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HOLLOW MESOPOROUS SILICA NANOPARTICLES FABRICATION FOR ANTICANCER DRUG DELIVERY

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Abstract. Mesoporous silica nanoparticles (MSNs) have attracted significant attention from researchers thanks to their high surface area and pore volume, which can increase drug loading capacity. Moreover, MSNs, with their biocompatibility and ease of surface functionalization, are seen as potential drug delivery system. However, the loading of drug into MSNs system still needs further improvement. In this study, hollow mesoporous silica nanoparticles (HMSNs) were fabricated in order to increase the drug loading capacity of nanosilica materials. The synthesized HMSNs possessed inner hollow cores that could remarkably raise the total pore volume and thus improve the capacity for cargo loading. HMSNs were synthesized according to the hard-template method with three main steps: (1) forming of solid SiO_2 nanoparticles as templates, (2) forming of core-shell structure by coating MSN layers onto the templates, and (3) forming of hollow core structure by etching away the solid template. The HMSNs product was characterized by TEM, XRD, TGA and FTIR. In addition, drug loading capacity of the material was evaluated with doxorubicin as model drug. The results indicated remarkable improvement in drug loading capacity, compared to MSN sample. These results demonstrated the potential of HMSNs in the delivery of anticancer agents.

Keywords: hollow mesoporous silica nanoparticles, silica, biomedicine.

Classification nmbers: 2.4.3, 2.7.1.

1. INTRODUCTION

One of the most researched approach in cancer treatment that has attracted tremendous attention from scientists is targeted therapy using nanoparticles as “delivery system” to ensure site-specific delivery and release of drugs [1-3]. Compared to traditional chemotherapy with non-specific distribution of drugs throughout the body, the use of nanocarriers could reduce undesired side effects and improve therapeutic outcome [4].

In recent years, inorganic nanomaterials have received lots of attention as drug carriers thanks to their stability and ease of surface modification [5]. Mesoporous silica nanoparticles are

among the materials with high potential in application [6]. Mesoporous nanomaterials have a great number of advantages, including physicochemical stability and biocompatibilicy [7, 8]. Moreover, porous structure provides room for carrying of active molecules [9, 10]. Researchers have been able to control the particle sizes to apply in anticancer drug delivery. However, the loading capacity of these systems has not been desirable, so a novel material with porous structure and a hollow inner core has been under development.

In this study, hollow mesoporous silica nanoparticles (HMSNs) were synthesized following the hard-template method. Particles morphology and size were evaluated by transmission electron microscope (TEM). Drug loading efficiency and capacity were determined with doxorubicin (DOX) as model drug. The results showed that HMSNs are a potential carrier for anticancer drugs.

2. EXPERIMENT

2.1. Materials and equipment

Tetraethyl orthosilicate (TEOS, 98%), Doxorubicin (DOX) were purchased from Sigma–Aldrich (USA), cetyltrimethylammonium bromide (CTAB, 99 %), ethanol and ammonia (NH_3 28 %) solution were bought from Merck. Deionized water (deH_2O) was used throughout the experiment.

TEM images were taken on Jem-1400 (Japan) at University of Technology – Ho Chi Minh city. XRD patterns were obtained by D2 Phaser (Bruker, German) at Customs Branch of Goods Verification No. 3 – Ho Chi Minh city. Surface area was evaluated by BET method (Barrett-Emmet-Taller) on Tristar 3020 at Tra Vinh University – Tra Vinh province. Surface charge was obtained on Zetasizer Nano ZS100 (Horiba, USA), UV-Vis spectrum was measured by UV 1800 (Shimazu, Japan), and FTIR spectra was measured by PerkinElmer Frontier (USA) at Institute of Applied Materials Science – Ho Chi Minh city.

2.2. Synthesis of HMSNs

HMSNs were synthesized via three steps: (1) Formation of solid silica nanoparticles (SiO_2) by Stober method [11]. In brief, ethanol (13.5 M) and NH_3 (0.38 M) were stirred in deH2O for 30 min at 50 ^oC. Then, TEOS (0.29 M) was added to the mixture and continued stirring for 6 h at 50 ^oC. The product was dialyzed (12-14 kDa membrane) and lyophilized. (2) Coating of MSN layers onto SiO_2 template (SiO_2-L). Briefly, a mixture of CTAB 0.05 M, ethanol/ammonia (1.43:0.05 M/M) and SiO_2 templates were stirred for 30 min at 50 ^oC. TEOS (0.27 M) was added to the solution and stirred for 6 h, followed by dialysis. (3) Etching of the core template to produce hollow structure (HMSN). In brief, Na_2CO_3 0.2 M was used as etching agent by stirring together with SiO_2-L solution for 9 h at 50 ^oC. The product was dialysed against acetic acid:ethanol (1:1, v/v) and washed with deH_2O, followed by freeze drying.

2.3. Encapsulation of DOX

To evaluate the amount of encapsulated drug, dialysis method was utilized. Doxorubicin loaded nanocarriers (HMSN-DOX, 1:4 m/m) were put into dialysis membrane, then let diffuse in an appropriate media. At pre-determined intervals, samples of dialysis media were taken to quantification by UV-Vis spectrophotometry at wavelength 544 nm.

Drug loading efficiency – DLE and drug loading capacity – DLC were determined via the following equations:

$$\text{DLE (\%)} = \frac{\text{Amount of encapsulated drug}}{\text{Initial amount of drug for loading}} \times 100\ \% \quad (1)$$

$$\text{DLC (\%)} = \frac{\text{Amount of encapsulated drug}}{\text{Total amount of drug and carriers}} \times 100\ \% \quad (2)$$

The products from each synthesis step were evaluated by TEM imaging and the final HMSN product was further characterized by FTIR, XRD and TGA.

3. RESULTS AND DISCUSSION

3.1. TEM imaging

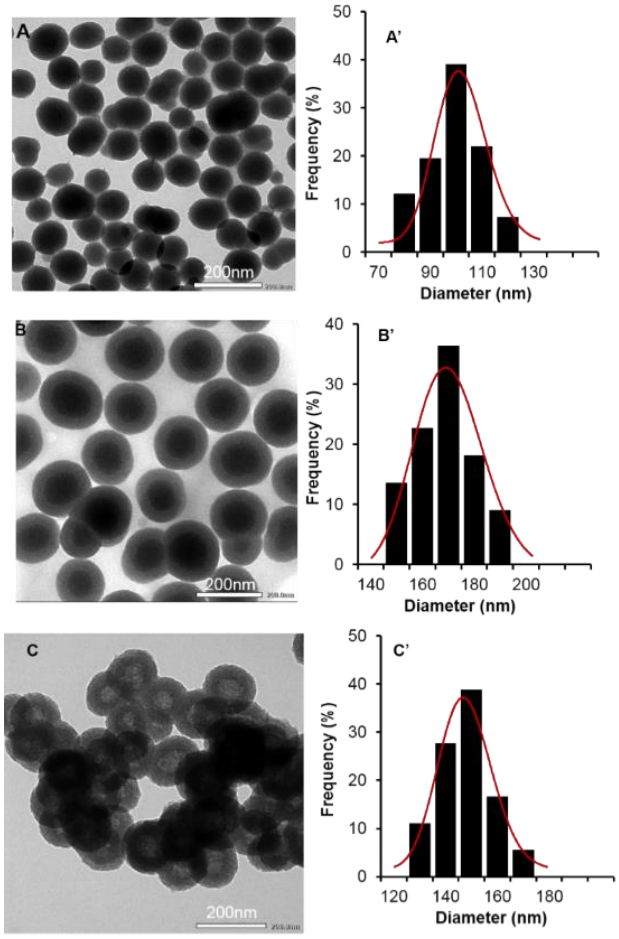

Figure 1. TEM images and size distributions from TEM of SiO_2 (A, A'), SiO_2-L (B, B') and HMSN (C, C').

Particle morphology and size were observed by transmission electron microscope (TEM). The results indicated that in all three steps of synthesis, the nanoparticles were in spherical shape

and had narrow distribution. According to TEM imaging, SiO_2 core templates were 104 ± 0.7 nm. The silica coat on the templates, formed via hydrolysis and condensation reactions of the precursor TEOS in presence of CTAB, was about 60 nm thick. In the last step, Figure 1 (C, C') showed that the core templates were successfully etched away to form hollow structure of around 134.0 nm.

3.2. Zeta potential

Surface charges from all synthesis steps were shown in Figure 2. Zeta potential indicates the surface charge and stability of the system. Larger value in zeta potential means higher electric repulsion between particles, thus reducing aggregation and improving the stability and dispersion of the particles. The charges of SiO_2 templates and HMSN with surface hydroxyl groups were found to be negative, -44.3 ± 0.6 mV and -25.0 ± 0.9 mV, respectively. At the second step when the coating required the presence of CTAB surfactant with positively charged CTA+ groups.

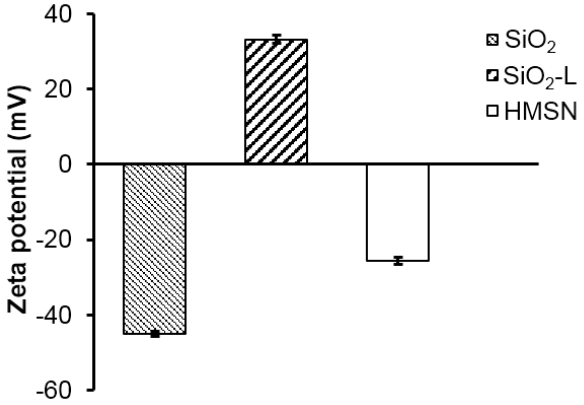

Figure 2. Zeta potential of SiO_2, SiO_2-L and HMSN.

3.3. FTIR spectrum analysis

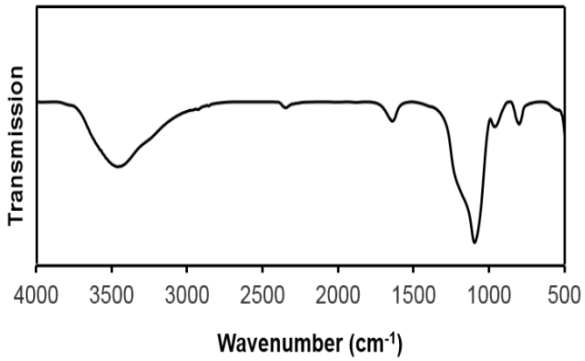

Figure 3. Fourier transform infrared spectrum of hollow mesoporous silica nanoparticle.

As can be seen from the FTIR spectrum of HMSN (Fig. 3), an absorption band from 3300 – 3500 cm^{-1} was to the silanol group on the surface of silica material. Other signals were assigned to Si-O-Si (1100 cm^{-1}), OH stretching of water in HMSN (1635 cm^{-1}), asymmetric bending and

stretching of Si–OH (960 cm^{-1} and 800 cm^{-1}) , respectively, proving the presence of inorganic SiO_2 [12].

3.4. TGA curve analysis

The heating process of HMSN (Fig. 4) was ranged from 25 ^oC to 80 0^oC [13]. At temperature lower than 170 ^oC, a loss of 12 % was from the evaporation of water physically adsorbed to the sample and part of the dehydroxylation of silanol groups on the surface of the material. A following 5 % loss at 170 ^oC – 500 ^oC was due to the degradation of trace organic, the evaporation of physically bonded water and the dehydroxylation of part of silanol groups within the material. At temperature over 500 ^oC, slight weight loss was attributed to the dehydroxylation of part of silanol groups within the structure. Raising the temperature to 800 ^oC did not completely break down the silanol groups.

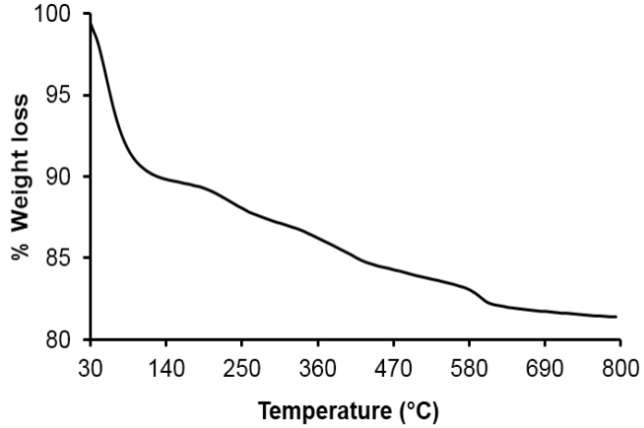

Figure 4. Thermogravimetric analysis (TGA) diagram of hollow mesoporous silica nanoparticle. TGA was performed in air with the temperature ramped from 25 – 800 °C at a rate of 10 °C/min.

3.5. Surface area via BET method

The surface area of the material was evaluated by nitrogen adsorption-desorption and BET method to be 983.7 m^2/g. As can be seen from Fig. 5, the nitrogen adsorption-desorption isotherms of HMSN belongs to type IV and hysteresis loop of H4 type, according to IUPAC classification. Capillary condensation occurring at the relative pressure of 0.42 indicates that the outer layer of HMSN had small to average capillary structure.

3.7. Drug loading efficiency and capacity

DLE and DLC are essential parameters in design of drug delivery system since they have a direct impact on the effectiveness of the system. These parameters were determined directly from the amount of DOX encapsulated within the particles via aforementioned equations. The DLE and DLC of synthesized HMSN were 22.70 ± 0.77 % and 5.40 ± 0.17 %, respectively. In comparison with other studies, Cheng *et al*. developed novel pH-sensitive delivery vehicles, DOX-loaded folic acid-conjugated polydopamine modified HMSN, to improve their long-term blood circulation. The results showed that the DLE of HMSNs-DOX was 10.53 ± 0.3 % [14]. In another previous study by Moghaddam *et al*., the tunable glutathione (GSH)-sensitive hollow mesoporous silica nanoparticles ($HMSiO_2$ NPs) were synthesized and DOX is loaded into the

pores of $HMSiO_2$ NPs. The GSH-sensitive DOX-loaded $HMSiO_2$ NPs were successfully prepared with DLE for GSH-sensitive and TEOS $HMSiO_2$ NPs were 12 ± 0.7 % and 11 ± 0.5 %, respectively [15]. These results demonstrated that the prepared HMSN with the high DLE has the potential to be delivered more efficiently to cancer cells.

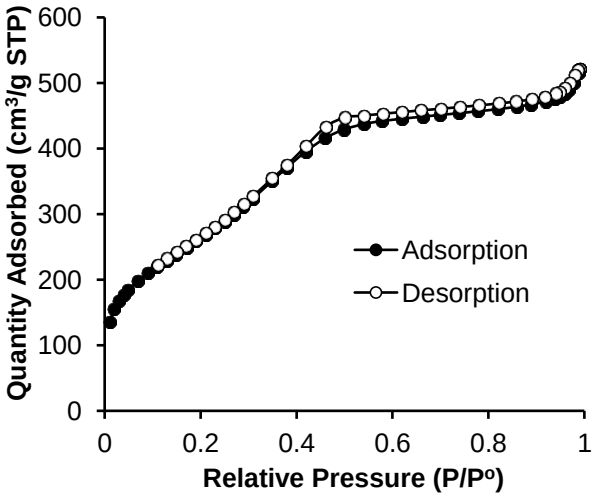

Figure 5. Nitrogen adsorption-desorption isotherms of HMSN.

4. CONCLUSION

In this study, hollow mesoporous silica nanoparticles were successfully synthesized via hard-template method with three steps. TEM imaging showed that the synthesized particles had spherical shape and possessed a hollow core structure after the etching of SiO_2 templates. This hollow structure was the key to the high drug loading capacity of HMSN. It is also important to note that HMSN retains the advantages of mesoporous silica nanoparticles (MSN), such as high drug loading capacity, physicochemical stability, etc. and thus is a potential material for biomedical application as drug delivery system.

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