Structure of Charged Dendrimer Solutions As Seen by Small-Angle Neutron Scattering

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ABSTRACT: We present results of small-angle neutron scattering (SANS) investigations into the characteristics of charged poly(amidoamine) (PAMAM) dendrimer solutions in deuterium oxide. The study shows that ionic strength, surface charge density, and concentration are the crucial parameters controlling the degree of structural organization of the solution. Upon the addition of acid, HCl, we observe a peak in the scattering intensity. The results indicate that increasing ionization of terminal amines results in interparticle interactions giving rise to local, liquid-like ordering. These interactions can be screened by adding monovalent salt (NaCl) in large quantities. The short-range interactions were studied by systematically varying dendrimer and salt concentration (up to 2 M). Contrary to most weak polyelectrolyte systems, we did not observe a negative second virial coefficient, even for the highest salt concentrations.

Introduction

Dendrimers are highly branched macromolecules consisting of a multifunctional core from which successive branched repeat units extend radially outward. The resulting molecules have a well-defined number of end groups and narrow molecular weight distribution.1,2 These molecules are synthesized in a stepwise manner so that each new step, or generation, doubles the molecular weight, the number of end groups, and the number of branch points. In addition, it was recently found that with successive generations dendrimers become increasingly spherical, their form factor evolving from starlike at low generation to dense sphere at higher generations.3 The most frequently studied dendrimers contain amine-terminated end groups. In this case the surface charge densities of these molecules can be manipulated by varying the pH of the solution, and the dendrimers can be viewed as nanoscopic polyelectrolyte particles.

The nature of the interactions between macromolecules in solution has received considerable experimental and theoretical attention through the years.4 Often, experimental efforts are directed toward a search for a suitable model system that allows direct comparison with theories. In general, model systems should consist of molecules with (1) uniform size and shape, (2) ionizable groups able to support large variations of charge density, and (3) solubility over a wide range of conditions to allow sampling a large parameter space. Dendrimers are good candidates for exploring the physics of macrorions.

Aside from considerations of a theoretical nature, a number of potential applications for dendrimers, such as "smart" drug delivery agents, are expected to involve drastic changes in their aqueous environment. It is therefore important to assess the response of dendrimers to such changes. The goal of this study is to systematically vary the degree of ionization of the dendrimers as well as the ionic strength of the solution in order to probe dendrimer-dendrimer interactions over a wide range of ionic conditions.

Experimental Section

Materials. Generation 5 (G5) polyamidoamine (PAMAM) dendrimers were obtained from Dendritech, Inc., Midland, MI.3 The G5 dendrimer solutions were prepared from a 0.22 mass fraction aqueous stock solution as follows. Aliquots of the stock solution were diluted with ultrapure H2O (18 MΩ·cm, Barnstead) and dialyzed to remove low molecular weight impurities. Dialysis was performed using a Spectrum microdialyzer with a 10 mL half-cell volume and a 12–14 kDa molecular weight cutoff (MWCO) cellulose membrane (Spectra/Por, Spectrum). H2O solvent was exchanged by dialysis against 99.9% (low paramagnetic) D2O (Cambridge Isotopes, Inc.).5,6 The final D2O volume fraction of the solvent was >96.5%, as measured from the scattering intensity of the solution. The G5 PAMAM dendrimers have 128 terminal amine groups that can be ionized to the −NH3+ form through the addition of a (strong) acid. The stoichiometric ratio of added HCl to the total number of terminal groups in solution is noted α = [HCl]/[−NH3+], and it is proportional, but does not correspond exactly, to the extent of the ionization of the terminal amine groups.

The values of dendrimer volume fraction, φ, and α were calculated from the weight of the added components and values of density, molecular weight, and functionality reported in the literature.7 The main characteristics of the samples studied are listed in Tables 1–3.

SANS. SANS data were obtained on the 8 and 30 m instruments located at the Center for Neutron Research at the National Institute of Standards and Technology in Gaithersburg, MD.8 The 8 m measurements were performed using a sample-detector distance of 360 cm and an average neutron wavelength of 9 Å with a spread Δλ/λ = 0.25. These conditions yielded a q range of 0.008–0.123 Å−1, where q denotes the amplitude of the scattering vector. The 30 m SANS experiments were conducted using several detector configurations and 6 Å (Δλ/λ = 0.10) neutrons, corresponding to a q range from 0.004 to 0.31 Å−1. All the samples were contained in quartz cells having circular windows and path lengths of 1.2, or 5 mm. The raw (2-D) data were reduced following the standard data reduction software available at the Cold Neutron Research Facility.10 The incoherent background level in the samples was estimated using two independent procedures. In the first one, a constant was subtracted so that the SANS data at high q values was adjusted to a q−4 slope in a log–log plot, following Glatter11 and Chen.12 In the cases when the data
at sufficiently high \( q \) was not available, the scattering profiles were fitted to a Guinier functional form with a floating baseline, which was shown by Prosa et al. to fit PAMAM dendrimers satisfactorily.\(^3\) In the second procedure, the incoherent intensity was interpolated from the transmissions of the samples and the scattering intensities of known \( \text{H}_2\text{O} \) and \( \text{D}_2\text{O} \) mixtures.\(^{13}\) In all cases, the resulting background corrections from these procedures were in good agreement (relative standard deviation \(<5\%\)). The corrected intensities were then converted to an absolute scale and were radially averaged yielding the \( I \) vs \( q \) plots for each sample.\(^{10}\) Experimental standard deviations in the scattered intensities were \(<1\%\), yielding uncertainty bars smaller than the plotted data symbols, and were not displayed for clarity.

The total scattering intensity \( I(q) \) from a homogeneous solution of monodispersed spherical particles can be written as\(^{14}\)

\[
I(q) = KP(q)S(q)
\]

where \( K \) is the contrast factor, \( P(q) \) is the single particle form factor arising from intraparticle interference (Figure 1a), and \( S(q) \) is the solution structure factor corresponding to interparticle interference (Figure 1b). The contrast factor is proportional to the particle volume fraction and depends on the difference of scattering length densities between the particles and the solvent.\(^{15}\) Expression 1 is exact in the case of spherical scattering objects and can be extended to the case of nonspherical particles by using an effective structure factor. The form factor corresponding to a given geometry and segment density distribution can be readily calculated.\(^{11,14}\) The calculation of the structure factor \( S(q) \) in the case of charged colloids requires complex models describing the effects of the long-range Coulombic interactions.\(^{8}\) Generally, when the particles interact, the changes in \( S(q) \) reflected in the overall scattering intensity arise from interparticle spatial correlations.\(^{11,14}\) When \( q \gg R_g^{-1} \), the structure factor is a constant \( S(q) = 1 \), and the total scattering reflects the nature of the individual particles (Figure 1b). The other interesting regime occurs when \( q \ll R_g^{-1} \); in this regime the scattering behavior is dominated by the osmotic compressibility of the solution. As \( q \to 0 \), the scattering intensity reduces to \( I(0) \sim k_B T \ln(b/\Pi r) \sim \phi(1 + A_2 \phi) \), where \( k_B \) is the Boltzmann constant, \( \Pi \) is the osmotic pressure of the solution, and \( A_2 \) is the second virial coefficient.\(^{14}\)

**Results and Discussion**

In Figure 2 we display the excess scattering intensity of aqueous (\( \text{D}_2\text{O} \)) dendrimer solutions prior to addition of salt or acid (i.e., in the preparation state) as a function of dendrimer concentration. The \( I(q) \) data are normalized by the overall dendrimer volume fraction, and a Guinier functional form \( I(q) = I_0 \exp(-q^2R_g^2/3) \) could be satisfactorily fit to the data, independent of the dendrimer concentration, yielding the apparent radius of gyration of the molecules.\(^{3,15}\) The value of the radius of gyration extrapolated at zero concentration from these measurements was \( 24.3 \pm 0.5 \, \text{Å} \),\(^{16}\) in agreement with previous SAXS experiments performed in methanol.\(^3\) The concentration dependence of the apparent radius of gyration follows the same trend observed by Topp et al. in similar experiments performed on poly(propyleneimine) dendrimers.\(^{15}\) We could also evaluate the (apparent) values of \( R_g \) in the case of charged dendrimer solutions in the presence of salt. The data reported in Figure 2b show that, for charged dendrimers with \( \alpha = 0.6 \) and for salt concentrations ranging from \( 50 \, \text{mM} \) to \( 2 \, \text{M} \),\(^{17}\) we do not find significant variations of the radius of gyration. The influence of \( \text{pH} \) and salt concentration on the dendrimer size in dilute solution will be discussed in more detail in a forthcoming paper.\(^{18}\)

Figure 3 shows the excess scattering profiles from solutions in which the dendrimer volume fraction was fixed at \( \phi = 0.042 \) and \( \alpha \) was varied from 0.0 to 1.0 (one HCl per terminal unit or primary amine group). As the charge density of the dendrimers is increased by the addition of acid, the total osmotic pressure of the

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**Table 1. Effect of Charge Density**

<table>
<thead>
<tr>
<th>sample</th>
<th>( \alpha )</th>
<th>( \phi \times 10^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>d4-0-0</td>
<td>0.010</td>
<td>2.85</td>
</tr>
<tr>
<td>d4-0-6</td>
<td>0.048</td>
<td>2.79</td>
</tr>
<tr>
<td>d4-0-1</td>
<td>0.072</td>
<td>2.80</td>
</tr>
</tbody>
</table>

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**Table 2. Effect of Salt Concentration**

<table>
<thead>
<tr>
<th>sample</th>
<th>( \alpha )</th>
<th>( \phi \times 10^{-2} )</th>
<th>( C_s ) (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1-0-5</td>
<td>0.57</td>
<td>0.61</td>
<td>0</td>
</tr>
<tr>
<td>D1-5-5</td>
<td>0.59</td>
<td>0.56</td>
<td>0.0050</td>
</tr>
<tr>
<td>D1-25-5</td>
<td>0.58</td>
<td>0.61</td>
<td>0.0250</td>
</tr>
<tr>
<td>D1-50-5</td>
<td>0.58</td>
<td>0.61</td>
<td>0.0500</td>
</tr>
<tr>
<td>D1-100-5</td>
<td>0.583</td>
<td>0.64</td>
<td>0.100</td>
</tr>
<tr>
<td>D1-1000-5</td>
<td>0.61</td>
<td>0.57</td>
<td>0.991</td>
</tr>
</tbody>
</table>

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**Table 3. Effect of Dendrimer Concentration**

<table>
<thead>
<tr>
<th>sample</th>
<th>( \alpha )</th>
<th>( \phi \times 10^{-2} )</th>
<th>( q^* ) (Å(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2-0-5</td>
<td>0.574</td>
<td>1.19</td>
<td>0.052</td>
</tr>
<tr>
<td>D1-0-5</td>
<td>0.574</td>
<td>0.61</td>
<td>0.044</td>
</tr>
<tr>
<td>D05-0-5</td>
<td>0.572</td>
<td>0.31</td>
<td>0.035</td>
</tr>
<tr>
<td>D02-0-5</td>
<td>0.574</td>
<td>0.11</td>
<td>0.025</td>
</tr>
</tbody>
</table>

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**Figure 1.** (a) Guinier functional form approximating the form factor \( P(q) \) of G5 PAMAM dendrimers.\(^3\) (b) Example of structure factor \( S(q) \) of charged spherical colloids in suspension calculated using the RMSA approximation.\(^4,27\)
solution increases accordingly, being essentially driven by the translational entropy of the "free" counterions. The progressive decrease of the scattering intensity at low scattering angles correlates well with the expected increase of the osmotic compressibility. However, the most notable effect of the addition of acid is the appearance of a peak in the scattering profiles, which becomes sharper upon increasing $R$. This peak is a common feature of polyelectrolyte solutions and charged colloidal suspensions and is consistent with the previous observations of charged dendrimers by Briber et al., Valachovic, and Ramzi et al. This peak can originate both from a depletion of the scattering intensity due to the increased osmotic compressibility of the solution and also from the presence of a peak in the structure factor, indicating a preferential wavelength for spatial density-density fluctuations. In general, it is very difficult to separate unambiguously the $P(q)$ and $S(q)$ contributions to the overall scattering. There exist robust numerical methods to perform this task [Glatter], but they are still model dependent. The most reliable data can in principle be obtained experimentally, using zero-average contrast for instance, but these experiments require deuterated samples, which are not always available.

In Figure 3b we plot the ratio of the scattering intensity and the form factor of the dendrimers extrapolated at zero concentration in the "uncharged conditions" (cf. Figure 2). (c) Plot of the scattering intensities normalized by the scattering profile of the dendrimer solution with $\alpha = 0$ (cf. Figure 3a). The symbols are the same as in (a).
the same finite concentration solution without added acid. This is shown in Figure 3c. Again, the peak position is independent of as is expected for a progressively more pronounced particle–particle spatial correlation.

Further insights into the origin of the peak in the scattering profile are given by evolution of the peak position as a function of dendrimer concentration (filled circles). The solid line is a power law fit to the data. For comparison, the expected results for the nearest-neighbor distance corresponding to simple cubic (circles), body-centered cubic (squares), and face-centered cubic (diamonds) packing for spheres with radius \( R = 31 \text{ Å} \) are also shown.

Figure 4. (a) Effect of dendrimer concentration on the scattering profiles for G5 PAMAM dendrimers with \( \alpha = 0.6 \). The scattering data are normalized by the dendrimer volume fractions of the solutions. Symbols represent \( \phi = 0.11 \) (crosses), 0.31 (diamonds), 0.61 (squares), and 1.19 (circles). (b) Evolution of the peak position \( q^* \) as a function of dendrimer concentration (filled circles). The solid line is a power law fit to the data. For comparison, the expected results for the nearest-neighbor distance corresponding to simple cubic (circles), body-centered cubic (squares), and face-centered cubic (diamonds) packing for spheres with radius \( R = 31 \text{ Å} \) are also shown.

The peak position is related to the average interparticle distance and is indicative of a local liquidlike ordering of the macroions. It should also be mentioned that the average interparticle distances \( \sim 2\pi/q^* \) range from 120 to 250 Å over the maximum concentration range in this study (cf. Figure 4b), which is considerably greater than the particle diameter, so that the origin of the scattering peak cannot be related to the swelling of the dendrimers. Therefore, the origin of this local ordering is not the volume excluded by neutral dendrimers in solutions, as observed at higher concentrations, but is due to the presence of long-range interactions. As the charge on each dendrimer increases, electrostatic repulsions give rise to an effective electrostatic excluded volume, resulting in an average distance of closest approach between the dendrimers.

The electrostatic origin of this liquidlike ordering can be further ascertained by considering the effect of salt on the scattering profiles, as dendrimer–dendrimer interactions ought to be screened by the addition of salt. This fact is illustrated in Figure 5, which shows that with the addition of NaCl we recover progressively the scattering profile characteristic of uncharged dendrimer solutions. The screening effect is not very surprising as it is a common feature of colloidal and polyelectrolyte solutions. However, Figure 5 also shows what is perhaps the most interesting feature of these dendrimer solutions, namely the fact that even at high salt concentrations, we do not observe the onset of attractive interactions, which would lead to an increase of the scattered intensity at low \( q \).

Attempts to fit the data with a renormalized mean spherical approximation (RMSA) model including a spherical approximation for the dendrimer form factor yielded unphysical results in most cases. It was possible to find reasonable fitting parameters in better agreement with the experimental data, but only by assuming size and shape (ellipticity) variations of the dendrimers which are not consistent with the quantities measured experimentally by SAXS. Even in these cases, the overall fitted charge density was on the order of 30%–50% of the charge parameter \( \alpha \), which is consistent with previous comparisons between this model and experi-

Figure 5. Effect of the addition of NaCl to charged dendrimer solutions with \( \alpha = 0 \) and \( \phi = 0.04 \). Symbols correspond to \( C_0 = 0 \) (crosses), 5 mM (triangles down), 25 mM (triangles up), 50 mM (diamonds), 100 mM (squares), and 1000 mM (circles). Note that at 1 M of NaCl we recover the scattering profile of dendrimer solutions prior to addition of HCl.
The data are normalized by the common intercept at \( \phi = 0, I(0) \). Solid lines are linear fits to the data. Symbols correspond to samples with \( \alpha = 0.6 \) and \( C_S = 5 \text{ mM} \) (filled circles), 25 mM (squares), 50 mM (diamonds), 100 mM (triangles up), 1000 mM (triangles down), and 2000 mM (cross). Note that the circles refer to data from dendrimer in pure D_2O (\( \alpha = 0 \), no added salt). The slopes correspond to the second virial coefficient; a positive slope indicates repulsive interactions.

Figure 6. Inverse of I(0) as a function of dendrimer volume fraction ("Debye" plot). The data are normalized by the common intercept at \( \phi = 0, I(0) \). Solid lines are linear fits to the data. Symbols correspond to samples with \( \alpha = 0.6 \) and \( C_S = 5 \text{ mM} \) (filled circles), 25 mM (squares), 50 mM (diamonds), 100 mM (triangles up), 1000 mM (triangles down), and 2000 mM (cross). Note that the circles refer to data from dendrimer in pure D_2O (\( \alpha = 0 \), no added salt). The slopes correspond to the second virial coefficient; a positive slope indicates repulsive interactions.

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References and Notes

Certain commercial material and equipment are identified in this publication in order to specify adequately the experimental procedure. In no case does such identification imply recommendation by the National Institute of Standards and Technology nor does it imply that the material nor equipment identified is necessarily the best available for this purpose.

The parameters reported here were supplied by the manufacturer and were not measured at NIST.


The maximum total uncertainties can be estimated to be within 10% of the shown values.

The data were also normalized to take into account the effect of dendrimer concentration.

Since we cannot totally discard the influence of $P(q)$ at low-$q$ vectors, we did not attempt to quantify the virial coefficients. However, the $P(q)$ of the dendrimers is a constant, as shown in a forthcoming paper, so that the qualitative trend is correct.

The accepted SI unit of concentration, mol/L, has been represented by the symbol $M$ in order to conform to the conventions of this journal.

Swelling Response of PAMAM Dendrimers in Aqueous Solutions.

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