Adsorption and Release of Ampicillin antibiotic from Ordered Mesoporous Silica

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ABSTRACT

In this work the adsorption and the release of ampicillin - a β-lactam penicillin-like antibiotic - from MCM-41, SBA-15, and (amino functionalized) SBA-15-NH_2 ordered mesoporous silica (OMS) materials were investigated. The silica matrices differ for their pore size (SBA-15 vs. MCM-41) mainly, and also for surface charge (SBA-15 and MCM-41, vs. SBA-15-NH_2). OMS samples were characterized through small-angle X-rays scattering (SAXS), transmission electron microscopy (TEM), N_2 adsorption–desorption isotherms, Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and potentiometric titrations. The quantification of immobilized and released ampicillin was monitored by mean of UV-Vis spectroscopy. Experimental adsorption isotherms evidenced that ampicillin's loading is not related to the pore size (d_{BJH}) of the adsorbent. Indeed the maximal loadings were 237 mg/g for SBA-15 (d_{BJH} = 6.5 nm), 278 mg/g for MCM-41 (d_{BJH} = 2.2 nm), and 333 mg/g for SBA-15-NH_2 (d_{BJH} = 5.6 nm). Loading seems, instead, to be related to the surface charge density (σ) of the sorbent surface. Indeed, at pH 7.4 ampicillin drug is negatively charged and likely prefers to interact with SBA-15-NH_2 ($\sigma_{SBA\text{-}15\text{-}NH2}$ = +0.223 C m^{-2}) rather than the slightly negatively charged silicas ($\sigma_{SBA\text{-}15}$ = -0.044 C m^{-2} and $\sigma_{MCM\text{-}41}$= -0.033 C m^{-2}). Similarly, ampicillin release is affected by interfacial interactions. Indeed, we found a burst release from pure silica samples (SBA-15 and MCM-41), whereas a sustained one from SBA-15-NH_2 sample. We explain this behavior as a result of an attractive interaction between the protonated amino group of SBA-15-NH_2 and the negatively charged carboxylate group of ampicillin. In summary, in order to obtain a sustained drug release, the chemical nature of the matrix's surface plays a role which is more important than its textural features. SBA-15-NH_2 matrix is hence a suitable candidate for local sustained release of antibiotic drugs.

KEYWORDS: ampicillin; ordered mesoporous silica; functionalization; adsorption; drug delivery.

1. Introduction.

Osseointegration was discovered in 1969 when Branemark observed that a piece of titanium inserted in a rabbit bone was not easily removed because anchored in the tissue [1]. The osseointegration process begins after the surgical insertion of an implant and is possible when the material is biocompatible, and allows for the spontaneous formation of hydroxyapatite layers [2]. However, after the implant insertion inflammatory processes (as peri-implantitis) can occur [3]. This can cause the loss of the supporting bone [1] and different types of osteomyelitis [4]. These diseases can be treated with systemic administration of antibiotic or anti-inflammatory drugs (via injection or ingestion) to prevent necrosis and bone neoformation that can degenerate in a chronic state [4,5]. This type of antibiotic therapy is highly inefficient. Indeed, only a low amount of drug can reach the infected region, so that the therapy needs to be repeated several times. Moreover, large amounts of administrated drugs can cause several drawbacks such as sensitivity, bacterial resistance, or superinfections. The proposed solution to all these problems is the *'in situ'* drug administration [5,6]. Nowadays different materials - i.e. antibiotic-impregnated cements, pastes, powders, degradable sponges or fleeces - are used in orthopedic surgery [7]. Nevertheless, only few nanomaterials can both easily be osseointegrated and slowly release antibiotic or anti-inflammatory drugs [5,8–11].

Ordered mesoporous silicas (OMSs), firstly synthesized in the early 90s [12,13], are interesting matrices for drug delivery [4,14–23]. Relevant features of OMSs are the high surface area (up to 1000 m^2/g), the narrow distribution of the pores size (2-30 nm), the high pore volume (1-3 cm^3/g) and the possibility to introduce several types of surface functional groups [24–30]. Moreover, OMSs display high biocompatibility [19,31–34] and allow for osseointegration if placed in a simulated body fluid [9,11]. All these features make OMSs very interesting matrices for biomedical applications as depot carriers for *'in situ'* controlled drug release. The use of OMS for drug delivery, dates back to 2001, when Vallet-Regì et al. studied the immobilization of ibuprofen on MCM-41

[35]. After that pioneering work the drug-loading capacity, and the sustained drug release from OMSs (i.e. MCM-41 and SBA-15) with different antibiotic or anti-inflammatory drugs - i.e. ibuprofen [36–38], gentamicin [39] and amoxicillin [25,40,41] - were widely investigated. Moreover, those studies showed how to modulate the drug loading and the release kinetics by functionalizing the surface of OMS samples [14,17,25,36,37,42,43], or by changing the pH, at which the drug loading is carried out [14,38,42,44,45].

Ampicillin is a penicillin-like molecule belonging to the class of β-lactam antibiotics, useful for the treatment of infections mediated by both gram-negative and gram-positive bacteria [5,7]. Ampicillin is usually administered in combination with sulbactam [46–48], a powerful and highly specific inhibitor of β-lactamases [49]. In particular, the therapeutic ampicillin dosage is 1.5-12 g/day for adults and 150 mg/kg/day for children [46]. After the administration, the maximal concentration of ampicillin in blood serum mainly depends on the patient's age. For adults (from 20 to 65 years) who have been administered with 2 g of ampicillin, the maximal measured concentration is about 80-110 μg/mL [50]. While for children (from 1 to 12 years) a dose of 40-80 mg/kg every 6 h results in an ampicillin concentration of 177-200 μg/mL [51]. Due to its high purity and low cost, ampicillin can be used as a model drug for a better understanding of the processes that regulate the adsorption and the release of therapeutic molecules from OMS-based depot systems. Recently, the release of ampicillin from different materials - amorphous silica, calcium silicate, silica/polycaprolactone, and $xCaO{\cdot}SiO_2$ - was studied [5,52]. These studies showed that the rate of release is affected by the chemical composition of the used material. Singh et al. used silica-based nanotubes for the adsorption and release of ampicillin [53]. They found that both adsorption and release rates are determined by the pore size of the carrier.

The aim of this work is to understand which, between the textural features and the chemical nature of the surface, plays the most important role to address the adsorption and release phenomena of ampicillin antibiotic from ordered mesoporous silica. To this purpose we compared the behavior of

three different OMS samples, namely, SBA-15, SBA-15-NH_2 and MCM-41. In particular, we investigated the effect of pore size (SBA-15 versus MCM-41) and surface charge (SBA-15 versus SBA-15-NH_2) toward ampicillin adsorption (isotherms and kinetics) and release in simulated physiological conditions.

2. Materials and methods.

2.1 Chemicals.

Tetraethoxysilane (TEOS, 98%), pluronic copolymer 123 ($EO_{20}PO_{70}EO_{20}$), cetyltrimethylammonium bromide (CTAB, > 99 %), (3-aminopropyl)triethoxysilane (APTES, > 98 %), sodium hydroxide, hydrochloride acid (37 %), ampicillin sodium salt, acetic acid (> 99.7 %), sodium acetate (> 99 %), sodium dihydrogen phosphate (99.0 %), and sodium phosphate dibasic (≥ 99.5 %) were purchased from Sigma-Aldrich (Milan, Italy). Standard buffers at pH 1, 4, 6, 9, and 10 were purchased from Hanna Instruments (Szeged, Hungary). All chemical reagents were used without further purification.

2.2 Synthesis and characterization of SBA-15, MCM-41, and SBA-15-NH_2 samples.

SBA-15 and MCM-41 were synthesized according to the methods reported in refs. [27] and [54,55] respectively. We just remind that the synthesis occurs with a cooperative templating mechanism between TEOS and either Pluronic copolymer 123 (SBA-15) or CTAB (MCM-41). The organic surfactants were removed by calcination at 550°C for 5 h. The functionalized SBA-15-NH_2 was prepared using APTES through a post-functionalization method described in ref. [27]. The resulting solid was filtered and washed with acetone and dried overnight under vacuum at room temperature.

All materials were characterized through N_2 adsorption–desorption isotherms (texture), SAXS and TEM (structure), FTIR (functional groups), potentiometric titrations (surface charge density, σ), and thermogravimetric analysis (TGA). Textural analysis was carried out on an ASAP 2020

instrument, by determining the N_2 adsorption/desorption isotherm at 77 K. Before analysis MCM-41 and SBA-15 samples were heated at 250°C at a rate of 1°C/min under vacuum for 12 h, while SBA-15-NH_2 was heated at 110°C at the rate of 1°C/min under vacuum for 24h. The Brunaeur-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) method, calculated from the desorption branch of N_2 isotherm, were used to calculate surface area, pore volume and pore size distribution respectively. Small-angle X-ray scattering (SAXS) pattern was recorded with a S3-MICRO SWAXS camera system (HECUS X-ray Systems, Graz, Austria), as elsewhere reported [27]. Thin-walled 2 mm glass capillaries were filled with the sample for the scattering experiments. The scattering patterns were recorded for 1 h. Transmission electron microscopy (TEM) images were obtained on a JEOL 100S microscope, finely ground samples were placed directly onto formvar-coated electron microscopy nickel grids. Fourier transform infrared (FTIR) studies were conducted with a Bruker Tensor 27 spectrophotometer equipped with a diamond-ATR accessory and a DTGS detector. A number of 256 scans at a resolution of 2 cm^{-1} were averaged from wave number 4000 to 400 cm^{-1}. Surface charge density (σ vs. pH curves) of all samples was determined through potentiometric titrations which were carried out through an automatic titrator, Titrando 836 from Methrom (Herizau, Switzerland). The titration method is described in detail in ref. [56]. Thermogravimetric analysis (TGA) was carried out in the range 25−1000 °C (heating rate = 10 °C·min^{-1}), and under oxygen flow (flow rate = 50 mL·min^{-1}) by mean of a Mettler-Toledo TGA/STDA 851.

2.3 **Ampicillin adsorption and release from mesoporous silica samples**

The adsorption isotherm of ampicillin on mesoporous silica (SBA-15, MCM-41, SBA-15-NH_2) was carried out by suspending different samples (50 mg each) of mesoporous silica in 50 mM phosphate buffer (pH 7.4) solutions having ampicillin concentrations in the range between 0.5 and 25 mg/mL. The suspensions were kept under rotation for 24 h at 298 K. The adsorbed amount of ampicillin was

obtained by calculating the difference between the initial and the final (equilibrium) concentration of ampicillin in the adsorption solution. The concentration of ampicillin was determined through a calibration curve (in the concentration range 0.1-2.0 mg/mL) by measuring the absorbance at λ = 268 nm through a Cary UV-Vis spectrophotometer. After the immobilization ampicillin-loaded silica samples were collected through centrifugation, dried under vacuum, and analyzed through ATR-FTIR to assess the drug immobilization. The adsorption kinetics experiments of ampicillin adsorption on OMS samples were carried out similarly to what done for the isotherms. The only difference is that liquid samples were withdrawn at different fixed times (in the range 0 - 24 h) from the OMS/ampicillin suspension and analyzed through UV-Vis to assess the residual ampicillin concentration in the liquid phase.

In vitro drug release was carried out by suspending 50 mg of ampicillin-loaded mesoporous silica in 25 mL of 50 mM phosphate buffer saline solution (PBS: pH 7.4 and 0.15 M NaCl) at 37°C in an orbital shaker (120 rpm). At fixed time intervals, a volume of 5 mL of solution was withdrawn and the same volume of PBS was replaced to maintain sink conditions. The amount of released ampicillin was quantified through UV-Vis spectrophotometry, using a calibration curve in the concentration range 0.1 - 2.0 mg/mL (100 - 2000 μg/mL). The amount of ampicillin released was calculated taking into account the dilution caused by the introduction of fresh PBS after each withdrawal [57].

3. Results and discussion

3.1 Characterization of OMSs

Fig.1 shows the adsorption/desorption N_2 isotherms of SBA-15, SBA-15-NH_2 and MCM-41. SBA-15 and SBA-15-NH_2 exhibit a type IV isotherm with a sharp increase of N_2 volume adsorption at the relative pressure of p/p°= 0.6-0.7. The H1 hysteresis cycle is associated to a channel-like

mesoporous structure. Respect to the curve of SBA-15, that of SBA-15-NH_2 is shifted toward low adsorbed nitrogen volumes. This result is consistent with a decrease of the pore volume due to the functionalization step with APTES.

MCM-41 isotherm strongly differs from that of SBA-15 and SBA-15-NH_2. It shows a similar increase of N_2 volume adsorption but at a lower relative pressure, between 0.2 and 0.4 p/p°, as expected by the smaller pores of MCM-41. In this case the isotherm is reversible and does not show any appreciable hysteresis loop. Table 1 reports the values of surface area, pore size, and pore volume for the three mesoporous silica samples.

Table 1. Surface area, pore volume, and pore size of SBA-15, SBA-15-NH_2 and MCM-41.

Sample	[a]S_{BET} (m^2/g)	[b]Pore volume (cm^3/g)	[c]$d_{BJH\text{-}Des}$ (Å)	[d]*a* (Å)
SBA-15	867	1.23	64.5	113.4
SBA-15-NH_2	408	0.69	55.7	113.4
MCM-41	1169	0.91	22.3	40.1

[a] Surface area calculated by the BET method (pressure range 0-1 p/p°).
[b] Pore volume (calculated at p/p°=0.99).
[c] Pore diameter calculated by applying the BJH method to the desorption branch of the isotherm.
[d] Lattice parameter obtained by SAXS.

Samples were also characterized through SAXS technique (Fig. 2a). SBA-15 and SBA-15-NH_2 show the typical pattern of hexagonal phases, with an intense peak and two weak peaks due to the reflections of (100), (110) and (200) planes, respectively. This confirms that the functionalization of SBA-15 with APTES does not modify the ordered hexagonal structure. Also MCM-41 shows a SAXS pattern with the three typical peaks of hexagonal phases but, in this case, they occur at higher *q* (scattering vector) values than those recorded for the other two OMSs. This is due to the reciprocal relationship which occurs between plane distance, *d*, and the scattering vector, *q*, ($d=2\pi/q$). The lattice parameter (*a*) determined by SAXS measurements was 113.4 Å for SBA-15 and SBA-15-NH_2, and 40.1 Å for MCM-41 (Table 1).

Figures 2b-d show TEM micrographs of the three mesoporous silica samples. It can be seen that MCM-41 sample is constituted by quasi-spherical nanoparticles with diameters in the range 60-100 nm (Fig. 2b), whereas SBA-15 and SBA-15-NH_2 samples have bigger particles with sizes around 1.0 μm (Fig. 2c and 2d). The TEM images show the cylindrical channels for all samples.

The FTIR spectra of SBA-15 and MCM-41 (Fig. 3a) show two intense peaks at 1060 and 800 cm^{-1} assigned to the asymmetric and symmetric stretching vibrations of Si-O-Si group, respectively. Moreover, a peak at 450 cm^{-1} is due to the deformation modes of the O-Si-O group [25]. The peak at 960 cm^{-1} is attributed to Si-OH stretching [58,59]. SBA-15-NH_2 sample shows a spectrum similar to those of SBA-15 and MCM-41 (Fig. 3a) but, in addition, we observe a weak peak around 1558 cm^{-1} (Fig. 3b) attributed to the bending of -NH_2, which confirms the amino-functionalization [25,60,61]. Thermogravimetric analysis (TGA) of OMS samples was then carried out. Figure 4 shows the comparison among the percentage mass loss (Δm) profiles as a function of temperature for OMS samples. The mass loss observed for all samples at T < 200 °C can be ascribed to the removal of humidity and to the condensation of surface silanols. This last likely occurring for the pure silica samples (i.e. MCM-41 and SBA-15). Above 200 °C only SBA-15-NH_2 has a consistent mass loss (Δm = 9.4 %) due to the burning of organic groups. This is a further confirmation of the occurred functionalization with APTES.

Figure 5 shows the surface charge density (σ) versus pH curves obtained for the three OMS samples. SBA-15 and MCM-41 samples show a very similar trend, that is, the surface charge is close to zero at acidic pH and becomes negative for pH > 6. It can be noticed that for pH > 8, SBA-15 surface charge is more negative than that of MCM-41. This may be due to a slightly more acidic behavior of SBA-15 silanols compared to those of MCM-41 [62]. As a result of the functionalization, the surface charge density of SBA-15-NH_2 becomes highly positive at acidic pH values. σ remains positive up to pH = 9.7, which is the point of zero charge of that material. Consequently, at the pH value used to immobilize the ampicillin (pH = 7.4) SBA-15 and MCM-41

carry a slightly negative surface charge ($\sigma_{SBA\text{-}15}$ = -0.044 C m^{-2} and $\sigma_{MCM\text{-}41}$= -0.033 C m^{-2}) while SBA-15-NH_2 has a highly positive surface charge ($\sigma_{SBA\text{-}15\text{-}NH2}$ = +0.223 C m^{-2}). At that pH, ampicillin carries a negative net charge (pk_{aCOOH} = 2.96, pk_{aNH3} = 7.22)[63]. Hence its adsorption on SBA-15-NH_2 would involve attractive electrostatic interactions. This will be discussed further in next paragraphs.

3.2 Determination of the adsorption isotherms and kinetics of ampicillin on OMSs

The different OMS samples, SBA-15, SBA-15-NH_2 and MCM-41, were then used as carriers for the adsorption of ampicillin antibiotic. The adsorption isotherms (T = 298 K) were determined plotting the loading of ampicillin on OMS (L_A, $mg_{ampicillin}/g_{OMS}$) versus the equilibrium concentration of ampicillin in the adsorbing solution (C_r, $mg_{ampicillin}$/mL). We used three different models - namely: Freundlich, Langmuir, and Temkin - to fit the experimental adsorption data. Figure 6a shows the adsorption isotherm fitted by the Freundlich equation [64–67]:

$$L_A = K_F C_r^{1/n} \qquad (1)$$

Where, K_F (mL/g) and 1/n (dimensionless) are the Freundlich constants, that is, empirical parameters specific for each adsorbent-adsorbate pair at a given temperature. The Freundlich model considers a heterogeneous adsorbent surface with non-identical adsorption sites. This results in the formation of a multi layer of adsorbed molecules. K_F is the support capacity, while l/n is the heterogeneity factor. The closer the latter is to 0 the more heterogeneous the adsorbent surface. Figure 6b shows the fitting obtained with the Langmuir model:

$$L_A = \frac{L_0 K_L C_r}{1+K_L C_r} \qquad (2)$$

Where, L_0 and K_L are the maximum mono-layer coverage capacity and the Langmuir constant, respectively. Differently by the previous model, here only a monolayer of adsorbed molecules is allowed. Moreover, Langmuir isotherm refers to homogeneous adsorption, that is, it can only occur

at a finite number of identical and equivalent localized sites, with no lateral interaction and steric hindrance between the adsorbed molecules, even on adjacent sites.

Finally, Figure 6c shows the fitting obtained with the Temkin model:

$$L_A = \frac{RT}{b_T} lnA_T C_r \qquad (3)$$

Where, b_T is the Temkin constant and A_T is the isotherm equilibrium binding constant.

These models were chosen among many available since they resulted in quite good fittings of our experimental results. In particular, as shown in Table 2, Freundlich and Lamgmuir fittings were better than Temkin as confirmed by their higher correlation coefficients (R). In Figure 6, adsorption isotherm curves have higher slopes for MCM-41 respect to SBA-15. This resulted, in our experimental conditions, in the maximal loadings of 278 mg/g and 237 mg/g for MCM-41 and SBA-15 respectively. The materials are both pure silica, but with different particle and pore size (see Figure 2 and Table 1). This result seems to mean that the wider pore size of SBA-15, compared to that of MCM-41, does not necessarily result in a higher ampicillin loading.

Table 2. Isotherm parameters for ampicillin adsorption on OMS samples.

	Freundlich			**Langmuir**			**Temkin**		
Sample	**K_F (mL/g)**	**1/n**	**R**	**L_0 (mg/g)**	**K_L (dm^3/mg)**	**R**	**b_T**	**A_T (L/g)**	**R**
SBA-15	10	0.9	0.985	1588	0.006	0.986	38	0.6	0.906
SBA-15-NH_2	18	0.85	0.988	1035	0.015	0.991	26	0.6	0.949
MCM-41	16	0.9	0.986	495	0.04	0.984	37	1.5	0.976

On the contrary, a lower pore size results in a higher surface area which is, evidently, widely available for ampicillin adsorption. However, also surface area does not seem to be the distinctive feature for obtaining a high loading. Indeed, SBA-15-NH_2 sample, with a S_{BET} = 408 m^2/g 'only', resulted in the highest loading (333 mg/g). Our hypothesis is that this result is due to the chemical nature of SBA-15-NH_2 surface. This gives rise to attractive forces between the adsorbent and the adsorbate. Indeed at pH 7.4 SBA-15-NH_2 carries a positive surface charge (Figure 5), hence we

expect a favorable electrostatic interaction with the carboxylate group of ampicillin. Singh et al. immobilized ampicillin on aminated mesoporous silica nanotubes (mSiNTs) [53]. They found that the loading capacity was affected by the presence of CTAB during the synthesis since it increased both pore size and surface area of the obtained material. In particular a loading of 110 mg/g was obtained for the material synthesized without CTAB, whereas a loading capacity of 180 mg/g in the presence of CTAB. Both values were, nevertheless, lower than those obtained with our adsorbents.

We also investigated the adsorption kinetics of ampicillin on OMSs samples (Figure 7). The slope of the curve gives an estimation of the adsorption rate. This follow the series MCM-41 > SBA-15 > SBA-15-NH_2. The adsorption of molecules on mesoporous silica particles is a multistep process (1. external diffusion; 2. internal diffusion; 3. adsorbent-adsorbate interaction). We ascribe the trends shown in Figure 7 to the lower particle size of MCM-41 which permits a faster diffusion, likely both external and internal (shorter channels). Nevertheless, ampicillin adsorption on SBA-15-NH_2, although occurred with a slower rate, reached the highest loading.

3.3 FTIR characterization of ampicillin-loaded OMS samples.

The adsorption of ampicillin on OMS samples was also confirmed by FTIR spectra (Figure 8). Figure 8a shows the chemical structure of ampicillin molecule while the relative FTIR spectrum, in the whole spectral range 4000-400 cm^{-1}, is shown in Figure 8b. Figure 8c shows a comparison among the FTIR spectra of free ampicillin and the ampicillin-loaded OMS samples in the range between 1900 cm^{-1} and 1300 cm^{-1}. In that figure, the peaks at 1764 cm^{-1} and 1693 cm^{-1} are attributed to the -C=O of the beta-lactam ring and of the amide bond respectively, those at 1640 cm^{-1} and 1378 cm^{-1} are instead related to the symmetric and asymmetric stretching of -COOH. Finally, the peaks at 1582 cm^{-1}, 1525 cm^{-1}, and 1455 cm^{-1} are assigned to the N-H deformation of the amide bond, the -NH_2 bending, and the –C=C–C stretching of aromatic ring, respectively [25,63,68]. Figure 8c displays also the FTIR spectra of the ampicillin-loaded OMS samples (MCM-41, SBA-

15, and SBA-15-NH_2). We notice that most peaks occurring in the spectrum of the free ampicillin occur also in the FTIR spectra of ampicillin-loaded OMS samples. In particular, we observe the peaks at 1765 cm^{-1} (-C=O of the beta-lactam ring) and the shoulder at 1685 cm^{-1} (amide bond) [63], and those at 1640 and 1395 cm^{-1} (symmetric and asymmetric stretching of –COOH) [27,37] and the shoulder at 1590 cm^{-1} (N-H deformation) [25].

Finally, we observe the peaks in the range 1525-1540 cm^{-1} (–NH_2 bending) and that at 1460 cm^{-1} (C=C-C stretching of the aromatic ring) [25,63]. We also notice that the spectrum of ampicillin loaded on SBA-15-NH_2 looks different from those of the drug loaded on SBA-15 and MCM-41. In particular, the broad band between 1700-1500 cm^{-1} shows additional peaks, like that at 1600 cm^{-1} and 1580 cm^{-1}, likely due to an electrostatic interaction between the protonated amino group of SBA-15-NH_2 and the carboxylate of ampicillin [25].

In summary, FTIR measurements confirm that ampicillin is successfully adsorbed on all OMS samples and that the chemical nature of the surface groups, that is, silanols (MCM-41 and SBA-15) or amino (SBA-15-NH_2), results in a different intermolecular interaction between the drug and the adsorbent. Indeed, at pH 7.4 the carboxylate of ampicillin is fully dissociated (pK_{aCOOH} = 2.96), whereas the -NH_2 group is only partially protonated (pK_{aNH2} = 7.22)[63]. Therefore we expect an attractive electrostatic interaction between the carboxylate of ampicillin with the protonated amino group of SBA-15-NH_2 (see Figure 5). On the contrary, electrostatics would be much less important for the interaction between the only partially charged amino group of ampicillin and the slightly negatively charged silica (SBA-15, MCM-41) surface.

3.4 Ampicillin release from OMS samples

The release of ampicillin from SBA-15, SBA-15-NH_2 and MCM-41 matrices was then studied. The ampicillin-loaded OMS samples, with similar ampicillin loadings around 200 mg/g, were suspended in PBS (pH 7.4) at 37°C, and the concentration of released ampicillin was measured at different

times according to the procedure described above (see par. 2.3). We should specify that loadings cannot exactly be the same for the three samples, since they can only approximately be controlled due to the different ampicillin/OMS affinity. Nonetheless, any difference in the initial loading becomes not important since the release kinetics is expressed in mass % according to the following equation [26,57,69]:

$$\frac{M_t}{M_0}\% = A_{Max}(1 - e^{-kt}) \qquad (4)$$

where M_t is the mass released at time t, M_0 is the total mass of ampicillin adsorbed in the material, A_{Max} is the maximal mass (%) released, k is the release rate constant, and t is the time. Figure 9 shows the kinetics of ampicillin release from the different OMS samples.

MCM-41 and SBA-15 materials gave rise to a burst release, reaching a maximal amount of released drug of A_{Max} = 56 % and 42 % respectively within the first two hours (Figure 9). Differently, SBA-15-NH_2 resulted in a more gradual release, reaching a release maximum A_{Max}= 30 % after about 8 hours. The maximal amount of released drug corresponds to an ampicillin concentration in the release solution of 327 μg/mL, 210 μg/mL, and 145 μg/mL for MCM-41, SBA-15, and SBA-15-NH_2, respectively. We observe that the latter value (SBA-15-NH_2) is comparable to the concentration of ampicillin in the serum of both adults and children [50,51]. Similar release kinetics were reported for amoxicillin release from pure and functionalized SBA-15 and MCM-41 [25,41]. Also in those cases the maximal amount of drug was released in the first hours. The authors justified their results considering that the sustained release is regulated by a diffusion mechanism which is affected by steric hindrance. We propose that in our system, the release is affected by the competition between the affinity of ampicillin for the adsorbent surface and its solubility in the release medium (PBS) [70]. This results in a quick desorption particularly from the silica surfaces as a result of a weak electrostatic interaction (both attractive and repulsive). Differently, the electrostatic attraction between ampicillin and SBA-15-NH_2 surface, prevents a quick and

significant release. Indeed the occurrence of an attractive electrostatic interaction between the protonated amino groups of SBA-15-NH_2 and the carboxylate groups of ampicillin is clearly seen in the FTIR spectra in Figure 8c.

4. Conclusions

In this work the adsorption and the release of ampicillin from mesoporous silica matrices, differing in pore size and chemical proprieties due to surface functionalization, were studied. Results show that complex mechanisms and interactions address drug loading and release. The materials we compared had either different pore size but similar surface charge (SBA-15 and MCM-41), or similar pore size but different surface charge (SBA-15 and SBA-15-NH_2). It emerges that both ampicillin adsorption (isotherms and kinetics) and release are affected by the surface charge more than by the pore size. The interaction between a specific drug and a specific adsorbent is a very peculiar phenomenon. To the best of our knowledge, what found here for ampicillin/OMS systems was not previously available in the literature. The low negative surface charge of the two pure silica SBA-15 and MCM-41 matrices favors a rapid and efficient release at pH 7.4, this being slightly higher for MCM-41. Indeed, MCM-41 particles are much smaller than those of SBA-15 therefore a larger amount of adsorbed drug is likely to be located on the external surface or close to the pore openings. In the case of SBA-15-NH_2 matrix, electrostatic interactions favor a sustained release of ampicillin antibiotic. Indeed release from amino-functionalized material is slower than that measured for pure silica. The sustained release is likely due the presence of protonated amino groups that favorably interact with the negatively charged carboxylate of ampicillin. Future work will be necessary to investigate the use of SBA-15-NH_2 matrix for the fabrication of a depot system for a locally sustained release of ampicillin.

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References

[1] A.D. Pye, D.E.A. Lockhart, M.P. Dawson, C.A. Murray, A.J. Smith, A review of dental implants and infection, J. Hosp. Infect. 72 (2009) 104–110. doi:10.1016/j.jhin.2009.02.010.

[2] H.-A. Hansson, T. Albrektsson, P.-I. Branemark, Structural aspects of the interface between tissue and titanium implants, J. Prosthet. Dent. 50 (1983) 108–113.

[3] A. Mombelli, P.N. Lang, The diagnosis and treatment of peri-implantitis, Periodontology. 17 (1998) 63–76.

[4] D. Molina-Manso, M. Manzano, J.C. Doadrio, G. Del Prado, A. Ortiz-Pérez, M. Vallet-Regì, E. Gòmez-Barrena, J. Esteban, Usefulness of SBA-15 mesoporous ceramics as a delivery system for vancomycin, rifampicin and linezolid: A preliminary report, Int. J. Antimicrob. Agents. 40 (2012) 252–256. doi:10.1016/j.ijantimicag.2012.05.013.

[5] M. Catauro, F. Papale, G. Roviello, C. Ferone, F. Bollino, M. Trifuoggi, C. Aurilio, Synthesis of SiO2 and CaO rich calcium silicate systems via sol-gel process: Bioactivity, biocompatibility, and drug delivery tests, J. Biomed. Mater. Res. - Part A. 102 (2014) 3087–3092. doi:10.1002/jbm.a.34978.

[6] S. Kamath, S. Sinha, E. Shaari, D. Young, A.C. Campbell, Role of topical antibiotics in hip surgery: A prospective randomised study, Injury. 36 (2005) 783–787. doi:10.1016/j.injury.2005.01.001.

[7] P. Huiras, J.K. Logan, S. Papadopoulos, D. Whitney, Local antimicrobial administration for prophylaxis of surgical site infections, Pharmacotherapy. 32 (2012) 1006–1019. doi:10.1002/phar.1135.

[8] A. Chaudhari, M. V Cardoso, J. Martens, K. Vandamme, I. Naert, J. Duyck, Bone tissue response to BMP-2 adsorbed on amorphous microporous silica implants, J. Clin. Periodontol. 39 (2012) 1206–1213. doi:10.1111/jcpe.12005.

[9] A.J. Salinas, M. Vallet-Regí, Bioactive ceramics: from bone grafts to tissue engineering, RSC Adv. 3 (2013) 11116–11131. doi:10.1039/c3ra00166k.

[10] M. V. Cabañas, J. Peña, J. Román, C. Ramírez-Santillán, M.C. Matesanz, M.J. Feito, M.T. Portolés, M. Vallet-Regí, Design of tunable protein-releasing nanoapatite/hydrogel scaffolds for hard tissue engineering, Mater. Chem. Phys. 144 (2014) 409–417. doi:10.1016/j.matchemphys.2014.01.011.

[11] M. Vallet-Regí, Nanostructured mesoporous silica matrices in nanomedicine, J. Intern. Med. 267 (2010) 22–43. doi:10.1111/j.1365-2796.2009.02190.x.

[12] C.T. Kresge, M.E. Leonowicz, W.J. Roth, J.C. Vartuli, J.S. Beck, Ordered mesoporous molecular sieves synthesized by liquid-crystal template mechanism, Nature. 359 (1992) 710–712. doi:10.1038/355242a0.

[13] C.J. Brinker, Y. Lu, A. Sellinger, H. Fan, Evaporation-Induced Self-Assembly: Nanostructures Made Easy, Adv. Mater. 11 (1999) 579–585.

[14] A. Baeza, M. Colilla, M. Vallet-Regí, Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery, Expert Opin. Drug Deliv. 12 (2015) 319–337. doi:10.1517/17425247.2014.953051.

[15] D. Berger, L. Bajenaru, S. Nastase, R.-A. Mitran, C. Munteanu, C. Matei, Influence of structural, textural and surface properties of mesostructured silica and aluminosilicate carriers on aminoglycoside uptake and in vitro delivery, Microporous Mesoporous Mater. 206 (2015) 150–160. doi:10.1016/j.micromeso.2014.12.022.

[16] N.Ž. Knežević, J.-O. Durand, Large pore mesoporous silica nanomaterials for application in delivery of biomolecules, Nanoscale. 7 (2015) 2199–2209. doi:10.1039/C4NR06114D.

[17] A.L. Doadrio, J.M. Sánchez-Montero, J.C. Doadrio, A.J. Salinas, M. Vallet-Regí, A molecular model to explain the controlled release from SBA-15 functionalized with APTES, Microporous Mesoporous Mater. 195 (2014) 43–49. doi:10.1016/j.micromeso.2014.04.019.

[18] V. Mamaeva, C. Sahlgren, M. Lindén, Mesoporous silica nanoparticles in medicine-Recent advances, Adv. Drug Deliv. Rev. 65 (2013) 689–702. doi:10.1016/j.addr.2012.07.018.

[19] L. Maggini, I. Cabrera, A. Ruiz-Carretero, E.A. Prasetyanto, E. Robinet, L. De Cola, Breakable mesoporous silica nanoparticles for targeted drug delivery, Nanoscale. 8 (2016) 7240–7247. doi:10.1039/C5NR09112H.

[20] A. Bertucci, E.A. Prasetyanto, D. Septiadi, A. Manicardi, E. Brognara, R. Gambari, R. Corradini, L. De Cola, Combined Delivery of Temozolomide and Anti-miR221 PNA Using Mesoporous Silica Nanoparticles Induces Apoptosis in Resistant Glioma Cells, Small. 11 (2015) 5687–5695. doi:10.1002/smll.201500540.

[21] T. Kjellman, X. Xia, V. Alfredsson, A.E. Garcia-Bennett, Influence of microporosity in SBA-15 on the release properties of anticancer drug dasatinib, J. Mater. Chem. B. 2 (2014) 5265–5271. doi:10.1039/C4TB00418C.

[22] M. Monduzzi, S. Lampis, S. Murgia, A. Salis, From self-assembly fundamental knowledge to nanomedicine developments, Adv. Colloid Interface Sci. 205 (2014) 48–67. doi:10.1016/j.cis.2013.10.009.

[23] M. Piludu, L. Medda, F. Cugia, M. Monduzzi, A. Salis, Silver Enhancement for Transmission Electron Microscopy Imaging of Antibody Fragment-Gold Nanoparticles Conjugates Immobilized on Ordered Mesoporous Silica, Langmuir. 31 (2015) 9458–9463. doi:10.1021/acs.langmuir.5b02830.

[24] Z. Tao, Mesoporous silica-based nanodevices for biological applications, RSC Adv. 4 (2014) 18961. doi:10.1039/c3ra47166g.

[25] F. Sevimli, Y. Aysen, Surface functionalization of SBA-15 particles for amoxicillin delivery, Microporous Mesoporous Mater. 158 (2012) 281–291. doi:10.1016/j.micromeso.2012.02.037.

[26] A. Nieto, M. Colilla, F. Balas, M. Vallet-Regì, Surface electrochemistry of mesoporous silicas as a key factor in the design of tailored delivery devices, Langmuir. 26 (2010) 5038–5049. doi:10.1021/la904820k.

[27] L. Medda, M.F. Casula, M. Monduzzi, A. Salis, Adsorption of Lysozyme on Hyaluronic Acid Functionalized SBA-15 Mesoporous Silica: A Possible Bioadhesive Depot System, Langmuir. 30 (2014) 12996–13004. doi:10.1021/la503224n.

[28] Y. Liu, X. Ding, J. Li, Z. Luo, Y. Hu, J. Liu, L. Dai, J. Zhou, C. Hou, K. Cai, Enzyme responsive drug delivery system based on mesoporous silica nanoparticles for tumor therapy in vivo, Nanotechnology. 26 (2015) 145102–14115. doi:10.1088/0957-4484/26/14/145102.

[29] B. Lebeau, A. Galarneau, M. Linden, Introduction for 20 years of research on ordered mesoporous materials., Chem. Soc. Rev. 42 (2013) 3661–3662. doi:10.1039/c3cs90005c.

[30] P. Kipkemboi, A. Fogden, V. Alfredsson, K. Flodström, Triblock copolymers as templates in mesoporous silica formation: Structural dependence on polymer chain length and synthesis temperature, Langmuir. 17 (2001) 5398–5402. doi:10.1021/la001715i.

[31] Z. Chen, Z. Li, Y. Lin, M. Yin, J. Ren, X. Qu, Bioresponsive hyaluronic acid-capped mesoporous silica nanoparticles for targeted drug delivery, Chem. Eur. J. 19 (2013) 1778–1783. doi:10.1002/chem.201202038.

[32] S. Williams, A. Neumann, I. Bremer, Y. Su, G. Dräger, C. Kasper, P. Behrens, Nanoporous silica nanoparticles as biomaterials: evaluation of different strategies for the functionalization with polysialic acid by step-by-step cytocompatibility testing, J. Mater. Sci. Mater. Med. 26 (2015). doi:10.1007/s10856-015-5409-3.

[33] S.P. Hudson, R.F. Padera, R. Langer, D.S. Kohane, The biocompatibility of mesoporous silicates., Biomaterials. 29 (2008) 4045–55. doi:10.1016/j.biomaterials.2008.07.007.

[34] K. Braun, A. Pochert, M. Beck, R. Fiedler, J. Gruber, M. Lindén, Dissolution kinetics of mesoporous silica nanoparticles in different simulated body fluids, J. Sol-Gel Sci. Technol. 79 (2016) 319–327. doi:10.1007/s10971-016-4053-9.

[35] M. Vallet-Regi, A. Rámila, R.P. Del Real, J. Pérez-Pariente, A new property of MCM-41: Drug delivery system, Chem. Mater. 13 (2001) 308–311. doi:10.1021/cm0011559.

[36] A. Szegedi, M. Popova, I. Goshev, S. Klébert, J. Mihály, Controlled drug release on amine functionalized spherical MCM-41, J. Solid State Chem. 194 (2012) 257–263. doi:10.1016/j.jssc.2012.05.030.

[37] A. Szegedi, M. Popova, I. Goshev, J. Mihály, Effect of amine functionalization of spherical MCM-41 and SBA-15 on controlled drug release, J. Solid State Chem. 184 (2011) 1201–1207. doi:10.1016/j.jssc.2011.03.005.

[38] Q. Gao, Y. Xu, D. Wu, W. Shen, F. Deng, Synthesis, characterization, and in vitro pH-controllable drug release from mesoporous silica spheres with switchable gates, Langmuir. 26 (2010) 17133–17138. doi:10.1021/la102952n.

[39] A.L. Doadrio, E.M.B. Sousa, J.C. Doadrio, J. Pérez Pariente, I. Izquierdo-Barba, M. Vallet-Regí, Mesoporous SBA-15 HPLC evaluation for controlled gentamicin drug delivery, J. Control. Release. 97 (2004) 125–132. doi:10.1016/j.jconrel.2004.03.005.

[40] M. Vallet-Regì, J.C. Doadrio, A.L. Doadrio, I. Izquierdo-Barba, J. Pérez-Pariente, Hexagonal ordered mesoporous material as a matrix for the controlled release of amoxicillin, Solid State Ionics. 172 (2004) 435–439. doi:10.1016/j.ssi.2004.04.036.

[41] Z. Li, K. Su, B. Cheng, Y. Deng, Organically modified MCM-type material preparation and its usage in controlled amoxicillin delivery, J. Colloid Interface Sci. 342 (2010) 607–613. doi:10.1016/j.jcis.2009.10.073.

[42] Y. Jiao, S. Shen, Y. Sun, X. Jiang, W. Yang, A Functionalized Hollow Mesoporous Silica Nanoparticles-Based Controlled Dual-Drug Delivery System for Improved Tumor Cell Cytotoxicity, Part. Part. Syst. Charact. 32 (2015) 222–233. doi:10.1002/ppsc.201400115.

[43] E.I. Basaldella, M.S. Legnoverde, Functionalized silica matrices for controlled delivery of

cephalexin, J. Sol-Gel Sci. Technol. 56 (2010) 191–196. doi:10.1007/s10971-010-2293-7.

[44] M.-Y. Yang, L. Tan, H.-X. Wu, C.-J. Liu, R.-X. Zhuo, Dual-stimuli-responsive polymer-coated mesoporous silica nanoparticles used for controlled drug delivery, J. Appl. Polym. Sci. 132 (2015) 42395–42403. doi:10.1002/app.42395.

[45] Y. Yan, J. Fu, X. Liu, T. Wang, X. Lu, Acid-responsive intracellular doxorubicin release from click chemistry functionalized mesoporous silica nanoparticles, RSC Adv. 5 (2015) 30640–30646. doi:10.1039/C5RA00059A.

[46] H.M. Lode, Rational antibiotic therapy and the position of ampicillin/sulbactam, Int. J. Antimicrob. Agents. 32 (2008) 10–28. doi:10.1016/j.ijantimicag.2008.02.004.

[47] H. Lode, Role of sultamicillin and ampicillin/sulbactam in the treatment of upper and lower bacterial respiratory tract infections, Int. J. Antimicrob. Agents. 18 (2001) 199–209. doi:10.1016/S0924-8579(01)00387-9.

[48] J.D. Williams, β-Lactamases and β-lactamase inhibitors, Int. J. Antimicrob. Agents. 12 (1999) S3–S7. doi:10.1016/S0924-8579(99)00085-0.

[49] J.D. Williams, β-Lactamase Inhibition and In Vitro Activity of Sulbactam and Sulbactam/Cefoperazone, Clin. Infect. Dis. 24 (1997) 494–497. doi:1058-4838/97/2403-0030$02 .00.

[50] B.R. Meyers, P. Wilkinson, M.H. Mendelson, S. Walsh, C. Bournazos, S.Z. Hirschman, Pharmacokinetics of aztreonam in healthy elderly and young adult volunteers, Antimicrob. Agents Chemother. 35 (1991) 2098–2101. doi:0066-4804/91/102098-04$02.00/0.

[51] M.C. Nahata, V.I. Vashi, R.N. Swanson, M.A. Messig, M. Chung, Pharmacokinetics of ampicillin and sulbactam in pediatric patients, Antimicrob. Agents Chemother. 43 (1999) 1225–1229. doi:10.1016/0002-9378(93)90515-K.

[52] F. De Gaetano, L. Ambrosio, M.G. Raucci, A. Marotta, M. Catauro, Sol-gel processing of drug delivery materials and release kinetics, J. Mater. Sci. Mater. Med. 16 (2005) 261–265. doi:10.1007/s10856-005-6688-x.

[53] R.K. Singh, T.-H. Kim, J.-J. Kim, E.-J. Lee, J.C. Knowles, H.-W. Kim, Mesoporous silica tubular nanocarriers for the delivery of therapeutic molecules, RSC Adv. 3 (2013) 8692–8704. doi:10.1039/c3ra22975k.

[54] I.I. Slowing, B.G. Trewyn, V.S. Lin, Mesoporous Silica Nanoparticles for Intracellular Delivery of Membrane-Impermeable Proteins, (2007) 8845–8849.

[55] A. Salis, M. Fanti, L. Medda, V. Nairi, F. Cugia, M. Piludu, V. Sogos, M. Monduzzi, Mesoporous Silica Nanoparticles Functionalized with Hyaluronic Acid and Chitosan Biopolymers. Effect of Functionalization on Cell Internalization, ACS Biomater. Sci. Eng. 2 (2016) 741–751. doi:10.1021/acsbiomaterials.5b00502.

[56] A. Salis, D.F. Parsons, M. Boström, L. Medda, B. Barse, B.W. Ninham, M. Monduzzi, Ion specific surface charge density of SBA-15 mesoporous silica, Langmuir. 26 (2010) 2484–2490. doi:10.1021/la902721a.

[57] D. Steri, M. Monduzzi, A. Salis, Ionic strength affects lysozyme adsorption and release from SBA-15 mesoporous silica, Microporous Mesoporous Mater. 170 (2013) 164–172. doi:10.1016/j.micromeso.2012.12.002.

[58] B.A. Morrow, A.J. McFarlan, Surface vibrational modes of silanol groups on silica, J. Phys. Chem. 96 (1992) 1395–1400. doi:10.1021/j100182a068.

[59] D. Scarano, A. Zecchina, S. Bordiga, F. Geobaldo, G. Spoto, G. Petrini, G. Leofanti, M.

Padovan, G. Tozzola, Fourier-Transform Infrared and Raman-Spectra of Pure and Al, B-, Ti- and Fe-Substituted Silicalites: Stretching-Mode Region, J. Chem. Soc. Trans. 89 (1993) 4123–4130. doi:10.1039/ft9938904123.

[60] S.W. Song, K. Hidajat, S. Kawi, Functionalized SBA-15 materials as carriers for controlled drug delivery: Influence of surface properties on matrix-drug interactions, Langmuir. 21 (2005) 9568–9575. doi:10.1021/la051167e.

[61] E. Gianotti, V. Dellarocca, L. Marchese, G. Martra, S. Coluccia, T. Maschmeyer, NH3 adsorption on MCM-41 and Ti-grafted MCM-41. FTIR, DR UV-Vis-NIR and photoluminescence studies, Phys. Chem. Chem. Phys. 4 (2002) 6109–6115. doi:10.1039/b207231a.

[62] J. Grams, N. Potrzebowska, J. Goscianska, B. Michalkiewicz, A.M. Ruppert, Mesoporous silicas as supports for Ni catalyst used in cellulose conversion to hydrogen rich gas, Int. J. Hydrogen Energy. 41 (2016) 8656–8667. doi:10.1016/j.ijhydene.2015.12.146.

[63] O. Rozas, D. Contreras, M.A. Mondaca, M. Pérez-Moya, H.D. Mansilla, Experimental design of Fenton and photo-Fenton reactions for the treatment of ampicillin solutions, J. Hazard. Mater. 177 (2010) 1025–1030. doi:10.1016/j.jhazmat.2010.01.023.

[64] Y.-S. Ho, Selection of optimum sorption isotherm, Carbon N. Y. 42 (2004) 2115–2116. doi:10.1016/j.carbon.2004.03.019.

[65] K. Gul, S. Sohni, M. Waqar, F. Ahmad, N.A.N. Norulaini, M. Omar, Functionalization of Magnetic Chitosan with Graphene Oxide for Removal of Cationic and Anionic Dyes from Aqueous Solution, Carbohydr. Polym. 152 (2016) 520–531. doi:10.1016/j.carbpol.2016.06.045.

[66] J. Zhu, X. Zhu, J. Gu, L. Zhao, L. Jiang, Y. Qiu, Effective adsorption and concentration of carnosine by nickel species within mesoporous silica, LWT - Food Sci. Technol. 74 (2016) 211–218. doi:10.1016/j.lwt.2016.07.016.

[67] E.A. Dil, M. Ghaedi, A. Asfaram, S. Hajati, F. Mehrabi, A. Goudarzi, Preparation of nanomaterials for the ultrasound-enhanced removal of Pb2+ ions and malachite green dye: Chemometric optimization and modeling, Ultrason. Sonochem. 34 (2017) 677–691. doi:10.1016/j.ultsonch.2016.07.001.

[68] J. Coates, Interpretation of Infrared Spectra , A Practical Approach, Encycl. Anal. Chem. John Wiley& Sons Ltd, Chichester, 2000. (2000) 10815–10837.

[69] M.S. Bhattacharyya, P. Hiwale, M. Piras, L. Medda, D. Steri, M. Piludu, A. Salis, M. Monduzzi, Lysozyme adsorption and release from ordered mesoporous materials, J. Phys. Chem. C. 114 (2010) 19928–19934. doi:10.1021/jp1078218.

[70] H. Liu, K.K. Leonas, Y. Zhao, Antimicrobial Properties and Release Profile of Ampicillin from Electrospun Poly (ε -caprolactone) Nanofiber Yarns, J. Eng. Fiber. Fabr. 5 (2010) 10–19.