Mechanism study on pH-responsive cyclodextrin capped mesoporous silica: effect of different stalk densities and the type of cyclodextrin

Ling Bai¹, Qinfu Zhao¹, Jian Wang³, Yikun Gao², Zhou Sha¹, Donghua Di¹, Ning Han¹, Ying Wang¹, Jinghai Zhang² and Siling Wang¹

¹ Liaoning Provincial Key Laboratory of Studying the Modern Drug preparations, Shenyang Pharmaceutical University, No.103 Wenhua Road, Shenyang 110016, People’s Republic of China
² School of Medical Devices, Shenyang Pharmaceutical University, No.103 Wenhua Road, Shenyang 110016, People’s Republic of China
³ Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, No.103 Wenhua Road, Shenyang 110016, People’s Republic of China

E-mail: silingwang@syphu.edu.cn

Received 27 November 2014, revised 14 February 2015
Accepted for publication 26 February 2015
Published 1 April 2015

Abstract

Cyclodextrin (CD)-capped mesoporous silica nanoparticles (MSN) with pH-responsive properties were synthesized, but little research has been carried out to evaluate the impact of critical factors such as the stalk density and the type of CD on the pH-responsive release behavior. Here, the effect of different stalk densities on the pH-responsive release behavior was investigated. Either too low or too high density of the grafted p-anisidine stalk could result in poor cargo release, and the optimum stalk density for MSN was measured by thermal analysis, and found to be approximately 8.7 stalks nm⁻². To achieve effective release control, the CD capes, α-CD and β-CD, were also investigated. Isothermal titration calorimetry (ITC) analysis was employed to determine the formation constants (Kf) of the two CD with p-anisidine at different pH values. The results obtained showed that the complex of β-CD with p-anisidine had excellent pH-responsive behavior as it exhibited the largest changed formation constant (ΔKf) in different pH media. Furthermore, the pH-responsive mechanism between CD and p-anisidine molecules was investigated through ITC and a molecular modeling study. The release of antitumor drug DOX presents a significant prospect toward the development of pH-responsive nanoparticles as a drug delivery vehicle.

Keywords: mesoporous silica nanoparticles, pH-responsive, stalk density, cyclodextrin, controlled release system

(Some figures may appear in colour only in the online journal)

1. Introduction

Mesoporous silica nanoparticles (MSNs) have been widely studied as a vehicle in controlled release systems due to their favorable properties, such as a large surface area, ordered pore structure and adjustable pore diameter [1]. They have large numbers of silanol groups which allow easy modification...
with a variety of organic moieties to diversify their design [2, 3]. Based on the above advantages, stimuli-responsive end-capped MSNs have attracted much attention for application in controlled release systems by capping silica pores with supermolecules or nanoparticles, thereby endowing the silica with controlled-release ability [4, 5]. The caps, as the moving parts of the functionalized silica, can completely block and open the pore outlets automatically under various triggers irrespective of extrinsic or intrinsic factors, such as pH [6, 7], light [8, 9], magnetic field [9, 10] and redox potential [11, 12]. Among a variety of stimuli that have been used in capped MSN systems, the pH is attracting growing interest. A good pH-responsive system should allow precise release control with only slight change in the pH media. Previous studies have described the pH-responsive capped MSNs with various caps, including organic macromolecules, nanoparticles and biomolecules [7, 13, 14]. In recent years, several exciting designs have been investigated using this approach. For example, Rui Liu et al. attached Au nanoparticles to the silica surface as caps through acid degradable acetal linkers [7]. Chiyoung Park and co-workers have developed a pH-responsive polypseudorotaxane, which consisted of cyclodextrin (CD) and linear PEI. The system was controlled by the change in pH value due to the reverse complexation and decomplexation of PEI chain and CD molecules [15]. In addition, biomolecules such as lysozyme have also been chosen as caps by the Mengjun Xue group. The pore entrance opening/closing mechanism was related to the conformational change which led to a change in size of the lysozyme molecules induced by a change in pH [16]. However, as far as applications are concerned, it is generally expected that the pH-responsive mechanized nanoparticles will have the advantages of a simple design and production, offering straightforward operation, and effective action.

In the work reported here, we constructed a simple and effective pH-responsive end-capped MSN controlled release system that involved CD as the pore cap to regulate the release of the cargo trapped in the silica channels. This pH-responsive system consisted of p-anisidine stalks and CD caps based on MSNs. The caps surround the p-anisidine stalks tightly, thereby trapping the outlets efficiently when a suitable stalk density was employed at a neutral pH. While with the addition of acid, the nitrogen of p-anisidine (on the stalk) became protonated and this resulted in the spontaneous dethreading of the CD caps and unblocking of the silica outlets. The working principle of the system is illustrated in figure 1.

Although a variety of pH-responsive MSNs with supermolecular caps based on CD have been synthesized [13, 17], several critical factors, such as the stalk density and the type of CD which may play a vital role in the release behavior, have not yet been studied in detail. Furthermore, detailed analysis of the thermodynamic parameters and energetics involved in the process of inclusion are lacking. A better understanding of these processes would lead to design suitable functionalized MSNs. So, the study, for the first time, provided insights regarding the thermodynamic parameters and energetics involved in the inclusion process. Hence, much of our present work has been aimed tailoring the functionalized MSNs properly in order to improve the efficiency of controlled release.

As the host material, we selected MCM-41 as the carrier due to its tubular pore passages with a 2D hexagonal porous structure [18, 19], and the single connection between individual porous channels allowed it to be an independent reservoir for cargos with a ‘no-leaking’ capability, even in the event of incomplete capping. A water-soluble biological stain, Hoechst 33342 was employed as the cargo model, with the main objective of demonstrating the working principle of the pH-responsive operation of CD-equipped MSNs. Moreover, the antitumor drug DOX was chosen to verify the potential of the vehicle for pH-responsive drug release.

2. Materials and methods

2.1. Materials

The following reagents were purchased from Sigma–Aldrich and used without purification: tetraethyl orthosilicate (TEOS, 99%), hexadecyl trimethyl ammonium bromide (CTAB, 99%), 3-iodomethyltrimethoxysiliane (IPTMS, 95%), p-anisidine (99%), Hoechst33342 (98.0%), α-CD (95%), β-CD (95%), triethylamine (99%). Toluene (99.5%), methanol (99.5%), hydrochloric acid (HCl, 36 wt%) and sodium hydroxide (NaOH, 96%) were purchased from Yu Wang Reagent Company (Shandong, China). Doxorubicin hydrochloride was a gift from Zhejiang Hisun Pharmaceutical, Deionized water in all experiments was prepared by ion exchange.

2.2. Characterization

The porous structure of the silica nanoparticles was characterized using transmission electron microscopy (TEM) on a Tecnai G2 F30 instrument (FEI, USA). Zeta potential values of the samples were measured using ZetaSizer Nano (Malvern Instruments, UK) and used without purification. The porous structure of the silica nanoparticles was characterized using transmission electron microscopy (TEM) on a Tecnai G2 F30 instrument (FEI, USA). Zeta potential values of the samples were measured using ZetaSizer Nano (Malvern Instruments, UK).
Instruments, UK). Nitrogen adsorption–desorption isotherms were measured on a V-Sorb 2800P surface area and pore size analyzer. The Brunauer–Emmett–Teller (BET) model was applied to evaluate the specific surface areas. Pore size and pore volume were determined from the adsorption data by the BJH method. UV–vis spectra were measured using a Unic-2000 UV spectrophotometer (Unic Company, Shanghai). Elemental analyses of Si, O and N element were carried out using a PHI Quantera (ULVAC-PHI Company, Japan). Thermo-gravimetric analysis (TGA) was performed using a NETZSCH STA 449C thermal gravimetric instrument (Germany) by heating the sample at 10 °C min⁻¹ from room temperature to 900 °C under an N₂ atmosphere. Isothermal titration calorimetry (ITC) experiments were carried out on a fully computer-operated isothermal titration calorimeter (Microcal ITC 200) instrument. The raw data from ITC were analyzed using Origin 8.0 software with a 'one set of binding sites' model. All experiments were performed at 25 °C and all solutions were degassed for 10 min before use.

### 2.3. Preparation of MCM-41 MSN

A mixture of CTAB (1.0 g), 2.0 M of NaOH (aq) (3.5 mL) and H₂O (480 mL) were added with constant stirring and heated at 80 °C. After 30 min of stirring, TEOS (5 mL) was slowly added to the solution and the mixture was kept at 80 °C with stirring for another two hours. Following the addition of TEOS, a white precipitation was observed. To reduce the aggregation of nanoparticles, the obtained suspension was homogenized by an ATS AH100D homogenizer at 700 bar for six cycles (ATS Engineer, Shanghai, China). Then obtained solid was collected by filtration and washed with water and methanol. To extract the templating agents, an acid extraction was accomplished in a mixture of methanol (480 mL), concentrated hydrochloric acid (27 ml) and as-made materials at 75 °C for 24 h. The as-synthesized MCM-41 silica particles were centrifuged at 6000 rpm and then washed with water and methanol for several times, finally dried under vacuum.

### 2.4. The surface modification of MCM-41 MSN

Silica nanoparticles were dried under 100 °C for 10 h before the functional modification. To construct a p-anisidine modified silica surface, the dried nanoparticles (100 mg) in dry toluene (10 mL) were reacted with IPTMS (0.05 mmol) in an N₂ atmosphere and refluxed for one day to form MCM-41-I nanoparticles. The procedure was carried out under water-free conditions in toluene to avoid unwanted intermolecular polymerization among IPTMS molecules. After this procedure, the obtained MCM-41-I nanoparticles were washed with dry toluene and methanol. Next, p-anisidine (0.5 mmol) and triethylamine (1.5 mmol) were added to the MCM-41-I particles in toluene solution and reacted at 80 °C under N₂ gas for 48 h. Finally, the product, named MCM-41-p-anisidine, was collected by filtration and washed thoroughly with toluene and methanol, and then dried under vacuum.

### 2.5. Cargo loading and β-CD capping

The synthesized MCM-41-p-anisidine sample (50 mg) was added to Hoechst33342 solution (5 mL, 1 mM water solution). An excess of β-CD was added to the solution which contained the Hoechst 33342 loaded nanoparticles, then sonicated for 30 min and stirred at RT for another three days. Finally, to remove excess Hoechst 33342 and β-CD, the dye-loaded solids were centrifuged and washed with water until no dye was detected in the washing supernatant. The capped particles were then dried under vacuum for later stimulated release studies.

### 2.6. Controlled release experiment

The Hoechst 33342 loaded, CD-equipped silica nanoparticles (10 mg) were suspended in 10 mL pH 7.4 phosphate buffer solution. To measure the concentration of dye release, 3 mL buffer solution with the dispersed particles was removed and centrifuged at 6000 rpm. The supernatant without nanoparticles was subjected to UV–vis spectroscopy at an absorption peak 335 nm to determine the concentration of the dye released. Adjusting the pH of the solution to 5.0 was accomplished by the addition of HCl (0.1 M), which fully activated the release of dye molecules from the nanoparticles.

### 2.7. Molecular modeling

Structure of p-anisidine stalk was created using Chembiodraw software. The α-CD and β-CD structure were extracted from the crystal structure of α-CD and β-CD complexes obtained from Cambridge Crystallographic Data Centre (Nos. CCDC-136109 and CCDC-798968). Complexes between p-anisidine stalk with α-CD and β-CD under the different simulated conditions were predicted by molecular docking, using Discovery Studio 4.0 software.

### 2.8. DOX loading and release

The p-anisidine modified MCM-41 nanoparticles (20 mg) were suspended in an aqueous solution of DOX (1 mM, 2 mL) at pH 7.0 and stirred overnight. An excess of β-CD was added to the solution, and then adjusted the pH of the solution mixture to be neutral. The resulting mixture was stirred for three days at room temperature. The DOX-loaded, CD-capped MSN were collected by centrifugation and washed with water until no DOX was detected in the washing supernatant. To investigate the pH responsive DOX release, the DOX-loaded, CD-capped MSN were prepared in 10 mL aqueous solution at pH 7.4 and pH 5.0. The emission of DOX was measured at different time intervals using UV–vis spectroscopy with the absorption maximum of DOX at 480 nm.
3. Results and discussion

3.1. Characterization of the p-anisidine-functionalized MSNs

Bare MCM-41 templated by CTAB was synthesized through a well-established procedure [20, 21], and then solvent extraction was carried out on bare MCM-41 to obtained empty nanopores by removal of templating agents. Construction of the p-anisidine-modified silica involved derivatizing the silanol functional groups on the bare MCM-41 silica with IPTMS to afford iodo-functionalized porous silica. This provided target sites for the following functionalization with p-anisidine through a nucleophilic substitution reaction to yield MCM-41-p-anisidine. The p-anisidine-functionalized silica nanoparticles were characterized by TEM, N₂ adsorption–desorption analysis and zeta potential measurements. The size and morphology of the silica nanoparticles, before and after functionalization, were investigated by TEM. As shown in figure 2, the TEM images of MCM-41 nanoparticles were spherical in shape and about 100 nm in diameter, with highly ordered streak structural features. It should be noted that the particles, with or without functionalization, exhibited no obvious differences in shape, and they both had ordered tubular pores with a spherical shape, which indicated that the organic stalk functionalized silica retained the characteristics of MSN even after grafting p-anisidine groups to the outlets of the pores.

The structural parameters of the bare silica particles and functionalized silica particles were calculated by the BET method (table 1). Nitrogen physisorption analysis showed similar type IV adsorption isotherms (figure 3(a)) [22]. For the template-removed MCM-41, the BET surface area, pore volume and pore diameter were 1067 m² g⁻¹, 1.223 cm³ g⁻¹ and 2.9 nm, respectively. After functionalization with p-anisidine, the surface area, pore volume and pore diameter values were reduced slightly, to 959 m² g⁻¹, 1.196 cm³ g⁻¹ and 2.8 nm, respectively. This indicated that discrete stalks did not markedly influence the pore size due to their low concentration on the silica walls, indicating that the stalks were present predominantly on the outer surface of the particles rather than on the inner surface of the pore channels. Also, the capped MSNs had a BET surface area of 636 m² g⁻¹, pore volume of 0.583 cm³ g⁻¹ and a pore diameter of 2.5 nm, which indicated that most of the pore entrances of the capped MSNs were indeed blocked by β-CD molecules.

Zeta potentials of bare MCM-41 nanoparticles and p-anisidine-modified silica at different pH values were measured, as the charge of the outer functional groups of p-anisidine was influenced by the change in pH (figure 3(b)). At pH 7.4, the zeta potential of bare MCM-41 was −22 ± 1.3 mV, while the zeta potential of p-anisidine modified silica increased to +28 ± 1.2 mV at pH 5.0 from −26 ± 0.9 mV at pH 7.4, demonstrating the successful attachment of the p-anisidine groups to the mesoporous silica. At the neutral pH, only silanol group with an isoelectric point which was 2–3 deprotonated and was in the way of the negatively charged Si–O⁻ [23]. When the pH value was reduced to 5.0, the functionalized nanoparticles exhibited a positive zeta potential which might attribute to the protonation of nitrogen on the p-anisidine stalk at this pH. These results indicated that the p-anisidine-modified silica exhibited promising pH-responsive properties.

3.2. pH-responsive release of cargo molecules at different pH values

To examine the pH sensitivity of the functionalized nanoparticles, the influence of pH on release was investigated. We used β-CD as the cap and three different pH values (pH 7.4, pH 6.5 and pH 5.0) were selected. The pH of the system was adjusted to 6.5 and 5.0 by the addition of 0.1 M HCl and the release was measured by UV–vis absorption spectroscopy.
From the release curves of Hoechst 33342-loaded, β-CD-capped MSNs, it could be seen that the amount of released Hoechst 33342 increased as the pH fell over a certain value, reflecting pH-dependent behavior (figure 4). The cumulative release was significantly higher at pH 5.0 than at pH 6.5 or pH 7.4, from 0.7% at pH 7.4 and 3.6% at pH 6.5 to 84.2% within 4 h with a medium pH of 5.0. The above results indicated that there were no significant differences in the release between pH 7.4 and pH 6.5, which meant that the system was not fully activated [24]. At pH 7.4 and pH 6.5, the p-anisidine stalks remained hydrophobic and, therefore, could bind to the CD caps tightly. As a result, the CD caps blocked the nanopores and trapped the cargo molecules inside the pores. When the pH of the external medium fell below the pKa (pKa = 6) of p-anisidine, the interactions between the stalks and β-CD decreased and forced the separation of the CD molecules [24]. After removal of the CD molecules, the cargo was able to be released by diffusion. Therefore, the β-CD capped MSNs showed a good pH-responsive release capability.

3.3. pH-responsive release of cargo molecules with different stalk densities

Once the pH-dependent release characteristics were confirmed, the separate effects of stalk density and the type of CD on the release of the cargo Hoechst 33342 were investigated. The variable stalk densities were obtained by tuning the amount of IPTMS, and the experiment was performed to confirm the effect of the amount of IPTMS on the pH-responsive behavior. To optimize the stalk density, 100 mg MCM-41 particles were treated with different amounts of IPTMS (0.025 mmol, 0.05 mmol and 0.1 mmol), and subsequently reacted with an excess of p-anisidine to yield p-anisidine-grafted MCM-41 with low, medium and high stalk densities, giving rise to different functionalization levels. Loading was carried out by soaking 50 mg of the p-anisidine-grafted MCM-41 particles in 5 mL Hoechst 33342 solution followed by capping with β-CD. A pH of 5.0 was chosen as the trigger pH to release the cargo. The percentage of released cargo with different stalk densities is plotted in figure 5. Prior
to pH trigger, the premature release percentage of the system with low stalk density and high stalk density was 33.8% and 20.0% within 4 h, that was in stark contrast to the case with medium stalk density which showed no premature release. Upon lowering of the pH, the release percentage increased from 50.3% at a low stalk density to 85.4% at the medium stalk density but fell to 63.8% at the high stalk density. Thus, we were able to conclude that the optimum release was achieved while the silica functionalized with the optimum stalk density corresponded to 0.05 mmol IPTMS. A possible reason for this might be the followings. At a low concentration of IPTMS groups on the silica surface, the functional groups of iodo (I-) reacted almost completely with the p-anisidine molecules, forming silica particles surrounded by p-anisidine stalks. However, the stalk density was not enough to completely block the orifices of the particles, although all the stalks were capped with CD molecules, and the existence of the gaps between the stalks was considered not to be effective enough to prevent leaking of the cargo from the channels. By increasing the concentration of IPTMS groups on the silica surface, the functional groups of iodo would increase the interaction with p-anisidine molecules and, hence, increase the stalk density. Also, the p-anisidine stalks would be completely capped with the CD molecules, resulting in blocking of the pore entrances efficiently, thus preventing the cargo from leaking. However, above the critical concentration of the IPTMS groups, the stalk density would be increased, but the capping procedure was not so effective, possibly due to the steric hindrance between the dense stalks and the CD molecules, and this could reduce the accessibility and reactivity of the p-anisidine groups on the stalks. Therefore, there might be an optimum stalk density on the silica surface, at which an effective pH-responsive behavior would be exhibited. Furthermore, \( N_2 \) adsorption–desorption analysis, elemental analysis and TGA were used to evaluate the properties of the mesoporous silica with different stalk densities.

In contrast to bare silica particles, the surface area, pore volume and pore diameter of different stalk densities were reduced by different degrees, as shown in table 2. For the functionalized silica with a low and medium stalk density, the surface area, pore volume and pore diameter values decreased slightly compared with the bare MCM-41, which showed that the stalks around the pore rim had an appropriate density so the stalks could not significantly influence the pore size. However, for the functionalized silica with a high stalk density, the surface area, pore volume and pore diameter values fell progressively, to 678 m\(^2\) g\(^{-1}\), 0.763 cm\(^3\) g\(^{-1}\) and 2.6 nm respectively, demonstrating that most of the pores were sealed by stalks.

To further confirm the different stalk densities, elemental analysis was carried out to determine the N percentage of p-anisidine-modified nanoparticles as the p-anisidine groups attached mainly on the external surface of the silica particles. From the results of the elemental analysis, no N element was detected indicating that there was no residual template agent on the silica surface. After modification, the elemental N could be detected in all particles, although the values were very low, possibly because the percentage of N was very low when compared with other elements such as Si and O [25]. The number of p-anisidine stalks per gram of nanoparticles was calculated and given in table 3. The elemental analysis of low, medium and high stalk density modified nanoparticles revealed the presence of 0.257 mmol g\(^{-1}\), 0.321 mmol g\(^{-1}\) and 0.42 mmol g\(^{-1}\) of stalks anchored to the silica.

The stalk density could also be obtained by TGA. The thermal analysis curves of bare silica and functionalized silica with different stalk densities are shown in figure 6. TGA graphs of the samples all showed a sharp weight loss at temperatures below 100 °C corresponding to the loss of physisorbed water [26, 27]. For the bare silica (figure 6(a)), there was no weight loss below 600 °C, while a weight loss of 6.2% was observed in the temperature range 600–900 °C, which was attributed to the present of template. However, the TGA spectrum of functionalized silica showed three steps of weight loss, the first step was below 100 °C, the second step was in the region 155–292 °C and the last step was in the region 292–600 °C(figures 6(b)–(d)). As mentioned earlier, the weight loss of first step might be due to the volatility of the water. The second weight loss could be the loss of unreacted IPTMS on the silica surface, which was 1.62%, 2.21% and 3.87% for the silica with a low, medium and high stalk density, respectively. The third degradation range (292–600 °C) observed in the TGA curve was a feature of the modified portion of the total amount of p-anisidine stalk and the weight loss was 6.78%, 10.93% and 16.23% for the silica with a low, medium and high stalk density, respectively. On the basis of calculations, the stalk densities of the p-anisidine groups on the silica particles for low, medium and high stalk densities were around 5.4, 8.7 and 12.8 stalks nm\(^{-2}\), respectively [28]. The detailed calculations were as follows.

Because MCM-41 mesoporous silica had an array of hexagonal tubes [29], we considered that the silica was constructed by numbers of hexagonal units. The hexagonal pore model of MCM-41 mesoporous silica is shown in figure 7. In this model, the lattice spacing \( (d_{lattice}) \) was 4 nm and the density of amorphous silica was around 2.5 g cm\(^{-3}\), which was obtained from the [28, 30]. The pore diameter \( (r) \) of the MSNs was 2.9 nm, obtained from BET analysis, and the size

| Sample          | BET surface area (m\(^2\) g\(^{-1}\)) | Pore volume (cm\(^3\) g\(^{-1}\)) | Pore size (nm) |
|-----------------|--------------------------------------|----------------------------------|----------------|
| Low stalk density | 1024                                 | 1.208                            | 2.9            |
| Medium stalk density | 959                                  | 1.196                            | 2.8            |
| High stalk density | 678                                   | 0.763                            | 2.3            |
The total percentage of the silica wall in a single nanoparticle

\[ P_{\text{wall}} = \left(1 - S_{\text{pore}}/S_{\text{hex}}\right) \times 100\%. \]  

The mass of one particle

\[ m_{\text{particle}} = P_{\text{wall}} \times \rho_{\text{silica}} \times V_{\text{particle}}. \]  

The area of one nanoparticle

\[ S_{\text{particle}} = 4\pi (1/2R)^2. \]  

The number of pores on one nanoparticle

\[ N_{\text{pore}} = S_{\text{particle}}/S_{\text{hex}}. \]  

The total area of the pore openings on the surface of one single nanoparticle

\[ S_{\text{total pore}} = N_{\text{pore}} \times S_{\text{pore}}. \]  

The total surface area for the modification of silica agent

\[ S_{\text{surface silica}} = S_{\text{particle}} - S_{\text{total pore}}. \]  

The number of the stalks on each nanoparticle

\[ N_{\text{stalk}} = \left( m_{\text{particle}} \times n \% \right) / M_{\text{w(stalk)}} \times N_{\text{A}} \times (n = 6.78\%, 10.93\%, 16.23\%). \]  

The average stalk density of p-anisidine group on the silica particle

\[ \delta_{\text{stalk density}} = N_{\text{stalk}} / S_{\text{surface silica}}. \]  

In addition, the template of CTAB exhibited a weight loss in TGA while no template was detected using elemental analysis, which might be because the residual template was inside the pore channels instead of on the silica surface.

### 3.4. pH-responsive release of cargo molecules with different types of CD

Unless otherwise specified, functionalized silica with 0.05 mmol (100 mg silica) IPTMS was chosen in the subsequent research as the optimum stalk density. As mentioned above, another key factor was the capping ability of different types of CD. As is well known, CD had a hydrophobic internal cavity with appropriate dimensions, which allowed the binding of different molecules no matter organic or inorganic molecule [31]. Based on the unique nature of CD, the CD families have attracted much attention for application in silica delivery systems [4, 32–34]. The CD molecules were capable of blocking the pore outlets of silica, thus blocking the cargo molecules inside the silica channels until they were released from the stalks and left the pore entrance by stimulation under an appropriate environment. In this section, we selected α-CD and β-CD as representative capped molecules to investigate how they affected the release behavior of the cargo molecules. As a comparison, cargo release experiments based on p-anisidine-modified silica capped with different types of CD were performed. As shown in figure 8, a clear difference in the release percentage was observed within 4 h. At pH 5.0, the release percentage of β-CD and α-CD capped...
silica exhibited a decreasing trend, and the corresponding release percentages were 86.5% and 60.8%, respectively. A plausible explanation for these differences was the different formation constants ($K_f$) between the CD and the p-anisidine stalls in media of different pH. In order to quantify the formation constants of different CD with p-anisidine unit on the stall, we assumed the formation constants for complexes of CD with p-anisidine site at 25 °C in the phosphate buffer.

| pH  | Parameters       | $\alpha$-CD | $\beta$-CD |
|-----|------------------|--------------|------------|
| 7.4 | Ka/L mol$^{-1}$  | 750 ± 39     | 1020 ± 105 |
|     | $\Delta H^o$/(kJ mol$^{-1}$) | -16.32 ± 0.02 | -4.98 ± 0.02 |
|     | $\Delta S^o$/(kJ mol$^{-1}$) | 0.12 ± 0.01   | 12.18 ± 0.01 |
| 5.0 | Ka/L mol$^{-1}$  | 430 ± 16     | 268 ± 10   |
|     | $\Delta H^o$/(kJ mol$^{-1}$) | -9.52 ± 0.01  | -12.18 ± 0.02 |
|     | $\Delta S^o$/(kJ mol$^{-1}$) | 5.50 ± 0.01   | 1.67 ± 0.03 |
|     | $\Delta G^o$/(kJ mol$^{-1}$) | -15.02 ± 0.02 | -13.85 ± 0.01 |
|     | $\Delta K_f$/(L mol$^{-1}$) | 320 ± 25     | 752 ± 63   |

* $\Delta K_f = Ka(7.4)/Ka(5.0)$

respectively. Thereby the changed $K_f$ ($\Delta K_f$) values for $\alpha$-CD and $\beta$-CD with p-anisidine were 320 M$^{-1}$ and 752 M$^{-1}$, respectively. These results indicated that the acidity of the medium had a marked impact on the complexation process [35]. One plausible explanation was that the phenyl ring of p-anisidine exhibited stronger hydrophobic interaction with $\beta$-CD than with $\alpha$-CD when under a neutral environment, since the $\beta$-CD cavity was bigger to allow the aromatic ring to exert deeply [36]. However, in acidic medium, ionized p-anisidine exhibited a much weaker binding to $\beta$-CD than that with $\alpha$-CD due to their enhanced hydrophilic character. In addition, the larger the change in $K_f$ at different pH values, the more the improvement in the pH-responsive release behavior. Therefore it could be inferred that the ability of CD to produce a better pH-responsive release was in the order $\beta$-CD > $\alpha$-CD.

Figure 9 showed the schematic illustration of $\alpha$-CD capped MSNs and $\beta$-CD capped MSNs on the cargo release. Furthermore, the driving energies for the formation of the inclusion complexes were also investigated. The formation constants obtained at different pH values were related to the strength that driven the formation of host–guest complex. The thermodynamic parameters $\Delta H^o$, $\Delta G^o$ and $\Delta S^o$ for complexes at pH 7.4 and pH 5.0 calculated from the ITC experiments are shown in table 4. It should be noted that the thermodynamic parameters were mainly influenced by the type of CD and the pH of the media. These parameters could be used to gauge the interactions between p-anisidine and CD. It was confirmed that all the associations of p-anisidine with the CD were exothermic and spontaneous with negative enthalpies and negative Gibbs energies [37, 38]. Generally, the complexation formed by CD was believed to be driven mostly by hydrophobic forces, van der Waals forces, hydrogen bonding, electrostatic interaction, release of conformational strain, exclusion of high energy water in the CD cavity and charge-transfer interactions, these main forces were usually combined in the inclusion process [38]. In general, the value of $\Delta G^o$ was reflected by enthalpy and entropy, and a large and negative value of $\Delta H^o$ for complexation of CD was mainly attributed to van der Waals forces and hydrogen bonding, while a large and positive value of $\Delta S^o$ was mainly attributed to hydrophobic forces [39]. For the p-anisidine/$\alpha$-CD system, the inclusion process was associated with a relatively large negative value of $\Delta H^o$ and positive $\Delta S^o$ value but with a very small extent, so, the inclusion process was enthalpy driven. At the same time, the thermodynamic results suggested that the inclusion complex formed at pH 7.4 with $\Delta H^o$ of $-16.32$ kJ mol$^{-1}$ was more stable than that the complex formed at pH 5.0 ($\Delta H^o = -9.52$ kJ mol$^{-1}$). On the other hand, large negative enthalpy changes were mainly attributed to van der Waals forces and hydrogen bonding, therefore, the main driving force for the complex formed by p-anisidine with $\alpha$-CD either at pH 7.0 or pH 5.0 was van der Waals forces and hydrogen bonding. While for the p-anisidine/$\beta$-CD system, the opposite sign of large positive $\Delta S^o$ was observed which might be related to a rather different complexation process [40]. At pH 7.0, the positive entropic contribution to the $\Delta G^o$ was notably larger than the enthalpic one. A slightly $\Delta H^o$ and large positive $\Delta S^o$ indicating the process was entropy driven,
and the main driving force was hydrophobic. The positive $\Delta S^\circ$ value was due to the breaking of highly ordered aqueous microenvironments surrounding the hydrophobic part of the p-anisidine upon binding the $\beta$-CD, and the p-anisidine fitted well in the hydrophobic CD cavity [41]. But for the case of pH 5.0, $\Delta S^\circ$ and $\Delta G^\circ$ decreased as $K_f$ decreased, which can be attributed to weak interaction of the protonated p-anisidine with the hydrophobic cavity of the CD. It was clear, however, that the changed $K_f$ values for $\beta$-CD was larger than that for $\alpha$-CD with p-anisidine, which indicated that the interaction existing in this system was probably influenced less by van der Waals forces or hydrogen bonding and more by hydrophobic ones, and the system of p-anisidine/$\beta$-CD exhibited better pH-responsive property than the p-anisidine/$\alpha$-CD one which was in a good agreement with the release experiment. Moreover, a larger formation constant was found at pH 7.4 than at pH 5.0, this observation was consistent with the other rules seen in the literature [42], where a charged guest exhibited lower formation constants for CD than its corresponding unionized state. The identification of this phenomenon at atomic level will be further discussed in the molecular modeling studies.

3.5. Molecular modeling

Inclusion complexes between p-anisidine and the two types CD were prepared and discussed by experimental studies. We found that molecular modeling was an important tool to clarify mechanism of binding and to confirm binding forces between p-anisidine and the two types CD, the structure of neutral and ionized p-anisidine stalk with $\alpha$-CD and $\beta$-CD was further investigated by molecular docking. The representative conformation for this binding mode is presented in figure 10. Once again, the results well agreed with the experimental data. For p-anisidine stalk/$\alpha$-CD, it showed that the main driving force for the complex formation constituted a hydrogen bond interaction between NH group of the p-anisidine stalk and the oxygen atom from $\alpha$-CD no matter the stalk was ionized or not. The interaction energies for the complexes formed by unionized and ionized p-anisidine stalk with $\alpha$-CD corresponded respectively to $-3.33$ and $-4.05$ kJ mol$^{-1}$ which suggested that the unionized stalk was more stable than that of the ionized one. As the case for p-anisidine stalk/$\beta$-CD in neutral pH, marked fit phenomena took place when the p-anisidine part on the stalk was totally included in the hydrophobic cavity of $\beta$-CD with a strong hydrophobic interaction, which were in agreement with the experimental observations obtained by ITC. When the binding mode of the ionized form of stalk was analyzed, a weak hydrogen bond occurred, which exhibited a lower interaction energies than the neutral form ($-4.15$ and $-2.25$ kJ mol$^{-1}$, respectively). As predicted in the experimental section, hydrophobic interaction was the main driving force for complexation formed by p-anisidine stalk and $\beta$-CD. From the results above, we can hypothesize that the affinity of p-anisidine stalk diminished as the ionization of the guest increased as a consequence of the higher energy required to desolvate the ionized p-anisidine stalk [42]. To sum up, the ionization state of the stalk caused its affinity for $\alpha$-CD and $\beta$-CD to be lowered, which exhibited excellent pH-responsive property.

3.6. Drug release

In order to prove that the CD-capped nanoparticles can be applied for potential drug delivery system, the antitumor drug DOX was used to investigate the pH-responsive property of the CD-capped nanovalve system. The DOX loading efficiency was 4.5% which was determined on an UV–vis spectroscopy. The cumulative release curves of DOX from $\beta$-CD-capped MSN are shown in figure 11. Compared to the release behavior of Hoechst 33342 loaded particles, the release of DOX from MSN was slower in pH 5.0 releasing media. This observation raised the possibility that the cationic property of DOX (pKa = 8.2) made it to interact electrostatically with the Si−O$^-$ (negative charge) groups in the silica nanopores and thus this relatively strong interaction led to the slow release of DOX upon the opening of nanovalve [43]. The obvious release of DOX was observed at pH 5.0 in comparison to the case at pH 7.4 within 6 h, the accumulated release percentage of the capped silica at pH 5.0 was less than 60%, while the case at pH 7.4 showed no release within 6 h. These results indicated that the CD-capped MSNs exhibited pH-responsive release behavior and had promising applications in drug delivery system.

4. Conclusions

In this study, we successfully constructed a pH-responsive CD capped MSN controlled release system by a relatively simple method. The organic component of the stalk was confirmed by a variety of experimental characterizations, and the system exhibited good pH-responsive controlled release. Different release percentages were investigated when the stalk density was changed or the type of CD was changed. Detailed examinations of the pH-responsive behavior of the CD capped MSNs with differing stalk densities were carried out. The
results obtained indicated that either too low or too high a stalk density would adversely affect the amount of released cargo, and only an optimum stalk density could produce an ideal pH-responsive behavior and, therefore, an optimum stalk density was selected. The application of TGA gave an optimum stalk density of ∼8.7 stalks nm⁻². Moreover, different release percentages were observed resulting from the different degrees of changed formation constants of the complexes formed by CD with p-anisidine molecules at different pH values and we found that the β-CD capped MSNs exhibited a good responsiveness to changes in external pH. In addition, the thermodynamic parameters calculated from the ITC experiment showed that the complexation process of p-anisidine/α-CD were predominantly enthalpy-driven with the main driving force of van der Waals forces and hydrogen bonding under neutral or acidic media, while for p-anisidine/β-CD, the complexion process was entropy driven with strong hydrophobic interaction under neutral pH and weak hydrogen bonding under acidic media. The influence of pH on the affinity of the p-anisidine stalk with CD was carefully analyzed and discussed at molecular level by applying molecular modeling study, which was in good agreement with ITC results. In short, both the stalk density and the type of CD played a central role in the pH-responsive controlled release behavior. All the above results were encouraging and provided important information about the design of functional mesoporous silica to be applied in a variety of applications, especially drug delivery.

Acknowledgments

This work was supported by National Basic Research Program of China (973 Program) (NO.2015CB932100), National Natural Science Foundation of China (NO.81473165), Liaoning Provincial Key Laboratory of Drug Preparation Design & Evaluation of Liaoning Provincial Education Department (LZ2014045).

References

[1] Slowing I I, Vivero-Escoto J L, Wu C-W and Lin V S-Y 2008 Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers Adv. Drug Deliv. Rev. 60 1278–88
[2] Vallet-Regí M, Colilla M and González B 2011 Medical applications of organic–inorganic hybrid materials within the field of silica-based bioceramics Chem. Soc. Rev. 40 596–607
[3] Tang F, Li L and Chen D 2012 Mesoporous silica nanoparticles: synthesis, biocompatibility and drug delivery Adv. Mater. 24 1504–34
[4] Zhao Y, Vivero-Escoto J L, Slowing I I, Trewn B G and Lin V S 2010 Capped mesoporous silica nanoparticles as stimuli-responsive controlled release systems for intracellular drug/gene delivery Expert Opin. Drug Deliv. 7 1013–29
[5] Chen X, Cheng X, Soeriyadi A H, Sagnella S M, Lu X, Scott J A, Lowe S B, Kavallaris M and Gooding J J 2014 Stimuli-responsive functionalized mesoporous silica nanoparticles for drug release in response to various biological stimuli Biomater. Sci. 2 121–30
[6] Zhou L, Li Z, Liu Z, Ren J and Qu X 2013 Luminescent carbon dot-gated nanovehicles for pH-triggered intracellular controlled release and imaging Langmuir 29 6396–403
[7] Liu R, Zhang Y, Zhao X, Agarwal A, Mueller L J and Feng P 2010 pH-responsive nanogated ensemble based on gold-capped mesoporous silica through an acid-labile acetal linker J. Am. Chem. Soc. 132 1500–1
[8] Yuan Q, Zhang Y, Chen T, Lu D, Zhao Z, Zhang X, Li Z, Yan C-H and Tan W 2012 Photon-manipulated drug release from a mesoporous nanocarrier controlled by azobenzene-modified nucleic acid ACS Nano 6 6337–44

[9] He D, He X, Wang K, Cao J and Zhao Y 2012 A light-responsive reversible molecule-gated system using thymine-modified mesoporous silica nanoparticles Langmuir 28 4003–8

[10] Gan Q, Lu X, Yuan Y, Qian J, Zhou H, Lu X, Shi J and Liu C 2011 A magnetic, reversible pH-responsive nanogated ensemble based on Fe3O4 nanoparticles-capped mesoporous silica Biomaterials 32 1932–42

[11] Luo Z, Cai K, Hu Y, Zhao L, Liu P, Duan L and Yang W 2011 Mesoporous silica nanoparticles end-capped with collagen: redox-responsive nanoreservoirs for targeted drug delivery Angew. Chem., Int. Ed. Engl. 50 640–3

[12] Luo Z, Cai K, Hu Y, Li J, Ding X, Zhang B, Xu D, Yang W and Liu P 2012 Redox-responsive mesoporous nanoreservoirs for controlled intracellular anticancer drug delivery based on magnetic nanoparticles Adv. Mater. 24 4293–5

[13] Xue M, Zhong X, Shaposhnik Z, Qu Y, Tamanofi F, Duan X and Zink J I 2011 pH-operated mechanized porous silicon nanoparticles J. Am. Chem. Soc. 133 8798–801

[14] Azzar E, Coli C, Marcos M D, Martínez–Máñez R, Sancenón F, Soto J, Amorós P, Cano J and Ruiz E 2009 Borate-driven galitek scaffolding using mesoporous materials functionalised with saccharides Chem. Eur. J. 15 6877–88

[15] Park C, Oh K, Lee S C and Kim C 2007 Controlled release of guest molecules from mesoporous silica particles based on a pH-responsive polyisoprostaxane motif Angew. Chem., Int. Ed. Engl. 46 1455–7

[16] Xue M and Findenegg G H 2012 Lysozyme as a pH-responsive valve for the controlled release of guest molecules from mesoporous silica Langmuir 28 17578–84

[17] Guo W, Wang J, Lee S J, Dong F, Park S S and Ha C S 2010 A general pH-responsive supramolecular nanovalue based on mesoporous organosilica hollow nanospheres Chem. Eur. J. 16 8641–6

[18] Li W and Zhao D 2013 An overview of the synthesis of ordered mesoporous materials Chem. Commun. 49 943–6

[19] Guo Z, Liu X-M, Ma L, Li J, Zhang H, Gao Y-P and Yuan Y 2013 Effects of particle morphology, pore size and surface coating of mesoporous silica on Naproxen dissolution rate enhancement Adsorpt. Surf. B 91 405–12

[20] Li Z, Barnes J C, Bosoy A, Stoddart J F and Zink J I 2012 Mesoporous silica nanoparticles in biomedical applications Chem. Soc. Rev. 41 2590–605

[21] Lu J, Liovig M, Li Z, Zink J I and Tamanoff F 2010 Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals Small 6 1794–805

[22] Nastase S, Bajenaru L, Matci C, Mitran R A and Berger D 2013 Ordered mesoporous silica and aluminosilicate-type matrix for amikacin delivery systems Microporous Mesoporous Mater. 182 32–9

[23] Zhu Y, Shi J, Shen W, Dong X, Feng J, Ruan M and Li Y 2005 Stimuli-responsive controlled drug release from a hollow mesoporous silica sphere/polyelectrolyte multilayer core–shell structure Angew. Chem. 117 5213–7

[24] Du L, Liao S, Qhatib H A, Stoddart J F and Zink J I 2009 Controlled-access hollow mechanized silica nanocounters J. Am. Chem. Soc. 131 15136–42

[25] Zhou Y, Tan L L, Li Q L, Qiu X L, Qi A D, Tao Y and Yang Y W Acetylcholine-triggered cargo release from supramolecular nanovales based on different macrocyclic receptors Chem. Eur. J. 20 2998–3004

[26] Rivera-Jiménez S M and Hernández-Maldonado A J 2008 Nickel (II) grafted MCM-41: a novel sorbent for the removal of naproxen from water Microporous Mesoporous Mater. 116 246–52

[27] Shyylesh S, Sharma S, Mirajkar S and Singh A 2004 Silica functionalised sulphonic acid groups: synthesis, characterization and catalytic activity in acetalization and acetolysis reactions J. Mol. Catalysis A 212 219–28

[28] Xue M and Zink J I 2014 Probing the microenvironment in the confined pores of mesoporous silica nanoparticles J. Phys. Chem. Lett. 5 839–42

[29] Ariga K, Ji Q, Hill J P, Kawazoe N and Chen G 2009 Supramolecular approaches to biological therapy Expert Opin. Biol. Therapy 9 307–20

[30] Devynck F, Giustinio F, Broqvist P and Pasqualotto A 2007 Structural and electronic properties of an abrupt H-SiC (0001)/SiO2 interface model: classical molecular dynamics simulations and density functional calculations Phys. Rev. B 76 075351

[31] Loftsson T and Duchene D 2007 Cyclodextrins and their pharmaceutical applications Int. J. Pharmaceutics 329 1–11

[32] Park C, Kim H, Kim S and Kim C 2009 Enzyme responsive nanocarriers with cyclodextrin gatekeepers and synergistic effects in release of guests J. Am. Chem. Soc. 131 16614–5

[33] Ambrogio M W, Thomas C R, Zhao Y-L, Zink J I and Stoddart J F 2011 Mechanized silica nanoparticles: a new frontier in theranostic nanomedicine Acc. Chem. Res. 44 903–13

[34] Kim H, Kim S, Park C, Lee H, Park H J and Kim C 2010 Glutathione-induced intracellular release of guests from mesoporous silica nanocounters with cyclodextrin gatekeepers Adv. Mater. 22 4280–3

[35] Zheng Y, Dong L-N, Liu M, Chen A, Feng S, Wang B and Sun D 2013 Effect of pH on the complexation of kaempferol-4′-glucoside with three β-cyclodextrin derivatives: isothermal titration calorimetry and spectroscopy study J. Agric. Food Chem. 62 244–50

[36] Liu L and Guo Q-X 1999 Novel prediction for the driving force and guest orientation in the complexation of α- and β-cyclodextrin with benzene derivatives J. Phys. Chem. B 103 3461–7

[37] Venkatesh G, Sivasankar T, Karthick M and Rajendiran N 2013 Inclusion complexes of sulphanilamide drugs and β-cyclodextrin: a theoretical approach J. Inclusion Phenom. Macrocyclic Chem. 77 309–18

[38] Fielding L, McKellar S C and Florence A J 2011 Precision studies in supramolecular chemistry: a 1H NMR study of hydroxymethylacetophenone/β-cyclodextrin complexes Magn. Reson. Chem. 49 117–25

[39] Ikeda T, Hirota E, Ooya T and Yui N 2001 Thermal analysis of inclusion complexation between α-cyclodextrin-based molecular tube and sodium alkyl sulfonate Langmuir 17 234–8

[40] Chakraborty S, Basu A, Lahiri A and Basak S 2010 Inclusion of chrysirin in β-cyclodextrin nanocavity and its effect on antioxidant potential of chrysirin: a spectroscopic and molecular modeling approach J. Mol. Struct. 977 180–8

[41] Sancho M I, Gasull E, Blanco S E and Castro E A 2011 Inclusion complex of 2-chlorobenzophenone with β-cyclodextrin inclusion complex. A spectroscopic study J. Mol. Struct. 977 180–8

[42] Sancho M I, Gasull E, Blanco S E and Castro E A 2011 Inclusion complex of 2-chlorobenzophenone with β-cyclodextrin J. Mol. Struct. 977 219–26

[43] Ikeda T, Hirota E, Ooya T and Yui N 2001 Thermodynamic analysis of inclusion complexation between α-cyclodextrin-based molecular tube and sodium alkyl sulfonate Langmuir 17 234–8

[44] Chakraborty S, Basu A, Lahiri A and Basak S 2010 Inclusion of chrysirin in β-cyclodextrin nanocavity and its effect on antioxidant potential of chrysirin: a spectroscopic and molecular modeling approach J. Mol. Struct. 977 180–8

[45] Sancho M I, Gasull E, Blanco S E and Castro E A 2011 Inclusion complex of 2-chlorobenzophenone with cyclomaltoheptaose (β-cyclodextrin): temperature, solvent effects and molecular modeling Carbohydrate Res. 346 1978–84

[46] Oohara Y, Schenfeld E M, Quevedo M A, Fernández M A, Longhi M B and Granero G E 2012 Characterization of the hydrochlorothiazide-β-cyclodextrin inclusion complex. Experimental and theoretical methods J. Phys. Chem. B 117 206–17

[47] Meng H, Xue M, Xia T, Zhao Y-L, Tamanoff F, Stoddart J F, Zink J I and Nel A E 2010 Autonomous in vitro anticancer drug release from mesoporous silica nanoparticles by pH-sensitive nanovales J. Am. Chem. Soc. 132 12690–7