Nanocages Derived from Shell Cross-Linked Micelle Templates

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Over the past decade, there has been a surge of interest in nanomaterials, which include structures with at least one dimension under 100 nm. It has been found that the ability to tailor the composition, structure, properties, and function of organic materials with control on the nanometer scale is leading to the production of nanomaterials that exhibit interesting properties, and which may be applicable in broadly ranging technologies. Of particular recent interest are hollow-sphere structures, due to their potential for encapsulation of large quantities of guest molecules or large-sized guests within the "empty" core domain. For example, the microencapsulation of biologically active components has been investigated for the development of artificial cells.[1]

The preparation of hollow particles of micrometer dimensions is accomplished through the formation of microemulsions;[2] however, the preparation of hollow spheres of <100 nanometer diameters offers significant synthetic challenges. The difficulties encountered in the preparation of cavity-containing nanostructures, when macroscale reduction reaches a limit that is still beyond the construction capabilities of molecular chemistry, have been overcome with the development of self-assembly and templating approaches. For example, the phase separation properties of diblock copolymers were employed for the preparation of vesicular structures, using amphiphilic flexible coil−coil block copolymers of drastically unequal block lengths[3] or rod−coil block copolymers.[4] In general, the stability of self-assembled structures can be increased through the incorporation of ionic interactions, involving the assembly of polyelectrolytes.[5] Therefore, an alternate approach for the preparation of hollow polymer shells of uniform micrometer-sized diameter and nanometer-scale shell thickness included the layer-by-layer deposition of polyelectrolytes upon a spherical microparticle,[6] serving as a template, followed by dissolution of the template−core particle.[7] Although the ionic interactions within the polyelectrolyte shells provide for structural stability, charged functional groups remain on the surface of and throughout the shell. The use of a spherical particle template for adsorption of noncharged species, however, has led to difficulties in complete coverage of the template[8] or to overcoverage, aggregation, and the formation of micronetworks.[9] Herein, we describe the preparation of stable, individual, nanometer-sized, hollow, water-miscible spheres by using a combination of these two approaches of self-assembly and templating. This technique relies upon the micellization of diblock copolymers into spherical particles of core−shell morphology, followed by network formation selectively throughout the shell layer, and then degradation and extraction of the core material to leave the membrane-like shell as the hollow nanosphere (nanocage) product.

As illustrated in Figure 1, condensation reactions between diamino cross-linkers[10] and pendant carboxylic acid groups along the poly(acrylic acid) segments located in the periphery of the polymer micelles of isoprene and acrylic acid diblock copolymers, (PI-*b*-PAA),[11] yielded the amphiphilic shell cross-linked knedel-like (SCK)[12] nanostructures **1** and **2**. These SCKs, composed of a poly(*cis*-1,4-isoprene) core domain that is surrounded by a cross-linked polyacrylamide shell, served as the precursors to the complex hollow nanostructures **3** and **4**. The double bonds present along the backbone of the *cis*-1,4-polyisoprene contained within the SCK cores underwent oxidative scission upon exposure to ozone[13] for 30 min, followed by reduction of the ozonides by reaction with sodium sulfite.[14] Finally, the hollow nanospheres were obtained after the small molecule fragmentation products were extracted by diffusion through the cross-linked shell in a dialysis step.[15]

Characterization by dynamic light scattering (DLS)[16] in aqueous solutions and visualization by transmission electron microscopy (TEM) and atomic force microscopy (AFM) in the solid state confirmed the necessity for the use of the SCK structure for the formation of hollow nanospheres and allowed for determination of the sizes, shapes, and structures of the materials. As expected, the polymer micelles, which lack covalent shell cross-linking and are held together by only hydrophobic interactions between the polyisoprene segments within the core domain, disintegrated upon ozonolysis (Figure 2a and d). Additionally, the mean number-average hydrodynamic diameter (D_h) for ozone-treated polymer micelles was measured as 3 ± 2 nm by DLS. In contrast, the cross-linked shell of the SCK allowed for the preparation of stable nanostructures. The mean number-average D_h of SCK **1** was 27 ± 9 nm, and the D_h increased to 133 ± 1 nm after ozonolysis to

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(11) Polymer micelles were obtained by adding a concentrated solution of diblock copolymer (PI$_{130}$-*b*-PAA$_{170}$) (∼20 mg/mL) in tetrahydrofuran (THF) dropwise to a magnetically stirred 1:1 (v:v) mixture of THF and water. An aqueous solution of the polymer micelles (∼1 mg/mL) was then obtained by dialysis against distilled water. Poly(isoprene-*b*-acrylic acid), PI$_{130}$-*b*-PAA$_{170}$ (subscripts denote the numbers of monomer repeat units in each block), was prepared by the hydrolysis of poly(isoprene-*b*-*tert*-butyl acrylate) copolymer precursor, which was synthesized by sequential anionic polymerization of isoprene using *sec*-butyllithium initiator in hexane at room temperature, followed by addition of diphenylethylene and then polymerization of *tert*-butyl acrylate in hexane/tetrahydrofuran containing lithium chloride at −78 °C. Approximately 95% of the isoprene repeat units were of *cis*-1,4-microstructure. The hydrolysis of the *tert*-butyl esters by heating the diblock polymers in 1,4-dioxane containing concentrated HCl at reflux caused about 30% of the isoprene double bonds to undergo hydrochlorination, with 60% of the *cis*-1,4-isoprene repeat units remaining intact.

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(15) Comparison of the IR spectra of the SCK and the core-eroded nanocage revealed that the polyisoprene core was indeed degraded and removed. Indicative IR absorbances included significant reduction in the intensities of the bands from C−H stretching modes at 3000−2850 cm^{-1}, a decrease in the observed bending modes of the CH$_2$ and CH$_3$ groups at 1450 and 1380 cm^{-1}, and the complete disappearance of the C=C−H out-of-plane bend at 800 cm^{-1}.

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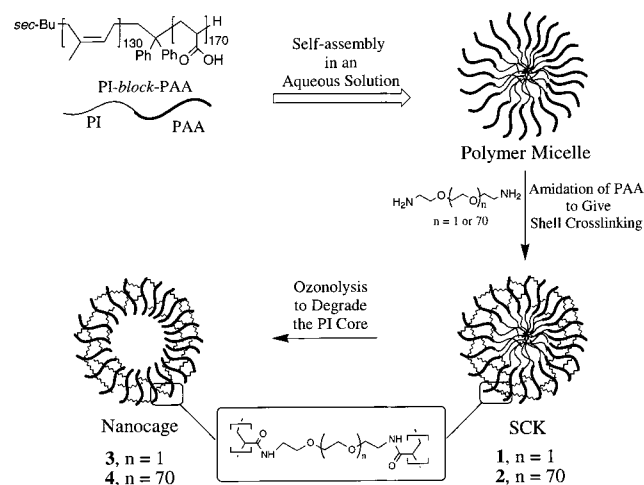
Figure 1. Schematic illustration of the procedure for the formation of hollow nanocapsules starting from poly(isoprene-*b*-acrylic acid) and using self-assembly of the amphiphilic block copolymers to generate the shell cross-linked micelle template, followed by oxidative cleavage of the core. The final nanocages are composed of polyacrylamide cross-linked through triethylene oxide (**3**) or poly(ethylene oxide) (**4**) chains.

form **3**.[17] This increase in D_h despite the removal of the core material can be explained by the cross-linked polyacrylamide shell acting like a hydrogel material and undergoing expansion as the core domain fills with solvent after the removal of the hydrophobic polyisoprene core. The extent of this expansion is significant, which suggests that the hollow nanospheres consist of thin shells of high porosity, and thus are most accurately described as nanocages. TEM evaluation of the structures and diameters of SCK **1** (31 ± 3 nm) and the nanocage **3** (75 ± 10 nm) adsorbed onto carbon surfaces (Figure 2b and e, respectively) agrees with the DLS findings in aqueous solution. The smaller diameter measured in the dry state by TEM (75 ± 10 nm), in comparison to the DLS data (133 ± 1 nm), further supports the swelling behavior of the polyacrylamide nanocage when in an aqueous solution.

The diameter of the nanocage was found to be dependent upon the length of the cross-linkers used for the SCK preparation. When diamino poly(ethylene oxide) chains were used, SCK **2** (83 ± 32 nm by TEM, Figure 2c) gave nanocage **4** (130 ± 35 nm by TEM, Figure 2f), where again the increased diameter after ozonolysis implies that the cross-linked shell expands to an extent that is limited by the cross-links and the nanocages collapse on the surface to occupy a larger substrate surface area than do the SCKs.

The presence of circular two-dimensional structures in the TEM images of the nanocage samples supports the persistence of the spherical shells after degradation of the cores; however, ruptured regions of the shell due to the osmotic pressure increases associated with the core dissolution cannot be entirely ruled out. AFM imaging provides further evidence for intact cage-like nanostructures, as well as their size and three-dimensional shape when adsorbed onto the polar mica substrate. As seen in Figure 2g and h, the two nanocage structures, **3** and **4**, appear as intact flattened spheres with diameters of 85 ± 20 and 210 ± 90 nm, respectively. The thickness of the hollow nanoshells derived from

(17) The SCK sample also contained a trace amount (<1% by number) of larger sized particles (ca. 160 nm D_h), which was no longer detected after ozonolysis. The loss of the larger particles was likely due to swelling of the resulting nanocapsules to a diameter greater than 200 nm the cutoff of the membrane used for filtration (0.2 μm filter) prior to DLS and TEM analysis.
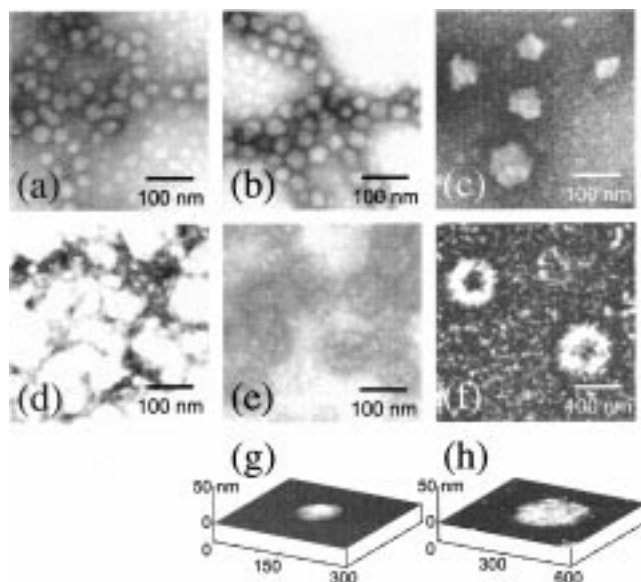

Figure 2. The nanocage products were imaged and compared with their SCK precursors by TEM (a–f) and tapping mode AFM (g and h). TEM samples were prepared by negative staining of 1:1, v:v ratio of sample and 2.5% uranyl acetate solution deposited on a carbon-coated copper grid. AFM samples were prepared by depositing a 1–2 μL drop of dilute aqueous solutions of **3** and **4** on freshly cleaved mica. The images shown include: (a) PI$_{130}$-*b*-PAA$_{170}$ polymer micelles, D_{av} = 33 ± 5 nm; (b) SCK **1**, D_{av} = 31 ± 3 nm; (c) SCK **2**, D_{av} = 83 ± 32 nm; (d) ozone-treated polymer micelles; (e) nanocage **3**, D_{av} = 75 ± 10 nm; (f) nanocage **4**, D_{av} = 130 ± 35 nm; (g) single nanocage **3**, D_{av} = 85 ± 20 nm; (h) single nanocage **4**, D_{av} = 210 ± 90 nm. The average diameters (D_{av}) are based on 20–30 direct measurements from a larger set of TEM and AFM micrographs. In all cases, the shells appeared as highly regular circular objects. Such shape is an indication that, upon removal of the core, the shells retained their spherical shape. In addition, the AFM images indicate that after deposition on substrates, the shells collapsed into highly flattened objects (their diameter:height ratios typically exceeded 10:1), which is consistent with their hollow-core nature. Notice also, that the average diameters of nanocages **3** and **4** (e and f) are about twice as large as their SCK precursors **1** and **2** (b and c). This implies that upon removal of constraints imposed by the core, the shells underwent significant expansion (swelling).

volume and diameter measurements was in both cases equal to approximately 1 nm.

In summary, the cross-linked shell of SCKs constitutes a stable network that expands after removal of the core to give hollow nanocages. In contrast, the organized assembly of polymer micelles is destroyed upon removal of the nucleating core domain. Therefore, by using shell cross-linked micelles as a template, we have developed a new method to obtain robust nanostructures composed of a spherical, covalently bound polymer membrane surrounding a nanoscale cavity. Additionally, the unique method for their production should provide for exceptional control over the composition of the nanocages from the outer surface, through the shell, and including the inner surface.