Dendrimers with Hydrophobic Cores and the Formation of Supramolecular Dendrimer−Surfactant Assemblies

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The structure of a series of poly(amidoamine) dendrimers Gn(C12) generated from a diaminododecane core have been investigated using the photophysical properties of an external dye, nile red. The modified dendrimers Gn(C12) show the ability to host the hydrophobic dye, nile red, in aqueous solution. The ability of Gn(C12) to host nile red has been compared to corresponding amino-core Gn(NH_3) and diaminoethane-core Gn(C2) dendrimers of the same generation size. The emission of nile red in aqueous media is significantly enhanced in the presence of Gn(C12) and not at all for Gn(NH_3) and Gn(C2). These results imply a strong tendency for the nile red probe to associate with the long methylene chain of the modified dendrimers in aqueous solutions. Moreover, the interactions of these dendrimers with anionic surfactants generate supramolecular assemblies which greatly enhance their ability to accomodate the nile red. Fluorescence polarization and emission as a function of pH were also studied in an effort to elucidate the interaction of the nile red probe with the dendrimer−surfactant assemblies.

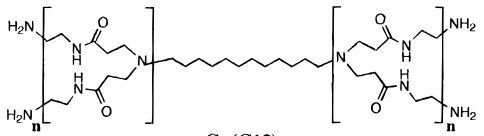
Introduction

The structure and properties of several poly(amidoamine) dendrimers have been studied extensively.[1−6] In general, these investigations report highly ordered hyperbranched arrays with an open structure for early generation dendrimers and a more dense, spheroid topology for the later generations.[1−3] In the studies reported here, we explore the effect of modifications to the dendrimer core on the overall structure and properties of macromolecules Gn(C12) and Gn(C2). The class of dendrimers composed of poly(amidoamine)s was modified, by replacing the diaminoethylene core with diaminoalkanes, i.e., Gn(C12) and Gn(C2), Scheme 1.

This modification may dramatically alter the morphology and properties of these macromolecules, by (1) incorporating a hydrophobic "site" at the core of a hydrophilic periphery, (2) making the overall morphology less compact, and thus (3) allowing access to internal amines even at larger generation sizes, through the "wedge" created by the the core.

Moreover, we expect that the modified dendrimers have potential applications in host−guest interactions. Host−guest systems made from dendrimers have enormous possibilities in the areas of membrane transport and drug delivery.[7−9] The interest in dendrimeric macromolecules Gn(C12) and Gn(C2) for such applications stems from their distinctive dual-natured properties, an external periphery bearing multiple functional groups for solubility in aqueous media combined with an internal hydrophobic core for large molecule transport. While the internal core of typical NH_3-core poly(amidoamine) dendrimers is large enough to contain small molecules,[10] it is unable to incorporate larger organic molecules potentially useful to drug delivery. Hence, in the studies reported here, the elongated methylene-based cores possess enhanced hydrophobic character with the possibility of encapsulating larger molecules. We explore this possibility by employing

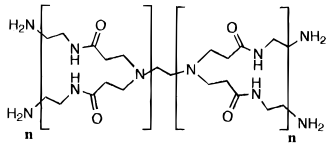
Scheme 1



Gn(C12)

C = chain length = 12
G = generation number

		Molecular Weight	Surface Groups			Molecular Weight	Surface Groups
G0(C12)	n=1	656	4	**G4(C12)**	n=5	14355	64
G2(C12)	n=3	3396	16	**G5(C12)**	n=6	28952	128
G3(C12)	n=4	7049	32	**G6(C12)**	n=7	58189	256



Gn(C2)

C = chain length = 2
G = generation number

		Molecular Weight	Surface Groups			Molecular Weight	Surface Groups
G0(C2)	n=1	517	4	**G4(C2)**	n=5	14215	64
G2(C2)	n=3	3256	16	**G5(C2)**	n=6	28826	128
G3(C2)	n=4	6909	32	**G6(C2)**	n=7	58048	256

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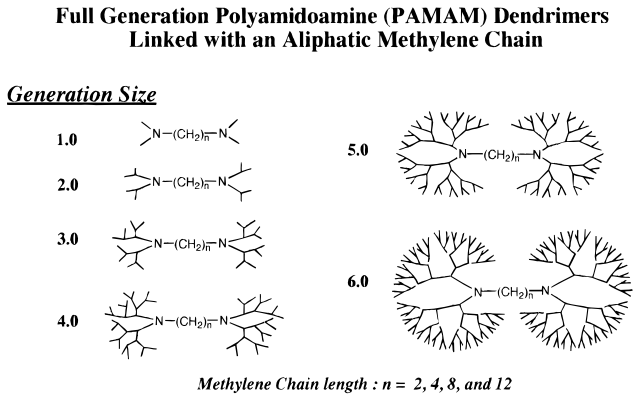


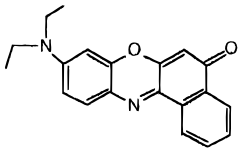
Figure 1. Structural representation of polyamidoamine (PAMAM) dendrimers modified with a methylene chain.

the photophysical properties of the hydrophobic molecule nile red as a probe.

The experiments described here probe not only the nature of the hydrophobic core in these macromolecules and morphology changes as a function of generation but also the accessibility of internal amines through examination of their electrostatic interactions with oppositely charged surfactant molecules.

Experimental Section

Materials. The synthesis of the Starburst dendrimers (SBDs) employed in this study was described in a previous paper.[3] The Gn(C12) samples used in this report were stored in methanol solution.[11] Stock solutions of the dendrimers were prepared by removing the methanol under vacuum overnight and rediluting the dendrimer in a known amount of deionized water. Sodium dodecyl sulfate (SDS) (Fisher) was recrystallized from methanol. Dodecyltrimethylammonium bromide (DTAB) (Fisher) was recrystallized from methanol. Phenoxazon-9 (trivial name: nile red) (Lambda Physik) was used as received.



(**1**) Phenoxazon-9 (trivial name: nile red)

Methods. Fluorescence spectra were recorded with a SPEX Flouromax-2 spectrofluorimeter. Fluorescence polarization was measured using a Gland Thomson Polarizer on the SPEX Fluormax-2. Absorption spectra were recorded on a Hewlett-Packard 8452A Diode Array Spectrometer. pH measurements were recorded on a Corning Combination Electrode pH meter.

Results and Discussion

1. Enhanced Emission from Hydrophobic Pocket. The macromolecules Gn(C12) and Gn(C2) were prepared via the in situ construction of branched amido amines, with varying generation sizes (0−6), around a diaminoalkane core.[12] Insertion of the chain modifies the normal globular shape of the dendrimer (Figure 1).

We speculated that the methylene chain serves as a hydrophobic environment between the two hydrophilic dendrons. To test this hypothesis, we used the hydrophobic fluorescence probe phenoxazone-9, trivially known as nile red.[13] Nile red is a hydrophobic dye that fluoresces intensely in the presence of hydrophobic lipids and organic solvents, but displays weak emission in aqueous media.[14] This property serves as key tool for analyzing the methylene-linked dendrimers Gn(Cn).

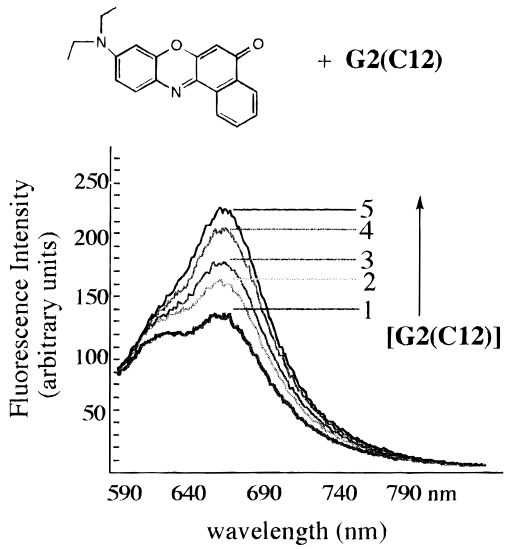


Figure 2. Emission spectra of nile red ($\lambda_{ex} = 570$ nm) following addition of dendrimer G2(C12). (1) Nile red only; (2) nile red + 6×10^{-5} M G2(C12); (3) nile red + 2×10^{-4} M G2(C12); (4) nile red + 6×10^{-4} M G2(C12); (5) nile red + 1×10^{-3} M G2(C12).

Fluorescence measurements were conducted by taking the emission spectra of nile red dissolved in aqueous solution (10^{-5} M) following each addition of an aqueous dendrimer aliquot (10^{-5}−10^{-3} M). Representative emission spectra from the addition of G2(C12) at various concentrations to nile red are shown in Figure 2. Figure 2 also shows a marked increase in the nile red fluorescence intensity upon addition of G2(C12).

Dendrimer G3(C12) also significantly enhances nile red emission, while the addition of G0(C12) and G4(C12)−G6(C12) provides a comparatively smaller emission increase. These results are consistent with the hypothesis that the methylene chain provides a hydrophobic environment for the nile red probe. However, it appears that the effective extent of hydrophobicity provided to the probe depends on the length of the chain as well as the generation size of the dendrimer added. For example, the dendrimers Gn(C8) and Gn(C4), analogous to Gn(C12), but with chain lengths of 4 and 8, for all generation sizes show no increase in emission intensity with the nile red dye, Figure 3.

Dendrimers G4(C12)−G6(C12) with the same chain length but increasing generation size, provide very little increase in emission intensity of nile red for generation sizes larger than 2.0. Presumably the larger generation sizes prevent the nile red from accessing the methylene chain core.

2. Effect of Adding Anionic Surfactants to Cationic Dendrimers. Photophysical studies of the interaction between carboxyl-terminated starburst dendrimers (NH_3-core) and cationic surfactants have been thoroughly investigated using the pyrene-probe technique.[15,16] In the NH_3-core dendrimers, close packing of surface groups on

(11) The composition of dendrimers Gn(C12) were determined by NMR analysis: 80% pure dendrimer, 20% degraded impurity. Mass spectrometry analysis indicates that the impurity is an intramolecular Michael addition of the dendrimer repeat unit with the primary amine groups of other dendrimers.

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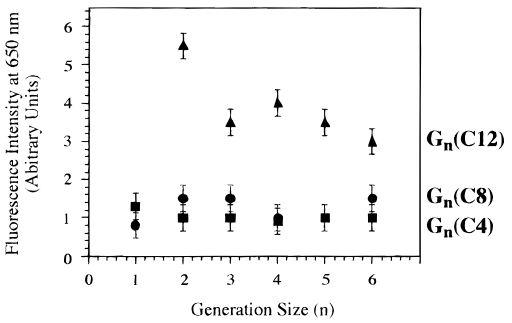


Figure 3. Fluorescence intensity of nile red at 650 nm versus generation size of dendrimers Gn(C12), Gn(C8), and Gn(C4).

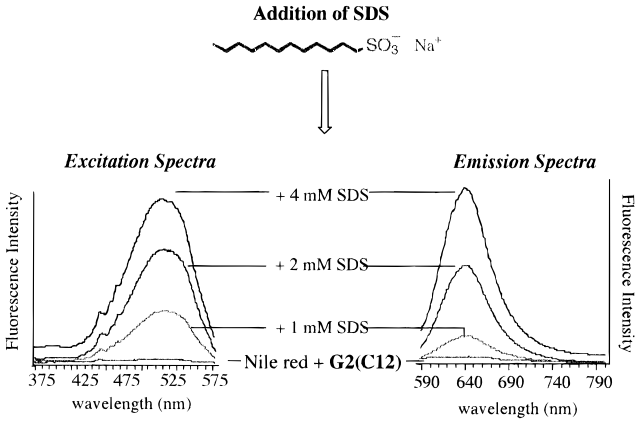
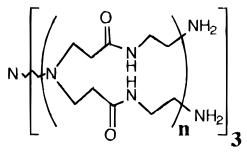


Figure 4. Emission spectra (λ_{ex} = 570 nm) and excitation spectra (λ_{em} = 650 nm) of nile red following addition of 1, 2, and 4 mM SDS.

later generation dendrimer surfaces resembled that of micelles.[15] Analogous experiments with nile red as the hydrophobic probe are used here to study dendrimers Gn-(C12) and Gn(C2) and to compare their interactions to (NH_3-core) Gn(NH_3) amine−terminated dendrimers of similar generation size. Preliminary experiments were carried out using the anionic surfactant SDS and Gn-(C12). These were followed with experiments involving SDS and amino-core dendrimers Gn(NH_3), as well as with Gn(C2) bearing a chain length of 2.



Gn(NH3)

G = generation

	Molecular Weight	Surface Groups
G1(NH3): n = 1	359	3
G2(NH3): n = 3	2414	12
G3(NH3): n = 3	5154	24
G4(NH3): n = 3	10633	48
G5(NH3): n = 3	21591	96
G6(NH3): n = 3	43508	192

The results of typical fluorescence emission and excitation spectra versus concentration of SDS added to G2-(C12) are shown in Figure 4. The cmc of SDS in aqueous solution is 8×10^{-3} M.

In this experiment, aliquots of SDS were added to an aqueous solution of nile red (5×10^{-5} M) and G2(C12) (8×10^{-4} M, in terminal groups, i.e., the concentration of functional groups remained the same throughout the series of different generations). Each spectrum exhibits a significant increase in nile red intensity with each increase in SDS concentration (1−6 mM). A plateau was reached at 7 mM SDS.

Performing the same experiments with dendrimers Gn-(NH_3) and Gn(C2), then converting all of the spectra into a plot of intensity (at the λ_{max} of nile red: 650 nm) versus the generation size of Gn(C12) at each concentration of SDS added is shown in Figure 5.

In Figure 5A, there is minimal change in nile red intensity with SDS and Gn(NH_3) across the series of generation sizes from 1.0 to 6.0. This same trend is seen in Figure 5B for the addition of SDS to aqueous solutions of nile red (5×10^{-5} M) and Gn(C2).

In Figure 5C, the plot shows dramatic increases in the nile red intensity in the presence of Gn(C12) upon addition of SDS. At 1 mM concentration of SDS [well below the critical micelle concentration (8mM)], the overall intensity of nile red is 10 times greater than with the addition of Gn(NH_3) or Gn(C2). The intensity continues to increase with a final plateau at 7 mM SDS. This dramatic increase suggests that the methylene chain of Gn(C12) lends additional sites of aggregation to the SDS. Most probably these sites are from the interaction of the hydrophobic chains of the SDS with the hydrophobic core. In dendrimers Gn(C12), the methylene chain prevents close packing of the surface groups causing them to manifest properties different from dendrimers Gn(NH_3) or Gn(C2).

The trend across the generation series exhibits a sharp increase at generation sizes beyond $G = 3.0$, followed by a decrease at $G = 4.0$, and leveling out at $G = 5.0$. This breaking point at G3(C12) indicates that this structure allows for the greatest amount of SDS molecules to access the methylene core. Beyond G3(C12), the methylene core is not as accessible as the generation size increases and the structure becomes more tightly packed. It is important to note however, that the intensity at generation size 3 for Gn(C12) is still significantly larger than that of dendrimers Gn(NH_3) or Gn(C2) of the same generations. This indicates that the methylene core of Gn(C12) is accessible, even at higher generations.

These results were compared with the change in intensity upon addition of a cationic surfactant, dodecyltrimethylammonium bromide (DTAB). DTAB's positive charge on the tertiary amine is likely to be repelled by the cationic dendrimers G2(C12), but it's hydrophobic dodecyl chain is inclined to be weakly attracted to the hydrophobic chain linking the dendrons. As predicted, we see only a slight increase (1% compared to that of SDS) of nile red intensity upon addition of DTAB. This slight increase over that of the dendrimer alone is surmised to result from the interaction of nile red with the DTAB and not from an interaction of DTAB with the dendrimer.

3. Polarization Experiments. Steady-state fluorescence polarization experiments with polarized excitation light provide information concerning the rotational dynamics of molecules in a restrained or polarized environment.[17,18] With this tool we can probe the rigidity of the environment (on the time scale of the emission) provided to the nile red by either the dendrimer, or the combination of dendrimers and micelles as discussed in section 2. Thus, the degree of polarization will serve as a measure of the nile red molecules bound rigidly and aligned with either the dendrimer or the dendrimer-micelle complex.

The steady-state polarization is given by the equation[18]

$$P = \left[\left(\frac{R_v}{R_h} - 1\right)\Big/\left(\frac{R_v}{R_h} + 1\right)\right]$$

where $R_v = I_{vv}/I_{hv}$ and $R_h = I_{vh}/I_{hh}$. The terms I_{vv} and I_{vh} are the intensities of the vertically polarized emission from

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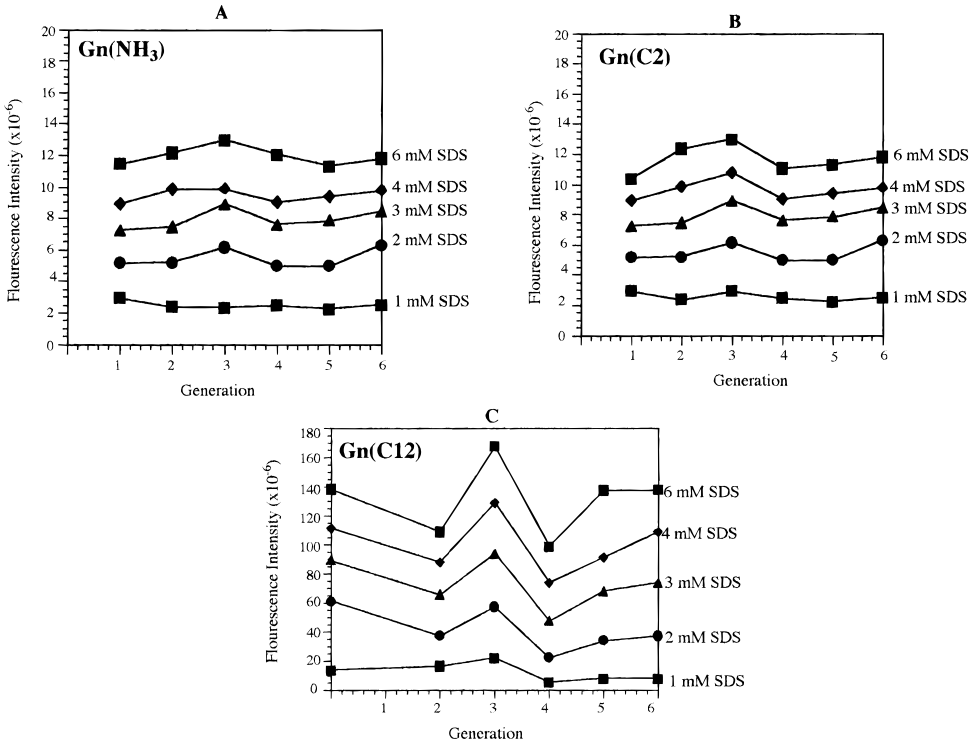

Figure 5. Fluorescence intensity of nile red [$\lambda_{ex} = 570$ nm, $\lambda_{em} = 650$ nm)] versus generation size at varying concentrations of SDS. (A) nile red + Gn(C2) + SDS; (B) nile red + Gn(C12) + SDS.

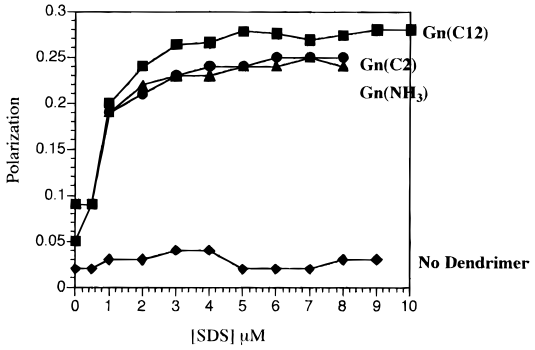

Figure 6. Polarization of nile red emission (in the presence of dendrimer, 8×10^{-4} M) versus concentration of SDS added: (1) SDS only; (2) SDS + G2(NH_3); (3) SDS + G2(C2); (4) SDS + G2(C12).

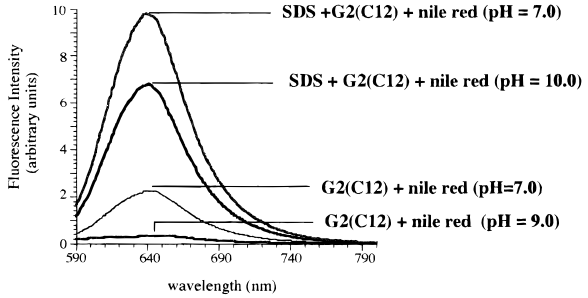

Figure 7. Fluorescence intensity of nile red ($\lambda_{ex} = 570$ nm) in the presence of SDS + G2(C12) at pH 7 and 10.

irradiation with vertically and horizontally polarized light, respectively. I_{hv} and I_{hh} are the intensities of the horizontally polarized emission from irradiation with vertically and horizontally polarized light, respectively. The numerical polarization value was automatically calculated in the SPEX polarization software program and is used directly in Figure 6, comparing dendrimer concentration and polarization.

For free nile red in water, the value of P is low, i.e., approximately 0.02−0.05. Upon addition of dendrimer, G2(C12), the polarization increased to 0.09. Following addition of a 1 mM aliquot of SDS, the polarization increased dramatically from 0.09 to 0.2, and increased gradually to a plateau at 0.3, as shown in the curve with ■, Figure 6.

The change in polarization of G2(C12) was compared to dendrimers Gn(NH_3) and G2(C2), curves with ▲ and ●, respectively. In both cases, we see no increase in polarization upon addition of the dendrimer, only a marked increase upon addition of SDS. This increase, however, is still less than that for G2(C12).

4. pH Studies. Because the dendrimers discussed here have a high surface coverage of primary amines, they are highly soluble in aqueous solutions, which also makes them highly sensitive to the pH of their environment.[1,19] For example, aqueous solutions of amine terminated PAMAM dendrimers having a typical pH of 11−11.5 contain a mixture of primary (NH_2) and ammonium (NH_3^+) amines.[20] At a pH of 7.0, the environment is acidic enough to protonate all (1000 protonated:1 nonprotonated) of the primary amines to their ammonium form. In the case of the dendrimers with a methylene chain linking the two wedges, the number of cationic (ammonium) groups on the surface of the dendrimer will have a direct effect on the overall macromolecular structure; full protonation should elongate the chain via repulsive forces from the two wedges, while partial protonation does not. Such structural features can relate directly to the function of the macromolecule. Thus, fluorescence emission from dendrimers at pH 7 show a higher emission than those at pH 9 since the lower pH affords a more elongated dendrimer morphology able to accomodate more dye molecules. At pH 9, the "contracted" dendrimer morphology inhibits a large amount of dye molecules from entering the interior of the dendrimer. Hence, fluorescence ex-

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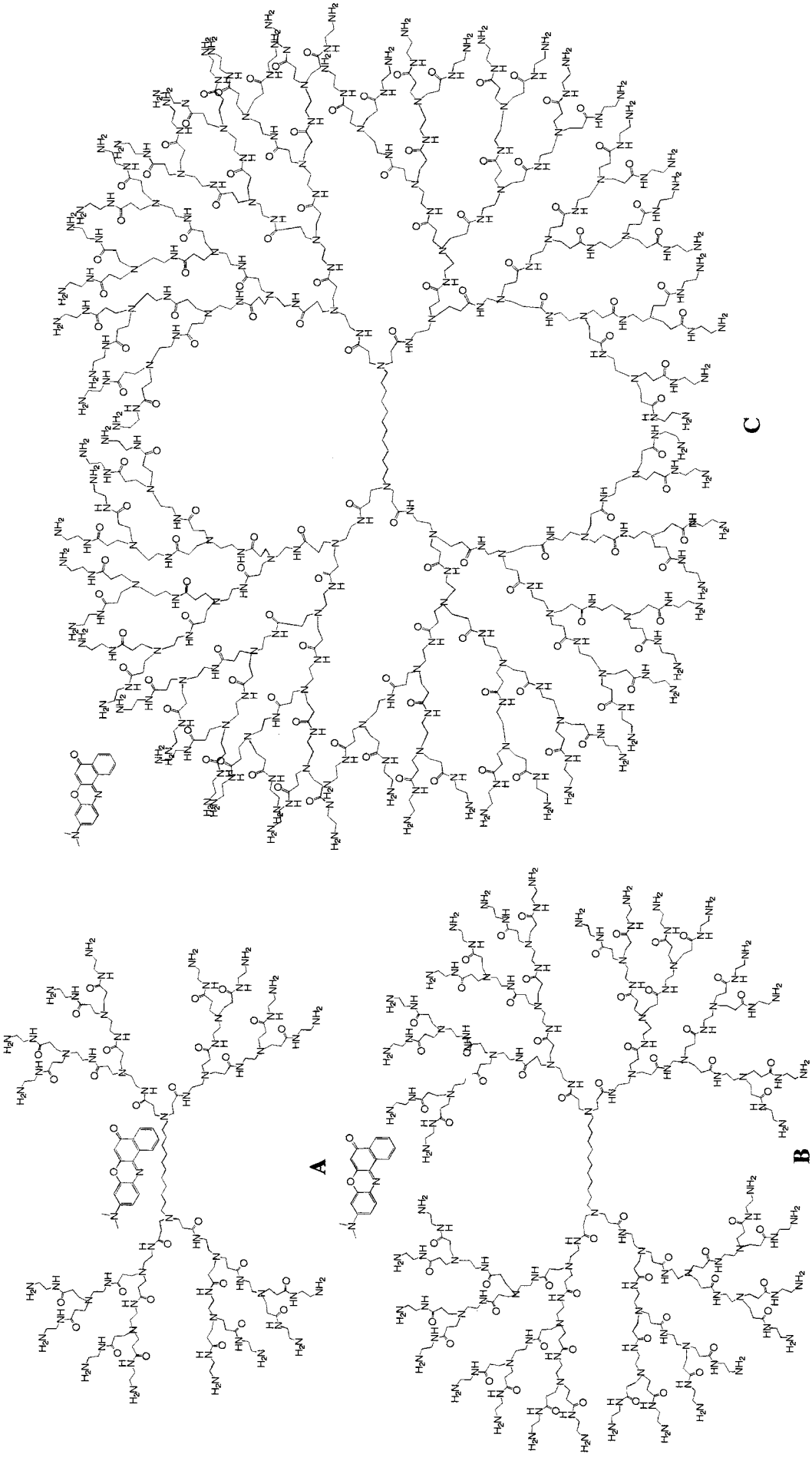

Figure 8. Two-Dimensional structures of dendrimers (A) G2(C12), (B) G3(C12), and (C) G4(C12) and their proposed interaction with the nile red probe.

periments with nile red were carried out at pH 7.0 to test the effect of pH on macromolecular structure and its interaction with the hydrophobic probe nile red. Comparisons of the emission from nile red, at pH 10−7, show a 20% enhancement of the emission at the lower pH, Figure 7. By comparison, the emission of nile red with SDS alone at pH 7 and 10 is similar to that of G2(C12) at pH 10.

Control experiments verify that the emission of nile red in the absence of dendrimer is the same at both pH values. The pK_a of the primary amines is 10, whereas the pK_a of the tertiary amines is 4.5; hence, we know that the interior amines are fully protonated at pH 7.0. Protonation of the internal amines provides additional sites for the SDS to bind electrostatically. Thus, the interaction of the internal amines and SDS supply additional hydrophobic sites for the nile red to occupy that are not available to the closed shell dendrimers Gn(NH_3) or Gn(C2).

Conclusions

The difference in emission spectra in Figure 3 gives strong evidence that the methylene chain of dendrimers Gn(C12) provides a hydrophobic "environment" to the nile red, for the longest chain (C = 12), but not for the shorter chains (C = 4, 8). This unique feature provides a new type of dendrimer capable of hosting small organic molecules in aqueous media. Such properties are key components to the transport of biological molecules. A schematic showing two-dimensional models of dendrimers G2(C12), G3(C12), and G4(C12) and their proposed interaction with the nile red probe is shown in Figure 8. In this drawn-to-scale model, the nile red probe is able to access the long methylene chain for G2(C12), Figure 8A, but not for G3(C12) and G4(C12), Figure 8, panels B and C. For the latter two dendrimers, the larger generation dendrimer dendrons effectively close and block access of the nile red probe to the methylene chain. Hence, these dendrimers are sterically less capable of hosting hydrophobic molecules than those of generation 2.

Combining dendrimers Gn(C12) with anionic surfactants, we have created noncovalently bound supramolecular assemblies with an even greater ability for hosting organic molecules in aqueous media, than the dendrimers themselves. Some proposed structures of these supramolecular assemblies are shown in Figure 9. In comparison with Gn(NH_3) and Gn(C2), dendrimers Gn(C12) exhibit 10 times greater enhanced emission of the hydrophobic dye nile red. This is rationalized in the structure of the proposed assemblies, Figure 9, panels A and B, for G2(C12) and G2(C2), respectively, with SDS in the presence of nile red. Since the exterior number of functional groups of Gn(C2) available for electrostatic binding is the same as in Gn(C12), we attribute the enhancement in emission with Gn(C12) to its more open core structure (Figure 8A). This open structure provides anionic surfactants access to interior tertiary amines not accessible in dendrimers Gn(NH_3) and Gn(C2).

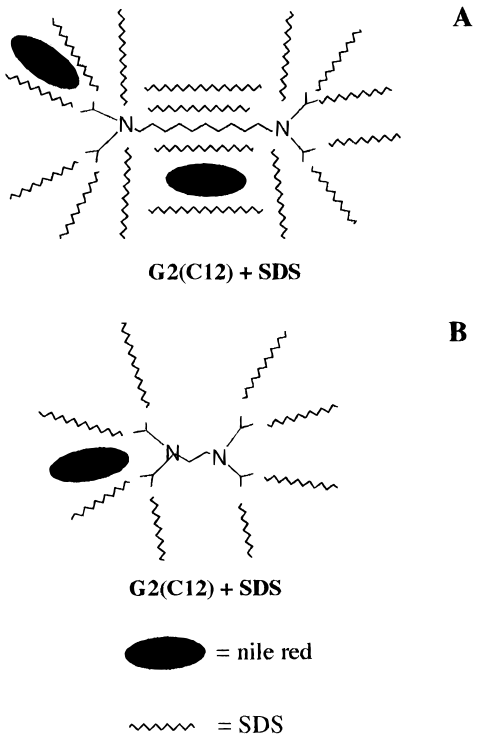

Figure 9. Proposed structures for interaction between dendrimers Gn(C12) or Gn(C2) and SDS with the nile red probe. (A) Gn(C12) + SDS + nile red and (B) Gn(C2) + SDS + nile red.

We attribute the excess polarization of G2(C12) to the chain linking the two dendrimer wedges. Ultimately, binding of the probe to the chain provides restriction of motion and a degree of polarization to the nile red dye, not available in the case where the chain is absent.

The enhanced emission at lower pH values suggests that pH is a useful tool to adjust the macromolecular structure of dendrimers Gn(C12). In their "all-protonated" form (at pH 7.0), the wedges of dendrimers Gn(C12) are repelled to a greater degree than at pH 10, allowing for an expanded form of the dendrimer and greater access to the methylene chain core. In interactions with surfactants, the lower pH serves to create more positively charged sites for interaction with the anionic head groups.

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