Spectroscopic Investigations of Core-Based, Randomly Hyperbranched Polymers and Comparison with their Dendrimeric Counterparts

Katrina K. Kline and Sheryl A. Tucker*

Department of Chemistry, 125 Chemistry Building, University of Missouri, Columbia, Missouri 65211

Received: July 13, 2009; Revised Manuscript Received: September 22, 2009

Randomly hyperbranched polymers containing a core region are a relatively new subclass of materials. In comparison to dendrimeric polymers, there are many similarities, including their proposed applications. Because the core-based hyperbranched polymers can be prepared in a one-pot synthesis, they are an attractive alternative to the dendrimers, which require a laborious multistep process. This research is centered around the comparison of core-based, randomly hyperbranched poly(ethyleneimine) with the dendrimeric poly(propyleneimine) to better understand their host−guest properties. Two sizes of each polymer class were examined, and studies were carried out using the solvatochromic reporter molecule phenol blue. Absorbance and fluorescence measurements (emission and anisotropy) were utilized to determine the location of the fluorophore within the polymer. Results indicate that the phenol blue does associate with both the hyperbranched poly(ethyleneimine) and the dendrmeric poly(propyleneimine), although the association is not the same for the two polymer classes. The loading capacity of both polymer classes was also determined.

Introduction

Traditional randomly hyperbranched polymers have limited applications due to their large molecular weight distributions and wide range of polydispersities. Such polymers generally have a degree of branching between 50 and 75% and are nonsymmetrical molecules.[1−4] Recently, a new subclass of randomly hyperbranched polymers, containing an initiator core molecule similar to that of the dendrimeric polymers, has been synthesized on an industrial scale.[5] Understanding these core-based randomly hyperbranched polymers (CBHP) is important because their unique structure results in many of their proposed applications being very similar to those of their dendritic cousins.

Dendrimers are a class of polymers that have well-defined macromolecular structures. They are synthesized in a labor-intensive process of iterative additions of monomeric branching units, called generations (G), which emanate from or are later attached to a core atom or molecule. In contrast to the hyperbranched polymers, dendrimers have a degree of branching of 100%.[6] There is a wide range of applications for dendrimers, including drug transport,[7] gene transport systems,[8] high-loading supports for organic synthesis,[9] water purification systems,[10] and molecular nanocarriers.[11] However, due to their labor-intensive synthesis and the resulting limited availability in bulk quantities, large-scale use of dendrimers is limited.

Core-based hyperbranched polymers are a potentially attractive alternative to dendrimers because they have been and can be prepared using a one-pot synthesis with a maximum of two steps with a polydispersity range of 1.3 to 2.5. A higher polydispersity number indicates a wider range of molecular weights, with dendrimers having a polydispersity of one.[12] Pictorially, they can be thought of as "irregular" dendrimers — those with some branches missing (Figure 1). Core-based hyperbranched polymers have similar structural features as compared to dendrimers, which makes them an interesting material. Aside from both containing a core region, the CBHP also have a globular three-dimensional architecture, "interior cavities", and the availability of a large number of functional groups.[13−19] These architectural features are responsible for the larger number of proposed applications of these advanced materials that mirror those of dendritic polymers.

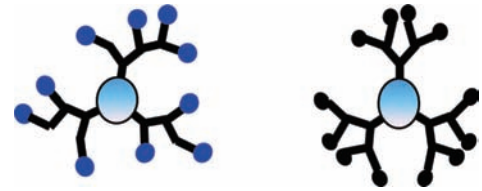

Figure 1. Representation of a core-based hyperbranched polymer (left) and dendrimer (right).

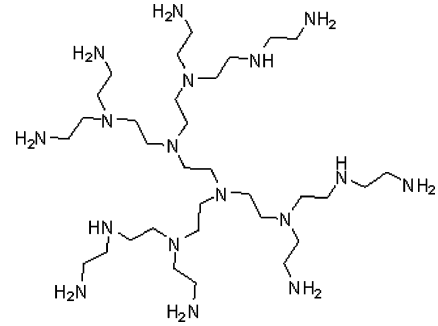

Figure 2. Molecular structure of the CBHP poly(ethyleneimine), PEI.

Using spectroscopic investigations, this research compares the host−guest properties of CBHP poly(ethyleneimine) (PEI, Figure 2) to its dendritic counterpart, poly(propyleneimine) (PPI, Figure 3) to determine if the hyperbranched PEI is, indeed, a possible alternative for the significantly more expensive dendritic PPI. The widespread use of materials is driven by their availability. Although dendritic poly(ethyleneimine) has been synthesized,[35] the PPI dendrimers were chosen for comparison with hyperbranched PEI due to commercial availability, as well as their structural similarities. For example, both polymers

* Corresponding author. TuckerS@missouri.edu.

10.1021/jp906609e CCC: $40.75
Published on Web 10/13/2009

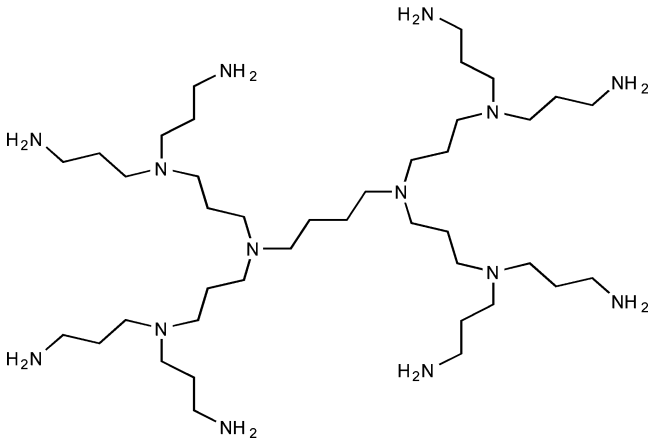

Figure 3. Molecular structure of dendritic poly(propyleneimine), PPI.

TABLE 1: Polymer Properties for Comparison of Hyperbranched PEI to Dendritic PPI

polymer	MW (g/mol)	core size	surface groups	mmonomer units	polydispersity
dendritic PPI G4	3513	C4	32	50	1
hyper PEI-5	~5000	C2	35	115	1.3
dendritic PPI G5	7161	C4	64	114	1
Hyper PEI-25	~25 000	C2	174	580	2.5

consist of repeating amine units, although the dendritic PPI has a longer carbon chain both at the core and in the branching units. Both polymer families are available in a few sizes (generations, G) with amine terminal groups. On the basis of availability and similarities, hyperbranched PEI-5 and -25 are compared to dendritic PPI G4 ([core: diaminobutane (DAB)]; ($G = 4$); {dendri-poly(propyleneimine)-$(NH_2)_{32}$}) and G5 ([core: DAB]; ($G = 5$); {dendri-poly(propyleneimine)-$(NH_2)_{64}$}). Table 1 denotes the abbreviations used and shows the relative relationship between polymer properties. For example, PEI-5 and PPI G4 have similar numbers of surface groups; PEI-5 and PPI G5 have comparable molecular weights and monomer units; and with its smaller core (C2 vs. C4), PEI-25 may afford similar interior space to PPI G5. Moreover, the host−guest properties of the PPI dendrimers have already been extensively studied.[20,28,31,34] Understanding how the unique chemical and physical properties of CBHP affect their interactions with guest molecules could provide critical information regarding proposed applications.

Studies are carried out using the fluorescent probe phenol blue (PB), which has been previously shown to associate with the core region of the dendritic PPI.[20] The solvatochromic nature of PB makes it very useful for reporting on the interior polarity regions of the polymers, and this property is exhibited in both the absorption and fluorescence emission spectra. The physical[21−23] and spectroscopic absorption properties[24−27] of PB have been well-characterized, and the dye is believed to exist as the neutral quinoneimine[21] in both protic and aprotic solvents of varying polarities. As reported previously,[28] the solvatochromic absorption band of PB shifts to longer wavelengths with increasing solvent polarity and has a λ_{max} range from 652 nm in polar water to 541 nm in nonpolar *n*-heptane. The solvatochromic fluorescence emission band also shifts to longer wavelengths with increasing polarity and has a λ_{max} range from 620 nm in methanol to 602 nm in acetone.[28] The dye's fluorescence quantum yield decreases at the solvent polarity extremes, which leads to the signal's becoming virtually absent under typical experimental conditions.

Previous studies with PB compared similar dendritic polymer families to understand class-specific properties.[20] This work extends those studies to a structurally similar material: core-base hyperbranched polymers. Although there has been considerable investigation into the ability of dendrimers to act as host molecules, to date, there have been no parallel investigations into the unmodified CBHP PEI.

Experimental Section

Materials. Materials were obtained from the following suppliers and used as received: PB and amine-terminated PPI dendrimers with tetrafunctional diaminobutane cores (Aldrich, Milwaukee, WI); tetrahydrofuran, toluene and HPLC-grade water (Fisher, Pittsburgh, PA); and CBHP PEI (Hyperpolymers, North Rhine-Westphaila, Germany). The polymers were obtained as neat liquids and stored at 5 °C.

Sample Preparation. Stock solutions of PB were prepared by dissolving the dye in tetrahydrofuran and were stored in the dark at room temperature. Samples were prepared by quantitatively transferring known aliquots of the PB stock solution into volumetric flasks, where the solvent was stripped off under ultrahigh purity nitrogen. Appropriate volumes of hyperbranched PEI or dendritic PPI were then quantitatively transferred to the flask and diluted to volume with HPLC-grade water. The samples can be divided into two concentration groups: excess polymer and excess dye. Final concentrations of the excess polymer samples are [PB] $= 1 \times 10^{-6}$ M and [polymer] $= 1 \times 10^{-4}$ M. Final concentrations of the excess dye samples are [PB] $= 5 \times 10^{-5}$ M and [polymer] $= 5 \times 10^{-6}$ M. In the dark at room temperature, samples were stirred for ~24 h to facilitate dye and polymer solubilization, followed by an additional ~24 h equilibration period. For samples containing excess PB, the dye was extracted using three 0.5 mL aliquots of toluene after initial measurements were made. Postextraction samples were allowed to re-equilibrate ~24 h in the dark at room temperature.

Methods. ***UV/vis Absorption Measurements.*** Absorption spectra were collected using 1-cm^2 Suprasil quartz cuvettes (Hellma, Forest Hills, NY) on a Hitachi U-300 (Hitachi Instruments, Danbury, CT) double-beam spectrophotometer with a scan rate of 120 nm/min, slit width of 1 nm, and a thermostatted cell temperature of 25 °C. Spectra were blank-corrected for possible absorption of the solvent and aqueous polymers, although there was no visible background signal at the wavelength of interest due to the polymers.

PeakFit 4.0 for Windows (AISN Software, SPSS Science, Chicago, IL) was used to deconvolute the overlapping bands present in the absorption spectra of samples containing excess PB. The absorption bands of aqueous and polymer-associated PB were set at 654 and 556 nm, respectively. A two-peak model was used for most of these samples, and the width, amplitude, and shape of the bands were allowed to vary according to best fit.

Fluorescence Measurements. The fluorescence emission spectra and anisotropy data were collected in the same cuvettes on an SLM 48000 DSCF/MHF spectrofluorometer (Jobin Yvon, Edison, NJ) at a thermostatted cell temperature of 25 °C. The excitation source was an Ion Laser Technology (Salt Lake City, UT) RPC-50-220 argon ion laser operated at 514 nm and 30 mW.

For fluorescence emission spectra, the emission monochromator slit widths (entrance and exit) were set at 16 and 8 nm, respectively. The emission scan interval was 1 nm, and measurements were recorded from an internal average of five signal samplings per emission wavelength. The fluorescence emission was passed through a Glan Thompson polarizer set at

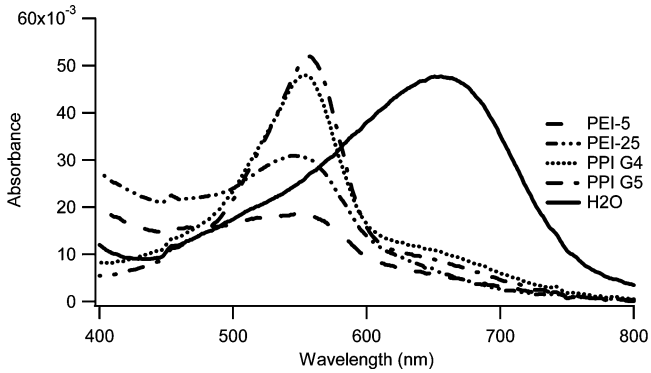

Figure 4. Representative absorption spectra of [1 × 10^{-6} M] PB in aqueous [1 × 10^{-4} M] dendritic PPI and hyperbranched PEI.

0° (vertical) to correct for the Woods anomaly.[29] Spectra were also absorption- and solvent-blank corrected.

Anisotropy measurements were collected in "L" format[30] using Glan Thompson polarizers, a 550-nm long pass filter (KV550 Schott Glass Technologies, Duryea, PA), and a 610-nm short pass filter (03 SWP 610 Melles Griot, Irvine, CA). The means and standard deviations were calculated from five sample replicates, each containing an internal average of five signal samplings at each of the four polarizer orientations (0−0°, 0−90°, 90−0°, and 90−90°).

Results

Samples can be divided into two categories on the basis of the polymer/dye ratio examined: excess polymer and excess dye—100:1 and 1:10, respectively. These ratios were chosen on the basis of the experimental studies with the aforementioned dendrimer families, the solubility of the solution components, and experimental conditions suitable for spectroscopic studies. The results obtained are discussed below.

Excess Polymer. ***UV/vis Absorption Spectroscopy.*** Representative absorption spectra of PB in water and aqueous polymer solutions are shown in Figure 4. All polymer-containing samples show one main absorption band centered around 550 nm, which is due to a relatively nonpolar nanoenvironment and is similar to that of PB in neat *n*-heptane. The absorption spectrum of PB in water consists of a single band centered around 650 nm, a much more polar nanoenvironment than is seen with the polymer-associated PB. This wavelength is indicative of only aqueous PB, in agreement with the solvent polarity scale of PB. In the presence of the dendritic polymers, the PB absorbance is significantly enhanced compared to the hyperbranched samples. In addition, a previously noted, potential ground-state interaction with amine groups (absorbance ∼400 nm) is also more prominent in the presence of the hyperbranched PEI polymers.[28]

Fluorescence Emission Spectroscopy. Representative fluorescence spectra, corrected for the solvent and differential absorption at the excitation wavelength, are shown in Figure 5. In the presence of the polymers, a single, broad solvatochromic fluorescence emission band is present for PB. There is a blue shift (∼15 nm) of this band in both PPI generations, indicating decreased nanoenvironmental polarity, as compared to that in both PEI samples (∼580 vs ∼595 nm). There is also a pronounced increase in the relative fluorescence intensity for the dendritic PPI samples.

Fluorescence anisotropy, which is an indication of the rotational freedom of a fluorophore during the excited state, is an important tool in understanding host−guest chemistry.[30]

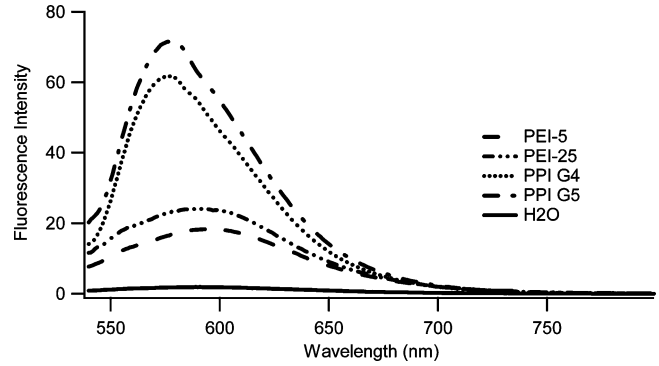

Figure 5. Representative fluorescence emission spectra of [1 × 10^{-6} M] PB in aqueous [1 × 10^{-4} M] dendritic PPI and hyperbranched PEI (HV = 795).

TABLE 2: Fluorescence Anisotropy Values of PB [1 × 10^{-6} M] in Aqueous Dendritic PPI [1 × 10^{-4} M] and Hyperbranched PEI [1 × 10^{-4} M], $n = 3$ Measurements

solvent	anisotropy, r ($\pm\sigma$)
water	0.087 (±0.003)
dendritic PPI G4	0.235 (±0.007)
hyperbranched PEI-5	0.208 (±0.005)
dendritic PPI G5	0.275 (±0.008)
hyperbranched PEI-25	0.246 (±0.004)

Anisotropy values of the PB solutions are shown in Table 2. For PB, values approaching zero indicate an unhindered rotator, whereas values approaching ∼0.32 represent a highly constrained nanoenvironment.[30] Consistent with prior studies, these values range from 0.087 for uncomplexed PB in water to 0.275 for PB associated in the core region of dendritic PPI.[28] As shown, there is an increase in anisotropy values as the size of the polymer increases for both polymer types; however, in general, PB in the PPI dendrimers has overall higher anisotropy values than PB in the hyperbranched PEI polymers.

Excess Dye. To examine the maximum uptake capacity of the polymers under normal experimental conditions, dye/dendrimer association was allowed to occur in the presence of a 10-fold excess of PB.

UV/vis Absorption Spectroscopy. Representative absorption spectra of PB in water and aqueous polymers are shown in Figure 6. The initial absorption spectra of all samples under investigation contain one broad peak centered around 654 nm, indicative of aqueous PB, which is in 10-fold excess relative to the polymers. The absorbance spectrum of hyperbranched PEI-25 contains the only evidence of associated PB, a slight peak centered around 551 nm, noted during deconvolution.

Fluorescence Emission Spectroscopy. Representative initial fluorescence spectra, corrected for the solvent and differential

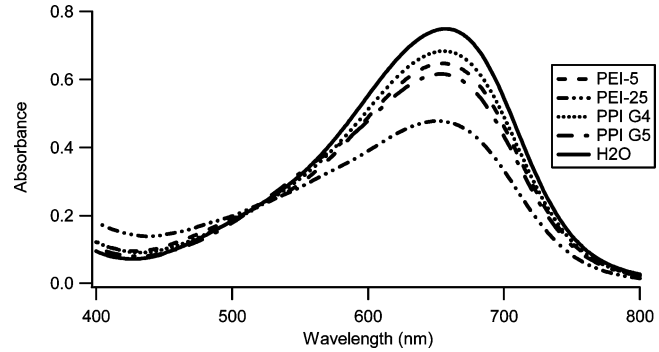

Figure 6. Representative initial absorbance spectra of [5 × 10^{-5} M] phenol blue in aqueous [5 × 10^{-6} M] polymers.

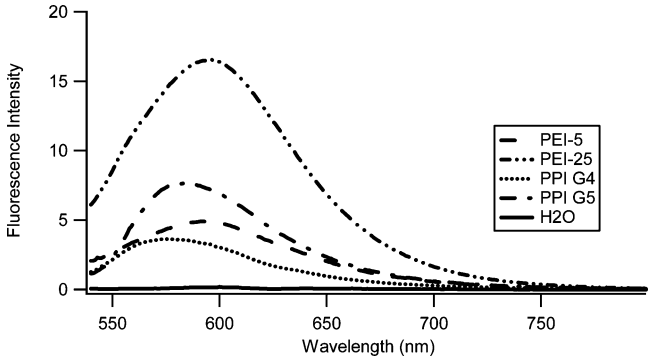

Figure 7. Representative initial fluorescence emission spectra of [5 × 10^{-5} M] PB in aqueous [5 × 10^{-6} M] dendritic PPI and hyperbranched PEI (HV = 445).

TABLE 3: Initial and Postextraction Anisotropy Values for PB [5 × 10^{-5} M] in Aqueous Dendritic PPI [5 × 10^{-6} M] and Hyperbranched PEI [5 × 10^{-6} M], $n = 3$ Measurements

aolvent	pre-extraction anisotropy, r ($\pm\sigma$)	postextraction anisotropy, r ($\pm\sigma$)
water	0.083 (±0.003)	0.068 (±0.004)
dendritic PPI G4	0.221 (±0.004)	0.22 (±0.01)
hyperbranched PEI-5	0.209 (±0.006)	0.213 (±0.008)
dendritic PPI G5	0.254 (±0.004)	0.237 (±0.003)
hyperbranched PEI-25	0.241 (±0.009)	0.231 (±0.007)

absorption at the excitation wavelength are shown in Figure 7. The fluorescence emission spectra all consist of a single broadband, indicating association of PB has occurred for all polymers. As with the excess polymer samples, the blue shift in the dendritic samples (~15 nm) is also observed relative to the emission of the CBHP (~580 vs ~595 nm). However, the fluorescence trend has changed relative to that seen in the excess polymer ratio studied. The dendrimers no longer have the highest fluorescence emission; instead, it occurs in the hyperbranched PEI-25 sample.

Initial anisotropy values of the PB solutions are shown in Table 3. These values range from 0.083 for aqueous PB to 0.254 in dendritic PPI G5. Again, the same trends are observed as seen in the excess polymer study; however, the anisotropy value for the larger hyperbranched PEI-25 is much closer to that of dendritic PPI G5, with a difference of only 0.013 for the excess dye samples as compared to a difference of 0.031 seen in the excess polymer study.

Excess Dye Postextraction. To examine the nature of the dye/dendrimer association, the samples containing excess dye were extracted with toluene. Toluene was chosen on the basis of previous work that determined that PB is readily soluble in this solvent, but the polymers are not.[34]

UV/vis Absorption Spectroscopy. Representative postextraction absorption spectra of PB in water and aqueous polymers are shown in Figure 8. After the removal of unassociated dye with toluene, the absorbance spectra appear slightly different from those of the excess polymer study: a peak centered around 550 nm, indicative of associated PB, is seen for all samples. However, in the presence of the smaller polymers, hyperbranched PEI-5 and dendritic PPI G4, both PB samples contain a second peak centered around 650 nm, indicative of aqueous PB. The appearance of this second peak implies that not all of the aqueous PB was removed from the sample during extraction. The ground state interaction of PB with the amine groups of the polymers (~400 nm) is still in evidence and is similar to that seen in the excess polymer studies for all samples under investigation. Deconvolution of the spectra reveals that there is some contribution from toluene-solvated PB (~570 nm) to the overall absorption spectra.

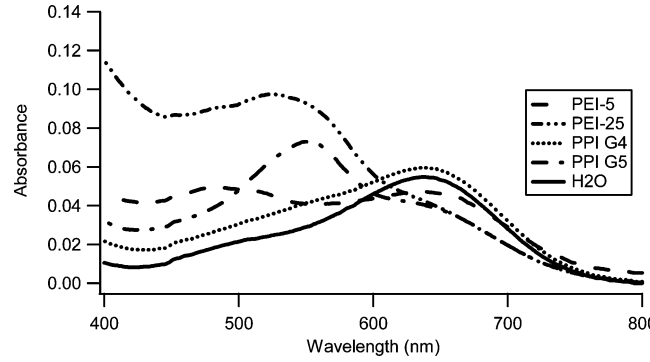

Figure 8. Representative postextraction absorption spectra of [5 × 10^{-5} M] PB in aqueous [5 × 10^{-6} M] dendritic PPI and hyperbranched PEI.

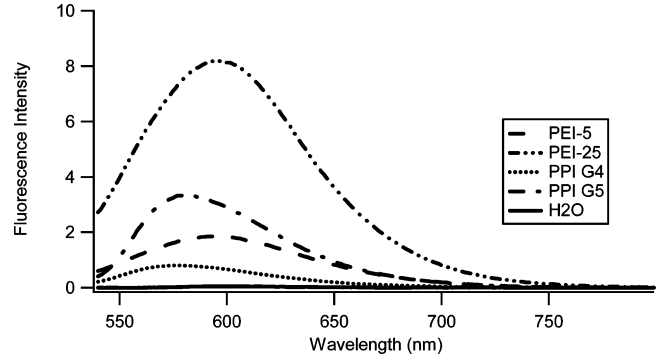

Figure 9. Representative postextraction fluorescence emission spectra of [5 × 10^{-5} M] PB in aqueous [5 × 10^{-6} M] dendritic PPI and hyperbranched PEI (HV = 445).

Fluorescence Emission Spectroscopy. Representative postextraction fluorescence spectra, corrected for the solvent and differential absorption at the excitation wavelength are shown in Figure 9. After removal of unassociated PB with toluene, fluorescence emission spectra are similar to that of the samples prior to extraction, although all show a slight intensity decrease from the initial measurements, most likely due to aging of the sample. The wavelength of emission of PB in the polymers remains the same as in the initial measurements, with the blue shift of the dendrimers as compared to the CBHP (~580 vs ~595 nm) still evident. The hyperbranched PEI-25 continues to have the highest fluorescence emission of the polymers under investigation.

Postextraction anisotropy values of the PB solutions are also shown in Table 3. Anisotropy values range from 0.068 for aqueous PB to 0.231 for dendritic PPI G5. Although the overall trends in the data are similar, these older samples have decreased anisotropy values, which could be attributed to contributions from aqueous or toluene-solvated PB. The previously observed difference in anisotropy values for comparably sized dendritic and CBHP is no longer present: they are statistically the same. For example, the CBHP PEI-5 has an anisotropy value of 0.21, which is very similar to that of dendritic PPI G4 at 0.22.

Using Beer's Law as previously described,[20,34] the number of PB molecules encapsulated in the polymers was quantified. The molar absorptivities were calculated for associated dye from Beer's Law plots of varying dye concentrations in dendritic PPI G5 and hyperbranched PEI-5 and 25, as described in the literature.[20,34] The number of PB molecules encapsulated with both the dendritic PPI and hyperbranched PEI, as well as their

TABLE 4: Ratio of PB molecules entrapped in a single polymer and binding constants[a]

polymer	calcd PB concn	PB/polymer ratio	K_D/1000
dendritic PPI G4	9.84×10^{-6}M	2:1	40
hyperbranched PEI-5	1.07×10^{-5}M	2:1	43
dendritic PPI G5	1.59×10^{-5}M	3:1	64
hyperbranched PEI-25	2.62×10^{-5}M	5:1	105

[a] Molar absorptivities: $\varepsilon_{\text{PEI-25,556 nm}} = 4700$ L·mol^{-1}·cm^{-1}; $\varepsilon_{\text{PEI-5, 556 nm}} = 3800$ L·mol^{-1}·cm^{-1}; $\varepsilon_{\text{PPI, 554 nm}} = 6800$ L·mol^{-1}·cm^{-1}, $\varepsilon_{\text{PB, 654 nm}} = 17500$ L·mol^{-1}·cm^{-1}).

binding constants are found in Table 4. All polymers show a dye/polymer ratio greater than 1:1. Previous investigations of PAMAM dendrimers illustrated their ability to encapsulate multiple PB molecules.[34] The larger-generation PAMAM dendrimers, G5 and G7, were able to encapsulate 3 and 6 PB molecules, respectively.[34] Dendritic PAMAM G7 has 128 surface groups, making it the closest in size to the hyperbranched PEI-25 investigated here, and the amount of PB encapsulated per polymer is very similar for the two. It has been previously reported that loading multiple PB molecules in PAMAM dendrimers resulted in the dye's associating with the branches as well as the core region.[34] Because the branching units are longer for PAMAM than either PPI or the CBHP PEI, it makes sense that the larger generation of PAMAM (G7) would be able to encapsulate the most PB molecules of these three polymer families studied.

The binding constants (K_D) calculated for the polymer-associated dye increase as the size of the polymer increases. This is to be expected because the larger polymers are able to encapsulate more dye molecules than the smaller polymers, as evidenced by their higher fluorescence emission intensity, increased absorption in the wavelength region of interest, and the concentrations of associated PB obtained using Beer's Law.

Discussion

Excess Polymer. Comparing the association of PB with dendritic and hyperbranched polymers, the experimental results reveal similar host−guest properties. Previous studies with excess dendritic polymer indicate that association of PB is polymer-size-dependent: PB association increases as the generation increases.[20] The same trend is observed in the CBHP. For example, the absorption and emission spectra in Figures 4 and 5 reveal increased association in larger PEI-25 as compared to PEI-5. From the aforementioned studies, it is also known that PB associates with dendritic PPI in a very nonpolar nanoenvironment at or near the core region, regardless of the generation of dendrimer under investigation.[20,28] On the basis of the experimental results presented here, it also appears that the PB guest molecule is located in a similar core region in the CBHP PEI, even though these materials are much more irregular and polydisperse. The nanoenvironmental polarity observed for PB when associated with the CBHP is nearly identical for all polymers studied. For example, the absorption band is centered around 556 nm for both hyperbranched PEI-5 and -25 and 554 nm for dendritic PPI G4 and -5. The fluorescence emission results are analogous, with a shift in fluorescence emission wavelength to a nonpolar region. The nanoenvironment is slightly more nonpolar for the dendrimer-containing samples as compared with those with the hyperbranched polymers. These observations indicate that the dye associates in a similar location, irrespective of the polymer identity or polydispersity. In addition, the fluorescence emission intensity increases in the presence of all the polymers, with the highest emission intensities seen in both dendrimers. The enhanced intensity indicates that the PB is in a more protected nanoenvironment when encapsulated in either polymer type, as compared with aqueous solution. However, given the higher emission intensity, after correction for sample concentration, the dendritic PPI polymers provide more protection from excited-state deactivation than the hyperbranched PEI.

Anisotropy trends in Table 2 are consistent with the aforementioned results and previous studies:[20,31] there is an increase in anisotropy with increasing polymer size within both polymers classes. Moreover, when comparing the CBHP with dendrimers with similar properties, PB in the dendrimers results in higher overall anisotropy values. Steady-state anisotropy values result from a weighted average of both the rotational motion of the fluorophore in a local nanoenvironment and in the host−guest complex. In previous work,[20] there was a linear relationship with increasing dendrimer generations and PB anisotropy values for PPI and PAMAM, poly(amidoamine), dendrimer families. This trend was attributed to the anisotropy values primarily reflecting the molecular volume of the dendrimers. In addition, compared with PAMAM, which has a smaller core (C2 vs C4 for PPI), PB was found to have a better fit within the core of the PPI dendrimer family in general. Given their smaller C2 core, potentially more open structure, and expected size difference between the largest PPI studied (G5, 114 branching units, MW $\approx$ 7000) and the largest PEI polymer (25, ~580 branching units, MW $\approx$ 25 000), it is anticipated that the lower anisotropy values observed for the hyperbranched polymers also results from the fit of the guest PB in the host polymer core region.

Excess Dye. For the excess dye study, the PB concentration is 10 times higher than the polymer concentration. As mentioned previously, the initial absorption spectra (Figure 6) of the samples primarily reveal only aqueous PB due to the large excess of dye. As such, with PB in water being marginally fluorescent under these conditions, the fluorescence emission data provide insight as to the polymer−dye association prior to extraction. The fluorescence emission (Figure 7) reveals association at the expected wavelength region based on data obtained from the excess polymer study. However, there is one notable difference when comparing with the excess polymer study: the fluorescence emission intensity for the CBHP is significantly enhanced compared with that of the dendrimers. In the presence of excess dye, the hyperbranched PEI-25 has the highest fluorescence emission, indicating that it is able to entrap the most PB molecules. This is not surprising, considering the much larger size of the hyperbranched PEI-25 as compared with the other polymers under investigation: PEI-25 has the highest molecular weight as well as the largest number of surface groups and monomer units. It appears that the fluorescence emission intensity increases with increasing polymer size, regardless of polymer class (CBHP vs dendrimers).

Initial anisotropy values (Table 3) of the excess dye samples are very similar to those seen in the excess polymer study. However, the anisotropy values for dendrimer-associated PB are lower as compared to the excess polymer study. This is most likely due to the increased contribution of unassociated PB because the measured anisotropy is a weighted average of both polymer-associated and unassociated dye. In addition, anisotropy measurements, with the broad emission windows used, are more sensitive to the weakly fluorescent, aqueous PB. Given the aforementioned absorption and emission data and that there is little change in the anisotropy values for the PB associated with the CBHP, it appears that hyperbranched polymers are better

able to accommodate the increased dye population. As with the previous results[20,28,31] and those of the excess polymer study presented here, there is an increase in anisotropy with increasing polymer size, and the dendritic polymers have higher anisotropy values overall.

Excess Dye Post Extraction. After extraction of unassociated dye with toluene, evidence of the PB−polymer association is observed for all measurements collected: absorption, emission, and anisotropy. Postextraction CBHP PEI-25 and dendritic PPI G5 absorption spectra (Figure 8) reveal a single band present in the region of associated PB for both of these larger polymers. Absorption spectra of the smaller polymers, PEI-5 and PPI G4, each contain two peaks: one indicative of polymer-associated PB, and the other, of aqueous PB. The presence of aqueous-solvated PB in the postextraction data indicates that not all of the unassociated dye was removed during the extraction process. Deconvolution of the absorption spectra reveals that not all of the toluene was removed from the system because there is evidence of toluene-solvated PB.

The postextraction fluorescence emission data (Figure 9) follows the same trend observed in the initial data: the intensity increases with increasing polymer size, irrespective of polymer type. This is to be expected, as the weakly fluorescent, unassociated dye would not have contributed to the overall fluorescence emission signal. This trend indicates that the larger polymers are able to entrap more dye, as compared with the smaller polymers, as expected. Moreover, the emission intensity is significantly higher for all the samples studied compared with the excess polymer studies when the detector sensitivity (HV = 795 excess polymer vs 445 excess dye) is taken into account. This observation illustrates the ability to effectively load up the polymers, particularly PEI 25. However, when comparing pre- and postextraction spectra taken under the same experimental conditions, there is an overall decrease in the emission intensities across all polymer samples postextraction. Whereas minor decreases may be due to sample aging from repeated exposure to the excitation source or the time lapse between measurements, an attenuation in the emission intensity of the magnitude, as seen in Figure 9, is attributable to the removal of loosely associated dye.

Anisotropy measurements postextraction are similar to or slightly lower than the initial values observed in the excess dye study. Although some sample aging occurs, the most likely explanation is the presence of minute amounts of toluene remaining postextraction. The presence of toluene will increase the solubility of unassociated PB, and the lower viscosity of the toluene affords faster rotational motion of the dye, resulting in lowered anisotropy values. The significant change in the anisotropy value for PB in water (0.083 vs 0.068 postextraction), supports this assumption. This control sample was also extracted with toluene, and PB is much more readily soluble in it, as compared to water. It is also possible that the contribution from dye molecules in differing parts of the polymers (i.e., branches vs core) could lead to lowered anisotropy values because certain areas of the polymers will have less hindered dye rotation.

Given the experimental results from the excess dye study, it is clear that it is possible to load the polymer hosts with multiple guest molecules. Indeed, dendrimers have been previously shown to entrap several dye molecules,[32−34] although the conditions necessary to achieve such results are considerably different, synthetic modification. For example, previous work using dendritic PPI to encapsulate multiple Bengal rose molecules involved extensive chemical treatment, including structural modification of the dendrimers and relatively severe conditions.[32] The parameters for achieving multiple-dye encapsulation with dendritic PPI and hyperbranched PEI were mild, requiring only 24 h of equilibration.

Conclusion

The physical properties of the interior regions of the hyperbranched PEI polymers were studied and compared with those of dendritic PPI through the association of the solvatochromic probe phenol blue. The experimental results clearly indicate that the hyperbranched PEI is able to entrap PB as a guest molecule in a manner similar to that of dendritic PPI and PAMAM, although not the focus of this work. Both polymer families appear to encapsulate the dye in a relatively nonpolar, tightly held nanoenvironment. The polarity within the nanocavities of the polymers was shown to be similar to that of PB in *n*-heptane, which is in agreement with previous studies of dendritic PPI with PB.[20,31,34] As with the PPI dendrimers, the association of phenol blue with the CBHP occurs relatively rapidly, even in the smallest generation.

Although the association types of the two polymer families are similar, there are some notable differences in the association of PB with the CBHP, as compared with the dendrimers. The data obtained from PB associated with hyperbranched PEI indicates that the dye is in a more polar nanoenvironment and more loosely held than when associated with the dendritic PPI. This can be attributed to the structural differences in the two polymer families and the ability of PB to sense those differences: the hyperbranched PEI have a shorter core length (C2 vs C4 for the dendrimers), shorter branching units (C2 vs C3 for the dendrimers), increased polydispersity of the CBHP vs dendrimer, and chemical functionality (increased amine population in CBHP leading to a slightly more polar microenvironment). However, even with the host−guest differences present, encapsulation of PB appears to be complete and occurs in the interior cavities of the CBHP as well as in the dendrimers.

The polymer−dye complexes of both the hyperbranched PEI and dendritic PPI with PB were found to be relatively stable and fairly robust. Extractions of excess aqueous dye with toluene appeared to have little or no effect on the encapsulated PB molecules. This is particularly significant considering that some level of stability is required for many of the proposed host−guest applications of the polymers; a host incapable of retaining guest molecules would not be desirable.

References and Notes

(1) Seiler, M. *Chem. Eng. Technol.* **2002**, *25*, 237–253.
(2) Sunder, A.; Heinemann, J.; Frey, H. *Chem.−Eur. J.* **2000**, *6*, 2499–2506.
(3) Yates, C. R.; Hayes, W. *Eur. Polym. J.* **2004**, *40*, 1257–1281.
(4) Frey, H.; Haag, R. *Rev. Mol. Biotechnol.* **2002**, *90*, 257–267.
(5) HyperPolymers. http://www.hyperpolymers.com/prodinf.html (accessed August 8, 2007).
(6) Hanselmann, R; Hölter, D.; Frey, H. *Macromolecules* **1998**, *31*, 3790–3801.
(7) Suttiruengwong, S.; Rolker, J.; Smirnova, I.; Arlt, W.; Seiler, M.; Lüderitz, L.; Pérez de Diego, Y.; Jansens, P. *Pharm. Dev. Technol.* **2006**, *11*, 55–70.
(8) Paleos, C. M.; Tsiourvas, D.; Sideratou, Z. *Mol. Pharm.* **2007**, *4*, 169–188.
(9) Haag, R. *Chem.−Eur. J.* **2001**, *7*, 327–335.
(10) Arkas, M.; Tsiourvas, D.; Paleos, C. M. *Chem. Mater.* **2005**, *17*, 3439–3444.
(11) Krämer, M.; Stumbé, J.; Türk, H.; Krause, S.; Komp, A.; Prokhorova, S.; Kautz, H.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 4252–4256.
(12) Krämer, M.; Stumbé, J.; Grimm, G.; Kaufmann, B.; Krüger, U.; Weber, M.; Haag, R. *ChemBioChem* **2004**, *5*, 1081–1087.
(13) Möck, A.; Burgath, A.; Hanselmann, R; Frey, H. *Macromolecules* **2001**, *34*, 7692–7698.

(14) Haag, R.; Sunder, A.; Stumbé, J. *J. Am. Chem. Soc.* **2000**, *122*, 2954–2955.
(15) Radke, W.; Litvinenko, G.; Müller, A. H. E. *Macromolecules* **1998**, *31*, 239–248.
(16) Sunder, A.; Mülhaupt, R.; Haag, R.; Frey, H. *Macromolecules* **2000**, *33*, 253–254.
(17) Knischka, R.; Lutz, P. J.; Sunder, A.; Mülhaupt, R.; Frey, H. *Macromolecules* **2000**, *33*, 315–320.
(18) Haag, R.; Stumbé, J.; Sunder, A.; Frey, H.; Hebel, A. *Macromolecules* **2000**, *33*, 8158–8166.
(19) Maier, S.; Sunder, A.; Frey, H.; Mülhaupt, R. *Macromol. Rapid Commun.* **2000**, *21*, 226–230.
(20) Richter-Egger, D.; Tesfai, A.; Tucker, S. A. *Anal. Chem.* **2001**, *73*, 5743–5751.
(21) Morley, J. O.; Fitton, A. L. *J. Phys Chem. A* **1999**, *103*, 11442–11450.
(22) Figueras, J. *J. Am. Chem. Soc.* **1971**, *93*, 3255–3263.
(23) Kolling, O. W. *J. Phys. Chem.* **1991**, *95*, 3950–3954.
(24) Kolling, O. W. *Anal. Chem.* **1981**, *53*, 54–56.
(25) Kolling, O. W.; Goodnight, J. L. *Anal. Chem.* **1973**, *45*, 160–164.
(26) Kolling, O. W.; Goodnight, J. L. *Anal. Chem.* **1974**, *46*, 482–485.
(27) Kolling, O. W. *Anal. Chem.* **1978**, *50*, 212–215.
(28) Richter-Egger, D. L.; Landrey, J. C.; Tesfai, A.; Tucker, S. A. *J. Phys. Chem. A* **2001**, *105*, 6826–6833.
(29) Kessel, D. *Photochem. Photobiol.* **1991**, *54*, 481–483.
(30) Lackowitz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.
(31) Morgan, E. Ph.D. Dissertation, University of Missouri, Columbia, MO, 2005.
(32) Jansen, J. F. G. A.; Meijer, E. W. *J. Am. Chem. Soc.* **1995**, *117*, 4417–4418.
(33) Jansen, J. F. G. A.; de Brabander-van den Berg, E. M. M.; Meijer, E. W. *Recueil des Travaux Chimiques des Pays-Bas* **1995**, *114*, 225–230.
(34) Kline, K. K.; Morgan, E. J.; Norton, L. K.; Tucker, S. A. *Talanta* **2009**, *78*, 1489–1491.
(35) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. *Angew. Chem., Int. Ed., Engl.* **1990**, *29*, 138–175.

JP906609E