Nanosphere−Microsphere Assembly: Methods for Core−Shell Materials Preparation

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Received February 9, 2001. Revised Manuscript Received April 3, 2001

Core−shell composite materials consisting of a silica core and a polystyrene (PS) shell were prepared by colloidal assembly of PS nanospheres onto silica microspheres. The assembly process was controlled by specific chemical (amine−aldehyde) or biochemical (avidin−biotin) interactions between the nanospheres and microspheres. Colloidal assembly was performed using polymer nanoparticles (100−200 nm diameter) and silica particles (3−10 μm diameter). Heating the assembled materials to temperatures above the glass transition (T_g) of the polymer nanoparticles allows the polymer to flow over the microsphere surfaces, resulting in uniform core−shell materials. Nanosphere packing density on the microsphere surfaces influenced the uniformity of the resulting polymer shell. Scanning electron microscopy, transmission electron microscopy, scanning force microscopy, and Fourier transform infrared spectroscopy were used to characterize the materials presented in this paper.

Introduction

Core−shell materials consist of a core structural domain covered by a shell domain. The core and shell domains may be composed of a variety of materials including polymers, inorganic solids, and metals. Core−shell materials are typically spherical in shape; however, other shapes are possible. The shell is tailored to keep the core dispersed in a solvent or to protect it from dissolution or hydrolysis. Core−shell materials have been prepared with polymer shells to protect medicines or other materials from dissolution or hydrolysis.[1] Polymer shells are frequently used to stabilize pigments in paints. Core−shell materials can also be used to strengthen polymeric materials.[2] Other areas of application include the preparation of stationary phases for chromatography or the preparation of sensing materials. Core−shell nanoparticles loaded with gadolinium have recently been used as new contrast agents for magnetic resonance imaging.[3]

Techniques for the preparation of core−shell materials on both nanometer and micrometer scales include intramicellar polymerization,[4,5] copolymerization of hydrophobic monomer core−hydrophilic shells,[6] template-directed self-assembly,[7−10] template-directed living polymerization,[11,12] and encapsulation of silica nanoparticles by in situ polymerization.[13] Core−shell-microstructured materials have also been made by self-assembly of amphiphilic rod−coil diblock copolymers.[14,15] Advances in the preparation of core−shell materials have been reviewed recently.[16] Sequential deposition of oppositely charged silica nanoparticles and polymers was used to create core−shell materials with shells of predetermined thickness.[17−19] Ball-like composite aggregates and microstructured hollow spheres have also been produced using electrostatic interactions to assemble particles onto emulsion droplets.[20,21] Another example of colloidal assembly involves the heterocoagulation of oppositely charged core and shell particles.[22−26] Heterocoagulation

(1) Lee, J. H.; Park, T. G.; Choi, H.-K. *J. Microencapsulation* **1999**, *16*, 715−729.
(2) Schirrer, R.; Lenke, R.; Boudouaz, J. *Polym. Eng. Sci.* **1997**, *37*, 1748−1760.
(3) Reynolds, C. H.; Annan, N.; Beshah, K.; Huber, J. H.; Shaber, S. H.; Lenkinski, R. E.; Wortman, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 8940−8945.
(4) Thurmond, K. B.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *199*, 6656−6665.
(5) Ding, J.; Liu, G. *Macromolecules* **1998**, *31*, 6554−6558.
(6) Serizawa, T.; Takehara, S.; Akashi, M. *Macromolecules* **2000**, *33*, 1759−1764.
(7) Hall, S. R.; Davis, S. A.; Mann, S. *Langmuir* **2000**, *16*, 1454−1456.
(8) Marinakos, S. M.; Novak, J. P.; Brousseau, L. C., III; House, A. B.; Edecki, E. M.; Feldhaus, J. C.; Feldheim, D. L. *J. Am. Chem. Soc.* **1999**, *121*, 8518−8522.
(9) Barthet, C.; Armes, S. P.; Lascelles, S. F.; Luk, S. Y.; Stanley, H. M. *Langmuir* **1998**, *14*, 2032−2041.
(10) Marinakos, S. M.; Brousseau, L. C., III; Jones, A.; Feldheim, D. L. *Chem. Mater.* **1998**, *10*, 1214−1219.
(11) Mandal, T. K.; Fleming, M. S.; Walt, D. R. *Chem. Mater.* **2000**, *12*, 3481−3487.
(12) von Werne, T.; Patten, T. E. *J. Am. Chem. Soc.* **1999**, *121*, 7409−7410.
(13) Sondi, I.; Fedynyshyn, T. H.; Sinta, R.; Matijevic, E. *Langmuir* **2000**, *16*, 9031−9034.
(14) Jenekhe, S. A.; Chen, X. L. *Science* **1998**, *279*, 1903−1907.
(15) Chen, X. L.; Jenekhe, S. A. *Langmuir* **1999**, *15*, 8007−8017.
(16) Caruso, F. *Adv. Mater.* **2001**, *13*, 11−22.
(17) Caruso, F.; Caruso, A.; Mohwald, H. *Science* **1998**, *282*, 1111−1114.
(18) Caruso, F.; Lichtenfeld, H.; Giersig, M.; Mohwald, H. *J. Am. Chem. Soc.* **1998**, *120*, 8523−8524.
(19) Caruso, F.; Trau, D.; Mohwald, H.; Renneberg, R. *Langmuir* **2000**, *16*, 8932−8936.
(20) Velev, O. D.; Furusawa, K.; Nagayama, K. *Langmuir* **1996**, *12*, 2374−2384.
(21) Velev, O. D.; Furusawa, K.; Nagayama, K. *Langmuir* **1996**, *12*, 2385−2391.
(22) Kawahashi, N.; Matijevic, E. *J. Colloid Interface Sci.* **1990**, *138*, 534.

10.1021/cm010168z CCC: $20.00
Published on Web 05/25/2001

of anionic polystyrene (PS) particles with cationic poly(butyl methacrylate) (PBMA) particles, followed by heating of the product at temperatures above the T_g of PBMA, results in a core−shell material with a PS core and a PBMA shell.[27]

In this paper, we demonstrate that this technique can be expanded to include specific chemical and biochemical interactions as a way to control the particle assembly. In this method, polymer nanospheres are assembled onto the surface of silica microspheres. The assembled composite is subsequently heated at temperatures above the glass transition (T_g) of the polymer nanospheres, allowing the polymer to flow over the silica microsphere surface and resulting in a uniform core−shell composite. We demonstrate colloidal assembly using 100 and 200 nm diameter amine-modified PS nanospheres assembled onto 3−10 μm diameter glutaraldehyde-activated silica microspheres. Scanning force microscopy (SFM) was used to estimate the packing density of polymer nanospheres on the silica microsphere surfaces. The biospecific interaction between avidin and biotin was also used to control the assembly of PS nanospheres onto silica microspheres. Avidin, a 40 kD glycoprotein, is known to have four high-affinity binding sites for the vitamin derivative biotin. When avidin-labeled PS nanospheres were mixed with biotin-labeled silica microspheres in the appropriate number ratio, PS nanospheres assembled onto the microsphere surfaces, covering the microspheres. Thermally annealed composites, produced by heating PS nanosphere−silica microsphere assemblies at temperatures higher than the T_g of PS, were characterized using several analytical techniques. The compositions of the resulting core−shell materials were confirmed by Fourier transform infrared (FTIR) spectroscopy to be PS−silica composites. The uniformity of the shell material coating was confirmed by scanning electron microscopy (SEM). Core silica particles were etched with hydrofluoric acid to confirm the existence of the shell structure. The resulting hollow polymer microspheres were characterized by transmission electron microscopy (TEM).

Materials and Methods

Materials. Amine-labeled porous silica microspheres (~3 and ~5 μm diameter) were from Phenomenex Inc. (Torrance, CA). Amine-modified PS nanospheres (100 and 200 nm mean diameter) were from Polysciences Inc. (Warrington, PA). A 25% glutaraldehyde solution (aqueous), ethanol, and ethylene glycol were from Sigma−Aldrich Chemical Co. (Milwaukee, WI). Avidin (neutravidin) and biotin sulfosuccinimdyl ester (biotin-SSE) were from Molecular Probes Inc. (Eugene, OR). All other reagents were from Fisher Scientific (Pittsburgh, PA). All solutions were prepared using ultrapure water (Barnstead/Thermolyne, Dubuque, IA).

Methods. ***Nanosphere−Microsphere Preparation.*** Amine-labeled silica microspheres were prepared for assembly by first activating with glutaraldehyde. Prior to activation, approximately 2−6 mg of dry microspheres were placed into an eppendorf tube and washed five times with 1.0 mL of ultrapure water. Microspheres were cleaned using five cycles of centrifugation (5000*g*), supernatant removal, and resuspension in 1.0 mL of ultrapure water. The microspheres were then washed two times with 1.0 mL of a 50 mM phosphate buffer (pH 6.9). The microspheres were then centrifuged again (5000*g*), the supernatant was removed, and 1.0 mL of a 2.5% glutaraldehyde solution in 50 mM phosphate buffer (pH 6.9) was added. The microspheres were suspended, covered with foil, and mixed on a vortex shaker for 2 h at 4 °C. After 2 h the microspheres were washed five times with 1.0 mL of ultrapure water. Microspheres were washed another five times to exchange them into a 50 mM phosphate buffer (pH 7.4) (PBS). The microspheres were stored at 4 °C protected from light until needed for the assembly process. Amine-modified PS nanospheres were similarly prepared for assembly by washing (centrifuging at 18 000*g* and resuspending in water) five times with 1.0 mL of ultrapure water and two times with 1.0 mL of 50 mM PBS. Nanospheres were stored at 4 °C until used in the assembly process. Typically, 100 μL of a 2.7% (w/v) suspension of nanospheres was prepared using this procedure.

Biotin-labeled silica microspheres were prepared by treating 2−4 mg of amine-labeled silica microspheres with 1.0 mL of a 5 mM solution of biotin-SSE in 0.13 M sodium bicarbonate (SBB) buffer (pH 8.3). The microspheres were suspended and shaken on a vortex shaker for 1 h at 4 °C. Excess biotin-SSE is removed with several cycles of centrifugation (5000*g*), supernatant removal, and resuspension with 1.0 mL of 50 mM PBS. The microspheres were stored at 4 °C until used in the colloidal assembly process. Alternatively, silica microspheres were labeled with avidin by treating 2−4 mg of glutaraldehyde-activated silica microspheres with 1.0 mL of a 2 mg/mL avidin in phosphate buffer (pH 6.9) for 2 h at 4 °C. Excess avidin was removed by several cycles of centrifugation and resuspension in 50 mM PBS.

Biotin-labeled nanospheres were prepared as follows: 100 μL of a 2.7% (w/v) suspension of amine-modified PS nanospheres was washed four times with 1.0 mL of ultrapure water and then with 1.0 mL of 0.13 M SBB. Biotin-SSE was then added to a final concentration of 5 mM. The nanospheres were shaken on a vortex mixer for 1 h at 4 °C. Subsequently, excess biotin-SSE was removed with three cycles of centrifugation/resuspension in 1.0 mL of 50 mM PBS. When avidin-modified nanospheres were needed for the assembly process, biotin-modified nanospheres (in 50 mM PBS) were treated with 1.0 mL of a 0.1 mg/mL solution of avidin in 50 mM PBS. The nanosphere/avidin suspension was gently mixed and then shaken for 2 h at 4 °C on a vortex shaker. Subsequently, the nanospheres were washed seven times with 1.0 mL of 50 mM PBS. Avidin-modified nanospheres were stored at 4 °C until they were used in the assembly process.

Nanosphere−Microsphere Assembly. The colloidal assembly process that we describe was controlled by either specific chemical or biochemical interactions. The reactions of amine-modified PS nanospheres with glutaraldehyde-activated silica microspheres and avidin/biotin-labeled PS nanospheres with avidin/biotin silica microspheres were used to direct the assembly. Amine-modified PS nanospheres were assembled onto aldehyde-activated silica microspheres as follows: Aldehyde-activated silica microspheres (2−4 mg) were suspended in 50 mM PBS. Depending on the particle sizes, this suspension contained a microsphere concentration of approximately 6.0×10^8 particles/mL. An appropriate volume of a suspension of amine-modified PS nanospheres in PBS was then added so that a 5000:1 number ratio of nanospheres to microspheres was achieved. The suspension was shaken at 4 °C for 12−18 h on a vortex mixer. Subsequently, the product was purified by alternately centrifuging (200*g*) and resuspending the assembled product in ultrapure water. An identical process was followed when assembly was controlled by the biospecific interaction of avidin- and biotin-labeled nanospheres and microspheres.

(23) Furusawa, K.; Nagashima, K.; Anzai, C. *Colloid Polym. Sci.* **1994**, *272*, 1104.
(24) Harding, R. D. *J. Colloid Interface Sci.* **1972**, *40*, 164−173.
(25) Okubo, M.; Miyachi, N.; Lu, Y. *Colloid Polym. Sci.* **1994**, *272*, 270.
(26) Ottewill, R. H.; Schofield, A. B.; Waters, J. A. *Colloid Polym. Sci.* **1996**, *274*, 763.
(27) Ottewill, R. H.; Schofield, A. B.; Waters, J. A.; St. J. Williams, N. *Colloid Polym. Sci.* **1997**, *275*, 274−283.

Nanosphere−Microsphere Assembly Melting. To produce a material with a core−shell morphology, the nanosphere−microsphere assemblies were heated at 170−180 °C in ethylene glycol using a temperature-controlled hot plate with a silicone oil bath. As the temperature increased above the glass transition (T_g) of the polymer nanospheres, the polymer melted and then flowed over the surface of the silica microsphere templates. As a result, uniform core−shell materials consisting of a silica core and polymer shell were produced. To prepare the nanosphere−microsphere assemblies for melting, 2 mg of the assembled product was suspended in 250 μL of ethylene glycol. Ethylene glycol was chosen as the solvent because it has a high boiling point and PS is insoluble in it. This suspension was then added to 750 μL of ethylene glycol in a glass vial maintained at 170−180 °C (silicone oil bath). The mixture was stirred vigorously for 5−10 min. The mixture was then removed from the oil bath and sonicated while cooling in room temperature water. The suspension was then centrifuged and resuspended in ethanol two times. The suspension was dried on a piece of aluminum and subsequently was brought to a temperature of 170−180 °C. The composite was heated at this temperature for 20 min. After this time the product was allowed to cool, removed from the metal, and resuspended in ultrapure water. The product was sonicated for 2 min, then centrifuged, and resuspended in ultrapure water an additional two times. To melt the avidin/biotin-directed assembly, the composite was first washed with ethanol and then applied in a thin layer on an aluminum metal block. The block was then heated at 170−180 °C for 20−30 min to allow the assembled PS nanoparticles to melt and flow over the silica microsphere surfaces.

Electron Microscopy. SEM and TEM analyses were performed using standard techniques and instrumentation.

FTIR Spectroscopy. FTIR (Nicolet Magna-760, Nicolet Instrument Corp., Madison, WI) spectroscopy was used to identify the polymer on the microsphere surfaces. Spectra were obtained at a resolution of 2 cm^{-1}, and averages of 64−100 spectral/scans (for an enhanced signal) were obtained in the wavenumber range of 400−4000 cm^{-1}. All samples were prepared for analysis using a KBr pellet. Pellets were prepared using a 50:1 weight ratio of KBr to sample. All spectra were acquired at room temperature.

SFM. Surfaces of composite microspheres were imaged using a Digital Instruments Nanoscope IIIa scanning probe microscope (Digital Instruments Inc., Santa Barbara, CA). Images were acquired in tapping mode under standard conditions.

Nitrogen Analysis. Weight percent nitrogen was determined by Galbraith Laboratories Inc. (Knoxville, TN). Oven-dried samples of amine-labeled silica microspheres and amine-modified polymer nanospheres were submitted for analysis.

Chemical Etching of Core−Shell Composites. The silica cores were etched using an 8% aqueous solution of hydrofluoric acid (**Caution! Hydrofluoric acid solutions are extremely corrosive**). Approximately 1 mg of a PS−silica composite was suspended in 1.0 mL of ultrapure water. Concentrated hydrofluoric acid (50% w/v) was then added to bring the total HF concentration to 8%. The suspension was allowed to stand for 20 min to ensure complete removal of the silica cores. The composite was then washed five times with 1.0 mL of ultrapure water. The resulting hollow polymer microspheres were then air-dried on glass slides prior to SEM or TEM analysis.

Results and Discussion

Specific chemical and biological interactions between colloidal particles were used to control the assembly of polymer nanospheres onto the surfaces of silica microspheres. The general procedure for the colloidal assembly of polymer nanospheres with silica microspheres is shown in Figure 1. The assembly process was performed by mixing a suspension of complementary types of nanospheres and microspheres at 4 °C for 12−18 h. The goal of the assembly process was to pack as many nanospheres onto the microsphere surface as possible. We estimated the numbers of nanospheres that could pack onto the microsphere surfaces by dividing the theoretical microsphere surface area by the cross-sectional area of a plane bisecting a nanosphere. The resulting value was used to determine the minimum number of nanospheres needed in suspension for each microsphere present. For example, to assemble 100 nm PS nanospheres onto 3 μm diameter silica microspheres, we calculated that approximately a 5000:1 number ratio of nanospheres to microspheres should be used. Similar estimates were made when nanospheres and microspheres with different diameters were assembled. The number of nanospheres required to completely cover a microsphere can also be calculated from theory.[27,28] Our estimates agree well with this theory, which assumes hexagonal close packing of the nanospheres onto a planar surface.

Following assembly, the composites were heated at 170−180 °C in order to melt the polymer nanospheres (see Figure 1). The polymer melts and flows over the microsphere surfaces to yield uniform core−shell materials consisting of a silica core and a polymer shell. Representative SEM images of these materials are shown in Figures 2a−d. An SEM image of 100 nm amine-modified PS particles assembled onto 3 μm diameter glutaraldehyde-activated silica microspheres is shown in Figure 2a. Figure 2b is an SEM of 200 nm diameter amine-modified PS particles assembled onto 5 μm diameter silica microspheres. An SEM of 100 nm avidin-labeled nanospheres assembled onto 3 μm diameter biotin-labeled silica microspheres is shown in Figure 2c. Tapping mode SFM was used to image the surfaces of composite microspheres (5 μm diameter) that were assembled with 200 nm PS nanospheres. The SFM scan shows that the 200 nm PS particles are assembled in a dense array on the surface of the microspheres (Figure 2e). As described previously,[27] the packing density of the polymer nanospheres on the surface of the silica microspheres is an important variable in the formation of a uniform polymer shell around the silica microspheres. Packing density was easy to control when the assembly process was controlled by amine−aldehyde chemistry. The packing density was, however, more difficult to control when assembly was controlled by the interactions of avidin- and biotin-labeled colloidal particles. By comparing parts a and b of Figure 2 with Figure 2c, one can see that the coverage of the silica microsphere surface with PS particles was not as uniform when avidin/biotin assembly was employed as when amine−glutaraldehyde chemistry was used to link the polymer nanospheres to the silica microsphere surfaces. The less uniform packing of nanospheres on the silica with avidin/biotin chemistry may be due to some slight aggregation of the avidin-labeled nanospheres prior to performing the assembly with biotin-labeled silica. Aggregation may arise during the synthesis of the avidin-labeled nanospheres, because the nanospheres are first labeled with biotin and then subsequently treated with an excess of avidin. Cross-

(28) Roulstone, B. J.; Waters, J. A. European Patent No. 549,163, 1994.

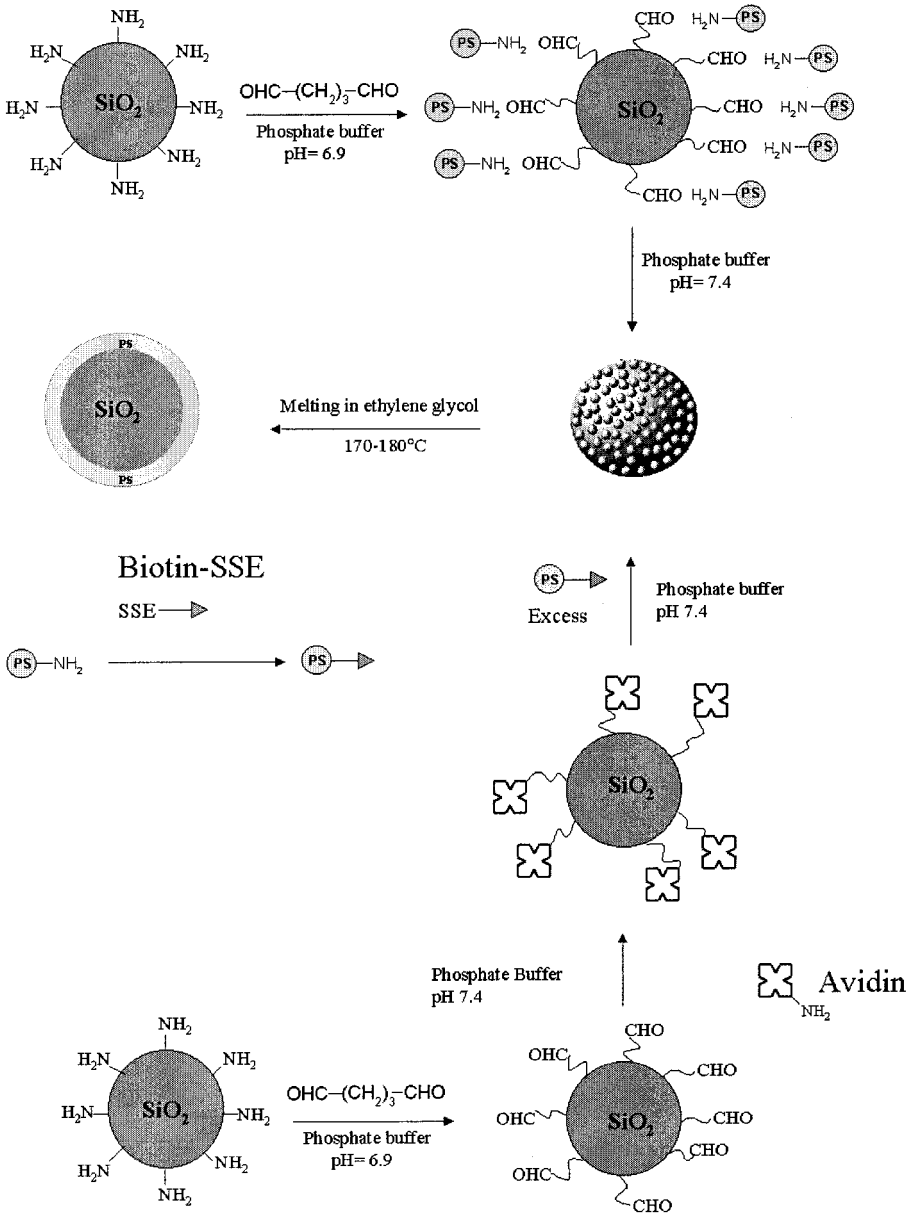

Figure 1. Scheme for assembling composite materials via both glutaraldehyde chemistry and biospecific interactions. The top section illustrates assembly starting with amine-labeled silica. Glutaraldehyde treatment of the labeled silica, followed by reaction with amine-modified PS nanospheres, results in an assembled material. This material is heated at 170−180 °C to melt the PS, resulting in a core−shell material composed of a silica core and PS shell. The bottom section illustrates assembly of biotin-labeled PS nanoparticles onto avidin-coated silica microspheres.

linking of nanospheres may occur if the concentration of nanospheres in the suspension is too high relative to the amount of avidin used. Aggregation may also occur when insufficient numbers of biotin-labeled nanospheres are mixed with avidin-labeled silica microspheres. Despite the disadvantages to the use of avidin/biotin, the assembled composites are comparable to those formed when amine−glutaraldehyde is used to control the assembly process.

Because the methods we used to control the assembly process involve specific chemical and biochemical interactions, it was necessary to verify that the assembled composites were the result of these specific interactions between the particles and not of nonspecific interactions. Nonspecific binding during the assembly process was minimal for both the amine−glutaraldehyde and avidin/biotin methods. Percentages of nonspecific binding were estimated based on the theoretical maximum number of nanospheres that could cover half of a microsphere surface. The number of nanospheres visible in SEM images of controls assembled with nonspecific binding were counted and taken as the percentage of the theoretical maximum. Approximately 10−12% nonspecific binding was observed when amine-modified nanospheres were mixed with amine-coated silica microspheres. The weight percents of nitrogen for the amine-labeled silica microspheres and amine-modified PS nanospheres, that were used in the nonspecific binding control experiment, were 0.73% and <0.5%, respectively. Because both microspheres and nanospheres were labeled with amine groups, they should have roughly the same net charge at pH 7.4. Any binding observed is, therefore, likely due to nonspecific interactions that overcome the repulsive electrostatic forces. An SEM

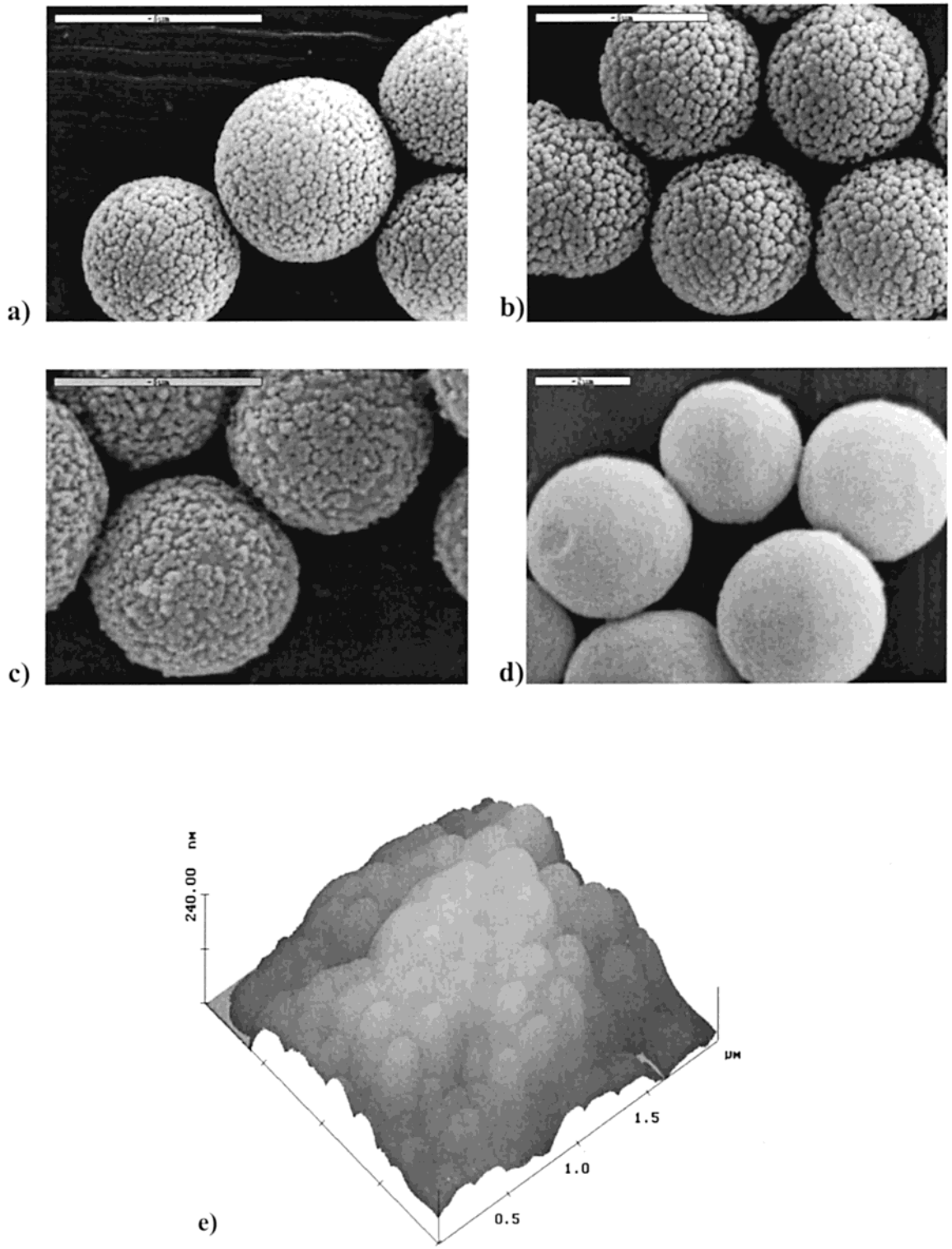

Figure 2. (a) 3 μm mean diameter glutaraldehyde-coated silica assembled with 100 nm mean diameter amino-functionalized PS particles. (b) 5 μm mean diameter glutaraldehyde-coated silica assembled with 200 nm mean diameter amino-functionalized PS nanoparticles. (c) 5 μm mean diameter avidin-coated silica assembled with 100 nm mean diameter biotin-coated PS nanoparticles. (d) PS-coated silica particles obtained after heating of the assembled material shown in part a at 170−180 °C in ethylene glycol. (e) Tapping mode SFM scan of the surface of the composite produced when 200 nm PS nanospheres are assembled on 5 μm diameter silica. The scale marker bars in parts a−c correspond to 5 μm. The scale marker bar in Figure 2d corresponds to 2 μm.

image of microspheres prepared using nonspecific binding is shown in Figure 3. Other nonspecific binding controls included mixing unmodified PS nanospheres with aldehyde-activated silica microspheres, amine-modified PS nanospheres with unmodified silica microspheres, and unmodified PS nanospheres with unmodified silica microspheres. In each of these cases, nonspecific binding was <1%. A nonspecific binding control for an avidin/biotin-directed assembly was performed by mixing avidin-labeled nanospheres with avidin-labeled silica microspheres. Approximately 1% nonspecific binding was observed.

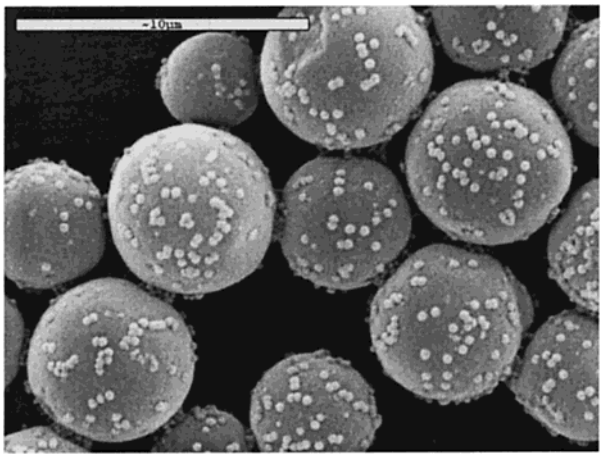

Figure 3. SEM of a nonspecific binding control. 100 nm amine-modified nanospheres were mixed with amine-labeled silica microspheres under conditions identical with those in the assembly process.

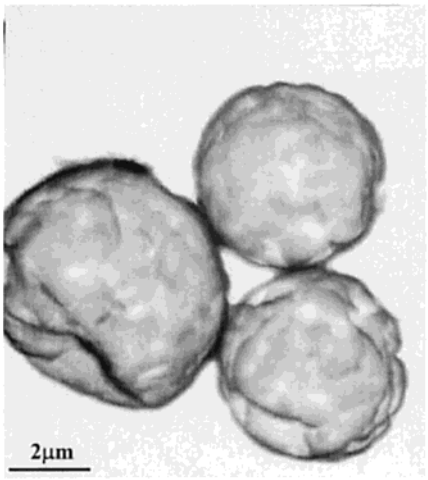

Figure 4. TEM of hollow microspheres after chemical etching of core−shell materials produced by assembly/melting of 200 nm PS nanospheres with 5 μm diameter silica microspheres.

The assembled composites prepared by either the amine−glutaraldehyde or avidin/biotin methods were very stable (as observed by SEM). No noticeable changes in the surfaces of the materials were observed upon several weeks of storage in solution at room temperature or at 4−8 °C. Suspension in ethanol or ethylene glycol had no effect unless the temperature was increased above the glass transition (T_g) of PS. The stability of the assembled composites in ethylene glycol was important because the melting procedure was performed at elevated temperature in this solvent.

The PS nanosphere−silica microsphere assemblies were heated in ethylene glycol under the premise that the polymer nanospheres would melt and the polymer would flow over the silica microsphere surfaces, producing a core−shell composite with a uniform polymer coating. Ethylene glycol was chosen as the solvent for heating the materials because it has a high boiling point and because PS and many other polymers are insoluble in it. Microsphere aggregation during the heat treatment was minimized by controlling the concentrations of microspheres in solution. Annealing the composites on an aluminum metal block after the initial heating in ethylene glycol helped to improve the uniformity of the polymer coating. Melting of the 100 nm PS−3 μm silica microsphere assembly (Figure 2a) at high temperature in ethylene glycol, followed by heating on an aluminum block results in the uniform PS−silica core−shell composite shown in Figure 2d. Avidin/biotin-assembled composites could only be melted on an aluminum metal surface because melting in an ethylene glycol solution did not result in uniformly coated core−shell composites. This result may be due to the instability of the avidin/biotin linkage in solution at the high temperatures used to melt the nanospheres, which causes them to dissociate. To verify that PS was coating the silica microspheres, two separate experiments were performed. A time study was conducted by heating the PS nanoparticle-coated silica microspheres in ethylene glycol and then removing aliquots of assembled microspheres at various times during the course of the 30 min heating. SEM images showed that nanoparticles remained attached to the silica microsphere surface after 5 min. As the heating time increased, the spherical

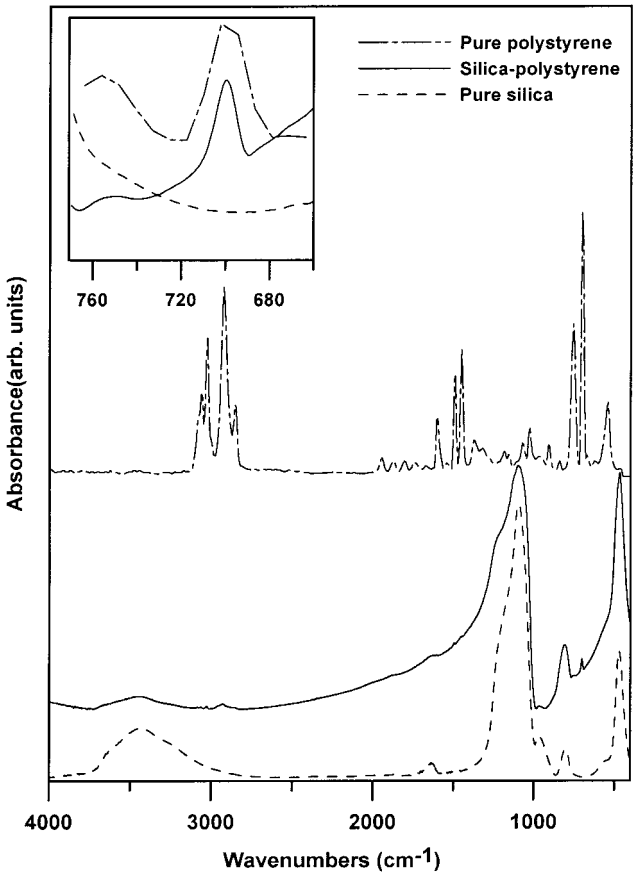

Figure 5. FTIR spectroscopy of pure PS (top spectrum), a melted PS−silica composite (middle spectrum), and pure silica (bottom spectrum). The inset is a comparison of the spectra in the range of 650−780 cm^{-1}. Bands at 750 and 697 cm^{-1} correspond to PS−benzene C−H out-of-plane bending and ring bending vibrations, respectively.

nanoparticles melted and gradually filled in the spaces between nanoparticles until the surface was uniformly coated. SEM images of the time series are included in the Supporting Information. The integrity of the polymer shells was determined by removing the silica cores by chemical etching with hydrofluoric acid. After chemical etching, hollow polymer shells were all that remained of the composite. Most of the hollow polymer

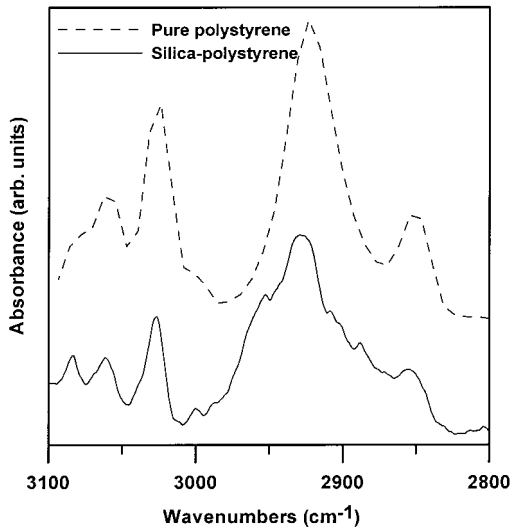

Figure 6. Comparison of FTIR spectra of pure PS and a PS−silica composite. Peaks in the wavenumber range (2850-2950 cm^{-1}) correspond to the aliphatic C−H stretching of PS.

microspheres produced remained intact after sonication in ultrapure water and centrifugation at 2000*g*. This result provides additional evidence of polymer coating, because the silica microspheres do not survive such a treatment. A representative TEM of hollow polymer shells produced by assembling 200 nm PS nanospheres onto 5 μm silica microspheres, followed by annealing and chemical etching, is shown in Figure 4. The hollow spheres in Figure 4 do not appear perfectly uniform because of deformation during purification. Uniformity was also affected by the heating time. FTIR spectra (Figure 5) of the PS−silica core−shell composites provide additional evidence that the melting procedure results in polymer-coated microspheres. The spectra reveal bands at 750 and 697 cm^{-1}, which correspond to the phenyl C−H out-of-plane bending and benzene out-of-plane ring bending, respectively. Both of these resonances are characteristic of PS and are absent from the starting silica microspheres. Aliphatic C−H stretching resonances of PS (2850-2950 cm^{-1}) can be seen in the PS-silica composite spectrum shown in Figure 6.

Conclusions

Nanosphere−microsphere assembly accesses novel materials through a simple yet flexible procedure. By selection of the compositions of the particles used in the assembly procedure, considerable control can be gained over the physical and chemical properties of the resulting composites. Additional control over physical−chemical properties is achieved by the ability to melt assembled polymer particles, yielding uniform silica core−polymer shell composite materials. The use of specific chemical−biochemical interactions to control the assembly process of colloidal particles may have several advantages over the use of electrostatic interactions or heterocoagulation to prepare core−shell composites. One advantage is that a wider range of materials may be assembled when specific interactions are used. For example, particles that are not charged or have the same charge may still be assembled using this technique. Amine-modified PS nanospheres are assembled onto amine-labeled silica by simply activating the silica surface with a cross-linking dialdehyde. Another advantage is the improved stability of the assembled products when covalent or strong biospecific interactions are employed. The stability of the bonds between the particles may allow the use of a wider range of pHs, ionic strengths, and solvents in the assembly process.

We have shown that assembled materials can be used to produce core−shell composites consisting of a silica core and a PS shell. Such materials may have many applications in both analytical and materials chemistry development. Core−shell composite materials may have application, for example, in the design of layered sensing materials, the production of stationary phases for chromatographic separations, or the development of drug delivery systems. It has been reported that a thin polybutadiene film can be physically adsorbed onto zirconia surfaces and then cross-linked, resulting in a stationary phase for reversed-phase chromatography with exceptional stability at high pH.[29] In the future we plan to extend the range of particle sizes with which we perform the particle assembly. It may also be possible to create core−shell materials with the shells consisting of two or more polymers by simply mixing polymer nanospheres in the desired ratios prior to assembly and annealing.

Acknowledgment. This research was supported by grants from NIH (GM 48142) and DARPA.

Supporting Information Available: SEM images of the PS−silica heating over time, 200 nm PS nanospheres assembled on 5 μm silica microspheres (SEM), and additional SEM images of PS−silica composites (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) Rigney, M. P.; Weber, T. P.; Carr, P. W. *J. Chromatogr.* **1989**, *484*, 273.