# Shell Thickness Control of Mesoporous Silica Hollow Capsules and Their Release Behavior 

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#### Abstract

Due to their biocompatibility and mechanical robustness, the mesoporous silica hollow capsules (MSHCs) are good candidates for drug carriers in drug delivery system. So far, we had developed the preparation method based on the template-assisted method to obtained MSHCs with an excellent monodispersity and a desired inner diameter. Here, we propose a method to control the shell thickness of the MSHCs by repeating the sol-gel reaction that forms the shell structure. Because of the simple repetition of the reaction, the shell thickness was proportional to the number of the reaction repeated. The dependence of the release rate on the shell thickness was also investigated, and it was demonstrated that the thicker the shell, the slower the release rate was.


Keywords: mesoporous silica, hollow capsule, shell thickness, release

## 1 INTRODUCTION

Drug delivery systems are the technologies for the targeted deliver and release of a minimum required amount of pharmaceutical agents to an affected part and optimize the administration of agents. Thus, the aim of the systems is to maximize the medical efficacy of drugs and to minimize the side effect of drugs simulutaneously [1].

Particular drug carriers were utilized to prevent drugs from corrosion under physiological conditions, to achieve the targeted delivery of drugs, and to prolong the circulation of drugs within vasculature [1-3]. As the carriers, micelles, polymers, liposomes, mesoporous particles, and hollow capsules have been proposed [1-3]. Specifically, the mesoporous silica particles have been proven to show good biocompatibility and high thermal/chemical stability and are good candidates as the drug carriers [3,4]. A further improvement to have a larger capacity for loading drugs was made, and mesoporous silica hollow capsules (MSHCs) were prepared by the template-assisted method [3,5]. Figure 1 summarizes our procedure to prepare the MSHCs [5]. The polystyrene (PS) particles and the cylindrical micelles of cetyl trimethyl ammonium bromide (CTAB) were used as templates for the hollow compartments of the capsules and for the pores across the
silica shell, respectively. The sol-gel reaction was made to form the silica/micelle complex on the PS particles followed by the pyrolysis of the organic templates, resulting in the MSHCs. Besides the pyrolysis, the dissolution was also applied to remove the templates [6,7]. In addition to the control in the size and shape of the capsules, the control of the shell thickness of the MSHCs is crucial, because the thickness affects the release rate of the loaded materials. The control by the concentrations of CTAB and the silica precursor had proposed $[7,8]$ and the dependence of the release rate on the shell thickness had been investigated [7].

Here, we report another strategy to control the shell thickness of the MSHCs, i.e., the shell thickness can be controlled by repeating the reaction for the formation of the silica/micelle complex on the particle before the calcination (Fig. 1). In principle, this method can form the shell as thick as one wants by increasing the number of the repetition. Furthermore, the alizarin dye was loaded to the MSHCs and the dependence of the release rate of alizarin on the shell thickness was observed.


Figure 1: Preparation of mesoporous silica hollow capsule by template-assisted method.

## 2 EXPERIMENTAL

### 2.1 Materials

The dispersion of the PS particles of 80 nm in average diameter was purchased from Magsphere Inc. and the concentration and the standard deviation of the diameter were $10 \% \mathrm{w} / \mathrm{v}$ and 11 nm , respectively. The cationic surfactant (CTAB, > 99\%) and the silica precursor, tetraethoxysilane (TEOS > 99.9\%), were obtained from Sigma-Aldrich and Alfa Aesar, respectively. Ethanol (EtOH, $>99.5 \%)$ and aqueous ammonia $\left(\mathrm{NH}_{3} \mathrm{aq}, 25 \mathrm{wt} \%\right)$ used for the sol-gel reaction and alizarin and its solvent (acetone, >
$99 \%$ ) used for the loading and release experiments were purchased from Wako Pure Chemical Industries, Ltd. Pure water ( $>18 \mathrm{M} \Omega \mathrm{cm}$ ) was prepared in a Milli-Q system (Elix Advantage 3).

### 2.2 Characterizations

A transmission electron microscope (TEM, JEM-1200 EXII, JEOL) was used to observe the MSHCs and was operated at an acceleration voltage of 80 kV . The sample was prepared by placing $0.25 \mu \mathrm{~L}$ of the dispersion at the center of the capper grid coated by collodion film (Nisshin EM Corporation) followed by the evaporation of the disperse medium at room temperature.

From the TEM image, five capsules were chosen randomly, and the outer and inner diameters and the shell thickness were obtained in four different directions from each of the five capsules. The three sets of $5 \times 4$ values for the outer and inner diameters and the shell thickness, respectively, were then averaged over the five capsules.

During the sol-gel reaction, not only the formation of the silica/micelle complex on the PS particles but also the formation of particular silica/micelle complexes occurred, and the latter ones became the unwanted solid mesoporous silica particles after the calcination. The fraction of these byproducts was also evaluated from the TEM image and was defined as the number of the byproducts divided by the number of the byproducts and the capsules.

The release profile was also observed by the TEM image. After the release was triggered, the dispersion was incubated for certain period and $0.25 \mu \mathrm{~L}$ of the dispersion was taken to prepare the grid as mentioned above. From the TEM image, we counted the total number of capsules and the number of the empty capsules, which did not exhibit any indications of loaded materials at all in their hollow part, and defined the value of the latter number divided by the former number as the fraction of the empty or released capsules. Note that we treated the capsule as the loaded capsule, even if its hollow part was not fully filled with materials.

## 3 RESULTS AND DISCUSSION

### 3.1 Control of Shell Thickness

Flow chart of the procedure for the MSHC synthesis is indicated in Fig. 2. The procedure by the single sol-gel reaction (without the repetition of the reaction) as in the previous report [5] is explained briefly and the MSHC obtained by this procedure is termed as "MSHC1". Solution1, the mixture of the materials indicated in Fig. 2, was stirred at 500 rpm at $30{ }^{\circ} \mathrm{C}$ for 10 min . The concentration of CTAB in Solution1 was 3.1 mM and the volume fraction of EtOH in Solution1 was 0.25 . $50 \mu \mathrm{~L}$ of TEOS was added drop by drop and the solution was stirred at 500 rpm at $30{ }^{\circ} \mathrm{C}$ for another $2 \mathrm{~h}(x=2 \mathrm{~h}$ in Fig. 2) to let the sol-gel reaction complete.

After the 2-h reaction, the byproducts that were smaller than the silica/micelle coated PS particles were carefully removed by repeating centrifuging, removing the supernatant, and resuspending in water (Step $b$ in Fig. 2). The dispersed medium was exchanged from water to EtOH and the dispersion was dried in a ceramic crucible. The silica/micelle coated PS particles then calcined at $550{ }^{\circ} \mathrm{C}$ for 6 h . After the calcination, the sample was redispersed in water by sonication, followed by the sedimentation of aggregated capsules (Step $c$ in Fig. 2), and the supernatant was collected and water was added to it to obtain the 1 mL aqueous dispersion of MSHC1.


Figure 2: Flow chart of the MSHC synthesis.
Based on this procedure, the sol-gel reaction was repeated to obtain the thicker shell than MSHC1. The 1st sol-gel reaction was made for $1 \mathrm{~h}(x=1 \mathrm{~h}$ in Fig. 2) and the centrifugation was made one time to remove the byproducts (Step $a$ in Fig. 2). In Step $a$, the supernatant of $14.5 \sim 15.5$ mL was removed after the centrifugation and the sediment was redispersed (total amount of $1.5 \sim 0.5 \mathrm{~mL}$ ). After Step $a$, Solution2 was added and the 2 nd sol-gel reaction was made for 2 h ( $y=2 \mathrm{~h}$ in Fig. 2) with stirring at 500 rpm at 30 ${ }^{\circ} \mathrm{C}$, followed by Step $b$. From Step $b$, the procedure was the same as mentioned above, and the resultant sample is termed as MSHC2.

When the reaction was repeated three times, Solution3 was added after the 2 nd sol-gel reaction was made for $y=$

1 h , and the 3rd sol-gel reaction was carried out for $z=2 \mathrm{~h}$, followed by Step $b$. From Step $b$, the procedure was the same, and the resultant sample is termed as MSHC3.

When the reaction was repeated four times, Solution4 was added after the 3 rd sol-gel reaction was made for $z=$ 1 h , and the 4 th sol-gel reaction was carried out for 2 h . From Step $b$, the procedure was the same, and the resultant sample is termed as MSHC4.

The TEM images of MSHC1 to 4 are shown in Figs. 3a to d, respectively. It can be clearly seen that, as the number of the repetition of the sol-gel reaction increased, the shell thickness increased. The dependences of the outer and inner diameters and the shell thickness on the number of the repetition are indicated in Fig. 4. The outer diameter and the shell thickness increased almost linearly as the number of the reaction increased. While, the inner diameter did not depend on the repetition number and was almost constant, indicating that the template-assisted method worked properly even when the sol-gel reaction was made more than one time.


Figure 3: TEM images of the capsules prepared according the flow chart shown in Fig. 2. (a) The capsules obtained by the single sol-gel reaction (MSHC1). (b) The capsules obtained by repeating the reaction two times (MSHC2), (c) three times (MSHC3), and (d) four times (MSHC4).


Figure 4: Outer and inner diameters and shell thickness of the capsules as a function of the repetition number of the sol-gel reaction.


Figure 5: Fraction of byproducts with and without Step $a$ shown in the flow chart in Fig. 2 as a function of the repetition number of the sol-gel reaction.

We had to separate the byproducts from the capsules, and the fraction of the byproducts can be reduced at Step $b$ in Fig. 2 for the sample MSHC1 [5]. When the reaction was repeated to obtain the thicker shell, we also faced with the problem of the byproduct separation. When we made Step $b$ before the 2 nd sol-gel reaction, almost no sol-gel reaction occurred on the silica/micelle complex formed at the 1st reaction, resulting in almost no increase in the shell thickness on the 2 nd reaction. This result indicates that the repeating centrifuging and exchanging the supernatant to water at Step $b$ reform the surface of the silica/micelle complex from active to inactive surface against the sol-gel reaction. On the other hand, when the reaction was repeated without removing the byproducts, not only the thickness of the silica/micelle complexes on the PS particles but also the diameter of the byproducts increased and the difference in the diameter between the capsules and the byproducts was reduced. Therefore, as the repetition number increased, the separation became more difficult and the fraction of the byproducts increased as shown in Fig. 5 (circle). To reduce the fraction of the byproducts, we inserted Step $a$ (Fig. 2, shaded box), i.e., one centrifugation followed by a removal of the supernatant. As Fig. 5 (triangle) shows, the fraction was significantly reduced with the increase in shell thickness shown in Figs. 3 and 4. Thus, Step $a$ was the key procedure to reduce the byproducts and to increase the shell thickness, simultaneously.

### 3.2 Dependence of Release Rate on Shell Thickness

The alizarin dye (inset of Fig. 6a) was loaded in MSHCs. The loading procedure is in brief described in the followings. From the resultant MSHC dispersion ( 1 mL ), $50 \mu \mathrm{~L}$ was taken and dried in the centrifuge tube. $50 \mu \mathrm{~L}$ of the 14 mM alizarin solution in acetone was added to the tube and sonicated for 1 h followed by a 1-day incubation to let alizarin diffuse into the capsules. After the incubation, $20 \mu \mathrm{~L}$ of the dispersion was transfer to the Teflon vessel with the inner diameter of 25 mm , and 5 mL of the 14 mM alizarin solution in acetone was added. The acetone was then slowly evaporated under air at room temperature. Three days were spent for the evaporation. After the 3-day
evaporation, 1 mL of the saturated aqueous solution of alizarin $(10 \mu \mathrm{M})$ was added to the vessel and the dried sample was redispersed by sonication. To separate the loaded capsules from the alizarin precipitations dispersed outside of the capsules, the obtained dispersion was filtrated ( $0.8 \mu \mathrm{~m}$ pore size) and centrifuged and the supernatant was removed until $10 \mu \mathrm{~L}$ remained. The remained $10 \mu \mathrm{~L}$ of the supernatant and the sediment was redispersed and $0.25 \mu \mathrm{~L}$ was took from the dispersion and placed on the TEM grid. The TEM image of MSHC1 after loading is indicated in Fig. 6a. When the TEM images of MSHC1 before and after loading are compared, the contrast of the hollow part, which appeared to be light gray in Fig. 3a, went dark in Fig. 6 a , indicating that the electron density of the hollow part increased due to the loading of alizarin.


Figure 6: TEM images of (a) MSHC1 after the loading of alizarin, (b) after the $60-\mathrm{min}$ incubation after the release was triggered, and (c) after the $300-\mathrm{min}$ incubation.


Figure 7: The fractions of empty capsules as a function of incubation time obtained by the capsules with the three different shell thicknesses. The shell thicknesses of MSHC1, MSHC2, and MSHC3 were 32, 55, and 95 nm , respectively.

The remained dispersion of the loaded capsules in the saturated aqueous solution of alizarin $(9.75 \mu \mathrm{~L})$ was mixed with $300 \mu \mathrm{~L}$ of pure water to trigger the release of alizarin from the capsules. After each certain period of incubation
( 30 min to 300 min ) from the start of the release, $0.25 \mu \mathrm{~L}$ of the dispersions was taken and placed on the TEM grid. For examples, the TEM images of MSHC1 after the 60 -min and the $300-\mathrm{min}$ incubations are shown in Figs. 6b and c. When the images of Figs. 6a to c are compared, it can be seen that the fraction of the empty capsules increased as the incubation period increased. The dependences of the fraction of the empty capsules on the incubation period were observed using MSHC1, MSHC2, and MSHC3, and are indicated in Fig. 7. Before triggering the release (at the incubation time of 0 min in Fig. 7), the fractions of the empty capsules were around $3 \%$ among the three cases. As the incubation time increased, the fractions increased indicating that the release of alizarin progressed, and it is clearly shown that the thicker the shell, the slower the release rate was.

## 4 SUMMERY

By repeating the sol-gel reaction, the thickness of the mesoporous silica shell was successfully controlled in a linear manner. When the reaction was repeated, the removal of the byproducts at Step $a$ in Fig. 2 is important to reduce the fraction of the byproducts (Fig. 5). However, upon the removal of the byproducts at Step $a$, the PS particles coated by the silica/micelle complex were also wasted together with byproducts, and the yield of the capsules became less than half of the yield of MSHC1. Thus, the relationship between the decrease in the fraction of byproducts and the increase in the yield of the capsules prepared by the repetition of the reaction is trade-off.

The materials were loaded into the capsules with the different shell thicknesses and it was demonstrated that the release rate went slower as the shell thickness increased (Fig. 7). This result coincides with the report [7] and further development in the controlled release strategy using our MSHCs is ongoing.

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