

Synthesis of Fluorescent Silica Hollow Nanoparticles Using Coumarin Fluorophore

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Abstract: There have been a lot of studies on fluorescent silica nanoparticles as a bioimaging reagent. Almost all of them, however, are based on dense silica nanoparticles. Recently, increasing number of studies have been directed to fluorescent silica hollow nanoparticles, because hollow nanoparticles can be employed as *theranostic* (**therapeutic** + **diagnostic**) particles. In this study, the fluorescent silica hollow nanoparticles (FSHN) have been synthesized by labelling coumarin dye on the surface of silica hollow nanoparticles (SHN). SHN has been prepared by utilizing the triblock copolymeric micelles of poly(styrene-*b*-2-vinylpyridine-*b*-ethylene oxide) (PS-PVP-PEO) as a template and tetramethoxysilane (TMOS) has been added as a precursor. The micelles exhibit a core-shell-corona (CSC) structure in aqueous solutions. The prepared SHN has been thoroughly characterized by transmission electron microscopy (TEM) and X-ray diffraction spectroscopy (XRD). According to TEM results, the size of the silica hollow nanoparticles is about 33 nm, the size of the cavity part is about 10 nm, and the size of the shell is about 12.5 nm. The SHN is amorphous in nature. FSHN has been synthesized by labelling silane coupling reagent, 4-methyl-7-trimethylsilyloxycoumarin (MTSC) on SHN. The synthesized FSHN has been examined by transmission electron microscopy (TEM). The size of the synthesized hollow silica nanoparticles is 35±1 nm and the size of the cavity part is 10±1 nm, and the size of the shell is 12.5±1 nm. Fluorescence properties of FSHN are elucidated by observing various fluorescence parameters such as intensity, fluorescence lifetime and quantum yield. The fluorescence life time is 2.08 ns and the quantum yield is 0.637. The results demonstrate that the FSHN can be successfully prepared.

Keywords: Fluorescent, hollow, silica, nanoparticles, coumarin

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1. Introduction

In recent years, hollow inorganic nanoparticles have received greater attention than dense inorganic nanoparticles because of their intriguing properties, e.g., larger specific area, lower density, greater surface permeability, etc. They give a new platform for a range of applications in biomedical diagnosis, sensors, catalysis, photonic crystals, controlled drug delivery and therapy, ion batteries and so on [1]. The production of inorganic hollow nanospheres using hard templates can cause low product yield and shell instability upon template removal [2]. The amphiphilic block copolymers have been utilized as versatile templates to synthesize hollow nanostructures with well-defined shape and ordered mesoporous materials since last decade. Amphiphilic molecules self-assemble into nanostructures including spherical micelles [3]. The AB diblock or ABA triblock copolymeric micelles so far employed for the synthesis of inorganic materials provide a core-corona type architecture. In these systems, the corona of the micelles acts as a reservoir of the inorganic precursors, and the core acts as a template of the hollow [4]. In this approach, however, the template micelles become very unstable when the precursor is sorbed into the corona, leading to the formation of aggregates. To overcome these problems, we propose using ABC triblock copolymer micelles with a core-shell-corona structure as a soft template [5]. Newly developed three-component ABC triblock copolymers may solve this problem. Micelles formed from ABC triblock copolymers that dissolve only one or two of the blocks in selective solvents provide templates for these improved nanoassemblies [6]. By handling individual polymer blocks, additional features at the molecular level can be encoded [7]. Recently, increasing number of studies are directed to fluorescent silica hollow nanoparticles, because hollow nanoparticles can be employed as *theranostic* (= **therapeutic** + **diagnostic**) particles which play both therapeutic and diagnostic roles. The particles have fluorescent dyes on the silica surface and therapeutic drug inside the void space. The fluorescent dyes so far employed are mainly organic dyes including porphyrin, acridine orange, rhodamine dyes, and fluorescein [8-10]. Coumarin dyes have never been used for this purpose. In this study, the fluorescent silica hollow nanoparticles (FSHN) have been synthesized by labelling coumarin dye on the surface of silica hollow nanoparticles (SHN). SHN has been prepared by utilizing the triblock copolymeric micelles of poly(styrene-*b*-2-vinylpyridine-*b*-ethylene oxide) (PS-PVP-PEO) as a template and tetramethoxysilane (TMOS) has been added as a precursor. The micelles exhibit a core-shell-corona (CSC) structure in aqueous solutions. The protonated PVP block acts as an acid catalyst for the hydrolysis of tetramethoxysilane (TMOS). The hydrolyzed silica precursor species have a negative charge at pH 3 and thus it can strongly bind to the protonated PVP block. PS core acts as a template for hollow void, PVP-shell domain serves as a reaction site for sol-gel reaction, and PEO corona stabilizes

the micelles. Figure 1 shows a typical procedure for fabricating hollow silica nanoparticles with a template of the PS-PVP-PEO micelles using sol-gel techniques. FSHN has been synthesized by labelling silane coupling reagent, 4-methyl-7-trimethylsiloxycoumarin (MTSC) on SHN. Fluorescence properties of FSHN are elucidated by observing various fluorescence parameters such as intensity, fluorescence lifetime and quantum yield.

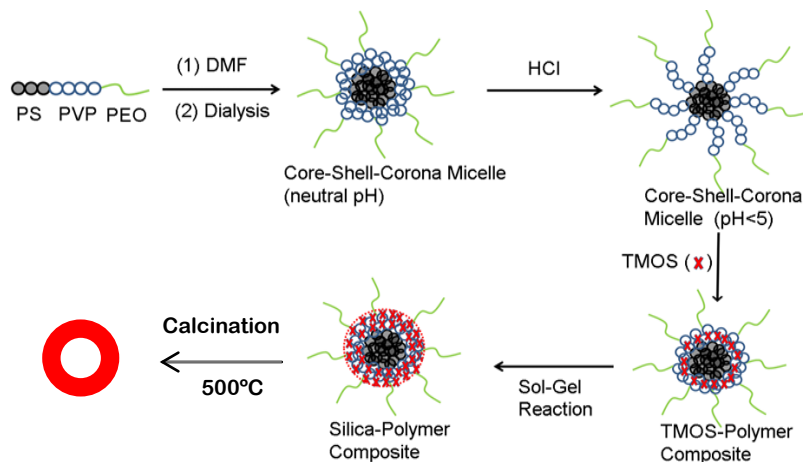


Figure 1. A typical procedure for preparation of silica hollow nanoparticles with a template of the PS-PVP-PEO micelles using sol-gel techniques.

2. Materials and Methods

Materials

The triblock polymer poly(styrene-*b*-2-vinylpyridine-*b*-ethylene oxide) (PS_(45k)-PVP_(26k)-PEO_(82k)) was kindly provided by Professor Yusa, Department of Materials Science and Chemistry, University of Hyogo, Japan. The numbers in parentheses are the molecular weights of the block chains (42k, for example, denotes 42000). Tetramethoxysilane (TMOS, >98%), dimethylformamide (DMF), and hydrochloric acid were obtained from Wako Pure Chemicals and 4-methyl-7-trimethylsiloxycoumarin (MTSC) from Gelest Inc.

Preparation of CSC Micelles

The micelles of PS-PVP-PEO were prepared as described in the previous paper published by our group [11]. The micelle solution was stirred for one hour and then the solution was dialyzed against water. Finally, the micelle solution was transferred to 100 mL standard flask and diluted with water to obtain a polymer concentration of 1 g L⁻¹.

Fabrication of Silica Hollow Nanoparticles Templated by the CSC Micelles

In a typical synthesis of silica hollow nanoparticles, a certain amount of a CSC micelle solution with polymer concentration of 1 g L^{-1} was taken in a conical flask. The pH was adjusted to around 3 using dilute HCl and the micelle solution was constantly stirred using a magnetic stirrer. Then the desired amount of silica precursor (TMOS) was added. The mixture was stirred at room temperature and aged for a few days under static condition for extensive condensation of silica species. The polymer/silica composite was separated and the solid was washed repeatedly with distilled water followed by calcination of composite particles.

Synthesis of Fluorescent Silica Hollow Nanoparticles

Ethanol/water mixed solution (95 % v/v) was prepared. The pH of the solution was adjusted to 4.5 – 5.5 using dilute acetic acid. A certain amount of MTSC was added to 10 mL of ethanol/water mixed solution under agitation. Silica hollow nanoparticles (20 mg) were added to the above solution and agitated for 5 min. The silica hollow particles were separated from the solution by centrifugation, followed by washing repeatedly with ethanol. Coumarin added silica hollow particles were first dried at room temperature, then at 110°C for 5 – 10 minutes, and finally at room temperature for 24 hours.

Characterization of SHN by Spectroscopic Methods

TEM pictures were recorded on a JEOL JEM-1210 electron microscope operating at 80 kV. The TEM samples were prepared by casting a drop of silica added micelle solution at pH 3 on a copper grid followed by staining with 1 wt% phosphotungstic acid. The samples were finally dried at room temperature. Powder X-ray diffraction (XRD) patterns were measured on a Rigaku RINT-2200 diffractometer with $\text{CuK}\alpha$ radiation (40 kV, 30 mA) at a scan speed of 1° min^{-1} .

Fluorescence Spectroscopy

Fluorescence excitation and emission spectra were recorded on JASCO FP-6500 fluorescence spectrophotometer [12]. The temperature of the water-jacketed cell holder was controlled with a circulated thermostatic water bath. Fluorescence polarization measurements were performed with Hitachi F-4000 fluorescence spectrophotometer.

3. Results and Discussion

The formation of CSC PS–PVP–PEO triblock copolymeric micelles has been systematically studied by DLS and SEM. Figure 2 shows the hydrodynamic diameters (D_h) of the CSC micelles at pH 7. The average

hydrodynamic diameter of the mixed micelles is found to be 58 nm and 101 nm at pH 7 and 3 respectively. The increased micelle size in acidic medium is ascribed to extended conformation of the PVP block due to repulsion between the protonated pyridine units of the CSC micelles. The representative SEM image of CSC micelles is shown in Figure 3. A spherical morphology of micelle particles can be clearly seen in the SEM image. Figure 4 (a) and (b) exhibit the representative TEM images of silica nanoparticles before and after labelling with coumarin dye where hollow structures are clearly visible. The TEM images of hollow nanoparticles depict a uniform spherical hollow structure with a similar shell thickness. The features of the silica hollow nanoparticles including particle diameter, cavity size, and shell thickness were estimated from the TEM pictures. The average particle size was calculated by selecting about 50 particles for each sample. The size of the synthesized silica hollow nanoparticles is about 33 nm, the size of the cavity part is about 10 nm, and the size of the shell is about 12.5 nm. Not much difference in size of particle diameter, cavity size, and shell thickness has been found before and after labelling of SHN with coumarin dye except for the slightly darker colour. The SHN is amorphous in nature according to the XRD result. Wang et al. observed that both SHN and FSHN exhibit a discrete, and hollow spherical shape with an average diameter of 80 nm by labelling of 4-formyl-4'-formaloximetriphenylamine on polyacrylic acid (PAA) [13]. In comparison with other results, SHN prepared by our route, utilizing the CSC micelles of (PS-PVP-PEO) as a template gives smaller size and well-defined hollow nanoparticles [14].

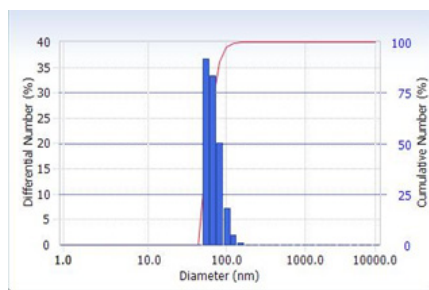


Figure 2. Particle size distribution of PS-PVP-PEO triblock polymer micelles at pH 3.

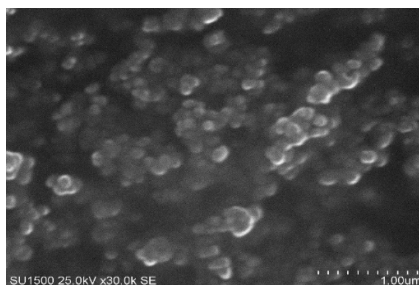


Figure 3. Representative SEM image of polymeric micelles in aqueous solution containing (PS(42k)-PVP(26k)-PEO(80k)) triblock polymer (1.0 g L⁻¹) at pH 3 and at 25 °C.

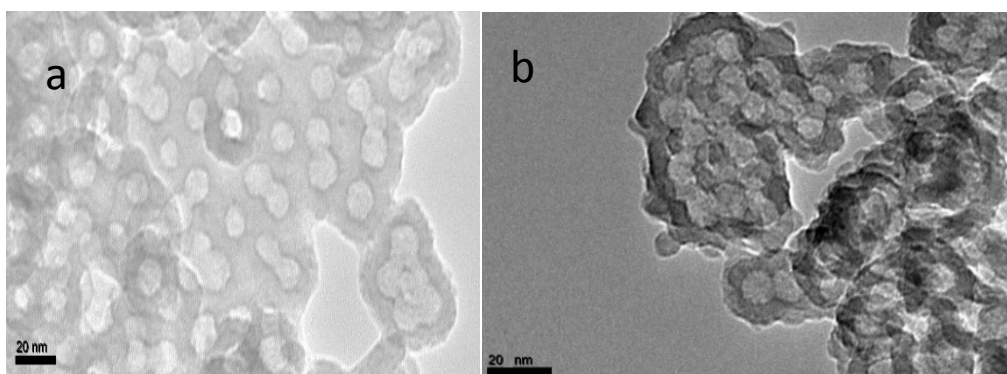


Figure 4. Representative TEM image of (a) hollow silica nanoparticles templated by micelles of (PS(42k)-PVP(26k)-PEO(80k)) (b) hollow silica nanoparticles after labelling with coumarin.

Fluorescence properties of FSHN are elucidated by observing various fluorescence parameters such as intensity, fluorescence lifetime and quantum yield. Figure 5 shows the fluorescence spectra of coumarin, SHN, and coumarin dye labelling SHN. As shown in the figure, no or very weak fluorescence spectra is observed for individual coumarin and SHN. The fluorescence spectra is augmented, however, for coumarin dye labelling SHN. Fluorescence lifetime is an intrinsic property of a fluorophore. The fluorescent lifetime of FSHN is 2.08 ns and the quantum yield is 0.637. It reveals that the prepared SHN can be fluorescent by labelling with coumarin dye. Due to unique hollow structure and intense fluorescence nature, the synthesized FSHN can be used in the field of *theranostic*. The results demonstrate that the FSHN can be successfully prepared.

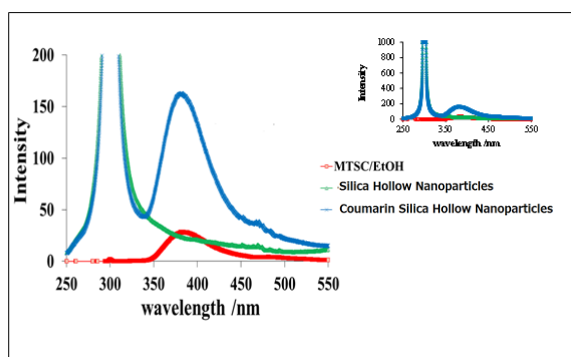


Figure 5. Representative fluorescence emission spectra of coumarin, SHN, coumarin labelling SHN. $\lambda_{em} = 384\text{nm}$, $\lambda_{ex} = 323\text{nm}$.

4. Conclusion

In summary, we have successfully prepared fluorescent silica hollow nanoparticles with a high uniformity in size using a template of the PS-PVP-PEO triblock copolymer micelles with a core-shell-corona architecture. This method is simple when compared to other techniques like the layer-by-layer method. Because of the versatile surface modification and fluorescent properties, FSHN are highly desirable for further targeting as a *theranostic* and bioimaging reagent.

Acknowledgments

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