Supplementary Information

High-efficiency loading in small mesopores (2-3 nm) forming a matrix type controlled drug delivery nanosystem

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Experimental

Synthesis of Mesoporous Silica MCM-41 nanoparticles:

With cetyltrimethylammonium bromide (CTAB) as the template and sodium hydroxide as the catalysis, MCM-41 was simply prepared in water solution. Firstly, 1.0 g CTAB was dissolved in 480 mL deionized water and heated to 358 K with vigorous stirring. Then 3.5 mL NaOH (2 M) was dropped into the solution to adjust the pH value. After a while, 5 mL TEOS was injected into the mixture slowly and reacted at 358 K for 3 h with continuous stirring at 600 rpm. The final suspension were filtered and washed by absolute ethyl alcohol and deionized water alternately for 3 times. Then the product was dried in an oven at 323 K for 6 h. The MCM-41 can be finally obtained after calcinated in air under 873 K to clear the template for 4 h.

Synthesis of Enro-MCM-41 Delivery System:

0.2 g Enro·HCl was dissolved in 35 mL deionized water and then 0.12 g MCM-41 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 (2 M) 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.

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

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

We can gain samples with different drug loading rates (3.89%, 14.52%, 26.10%, 40.55% and 62.31% were chosen to evaluate in release experiment), which are called as 3.89%, 14.52%, 26.10%, 40.55% and 62.31% for abbreviation in later expression, via adjusting the amount of

NaOH (2 M) added which means adjusting pH of the solutions or changing the dosage of carrier. The recrystallized Enro·HCl was prepared by the same process just as above without the addition of MCM-41 and was called Re-Enro for abbreviation.

What's more, three samples with loading capacity of 61.76% ± 5.27% were prepared for contrast. Solid SiO_2 (synthetized via the classical approach[1]) loaded Enro using our vacuum-recrystal method was called SS for abbreviation. Sample with MCM-41 as the carrier was prepared via the vacuum process and then used a rotavapor loaded method instead of the recrystal process called RL for abbreviation. Drug loaded sample use MCM-41 as the carrier was synthetized according to the recrystal method without vacuum process called WV for abbreviation.

***In Vitro* Drug Release:**

To profile the extended-release effect of the drugs, 10 mg of Enro-MCM-41 (the 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 solutions (0.01 M, pH 7.4) under atmospheric pressure and 37 °C. Meanwhile, a slow stir was also kept at 100 rpm. At regular intervals, 3.0 mL of the sustained-release solutions was transferred to be detected by a UV-Vis spectrophotometer (UV-2550, SHIMADZU) at the wavelength of 271 nm. After measured, the solutions was then returned back to the flask to keep the volume of sustained-release solutions unchanged.

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-MCM-41 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:

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

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:

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

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.

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

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.

Discussion

Enrofloxacin, a second-generation fluoroquinolone, is a broad-spectrum, concentration dependent bactericidal antibiotic. It is usually used to cure enteritis, pulmonitis, mastitis, skin and soft tissue bacterial infections in clinical application. As its antibacterial property depends on plasma drug peak concentration, developing a delivery system with high loading capacity to keep a lasting effective concentration can effectually enhance the pesticide effect and reduce the

toxicity. Because of the poorly soluble[2] in water, enrofloxacin hydrochloride was chosen to be the target drug for an example of fluoroquinolone hydrochlorides to verify the practical performance of our controlled delivery nanosystem.

As shown in Fig. S2 (ESI†), MCM-41 didn't display obvious peaks at large-angle degrees, however, a strong wide peak appeared after drug loaded between 16.91° and 24.50° which could be explained as the potentiation caused by the diffraction of drug crystallization. Two kinds of drug loaded samples with a rigorously coincident capacity of 61.76%±5.27% were tested in this characterization. Sample WV displayed stronger and sharper peaks of Enro especially at 21.41°, 22.60°, 23.39° and 27.37° compared with sample 62.31%. This declared that less drug molecules recrystallized on the surface of the carrier using vacuum-recrystal method and much of drugs were loaded in the channels. Contacting with our drug loaded process and loading capacities at different pH (provided in Fig. 2), it proved that drug molecules could not be kept in the pores without the recrystal process. So it was possible that quite a few of drug molecules have recrystallized within the pores in our study. However, XRD diffractograms could not convincingly prove the majority of the drug resided within the pores in its crystalline state and the peaks shown for the MCM-41 sample loaded with Enro could just be due to the drugs on the external surface. We hope that the crystalline packing within the pores could be completely demonstrated by other more powerful supports in our future research.

Small mesopores materials have many limitations when used as drug carriers. As shown in Table S1 (ESI†), it is very difficult to encapsulate drug molecules in small mesopores via usual immersion method (without the vacuum and recrystal processes) due to the air detained in the channels. However, considering the cost and possible toxic potential of the carrier, and the high entrapment efficiency of drugs which can reduce unnecessary waste, we think a high loading capacity for Enrofloxacin in this release system is available. The loading capacity was not much enhanced even after reloaded 4 times repeating the immersion situation while it could be improved to 62.31% using vacuum-recrystal method.

Meanwhile, although the great loading capacity was obtained, it meant nothing if drug molecules could not be loaded deep into the channel in the controlled-release system. This is another factor that hinders the application of small mesopores materials in drug release area. In this work we have solved this problem. The results of TG (shown in Fig. S4 (ESI†)) and the release curves shown in Fig. S5 (ESI†) have certified the effective drug encapsulation in the depths of the channels via our vacuum-recrystal loaded method.

As shown in Fig. S4 (ESI†), drug molecules thermostability increased as sample WV (loaded via the recrystal method without vacuum process) < RL (prepared via the a rotavapor method instead of the recrystal process) < 62.31%, at a same drug loading capacity of 61.76% ± 5.27%, which could be interpreted as the gradually increasing amounts of loading drug in the channel. The complete disparity of weight loss between sample SS (solid SiO_2 loaded drug via our vacuum-recrystal method) and 62.31%, with consistent loading capacity, which was 43.68% and 26.19% respectively, further certified that it was the mesoporous channels of MCM-41 that protected loaded drug molecules from a violent pyrolyzation. Moreover, the obvious slower falling rate over 356 °C in weight loss curves of sample 62.31% further certified the effective drug encapsulation in the depths of the channels via our vacuum-recrystal loaded method.

In addition, a detailed comparison of release behaviors between MCM-41-Enro (62.31%) and solid SiO_2-Enro (SS), as well as samples prepared via different loading methods, with a rigorously

coincident content of Enro loaded (61.76%±5.27%) was also made and the results are shown in Fig. S5 (ESI†). It could be judged that SS acted an obvious burst release of 45.16% at 1.5h while it was only 10.77% in mesoporous sample and its release behavior had ceased at the 1st day while only 63.94% of drugs gave off from the mesoporous sample. This obvious difference certified the peculiarity of the drug loaded method in this study that the drug molecules were not simply deposited on the surface of the carriers but crystallized largely in the channel of MCM-41 nanospheres. Moreover, the obvious burst release was observed for the sample using rotavapor loaded method (RL) and the recrystal loaded method without vacuum (WV) during the first 3 h, and quickly reached the release plateau as well. As compared with the slow release rate of 16.78% in the first 3 h and the extended release lasting for more than 5 days expressed in the sample 62.31% obtained via our vacuum-recrystal method, it was confirmed that vacuum treatment was very necessary to get a preferable controlled release property in the drug loading process, and recrystal process made a great contribution to immobilizing drug molecules deep into the channel as much as possible which could never be replaced by rotavapor. The irreplaceable craft of loaded method in our current report had also been emphasized.

However, we insist that it is of great significance to explore an efficiently drug encapsulation method for small pore mesoporous materials in drug delivery application because the narrow channels are the natural speed governor in the release behavior. The release system can realize a prolonged delivery easily with small mesopores materials as drug carriers. Owing to the approximate microporous size, a preferable matrix type release was realized which could make a great contribution to improving the delivery property as drug carrier as shown in Fig. 4b. The extremely confined channels of small mesopores materials formed the framework which hindered drug molecules directly dissolved out into the external medium solvents but dissolved into its permeating fluid phase within the matrix firstly and then diffused out to the external medium.

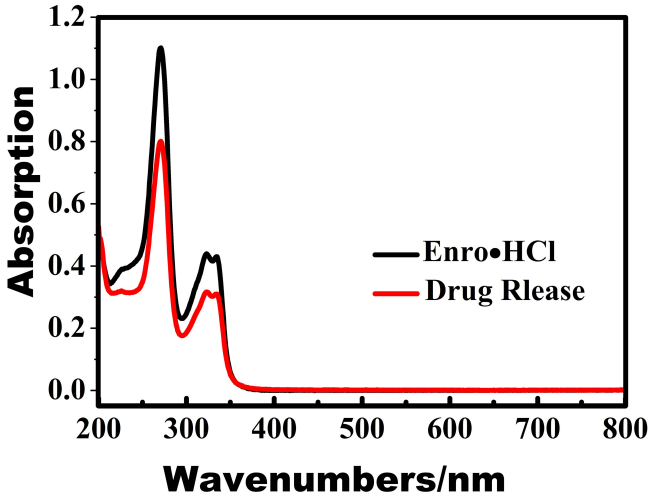

Fig. S1 UV-vis spectra of standard Enro·HCl and released drugs.

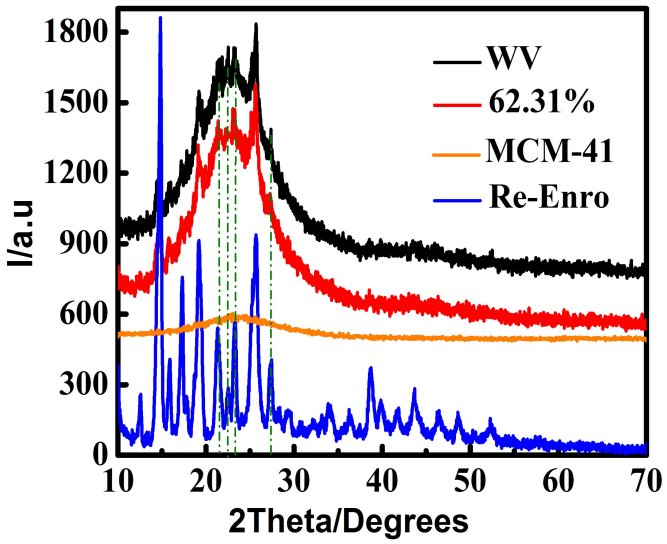

Fig. S2 The high-angle XRD patterns of recrystallized Enro (Re-Enro), blank MCM-41, drug loaded sample of 62.31% and sample WV (loaded via the recrystal method without vacuum process) with a rigorously coincident capacity of 61.76%±5.27%.

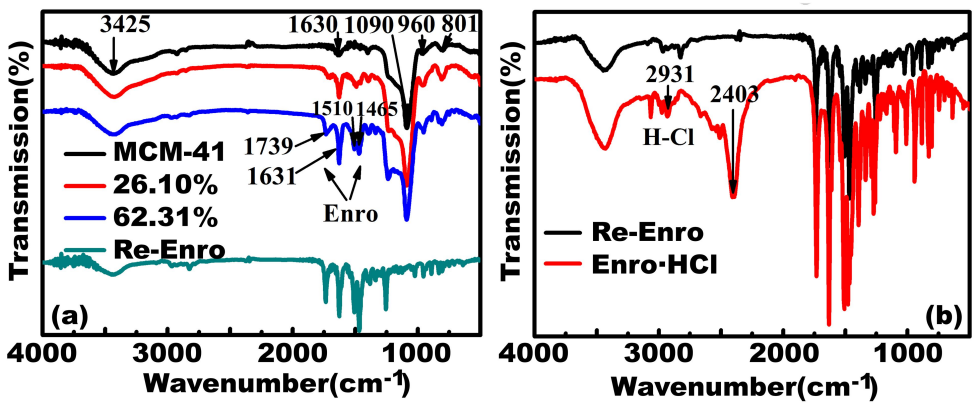

Fig. S3 (a) Infrared spectrum of MCM-41 before and after drug loaded. (b) Infrared spectrum of Enro·HCl before and after recrystallized.

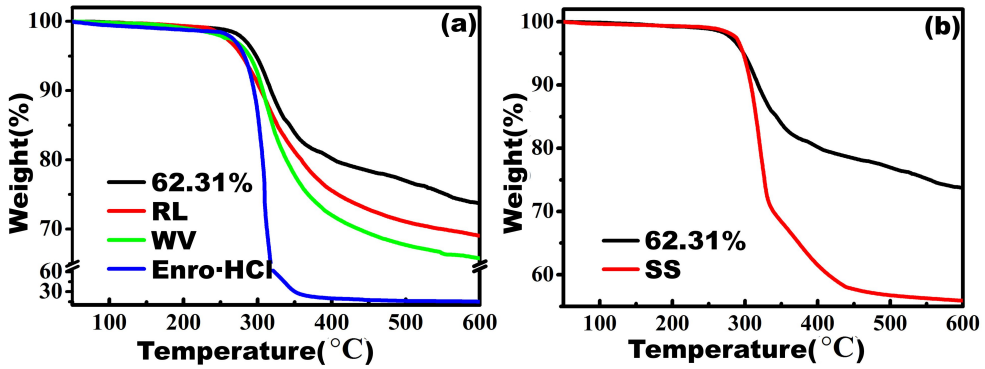

Fig. S4 TG weight loss curves of Enro-MCM-41 delivery nanosystem and the contrast samples with a rigorously coincident capacity of 61.76%±5.27%.

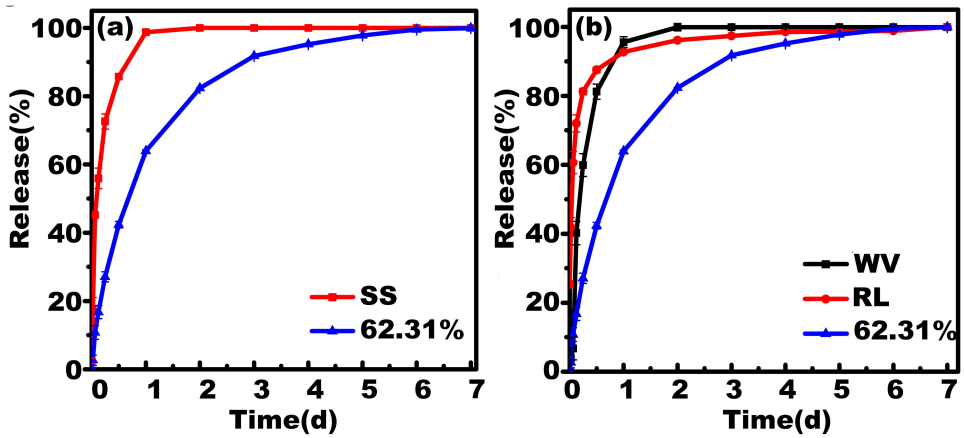

Fig. S5 The release curves of Enro-MCM-41 delivery nanosystem and the contrast samples with a rigorously coincident capacity of 61.76%±5.27%.

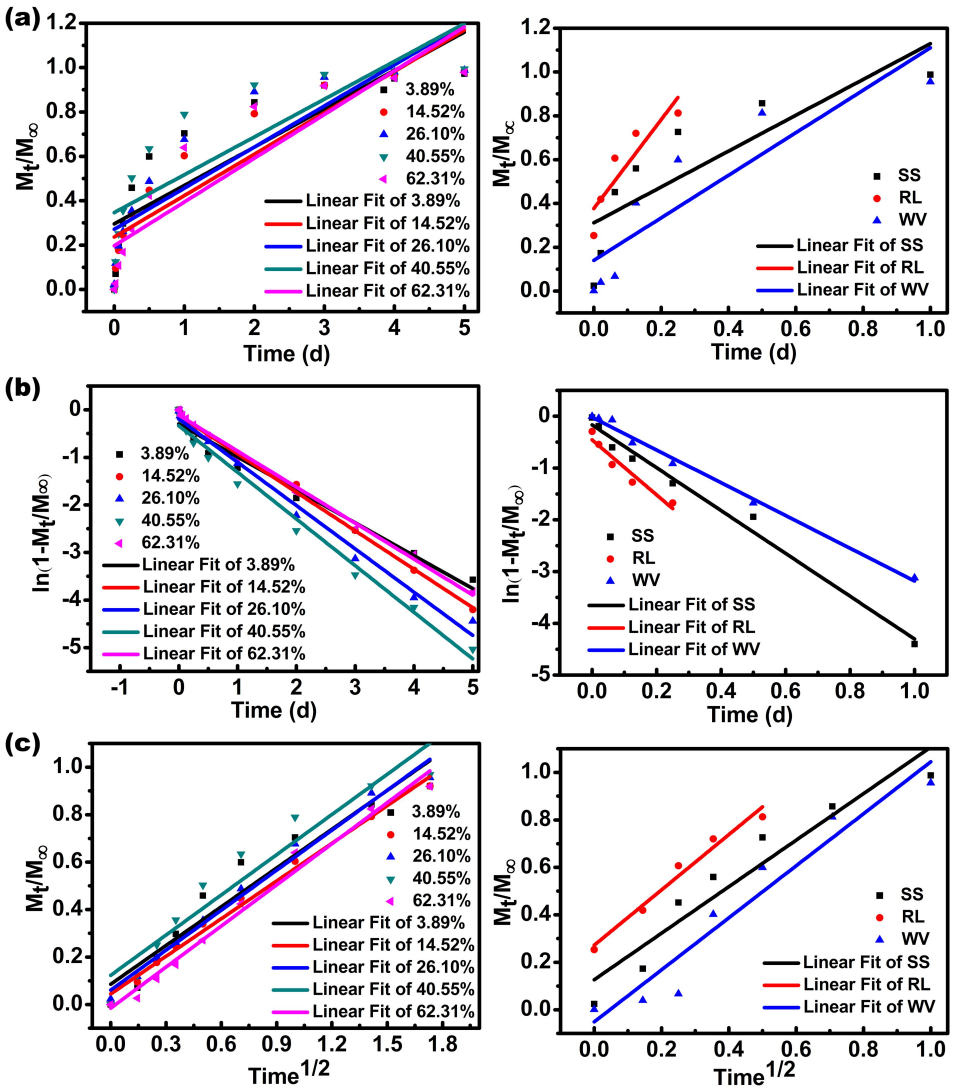

Fig. S6 Fitted dissolution-diffusion kinetic models of zero-order(a), First-order(b), and Higuchi(c)

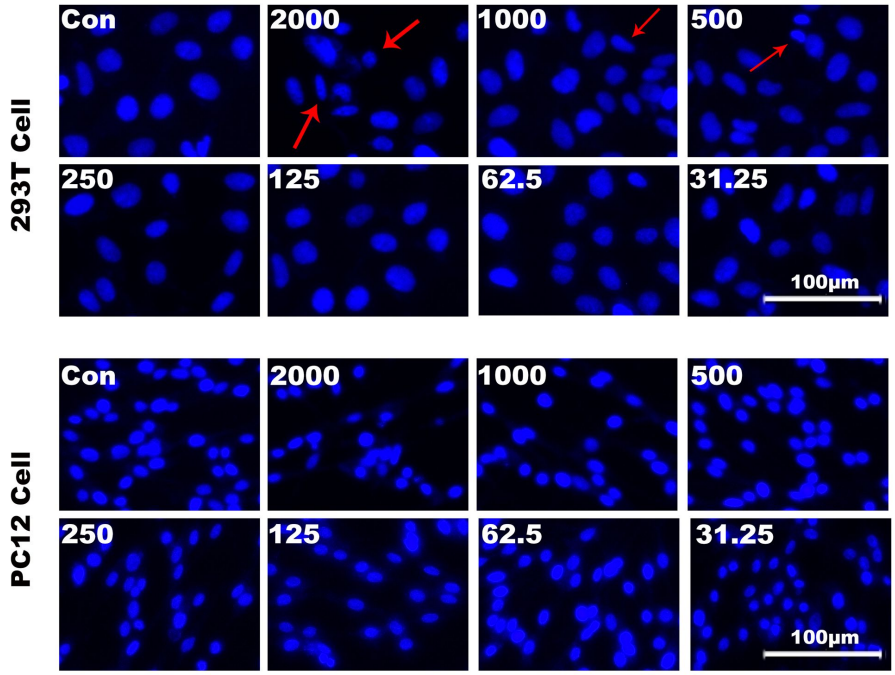

Fig. S7 Immunofluorescence microscopy analysis of apoptosis in 293T cells and PC12 cells induced by MCM-41 at different concentrations(ranging from 31.25 to 2000 μg/mL) for 72 h. Red arrows indicated apoptosis cells. The MCM-41 nanospheres did not have any significant effect on the nucleuses (blue) stained with hoechst33342. Scale bar: 100 μm.

Table S1. Loading capacities varied as different reloading times via usual immersion method

Reloading Times	0	1	2	3	4	vacuum-recrystal
Loading Capacity(%)	3.59±1.32	4.30±0.90	5.13±0.73	6.31±1.16	6.35±0.89	62.31±2.35

Table S2. BET surface area and aperture parameters before and after drug loading

Sample	BET Surface Area	Pore Volume	Average Pore Size
MCM-41	1051.75 m^2/g	1.39 cm^3/g	2.64 nm
Loaded	373.95 m^2/g	0.41 cm^3/g	38.71 nm

Table S3. Fitted Kinetic Release Parameters for Enro-MCM-41 nanosystem.

	$M_t / M_\infty = kt + K$ (Zero-order model)			$\ln(1-M_t / M_\infty) = -kt + K$ (First-order model)			$M_t / M_\infty = kt^{1/2} + K$ (Higuchi mod)		
Sample	Slope(k)	Intercept(K)	R^2	Slope(k)	Intercept(K)	R^2	Slope(k)	Intercept(K)	R^2
3.89%	0.17284	0.29548	0.69808	0.69197	-0.3008	0.97195	0.54463	0.08475	0.92191
14.52%	0.18662	0.23583	0.82208	0.80897	-0.11632	0.99674	0.52916	0.04367	0.99021
26.10%	0.18528	0.2707	0.77301	0.91068	-0.19194	0.9906	0.56188	0.0597	0.98323
40.55%	0.17034	0.34612	0.6544	0.98107	-0.33394	0.98484	0.56474	0.12248	0.9147
62.31%	0.19728	0.19631	0.80083	0.75666	-0.11262	0.99441	0.57753	-0.01628	0.98238
SS	0.81856	0.31086	0.64748	4.13855	-0.16741	0.98442	0.98057	0.12592	0.90191
RL	2.02797	0.37658	0.74714	5.31734	-0.45607	0.90884	1.16558	0.27158	0.96274
WV	0.97123	0.13939	0.76815	3.16477	-0.01939	0.99182	1.09681	-0.05191	0.91873

Reference:

1 K. D. Hartlen, A. P. T. Athanasopoulos and V. Kitaev, *Langmuir : the ACS journal of surfaces and colloids*, 2008, **24**, 1714.

2 (a) R. Mellaerts, E. J. Fayad, G. V. Mooter, P. Augustijns, M. Rivallan, F. Thibault-Starzyk and J. A. Martens, *Molecular Pharmaceutics*, 2013, **10**, 567; (b) X. Xia, C. Zhou, L. Ballell and A. E. Garcia-Bennett, *ChemMedChem*, 2012, **7**, 43; (c) G. T. Rengarajan, D. Enke, M. Steinhartc and M. Beiner, *J. Mater. Chem.*, 2008, **18**, 2537; (d) R. Prasad and S. Lele, *Philos. Mag. Lett.*, 1994, **70**, 357; (e) C. L. Jackson and G. B. McKenna, *Chem. Mater.*, 1996, **8**, 2128.