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Interaction of Nanosized Copolymer Networks with Oppositely Charged Amphiphilic Molecules

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ABSTRACT

A new class of nanocomposite materials is synthesized by reacting nanoscale networks of cross-linked hydrophilic nonionic and cationic polymers, poly(ethylene oxide)-*cl*-polyethyleneimine, with anionic surfactants. Formation of hydrophobic domains from polyethyleneimine-surfactant complexes leads to a network collapse exhibited in a decrease in the particle size from ca. 300 nm to ca. 50 nm. Due to their cross-linked architecture, the poly(ethylene oxide)-*cl*-polyethyleneimine-based complexes are more resistant to the changes in the environmental characteristics, such as pH and salt concentration, compared to complexes formed by a graft copolymer, poly(ethylene oxide)-*g*-polyethyleneimine and surfactants. Poorly soluble biologically active molecules, retinoic acid and indomethacin, were immobilized in the network complexes forming stable aqueous dispersions. The release of the drug from the network dispersion has been demonstrated. These materials are potentially useful as carriers in pharmaceutical drug delivery applications.

Introduction. Self-assembled nanosized materials have recently been synthesized by reacting block or graft copolymers containing ionic and nonionic water-soluble segments (block ionomers) with oppositely charged surfactants.[1−3] In such systems, the ionic headgroups of the surfactant form salt bonds with the units of the polyion segment, while the surfactant tails segregate into hydrophobic domains. Nonionic segments of the block copolymers (e.g., poly(ethylene oxide), PEO) prevent macroscopic phase separation and stabilize the complexes in aqueous dispersions. Previous studies demonstrated that these systems can be potentially used in drug delivery because they can incorporate a variety of hydrophilic drugs (in the aqueous cavity of vesicles) or hydrophobic drugs (in the hydrocarbon regions).[2,4] Small size of the particles formed in such systems (less than 100 nm) is essential for drug delivery, because such particles need to penetrate small capillaries and enter the cells through the endocytosis route to exhibit their physiological function.[5] However, the complexes formed by single tail surfactants are not sufficiently stable in the biological milieu, as they can disintegrate during dilution that occurs in biological fluids. One way to stabilize them is to chemically link surfactant components with each other, which forms robust surfactant arrays covered by the block ionomer chains.[6] The other possibility is to cross-link the polymer chains with each other and form a network in which small amphiphilic molecules can be immobilized. Recently, dispersed cross-linked networks of polyethyleneimine (PEI) and PEO (PEO-*cl*-PEI) of nanoscale size were synthesized and evaluated as carriers for delivery of antisense oligonucleotides.[7] The PEO-*cl*-PEI nanoparticles ("nanogels") are structurally related to the previously studied graft copolymer PEO-*g*-PEI.[4] The present work explores the potential use of the dispersed block ionomer networks for immobilization of oppositely charged low molecular weight amphiphilic molecules including biologically active compounds such as all-*trans*-retinoic acid and indomethacin.

Experimental Section. PEO-*cl*-PEI networks were synthesized by cross-linking PEI, $M \approx 25000$ with double-end *N*,*N*′-carbonyldiimidazole-activated PEO, $M_n \approx 8000$ using an emulsification/solvent evaporation technique as described previously.[7] Following the synthesis, the nanogel particles were fractionated by gel-permeation chromatography, and a fraction with an average particle diameter of ca. 250 nm was used in subsequent experiments. This sample contained approximately seven PEO chains per one PEI chain (as determined from ^{1}H NMR spectra). Nitrogen concentration in the PEO-*cl*-PEI sample was estimated by elemental analysis. Synthesis of PEO-*g*-PEI was described in ref 4. Sodium dodecyl sulfate (SDS), sodium tetradecyl sulfate (TDS), bis(2-ethylhexyl)sulfosuccinate (AOT), sodium oleate (OA), all-*trans*-retinoic acid (RA), and indomethacin were used as anionic components. The stock solution of OA and indomethacin were prepared in methanol and dimethylformamide, respectively. To prepare the sodium salt of RA, initial crystalline powder was dissolved in methanol and

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mixed with equivalent amount of 1 M NaOH. The resulting solution was used as a stock solution for further experiments. 1-(*m*-Tolylazo)-2-naphthylamine (Yellow OB) was used as a model hydrophobic molecule for the solubilization experiments.

The degree of conversion (θ) in the reaction between PEO-*cl*-PEI and alkyl sulfates was determined by potentiometric titration.[8] Fluorescence measurements were performed at 25 °C using a Shimadzu P5000 spectrofluorophotometer. Pyrene was used as a fluorescence probe (excitation wavelength was 333 nm). The sample solutions were prepared by adding known amounts of pyrene in acetone to each of series of empty vials. After acetone evaporation, the measured amounts of surfactant solution or copolymer/surfactant mixture were added to the vials. Pyrene concentration in the final solution was 6×10^{-7} M (slightly below pyrene solubility in water). The sample solutions were stirred overnight at room temperature. Electrophoretic mobility measurements were performed using a "ZetaPlus" analyzer (Brookhaven Instrument Co.) with 15 mW solid-state laser operated at a laser wavelength of 635 nm. The zeta-potential (ζ) of the particles was calculated from the electrophoretic mobility values using the Smoluchowski equation. Effective hydrodynamic diameters (D_{eff}) of the particles were measured by photon correlation spectroscopy in a thermostatic cell at a scattering angle of 90° using the same instrument equipped with the multiangle sizing option (BI-MAS). All measurements were performed at 23 °C. Software provided by the manufacturer was used to calculate D_{eff} values.[9] Particle size measurements were performed in the relatively wide range (from 0.5 to 42 mg/mL) of the concentration of PEO-*cl*-PEI and their mixtures with surfactants. D_{eff} values were independent of the concentration of PEO-*cl*-PEI in the entire range of concentration used. The negative staining (uranyl acetate) technique was used for transmission electron microscopy (TEM) study. A drop of the sample solution was allowed to settle on a Formvar precoated grid for one minute. Excess sample was wicked away with filter paper, and a drop of 1% uranyl acetate solution was allowed to contact the sample for one minute. The samples were analyzed using a Hitachi H-7000 microscope.

Results and Discussion. Interaction between polyions and oppositely charged surfactants is a cooperative process in which the ionic headgroups of the surfactant bind to the polyion repeating units while the surfactant alkyl groups segregate into hydrophobic domains.[10] This process is characterized by a "critical association concentration" (CAC), indicating the onset of surfactant binding to the polymer. Formation of the complexes between the cationic polymers and anionic surfactants was characterized in this work by steady-state fluorescence using pyrene as a probe.[11] Typical dependences of I_1/I_3 vs surfactant concentration, observed in this work with all surfactants, are presented in Figure 1 using TDS/PEO-*cl*-PEI and TDS/PEO-*g*-PEI mixtures as examples. The onsets of sharp decrease in the I_1/I_3 values in these dependencies indicate the formation of nonpolar microphases and determine the corresponding values of CAC. The dependence of the I_1/I_3 ratio on the TDS concentration

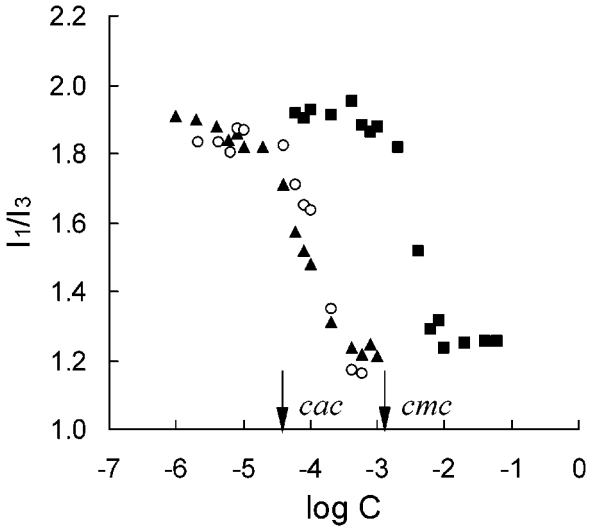

Figure 1. Variation of the I_1/I_3 ratio for the (■) TDS, (▲) PEO-*g*-PEI/TDS, and (○)PEO-*cl*-PEI/TDS mixtures as a function of surfactant concentration. Vertical arrows indicate CMC value and CAC value in the presence of PEO-*cl*-PEI, respectively. 10 mM phosphate buffer, pH 7.0, 25 °C.

in the polymer-free system is also shown in Figure 1 for comparison. Based on this dependence the critical micelle concentration (CMC) of TDS was determined. As is seen in the figure, the CAC values were practically the same for both PEO-*cl*-PEI and PEO-*g*-PEI copolymer-based systems: 3.2×10^{-5} M and 2.6×10^{-5} M, respectively. However, both these values were about 2 orders of magnitude lower than the corresponding CMC (2.2×10^{-3} M), suggesting that binding with the polycation templates promoted the surfactant aggregation.

The binding equilibrium in the cationic copolymer and surfactant mixtures was further studied by potentiometric titration. Interaction between an anionic surfactant (S^-) and a polybase represents an ion exchange reaction resulting in the release of the hydroxyl ions in accordance with the following scheme:

$$(|\text{-N(R)H})_n + n\text{S}^- + n\text{H}_2\text{O} \leftrightarrow [|\text{-N(R)H}_2{}^+\ \text{S}^-]_n + n\text{OH}^- \quad \text{(I)}$$

where R is the $-$H or $-$CH$_2-$ group of the PEI chains.[12] The acid titration curves were obtained for the mixtures of the surfactants with (1) PEO-*cl*-PEI and (2) PEO-*g*-PEI. Figure 2 represents typical θ-pH curves for AOT/PEO-*cl*-PEI (curve 1) and AOT/PEO-*g*-PEI (curve 2) mixtures as well as ionization curves (α-pH dependencies) for the free copolymers and PEI homopolymer (curves 3−5). As is seen from Figure 2, for both systems studied the θ values increased sharply over relatively narrow intervals of pH. This demonstrated that the interaction AOT and PEI segments had a distinct cooperative character and resulted in the formation of the polyion−surfactant complexes. The θ-pH curves for the copolymer−surfactant mixtures were shifted to higher pH compared to the α-pH curves for the corresponding copolymers and PEI homopolymer. The observed

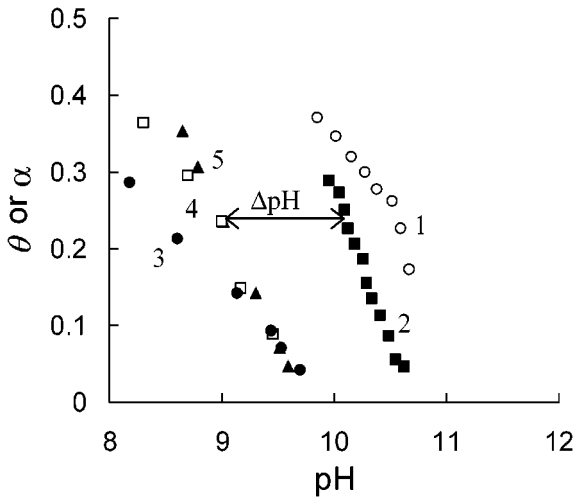

Figure 2. θ-pH dependencies for the equimolar mixtures PEO-*cl*-PEI/AOT (1,○), PEO-*g*-PEI/AOT (2,■), and α-pH dependence for PEO-*cl*-PEI (3,●), PEO-*g*-PEI (4, □), and PEI homopolymer (5,▲) in salt-free solutions. The pH shift, ΔpH, between θ-pH and α-pH dependencies is shown for PEO-*g*-PEI complex as an example. Concentration of AOT was 0.002 M, 23 °C.

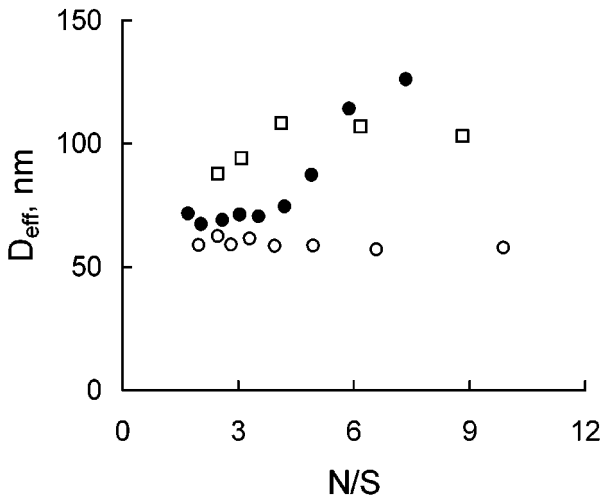

Figure 3. Effective diameters of PEO-*cl*-PEI complexes with AOT (○), OA (□), and indomethacin (●) at various compositions of mixture, N/S. Complexes prepared in 10 mM phosphate buffer, pH 7.0.

pH shift was a result of the hydroxyl ion release in the ion-exchange reaction (I). The pH difference, ΔpH(α) between θ-pH curves and α-pH curves at each $\theta = \alpha$ provided a differential measure of a free energy of cooperative stabilization of the polycation−surfactant complexes.[8,13] Greater shift of the θ-pH curves to the alkali pH compared to the α-pH curves corresponded to more stable complexes. Therefore, data in Figure 2 (curves 1 and 2) suggest that the complexes of cross-linked PEO-*cl*-PEI with anionic surfactants were more stable than the complexes formed by the graft copolymer. Such difference in the stability can be attributed to the effect of the copolymer architecture on the self-assembly of the cross-linked and graft copolymer based complexes. Interaction of surfactants with the graft copolymers is a two-stage process. First, the surfactant molecules bind with the individual graft copolymer chains, and second the formed complexes self-assemble into the micelle-like aggregates.[3] In contrast, multiple PEI segments in the PEO-*cl*-PEI are already assembled within the gel particles, i.e., such systems do not exhibit an unfavorable entropy contribution resulting from self-assembly of the individual graft copolymer-surfactant complexes into the micelle-like aggregates.[14]

The complexes of PEO-*cl*-PEI with surfactants were further characterized on a macroscopic level by a laser microelectrophoresis technique and photon correlation spectroscopy at various compositions of the mixture. The composition of the mixture, N/S, is expressed as a ratio of the total concentration of amino groups of PEO-*cl*-PEI to the concentration of the surfactant the in the system. In all experiments the concentration of amino groups (N) was constant while the concentration of the surfactant (S) was varied. Because only a fraction of amino groups could be protonated at pH 7.0,[12] the stoichiometric compositions of the mixtures at this pH corresponded to N/S ≈ 2.5. The net charge of the unloaded PEO-*cl*-PEI particles was slightly positive (ca. 4−5 mV). Such small value of the zeta-potential is obviously a result of localization of a significant portion of the charged amino groups and the condensed counterions within the network particles. This should lead to a decrease in the electric potential at the surface of shear of such particles. Incorporation of surfactant molecules into PEO-*cl*-PEI resulted in a decrease of ζ-potential almost to zero. The particles remained practically electroneutral in the range of N/S from 2.5 to 10. When an excess of surfactant was added to the system (N/S < 2.5), the net charge of the particles became slightly negative. The change in the sign of ζ-potential can be attributed to incorporation of the excess of the surfactant into the PEO-*cl*-PEI/surfactant complexes. Surfactant molecules can bind with the stoichiometric complexes through interactions with the hydrophobic domains and/or PEO segments of the network.[10,15] Similar behavior was previously reported for the complexes of anionic surfactants with PEO-*g*-PEI.[3]

Effective diameters of the particles formed in PEO-*cl*-PEI and AOT mixtures at pH 7.0 are presented in Figure 3. (The same figure also presents the data for the mixtures of PEO-*cl*-PEI with OA and indomethacin, which will be discussed further.) The binding of AOT to the amino groups of ionic segments of PEO-*cl*-PEI networks resulted in a significant decrease in the particle size (compare to initial diameter of ca. 300 nm, not shown). A sharp decrease in the size of the particles was observed even under the conditions of the deficiency of the surfactant in the reaction mixture (N/S > 2.5). The contraction of PEO-*cl*-PEI networks as a result of surfactant binding was also confirmed by electron microscopy. Typical electron micrographs are presented in Figure 4. The images revealed close to spherical PEO-*cl*-PEI particles (A), which collapsed upon the interaction with amphiphilic molecules (B). Based upon the changes of diameters of the particles before and after addition of surfactants, as revealed by both light scattering and electron microscopy, the volume of the particles decreases more than 100-fold upon interaction of the network with surfactants.

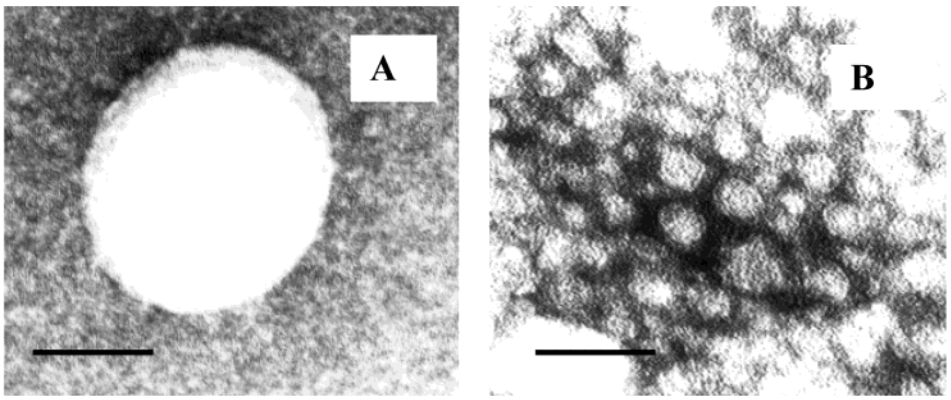

Figure 4. TEM micrographs of PEO-*cl*-PEI (A) and PEO-*cl*-PEI/OA complex (B) formed at N/S = 3.0 in 10 mM phosphate buffer, pH 7.0. Bar corresponds to 100 nm.

Table 1: Effective Diameters of PEO-cl-PEI/AOT Particles (N/S = 2.5) at Various pH

pH	4.1	5.0	6.0	7.0	8.8	9.5
D_{eff}, nm	45	43	54	55	54	53

A network collapse is typical for polyelectrolyte gels upon their interaction with oppositely charged surfactants.[16] It is noteworthy, however, that in the case of PEO-*cl*-PEI nanogels the network collapse is not accompanied by phase separation and stable dispersions are formed.

The effective diameters of the PEO-*cl*-PEI particles were further examined in the presence of AOT at various pH values and a constant N/S ratio of 2.5 (Table 1). It is noteworthy that the initial unloaded PEO-*cl*-PEI particles exhibited relatively little size variation upon changes in pH from 4 to 10. The particle size was ca. 300 nm in the range of pH from 4.0 to 8.5 and decreased to ca. 260 nm at pH 10. This behavior was, probably, due to a relatively high degree of cross-linking of the PEO-*cl*-PEI network that limits the extent of its swelling. In the presence of the surfactant, the smallest particle size (ca. 45 nm) was observed at pH 4.0 to 5.0, when the amino groups of PEI segments are ionized completely and the degree of conversion in the reaction between PEI chains and surfactant is maximal. Interestingly, addition of AOT resulted contraction of PEO-*cl*-PEI particles even at pH 10 when the degree of conversion was relatively low. Therefore, binding of the surfactant with even a small portion of amino groups in PEO-*cl*-PEI resulted in a collapse of the networks.

Despite charge neutralization PEO-*cl*-PEI/surfactant complexes formed fine, slightly opalescent dispersions that exhibited no precipitation in the entire ranges of N/S and pH examined. However, in contrast to the complexes formed by the PEO-*g*-PEI graft copolymer, the PEO-*cl*-PEI-based complexes were less stable at the stoichiometric compositions. The PEO-*cl*-PEI/surfactant complexes prepared at N/S = 2.5 aggregated and precipitated after 3−4 days. In contrast, dispersions of stoichiometric PEO-*g*-PEI/surfactant complexes were stable for many weeks of observation.

Addition of simple salts to the mixtures of ionic surfactants with polyions leads to destabilization of the system of the salt bonds in polymer−surfactant complexes.[10] This is usually

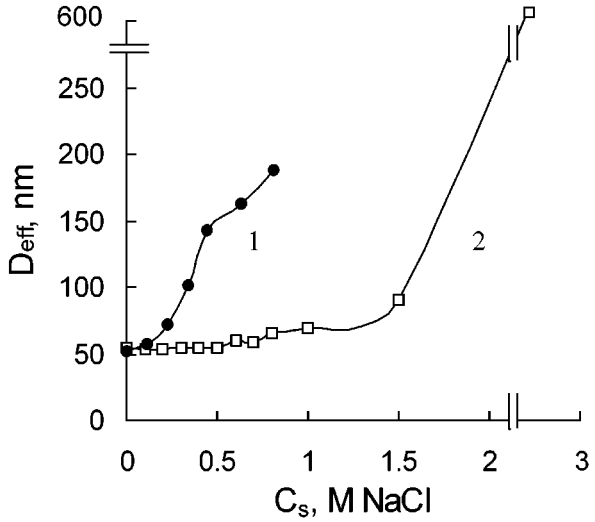

Figure 5. Effective diameters of PEO-*cl*-PEI/AOT (curve 1) and PEO-*cl*-PEI/AOT (curve 2) complexes at various concentration of added salt. Complexes prepared at N/S = 2.5 in 10 mM phosphate buffer, pH 7.0.

accompanied by an initial aggregation of the particles followed by a complete disappearance of the complex species at high salt concentration. The complexes of cationic polymers with alkyl sulfates usually disintegrate at very high concentration of NaCl of ca. 1.7 M.[16] However, PEO-*g*-PEI/AOT complexes aggregated at much lower NaCl concentration, ca. 0.2 M (Figure 5). In contrast, in the mixtures of PEO-*cl*-PEI and AOT (curve 2 in Figure 5), the particle size did not change upon elevating NaCl concentrations up to 1.5 M. At higher concentrations of NaCl (1.5−3.0 M) the progressive aggregation was observed, possibly, due to the salting out of the PEO-*cl*-PEI species. These data suggest that dispersions from nanosized networks and surfactants are more stable in the presence of salt than corresponding block ionomer complexes. It is likely that in the case of PEO-*g*-PEI-based complexes salt-induced aggregation includes rearrangement of the graft copolymer chains among complex particles. Conversely, such rearrangement is hindered in the case of cross-linked copolymer, which results in a higher stability of PEO-cl-PEI/surfactant complexes in the presence of the simple salt.

Many biologically active compounds are characterized by relatively low solubility, which hinders their pharmacological use. At the same time, some of these compounds are ionogenic surfactants that can be used as components of block ionomer complexes for drug delivery.[4] Incorporation of amphiphilic molecules with low solubility into PEO-*cl*-PEI nanogels was evaluated using OA, indomethacin, and RA. In the case of OA and indomethacin, the complexes were prepared by mixing small amounts of concentrated solutions of the surfactants in organic solvent (ethanol, dimethylformamide) with aqueous dispersions of PEO-*cl*-PEI in 10 mM phosphate buffer, pH 7.0 (in all cases the final content of organic solvent did not exceed 5−8 vol %). For both studied compounds, the fine dispersions of the complex particles were obtained in the wide range of compositions of the mixture (Figure 3). These formulations were stable over relatively long periods of time (at least a week). However, this protocol was not successful in the case of RA. Mixing of PEO-*cl*-PEI and RA at pH 7.0 resulted in immediate precipitation. Therefore, the complexes were prepared by mixing of the components at pH 10, under the conditions when RA is completely ionized and stable (N/S ratio 3.0). This procedure produced a practically transparent and stable dispersion with the particle size of ca. 70 nm. After formation at pH 10.0 the pH of dispersion was adjusted to 7.0. The pH adjustment did not result in a change of the size of the particles. Furthermore, the dispersion remained stable in 0.15 M NaCl. In the absence of added salt this dispersion could be lyophilized, stored in lyophilized form for several days, and then redispersed. The particle size in the redispersed system (10 mM phosphate buffer, pH 7.0) was higher (120 nm) than that in the original dispersion. However, this system remained stable for over a week. This suggested that a useful pharmaceutical formulation of RA can be prepared by its immobilization in the nanogel system, which is stable at physiological pH and ionic strength.

The hydrophobic regions of PEO-*cl*-PEI/surfactant complexes can also serve as nonaqueous reservoirs for solubilizing water-insoluble molecules. For example, PEO-*cl*-PEI/OA complexes incorporated a significant amount of water-insoluble dye, Yellow OB. The dye-containing PEO-*cl*-PEI/OA dispersions remained stable for several days. The solubilization efficiency of network/surfactant complexes (ca. 10 μg dye per mg of complex) was significantly higher compared to the previously studied block ionomer complexes of the same composition (ca. 1.5 μg dye per mg of complex).[4] These data suggested that PEO-*cl*-PEI/surfactant dispersions can potentially be used for solubilization of hydrophobic drugs.

To examine drug release kinetics, the dispersion of nanogel loaded with indomethacin (at 1 mg of drug per 1 mg of polymer) was dialyzed against 10 mM phosphate buffer, pH 7.0 using SpectraPore 3.5 kDa membrane tubing.[17] The amount of the drug in the external solution was determined by UV−vis spectroscopy.[17] This experiment suggested that during the first hour of dialysis over 17.5% of the drug was released in the external solution. After 24 h, ca. 82% of indomethacin was found in the external solution. The release of the drug from PEO-*cl*-PEI nanogels results in the restoration of their size. Indeed, the estimated effective diameter of the PEO-*cl*-PEI particles after dialysis was ca. 270 nm. This demonstrates that the biological agents immobilized within the nanogel can be released from it and suggests that such systems may be useful for drug delivery applications.

Conclusions. This paper reports the studies on a new family of nanoscale materials on the basis of dispersed networks of cross-linked cationic polyelectrolyte and nonionic hydrophilic polymer. Interaction of oppositely charged amphiphilic molecules with dispersed PEO-*cl*-PEI networks resulted in the formation of nanocomposite materials in which the hydrophobic regions from a polyion−surfactant complex are joined by the hydrophilic PEO chains. The formation of polyion−surfactant complexes led to the collapse of the nanogel particles exhibited in over 100-fold decrease in the particle volume. However, surfactant-neutralized particles formed stable dispersions and did not aggregate for many days. Due to their cross-linked polymer architecture, the network-based complexes are more resistant to the changes in environmental characteristics (such as pH and salt concentration) compared to block ionomer complexes. These systems allowed immobilization of biologically active compounds such as amphiphilic molecules of opposite charge (bound to polycation chains) or hydrophobic molecules (incorporated into nonpolar regions of polyion−surfactant complexes). These nanocomposite materials combine the properties of nanoparticles, such as small size and stability, with the properties of polymer−surfactant complexes, such as simplicity of preparation and modification of characteristics by variation of composition and structure of a surfactant component. The results presented in this work extend the area of polymer micelles and other self-assembled nanoscale systems that have recently attracted significant attention in biology and pharmaceutics.[18]

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References

(1) Bronich, T. K.; Kabanov, A. V.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *Macromolecules* **1997**, *30*, 3519.
(2) Kabanov, A. V.; Bronich, T. K.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *J. Am. Chem. Soc.* **1998**, *120*, 9941.
(3) Bronich, T. K.; Cherry, T.; Vinogradov, S. V.; Eisenberg, A.; Kabanov, V. A.; Kabanov, A. V. *Langmuir* **1998**, *14*, 6101.
(4) Bronich, T. K.; Nehls, A.; Eisenberg, A.; Kabanov, V. A.; Kabanov, A. V. *Colloids Surf., B* **1999**, *16*, 243.
(5) Kabanov, A. V. *Pharm. Sci. Technol. Today* **1999**, *2*, 365.
(6) Bronich, T. K.; Ouyang, M.; Eisenberg, A.; Kabanov, V. A.; Szoka, F. C.; Kabanov, A. V. *Polym. Prepr.* **2000**, *41* (2), 1642.
(7) Vinogradov, S. V.; Batrakova, E. V.; Kabanov, A. V. *Colloids Surf. B* **1999**, *16*, 291.
(8) Kabanov, V. A. *Polym. Sci. Russ.* **1994**, *36*, 143 (translated from *Vysokomol. Soedin.* **1994**, *36*, 183).
(9) "ZetaPlus" analyzer with multiangle sizing option is equipped with digital autocorrelator BI-9000 for data collection. The autocorrelation function is analyzed using the method of cumulants. The BI-MAS

software automatically fits the autocorrelation function, determines the translational diffusion coefficient of the particles, and then reports their hydrodynamic diameters calculated using the Stock's−Einstein equation for hard spheres.

(10) *Interactions of Surfactants with Polymers and Proteins*; Goddard, E. D., Ananthapadmanabhan, K. P., Eds.; CRC Press: Boca Raton, FL, 1993.

(11) The ratio of intensities of the first and third highest energy emission peaks in the pyrene spectrum (known as the I_1/I_3 ratio) has been shown to correlate with the polarity of the immediate environment of pyrene molecule. In aqueous solution, the I_1/I_3 value is about 1.7−1.9, while in a nonpolar solvent such as hexane, it is about 0.6. In a typical surfactant solution below CMC, the I_1/I_3 value is the same as that in water. When the concentration of surfactant exceeds CMC, the I_1/I_3 ratio drops significantly, indicating the incorporation of pyrene into the nonpolar interior of the micelle (Becker, R. S.; Singh, I. S.; Jackson, E. A. *J. Chem. Phys.* **1963**, *38*, 2144; Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039).

(12) The degree of conversion, θ, in the ion exchange reactions (I) can be defined as the ratio of the current number of salt bonds formed by the polyion to the ultimate one. This parameter was determined from the original titration curves using the following expression: $\theta = (m_a/V + [OH^-] - \sqrt{K_b C_o})/C_o$, where m_a is the number of moles of the added acid, V is the current volume of the reaction system, K_b is the characteristic dissociation constant, C_o is the base-molar concentration of the polybase. The branched PEI used in this work contained primary, secondary, and tertiary amino groups. It was estimated from the potentiometric titration curve that only approximately 60% of the total amount of amino groups could be protonated. It appeared that some part of higher order amino groups does not participate in the ion-exchange reactions with anionic surfactant molecules. Only a concentration of ionizing amino groups was used as a C_o value.

(13) Kabanov, A. V.; Bronich, T. K.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *Macromolecules* **1996**, *29*, 6797.

(14) It is important to note the nonmonotonic character of the α-pH curve for PEO-*cl*-PEI network and θ-pH curve for the PEO-*cl*-PEI/AOT mixture. Particularly, at low α values ($\alpha < 0.15$) the ionization curve for the network (curve 3) superimposed with α-pH curves for the PEO-*g*-PEI copolymer (curve 4) and PEI homopolymer (curve 5). However, these curves were distinct at higher α, suggesting that in the case of the network the same degrees of ionization were achieved at lower pH than in the cases of the homopolymer and graft copolymer. This difference can be attributed to the cross-linked architecture of the PEO-*cl*-PEI copolymer. It is likely that high density of cross-linking of the network (ca. 1 PEO chain per 80 units of PEI) leads to a relatively high local concentration of the amino groups in the interior of the PEO-*cl*-PEI species. As a result protonation of the inner amino groups should increase the electrostatic repulsion and result in reduced basicity of the network at higher degrees of ionization. The electrostatic repulsion effect should be less pronounced in the case of the homopolymer and graft copolymer. The peculiarities in the PEO-*cl*-PEI ionization could also contribute to the nonmonotonic behavior of the θ-pH curve observed for the PEO-*cl*-PEI/AOT mixture.

(15) Brackman, J. C.; Engeberts, J. B. F. N. *Chem. Soc. Rev.* **1993**, *22*, 85.

(16) Kabanov, V. A.; Zezin, A. B.; Rogacheva, V. B.; Khandurina, Y. V.; Novoskoltseva, O. A. *Macromol. Symp.* **1997**, *126*, 79.

(17) Complex of PEO-*cl*-PEI and indomethacin was prepared at N/S = 2.5 in 10 mM phosphate buffer, pH 7.0. The particle size in the prepared dispersion was ca. 55 nm. Concentration of indomethacin in the final dispersion was 0.96 mg/mL. Concentration of indomethacin was determined by measuring the absorbance at 320 nm. For this analysis, we obtained the calibration curve of standard solutions containing 0−30 μg/mL of indomethacin. The extinction coefficient $\epsilon_{320} = 0.019$ mL/μg was used for estimation of the drug concentrations in the solution.

(18) *Polymeric Micelles in Biology and Pharmaceutics (Special Issue: Colloids and Surfaces B: Biointerfaces)*; Kataoka, K., Kabanov, A. V., Eds.; Elsevier: Amsterdam, Lausanne, New York, Oxford, Shannon, Tokyo, 1999.

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