Study of Interpolymer Complexes of Oppositely Charged Macromolecules with Different Affinity to Solvent

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ABSTRACT: We consider the new type of water-soluble stoichiometric interpolymer polyelectrolyte complexes (IPEC) consisting of macromolecules having different affinities to solvent and different densities of ionic groups along the chain. It was proposed that complexes of such macromolecules have a core−shell structure. The inner part of complex with a radius r contains monomer units of both macromolecules, while exterior part with a radius R consists exclusively of monomer units of hydrophilic macromolecules. With such complex organization, the external charged hydrophilic shell effectively protects the complex from precipitation and fusion with other complex assemblies. These theoretical conclusions allow to explain the experimental data on the stability of IPECs containing poly(L-lysine), poly(L-lysine citramide), poly(L-lysine citramide imide), and poly(acrylic acid), and can be of interest in the field of DNA−polycation complexes aimed at transfecting genes to cell nuclei.

1. Introduction

Intermolecular polyelectrolyte complexes (IPECs) have attracted considerable attention for a long time starting from their first studies in the middle of the 20th century.[1−5] IPECs are formed spontaneously in solution upon mixing positively and negatively charged polyions. The association between these polyions occurs thanks to strong cooperative electrostatic interactions.[1−4] IPECs play an essential role 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 like DNA−enzyme−protein interactions occurring in cell machineries.[5,6] On the other hand, and somewhat related, IPECs dispersed in water are very prospective for various applications in biomedical fields, including the encapsulations of biological substances, drug delivery systems, or gene transfection and gene therapy.[5−8]

A great number of comprehensive experimental studies of complexes including their preparation and structure analysis have been performed during last decades.[2−4] It has been found that IPEC properties considerably depend on the positive and negative charge ratios within mixtures. Nonstoichiometric complexes appear if there is an excess of charges of one sign in the solution. These complexes are generally in the form of hydrodispersed tiny particles or micellar aggregates stabilized by the presence of a surface net charge. In contrast to nonstoichiometric IPECs, stoichiometric IPECs contain equal amounts of opposite charges. The net charge is thus zero, and macroscopic phase separation is observed.

Recently, it was shown that the phase separation in stoichiometric mixtures could be prevented, if a hydrophilic nonionic block is attached to at least one polyelectrolyte.[9,10] The interactions between monomeric units of this block and the solvent stabilize the formation of finite-size aggregates in the solution. The aggregates have a well-defined micellar core−shell structure where the core of the micelle consisting of polyelectrolyte complex is surrounded by hydrophilic corona of nonionic block. This type of complexes is called block−ionomer complexes. Theories have been proposed for such complexes.[11,12]

In parallel, some of us found recently that cationic macromolecules such as poly(L-lysine) and poly(amino serinate) form water-soluble stoichiometric IPECs with anionic macromolecules of poly(L-lysine citramide) and poly(L-lysine citramide imide), two polyelectrolytes of the polycarboxylate-type whose backbone bears one hydrophilic OH group in each lysyl−citramide repeating unit (Scheme 1).[13] We suggested that the apparent solubility of such complexes result from the presence of hydrophilic hydroxyl groups. Indeed the mixture of cationic macromolecules of poly(L-lysine) with poly(acrylic acid) having a hydrophobic main chain always led to macroscopic phase separation.[13] In the case of PLCA and PLCAI polycarboxylates, the absence of precipitation could be due to the interactions of the hydrophilic OH groups with aqueous surroundings.

Accordingly, one can expect that PLCA and PLCAI complexes are heterogeneous and composed of an inner part with radius r that contains monomer units of both macromolecules and a exterior part (with radius R) that consists exclusively of monomer units of longer hydrophilic macromolecules. The external charged hydrophilic shell would thus protect the complex elementary entities to aggregate with other similar entities. Such 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. In contrary, the factor preventing the formation of such layer is the Coulomb attraction between excess of negative and positive groups in internal and external parts of IPEC, respectively. The balance of these two main factors determines availability and width of protective external layer. It is clear that such IPECs will be stable upon aggregation only in the case of $r < R$.

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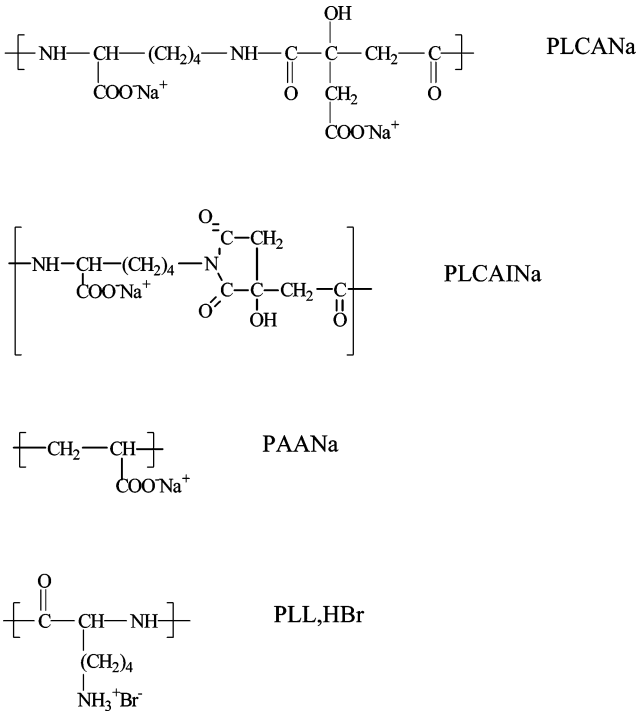
Scheme 1. Chemical Structure of PLCANa, PLCAINa, PAANa, and PLL,HBr



In this article, we analyze the factors that govern the stability of complexes between oppositely charged macromolecules having different affinity to solvent and we propose a thermodynamic approach that describes such complexes. The theoretical calculations are compared with additional experimental data on the stability of IPECs between poly(L-lysine) and three polycarboxylates, namely poly(L-lysine citramide), poly(L-lysine citramide imide), and poly(acrylic acid). It is important to note that the distance between charged groups in poly(L-lysine) and poly(acrylic acid) is at least twice smaller than in poly(L-lysine citramide) and poly(L-lysine citramide imide). Analysis shows that this is an additional factor contributing to the stability of their complexes.

The second section will present the materials and methods used. Section 3 is aimed at reporting experimental data. In a fourth section, we derive the free energy of complexations between oppositely charged macromolecules having different affinity to solvent and analyze the conditions of their stability. The comparison between experimental data and theoretical results are discussed in section 5.

2. Methods and Materials

Raw Materials. Poly(L-lysine) hydrobromide (PLL,HBr) (average $M_w = 30\,000$ g/mol) was purchased from Sigma. Poly(L-lysine citramide) ($M_w = 42\,000$ g/mol) and poly(L-lysine citramide imide) ($M_w = 48\,000$ g/mol) sodium salts, respectively PLCANa and PLCAINa, were synthesized as previously described.[14] PLCA was issued 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). Linear sodium poly(acrylate) (PAANa, $M_w = 81\,000$ g/mol) was prepared in two steps. First, poly(benzyl acrylate) was synthesized by radical polymerization in toluene at 70 °C with AIBN as the initiator. PAA was then obtained by hydrogenolysis of poly(benzyl acrylate) using Pd/C as catalyst followed by neutralization of carboxyl groups with NaOH. PAA molecules contain 1 negative charge per unit (Scheme 1). 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.

IPEC Formation. Typically, IPECs were obtained by adding successively an appropriate volume of a 1 M PLL solution to a strongly stirred polyanion solution (20 mg in 1 mL of water) in a plastic vial at room temperature.[15] Three additions were successively carried out until the global N_{PC}/N_{PA} ratio reached 1. We order to reach $N_{PC}/N_{PA} = 0.3$, 0.6 and finally 1. Precipitates were collected after each addition, washed with deionized water and finally freeze-dried.

Methods. The molecular weight of the polyanion and polycation engaged in the precipitates was determined by size exclusion chromatography (SEC) after separation of the two components by gel filtration on CM-CL6B Sepharose gel.[15] At 1 M NaCl, PLL was retained on the gel while the polyanion was eluted. The retained PLL was then released from the gel using 2 M NaCl.

SEC experiments were performed using a LCC-501 System (from Pharmacia Biotech) equipped with a UV−visible detector and a column packed with the anionic CM-CL6B Sepharose gel. The mobile phase was a solution of 0.15 M NaH_2PO_4 and 1 M NaCl at pH = 7.4. The detector operated at 214 nm. The elution rate was 0.25 mL/min. PAA standards (from Polymer Laboratories) or PSS standards (from Wheaton) were used for calibration. The significance of the calibration based on PSS standards for determining the PLCA and PLCAI molecular weight was controlled using three PLCA whose molecular weights were respectively 56 000, 34 000, and 26 000 g/mol according to static light scattering. The correction factor $\log[\overline{M_w}\text{ (PLCA)}]/\log[\overline{M_w}\text{ (PSS)}]$ was 1.030. The molecular weight of the PLL polycation engaged in IPECs was determined by SEC using the same system but with a column packed with a cationic DEAE Sepharose gel. The mobile phase was a solution of 1 M NH_4Cl and 1 M NaCl at pH = 4.0. The detector operated at 214 nm. The elution rate was 0.25 mL/min. P2VP standards (from Fluka) were used for calibration. The significance of the calibration based on P2VP standards for determining the PLL molecular weight was controlled using four PLL whose molecular weights were respectively 73 500, 51 000, 26300, and 12 700 g/mol according to static light scattering. The correction factor $\log[\overline{M_w}\text{ (PLL)}]/\log[\overline{M_w}\text{ (P2VP)}]$ was 1.065.

The values of the hydrodynamic radius R_H of IPEC particles were determined in NaCl solutions by dynamic light scattering (DLS). Vertically polarized DLS measurements were performed at 25 °C on a commercial BI-200SM instrument (from Brookhaven Instrument Corporation) equipped with a 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 from the average diffusion coefficient D using the Stokes−Einstein equation:

$$R_H = \frac{kT}{6\pi\eta D} \tag{1}$$

where k is the Boltzmann constant, T is the absolute temperature and η is the viscosity of the measured liquid at 25 °C. The viscosity of the polymer solutions used for DLS measurements was determined using a rheometer Rheostress RS 100 (from Haake) at 25 °C. The refractive index increments were determined for the various polymers using a Brice-Phoenix refractometer at 633 nm at 25 °C.

IPECs were first dissolved in 2.5 M NaCl at a concentration of 0.4 mg/mL. 65 μL of the resulting solution were then added in a Pyrex tube to a strongly stirred NaCl solution whose concentration was calculated to allow setting at the appropriate final NaCl concentration. The final amounts of precipitates and the total volume were 13 μg/mL and 2 mL, respectively. DLS measurements were performed 24 h after IPEC preparation.

3. Experimental Results

Characteristics of the Complexed Polyelectrolytes. The protocol consisting in pouring the polycation PLL into the polyanion, all the polycation molecules were engaged in the complex and thus the PLL molecular weight was unchanged

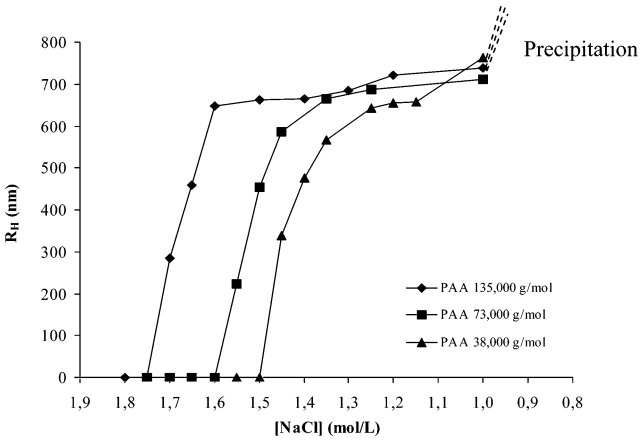


Figure 1. R_H dependence of different PLL−PAA complexes on NaCl concentration. [IPEC] = 13 μg/mL and M_w (PLL) = 30 000 g/mol.

Table 1. Chemical Data of the Polyanions PLCA, PLCAI, and PAA Complexed with the Polycation PLL

polymer	M_w	M_n	DP_n	I_p
PLCA	55 000	32 000	107	1.7
	27 000	15 000	50	1.8
	18 000	10 000	33	1.8
PLCAI	69 000	34 000	120	2.0
	38 000	24 000	85	1.6
	16 000	9000	32	1.8
PAA	135 000	40 000	563	3.4
	73 000	21 000	296	3.5
	38 000	8000	113	4.8

(26 000 g/mol), as expected and already observed.[15] In contrast, selectivity was observed in the case of the polyanion molecules. The average molecular weight (M_w) and weight-average degree of polymerization (DP_w) of the polyanions issued from the different IPECs prepared are presented in Table 1.

Influence of Salt. The influence of NaCl on the interactions between the various polyanions and PLL was investigated by dilution starting from a NaCl concentration high enough to decomplex IPECs, namely 2.5 M. DLS was used to monitor the presence of complex in the media at various salt concentration and with a constant 13 μg/mL polymer concentration. In the case of 2.5 M NaCl, the sizes of the scattering species were around 10 nm in agreement with dimensions of separated polyions. Under these conditions, decreasing the salt concentration led sooner or later to the appearance of much greater scattering species with sizes larger than 100 nm. The salt concentration at which the formation of these rather large scattering species became detectable was named C_{recomp}, with "recomp" standing for recomplexation. Because of the fact that antagonist polyelectrolytes were initially in 2.5 M NaCl, it was not possible to decrease the salt concentration below 0.1 M NaCl by dilution.

In the case of the PLL−PAA complexes (Figure 1), recomplexation was detected for C_{recomp} values decreasing with decreasing the PAA molecular weight. When the salt concentration was decreased, a sharp size increase was observed, followed by a zone where the size variations leveled off. Below 1 M in NaCl, macroscopic flocculation was observed.

The PLL−PLCA and PLL−PLCAI complexes led to profiles composed of onsets and plateaux as shown in Figure 2 and Figure 3, respectively. There was no flocculation in contrast to what was observed for PLL−PAA complexes. The plateau zones were extended up to the lower 0.1 M limit of the salt concentration. For a given PLL/polyanion couple, the curve leveled off to almost the same R_H value which was found to be

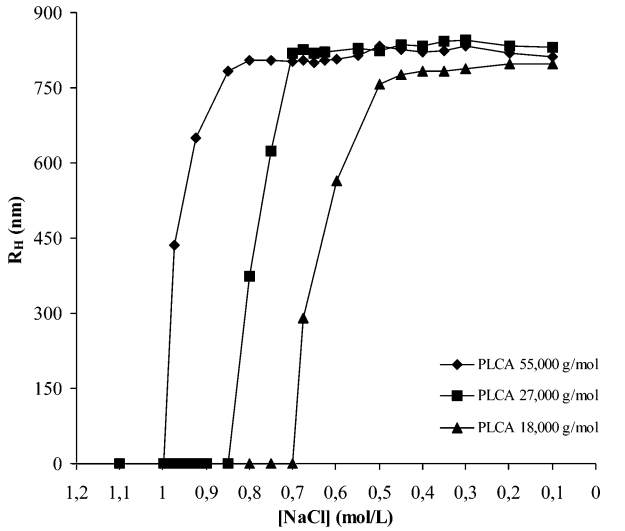


Figure 2. R_H dependence of different PLL−PLCA complexes on NaCl concentration. [IPEC] = 13 μg/mL and M_w (PLL) = 30 000 g/mol.

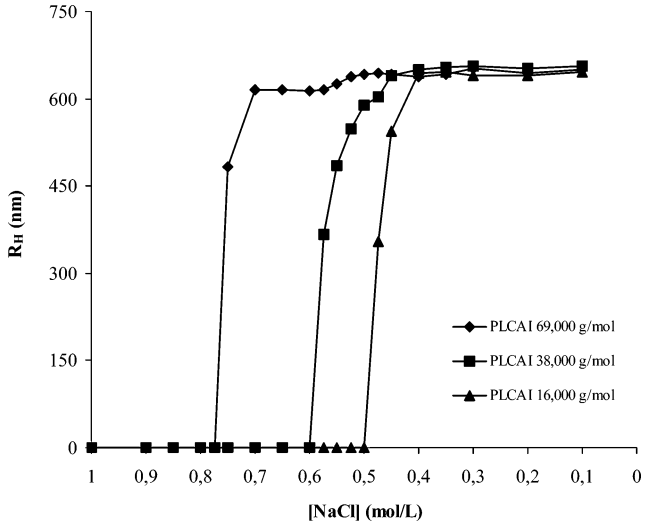


Figure 3. R_H dependence of different PLL−PLCAI complexes on NaCl concentration. [IPEC] = 13 μg/mL and M_w (PLL) = 30 000 g/mol.

relatively independent of the polyanion molecular weight. This R_H value was lower for PLCAI than for PLCA. The C_{recomp} values decreased with decreasing the polyanion molecular weight, as in the case of PLL−PAA complexes.

It was tentatively concluded that nanoparticles of similar size were obtained from macromolecules of different molecular weights. However, if similar sizes were obtained for complexes composed of macromolecules of different molecular weights, the NaCl concentration required to destabilize such IPECs increased when the molecular weight increased. As previously suggested, the difference of behavior between the PLCA or PLCAI and PAA systems can be assigned both to the presence of hydrophilic OH groups and the amide dipoles present in PLCA and PLCAI polyanions which stabilized the IPEC particles against dramatic increase of hydrophobicity and to the higher charge density of PAA molecules (as compared with PLCA and PLCAI ones) which increased the attractive electrostatic interactions with the polycation.[13] This assignment well agrees with the higher C_{recomp} observed for the PLL−PAA complexes as compared with the PLL−PLCA and PLL−PLCAI complexes. In order to verify this hypothesis, theoretical calculations were carried out by considering the macromolecules of PLL and PAA as hydro-

phobic and the macromolecules of both PLCA and PLCAI as hydrophilic.

4. Theoretical Part

The interpolymer complex described in above section were formed by oppositely charged macromolecules which differ significantly in terms of molecular weights, affinity to solvent, density of ionic groups along the chain, and molecular weight distribution.

Describing such mixture in detail is rather complicated. Let us consider a simplified model that takes into account only the main features of systems under consideration, namely, the different affinity of macromolecules to solvent and the different distance between ionic groups along the macromolecule backbone.

First, let us consider the case of diluted solution of oppositely charged macromolecules **A** and macromolecules **C** (i) that have hydrophilic and hydrophobic backbone, respectively; (ii) that bear equal numbers of ionic groups; (iii) that have different degree of polymerization (hydrophilic **A** macromolecules being longer). Furthermore, mixture composition is stoichiometric, i.e., the number of positive charges in the solution is assumed equal to the number of negative charges.

Unimer Complex. To start, let us consider the construction of a 1−1 complex of macromolecules **A** and **C** with different degree of polymerization. **A** and **C** polyions carry charges of the opposite sign. The charged groups are distributed randomly along the chain and the total numbers of charged groups per each macromolecule are equal and amounts to Q regardless of charge signs. To be definite, let us propose that macromolecules **A** are negatively charged and twice as long as positively charged macromolecules **C**. Furthermore, let the long backbone of **A** macromolecules have affinity with solvent, i.e., water, while shorter cationic macromolecules **C** do not have it.

Let us denote as N the degree of polymerization of cations **C**, and as f its degree of ionization: $f = Q/N$. Then the degree of polymerization N_A of anion **A** is equal to $2N$, and its degree of ionization is $f/2$. We assume that both macroions are flexible and have monomer unit of equal typical size a.

As stated in the introduction, we assume that the oppositely charged macroions **A** and **C** form IPECs having core−shell structure. The inner part of the complex with radius r contains monomer units of both macromolecules while exterior part (having radius R) consists exclusively of monomer units of longer hydrophilic macromolecule **A**.

It was previously shown that the stoichiometric polyelectrolyte complex can be considered as a globule of a polyampholyte macromolecule at isoelectric point and described within the framework of volume approximation.[16] We assume that the volume approximation is valid for the case under consideration as well.

Following this approach, the total free energy F of core−shell IPEC can be presented as sum of three contributions—the free energy F_{int} of non-Coulomb van der Waals type forces, the free energy F_{DH} of Debye−Hukkel electrostatic interactions due to the fluctuation of charges, and the free energy F_{cond} of electrostatic interactions between excess of charges of internal and external parts of complex:

$$F = F_{int} + F_{DH} + F_{cond} \tag{2}$$

The internal part of the complex contains all N monomer units of cation **C**, and $N_A^{in} \leq 2N$ monomer units of anion **A**. Thus, the total number of charged monomer units of both signs in internal part of complex is $Nf + N_A^{in} f/2 = f(N + N_A^{in}/2)$, and free energy F_{HD} can be written as

$$\frac{F_{DH}}{kT} = -\frac{2}{3}\sqrt{\pi}u^{3/2}f^{3/2}\frac{\left(N + \frac{N_A^{in}}{2}\right)^{3/2}}{V_r^{1/2}} \tag{3}$$

where V_r is the volume of the internal part of complex ($V_r = (4\pi/3)r^3$); $u = e^2/\epsilon aT$ is a characteristic dimensionless parameter (T, temperature; ϵ, dielectric constant of medium; e, elementary charge). In the case of aqueous solution ($\epsilon \sim 80$) at room temperature ($T \sim 300$ K) and for size of monomer units $a \sim 1$ nm, the parameter u is approximately equal to unity: $u \sim 1$.

The contribution F_{cond} of interaction of noncompensated charges of internal and external parts of complex can be written within the model of spherical capacitor having charge dQ. The charge dQ of such capacitor is equal to difference between the total numbers Nf of positively charged monomer units and the total number $N_A^{in} f/2$ of negatively charged monomer units in the internal part of the complex:

$$\frac{F_{cond}}{kT} = (dQ)^2 u\left(\frac{a}{r} - \frac{a}{R}\right) = \left(\frac{4\pi}{3}\right)^{1/3} f^2 u\left(N - \frac{N_A^{in}}{2}\right)\left(\frac{a}{V_r^{1/3}} - \frac{a}{V_R^{1/3}}\right) \tag{4}$$

where V_R is the total volume of the complex ($V_R = 4\pi/3\ R^3$).

Finally, the contribution F_{int} can be written as a sum of free energy of interactions in internal F_{int}^{in} and external F_{int}^{out} parts of the complex:

$$F_{int} = F_{int}^{in} + F_{int}^{out}$$

In Flory−Huggins approximation the term F_{int}^{in} is

$$\frac{F_{int}^{in}}{kTV_{in}} = \chi_{AC}\varphi_C\varphi_A^{in} + \chi_{CS}\varphi_C(1 - \varphi_C - \varphi_A^{in}) + \chi_{AS}\varphi_A^{in}(1 - \varphi_C - \varphi_A^{in}) + (1 - \varphi_C - \varphi_A^{in})\ln(1 - \varphi_C - \varphi_A^{in}) \tag{5}$$

where $\varphi_C = Na^3/V_r$, $\varphi_A^{in} = N_A^{in}a^3/V_r$ are volume fraction of monomer units **A** and monomer units **B** in the inner part of complex, correspondingly; χ_{AC}, χ_{AS}, and χ_{CS} are Flory−Huggins parameters of interaction of monomer units (**A**, **C**) between each other and with molecules of solvent (**S**).

Using the same approximation, we should write free energy F_{out}^{in} of external part of complex in the following form:

$$\frac{F_{int}^{out}}{kTV_{out}} = \chi_{AS}\varphi_A^{out}(1 - \varphi_A^{out}) + (1 - \varphi_A^{out})\ln(1 - \varphi_A^{out}) \tag{6}$$

where $\varphi_A^{out} = (N_A - N_A^{in})/(V_R - V_r)a^3 = (2N - N_A^{in})/(V_R - V_r)a^3$ is the volume fraction of polymer **A** in shell of IPEC.

The equilibrium values of volume V_r and V_R, as well as the concentration of monomer units in both parts of IPEC, are determined by conditions of equality of osmotic pressures and chemical potentials of the coexisting parts of the complex. In the case under consideration, this condition is equivalent to the following condition for the derivatives of total free energy F with respect to the variables V_R, V_r and N_A^{in}:

$$\frac{\partial F}{\partial V_R} = 0; \frac{\partial F}{\partial V_r} = 0; \frac{\partial F}{\partial N_A^{in}} = 0 \quad (7)$$

The system of equations written above was solved numerically. For simplicity in our calculations the value of χ_{AC} was taken equal to zero, and we set $\chi_{AS} = -\chi_{CS} = \chi \leq 0$.

Figure 4A shows the dependence of radii of complex R and of internal part r on the degree of ionization f for different values of χ. One can see that for macroions with equal affinity to solvent ($\chi = 0$), radii R and r are equal to each other: $R = r$. This indicates that the protecting layer is absent and IPEC contains the only phase with homogeneous distribution of monomer units of both macroions. With increase of degree of ionization f the electrostatic interaction becomes stronger and complex diminishes its size.

In the case of negative values of parameter χ ($\chi \leq 0$), when complex composed of macroions with different affinity to solvent, IPEC indeed has a core−shell structure with hydrophobic core and hydrophilic shell. The calculations show that the protective layer of IPEC is rather narrow, its width corresponds to the size of few monomer units and its total charge is about few elementary charges e (Figure 4B). The width of layer Δ, $\Delta = R - r$, increases with increase of hydrophilicity of anion and with decrease of degree of ionization f of macroions. Thus, due to the strong electrostatic interaction anionic monomer units are mainly concentrated within internal part of IPEC. The number of hydrophilic anionic groups is twice larger than the number of hydrophobic groups of polycations, as a result in spite of strong hydrophobicity of polycations (the low negative values of interaction parameter χ_{AS}) the size of polymer complex R varies approximately inversely proportionally to degree of ionization f: $R \sim f^{-1}$. It should be reminded that such dependence is characteristic for amphiphilic macroions immersed in good solvent; in ideal solvent $R \sim f^{-1/3}$ and radius of macroion R shows no dependence on i in poor solvent: $R \sim f^{0.16-17}$ The dependence of the total concentration of monomer units within inner part of IPEC $c_{in} = \varphi_A^{in} + \varphi_C$ on degree of ionization f is shown in Figure 4C.

Because of the same reasons, the size of IPEC, i.e., radius R, is larger than the size of complex with $\chi = 0$, and it increases with the increase of $|\chi|$.

The core−shell structure of IPEC is also observed for macromolecules with high degree of polymerization N (Figure 5A). The width of protective layer decreases with N, however, its charge δQ increases (Figure 5B). The scaling dependence of radius R of a complex on its degree of polymerization giving $R \sim N^{1/3}$ is typical for a polyampholyte globule stabilized by attractive electrostatic interactions.[17]

Figure 6 shows the dependence of the free energy $f_s = F/N$ per monomer unit on the degree of polymerization N. As it can be seen, the free energy f_s per monomer unit increases with increasing of N. This fact lets us to conclude that the formation of complex containing few macromolecules of cations and anions is energetically unfavorable.

Complex with *m* Macromolecules of Cations and Anions. Indeed, when writing the free energy of complex of few cationic and few anionic macromolecules in the case of long macromolecules, we can neglect the entropy contribution of polymer chains. Then the free energy of complex containing m macromolecules of cations and m macromolecules of anions is equal to the free energy of unimer complex described above ($m = 1$) between single cation and single anion given by eqs 2−6 with the degrees of macroion polymerization N_C and N_A replaced by total numbers of monomer units in

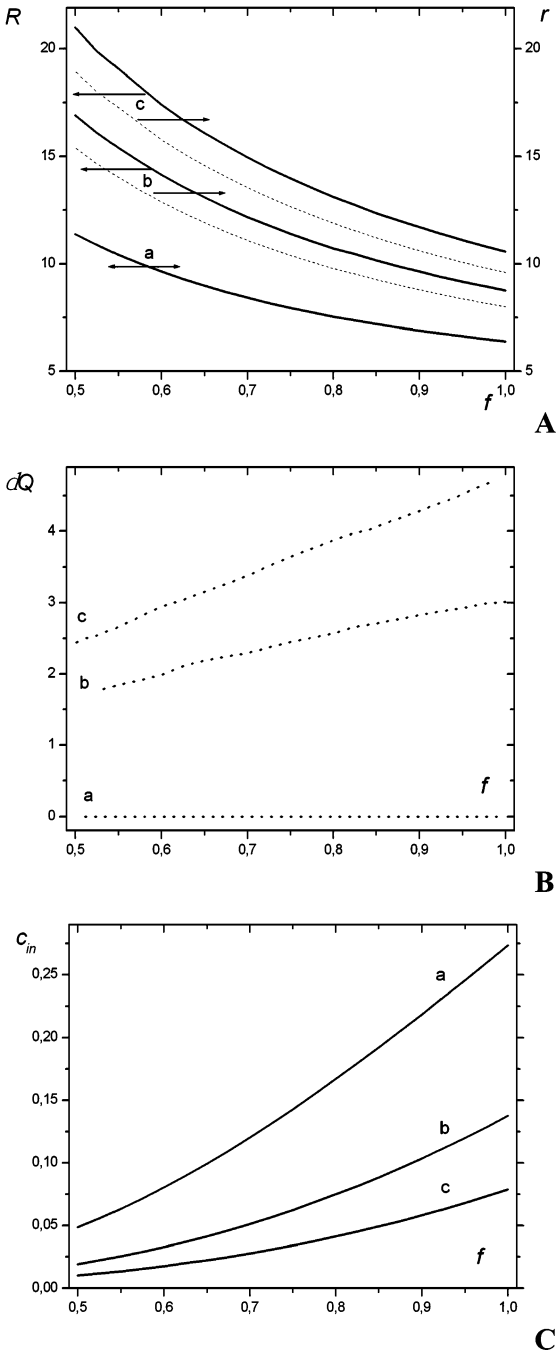


Figure 4. Dependences of the radii r (dashed line) and R (solid line) (A), charge dQ (B), and concentration c_{in} (C) on degree of ionization f for different affinity of macromolecules to solvent $\chi = 0$ (a), −1 (b), and −2 (c). $P = 100$.

cationic and anionic macromolecules: $N_C = N \rightarrow mN$; $N_A = 2N \rightarrow 2mN$. All other calculations remain the same. It is clear that in this context the formation of complex having m macromolecules leads always to the growth of free energy per macromolecule since the free energy f_S per monomer unit increases monotonously with the increase of number of monomeric units forming the complex. Thus, the free energy of IPEC of m cationic and m anionic macromolecules is always higher than free energy of 1−1 complex and therefore the formation of complex with few macromolecules is energetically unfavorable.

Macrophase Separation. Another possibility for phase behavior of the diluted solution of considered above oppositely charged macromolecules is the precipitation instead of the

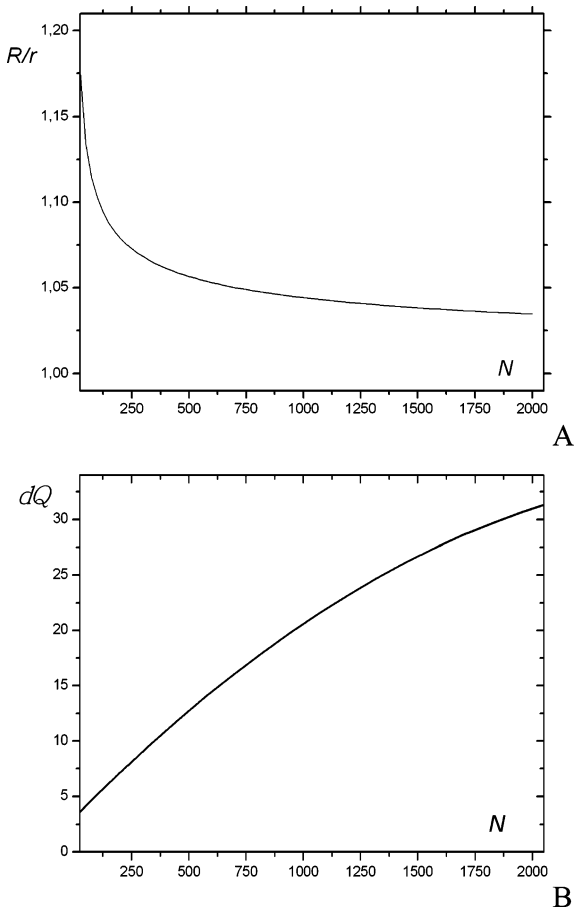


Figure 5. Dependences of ratio R/r (A) and of charge dQ (B) on degree of polymerization N. $f = 1$, $\chi = -2$.

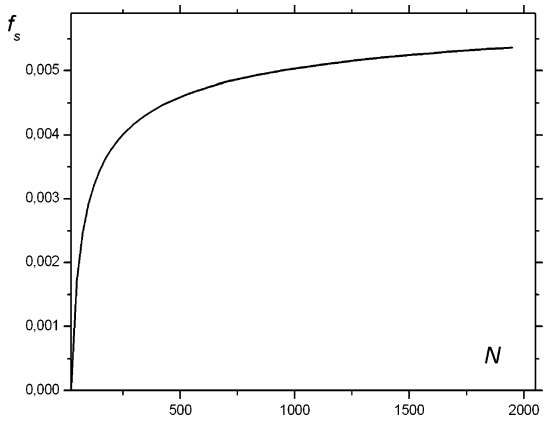


Figure 6. Free energy f_s per monomer unit as a function of degree of polymerization N. $f = 1$, $\chi = -2$.

formation of unimer ($m = 1$) **A**−**C** complex. However, it is easy to show that the precipitation is also unfavorable as soon as two-phase complexes are formed.

Indeed, the free energy f_P per one monomer unit in precipitant is $f_P = 2\varphi_P/3N \ln 2\varphi_P/3N + f_V$, where φ_P is volume fraction of polymer in precipitant and f_V is the energetic contribution to free energy f_P. The contribution f_V coincides with written-above free energy per one monomer unit in the complex f_s (eq 1) if both radius are assumed to be equal to each other: $R = r$. It is clear that the following inequality should be fulfilled $f_V > f_S$, as soon as the formation of two-phase complex is favorable. This allows to conclude that in dilute solution the 1−1 complexes are stable upon precipitation.

5. Discussion

Thus, theoretical calculation shows that in solution of oppositely charged hydrophobic macromolecules **C** and hydrophilic macromolecules **A** carrying equal numbers of ionic groups and having different degree of polymerization, the core−shell 1−1 complexes are formed. These complexes are stable upon aggregation, formation of larger complexes and precipitation.

It is clear that the stoichiometric complex of oppositely charged macromolecules having different affinity to solvent and carrying out *not* equal number of charged groups per chain should *not* include equal number of macromolecules. In this case the ratio, between positively and negatively charged macromolecules within the complex differs from unity and fulfils the conditions of electroneutrality. However, all other conclusions of proposed theoretical model for the structure of complexes should remain unchanged. Namely, the complexes with minimum number of oppositely charged macromolecules required to preserve the electroneutrality condition should be formed in such solutions. These complexes have a core−shell structure with hydrophilic shell, and due to such structure they are stable with respect to macrophase separation and fusion with other complexes.

Actually, this is the case in our experimental study. We found that the stoichiometric complexes of oppositely charged macromolecules with different affinity to the solvent are stable upon aggregation and precipitation. On the other hand, the complexes of PLL−PAA macromolecules, both having hydrophobic backbone, macroscopically precipitate.

The complexes under investigation are formed by oppositely charged macromolecules having different lengths, different charge densities and different molecular weight distributions. Interesting feature is that the size of complexes formed by polycation PLL of almost the same molecular weight and polyanions (PAA, PLCA, PLCAI) with broad molecular weight distribution and different average molecular weights have almost the same average size. Apparently the very broad distribution of polyanions over molecular weights allows fit the numbers of oppositely charged macromolecules in interpolymer complex, so that the total charge of the complex is approximately equal to zero and this complex contains the minimum possible amount of macromolecules. The detailed study of the mechanism promoting the formation of aggregates with optimum size in mixtures of oppositely charged macromolecules with broad distribution of macromolecules over molecular weight, as well as of the dependence of such IPEC on the salt concentration, will be the subject of a forthcoming publication.

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