

Hydrogel-Coated Glassy Nanospheres: A Novel Method for the Synthesis of Shell Cross-Linked Knedels

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Abstract: A novel method was developed to prepare shell-cross-linked knedel-like structures (SCK's), which are double-layered polymer nanospheres, in a convenient three-step procedure: (1) polystyrene-*b*-poly(acrylic acid) ((DP_n)_{PS} = 130, (DP_n)_{PAA} = 120) copolymer was prepared by sequential anionic polymerization of styrene and *tert*-butyl acrylate, followed by hydrolysis; (2) the amphiphilic block copolymer was allowed to self-assemble into spherical multimolecular micelles in a water and THF solvent mixture; (3) the poly(acrylic acid), comprising the shells of micelles, was cross-linked by amidation using several di- and multiamino linkers (2,2'-(ethylenedioxy)-bis(ethylamine), hexakis(ethylene glycol)diamine, hexamethylenediamine, and triethylenetetramine). The extent of cross-linking was controlled by the relative amount of cross-link reagent used. The SCK's were spherical, core-shell structures composed of polystyrene cores and cross-linked hydrogel-like shells. The sizes, shapes, and structural differences between the polymer micelles and the SCK's were studied by atomic force microscopy (AFM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) in aqueous solution. The non-cross-linked polymer micelles deformed substantially upon adsorption onto mica and became ellipsoidal upon drying on a carbon surface, whereas the SCK's remained as stable, spherical structures under all conditions. The polymer micelles had a number average height of 17 nm from AFM and 26 nm diameter from TEM, while the SCK's prepared from 2,2'-(ethylenedioxy)bis(ethylamine) cross-linkers had a number average height of 24 nm from AFM and a number average diameter of 28 nm from TEM. The SCK shell thickness swelled 2–3-fold in water, as determined from comparison of the number average diameter obtained by DLS (37 nm) with that by TEM. Spectroscopic methods and thermal analysis were utilized to further characterize the SCK's.

Introduction

Shell-cross-linked knedel-like macromolecular structures¹ (SCK's) have been synthesized by a novel three-step approach, to produce spherical, nanometer-sized, core-shell particles composed of a polystyrene (glass) core surrounded by and covalently bound to a shell of cross-linked poly(acrylic acid)/polyacrylamide (hydrogel). Hydrogel materials are cross-linked networks of hydrophilic polymers that swell in the presence of water, to contain 20–95% water, while maintaining their overall shape. Due to the surface mobility and the high degree of swelling, which can be made environment responsive, hydrogels are receiving considerable attention for a range of applications in biomedicine and biotechnology.² The volume expansion of a hydrogel upon swelling is dependent upon the nature of the hydrophilic polymer, the degree of cross-linking, and the initial size and shape of the hydrogel sample. As the size (or film thickness) of the hydrogel decreases, the volume expansion factor increases,³ and hydrogel foams⁴ have been found to

possess high swelling ratios and rates of swelling. The incorporation of a hydrogel surface layer into the SCK structure allows for a dramatic increase in the surface-to-volume ratio by decreasing the size of hydrogel particles, while accurately controlling their shape and mechanical strength.

SCK's¹ are a new class of nanomaterials, which resemble polymer micelles⁵ in their amphiphilic core-shell morphology but are similar to dendrimers⁶ and arborescent polymers⁷ in being covalently bound and stable structures. As an extension of the previous work, highly water soluble units (poly(acrylic acid) and polyacrylamide) have now been incorporated into the shells of the SCK's. In addition, the chemistry used for the preparation of SCK's involved addition of multifunctional cross-linking agents rather than radical addition polymerization of side groups.¹ The versatility of this method was demonstrated by using different types of linkers and varying the extent of cross-

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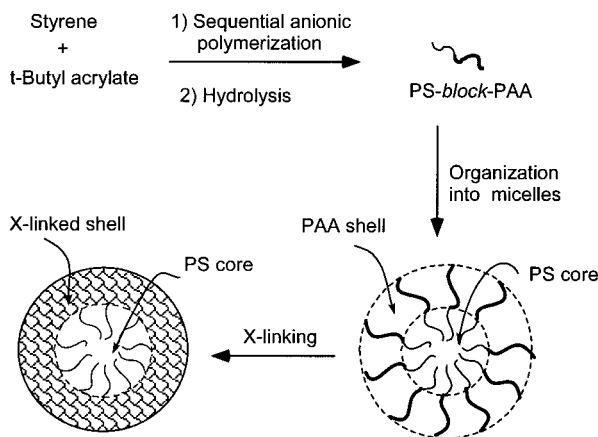


Figure 1. Illustration of the three-step synthesis to prepare SCK's: (1) formation of an amphiphilic block copolymer, PS-*b*-PAA; (2) self-assembly of the block copolymer into polymer micelles; (3) cross-linking of the shell layer by reaction of functionalities located along the backbone of the hydrophilic segment of the block copolymer.

linking to obtain a series of SCK's with different dimensions, compositions, and surface-layer charge densities.

Results and Discussion

As shown schematically in Figure 1, the three main steps for the preparation of SCK's include: (1) synthesis of the amphiphilic block copolymer, containing reactive functionalities along the hydrophilic block; (2) placement of the amphiphilic block copolymer into a solvent system at a concentration to allow for micellization to form spherical nanospheres, but at low enough concentration to prevent intermicellar reactions, and (3) cross-linking throughout the peripheral layer by reactions of side groups along the hydrophilic backbone. In the present SCK's, the block copolymer is polystyrene-*b*-poly(acrylic acid)⁸ (PS-*b*-PAA) and the cross-linking is accomplished by coupling reactions of the carboxylic acid functionalities with multifunctional linkers. Polymerizations of the exterior head groups of micelles formed from low molecular weight surfactants in aqueous media have been reported to be difficult,⁹ due to the instability of the micelles and the high critical micelle concentration (cmc). In contrast, amphiphilic polymers have lower cmc's and form micelles of greater stability, which allow for intramicellar polymerization and cross-linking to occur.¹⁰

The PS-*b*-PAA was prepared by sequential anionic polymerization of styrene and then *tert*-butyl acrylate, followed by hydrolysis.¹¹ An aqueous solution of polymer micelles was prepared by dissolving PS-*b*-PAA in THF, a common solvent for both blocks, and adding water (0.5 mL/min), which is a good solvent for PAA and a nonsolvent for PS, to induce

micellization. The THF was then removed by dialysis against distilled water.

Initially, a diamino oligo(ethylene glycol), 2,2'-(ethylene-dioxy)bis(ethylamine) (**1**), was selected as the cross-linker because of its solubility in water and the ability to perform amidation cross-linking reactions between the acrylic acid repeat units at room temperature in an aqueous solution of the PS-*b*-PAA to yield a cross-linked poly(ethylene glycol)-acrylamide shell layer. The carboxylic acids on the PAA block of PS-*b*-PAA micelles in an aqueous solution were activated by addition of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide methiodide (**2**).¹² The linker, **1**, was then added to cross-link the PAA domain by formation of amide bonds. The extent of cross-linking was varied, as 15% and 100%,¹³ by addition of the appropriate amounts of the carbodiimide and the cross-linker to give SCK's **3** and **4**, respectively (Scheme 1).

The progress of the cross-linking was monitored by the rate of disappearance of resonances due to carbodiimide methyl protons at 1.20 ppm and appearance of the urea methyl protons at 1.05 ppm, in D₂O. Interestingly, at 1:1 stoichiometry of the acrylic acid repeat units and **2**, only one-half of the acrylic acid units on the block copolymer reacted initially, and the reaction proceeded further after the addition of the linking reagent **1**. Presumably, after one acrylic acid unit reacts with **2**, the neighboring unit becomes sterically hindered. Replacing the bulky leaving group by a linear linker allows the adjacent unreacted acrylic acid to be activated by **2**. This facilitates a smooth conversion from micelles to SCK's without a sudden change of the amphiphilic nature of the spheres. As a result, the SCK's inherit the globular morphology of the micelles, avoiding possible precipitation or aggregation of the intermediate polymer micelles.

To demonstrate the versatility of this approach and to obtain SCK's with different compositions and properties, several other linkers were employed. By cross-linking the polymer micelles with hexakis(ethylene glycol)diamine, hexamethylenediamine, and triethylenetetramine, under the same conditions as for **3** and **4**, SCK's **5**, **6**, and **7** were prepared, respectively (Scheme 1). In all cases, the SCK formation was performed at 0.1–0.8 mg/mL micelle concentration and was complete within 2 h. Dialysis of the aqueous reaction mixture against water removed the urea byproduct and allowed for isolation of the SCK products. Aqueous solutions of the SCK's appeared clear with a bluish tint, and they remained stable for months at room temperature when at low concentrations.

Characterization of the entire compositions of the SCK's by solution NMR was not possible because the SCK's were "invisible" in the solution (D₂O) ¹H NMR spectra, despite the appearance of solubility. However, upon addition of an excess of THF-*d*₈, resonances from the PS core were observed. This indicated that the cross-linked shell was permeable and the PS core was swelled by THF-*d*₈.

Therefore, the compositions of the SCK's were characterized by IR, solid-state ¹³C NMR, and elemental analysis. Solid samples of PS-*b*-PAA micelles and resulting SCK's were obtained by lyophilizing dialyzed aqueous samples. For the micelle sample, a broad OH band at 3600–2500 cm⁻¹ and C=O stretch at 1713 cm⁻¹ from the carboxylic acid groups are observed. Upon amide formation, the carbonyl band blue shifts, and amide I and II bands appear at *ca.* 1650 and 1560 cm⁻¹.

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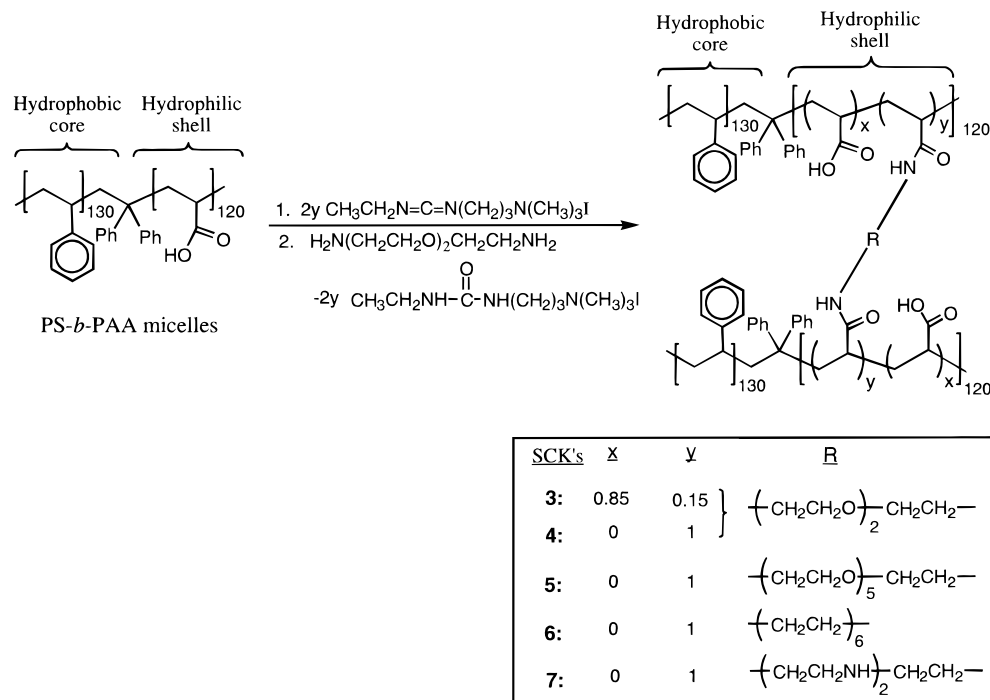
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Scheme 1. Formation of SCK's 3–7 by Cross-Linking of the PAA Peripheral Layer of PS-*b*-PAA Micelles

IR absorption bands for the carboxylic acid and amide functionalities are seen for **3**, while only the amide I and amide II bands are observed for **4–7**. In the solid-state ¹³C NMR spectrum of **4**, the amide carbon has a resonance centered at 162 ppm, the ethylene oxide linker carbons resonate at 70 ppm, and the resonances of the backbone and aromatic styrenyl carbons are observed at 30–50, 130, and 145 ppm. The elemental analysis also confirms the extent of cross-linking; the percentages of C, H, and N agreed with the calculated values.

Differential scanning calorimetry (DSC) shows two glass transition temperatures (*T_g*'s), at 97 and 128 °C, for the lyophilized PS-*b*-PAA micelles, indicating phase separation into domains for polystyrene and poly(acrylic acid). For each of the solid SCK samples, only one *T_g* is observed, corresponding to the PS core. Although single *T_g*'s for the SCK's are observed, separate domains for the core and shell are supported by experiments involving plasticization of the shell by polar small organic molecules. In these experiments,¹⁴ the shell *T_g* for **4** decreased to 15 °C, whereas the core maintained the *T_g* of PS (~100 °C). Without plasticizer present, the glass transitions for the shells of the SCK's are not observed prior to the onset of thermal degradation of the materials. The thermal stabilities of the freeze-dried micelles and SCK's were examined by thermogravimetric analysis (TGA). Incorporation of the ethylene glycol linker decreases the thermal stability; the micelles lost 60% of mass at 410 °C, whereas **3**, **4**, and **5** experienced 60% mass loss at 390, 370, and 365 °C, respectively. SCK's with other linkers also exhibited lower thermal stabilities; **6** and **7** experienced 60% mass loss at 380 and 360 °C, respectively.

(14) The plasticizer was 1-(3-(dimethylamino)propyl)-3-ethylurea methiodide, a byproduct which remains present within the SCK's unless removed by dialysis. For comparative purposes, cross-linking experiments involving homoPAA were performed at similar concentration to that used in the preparation of the SCK's. As expected, the *T_g* of homoPAA after being cross-linked with **1** depends upon the extent of cross-linking and the presence of the plasticizing urea byproduct. In experiments with PAA (*M_w* = 5000 amu) at 0.5 mg/mL, reaction of 15% of the acrylic acid groups with **2** and then **1** gave a product that exhibited a *T_g* = 83 °C prior to dialysis and *T_g* = 120 °C after removal of the urea byproduct; conversion of 100% of the homoPAA acid functionalities resulted in a tacky material with *T_g* = 17 °C prior to dialysis, and following removal of the urea by dialysis, no *T_g* was observable below 160 °C.

The size and shape of the SCK's were measured in the solid state by atomic force microscopy (AFM) and transmission electron microscopy (TEM) and in solution by dynamic light scattering (DLS). Interesting contrasts were found between the PS-*b*-PAA micelles and the SCK's, which demonstrate the stability induced through the cross-linked shell. As shown in Figure 2a, the PS-*b*-PAA micelles are dispersed upon the mica surface, with a corona surrounding each particle (see inset). The number average height of micelles adsorbed on mica was 17 nm. The SCK's form regularly-packed aggregates of spherical particles (Figure 2b), with no visible coronas, and the number average height of **4** was 24 nm. Although the difference in aggregation behavior is consistent with differing surface polarities, the difference in height between the micelles and SCK's cannot be accounted for by the increase in volume of material due to the linker or by the change in the polarity due to the transformation from the acid to the amide. This is supported by functionalization of the PS-*b*-PAA with 2-methoxyethylamine, a monofunctional amine analogue of **1** that cannot form cross-links, which gave modified micelles (**8**) having a number average height of 17 nm under the same AFM conditions. This indicates that the non-cross-linked polymer micelles spread on the mica surface, whereas the SCK's maintain spherical shape. Comparison of the histograms for the PS-*b*-PAA micelles, non-cross-linked functionalized micelles **8**, and SCK **4** (Figure 3) demonstrates that the shell cross-linking improves the mechanical stability of the structures.

Evaluation of the diameters of micelles and SCK's by TEM agreed with the AFM heights and with the conclusion of deformation of the micelles. Figure 4 contains TEM images of the micelles and SCK's **4–7** on a carbon surface. The surface interactions with the carbon film are different from those of the polar mica surface used for AFM, causing the micelles and **7** (containing positive charges from protonated amino groups) to aggregate on the carbon, whereas the neutral SCK's are more dispersed. The micelles are somewhat ellipsoidal, and size distribution analysis (from measurement of both the long and short axes) gives an average diameter of 26 nm. From the size of the micellar particles and the contents of PS and PAA, the

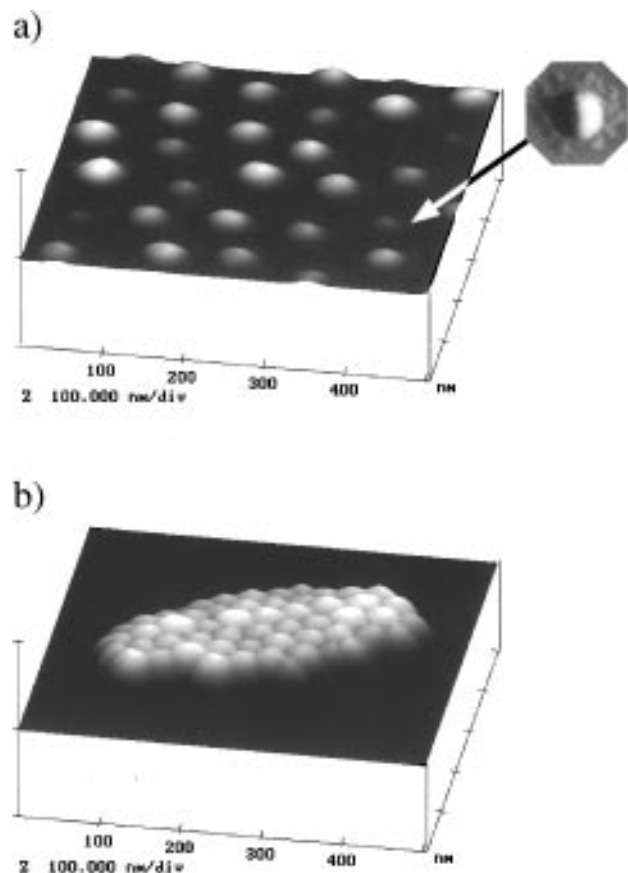


Figure 2. Tapping-mode AFM images of (a) PS-*b*-PAA micelles and (b) **4**, deposited on freshly cleaved mica. The lateral sizes of the particles are distorted due to the finite size of the AFM probe tip; therefore, the particle diameters were measured from the particle heights. The enlargement in a shows a typical corona around PS-*b*-PAA micelles observed in error signal (cantilever amplitude) mode.

number of PS-*b*-PAA chains in each micelle is calculated as 270. The average diameter for **4** was found to be 28 nm, which is expected upon the basis of the increase in volume from the addition of **1**. From comparison of Figure 4a with Figure 4b–e, it is clearly seen that the SCK's maintain a spherical shape. The average diameters of **5–7** were determined by TEM as 36, 29, and 26 nm.

Considering the solid-state diameter of 28 nm (obtained from TEM) for **4**, along with its composition and spherical shape, the core diameter and shell thickness can be calculated to be 22 and 3 nm, respectively. The diameter of **4** in an aqueous solution from DLS measurements was determined to be 37 nm,¹⁵ which corresponds to swelling of the shell to 7.5-nm thickness, or a 2–3-fold increase in hydrogel layer thickness. The extent of swelling of **4** was also evident by comparison of AFM images of a dried thin film of **4** with the same film covered with water (Figure 5). AFM imaging was performed under water immediately after wetting and after 1 h, with little change. This indicates that the hydrogel layer associated with the SCK's absorbs water quickly.

Conclusion

Although polymer micellar systems have been studied extensively for their physical properties, little work has been

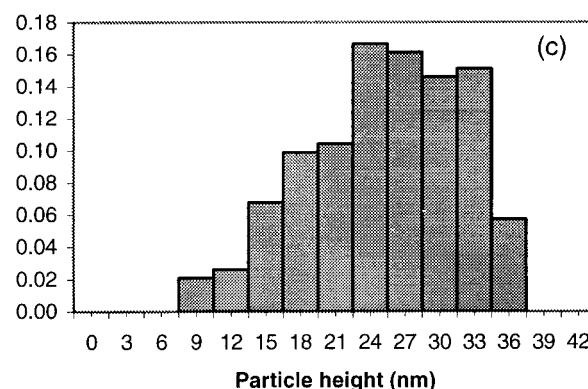
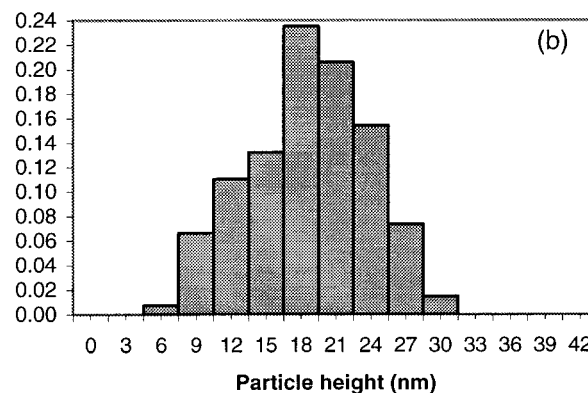
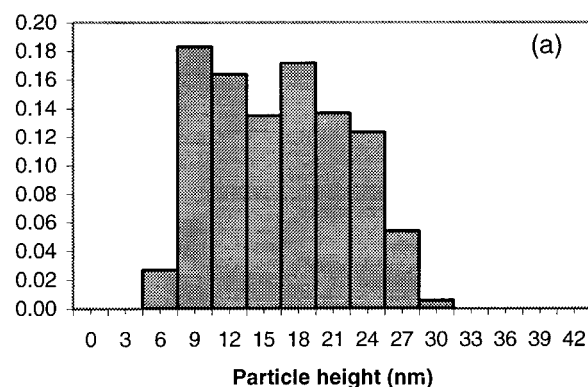


Figure 3. Histograms of fractions of particles *vs* particle heights (nm) of (a) PS-*b*-PAA micelles, (b) micelle **8**, and (c) SCK **4**, measured by AFM.

done to chemically modify the micelles.¹⁶ The combination of hydrogel properties and polymer micelle architecture has generated materials that have great potential for applications such as specific binding and molecular recognition, controlled release of drugs and other agents, gene therapy, water-borne coatings, pollutant removal, catalysis, and sensors, among others. The properties of SCK's can be modified by varying the block copolymer composition, cross-link reagent, and surface functionality to produce a broad range of nanoparticles with different sizes and charges from the same micellar precursor simply by using different types of linkers. Although the amount of linker incorporated can be easily determined, the actual extent of cross-linking is not known. Evidence for cross-linking is clearly seen in the enhanced stabilities of the SCK's in comparison to the polymer micelle precursor, and quantification of the cross-link density is being studied by selective isotopic labeling combined with sophisticated solid-state NMR experiments.

(15) DLS measurements upon **4** detected two particle distributions centered at 37 and 93 nm, which gave ratios of 99:1 by number, 90:10 by volume, and 43:57 by intensity, for the 37 and 93 nm particles, respectively.

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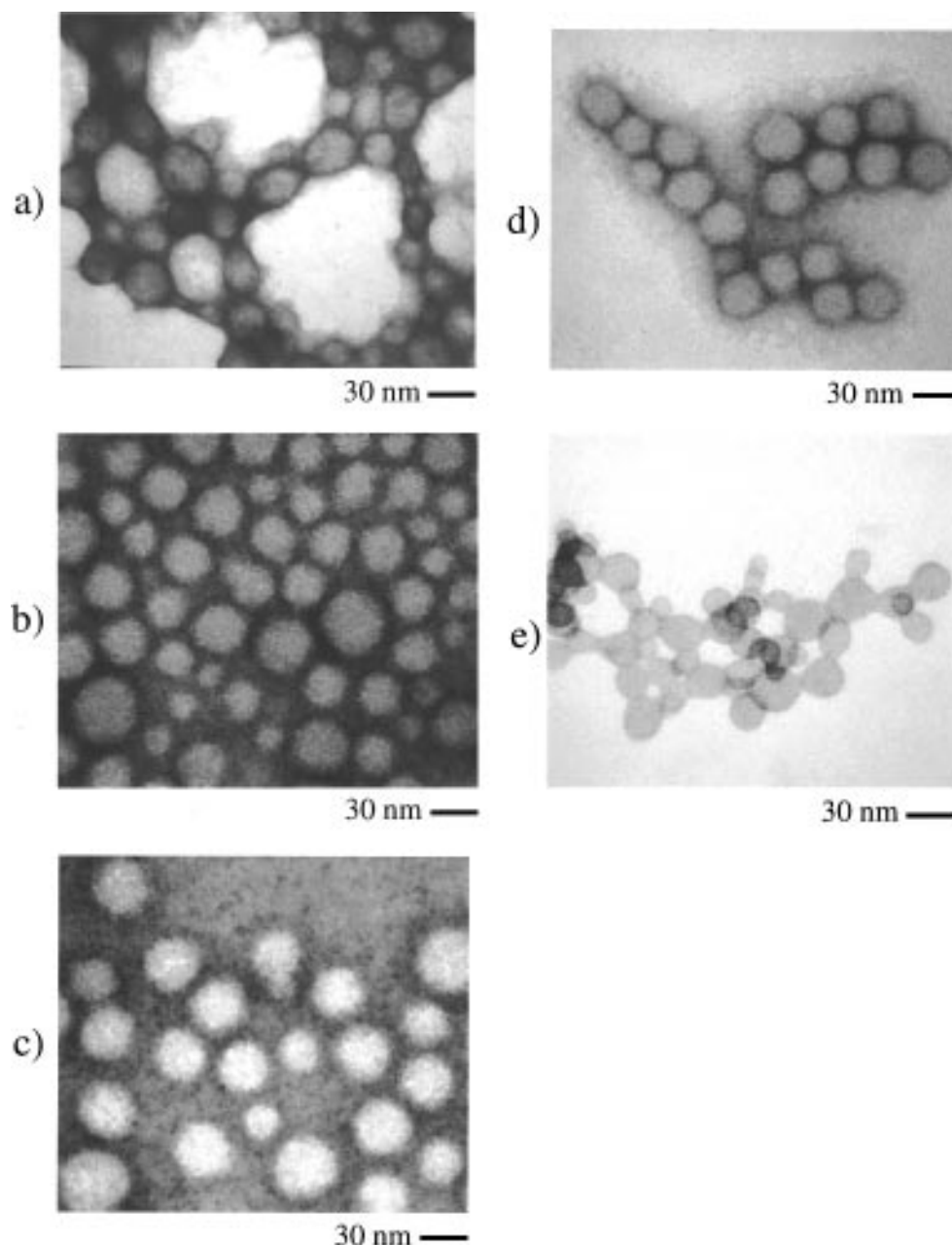


Figure 4. Negatively-stained transmission electron microscopy images at 300 K magnification of (a) PS-*b*-PAA micelles, (b) **4**, (c) **5**, (d) **6**, and (e) **7**, prepared by dropping aqueous solutions, containing the SCK sample and uranyl acetate, upon a carbon-coated copper grid and allowing it to dry.

Experimental Section

Measurements. IR spectra of samples in the solid state as KBr disks were obtained on a Mattson polaris spectrometer. ^1H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer with the solvent peak used as a reference. Cross-polarization magic-angle-spinning ^{13}C NMR spectra were obtained at room temperature on a DNP CPMAS spectrometer built around a horizontal 6-in. bore Oxford superconducting solenoid operating at a proton Larmor frequency of 60 MHz, 15.1 MHz for carbons. Lyophilized samples (*ca.* 200 mg) were spun at 1859 Hz, and experiments began with 1-ms matched spin-lock cross-polarization transfers from protons at 50 kHz followed by proton decoupling at 90 kHz. The sequence repetition time for all experiments was 1 s.

Size exclusion chromatography was conducted on a Hewlett Packard series 1050 HPLC with a Hewlett Packard 1047A refractive index detector, Wyatt MiniDawn low-angle laser light scattering detector, and a Viscotek model 110 differential viscometer: data analysis was done by Viscotek (Houston, TX) Trisec GPC Software, v. 2.70. Two 5- μm Polymer Laboratories PL gel columns (300 \times 7.7 mm) connected

in series, in order of increasing pore size (500 \AA , mixed bed E), were used. The eluent was THF distilled over CaH_2 .

Glass transition temperatures were measured by differential scanning calorimetry on a Perkin-Elmer DSC-4 differential scanning calorimeter. Heating rates were 10 $^\circ\text{C}/\text{min}$, and T_g was taken as the midpoint of the inflection tangent, upon the third or subsequent heating scan. Thermogravimetric analyses were done on a Perkin-Elmer TGS-2 thermogravimetric analyzer. For both DSC and TGA, the Perkin-Elmer instruments were upgraded with Instrument Specialists, Inc. (Antioch, IL) temperature program interface PE, and data were acquired and analyzed using TA-PC software v. 2.11 (Instrument Specialists, Inc.).

Samples for transmission electron microscopy (TEM) imaging were prepared by placing a 5- μL drop of aqueous solution containing micelles or SCK's (*ca.* 0.1 mg/mL) and an equal volume of 2.5 wt % of uranyl acetate (a negative stain) on a carbon-coated copper grid and allowing it to dry in the air for 1 min before blotting off extra solution. TEM imaging was performed on a Hitachi H-600 TEM.

Samples for atomic force microscopy (AFM) imaging were prepared by placing a 2- μL drop of suspension of micelles or SCK's (typical

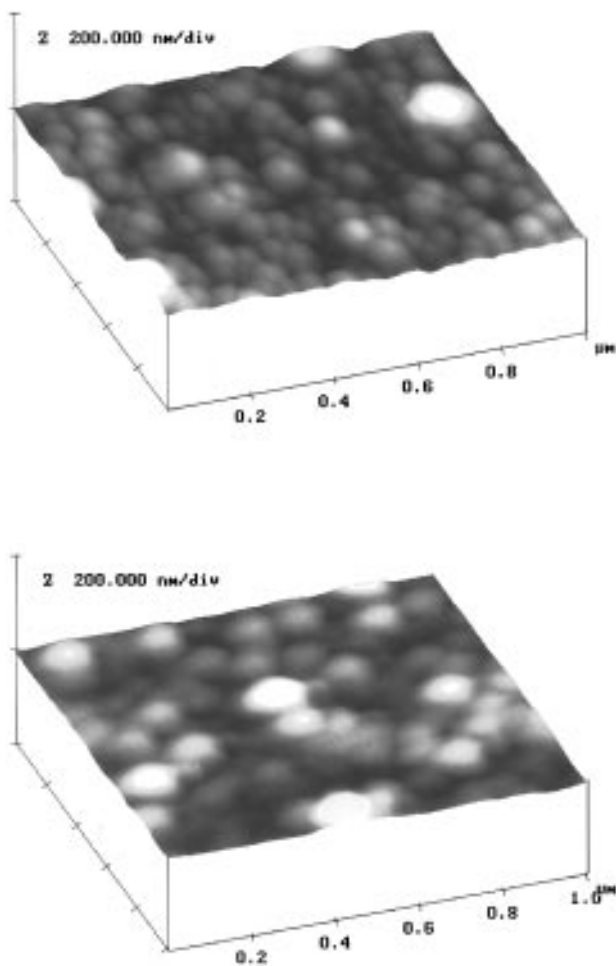


Figure 5. AFM evidence for swelling of SCK 4 in water, based on a comparison of tapping-mode images of a thick film deposited on glass and imaged (a, top) in air and subsequently (b, bottom) under water.

concentration about 0.1 mg/mL) on freshly cleaved mica (Ruby clear mica, New York Mica Co.) and allowing it to dry in the air. The samples thus prepared were imaged immediately after deposition as well as after several days without noticeable changes in appearance and average dimensions. Thick films of SCK's for comparison of dry and fully hydrated SCK's were prepared by placing $\sim 100 \mu\text{L}$ of concentrated aqueous solution of SCK's ($\sim 1 \text{ mg/mL}$) on glass slides followed by overnight drying in air. AFM imaging was performed using a Nanoscope III-M system (Digital Instruments, Santa Barbara, CA) equipped with a J-type vertical engage piezoelectric scanner and operated in tapping mode in air¹⁷ and under water.¹⁸ In tapping mode imaging, the cantilever is oscillated above the surface at a frequency close to its resonance frequency and touches it intermittently at the peak of each oscillation cycle. The decrease of cantilever oscillation amplitude due to intermittent contacts is used as a control signal for a feedback loop to track the surface topography. Throughout this study the typical values of the set point (ratio of dampened to free oscillation amplitude) were between 0.90 and 0.95. High-resolution imaging in air was performed in tapping mode with the aid of standard etched silicon probes ($l = 120 \mu\text{m}$; spring constant, $\sim 50 \text{ N/m}$; resonance frequency, $\sim 300 \text{ kHz}$). A typical raw signal corresponding to cantilever oscillation was $\sim 1.0 \text{ V}$. Comparison of dry and fully hydrated SCK's was carried out in tapping mode with $100\text{-}\mu\text{m}$ long, wide-legged silicon nitride cantilevers (nominal spring constant, $\sim 0.58 \text{ N/m}$; resonance frequency, $\sim 45 \text{ kHz}$) mounted in a fluid-tapping glass cell equipped with a piezoelectric actuator. Due to their low spring constants, silicon

nitride cantilevers tend to be easily trapped by the attractive forces on the sample surface and thus are not routinely used for tapping mode operation in air. We have determined however that stable imaging was possible when the cantilever was oscillated at a high amplitude (typical raw signal $> 8 \text{ V}$) close to its resonance frequency. Tapping mode imaging in water was performed at frequencies of $\sim 10 \text{ kHz}$ with the raw signal of cantilever oscillation amplitude of about 0.5 V and a set point corresponding to 90–95% of the “free” oscillation amplitude. Typical scan frequencies were between 1.5 and 5 Hz, depending on scan areas, which varied from $4 \times 4 \mu\text{m}$ to $500 \times 500 \text{ nm}$. The SCK diameters were estimated by the heights of the SCK's, which were measured with the aid of a bearing analysis procedure, using the Nanoscope III software package. Distributions of heights of the SCK's were obtained from individual measurements performed on *ca.* 300 particles.

The size distribution for SCK's in solution was determined by dynamic light scattering (DLS). The DLS instrumentation was a Brookhaven Instruments Co. (Holtville, NY) system consisting of a model BI-200SM goniometer, a model EMI-9865 photomultiplier, and a model BI-9000AT digital correlator. Incident light was provided by a model 95-2 Ar ion laser (Lexel Corp., Palo Alto, CA) operated at 514.5 nm . All measurements were made at $16 \pm 1 \text{ }^\circ\text{C}$. Prior to analysis, solutions were centrifuged in a model 5414 microfuge (Brinkman Inst. Co., Westbury, NY) for 6 min to sediment dust particles.

Scattered light was collected at a fixed angle of 90° . The digital correlator was operated with 200 channels, a dual sampling time of 100 ns, a $5\text{-}\mu\text{s}$ ratio channel spacing, and a duration of 5 min. A photomultiplier aperture of $200 \mu\text{m}$ was used, and the incident laser intensity was adjusted to obtain a photon counting rate of 83 kcps. Only measurements in which measured and calculated baselines of the intensity autocorrelation function agreed to within 0.1% were used to calculate particle size. The calculation of particle size distribution and distribution averages was performed with the ISDA software package (Brookhaven Instruments Co., Holtville, NY) which employed single-exponential fitting, cumulants analysis, and non-negatively constrained least-squares particle size distribution analysis routines.¹⁹

Materials. THF (Aldrich) was dried over calcium hydride and distilled from potassium/benzophenone ketyl. Styrene was purified by distillation over calcium hydride and then distilled from dibutylmagnesium under vacuum. 1,1-Diphenylethylene (DPE) was degassed three times by freeze–thaw, *s*-BuLi was then added until a cherry red color was obtained, the solution was allowed to stir for 30 min, and then the DPE was distilled under vacuum. *tert*-Butyl acrylate (tBuA) was dried by distillation over calcium hydride, AlEt_3 was then added until a green-yellow color persisted, and then the tBuA was distilled under vacuum prior to polymerization. *sec*-Butyllithium (Acros, 1.3 M in cyclohexane/hexane) was used as received. The concentration of *sec*-butyllithium was determined by titration with 2,5-dimethoxybenzyl alcohol in dry, freshly-distilled THF. 2,2'-(Ethylenedioxy)bis(ethylamine) (**1**), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide methiodide (**2**), hexamethylenediamine (98%), and triethylenetetramine hydrate (98%) were purchased from Aldrich Co. Hexakis(ethylene glycol)diamine (99%) was purchased from Shearwater Polymers, Inc. These chemicals were used in the cross-linking reaction without further purification. Spectra/Por Membrane (MWCO: 12 000–14 000) used for dialysis was obtained from Spectrum Medical Industries, Inc. (Laguna Hills, CA).

Polystyrene-block-poly(acrylic acid). The diblock copolymer of styrene and *tert*-butyl acrylate was prepared by sequential anionic polymerization in THF at $-78 \text{ }^\circ\text{C}$ using *sec*-butyllithium as the initiator. The procedure summarized here is similar to that reported elsewhere²⁰ with slight alterations. The polymerization was done on a double manifold connected to high-vacuum line (10^{-6} mmHg) and argon (99.9995%).

To the purified THF (350 mL) was added lithium chloride (1.6 g, 38 mmol) and initiator *sec*-butyllithium (1.4 mL of 1.25 M hexane solution, 1.75 mmol). The purified styrene (28 mL, 25 g, 0.24 mol) was added to the initiator solution. An aliquot of the reaction mixture was taken after the formation of the polystyrene block and quenched

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in degassed methanol for the determination of its molecular weight and molecular weight distribution by GPC. To every living polystyryl anion was added a stoichiometric amount of diphenylethylene (1.75 mmol) to modify its reactivity. To the polymerization mixture was then added slowly *tert*-butyl acrylate (18 mL, 16 g, 0.12 mol) in THF (20 mL) to form the second block. Degassed methanol was added to terminate the polymerization. The block copolymer, polystyrene-*block*-poly(*tert*-butyl acrylate) was precipitated from the THF reaction mixture into a 8:2 methanol/water mixture. The poly(*tert*-butyl acrylate) block was hydrolyzed in toluene at 90 °C, using *p*-toluenesulfonic acid (5%) as the catalyst. After the solvent was evaporated *in vacuo*, the product was dissolved in THF and purified by precipitation into hexane. Subsequent filtering and drying yielded polystyrene-*block*-poly(acrylic acid) as a white powder. This block copolymer had a polydispersity index (M_w/M_n) *ca.* 1.4, which was determined by GPC. The numbers of styrene units and acrylic acid units were 130 and 120, respectively.¹¹

Formation of Micelles. The polymer micelle size has been reported to depend on the preparation history.^{8a,21} Direct dissolution of the diblock polymer into THF/H₂O gave a combination of nanometer-sized particles of different shapes, including spherical, cylindrical, and ellipsoidal. However, formation of micelles by first dissolving the block polymer in a good solvent (THF) for both blocks and then gradually adding a nonsolvent (H₂O) for PS block to induce micellization²² worked well for the PS-*b*-PAA system. Spherical micelles of narrow size distribution were obtained by this method. A typical procedure to prepare an aqueous solution of micelles is outlined below. The PS-*b*-PAA was first dissolved in THF (1 mg/mL). An equal volume of water was added dropwise (0.5 mL/min) to induce micellization. Micellization occurred when the water content exceeded 10%, and the solution had a bluish tint at this time. An extra amount of water was then added to the micellar solution to obtain the desired concentration. The THF was removed by dialysis against distilled water.

SCK 3 (15% Cross-Linked). To a stock solution of PS-*b*-PAA aqueous micellar solution (0.50 mg/mL, 100 mL, 0.26 mmol acrylic acid unit) was added carbodiimide (**2**) (11.5 mg, 0.038 mmol, 15% of the total amount of acrylic acid units). The mixture was allowed to stir for 30 min before the cross-linking reagent 2,2'-(ethylenedioxy)-bis(ethylamine) (**1**) (2.8 mg, 0.019 mmol, 7.5% of the total amount of acrylic acid units) was added. The reaction mixture was stirred for 2 h at room temperature and then transferred to a dialysis bag and dialyzed against distilled water for 24 h to remove small byproducts. A solid sample of the SCK (**3**) was obtained by lyophilization. IR: 3500–2500, 3050, 2950, 1717, 1601, 1560, 1492, 1452, 1280, 780, 690 cm⁻¹. ¹³C NMR (solid state): δ 30–50 (aliphatic C's of PS and PAA), 70 (ethylene oxide), 130, 145 (styrene Ar C's), 158 (C=O of amide), 175 (C=O of carboxylic acid). Anal. Calcd: C%, 75.52; H%, 6.96; N%, 1.08. Found: C%, 75.47; H%, 7.07; N%, 0.90. DSC: $T_g = 102$ °C. TGA: 10% weight loss below 280 °C; 70% weight loss 280–400 °C; 20% weight loss 400–420 °C.

SCK 4 (100% Cross-Linked). To a stock solution of PS-*b*-PAA aqueous micellar solution (0.50 mg/mL, 100 mL, 0.26 mmol acrylic acid unit) was added carbodiimide (**2**) (76.0 mg, 0.256 mmol, 100% of the total amount of acrylic acid units). The mixture was allowed to stir for 30 min before the cross-linking reagent 2,2'-(ethylenedioxy)-bis(ethylamine) (**1**) (19.0 mg, 0.128 mmol) was added. The reaction mixture was stirred for 2 h at room temperature, and then an aliquot of solution was transferred to a dialysis bag and dialyzed against distilled water for 24 h to remove small byproducts. A solid sample of the SCK (**4**) was obtained by lyophilization. IR: 3500–3200, 3050, 2950, 1649, 1559, 1492, 1451, 1100, 770, 690 cm⁻¹. ¹³C NMR (solid state): δ 30–50 (aliphatic C's of PS and PAA), 70 (ethylene oxide), 130, 145 (styrene Ar C's), 162 (C=O of amide). Anal. Calcd: C%, 69.20; H%, 7.59; N%, 5.46. Found: C%, 69.54; H%, 7.52; N%, 4.96.

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DSC: dialyzed sample $T_g = 101$ °C; undialyzed sample which contained 1-(3-(dimethylamino)propyl)-3-ethylurea methiodide ($(T_g)_1 = 15$ °C, $(T_g)_2 = 102$ °C. TGA: 10% weight loss below 280 °C; 60% weight loss 280–380 °C; 30% weight loss 380–480 °C. An average diameter of 28 nm was obtained by TEM.

SCK 5. To a stock solution of PS-*b*-PAA aqueous micellar solution (0.50 mg/mL, 60 mL, 0.15 mmol of acrylic acid unit) was added carbodiimide (**2**) (45.8 mg, 0.154 mmol, 100% of the total amount of acrylic acid units). The mixture was allowed to stir for 10 min before the cross-linking reagent hexakis(ethylene glycol)diamine (21.6 mg, 0.077 mmol) was added. The reaction mixture was stirred for 2 h at room temperature and then transferred to a dialysis bag and dialyzed against distilled water for 24 h to remove small byproducts. A solid sample of the SCK (**5**) was obtained by lyophilization. IR: 3500–3200, 3026, 2924, 1637, 1560, 1491, 1452, 698 cm⁻¹. Anal. Calcd: C%, 69.88; H%, 8.03; N%, 4.43. Found: C%, 68.18; H%, 7.84; N%, 4.70. DSC: $T_g = 104$ °C. TGA: 10% weight loss below 250 °C; 60% weight loss 250–390 °C; 15% weight loss 390–500 °C. An average diameter of 36 nm was obtained by TEM.

SCK 6. To a stock solution of PS-*b*-PAA aqueous micellar solution (0.50 mg/mL, 60 mL, 0.154 mmol acrylic acid unit) was added carbodiimide (**2**) (45.8 mg, 0.154 mmol, 100% of the total amount of acrylic acid units). The mixture was allowed to stir for 10 min before the cross-linking reagent hexamethylenediamine (9.0 mg, 0.077 mmol) was added. The reaction mixture was stirred for 2 h at room temperature and then transferred to a dialysis bag and dialyzed against distilled water for 24 h to remove small byproducts. A solid sample of the SCK (**6**) was obtained by lyophilization. IR: 3500–3200, 3100, 3075, 3050, 2950, 1649, 1560, 1492, 1452, 1096, 698 cm⁻¹. DSC: $T_g = 105$ °C. TGA: 10% weight loss below 275 °C; 55% weight loss 275–400 °C; 20% weight loss 400–500 °C. An average diameter of 29 nm was obtained by TEM.

SCK 7. To a stock solution of PS-*b*-PAA aqueous micellar solution (0.50 mg/mL, 60 mL, 0.15 mmol acrylic acid unit) was added carbodiimide (**2**) (45.8 mg, 0.154 mmol, 100% of the total amount of acrylic acid units). The mixture was allowed to stir for 10 min before the cross-linking reagent triethylenetetramine (11.3 mg, 0.077 mmol) was added. The reaction mixture was stirred for 2 h at room temperature and then transferred to a dialysis bag and dialyzed against distilled water for 24 h to remove small byproducts. A solid sample of the SCK (**7**) was obtained by lyophilization. IR: 3500–3200, 3026, 2927, 1650, 1560, 1491, 1452, 1406, 699 cm⁻¹. DSC: $T_g = 110$ °C. TGA: 20% weight loss below 300 °C; 50% weight loss 300–375 °C; 15% weight loss 375–500 °C. An average diameter of 26 nm was obtained by TEM.

Micelle 8. To a stock solution of PS-*b*-PAA aqueous micellar solution (0.50 mg/mL, 10 mL, 0.026 mmol of acrylic acid unit) was added carbodiimide (**2**) (7.6 mg, 0.026 mmol, 100% of the total amount of acrylic acid units). The mixture was allowed to stir for 10 min before monofunctional amine 2-methoxyethylamine (2.0 mg, 0.026 mmol) was added. The reaction mixture was stirred for 2 h at room temperature and then transferred to a dialysis bag and dialyzed against distilled water for 24 h to remove small byproducts. A solid sample of the micelle (**8**) was obtained by lyophilization. IR: 3500–3200, 3100, 3050, 2950, 1649, 1560, 1492, 1452, 1096, 690 cm⁻¹. DSC: $(T_g)_1 = 85$ °C, $(T_g)_2 = 100$ °C.

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