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Interaction of Astramol Poly(propyleneimine) Dendrimers with Linear Polyanions

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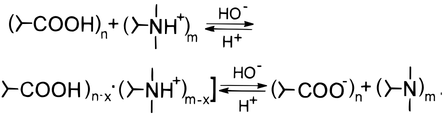
ABSTRACT: Interaction between Astramol poly(propylene imine) dendrimers of five generations, DAB-*dendr*-$(NH_2)_x$ (where x is equal to 4, 8, 16, 32, or 64) and linear poly(sodium acrylate), poly(acrylic) acid, poly(sodium styrenesulfonate), or native DNA was studied in salt-free solutions by means of potentiometric, argentometric, and turbidimetric titrations. In addition to x outer primary amine groups the dendrimer molecule contains $x - 2$ inner tertiary amine groups. It is found that the pH-controlled interpolyelectrolyte coupling reaction resulting in formation of the corresponding interpolyelectrolyte complexes occurs on mixing of the dendrimer and the polyanion solutions. A peculiar finding was that all dendrimer amine groups being protonated can form ion pairs with carboxylate or sulfonate groups of the polyanions. In other words DAB-*dendr*-$(NH_2)_x$ molecules are fully penetrable for rather flexible oppositely charged polyelectrolyte chains. However, this is not the case for rigid negatively charged DNA double helixes which apparently bind only to the dendrimer shell. The complexes of dendrimers, with polyanions containing equal amounts of cationic and anionic groups mostly ion-paired (stoichiometric complexes), are insoluble in water. At the same time water soluble nonstoichiometric interpolyelectrolyte complexes can be obtained if the dendrimer is a deficient component of the complex species. This behavior is similar to that of complexes formed by oppositely charged linear polyions.

Introduction

It is well-known that two oppositely charged polyelectrolytes undergo a cooperative interpolyelectrolyte coupling reaction in aqueous solution to form an interpolyelectrolyte complex (IPEC). Such complexes formed by linear polyelectrolyte pairs have been the subject of extensive studies for years.[1−6] IPEC can be obtained by direct mixing of solutions of polycationic and polyanionic components:



$$(\succ\!-COOH)_n + (\succ\!-\overset{|}{\underset{|}{N}}H^+)_m \underset{H^+}{\overset{HO^-}{\rightleftarrows}}$$

$$[(\succ\!-COO^-\ {}^+H\overset{|}{\underset{|}{N}}\!-\!\prec)_x \cdot (\succ\!-COOH)_{n-x} \cdot (\succ\!-\overset{|}{\underset{|}{N}}H^+)_{m-x}] \underset{H^+}{\overset{HO^-}{\rightleftarrows}} (\succ\!-COO^-)_n + (\succ\!-\overset{|}{\underset{|}{N}})_m$$

(low molecular counterions are omitted).

The degree of conversion, Θ, in the above reaction is determined as the ratio of a current or equilibrium number of interpolyion salt bonds to the ultimate one ($\Theta = x/m$ when $n \geq m$, and $\Theta = x/n$ when $n < m$). If the interacting polyelectrolytes represent a weak polyacid and (or) a weak polybase, Θ can be easily controlled by variation of the pH as it is shown in the above scheme. IPECs formed by mixing of linear polyelectrolytes in the ratio corresponding to equality in mol amount of cationic (N_+) and anionic (N_-) groups ($z = N_+/N_- = 1$) are water insoluble at Θ close to 1 (stoichiometric IPECs), but undergo limited swelling. However, partly complexed components can be retained in basic solution at $\Theta < 0.5$ depending on the nature of the interacting polyions and reaction conditions.

Recently we have shown that Astramol poly(propylene imine) dendrimers, being symmetrical treelike branched polyamines, reveal some typical polyelectrolyte behavior when protonated.[7,8] In particular the apparent ionization constant strongly depends on the degree of ionization in salt-free solution, so that the pH intervals of protonation of the primary amine groups (D_x-NH_2) on the outer shell and the tertiary amine groups ($D_x \rightarrow N$) in the core are considerably overlapped for the higher dendrimer generations. Both these effects are markedly suppressed in the presence of a shielding simple salt. The fact that the shell and the core amine groups can be fully protonated, of course, means that the dendrimer structure is penetrable for the low molecular counterions. The question is whether and to what extent it is penetrable for linear polyanions to form IPECs. This is the subject of the present study.

Experimental Section

Materials. Astramol poly(propylene imine) dendrimers were produced at DSM. They are commercially available from DSM or Aldrich. The synthesis involves a repetitive reaction sequence of Michael additions of acrylonitrile to primary amine endgroups followed by catalytic hydrogenation of the nitrile groups. Diaminobutane was used as the core molecule. Detailed information on the synthesis was published elsewhere.[9,10] The Astramol poly(propylene imine) dendrimers of five generations from DAB-*dendr*-$(NH_2)_4$ to DAB-*dendr*-$(NH_2)_{64}$ were used.

Poly(acrylic acid) (PAA) was prepared by radical polymerization of the monomer. Acrylic acid, doubly distilled in a vacuum, was polymerized in the form of a 30 wt % solution in dioxane with 0.5 wt % of AIBN at 65 °C during 18 h in argon atmosphere. The polymer obtained was precipitated twice from the solution in dioxane by toluene, then redissolved in dioxane and lyophilized. The PAA sample was fractionated by fractional precipitation from methanol solution by ethyl acetate. The fraction with M_w ca. 10^5 was used (measured by light scattering using a small-angle light-scattering photometer KMX-6, Milton Roy). Poly(sodium acrylate) (NaPA) aqueous

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solutions were prepared by neutralization of PAA solutions in distilled water with the equivalent amount of NaOH. The pH of 0.005 mol/L NaPA salt-free solution was 8.7.

Poly(sodium styrenesulfonate) (NaPSS) with *M* ca. 10^5 purchased from Serva and native DNA from salmon sperm, 300−500 b.p., purchased from GosNIIOHT (Moscow) were used as received.

Measurements. The potentiometric titration of aqueous solutions of the dendrimers, PAA, and mixtures of the dendrimers with PAA, NaPA, and NaPSS was performed using a RTS 822 Radiometer titrator with a Sigma glass calomel combination electrode at 20 °C. The 5 mL solutions containing 0.005 mol/L of the dendrimer in free base form or an equimolar mixture of the dendrimer with NaPA or NaPSS in distilled water was titrated with 0.1 N HCl solution. In reverse experiments 5 mL of solution containing 0.005 mol/L PAA or an equimolar mixture of PAA with the DAB-*dendr*-$(NH_2)_x$·HCl, i.e., the fully protonated dendrimer in distilled water, was titrated with 0.1 N NaOH solution. Using a micropipet, the 25 μL portions of the titrants were added dropwise with continuous stirring at 5 min time intervals to achieve the constancy in measured pH values.

Turbidimetric titration of NaPA in salt-free aqueous solutions with aqueous solutions of the DAB-*dendr*-$(NH_2)_x$·HCl was performed using a UV Hitachi 150−20 spectrophotometer. The 2.5 mL solutions of NaPA with concentrations of 0.0015−0.0020 mol/L were titrated by adding dropwise 20 μL portions of 0.020−0.025 mol/L salt-free solutions of DAB-*dendr*-$(NH_2)_x$·HCl. A time interval of 1 min was kept between successive additions. The titration was carried out at 20 °C in a 1 cm rectangular quartz cell at continuous stirring. The optical density of the solutions was measured at the wavelength λ = 500 nm.

The same spectrophotometer was used to measure the NaPSS concentration in aqueous solutions at the wavelength λ = 262 nm, using an extinction coefficient ϵ = 450 $L{\cdot}mol^{-1}{\cdot}cm^{-1}$.

The concentration of Cl^- ions in aqueous solutions was measured by argentometric titration using a pH-673 pH-meter with glass pAg electrode ASL-51-07 and Ag/AgCl reference AVL-1M3 electrode at 20 °C.

The precipitates were separated from the supernatants using a preparative Beckman J2 ultracentrifuge with rotor type JA-21 at 18 000 rpm during 40 min.

Results and Discussion

Interaction of DAB-*dendr*-$(NH_2)_x$ with NaPA, PAA, and NaPSS was investigated using potentiometric titration. The diluted salt-free 0.005 mol/L aqueous solutions of DAB-*dendr*-$(NH_2)_x$ in the form of free base or fully protonated were mixed with solutions of the equivalent amount of NaPA or PAA correspondingly and then titrated with HCl or NaOH. The potentiometric titration curves of equimolar mixtures of DAB-*dendr*-$(NH_2)_x$ with NaPA are shown in Figure 1 as the dependence of pH on the volume of added 0.1 N HCl (curves 1−5). The potentiometric titration curves (6−10) of DAB-*dendr*-$(NH_2)_x$ solutions in the absence of the NaPA are also shown in Figure 1. The comparison of the corresponding experimental points for DAB-*dendr*-$(NH_2)_x$ and DAB-*dendr*-$(NH_2)_x$−NaPA mixture solutions located on the ordinate reveals a noticeable pH jump when the original DAB-*dendr*-$(NH_2)_x$ and NaPA solutions are mixed. This jump evidences that the interpolyelectrolyte reaction apparently including the following steps

$$D_x\text{-}NH_2 + \overset{\ominus}{O}OC\text{-}PA \underset{}{\overset{H_2O}{\rightleftarrows}} D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{O}OC\text{-}PA + OH^{\ominus} \qquad (1^*)$$

$$D_x{>}N + \overset{\ominus}{O}OC\text{-}PA \overset{H_2O}{\rightleftarrows} D_x{>}\overset{\oplus}{N}H\overset{\ominus}{O}OC\text{-}PA + OH^{\ominus} \qquad (1^*a)$$

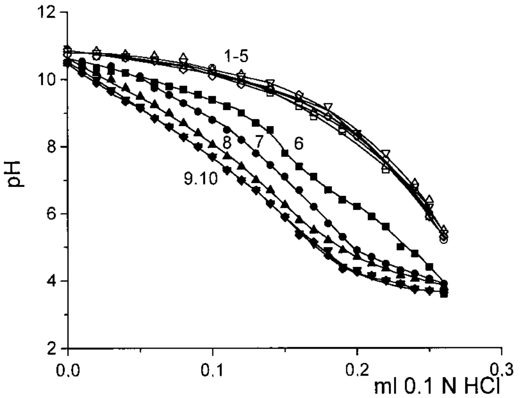


Figure 1. Potentiometric titration curves for equimolar mixtures DAB-*dendr*-$(NH_2)_4$−NaPA (1), DAB-*dendr*-$(NH_2)_8$−NaPA (2), DAB-*dendr*-$(NH_2)_{16}$−NaPA (3), DAB-*dendr*-$(NH_2)_{32}$−NaPA (4), and DAB-*dendr*-$(NH_2)_{64}$−NaPA (5) and for DAB-*dendr*-$(NH_2)_4$ (6), DAB-*dendr*-$(NH_2)_8$ (7), DAB-*dendr*-$(NH_2)_{16}$ (8), DAB-*dendr*-$(NH_2)_{32}$ (9), DAB-*dendr*-$(NH_2)_{64}$ (10) in salt-free aqueous solutions. [DAB-*dendr*-$(NH_2)_x$] = 0.005 mol/L, *T* = 20 °C.

proceeds to some extent even when HCl is still not added. Addition of HCl shifts the equilibria 1* and 1*a further to the right, i.e., to progressive formation of the IPEC. The reaction mixtures remain homogeneous in the range of 0−0.075 mL 0.1 N HCl added but progressively turn hazy at higher amounts of the titrant because of microphase separation of the formed IPEC. Here as well as in the further experiments titration is followed by an increase of ionic strength due to release of NaCl. However, such an increase not exceeding 5 × 10^{-3} M is 1.5−2 orders of magnitude lower than the salt concentration affecting stability of formed IPECs.

The potentiometric titration curves of the solutions obtained by mixing PAA with equal molar amounts of fully protonated dendrimers (curves 1−5) and that of free PAA (curve 6) are represented in Figure 2 as the dependence of pH on the volume of added 0.1 N NaOH. The pH drop observed when the original PAA and DAB-*dendr*-$(NH_2)_x$·HCl solutions are mixed is also due to formation of interpolyelectrolyte salt bonds:

$$D_x\text{-}NH_3^{\oplus} + HOOC\text{-}PA \rightleftarrows D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{O}OC\text{-}PA + H^{\oplus} \qquad (2^*)$$

$$D_x{>}NH^{\oplus} + HOOC\text{-}PA \rightleftarrows D_x{>}\overset{\oplus}{N}H\overset{\ominus}{O}OC\text{-}PA + H^{\oplus} \qquad (2^*a)$$

Such an IPEC formed in acidic conditions immediately microphase separates so that the reaction mixtures are hazy in the whole range of titration. The further addition of NaOH also shifts these equilibria to the right.

As is seen in Figure 1, the potentiometric titration curves of all generation dendrimers interacting with NaPA are superimposed and located at higher pH values than those of free dendrimers. At the same time the potentiometric titration curves of PAA in the presence of protonated dendrimers are located at lower pH than that of free PAA (Figure 2). In other words the dendrimers in the base form behave like a stronger base in the presence of NaPA, while PAA behaves like a stronger acid in the presence of protonated dendrimers. Such a behavior is caused by the cooperative character of multisite bonding of the oppositely charged units within the IPEC formed.

Each step of 1* and 1*a or 2* and 2*a results in formation of one interpolyelectrolyte salt bond (ion pair)

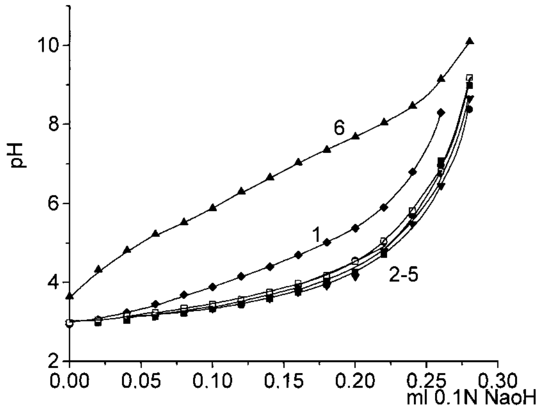


Figure 2. Potentiometric titration curves for equimolar mixtures of DAB-*dendr*-$(NH_2)_4$·HCl−PAA (curve 1), DAB-*dendr*-$(NH_2)_8$·HCl−PAA (curve 2), DAB-*dendr*-$(NH_2)_{16}$·HCl−PAA (curve 3), DAB-*dendr*-$(NH_2)_{32}$·HCl−PAA (curve 4), and DAB-*dendr*-$(NH_2)_{64}$·HCl−PAA (curve 5) and for PAA (curve 6) in salt-free aqueous solutions. [DAB-*dendr*-$(NH_2)_x$·HCl] = 0.005 base-mol/L, T = 20 °C.

and release of one OH^- or one H^+ ion into the surroundings. Therefore the degree of conversion, Θ, in the reactions can be expressed as

$$\Theta = C_c/C_o = (q/v + 10^{-14}/[H^+] - 10^{-14}/[H^+]_{D_x})/C_o \quad (1)$$

$$\Theta = C_c/C_o = (q/v + [H^+] - [H^+]_{PAA})/C_o \quad (1')$$

for alkaline or acidic pH regions, respectively.[6,11] Here C_c is the current concentration of interpolyelectrolyte salt bonds, C_o is the initial concentration of ionizing polymer component: (DAB-*dendr*-$(NH_2)_x$ or PAA, q is the number of equivalents of the titrant (HCl or NaOH) added, v is the volume of the solution, $[H^+]$ is the measured total concentration of hydrogen ions, and $[H^+]_{D_x}$ or $[H^+]_{PAA}$ is the concentration of hydrogen ions in the solution of free (DAB-*dendr*-$(NH_2)_x$ or PAA. In the range pH > pH_{D_x}, $[H^+]_{D_x}$ can be estimated using the following equation.[6,11]

$$[H^+]_{D_x} = 10^{-14}/\sqrt{K_b(1 - \Theta)C_o} \quad (2)$$

Correspondingly at pH < pH_{PAA}

$$[H^+]_{PAA} = \sqrt{K_a(1 - \Theta)C_o} \quad (2')$$

where K_b is the intrinsic basicity constant of the dendrimer amine group and K_a is the intrinsic acidity constant of PAA.[11]

The Θ values were calculated from the titration curves of Figures 1 and 2 using eqs 1 and 2 or eqs 1′ and 2′ by iteration in the pH ranges, where such a calculation is of a sufficient accuracy. The Θ−pH dependencies represented in Figure 3 show the above-mentioned cooperative character of the interpolyelectrolyte reactions following from the higher steepness of the reaction profiles and the significant pH shifts in comparison with neutralization curves (α−pH) for free dendrimers (curves 7−9) and free PAA (curve 6).[12] The IPECs formed exist in the pH region enclosed by curves 1 and 2. They dissociate to the original polyelectrolyte components at pH < 3 and at pH > 11.

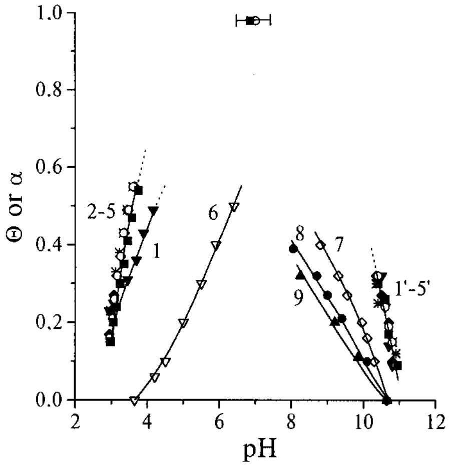


Figure 3. Θ−pH dependencies for equimolar mixtures DAB-*dendr*-$(NH_2)_4$-PAA (1, 1′), DAB-*dendr*-$(NH_2)_8$−PAA (2, 2′), DAB-*dendr*-$(NH_2)_{16}$−PAA (3, 3′), DAB-*dendr*-$(NH_2)_{32}$−PAA (4, 4′), and DAB-*dendr*-$(NH_2)_{64}$−PAA (5, 5′) and α−pH dependencies for PAA (6), DAB-*dendr*-$(NH_2)_8$ (7), DAB-*dendr*-$(NH_2)_{16}$ (8), and DAB-*dendr*-$(NH_2)_{32}$ (9) in salt-free aqueous solutions. [DAB-*dendr*-$(NH_2)_x$] = [PAA] = 0.005 mol/L. The Θ values at pH = 7 were obtained by direct chemical analysis (see text).

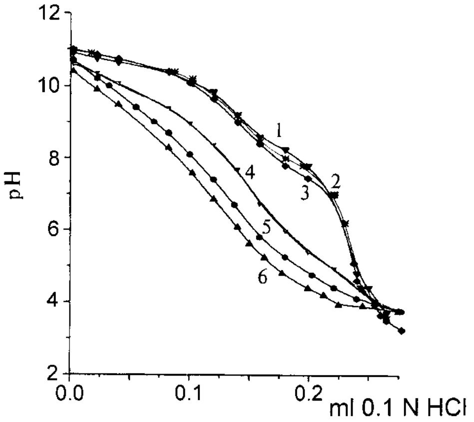
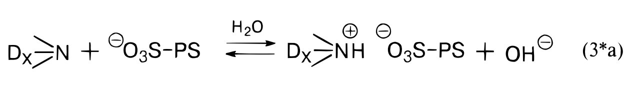


Figure 4. Potentiometric titration curves for equimolar mixtures of DAB-*dendr*-$(NH_2)_8$−NaPSS (1), DAB-*dendr*-$(NH_2)_{16}$−NaPSS (2), and DAB-*dendr*-$(NH_2)_{32}$−NaPSS (3) and for DAB-*dendr*-$(NH_2)_8$ (4), DAB-*dendr*-$(NH_2)_{16}$ (5), and DAB-*dendr*-$(NH_2)_{32}$ (6) in salt-free aqueous solutions. [DAB-*dendr*-$(NH_2)_x$] = 0.005 mol/L, T = 20 °C.

Similar results were obtained in the potentiometric study of protonation of DAB-*dendr*-$(NH_2)_x$ in the presence of NaPSS. The potentiometric titration curves of equimolar mixtures of DAB-*dendr*-$(NH_2)_x$ with NaPSS by 0.1 N HCl are shown in Figure 4. These curves describe protonation of DAB-*dendr*-$(NH_2)_x$ molecules interacting with the PSS-polyanions to form the corresponding IPECs:



$$D_x\text{-}NH_2 + {}^{\ominus}O_3S\text{-}PS \xrightleftharpoons{H_2O} D_x\text{-}\overset{\oplus}{N}H_3\ \overset{\ominus}{O_3}S\text{-}PS + OH^{\ominus} \quad (3^*)$$

$$D_x{>}N + {}^{\ominus}O_3S\text{-}PS \xrightleftharpoons{H_2O} D_x{>}\overset{\oplus}{N}H\ \overset{\ominus}{O_3}S\text{-}PS + OH^{\ominus} \quad (3^*a)$$

The reaction mixtures undergo microphase separation on mixing of the NaPSS and dendrimer solutions progressively getting hazy on further titration. The Θ−pH profiles calculated from the data of Figure 4 using eqs 1 and 2 are represented in Figure 5. They look similar to those in Figure 3, bounding the area of IPEC stability in the basic region, and also reveal a cooperative character of the interpolyelectrolyte coupling reaction. However, in contrast to the poly(acrylate anion), ionization of poly(styrenesulfonate anion) is not sup-

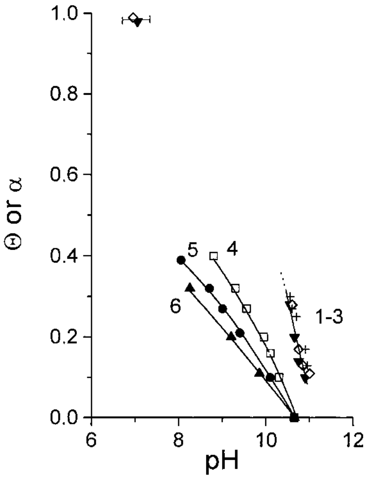


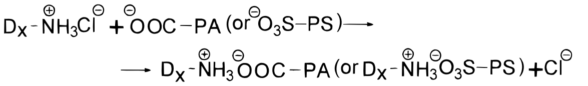
Figure 5. Θ−pH dependencies for equimolar mixtures DAB-*dendr*-$(NH_2)_8$−NaPSS (1), DAB-*dendr*-$(NH_2)_{16}$−NaPSS (2), and DAB-*dendr*-$(NH_2)_{32}$−NaPSS (3) and α−pH dependencies for DAB-*dendr*-$(NH_2)_8$ (4), DAB-*dendr*-$(NH_2)_{16}$ (5), DAB-*dendr*-$(NH_2)_{32}$ (6) in salt-free aqueous solutions. [DAB-*dendr*-$(NH_2)_x$] = [NaPSS] = 0.005 mol/L. The Θ values at pH = 7 were obtained by direct chemical analysis (see text).

pressed in the acidic region. Therefore IPECs formed by DAB-*dendr*-$(NH_2)_x$ and NaPSS are stable in the whole pH range to the left of Θ−pH profiles of Figure 5.

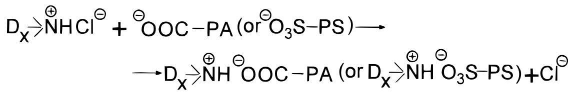
However, it is worthwhile to point out some differences in the potentiometric behavior of DAB-*dendr*-$(NH_2)_x$−NaPSS and DAB-*dendr*-$(NH_2)_x$−NaPA mixtures at relatively high degrees of dendrimer protonation. It is seen that potentiometric curves of the mixtures of DAB-*dendr*-$(NH_2)_x$ with NaPA (Figure 1) are rather smooth and do not have any peculiarities. At the same time the curves corresponding to the mixtures of DAB-*dendr*-$(NH_2)_x$ with NaPSS (Figure 4) have explicit inflection points in the mid titration region. Such a difference might be attributed to some higher specific affinity of sulfonate groups to primary amine groups of the dendrimer shell in comparison with tertiary amine groups of the core. Then the above-mentioned polyelectrolyte overlapping of the pH ranges of shell and core protonation is somewhat suppressed, so that the reactions 3* and 3*a become distinguishable by potentiometric titration. It is noteworthy that the positions of the inflection points are exactly reproduced on the reverse titration curves of the DAB-*dendr*-$(NH_2)_x$−NaPSS mixtures with NaOH, indicating their equilibrium origin.

It is evident that the degree of conversion, Θ, achieved in the interpolyelectrolyte reaction, actually characterizes the extent of permeability of a dendrimer structure for oppositely charged linear polyanions. Unfortunately eqs 1 (1′) and 2 (2′) are not applicable at a pH range close to neutrality (i.e. at high Θ), because it is impossible to discriminate between the functional groups of titrated weak polyelectrolytes, which being ionized are not involved in IPEC's ion pairs.

Therefore we used another approach based on the experimental fact that IPECs obtained by mixing of equimolar solutions of the DAB-*dendr*-$(NH_2)_x$ hydrochlorides and NaPA or NaPSS are insoluble in water and can be separated from the reaction mixture. Then Θ is experimentally determined simply by measuring the amount of Cl^- ions released in the surroundings due to the reactions



$$D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{Cl} + \overset{\ominus}{O}OC\text{-}PA\ (\text{or}\ \overset{\ominus}{O}_3S\text{-}PS) \longrightarrow D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{O}OC\text{-}PA\ (\text{or}\ D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{O}_3S\text{-}PS) + \overset{\ominus}{Cl} \tag{4*}$$



$$D_x{\to}\overset{\oplus}{N}H\overset{\ominus}{Cl} + \overset{\ominus}{O}OC\text{-}PA\ (\text{or}\ \overset{\ominus}{O}_3S\text{-}PS) \longrightarrow D_x{\to}\overset{\oplus}{N}H\,\overset{\ominus}{O}OC\text{-}PA\ (\text{or}\ D_x{\to}\overset{\oplus}{N}H\,\overset{\ominus}{O}_3S\text{-}PS) + \overset{\ominus}{Cl} \tag{4*a}$$

and that remained in the supernatant after IPEC separation. To perform the experiments the 0.002−0.005N DAB-*dendr*-$(NH_2)_x$ aqueous solutions were neutralized by equivalent amounts of HCl and mixed with equimolar solutions of NaPA or NaPSS. The insoluble IPECs formed were separated by ultracentrifuging. Neither dendrimers nor polyanions were detected in supernatants by means of potentiometric titration or UV spectrophotometry (in the case of NaPSS), indicating that precipitated IPECs contain an equal molar amount of each polyelectrolyte component. As mentioned above, such insoluble stoichiometric IPECs are always formed on mixing oppositely charged linear polyelectrolytes at a molar ratio $z = 1$, if Θ is close to 1. The molar amount of Cl^- ions in supernatant, ν_s, was measured by argentometric titration. The typical values of Θ at $z = 1$, determined as $\Theta_{max} = \nu_s/\nu_o$, where ν_o is the total mol amount of Cl^- ions in the original dendrimer solution, are represented in Table 1. These values were several times reproduced and double checked by determing the amount of a residual Cl^- ions, ν_r, in the IPEC precipitates. The data of Table 1, namely the fact that $\Theta_{max} = 1$, undoubtedly imply that all amine groups of studied dendrimers being protonated form ion pairs (salt bonds) with carboxylate or sulfonate groups of the polyanions. The above conclusion is not trivial and actually means that all primary amine groups of the dendrimer shell as well as the tertiary amine groups of the dendrimer core are available for the anionic groups attached to the linear polymer chains.

In other words, DAB-*dendr*-$(NH_2)_x$ molecules, at least up to their fifth generation, are fully penetrable for the flexible linear polyanions and are able not only to wind around the dendrimer species but also to enter into the interior. Therefore, the studied dendrimer molecules cannot be treated simply as surface charged compact stiff particles. This result is different from obtained by Dubin et al.[13] for carboxylated shell dendrimers interacting with the linear polycations. However, the dendrimers they used in addition to the exterior carboxylate groups also contained tertiary amine groups in the core making it not attractive for polycations.

The above conclusion on DAB-*dendr*-$(NH_2)_x$ penetrability valid for rather flexible linear polyanions may not be true for rigid rode-like chains. It is likely the case of the negatively charged DNA double helixes that actually reveals this by comparing the potentiometric titration curves of DAB-*dendr*-$(NH_2)_{32}$-DNA (curve 1) and DAB-*dendr*-$(NH_2)_{32}$−NaPA (curve 2) equimolar mixtures with that of the free dendrimer solution (curve 3), represented in Figure 6. The shift of curve 1 to higher pH range in comparison with curve 3 definitely indicates that dendrimer anine groups protonated in the presence of DNA do form ion pairs with the DNA phosphate groups; i.e., the DAB-*dendr*-$(NH_2)_{32}$-DNA IPEC is formed.

Table 1. Argentometric Titration Data for the Cl^- Ions Distribution between Supernatant and IPEC Phases

system	ν_o, mol	ν_s, mol	ν_r, mol	$\Theta_{max} = \nu_s/\nu_o$
DAB-*dendr*-$(NH_2)_{32}$·HCl−NaPA	1.0×10^{-4}	9.4×10^{-5}	0.5×10^{-5}	0.94
DAB-*dendr*-$(NH_2)_{64}$·HCl−NaPA	9.0×10^{-5}	8.6×10^{-5}	0.2×10^{-5}	0.96
DAB-*dendr*-$(NH_2)_4$·HCl−NaPSS	3.5×10^{-5}	3.5×10^{-5}	less than 0.05×10^{-5}	1.0
DAB-*dendr*-$(NH_2)_{32}$·HCl−NaPSS	8.7×10^{-5}	8.75×10^{-5}	less than 0.05×10^{-5}	1.0
DAB-*dendr*-$(NH_2)_{64}$·HCl−NaPSS	8.7×10^{-5}	8.7×10^{-5}	less than 0.05×10^{-5}	1.0

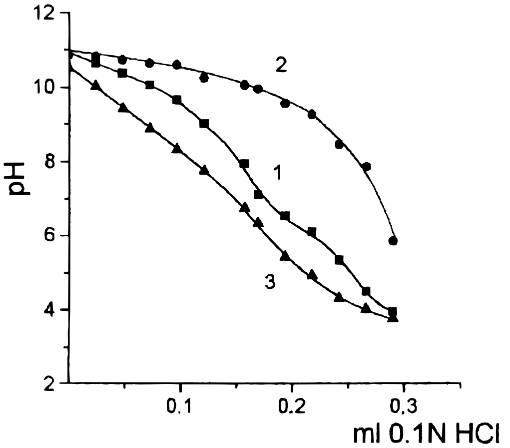


Figure 6. Potentiometric titration curves for equimolar mixtures of DAB-*dendr*-$(NH_2)_{32}$ with DNA (1), DAB-*dendr*-$(NH_2)_{32}$ with NaPA (2), and DAB-*dendr*-$(NH_2)_{32}$ (3) in salt-free aqueous solutions. [DAB-*dendr*-$(NH_2)_{32}$] = 0.005 mol/L, T = 20 °C.

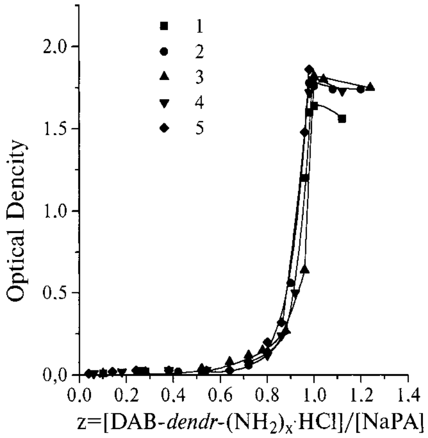
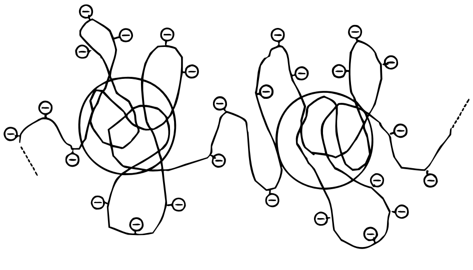


Figure 7. Turbidimetric titration curves of NaPA solution by DAB-*dendr*-$(NH_2)_4$·HCl (1), DAB-*dendr*-$(NH_2)_8$·HCl (2), DAB-*dendr*-$(NH_2)_{16}$·HCl (3), DAB-*dendr*-$(NH_2)_{32}$·HCl (4), and DAB-*dendr*-$(NH_2)_{64}$·HCl (5) in salt-free aqueous solutions. [NaPA] = 0.002 mol/L; [DAB-*dendr*-$(NH_2)_8$·HCl] = 0.02 mol/L, T = 20 °C.

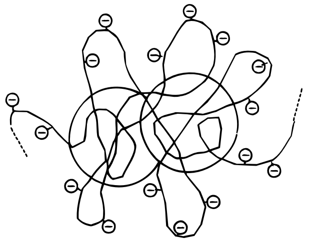
However this shift is considerably smaller than that observed for curve 2, corresponding to the case of complete dendrimer penetrability with respect to a linear flexible polyanion. The detailed study of DAB-*dendr*-$(NH_2)_x$ interaction with native DNA, being of special interest, will be reported elsewhere.

The above results were obtained by studying IPECs formed in the dendrimer−polyanion mixtures containing equal mol amounts of oppositely charged polyelectrolyte units ($z = 1$). Such IPECs are found insoluble in water at $\Theta = 1$ similar to stoichiometric IPECs formed by linear polyelectrolytes. At the same time it is known that coupling of linear polyelectrolytes mixed in nonequimolar ratios ($z \neq 1$) results in formation of nonstoichiometric IPECs water soluble at $\Theta = 1$, if the deficient polyelectrolyte has a lower degree of polymerization (guest polyelectrolyte) than that of the excessive one (host polyelectrolyte).$^{4-6}$

Similar behavior proves to be typical also for the nonstoichiometric IPECs formed on mixing DAB-*dendr*-$(NH_2)_x$.HCl and NaPA solutions where the protonated dendrimer plays a part of a guest with respect to the host PA−linear polyanion. It follows from the data on turbidimetric titration of diluted NaPA salt-free solution (pH = 8.7) by salt-free solutions of fully protonated dendrimers (pH = 4.5). The turbidimetric titration curves are presented in Figure 7 as the dependence of optical density of the resulting system on its composition, $z = N_+/N_-$. It is seen that the reaction solutions remain homogeneous in the whole range of compositions $0 < z < 0.6$. Then the turbidity increases reaching the maximum value at $z = 1$. The change of pH from 8.7 to 7.0 proceeding on titration is insignificant with respect to the Θ value, which remains close to 1 in the whole range of z. In other words the nonstoichiometric DAB-*dendr*-$(NH_2)_x$−PA IPECs formed are actually soluble in water, if the host polyanion is populated by the guest dendrimer polycations by less than 60%. The structure of such IPEC can be schematically represented as threads carrying separated or aggregated beads:



or



Each bead represents a protonated dendrimer fully neutralized by interpenetrated PA units. The free parts of a thread represent the negatively charged hydrophilic PA segments responsible for solubility of the whole nonstoichiometric IPEC species.

References and Notes

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(11) Applicability of eqs 1′ and 2′ is clarified by the following considerations. Taking into account equilibria 2*, 2*a and also PAA ionization equilibrium

$$\mathrm{PA{-}COOH} \rightleftarrows \mathrm{PA{-}COO^-} + \mathrm{H^+}$$

we come to the conclusion that $[H^+]$ measured on mixing PAA and the solutions of protonated dendrimers is a sum of

$$[\mathrm{H^+}]' = [\mathrm{D}_x{-}\mathrm{NH_3}^{+-}\mathrm{OOC{-}PA}] + [{>}\mathrm{D}_x{-}\mathrm{NH}^{+-}\mathrm{OOC{-}PA}]$$

released due to the interpolyelectrolyte coupling reaction, and $[H^+]''$ resulting from ionization of free (noncomplexed) PAA units. Hence

$$\Theta_o = [\mathrm{H^+}]'/C_o = ([\mathrm{H^+}] - [\mathrm{H^+}]'')/C_o$$

where Θ_o is the initial degree of conversion and C_o is the base molar concentration of PAA. For PAA ionization equilibrium in dilute solution one has

$$\frac{[\mathrm{H^+}][\mathrm{PA{-}COO^-}]}{[\mathrm{PA{-}COOH}]} = \frac{[\mathrm{H^+}]^2}{C_o(1-\beta-\Theta)} = \mathrm{K}(\beta) \quad (\beta + \Theta < 1)$$

where $K(\beta)$ is the ionization equilibrium constant at a given degree of ionization, β, of noncomplexed PAA units. In the range of pH lower than the pH value established in a salt-free solution of pure PAA, one has $\beta \ll 1$ and $K(\beta) \approx K_a = 1.6 \times 10^{-5}$. Therefore

$$[\mathrm{H^+}]' \approx [\mathrm{H^+}]_{\mathrm{PAA}} \approx \sqrt{K_a(1-\Theta_o)C_o}$$

and

$$\Theta_o \approx ([\mathrm{H^+}] - \sqrt{K_a(1-\Theta_o)C_o})/C_o$$

At $\Theta_o = 0.1$ the contribution of $[H^+]_{PAA}$ in total $[H^+]$ is less than 10%. Addition of NaOH is followed by a further shift of equilibria 2 and 2* to the right. Then the current degree of conversion, Θ, is expressed as

$$\Theta \approx ([\mathrm{H^+}] + q/v - \sqrt{K_a(1-\Theta)C_o})/C_o$$

so that the contribution of the $[H^+]_{PAA}$ term becomes even smaller and can be simply neglected at pH > pH_{PAA}, where eq 2′ is not valid any more. However, at pH > pH_{PAA}, NaOH may be markedly consumed for titration of PAA carboxyl groups that are not then necessarily involved in formation of the IPEC salt bonds. Therefore the validity of the simplified eq 1′ should be checked by some independent measurement. Similar considerations are valid to clarify the applicability of eqs 1 (page 6) and 2 (page 7).

(12) Degree of neutralization, α, of DAB-*dendr*-$(NH_2)_x$ in Figures 3 and 5 is traditionally determined as a ratio of the molar amount of added HCl, m_{HCl}, to the molar amount of the dendrimer amine groups, m_a, in solution assuming that α approaches 1 at $m_{HCl} \geq m_a$. Correspondingly, the degree of PAA neutralization, α, in Figure 3 is determined as the ratio of the mol amount of added NaOH, m_{NaOH}, to the mol amount of PAA carboxyl groups, m_{COOH}, assuming that $\alpha = 1$ at $m_{NaOH} \geq m_{COOH}$.

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