“Using evaporation induced self-assembly for the direct drug templating of therapeutic vectors with high loading fractions, tunable drug release and controlled degradation”

Authors: T. Fontecave, C. Boissière, N. Baccile, F.J. Plou, C. Sanchez

Published in:

Chemistry of Materials 25 (23), pp. 4671–4678 (2013)

<http://dx.doi.org/10.1021/cm401807m>

Using Evaporation Induced Self Assembly for the Direct Drug Templating of therapeutic vectors with high loading fractions, tunable drug release and controlled degradation

Thomas Fontecave^a, Niki Baccile^a, Clement Sanchez^a, Francisco J. Plou^b, Cedric Boissiere^a

^aUPMC Univ. Paris 06, CNRS, UMR 7574 Laboratoire Chimie de la Matière Condensée de Paris
Collège de France, 11 place Marcelin Berthelot, 75005 Paris (France) F-75005, Paris, France

^bInstituto de Catalisis y Petroleoquimica, CSIC, 28049 Madrid, Spain

ABSTRACT: A novel one-step approach for designing non aggregated silica-based mesostructured therapeutic vectors is presented. Evaporation Induced Self Assembly was used in combination with amphiphilic drugs for preparing already loaded class I and class II hybrid materials containing from 50 to 60 wt% (up to 75 vol%) of drug. A good mesostructuration was able to promote an interfacial control of the drug release in PBS medium with a complete absence of the initial burst release very often described in literature for water soluble drug release experiments. The investigation of both release mechanisms and matrix dissolution was conducted via *in situ* ellipsometry and NMR. It proved that the nature of the drug/matrix interaction, the chemical composition of the drug/matrix interface, as well as the mesostructuration quality parameters must be taken into account at the same time for tuning the drug release rate, while maintaining the dissolution rate of silica at a reasonable level for limiting its toxicity. It proved also that non-calcined as made silica-based class I hybrid materials can be efficiently used for tuning drug release kinetics from one hour to day scale.

INTRODUCTION

The design of nanomaterials for drug transport and controlled release is a matter of utmost importance in biomedical science. Such therapeutic vectors, developed and tested in the last ten years, are aimed for accumulating in diseased tissues and deliver drug molecules at a tunable rate (Gref 1994, Brigger 2002, Horcajada 2008, Cheng 2009). The biodegradability of the employed material is a key parameter because of the potential risk of bioaccumulation and because of the influence of degradation kinetics on drug release rates (Pahaku Mitchell 2012, Soppimath 2001). Drug release from mesoporous silica-based vectors is particularly promising (Giri 2005, Vallet-Regi 2007, Rosenholm 2009, Liu 2009). Their tunable pore size and surface chemistry allow in principle a good drug/material surface interaction. Moreover, the easy functionalization of their external surface by organic moieties or polymers is a major advantage for site-specific targeting (Rosenholm 2010). and their degradation kinetics were proven accurately modulable in a large range by chemical modification of the silica matrix (Izquierdo-Barba 2010, Fontecave 2012, Pohaku-Mitchell 2012).

In general, their mesoporous network is built by self-assembly of surfactant molecules and condensation of the inorganic matrix around this assembly. The resulting hybrid material is either washed (Vallet-Regi 2001) or calcined (Colilla 2010) so that mesopores are formed and are accessible for drug loading. The drug loading is then achieved by successive incubations of the particles in a concentrated solution of drug (Charnay 2004). Higher drug loads are usually achieved within highly porous silica carriers presenting mesopores larger than 4 nm. However, this method leads to a part of the drug molecules adsorbed onto their internal surface, and to another part weakly entrapped at the heart of mesopores. These latter are rapidly released by diffusion and cause an initial burst of drug release which is difficult to control (Song Langmuir 2005). In order to maintain high drug loads and to avoid the presence of these weakly bound molecules which release is hard to control, the mesopores size has to be finely tuned for each specific drug, which obliges materials scientist to design and optimize a specific inorganic vector for each drug (Horcajada 2004).

Building a system in which a molecule is at the same time the structuring agent and the therapeutic agent represents an original approach in this field for it would allow the direct mesostructuration and encapsulating of high loads of drug, while insuring that each drug molecules interact in a similar way with the silica-based matrix. If the drug displays amphiphilic character, uncontrolled phase separation during sol-gel encapsulation can be avoided and the homogeneity of the material at the nanoscale is ensured by controlled nanosize separation of drug micelles within the inorganic or hybrid network. The three main advantages of this strategy are the

following: (i) a much simpler one step synthesis method in which no calcination and no post drug loading is required (obviously favorable in terms of atom economy), (ii) high drug loading can be achieved (more than 50 vol % according to usual syntheses of mesostructured silica with common structuring agents such as CTAB or nonionic surfactants), which decreases the amount of inorganic matrix in proportion, contributing to decrease its intrinsic toxicity, (iii) each drug molecule should interact in a similar manner with the silica-based wall. The release kinetic is thus in principle very easily tunable by modulating the drug-wall interaction and the degradation rate of the matrix. In this work, we investigated the validity of this concept by using three types of amphiphilic drug molecules: stearyl choline iodide (SC), glucosyl resveratrol (GR) and sophorolipids (SL). Choline esters were shown to be potentially useful for the treatment of Alzheimer disease (Brevet Patel 2005, Alcaro 2010). Resveratrol is a natural molecule contained in red wine and may be related with the decrease in coronary heart disease observed among wine drinkers (French paradox) (Frémont 2000). Glycosylation of resveratrol protects it from enzymatic oxidation, increases its absorption and thus extends its lifetime in the cell (Regev-Shoshani 2001, Biasutto, 2009). Sophorolipids are microbial glycolipids obtained from the culture broth of *Candida bombicola* in presence of glucose and fatty acids (Van Bogaert 2007). Anticancer properties of these compounds have been proposed in the late 90's (Scholz, 1998), and confirmed in more recent studies, sophorolipids influence the growth and apoptosis of H7402 human liver cancer cells (Chen, 2006). Their self-assembling properties have been previously investigated in (Baccile 2010) and (Baccile 2012). These molecules have surfactant properties (Figure 1) and were used in the present work for synthesizing mesostructured silica or class II hybrid materials by Evaporation Induced Self Assembly (EISA). For comparing the properties of mesostructured materials made out of those molecules, we prepared both particles and films by evaporation Induced Self Assembly (EISA) (particles were made through aerosol route, and thin films were made by dip-coating) (Brinker 1999, Boissiere 2011). Thin films were used as model systems for monitoring precisely by *in situ* ellipsometry the drug release and the degradation kinetics of the sol-gel matrix in undersaturation conditions of silica. Thanks to the low amount of matter involved with thin films experiments, we avoided the silica saturation and renucleation of silica particles or gel network detrimental for the comparison of the different drug systems investigated (details of the experimental protocol is described in the reference Fontecave 2012).

EXPERIMENTAL SECTION

Sophorolipids synthesis. In this work we used the open acidic form of sophorolipids, as shown in Figure 1. The detailed synthesis of these compounds was described in a previous work [Baccile et al., Green Chem., 2010].

Glucosyl-Resveratrol biosynthesis: A mix of (3-OH)- α -glucosylated resveratrol and (4'-OH)- α -glucosylated resveratrol was synthesized by scaling-up the enzymatic reaction described in reference (Torres 2011).

Figure 1: Drug molecules used for mesostructuring silica materials, A) stearylcholine Iodide, B) 3-O- α -D-glucosyl resveratrol C) 4'-O- α -glucosyl resveratrol, D) open acidic form of sophorolipid

Thin film synthesis:

Thin films were made by sol-gel process. Initial sol compositions are described in the table 1. The sols were coated on silicon wafers by dip-coating, at a constant rate of 6 mm.s⁻¹, in a 40% relative humidity atmosphere. Right after withdrawal, the sample is exposed to a 75% relative humidity atmosphere for 5 minutes. Films were then treated at 70°C for 2 days. For GISAXS, ellipsometry and TEM analyses, films were calcined 30 minutes at 200°C and 10 minutes at 300°C, in the case of GR, 30 minutes at 200°C and 10 minutes at 400°C, in the case of SL and 30 minutes at 200°C and 20 minutes at 550°C, in the case of SC.

Aerosol-made particles synthesis:

Initial sol compositions are described in table 1. For stearyl choline and sophorolipids, the home-made aerosol apparatus described in reference (Julian-Lopez 2007) was used. Briefly, droplets are generated by a TSI-6 jet atomizer. They are led by an air flow through 50 cm-long 250°C-heated quartz tube in which they stay a few

seconds. Resulting solid particles were collected with a PTFE filter. For glucosyl-resveratrol, a low-temperature aerosol process was developed. The aerosol is generated from the sol and led by dry air to a 3 meter long - 0.6 cm large - helical glass tube. The tube is thermostated at 60°C thanks to circulating warm oil. Before collection, the aerosol is bubbled in a 0.25 mol.L⁻¹ solution of ammonia in water in order to improve the condensation of the outer silica part of the particles, so that the particles do not stick one to another onto the filter.

*Table 1 : **Optimized** Composition of solution used for the preparation of film **and aerosol** materials in presence of different drugs.*

	GR film	SL film	SL-MTEOS film	SC film	GR aerosol	SL aerosol	SC aerosol
TEOS	1	1	0,7	1	1	1	1
MTEOS	-	-	0,3	-	-	-	-
Drug	0,17	0,06	0,06	0,18	0,17	0,1	0,18
H ₂ O	5	5	5	5	20	25	50
EtOH	60	60	60	60	20	25	600
HCl	1,1	1,1	1,1	1,1	0,36	0,0046	11

Characterizations. Grazing Incidence Small-Angle X-ray Scattering (GI-SAXS) was recorded using a Rigaku S-MAX 3000. Spectroscopic ellipsometry and Environmental Ellipsometric Porosimetry analyses were recorded with a M2000 Woolam ellipsometer equipped with a controlled atmosphere cell in which relative humidity was controlled by mass flow controllers (the setup and analysis protocol are described in reference Boissiere 2005). The analysis beam incidence angle was fixed at 70°. The modeling of the deposited sol-gel layers was performed with a simple Cauchy model. Transmission electron microscopy (TEM) was carried out using a JEOL 100 CX II microscope. TEM micrographs were processed with a slow scan CCD camera and analyzed with the Digital Micrograph program

Degradation study. A dedicated in-situ ellipsometric analysis cell was developed for the degradation study. Briefly, the aim of this analysis being to directly monitor the degradation kinetic of mesoporous model layers in simulated biological medium, we built a double layer liquid cell containing 50 mL of PBS medium. The PBS temperature was controlled and maintained by liquid circulation of hot water within the walls of the liquid cell via an external thermostatic circulation pump. Two non-birefringent windows were adapted for allowing the light beam of the ellipsometer to pass. The real temperature of PBS was controlled by a thermocouple. For each experiment, the 1 cm² film was introduced into the chamber after thermal equilibration and analysed

automatically every 60 seconds. All details of the experimental setup are given in the ref [described in paper of Thomas].

Drug release study. GR and SL: Drug release studies were made by UV spectroscopy by immersing 0.25 cm² of thin film at the bottom of a quartz cell containing 3 mL of PBS (temperature is controlled with a thermostatic bath). The absorbance is measured in the PBS at 315 nm to quantify the glucosyl-resveratrol released in the aqueous medium, and at 260 nm for the sophorolipids. SC: 2 cm² of thin film were immersed in 15mL of thermostated PBS. For each measurement, 0.5mL of the solution was taken off, dried and dissolved in deuterated methanol. ¹H-NMR was performed on a Bruker AVIII 300 Spectrometer. Integration of SC terminal methyl peak (at 0.9 ppm) was compared to integration of CD₂H-OD peak (at 3.35 ppm).

In order to monitor silica dissolution, the volume fraction of silica was computed at each time of the analysis. At each time, a system of three equations with three unknowns was resolved. The three unknowns are $f_{\text{SiO}_2}(t)$, $f_{\text{Drug}}(t)$ and $f_{\text{H}_2\text{O}}(t)$ which are the volume fractions of silica, Glucosyl-Resveratrol and water, respectively. The three equations are:

- (i) BEMA [$f_{\text{SiO}_2}(t)$, $f_{\text{Drug}}(t)$, $f_{\text{H}_2\text{O}}(t)$] = $n(t)$
- (ii) $f_{\text{Drug}}(t) = f_{\text{Drug}}(0) \cdot [1 - n f_{\text{Drug}}(t)]$
- (iii) $f_{\text{SiO}_2}(t) + f_{\text{Drug}}(t) + f_{\text{H}_2\text{O}}(t) = 1$

where BEMA is the Bruggeman Effective Medium Approximation, $n(t)$ is the refractive index of the thin film measured by ellipsometry and $n f_{\text{Drug}}(t)$ is the normalized molar fraction of drug released, measured by *in situ* UV spectroscopy. The refractive index of the drug was computed with a standard Bruggemann Effective Medium Approximation between drug and silica, considering that the volume fraction could be deduced from the mass fraction and the densities of each component of the films. A dense silica film was made with the same low-temperature thermal treatment for determining the refractive index of silica in the class I hybrid thin film. The variations of thickness were then considered to compute the remaining silica quantity, at each time.

RESULTS

Structuration by Stearoyl Choline

Figure 2: TEM pictures of silica mesostructured by Stearoyl Choline via EISA in A) film and B) particle shapes. C) N₂ physisorption isotherm of calcined particles.

Stearoyl Choline (SC) templated very efficiently silica as thin films exhibiting a good quality 2D hexagonal mesostructure was obtained (as confirmed by GISAXS and TEM after calcination) (Figure 2A and SI 1). The d-spacing value for the p6m phase is 4.2 nm and is consistent with TEM image. For measuring the size of the drug domains issued from the EISA processing of these drugs, we calcined the film at 550°C for 20 minutes and performed water adsorption-desorption followed by environmental ellipsometric porosimetry (EEP). The calcined matrix exhibited pore size of 2.1 nm with a narrow pore size distribution, proving that this drug is able to form very homogeneous domains

within the silica matrix (SI 2). Both the pore size and the 2D hexagonal mesostructure observed by TEM are very similar to those obtained at the same concentration with

CTAB surfactant, whose chemical structure is very close to that of stearoyl choline molecule (Grosso 2004). The templating efficiency of SC is thus very high. The same process of structuration by EISA was used for preparing mesostructured particles by spray drying. The spherical nanosize particles obtained exhibited a worm like type mesostructure (Fig 2). The drug loading, directly controlled by the initial SC/TEOS molar ratio of the solution is, in this case, fairly high (60 wt%) if compared with drugs post impregnation in mesostructured materials of similar pore size (Vallet-Regi 2007 Angewandte). If we assume that this drug behaves in a similar way than CTAB surfactant, the theoretical maximum load of such a vector (according to the maximum CTAB/Si molar ratio at which a mesostructure is reported by spray drying processing in literature) could be as high as 73wt %. After calcination of the powder, the narrow pore size distribution observed by nitrogen physisorption analysis (Fig. 2C and SI 3) proved that SC still efficiently templates silica matrix as expected. As a consequence, the concept of direct drug templating is valid for making therapeutic silica-based nanovectors.

Structuration by Glucosyl-Resveratrol

Glucosyl-Resveratrol (GR) templated silica thin films exhibit a poorly structured worm-like network. The pore size distribution obtained from water adsorption is broad (mainly between 2 and 6 nm) (SI 2). The d-spacing measured by GI-SAXS is around 7.2 nm (SI 1). The low quality of the mesostructuration is confirmed on TEM pictures by the presence of small stacks of short porous channels with a spatial average period of 7.5 nm.

Figure 3: TEM pictures of silica mesostructured by Glycosyl-Resveratrol via EISA in A) film and B) particle shapes. C) N₂ physisorption isotherm of calcined particles.

However, as nanosize domains observed are still in a few nanometers in size, the encapsulation mechanism is still likely to be due to a cooperative structuration of silica and GR whose micellization properties have been reported elsewhere (Torres 2011). The same process of structuration by EISA was used for preparing mesostructured particles by spray drying with high drug loading (52 wt %). The spherical nanosize particles obtained exhibited no apparent mesostructuration by TEM or SAXS (Fig. 3C). After calcination of the powder, a broad pore size distribution is observed by nitrogen physisorption analysis proving that aerosol processing of such molecule is perfectible (SI3). The origin of the low efficacy of GR in mesostructuring aerosol made particles comes probably from the two phenol groups of the hydrophobic tail which tend to interact with

silica oligomers by hydrogen bonding, and make more difficult the formation of micelles in such synthesis conditions. Moreover, the formation of pre-micellar aggregates common for such molecules [Takaya Sakai,^{*,†} Youhei Kaneko,[†] and Kaoru Tsujii *Langmuir* 2006, 22, 2039-2044 et *Adv. Synth. Catal.* 2011] is likely to slow down the formation of micelles during the EISA process. Finally, the solution evaporation being faster in spray drying (due to the presence of a significant amount of ethanol in the solution and dry air) than in film formation, the silica/GR seems to achieve mesostructuration only under slow evaporation conditions. As a conclusion, if the efficient encapsulation of high amount of GR is possible by EISA, the efficacy of using Glucosyl-Resveratrol

drugs for creating well defined drug domains with drug/matrix interactions-tunable release is still unclear from our data.

Structuration by Sophorolipid

Figure 4: TEM pictures of silica mesostructured by Sophorolipid via EISA in A) film and B) particle shapes. C) N₂ physisorption isotherm of calcined particles.

Sophorolipid (SL) templated silica thin films exhibit a typical worm-like mesostructure. This was studied before and this is shown by the continuous ring-shaped signal observed on the GI-SAXS pattern (*cf.* SI 1) which indicated a correlation distance of 5 nm in the in-plane direction. The wormlike structure is confirmed by the corresponding TEM images on Figure 4A. The encapsulation by cooperative mesostructuration is confirmed by the narrow pore size distribution of 2.4 nm obtained by water physisorption of the calcined film (SI 2). The same process of structuration by EISA was used for preparing mesostructured particles by spray drying. The SL-templated particles exhibit a worm-like

mesostructure (see Figure 4B), as observed for the thin films. The drug loading of this vector is in such conditions

of 51 wt% and can theoretically be increased between 56 and 60 wt% according to the SL/TEOS domain of mesostructuration reported in literature [Baccile 2010]. After calcination of the powder, nitrogen physisorption isotherm is characteristic of a super-microporous structure with pore size close to 1.8 nm diameter difficult to extract from BJH model (SI. 3). Hence in both film and aerosol materials, pore size distributions are compatible with an efficient templating of the silica matrix. As SL can efficiently form monodisperse nanodomains within aerosol particles, they can be used for testing the validity of drug/matrix interaction-controlled release.

Drug release and degradation of thin films

To compare drug release rates of different films produced in this study, we used Hill's equation with good accuracy (results given in table 2). From these data we could simply analyse the various parameters and interdependencies of the drug/matrix/interaction/structuration system and extract the critical parameters.

Table 2: Half release times of silica and drug obtained from Hill's equation.

	GR	SL	SL-MTEOS	SC	GR aerosol
$t_{1/2}$ drug release (min)	16	30	41	300	15
$t_{1/2}$ silica dissolution (min)	25	29	350	83	-

1) Mesostructuration impacts drug release rates.

Glucosyl-Resveratrol and Sophorolipids have the same type of hydrophilic glucose derivated head. They interact with the silica surface through hydrogen bonding. However, the SL release in PBS (pH=7.4) is about two times slower than GR release. It appears clearly that the mesostructuration quality has a strong impact on both drug release profile and kinetics (Fig. 5). The presence of aggregates in the GR-SiO₂ architecture (evidences by the broad pore size distribution obtained from physisorption isotherms of powder and film) promotes an irregular

Figure 5 : Drug and silica release profiles obtained in PBS solution at 37°C. Glucosyl-Resveratrol (GR), Stearoyl Choline (SC), Sophorolipid (SL)

release profile characterized by first bursts of GR release, followed by a more regular release profile that can be fitted by a standard Hill's equation. Conversely, SL was shown to have a well-structured vermicular network promoting narrow domains size distribution within the silica matrix. In such a case, each SL molecule *a priori* interacts directly with the silica walls. It is remarkable to observe that the slower and more controlled SL release resulting only from the nanostructuration homogeneity can be observed for such a high drug loading (51 wt%).

2) Drug/material interaction impacts drug release rates.

Sophorolipid and Stearoyl-Choline templated silica thin films have the same type of cylindrical drug networks. In both cases, the great majority of drug molecules are in direct interaction with the inorganic surface. However, the type of interaction between drug and silica is completely different in each case. In the case of SL, the sugar moiety is very likely to interact with the inorganic matrix by hydrogen bonding. In the case of SC, at pH=7.4 a strong electrostatic interaction exists between the positively charged quaternary ammonium head of the molecules and the negatively charged silanolate groups of the surface. This interaction leads to extremely slow drug release, with a half-time of 5 hours, and a total release after two days. Although not surprising, the strong impact of the drug/matrix interaction onto the release time is very interesting because it allows day scale release times with poorly condensed (non calcined) silica network, proving that the direct-drug-templating approach is a robust method for tailoring silica-based vectors with controlled dissolution/release kinetics.

3) Drug/material composition impacts drug release rates

By looking at the pure silica/SL system, one sees that half-times of silica dissolution and drug release have the

same Hill's equation release profile, suggesting that the progressive dissolution of the matrix is partially responsible for the SL release. Such a silica dissolution profile contrasts a lot with the linear dissolution profiles observed on pure mesoporous silica materials, proving that the drug/matrix interaction and/or the film composition and structure strongly impact the vector dissolution profile (Fontecave 2012). As already demonstrated in the same reference, the 30% methylated hybrid silica

Figure 6 : In situ monitoring of film relative thickness as a function of PBS soaking time

matrix is much more stable towards hydrolysis than pure silica matrix. The experiments here reported confirm that this trend is maintained in presence of mesostructured drug domains, with dissolution of the SL-MTEOS30 matrix being one order of magnitude slower than that of SL-TEOS. Although the interaction between SL and the 30% mol methylated matrix can be expected to be weaker, the SL release from SL-MTEOS30 is also found slower (by about 35%) than from SL-TEOS, while still exhibiting a regular release profile as expected from a drug-mesostructured material with a narrow drug domains size distribution (SI 2). As a consequence, the drug/material interaction is not the only parameter influencing the drug release. If now one compares the evolution of the film thicknesses versus time in PBS, one realises that drug release mechanisms are more complex than “simple” diffusion-limited and/or dissolution assisted mechanisms (Fig. 6). Indeed, the soaking in PBS medium promotes the swelling of the SL-based films while SC-based film thickness decreases with time. This strongly suggests that water molecules diffuse within SL-based films via the large silica/sophorose interphase which is able to dissolve high amount of water molecules. In a previous work, we have shown that the SL micelle/H₂O interface is constituted by 90% of water (Baccile 2012). Under such conditions, water molecules are likely to be good competitors for sophorose/silanolates hydrogen bonds and thus to promote the fast release of sophorolipid molecules (Fig. 7). Such mechanism is coherent with the fact that the methylation of 30% of silica centres slows down the swelling of the film (thus the amount of water diffusing at the SL/silica interface) and slows down the release of sophorolipids molecules. A similar mechanism is probably responsible for the fast release of many hydrophilic species adsorbed within mesoporous silica materials in literature (captopril (Qu

Figure 7: Scheme of water diffusion triggered release mechanism for Sophorolipids and StearoylCholine structuring agents.

2006), gentamicin (Doadrio 2004)). By extension, this fast diffusion of water at the heart of the mesostructured material is likely to promote early dissolution of the silica matrix thus faster drug release. Saccharide molecules can condense with silica to form Si-O-C bonds when the hybrid material is treated above 110°C (Abdelmouleh 2002, Castellano 2004). It was not observed in this study because we performed low-temperature thermal stabilization (70°C), but this effect can be considered for tailoring the release kinetics of saccharide derivatives. Conversely, the

hydrophilic Stearoylcholine/silica interface which is much narrower and less eager for water molecules does not facilitate their diffusion within the film (Baccile 2007). The progressive decrease of thickness suggests that the drug release mechanism at stake here is more related with a classical desorption of SC, probably followed by diffusion-triggered rearrangement of SC within the silica network. In such conditions, the dissolution of silica is slow at short time (dissolution at the external surface of the material) and progressively quickens as more internal surface is available for water. From the comparison of both systems, we learnt that if the strength of the drug/matrix interaction plays a major role in the drug release kinetic, strong modulations can be observed if water can diffuse and compete with it.

In this work, we proved that EISA strategy was a simple, fast and efficient process for producing silica- based particles directly mesostructured by amphiphilic therapeutic molecules. In this context, many biologically active compounds (vitamins, hormones, flavonoids, antibiotics, etc.) contain glycosidic moieties and exhibit surfactant properties. When prepared by spray drying, already loaded and non-aggregated spherical particles were obtained in one step. Very high drug loads could be obtained (up to 60 wt% in this work) while maintaining sophorolipids and stearyl choline structuring agents in monodisperse micellar domains periodically organized within the silica matrix. Even if the periodicity of the matrix is not necessarily the goal one should aim for in vectorization application, in this case it was shown to be a good quality criteria for promoting at the same time high amphiphilic drug loading and homogeneous drug/matrix interaction. This constitutes a strong improvement if compared with conventional silica-based mesoporous vectors that can hardly achieve both high loading and homogeneous drug/matrix interaction (Azais 2006). In addition, reaching very high drug loading impacts positively the global toxicity of the vector because much less inorganic material is used for their constitution. In parallel, we studied *in situ* both drug release kinetic and matrix dissolution in PBS medium. For that, drug-templated films of similar composition were prepared by EISA. By coupling ellipsometry investigations and NMR analysis of PBS solution versus time, we could decorrelate silica dissolution and drug release and studied the influences and interdependency of the matrix mesostructure, structure and composition onto dissolution and release kinetics. We proved that a good mesostructuration quality coupled with the drug-templating approach (thus homogeneous drug/silica interaction) is able to eliminate completely the initial burst release very often described in literature in release experiments of water soluble drugs. In a similar way than with class II hybrid mesoporous silica, we showed that non covalent drug/silica interactions of such mesostructured class I hybrid material could present a wide range of release kinetics, from minutes in the case of sugar-based hydrophilic head

interacting with silica-based surfaces, to hours, in the case of strong electrostatic interaction. Both release and dissolution kinetics were found impacted by the structuring capacity of the drug, the capacity of the organic/inorganic interface to solubilize water and the composition of the matrix proving that the controlled design of a therapeutic vector has to be considered globally for tuning the drug release rate and the dissolution kinetic of the matrix.

The direct drug templating approach presented here can be generalized to hydrophilic drug which can be functionalized with hydrophobic tails. Resulting molecules can have self-assembly properties (Couvreur 2008) and are also potential candidates for this novel strategy. Hydrophobic drugs are compatible with this approach as well. They can be solvated in the hydrophobic cores of bio-compatible surfactant micelles during the EISA process (Fatnassi 2010). Combined with the easy elaboration of class II hybrid materials and nano-composites by EISA, the direct drug templating is likely to promote in the near future a better method for mastering and tuning at the same time the complexity of drug/material interaction in liquid biological medium, and the intrinsic stability of the matrix that must be tuned for limiting its toxicity.

- (1) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Torchilin, V.; Langer, R. *Science* 1994, 263, 1600–1603.
- (2) Brigger, I.; Dubernet, C.; Couvreur, P. *Advanced drug delivery reviews* 2002, 54, 631–651.
- (3) Horcajada, P.; Serre, C.; Maurin, G.; Ramsahye, N. a; Balas, F.; Vallet-Regí, M.; Sebban, M.; Taulelle, F.; Férey, G. *Journal of the American Chemical Society* 2008, 130, 6774–80.
- (4) Cheng, K.; Peng, S.; Xu, C.; Sun, S. *Journal of the American Chemical Society* 2009, 131, 10637–44.
- (5) Pohaku Mitchell, K. K.; Liberman, A.; Kummel, A. C.; Trogler, W. C. *Journal of the American Chemical Society* 2012, 134, 13997–4003.
- (6) Soppimath, K. S.; Aminabhavi, T. M.; Kulkarni, a R.; Rudzinski, W. E. *Journal of controlled release : official journal of the Controlled Release Society* 2001, 70, 1–20.
- (7) Giri, S.; Trewyn, B. G.; Stellmaker, M. P.; Lin, V. S.-Y. *Angewandte Chemie (International ed. in English)* 2005, 44, 5038–44.
- (8) Vallet-Regí, M.; Balas, F.; Arcos, D. *Angewandte Chemie (International ed. in English)* 2007, 46, 7548–58.
- (9) Rosenholm, J. M.; Meinander, A.; Peuhu, E.; Niemi, R.; Eriksson, J. E.; Sahlgren, C.; Lindén, M. *ACS nano* 2009, 3, 197–206.
- (10) Liu, J.; Stace-Naughton, A.; Jiang, X.; Brinker, C. J. *Journal of the American Chemical Society* 2009, 131, 1354–5.
- (11) Izquierdo-Barba, I.; Colilla, M.; Manzano, M.; Vallet-Regí, M. *Microporous and Mesoporous Materials* 2010, 132, 442–452.
- (12) Fontecave, T.; Sanchez, C.; Azaïs, T.; Boissière, C. *Chemistry of Materials* 2012, 24, 4326–4336.
- (13) Vallet-Regí, M.; Rámila, A.; Del Real, R. P.; Pérez-Pariente, J. *Chemistry of Materials* 2001, 13, 308–311.
- (14) Colilla, M.; Manzano, M.; Izquierdo-Barba, I.; Vallet-Regí, M.; Boissière, C.; Sanchez, C. *Chemistry of Materials* 2010, 22, 1821–1830.

- (15) Charnay, C.; Bégu, S.; Tourné-Péteilh, C.; Nicole, L.; Lerner, D. A.; Devoisselle, J.-M. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V* 2004, 57, 533–40.
- (16) Song, S.-W.; Hidajat, K.; Kawi, S. *Langmuir : the ACS journal of surfaces and colloids* 2005, 21, 9568–75.
- (17) Horcajada, P.; Rámila, A.; Pérez-Pariente, J.; Vallet-Regí, M. *Microporous and Mesoporous Materials* 2004, 68, 105–109.
- (18) Patel, H. ; WO 2005/019157
- (19) Alcaro, S.; Arcone, R.; Costa, G.; De Vita, D.; Iannone, M.; Ortuso, F.; Procopio, A.; Pasceri, R.; Rotiroti, D.; Scipione, L. *Current pharmaceutical design* 2010, 16, 692–7.
- (20) Frémont, L. *Life Sciences* 2000, 66, 663–673.
- (21) Regev-Shoshani, G.; Shoseyov, O.; Bilkis, I.; Kerem, Z. *The Biochemical journal* 2003, 374, 157–63.
- (22) Van Bogaert, I. N. a; Saerens, K.; De Muynck, C.; Develter, D.; Soetaert, W.; Vandamme, E. J. *Applied microbiology and biotechnology* 2007, 76, 23–34.
- (23) Scholz, C.; Mehta, S.; Bisht, K.; Guilmanov, V.; Kaplan, D.; Nicolosi, R.; Gross, R. *Proc. Am. Chem. Soc. Polymer Preprints* 1998, 39, 168–169.
- (24) Chen, J.; Song, X.; Zhang, H.; Qu, Y.; Miao, J. *Applied microbiology and biotechnology* 2006, 72, 52–9.
- (25) Baccile, N.; Nassif, N.; Malfatti, L.; Van Bogaert, I. N. a.; Soetaert, W.; Pehau-Arnaudet, G.; Babonneau, F. *Green Chemistry* 2010, 12, 1564.
- (26) Baccile, N.; Babonneau, F.; Jestin, J.; Pehau-Arnaudet, G.; Van Bogaert, I. *ACS nano* 2012, 6, 4763–76.
- (27) Brinker, C. J.; Lu, Y.; Sellinger, A.; Fan, H. *Advanced Materials* 1999, 11, 579–585.
- (28) Boissière, C.; Grosso, D.; Chaumonnot, A.; Nicole, L.; Sanchez, C. *Advanced materials (Deerfield Beach, Fla.)* 2011, 23, 599–623.

- (29) Torres, P. ; Poveda, A. ; Jimenez-Barbero, J. ; Parra, J.L. ; Comelles, F. ; Ballestros, A. ; Plou, F. *Advanced Synthesis & Catalysis* 2011, 353, 1077-1086.
- (30) Julian-Lopez, B.; Boissière, C. ; Chanéac, C. ; Grosso, D. ; Vasseur, S. ; Miraux, S. ; Duguet, E. ; Sanchez, C. *Journal of Materials Chemistry* 2007, 17, 1563-1569
- (31) Boissière, C.; Grosso, D.; Lepoutre, S.; Nicole, L.; Bruneau, A. B.; Sanchez, C. *Langmuir : the ACS journal of surfaces and colloids* 2005, 21, 12362–71.
- (32) Grosso, D. ; Cagnol, F. ; Soler-Illia, G. ; Crepaldi, E. ; Amenitsch, H. ; Brunet-Bruneau, A. ; Bourgeois, A. ; Sanchez, C. *Advanced Functional Materials* 2004, 14, 309-322.
- (33) Vallet-Regi, M. ; Balas, F. ; Arcos, D. *Angewandte Chemie (International ed. In English)* 2007, 46, 7548-7558.
- (34) *Langmuir* 2006??
- (35) Abdelmouleh, M.; Boufi, S.; ben Salah, A.; Belgacem, M.N. ; Gandini, A. *Langmuir : the ACS journal of surfaces and colloids* 2002, 18, 3203-3208
- (36) Castellano, M.; Gandini, A.; Fabbri, P.; Belgacem, M.N. *Journal of colloid and interface science* 2004, 273, 505-511
- (37) Qu, F.; Zhu, G.; Huang, S.; Li, S. ; Sun, J. ; Zhang, D. ; Qiu, S. *Microporous and Mesoporous Materials* 2006, 92, 1-9.
- (38) Doadrio, A.L.; Sousa, E.M.B.; Doadrio, J.C.; Pérez Pariente, J.; Izquierdo-Barba, I. ; Vallet-Regi, M. *Journal of Controlled Release* 2004, 97, 125-132.
- (39) Azaïs, T.; Tourné-Péteilh, C.; Aussenac, F. ; Baccile, N. ; Coelho, C. ; Devoisselle, J-M. ; Babonneau, F. *Chemistry of Materials* 2006, 18, 6382-6390.
- (40) Couvreur, P.; Harivardan Reddy, L.; Mangenot, S. ; Poupaert, J.H. ; Desmaële, D. ; Lepêtre-Mouelhi, S. ; Pili, B. ; Bourgaux, C. ; Amenitsch, H. ; Ollivon, M. *Small* 2008, 4, 247-253.
- (41) Fatnassi M.; Tourné-Péteilh, Corine, Cacciaguerra, T.; Dieudonné, P. ; Devoisselle, J-M. ; Alonso, B. *New Journal of Chemistry* 2010, 34, 607-610