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Salt Effects on Complexes of Oppositely Charged Macromolecules Having Different Affinity to Water

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ABSTRACT: The influence of salt concentration on the size and on the thermodynamic stability of interpolymer complexes composed of oppositely charged macroions having different affinity to solvent was studied from a theoretical viewpoint. It was shown that increasing salt concentration causes changes in the structure of complex particles. At low salt concentration, the particles preserve their structure and size. At a critical salt concentration, n_S^{cr}, the particle size rises sharply to a slightly larger dimension. From this concentration, the macroions forming the interpolymer complex start to separate, and the complex is fully destroyed at a salt concentration n_S^*. After separation, the macroions coexist in solution and with further increase in salt concentration reduce their sizes according to the screening of polyion charges by salt ions. n_S^{cr} and n_S^* depend on physical parameters such as the degree of polymerization of macroions, their degree of ionization, and macroion−solvent interaction parameters. Experimental data collected in the particular cases of PLL-PLCA and PLL-PLCAI complexes with polylysine qualitatively agree with the trends indicated by the theoretical approach.

Introduction

Intermolecular polyelectrolyte complexes (IPECs) have attracted considerable attention for a long time starting from first studies in the middle of the 20th century.[1−5] IPECs are formed spontaneously in solution thanks to strong cooperative electrostatic interactions when positively and negatively charged polyions are mixed.[1−4] IPECs play essential roles in nature where many charged systems, namely, proteins, polysaccharides, and cells, are dispersed in fluids and tissues in the absence of macroscopic precipitation.[5] They are involved in many biological phenomena that condition life, some of them being very complex such as DNA−protein interactions occurring in cell machineries.[5,6] Somewhat related to this, IPECs dispersed in water have potentials for various biomedical applications, including encapsulation of biological substances for drug delivery, gene transfection, and gene therapy.[5−10]

A great number of comprehensive experimental studies of IPECs, including preparation and structure characterization, have been reported in recent decades.[2−7] It has been shown that IPEC properties depend considerably on the ratio of positive to negative charges within mixtures. Nonstoichiometric complexes appear if there is an excess of charges of one sign in the solution. They take on the form of hydrodispersed tiny particles or micellar aggregates stabilized by the presence of a surface net charge. In contrast with nonstoichiometric ones, stoichiometric IPECs contain equal amounts of opposite charges. The net charge of IPEC is zero; as a result, the different IPECs aggregate and precipitate.

Recently, it was shown that the phase separation in stoichiometric mixtures could be prevented if at least one of the polyelectrolytes is a diblock amphiphilic copolymer that includes a hydrophilic nonionic block, for example, poly(ethylene glycol).[11−14] The interactions between monomeric units of this block and the solvent stabilize the formation of finite-size particles in the solution. These particles have a well-defined core−shell structure where the core is surrounded by an hydrophilic corona formed by the hydrophilic block. Theories have been proposed for such complexes that are named block−ionomer complexes[15,16].

Some of us found recently that cationic macromolecules such as poly(L-lysine), PLL, and poly(amino serinate), PSA, form water nanodispersed (apparently soluble) stoichiometric IPECs with anionic macromolecules of poly(L-lysine citramide), PLCA, and poly(L-lysine citramide imide), PLCAI, two polycarboxylic acids whose backbone bears one hydrophilic hydroxyl group on each lysyl−curyl repeating unit[17]. Because the mixture of cationic macromolecules of poly(L-lysine) with poly(acrylic acid), whose backbone is hydrophobic, always leads to macroscopic phase separation (i.e., never shows nanodispersed particles in aqueous media),[17] the aqueous nanodispersion of the PLCA and PLCAI complex was assigned to the presence of hydrophilic hydroxyl groups along main chains that contributed to form a water-friendly corona thanks to interaction with the aqueous surrounding medium.

The theory of such IPECs was proposed by us in ref 18, where the factors that govern the stability of complexes between oppositely charged macromolecules having different affinity to solvent were analyzed. This theory was based on the assumption that such IPECs have core−shell structure. Accordingly, they were regarded as beign composed of an inner part with radius r that contains monomer units of both macromolecules and an external part (with radius R) that consists exclusively of monomer units of longer hydrophilic macromolecules. Consequently, the external charged hydrophilic shell prevents the complex elementary entities from aggregating and precipitating.

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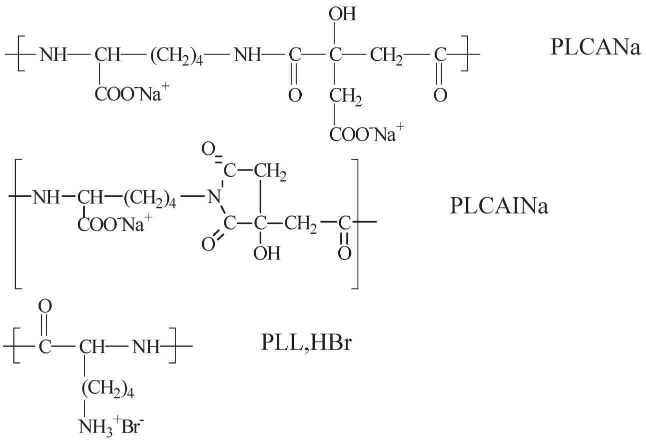
Scheme 1. Chemical Formula of PLCANa, PLCAINa, and PLL,HBr



The shell is formed thanks to the energetic gain for hydrophilic monomer units to be exposed in the surrounding solvent rather than into the hydrophobic core. The factor that acts against the formation of such a layer is the Coulomb attraction between the excess of negative and positive groups in the internal and external parts of the IPEC, respectively. The balance of these two main factors determines the availability and the thickness of the external protective layer.

It is well known that salt ions screen electrostatic interactions and can destabilize IPECs when their concentration is high enough. In the case of the core−shell complexes under consideration, the introduction of salt ions can play a double role. On one hand, the screening of electrostatic interaction does weaken the electrostatic attraction between oppositely charged ions and for sure destabilizes IPECs. On the other hand, the decrease in electrostatic interaction between oppositely charged core and shell of complex can lead to the increase in the thickness of the protective shell and thus serves to the stabilization of complexes with respect to the aggregation in the solvent within a certain range of salt concentration.

The aim of this article is to analyze the influence of salt on the stability, the structure and the solubility of interpolyelectrolyte complexes formed by macromolecules having different affinity to water from a theoretical viewpoint. In attempts to test the theory, a comparison is made with experimental data obtained in the case of PLL complexes with PLCA/PLL and PLCAI/PLL.

Experimental Section

Materials. Poly(L-lysine) hydrobromide (PLL,HBr) of different molecular weight (PLL12: M_w = 12 700 g/mol, PLL27: M_w = 26 300 g/mol, and PLL80: M_w = 73 500 g/mol) was purchased from Sigma. Poly(L-lysine citramide) (M_w = 39 000 g/mol) and poly(L-lysine citramide imide) (M_w = 51 000 g/mol) sodium salts, respectively, PLCANa and PLCAINa, were synthesized as previously described.[19] PLCA was generated from the alkaline hydrolysis of the cyclic imide groups of PLCAI. PLCA molecules contain two negative charges per unit, whereas PLCAI molecules contain only one negative charge per unit (Scheme 1). The calculated distance between neighboring charges in PLL is about 5 nm, which is almost 2 times less than the distance between charges in PLCAINa, equal to ~11 nm. In PLCANa macromolecules that contain two charges per unit, the calculated distances between neighboring charges varied from 10 to 3.5 nm according to main chain isomeries.

Deionized water obtained with a Milli-Q system from Millipore was further distilled once and filtered three times through a 0.22 μm filter from Millipore before use. Analytical grade NaCl was purchased from Merck.

Methods. *IPEC Formation.* Typically, IPECs were prepared at charge stoichiometry (cationic charges to anionic charges N_{PC}/N_{PA} ratio = 1) by direct addition of a polycation aqueous solution to a strongly stirred polyanion solution in water or NaCl at the desired concentration in a Pyrex tube at 25 °C. Experiments were conducted at 10^{-6} mol/L polyion concentrations.

Analyses. Vertically polarized dynamic light scattering (DLS) measurements were performed at 25 °C on a Brookhaven Instrument Corporation BI-200SM instrument equipped with an Ar−Kr laser (514.5 nm) and a multibit, multi-τ digital correlator (model BI-9000AT) covering more than 10 decades in delay time, τ. The apparatus was calibrated with toluene, whose absolute scattering value was 32×10^{-6} cm^{-1} at $\theta = 90°$. The hydrodynamic radius, R_H, was calculated using the Stokes−Einstein equation

$$R_H = \frac{kT}{6\pi\eta D} \qquad (1)$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the measured liquid at 25 °C, and D is the average diffusion coefficient. The viscosity of the polymer solutions was determined using a Rheometer Rheostress RS 100 (from Haake) at 25 °C. The refractive index increments were determined using a Brice-Phoenix refractometer at 633 nm at 25 °C.

Model of Intermolecular Polyelectrolyte Complexes Made of Polyelectrolytes with Different Solvent Affinities. Let us consider an interpolyelectrolyte complex consisting of two charged macromolecules in the presence of a 1−1 salt at concentration n_S. For the sake of simplification, the model takes into account the main features of IPECs only, that is, the affinity of the macromolecules to solvent and the distance between ionic groups along macromolecule backbones. Accordingly, the interpolyelectrolyte complex is formed by negatively charged A and positively charged C macromolecules that: (i) have different solvent affinities, the nonionic part of macromolecules being more hydrophilic for A than for C; (ii) bear equal numbers of ionic groups distributed at random along the chains, the total numbers of charged groups per macromolecule being equal and amounting to Q regardless of the charge sign e, and (iii) have different degrees of polymerization (hydrophilic A being longer than C). Furthermore, the mixture is neutral; that is, the number of positive charges in the solution is assumed to be equal to the number of negative charges according to stoichiometry.

To be definite, let us propose that macromolecules A are 2 times longer than C. Furthermore, let the nonionic part of the A backbone have affinity to water, whereas the nonionic C backbone does not like water.

Let us now denote N_C, the degree of polymerization of polycation C, and f_C, its degree of ionization; then, $f_C = Q/N_C$. Accordingly, the degree of polymerization, N_A, of anion A is equal to $2N_C$, and its degree of ionization is $f_A = f_C/2$. We assume that both macroions are flexible and have monomer unit of equal size a.

Let us propose that oppositely charged macroions A and C form spherical IPECs having a core−shell structure. The inner part of the complex with radius r contains monomer units of both macromolecules, whereas the exterior part (having radius R) consists exclusively of monomer units of the longer hydrophilic macromolecule A (Figure 1).

Finally, let us assume that macroions forming complexes are long enough to neglect surface effects (volume approximation). It is noteworthy that the volume approximation was already used to describe globular IPECs from a theoretical viewpoint.[20]

In volume approximation, the total free energy, F, of core−shell IPECs can be presented as the sum of three contributions: (i) free energy of the inner part of the complex F_{in}, (ii) free energy of the outer layer, F_{out}, and (iii) free energy of the external solution F_{ext}

$$F = F_{in} + F_{out} + F_{ext} \qquad (2)$$

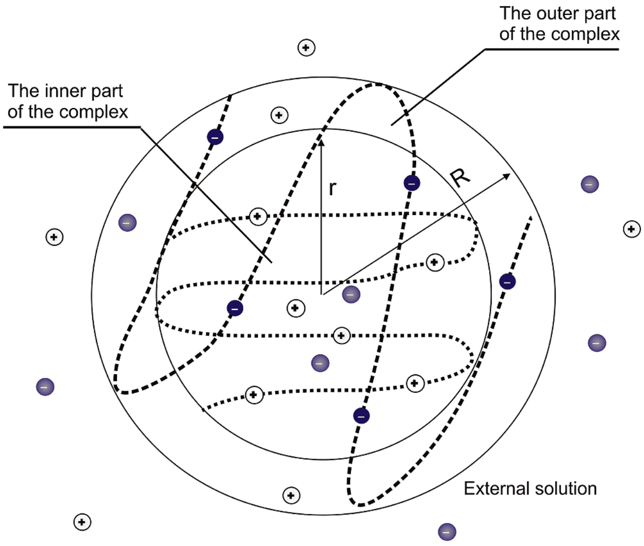


Figure 1. Schematic picture of interpolymer complex.

The free energy of the inner part, F_{in}, is the sum of four contributions

$$F_{\mathrm{in}} = F_{\mathrm{DH}} + F_{\mathrm{ci}}^{\mathrm{in}} + F_{\mathrm{int}}^{\mathrm{in}} + F_{\mathrm{el}} \tag{3}$$

where $F_{\mathrm{int}}^{\mathrm{in}}$ is the free energy of non-Coulomb van der Waals-type forces in the inner part of the interpolymercomplex particle; F_{DH} is the free energy of Debye–Hückel electrostatic interactions due to charge fluctuations; $F_{\mathrm{ci}}^{\mathrm{in}}$ is the contribution due to the translation entropy of counterions; and F_{el} describes the elasticity of macromolecules. In the internal part of the IPEC particles, N_{C} cationic species combine with $N_{\mathrm{A}}^{\mathrm{in}} \le 2N_{\mathrm{C}}$ polyanion charges. Therefore, the internal part of complex contains $N_{\mathrm{C}}f_{\mathrm{C}}$ positively charged monomer units and $N_{\mathrm{A}}^{\mathrm{in}}({}^{f_{\mathrm{c}}}/_{2})$ negatively charged monomer units. Also, the internal part of the IPEC contains salt ions. Let us denote $N_{\mathrm{S,C}}^{\mathrm{in}}$ and $N_{\mathrm{S,A}}^{\mathrm{in}}$ the total numbers of positively and negatively charged salt ions in the complex particle core, respectively.

The free energy F_{DH} can thus be written as[21,22]

$$\frac{F_{\mathrm{DH}}}{kT} = -\frac{2}{3}\sqrt{\pi}u^{3/2}\frac{\left(N_{\mathrm{C}}f_{\mathrm{C}} + N_{\mathrm{A}}^{\mathrm{in}}\frac{f_{\mathrm{C}}}{2} + N_{\mathrm{S,\,A}}^{\mathrm{in}} + N_{\mathrm{S,\,C}}^{\mathrm{in}}\right)^{3/2}a^{3/2}}{V_{\mathrm{in}}^{1/2}} \tag{4}$$

where V_{in} is the volume of the internal part of the particle of interpolymer complex ($V_{\mathrm{in}} = ({}^{4\pi}/_{3})r^3$); $u = {}^{e^2}/_{kT\varepsilon a}$ is a characteristic dimensionless parameter, where ε is the dielectric constant of medium and e is the elementary charge. In the case of aqueous solution ($\varepsilon \approx 80$) at room temperature ($T \approx 300$ K) and for ~1 nm monomer units ($a \approx 1$ nm), the parameter u is approximately equal to unity, $u \approx 1$.

In the Flory–Huggins approximation, the free energy of non-Coulomb interactions $F_{\mathrm{int}}^{\mathrm{in}}$ is

$$\begin{aligned}\frac{F_{\mathrm{int}}^{\mathrm{in}}}{kT} &= \chi_{\mathrm{AC}}N_{\mathrm{C}}\frac{N_{\mathrm{A}}^{\mathrm{in}}}{V_{\mathrm{in}}}a^3 + \chi_{\mathrm{CS}}N_{\mathrm{C}}\left(1 - \frac{N_{\mathrm{C}}a^3}{V_{\mathrm{in}}} - \frac{N_{\mathrm{A}}^{\mathrm{in}}a^3}{V_{\mathrm{in}}}\right)\\ &\quad + \chi_{\mathrm{AS}}N_{\mathrm{A}}^{\mathrm{in}}\left(1 - \frac{N_{\mathrm{C}}a^3}{V_{\mathrm{in}}} - \frac{N_{\mathrm{A}}^{\mathrm{in}}a^3}{V_{\mathrm{in}}}\right)\\ &\quad + \left(\frac{V_{\mathrm{in}}}{a^3} - N_{\mathrm{C}} - N_{\mathrm{A}}^{\mathrm{in}}\right)\ln\left(1 - \frac{N_{\mathrm{C}}a^3}{V_{\mathrm{in}}} - \frac{N_{\mathrm{A}}^{\mathrm{in}}a^3}{V_{\mathrm{in}}}\right)\end{aligned} \tag{5}$$

where χ_{AC}, χ_{AS}, χ_{CS} are the Flory–Huggins parameters of interaction of A and C monomer units between each other and with solvent molecules, S.

The contribution $F_{\mathrm{ci}}^{\mathrm{in}}$ due to translational entropy of salt ions can be written as

$$\frac{F_{\mathrm{ci}}^{\mathrm{in}}}{kT} = N_{\mathrm{S,\,A}}^{\mathrm{in}}\ln\left(\frac{N_{\mathrm{S,\,A}}^{\mathrm{in}}a^3}{V_{\mathrm{in}}}\right) + N_{\mathrm{S,\,C}}^{\mathrm{in}}\ln\left(\frac{N_{\mathrm{S,\,C}}^{\mathrm{in}}a^3}{V_{\mathrm{in}}}\right) \tag{6}$$

Finally, the elastic energy, F_{el}, that describes the changes in the set of possible conformational states of the macromolecules with respect to their ideal conformations can be noted as

$$\frac{F_{\mathrm{el}}}{kT} = \left(\frac{N_{\mathrm{c}}a^3}{V_{\mathrm{in}}}\frac{1}{\varphi_{\mathrm{o,\,C}}}\right)^{2/3} + \left(\frac{N_{\mathrm{A}}a^3}{V_{\mathrm{in}} + V_{\mathrm{out}}}\frac{1}{\varphi_{\mathrm{o,\,A}}}\right)^{2/3} \tag{7}$$

where $\varphi_{\mathrm{o},i}$ is the volume fraction of polymers in ideal coil (i = A, C).

In ideal conformation, which is a conformation with the end-to-end distance proportional to the square root of the degree of polymerization, $N_i^{1/2}$, the volume fraction of macromolecules within its effective volume is inversely proportional to $N_i^{1/2}$, with prefactor α depending on macromolecular flexibility. (For more details, see ref 23.) To be definite, in our calculation, we set α = 0.1 for both macroions, meaning the volume fractions of polymers A and C in their ideal coil are, respectively, equal to

$$\varphi_{\mathrm{o,\,C}} = \alpha N_{\mathrm{C}}^{-1/2}; \quad \varphi_{\mathrm{o,\,A}} = \alpha N_{\mathrm{A}}^{-1/2} \tag{8}$$

Analysis shows that in the shell the electrostatic interactions are small[24−27] and the free energy of the shell, F_{out}, is equal to the sum of two terms: (i) the translational entropy of salt ions $F_{\mathrm{ci}}^{\mathrm{out}}$ and (ii) the free energy of non-Coulomb interactions, $F_{\mathrm{int}}^{\mathrm{out}}$

$$F_{\mathrm{out}} = F_{\mathrm{ci}}^{\mathrm{out}} + F_{\mathrm{int}}^{\mathrm{out}} \tag{9}$$

with the contribution to free energy $F_{\mathrm{ci}}^{\mathrm{out}}$ equal to

$$\frac{F_{\mathrm{ci}}^{\mathrm{out}}}{kT} = N_{\mathrm{S,\,A}}^{\mathrm{out}}\ln\left(\frac{N_{\mathrm{S,\,A}}^{\mathrm{out}}a^3}{V_{\mathrm{out}}}\right) + N_{\mathrm{S,\,C}}^{\mathrm{out}}\ln\left(\frac{N_{\mathrm{S,\,C}}^{\mathrm{out}}a^3}{V_{\mathrm{out}}}\right) \tag{10}$$

where V_{out} is the shell volume ($V_{\mathrm{out}} = ({}^{4\pi}/_{3})R^3 - V_{\mathrm{in}}$) and $N_{\mathrm{S,C}}^{\mathrm{out}}$ and $N_{\mathrm{S,A}}^{\mathrm{out}}$ are the total amounts of positively and negatively charged ions in the shell, respectively.

The free energy, $F_{\mathrm{int}}^{\mathrm{out}}$, of non-Coulomb interactions in the shell is expressed as

$$\begin{aligned}\frac{F_{\mathrm{int}}^{\mathrm{out}}}{kT} &= \chi_{\mathrm{AS}}(N_{\mathrm{A}} - N_{\mathrm{A}}^{\mathrm{in}})\left(1 - \frac{N_{\mathrm{A}} - N_{\mathrm{A}}^{\mathrm{in}}}{V_{\mathrm{out}}}a^3\right)\\ &\quad + \left(\frac{V_{\mathrm{out}}}{a^3} - N_{\mathrm{A}} - N_{\mathrm{A}}^{\mathrm{in}}\right)\ln\left(1 - \frac{N_{\mathrm{A}} - N_{\mathrm{A}}^{\mathrm{in}}}{V_{\mathrm{out}}}a^3\right)\end{aligned} \tag{11}$$

Finally, the free energy F_{ext} of the external solution contains only contribution due to the translation entropy of salt ions. It is read as

$$\frac{F_{\mathrm{ext}}}{kT} = 2N_s^{\mathrm{ext}}\ln\left(\frac{N_{\mathrm{s}}^{\mathrm{ext}}a^3}{V_{\mathrm{ext}}}\right) \tag{12}$$

where $N_{\mathrm{s}}^{\mathrm{ext}}$ stands for the total amount of similarly charged salt ions out of complex particles, and V_{ext} is the volume of the solution per one interpolymer particle.

To ensure electroneutrality at the macroscopic level, salt ions within the complex are redistributed to compensate the core charge $\delta Q = N_{\mathrm{c}}f_{\mathrm{c}} - N_{\mathrm{A}}^{\mathrm{in}}(f_{\mathrm{c}}/2)$ and the shell charge in excess in such a way that the total amount of salt cations, $N_{\mathrm{S,C}}^{\mathrm{in}}$, in the particle core is a little bit smaller than the total amount of salt anions, $N_{\mathrm{S,A}}^{\mathrm{in}}$. Conversely, in the shell, the total amount of salt cations, $N_{\mathrm{S,C}}^{\mathrm{out}}$, is slightly greater than the total amount of salt anions, $N_{\mathrm{S,A}}^{\mathrm{out}}$.

It is easy to show that in the case of dilute solutions, that is, when the volume per single interpolymer complex noticeably exceeds the volume of the particle, the addition of relatively large amounts of salt into the solution changes the distribution of charges within the core and the shell and causes macroion redistributions that are totally compensated by appropriate salt ion redistributions according to

$$N_{\mathrm{S,A}}^{\mathrm{out}} - N_{\mathrm{S,C}}^{\mathrm{out}} = N_{\mathrm{S,C}}^{\mathrm{in}} - N_{\mathrm{S,A}}^{\mathrm{in}} \tag{13}$$

$$N_{\mathrm{S,A}}^{\mathrm{in}} \approx N_{\mathrm{S,C}}^{\mathrm{in}} - N_{\mathrm{C}} f_{\mathrm{C}} + N_{\mathrm{A}}^{\mathrm{in}} \frac{f_{\mathrm{C}}}{2} \tag{14}$$

$$N_{\mathrm{S,A}}^{\mathrm{out}} \approx N_{\mathrm{S,C}}^{\mathrm{out}} + N_{\mathrm{C}} f_{\mathrm{C}} - N_{\mathrm{A}}^{\mathrm{in}} \frac{f_{\mathrm{C}}}{2} \tag{15}$$

The equilibrium values of the outer, V_{out}, and the inner, V_{in}, volume of the interpolymer complex, the salt concentrations in its core, $n_{\mathrm{s}}^{\mathrm{in}} = (N_{\mathrm{S,A}}^{\mathrm{in}} + N_{\mathrm{S,C}}^{\mathrm{in}} a^3)/V_{\mathrm{in}}$, and in the particle shell, $n_{\mathrm{s}}^{\mathrm{out}} = (N_{\mathrm{S,A}}^{\mathrm{out}} + N_{\mathrm{S,C}}^{\mathrm{out}} a^3)/V_{\mathrm{out}}$, are determined by the equilibrium conditions of osmotic pressures and chemical potentials of the coexisting phase

$$\begin{gathered} \frac{\partial F_{\mathrm{out}}}{\partial V_{\mathrm{out}}} = \frac{\partial F_{\mathrm{ext}}}{\partial V_{\mathrm{ext}}}; \quad \frac{\partial F_{\mathrm{out}}}{\partial V_{\mathrm{out}}} = \frac{\partial F_{\mathrm{ext}}}{\partial V_{\mathrm{ext}}} \\ \frac{\partial F_{\mathrm{in}}}{\partial N_{\mathrm{S,C}}^{\mathrm{in}}} = \frac{\partial F_{\mathrm{out}}}{\partial N_{\mathrm{S,C}}^{\mathrm{out}}}; \quad \frac{\partial F_{\mathrm{out}}}{\partial N_{\mathrm{S,C}}^{\mathrm{out}}} = \frac{\partial F_{\mathrm{ext}}}{\partial N_{\mathrm{S,C}}^{\mathrm{ext}}} \end{gathered} \tag{16}$$

The amount of anionic monomer units entering into the complex core, $N_{\mathrm{A}}^{\mathrm{in}}$, is determined by minimization of the full free energy, F

$$\frac{\partial F}{\partial N_{\mathrm{A}}^{\mathrm{in}}} = 0 \tag{17}$$

The system of eqs 16 and 17 was numerically solved for different values: (i) of the interaction parameters χ_{AS} and χ_{CS}, assuming $\chi_{\mathrm{AC}} = 0$; (ii) of macromolecule lengths N_{C} (and therefore N_{A}), with $N_{\mathrm{A}} = 2N_{\mathrm{C}}$; (iii) of the degree of ionization f_{C}; and (iv) of the salt concentration multiplied by the volume of monomer units a^3, $n_{\mathrm{s}} = (N_{\mathrm{s}}^{\mathrm{ext}} a^3)/V_{\mathrm{ext}}$. Note that because of this definition of salt concentration, $n_{\mathrm{s}} < 1$. As a result, we defined a volume fraction of polycations in a complex core, $\varphi_{\mathrm{C}} = (N_{\mathrm{C}} a^3)/V^{\mathrm{in}}$, a volume fraction of polyanions in the inner part, $\varphi_{\mathrm{A}}^{\mathrm{in}} = (N_{\mathrm{A}}^{\mathrm{in}} a^3)/V^{\mathrm{in}}$, and in the outer part, $\varphi_{\mathrm{A}}^{\mathrm{out}} = ((N_{\mathrm{A}} - N_{\mathrm{A}}^{\mathrm{in}}) a^3)/V^{\mathrm{out}}$, of the particle, and also a fraction of monomer units forming the shell, $\Delta N = (1 - N_{\mathrm{A}}^{\mathrm{in}})/N_{\mathrm{A}}$.

Note that these calculations simultaneously allow us to check the validity of the core–shell model. Indeed, our calculation confirm core–shell if the fraction of monomer units in the shell is $0 < \Delta N < 1$ and the volume fraction of polyanion in the inner and outer parts are not equal to each other: $\varphi_{\mathrm{A}}^{\mathrm{in}} \neq \varphi_{\mathrm{A}}^{\mathrm{out}}$. It is also noteworthy that χ_{AS} and χ_{CS} values were primarily selected to reflect trends.

Results are shown in the next section.

Results and Discussion

Theoretical Approach. Figure 2A presents the polycation volume fraction, φ_{C}, and the polyanion volume fractions in the core, $\varphi_{\mathrm{A}}^{\mathrm{in}}$, and in the outer shell, $\varphi_{\mathrm{A}}^{\mathrm{out}}$, as the function of salt concentration, n_{s}, for fully ionized long macromolecules ($N_{\mathrm{C}} = 1000, f_{\mathrm{C}} = 1$), and for $\chi_{\mathrm{CS}} = -\chi_{\mathrm{AS}} = 1.0$. Figure 2B shows the corresponding dependency of the fraction ΔN of anionic monomer units forming the shell. At relatively low n_{s} salt concentrations, the $\varphi_{\mathrm{A}}^{\mathrm{in}}$ volume fraction in the core counts for approximately twice that of the polycation φ_{C}, and only a small amount of units (about 0.1%) are involved in the particle shell. The fraction of anionic monomer units present in the particle shell, ΔN, remains very small although it increases linearly with salt concentration: $\Delta N \approx n_{\mathrm{S}}$. In parallel, $\varphi_{\mathrm{A}}^{\mathrm{in}}$ and φ_{C} remain practically constant up to a critical salt concentration, $n_{\mathrm{S}}^{\mathrm{cr}}$. At this point, the complexed chains undergo a sharp increase. After this sharp transition referred to as "decollapse", macromolecules are still complexed, but ~5% of the anionic monomer units move from the inner part of the complex to the shell. Above $n_{\mathrm{S}}^{\mathrm{cr}}$, more anionic monomer units are leaving the particle core, and ΔN increases rapidly ($\Delta N \approx n_{\mathrm{S}}^{4/3}$) to reach finally a salt concentration n_{S}^{*}, at which ΔN becomes close to 1. At this point, polyanionic and polycationic macromolecules are practically separated, and the interpolymer complex is destroyed. Both macromolecules are coiled according to their highly charged polyelectrolyte structural characteristics. Above n_{S}^{*}, high salt concentrations screen electrostatic charges, and polyelectrolyte macromolecules are relaxed until the size of coiled uncharged macromolecule.

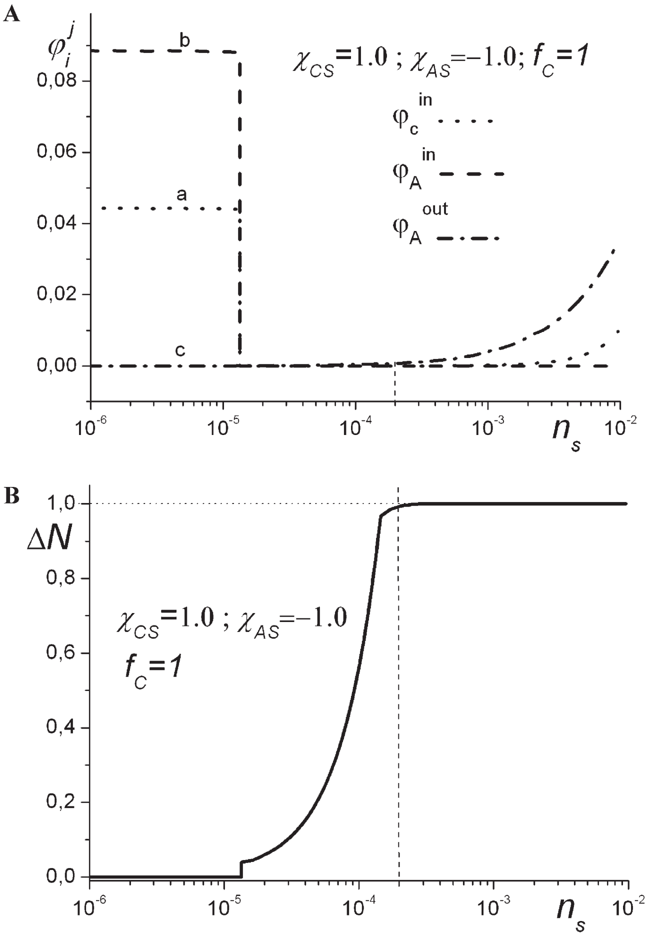


Figure 2. (A) Volume fractions of polycation φ_{C} and polyanion $\varphi_{\mathrm{A}}^{\mathrm{in}}$ in the complex core and of polyanion in the shell $\varphi_{\mathrm{A}}^{\mathrm{out}}$ and (B) fraction of monomer units forming the shell ΔN as a function of salt concentration, n_{s}, at $N_{\mathrm{C}} = 1000, f_{\mathrm{C}} = 1, \chi_{\mathrm{CS}} = 1$, and $\chi_{\mathrm{AS}} = -1$.

In Figure 3A, the dependences of the polycation volume fraction, φ_{C}, and of the polyanion volume fraction in the inner part of the IPECs, $\varphi_{\mathrm{A}}^{\mathrm{in}}$, on the salt concentration, n_{s}, for sufficiently long macromolecules ($N_{\mathrm{C}} = 1000$) are given for different values of degree of ionization, f_{C}. Figure 3B

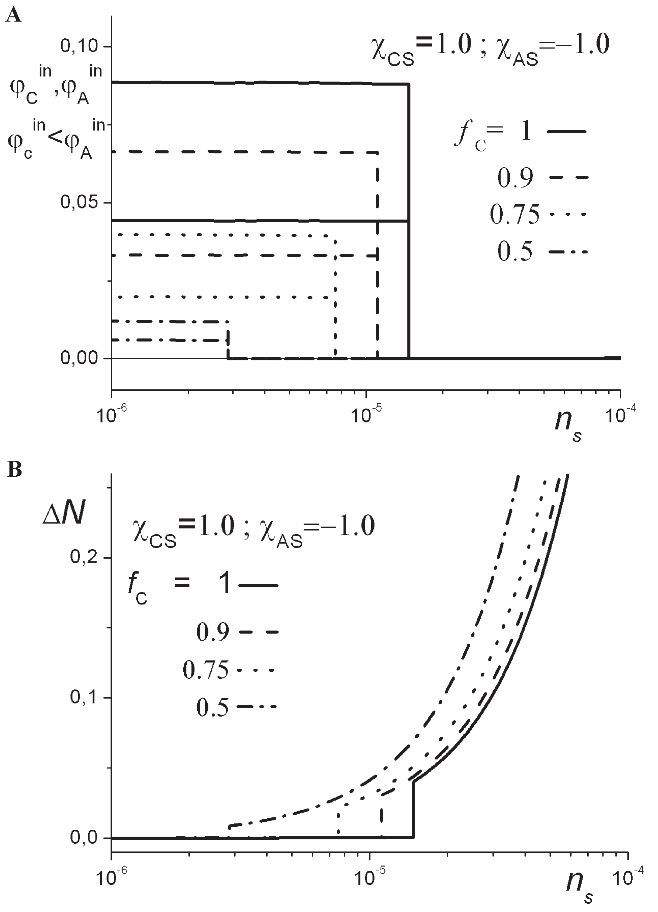


Figure 3. (A) Volume fractions of polycation φ_C and of polyanion φ_A^{in} in the complex core and (B) fraction of monomer units forming the shell ΔN as a function of the salt concentration, n_s, at $N_C = 1000$, $\chi_{CS} = 1$, and $\chi_{AS} = -1$ for different degrees of ionization, f_C.

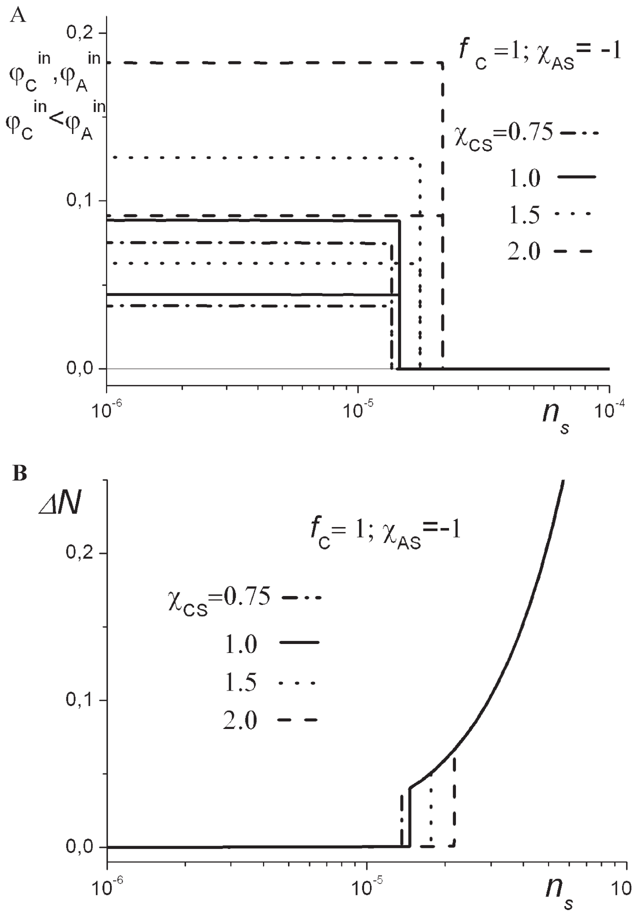


Figure 4. (A) Volume fractions of polycation φ_C and of polyanion φ_A^{in} in the complex core and (B) fraction of monomer units forming the shell ΔN as a function of salt concentration n_s at $N_C = 1000$, $f_C = 1$, and $\chi_{AS} = -1$ for different values of parameter χ_{CS}.

presents the corresponding dependency of ΔN, the fraction of anionic monomer units involved in the shell. When the salt concentration is low, φ_A^{in} is up to 2 times greater than φ_C, and only a small amount of anionic units are included within the protective shell according to ΔN. The trend depends on the degree of ionization. The smaller the degree of ionization, the greater the value of ΔN. The particle size remains the same up to n_S^{cr}, at which "decollapse" occurs. The higher the degree of ionization, f_C, the greater the critical salt concentration, n_S^{cr}, and the larger the change of ΔN at the point of complex "decollapse". It should be mentioned that in the case of strongly charged polyions ($f_C \approx 1$), the total complex destruction (when ΔN approaches 1) occurs at a higher n_s salt concentration than for weakly charged macroions, that is, when f_C is small.

Figure 4 shows the evolution of φ_C and φ_A^{in} (Figure 4A) and of ΔN(Figure 4B) with increasing n_s for macromolecules with $N_C = 1000$, $f_C = 1$, and $\chi_{AS} = -1$ and different values of χ_{CS}. With increasing χ_{CS}, that is, for greater polycation hydrophobicity, φ_C and φ_A^{in} both increase. In parallel, the "decollapse" occurs at greater n_s salt concentration, and the change of ΔN at "decollapse" is larger. It is noteworthy that before and after "decollapse", variations are totally congruent. This means that one observes particle destabilization (ΔN approaches 1: $\Delta N \approx 1$) at the same salt concentration.

The $\Delta N(n_s)$ dependences for particles differing only by the affinity of the polyanion to the solvent (i.e., χ_{AS}) are also congruent (Figure 5B). In this case, the greater the affinity of the polyanion to the solvent (i.e., the greater the χ_{AS} absolute value), the lower the density in monomer units within the particle core at low salt concentration, and the lower the salt concentration at which "decollapse" occurs (Figure 5).

Figure 6 presents results of calculation of φ_C and φ_A^{in} (A) and of ΔN (B) as a function of n_s for different degrees of polymerization, N_C. One can see that at low n_s values, increasing the degree of polymerization, N_C, causes a decrease in the density of monomer units in the particle core. However, in the case of sufficiently long macromolecules having different large N_C, the variations of the monomer unit density in the core of a complex are practically congruent. The shorter the macromolecule, the higher the values of n_s at which "decollapse" occurs. Furthermore, the bigger the ΔN leap at "decollapse", the smaller the zone where the swollen interpolymer complex is stable. Analysis shows that at any salt concentration (before and after "decollapse" transition), the free energy per one monomer unit of complexes grows with an increase in the macroion degree of polymerization. This implies that the IPECs are stable with respect to aggregation with each other.

Therefore, by monitoring the solution of the above-described macromolecules with an increase in salt concentration, one can see at relatively low salt concentration the interpolymer complexes: first, they are collapsed; then, they are swollen. At high salt concentration, one observes the solution of individual macroions. For the sake of comparing theory and experiment, it is useful to plot the evolution of size R_{cl} of particles (interpolymer complexes or macroions) with salt concentration.

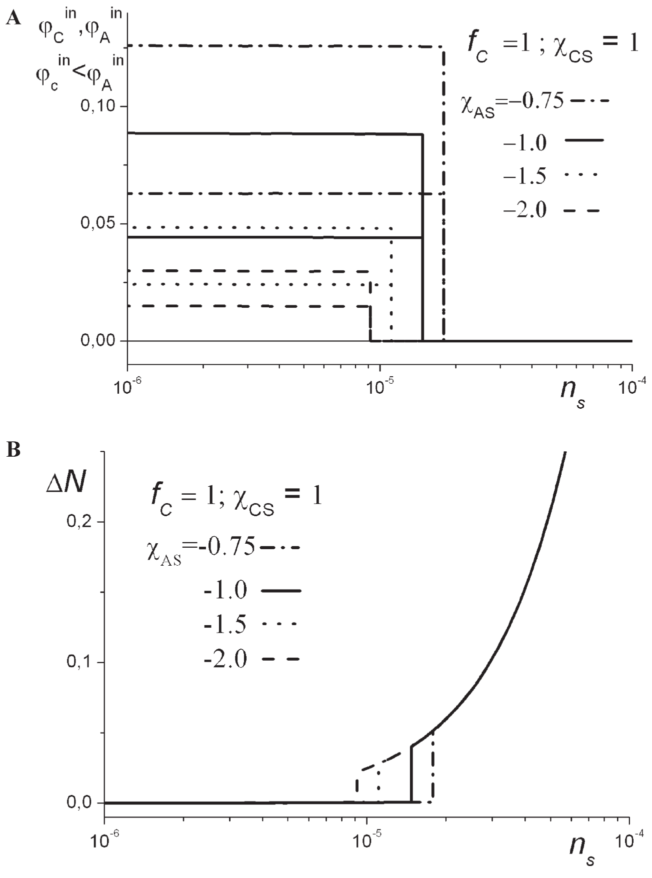


Figure 5. (A) Volume fractions of polycation φ_C and of polyanion φ_A^{in} in the complex core and (B) fraction of monomer units forming the shell ΔN as a function of the salt concentration, n_s, at $N_C = 1000, f_C = 1$, and $\chi_{CS} = 1$ for different values of interaction parameter, χ_{AS}.

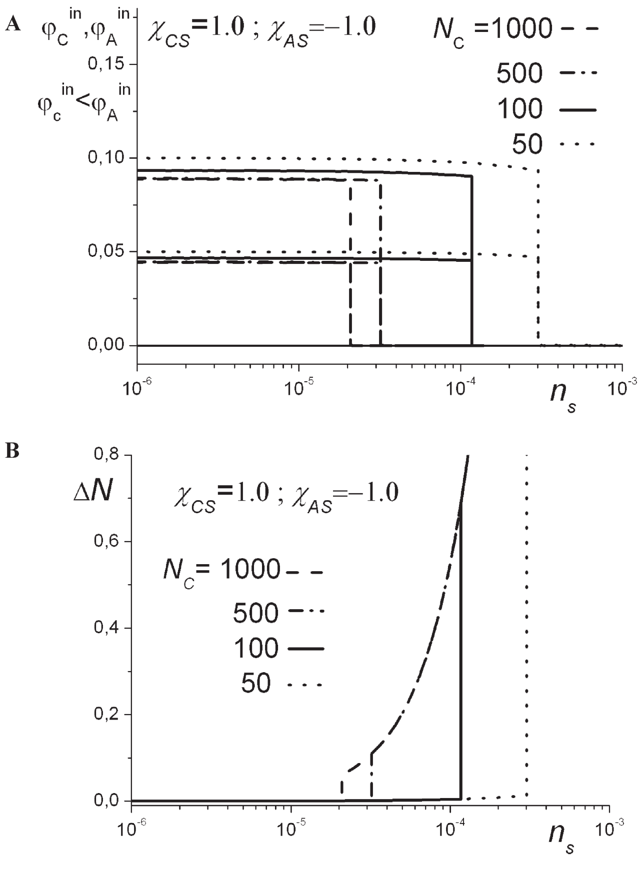


Figure 6. (A) Volume fractions of polycation φ_C and of polyanion φ_A^{in} in the complex core and (B) fraction of monomer units forming the shell ΔN as a function of salt concentration, n_s, at $f_C = 1, \chi_{CS} = 1$, and $\chi_{AS} = -1$ for different degree of polymerization, N_C.

We assume that at salt concentration smaller than n_S^*, that is, before the total destruction of the interpolymer complex, the particle size R_{cl} is equal to that of the anion macromolecules forming the external shell: $R_{cl} = R$. After destruction, the average particle size is proposed to be equal to

$$R_{cl} = \frac{R_C + R_A}{2} \tag{18}$$

where R_C and R_A are the sizes of separately moving polycation and polyanion.

To evaluate R_C and R_A, we use the theory of individual polyelectrolyte macromolecules with the degree of polymerization, N_j, and the degree of ionization, f_j, in solution of salt at concentration, n_s. Here and later on, index j is attributed either to polycationic ($j = C$) or polyanionic ($j = A$) macromolecules. The concentration of macroions is low, and one can neglect interactions of macromolecules. Also, we propose that the total charge, Q, of each macromolecule is totally compensated by the excess of oppositely charged small ions in the effective volume $V_j = (^{4\pi}/_3)R_j^3$. In other words, the number of salt ions, $N_{S,j}^{in,op}$, having opposite sign to the polymer chain charges exceeds the number of salt ions with the same charge $N_{S,j}^{in}$ by Q: $N_{S,j}^{in,op} = N_{S,j}^{in} + Q$.

The free energies of macroions (both polycations and polyanions) are presented as the sum of three contributions

$$F^j = F_{int}^j + F_{ci}^j + F_{el}^j \tag{19}$$

where F_{int}^j is the free energy of non-Coulomb van der Waals type forces, F_{ci}^j is the free energy due to the translation entropy of counterions, and F_{el}^j is that corresponding to the elasticity of macromolecules.

Using the same approximation as that discussed in the above theory of interpolyelectrolyte complexes, we should write the interaction free energy, F_{int}^j, in Flory−Huggins approximation

$$\frac{F_{int}^j}{kT} = \chi_{jS} N_j \left(1 - \frac{N_j}{V_j} a^3\right) + \left(\frac{V_j}{a^3} - N_j\right) \ln\left(1 - \frac{N_j}{V_j} a^3\right) \tag{20}$$

where χ_{jS} is the Flory−Huggins parameter of interaction j monomer units ($j = A$ or C) with solvent molecules, S.

The contribution to free energy F_{ci}^j due to the translational entropy of salt ions is

$$\frac{F_{ci}^j}{kT} = (N_{S,j}^{in} + N_j f_j) \ln \frac{(N_{S,j}^{in} + Q)a^3}{V_j} + N_{S,j}^{in} \ln\left(\frac{N_{S,j}^{in} a^3}{V_j}\right) + 2N_S^{ext} \ln\left(\frac{N_S^{ext} a^3}{V^{ext}}\right) \tag{21}$$

Finally, the elastic free energy F_{el}^j is

$$\frac{F_{el}^j}{kT} = \left(\frac{N_j a^3}{V_j \varphi_{o,j}}\right)^{2/3} \tag{22}$$

where $\varphi_{o,j}$ is a volume fraction of a polymer in ideal coil state (eq 8).

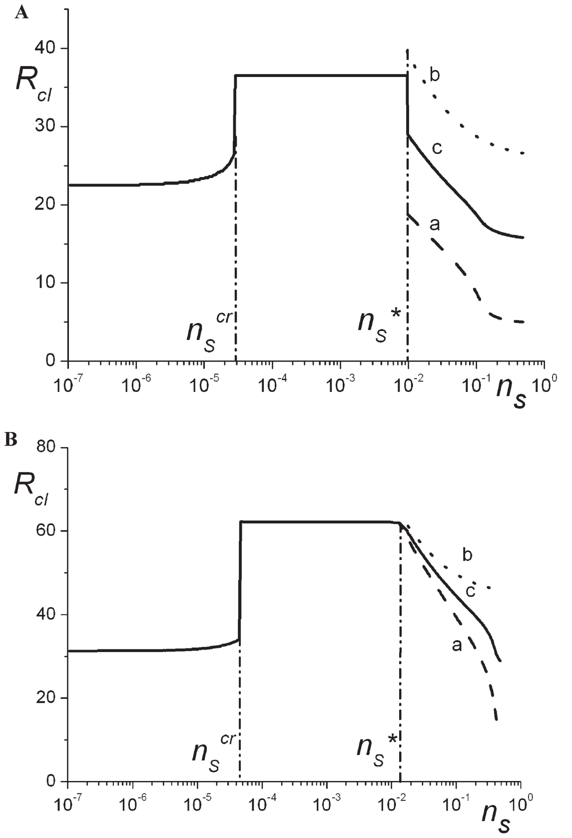


Figure 7. Calculated size R_{cl} of particles (c) as a function of salt concentration, n_s, for $f_C = 0.6$, $\chi_{CS} = 0.75$, $\chi_{AS} = -0.75$, and N_C = (A) 200 and (B) 1000. Corresponding radii of individual polycation R_C (a) and polyanion R_A (b) are shown by dashed lines.

At equilibrium, salt osmotic pressures within a macroion and in the external solution are equal to each other and chemical potentials as well. Both equilibriums condition the size of macromolecules. The equations were solved numerically using the parameter values used in the case of interpolymer complexes above.

The evolution of the averaged radius of interpolymer complex particles, R, and those of the averaged radii of separate macroions, $R_j = ((^3/_{4\pi})V_j)^{1/3}$, on the salt concentration, n_s, are shown in Figures 7 and 8. The salt concentration-dependency of the size R_{cl} of particles made of oppositely charged macroions with different affinity to solvent is nonmonotonic. At low salt concentration, interpolymer complexes are compact. At the critical salt concentration, n_S^{cr}, R_{cl} increases sharply. For higher values of the salt concentration, the complex size (i.e., calculated size of particles) remains the same up to the point where segregation between polyanions and polycations is almost completed. At this point, $n_s = n_S^*$ and the averaged size, R_{cl}, is determined as the average of polyanion, R_A, and polycation, R_C, sizes. (See eq 18.) The polyion sizes within the complex (curve c) differ from those in free conformation (curves a and b). In other words, the polycation size, r, within the interpolymer complex (not shown in Figures) is somewhat larger than that of the separate polycation, R_C. In contrast, the polyanion size R, within the complex is smaller than the size R_A of the freely moving corresponding macromolecule. (See the difference between the complex size and curve *a* at n_S^* in Figures 7 and 8.) Respectively, the change of average size R_{cl} of particles at point n_S^* is determined by the relative difference between the sizes of macroions in free state and within the complex.

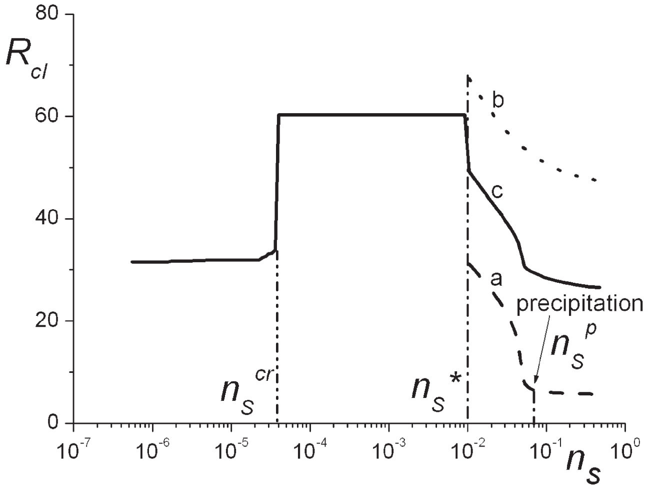


Figure 8. Calculated size R_{cl} (c) as a function of the salt concentration, n_s, for $f_C = 0.6$, $\chi_{CS} = 1.0$, $\chi_{AS} = -1.0$, and $N_C = 1000$. Corresponding radii of individual polycation R_C (a) and polyanion R_A (b) are shown by dashed lines.

Figure 7 shows the variations of averaged radius, R_{cl}, calculated for $\chi_{CS} = -\chi_{AS} = 0.75$ and for two different degrees of polymerization of macromolecules N_C and N_A. In the case of the complex consisting of macromolecules with a relatively small degree of polymerization ($N_C = 200$, $N_A = 400$) at the salt concentration, n_S^*, one could see a sharp steplike change of R_{cl} corresponding to the separation of oppositely charged polyions (Figure 7A). In contrast, for longer macromolecules ($N_C = 1000$, $N_A = 2000$), there was no significant R_{cl} changes observed for the polycation and polyanion sizes within the complex and in the free state (Figure 7B). In both cases, R_{cl} decreased smoothly for salt concentration greater than n_S^*.

Figure 8 shows the calculated sizes R_{cl} of particles composed of long macromolecules ($N_C = 1000$) for the case of a more hydrophobic polycation and a more hydrophilic polyanion than that in Figure 7B ($\chi_{CS} = -\chi_{AS} = 1.0$ instead of 0.75). Under these conditions, the sizes of free macroions R_A and R_C differ from those in the complex, and there is a stepwise decrease in R_{cl} at macroion separation. For $n_s > n_S^*$, the separate polycation, in its turn, undergoes a coil–globule transition, and thus above the salt concentration, n_s^p, shown in Figure 8 by arrow, the polycations can precipitate, and if so, the average size of particles in the soluble fraction is determined by the size of polyanions floating in the supernatant (curve b).

In conclusion, in the case under consideration, the calculated size, R_{cl}, is a nonmonotonic function of salt concentration, n_s. One can distinguish two critical points on this dependency: the "decollapse" transition of interpolymer complex, n_S^{cr}, and the point of total separation of macroions, n_S^*. In the point of macroion separation, the observed change in averaged sizes of particles depends on the degree of polymerization N_A (and N_C) and on parameters of macroions interaction with solvent χ_{CS} and χ_{AS}. For fixed values of χ_{CS} and χ_{AS}, the higher the degree of polymerization, the smaller the change of particle size upon macroion separation. We would also like to mention here that experimentally determined hydrodynamic radius or radius of gyration differ from what we described here as a calculated size, R_{cl}, of the particle and what for the case of macroions is more close to the chain end-to-end distance. However, it is known that all of these parameters differ from each other by a factor of the order of unity.[23] Therefore, one can observe qualitatively

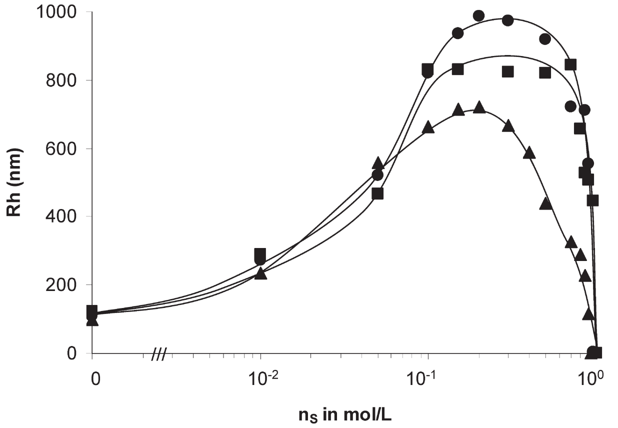


Figure 9. Experimental average hydrodynamic radius, R_H, of PLCA/PLL complex particles as a function of salt concentration, n_S. [polymer] = 10^{-6} mol/L. ■, PLL80-PLCA; ●, PLL27-PLCA; ▲, PLL12-PLCA.

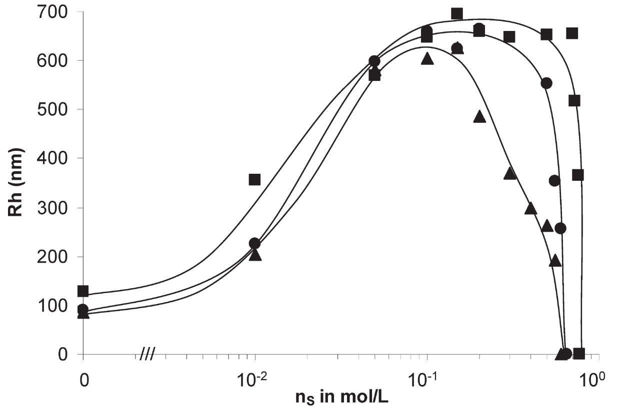


Figure 10. Experimental average hydrodynamic radius, R_H, of PLCAI/PLL complex particles as a function of salt concentration, n_S. [polymer] = 10^{-6} mol/L. ■, PLL80-PLCAI; ●, PLL27-PLCAI; ▲, PLL12-PLCAI.

similar dependences on the experimental techniques used to determine the characteristic size of particles.

Comparison with Experimental Data. The values of the hydrodynamic radius, R_H, of IPEC particles of PLCA/PLL and PLCAI/PLL complexes obtained after the addition of the polycation into the polyanion solution were determined in NaCl solutions by DLS.

Figure 9 shows the dependency of R_H for PLL/PLCA complexes involving a PLCA sample combined with PLL of different molecular weights to mimic the situation described in Figure 7A. Experiments were conducted at very low polyion concentrations (~10^{-6} mol/L in repeating units) selected to be in the range generally used in the case of the administration of macromolecular prodrugs or of complexes for gene transfection in cell cultures.[8,9] In pure water, IPEC particles of 100−150 nm size were dispersed in crystal clear aqueous media. When the salt concentration increased from 0 to 0.1 M, IPEC particles started swelling, leading to a sharp increase in their size and appearance of turbidity. Accordingly to curve profiles, it seems that at very low concentration in the $0 < n_s < 0.02$, the increase looks moderate compared with the zone $0.02 < n_s < 0.1$. It is worth noting that experimentally it was not possible to collect R_H data for salt concentrations lower than 0.02 M. In the 0.1 M $< n_s <$ 0.2 M zone, R_H increased rather rapidly but not as sharply as the theoretical curves in Figure 7A suggest, probably because of the polydispersity of the real polyions. Theory yields radii of ideal complex particles with minimal free energy for each set of system parameters (salt concentration, solvent quality, etc.). In the case of a sharp first-order transition, two objects can be present[28] that lead to bimodal size distributions corresponding to "collapsed" and "swollen" interpolymer complexes whose relative numbers change with system parameters, a phenomenon that can contribute to broaden the transition as well. Beyond 0.2 M, particles size decreased more or less rapidly depending on the PLL molecular weight, and this up to a salt concentration where a slop change is observed with a sharper decrease also depending on PLL molecular weight. The higher the molecular weight, the flatter the first decrease and the steeper the last decrease. The slope change in the low salt concentration range can be seen as n_S^{cr}, and the beginning of the fast decrease at high salt concentration can be seen as n_S^*, with n_S^{cr} being relatively independent of the PLL molecular weight, in contrast with n_S^*. Indeed, the NaCl concentration required to destabilize the IPEC increased from 0.85 to 1 M, and the IPEC particle maximal size increased from 725 to 840 nm when PLL molecular weight varied from 12 700 to 73 500 g/mol, that is, when the number of positive charges per macromolecule increased. At high salt concentration, R_H values are similar to those obtained for solutions of the initial polyions taken separately at high salt concentrations (5−10 nm). This behavior, which reflects the ionic strength-promoted IPEC dissociation, depended on PLL molecular weight. The same trends were observed in the case of PLCAI complexes (Figure 10). Particle size increased with the polyanion charge density and gained ~100−200 nm when comparing PLCAI and PLCA data, with PLCA having a higher charge density than PLCAI (Figure 9 and 10). n_S^{cr} and n_S^* critical salt concentrations were lower in the case of PLCAI than in the case of PLCA, probably because of the greater number of electrostatic interactions per chain length unit in the latter, a feature that made PLCA/PLL complexes more stable than PLCAI/PLL complexes.

It should also be mentioned that experimentally we have studied a mixture of macromolecules with different ratios of molecular weight, that is, the degree of polymerization, and the total number of charges per oppositely charged macroions. Actually, PLCAINa contains ~165 monomer units, and PLCANa has 112 monomer units with a two-fold larger number of negative charges. The total numbers of monomer units, and simultaneously, the total numbers of positive charges in PLL12, PLL27, PLL80, are 61, 126, and 352, correspondingly. Therefore, one can expect that to compensate the total charge, the separate interpolymer complexes contain different numbers of oppositely charged macroions. These facts as well as various facts related to polymer complexity in terms of polydispersity, molecular weights distribution, and intrachain variation of charge density when one deals with more or less random copolymers limited the present comparisons.

For the comparison of the theory with the experiments, we considered the presence of OH groups along the PLCA and PLCAI main chains to be a good way to provide the extra hydrophilicity required to form a core−shell structure with respect to PLL or PAA chains. One can see that the shape of the dependence of hydrodynamics radii, R_H, on the salt concentration qualitatively corresponds to the theoretically calculated one.

Conclusions

The thermodynamic stability of nanodispersed, stoichiometric polyelectrolyte complexes (IPECs) consisting of oppositely charged macromolecules having different affinity to water were considered in the presence of increasing salt concentration. It was shown that such complexes have a core–shell structure, the shell accommodating the polyion with the higher affinity to water preferentially.

According to the theory, at low salt concentrations, the fraction of hydrophilic units forming the complex shell is rather small and grows smoothly with the salt concentration up to a critical salt concentration, n_S^{cr}, where the complex size increases sharply. This change is associated with a decollapse transition undergone by the complexed macromolecules by analogy to corresponding transitions in polyelectrolyte gels and designated by the term "decollapse". After the "decollapse" transition, macroions are still complexes. At this point, the separation is unfavorable from the entropic viewpoint. Indeed, separating both macroions requires much higher concentrations of oppositely charged counterions to compensate the total charge. Separation occurs at salt concentration, n_S^*, when polyions separate totally; that is, each becomes freely moving in the aqueous medium. For concentrations higher than n_S^*, polyions undergo normal charge screening that can go up to precipitation.

The volume fraction of polymer in inner and external parts of the polyelectrolyte complex composed of polyions with sufficiently long chains is almost independent of the chain length, N_C, and primarily depends on the salt concentration. The critical salt concentration, n_S^{cr}, rises when the degree of ionization, f_C, of the macromolecules increases and when the hydrophobicity of the polycation increases, whereas it decreases when the degree of polymerization, N_C, increases or when the affinity of the anionic hydrophilic sites with water increases. The higher the degree of ionization of the macroions, f_C, the higher the concentration, n_S^*, at which total separation occurs; this concentration does not depend on interaction parameters or on the degree of polymerization of macroions. It was shown that in both the collapsed and swollen states, the interpolymer complexes are stable with respect to their aggregation.

The evolution of size, R_{cl}, of particles (the interpolymer complexes at low salt concentration or separate macroions at high concentration of salt) in solution of such macromolecules with an increase in salt concentration, n_s, was calculated. $R_{cl}(n_s)$ was shown in the nonmonotonic function with a sharp increase and decrease in the size, R_{cl}, at n_S^{cr} and n_S^*, respectively. The calculated change of particle sizes, R_{cl}, at n_S^* is determined by the relative difference between the sizes of macroions in the free state and within the complex. It depends on all physical parameters, such as the degree of polymerization, N_C, the degree of ionization f_C, and the parameters of macroions interaction, χ_{CS} and χ_{AS}.

Experimental data collected in the particular cases of PLL/PLCA and PLL/PLCAI complexes agree well with the trends indicated by the theoretical approach, although critical salt concentrations, n_S^{cr} and n_S^*, are not as sharply defined, a feature tentatively assigned to the polydispersity of real systems and to averaged sizing due to the coexistence, in the first-order transition, of IPEC of different sizes with characteristics depending on system parameters (salt concentration, solvent quality, etc.).

We would like to emphasize that the sharp increase in IPEC particle size with an increase in salt concentration was not due to aggregation, that is, to an increase in the number of macromolecules forming the complexes, but rather to the ≪decollapse≫ of complex particles themselves. One can expect that this phenomenon will be of interest in the case of the polyelectrolytes main chain bearing hydrophilic groups such as proteins, polysaccharides, and other biopolyelectrolytes.

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