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A study of some fundamental physicochemical variables on the morphology of mesoporous silica nanoparticles MCM-41 type

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Abstract

All variables affecting the morphology of Mesoporous Silica Nanoparticles (MSN) should be carefully analyzed in order to truly tailored design their mesoporous structure according to their final use. Although complete control on MCM-41 synthesis has been already claimed, reproducibility and repeatability of results remain a big issue due to the lack of information reported in literature. Stirring rate, reaction volume and system configuration (*i.e.* opened or closed reactor), are three variables that are usually omitted, making difficult the comparison of product characteristics. Specifically, the rate of solvent evaporation is seldom disclosed, and its influence has not been previously analyzed. These variables were systematically studied in this work and they were proven to have a fundamental impact on final particle morphology. Hence, a high degree of circularity (C = 0.97) and monodispersed particle size distributions were only achieved when a stirring speed of 500 rpm and a reaction scale of 500 mL were used in a partially opened system, for a two hours reaction at 80°C. Well-shaped spherical mesoporous silica nanoparticles with a diameter of 95 nm, a pore size of 2.8 nm, and a total surface area of 954 m^2 g^{-1} were obtained. Final characteristics made this product suitable to be used in biomedicine and nanopharmaceutics, especially for the design of drug delivery systems.

Keywords: MSN, partially opened system, reaction volume, stirring rate, MCM-41.

Introduction

Mesoporous Silica Nanoparticles (MSN) are spherical silica particles with diameters smaller than 200 nm, and pore sizes ranging from 2 to 50 nm (IUPAC 2014). The most common type is known as MCM-41 (*Mobile Catalytic Material No. 41*) and it is conventionally synthesized by hydrolysis-condensation reactions of tetraethyl orthosilicate (TEOS), in the presence of a surfactant (Kresge et al. 1992). The packing of surfactant micelles in solution provides the template for the mesoporous structure of MSN. Then, a highly ordered mesostructure of unidirectional cylindrical channels organized in hexagonal arrangements is formed inside the particles, which provides them with very high surface areas (1000 $m^2\,g^{-1}$) and pore volumes (from 0.5 to 2 $cm^3\,g^{-1}$) (Yang and Yu 2015). One of the most promising applications for these unique characteristics are in the biomedical and nanopharmaceutical fields, including: biosensing (Zhang et al. 2015), biological imaging (Chen et al. 2015a; Xu et al. 2015a, 2016), gasotransmitters (Chakraborty and Mascharak 2016), tissue engineering (Chen et al. 2013), gene transfer (Brevet et al. 2014), vaccine adjuvants (Oliveira et al. 2016) and, particularly drug delivery systems. In this field, latest advances includes MSN functionalization with polyelectrolytes (Varga et al. 2015), lipid bilayers (Han et al. 2016), and polymeric shells (Hu et al. 2014; Xu et al. 2015b), or mechanized MSN with different gate keepers (Cheng et al. 2016; Rafi et al. 2016; Xu et al. 2016), PAMAM dendrimers (Xu et al. 2015a) or β-cyclodextrin (Chen et al. 2015b) for the design of pH-responsive delivery vehicles, notably for cancer treatment (Cheng et al. 2016; Han et al. 2016; Hu et al. 2014; Rafi et al. 2016; Xu et al. 2015b). Excellent reviews may be found illustrating a variety of novel synthesis technologies for MSN and their most promising and leading-edge applications in biomedicine (Beltrán-Osuna and Perilla 2016; Sun et al. 2015; Wang et al. 2015).

MSN were proposed for the first time as a drug delivery system in 2001 (Vallet-Regi et al. 2001) and, since then, reports have been exponentially growing, but only until 2008 MSN started to be use *in vitro* and *in vivo* assays on animals (Chen et al. 2013).

Recently, silica spheres of 6 nm, surface-functionalized with PEG (*polyethylene glycol*) chains and peptides were synthesized (Phillips et al. 2014). These are known as C-dots (*Cornell dots*), the first nanoparticle approved by the FDA tested in human trials, and the only one that has been used in clinical stage so far. Currently, all studies of MSN are in preclinical stages of development. Despite the enormous potential of MSN, there are still many challenges to overcome for the use of MSN as delivery vehicles. According to the U.S. Department of Health and Human Services (2015), one of these challenges is associated with the modulation of MSN size, shape, pore size and surface chemistry, and the determination of their influence in biodistribution, toxicity and drug release. Access to targets and diffusion kinetics and, therefore, efficiency of the drug carrier, are related directly with MSN morphology and size particle distribution, which must be precisely controlled to allow a proper release (Crommelin and Florence 2013). Particle shape is one of the most important aspects in terms of cellular internalization (Vallet-Regi and Arcos Navarrete 2016; Slowing et al. 2008), and it has been also recognized that even length and curvature of nanopores may affect load capacity and drug release profiles (Sun et al. 2015). Therefore, precise modulation of shape and size are of great importance for the design of safe and efficient drug delivery systems (Mamaeva et al. 2013).

Although precise control to obtain spherical MCM-41 monodispersed nanoparticles has been already claimed in some reports (Barrabino 2011; Chen et al. 2012; Lv et al. 2016; Wanyika et al. 2011), reproducibility of results and comparison of product characteristics may be considered a critical factor of study. MSN synthesis is still far from being completely understood, since there are so many variables that must be taken into account, not all of them have been carefully studied, and some of them are often omitted. Besides, there is no agreement on the optimal conditions to achieve a specific morphology. For example, some studies have analyzed systematically the influence of variables affecting MCM-41 morphology, such as: alkoxysilane precursor (Huh et al. 2003), surfactant type (Ikari et al. 2006; Morishige et al. 1997) and surfactant concentration (Cai et al. 2001; Lelong et al. 2008; Shi et al. 2010), molar ratio of

reactants (Bastos et al 2011; Cai et al. 2001; Manzano et al. 2008; Wanyika et al. 2011), temperature and catalyst concentration (Chen et al. 2012), and other synthesis procedures (Guo et al. 2013; Qu et al. 2006). But particularly, there are three variables of utmost importance that are not usually reported. First, stirring rate is almost always mentioned as being continuous or vigorous, but quantitative values used are not disclosed, as it is shown in our previous review (Beltrán-Osuna and Perilla 2016). Two reports were found that actually studied its influence on reaction. One of them indicates that particle size diminished from 111 to 38 nm when stirring speed was varied between 100 and 1000 rpm, but for MSN where the desired hexagonal arrangement of mesopores was lost (Lv et al. 2016). For MCM-41, only one study shows qualitatively that particle size distribution narrowed when stirring rate was increased (from 200 to 500 rpm), without further implications. Clearly, the strength of agitation should be enough to facilitate TEOS solubilization (mostly at the beginning of the reaction) and to promote the transport of reactants throughout the mesoporous structure formation. At the same time, mixing speed should not be too high to avoid a big vortex that would allow the formation of eddies in a turbulent flow. Therefore, it may be anticipated that stirring rate will affect not only size but also shape of nanoparticles. In the second place, the volume of reactants is sometimes replaced by the molar ratios between them, but reaction scale is rarely mentioned, and its influence on particle size and morphology has not been analyzed. It would not be surprising that the total volume as well as the reactor geometry influence the flow patterns in the medium, affecting the MSN shape at the mesoscopic scale. Finally and foremost, system configuration (*i.e.* an opened or a closed reactor) is never specified. Certainly, allowing evaporation of ethanol and water will change TEOS hydrolysis and condensation kinetics, molar ratios between reagents, and the rate of evaporation would affect the final size and shape of the particles. Knowingly or not, all these variables are commonly fixed by researchers in order to achieve the desired repeatability, but truly reproducibility is not easy to achieve due to the lack of detailed information in literature. Finally, it is worth to mention that only a scarce number of companies in the world supply this product, and there are many efforts focused in the comprehension of MSN synthesis to be indeed able to tailor MCM-41 characteristics according to final use.

This work aims to study in detail the influence of such variables in the final nanoparticle morphology, specifically for MCM-41, since optimum conditions must differ between other types of MSN (MCM-48, SBA-15, KIT-6, etc.). To the best of our knowledge, these physical variables have not been explicitly reported; expressly the influence of the solvent evaporation rate on MSN morphology, and their influence seem absent in specialized MSN reviews (Bharti et al. 2015; Wu et al. 2013). Thus, all variables must be taken into account and reported, to completely control the MSN shape, size and size distribution. This will also allow proficiently assessing and comparing their final characteristics among different protocols and products in literature. The goal is to obtain well-shaped spherical particles in the nanometric scale (*e.g.* 100 nm) with narrow particle size distribution, to achieve a repeatable and reproducible method to produce MSN type MCM-41.

Experimental

MSN synthesis

TEOS (99%), hexadecyltrimethylammonium bromide (CTAB, 98%) and sodium hydroxide (NaOH) were employed as silica precursor, surfactant template, and catalyst, respectively. Distilled water was used in all experiments. All reagents were purchased from Sigma-Aldrich Co. and they were used as provided. In all cases, MSN synthesis was carried out in a cylindrical glass reactor. In a typical procedure (*i.e.* for a volume reaction of 500 mL), 1 g CTAB was added to 480 mL water at 30°C, and mixed at 150 rpm, avoiding the formation of any kind of foam. After complete dissolution, 3.5 mL NaOH aqueous solution (2 M) was added to the homogeneous solution, the reaction temperature was raised until 80°C and waited for its stabilization. Then, stirring speed was increased up to 500 rpm and the reaction started immediately when 5 mL of TEOS were added dropwise (at a rate of 1 mL min^{-1}). Reaction time varied between 2 and 4 hours, depending on the chosen system configuration. Once the reaction had finished, the temperature of the medium was quickly decreased to 20°C using an ice-water bath.

Several wash-centrifugation cycles (5000 rpm, 10 min) were done until water reached a neutral pH and then, MSN were collected. The white precipitate was dried in oven at 80°C for 8 hours, and grounded in a ceramic mortar to reduce the size of the agglomerate and to homogenize the sample. To remove the surfactant from the mesoporous structure, a calcination process was used, going from room temperature to 723 K ($10°C$ min^{-1}), holding it up for 6 hours, and stopping the heating afterwards (the sample was removed from oven after cooling). The obtained product was a fine white powder that was kept at room conditions until characterization.

Characterization

The morphology of the calcinated MSN samples was observed via Field Emission Scanning Electron Microscopy (FESEM), using a Zeiss Oxford Instruments Ultra 55 electron microscope coupled with an in-lens detector. The mesostructure of the product was observed through Transmission Electron Microscopy (TEM) using a JEM 2100f electron microscope with an accelerating voltage of 200 kV. Sample preparation included sonication of 0.1-1.0 mg MSN in 1 mL of pure ethanol for 15 minutes. A drop of 5 μL of this dispersion was directly placed on the nickel grid for TEM and on the aluminum tip for FESEM, and left to dry under room conditions. FESEM samples were gold-palladium plated before taking the micrographs. The mesoporous structure was characterized by Small-Angle X-Ray Diffraction (SAXRD), using a Bruker Axs D8 Advance Diffractometer, with Cu-Kα radiation (33 kW, 50 mA, 0.154085 nm), a step size of 0.04° and a scanning rate of 3s by step, over the range $0.73° < 2θ < 10°$. MSN porosity was characterized by carrying out nitrogen adsorption/desorption isotherm measurements on a Micromeritics TriStarII PLUS analyzer at 77 K. Each sample was outgassed at 473 K for 10 hours before measurement. The specific surface area was calculated by the Brunauer-Emmett-Teller (BET) method and the average pore size was calculated by using Barrett-Joyner-Halenda (BJH) model. Thermogravimetric analysis (TGA) was carried on MSN materials using a Mettler Toledo TGA/DSC, following a three-step method: a) fast heating from 30°C to 100°C, b) isothermal at 100°C for one hour, and c) heating to 800°C at 10°C/min.

Hydrodynamic particle diameter (d_h) and size distribution of MSN were measured by Dynamic Light Scattering (DLS), using Malvern ZetaSizer Nano ZS ('red' laser, 632.8 nm, 4 mW, 175°). Sample dispersions were made at very low concentrations (< 0.1 mg mL^{-1}) to assure Brownian motion. Dispersions were mixed in a sonication bath for 30 minutes before each experiment. Five measurements at 20°C were carried out for each sample, using water and ethanol as solvents. Nanoparticle size and size distributions of the samples were also calculated by measuring the particle diameter on the micrographs, using the software Image J for a counting of at least 500 particles for each reported value. Average diameter of the particle (d_p) and circularity (C) were measured by Image J. For the non-spherical particles, d_p was calculated as the arithmetic mean between the shortest and largest diameter observed in the micrographs. Circularity indicates how close the particle shape projection is to that of a perfect circle (for which C = 1). It is defined by Equation (1):

$$C = 4A/\pi L^2 \qquad (1)$$

where A is the area of each particle on the micrograph, and L the length of the particle (Merkus 2009). In this study, L was replaced by L_F, known as the Feret diameter, which is the distance between two parallel tangents on opposite sides of the particle profile. All particles were measured in the same direction (regardless of particle orientation) to ensure statistical significance. Histograms of particle size distributions and Gaussian fitting for all samples are presented in supplementary material.

Results and Discussion

Four experimental variables, at three level each one, were analyzed during MSN synthesis: stirring speed, reaction volume, system configuration and NaOH/TEOS molar ratio. The influence of each physicochemical variable on MSN shape was quantitatively analyzed by two means (d_p and C). The product displaying the best spherical

morphology under the electronic microscope was finally chosen and fully characterized.

Influence of the stirring speed

First, volume reaction was kept at 50 mL, using a closed system and maintaining the same molar ratios of reactants as detailed before in the experimental section (for 500 mL). A reaction time of 4 hours was established from a previous follow-up (not shown) where small samples were taken every 30 minutes from the reactor. It was concluded that shorter times do not allow a complete formation of the expected MSN morphology. In this first set of experiments, different MSN products were obtained by varying the magnetic stirring speed of the reaction at 250, 500 and 700 rpm. Since sonication is a well-established method in the synthesis of nanoparticles, an additional experiment was carried out. The reactor was placed inside a sonication bath during the whole reaction to evaluate its influence in the final state of particle aggregation. MSN morphologies found by FESEM for these four experiments are shown in Figure 1. It can be seen that at low speed rates (250 rpm, Figure 1a) shapeless particles (C = 0.72 ± 0.18) with very dispersed diameters reaching the micrometric scale (d_p = 267 ± 131 nm) were obtained. Both, circularity and particle size were improved (C = 0.82 ± 0.26 and d_p = 184 ± 64 nm) when the stirring speed was increased (500 rpm, Figure 1b). But, although particle size dropped significantly (d_p = 141 ± 35 nm) for the highest stirring rate (750 rpm, Figure 1c), bean-shaped particles were still obtained (C = 0.83 ± 0.17). Well-shaped spherical nanoparticles at this scale were only achieved when sonication was used with the aim of helping MSN dispersion (Figure 1d). However, the mesoporous structure was clearly affected in that case. As shown in Figure 2, the parallel channels observed for MCM-41 when magnetic stirring was used (Figure 2a), appeared widely disordered with sonication (Figure 2b), probably due to the high energy exert during the cavitation phenomenon. Since the smallest particles were obtained at the highest speed, a value of 750 rpm was chosen for the next stage.

Influence of the reaction volume

Reaction volume was varied in 50, 250 and 500 mL, for a 4 hours synthesis, and its

influence on MSN morphology is shown in Figure 3. It is seen that shape and size were improved when scaling the reaction from 50 mL (C = 0.81 ± 0.21, d_p = 148 ± 47 nm, Figure 3a), to 250 mL (C = 0.87 ± 0.21, d_p = 106 ± 39 nm, Figure 3b). For 500 mL, more rounded particles with less size dispersion were obtained (C = 0.90 ± 0.13, d_p = 89 ± 18 nm, Figure 3c). These results were attributed to the different pattern flows achieved in each scale. Indeed, previous experiments (data not shown) indicated that not only the geometry of the reactor, but even the size of the magnetic stir bar exert a significant influence on the final MSN morphology. It is worth to notice then, that results shown in Figure 3 were obtained using cylindrical glass reactors of different sizes but preserving the same height-to-diameter ratio (HDR=1.2). The length of the magnetic bars was varied to keep a constant relation between both, the stir bar and reactor diameter. Although spherical shaped particles were obtained for the highest tested dilution (500 mL), Figure 3c shows some other nanoparticles (d_p < 20 nm, not considered for C and d_p values previously reported) suggesting a possible Ostwald ripening. This could have been originated because of excessive stirring, leading to a turbulent flow where small particles are prone to be formed. Therefore, a feedback experimental set was carried out to test three new levels of the stirring speed at a reaction volume of 500 mL, as shown in Figure 4. It is seen that a value of 375 rpm (Figure 4a) is not high enough to obtain a homogeneous spherical shape, as it was better achieved at 500 or 650 rpm (Figures 4b and 4c). The effect of stirring rate on C and d_p is shown in Figure 5, where the highest value of circularity is reached for the highest stirring speed. However, it is obtained at the expense of broadening the particle size distribution, as seen in the error bars. Therefore, considering the closer values for C and d_p between the last two stirring rates values, 500 mL and 500 rpm were the conditions selected for the next stage.

Influence of the system configuration

Until here, all experiments have been carried out in a closed system (where loss of mass toward the atmosphere was not possible). Two more experiments were made, now in an opened system (allowing complete release of the evaporated solvent) and a partially

opened system (restricting a percentage of the opening area). In both cases, reaction time was reduced to 2 hours, because solvent evaporation diminished the reaction volume to ¼ and ½ of the initial value for the opened and partially opened systems, respectively. It may be observed that when no restriction was placed on the opening exit (with an area of 64 cm^2), not only particle size increased drastically from $d_p = 111 \pm 13$ nm (500 rpm, Figure 5) to $d_p = 406 \pm 89$ nm (Figure 6a) but also angular profiles were developed at the mesoscopic scale, reshaping the MSN surface ($C = 0.75 \pm 0.27$). These changes were probably due to the loss of the ethanol evaporated during the reaction, displacing the TEOS hydrolysis equilibrium and gradually changing the polarity of the medium. In this way, more TEOS was hydrolyzed but became less soluble, modifying the mesoporous structure evolution compared to the closed system. The hydrolyzed TEOS polymerized preferably on the surface of already formed MSN, in order to minimize surface energy. Thus, less particles, but with larger sizes, were formed. Then, CTAB and TEOS interactions forming the mesoporous template were also affected by different transport phenomena, such as ethanol diffusion gradient, along with convection and flow patterns. As a result of this very complex dynamic phase behavior, edges on the MSN surfaces were formed. Since dilution plays a fundamental role in phase behavior, further experiments would be required to gather information about a possible formation of a lamellar phase of the surfactant. This might be influencing the mesostructure evolution of the particle and, therefore, the appearance of edges on the final MSN morphology. From these results, it is concluded that a partially opened system (i.e. covering 4/5 of the opening area) becomes the most adequate alternative, producing particles with remarkable spherical shape and nanometric size ($C = 0.97 \pm 0.09$, $d_p = 95 \pm 15$ nm) as shown in Figure 6b. Clearly, ethanol evaporation rate is in this way set to a proper value where it enhances TEOS hydrolysis, while allowing the formation of well-shaped and monodispersed nanoparticles in a proper diluted medium. This result was obtained using a NaOH/TEOS molar ratio of 0.3. The influence of this value was also studied and presented as follows.

Influence of the NaOH/TEOS molar ratio

The NaOH/TEOS molar ratio was varied in three levels (0.03, 0.3 and 0.5) for a partially opened system, to study the influence of the catalyst on MSN morphology. It was observed that nanoparticle size was directly dependent on catalyst concentration. The size doubled from 40 ± 5 nm (Figure 7a) to 95 ± 15 nm (Figure 7b, same sample shown in Figure 6b), when NaOH/TEOS molar ratio was 10 times increased. However, when this value was increased from 0.3 to 0.5 (Figure 7c), a non-proportional trend was observed with final particle sizes reaching almost half micron ($d_p = 480 \pm 112$ nm). Besides these widely dispersed sizes, the appearance of edged morphologies ($C= 0.79 \pm 0.11$) was also evident. NaOH/TEOS molar ratios values were chosen based on the study of Chen and coworkers (Chen et al. 2012), which reports a perfect linear relationship between particle diameters (40 ± 4, 80 ± 7, 120 ± 13, and 160 ± 20 nm) and NaOH/TEOS molar ratios (0.2, 0.3, 0.4 and 0.5). The authors claimed the complete and precise control for nanosized MCM-41 and selected 200-300 rpm as the best stirring speed. However, equally important variables were not mentioned, such as system configuration, reactor geometry or TEOS addition rate that could lead to the reproducibility and fair comparison among literature results.

Physicochemical characterization of MCM-41

In order to corroborate the nature of the MCM-41 material, the sample with better circularity (Figures 6b and 7b) was fully characterized. First, N_2 adsorption-desorption isotherm measurements (Figure 8) show an isotherm type IV according to the IUPAC classification (IUPAC 1985). Four zones are observed: (i) a linear region at a low relative pressure, *P/P₀*, where BET model was applied (assigned to the formation of a N_2 adsorbed monolayer), (ii) an increase step at a medium relative pressure ($P/P_0 = 0.2$ - 0.4) due to capillary condensation, (iii) a long plateau, and (iv) a final increase at a high relative pressure ($P/P_0 > 0.8$) corresponding to the filling of voids among particles. An important disagreement among literature is related with the appearance of a hysteresis loop between adsorption and desorption curves. Many authors attribute this loop as a proof of the existence of mesoporosity for MCM-41 (Ukmar et al. 2011; Zhou et al.

2016), while others reports indicate that it is actually the absence of hysteresis, which suggests the formation of uniform cylindrical mesopores inside the nanoparticle (Bernardos et al. 2010; Khezri et at. 2014). Original IUPAC classification describes indeed the existence of a hysteresis loop in the definition of a type IV curve. But although currently applied by many, it has to be noticed that this classification is dated from more than three decades ago, when MCM-41 materials were not yet on scene (firstly reported in 1992 by Beck et al. (1992) and Kresge et al. (1992)). Indeed, current studies have demonstrated that the same MCM-41 material may exhibit nearly reversible isotherms or well-defined hysteresis loops, depending on test operational conditions such as temperature (Rouquerol et al. 2014), pore size distribution or the nature of the gas adsorbed (Selvam et al. 2001). In this study, the corresponding total surface area (A_s), average pore diameter (ϕ_{pore}) and pore volume (v_{pore}) were obtained using BET and BJH models, and the results are summarized in Table 1. A very narrow pore size distribution (as shown in insert Figure 8) was also observed, indicating a homogeneous mesoporous structure inside the MSN product.

Although silicon dioxide is an amorphous material, mesoporous inside MSN may be analyzed as if they were organized in a unit cell and measured by SAXRD, as shown in Figure 9a. Three well resolved peaks were found: one sharp and intense peak around 2θ from 2.0° to 2.5°, and two other less intense peaks between 2θ from 3.5° to 5.0°, as expected for MCM-41 (Khezri et at. 2014). The main peak ($2\theta = 2.128°$) is shifted around $2\theta = 0.16°$ for the calcinated sample, which corresponds to the contraction of the unitary cell (due to silanol group condensation along heat treatment). This decrease in the interplanar space is also associated to CTAB decomposition inside the pores (Shibata et al. 2010). These three well-resolved peaks shown in Figure 9a may be assigned to the reflections of (100), (110) and (200) diffraction planes for a 2-D hexagonal lattice ($p6\ mm$). From the principal interplanar spacing, $d_{(100)} = 38,605$ Å (obtained from Bragg's law), the lattice parameter for the hexagonal arrangements of MCM-41 was calculated as $a_0 = 2d_{(100)}/\sqrt{3} = 44,6$ Å. Wall thickness (w) was also estimated from pore diameter ($\phi_{pore} = 2.8$ nm, obtained by BJH model) as: $w = a_0 - \phi_{pore}$

= 16.6 Å, which falls within the expected values, between 10-27 Å for a calcinated silica type MCM-41 (Edler 1997).

Hexagonal arrangements were also clearly verified by TEM images (Figure 9b), and MCM-41 pore measurements by ImageJ software gave as result an average value of ϕ_{pore} = 2.7 nm, which is in excellent agreement with the average pore diameter obtained from SAXRD patterns and adsorption isotherms. Also, it must be noticed that heat treatment has a big influence on the structure of mesoporous materials, which are conventionally calcinated at 823 K (Beltrán-Osuna and Perilla 2016). However, this temperature could be excessive, since complete removal of CTAB from pores has been proven to take place at 613 K, and sintering of silica walls occurs at temperatures higher than 823 K (Shibata et al. 2010). This phenomenon could reinforce the mechanical properties of the material, but diminishing the pore volume, which is not desired if MSN are used as delivery vehicles. For this reason, an intermediate temperature (723 K) was used in this study, and it was proved that the hexagonal mesoporous structure was always conserved. Also, CTAB removal from pores was verified by thermogravimetric analysis, since a small weight loss ($< 2\%$) was calculated for the calcinated sample (see Supplementary material). This drop was expected due to the loss of water of hydration at high temperatures (Iliade et al. 2012).

Finally, d_h of MSN (dispersed in water and ethanol) was obtained by DLS technique, and the results are shown in Figure 10. Larger particles were detected using water as a solvent (d_h = 201.4 nm ± 44 nm) suggesting particle agglomeration. Instead, the lower sizes were measured when using ethanol (d_h = 115.5 nm ± 10 nm) since a less polar medium allows disruption of particle association due to hydroxyl groups left on MSN surface. Only one peak was obtained in both cases, showing good repeatability. These results are in agreement with particle size measured on FESEM images by the ImageJ software (d_p = 95 ± 15 nm, Figure 6b), taking into account that d_h values are always higher than d_p because of particle solvation and other hydrodynamic effects. All the results are summarized in Table 1. According with their size, pore diameter and pore

morphology, the MSN synthesized could be used in several biomedical applications, especially for the design of drug delivery systems.

Conclusions

Well-shaped spherical mesoporous silica nanoparticles were obtained, with particle size of 95 nm and pore size of 2.8 nm. The high degree of order in the mesopores arrangement expected for a type MCM-41 silica material was corroborated by SAXRD and a total surface area of 954 m^2 g^{-1} and a volume pore of 0.66 cm^3 g^{-1} were calculated from nitrogen adsorption-desorption isotherms. Physical parameters such as stirring speed, reactor geometry, reaction scale and system configuration were proved to have a significant influence on final MSN morphology. It is concluded that transport phenomena associated with flow patterns, convection and diffusion gradients, along with a complex phase behavior, control the final particle shape at the mesoscopic scale. Almost perfect spherical morphology with low size dispersity was only obtained for a partially open system, for which a moderate ethanol evaporation rate promotes TEOS hydrolysis, but allowing particle formation at an appropriate dilution. Catalyst concentration was found to be the principal variable affecting particle size, following a direct but not proportional relationship between particle diameter and NaOH/TEOS molar ratio. Clearly, a complete report of all reaction conditions is essential in literature to achieve the desired repeatability and reproducibility on MSN synthesis.

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Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1 Characterization of MCM-41 synthesized nanoparticles

Property	Value	Units
Surface area, A_s	954.3	$m^2\,g^{-1}$
Pore volume, v_{pore}	0.658	$cm^3\,g^{-1}$
Pore size, ϕ_{pore}	2.8	nm
Lattice parameter, a_0	44.58	Å
Wall thickness, w	12.08	Å
Particle size, d_p	95	nm
Hydrodynamic diameter, d_h	115	nm

Figure captions

Fig. 1 MSN morphology obtained at different stirring speeds for a reaction volume of 50 mL: a) 250 rpm, b) 500 rpm, c) 750 rpm, d) with a sonication bath

Fig. 2 Mesoporous structure obtained using a reaction volume of 50 mL: a) magnetic stirring (750 rpm), b) sonication stirring

Fig. 3 MSN morphology obtained at different reaction volumes for a stirring speed of 750 rpm: a) 50 mL, b) 250 mL c) 500 mL

Fig. 4 MSN morphology obtained at different stirring speeds for a reaction volume of 500 mL: a) 350 rpm, b) 500 rpm, c) 650 rpm

Fig. 5 Influence of stirring speed on circularity (C) and particle size (d_p) for MSN (reaction volume: 500 mL). Error bars represent one standard deviation

Fig. 6 MSN morphology obtained at 500 rpm using: a) a complete opened system, b) a partially opened system (80% of opening area covered, compared to the closed system)

Fig. 7 Influence of catalyst concentration on MSN morphology for a partially opened system, varying NaOH/TEOS molar ratio between: a) 0.03, b) 0.3, c) 0.5

Fig. 8 Adsorption-desorption isotherms of nitrogen at 77 K for MSN type MCM-41. Insert shows sample pore size distribution obtained from BJH model

Fig. 9 Mesoporous arrangement for MCM-41: a) SAXRD measurements for native and calcinated material, b) TEM micrograph showing the hexagonal alignment of mesopores

Fig. 10 Hydrodynamic particle measurements obtained from DLS for MSN type MCM-41 using: a) water, b) ethanol, as solvent (Measurement temperature: 20°C)

Fig. 1

100 nm

Fig. 2

Fig. 3

(a) 200 nm

(b) 200 nm

(c) 200 nm

Fig. 4

(a) (b) (c)

— 100 nm

Fig.5

Fig. 6

200 nm

Fig. 7

Fig. 8   Click here to download Figure Fig 8.tiff

Fig. 9

Fig. 10

Click here to download Figure Fig 10.tiff ⬇

(a)

	Size (d.nm):	% Intensity:	St Dev (d.nm)
Peak 1:	201,4	100,0	44,80
Peak 2:	0,000	0,0	0,000
Peak 3:	0,000	0,0	0.000

(b)

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Peak 1:	115,5	100,0	15,05
Peak 2:	0,000	0,0	0,000
Peak 3:	0,000	0,0	0,000

Intensity (%)

Particle diameter (nm)