Microporous and Mesoporous Materials 225 (2016) 399–410

Contents lists available at ScienceDirect

Microporous and Mesoporous Materials

journal homepage: www.elsevier.com/locate/micromeso

# High resolution transmission electron microscopy: A key tool to understand drug release from mesoporous matrices

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ARTICLE INFO

*Article history:*
Received 29 September 2015
Received in revised form
8 January 2016
Accepted 13 January 2016
Available online 20 January 2016

*Keywords:*
Silica-based mesoporous materials
Functionalization
Mesostructural order
High resolution transmission electron microscopy
Drug delivery

ABSTRACT

This work demonstrates that high resolution transmission electron microscopy (HRTEM) is an essential tool to understand drug delivery performance of mesoporous silica materials, mainly those submitted to functionalization processes involving harsh conditions that may affect the mesostructure. Herein an SBA-15-type mesoporous material bearing $\equiv Si(CH_2)_2P(O)(OCH_2CH_3)_2$ groups was synthesized following the co-condensation route. Then, the resulting material was treated with 37 wt% HCl to convert ethylphosphonate groups to ethylphosphonic acid groups. The proper dealkylation of ethoxy groups following acid treatment was confirmed by FTIR and CP-MAS $^1H \rightarrow {}^{13}C$ solid state NMR, which indicated the presence of $\equiv Si(CH_2)_2P(O)(OH)_2$ functionalities in the treated sample. Characterization of mesoporous materials by XRD diffraction and $N_2$ adsorption points to well-ordered SBA-15 structures in both untreated and acid-treated samples. Nonetheless, a deep study by HRTEM reveals that the acid-treatment provokes noticeable loss of mesostructural order, only remaining small crystalline domains. This structural damage does not influence cargo loading but it severely affects the release of molecules confined into the mesopores, as concluded from *in vitro* delivery tests using cephalexin as model drug. Thus, whereas untreated sample showed a sustained diffusion-controlled drug release during more than 2 weeks, 100% of the loaded drug was released only after 10 h from treated sample. This abrupt burst effect cannot be explained on the basis of the existing matrix–drug interactions, whose nature and extension is quite similar under the release conditions for both samples. Thus, it can be only understood on the basis of the mesostructural damage revealed by HRTEM studies.



## 1. Introduction

Since silica-based ordered mesoporous materials entered the drug delivery landscape back in 2001 [1], they have received growing attention by the scientific community [2–14]. Mesoporous silicas are attractive drug carriers due to their outstanding properties, including: *i*) *low toxicity and biocompatibility*; *ii*) an *ordered pore arrangement*, with narrow pore size distributions that allow controlling drug loading and release kinetics; *iii*) *high surface area* that provides high potential for drug adsorption; *iv*) *great pore volume* to house high amount of therapeutic molecules; *v*) a *silanol-containing surface*, which can be functionalized to achieve higher control over drug loading and release.

Understanding drug delivery profiles from mesoporous matrices is essential to exploit their potential as controlled release systems. Drug release process obeys four sequential steps [15]: penetration of the release medium into the pore network, which is governed by osmotic pressure derived from concentration gradients; drug dissolution in the release medium; drug diffusion through the porous cavities due to concentration gradients; and drug diffusion and convection within the delivery medium. The structural and textural properties of mesoporous materials

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http://dx.doi.org/10.1016/j.micromeso.2016.01.019
1387-1811/

together with the chemical nature of their surface are the driving factors that govern the release of molecules and determine drug delivery profiles [2,5]. Thus, pore diameter is a limiting factor for the diffusion of the molecules to the delivery medium, thus regulating the release rate [1,16]. Pore connectivity and structure also influence drug release kinetics [17,18,20]. For instance 3D-bicontinuous cubic mesostructures allow easy fluid accessibility and fast molecular transport than 2D-hexagonal array of pores [17]. However, organic modification or functionalization of mesoporous matrices is cornerstone in the performance of these materials as drug delivery systems [2,5,19,20]. Commonly, functionalization strategies of mesoporous silicas rely on the covalently grafting of organic silanes ($(RO)_3SiR'$) [21]. This process permits the modification of the silica surface by grafting organic groups selective to the chemical nature of the drug to be hosted. In most cases, organic modification allows increasing host–guest interaction between the mesoporous matrix and the drug molecule. Matrix–drug interactions are usually established through electrostatic or Coulombic interaction, hydrogen bonding, and apolar interaction [5,22,23], offering many possibilities to control drug adsorption and release. Some functionalization processes require post-synthesis treatments under rather harsh conditions, highlighting those involving strong acids, which could affect the mesostructural order [24]. For instance, the treatment of SBA-15 functionalized with –CN groups followed by treatment with 48 wt.% $H_2SO_4$ to oxidize cyanide groups to carboxylic acid groups (–COOH) [25,26]; or the treatment of SBA-15 functionalized with $-P(O)(OCH_2CH_3)_2$ groups followed by the treatment with 37 wt.% HCl to dealkylate phosphonate groups to phosphonic acid groups ($-P(O)(OH)_2$) [27]. Whatever the functionalization method used to organically modify the surface of mesoporous matrices, but highlighting those involving aggressive conditions, it is indispensable to deeply study the properties of the resulting materials by using suitable characterization techniques. Actually, any alteration in the mesostructural order could strongly influence drug release process leading to unforeseen delivery profiles, albeit, to the best of our knowledge, this has not been reported yet.

Herein we demonstrate that high resolution transmission electron microscopy (HRTEM) is an essential tool to understand drug release kinetics from mesoporous matrices. For this purpose, SBA-15 incorporating ethylphosphonate functions ($-(CH_2)_2P(O)(OCH_2CH_3)_2$) was synthesized by co-condensation route and then was treated with concentrated HCl under reflux to achieve ethylphosphonic acid moieties ($-(CH_2)_2P(O)(OH)_2$). Although X-ray diffraction (XRD) and $N_2$ adsorption studies point to well-ordered 2D-hexagonal structures in both samples, *in vitro* drug delivery profiles are dramatically different, despite of exhibiting similar host–guest interactions under the release conditions. This behavior can be only explained if a deep characterization of samples by HRTEM is performed. Such study reveals that there is a noticeable loss of mesostructure in acid-treated sample, which would account for the rapid and uncontrolled drug release kinetics.

## 2. Materials and methods

### 2.1. Synthesis of materials

SBA-15 type mesoporous silica material containing nominal 15 mol% (based on silicon) phosphonic acid diethyl ester groups ($SBA15_{DPT}$) was synthesized by the co-condensation route using diethylphosphatoethyltriethoxysilane (DPT, 92%, ABCR) (Scheme 1). Briefly, 8.0 g of Pluronic® P123 block copolymer ($PEO_{20}PPO_{70}PEO_{20}$ kindly provided by BASF Co.) was added to a mixture of 276 mL of $H_2O$ and 20.6 mL of concentrated HCl (37 wt.%, Aldrich) [28]. The solution was moderately stirred at 35 °C until total surfactant dissolution. Then, 14.1 mL of tetraethyl orthosilicate (TEOS, 98 wt.%, Aldrich) was added. After *ca.* 20 min a white powder appeared. Then, 3.6 mL of DPT previously dissolved in 18 mL of isopropanol ($C_3H_7OH$, 99.5 wt.%, Aldrich) was dropwise added to the suspension. The molar composition of the reaction mixture was: 0.85TEOS:0.15 DPT:0.017P123:3.4HCl:208$H_2O$:3.4$C_3H_7OH$. Mixtures were kept under magnetic stirring at 35 °C for 24 h and then sealed in glass beakers and heated at 100 °C for further 24 h. Then, the obtained products were filtered, washed with deionized water, and then dried at 60 °C for 24 h. Finally, the surfactant was removed by a previously reported solvent extraction method [29]. Briefly, 1 g of material was soaked into a beaker containing 100 mL of an isooctane–ethanol (3:2) mixture (isooctane, ≥99.5 wt.%, Aldrich; ethanol, 95%, Panreac) and kept under magnetic stirring at room temperature for 48 h. Afterward, the sample was filtered and then subjected to a second extraction process by soaking the powder into 120 mL of an acetone–water (1:1) mixture (acetone, QP, Panreac) for 24 h at room temperature under magnetic stirring. After the extraction processes, samples were left to dry in a vacuum oven at 40 °C for 24 h to ensure total solvent removal.

Phosphonate ester groups can be transformed readily to phosphonic acid groups by acid-catalyzed hydrolytic dealkylation in concentrated HCl. Thus, SBA-15 type mesoporous silica material containing nominal 15 mol% (based on silicon) phosphonic acid groups ($HCl\text{-}SBA15_{DPT}$) was obtained from $SBA15_{DPT}$ by using a previously reported procedure consisting on treating the $SBA15_{DPT}$ sample with concentrated HCl (Scheme 1) [27]. Briefly, 0.5 g of $SBA15_{DPT}$ sample was refluxed in 50 mL of concentrated HCl (37%, Aldrich) at 100 °C for 24 h. Then sample was filtered, gently washed with deionized water and dried at 60 °C for 24 h.

### 2.2. Characterization of materials

The structural characterization of materials was performed by powdered X-ray diffraction (XRD) in a Philips X'Pert diffractometer (Eindhoven, The Netherlands) equipped with $CuK_\alpha$ (40 kV, 20 mA) over the range from 0.6 to 6.0° with a step of 0.02° and a contact time of 5 s. Electron microscopy was carried out in a JEOL 3000FEG transmission electron microscope operating at 300 kV and fitted with an X-ray energy dispersive spectrometer (EDS) (Oxford Instruments).

The textural properties of materials were determined by $N_2$ adsorption porosimetry. The $N_2$ adsorption/desorption analyses were carried out at −196 °C on a Micromeritics ASAP2020 analyzer (Micromeritics Co., Norcross, USA). In all cases, 50–70 mg of material was degassed at 80 °C for 24 h under a vacuum lower than 0.3 kPa before the analysis. The surface area ($S_{BET}$) was determined from adsorption data obtained at $P/P_0$ between 0.05 and 0.2 by using the Brunauer–Emmett–Teller (BET) method [30]. The total pore volume was estimated from the amount of $N_2$ adsorbed at a relative pressure of 0.97, assuming that adsorption on the external surface was negligible compared to adsorption in pores. To assess the possible existence of micropores (pore diameter <2 nm) in samples, the *t*-plot method was employed [31], which allowed the estimation of the microporous fraction contributions, $V_{\mu P}$ and $S_{\mu P}$, to the total pore volume and surface area, respectively. The average mesopore size ($D_P$) was obtained from the maximum of the pore size distribution calculated from the adsorption branch of the isotherm using the BJH method [32]. The wall thickness ($t_{wall}$) was calculated by using the expression $t_{wall} = a_0 - D_P$, where $a_0$ is the unit cell parameter calculated from the $d_{10}$ value of XRD using the expression $a_0 = d_{10} \times 2/\sqrt{3}$ [33].

The existence of functional groups and their chemical nature were investigated by Fourier transform infrared spectroscopy (FTIR) in a Thermo Nicolet Nexus spectrometer equipped with a