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Synthesis of Mesoporous Materials as Nano-carriers for an Antimalarial Drug†

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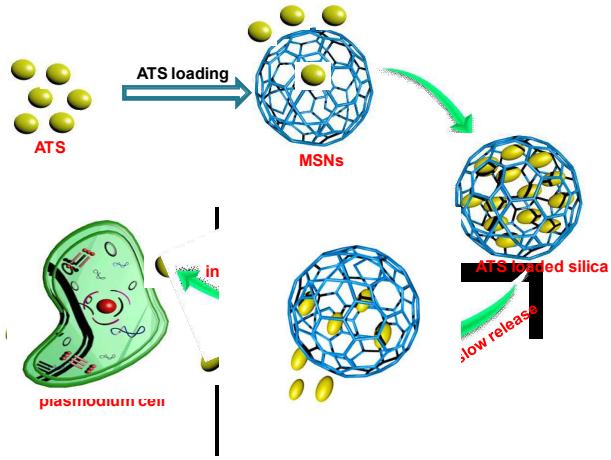
An antimalarial drug artesunate (ATS) was encapsulated in both functionalized MCM-41 and ordinary MCM-41 with an excellent loading capacity and sustained release behavior for possible biomedical application.

Plasmodia are responsible for malaria particularly in Sub-Saharan Africa and have caused human deaths.[1] Unfortunately, effort to put this disease under control has suffered from many drawbacks despite the launch of several antimalarial drugs from quinine to the present artemisinin combined therapy[2].The poor aqueous solubility of many antimalarial drugs is one of the problems responsible for the aforementioned. To increase the bioavailability in oral administration capable to decrease the chances of drugs resistance by the parasites, the recommended standard drug dose is required at the target cells. Artesunate drug (ATS) (see Fig. S1) is a particularly hydrophobic compound and has limited aqueous solubility.[3] Therefore, new strategies to circumvent this problem is in high demand

Nano carriers including clay, zeolites as well as some porous inorganic polymers have been widely explored as drug delivery vehicles.[4,5,6] Recently, the utilization of mesoporous silica nanoparticles (MSNs) e.g. MCM-41 as a drug delivery system (DDS) has attracted increasing attention due to their high loading capacity, ease of scalable synthesis, adjustable pore properties, high intracellular uptake and excellent biocompatibility etc.[7,8] It is shown that the encapsulation of insoluble drugs using MSNs with controlled pore size increase the drug solubility for gainful biological activity.[9,10] Recently, installation of stimuli responsive MSNs drug delivery systems is an excellent and safe strategy for the release of biomolecules (like genes, drugs, proteins etc) towards the targeted intracellular microenvironment.[11]

However, there is no report with regard to MSNs as the drug delivery system for ATS to the best of our knowledge. Hence, we conceived an idea (see scheme 1) of ATS loaded mesoporous silica nano carriers MSNs for the possibility as enhanced malaria therapy drug release system.



Scheme 1: ATS release strategy into malaria cell from MSNs

In this work, we carry out the research on MCM-41 and its hybrids as matrices for loading and release of ATS. The effect of different synthetic designs on the morphology and pore properties of modified and unmodified MSNs is demonstrated. ATS is encapsulated in the silica pores which gives u-tube and bean shape, and we also discuss the drug improved slow dissolution rate from the porous silica that could serve as

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interesting vehicle for delivering poorly water-soluble ATS when orally administered.

Sol-gel synthetic method as previously reported with slight modification was adopted to prepare as-synthesized MCM-41.[7a] Removal of the oligomeric surfactant CTAB was carried out by calcination at 550 °C which yielded mesoporous structure of MCM-41 (**1**) while acid solvent extraction technique i.e extraction method in acidified ethanol by reflux was used to remove the surfactant in the **2** and **3** (see Scheme 1, supporting information). During calcination process, the silanol groups are converted into siloxane (Si-O-Si) bridges.[12a] Organically modified silica hybrids with 3-aminopropyltriethoxysilane, and trichloro(3-phenylpropyl) silane (**2** and **3**) were synthesized using a one-pot co-condensation method. Impregnation of all the silica materials with ATS was successfully carried out and labelled as **1d**, **2d** and **3d** accordingly (d stands for ATS acronyms). For ATS release study, a method previously described was used[12] (see the SI for the loading and release experimental details).

The powder X-ray diffraction (PXRD) pattern of MCM-41, functionalized MCM-41 and their ATS loaded silica were examined (Fig. S2). MSNs show the characteristic reflection peaks of MCM-41 confirming the well-ordered 2D hexagonal mesostructure (*p6mm*). The molecular state of all the loaded silica materials was in amorphous state due to disordering of the ATS crystallinity when adsorbed or coated inside the nano silica pore.[8,12] DSC measurement confirms the structural phase transition of the free ATS (Fig. S3), a peak was observed for the endothermic change at 420 K corresponds to the ATS enthalpy melting point, but the peak disappeared in silica encapsulated ATS which signifies the amorphous state of the ATS in loaded silica obtained from the PXRD and Fourier transform infrared (FT-IR) spectroscopy (Fig S4a)[13]

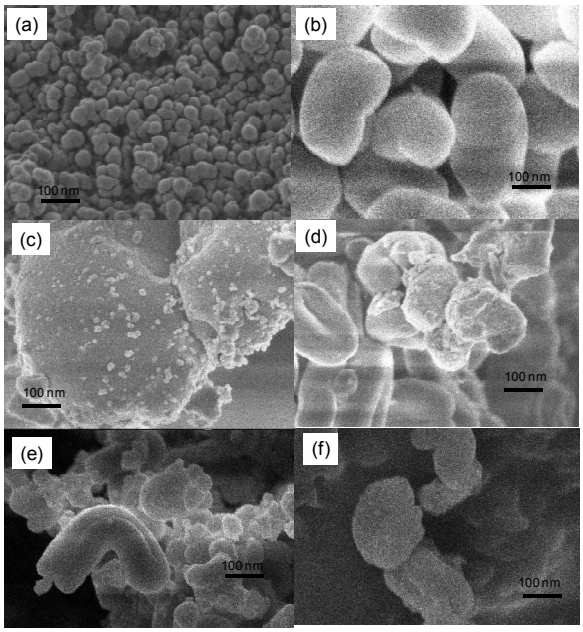

Fig. 1 SEM images of (a) **1**, (b) **1d**, (c) **2**, (d) **2d**, (e) **3** and (f) **3d**.

The well-ordered hexagonal MCM-41 with uniform pore sizes was measured by TEM (Fig. S5) while SEM image (Fig. 1) shows that **1** has spherical shape, while **2** and **3** exhibit long and curved hexagonal shape and **1d** and **3d** are bean like morphology, but **3d** shows a unique u tube shape.

The FT-IR spectra show as-synthesized, C-H stretching vibrational frequency 2925-2854 cm^{-1}. due to surfactant attachment In template free MCM-41, the characteristic stretching vibrational frequency around 1080 cm^{-1} and bending 900 cm^{-1} assigned to -Si-O-Si- fragment while 3395-3440 cm^{-1} is the isolated silanol υ(O-H) group assigment. Free MCM-41 has increased silanol density compared with the modified hybrids. The appearance of asymmetric bending NH_2 frequency around 1640 cm^{-1} further suggests the organo-amine grafting on silica frame work. Strong hydrogen bonding in the spectra of ATS loaded amine silica hybrids (**2d**) indicates amine from the silica and carboxyl group of the ATS interaction.[10a] Furthermore, disappearance of the 1759 cm^{-1} vibrational frequency (the carbonyl groups) in the loaded silica spectra attributed to ATS doping inside the silica pore.

X-ray Photoelectron Spectroscopy (XPS) further confirms the surface silanol functionalization with organosilica agents present in the samples. Sample **2** indicates significant amount of carbon (13.79 % C), oxygen (48.80 % O), silicon (30.11 % Si) and nitrogen (1.99 % N) elements respectively but no presence of (N) as expected in the sample **3,** carbon (12.68 % C), oxygen (57.09 % O), silicon (30.23 % Si) and sample **1,** carbon (3.72 % C), oxygen (61.63 % O), silicon (34.65 % Si) and 400 eV, 285 eV, and 104 eV nitrogen, carbon and silicon binding energies respectively(Fig S4b).

The N_2 adsorption/desorption analysis was used to characterize the silica interior pore properties (Table 1, Fig. S6), **1** has the largest surface area 1.53 × 10^3 m^2 g^{-1} and pore volume 7.83 × 10^{-1} cm^3 g^{-1}. Calcination at 550 °C enhances the MCM-41 particle surface area and its volume as this follows the usual concept in physical properties of matter volume (V) vs temperature (T).[14] We observed slight decrease in the surface areas and pore volumes of **2** and **3** (see Table 1) considered due to the silylating agent interaction with the silanol hydroxyl groups and drastic decrease in pore properties of **1d, 2d, 3d** ATS loaded silica materials in accordance with previous reports.[15]

Table 1 N_2 adsorption/desorption analysis of the unmodified and modified MSNs.

Sample	S_{BET} / m^2 g^{-1}	W_{BJH} / nm	Vp / cm^3 g^{-1}
1	1528	2.33	0.78
1d	377	2.10	0.20
2	233	2.36	0.14
2d	217	2.22	0.12
3	262	2.37	0.16
3d	93	2.30	0.05

Loading capacity and entrapment efficiency of the silica materials for ATS are calculated using UV-Vis spectrophotometry[10c] (see SI for detail equations). The wt. % of ATS loaded in the MCM-41 and modified MSNs are presented in **Table 2.** Drug loading capacity % of the modified silica **2** is 66.7 %. with its entrapment efficiency 100% This is attributed to strong bond interaction between amine and carboxyl group of the modified silica and ATS and its relatively large surface area unlike the unmodified silica **1** with more surface area and pore volume but could cargo 61.9 %. Nevertheless, **3** has the least loading capacity of 59.1 %. being the least surface area Organosilica functionalization through the covalent amide bond between the surface amino group of APTES and the phenyl of PPTCS intermolecular interactions with the carboxyl group of ATS serve as adsorptive platform for loading more ATS despite their reduced pore properties compared with calcined MCM-41(sample **1**) which has greater surface area and pore volume but loaded less ATS to the expectation.[16]

The TGA data expresses the weight loss of ATS (Fig. S7). Below 100 °C, the weight loss include ethanol loading solvent effect but above this temperature is due to the drug partial decomposition in the silica pore coat In other words, incomplete weight loss of the encapsulated ATS (less than 40%) up till 500°C heating could be attributed to the silica coat which is thermally stable and protects ATS from complete decomposition. The amount of ATS loss can be related to the quantity of the adsorbed drug which has been calculated with UV-Vis absorption technique using Beer-Lambert principle (Fig. S8).[17]

Table 2 ATS Drug loading and entrapment for Unmodified/Modified MSNs.

Sample	Loading capacity / %	Entrapment efficiency / %	ATS loading / mg
1d	61.9	93	9.3
2d	66.7	100	10
3d	59.0	72	7.2

The ATS release for all the modified silica loaded drugs followed a slow release pattern[18] (Fig. 2) attributed to their pore size properties, strong hydrogen bond affinity between the amine of the silica and carboxyl group of the ATS and silica loaded morphology.[10a] We strongly believe that the two latter points: existence of covalent bond between the silica functional end groups (amino /phenyl) and ATS carboxyl group with the drug's encapsulation morphologies had controlled the release rate of the drug. Within 3h, **1d** had released 73.9 wt % drug considering the silica channel width and large pore size whereas **2d** and **3d** released only 47.9 wt %, 54.4 wt % respectively at the same rate time. This behaviour of slow drug release is maintained for the modified silica materials till 24 h. Comparatively, **2d** has 69.9 % due to the chemical bond, **3d** is least release 63.4 % considering its small pore size and the phenyl ring hindrance. ATS release experiment was investigated at pH = 3.5, but no ATS release suspected to be due to the structural rearrangement of the drug.

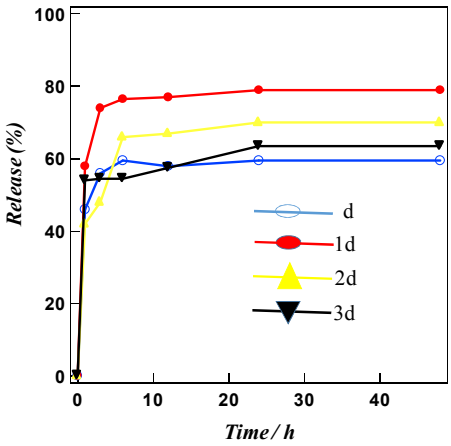

Fig. 2 Dissolution profiles of ATS from MSNs (2:1 w/w) system in 0.5 % SLS buffer at 37˚C, pH = 7.4.

In conclusion, we succeeded to prepare mesoporous silica sphere (MCM-41) and its amine, phenyl organically surface functionalized with long and curved hexagonal morphology respectively. The ATS inside the nano silica pore walls carrier controlled both the spherical and hexagonal silica morphologies into bean or novel u-tube shapes. The amount of the ATS loading was directly related to the pore surface area, the pore volumes and host-guest interaction while amine modified MCM-41 shows highest loading capacity of 66.7 % and 100 % entrapment efficiency. On the other hand, the strong hydrogen bond affinity in host-guest interaction for amine functionalized silica and phenyl ring steadily slow down the release rate compared with unmodified MCM-41. These aforementioned behaviors / properties of the biomaterials sound relevant in pharmacokinetics. Hence, we hope that the nano silica ATS medicine would gain due attention as the next high potent antimalarial oral delivery therapy supporting the World Health Organization effort towards ameliorate malaria disease.

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Notes and references

1 (a) World Health Organization, A global strategy for malaria 1993, control, http://www.who.int/malaria/publications /atoz/9241561610/en/ (b)I. Kleinschmidt, C. Schwabe, L. Benavente, M. Torrez, F. C. Ridl, J. L. Segura, P. Ehmer, and G. N. Nchama, *Am. J. Trop. Med. Hyg.,* 2009, **80,** 882.

2 (a) P. H. Van vianen, D. L. Klayman, A. J. Lin, C. B. Lugt, A. L. Van engen, H.J. Van Der Kaay, B. Mons, *Experimental Parasitology,*1990, **70**, 115; (b) N. J. White, *Malar J.* 2008, 1-6.

3 (a) R. Kannan, K. Kumar, D. Sehal, 2005, *Biochem J.,* **385,** 409. (b) H. H. P. Yiu, P. A. Wright, N. P. Botting, *J. Mol. Catal. B. Enzym.*, 2001, **15**, 81.

4 (a) T. M. Allen, P.R. Cullis, *Science,* 2004, **303**, 1818; (b) Y. Tozuka, E. Yonemochi, T. Oguchi, K. Yamamoto, *J. Colloid*

Interface Sci., 1998, **205**, 510; (c) T. Oguchi, T. Tozuka, S. Okonogi, E. Yonemochi, K. Yamamoto, *J. Pharm. Sci. Tech. Jpn.*, 1997, **57**, 168.
5 C. V Uglea, I. Albu, A. Vatajuna, M. Croitoru, S. Antoniu, L. Panaitescu, R. Ottenbrite, *J. Biomater. Sci. Polym. Ed.* 1994, **6** 633. (b) L.Xiong, X. Du, B. Shi, J. Bi, F. Kleitz, S.Z. Qiao, J. Mater. Chem. B., 2015, 3, 1712.
6 (a) K. Knop, R. Hoogenboom, D. Fischer, U. S. Schubert, *Angew. Chem. Int. Ed.*, 2010, **49**, 6288. (b) X. Du, S.Z. Qiao, small 2015 **11,** 392. (c)X. Du, L. Xiong, S.Z. Qiao, Adv. Healthcare Mater. 2015, **4,**771
7 (a) 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; (b) C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature*, 1992, **359**, 710.
8 (a) S.-W. Song, K. Hidajat, S. Kawi, *Langmuir,* 2005, **21**, 9568; (b) A. Yu, Y. Wang, E. Barlow, F. Caruso, *Adv. Mater,* 2005, **17**, 1737; (c) Y. L. Cheng, B. G. Trewyn, M. Dusan, J. Ksenija, X. Shu, J. Srdija, S. Y. Victor, *J. Am. Chem. Soc.,* 2003, **125**, 4451. (d) S.K. Das, M.K. Bhunia, D. Chakraborty, A.R. Khuda-Bukhsh, A. Bhaumik, Chem Commun. 2012, **48,** 2891.
9 (a) K. Cheng, C. C. Landry, *J. Am. Chem. Soc.* 2007, **129**, 9674; (b) D. S. Shephard, W. Zhou, T. Maschmeyer, J. M. Matters, C. L. Roper, S. Parsons, B. F. G. Johnson, M. J. Duer, *Angew. Chem., Int. Ed.* 1998, **37**, 2719.
10 (a) Y. Zhang, Z. Zhi, T. Jiang, J. Zhang, Z. Wang, S. Wang, *J. Control. Release,* 2010, **145**, 257. (b) S. Sadasivan, D. Khushalani, S. Mann, *J. Mater. Chem.,* 2003, **13,** 1023. (c) L. Palanikumar, E. S. Choi, J. Y. Cheon, S. H. Joo, and J-H. Ryu, *Adv. Funct. Mater.,* 2015, **25**, 957. (d) X. Du, B. Shi, J. Liang, J. Bi, S. Dai, S.Z. Qiao, Adv. Mater., 2013, **25,** 5981.
11 (a) X. Du, L. Xiong, S. Dai, F. Kleitz, S.Z. Qiao, Adv. Funct. Mater., 2014, **24,** 7627. (b) N. Niu. F. He, P. Ma, S. Gai, G. Yang, F. Qu, Y. Wang, J. Xu, ACS Appl. Mater. Interfaces, 2014, **6,** 3250 (c) T. Zhang, Z. Ding, H. Lin, L. Cui, C. Yang, X. Li, H. Niu, N. An, R. Tong, F. Qu, Eur. J. Inorg. Chem. 2015, 2277.
12 (b) C.Y. Chen, S. L. Burkett, H-X. Li, M.E. Davis, *Microporous mater* 1993, **2**, 27. (b) S. Jambhrunkar, S. Karmakar, A. Popat, M. Yu, C. Yu, *RSC Adv.*, 2014, **4**, 709.
13 F. Qu, G. Zhu, S. Huang, S. Li, J. Sun, D. Zhang, S. Qiu, *Microporous and Mesoporous Materials,* 2006, **92**, 1.
14 (a) V.G. Baonza, M. Taravillo, M. Caceres, J. Nunez, *Phys. Rev. B*, 1996, **53**, 5252. (b) J. R. MacDonald, *Rev. Mod. Phys.*, 1969, **41**, 316.
15 (a) H. Rao, X. Wang, X. Du, ZC. Xue, *J. Porous mater.*, 2013, **20**, 1231 (b) F. Qu, G. Zhu, H. Lin, W. Zhang, J. Sun, S. Li, S. Qiu, *J. Solid State Chem.*, 2006 **179**, 2027
16 (a) R. Mellaerts, C.A. Aerts, J. V Humbeeck, P. Augustijns, G. V Mooter, J. A. Martens, *Chem. Commun.*, 2007, ***13,*** 1375. (b) J. Andersson, J. Rosenholm, S. Areva, M. Linden, *Chem. Mater.* 2004, **16**, 4160. (c) P. Horcajada, A. Ramila, J. Pérez-Pariente, M. Vallet-Regı, *Microporous Mesoporous Mater.,* 2004, **68**, 105.
17 C. D. Nunes, P. D. Vaz, A. C. Fernandes, P. Ferreira, C. C. Romao, M. J. Calhorda, *Eur. J. Pharm. Biopharm.*, 2007, **66**, 357.
18 X.P Qiu, S. Leporatti, E. Donath, H. Mohwald, *Langmuir,* 2001, **17**, 5375.