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Size-TunableMesoporous Silica for Macromolecular Drug Delivery

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Abstract. A series of mesoporous silica nanospheres(MSNs)and different particle sizes rangingfrom about48 to 215nm has been successfully synthesized through modified base-catalyzed sol–gel method.The particle size distribution, structural and morphological of the samples were well characterized by dynamic light scattering technique (DLS), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR),X-ray diffraction (XRD) and thermalgravimetry (TG).The results reveal that this MSNs exhibits good physicalproperties, including narrow size distribution,uniform pore texture in the nanospheres and good thermal stability.What's more, the size canbetuned by altering the concentration of aqueous ammonia in the silica precursor systems.The obtained MSNs are further used as a drug delivery carrier toinvestigate the in vitro drug release properties usingbovine serum albumin (BSA) as a macromolecularrepresentative model drug.It is found that this MSNsexhibit good obvious sustained release properties for macromolecular drug, suggesting its potential application in biological fields.

1. Introduction

Protein therapeutics are promising candidates for disease treatment due to their high specificity and minimal adverse side effects, such as those used in the treatment of diabetes type I and II provided by insulin, are extremelybeneficialtreatments[1]. However, effective delivery ofproteins to target sites has proven monumental challenge [2].Proteins are rapidly cleared from the body, unable to survive high and low pH environments or denaturing enzymes (proteases) [3].One of the main functions of deliveryvehicles in these systems is to protect proteins from premature degradation in a biological environment.

Mesoporous silica nanoparticles (MSNs) have been proven to be ideal candidatesfor this application due to unique characteristic such as ordered mesoporousstructure [4, 5, 6],high surface area (>800 m^2 g^{-1}), large porevolume (≈1 cm3 g-1), tunable pore diameters (2–10 nm), good biocompatibility [7, 8] and well-defined surface properties for modification [9, 10],which endow them with great potential inthe fields of protein delivery [11]. To date, there are two important kinds of MSNs inprotein delivery, SBA-15 and MCM-41. SBA-15 particles have larger pore sizes (5–30 nm), allowing them to accommodate and deliver large, therapeuticmolecules [12]. However, the particle sizes of SBA-15 aregenerally in the range of 800 nm to 2 μm, which leading to limitations in vivo due

to small particle size (<200 nm) is required for efficient cellular uptake[4]. On the other hand, MCM-41-type MSN materials possess tunable sizes, with an average distribution of pores in nanoparticles. For the effective delivery of proteins in particular, special attention must be paid to the appropriate size. These developments are encouraging forfuture applications; whereas new breakthroughs are still neededto further extend the applications of mesoporous silica materials.

Furthermore, it is well known that the size and structure of MSNs have effect on the interface between the drug-carrying particle and body fluids, and could thereby affect the drug releasekinetics. Therefore, it is significant to studythe effect of different particle sizes on the loading and release of protein drugs. In particular, some recent reports indicated there are alternate strategies for controlling the particle size. The particle size,pore size, and pore structure is tunedby the concentrationof aqueous ammonia (NH_4OH) in ethanol [13]. Slowing et aldemonstratedMCM-41 with alarge average pore diameter were able to host Cyt*c*, thenrelease the protein under physiological conditions[5].Thus, the design and fabrication ofMSNs with different particle sizesshould beimportant for understanding the protein drug release behaviours fromsilica carriers.

Herein, a series of mesoporous silica nanospheres(MSNs) with tunable particle size, high thermal stability, high specific surface area and open porechannels has been obtained by modified base-catalyzed sol–gel method. For the purpose of investigating the morphology and nanostructure of scaffolds MSNs, the MSNs fabricated in different conditions were observed by TEM, and DLS confirmed the particle size and distribution properties. Futhermore, the aggregation structure and thermal stability were studied by FTIR, XRD and TG. Morever, the macromolecular drug load and release feature into/from a series of MSNs were investigated in detail.

2. Experimental Section

2.1. Material
Tetraethyl orthosilicate (TEOS)andHexadecyltrimethyl ammonium bromide (CTAB)were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Ammonium hydroxide, ethanol, methanol and hydrochloric acid were purchased fromSinopharmChemical Reagent Co. Ltd (Shanghai, China).Bovine serum albumin (BSA), and BCA Protein Assay Kit was purchased fromBeyotime Biotechnology Co. Ltd (Shanghai, China).

2.2. Synthesis of MSNs
All chemicals were of analytical grade and used without furtherpurification.MSNs used in this experiment wereprepared by the CATB-templated, base-catalyzed sol–gelmethod[14]. Briefly, different volumesof29 wt.% $NH_3 \cdot H_2O$was added to 200 ml deionized water. And then 0.279 g of CTAB was added at 50 ℃, under the same temperature, 1.394 ml TEOS was added for the synthesis reaction. After 6 h reaction and 2 h aging process, the sample was centrifuged and washed twice with deionized water and ethanol respectively. Thirdly, thesurfactant templates were removed by extraction in acidicmethanol (2 mL of HCl/100 mL of methanol, 36 h) at80 ℃. The final samples were washed thoroughly by deionized water and ethanol again, and then dried in the vacuum drying chamber overnight. These samples were named as MSNs-2, MSNs-4, MSNs-6, MSNs-8 according to amount of $NH_3 \cdot H_2O$ (Table 1). The final dried sample was weighed and the yield was calculated according to the mass percentage.

Table 1. The codes of MSNs according to amount of $NH_3 \cdot H_2O$

Sample	MSNs-2	MSNs-4	MSNs-6	MSNs-8
$NH_3 \cdot H_2O$ (ml)	2	4	6	8

2.3. Characterization
15 mg of samples was added into 6 ml anhydrous ethanol, and then dispersed by ultrasonic oscillation for 30 minutes. Then, the transmission electron microscopy (TEM) wasused to observe sizes and

morphologies. TEM was performed on a transmission electronmicroscope (JEM-2100, Japan) with a field emission gunoperating at 200 kV.FT-IRspectra were performed using a FTIR spectrophotometer (Nicolet 6700, USA) using KBr technique, for each measurement, each spectrum was obtained in the wave number ranging from 400 to 4000 cm^{-1}. X-ray diffraction (XRD) patterns were obtained in a X-Ray Diffractometer (Bruker D8 Advance, Germany) using Cu Kα irradiation. Scattering patterns were collected from 0.8 to 8.0 ° with a scan time of 0.1 s per 0.002 ° step. Thermogravimetry (TG) wascarried out on a thermo-analyzer(Netzsch STA 409, Germany) with a heatingrate of 10 ℃ min^{-1}.

2.4. Drug loading and releasing in vitro
100mg of MSNs (MSNs-2, MSNs-4, MSNs-6 and MSNs-8) were soaked in 2mlof 10mg/ml BSA solution at room temperature for 4 hand the solutions wereoscillatedat 200rpm. After the loading, suspensions were centrifuged for 10min at 5000r/min, and the MSNs were freeze-dried for 24 h.The BSA concentration of supernatant was measured by BCA Protein Assay Kit and the loading rate quantification of drug loading rate was performed and was expressed as w/w% unless otherwise specified.

The MSNs loaded with BSA were added to 8 ml 0.1 mM PBS solution. The suspension was put into the shaking table with rotating speed of 110 rpm 37 ℃.At specific time points (0.5 h,1 h,2 h,4 h,8 h,12 h,24 h,48 h,72 h and 96 h), 1ml solution was taken out for teat the concentration, and 1.0 mL fresh PBS was added into released fluid immediately. The concentrations of BSA in the released sample fluid were measured via BCA protein assay kit.

3. Results and Discussion

3.1. Preparation and Characterizations of MSNs
In order to prepare MSNs with different sizes, wemodified the synthesis method of MCM-48 silica particles reported by Kim et al [14].In this experiment, the yield of MSNs increased gradually with the increase of the amount of NH$_3$·H$_2$O. The yield of MSNs-6 and MSNs-8 was very similar and the yields of MSNs-6 and MSNs-8 were about 56% and 58% respectively, which were slightly higher than the other two groups (Figure.1). It can be seen from these results that withaugment of the amount of NH$_3$·H$_2$O in the reaction systems, the yieldofMSNsalso increased gradually.

The morphologies and microstructures of the MSNs are determined by TEM, asshown in Figure 2. TEM and the corresponding inserted HRTEMimage confirmed the hexagonal array of mesoporous channels in the MSNs.It can be seen that MSNs-2 and MSNs-4 consist of monodisperse spherical particles and these particles are non-aggregated with narrow size, but with the increase of NH$_3$·H$_2$O amount, the size of particles increased and tend to aggregated. The average diameters of these MSNs are calculated to be about 48, 110, 185 and 215 nm for MSMs-2, MSMs-4, MSMs-6, and MSMs-8, respectively.
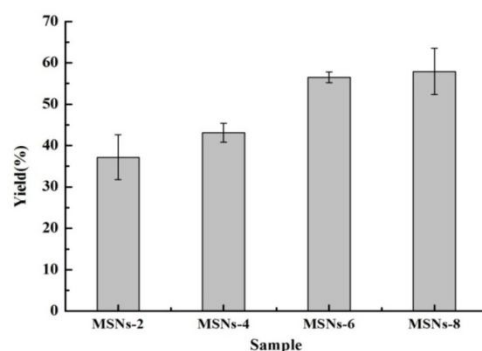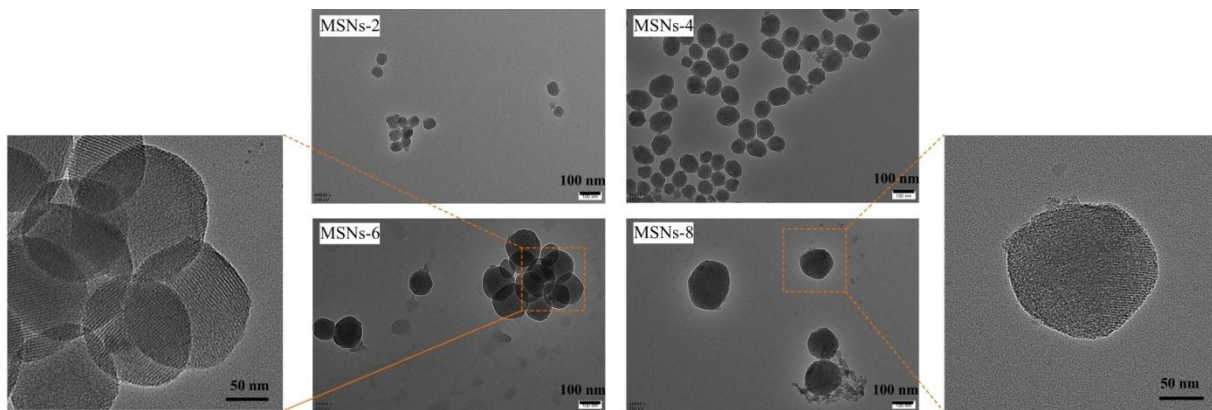

Figure1. Yield of different sample

Figure 2. TEM of mesoporous silica nanoparticles of samples MSNs-2, MSNs-4, MSNs-6 and MSNs-8. The inset in micrograph is HRTEM image of the sample.

The distributional stability of these samples in water were investigated and monitored by dynamic light scattering (DLS). Hydrodynamic diameter distributions are shown in Figure 3. Volume-weighted data of MSNs-2 and MSNs-4 showed a narrow hydrodynamic particle size distributions. These data indicated that these two samples could bethoroughly suspended and well-dispersed in water. But there appeared two peaks in the MSNs-8, which indicating obvious interparticle aggregation occurred in their suspension. The particle size of MSNs in water was further measured, It was found the particle size of sample were was 123, 142, 337 and 579 nm for MSNs-2, MSNs-4, MSNs-6 and MSNs-8respectively, which were slightly larger than the core particle sizes observed by TEM (Figure 2), which is usually the case taking into account the hydration corona around the particles and aggregation or flocculation.
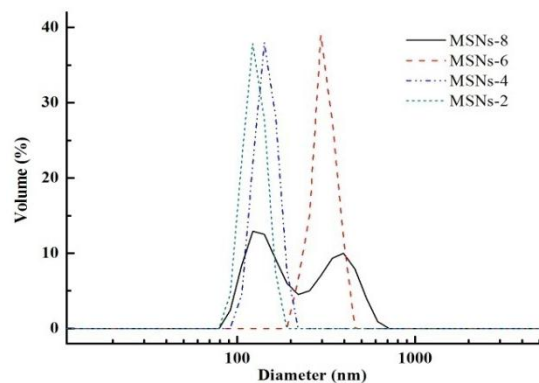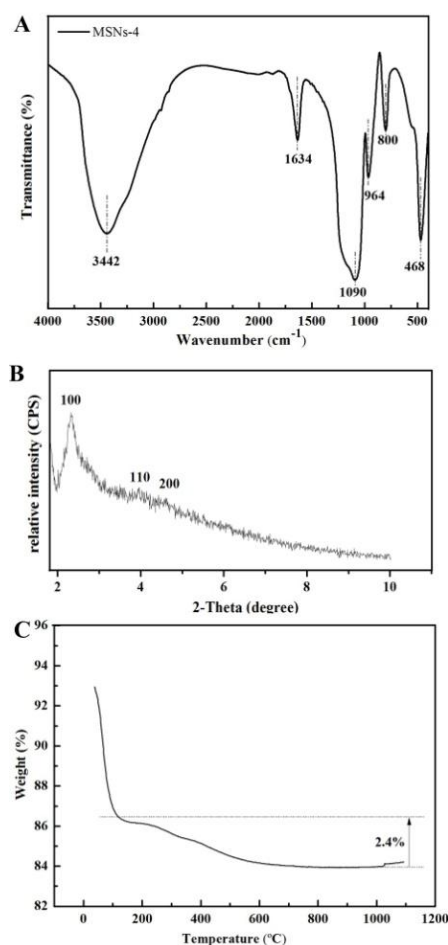

Figure 3. DLS results of samples

Figure 4. FTIR spectra (A), XRDpattern (B), TG profile of MSNs-4

In experiment, the structure properties and thermal stability of nanoparticles were investigated with MSNs-4 as the representative. It can be seen from the FTIR spectra (Figure 4, A), several broad absorption bands assigned to OH (3442 cm^{-1}), Si–O–Si (1090 cm^{-1}), Si–OH (800 cm^{-1}), and Si–O (468 cm^{-1}) bands can be seen clearly [15]. The strong band of OH indicates a large number of OH groups present on the surface, which are important for bonding drug molecules. The MSNs showed an ordered mesostructure like MCM-48type nanoparticles as revealed by low-angle XRD patterns (Figure 4, B), hexagonal mesoporous structure with the characteristic (100) peak between 2.14 and 2.30 ° and the d_{100} spacings were between 3.8 nm and 4.2 nm. The thermal stability of MSNs-4 was examined by TG analysis (Figure 4, C). It can be seen that at the beginning of the heating, there was a rapid decline with apparent weight loss, which may be due to the adsorption of a certain amount of free water on the surface of the material. After 110 ℃, only slight weight loss up to2.4% is observed in the temperature range from room temperature to 1100 ℃, which can mainly be due to the physically adsorbed water and hydroxyl groups in the materials, indicating the high stability of this material.

3.2. Macromolecular Drug Loading and Release Properties

To study the effect of MSNs size on the drug loading capacity and drug release, BSA was used as a model of macromolecular agent. Figure 5 showed the adsorption of BSA into the mesoporous matrices with different particle sizes. The experimental results showed that the drug loading rate of mesoporousnanospheres increased with the increase of argument of MSNs particle size. It can be found in Figure5 (A) that the drug loading rate of MSNs-2 was about 1.7%, the drug loading rate of MSNs-4 significantly increased to 6.1%, and the drug loading rate of MSNs-6 and MSNs-8 increased gradually to 8.3% and 10.3%, respectively.
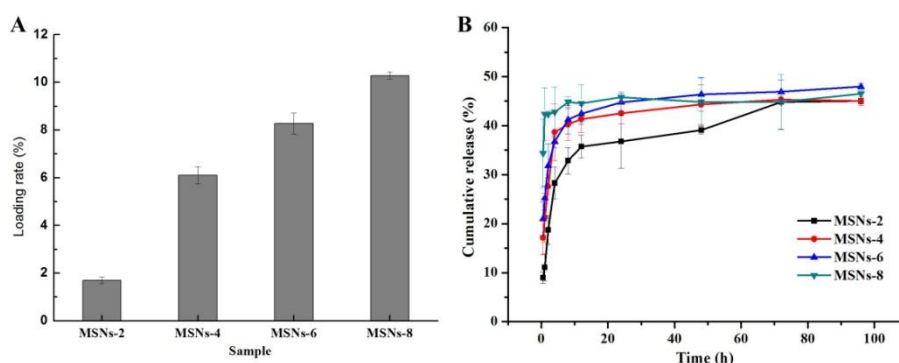
Figure 5. The loading rate of samples (A) and cumulative release curves of BSA from different MSNs

Figure 5 (B) showed the cumulative release profiles of the BSA from MSNs as a function of time in 0.1 mM PBS solution (pH=7.4).A relatively fast release within the first 12 h followed by a sustained release over 96h was observed, indicating the good sustained release properties of all the samples. In the initial 24h, the smaller particle size MSNs showed slower drug release rates, which may be due to the bigger particle size MSNs loaded more drugs. The slowest drug release rates assigned to MSNs-2, around 37 wt.% of total drug loading, while the drug release rates from MSNs-4, MSNs-6, and MSNs-8 were 43 wt.%, 44 wt.%, 45 wt.%. After 96 h, the amount of released BSA reached to 45 wt.%, 45 wt.%, 48 wt.% and 47 wt.% for MSNs-2, MSNs-4, MSNs-6, and MSNs-8, respectively.

4. Conclusion

In summary, a family of mesoporous silica nanospheres with different size has been synthesized via a facile and mild sol-gel method. The obtained MSNs possesses high thermal stability, tuneable particle size and special open pore channels, which allow easy access to the high specific area inside the pores. BSA release tests indicate this kind of MSNsexhibits good sustained release properties and the drug release rates are related to the particle size of the samples. Based on these results, it can be concluded that the MSNs-4 and MSNs-6 are better choicesfor macromolecular drug delivery system, this nanospheres presented here may have a good potential due to their perfect morphology, narrow and uniform pore size, good dispersancy and higher drug loading rate. This mesoporous silica should be promising in macromolecular drug release fields based on their good properties.

5. Acknowledgements

6. References

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