Encapsulation and Controlled Release of Antimalarial Drug Using Surface Functionalized Mesoporous Silica Nanocarriers

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Experimental Section

Chemicals

All the materials and chemicals were of analytical grade and were used as purchased. Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), and sodium lauryl sulphate (SLS) were obtained from Wako, while dialysis membrane and quinine were purchased from TCI. Deionized water, AR grade was used for all preparations and purifications.

Methods

The mesoporous silica nanoparticles were synthesized by the procedure of Beck *et al.*[1] with slight modification. In a typical synthesis, cetyltrimethylammonium bromide (CTAB) (1.34 g, 3.66 mmol) was suspended in distilled water (480 mL) and the temperature raised to 50 ℃ to form a clear solution. Then, NaOH (2 M, *ca*. 4 mL) was added to the solution with constant stirring to yield a pH = 12-13; heating was continued until the temperature reached 80 ℃. Tetraethyl orthosilicate (TEOS) (6.7 ml, 30mmol) was added dropwise and the solution was stirred for 2 h. The resulting product was removed by filtration and washed several times with distilled water and methanol until the pH of the aqueous wash solution was 7.0. The product was dried *in vacuo* overnight. The as-synthesized MCM-41 (1.0g) had its surfactant template removed by treatment with a 1% solution of concentrated HCl in methanol (100 mL) under reflux for 6 h. The resulting surfactant-removed product was removed by filtration and washed with water then methanol and dried i*n vacuo*, the product

is **1**. Post synthetic method was used to modify MCM-41 surface silanol with ammine and phenyl silylating agents using 3-aminopropyltriethoxysilane (APTES) and 3-phenylpropyltrichlorosilane (3-PPTCS), respectively to prepare **2,3**.

Characterization

Fourier Transform Infrared (FT-IR) spectra of the MSN were recorded on a FT-IR 8700 spectrometer employing KBR pellets. The pore properties of the MSNS (surface area, pore size and volume) were determined using nitrogen physisorption measurements at -196 ℃ employing a Micrometrics Tristar 3000 system and their QN encapsulated samples were degassed respectively at 120 ℃ for 6 h on a vacuum line. The pore-size distribution was measured from the desorption branch of the isotherm using a BJH model, followed by Gaussian fitting, pore volume, and surface area of the samples were calculated by using the Brunauer–Emmett–Teller (BET) equation. The DSC studies were performed on a NETZSCH DSC 200F3 Maia apparatus. The measurement was carried out from 293 to 473 K at heating rate of 10 K/min under a nitrogen gas flow for two cycles to check the structural phase transition peak of the samples' molecular state. Powder X-ray diffraction (PXRD) patterns of the silica and silica-loaded drugs were recorded on a Smart Lab Rigaku X-ray diffractometer with Fe-filtered Co radiation (Rigaku Corporation, Japan). The measurement conditions were as follows: target, CuKα; filter, Ni; voltage, 30 kV; current, 15 mA; scanning range, 2-30˚. Transmission electron microscopy (TEM) structural images were obtained using a JEOL 1010 instrument operated at 100 kV, while a JEOL-JSM 7600F instrument operating at an accelerating voltage of 5.0kV was used to obtain field-emission Scanning Electron Microscope (SEM) micrographs/morphologies of MSN and its encapsulated QN. At first, the silica nanoparticles were dropped onto the carbon-coated copper grid and then sputtered with a thin film of platinum and gold to initiate conductivity of the silica samples before scanning. The amount of QN loaded and released from the mesoporous silica was determined with the aid of a UV-3600 Shimadzu UV-Vis spectrometer.

Similar reported procedure was followed with slight change for loading the QN drug in the mesoporous silica nanoparticles. QN 10 mg was soaked in 10 mL of ethanol solution of MCM-41(**1**) (40 mg) (1:4 w/w) for 72h with constant mixing to allow full absorption of the drug inside the silica, the mixture flask was covered with aluminum foil to protect the drug from light. The **2** and **3** were also loaded with QN to get **4**, **5** and **6**. The drug loaded samples were separated from the solvent mixture by centrifugation for 30 minutes at 4000 rpm and washed with about 5 mL of ethanol to remove surface attached drugs. The residue i.e. adsorbed drug was dried in vacuum while the supernatant which contained small unabsorbed drug was determined after dilution using Beer-Lambert UV-Vis absorption spectrophotometer at 331.5 nm wavelength standard calibration curve. However, the actual amount of QN loaded in the MSNs was calculated by removing the weight of QN in the supernatant from the initial amount of QN used.

Equation formula for Drug Loading

Drug loading capacity (DLC %) = Weight of QN in MSNs / weight of QN-silica composite × 100%

Entrapment efficiency (EE %) = Weight of QN in MSNs / weight of initial QN loaded × 100%

In-vitro quinine dissolution procedure

QN loaded silica equivalent to 0.2 mg of the QN drug was singly weighed (4,5 & 6) and suspended in 1 ml of 0.5 % SLS buffer. This suspension was then placed in dialysis bag (Sigma Aldrich) with 10 kDa molecular weight cutoff and was immersed into 9 ml of 0.5 % SLS with continuous stirring at different pH (6, 7 & 8) and temperature of 25, 35 & 45 ˚C. At predetermined time intervals, 2 mL of the samples were withdrawn and analyzed for QN content at 331.5 nm using UV-Vis spectrophotometer. After the measurement, the solution was returned to the container. Pure QN release was studied along with silica-drug composite to compare the in vitro drug release profile by weighing 0.2 mg of pure QN and suspending it in 0.5 % SLS similar to that of MCM-41-QN. These samples were then properly diluted and analyzed for QN content at 331.5 nm using UV-Vis spectrophotometer.

Equation used for Calculating Drug Loading

Drug loading capacity (**DLC %**) = Weight of QN in MSN /weight of QN-silica composite employed x 100.

Entrapment efficiency (**EE %**) = Weight of QN in MSN / weight of initial QN present x 100.

In vitro **procedure for release of quinine**

Amounts of **4, 5, 6** corresponding to 2 mg of loaded QN were each suspended in 1 mL of 0.5 % SLS buffer solution. Each suspension was then placed in a dialysis bag (Sigma Aldrich) with a 10 kDa molecular weight cutoff. Individual bag was then immersed in 9 ml of the 0.5 % SLS buffer solution with continuous stirring at pH values of 6, 7, 8 and temperatures of 25, 35 and 45 $^{\circ}$C.

At predetermined time intervals, 1 mL of the samples were withdrawn and immediately replaced with an equal volume of buffer solution to keep the volume constant. All aliquots were then diluted and the concentration of QN determined via UV-Vis spectrophotometry at λ = 331.5 nm.

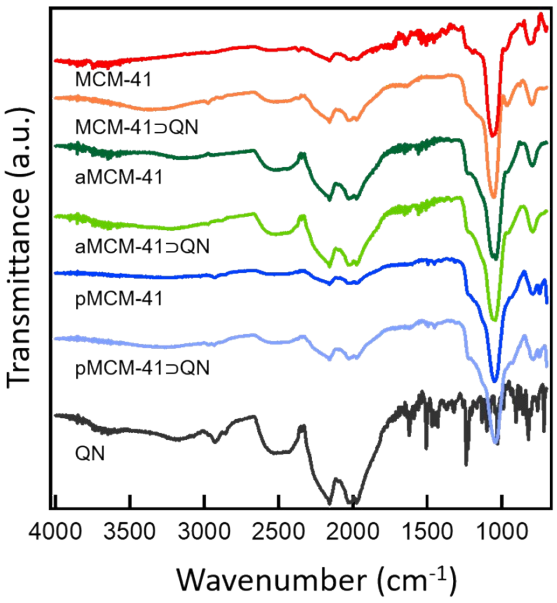

Figure S1: FT-IR spectra of **1-6** and **QN**

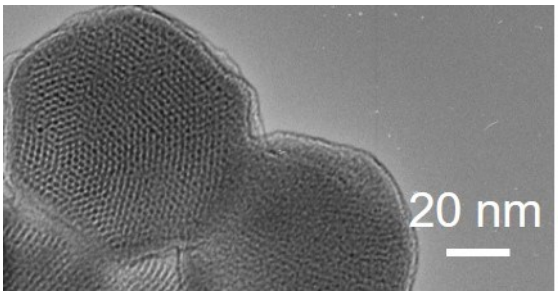

Figure S2: Hexagonal 2-D ordered structure of **1**

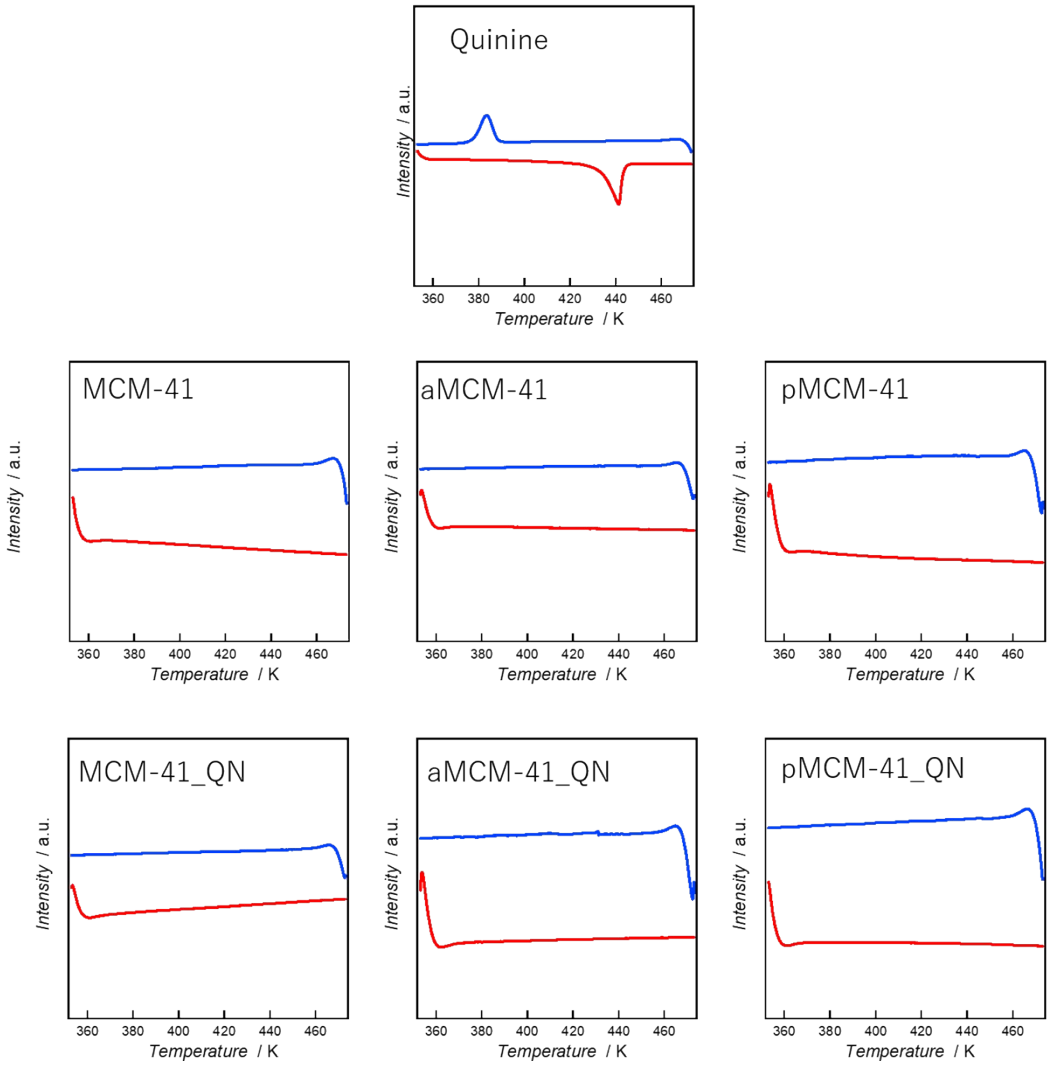

Figure S 3: DSC curves of **1-6** and **QN**

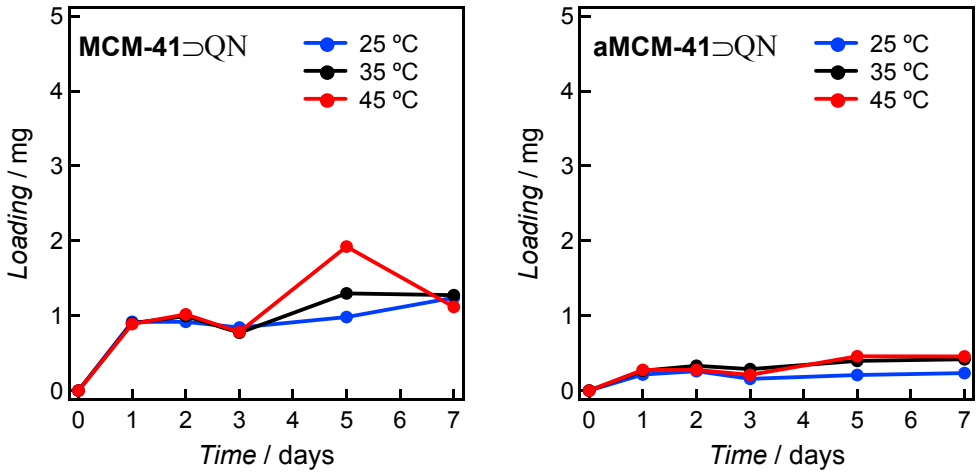

Figure S4: Temperature dependent QN loading of **4** and **5.**

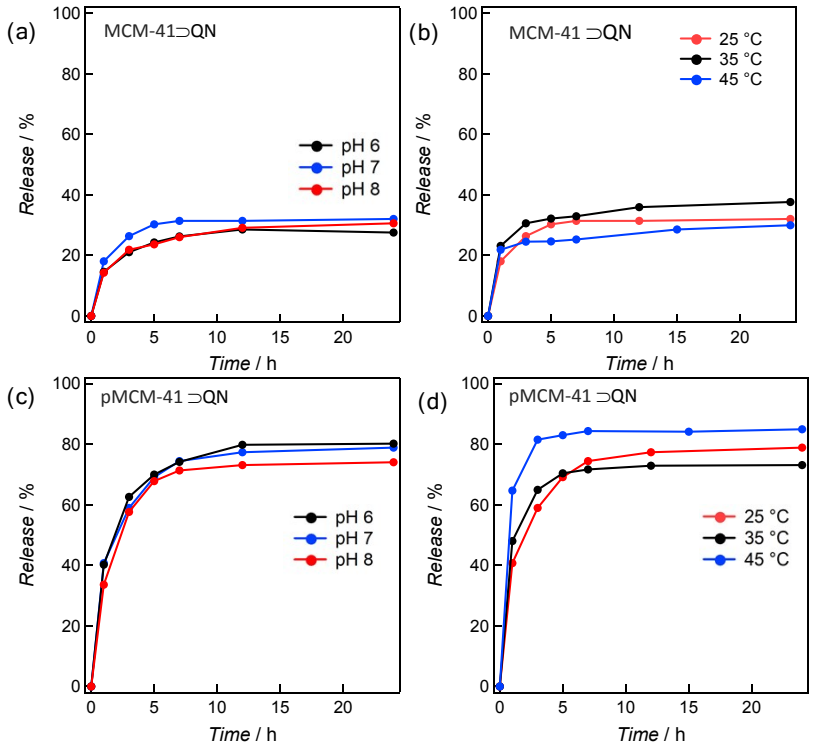

Figure S5: pH (a, c) and the temperature (b, d) dependent release of **4** and **6.**

Table S1: Quinine Drug Loading capacity and Entrapment efficiency of **1-3**

	conditions		DLC (%)	Average	EE (%)	Average
	pH	Temperature				
MCM-41	6	25	2.85	2.73	13.14	12.50
	7	25	2.44		10.81	
	8	25	2.96		13.77	
	7	35	2.62		11.67	
	7	45	2.78		13.09	
aMCM-41	6	25	0.50	1.06	2.06	2.74
	7	25	0.53		2.18	
	8	25	0.60		2.51	
	7	35	1.82		3.50	
	7	45	1.84		3.45	
pMCM41	6	25	7.17	7.48	46.60	49.72
	7	25	7.26		48.20	
	8	25	7.59		51.62	
	7	35	8.07		54.48	
	7	45	7.32		47.69	

References

1. J. S. Beck, J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T. W. Chu, D.H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, J. L. Schlenker, *J. Am. Chem. Soc.,* 1992, **114,** 10834.