Location of the Outer Shell and Influence of pH on Carboxylic Acid-Functionalized Poly(propyleneimine) Dendrimers

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Introduction

In this note the synthesis of a fourth- and fifth-generation carboxylic acid-functionalized poly(propyleneimine) dendrimer with a deuterated exterior shell is presented to study conformational changes. The fifth generation has been used to investigate the location of the outer shell. Next to the location of the outer shell the influence of the pH on the size of the dendrimer has been studied. Results from both SANS and NMR diffusion measurements are presented.

Experimental Section

Synthesis. To selectively deuterate the outer shell of carboxylate-functionalized poly(propyleneimine) dendrimers, a two-step method is necessary using deuterated agents, but which uses conditions similar to the synthesis of the "fully hydrogen" carboxylate-functionalized poly(propyleneimine) dendrimers.[1] First a Michael addition is performed of a third- and fourth-generation amine-functionalized poly(propyleneimine) dendrimer to 2 equiv of acrylonitrile-d_3. Pure acrylonitrile-*d*-functionalized dendrimers have been obtained by evaporation of the (small) excess of acrylonitrile-d_3. This was evidenced by ^{1}H NMR and ^{13}C NMR.

Subsequently, the obtained acrylonitrile-*d*-functionalized poly(propyleneimine) dendrimers have been hydrolyzed in a 6 M solution of hydrochloric acid, yielding a fourth- and fifth-generation carboxylate-*d*-functionalized poly(propyleneimine) dendrimer with 32 and 64 carboxylate end groups, respectively (Figure 1).

Synthesis of DAB-*dendr*-(COOH)$_{64}$-*d*$_{192}$. Acrylonitrile-d_3 (2.48 g, 44.2 mmol) was added to a stirred and cooled (ice/water) solution of DAB-*dendr*-$(NH_2)_{32}$ (1.55 g, 0.441 mmol) in water (10 mL) in a dropwise fashion. The reaction mixture was stirred overnight at 85 °C. The solvent was evaporated in vacuo, and the residue was stripped with dichloromethane to remove excess acrylonitrile, yielding the intermediate nitrile-functionalized dendrimer DAB-*dendr*-$(CN)_{64}$-d_{192} as a transparent colorless oil (3.07 g, 98%). ^{1}H NMR ($CDCl_3$): 2.56 (t, 64H, CH_2N), 2.47 (64H, NCD$_2$C*H*DCN), 2.42 (m, 180H, NCH_2), 1.61 (m, 120H, NCH$_2$CH_2CH$_2$N), 1.47 (m, 4H, CH_2-CH$_2$N).

DAB-*dendr*-$(CN)_{64}$-d_{192} (2.0 g, 0.281 mmol) was dissolved in 10 mL of 6 M HCl and stirred overnight at room temperature. Subsequently, the solution was refluxed for several hours and precipitated in acetone to yield DAB-*dendr*-$(COOH)_{64}$-d_{192} as a white powder after drying in vacuo (1.99 g, 85%).^{1}H NMR (D_2O)$_3$: δ = 3.50−3.10 (br, 244H, NCH_2CH$_2$CH_2N {240H} + NCH_2CH$_2$CH$_2$CH_2N {4H}), 2.84 (s, 64H, NCD$_2$C*H*DCOOH), 2.05−2.42 (br, 124H, NCH$_2$CH_2CH$_2$N), 1.78 (4H, NCH$_2$CH_2CH_2-CH$_2$N). IR (KBr): ν (cm^{-1}) = 1695 (C=O).

Analysis of DAB-*dendr*-(CN)$_{32}$-*d*$_{96}$. ^{1}H NMR ($CDCl_3$): 2.56 (t, 32H, CH_2N), 2.47 (32H, NCD$_2$C*H*DCN), 2.42 (m, 84H, NCH_2), 1.61 (m, 56H, NCH$_2$CH_2CH$_2$N), 1.47 (m, 4H, CH_2-CH$_2$N). ^{13}C NMR ($CDCl_3$): 118.9 (*C*N), 54.3/52.4/52.3/52.1/51.7/51.4 (NCH$_2$*C*H$_2$*C*H$_2$CH$_2$N + NCH$_2$*C*H$_2$CH$_2$N), 48.7 (*C*H$_2$N(CD$_2$-CHDCN)$_2$), 43.4 (N*C*D$_2$CHDCN), 25.0/24.4/24.3 (NCH$_2$*C*H$_2$-*C*H$_2$CH$_2$N + NCH$_2$*C*H$_2$CH$_2$N), 16.3 (NCD$_2$*C*HDCN).

Analysis of DAB-*dendr*-(COOH)$_{32}$-*d*$_{96}$. ^{1}H NMR (D_2O)$_3$: δ = 3.50−3.10 (br, 116H, NCH_2CH$_2$CH_2N {112H} + NCH_2-CH$_2$CH$_2$CH_2N {4H}), 2.84 (s, 32H, NCD$_2$C*H*DCOOH), 2.05−2.42 (br, 60H, NCH$_2$CH_2CH$_2$N), 1.78 (4H, NCH$_2$CH_2CH_2-CH$_2$N).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer at 400.13 and 100.62 MHz, respectively. Chemical shifts were recorded in ppm (δ) relative to TMS. IR spectra were recorded on a BioRad FTS 175.

The third- and fourth-generation amine-functionalized poly(propyleneimine) dendrimers, DAB-dendr-$(NH_2)_n$ (n = 16 and 32), were obtained from DSM. The deuterated acrylonitrile was purchased from Cambridge Isotope Laboratories (Andover, MA) with a degree of deuteration of 98%.

Measurements. SANS experiments were carried out using the LOQ instrument[2] at ISIS (Didcot, UK). The scattering angle q ranged from 0.04 to 1 Å^{-1}. Counting times were approximately 1 h for each sample.

By varying the D/H composition of the outer shell of the dendrimer and the composition of the solvent, different contrasts were generated to look at different parts of the dendrimer. The measurements were performed with three different contrasts: (1) "whole dendrimer"—all H-dendrimer in D_2O (99.9% D, Aldrich Chemicals), (2) "core"—outer shell deuterated dendrimer in D_2O, and (3) "outer shell"—outer shell deuterated dendrimer in H_2O/D_2O to match the inner part of the dendrimer (to observe the deuterated shell). The measurements were performed at mass percentages of 2% and some at 1% and 4% to check the effects of the interparticle structure factor $S(Q)$ of the scattering.

The measurements were performed at pH values of 2, 6, and 11. NaCl was added to a concentration of 1.0 M to screen the effect of the charge on the dendrimers on the interparticle structure factor. HCl and NaOH (or equivalent amounts of DCl and NaOD depending on the contrast situation) were used to adjust the pH.

The background was determined by subtracting the measured scattering of the appropriate solvents. In most cases a constant background was still visible, which was taken into account in the fits of the measurements. The FISH data analysis program of Dr. R. H. Heenan was used for the evaluation of the measurements.

The self-diffusion measurements were performed with the pulsed field gradient NMR method. Experimental details can be found in ref 3. The measurements were performed using concentrations of 0.5−3 wt % for the three pH values of 2, 6, and 11 at a salt concentration of 1.0 M NaCl.

Results and Discussion

In Figure 2 the Guinier fits[4] can be found of the solutions containing 2 wt % dendrimers. In Table 2 the respective Guinier radii are found. The Guinier fits were obtained following Glatter,[5] where the applicable fit region is $q < \pi/(2R_g)$. The Guinier fits were determined with a simultaneously fitted baseline. The data of the outer shell were only measured from $0 < q < 0.3$ Å^{-1},

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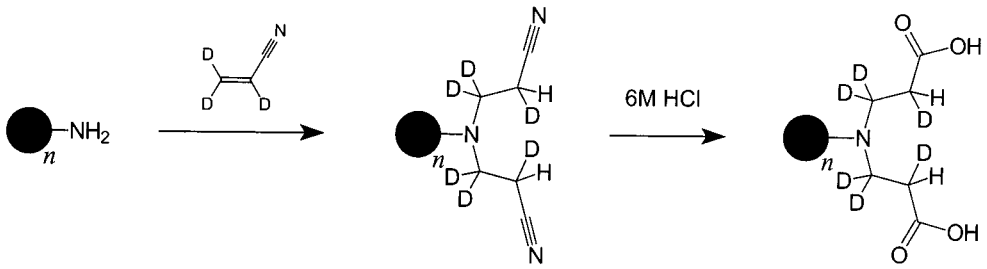

Figure 1. Two-step synthesis of carboxylated poly(propyleneimine) dendrimers with deuteration in the outer shell only. The synthesis starts from the third- and fourth-generation amine-functionalized dendrimer.

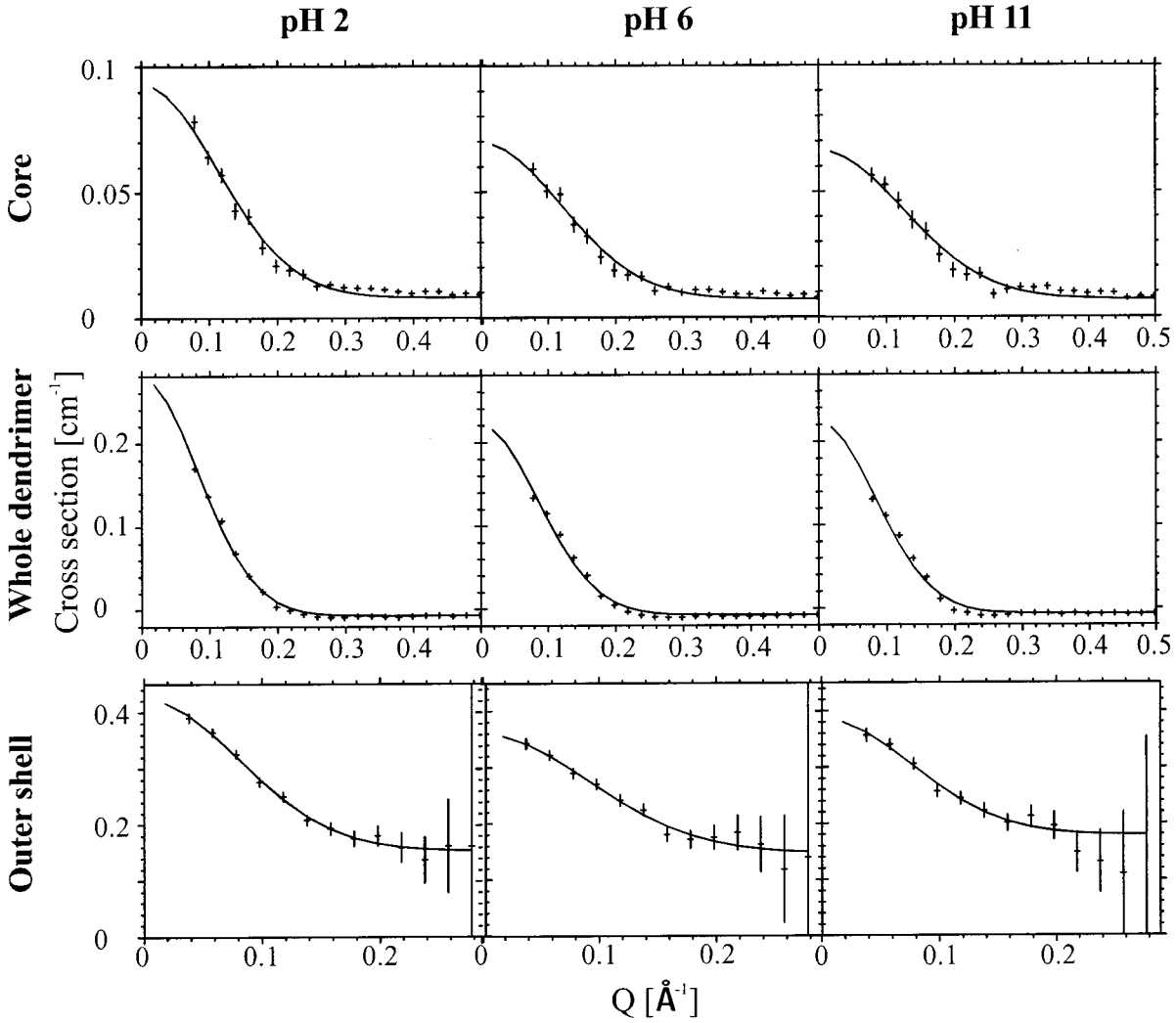

Figure 2. Guinier fits according to King[4] of the 2 wt % solutions. From left to right pH 2, pH 6, and pH 11. Going down, the following contrast situations are respectively shown: "core", "whole dendrimer", and "outer shell". The fits have been corrected for the background. For clarity, only $q = 0-0.5$ Å^{-1} is shown.

Table 1. Measured and Calculated Intensities (cm^{-1}) for the 2% Solutions at pH 2 in the Four Contrast Situations; Calculations Were Performed without and with Taking an Excess Salt Concentration in or around the Dendrimer

contrast	measd	calcd without salt	calcd with salt
core	0.12± 0.03	0.53	0.12
whole dendrimer	0.32 ± 0.04	0.95	0.36
outer shell	0.54 ± 0.15	0.12	0.54
nothing	0.02 ± 0.02	0.08	0.02

due to technical problems. Therefore, the error in the outer shell is much higher (10−20%) than for the other contrast situations. The different contrast variations that have been measured are presented in Figure 2. The scattered intensities are rather low, which limits data interpretation. Moreover, from the SANS intensity at zero momentum transfer it can be deduced that the 1 M salt in the mixtures gives rise to an electric double layer around the charged dendrimer (for example, ref 6).

For the density of the dendrimer we take the bulk value $D = 1.06 \times 10^6$ g m^{-3}. Densities have been determined by measuring the density as a function of concentration.[7] The resulting specific partial density has been used for the calculations. The fifth-generation dendrimer with the structure formula $C_{376}H_{816}N_{62}O_{128}$ has a total neutron scattering length $\rho = 0.5847 \times 10^{14}$

Table 2. Guinier Radii (nm) of 1, 2, and 4 wt % for the Measured Contrast Situations, "Core", "Whole Dendrimer", and "Outer Shell"[a]

wt %	contrast	pH 2	pH 6	pH 11
2	core	1.10 ± 0.04	1.03 ± 0.04	0.99 ± 0.04
2	whole dendrimer	1.47 ± 0.02	1.43 ± 0.04	1.48 ± 0.04
2	outer shell	1.58 ± 0.06	1.33 ± 0.11	1.72 ± 0.15
4	whole dendrimer	1.38 ± 0.013	1.15 ± 0.04	1.20 ± 0.05
4	outer shell	1.70 ± 0.04	1.28 ± 0.18	1.29 ± 0.09
1	whole dendrimer	1.39 ± 0.02	1.31 ± 0.02	

[a] Errors are least-square residuals. The actual errors may be higher; especially for the outer shell, the error will be around 10−20% of the radius for reasons explained in the text.

m^{-2}, the nucleus of the dendrimer with the structure formula $C_{164}H_{496}N_{62}$ has a neutron scattering length $\rho = -0.09159 \times 10^{14}$ m^{-2}, and the deuterated outer shell with the structure formula $(C_3H_2D_3O_2)_{64}$ has a neutron scattering length density $\rho = 3.632 \times 10^{14}$ m^{-2}. The concentrations of H_2O and D_2O were chosen to match the densities of the nucleus of the dendrimer in the "outer shell" mixtures. Calculation of the zero momentum intensities for the different contrast mixtures gave large deviations from the experiment. The zero momentum intensity depends on the number concentration n and the volume of the dendrimer $V = 1.32 \times 10^{-26}$ m^3, which was calculated from the mass density and the scattering length density contrast $\delta\rho$[4]

$$I(q=0) = nV^2\delta\rho^2$$

In the "whole dendrimer" and "core" mixture in H_2O, which has a negative scattering length density, the measured intensities were lower, and in the "outer shell" contrast, which has a positive scattering length, the measured intensity is higher. Taking into account an increased concentration of NaCl, which has a positive scattering length, around the dendrimer gives a good description of the zero momentum intensities. A consequence of this electrical double layer is that one always observes the scattering of the dendrimer combined with the NaCl around it. The scattering length density contrast is thus determined by the total scattering length of the dendrimer (normal or partly deuterated) Σb_{dend}, the total scattering length of the excess salt present in or around the dendrimer Σb_{salt}, and the scattering length density of the solvent used ρ_{solv}, including the 1 M of salt

$$\delta\rho = (\Sigma b_{dend} + \Sigma b_{salt})/V - \rho_{solv}$$

A good description of the intensities in all four the contrast situations was obtained with an excess salt scattering length of $\Sigma b_{salt} = 3$ pm, which corresponds to 250 NaCl molecules, which is not unreasonable for the amount of charges on dendrimer. The measured and calculated intensities are presented in Table 1.

The excess salt does not prevent to draw conclusions about the location of the last generation shell from the radii of gyration as shown in Table 2. The only difference between the "whole dendrimer" and "core" contrast is the deuteration of the last generation shell. The radius of gyration is larger for the "whole dendrimer" than for the "core" for all different pH values. The radius of gyration in the "outer shell" contrast is even larger, but it is hard to interpret this value, since it will be determined by both the last generation shell and the increased salt concentration.

Table 3. Self-Diffusion Coefficients at Zero Concentration and Hydrodynamic Radii of the Carboxylic Acid-Functionalized Poly(propyleneimine) Dendrimers for pH 2, 6, and 11

pH	D ($\times 10^{-11}$ m s^{-2})	radius (nm)
2	9.29 ± 0.18	2.15 ± 0.04
6	9.77 ± 0.17	2.04 ± 0.04
11	9.18 ± 0.14	2.17 ± 0.03

The radii of gyration of the 1 and 2 wt % solutions do not show a large difference (within experimental error). Together with the high screening by 1 M salt, this indicates that mainly the form factor is obtained. Therefore, the radius of gyration obtained from the 2 wt % samples are fair estimates of the real values. This is confirmed by radii from similar dendrimers.[7,8]

For pH 2 (2 wt % samples) the size of the "core" is the largest and decreases with increasing pH. At pH 2 the core appears to be larger than for pH 6 and pH 11, where both radii can be considered equal. Since at pH 2 the core is charged, one can conclude that the charge causes an increase of the core of about 6−10%. The radii of the "whole dendrimer" show a minimum at pH 6. The charged dendrimer at pH 2 has a larger size than the neutral dendrimer (pH 6). In the case of the "outer shell" a minimum is found at pH 6 as well. Because the neutral dendrimer has positively as well as negatively charged groups,[9] partial backfolding of the outer shell into the core can occur. An indication for this is found by the size of the solid sphere radius of the core (calculated from the radius of gyration). It has the same value as the radius of gyration of the outer shell, therefore showing overlap. Some overlap cannot be excluded at pH 2 and pH 11 with the present data either.

In Table 3 the self-diffusion coefficients at zero concentration and the calculated hydrodynamic radius can be found. The hydrodynamic radius reflects the size of a molecule as it moves through solution. Therefore, not only the size of the molecule itself is determined but also a solvation layer that moves along with the molecule.

The self-diffusion coefficients of the different pH values show that the dimensions of the charged dendrimers are larger. The uncharged dendrimer at pH 6 has the lowest radius. One would expect that charging of the outer shell could explain the larger hydrodynamic radius for pH 11. The SANS data, however, are difficult to interpret since especially the "outer shell" results could be overestimated due to the presence of counterions. Furthermore, the increase of the radius will be smaller in comparison with the core because only one shell, the outer, will stretch. Therefore, the hydrodynamic radius probably shows the effect of an enlarged solvation layer, due to the charged outer shell and only to a lesser extent the effect of the stretched outer shell.

The errors in the SANS data are large (around 10%); however, especially the 2 wt % "core" and "whole dendrimer" contrast situations (errors around 5%) show a clear size change (6−10%). Together with the size change of 5% found with NMR, these results point out that size change of dendrimers with pH is occurring. This is tentatively presented in Figure 3. Although Nisato et al.[10] find a small size change as a function of pH (<5%), they conclude that this is negligible. Newkome et al. have found size changes as a function of pH for a carboxylic acid-functionalized dendrimer.[11] Therefore, one could conclude that the extent of the change

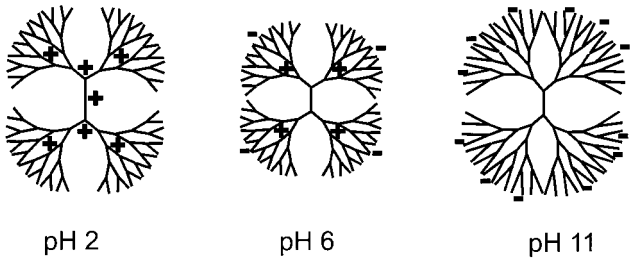

Figure 3. Different charge configurations of the dendrimers. At pH 2 the core is stretched due to the positive charge. At pH 6, there is a balance between the negative and positive charge. At pH 11, the outer shell is stretched due to the negative charges on the carboxylic acid groups.

depends on the length of the spacers, the functionality of the branching points, and the functional groups.

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