intensity from the dispersions of either of the larger polycations could indicate the formation of large complexes, whereas some fraction of the polymers is dissolved as individual chains.

Guest−Host Theory and the Molar Mass Effect. According to the classical guest−host theory of polyelectrolyte complexes, soluble PECs can be prepared at nonstoichiometric mixing ratios if the molar masses of the polyelectrolytes are significantly different.[17,28−30] In this case nonstoichiometric PECs have the charge of the uncomplexed host molecule. The stability of such PECs is determined by the minor component in the complex that is completely bound by the oppositely charged polyions.[27] The solubility of these PEC particles can be increased by the nonionic hydrophilic blocks and grafts.[9,31−32] In the case of the copolymers with the POE grafts or blocks not only the length of the charged blocks but also the length of the neutral POE blocks as well as the spatial distribution of the POE grafts affect properties of the PECs. In the present study, a high molar mass DNA was a host whereas a synthetic polyanion POE-*b*-PMANa was a guest molecule.

The process of the formation of a PMOTAC-*g*-POEs/DNA complex follows the guest−host scenario. Accordingly, DNA with a higher molar mass is a host molecule, and the polycations with lower molar masses act as guest molecules. With increase of the molar mass of the polycation, the difference of the molar masses of the guest PMOTAC-*g*-POEs and host DNA molecules decreases thus decreasing the stability of the complexes. In the case of PMOTAC-*g*-POE/POE-*b*-PMANa complexes, the molar mass of the polycation has an opposite effect. The increasing molar mass of the host molecule that is PMOTAC-*g*-POE in this case results in decreasing solubility and stability of these complexes. To explain such behavior, the effect of the ionic and neutral blocks ought to be considered separately.

Thus, Zintchenko et al.[8] studied the complexation of poly(diallyldimethylammonium chloride), PDADMAC, with POE-*block*-PAMPS focusing on the effect of the length of the stabilizing POE block. They found that the fraction of the neutral block in the copolymer has a great effect on the aggregation level of the PECs but does not play a dominant part in the stabilization of the particles against the salt-induced flocculation. In this respect, the length of the neutral block is important instead. The stability of the polyelectrolyte complexes was significantly improved in higher salt concentrations by using an anionic copolymer with long POE blocks in combination with PDADMAC.

In the frame of our investigation, PMOTAC-*g*-POE/POE-*b*-PMANa are shown to be smaller and more stable in comparison to PMOTAC-*g*-POE/DNA even in the dispersions with the stoichiometric mixing ratio, simply due to the larger amount of the POE chains in the shell of the PEC particles. The solubility and stability of the PMOTAC-*g*-POE/DNA and PMOTAC-*g*-POE/POE-*b*-PMANa complexes decrease with increasing M_{w} of the polycation. The longer polycations form larger PEC particles, which tend to precipitate at a lower ionic strength and at a lower mixing ratio than those observed for the shorter chains. The latter is especially pronounced for the complexes with DNA, which have a low POE content.

As previously shown, increasing the length of the anionic block increases the size of PMOTAC/POE-*b*-PMANa, due to the smaller molar fraction of the solubilizing POE blocks in the block copolymer.[18−20,33] A larger number of the POE blocks is needed for the stabilization of the PECs in the dispersions with the high salt concentration, and thus bigger particles are formed than in the dispersions with very low ionic strength. As has also been noticed, PMOTAC homopolymer and POE-*b*-PMANa form charged polyelectrolyte complexes both in the excess of the cationic host polyelectrolyte PMOTAC, and, most interestingly, in the excess of the anionic guest polyelectrolyte POE-*b*-PMANa.[33] In contrast, the PECs formed by two homopolyelectrolytes have not shown similar overcharging by the guest component, which underlines a key role of POE. Incorporation of the excess anionic guest POE-*b*-PMANa component to preformed stoichiometric PMOTAC/ POE-*b*-PMANa, and thus overcharging of polyelectrolyte complexes by the guest polyelectrolyte was studied by fluorescence and reported separately.[33] It has been demonstrated that the ability of polyelectrolyte complexes to incorporate an excess POE-*b*-PMANa increases with increasing ionic strength. A small excess of POE-*b*-PMANa induces an increase in the particle size.

Observation of the overcharging of the PMOTAC/POE-*b*-PMANa complexes by the guest agrees with our observations and reveals the ability of PMOTAC to bind more POE-*b*-PMANa chains than one may expect taking into account only the number of positive and negative charges. In the PMOTAC-*g*-POE/DNA dispersions with the cation-to-anion ratio below the stoichiometric one, PMOTAC-*g*-POE is a minor compound of the polyelectrolyte mixtures and expected to be completely bound to the polyions. However, in both cases, whether the PECs are formed by DNA as a host or by POE-*b*-PMANa as a guest at stoichiometric ratio, light scattering reveals the presence of the free chains of PMOTAC-*g*-POE, which are not incorporated into the PECs. That means that the polycation is in excess to the PECs.

The existence of the PMOTAC-*g*-POE free chains can be understood in terms of the intra- and intermolecular hydrogen bonding between POE and the acidic groups of PMANa-*g*-POE, which reduces the number of effective charges of the polyanion. Thus, POE-*b*-PMAA is a weak acid capable to form hydrogen bonds with the POE blocks; hydrogen bonding between POE and PMAA segments has been shown to take place at pH $<$ 5.5, corresponding to $\alpha < 0.3$, whereas in the mixed solutions of homopolymers, H-bonds form only at $\alpha < 0.1$.[19] In its turn, within the PEC particles, the dielectric properties of media are different in respect to the outer solution due to the high polymer content. In the aspect of the H-bond formation, the lowering of the dielectric constant is equivalent to the lowering of pH.

Conclusions

Formation of the poyelectrolyte complexes, PECs, between a homological series of the cationic PMOTAC-*g*-POE and the anionic POE-*b*-PMANa or DNA was investigated in aqueous dispersions as a function of varying the ionic strength, temperature, and the cation to anion mixing ratio of the polyelectrolytes. In the case of the PMOTAC-*g*-POE/POE-*b*-PMANa complexes only stoichiometric PECs were studied. The polyelectrolyte complexes of the PMOTAC-*g*-POE1 with either genomic DNA or synthetic POE-*b*-PMANa have extremely narrow size distributions. Though the narrow size distribution is not a novel observation for PECs,[11,27] this fact is surprising taking into account the high polydispersity of the polyelectrolytes studied. Formed PECs consist of a neutralized hydrophobic core stabilized by a shell of the uncomplexed polyelectrolyte and POE. The POE grafts and blocks enhance the solubility of PECs and promote the formation of colloidally stable PECs, of which size is smaller that that of PECs formed by homopolymers of the same mass.

Figure 1 schematically summarizes the effect of the ionic strength and temperature on the complexes of PMOTAC-*g*-POE/ POE-*b*-PMANa and PMOTAC-*g*-POE/DNA. Either of the PECs forms secondary aggregates upon increasing the ionic strength if temperature is kept constant. At high ionic strengths the polyelectrolyte complexes break and polymers dissolve as individual chains. However, large PECs are formed again when the solutions are heated above this temperature. Thermal energy of the counterions increases upon heating thus decreasing the effective screening of the charges of the polyions; more salt is required at higher temperature for the charge screening as well as for the decrease of the entropy gain upon PEC formation due to the release of the counterions. As a result, solutions of uncomplexed PMOTAC-*g*-POE and POE-*b*-PMANa as well as PMOTAC-*g*-POE and DNA turn cloudy upon heating, and the cloud point temperature depends on the ionic strength of the solvent.

In the present study it has been shown that the molar mass of the polycation as the minor component of PECs has a significant effect on the complex formation, as well as on the stability and size of the complexes. An increase in the polycation molar mass shifts the cloud point toward the lower polycation content in the complexes, and a macro phase separation occurs in the solutions with the cation to anion molar ratios much below than 1:1. For the PMOTAC-*g*-POEs/DNA complexes this observation is in accordance with the guest−host scenario: with increase of the molar mass of the guest (polycation), the difference between its molar mass and the molar mass of the host (polyanion) decreases thus decreasing the stability of the complexes. However, in the case of the PMOTAC-*g*-POE/ POE-*b*-PMANa complexes, the molar mass of the polycation as a host has an opposite effect. To explain such behavior, the effect of the neutral blocks ought to be considered as well as the ability of PMOTAC to bind more POE-*b*-PMANa chains than one may expect, taking into account only the number of positive and negative charges. Hydrogen bonding between POE and acidic groups of PMANa-*g*-POE reduces the number of effective charges of the polyanion.

The effect of the polycation molar mass on the re-formation of the PECs upon heating the solutions with 0.49 M NaCl can be understood as follows. Though the electrostatic interaction does not depend on the molar mass of the polyions, the intermolecular attraction of the oppositely charged polyions has an entropic origin and, therefore, is affected by the molar mass; polymers with a high molar mass have a smaller contribution to the entropy of the solution than shorter polymers dissolved in the solution of the same mass concentration. Therefore, longer polymers are less resistant to the intermolecular attraction upon heating the solutions than the low molar mass polymers. This results in the higher cloud point temperature of the shorter polymers and broader range of intermediate temperatures, i.e., between the temperature when PECs are formed and the cloud point temperature, at which the aggregates dominate. The higher molar mass polymers form the larger PEC particles and precipitate just above the temperature at which the PECs are re-formed.

References and Notes

(1) Dautzenberg, H. In *Physical Chemistry of Polyelectrolytes*; Radeva, T., Ed.; Marcel Dekker Inc.: New York, 2001; pp 743−792.

(2) Michaels, A. S.; Miekka, R. G. *J. Phys. Chem.* **1961**, *65*, 1765−1773.

(3) Dautzenberg, H.; Karibyants, N. *Macromol. Chem. Phys.* **1999**, *200*, 118−125.

(4) Sotiropoulou, M.; Cincu, C.; Bokias, G.; Staikos, G. *Polymer* **2004**, *45*, 1563−1568.

(5) Gohy, J.-F.; Varshney, S.; Jerome, R. *Macromolecules* **2001**, *34*, 3361−3366.

(6) Khutoryanskiy, V.; Nurkeeva, Z.; Mun, G.; Sergaziyev, A.; Ryskalieva, Z.; Rosiak, J. *Eur. Polym. J.*. **2003**, *39*, 761−766.

(7) Pergushov, D.; Buchhammer, H.; Lunkwitz, K. *Colloid Polym. Sci.* **1999**, *277*, 101−107.

(8) Zintchenko, A.; Dautzenberg, H.; Tauer, K.; Khrenov, V. *Langmuir* **2002**, *18(4)*, 1386-1393.

(9) Dautzenberg, H. *Macromol. Chem. Phys.* **2000**, *201* (14), 1765−1773.

(10) Virtanen J.; Tenhu H. *Macromolecules* **2000**, *33*, 5970−5975.

(11) Nisha, C. K.; Manorama, S. V.; Ganguli, M.; Maiti, S.; Kizhakkedathu, J. N. *Langmuir* **2004**, *20*, 2386-2396.

(12) Andersson, T.; Holappa, S.; Aseyev, V.; Tenhu, H. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 1904−1914.

(13) Dragan, S.; Schwartz, S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 2495−2505.

(14) Dragan, S.; Schwartz, S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5244−5252.

(15) Cooper, C. L.; Goulding, A.; Kayitmazer, A. B.; Ulrich, S.; Stoll, S.; Turksen, S.; Yusa, S.; Kumar, A.; Dubin, P. L. *Biomacromolecules* **2006**, *7*, 1025−1035.

(16) de Vasconcelos, C. L.; Bezerril, P. M.; dos Santos, D. E. S.; Dantas, T. N. C.; Pereira, M. R.; Fonseca, J. L. C. *Biomacromolecules* **2006**, *7*, 1245−1252.

(17) Kabanov, V. A.; Zezin, A. B. *Pure Appl. Chem.* **1984**, *56*, 343−354.

(18) Holappa, S.; Andersson, T.; Kantonen, L.; Plattner, P.; Tenhu, H. *Polymer* **2003**, *44* (26), 7907−7916.

(19) Holappa S.; Kantonen L.; Winnik F. M.; Tenhu H. *Macromolecules* **2004**, *37*, 7008−7018.

(20) Holappa S.; Karesoja M.; Shan J.; Tenhu H. *Macromolecules* **2002**, *35* (12), 4733−4738.

(21) Andersson, T.; Aseyev, V.; Tenhu, H. *Biomacromolecules* **2004**, *5* (5), 1853−1861.

(22) Bisht, H. S.; Manickam, D. S.; You, Y.; Oupicky, D. *Biomacromolecules* **2006**, *7*, 1169−1178.

(23) Gebhart, C. L.; Kabanov, A. V. *J. Controlled Release* **2001**, *73*, 401−416.

(24) Kabanov, A. V.; Gebhart, C. L.; Bronich, T. K.; Vinogradov, S. V. *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **2002**, *43* (2), 669−670.

(25) Oupicky, D.; Konak, C.; Ulbrich, K.; Wolfert, M. A.; Seymour, L. W. *J. Controlled Release* **2000**, *65*, 149−171.

(26) McAllister, K.; Sazani, P.; Adam, M.; Cho, M. J.; Rubinstein, M.; Samulski, R. J.; DeSimone, J. J. Am. Chem. Soc. **2002**, *124*, 15198−15207.

(27) Buchhammer, H.-M.; Mende, M.; Oelmann, M. *Colloids Surf. A: Physicochem. Eng. Aspects* **2003**, *218* (1−3), 151−159.

(28) Baranovskii, V. Y.; Litmanovich, A. A.; Papisov, I. M.; Kabanov, V. A. *Eur. Polym. J.* **1981**, *17* (9), 969−979.

(29) Tsuchida, E.; Osada, Y.; Sanada, K.. *J. Polym. Sci., Part A: Polym. Chem.* **1972**, *10* (11), 3397−404.

(30) Kabanov, V. A.; Zezin, A. B. A new class of complex water-soluble polyelectrolytes. *Macromol. Chem. Suppl.* **1984**, *6*, 259−76.

(31) Kabanov, A. V.; Vinogradov, S. V.; Suzdaltseva, Y. G.; Alakhov, V. Yu. *Bioconjugate Chem.* **1995**, *6* (6), 639−43.

(32) Karibyants, N.; Dautzenberg, H.; Coelfen, H. *Macromolecules* **1997**, *30* (25), 7803−7809.

(33) Holappa S.; Kantonen L.; Andersson, T.; Winnik F. M.; Tenhu H. *Langmuir* **2005**, *21* (24), 11431−11438.

BM0604911