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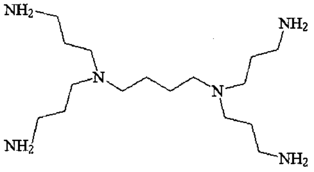
Polyelectrolyte Behavior and Interpolyelectrolyte Matching of Astramol™ Poly(propylene imine) Dendrimers

V.A. Kabanov

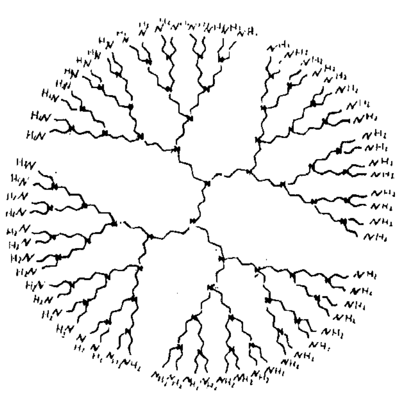
Polymer Science Department, Scool of Chemistry, Moscow Lomonosov State University, Leninskie Gori, Moscow V-234, Russia

Polyelectrolyte behavior of Astramol™ poly(propylene imine) dendrimers of five generations, G1-G5, namely DAB-*dendr*-$(NH_2)_x$ (where x is equal to 4, 8, 16, 32 or 64) was studied by means of potentiometric titration in salt-free water solutions and also in the presence of a shielding low molecular electrolyte (NaCl). In addition to x outer primary amine groups the dendrimer molecule contains x-2 inner tertiary amine groups. The repeating unit, the core molecule and the fifth generation dendrimer structure are shown in the following Scheme.

Repeating unit



G1



G5

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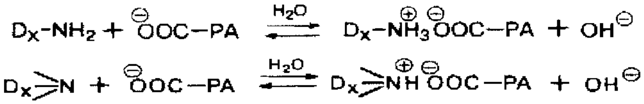
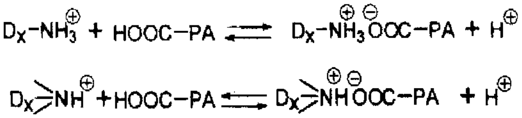
It was found that the presence of at least two types of amine groups with different basicity and electrostatic interaction of the protonated amine groups (the polyelectrolyte effect) are important factors in the peculiarities revealed on dendrimer ionization. The presence of the two types of amine groups is strongly manifested in the case of lower generation dendrimers: the two separate pH regions of dendrimer protonation were observed. The polyelectrolyte effect dominates in the case of higher generation dendrimers. It is manifested in the strong decrease of the pK(α) in the course of protonation of dendrimer amine groups due to the increasing electrostatic potential of the dendrimer polycations. In the latter case, both primary and tertiary amine groups are titrated in the markedly overlapping pH regions. The overlapping decreases when shielding simple salts are added to the aqueous dendrimer solutions [1].

It was also found [2] that interpolyelectrolyte coupling reaction resulting in formation of the corresponding interpolyelectrolyte complex (IPEC) occurred on mixing of the dendrimer and poly(acrylic acid) (PAA), or poly(styrenesulfonic acid) (PSSA), or DNA water solutions. In the case of dendrimer – PAA systems the interpolyelectrolyte reaction was pH - controlled both in alkaline and acidic pH regions.



$$D_x\text{-}NH_2 + \overset{\ominus}{O}OC\text{-}PA \xrightleftharpoons{H_2O} D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{O}OC\text{-}PA + OH^{\ominus}$$

$$D_x{>}N + \overset{\ominus}{O}OC\text{-}PA \xrightleftharpoons{H_2O} D_x{>}\overset{\oplus}{N}H\,\overset{\ominus}{O}OC\text{-}PA + OH^{\ominus}$$



$$D_x\text{-}NH_3^{\oplus} + HOOC\text{-}PA \rightleftharpoons D_x\text{-}\overset{\oplus}{N}H_3\overset{\ominus}{O}OC\text{-}PA + H^{\oplus}$$

$$D_x{>}NH^{\oplus} + HOOC\text{-}PA \rightleftharpoons D_x{>}\overset{\oplus}{N}H\,\overset{\ominus}{O}OC\text{-}PA + H^{\oplus}$$

The potentiometric titration curves of the dendrimer/PAA mixtures are represented in Figures 1 and 2, compared with those of free dendrimers and PAA.

It is seen that the dendrimers in the base form behave like a stronger polybase in the presence of poly(acrylate) anions (compare curves 1-5 with curves 6-10 in Figure 1) while PAA behaves like a stronger acid in the presence of protonated dendrimers (compare curves 1-5 with curve 6 in Figure 2). Such a behavior also typical for pairs of linear polyelectrolyte is caused by the cooperative character of multisite bonding of the oppositely charged units within the IPEC formed.

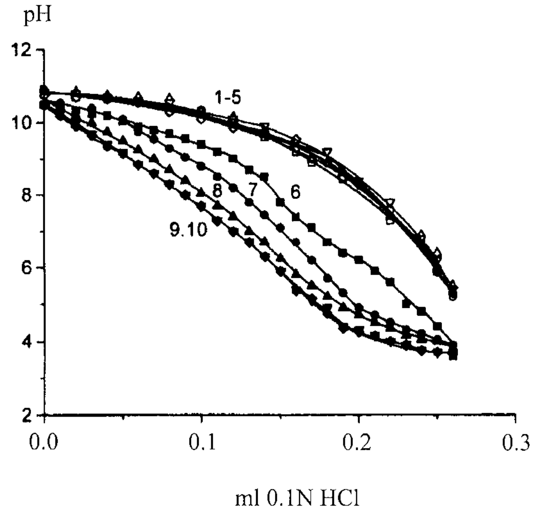


Figure 1. Potentiometric titration curves for equimolar mixtures DAB-*dendr*-$(NH_2)_4$-NaPA (1), DAB-*dendr*-$(NH_2)_8$-NaPA (2), DAB-*dendr*-$(NH_2)_{16}$-NaPA (3), DAB-*dendr*-$(NH_2)_{32}$-NaPA (4), and DAB-*dendr*-$(NH_2)_{64}$-NaPA (5) and for DAB-*dendr*-$(NH_2)_4$ (6), DAB-*dendr*-$(NH_2)_8$ (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.

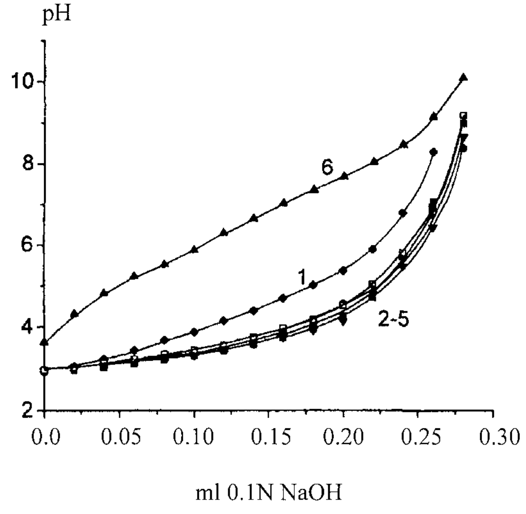


Figure 2. Potentiometric titration curves for equimolar mixtures of DAB-*dendr*-$(NH_2)_4$-HCl-PAA (curve 1), DAB-*dendr*-$(NH_2)_8$-HCl-PAA (curve 2), DAB-*dendr*-$(NH_2)_{16}$-HCl-PAA (curve 3), DAB-*dendr*-$(NH_2)_{32}$-HCl-PAA (curve 4), and DAB-*dendr*-$(NH_2)_{64}$-HCl-PAA (curve 5) and for PAA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

The current degree of conversion, $\Theta = C_c/C_0$ (where C_c is the current concentration of interpolyelectrolyte salt bonds, C_0 is the initial concentration of ionizing polymer component (DAB-*dendr*-$(NH_2)_x$ or PAA), was calculated from the above titration curves. The Θ-pH dependencies represented in Figure 3 reveal the above-mentioned cooperative character of the interpolyelectrolyte reactions manifested by 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 ionization curve of free PAA (curve 6). The IPECs formed are stable in the pH region enclosed by curves 1-5 and 1'-5'. However they dissociate to the original polyelectrolyte components at $pH < 3$ and at $pH > 11$.

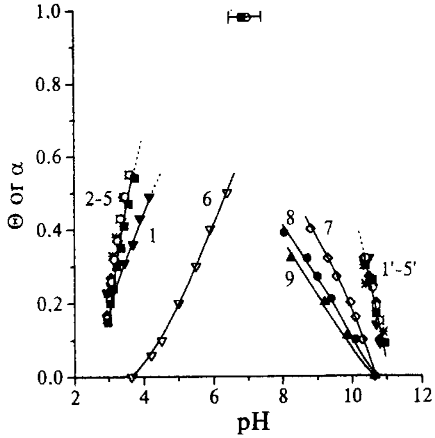


Figure 3. Θ-pH dependencies for equimolar mixtures DAB-*dendr*-$(NH_2)_4$-PAA (1, 1'*)*, DAB-*dendr*-$(NH_2)_8$-PAA (2, 2'), DAB-*dendr*-$(NH_2)_{16}$-PAA (3,3'), DAB-*dendr*-$(NH_2)_{32}$-PAA (4, 4'), and DAB-*dendr*-$(NH_2)_{64}$-PAA (5, 5') and α-pH dependencies for PAA (6), DAB-*dendr*-$(NH_2)_8$ (7), DAB-*dendr*-$(NH_2)_{16}$ (8), 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).

In contrast to PAA the degree of ionization of such a strong polyacid as PSSA doesn't depend on pH. Therefore in the case of dendrimer/PSSA systems the interpolyelectrolyte reaction was pH-controlled only in the alkaline pH region. In other words the IPECs formed from DAB-*dendr*-$(NH_2)_x$ and PSSA were stable against dissociation in the whole pH range below 11.

The dendrimer - polyanion IPECs containing equal amounts of cationic and anionic groups (stoichiometric complexes) were insoluble in water. At the same time water soluble nonstoichiometric IPECs could be obtained, if the dendrimer was a deficient component of the complex species. The turbidimetric titration curves for DAB-*dendr*-$(NH_2)_x.HCl/NaPA$ systems are represented in Figure 4 as the dependence of optical density of the resulting mixture on its composition, z = [DAB-*dendr*-$(NH_2)_x.HCl$] / [NaPA].

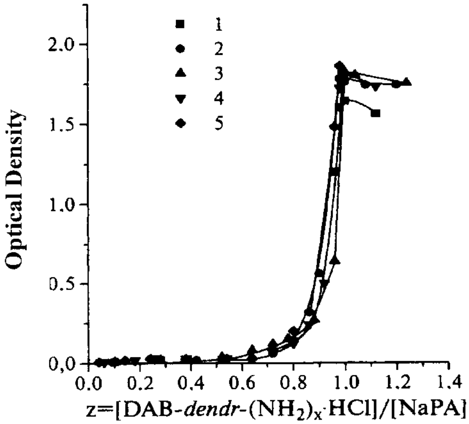
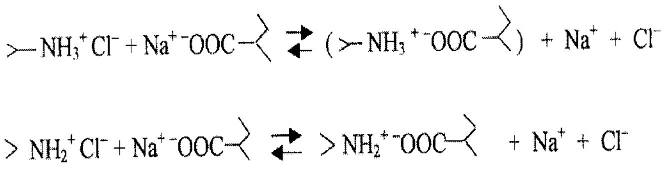


Figure 4. Turbidimetric titration curves of NaPA solution by DAB-*dendr*-$(NH_2)_4$-HCl (1), DAB-*dendr*-$(NH_2)_8$-HCl (2), DAB-*dendr*-$(NH_2)_{16}$-HCl (3), DAB-*dendr*-$(NH_2)_{32}$-HCl (4), and DAB-*dendr*-$(NH_2)_{64}$-HCl (5) in salt-free aqueous solutions. [NaPA] = 0.002 mol/L; [DAB-*dendr*-$(NH_2)_x$-HCl]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

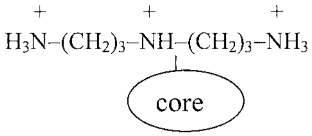
It is seen that the reaction solutions remain transparent and homogeneous in the whole range of compositions $0<z<0.6$. Then the turbidity has increased reaching the maximum value at $z = 1$. In other words the nonstoichiometric DAB-*dendr*-$(NH_2)_x$-PA IPECs formed were actually soluble in water, if the host polyanion was populated with the guest dendrimer polycations by less than 60%. This behavior is also similar to IPECs formed by oppositely charged linear polyions.

A peculiar finding was that practically all dendrimer amine groups being protonated could form ion pairs with carboxylate or sulfonate groups of the polyanions. It has been proved by direct measurement of NaCl release in the coupling reaction between the dendrimers and poly(sodium acrylate) or poly(sodium sulfonate) at equal concentrations of amine and anionic groups of the corresponding polyelectrolytes.



The molar amount of Cl⁻ ions in supernatant after separation of nonsoluble stoichiometric IPEC formed was measured by argentometric titration. The maximum degree of conversion determined as $\Theta_{max} = \nu_s/\nu_o$, has proved to be close to 1 (ν_s is the molar amount of Cl^- ions in supernatant, ν_o is the total molar amount of Cl^- ions in the original DAB-*dendr*-$(NH_2)_x$.HCl solution). This fact undoubtedly imply that all primary amino 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 polyelectrolyte 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.

However, this was not the case for rigid negatively charged DNA double helixes [2, 3]. The content of residual Cl^- (and consequently Na^+) ions in the thoroughly washed and dried IPEC sediment formed on mixing of DAB-*dendr*-$(NH_2)_{32}$ and salmon sperm DNA solutions at equal concentration of amine and phosphate groups was not negligible as in the cases mentioned above. Moreover it indicated that about one third of DNA phosphate groups has not reached the amine groups of the dendrimer to form intropolyelectrolyte salt bounds. This apparently means that only protonated primary and tertiary amine groups, belonging to the outer DAB-*dendr*-$(NH_2)_{32}$ branches,



$$\overset{+}{H_3N}\text{–}(CH_2)_3\text{–}\underset{\text{core}}{\overset{+}{NH}}\text{–}(CH_2)_3\text{–}\overset{+}{NH_3}$$

are sterically available for tight electrostatic binding to the phosphate groups of DNA double helix in solution. The rest of protonated tertiary amine groups located in the interior of the dendritic polycation remains neutralized by Cl^- counterions. Also, one third of sodium phosphate groups exposed on the surface of the DNA double helix in electroneutral 1:1 IPEC remains free to bind to protonated outer amine groups of the excess of dendrimer polycations. Estimation based on comparing the hydrodynamic radii of G4 and G5 dendrimers with the persistent length of the double helical DNA shows that at neutralization point there is still enough room to accomodate additional dendrimer polycations. This is why G4 and G5 dendrimers added to DNA in excess were able to form overcharged water soluble IPECs containing excess of positive charge that was in contrast with G1 and G2.

Importantly that solubility of these complexes has provided a unique opportunity to apply a scope of informative solution measurements for investigating the state of complexed DNA. In particular it has been shown using UV and CD spectroscopy combined with ultracentrifugation that the native DNA occupied by an excess of the G4 and G5 dendrimers compacts revealing wound double helical structure. Moreover, in the case of ultra-high molecular mass T4 DNA mixed with an excess of G4 dendrimer the compaction of individual DNA molecules and formation of the water soluble positively charged IPEC «unimers» was directly observed under a fluorecsence microscope [3].

It is likely that soluble positively charged compact complex species of this kind but formed with plasmid DNA play an important part in DNA translocation into cells and transfection phenomena.

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