

Supplementary Information

One-step synthesis of the magnetic hollow mesoporous silica (MHMS) nanospheres for drug delivery nanosystems *via* electrostatic self-assembly templated approach

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Experimental

Synthesis of Fe₃O₄ nanoparticles:

The Fe₃O₄ nanoparticles were synthesized *via* a coprecipitation method. 2.00 g of FeCl₂ and 2.70 g of FeCl₃ were dissolved in 13 mL hydrochloric acid solution with HCl/water (1/25), and then dropped into 90 mL of NaOH solution (1.5 M) until the black precipitation generated with vigorous stirring at 800 rpm in the atmosphere of nitrogen. The reaction was kept for 1 h and then the obtained Fe₃O₄ nanoparticles were separated by the magnetite, and then washed by deionized water for 3 times and finally re-dispersed in aqueous solution. The pH of the solution was adjusted to 6.0 by addition of 0.1 M of citric acid and the water-based ferrofluid was prepared with the concentration of 0.025 g/mL.

Synthesis of PS nanoparticles:

The PS nanoparticles were synthesized *via* a surfactant-free emulsion polymerization method. 10 g of styrene monomer, 0.0831 g of potassium persulfate and 0.005 g of sodium polystyrene sulfonate were dissolved in 90 mL deionized water with nitrogen bubbled for 30 min. The reaction was kept for 24 h under 70 °C with 800 rpm of stirring in the atmosphere of nitrogen. The PS nanoparticles were extracted by ethanol from the reaction media at last.

Synthesis of the magnetic hollow mesoporous nanospheres (MHMS):

The magnetic hollow mesoporous silica nanoparticles (MHMS) were one-step synthesized *via* the electrostatic self-assembly templated approach. 0.15 g of PS nanoparticles, 2.2 g of deionized water and 22 g absolute ethanol were mixed with ultrasound for 30 min to prepare solution A. 0.80 g of cetyltrimethylammonium bromide (CTAB), 9.60 g deionized water, 11.00 g absolute ethanol, 2 mL ammonium hydroxide (25%) and a certain amount of the water-based ferrofluid were mixed with ultrasound for 10 min to obtain solution B. The solution A was dropwise added into the solution B with ultrasound and kept for another 30 min before mechanical stirring. After stirring for 30 min, 3.3 mL of TEOS was injected into the mixture slowly and reacted at room temperature for 48 h. The product was collected by magnetite and washed by absolute ethanol and deionized water alternately for 3 times. Then the product was dried in an oven at 323 K for 6 h

and finally calcinated in air under 737 K to clear the template for 4 h. The hollow mesoporous silica nanoparticles (HMSN) and the magnetic sample (MHMS) can be prepared depending on whether the water-based ferrofluid was added. What's more, three kinds of MHMS nanospheres were finally prepared and named as MHMS₁, MHMS₂ and MHMS₃, respectively with 1 mL, 2 mL and 3 mL water-based ferrofluid injected in. This meant the mass ratios of Fe₃O₄/SiO₂ were 2.8%, 5.6% and 8.4%, respectively.

Drug loading:

Enrofloxacin (Enro) is a kind of fluoroquinolone antibiotics possessing a strong and wide antimicrobial activity. It is usually used to treat enteritis, pulmonitis, mastitis, skin and soft tissue bacterial infections in clinical application¹. Due to a significant concentration- and time-dependent bactericidal activity and the common phototoxicity of the fluoroquinolone hydrochlorides, it is necessary to introduce a high-loading and long-acting delivery system for higher maintained plasma levels, longer effective concentration duration and lower toxic or side effects to improve its efficacy. What's more, the target induction of the drugs, such as magnetism, could further enhance the therapeutic effect for the inner focus. Therefore, it is important to apply magnetic hollow mesoporous materials for the Enro drug release as the huge cavities for holding drugs, mesoporous channels for sustained release and the induced magnetism.

The drug loading method imported in this study was a vacuum-recrystal process which innovatively proposed in our previous articles².

0.2 g of Enro·HCl was dissolved in 35 mL of deionized water and then 0.12 g of vehicles was mixed into the solutions with ultrasound for 5 min. After that, the reactor was then vacuumized close to 0 MPa for 15 min by a vacuum pump. Next, a certain amount of NaOH (2M) was dropped into the reactor, and then the vacuum was kept for another 15 min. A magnetic stirring was maintained during the whole process and some crystalline particles can be observed gradually from the solutions after the addition of NaOH solutions.

Afterwards, the reactor was shocked for 48 h at room temperature to make drug crystals grow further. The final drug loading sample was filtered and washed with deionized water, then dried in an oven at 50 °C for 6 h. The filtrate was collected and the surplus drug was detected by a UV-Vis spectrophotometer (UV-2550, SHIMADZU) at the wavelength of 271 nm to determine the entrapment efficiency (EE) and loading capacity (LC). All measurements were performed in triplicate and averaged. The equations are shown below.

$$EE\% = \frac{m_{total} - m_{surplus}}{m_{total}} \times 100\%$$
$$LC\% = \frac{m_{total} - m_{surplus}}{m_{system}} \times 100\%$$

In this study, the loading capacities of different samples stabilized at 61.52% ± 4.43%.

As shown in Fig. S8, the carbonyl group of Enro·HCl associated with the inner surface hydroxyl group of the silica vehicles during the loading process. With the intrusion of NaOH (which led to the abrupt change of pH), the coordinated HCl was neutralized and the original bound carboxylic acid group (which can also combine with the hydroxyl group) was freed. Then it caused an increase of the binding sites between the drug and the carrier and a more orderly and compacter loading pattern. It means that drug molecules and the carrier can form an even tighter pair with a higher loading rate, which may bring great progress in delivery program as well.

Characterization

The FT-IR spectra were recorded in KBr pellets in the range 4000~500cm⁻¹ in a Fourier transform infrared spectrometer (360, Nicolet Avatar). Microscopic structure of MHMS before and after drug loading was detected by TEM (G220, Tecnai). The crystallization structures of HMSN and MHMS were detected by XRD (D8 Advance, BRUKER). The mesoporous structure of samples was confirmed by BET (GEMINI VII 2390, Micromeritics). The thermostability of HMSN and MHMS were tested by TGA (DTG-60, SHIMADZU) with a maximum temperature up to 800 °C in dry air. The surface aggregation of magnetic nanoparticles was analyzed by XPS (ESCALAB 250, Thermo-VG Scientific). The drug sustained release behavior of magnetic responsible systems was studied by UV-Vis Spectrophotometer (UV-2550, SHIMADZU). The magnetic separation process of MHMS in ethanol/water (1/1) was tested under the external magnetic field.

***In Vitro* Drug Release:**

To profile the extended-release effect of the drugs, 10 mg of drug loaded sample was dispersed into 2 mL PBS solutions (0.01 M, pH 7.4), and then poured into a sealed dialysis bag to ensure no solids bleed out. The sealed dialysis bag was placed in a conical flask filled of 500 mL PBS solution (0.01 M, pH 7.4) under atmospheric pressure and 37 °C. The release of the drug loaded HMSN was tested with a slow magnetic stir at 100 rpm as the blank control for loaded MHMS. At regular intervals, 3.0mL of the sustained-release solutions was transferred to be detected by a UV-Vis spectrophotometer (UV-2550, SHIMADZU) at the wavelength of 271nm. After measured, the solution was then returned back to the flask to keep the volume of sustained-release solutions unchanged.

The release of the drug loaded MHMS was measured under the intervention of the intermittent “on-off” alternating magnetic field (AMF) with 1200 mT at the alternative frequency of 100 rpm. The intermittence of “on-off” AMF was 10 min and 3.0 mL of the sustained-release solutions was transferred to be detected at every interval.

Kinetic Equation Models for Drug Release:

To determine the release mechanism *in vitro* test, three kinds of release kinetic statistic models were chosen to evaluate and describe the unique release profiles of Enro-HMSN delivery system.

Zero-order kinetics was dedicated to the controlled release system with a constant releasing rate which was independent of the drug concentration in the solvent. The equation for zero order release can be expressed as:

$$M_t / M_\infty = kt \quad (1)$$

With a reaction rate only depending on the concentration of drug molecules, first order kinetics was applied to describe the typical sustained-release system, the release mechanism of which follows Fick's law. The equation for first order release can be described as:

$$\ln(1-M_t / M_\infty) = -kt \quad (2)$$

The release kinetics of drugs delivered from insoluble porous materials was commonly described by an empirical Higuchi model which was well appropriate for matrix systems. The model was based on a square root of a time which was still dependent on the process of Fickian diffusion.

$$M_t / M_\infty = kt^{1/2} \quad (3)$$

In above equations, M_t and M_∞ represented the amount of drug release at time t and the final maximum cumulant, respectively. Meanwhile, k is the corresponding release rate constant.

***In Vitro* Release Pharmacokinetics:**

The fitting results of three pharmacokinetic models (Zero-order, First-order and Higuchi) were shown in Fig. S6, and the related kinetic parameters were also analyzed in Table S3. According to the fitting results, Zero-order model was not adequate to the release behavior in view of the visibly poor fit that reflected the release concentration was not constant. On the other hand, the release results showed preferable fit with First-order and Higuchi model, which implied the matrix type release mechanism conforming to the Fickian diffusion. It could be speculated that the drug molecules encapsulated inside of the hollow cavity were dissolved into the permeating fluid phase and released to the exterior through the cavity and the mesoporous channels gradually, which prolonged the pathway of drugs and postponed the later period of drug delivery .

***In Vitro* Cytotoxicity Test:**

Human embryonic kidney (HEK) 293T cells and rat pheochromocytoma PC12 cells were incubated to assess the cytotoxicity of HMSN and MHMS by the Cell Counting Kit-8 (CCK-8) and the cell apoptotic responses were also examined stained with Hoechst 33342 using an upright fluorescence microscope (Olympus, BX51, Tokyo, Japan). Meanwhile, the biocompatibility of HMSN and MHMS in the blood was also detected by the hemolytic experiment with the venous blood drawn from the SD rats and the hemolyzed samples were centrifuged, photographed, and the supernatants were measured at 570nm in a microplate reader (Bio Tek Instruments, Inc.).

Fig. S1 The XPS Fe2p spectrum of MHMS₃ as an example of MHMS.

Fig. S2 Infrared spectrum of drug loaded HMSN and MHMS (taking MHMS₃ as an instance). The

results proved that Enro had been encapsulated in the vehicles.

Fig. S3 TG weight loss curves of HMSN and MHMS (taking MHMS₃ as an instance).

Fig. S4 The TEM images of (a) PS and (b) Fe₃O₄ nanoparticles

Fig. S5 The release curves of HMSN, MHMS₁, MHMS₂ and MHMS₃ on a consecutive “on-off” operation under the external AMF with a coincident capacity of $61.52\% \pm 4.43\%$.

Fig. S6 Fitted dissolution-diffusion kinetic models of zero-order(a), First-order(b), and Higuchi(c).

Fig. S7 Immunofluorescence microscopy analysis of apoptosis in 293T cells and PC12 cells induced by HMSN or MHMS at different concentrations(ranging from 31.25 to 2000 $\mu\text{g/mL}$) for 72 h. Red arrows indicated apoptosis cells. The HMSN and MHMS both did not have any significant effect on the nucleuses (blue) stained with hoechst33342.

Fig. S8 Illustrations of molecular alterations during the vacuum-recrystal loaded process.

Table S1. BET surface area and aperture parameters of HMSN, MHMS₁, MHMS₂ and MHMS₃.

Sample	BET Surface Area	Pore Volume	Average Pore Size
HMSN	1103.32 m ² /g	0.75 cm ³ /g	2.64 nm
MHMS ₁	1026.10 m ² /g	0.74 cm ³ /g	2.64 nm
MHMS ₂	979.81 m ² /g	0.63 cm ³ /g	2.64 nm
MHMS ₃	854.02 m ² /g	0.61 cm ³ /g	2.64 nm

Table S2. Drug loading capacity (LC) and encapsulation efficiency (EE) parameters of HMSN, MHMS₁, MHMS₂ and MHMS₃.

Sample	HMSN	MHMS ₁	MHMS ₂	MHMS ₃
LC(%)	62.27 ± 3.68	61.68 ± 2.89	61.77 ± 2.35	60.26 ± 3.17
EE(%)	99.63 ± 5.89	98.69 ± 4.63	98.83 ± 3.76	96.42 ± 5.08

Table S3. Fitted Kinetic Release Parameters for Enro-HMSN nanosystem.

Model	Fuction	Slope(k)	Intercept(K)	R ²
Zero-order	$Mt / M_{\infty} = kt + K$	0.0048	0.26639	0.81361
First-order	$\ln(1-Mt / M_{\infty}) = -kt + K$	0.02419	-0.06748	0.96412
Higuchi	$Mt / M_{\infty} = kt^{1/2} + K$	0.07235	0.10813	0.96198

Reference:

- 1 (a) T. Yamane, H. Enokida, H. Hayami, M. Kawahara and M. Nakagawa, *Int J Urol*, 2012, 19, 360; (b) F. Lu, A. Popa, S. W. Zhou and J-J. Zhu, *Chem. Commun.*, 2013, 49, 11436; (c) G. Martelli, H. R. Zope, M. B. Capell and A. Kros, *Chem. Commun.*, 2013, 49, 9932.
- 2 F. Liu, H. Zhang, Q. Cao, X. Xiang, L. Wang, T. He, W. Liu, Y. Fang, D. Y.B. Deng and W. Zhou, *RSC Adv.*, 2014, 4, 8918.