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Narrowly Dispersed Double-Walled Concentric Hollow Polymeric Microspheres with Independent pH and Temperature Sensitivity

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Introduction

In recent years, hollow micro- and nanospheres have attracted a great deal of attention due to their potential applications in biomedicine, catalysis and paints, and as electronics materials.[1−3] Numerous physical and chemical methods have been developed to prepare organic, inorganic and hybrid hollow particles and spheres.[4−13] Efforts have also been made to broaden the range of potential applications by altering the properties of shell materials and by fabricating more sophisticated hollow structures, such as concentric hollow microspheres,[14−16] hollow spheres with controllable pore structure on surfaces,[17] and hollow polymer ellipsoids.[18] The inclusion of stimuli-responsive properties into these microstructures has allowed their unique application in biomaterials, for example, in anticancer drug delivery system, because of their increased functionality in comparison with the conventional inert structures.[19−21] In response to external stimuli, such as temperature, pH, ionic strength, electric field, and magnetic flux, the "smart" hollow spheres can undergo reversible structural transition and self-adjustment of their physicochemical properties.[22−31] Furthermore, with a better control of the size distribution of the spheres, it is possible to formulate more sophisticated delivery systems with improved selectivity and targeting efficiency. Herein, we describe the synthesis of double-walled concentric hollow polymeric microspheres of well-defined shape and narrow size distribution. The poly(methacrylic acid) (PMAA) inner shell and the poly(*N*-isopropylacrylamide) (PNIPMA) outer shell can respond independently to pH and temperature of the environment, respectively.

Results and Discussion

The synthesis procedures for the silica-polymer multilayer hybrid microspheres, and their corresponding hollow structures, are shown in Scheme 1. Initially, uniform silica-3-(trimethoxysilyl)propylmethacrylate (SiO_2-MPS) composite microspheres with an average diameter of 290 nm were synthesized via the sol−gel process, involving tetraethyl orthosilicate (TEOS) and MPS.[32,33] The carbon−carbon double bonds present on the surface of the microspheres allow distillation precipitation polymerization of methacrylic acid in acetonitrile, with 2,2′-azobisisobutyronitrile (AIBN) as initiator, to form an outer PMAA shell. After removal of the silica core from the resultant SiO_2/PMAA core−shell structures, pH-responsive hollow PMAA micropsheres were obtained (Supporting Information, Figure S1).[34] The SiO_2/PMAA/SiO_2 trilayer and SiO_2/PMAA/SiO_2/PNIPAM tetra-layer hybrids were synthesized subsequently from the SiO_2/PMAA core−shell microspheres by alternating the sol−gel and distillation precipitation polymerization processes. The concentrations of ethylene glycol dimethacrylate (EGDMA) and *N*,*N*′-methylene bisacrylamide (MBA) cross-linkers were controlled at 15 mol % and 20 mol %, respectively, for the formation of corresponding cross-linked PMAA and PNIPAM layers. Finally, nearly monodispersed double-walled concentric hollow PMAA-PNIPAM microspheres were obtained by selective removal of the inorganic silica core and interlayer by HF treatment for 48 h. The concentric hollow double-walled microspheres are stable in many organic solvents, allowing a wide range of applications.

The transmission electron microscopy (TEM) image of the SiO_2/PMAA/SiO_2 trilayer hybrid microspheres of uniform shape and size distribution is shown in Figure 1a. The PMAA interlayer has a lower contrast than the two silica layers. The size of the spheres has increased to about 506 nm in diameter, after incorporation of the outer SiO_2 layer. The FT-IR spectrum of the SiO_2/PMAA/SiO_2 trilayer hybrids shows an absorption peak at 1636 cm^{-1} (Figure 2a), attributable to the vinyl groups of MPS. Thus, carbon−carbon double bonds have again been introduced onto the outer surface of the SiO_2/PMAA/SiO_2 trilayer microspheres, allowing for the subsequent polymerization of *N*-isopropylacrylamide (NIPAM). Figure 1b shows the field emission scanning electron microscopy (FESEM) image of the well-defined SiO_2/PMAA/SiO_2/PNIPAM tetra-layer microspheres of narrow size distribution. The size of the tetra-layer microspheres has increased to about 556 nm in diameter. Thus, the thickness of the outer cross-linked PNIPAM shell is around 25 nm. The high magnification TEM image of a tetra-layer microsphere (Figure 1c) reveals the distinctive multilayer morphology of the hybrid structure. The successful formation of PNIPAM outer layer is further confirmed by FT-IR spectrum of the microspheres in Figure 2b. The characteristic absorption peaks at 1544, 1650, and 3300 cm^{-1} are associated with the vibration modes of the amide group in the PNIPAM shell. HF etching of the SiO_2/PMAA/SiO_2/PNIPAM hybrid microspheres removes the silica core and interlayer to produce the double-walled concentric hollow microspheres with a pH-responsive cross-linked PMAA inner shell and a temperature-responsive cross-linked PNIPAM outer shell. The structure of the resultant PMAA-PNIPAM concentric hollow microspheres with flexible or deformable shells is revealed by the FESEM and TEM images of Figure 1d and e, respectively. The dents in the hollow spheres indicate that the cross-linked PNIPAM outer shells remain very flexible due to the low degree of cross-linking. The unique hollow structure of concentric polymeric shells is clearly observed in the TEM image of Figure 1e. The FT-IR spectrum of the double-walled PMAA-PNIPAM hollow microspheres (Figure 2c) shows the disappearance of Si−O peak at 1100 cm^{-1} and the simultaneous increase in intensity of the peak characteristic of the PMAA and PNIPAM segments. The enhanced peak at 1729 cm^{-1} is due to the stretching vibration of the carbonyl moieties of the carboxylic acid segments in the PMAA inner shell. The barely discernible Si signal in the energy-dispersive X-ray (EDX) spectrum of the PMAA-PNIPAM

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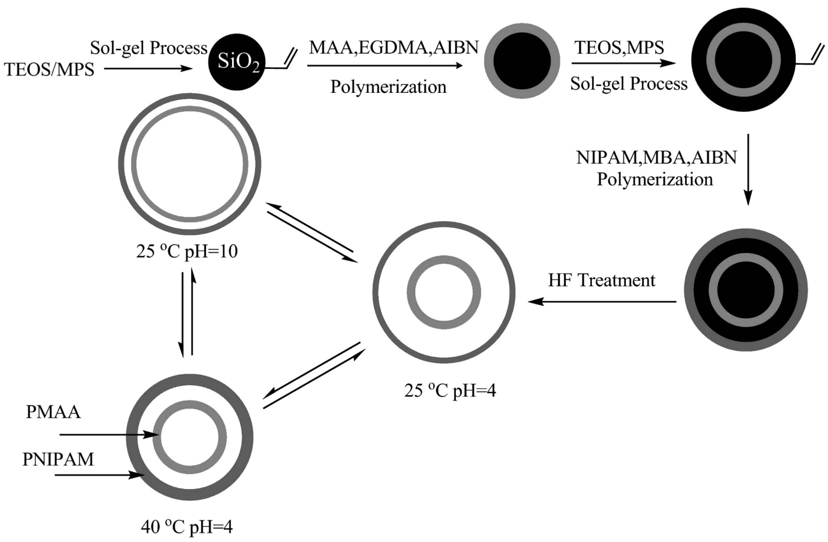
Scheme 1. Preparation of Polymer/Silica Multilayer Hybrid Microspheres and Double-Walled Concentric Hollow Polymeric Microspheres with Independent Sensitivity to pH and Temperature



TEOS: Si-$(OEt)_4$
MAA: CH_2=C(CH_3)COOH
MBA: CH_2-(NH-COCH=CH_2)$_2$
MPS: $(CH_3O)_3Si(CH_2)_3OCOC(CH_3)$=$CH_2$
NIPAM: H_2C=CHCONHCH$(CH_3)_2$
EGDMA: CH_2=C(CH_3)COO-CH_2CH_2-OCOC(CH_3)=CH_2

hollow microspheres (Supporting Information, Figure S2) is consistent with the successful removal of the silica core and interlayer by HF treatment to form the hollow double-walled microspheres.

The capsid of cowpea chlorotic mottle virus (CCMV) consists of a uniform protein coat for transporting viral genome through a reversible open/close gating mechanism, triggered by pH of the medium.[35] To illustrate the similarity in "intelligence" of the present microstructure, the stimuli-responsive properties of the double-walled PMAA-PNIPMA concentric hollow microspheres were investigated by dynamic laser scattering (DLS). At the constant pH of 10, the hydrodynamic diameter (D_h) of the double-walled hollow microspheres varies with temperature, as shown in Figure 3. The D_h of the double-walled PMAA-PNIPMA concentric hollow microspheres decreases considerably from 739 to 621 nm when the temperature of the medium increases from 25 to 40 °C and vice versa. According to the DLS results, the swelling ratio between 25 to 40 °C, defined as (D_{25}/D_{40}),[3] is calculated to be 1.69, confirming the thermo-responsive nature of the PNIPAM outer shell of the double-walled hollow microspheres. This behavior can be explained by the fact that PNIPAM exhibits a lower critical solution temperature (LCST, 32 °C) behavior in aqueous media.[25] Above the LCST, PNIPAM chains associated hydrophobically, leading to a decrease in effective D_h of the hollow microspheres. At a fixed temperature, the hydrodynamic diameter of the PMAA inner shell varies with pH of the medium. The cross-linked PMAA hollow microspheres obtained from the SiO_2/PMAA core−shell microsphere can be used to measure the size changes of PMAA inner shell as a function of pH value. The D_h of PMAA inner shell increases from 320 nm at pH = 4 to 540 nm at pH = 10 (Figure S3). This change is due to the Donnan osmotic swelling of the PMAA shell as the carboxylic acid groups are ionized at high pH values. The effect of pH on cross-linked PMAA inner wall can be observed directly in the TEM images. In comparison with that of the double-walled hollow microsphere exposed to pH = 10 (Figure 1e), the TEM image of the double-walled PMAA-PNIPAM concentric hollow microsphere exposed to pH = 4 (Figure 1f) reveals a decrease in size of the PMAA inner shell, while the size of PNIPAM outer shell remains almost unchanged. Despite the variation in size of the microspheres with pH and temperature of the medium, the polydispersity of double-walled hollow microspheres remains at about 1.20. The void fraction between the two shells and the thickness of the polymeric shell can be tuned through the simple adjustment of reaction time and/or initial monomer

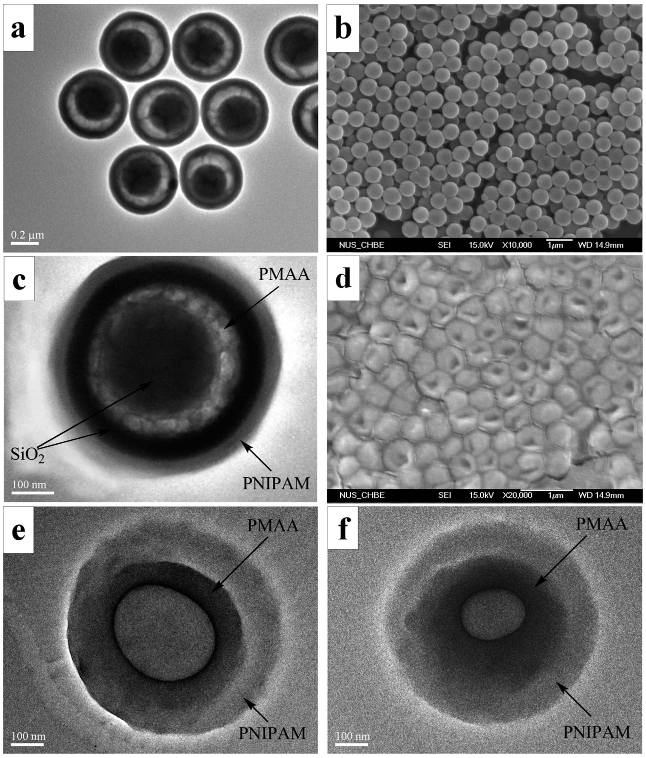


Figure 1. TEM and FESEM micrographs of (a) the SiO_2/PMAA/SiO_2 trilayer microspheres with the polymerization time of the outer layer fixed at 24 h, (b) and (c) the SiO_2/PMAA/SiO_2/PNIPAM tetra-layer microspheres, and the PMAA-PNIPAM double-walled concentric hollow microspheres (d) before and (e) after exposure to an aqueous medium of pH = 10 and 25 °C and (f) pH = 4 and 25 °C.

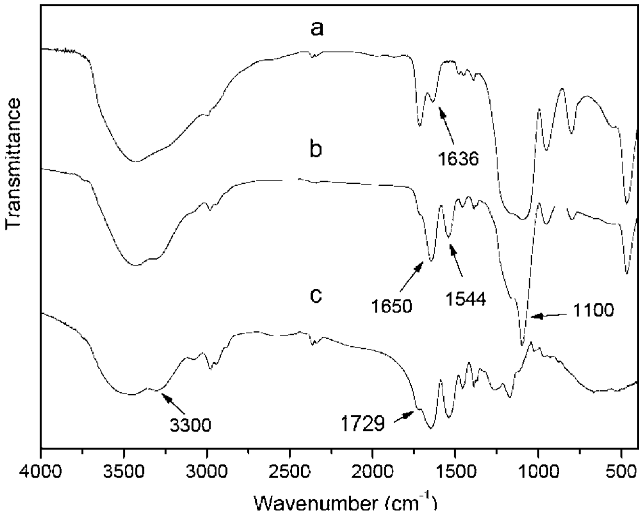


Figure 2. FT-IR spectra of (a) the SiO_2/PMAA/SiO_2 trilayer microspheres, (b) the SiO_2/PMAA/SiO_2/PNIPAM tetra-layer microspheres, and (c) the PMAA-PNIPAM double-walled concentric hollow microspheres.

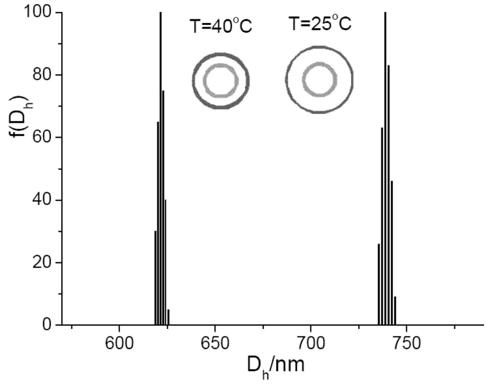


Figure 3. Hydrodynamic diameters (D_h) of the double-walled PMAA-PNIPAM concentric hollow microspheres and their size distribution in aqueous media of 25 and 40 °C at a constant pH of 10.

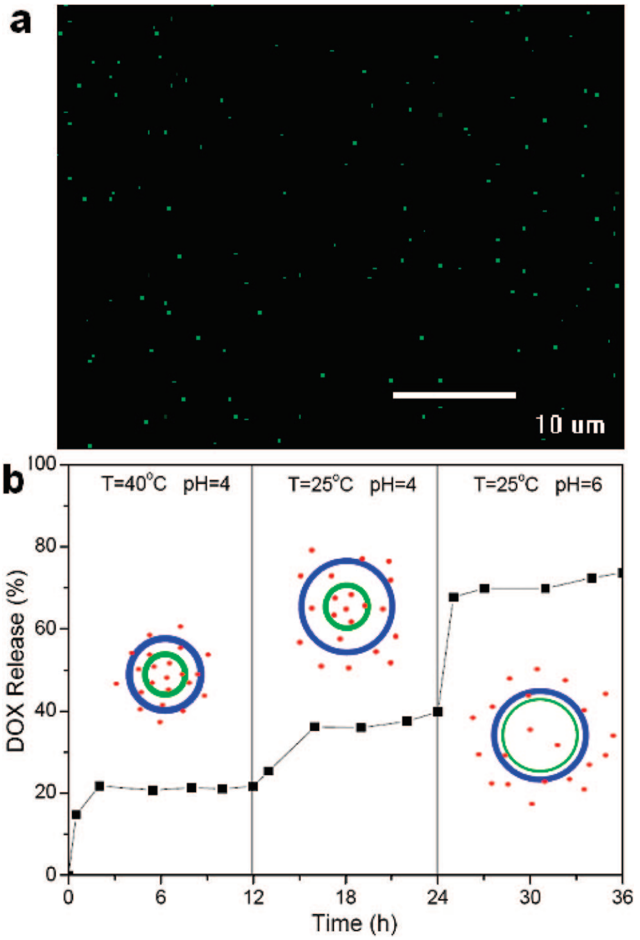


Figure 4. (a) CLSM image of the double-walled PMAA-PNIPAM concentric hollow microspheres loaded with doxorubicin hydrochloride (DOX), and (b) DOX release profiles from the double-walled PMAA-PNIPAM concentric hollow microspheres.

concentration (Figure S4). In addition, the swelling and deswelling behavior in a medium of constant pH or temperature can be easily controlled by the cross-linking density of the shell. The reversible changes in shell dimension, triggered by the external stimuli of pH and temperature, suggest that the double-walled PMAA-PNIPAM concentric hollow microspheres may be used to selectively encapsulate guest molecules for releasing in a controlled manner.

To study the stimuli-responsive delivery behavior of the double-walled PMAA-PNIPAM concentric hollow microspheres, the anticancer drug, doxorubicin hydrochloride (DOX), was loaded into the hollow microspheres. Figure 4a shows confocal laser scanning microscopy (CLSM) image of the double-walled PMAA-PNIPAM concentric hollow microspheres after loading of DOX (see Experimental Section). Figure 4b shows the release profile of the DOX-loaded PMAA-PNIPAM double-walled microspheres. Two modes of controlled release of DOX are discernible. At high temperature and low pH of the solution (0−12 h), the outer PNIPAM and inner PMAA cross-linked shells remained in a shrink state and the rate of release was at a low level. Upon lowering the solution temperature, while maintaining the same pH (12−24 h), swelling of the outer PNIPAM shell allowed the release of DOX from the void between the two shells. When the cross-linked PMAA inner shell was subsequently expanded by increasing the solution pH (24−36 h), DOX confined in the interior of PMAA shell was also released. The dual and independent sensitivity of the double-walled PMAA-PNIPAM concentric hollow microspheres are thus potentially useful for biomedical applications.

Conclusions

Nearly monodispersed silica/polymer multilayer hybrid composites and double-walled PMAA-PNIAPM concentric hollow polymeric microspheres with unique morphology and dual stimuli-responsive properties have been synthesized and characterized by FESEM, TEM, FT-IR, and EDX. The structures of polymer/silica multilayer hybrid microspheres and the double-walled hollow microspheres were shown to be well-defined. The respective PMAA inner shell and PNIPAM outer shell in the unique hollow structure can respond independently to changing pH and temperature of the environment. The change in hydrodynamic diameter (D_h) of the PMAA inner shell arises from the Donnan osmotic swelling, while the change in D_h of the PNIPAM outer shell is associated with the lower critical solution temperature behavior of the polymer. The doxorubicin-loaded PMAA-PNIAPM hollow microspheres exhibited temperature and pH-controlled release of anticancer drug. The double-walled PMAA-PNIAPM concentric hollow microspheres, with their unique structures and dual (independent) stimuli-responsive properties, prepared in this work are expected to be useful for stimuli-controlled release of drugs and functional molecules, and for mimicking transport barriers in biological systems.

Experimental Section

Synthesis of Silica/Polymer Multilayer Hybrid Microspheres and their Corresponding Hollow Microspheres. The MPS-modified silica templates were prepared according to the Stöber method.[32,33] About 9 mL of TEOS was added to a mixture of 150 mL of ethanol, 15 mL of DDW, and 3 mL of ammonia. The mixture was stirred vigorously at room temperature for 24 h. Then, 2 mL of MPS was injected into the silica sol over a reaction period of 24 h, to introduce the carbon−carbon double bonds onto the surface of silica microspheres. After the reaction, the resultant MPS modified silica particles (SiO_2-MPS) were purified by five cycles of centrifugation and redispersion in ethanol and doubly distilled water. The SiO_2/PMAA core−shell microspheres were prepared by distillation-precipitation polymerization of methacrylic acid in acetonitrile using EGDMA as a cross-linker.[34] Briefly, about 0.2 g of the SiO_2-MPS seeds was dispersed into 80 mL of acetonitrile under ultrasonication in a 100-mL flask, equipped with a fractionation column, a condenser and a receiver. A mixture of MAA (0.82 mL, 0.01 mol), EGDMA (0.28 mL, 0.0015 mol, 15 mol % relative to MAA) and 2,2′-azobisisobutyronitrile (AIBN, 0.02 g) was then introduced into the flask to initiate the polymerization. The reaction was allowed to proceed under reflux condition. The reaction was stopped within 1.5 h by distilling off 40 mL of acetonitrile. The resultant SiO_2−PMAA core−shell microspheres were purified by extraction with acetonitrile and ethanol five times to remove the unreacted monomers and oligomers. The SiO_2/PMAA/SiO_2 trilayer hybrid microspheres were synthesized by coating of an outer silica layer on SiO_2−PMAA core−shell microspheres via the Stöber reaction described above. About 0.15 g of the SiO_2−PMAA core−shell microspheres and 2.4 mL of ammonia were introduced into a water/ethanol (20 mL/160 mL) mixture under vigorous stirring. About 0.8 mL of TEOS was added dropwise into the flask over a period of 1 h at room temperature. The mixture was vigorously stirred at room temperature for 6 h. Then, 0.2 mL of MPS was injected into the silica sol to introduce the carbon−carbon double bonds onto the surface of silica microspheres over a period of 18 h. After the reaction, the resultant MPS modified SiO_2/PMAA/SiO_2 trilayer hybrids were purified by five cycles of centrifugation and redispersion in ethanol. The SiO_2/PMAA/SiO_2/PNIPAM tetra-layer hybrid microspheres were synthesized by distillation precipitation polymerization in acetonitrile with NIPAM and MBA as the monomer and cross-linker, respectively. The procedures were similar to those used for the synthesis of PMAA layer.

The pH-responsive hollow PMAA microspheres and the dual stimuli-responsive double-walled concentric hollow PMAA-PNIAPM were prepared by removal of the silica layer from the SiO_2/PMAA core−shell microspheres and the SiO_2/PMAA/SiO_2/PNIPAM tetra-layer hybrid mcirospheres, respectively. The multilayer hybrid microspheres were stirred in 10% HF for 48 h. The excess HF and SiF_4 were extracted from the hollow microspheres by five centrifugation-redispersion cycles in ethanol and water. The etching process was repeated two times to remove the silica layers completely. Finally, the hollow structures were dialyzed in double distilled water for two weeks.

Doxorubicin hydrochloride (DOX, Boryung, Pharmaceutical Co. Ltd., Seoul, Korea) was dissolved in 0.9 wt % NaCl solution to a concentration of 0.1 mg/mL. About 2.0 mg of the double-walled PMAA-PNIPAM concentric hollow microspheres was suspended in this DOX solution. The loading of DOX was allowed to take place for 48 h at room temperature and pH ∼ 7. The temperature and pH of the solution were then adjusted to 40 °C and 4, respectively, in the subsequent 2 h to induce shrinkage of the shells. The DOX-loaded microspheres were collected by centrifugation at 8000 rpm. The DOX-loaded hollow microspheres were then equilibrated in an aqueous medium to obtain the release profile. At the first 12 h, the temperature and pH were kept at 40 °C and 4, respectively. The temperature was subsequently adjusted to 25 °C, while maintaining the solution pH at 4, to release DOX through the outer PNIPAM shell in the following 12 h. Finally, the pH of the medium was adjusted to 6 to release DOX from the inner PMAA shell. The solution was removed periodically and characterized by measuring absorbance at the wavelength of 232 nm.

Characterization. FESEM and EDX analysis were performed on a JEOL JSM-6700 scanning electron microscope. TEM images were obtained on a JEOL JEM-2010 transmission electron microscope. The DLS measurements were performed on a Brookhaven 90 plus laser light scattering spectrometer at the scattering angle of $\theta = 90°$. The pH was adjusted by addition of HCl (0.01 M) or NaOH (0.01 M). NaCl was added to keep the solution under a constant ionic strength of 0.01 M. CLSM image was obtained from a Nikon digital eclipse C1 plus confocal system. The samples were placed on glass coverslips and viewed under a 100× oil immersion objective lens.

Supporting Information Available: TEM and DLS results of the pH-responsive PMAA hollow microspheres, EDX results of the SiO_2/PMAA/SiO_2/PNIPAM tetra-layer hybrid microspheres and the double-walled PMAA-PNIAPM concentric hollow microspheres, and TEM image of the SiO_2/PMAA/SiO_2 trilayer hybrid microspheres with a thick silica outer layer. This material is available free of charge via the Internet at http://pubs.acs.org.

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