Facile and simple preparation of pH-sensitive chitosan-mesoporous silica nanoparticles for future breast cancer treatment

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Abstract. The nanocarrier is linked to the core and shell by hydrogen bond. This drug delivery system represents a smart, biodegradable, and pH-sensitive nanocarrier for breast cancer therapy. These drug nanocarriers were linked by hydrogen bond from $-NH_2$ on chitosan and $-OH$ on mesoporous silica nanoparticle (MSN). And MSN was prepared by the cetyltrimethyl ammonium bromide (CTAB)-templated method *via* sol-gel and the loading of ibuprofen (IBU) into the pores of MSN was visualized by coprecipitation which was assessed by Fourier Transform Infrared Spectroscopy (FTIR) and nitrogen adsorption-desorption techniques. The wrapping of chitosan (CS) onto the surface of MSN was demonstrated by FTIR too. When the nanocarriers entered an acidic environment where large quantity cancer cells existed, the chitosan shell swell into loose random coil, exposing the drug and making them easy to be released. The results showed that the IBU could be successfully and effectively loaded into MSN and CS/MSN. The system was pH responsive. Drug release was much higher at pH 6.8 than at 7.4. This drug delivery system will represent a smart and biodegradable pH-responsive nanocarrier for breast cancer.

Keywords: smart polymers, chitosan, MSN, pH-sensitive nanoparticles, drug delivery system

1. Introduction

For cancer therapy, people have found that drug controlled delivery system [1] could control the release of drugs and significantly reduce the side effects in recent years [2–6]. Moreover, pH-responsive drug controlled delivery system [7–13] can release drug at certain part of body, because pH value is different at each part of the human body. The normal body fluid pH is about 7.4, while the pH value at cancer cell is approximately 6.8. Several biodegradable materials, such as chitosan [14], polylactic acid [15], gelatin [16], polycaprolactone [17] and poly-alkyl-cyanoacrylates [18] have been used as intelligent drug delivery systems. In the meanwhile, some inorganic nanoparticles like meso-

porous silica (MSN), Fe_3O_4 , carbon nanotubes, quantum dots [19–22] are also used as drug delivery systems.

MSN has been widely used in biomedical due to its good biocompatibility, non-toxic, tunable pore size, large surface areas and pore volume, modifiable surface, biodegradation, good chemical and thermal stabilities and other advantages [19, 23–26]. But unmodified MSN is not intelligent and has potential toxicity at high dose because of the interactions of surface silanols with cellular membranes [27, 28]. So it is necessary to wrap MSN with biomolecules, so that the system is intelligent and low toxicity [28, 29]. Chitosan has been widely used in intelligent drug delivery system because it has unique properties

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such as non-toxicity, biocompatibility, biodegradability, and so on [30]. The ionization of large quantities of amino groups on chains of chitosan provides it as a pH-sensitive ‘gatekeeper’.

Many polymer-coated and pH-sensitive MSN nanocarriers have been designed, while most of the system add poisonous solvent and the preparation process is complex. In the present work, we have developed a simple and facile approach to prepare an intelligent drug controlled delivery system by hydrogen bonding biopolymer as pH-responsive ‘gatekeeper’ and inorganic nanoparticles as drug carriers. Meanwhile, the system is sensitive to a narrow pH range. We described a pH-responsive drug nanocarrier based on MSNs, which was wrapped by chitosan. And drug controlled release was studied at pH 7.4 and 6.8 which used IBU as a model drug.

2. Experimental section

2.1. Materials

Cetyltrimethylammonium bromide (Tianjin Jinbei Fine Chemical Co., Ltd, China), tetraethyl orthosilicate (TEOS, analytical reagent, Tianjin Fuchen chemical reagent factory, China), mesitylene (Aladdin Industrial Corporation, China), chitosan (CS, $M_w = 110\,000$ – $150\,000$, Amresco, USA), sodium hydroxide (NaOH, Tianjin DeEn Chemical Co., Ltd, China), n-hexane (C_6H_{14} , Tianjin Fengchuan Chemical Co., Ltd, China), ethanol (CH_3CH_2OH , Tianjin Fengchuan Chemical Co., Ltd, China), ammonium nitrate (NH_4NO_3 , Tianjin Jinbei Fine Chemical Co., Ltd, China).

2.2. Characterization

Fourier transform infrared spectroscopy analysis was performed by using KBr in the region of 4000 – 400 cm^{-1} by Nicolet PROTE/GE/460. Surface analysis of the nanoparticles was performed by N_2 adsorption-desorption isotherm curves. Brunauer-Emmett-Teller (BET) surface areas were calculated by the BET method and the pore size distribution was calculated by the Barrett-Joyner-Halenda (BJH) method deduced from desorption data (Quadrachrome Instrument, USA). Transmission electron microscopy (TEM) images were measured with field-emission transmission electron microscopy (JEM-2100, Japan). Ultraviolet-visible (UV-vis) absorption spectra were analyzed by a SHIMADZU (UV-2401PC, Japan). The pH value was measured

with a pH meter (PHS-3C, Shanghai INESA Scientific Instrument Co. Ltd., China).

2.3. Preparation of mesoporous silica nanoparticles (MSNs)

Mesoporous silica nanoparticles were prepared by sol-gel method [31]. Briefly, a certain amount of CTAB and 0.28 g NaOH was dissolved in 480 mL deionized water. Then pore-expanding agent 7.0 mL mesitylene was added to the solution. The mixture was stirred vigorously and heated to 80°C . Afterwards, 5.0 mL TEOS was added dropwise at a proper rate to the solution. The reaction mixture was stirred vigorously at 80°C for another 2 h. The resulting white precipitate was isolated by filtration, washed with abundant ethanol and dried under vacuum at 100°C for 12 h.

The structure-templating CTAB and mesitylene molecules were removed from the composite material *via* solvent extraction. A suspension of 1.0 g of the as synthesized material was stirred for 5 h at 60°C in a 150 mL ethanol solution with 0.4 g NH_4NO_3 adding in. The template-removed nanoparticles were then isolated *via* filtration and dried under vacuum at room temperature.

2.4. Loading IBU into MSNs (MSN/IBU)

0.5 g MSNs were added to 30 mL IBU n-hexane solution (40 mg/mL). Then the mixture was stirred at room temperature for two days. After that, MSN/IBU was centrifuged and the resulting supernatant was analyzed by using UV-vis spectrophotometry at a λ_{max} of 264 nm to evaluate the absorbed IBU into MSNs.

2.5. Chitosan wrapping MSN/IBU (CS/MSN/IBU)

0.2, 0.4, 0.6 W/V CS/acetic acid (10% V/V) aqueous solution was prepared and the pH was adjusted to 6.0 with 1M NaOH. Then 0.2 g MSN/IBU were added to 40 mL CS/acetic acid aqueous solution and stirred for 36 h at room temperature. The mixture was centrifuged, washed with deionized water twice and dried under vacuum at room temperature.

2.6. *In vitro* release of IBU

The *in vitro* release of IBU was measured by preparing equal weight MSN/IBU and CS/MSN/IBU in pH 6.8 and pH 7.4 PBS buffer solutions at concen-

tration of 4 mg/mL. The mixture was keeping stirred at rate of 30 r/min and releasing temperature was kept at 37°C. Then release was determined by taking 3 mL of the IBU release medium for UV-vis analysis at 1 h intervals and replaced with the same volume of fresh PBS buffer solutions, which were preheated to 37°C.

3. Results and discussion

3.1. Morphologies and structures of MSNs and CS/MSN/IBU

In this study, four kinds of MSNs were prepared via different concentrations of CTAB (Figures 1–4, Table 1). When concentration of CTAB was 5.7 mM, the prepared MSN had the larger pore volume and

Figure 1. N_2 adsorption-desorption isotherms of mesoporous silica (a) and pore size distribution (b), (Concentration of CTAB is 1.9 mM)

Figure 2. N_2 adsorption-desorption isotherms of mesoporous silica (a) and pore size distribution (b), (Concentration of CTAB is 3.8 mM)

Figure 3. N_2 adsorption-desorption isotherms of mesoporous silica (a) and pore size distribution (b), (Concentration of CTAB is 5.7 mM)

Figure 4. N₂ adsorption-desorption isotherms of mesoporous silica (a) and pore size distribution (b), (Concentration of CTAB is 7.6 mM)

Table 1. Structure parameters of MSN and MSN/IBU

Samples	Concentration of CTAB [mM]	BET surface area [m ² /g]	Pore volume [cm ³ /g]	Small mean pore [nm]	Large mean pore [nm]
MSN1	1.9	267.1	0.29	3.8	9.3
MSN2	3.8	818.2	1.61	3.8	9.3
MSN3	5.7	948.1	1.34	3.8	9.8
MSN4	7.6	991.1	0.09	3.4	9.8
MSN/IBU	5.7	282.1	0.34	3.8	9.5

BET surface area. So in this study we chose MSN3 as drug nanocarrier. Figure 5 showed the average diameter of the prepared MSN was 200 nm. The MSN had regular array of mesoporous channels and large enough pores to load IBU inside. The TEM image of the chitosan wrapped MSN confirmed that chitosan successfully wrapped on the surface of MSN and CS/MSN/IBU still showed regular spherical structure.

As the concentration of CTAB increases, the size of the micelles formed in the solution became larger. CTAB might have been partially adsorbed inside the silica channel, with the result that template was not removed completely in the final and the BET surface area and pore volume decreased sharply. The N₂ adsorption-desorption isotherm curve of MSN showed a typical IV isotherm curve. The BET surface area is 948.1 m²/g and pore volume is

Figure 5. TEM images of MSN (a) and CS/MSN/IBU (b)

Figure 6. N₂ adsorption-desorption isotherm curve of MSN/IBU

1.34 cm³/g for MSN3 (Figure 3a, Table 1). The BJH method showed two pore size distributions centered on 3.8 nm (major) and 9.8 nm (minor) (Figure 3b, Table 1).

The structures of the nanoparticles were characterized by FTIR. As shown in Figure 7, the FTIR spectrums of MSN, MSN/IBU and CS/MSN/IBU show a hydroxyl absorption peak at 1636 cm⁻¹. And the typical absorption peaks at 1088 and 797 cm⁻¹ were the stretching vibration of Si–O–Si and Si–O [28]. While the adsorption spectrums of IBU, MSN/IBU, CS/MSN/IBU all showed the typical absorption peaks of IBU, which were the stretching vibration of C=O at 1710 cm⁻¹, C=C vibration and C–H of phenyl ring at 1510 and 1420 cm⁻¹, respectively. In addition, both the adsorption spectrums of CS and CS/MSN/IBU had the absorption peaks of amide I at 1652 cm⁻¹ and amide II at 1560 cm⁻¹. Carboxyl groups in IBU molecular and amino groups in CS

Figure 7. FTIR spectra of CS, IBU, MSN, MSN/IBU, CS/MSN/IBU

molecular interacted together during the preparation process of CS/MSN/IBU, so the characteristic peak (1710 cm⁻¹) did not appear any more. All these results proved that the IBU loaded in MSN and the CS wrapped MSN/IBU successfully. Furthermore, the loading of IBU into the pores of MSN could be demonstrated through N₂ adsorption-desorption analysis, which the BET surface area reduced from 948.1 to 282.1 m²/g and the pore volume reduced from 1.4 to 0.34 cm³/g after IBU loading into MSN (Figure 6, Table 1).

The loading efficiency of IBU into CS/MSN/IBU was dependent on the mass fraction of CS. In the meantime, the loading efficiency could be calculated by weighing method and ultra-violet photometer. The loading efficiency of MSN/IBU (LE_{MSN}) was deduced from Equation (1). The result was about 52.8%:

$$LE_{MSN} = \frac{m_1 - m_0}{m_1} \quad (1)$$

where m_0 and m_1 mean the weight of MSN before and after loading IBU into MSN, respectively.

In the process of CS wrapping MSN/IBU, there was IBU leaking out from the channels of MSN. The leaked IBU was measured by ultra-violet photometer (Figure 8). Equation (2) was used to calculate the loading efficiency of CS/MSN/IBU ($LE_{CS/MSN/IBU}$).

$$LE_{CS/MSN/IBU} = \frac{LE_{MSN} \cdot m_{MSN/IBU} - m_{IBU}}{m_{CS/MSN/IBU}} \quad (2)$$

where $m_{MSN/IBU}$ and m_{IBU} mean the weight of MSN/IBU and the weight of IBU leaked from the channels of MSN during the wrapping process.

Figure 8. Ultraviolet-visible spectrum of the released IBU during the CS wrapping process

The loading efficiency of IBU into CS/MSN/IBU was 22.2, 28.9, 38.3% respectively when the mass fraction of CS was 0.2, 0.4, 0.6%.

3.2. Release behavior of IBU

The absorption peak at 264 nm (*Abs*) of IBU was used as signal to study the release of IBU from MSN/IBU and CS/MSN/IBU at 37°C. The experiments were carried out in PBS buffer solutions at two different pH values (7.4 and 6.8), which were chosen to mimic the normal body fluid and cancer cell. As shown in Figure 9, the total amount of IBU released from MSN/IBU was about 85 and 93% at pH 7.4 and 6.8, respectively. The amount of IBU released from CS/MSN/IBU at pH 7.4 was about 9.8% for 1 h and 13% for 12 h, but the release amount had little increase during the whole release process. It indicated that chitosan wrapped MSN/IBU was stable for a long time and had lower side effect at physiological pH. While at pH 6.8, the release amount increased obviously (30% for 1 h and 53% for 12 h) and changed dramatically with the pH comparing with MSN/IBU. This phenomenon was caused by the change of molecular chain conformation of chitosan. Conformation of chitosan in solution is generally divided into spherical, random coil and rigid rod. One of the main factors influencing conformation is just the pH [32]. In acidic solution, the amino groups ($-\text{NH}_2$) on chitosan are protonated into $-\text{NH}_3^+$, electrostatic repulsion becomes strong between chitosan molecules and the hydrogen bonding becomes less effective. In this case, the molecular chain of chitosan turns into a swollen polymeric matrix which is like loose random coil,

Figure 9. Release profiles of IBU from MSN/IBU and CS/MSN/IBU drug delivery systems

Figure 10. Release profiles of IBU from CS/MSN/IBU with different chitosan mass ratio (pH 6.8)

which has less influence on the release of IBU. While at pH 7.4, the amino groups don't carry charge and weaker electrostatic repulsion exists, so the molecular chain gets together as a shrinking mode [10]. This structure blocks the release of IBU to some extent. So the release amount at pH 6.8 was higher than at pH 7.4.

As shown in Figure 10, with the increase of mass fraction of chitosan, the accumulated release amount of CS/MSN/IBU decreased greatly from 56 to 19%. With the increase of mass fraction of CS, solution viscosity increased, which resulted in the structure of CS/MSN/IBU becoming more closely, crosslinking degree increasing and the swelling capacity reducing. Eventually the drug release rate decreased with the increase of mass fraction of CS.

As shown in Figure 11, MSNs were wrapped by chitosan and chitosan presented different states under different pH conditions because of protonation [10]. In this system, chitosan acted as a pH-responsive 'gatekeeper' ensuring more drug release at pH 6.8, which was probably due to the formation of hydrogen bond between the $-\text{OH}$ on the MSN and the $-\text{NH}_2$ on the chitosan molecule.

Figure 11. The pH-responsive drug delivery system

4. Conclusions

In summary, we have developed a simple approach to prepare an intelligent drug controlled delivery system by hydrogen bonding biopolymer as pH-responsive ‘gatekeeper’ and inorganic nanoparticles as drug carriers. The ‘gatekeeper’ is biodegradable and nontoxic, which could be directly applied to medical environment. MSNs with largest BET surface area and pore width were chosen as drug carriers which could load more drugs. Drug loading demonstrated that IBU loading increased with the increase of mass fraction of chitosan. *In vitro* release tests demonstrated that IBU release from MSNs was not pH-responsive, yet drug release from CS/MSN was pH-responsive with much higher release amount at pH 6.8 than pH 7.4.

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