Functionalization of mesoporous materials with long alkyl chains as a strategy for controlling drug delivery pattern

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Mesoporous silica SBA-15 was prepared to evaluate its effectiveness as a matrix for the controlled delivery of macrolide-type antibiotics. Two types of material were used to evaluate the delivery: calcined samples and samples functionalized with octyltrimethoxysilane and octadecyltrimethoxysilane. The samples were charged with the macrolide antibiotic erythromycin and the release assays were carried out *in vitro*. It has been observed that the release rate decreases as the population of hydrophobic $-CH_2$ moieties in the host increases.

Introduction

A highly ordered hexagonal mesoporous silica structure SBA-15, has been synthesized using commercially available block-copolymer surfactants in strong acid media.[1] It possesses an ordered hexagonal arrangement of unidirectional mesoporous channels having diameters of 6.0–10.0 nm.[2] Indeed, the presence of micropores that connect the main mesopore channels has also been shown.[2] Its large pore size and high pore volume make this material suitable as a potential host for a variety of chemical compounds, including molecules with biological activity. In this context, it has been found that calcined SBA-15 can adsorb large quantities of several drugs inside the porous network and these are released under *in vitro* conditions in a quantitative manner.[3,4] However, in order to achieve an effective control of its release pattern it would be necessary to modify the chemical nature of the pore wall, as it has been shown for MCM-41.[5,6]

Several authors have used the modification of amorphous silica gel matrices with long alkyl chains for drug delivery systems. In one case, the release rate of dexmedetomidine is controlled by the degradation of the silica.[7] The functionalization of the silica with alkyl chains increases the hydrophobicity of the resulting material and decreases its degradation rate. Otsuka *et al.*[8] suggested that the affinity between the phytonadione, an "oily" drug, and the silica gel surface increases when the silica surface is modified with C18 chains, which affect the delivery rate.

Compared with amorphous silica drug carriers, the advantages of using ordered mesoporous materials are basically the presence of an ordered network of pores which are very homogeneous in size, a high pore volume, specific surface area and stability. Indeed, the very narrow pore size distribution, in the range 2.0–10.0 nm, together with the presence of a high concentration of silanol groups on the surface which can be functionalized to control pore size and surface properties, make ordered mesoporous materials suitable matrices for fine tuning drug delivery rate. Therefore, the drug delivery kinetics would be affected by the interactions between the drug and matrix, and would not be controlled by the degradation kinetics of the silica gel matrix.

In this work, we have modified the surface of the pore wall of SBA-15 with octyltrimethoxysilane (C8) and octadecyltrimethoxysilane (C18), in order to compare the release kinetics of the functionalized SBA-15 materials with those of calcined sample.

The functionalization of the pore wall with the long-chain hydrocarbon moieties C8 and C18 could have several effects. First, it will decrease the effective pore size; it will modify the chemical interaction between the pore surface and the adsorbed molecule, and will decrease the wettability of the surface by aqueous solutions.

We have chosen erythromycin as the model drug for release since it is a high molecular weight macrolide and we think that is more appropriate for the pore size of the SBA-15 (see Fig. 1 for its chemical 3D structure). Indeed, the most stable configuration of erythromycin in vacuum (Fig. 1), exposes a rather hydrophobic shell to the environment, in such a way

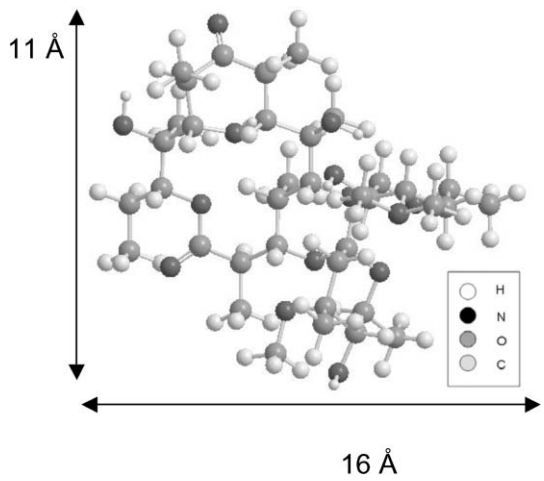

Fig. 1 Three dimensional picture of the erythromycin molecule.

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that an enhanced interaction with hydrophobic surfaces, like those resulting from the functionalization of the mesoporous surface with long alkyl chains, might be expected. Erythromycin belongs to the macrolide family of antibiotics, which are bacteriostatic agents that inhibit protein synthesis by binding reversibly to 50 S ribosomal subunits of sensitive microorganisms.[9]

Experimental

Materials preparation

SBA-15 materials were prepared according to the methods reported by Zhao.[1] In a typical procedure, 4g of triblock copolymer pluronic P123 [poly (ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol), M_{av} = 5800, Sigma-Aldrich] was dissolved in 138 g of water and 12.2 g of HCl (37 wt% solution) while stirring at 40 °C. After 2 h, 8.2 g of tetraethyl orthosilicate (TEOS, Sigma-Aldrich) was added. After aging with continuous stirring at 100 °C for 24 h in a Teflon® recipient hermetically closed, the solids were collected by filtration and dried in air at 40 °C. The surfactant was removed by calcination, which was carried out by increasing the temperature to 550 °C under a flow of nitrogen for 2 h, followed by 3 h in air. Chemical analysis shows that the surfactant is completely removed by this thermal treatment.

The SBA-15 materials were functionalized by anchoring hydrocarbons chains on the surface. This process consisted of the reaction between the calcined mesoporous SBA-15 and two alkoxysilanes with different chain size, octyltrimethoxysilane ($C_{11}H_{26}O_3Si$, Fluka) and octadecyltrimethoxysilane ($C_{21}H_{46}O_3Si$, Fluka). 1 eq (200 mg) of SBA-15 material were kept under inert atmosphere (argon) for 1 h and then refluxed with 4 meq of the silanes in 30 mL of toluene (T) or acetonitrile (ACE), for 24 h. The final samples were filtered, washed with toluene and acetonitrile, and dried at 60 °C.

In order to evaluate the delivery profile, the calcined SBA-15 and the samples modified with octyltrimethoxysilane (C8) and octadecyltrimethoxysilane (C18) with different solvents (toluene and acetonitrile) were formed into 0.1g disks (13 × 3 mm) by uniaxial (2.75 MPa) and then isostatic pressure (3 MPa). The different disks were immersed in a solution of erythromycin (30 mg mL^{-1}, drug/solid (w/w), 6 : 1) over a period of 3 days at room temperature while stirring. The erythromycin USP standard, was supplied by Sigma-Aldrich (ref. E0774). We have tested different solvents to evaluate the amount of drug absorbed into the SBA-15 matrices, particularly diethyl ether, methanol and acetonitrile. The use of acetonitrile leads to the highest amount of adsorbed drug, and therefore this has been the solvent of choice in this work.

Characterization

All the samples were characterized by X-ray diffraction (XRD), thermogravimetric analyses (TGA), elemental analysis, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and N_2 adsorption. The XRD patterns were obtained using a Philips XPert MDP (Cu Kα radiation) diffractometer with a multipurpose sample holder. The TGA was carried out between 30° and 800 °C in air (flow rate 100 mL min^{-1} with a heating rate of 10 °C min^{-1}) using a Perkin Elmer instrument. The surface area (BET) and pore size of the materials were determined by N_2 adsorption in a Micromeritics ASAP 2010 porosimeter. The pore size was calculated[10,11] using a method based on a simple geometric relation between the specific pore volume, obtained by N_2 adsorption (*t*-Plot Method), and the lattice spacing *d*, obtained from XRD data. Elemental Analysis was performed in a Macroanalyser Leco CNS-2000-I.

The surface modification was characterized by FTIR, with a Nicolet Nexus spectrophotometer in the range 4000–400 cm^{-1}. The FTIR spectra were recorded at room temperature in KBr pellets.

The hydrophobicity of calcined and functionalized SBA-15 was determined by measuring the amount of water adsorbed in the sample by using TGA. For this porpose, the samples were exposed for 3 days to 80% relative humidity and 0.34 kPa of vapor pressure at 30 °C by storing them in a vessel containing a saturated solution of KBr.

The amount of drug adsorbed on the samples was determined by TGA and elemental analysis.

Delivery procedure

The *In vitro* study of erythromycin release from the substrates was performed as follows. The release profile was obtained by soaking the disks in 30 mL of a simulated body fluid (SBF)[12] and maintained at 37 °C, while stirring. The erythromycin concentration in the liquid phase was evaluated by a HPLC method. RP-HPLC assays were performed with a liquid chromatographic system equipped with a Waters Alliance 2695 separations module (Waters, Milford, Massachusetts, USA), a variable-wavelength diode array detector, Waters 2996, and controlled by Millennium 32 software, at a 45 °C oven temperature. A X-Terra RP-18 reversed-phase column (5 μm, 3.9 × 150 mm), supplied by Waters, has been employed. The mobile phase consisted of 45 wt% acetonitrile, 15 wt% methanol and 40 wt% ammonium phosphate 0.01 M solution (v/v).[13] The flow rate was 1 mL min^{-1}. The effluent was monitored at 215 nm due to lower ε at other wavelengths and the injection volume was 50 μL. The erythromycin shows a peak at a retention time of 2.28 min (Fig. 2), with a 280 nm maximum (HPLC-Diode array detector measure) that is identical to an erythromycin measure in a conventional UV spectrophotometer.

Results and discussion

Characterization of calcined and functionalized SBA-15

Small-angle XRD of SBA-15 shows a well-resolved pattern with (100), (110) and (200) reflections, characteristics of the hexagonal structure of silica SBA-15. The XRD patterns of the samples after erythromycin adsorption and delivery showed no loss of structural ordering, with *d*(100) spacings of 8.7 nm for SBA-15 and SBA-15-Ery, and 9.4 nm for SBA-15-Ery after delivery.

The XRD patterns of both SBA-15-C8 and SBA-15-C18 indicate that the functionalization of the pore wall does not affect the structural order, showing *d*(100) values of

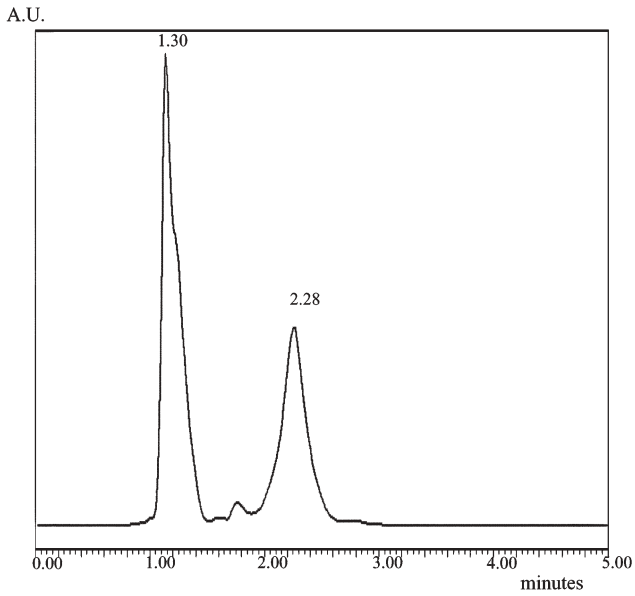

Fig. 2 Erythromycin chromatogram. t_r = 2.28 min. The peak at 1.3 min is due to the injection pressure.

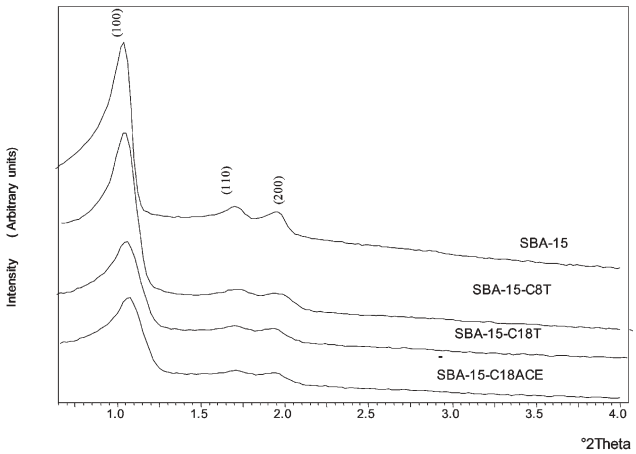

Fig. 3 XRD patterns of calcined SBA-15 and SBA-15 functionalized with C8 and C18 silanes in toluene (T) and acetonitrile (ACE).

$\sim$8.4 $\pm$ 0.2 nm. The XRD profiles are similar to that of calcined SBA-15, Fig. 3.

The presence of hydrocarbon chains attached to the pore wall after the silylation treatment was confirmed by FTIR (Fig. 4). In the SBA-15-C8 and SBA15-C18 spectra, the bands at 2926 cm^{-1} and 2854 cm^{-1} are attributed to the anti-symmetric and symmetric stretching vibration modes of the CH_2 groups, respectively,[14,15] which confirms the chemical modification of the SBA-15 with the different hydrocarbon

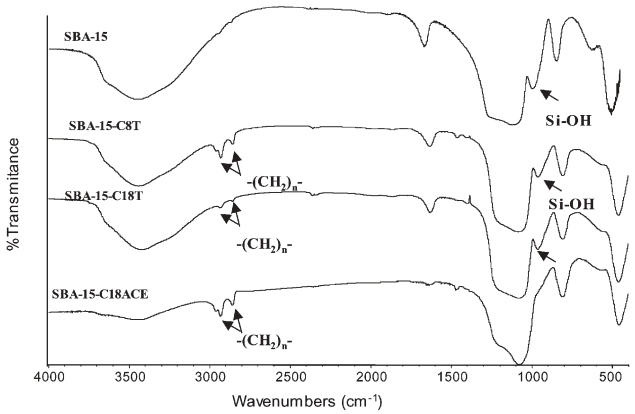

Fig. 4 FTIR spectra of SBA-15, SBA-15-C8T, SBA-15-C18T and SBA-15-C18ACE.

chains. Differences in the relative intensity of the CH_2 bands are observed as a function of the nature of the silane and solvent used in the functionalization procedure. The intensities of the bands can be compared because the weight of the samples in the IR analysis was the same. Indeed, Table 1 shows a similar variation in the carbon content. By using toluene as solvent, the anchoring of C8 moieties (0.99 mmol g^{-1} SiO_2) is more effective than that of C18 (0.15 mmol g^{-1} SiO_2). On the other hand, the high effectiveness of acetonitrile as solvent for the functionalization process is shown by the large amount of C18 attached to the pore surface (2.5 mmol g^{-1} SiO_2), one order of magnitude higher than that obtained for the less polar solvent toluene. It is believed that the polar surface of SBA-15 favours the penetration of the polar acetonitrile inside the pores.

Nitrogen isotherms of calcined and silylated SBA-15 are shown in Fig. 5, whereas Table 1 summarizes these results. It must be remarked that the microporosity exists in calcined SBA-15[1]. As expected, the introduction of the organic moieties leads to a decrease in pore diameter, surface area and pore volume, and this decrease is more noticeable for the matrix that contains the highest amount of organic material. Furthermore, the marked reduction in pore size shows that the pore wall is indeed decorated by organic moieties.

The effective uptake of erythromycin by the mesoporous materials when immersed in the acetonitrile solution of drug can be determined by TGA and elemental analysis. Table 2 shows the drug content of the matrices

The adsorption of erythromycin in different materials leads to a decreased in pore diameter, surface area, and pore volume (Fig. 5 and Table 2) and no micropores are detected. These facts indicate the presence of the drug in the pores after the adsorption of erythromycin. However, as can be seen by

Table 1 Amounts of the functional groups and the structural characteristics of SBA-15 mesoporous structure for different silanes and solvents

Sample	C/wt%	$mol_{chain}\ g_{SiO2}^{-1}$	mol_{chain}/mol_{SiO2}	$d_{(100)}$/nm	$S_{BET}/m^2\ g^{-1}$	D_p/nm	V_p/cm^3
SBA-15	—	—	—	8.7	787	8.8	1.06
SBA-15-C8T	7.2	0.99×10^{-3}	0.059	8.4	559	8.2	0.83
SBA-15-C18T	2.5	0.15×10^{-3}	0.009	8.5	535	8.3	0.83
SBA15-C18ACE	25.3	2.47×10^{-3}	0.148	8.2	71	5.4	0.19

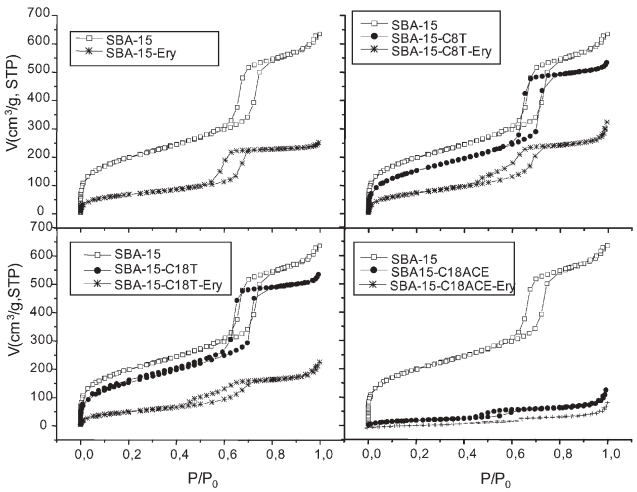

Fig. 5 Nitrogen adsorption isotherms of SBA-15, SBA-15-C8T, SBA-15-C18T and SBA-15-C18ACE before and after adsorption of erythromycin.

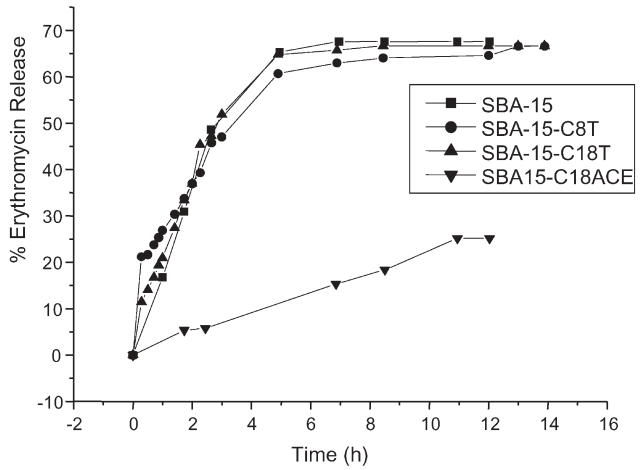

Fig. 6 Erythromycin release (%) from SBA-15, SBA-15-C8T, SBA-15-C18T and SBA-15-C18ACE.

analyzing the adsorption isotherms and BET results of the unmodified and functionalised SBA-15, the erythromycin molecules do not fully occupy the available space. On the other hand, the results of Table 2 show some differences among the substrates in the pore filling effectiveness of the erythromycin. These differences reflect changes in the affinity of the surface of the matrices for erythromycin. The adsorption of the drug is basically a surface phenomenon and it would be a function of the surface area of the material and the chemical affinity between the surface and the drug. It is interesting to observe that the sample with the lowest area (71 $m^2\ g^{-1}$, SBA-15-C18 ACE), contains as much erythromycin as those having surface areas above 500 m^2 g. This behavior suggests that the functionalization of the surface with the C18 chain strengthens the interaction between the erythromycin and matrix mesoporous surface.

Delivery process

The diffusion model that we have used to study the delivery of erythromycin from this system considers the leaching of the drug by the bathing fluid, which is able to enter the drug-matrix phase through pores. The drug is assumed to dissolve slowly into the fluid phase and diffuses from the system along the solvent-filled capillary channels.

For release from a planar system having a porous matrix, according to theoretical analysis by Higuchi,[16] the equation which can be used to predict the release rates is:

$$a = k\ t^{1/2} \quad (1)$$

Table 2 Loading (wt%) and textural properties of samples containing erythromycin

Sample	Erythromycin/ wt%	$d_{(100)}$/ nm	S_{BET}/ $m^2\ g^{-1}$	D_p/ nm	V_p/ cm^3
SBA-15-Ery	34	8.7	250	7.2	0.39
SBA-15-C8T-Ery	13	8.4	271	6.1	0.50
SBA-15-C18T-Ery	18	8.5	182	5.5	0.34
SBA-15-C18ACE-Ery	15	8.1	8	—	0.04

where a is the amount of drug released after time t and k is a release constant. When the drug is dispersed in the matrix and diffusion occurs through solvent-filled pores, the formulation of the constant is:

$$k = f(D,\varepsilon,\tau,C,A) \quad (2)$$

where D is the diffusivity of the drug in the solvent, τ is the tortuosity factor of the system, ε is the porosity of the matrix, A is the total amount of drug present in the matrix and C is the solubility of the drug in the solvent used.

The amount of erythromycin released was plotted against the square root of time and a linear regression was used to fit the data, with the origin included among the data (Fig. 6). It can be seen in the figure that the linear regression is obeyed only for release values lower than 50%, *i.e.*, this would be a threshold limit for valid application of the Higuchi model.

The values of the kinetic constant k of eqn. 1 for the matrices are presented in Table 3. A decrease of the rate constant brought about by functionalizing the matrix is found, but this effect is strongly dependent upon the silane and solvent used in the process. A decrease in the release rate by nearly one order of magnitude is achieved by anchoring 2.5 mmol of C18 per g of SiO_2. On taking into account the relative sizes of the erythromycin molecule (Fig 1) and pore matrices, the observed effect of the funtionalization over the release kinetic cannot be attributed to an eventual influence of the pore size. Nevertheless, in order to investigate an eventual influence of the pore size of the host material on the

Table 3 Kinetics constant (k), regression coefficient (r) and water adsorbed on the samples

Sample	Drug/wt%	k/r	Adsorbed water/wt%
SBA-15	34	18.5/0.999	6.5
	23	17.4/0.991	
	8.5	17.8/0.992	
SBA-15-C8T	13	13.1/0.927	3.0
SBA-15-C18T	18	17.0/0.991	4.4
SBA-15-C18ACE	15	2.2/0.996	2.0
MCM-41	29	12.6/0.996	—

erythromycin delivery, a different mesoporous material was tested. For this purpose, MCM-41 was used and compared with SBA-15. MCM-41 also presents a hexagonal arrangement of pores, and a pore size distribution centred at 3.8 nm.

The amount of erythromycin adsorbed in MCM-41 material (29 wt%), is close to that of SBA-15 (Table 2). Indeed, the delivery from MCM-41 is slightly slower than that shown by SBA-15 (Table 3). This decrease in the delivery rate may be due to the small pore size in MCM-41 compared to SBA-15, but the release is, nevertheless, much faster than observed for the sample functionalized with C18 moieties, which have larger pores than MCM-41. It seems, therefore, that such differences in pore size cannot be used to explain the differences observed in the delivery behavior of these funcionalized matrices. In fact, the chemical nature of the pore wall surface appears to play the most important role in determining erythromycin delivery rate . In this regard, it is interesting to observe that the kinetic constant decreases with the carbon content of the host matrix, *i.e.*, the sample containing 2.5 mmol of C18 moieties has the slower delivery pattern. Indeed, according to the data of Table 3, the rate constant is independent of the drug content. Therefore, it seems that anchoring the hydrocarbon chains to the pore surface reinforces the interaction between the matrix and the adsorbed erythromycin. The amount of water adsorbed in the sample has been determined by TG analysis (weight loss <120 °C) and taken as a measure of the relative hydrophobicity of the material. It can be seen in Table 3 that the surface becomes less hydrophilic as the carbon content of the solid increases, *i.e.*, the pattern observed for the kinetic constant k. It seems, therefore, that the observed influence of the organic groups present in the surface of SBA-15 on the release profile of erythromycin is probably due to the presence of hydrophobic interactions between the drug and the surface. However, it should be also taken into account that the wettability of the surface by the aqueous SBF solution also decreases as the surface becomes less hydrophilic, and this could also contribute to the observed release pattern.

Conclusions

The hexagonal mesoporous material SBA-15 is a suitable matrix for the adsorption and *in vitro* release of the macrolide antibiotic erythromycin. An effective control of the release rate of this antibiotic has been achieved by funtionalizing the surface of SBA-15 with hydrophobic long chain hydrocarbon moieties. For the sample containing the largest amount of $-CH_2$ groups, the release rate decreases by a factor of nearly one order of magnitude as compared with that of calcined SBA-15.

Acknowledgements

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