Spectroscopic investigations of pyrene butanol encapsulated in *C*-hexylpyrogallol[4]arene nanocapsules†

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Pyrogallol[4]arenes self-assemble to form stable hydrogen-bonded nanocapsules that have many unique properties making them potentially suitable for diverse applications, such as molecular transporters and nanoreactors. However, little is known about the host–guest behaviour of these materials. Utilizing nanoenvironment-dependent properties of the fluorescent reporter molecule, pyrene butanol (PBOH), the encapsulating abilities of the hexameric *C*-hexylpyrogallol[4]arene (PgC_6) nanocages are further explored. Solution-state, spectroscopic and spectrofluorometric investigations and solid-state, single-crystal X-ray diffraction studies are in agreement and indicate an ordered inner phase. Up to two PBOH guest molecules are sequestered within the nanocapsule along with encapsulation solvent. The nature of the PBOH–PgC_6 complex is found to change with time, as indicated by fluorophore leaching. These research findings provide additional insight into the encapsulation capabilities of PgC_n nanocapsules.

Introduction

In an effort to understand and mimic biologically important assemblies found in nature, such as proteins and viruses,[1] investigations of synthetic constructs with similar properties[1–4] have become increasingly useful. To date, self-assembling, noncovalent molecular structures have been shown to form a wide range of unique host structures, often geometrically resembling the high-symmetry Platonic and Archimedean polyhedra.[5] For example, *C*-methylresorcin[4]arene (CMRC) self-assembles into a hexameric nanocapsule that resembles a snub cube, contains eight structural water molecules, and has an internal volume of ~1300 Å^3.[6] CMRC nanocapsules are held together with 60 hydrogen bonds and are stable in nonpolar solvents.[6] Similar, but more stable, assemblies are formed by *C*-alkylpyrogallol[4]arenes (PgC_n). These nanocapsules have an interior volume of ~1250 Å^3, are seamed together with 72 hydrogen bonds, and are stable in nonpolar and moderately polar solvents.[6–11] The framework of the PgC_n nanocapsules is characterized in the solid phase by standard methods of X-ray crystallography and NMR spectroscopy.

Until recently, knowledge of the behaviour of these complex entities in solution has been fairly limited.[12–24] Our previous reports have provided critical insight through the use of molecular spectroscopy techniques, particularly steady-state and dynamic fluorescence measurements. The advantages of fluorescence spectroscopy are well known and include sensitivity, selectivity, and minimal sample perturbation. This groundbreaking work illustrated the potential of the PgC_n nanocapsules for wide ranging applications including molecular transport and sorting and catalysis.[11,12,25,26] Previous studies of pyrene butyric acid (PBA) encapsulated in *C*-hexylpyrogallol[4]arene (PgC_6) nanocapsules remarkably showed that the PBA guest remained entrapped within the PgC_6 host in solution.[12] X-Ray crystallographic data revealed that the nanocapsules were mainly doubly occupied by the guest. The fluorophores inside the cavity were separated by the sandwiched assembly solvent, acetonitrile. Crystallographic and spectroscopic investigations determined that the PBA interacted with the interior walls of the capsule both in solid and solution states. The encapsulation of acetonitrile, along with the guest probe, was also confirmed in solution by the addition of a fluorescence quenching agent, *N*,*N*-dimethylaniline (DMA). DMA addition to free PBA resulted in expected exciplex formation, apparent in the fluorescence emission spectra, due to charge transfer. However, the PBA–DMA exciplex emission was absent in the PBA–nanocapsule complex, thus demonstrating that the PBA was encapsulated and therefore not available for interaction with the DMA.[12,27,28]

In order to elucidate the role of guest functionality in encapsulation and stability of the resulting complex, the fluorescence reporter pyrene butanol (PBOH) was investigated. The PgC_6 host capsule easily accommodates PBOH guest molecules, since the molecular volume, ~260 Å^3,[29] is similar to that of PBA. The resulting supramolecular assembly is shown in Fig. 1.

Results and discussion

Solid-state studies

Single-crystal X-ray diffraction studies of the colorless crystals of PBOH show that the PgC_6 self-assembled into the expected hexameric capsule, and NMR spectroscopy reveals that the

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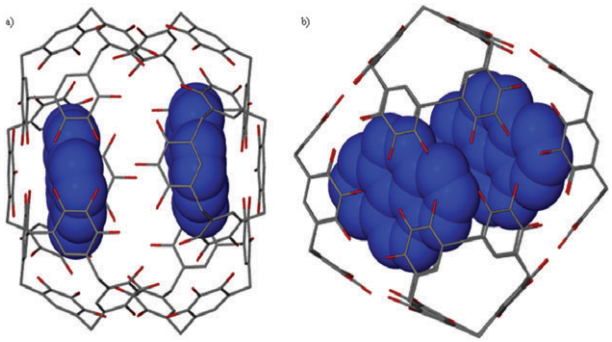

Fig. 1 Two different crystallographic perspectives (a and b) of PBOH encapsulated within PgC_6. For clarity, hydrogen and *n*-hexyl side chains of PgC_6 as well as the disordered butanol tail of PBOH have been removed.

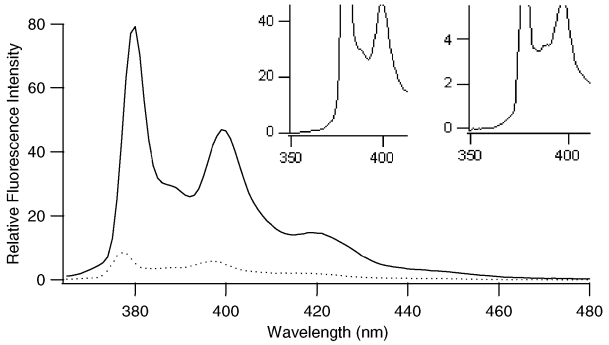

Fig. 2 Representative fluorescence emission spectra of free (dashed) and complexed PBOH (solid) in hexane. Inset is magnified to reveal changes in the second vibronic band of complexed (left) and free (right) PBOH.

nanocapsules contain 1.2 PBOH guest molecules.‡ As expected from the molecular structure of the guests, this occupancy is similar to that of PBA within the nanocapsules, *i.e.* there are an average of 1.5 PBA or 1.2 PBOH guest molecules per capsule.[12]

Despite the interior disorder, it was possible to model and refine the PBOH–pyrene moieties. The pyrene moieties are well separated from each other as evidenced by a pyrene centroid···centroid distance of 7.7 Å. Similar to the finding for PBA,[12] the capsule is spacious enough to contain two PBOH molecules, as well as encapsulation solvent molecules. Because of the crystallographic disorder, it was not possible to model the acetonitrile guest molecules.[12]

In the solid state, π interactions lock the PBOH molecules inside the nanocapsule. The pyrene rings interact with the capsule walls through π stacking and CH···π interactions. The position of the PBOH pyrene ring is very similar to that found in the PBA structure, therefore. With regard to the π-stacking interactions, the centroid-to-centroid distance in the PBOH structure is 3.87 Å, which compares to the 3.85 Å distance in the PBA structure. The PBA structure exhibited CH···π interactions with distances of 2.83 Å and 2.93 Å. It is likely that the butanol tail interacts with the interior wall of the capsule in some manner, but these interactions could not be ascertained because of the extensive disorder of the PBOH guests.

Solution studies

These unique host–guest complexes were also analysed by molecular spectroscopic techniques to better understand their state in solution. Such studies reveal information about the nanoenvironment and interactions between the host and the guest. Initial fluorescence spectra of the PBOH-containing nanocapsules and free PBOH are shown in Fig. 2. With more than an eight-fold increase in the fluorescence emission intensity compared to free PBOH, it is immediately evident that PBOH is still encapsulated. This intensity increase is due to the guest being in a protected environment within the nanocapsule, decreasing the incidence of collisional deactivation, which results in a radiationless, excited-state decay pathway. These results are quite similar to those observed for encapsulated PBA, which exhibited a five-fold increase in fluorescence emission intensity when compared to free PBA in solution.

Table 1 Representative fluorescence lifetimes (τ) and fractional intensities (α) of PBOH and PBA recovered using NLLS analysis[22]

Sample	τ_1	α_1
PgC_6–PBOH	110	0.99
Free PBOH	8.5	1.0
PgC_6–PBA	120	1.0
Free PBA	8.1	1.0

As seen in Table 1, fluorescence lifetime measurements also confirm the presence of PBOH within the nanocapsule. Lifetimes measure the time the fluorophore spends in the excited state prior to returning to the ground *via* the emission of a photon. For the aforementioned reasons, sequestered molecules also have significantly longer lifetimes compared to unbound fluorophores, as they are in a protected nano-environment. The increase in fluorescence lifetime for the encapsulated (110 ns) *versus* the free (8.5 ns) PBOH is consistent with previous results of encapsulated PBA, which also exhibited an increase of more than an order of magnitude.[12] These results are also consistent with the expected micro-environment of a constrained fluorophore.

In solution, there is also spectroscopic evidence of guest interaction with the host nanocapsule. A comparison of the absorption spectra of free PBA in hexane *versus* ensnared PBOH reveals spectral perturbations. The ground-state interaction of the PBOH with the capsule is manifested by a wavelength shift of ~10 nm upon encapsulation, the appearance of an additional peak centred around 354 nm, as well as an overall increase in signal intensity (Fig. 3). The host–guest interaction also persists in the excited state. As seen in the enlargement of the emission spectrum (inset of Fig. 2), the second band (λ = ~390 nm) changes with encapsulation. Given that the fluorescent moiety in both studies is the pyrene, it is not surprising that the nature of the spectral changes is the same for PBA and PBOH.

‡ Crystallographic data for PBOH encapsulated within PgC_6 nanocapsule, $C_{345.28}H_{450.21}N_4O_{74.72}$, M = 5852.18, a = 19.5278(6), b = 22.6384(7), c = 23.5862(7) Å, α = 69.145(2)°, β = 69.485(2)°, γ = 71.748(2)°, U = 8911.9(5) Å^3, triclinic, space group $P\bar{1}$, Z = 1, λ(Mo) = 0.71073 Å, T = 173 K, 43 305 reflections measured, 25 173 unique reflections, R_1 = 0.1810, wR_2 = 0.5175, R_{int} = 0.0271.

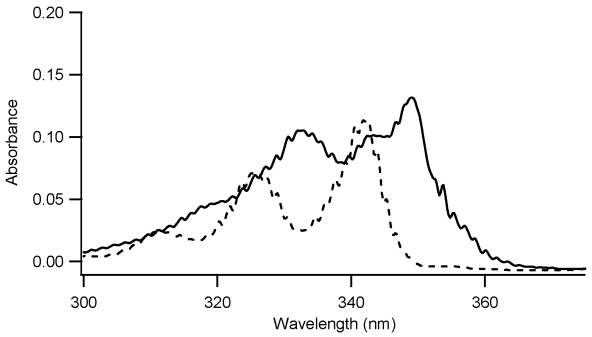

Fig. 3 Representative absorption spectra of free PBOH (dashed) and complexed (solid) in hexane.

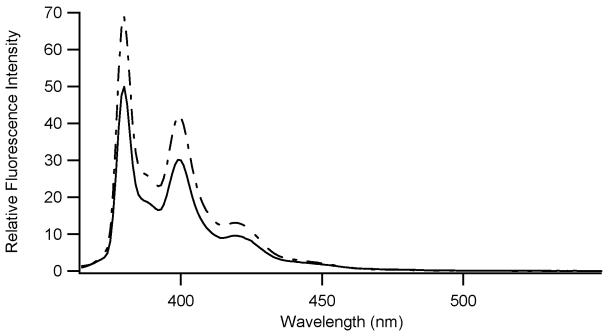

Fig. 5 Representative fluorescence emission spectra of encapsulated PBOH (dashed) with the addition of DMA (solid).

Also like the PBA study, the double occupation of the nanocapsule by two guest fluorophores cannot be confirmed in solution. However, the spatial separation between neighbouring fluorophores within the same nanocapsule seen in the solid state is supported in solution. Pyrene derivatives, such as PBA and PBOH, are known to exhibit excimer formation in solution.[30] Such formation is easily detectable in fluorescence emission spectra and occurs at longer wavelengths than monomer emission. Since the fluorescence spectra collected of encapsulated PBOH do not contain any evidence of excimer formation, it can be inferred that while there are two PBOH molecules within the same nanocapsule, they are separated enough to prevent any guest–guest interactions.

After confirming that the PBOH remains within the PgC6 nanocapsule in solution, the ability of small molecules to interact with the entrapped guest was tested and the presence of the encapsulation solvent was confirmed. A known fluorescence quencher, dimethylaniline (DMA), was chosen based on its ability to penetrate the hydrogen bonded seams of the capsule and to form a solvent-dependent exciplex with the PBOH guest,[12,27,28] as previously mentioned. In solution, free PBOH forms a fluorescent exciplex with DMA due to charge transfer between the molecules. In the presence of hexane, this exciplex is spectrally evident at ~445 nm (Fig. 4). Also note the expected attenuation of the emission intensity with quenching. Upon the addition of acetonitrile to the system with free PBOH, the charge-transfer band decreases in intensity and shifts wavelength approximately 10 nm. This change is expected because the PBOH–DMA charge-transfer exciplex is not detectable in the presence of pure acetonitrile. Upon addition of DMA to the PBOH–PgC_6 complex, the emission intensity decreases indicating quenching; however, no charge-transfer band was detected, signifying the formation of an *endo*-PBOH–DMA exciplex in the presence of acetonitrile (Fig. 5).[12,27,28]

Control studies were preformed to verify that DMA does indeed transverse the capsule seams and interact with PBOH in the presence of encapsulated acetonitrile. To ensure that the presence of the PgC_6 nanocapsule does not hinder the formation of the PBOH–DMA exciplex, *exo*-capsule PBOH was added to the hexane solution containing the complex.

As seen in Fig. 6, the addition of a small amount of fluorophore results in the expected charge-transfer exciplex at ~420 nm. Also, the addition of a small amount of acetonitrile to this sample diminishes the exciplex band, mirroring the original emission signal. These results confirm that the DMA interacts with *endo*-PBOH and the exciplex formation is suppressed by the presence of encapsulated acetonitrile.

This host–guest complex is remarkably similar to previous studies using PBA as a guest molecule with the PgC_6 nanocapsules. The only significant difference between the two guests appears to be the robustness of the assembly. PBA was found to have leached from the capsule only 10% over a 30 day period, while PBOH leached nearly 100% within 10 days. Encapsulation of the parent moiety pyrene was not as successful as the encapsulation of PBOH and PBA. Results

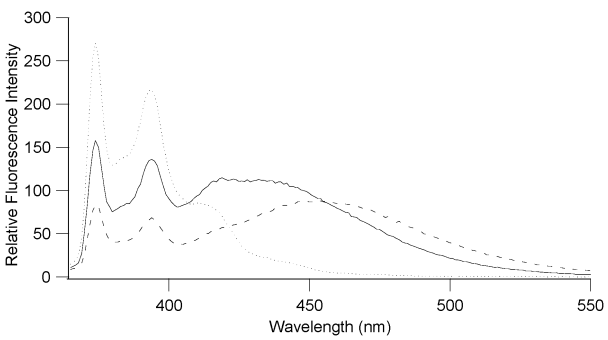

Fig. 4 Representative fluorescence emission spectra of free PBOH in hexane (dotted), with the addition of DMA (solid), and followed by the addition of acetonitrile (dashed).

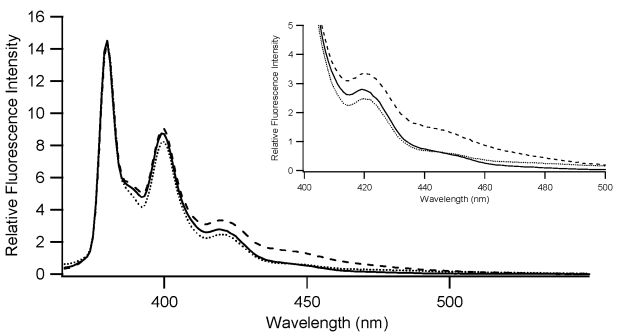

Fig. 6 Representative fluorescence emission spectra of encapsulated PBOH with DMA (solid), with the addition of *exo*-PBOH (dashed), followed by the addition of acetonitrile (dotted). Inset is magnified to reveal exciplex changes.

obtained were unclear on whether pyrene remained entrapped within the capsule in the solution state, as the intensity enhancements observed for encapsulated PBOH and PBA were not observed in the fluorescence emission spectra for encapsulated pyrene.[31] Moreover, the strong, systematic dependence on solvent polarity of pyrene in solution also did not confirm continued encapsulation.[32,33]

Given the data on three pyrene guests, it is hypothesized that the presence or functionality of the guest 'tail' "docks" the molecule in the host PgC_6 nanocapsule. Additional studies with ADMA (1-(9-anthryl)-3-(4-dimethylaniline)propane) also support this notion. As with PBOH and PBA, encapsulated ADMA also exhibited an increase in fluorescence emission intensity when compared to free ADMA in solution. The stability of the PBOH–PgC_6 complex is similar to that of encapsulated ADMA that demonstrated significant guest leaching in 12 days.[14] In the case of ADMA, the lack of long term stability was attributed to the conformational flexibility of the probe.[31] In PBA and PBOH studies, interactions are known to occur between the aryl groups of the capsule and the π system of the pyrene moiety. However, due to electron density, specific interactions, between the functional groups on the guest 'tail' and the interior of the capsule, could not be definitively resolved.

Conclusions

Solution and solid-state results are in agreement and indicate that both PBOH and acetonitrile are encapsulated and retained within the PgC_6 nanocapsule. It is clear from the spectral data that the nanocapsule provides a protective environment for PBOH guest molecules. The investigations of PBOH encapsulated within PgC_6 nanocapsules illustrate striking similarities with the previously investigated PBA. In both assemblies, the capsule is able to entrap up to two guest molecules, as well as the crystallisation solvent. For PBOH, double encapsulation occurred for ~20% of the capsules, while PBA exhibited double encapsulation of ~50%.[12] Inside the doubly occupied PBOH capsules, the guests were separated by 7.7 Å, which is remarkably similar to the 7.8 Å separation between PBA molecules in the doubly occupied PBA capsules.[12] The main difference between the two systems is their stability. PBOH leaches out of the capsule at a much faster rate than PBA, possibly due to the differing functionality of the guest "tail," aiding in "docking" the guest to the interior of the capsule walls.

These studies provide additional insight into the encapsulation capabilities of PgC_n nanocapsules. Future studies will focus on varying the nature and functionality of the guest molecule in order to elucidate the host–guest interaction, to understand how to customize the encapsulation process, and to control the stability of the resulting supramolecular assembly.

Experimental

Materials

C-Hexylpyrogallol[4]arene was synthesised according to literature procedures,[9] and PBOH was purchased from Aldrich, St. Louis, MO (99% purity) and used as received. The supramolecular structure 1 was assembled by sonication of a saturated solution of PgC_6 and PBOH in HPLC-grade acetonitrile (Fisher Scientific, Fair Lawn, New Jersey), using the same procedure that was reported by Dalgarno *et al.* in 2005.[12] Two to three weeks of slow evaporation yielded large, colourless crystals of the potential host–guest complex structure 1. Upon examination under a UV lamp (365 nm), the observation of strong fluorescence confirmed the presence of the fluorophore within the crystal. The composition of the nanocapsules was determined by single crystal X-ray diffraction and occupation was determined by dissolving the crystals of structure 1 in deuterated acetonitrile and integrating the ^1H NMR spectrum obtained.

Once the X-ray structure was obtained, the same crystal was dissolved in HPLC-grade hexane (Fisher Scientific) for absorption and fluorescence measurements. Stock solutions ($\sim 10^{-4}$ M PBOH) of the fluorophore or the complex were prepared by dissolving the dye or a single crystal of the complex in hexane. Diluted sample solutions ($\sim 10^{-6}$ M) were prepared by quantitatively transferring known aliquots of the stock solutions into vials and then diluting to volume. The final concentration of PBOH in solution was optically dilute ($\sim 10^{-6}$ M). Samples were examined immediately upon mixing, with the exception of those used in time-lapse studies, which were stored in the dark in sealed vials and interrogated at room temperature. Quenching studies were carried out by adding DMA (100%, Sigma-Aldrich, St. Louis, MO) in one microlitre aliquots to the samples until formation of an exciplex was spectrally evident. All solution state studies were performed at least in triplicate with three replicates collected and representative data are presented.

UV-Vis absorption measurements

All spectra were collected in 1 cm^2 Suprasil quartz cuvettes (Hellma), and absorption spectra were recorded on a Hitachi U-3000 double-beam spectrophotometer (Danbury, CT) with a scan rate of 120 nm min^{-1} and a slit width of 1 nm. Spectra were blank corrected for the possible absorption of the nanocapsules and the solvent in solution.

Fluorescence measurements

Spectra were measured using an SLM 48000 DSCF/MHF spectrofluorometer. The excitation source was a He–Cd Kimmon laser (Centennial, CO) operated at 325 nm and 10 mW. The emission was collected from 350–550 nm at 1 nm steps, with a slit width of 4 nm. All emission spectra were blank and absorbance corrected. Lifetimes were collected in the frequency domain on an SLM 48000 DSCF/MHF spectrofluorometer with multiharmonic, Fourier transform phase-modulation capabilities. In MHF mode, only a small fraction of the excitation power is used to interrogate the sample. A base frequency of 4.0 MHz and a cross-correlation frequency of 7.000 Hz were used; 10 pairs of sample–reference measurements were collected in triplicate for each sample, and each measurement contained 50 internal averages. The lifetime reference used was POPOP (τ_{ref} = 1.34 ns), 1,4-bis[5-phenyl-2-oxazolyl]benzene (99%, Sigma, St. Louis, MO) in ethanol (200 proof, AAPER Alcohol and Chemical, Shelbyville, KY).

The following filters were employed for emission collection: a 400 nm (80 nm band-pass, 03 FIV 044, Melles Griot, Irvine, CA), a 400 nm (40 nm band-pass, 03 FIV 026, Melles Griot). Lifetime data were analyzed by the SLM-Aminco nonlinear least-squares software.

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