Silica Nanobottles for Controlled Release and Applications to Vascular Injury

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Introduction

Humans have been making bottles for almost all of our history, with the earliest bottles dating back to 9000 BCE.[1] Bottles are used to contain and control the release of what is inside. Nanobottles are no different in that aspect. However, they exist on a much smaller scale. Nanobottles are characterized as colloidal particles, suspended in a fluid, in the size range of 100-10,000 nm. The key features of a nanobottle include a hollow interior with a single opening in the wall.[2] Nanobottles can be used in a multitude of applications for the encapsulation and controlled release of functional materials such as biomacromolecules, image contrast agents, medication, and cosmetics. The application of particular interest is the delivery and controlled release of medication for disease treatment. The release profile of the payload can be tuned by the size of the opening and the method of encapsulation.

Several methods of fabricating nanobottles, include the synthesis of polymer nanobottles through swelling and subsequently freeze-drying, synthesis of nanobottles based on silica (SiO_2) by extruding a solvent from hollow particles, and synthesis of SiO_2-based nanobottles through site-selected protection and deposition.[2] The issues with these methods are the lack of size uniformity of the product and the overall complexity of the processes. Herein a new method of synthesizing nanobottles by leveraging the swelling of a polymer encapsulated in a solid shell is developed. During swelling, the generated pressure will generate only a single opening in the shell.[3] After complete removal of the polymer, a nanobottle will be formed. The benefits of this method are the simplicity and potential for use in large-scale production.

The synthetic parameters and methods to tailor the properties of nanobottles to suit specific applications are discussed in this report. Specifically, the effects of tetraethyl orthosilicate (TEOS) precursor on shell thickness and the effect of quenching the swelling of polymer removal are investigated. Preliminary steps towards the application of SiO_2 nanobottles in the encapsulation of macromolecules for the potential treatment of vascular injury are discussed.

Methods and Materials

The SiO_2 nanobottles are fabricated by coating a layer of SiO_2 on the surface of commercial polystyrene (PS), followed by swelling and then removal of PS. Figure 1A shows the major steps in this process. Commercial PS beads with a diameter of 500 nm are coated with SiO_2 using TEOS as a precursor to SiO_2. Then the as-obtained PS@SiO_2 core-shell particles are swollen using a 1% toluene/water emulsion.

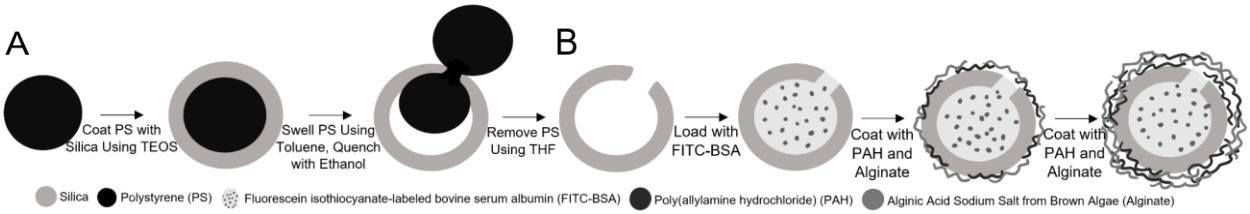

Figure 1. A) Overview of SiO_2 nanobottle synthesis and B) loading and sealing of nanobottles.

This swelling process will create a single opening in the SiO_2 shell and extrude the PS out through the opening, leading to the formation of a Janus particle. The PS is removed using tetrahydrofuran (THF). This leaves the SiO_2 shell with a hollow interior and a single opening in the wall. The overall size and wall thickness of the nanobottles can be tailored by using PS beads of different sizes and TEOS with different amounts, respectively, to best fit the application.

The process for loading of fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA) a fluorescent protein, into the nanobottle is shown in Figure 1 B. Firstly, the nanobottles are incubated in an aqueous solution of FITC-BSA. After the protein molecules have diffused into the cavities, the nanobottles are sealed using a polyelectrolyte double bilayer coating method. The double bilayer consisted of alternating layers of positively charged poly(allylamine hydrochloride) (PAH) and negatively charged alginic acid sodium salt from brown algae (alginate).[4] The samples are characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM), and fluorescence microscopy.

Results

Figure 2A shows the TEM images of a representative 500-nm PS colloidal sphere. Figure 2B shows the PS@SiO_2 core-shell particle obtained by incubation of 1 mL of 0.25% 500-nm PS colloidal spheres in ethanol with 80 µL ammonia and 5.0 µL TEOS. After swelling in 1% toluene, and removal of the PS with THF, the SiO_2 nanobottle is obtained (Figure 2C-D). Figure 3 shows an SEM image of the SiO_2 nanobottles. The nanobottles have a uniform size of 500 nm. Each nanobottle has a single opening or crack on the surface.

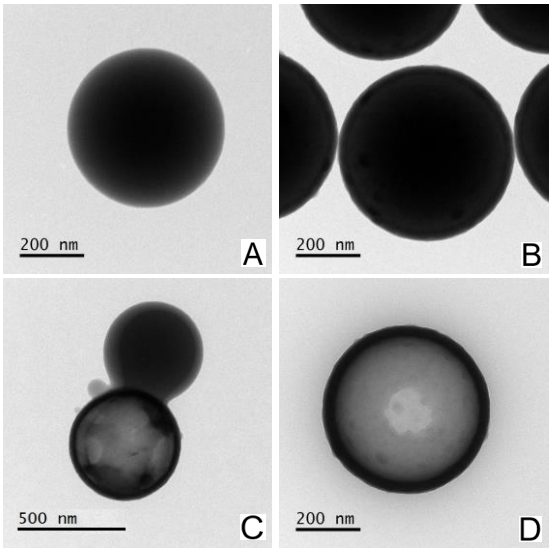

Figure 2. A) 500 nm PS bead TEM and B) 500-nm PS spheres coated with 5.0 µL TEOS. C) PS@SiO_2 Janus particle, after swollen with 1% toluene. D) SiO_2 nanobottle after removal of PS with THF.

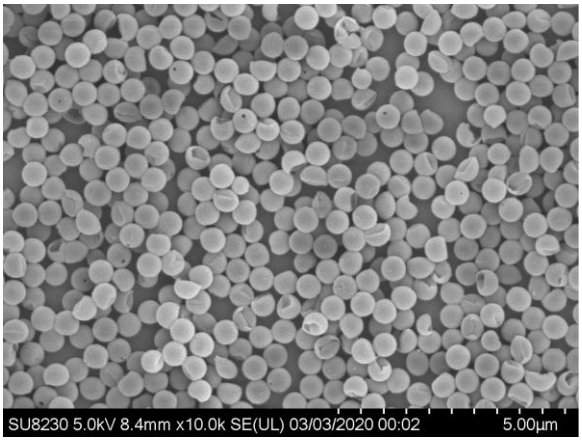

Figure 3. SiO_2 nanobottles after removal of PS.

Wall Thickness

The amount of TEOS was varied during the SiO_2 coating step leading to different shell thicknesses after deposition on and removal of the PS cores. Figure 3 shows TEM images of the samples at different stages during deposition with 1.5, 3.5, or 5.0 µLTEOS.

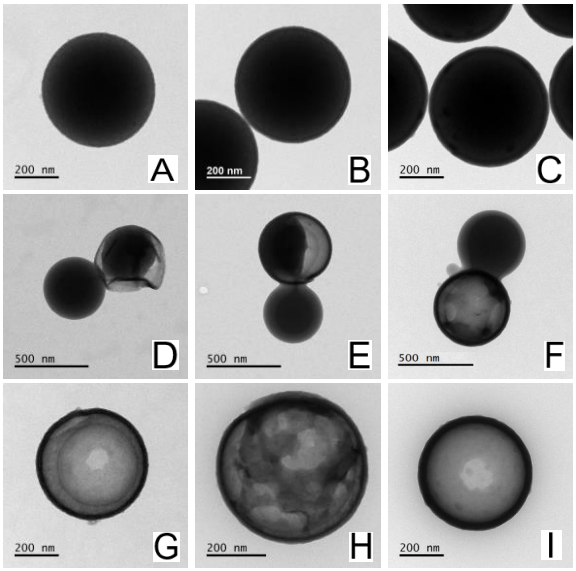

Figure 3. A-C) PS coated with (A) 1.5 µL, (B) 3.5 µL, and (C) 5.0 µL of TEOS. D-F) The corresponding PS@SiO_2 Janus particles after quenching the swelling. G-F) The corresponding SiO2 nanobottles.

When the amount of TEOS was 1.5 µL, the SiO_2 thickness was 12.25 nm (Figure 3A). Such a thin layer of SiO_2 resulted in shell deformities during swelling due to the weak mechanical properties. Comparatively, 2.5 and 5 µL of TEOS resulted in a 21.2 and 33.39 nm SiO_2 coating, respectively. The above results demonstrated that the shell thickness and thus the mechanical properties of the nanobottles can be tuned by varying the amount of TEOS.

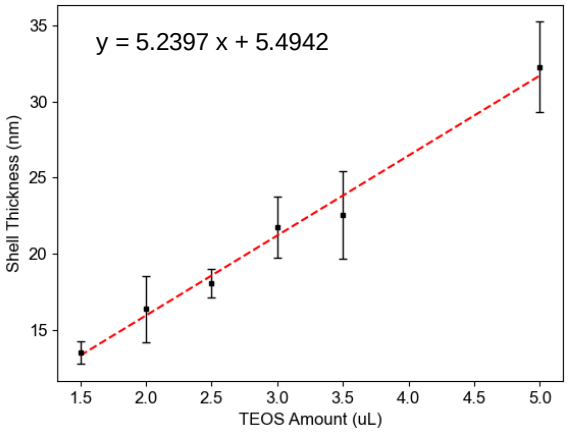

Figure 4. The effect of TEOS on shell thickness

To further investigate the correlation between shell thickness and the amount of TEOS, the shell thickness of 6 samples of nanobottle with different amounts of TEOS was measured. These values are shown in Figure 4. There is a linear trend within this range of data. The line of best fit (y = 5.2397 x + 5.4942) can be used to predict the shell thickness depending on the amount of TEOS used.

Polystyrene Removal

After the swelling process, the PS was removed using THF. A common occurrence is that not all of the PS was removed. To investigate the effect of quenching on the removal of PS, two alternative synthesis procedures were investigated. One directly removed the PS without quenching, while the other one quenched the swelling before removing PS. Figure 7 shows the TEM images of these two syntheses.

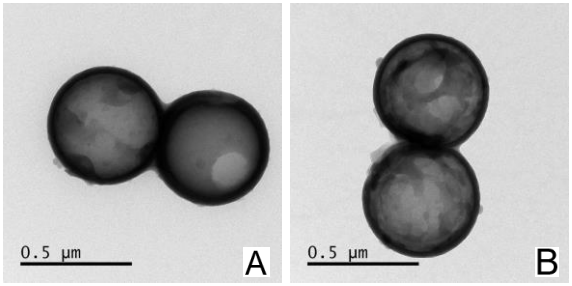

Figure 7. A) Removal of PS without quenching the swelling. B) Removal of PS with quenching of swelling

The nanobottles that did not have the swelling quenched with ethanol (Figure 7A) resulted in less remaining PS.

Loading Proteins into the Nanobottle

The nanobottles were loaded with FITC-BSA and coated with a PAH and alginate double bilayer. The resulting nanobottles can be seen in Figure 8, demonstrating the successful loading of the fluorescent proteins in the nanobottles.

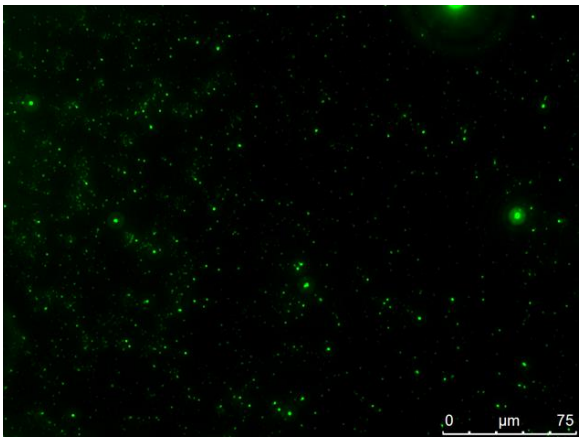

*Figure 8. A) Fluorescence microscopy of nanobottles loaded with FITC-BSA and coated with PAH and alginat*e.

Conclusions

The mechanical properties of the nanobottle can be tuned by changing the wall thickness of the nanobottle, through varying the amount of TEOS. For 500-nm PS beads, the wall thickness can be varied from 13 to 33 nm by using 1.5-5.0 µL of TEOS. Many of the hollow nanobottles contained some residual PS. When comparing the quenching of the swelling versus no quenching, it was found that no quenching resulted in less residual PS. However, it should be noted that quenching the swelling step is important to image the Janus particle stage. FITC-BSA was used as a model of biomacromolecules to evaluate the loading properties of these nanobottles. The nanobottles were sealed using a double bilayer of PAH and alginate. Fluorescent microscopy results confirmed that the FITC-BSA was successfully loaded into the nanobottles.

Future Applications

When the human vasculature is injured, it is very important that clotting occurs. Blood clots are formed when red blood cells and platelets clump and are held together by fibrin at the site of the injury. Fibrin is formed from fibrinogen. Factor VIII (fVIII) is a protein in the human body that promotes clotting. Individuals with Hemophilia A sometimes develop have anti-factor VIII antibodies that attack the fVIII, which severely reduces clotting. A treatment for hemophilia A is to intravenously infuse fVIII into the patient. However, for those who have the anti-factor VIII antibody, fVIII has a very short half-life.[5] Nanobottles can be loaded with fVIII, sealed with a polyelectrolyte double bilayer, and coated in fibrinogen. The nanobottle would protect factor VIII from the immune system. The factor VIII would only be released when platelets bind to the fibrinogen at the site of injury, and the resulting contractile forces would cause a rupture of the nanobottle. The thickness of the SiO_2 shell will be tuned to make sure the contractile forces can break the nanobottles.

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