Dendrimers Derived from Polyphosphazene−Poly(propyleneimine) Systems: Encapsulation and Triggered Release of Hydrophobic Guest Molecules

Song Yun Cho and Harry R. Allcock*

Department of Chemistry, The Pennsylvania State University, 104 Chemistry Research Building, University Park, Pennsylvania 16802

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ABSTRACT: Three novel polyphosphazene-functionalized diaminobutane poly(propyleneimine) dendrimers have been prepared and investigated for their properties as prospective hydrophobic drug delivery systems. The solubilization and release properties of these functionalized dendrimers have been investigated by absorption and fluorescence spectroscopy by using pyrene as a probe. The introduction of amphiphilic phosphazene groups at the external surface of the dendrimers affects the solubilization and guest release properties compared to the behavior of the parent dendrimers. The release of encapsulated guest molecules is triggered by the addition of sodium chloride solution. It has been established that these novel dendrimeric compounds exhibit useful protective and targeting properties. These properties render the new molecules promising candidates for salt-responsive controlled-release systems, possibly including prospective drug delivery applications.

Introduction

The targeted delivery of hydrophobic drug molecules to various sites in the body is a major challenge due to the insolubility of these species in aqueous media. The functionalization of dendrimers[1,2] is a preferred strategy for developing a variety of novel materials for various biological applications. In this respect, various functional groups have been introduced at the end groups and therefore at the external surface of the dendrimers to impart properties associated with the functional groups. Specifically, surface modification of the primary amino groups of diaminobutane poly(propyleneimine) dendrimers affords a diversity of compounds, as has recently been reviewed.[3] For instance, quaternary ammonium groups[4] or poly(ethylene glycol) chains[5] have been introduced at the external surface of dendrimers, with a view to the development of drug delivery systems.[6] Although this surface functionalizaion with poly(ethylene glycol) chains is a useful method for the stabilization of dendrimers in a biological environment and for targeting, most of PEGylated dendrimers share drawbacks of poor encapsulation and stabilization properties due to the chemically labile properties and highly linear structure of ethyleneoxy main chains of PEG.

Poly(organophosphazenes) are hybrid organic−inorganic polymers that contain a highly flexible phosphorus−nitrogen backbone to which are attached organic side groups. A wide range of properties can be obtained by variations of the organic substituents.[7,8] In particular, amphiphilic or water-soluble polyphosphazenes have attracted much attention due to their potential applications in biomaterials and drug delivery systems.[9,10] An ambient temperature, living cationic polymerization of phosphoranimines, initiated by PCl_5, has recently been developed.[11−14] This route not only provides molecular weight control and narrow polydispersities but also permits the synthesis of polymers with controlled architectures. Furthermore, the cationic polymerization of phosphoranimines provides a viable method for the formation of phosphazene−phosphazene and phosphazene−organic (or polysiloxane) block copolymers using various macroinitiators.[15]

We report here a model system based on hydrophobic diaminobutane poly(propyleneimine) dendrimers that bear amphiphilic polyphosphazene outer segments. The hydrophobic interior provides an environment for the stabilization of pyrene—a model for hydrophobic guest molecules. Efficient incorporation of the active molecule and its controlled release are prerequisites for effective drug delivery systems. With this in mind, we have investigated whether changes to the salt concentration in the environment of the dendrimers of our model system can affect their solubilizing ability, thus triggering the release of guest molecules.

Experimental Sections

Materials. Diaminobutane poly(propyleneimine) dendrimers with 4 (4-DAB), 8 (8-DAB), and 16 (16-DAB) amino groups at the external surface (Aldrich) were used as the starting compounds. Lithium bis(trimethylsilyl)amide (Aldrich) was used without further purification. Phosphorus pentachloride (Aldrich) was purified by sublimation under vacuum before use. 2,2,2-Trifluoroethanol and diethylene glycol methyl ether (Aldrich) were dried over calcium hydride and distilled before use. Tetrahydrofuran, dichloromethane and *n*-hexane (Aldrich) were distilled from sodium benzophenone ketyl under dry nitrogen. All glassware was flame-dried under vacuum before use. The reactions were performed under an atmosphere of dry argon or nitrogen.

Equipment. ^1H and ^{31}P NMR spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360 and 90.27 MHz, respectively. ^1H NMR spectra were referenced to solvent signals while ^{31}P NMR chemical shifts are relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. Molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å column, and calibrated vs polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt % solution of tetra-*n*-butylammonium nitrate (Aldrich) in THF (OmniSolv).

Synthesis of Bromophosphoranimine 1. A hexane (300 mL) solution of $LiN(SiMe_3)_2$ (18.28 g, 0.11 mol) was cooled to 0 °C,

* Corresponding author. E-mail: hra@chem.psu.edu.

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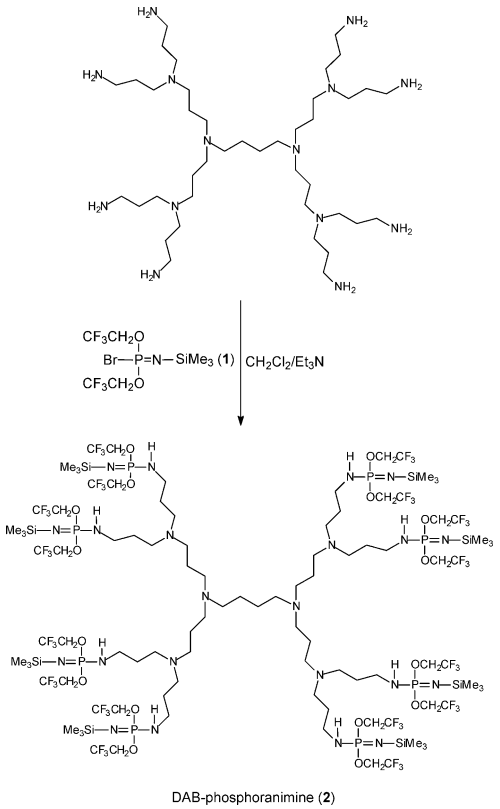
Scheme 1. Synthesis of DAB−Phosphoranimine (2)



and PCl_3 (15.00 g, 0.11 mol) was added slowly over 15 min. The reaction mixture was stirred at 0 °C for 1 h and allowed to warm to room temperature followed by stirring for 2 h. Then, a stock solution prepared from $NaOCH_2CF_3$ (0.22 mol; 21.81 g of $HOCH_2CF_3$ and 5.01 g of Na metal) was transferred to the reaction mixture at room temperature. After addition of the salt, the reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was centrifuged for 30 min to remove sodium chloride. The solution was transferred to a round-bottom flask and the solvent was removed at reduced pressure by rotary evaporation. The remaining solution was vacuum distilled to produce a colorless liquid. The product was dissolved in benzene (100 mL) and cooled to 10 °C. To this solution was added bromine (14.81 g) in benzene (30 mL) and the mixture was stirred at 10 °C for 2 h and allowed to warm to room temperature. The solvent was removed at reduced pressure and the crude product was vacuum distilled at least twice to remove unreacted bromine and produce a colorless liquid (17.34 g, yield 40%). ^{1}H NMR ($CDCl_3$) : δ 0 (s, 9H), 4.23 (q, 4H). ^{31}P NMR ($CDCl_3$) : δ −33.93.

Synthesis of Triethoxyphosphoranimine 3. To a solution of $Cl_3P{=}NSiMe_3$ (3.0 g, 13.36 mmol) in THF (50 mL) was added a solution of sodium ethoxide (44.09 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h and allowed to warm to room temperature. After completion of the reaction, the reaction mixture was passed through a pad of Celite to remove precipitated sodium chloride. The filtrate was collected and the solvent and excess ethanol were removed at reduced pressure. The crude product was purified by vacuum distillation (at 40 °C) to yield a yellow liquid (2.03 g, yield 60%). ^{1}H NMR ($CDCl_3$): δ 0.00 (s, 9H), 1.24 (t, 9H), 3.92 (q, 6 H). ^{31}P NMR ($CDCl_3$) : δ −4.03.

Synthesis of Chlorophosphoranimine 4.[16] A solution of $LiN(SiMe_3)_2$ (53.53 g, 0.32 mol) in 700 mL of diethyl ether was cooled to 0 °C, and PCl_3 (43.95 g, 0.32 mol) was added dropwise over 30 min. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h. Sulfuryl chloride (43.19 g, 0.32 mol) was added slowly and the reaction mixture was stirred at 0 °C for 3 h. After completion of the reaction, the salt was removed by filtration. The crude product was purified by vacuum distillation to yield a colorless liquid (25.15 g, yield 35%). ^{1}H NMR ($CDCl_3$): δ 0.00 (s, 9H). ^{31}P NMR ($CDCl_3$): δ −54.10.

Synthesis of Functional Dendrimers. A mixture of 8-DAB (0.050 g, 0.065 mmol) and triethylamine (0.055 g) in methylene chloride was cooled to 0 °C. To this solution was added dropwise $(CF_3CH_2O)_2BrP{=}NSiMe_3$ (**1**) (0.22 g, 0.54 mmol) over a 30 min period. The reaction mixture was stirred for 12 h at room temperature. In a separate reaction vessel, PCl_5 (0.24 g, 1.14 mmol) was dissolved in 50 mL of distilled CH_2Cl_2 at room temperature. The end-capper reagent $(CH_3CH_2O)_3P{=}NSiMe_3$ (**3**) (0.14 g, 0.57 mmol) was added to the solution, which was stirred for 2 h at room temperature. The monomer, $Cl_3P{=}NSiMe_3$ (**4**) (2.56 g, 11.39 mmol), was then added to the reaction mixture which was stirred for 12 h to generate "living" poly(dichlorophosphazene) chains. The polyphosphazene solution was then added to the solution of dendrimeric phosphoranimine in CH_2Cl_2 and the mixture was stirred for 48 h at room temperature to terminate the polymerization. The CH_2Cl_2 was removed from the reaction mixture under reduced pressure, and the polymer was redissolved in 25 mL of freshly distilled THF. An excess of $NaOCH_2CH_2OCH_2CH_2OCH_3$ (34.16 mmol) in THF was added to the dendrimer solution to replace the labile chlorine atoms in the phosphazene blocks. The reaction mixture was stirred at room temperature until ^{31}P NMR spectroscopy indicated complete replacement of the chlorine atoms. The dendrimer was purified again by dialysis against THF/H_2O (4/1 vol/vol), followed by drying under reduced pressure to give a viscous yellowish liquid. ^{1}H NMR ($CDCl_3$): δ 1.18 (m, $-POCH_2CH_3$), 1.36 (m, $-NCH_2CH_2CH_2N-$, $-NCH_2CH_2CH_2CH_2N-$, $-NCH_2CH_2CH_2NH-$), 2.20 (m, $-NCH_2CH_2CH_2N-$, $-NCH_2CH_2CH_2CH_2CH_2N-$, $-NCH_2CH_2CH_2NH-$), 3.08 (m, $-CH_2NH-$), 3.28 (broad s, $-CH_2OCH_3$), 3.44 (broad s, $-CH_2OCH_3$), 3.55 (broad s, $-CH_2OCH_2-$), 3.74 (broad s, $-POCH_2CH_3$), 3.98 (broad s, $-POCH_2CH_2O-$), 4.30 (broad s, $-POCH_2CF_3$). ^{31}P NMR ($CDCl_3$): δ −7.96.

Anal. Calcd for $C_{1800}H_{3936}N_{190}O_{1048}F_{48}P_{184}$: C, 41.88; H, 7.69; N, 5.16; F, 1.77; P, 11.04. Found: C, 41.76; H, 7.83; N, 5.53; F, 1.87; P, 11.46.

Sample Preparation. Nanopure water with a conductivity of 18.2 MΩ/cm (10 mL) was added dropwise to a stirred THF solution of the functional dendrimer (10 mL). The THF was removed on a rotary evaporator at 30 °C for 2 h. The dendrimer solution was diluted with Nanopure water to obtain a concentration range from 2.0×10^{-4} to 1.0×10^{-9} M. For the measurement of fluorescence spectra, a pyrene solution in THF (1.6×10^{-3} M) was added to Nanopure water to give a pyrene concentration of 16×10^{-7} M, and THF was removed using a rotary evaporator at 30 °C for 2 h. The pyrene solution was mixed with the dendrimer solutions to obtain dendrimer concentrations from 1.0×10^{-4} to 5.0×10^{-10} M. The pyrene concentration of the samples was 8.0×10^{-7} M. All the samples were sonicated for 15 min and were allowed to stand for 2 days before fluorescence measurements.

Fluorescence Measurements. The fluorescence spectra were obtained using a Perkin-Elmer LS 55 spectrofluorometer. The site of pyrene entrapment inside the dendrimers was probed by its fluorescence intensity (F/F^0) and I_1/I_3 ratio: F^0 is the total fluorescence intensity of aqueous pyrene solution, whereas F is the total fluorescence intensity of the same pyrene solution after addition of dendrimer which is lower than F^0 due to the resulting quenching (see Results and Discussion section). I_1 and I_3 are the fluorescence intensity of pyrene spectra at 373 and 383 nm, respectively, and their ratio is reflecting the polarity of the microenvironment that pyrene is sensing.[17]

Solubilization and Release Properties. The limiting solubilization of pyrene was measured by absorption spectroscopy (HP 8452 A diode array spectrophotometer) of 5.0×10^{-5} M dendrimeric solution incorporating 1.2×10^{-5} M of pyrene. For studying the release of solubilized pyrene as a function of added sodium chloride, an aqueous 5.0×10^{-5} M dendrimeric solution was used, incorporating 1.2×10^{-5} M of pyrene, and its absorption spectra was monitored as a function of NaCl concentration.

Scheme 2. Synthesis of DAB−PN Dendrimers

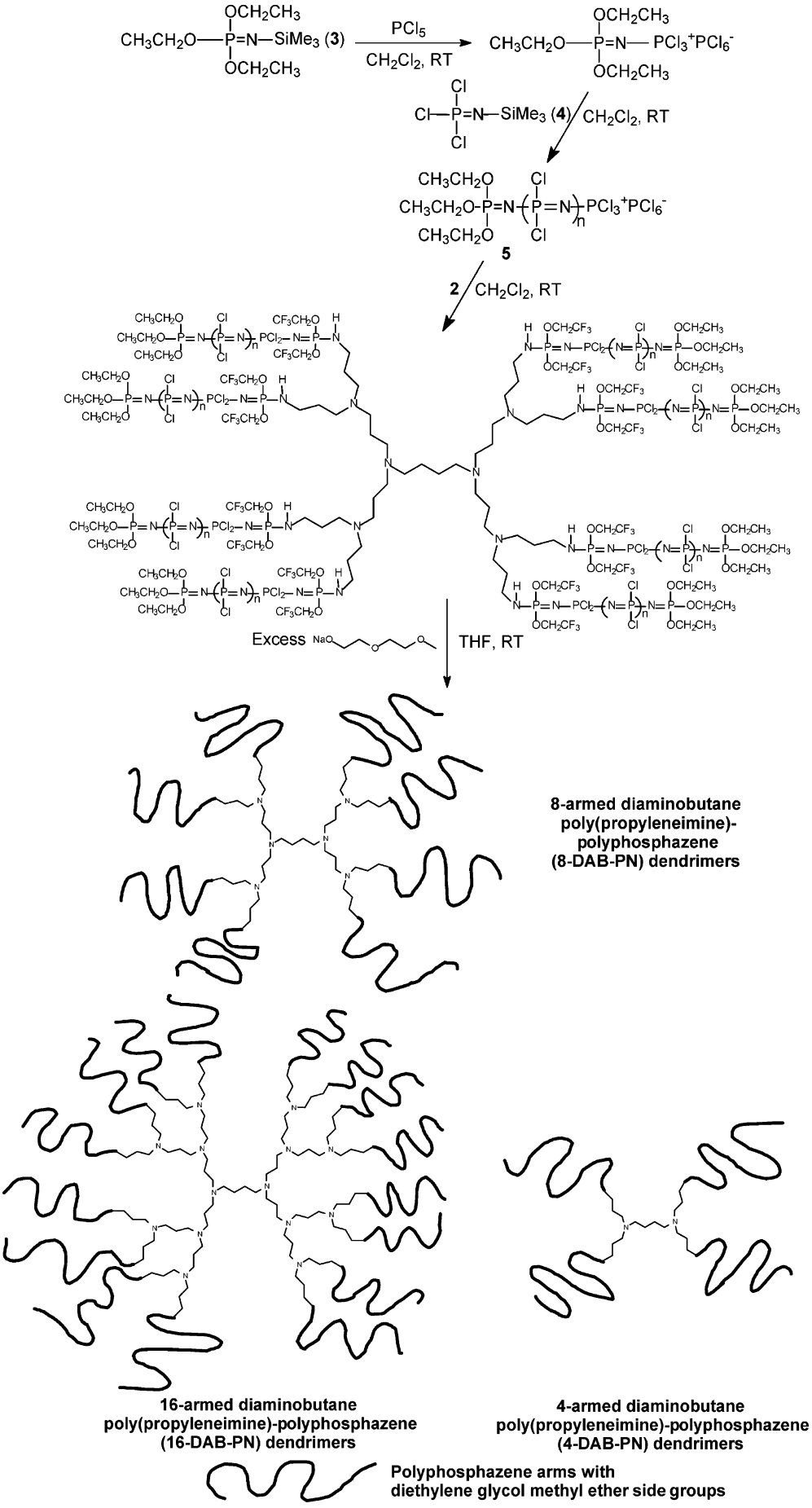


Table 1. Properties of DAB−PN Dendrimers

DAB−PN dendrimer	no. of repeating units per each arm	M_n (^{1}H NMR)[a]	M_n (M_w/M_n)[b]
4-DAB−PN	20	25 743	6 200 (1.33)
8-DAB−PN	20	51 610	26 800 (1.32)
16-DAB−PN	20	103 394	38 000 (1.21)

[a] Calculated from ^{1}H NMR spectra, by comparison of peaks at 3.98 ppm (−OC*H*$_2$CH$_2$O−) to peaks at 2.20 ppm (−NC*H*$_2$CH$_2$C*H*$_2$N−, −NC*H*$_2$CH$_2$CH$_2$C*H*$_2$N−, −NC*H*$_2$CH$_2$CH$_2$NH−). [b] Measured by GPC.

Results and Discussion

Terminology. The following terminology is used in this publication. The parent diaminobutane poly(propyleneimine) dendrimer starting materials are defined as 4-, 8-, or 16-DAB species, according to the number of arms present. The corresponding intermediates with phosphoranimine terminal units are then described as 4-, 8-, or 16-DAB−phosphoranimines. The final dendrimeric products with polyphosphazene side arms are 4-, 8-, or 16-DAB−PN species.

Synthesis of Diaminobutane Poly(propyleneimine)—Polyphosphazene (DAB—PN) Dendrimers. In earlier work in our program, triarmed phosphazene polymers were synthesized via the ambient temperature, cationic polymerization of phosphoranimines using tris(2-aminoethyl)amine as a starting core molecule.[12] Thus, it appeared that multi primary amines such as amine-terminated diaminobutane poly(propyleneimine) dendrimers should also be useful as core molecules to form a functional phosphoranimine. The synthetic procedure for preparation of functional dendrimers is illustrated in Schemes 1 and 2. A triethoxyphosphoranimine (**3**) was initiated with 2 equiv of PCl_5 at room temperature in CH_2Cl_2 to produce the cationic species $(CH_3CH_2O)_3P{=}N{-}PCl_3^+PCl_6^-$. Subsequent reaction of this initiator with given amounts of $Cl_3P{=}NSiMe_3$ (**4**) allowed the preparation of poly(dichlorophosphazene) with specific chain lengths (i.e., 20 repeating units). The addition of this living species to DAB−phosphoranimine (**2**) resulted in the formation of multiarmed diaminobutane poly(propyleneimine) dendrimers. Subsequent treatment of this species with $NaOCH_2CH_2OCH_2CH_2OCH_3$ yielded hydrolytically stable diaminobutane poly(propyleneimine)−polyphosphazene (DAB−PN) dendrimers (Scheme 2). The resulting dendrimeric polymers were soluble in THF, chloroform, acetone, and DMSO. The molecular weights and polydispersities of DAB−PN dendrimers, as estimated by GPC and ^{1}H NMR, are given in Table 1. The M_n values calculated by ^{1}H NMR showed good agreement with theoretical values derived from the reaction stoichiometries. The molecular weights of the DAB−PN dendrimers were determined by peak integration ratios of methyl protons (3.98 ppm) of the phosphazene arms to the methyl protons (2.20 ppm) of the core molecule (Table 1). Gel permeation chromatography showed polydispersities in the range 1.21−1.33 with unimodal distributions. Molecular weights measured by GPC were significantly lower than those calculated by ^{1}H NMR, possibly due to the highly spherical conformation of the dendrimers in THF solution and their subsequently small hydrodynamic volume.

Solubilization and Micropolarity Studies. It is well-established that the aqueous solubility of hydrophobic molecules can be dramatically enhanced in the presence of water-soluble surface active agents. Therefore, dendrimers, which resemble unimolecular micelles, are expected to increase the aqueous solubility of water-insoluble or weakly soluble organic compounds (Figure 1). The binding strength and the adsorption capacity of dendrimers may be altered depending on the available number of microcavities and the chemical composition of the repeating units. Although pyrene is not an active drug ingredient, it is a sensitive probe for determining the solubility of hydrophobic guest molecules and the hydrophobicity of the environment within a dendrimer. In the present study we have examined, by UV−vis absorption spectroscopy, aqueous dendrimer solutions of 4-, 8-, and 16-armed DAB−PN dendrimers, saturated with pyrene. The absorption spectra of pyrene in water in the presence of 4-, 8-, and 16-armed DAB−PN dendrimers are shown in Figure 2. In the absence of DAB −PN dendrimers, no soluble pyrene could be detected in the water as evidenced by the fact that the absorption spectrum of this solution is practically indistinguishable from the solvent baseline. However, in the presence of the dendrimers the spectra indicate that the DAB−PN dendrimers are capable of providing an apolar environment that sequesters hydrophobic pyrene molecules within its interior. Under these conditions, the maximum concentration of the solubilized pyrene was dramatically increased in proportion to the size of the corresponding dendrimer, either in terms of dendrimer molecular weight or the number of microcavities in each dendrimer (Table 2). Thus, the amount of solubilized pyrene, and presumably of other organic molecules, can be controlled to some desired value by using dendrimers of the appropriate generation. We conclude therefore that the generation level plays an important role in the association between pyrene and the dendrimer. In addition, the phosphazene-coupled dendrimer encapsulated significantly higher concentrations of pyrene than did the parent dendrimer (diaminobutane poly(propyleneimine) without the phosphazene component). This observed solubility increase is attributed to additional solubilization of the hydrophobic pyrene *in the polyphosphazene periphery*. Apparently polyphosphazene arms can stabilize pyrene by a hydrophobic interaction due to the amphiphilic nature of the ethyleneoxy functional groups attached to the polyphosphazene chains. Interestingly, in the case of the 4-armed DAB−PN dendrimers, which have quite small generations, the loading capacity is 13.6 mol %, which is roughly three times more than the loading capacity of the parent 4-armed dendrimeric solution (3.7 mol %). This is significantly beneficial for its application for a drug delivery system, and the loading enhancement is clearly attributed to the polyphosphazene functional groups introduced at the surface of the parent dendrimer.

Because the fluorescence spectrum of pyrene is sensitive to changes in its microenvironment, this technique is useful for investigating the polarity of the dendrimer interior that binds the hydrophobic guest molecule. The fluorescence emission spectra of pyrene at different concentrations of the 16-armed DAB−PN dendrimer are shown in Figure 3. The relative intensities of the first and the third emission peaks (I_1/I_3) in the emission spectrum of pyrene are known to be sensitive to the polarity of the microenvironment.[17] Figure 4 shows the variation of I_1/I_3 as a function of dendrimer concentrations in water saturated with pyrene. It is clear that the dependence of the ratio on the dendrimer concentration changes significantly from the 4- to the 16-armed DAB−PN dendrimer.

The experimental results shown in Figure 4 are rationalized in terms of the extent of water penetration into the dendrimer microcavities. Thus, the 4-armed DAB−PN dendrimer has only four repeat units and consequently possesses both the smallest radius and an open structure. It appears less able to protect pyrene molecules efficiently from the bulk aqueous phase than does the 8- or 16-armed DAB−PN dendrimer. The values of I_1/I_3 in the functionalized dendrimers decrease until they reach a value of about 1.0, which is close to that observed in a

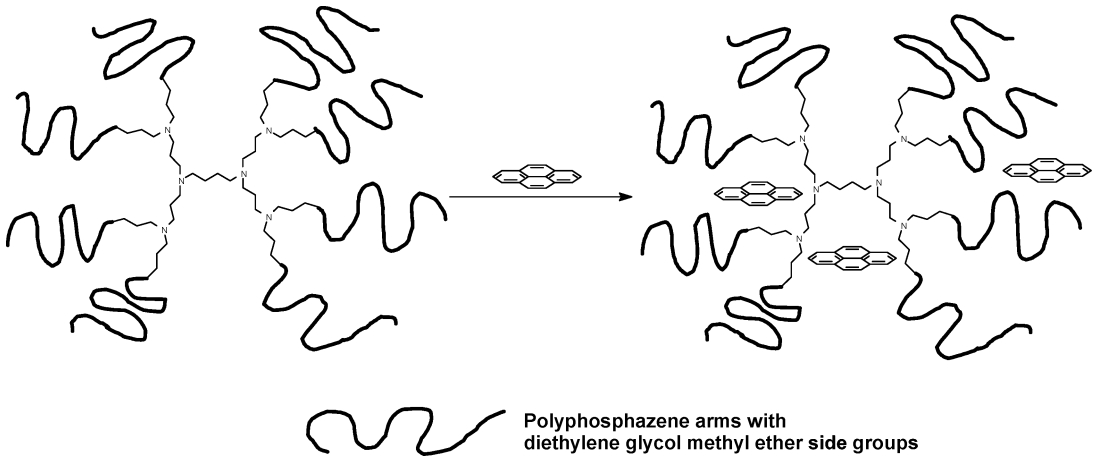


Figure 1. Schematic representation of the solubilization of pyrene in DAB−PN dendrimers.

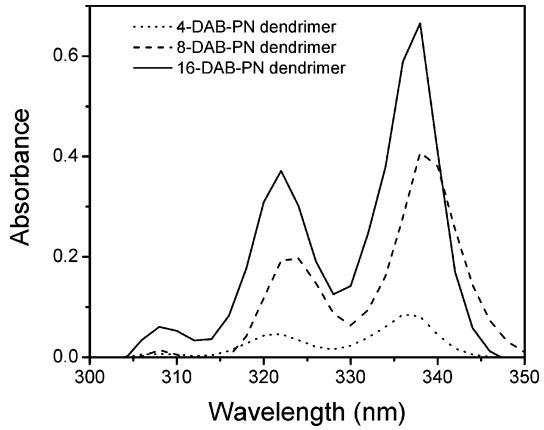


Figure 2. Absorption spectra of pyrene in aqueous solutions of DAB−PN dendrimers (5.0×10^{-5} M).

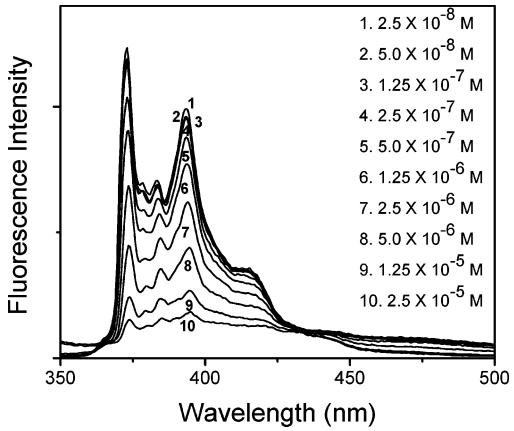


Figure 3. Emission spectra of pyrene in water (8.0×10^{-7} M) at different concentrations of 16-armed DAB−PN dendrimer.

Table 2. Comparative Solubility of Pyrene in DAB−PN Dendrimers and Parent Dendrimers

DAB−PN dendrimer	[dendrimer]/M	[PY]/M	PY/dendrimer molar ratio
4-DAB−PN	5.0×10^{-5}	6.80×10^{-6}	0.136
4-DAB	5.0×10^{-5}	1.86×10^{-6}	0.037
8-DAB−PN	5.0×10^{-5}	3.26×10^{-5}	0.652
8-DAB	5.0×10^{-5}	1.88×10^{-6}	0.038
16-DAB−PN	5.0×10^{-5}	5.31×10^{-5}	1.062
16-DAB	5.0×10^{-5}	1.95×10^{-6}	0.039

lipophilic medium. Thus, pyrene is mainly incorporated inside the dendrimers in order to avoid contact with the bulk water phase.

Pyrene Fluorescence Quenching. In a titration type addition of 4-, 8- or 16-armed DAB−PN dendrimer to an aqueous solution containing 8.0×10^{-7} M pyrene a strong quenching of fluorescence intensity of pyrene was observed, as shown in Figure 5. This fluorescence quenching was attributed[5] to the formation of a charge-transfer complex between pyrene and the tertiary amino groups as evidenced by the appearance of a weak exciplex fluorescence centered at approximately 480 nm.[18]

The association of pyrene with 4-, 8- and 16-armed DAB−PN dendrimer was assessed quantitatively by determining the pyrene-dendrimer binding constants $K_{\text{py/4-DAB-PN}}$, $K_{\text{py/8-DAB-PN}}$, and $K_{\text{py/16-DAB-PN}}$. Using the data from Figure 5, and fitting eq 1 for an assumed 1/1 pyrene/dendrimer complexation,[19] it was found that the binding (equilibrium) constants have the magnitudes $K_{\text{py/4-DAB-PN}} = 29\,946\ \text{M}^{-1}$ ($R^2 = 0.9746$), $K_{\text{py/8-DAB-PN}} = 64\,047\ \text{M}^{-1}$ ($R^2 = 0.9703$), and $K_{\text{py/16-DAB-PN}} = 64\,515\ \text{M}^{-1}$ ($R^2 = 0.9970$).

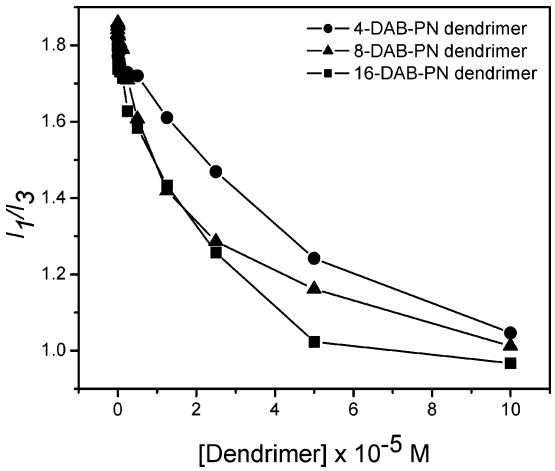


Figure 4. Variation of the intensity ratio of the first (I_1) to the third (I_3) pyrene fluorescence peaks vs the total added dendrimer concentration.

$$F/F^0 = 1 + \{(F^b/F^0) - 1\}K[\text{D}]/(1 + K[\text{D}]) \qquad (1)$$

The K values of the binding constants are dependent on the molecular weight and on the number of cavities in each dendrimer. It is also deduced that the maximum solubilities of pyrene in the three dendrimers follow an identical trend with respect to the dendrimer molecular weight, the number of cavities, and the corresponding binding constants.

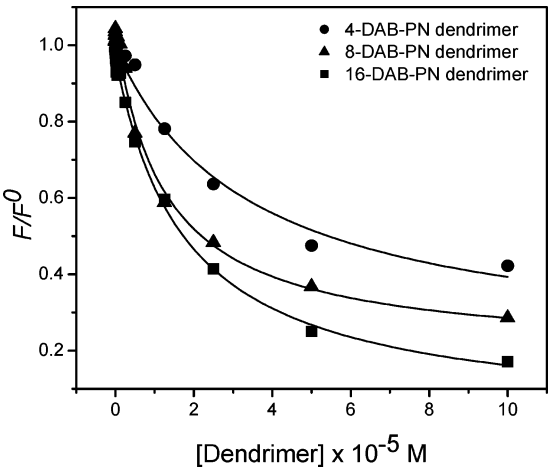


Figure 5. Plot of F/F^0 vs the total added dendrimer concentration. F^0 is the total fluorescence intensity of an aqueous pyrene solution (8.0×10^{-7} M), whereas F stands for the intensity after each addition of the dendrimer. The lines through the experimental points are the best fits according to eq 1.

Release Properties of DAB−PN Dendrimer. The release of encapsulated active ingredients and an understanding of how it is triggered are crucial parameters for the application of dendrimers as drug carriers and in other applications. Specifically, the release of the active ingredient from the carrier (dendrimer in this case) when it reaches a target site enhances its bioavailability and efficacy. The use of aqueous sodium chloride solution for triggering pyrene release has been established in independent studies.[20] It was reported that ions of alkali metals such as Na^+, K^+, and Cs^+ cationize ethyleneoxy moieties through complexation in aqueous media, and that increases in the salt concentration can increase the number of entrapped cations per ethyleneoxy unit.[21] The consequent increase in charge density causes expansion of the ethyleneoxy coils in polyphosphazene chains,[21] which can result in the swelling of the entire polyphosphazene periphery. The more open space in the dendrimer periphery, caused by changes in charge density, allows water to penetrate into the dendrimer. In this context, the amphiphilic polyphosphazene shell will be modified to be more hydrophilic and consequently less able to stabilize the hydrophobic guest molecules. Therefore, it should be possible to release solubilized pyrene into the bulk aqueous phase via the presence of metal cations. Indeed, by titrating the dendrimeric solutions with, for instance, sodium chloride, pyrene was released and dispersed in the bulk solution in the form of crystallites. This was shown visually by the turbidity of the sample. These particles were centrifuged. ^{1}H NMR spectra of the resulting solid material in $CDCl_3$ were measured and the spectra corresponded to that of pyrene. Figure 6 provided further proof by monitoring the pyrene concentration in the supernatant solution by UV spectroscopy. A gradual decrease of the concentration of solubilized pyrene was detected. It is noteworthy that sodium chloride in an extracellular environment can be complexed with ethyleneoxy moieties in polyphosphazene chains, which can affect the overall drug release profile. Hence, the possibility of drug release in the extracellular fluid should be considered for designing a targeted drug release system.

Conclusions

A novel functional dendrimeric system has been investigated which can possibly be utilized as a prospective drug delivery system. In addition to bearing protective polyphosphazene chains, this system also shows salt triggered release properties.

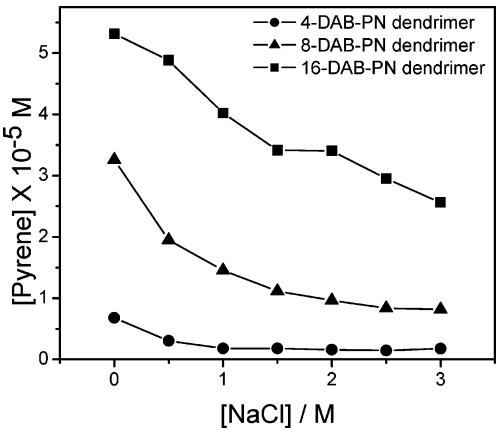


Figure 6. Plot of the concentration of pyrene in a 5.0×10^{-5} M dendrimeric solution as a function of added NaCl.

The introduction of the biocompatible polyphosphazene units at the surface of the poly(propyleneimine) dendrimers not only enhances the water solubility of pyrene, but also critically affects the release of the trapped molecules. Pyrene is released in the aqueous phase following addition of sodium chloride solution. The enhanced solubilization of pyrene molecules in DAB−PN dendrimers emphasizes their possible application as promising water-soluble controlled release drug carriers for hydrophobic molecules which can be protected by the polyphosphazene coat located at the dendrimer surface but released in aqueous media that contain sodium cations.

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References and Notes

(1) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons. Concepts, Syntheses, Perspectives*; Wiley-VCH: Weinheim, Germany, 2001; and references therein.
(2) Fréchet, J. M. J., Tomalia, D. A. Eds.; *Dendrimers and Other Dendritic Polymers*; Wiley Series in Polymer Science; John Wiley & Sons, Ltd.: New York, 2001; and references therein.
(3) Vögtle, F.; Gestermann, S.; Hesse, R.; Schwierz, H.; Windisch, B. *Prog. Polym. Sci.* **2000**, *25*, 987.
(4) Sideratou, Z.; Tsiourvas, D.; Paleos, C. M. *Langmuir* **2000**, *16*, 1766.
(5) Sideratou, Z.; Tsiourvas, D.; Paleos, C. M. *J. Colloid Interface Sci.* **2001**, *242*, 272.
(6) Liu, M. J.; Fréchet, J. M. J. *Pharm. Sci. Technol. Today* **1999**, *2*, 393.
(7) (a) Mark, J. E.; Allcock, H. R.; West, R. *Inorganic Polymers*; Prentice Hall: Englewood Cliffs, NJ, 1992. (b) Allcock, H. R. *Science* **1992**, *255*, 1106.
(8) Allcock, H. R. *Adv. Mater.* **1994**, *6*, 106.
(9) (a) Allcock, H. R.; Pucher, S. R.; Turner, M.; Fitzpatrick, R. *Macromolecules* **1992**, *25*, 5573. (b) Allcock, H. R.; Dudley, G. K. *Macromolecules* **1996**, *29*, 1313.
(10) (a) Allcock, H. R. *Biodegradable Polymers as Drug Delivery Systems*; Langer, R., Chasin, M., Eds.; Marcel Dekker: New York, 1990. (b) Allcock, H. R. *Chemistry and Applications of Polyphosphazenes*; Wiley-Interscience: Hoboken, NJ, 2003.
(11) Honeyman, C. H.; Manners, I.; Morrissey, C. T.; Allcock, H. R. *J. Am. Chem. Soc.* **1995**, *117*, 7035.
(12) Nelson, J. M.; Allcock, H. R.; Manners, I. *Macromolecules* **1997**, *30*, 1854.
(13) (a) Allcock, H. R.; Reeves, S. D.; Nelson, J. M.; Crane, C. A. *Macromolecules* **1997**, *30*, 2213. (b) Nelson, J. M.; Primrose, A. P.; Hartle, T. J.; Allcock, H. R. *Macromolecules* **1998**, *31*, 947.
(14) Allcock, H. R.; Reeves, S. D.; de Denus, C. R.; Crane, C. A. *Macromolecules* **2001**, *34*, 748.
(15) (a) Allcock, H. R.; Reeves, S. D.; Nelson, J. M.; Manners, I. *Macromolecules* **2000**, *33*, 3999. (b) Chang, Y.; Lee, S. C.; Kim, K. T.; Kim, C.; Reeves, S. D.; Allcock, H. R. *Macromolecules* **2001**, *34*, 269.
(16) (a) Wang, B.; Rivard, E.; Manners, I. *Inorg. Chem.* **2002**, *41*, 1690. (b) Allcock, H. R.; Cho, S. Y.; Steely, L. B. *Macromolecules* **2006**, *39*, 8334.

(17) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.

(18) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York and London, 1983.

(19) In eq 1, employed for the determination of the binding constants *K*, [D] is the concentration of the free dendrimer in solution while F^0 is the total fluorescence intensity of a pure aqueous pyrene solution, F^b is the total intensity of pyrene when completely bound, and *F* stands for the intensity of the latter at any moment during the titration procedure.

(20) Ambade, A. V.; Savariar, E. N.; Thayumanavan, S. *Mol. Pharm.* **2005**, *2*, 264.

(21) (a) Sartori, R.; Sepulveda, L.; Quina, F.; Lissi, E.; Abuin, E. *Macromolecules* **1990**, *23*, 3878. (b) Ananthapadmanabhan, K. P.; Goddard, E. D. *Langmuir* **1987**, *3*, 25.

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