Synthesis of discrete and dispersible hollow mesoporous silica nanoparticles with tailored shell thickness for controlled drug release†

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By employing poly (tert-butylacrylate) (PTBA) nanospheres as the dissolvable core templates, we develop a new method to synthesize hollow mesoporous silica nanoparticles (HMSN). Both the PTBA core and the structure-directing surfactant, cetyltrimethylammonium bromide (CTAB), can be easily and synchronously removed through solvent extraction in ethanol, which ensures the complete structure and ideal dispersibility of the products compared with the previous template synthesis that often needs calcination to remove the templates. Given that hollow core diameter and shell thickness are the key properties of HMSN, the hollow core diameter and shell thickness can be tailored precisely. In addition, as novel inorganic nanomaterials with a tuned structure, HMSN show notable biocompatibility and efficient doxorubicin (DOX) loading. In in vitro tests, the release rate of DOX-loaded HMSN exhibit a surprising shell-thickness-dependent and a pH responsive drug release character, suggesting that HMSN are a very promising drug delivery system for shell-thickness-controlled drug release. The results of intracellular tracking and cytotoxicity assays further demonstrate the potential and efficiency of HMSN as a drug delivery system.

Introduction

Mesoporous silica nanoparticles (MSNs) have attracted a lot of interest during recent years due to their combination of exceptional porosity with versatile decoration and high biocompatibility. They hold great promise for a diversity of applications, and have emerged as vehicles or reservoirs in a wide range of fields such as drug delivery,1–5 adsorption and heterogeneous catalysis.6–9 Efforts are ongoing to optimize their porous parameters, such as enhancement of pore volume or pore size,10,11 with the aim of significantly improving the storage capacity of cargoes, or facilitating the host-guest specific interaction for the intended purpose. From a technical point of view, introduction of a hollow cage into the core area of the MSNs is more feasible, and conducive to flexibly tuning the ratio of surface area to pore volume by modulating the hollow cavity size and mesoporous shell thickness. This advanced structure is of critical importance for mesoporous silica-based drug delivery systems, because more drug molecules can penetrate into a void core, rather than nonspecifically adsorb on pore walls resulting in a low accumulative release amount.12 In this respect, the hollow mesoporous silica nanoparticles (HMSN) are somewhat superior to their corresponding MSNs or other similar MSN composite microspheres. Additionally, they also possess other characteristics due to their hollow structure, for example, an increased active area for catalysis, the modulation of refractive index and a decreased particle density.13

One of the typical methods to make the hollow cavity in MSNs involves a template-eliminated process,14–16 where a hard sphere is used as a template to develop the silica shell with embedded surfactant molecules, and, in turn, they undergo calcination for removal of core template and surfactant, resulting in open mesopores and a hollow cavity. However, much attention in past reports has been paid to the creation of the intrinsic space, and little discussion has appeared to address the issue of particle dispersibility in solvents. As a matter of fact, an inevitable tendency of silica nanostructures, during high-temperature annealing, is to dehydrate and condense.17 Irreversible particle aggregation occurs, and probably leads to the formation of nondispensible and conglomerated materials. To obtain dispersible HMSN a second route has been reported making use of a soft template that is easily extracted under mild conditions. A variety of templates such as vesicles,18,19 gas bubbles,20,21 block copolymer aggregates,22–24 and oil-in-water emulsions,25,26 have been thoroughly explored to date, whereas they are often obsessed with uncontrollable sizes, and thus afford the resulting HMSN with a broad size distribution as well as a defective mesoporous structure.27,28 Therefore, a

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reliable and facile methodology for synthesis of discrete and dispersible HMSN with a tailor-made core–shell structure remains unexplored. These features are also superbly useful for many practical applications, especially as drug delivery systems (DDSs), because HMSN with improved dispersibility are amenable to versatile surface or inner modification by functional units.

DDSs with sustained drug release have more potential in clinical cancer therapy. Compared with organic carriers, inorganic materials possess high levels of drug encapsulation, superior chemical stability and excellent biocompatibility, all of which are well in line with the basic demands of DDSs.\textsuperscript{29,30} Among the known inorganic materials, MSNs are of greater appeal to us, due to their distinctive advantages including large surface area, high pore volume, chemically accessible surfaces and high biocompatibility.\textsuperscript{31–33} These features make MSNs very suitable as vehicles in DDSs. In our previous studies, we investigated the design of a sustainable, multiple stimuli-responsive controllable release system by applying modified MSNs with specific organic functional groups or polymers.\textsuperscript{34–37} However, we are aware that, since the physiological environment is so complicated, the organic-modified MSNs usually do not work ideally in vivo because of the presence of unexpected interactions with the organic moieties. Therefore, we speculate that the structure and components of MSNs should be as simple as possible so that their designed release behaviors remain as effective as expected. HMSN are such a kind of simple model, and show great potential in biomedicine due to their large surface area, high pore volume, high biocompatibility, exceptional guest-loading capacity, and tunable core and shell sizes.\textsuperscript{38,39} It is thus most likely that a rate-controlled and sustainable release behavior is obtainable by tuning the structural parameters of HMSN, rather than through chemical modification. This will lead to a more pragmatic delivery system. Along this line, Shi et al. have reported that HMSN with enlarged mesopores promise great potential in loading multiplex drugs for synchronic treatment.\textsuperscript{40} In this context, we have been wrapped up in the exploration of a reliable methodology for the structural regulation of HMSN, with a view to a better understanding of the structure–property relationship in DDSs. Also, it has rarely been studied in the MSNs-related reports thus far.

In this work, we report a facile and controllable route to synthesize uniform and structure-tailored HMSN using poly-(\textit{t}-butylacrylate) (PTBA) nanospheres as the core template and cetyltrimethylammonium bromide (CTAB) as the structure-directing surfactant. Both the PTBA core and CTAB can be simultaneously removed through solvent extraction in ethanol. The resulting HMSN possess a definite hollow core and mesoporous shell, and show a pronounced stability in water. The discrete and dispersible HMSN were employed as a novel drug vehicle to deliver a typical anticancer agent, doxorubicin (DOX). They displayed controllable drug release rates when the mesoporous shell thickness was varied. To the best of our knowledge, this is the first time the successful control of the DOX release rate through variation of shell thickness has been reported. Intracellular tracking and \textit{in vitro} cytotoxicity assays were used to evaluate the role of HMSN as drug carriers.

**Experimental**

**Materials**

\textit{t}-Butyl acrylate (\textit{t}-BA) and fluorescein isothiocyanate (FITC) were purchased from Aladdin (Shanghai, China), and \textit{t}-BA was purified through reduced pressure distillation. Phosphate-buffered saline (PBS) were obtained from Shanghai Qiangshun Chemical Reagent Company and (3-aminopropyl)triethoxysilane (APS) was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Tetraethoxysilane (TEOS), cetyltrimethylammonium bromide (CTAB), aqueous ammonia solution (25 wt%), ammonium nitrate (NH\textsubscript{4}NO\textsubscript{3}) and potassium persulfate (KPS) were obtained from Shanghai Chemical Reagents Company (Shanghai, China), and KPS was recrystallized from water. Doxorubicin in the form of its hydrochloride salt (DOX) was purchased from Beijing Huafeng United Technology Company (Beijing, China). C6 glioma cells were obtained from Chinese Academy of Sciences Cells Bank (Shanghai, China). Lyso-tracker Red was purchased from Molecular Probes (Eugene, OR, USA) and 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) was purchased from Beyotime (Shanghai, China). Dulbecco’s modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution and Trypsin–EDTA solution were obtained from Gibco (Tulsa, OK, USA).

**Characterization**

UV-vis spectra were obtained using a Perkin-Elmer Lambda 35 spectrophotometer. Nanoparticles were visualized using a JEOL 1230 transmission electron microscope (TEM). The size distribution of the nanoparticles was measured by dynamic light scattering (DLS) using an autosizer 4700 (Malvern). The zeta potential was measured using a Zetasizer Nano-ZS (Malvern). Fourier transform infrared spectroscopy (FTIR) measurements were performed with a NEXUS 470. Thermogravimetric analysis (TGA) was conducted on a Pyrisis 1 TGA thermal analyzer from 100 to 800 °C (10 °C min\textsuperscript{-1}) in a flow of nitrogen. Nitrogen adsorption–desorption isotherms were obtained on a Micromeritics Tristar 3000 pore analyzer at 77 K under continuous adsorption conditions. Intracellular tracking were subjected to observe with FV1000 confocal laser scanning microscopy (Olympus, Japan).

**Preparation of PTBA nanospheres**

PTBA nanospheres were prepared by conventional emulsion polymerization. 1 g of CTAB was dissolved in 89 mL of deionized water in a glass bottle and 2 mL of \textit{t}-butyl acrylate (\textit{t}-BA) was added to the solution. The mixture was stirred vigorously for 30 min under a dynamic nitrogen atmosphere at 60 °C. Then, 5 mL of KPS aqueous solution (0.08 M) was added in the mixture before 10 mL of \textit{t}-BA was dripped off in 2 h. The reaction was carried out for another 3 h and PTBA with a diameter of 84 nm (PTBA-84) was prepared. The molar ratio between \textit{t}-BA, KPS and CTAB was changed to 400 : 2 : 27, 1380 : 3.5 : 13.7 and 2410 : 3 : 13.7 respectively, then PTBA-36, PTBA-120 and PTBA-200 were obtained. All the samples were dialyzed against...
deionized water for 6 days using a Spectra/Por membrane ($M_w$ cutoff = 14 000 Da) before their solid contents were measured.

**Synthesis of HMSN**

0.51 g of PTBA-84 (11.8 wt% solid content) was dispersed uniformly in a mixed solution of ethanol (20 mL) and deionized water (50 mL). Then 10 mL of deionized water containing 0.3 g of CTAB was added. The mixture was stirred quickly for 30 min at 40 °C. Finally, 0.85 mL of aqueous ammonia solution (25 wt%), 1.16 mL of tetraethoxyxilane (TEOS) were successively added to the above mixture. The reaction was carried out for 24 h. After washing twice with water and ethanol, the products were refluxed in the ethanol solution of NH$_2$NO$_3$ (10 mg mL$^{-1}$) at 80 °C for 30 min. The synthesized product was centrifuged, washed with distilled water and ethanol several times. Then the samples were dispersed in deionized water before use. The other HMSN with different hollow core diameters and shell thickness were prepared using the same process, and detailed information on the amount of reactants are shown in Table S1 (in the ESI†).

**DOX loading and release**

DOX was dissolved in deionized water with a concentration of 1 mg mL$^{-1}$. 5 mg of HMSN was ultrasonically dispersed in 1.5 mL of the DOX solution. The mixture was stirred at room temperature for 24 h. Then the dispersion was centrifuged to collect the DOX-loaded HMSN and the products were washed with deionized water twice to remove the DOX absorbed on the surface. The mass of DOX loaded in the HMSN was analyzed by UV-vis at a wavelength of 480 nm. The drug loading content and entrapment efficiency were calculated using the eqn (1) and (2), respectively:

$$\text{Loading content (wt%)} = \frac{\text{Mass of drugs in the HMSN}}{\text{Mass of the drug loaded HMSN}}$$

(1)

$$\text{Entrapment efficiency (wt%)} = \frac{\text{Mass of drugs in the HMSN}}{\text{Initial mass of drugs}}$$

(2)

A series of DOX-loaded HMSN were respectively dispersed in 2 mL of phosphate buffer saline (PBS) solutions of different pH (5.5 and 7.4) and the samples were transferred into dialysis bags (molecular weight cut off 5000). Then, the dialysis bags were kept in a 250 mL lucifugal sink with 200 mL of PBS (pH 5.5 or 7.4) and gently stirred at 37 °C. At timed intervals, 2 mL of the solution was extracted periodically and then 2 mL of fresh PBS was added to keep the volume constant. The amount of released drug was analyzed by UV-vis and all released results were averaged over three measurements.

**Intracellular tracking of HMSN**

Fluorescein isothiocyanate (FITC) labeled HMSN (FITC-HMSN) were synthesized first. 10 mg of FITC was dissolved in 5.45 mL of ethanol, then 22 µL of 3-aminopropyltriethoxysilane (APS) was added to the solution. The mixture was stirred with a magnetic stirrer for 12 h at 25 °C. Then, 0.51 g of PTBA-84 (11.8 wt% solid content) was dispersed uniformly in a mixed solution of ethanol (20 mL) and deionized water (50 mL). Then 10 mL of deionized water containing 0.3 g of CTAB was added. The mixture was stirred quickly for 30 min at 40 °C. Finally, 0.85 mL of aqueous ammonia solution (25 wt%), 1.16 mL of TEOs and 100 µL of the above FITC mixture were successively added to the solution. The reaction was carried out for 24 h. After being washed with deionized water and ethanol several times, the products were washed in an ethanol solution of NH$_2$NO$_3$ (10 mg mL$^{-1}$) at 80 °C for 30 min, then FITC-HMSN were obtained through centrifugation. The samples were dispersed in deionized water and diluted to 1 mg mL$^{-1}$ before use.

C6 cells were plated on glass coverslips that were placed in 6-well plates. After 24 h, cells were preincubated in DMEM for 30 min, and then the cells were treated with FITC-HMSN and DOX loaded FITC-HMSN in DMEM for 2 h. Then the cells were washed with PBS (pH 7.4) three times, and fixed with 4% paraformaldehyde. The nuclei were stained with DAPI and the lysosomes of the cells treated with FITC-HMSN were stained with Lyso-tracker Red according to the operation manuals. Cells on the coverslips were washed with PBS (pH 7.4) and mounted in Aqua-Mount Slide Mounting Media (Thermo Fisher Scientific, MI, USA). The samples were subjected to observations with FV1000 confocal laser scanning microscopy (Olympus, Japan).

**In vitro cytotoxicity assays**

C6 cells were maintained in 10 cm tissue culture dishes in Dulbecco’s Modified Eagle Medium supplemented with 10% FBS (fetal bovine serum), penicillin (100 U mL$^{-1}$) and streptomycin (100 mg mL$^{-1}$) in a humidified atmosphere with 5% CO$_2$ at 37 °C.

For the MTT assays, C6 cells were seeded in 96-well plates with a density of 10 000 cells per well and allowed to adhere for 24 h prior to the assay, they were then treated with HMSN or DOX@HMSN in culture medium for 24 h. After each treatment, the cells were incubated with 0.5 mg mL$^{-1}$ MTT in DMEM for 4 h in dark and then mixed with dimethyl sulfoxide after the supernatant was removed. The OD value at 570 nm was read using the microplate reader (Synergy TM2, BIO-TEK Instruments Inc. USA). Cell viability was determined by the percentage of OD value of the study group over the control group.

**Result and discussion**

**Preparation and characterization of HMSN**

As is evident from the data compiled in Table S2 (in the ESI†), poly(tert-butylacrylate) (PTBA) nanospheres synthesized by conventional emulsion polymerization present controllable particle sizes from 36 to 200 nm, and relatively low polydispersity indexes as measured by Dynamic Light Scattering (DLS) in aqueous solution. The flexible regulation of the core sizes of HMSN is likely when using PTBA nanospheres of different sizes as the template. As shown in Scheme 1, a sol–gel nanocoating process was undertaken to achieve silica encapsulation with CTAB as the co-template.$^\text{4}$ The obtained composite particles were refluxed in an ethanol solution of NH$_2$NO$_3$ (10 mg mL$^{-1}$) to remove the PTBA in core and embedded CTAB in shell at the same time. The TEM image in Fig. 1a definitely displays the core–shell structure of the resulting particles. It can be seen that
The pore size is predominately centered at 2.6 nm in the mesoporous silica shell calculated from the adsorption branch by the Barrett–Joyner–Halenda (BJH) method (the inset in Fig. 1c). The Brunauer–Emmett–Teller (BET) surface area is 973 m$^2$ g$^{-1}$ and the pore volume is 0.95 cm$^3$ g$^{-1}$. In addition, the aqueous stability of these nanoparticles was thoroughly investigated. The zeta potential value of HMSN-C70-S40 is −27 mV at pH 7.4 resulting from the abundant Si–OH groups on the surface.$^{45}$ As shown in Fig. 1d (left), the particle dispersion in water is rather stable, and there is no obvious precipitation tendency over 5 days. Even a week later, no sediment was observed. In contrast, when HMSN were subject to calcination at 600 °C for 12 h to remove the template, their zeta potential value is only −11 mV since some of the Si–OH groups condense with one another at such high temperatures. The low surface charges of these HMSN lead to the decrease in their stability in aqueous solution,$^{46}$ as evidenced by the visible sediment at the bottom of the sample vial (Fig. 1d, right). As such, we highlight that the solvent-treating method of cleaning templates plays a vital role in preparation of discrete, highly uniform and very stable HMSN.

To further determine whether the PTBA template was removed entirely, FTIR analysis was conducted on the different samples before and after the template-cleaning step. As a control, the characteristic peaks of PTBA, appearing at 1731 and 1147 cm$^{-1}$, are assigned to the typical vibrations of C==O and C–O, respectively (Fig. 2a). As for the HMSN without the template removed, a series of absorption peaks, such as the C–H stretching vibrations at 2921 and 2850 cm$^{-1}$, the C==O stretching vibration of PTBA at 1731 cm$^{-1}$, the C–H bending vibration at 1486 cm$^{-1}$ and the Si–O stretching vibration at 1055 cm$^{-1}$, are observed, indicating the formation of the core–shell structure (Fig. 2b). After the template removal process HMSN-C70-S40 gives a much simpler FTIR spectrum as shown in Fig. 2c. The main peak appears at 1055 cm$^{-1}$ due to the Si–O stretching vibration, which implies that both CTAB and PTBA are extracted completely. Furthermore, the ethanol solution collected during the extraction process affords almost the same peaks as PTBA (Fig. 2d). It proves again that the PTBA core is dissolved in ethanol, and that these polymer chains then diffuse into the medium through the mesoporous shells. Also, as shown in

![Fig. 1](image1.png)

**Fig. 1** (a) TEM image of HMSN-C70-S40; (b) DLS curve of HMSN-C70-S40 in aqueous solution, PDI = polydispersity index; (c) adsorption–desorption isotherm of HMSN-C70-S40 and the inset is pores size distribution obtained by BJH method; (d) photographs of the dispersed solution of HMSN whose templates are removed with and without calcination.

they have a narrow size distribution, and are composed of a hollow cage with a diameter of 70 ± 5 nm and a uniform shell with a thickness of 40 ± 5 nm (HMSN-C70-S40). Furthermore, the polydispersity index of these HMSN is approximately 0.05 (Fig. 1b), suggesting a high size uniformity in these products.$^{42}$ It is found that the hollow core diameters are slightly smaller than the size of the PTBA template that was measured by DLS (84 nm). This is presumably due to the fact that a hydrate layer exists on the PTBA templates, thereby causing a larger hydrodynamic diameter than in its dried form. The N$_2$ adsorption–desorption results, as shown in Fig. 1c, give a typical type-IV curve, indicative of a mesoporous character for HMSN-C70-S40.$^{43}$ The adsorption curve shows a remarkable capillary condensation in the range of $P/P_0 = 0.25–0.40$, ascribed to the existence of mesopores in the shell, and another condensation step at $P/P_0 > 0.98$. The type I4 loop with parallel branches at relative pressures between 0.44 and 1.0 is attributed to the hollow structure.$^{44}

![Fig. 2](image2.png)

**Fig. 2** FTIR spectra of (a) PTBA nanospheres, (b) HMSN without the template removed, (c) the hollow mesoporous silica nanoparticles (HMSN-C70-S40), and (d) the ethanol solution collected during the extraction process.
Fig. 3, thermogravimetric analysis (TGA) was used to evaluate the organic residue in the resulting HMSN. The HMSN without the template removed give a weight loss of up to 43.2% as a result of the presence of the PTBA and CTAB templates. Upon treatment with the ethanol solution of NH₄NO₃ for 30 min, the obtained particles show a weight loss of 13.9%, and when the processing time was prolonged by 6 h, their weight loss is just 13.3% which is almost invariant when compared with the former. The additional weight loss is attributed to the dehydroxylation of Si–OH groups. As a result, it is believed that the template removal process, wherein the PTBA cores are dissolved in the solution accompanied by the CTAB removal through ion exchange, can be very quickly completed within 30 min.

To tune the hollow core diameter and shell thickness of the HMSN, the PTBA nanospheres with sizes of 36, 120, and 200 nm were used as core templates to prepare the well-structured HMSN. Also, the possibility of keeping the shell thickness constant was afforded by regulating the feed amount of TEOS according to the diameter of the PTBA nanospheres used. As shown in Fig. 4a–c, various HMSN with hollow cores of 30 ± 5, 110 ± 10, and 180 ± 10 nm and almost similar shells of 40 nm thick are obtained, which corresponds to the samples denoted HMSN-C30-S40, HMSN-C110-S40, and HMSN-C180-S40, respectively. Meanwhile, we prepared HMSN with different shell thickness by varying the amount of TEOS and CTAB. When PTBA-84 was employed as the core template, one can see that the resulting nanospheres, as shown in Fig. 4d–f, yield a homogeneous shell with the various thicknesses of 20 ± 5, 65 ± 5, and 95 ± 5 nm, and a uniform hollow core with a diameter of 70 ± 5 nm. Herein, they are denoted HMSN-C70-S20, HMSN-C70-S65 and HMSN-C70-S95, respectively. These particles all showed good size uniformity (see Table S3 in the ESI†). Afterwards, their porosity was tested by taking advantage of the N₂ adsorption–desorption measurement. The typical mesoporous structure for all the samples, i.e. HMSN-C70-S20, HMSN-C70-S40, HMSN-C70-S65 and HMSN-C70-S95, is obtained as shown by the fact that they all afforded type-IV sorption isotherms (see Fig. S1 in the ESI†). Meanwhile, their BET surface areas, mesoporous volumes and pore diameters were estimated. As listed in Table 1, all of the relevant porous parameters are nearly the same, which suggests that the change of shell thickness has no influence on their mesoporosity.

Fig. 4  TEM images of HMSN with various hollow core sizes and shell thicknesses: (a) HMSN-C30-S40, (b) HMSN-C110-S40, (c) HMSN-C180-S40, (d) HMSN-C70-S20, (e) HMSN-C70-S65 and (f) HMSN-C70-S95.

DOX loading and release

With the potential application of HMSN in drug delivery in mind, we have embarked on an investigation of the effect of mesoporous shell thicknesses on drug-release behaviour. Typically, the HMSN-C70 microspheres with shell thicknesses of 20 ± 5, 40 ± 5, 65 ± 5, and 95 ± 5 nm were loaded with DOX (1/0.3, w/w) for 24 h at 25 °C, and ultimately afforded DOX loading contents of 18.4 wt%, 19.1 wt%, 18.2 wt% and 18.4 wt%, respectively. Also, their entrapment efficiencies were estimated to be 79.7 wt%, 82.8 wt%, 78.8 wt% and 79.7 wt%, respectively, corresponding to HMSN-C70-S20, HMSN-C70-S40, HMSN-C70-S65 and HMSN-C70-S95. The N₂ adsorption–desorption results demonstrated that, compared with that of HMSN-C70-S40, the pore size of DOX loaded HMSN-C70-S40 only slightly changed from 2.6 to 2.5 nm (Table 1), and the surface area and pore volume also only decreased slightly, indicating that the effect of the loaded drugs on the porosity was limited. Thus, the drug was mainly stored in the hollow core, and was only partly adsorbed in the shell so that the DOX loaded HMSN still had the typical mesoporous structure. Obviously, there is no big difference among these samples in DOX loading content and entrapment efficiency. The reason is that the four kinds of HMSN with the similar sized cores provide similar surface areas and pore volumes which ensure an equal capacity for drug storage.

It is well known that the micro-environment around tumor tissues and other inflamed tissues in the body tends to be more acidic (pH 5.5–6.5) relative to the normal tissues (pH 7.4). As such, the release behavior of DOX-loaded HMSN-C70 with various shell thicknesses were investigated at pH 5.5 and 7.4. As a control, the release curves of pure DOX solution in the dialysis bag at pH 5.5 and 7.4 were determined first (Fig. 5a). The results show that the release trends of pure DOX in the dialysis bag at pH 5.5 and 7.4 have no distinct differences, and most of the DOX can be released in 1 h. So the dialysis bag did not interfere in the release trends of DOX-loaded HMSN-C70 at pH 5.5 and 7.4. As shown in Fig. 5b, in the neutral medium (pH 7.4), the amount of
the loaded DOX released increases in the first 2 h for all four HMSN, while it is found that the thicker shell is conducive to the inhibition of the release of the loaded DOX. For example, HMSN-C70-S95 gives rise to the release of 11.7% of the DOX, whereas HMSN-C70-S20 attains a release of 19.7%. As the process was prolonged by 24 h, an eventual equilibrium was reached, and the different HMSN show little difference in released content. They provide similar release amounts of 21.2%, 21.9%, 23.8% and 23.2%. The result may be ascribed to the solubility of DOX dropping rapidly as the pH of the aqueous solution increases. At high pH, the poor solubility becomes the main factor in the impedance of the drug release process, resulting in the released amounts of DOX being lower and level. At pH 5.5, the HMSN-C70 with various shell thicknesses accounts for the difference in amount of the loaded DOX released, it reaches 50.8% for HMSN-C70-S95, 58.8% for HMSN-C70-S65, 72.8% for HMSN-C70-S40 and 78.4% for HMSN-C70-S20 in 24 h. This implies that the release rate and amount of DOX is predominately influenced by the thickness of the shell. When the thickness decreases from 95 to 20 nm, it becomes much easier for the loaded DOX to diffuse out through the porous shell. It is believed that varying the thickness of the porous shell will become a more facile and practical method to effectively control the drug release amount and rate. This result demonstrates the mechanism of the drug release in the channels to be diffusion controlled, as the diffusion time of drug molecules in the shell is strongly linked to the diffusion distance. These results are also fitted to the Higuchi model in order to understand whether the diffusion was Fickian diffusion. The linear profiles of the release percentage versus the square root of time (see Fig. S2 in the ESI†) suggest that DOX release from the HMSN carriers is consistent with a Fickian diffusion mechanism. Also, we note that when the shell thickness is less than 40 nm, the total amounts released are almost the same for the two thin-shell samples (HMSN-C70-S40 and HMSN-C70-S20), meaning that a distance of 40 nm may not be sufficient to limit the diffuse of small drug molecules. Compared with previously reported drug delivery systems, this study is, for the first time, reporting the realization of sustainable drug release by regulating the shell thicknesses of hollow mesoporous nanoparticles. Additionally, since the amounts of DOX released at pH 5.5 are improved much more than that at pH 7.4, it reflects the efficacy of DOX against tumor tissues will be greater than that occurring in normal tissues.

**Intracellular tracking of HMSN**

The endocytosis properties of HMSN as drug carriers were further examined. Prior to the cellular experiments, fluorescein isothiocyanate (FITC) labeled HMSN (FITC-HMSN) were synthesized through fluorescent monomer cocondensation with TEOS around the PTBA cores. The TEM image (see Fig. S3 in the ESI†) shows that the introduction of FITC molecules doesn’t effect the production of the well-defined core–shell structure. Also, the resulting HMSN dispersion in water is capable of emitting a strong green fluorescence when irradiated by UV lamp. The fluorescent image (see Fig. S4 in the ESI†) shows that the FITC is localized in the core of the HMSN, whereas the PTBA is distributed in the shell. This is consistent with our previous reports. 

**Table 1** Properties of HMSN with different shell thicknesses and drug loaded HMSN

<table>
<thead>
<tr>
<th>Samples</th>
<th>BET surface area (m² g⁻¹)</th>
<th>Pore size⁴ (nm)</th>
<th>Mesoporous volume (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMSN-C70-S20</td>
<td>1022</td>
<td>2.3</td>
<td>0.98</td>
</tr>
<tr>
<td>HMSN-C70-S40</td>
<td>973</td>
<td>2.6</td>
<td>0.95</td>
</tr>
<tr>
<td>HMSN-C70-S65</td>
<td>932</td>
<td>2.5</td>
<td>0.88</td>
</tr>
<tr>
<td>HMSN-C70-S95</td>
<td>913</td>
<td>2.5</td>
<td>0.87</td>
</tr>
<tr>
<td>DOX loaded HMSN-C70-S40</td>
<td>803</td>
<td>2.5</td>
<td>0.74</td>
</tr>
</tbody>
</table>

⁴ Measured by N₂ adsorption at 77 K using the BJH method.

**Fig. 5** (a) Release curves of pure DOX in the dialysis bag at pH 5.5 (■) and 7.4 (○) and 7.4 (○). (b) DOX release from HMSN-C70-S20 (○), HMSN-C70-S40 (□), HMSN-C70-S65 (△) and HMSN-C70-S95 (◇) at pH 7.4, and HMSN-C70-S20 (●), HMSN-C70-S40 (■), HMSN-C70-S65 (▲), HMSN-C70-S95 (◇) at pH 5.5.
Effects on the survival of C6 cells of free DOX, DOX loaded HMSN-C70-S40 and blank HMSN-C70-S40. The corresponding concentrations of HMSN-C70-S40 are 0.09, 0.21, 0.43, 2.1, 8.5, 43 and 213 μg mL⁻¹, respectively.

**Conclusion**

In conclusion, PTBA nanospheres as a dissolvable core template were developed to fabricate uniform, discrete and dispersible HMSN, which avoids the influence of the surface properties and structure features of HMSN stemming from the conventional process of core template removal. The resulting HMSN showed good stability in aqueous solution due to the good preservation of the Si–OH groups during the process of template removal. The mechanism of the removal step was proved to be a fast dissolution and diffusion procedure. Furthermore, the hollow core diameter and shell thickness of the HMSN were successfully accurate tailored. The data of N₂ adsorption–desorption indicated that the change of shell thickness had no influence on their mesoporosity. The relation between the release behavior of DOX and the mesoporous shell thickness was investigated. The DOX release exhibited a shell thickness dependence. This novel mechanism between free DOX and DOX loaded nanospheres is more effective at entering the cells than free DOX. This study demonstrates well that the HMSN are endowed with the potential for application as DDSs.

**In vitro cytotoxicity assays**

Furthermore, the *in vitro* cytotoxicity against C6 cells was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays to evaluate the HMSN potential for application as DDSs (Fig. 7). HMSN-C70-S40 was selected as the model thanks to its representative structure and high synthetic yield. The results show that a 24 h incubation of C6 cells with HMSN-C70-S40 loaded with 0.02, 0.05, 0.1, 0.5, 2, 10 and 50 μg mL⁻¹ of DOX reduce the fraction surviving to 103.2%, 99.5%, 97.5%, 88.3%, 77.1%, 55.7% and 35.8%, respectively. As a control, the cytotoxicity assays of the blank HMSN-C70-S40 with the corresponding concentration are performed. The results indicate that HMSN-C70-S40 shows no cytotoxicity to the C6 cells at low concentrations.

**Fig. 6** Confocal laser scanning microscope images of C6 cells cultivated with FITC-HMSN (a–d) and DOX loaded FITC-HMSN (e–h) at 37 °C for 2 h. Blue: the nucleus (a and e); green: FITC (b and f); red: lysosome (c) and DOX (g); merging of image a, b and c (d); merging image e, f and g (h). All the scale bars are 10 μm.
the effectiveness of HMSN as drug carriers. We expect that this study may contribute to the promotion and advancement of research on HMSN for use as versatile nano-containers satisfying the demands of controlled drug delivery systems.

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Notes and references