Electronic Supporting Information (ESI)

Controlled Release of Cargo Molecules from Hollow Mesoporous Silica Nanoparticles based on acid and base dual-responsive Cucurbit[7]uril Pseudorotaxanes

Tao Chen[‡], Nianwang Yang[‡] and Jiajun Fu^{*}

School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, China.

‡ These authors contributed equally to this article.

*To whom correspondence should be addressed. E-mail: fujiajun668@gmail.com

Table of Contents

Experimental Section		
Materials	2	
Preparation of hollow mesoporous silica nanoparticles (HMSs)	2	
Functionalization of the surface of HMSs	3	
Loading of Gemcitabine (Gem)	3	
CB[7] capping	3	
Controlled release of Gem	3	
Characterization	4	
Analytical data	5	
XRD analysis	5	
N ₂ adsorption/desorption isotherms	6	
TEM and SEM images	7	
Element analysis	8	
²⁹ Si CP/MAS SSNMR spectra	9	
FTIR spectra	10	
TGA analysis	11	
Estimation of the loading amount of Gem	12	
Reference	12	

Experimental Section

Materials

Tetraethoxysilane (TEOS, 99.0%), chloromethyltrimethoxysilane (CMTES, \geq 95.0%), toluene (\geq 99.5%), 1,6-hexanediamine (HDA), [2-(acryloyloxy)ethyl] trimethylammonium chloride (AETAC, 80 wt% cetyltrimethylammonium bromide in water). (CTAB. >99.0%), 2'2-azobis(2-methylpropionamidine) dihydrochloride (V-50, > 97%), N,N'-dicyclohexylcarbodiimide (DCC, 99.0%), 4-dimethylaminopyridine (DMAP, 99.0%), ammonium hydroxide solution (28 wt%) were obtained from Aladdin Reagent Co., Ltd (China). Ferrocene dicarboxylic acid (FcCOOH) was purchased from J&K Scientific Co. Ltd. (China). Gemcitabine (Gem, > 95%) and cucurbit[7]uril (CB[7], > 95%) were purchased from Sigma-Aldrich Inc. All reagents were used without further purification unless otherwise stated. Phosphate buffered saline (PBS, 0.1 M, pH 6.5) was used in the experiments, and the pH of solution was adjusted with HCl and NaOH (0.1 M). Styrene (> 99.0%) was purified by washing with NaOH (10 wt%) and deionized water.

Preparation of hollow mesoporous silica nanoparticles (HMSs)

The hollow mesoporous silica nanoparticles (HMSs) were prepared using polystyrene latex as hard core templates as described in the previous literature with minor modification. The PS was prepared by emulsifier-free emulsion polymerization. Briefly, 1.0 g of AETAC (80 wt% in H₂O) was dissolved in deionized H₂O (390 mL) prior to the addition of styrene (40 g) and then stirred by a mechanical stirrer at room temperature for 30 min. The mixture was deoxidized by bubbling nitrogen for 30 min at 90 °C before V-50 solution (10 wt%, 10 mL) was added. Subsequently the polymerization was performed at 90 °C for 24 h under the atmosphere of nitrogen.

10 g of PS (~ 9%) was added dropwise to the mixture solution containing CTAB (0.8 g), H₂O (29 g), ethanol (12 g), and ammonia solution (1 mL) at room temperature under vigorous stirring, and dispersed by ultrasonic vibration for 15 min. 4.0 g of TEOS was injected into the mixture solution by an injection pump. After stirring for 48 h at room temperature, the product was collected by centrifugation with the speed of 10, 000 rmp for 15 min and washed with ethanol three times. Then, the sample was dried under vacuum overnight. Finally, the surfactant (CTAB) was removed by calcining at 550 °C for 8 h with a heating rate 3 °C min⁻¹. The samples (0.5 g) were stirred in anhydrous ethanol (80 mL) with concentrated HCl (4 g) at 50 °C for 5 h, providing abundant surface hydroxyl groups, and afterwards stirred in THF (50 mL) at room temperature overnight to completely remove template agents. The resulting solid was recovered by centrifugation, washed by MeOH

several times and dried at 60 °C under vacuum overnight.

Functionalization of the surface of HMSs

HMSs (200 mg) were suspended in dry toluene (15 mL) by stirring and ultrasonic vibration. CMTES (60 mL, 0.28 mmol) was added to the solution with magnetic stirring under argon. After the solution was refluxing overnight, the CMTES-modified HMSs (CMHMSs) were separated from the solution by centrifugation and washed with dry toluene and absolute MeOH. The nanoparticles were dried under vacuum at 60 $^{\circ}$ C overnight.

CMHMSs (100 mg) were added into a toluene solution of excess HDA, and then stirred and sonicated to dispersion. The mixture solution was refluxed overnight under argon. Afterwards, CMTES/HDA-modified HMSs (HDAHMSs) were collected by centrifugation and washing with MeOH. Finally, the powder was dried for further modification.

FcCOOH (100 μ mol, 27mg) was dissolved in dry DMF (10 mL), and then HDAHMSs (100 mg) were suspended in the mixed solution, followed by the addition of DCC (100 μ mol, 20 mg) and DMAP (150 μ mol, 20 mg). After stirred for 24 h under argon, the product (FcHMSs) were obtained by centrifugation and washed thoroughly with H₂O and absolute EtOH.

Loading of Gemcitabine (Gem)

The FcHMSs (50 mg) were soaked in Gem aqueous solution (10 mg mL⁻¹, 5 mL) and sonicated in solution to maximized dispersion. The suspended solution was stirred for 24 h under vacuum, and then the Gem-loaded FcHMSs were obtained by centrifugation and dried under vacuum overnight.

CB[7] capping

CB[7] (50 mg) was completely dissolved in PBS solution (pH 6.5, 4 mL) containing NaCl (0.1 wt%) and Gem (2 mg mL⁻¹) by ultrasonic vibration. Gem-loaded FcHMSs (50 mg) were suspended in the above solution, and the mixture solution was stirred for 3 d to form the entire Gem-loaded, CB7-capped FcHMSs at room temperature. The nanoparticles were collected by centrifugation and washed adequately with PBS solution (pH 6.5) and MeOH, and subsequently dried under vacuum overnight for further analysis.

Controlled release of Gem

In order to investigate the operation of acid and base dual-responsive mechanized HMSs in aqueous solution, UV-vis spectroscopy was used to monitor the release curves of Gem from CB[7]-capped FcHMSs as a function of time at 267 nm wavelength. Gem-loaded, CB[7]-capped FcHMSs (1 mg) were placed in the dialysis membrane at the top of the sealed quartz cuvette to prevent nanoparticles from dispersing into solution. The PBS solution (4 mL, pH 6.5) was carefully added into the cuvette in

order to ensure that the nanoparticles were completely immersed into the solution. The release of Gem from mechanized HMSs was realized by adjusting the aqueous solution to the desired pH with NaOH or HCl solution (0.1 M). The absorbance of the dissolved Gem was collected at one-second interval as a function of time during the course of the controlled release experiment.

Characterization

\

Transmission electron microscopy (TEM) images of as-prepared samples were observed on a JEOL JEM-2100 with an accelerating voltage of 200 Kv. The morphologies of samples were observed under scanning electron microscopy (SEM, S4800, Hitachi). Small-angle powder X-ray diffraction (XRD) analyses were performed by a Bruker D8 Advance diffractometer with Cu K α radiation ($\lambda \approx 1.54$ Å). Thermogravimetric analyses (TGA) were carried out on a TGA/SDTA851e from 50 to 800 °C at a heating rate of 20 $^{\circ}$ C min⁻¹ in nitrogen flow. N₂ adsorption-desorption isotherms at 77 K were measured on a Quantachrome Nova 1000 Micrometric apparatus. Before the measurements, the samples were degassed in vacuum at 433 K for 12 h. The surface area and pore volume were calculated by Brunauer-Emmett-Teller (BET) method. The pore size distribution was estimated by the Barrett-Jayner-Halenda (BJH) method. The solid state ¹³C and ²⁹Si CPMAS NMR spectra were collected on a Bruker DSX400 NMR spectrometer, operating at Larmor frequencies of 100.6 and 79.5 MHz and equipped with a 7 mm probe. Fourier transform infrared (FTIR) spectra of samples were collected by Tensor 27 FTIR system using the KBr pellet method. The concentrations of the Gem in solution were determined with a Shimadzu UV-1800 spectrometer. Elemental analysis was performed by Elemental Vario EL III. The carbon, hydrogen and nitrogen contents of each sample were determined using the thermal conductively detector.

Analytical data

XRD analysis



Figure S1. XRD patterns of HMSs, CMHMSs (red), HDAHMSs (blue) and FcHMSs (green).

The low-angle XRD pattern of HMSs shows an ordered 2D mesopore structure.¹ The HMSs have the diffraction peaks at around $2\theta = 2.2$, corresponding to a pore spacing of 4.0 nm. The XRD patterns of different functionalized samples are similar to that of the HMSs, which indicated the mesoporous structure is preserved.



N₂ adsorption/desorption isotherms

Figure S2. N_2 adsorption-desorption isotherm of HMSs (a), FcHMSs (b) and Gem-loaded, CB[7]-capped FcHMSs (c). The inserts show pore size distributions of HMSs (a), FcHMSs (b) and Gem-loaded, CB[7]-capped FcHMSs (c).

Table S1. BET and BJH parameters of HMSs, FcHMSs and Gem-loaded, CB[7]-capped FcHMSs.

Sample	Specific surface Area(m ² g ⁻¹)	BJH average pore diameter (nm)	Pore volume(cm ³ g ⁻¹)
HMSs	874.17	2.52	0.98
FcHMSs	602.88	2.26	0.67
Gem-loaded,			
CB7-capped	150.12	2.07	0.16
FcHMSs			

The distinct reduction in surface area, average pore diameter and pore volume indicated that Gem-loaded, CB[7]-capped FcHMSs were encapsulated with Gem molecules and CB[7] may block the pore opening to some degree.

TEM and SEM images



Figure S3. TEM (a) and SEM (b) images of FcHMSs.

Seen from the TEM images of HMSs and FcHMSs, the surface modification procedure hardly alters the overall morphology after FcCOOH attachment. The SEM image of FcHMSs shows no remarkable difference in the size compared with HMSs.

Element analysis

Type of silica	Nitrogen (%)	Carbon (%)	Hydrogen (%)
HMSs	0.122	0.391	0.289
CMHMSs	0.139	3.044	0.580
HDAHMSs	0.807	6.021	1.274
FcHMSs	0.239	10.75	2.331

Table S2. Element analysis of the HMSs, CMHMSs, HDAHMSs and FcHMSs.

Elemental analysis of the functional samples revealed a high proportion of carbon and the presence of nitrogen in HDAHMSs and FcHMSs confirmed the successful functionalization of HDA and FcCOOH on the surface of the HMSs. It can be concluded that the effective amounts of HDA and FcCOOH molecules attched to the surface of HMSs is 0.413 and 0.394 mmol g^{-1} , respectively.

²⁹Si CP/MAS SSNMR spectra



Figure S4. ²⁹Si CP/MAS SSNMR spectra of pure HMSs (a), HDAHMSs (b) and FcHMSs (c).

The ²⁹Si CP/MAS spectrum of HMSs shows three signals in the Q region at -92, -100 and -110 ppm, attributed to different silanol groups in the bulk silica. The spectra of HDAHMSs (b) and FcHMSs (c) show the peaks in the Q and T regions, which correspond to bulk silica and functionalized silica, respectively.

FTIR spectra



Figure S5. FTIR spectra of pure HMSs (a), CMHMSs (b), HDAHMSs (c), FcHMSs (d) and CB[7]-capped FcHMSs (e).

The infared bands related to HDA, FcCOOH and CB[7] were observed in the spectrum of CB[7]-capped FcHMSs powerfully validating successful installation process of acid and base dual-responsive nanovalves on the surface of the HMSs.

TGA analysis



Figure S6. TGA analysis of CMHMSs (a), HDAHMSs (b), FcHMSs (c) and Gem-loaded, CB[7]-capped FcHMSs (d).

Thermogravimetric analysis (TGA) of each functionalized samples shows weight losses of 8.3 %, 12.9 %, 18.0 % and 30.5 % for CMHMSs, HDAHMSs, FcHMSs and Gem-loaded, CB[7]-capped FcHMSs, respectively. For the CMHMSs, the weight loss is attributed to the decomposition of methyl chloride. The degree of degradation of HDAHMSs increases by 4.6% compared with the CMHMSs, indicating that HDA molecules were successfully anchored on the surface of HMSs. As for the FcHMSs, the weight loss is 5.1% more than that of HDAHMSs, suggesting the existence of FcCOOH molecules. Furthermore, Gem-loaded, CB[7]-capped FcHMSs are continually increased by 12.5 %, owing to the total weight loss of Gem and CB[7].

Estimation of the loading amount of Gem



Figure S7. Standard curve of UV absorption intensity of Gem as a function of its concentration (left) (Gem: $0-50 \ \mu g \ mL^{-1}$; PBS 10 mM, pH 6.5); The absorption spectrum of Gem released from mechanized HMSs before (blue), after the addition of base (black) and the mechanized conventional MCM-41 after complete release (red).

To determine the amount of Gem loaded in acid and base dual-responsive mechanized HMSs during the release experiment, 1 mg of the samples were placed into centrifugal tubes and 4 mL of PBS solution (0.1 M, pH 6.5) was added. The absorbance of Gem dissolved in aqueous solution was almost negligible (blue line), but was sharply increased after base stimuli (black line). Using Beer's Law, it can be calculated that 61 mg Gem is released per gram mechanized HMSs according to the absorption spectrum of Gem. For comparison, only a maximum of 2.4 wt% Gem is released for conventional MCM-41 assembled by the same conditions.

Reference

S. L. Gai, P. P. Yang, C. X. Li, W. X. Wang, Y. L. Dai, N. Niu and J. Lin, *Adv. Funct. Mater.*, 2010, 20, 1166.