Hybrid Nanocontainer with Dual Control Release System

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Abstract

The aim of this project was to develop novel hybrid mesoporous silica nanoparticles (MSNs) with a double pore system, i.e., containing pores of different sizes and/or structures and functionality. This constitutes a major challenge in the area of smart nanocarriers, allowing the simultaneous loading of different types of cargo, and their release under specified conditions.
To achieve this goal, we tested four different templates in the synthesis of mesoporous silica nanoparticles, using the surfactants hexadecyltrimethylammonium bromide (CTAB), 1-hexadecyl-3-methylimidazolium chloride (Met), 1-hexadecylpyridinium chloride (Pyr) and 1H1H2H2H-perfluorodecylpyridinium chloride (HFDePC). We thus obtained four types of nanoparticles: 1-MSNs-C using CTAB (diameter 69 ± 6 nm), 1-MSNs-Met using Met (diameter 73 ± 10 nm), 1-MSNs-Pyr using Pyr (diameter 64 ± 10 nm) and 1-MSNs-HFDePC using HFDePC (diameter 21 ± 2 nm). To increase the size of the last, several hypotheses were tested, achieving the goal with addition of sodium chloride (NaCl) (diameter 61±10 nm). Removal of the surfactants was evaluated by different methods: calcination, extraction with acidified ethanol (EtOH/HCl), pure tetrahydrofuran (THF), THF with lithium bromide (LiBr) and dichloromethane (DCM), in order to select a pair of templates for the preparation of MSNs with a double pore system with selective removal of each one. None of the extraction processes under study could selectively remove one of the surfactants efficiently. The best methods were 1-MSNs-Pyr extracted with THF and 1-MSNs-HFDePC extracted with EtOH/HCl. CTAB and HFDePC were used to prepare nanoparticles with a double pore system, one without and the other with NaCl, yielding MSNs with diameters of 35±4 and 34±3 nm, respectively. Overall, the novel materials are very promising to develop nanoparticles with a double pore system for selective release.

1. Introduction

Silica nanoparticles have properties that make them increasingly important in several areas. These NPs are robust, biocompatible, non-toxic, thermally and chemically stable, electronegative in aqueous media at neutral pH, mechanically stable, protect and stabilize embedded molecules, are easy to prepare, exhibit good monodispersity, have high specific surface area and, finally, their surface can be functionalized due to an high surface silanol concentration.[1–3]

Spherical silica nanoparticles can have diameters ranging from a few tens of nanometers to micrometers. By changing the reaction conditions, such as the concentration of reactants, the pH, and the reaction temperature, the sizes of the particles can be easily tuned.[2]These nanoparticles can be used in in compact form or with a mesoporous network. For the synthesis of compact NPs, the Sol-Gel method[4] or the Stöber method[5] can be used. For mesoporous nanoparticles the so-called modified Stöber method is used.[6]

The sol-gel process has been widely used to produce silica nanoparticles. In this process, the development of networks occurs through the arrangement of a colloidal suspension, which is called sol, and the formation of gel that forms a system in continuous liquid phase. That said, a sol is a stable colloidal dispersion of particles, usually 1-100 nm, in a liquid, whereas a gel is an interconnected solid porous network. [4,7]

In 1968, Stöber *et all.* Described a method to obtain spherical and compact silica particles, using silica alkoxides as precursors, ammonia as catalyst and water, in low molecular weight alcohols as solvent. Particles with sizes between 5-2000 nm were obtained, depending on the conditions used. [5]

The synthesis of the first silica mesoporous arrays with an ordered structure and a uniform pore size was made in 1992 by scientists from Mobil Corporation.[8] The synthesis of mesoporous silica nanoparticles is classified as a modified Stöber method, that is, the synthesis begins with the formation of cylindrical micelles that aggregate in a hexagonal form depending on the stability of the medium.

The less stable, the greater the aggregation and, consequently, the final size of the particles. This stability is modified by the addition of the basic catalyst in different concentrations, since it neutralizes the surface charges of the micelle polar part. After the formation of these structures, the precursor of silica is added, which has a greater affinity with the surface of the micelles than with itself due to the fact that in basic pH silica deprotons after hydrolysis, taking a negative form what causes it to bind to the positive charges of micelles and, therefore, the reactions of hydrolysis and condensation occur in these active centers, thus forming the MSNs. [9,10]

To make the pore network of the MSNs available it is necessary to remove the template. The two best known processes for removing the template are calcination and solvent extraction.

Calcination is the most used method for removing templates. It is typically completed under an air atmosphere at approximately 500°C and it is necessary a long time to complete it due to the low restrictive heating rates, which normally are 1°C/min. [11,12]

Solvent extraction it is usually made with an organic solvent such as ethanol acidified with hydrochloric acid. Organic solvents at low pH values cause the silica to protonate, which leads it to its neutral state, decreasing the silica-template interactions.[13,14]

Mesoporous silica nanoparticles with a double pore system are very interesting materials. Unlike nanoparticles with only one type of pore they can have pores of different sizes or structures, load several types of molecules, from drugs to proteins and nucleic acids, for example. These materials offer the possibility to functionalize the nanoparticle surface, as well as the surface of each type of pore selectively.[15,16]

The aim of this project is to develop a hybrid nanocontainer based on mesoporous silica nanoparticles with a double pore system for selective release control. To find the ideal pair of templates that would be selectively removed it is necessary to test several templates and to study different extraction methods. Firstly, MSNs with only one template will be prepared, using four surfactants with different characteristics, in order to be selectively removed: hexadecyltrimethylammonium bromide (CTAB), a cationic surfactant, 1-hexadecyl-3-methylimidazolium chloride (Met) and 1-hexadecylpyridinium chloride (Pyr), both ionic liquids (IL), and finally, a fluorinated surfactant, 1H1H2H2H-perfluorodecylpyridinium chloride (HFDePC). The fact that the surfactants are different is important because in the synthesis with two templates (2-MSNs) they will have higher probability of not forming mixed micelles. Also, increasing the probability of being selectively extracted. In order to achieve a selective template extraction, it is necessary to test different extraction methods to see which could extracts one of the templates, but not the others. Calcinations have to be performed as a basis of comparison. Various solvent extractions will also be performed using solvents from different families, i.e., ethers, alkanes, chlorinated chains, alcohols, etc. The most suitable solvents for this study are tetrahydrofuran (THF), hexane, dichloromethane (DCM) and acidified ethanol (EtOH/HCl). Finally, in order to make nanoparticles with a double pore system it is necessary to choose the pair of surfactants that will not form mixed micelles and that can be selectively.

1.1. Materials

The following materials were used without further purification: 1-hexadecyl-3-methylimidazolium chloride (98%, IOLITEC), 1-hexadecylpyridinium chloride (98%, IOLITEC) hexadecyltrimethylammonium bromide (99 % CTAB, Sigma), 1H, 1H, 2H, 2H-heptadecafluorodecyl Iodide (98%, TCI), pyridine (99% Pyridine, Sigma-Aldrich), diethyl ether (99.8%, ACS reagent, Honeywell), acetone (99% Acetone for spectroscopy, Acros Organics), methanol (ACS reagent, Sigma-Aldrich), Amberlite IRA-410 chloride form (Sigma-Aldrich), sodium hydroxide (Pure NaOH, PanReac AppliChem), sodium chloride (NaCl, PanReac AppliChem), tetraethoxysilane (98 % TEOS, Aldrich), hydrochloric acid (37 % HCl, HCl fuming, 37% ACS reagent, Sigma-Aldrich), absolute ethanol (99.9 % EtOH, Scharlau), lithium bromide (LiBr, Sigma-Aldrich), tetrahydrofuran (ACS reagent, THF, Carlo Erba Reagents) and dichloromethane (99.95% DCM, José Manuel Gomes dos Santos, Lda). The deionized (DI) water was generated using a Millipore Milli-Q system (≥18 MΩcm, Merck, NJ, USA).

1.2. Equipment

The nanoparticles were characterized by TEM and nitrogen adsorption. TEM images were obtained on a Hitachi transmission electron microscope (Hitachi High – technologies, Tokyo, Japan), model H-8100, with a LaB_6 filament (Hitachi) complemented with an accelerator voltage of 200 kV. A camera KeenView (Soft Imaging System, Münster, Germany) is a part of this equipment, which through iTEM software, allows acquiring TEM images. To analyze MSNs, the particles are dispersed in ethanol and then prepared and dried on a carbon grid. The size/dimension, polydispersity, and morphology of the particles were obtained by measuring at least 50 nanoparticles by Image J software. To characterize the MSNs and their pores it was performed nitrogen adsorption (BET). The N_2 adsorption–desorption isotherms were obtained at 77 K of the degassed samples, using a Micromeritics ASAP 2010.

1.3. Methods

1.3.1. Fluorinated Surfactant (HFDePC) Synthesis

The fluorinated surfactant, 1H, 1H, 2H, 2H-perfluorodecylpyridinium chloride (HFDePC), was synthesized according to the literature[17]. 5 g of 1H, 1H, 2H, 2H-heptadecafluorodecyl Iodide were dissolved in a pyridine solution and then the mixture was refluxed for 30 minutes. Afterwards, with the heating and stirring turned off, the mixture cooled down and formed yellow

precipitates. These precipitates were filtered with diethyl ether, recrystallized from acetone and filtered again with acetone. The filtered solids were left to dry in the oven for 24 hours so that the greatest amount of solvent would evaporate. Then, it was made an ionic exchange for 24 hours twice, joining resin (Amberlite IRA-410 chloride form) with the precipitated solid and with enough methanol so that constant agitation is possible. Finally, on a rotary evaporator as much methanol as possible was evaporated from the solution of the previous step and then it was left to dry in vacuum for 24h.

1.3.2.Single Template MSNs (1-MSNs)

1.3.2.1. Synthesis

1-MSNs were synthetized by the modified Stöber method reported in the literature[18]. In a 500 mL polypropylene flask, 240 mL of deionized (DI) water and surfactant were added. Depending on the final particles desired were used 500 mg of CTAB (1-MSN-C), 771 mg of HFDePC (1-MSN-HFDePC), 998 mg of HFDePC (1-MSN- HFDePC (7.2 mM)), 471 mg of Met (1-MSN-Met) or 466 mg of Pyr (1-MSN-Pyr). These mixtures were mechanically stirred during 1h at 40°C. Then, were added 1.75 mL of NaOH solution (1.6M) and after 15 minutes 2.5 mL of TEOS were added dropwise. The solutions were left stirring for, at least, 3 hours. The mesoporous nanoparticles were recovered by centrifugation at 80000 x g for 15 minutes at 20°C and were washed two times with a mixture of ethanol and water (50% v/v) and one time with absolute ethanol, discarding each time the supernatant. The MSNs were dried at 60°C overnight a ventilated oven. In the case of the synthesis with the fluorinated surfactant (HFDePC), 0.875 mL of a 4.8 M NaCl solution was added to increase the particle size in order to allow an analysis of these particles in TEM. To study the effect of the ratio between TEOS concentration and fluorinated surfactant concentration, it was made a synthesis in which was added 10 times more volume of TEOS, the other parameters remained equal.

1.3.2.2. Template removal

The template in all the MSNs were removed with various methods, such as, solvent extraction with acidified ethanol, solvent extraction with THF in reflux, solvent extraction with DCM in reflux and calcination. Extractions with the addition of a salt were also tried, i.e., extraction with THF at reflux and at room temperature with Lithium Bromide (LiBr).

In solvent extraction with acidified ethanol was used a polypropylene flask with an acidified ethanolic solution (0.5 M HCl, 10 mL for each 200 mg of MSNs), and stirred overnight at 50 ºC. Then the mixture was centrifuged at 9500 rpm for 10 minutes and washed three times with absolute ethanol. The MSNs were dried overnight at 60°C in a ventilated oven.

In solvent extraction with THF in reflux was used a 50 mL round-bottomed flask with 25 mL of THF and 500 mg of each sample MSNs and it was refluxed overnight at 80 ºC. Then the mixture was centrifuged at 9500 rpm for 10 minutes and washed three times with absolute ethanol. The MSNs were dried overnight at 60°C in a ventilated oven.

In solvent extraction with DCM in reflux was used was a 50 mL round-bottomed flask with 25 mL of THF and 500 mg of each sample MSNs and it was refluxed overnight at 45 ºC. Then the mixture was centrifuged at 9500 rpm for 10 minutes and washed three times with absolute ethanol. The MSNs were dried overnight at 60°C in a ventilated oven.

Calcination of all 1-MSNs was performed applying a 1°C/min temperature increase rate up to 500°C at a nitrogen atmosphere, following 6 h at 600°C in an air atmosphere, with an air flow of is 8 L air/g particles/h.

The extractions with THF and LiBr were made with 12.5 mL of a solution of 0.5 M LiBr in THF along with 250 mg of MSNs in a 50 mL round-bottomed flask, one was made at reflux temperature and the other at room temperature. Then both mixtures were centrifuged at 9500 rpm for 10 minutes and washed three times with absolute ethanol. The MSNs were dried overnight at 60°C in a ventilated oven.

1.3.3.Synthesis of Double Template MSNs (2-MSNs)

2-MSNs were synthesized by the same method as 1-MSNs, that is, by the modified Stöber method, differing only in the fact that two surfactants were added instead of one. In a 500 mL polypropylene flask, 240 mL of deionized (DI) water and the two surfactants were added. Two syntheses were performed with 771 mg of HFDePC and 500 mg of CTAB. These mixtures were mechanically stirred during 1h at 40°C. Then, were added 1.75 mL of NaOH solution (1.6M) in one of the mixtures and in the other mixture were added 1.75mL of the same NaOH solution and 0.875 mL of NaCl (4.8M), and after 15 minutes 2.5 mL of TEOS were added dropwise. The solutions were left stirring for, at least, 3 hours. The mesoporous nanoparticles were recovered by centrifugation at 80000 x g for 15 minutes at 20°C and were washed two times with a mixture of ethanol and water (50% v/v) and one time with absolute ethanol, discarding each time the supernatant. The MSNs were dried at 60°C overnight a ventilated oven.

2. Results and Discussion

The aim of this novel project was to develop a hybrid nanocontainer based on mesoporous silica nanoparticles with a double pore system. The study was based on four surfactants with different characteristics, hexadecyltrimethylammonium bromide (CTAB), 1-hexadecyl-3-methylimidazolium chloride (Met), 1-hexadecylpyridinium chloride (Pyr) and 1H1H2H2H-perfluorodecylpyridinium chloride (HFDePC), in order to obtain a system with a double pore system in which both templates are selectively removed. For this, syntheses of single templated nanoparticles were performed and then, several template extraction methods were studied, in order to determine the best pair of surfactants for selective extraction. In Figure 1, is the illustrative summary of our strategy.

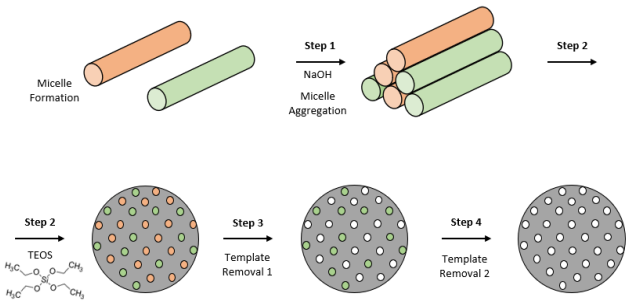

Figure 1: Schematic illustration of a hybrid mesoporous silica nanoparticles with a double pore system and selective release control.

2.1. Synthesis and Characterization of Single Template MSNs (1-MSNs)

In order to determine the best pair of surfactants to use in the synthesis of MSNs with two pore systems, independent syntheses were made with CTAB and three new templates: 1-hexadecyl-3-methylimidazolium chloride (Met), 1-hexadecylpyridinium chloride (Pyr) and 1H1H2H2H-perfluorodecylpyridinium chloride (HFDePC), where the first two are ionic liquids and the last is a fluorinated surfactant. The syntheses were performed under the same conditions for all surfactants, with a 5.7 mM concentration for all of them. Syntheses were performed with CTAB to serve as a reference. Table 1 presents a summary of synthesis details concerning each template, including their CMC.

Table 1: Quantities of surfactants for each synthesis and respective CMC.

Surfactant	Molecular Weight (g/mol)	Quantity (mg) (5.7 mM concentration)	CMC (mM)
CTAB	364.5	500	0.92 – 1.0[19,20]
Met	343	471	1.0[21]
Pyr	340	466	0.9[22]
HFDePC	561.7	771	2.5[17]

The synthesis of 1-MSNs starts by mixing the template with water, which is stabilized at the same temperature (40°C) for about an hour, then the base is added, in this case NaOH and, finally, TEOS is added drop by drop. The synthesis is completed after 3 hours. The same concentration of NaOH was used in all syntheses (1.6 M) to analyze the differences in sizes at the end.

2.1.1. Nanoparticle Morphology

Before removing the template, the diameter distributions of the 1-MSNs were determined by TEM. Samples of MSNs with CTAB (1-MSNs-C), with Met (1-MSNs-Met), with Pyr (1-MSNs-Pyr) and with HFDePC (1-MSNs-HFDePC), were analyzed. In Figure 2 are show the TEM images of each sample and their respective size distribution. The mean diameters obtained were: 69 ± 6 nm for 1-MSNs-C, 73 ± 10 nm for 1-MSNs-Met, 64 ± 9 nm for 1-MSNs-Pyr, 21 ± 2 nm for 1-MSNs-HFDePC, 61 ± 10 nm for 1-MSNs-HFDePC-NaCl and 17 ± 3 nm for 1-MSNs-HFDePC(+TEOS).

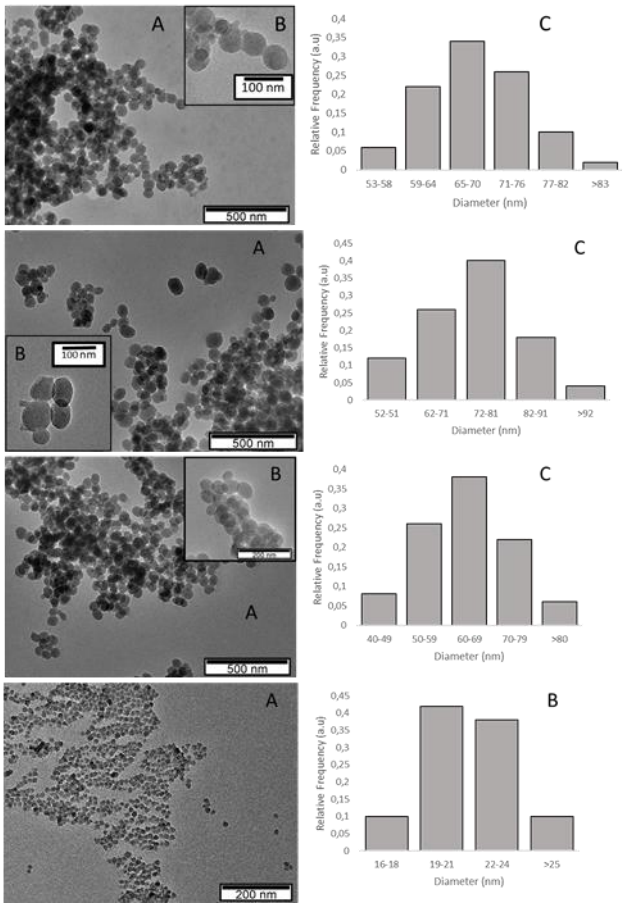

Figure 2: TEM images of samples 1-MSNs-C (69 ± 6 nm), 1-MSNs-Met (73± 10 nm), 1-MSNs-Pyr (64 ± 9 nm) and 1-MSNs-HFDePC (21 ± 2 nm), respectively, A – 500 or 200 nm scale; B-100 or 200 nm scale, and respective size distribution (C).

As we can see, in samples 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr, Figure 2, the size distribution is similar with low dispersity and their pore system is well defined, that is, the pores are more well packed. All of these three surfactants have a hydrocarbon chain of sixteen carbons, so the only difference between them is the cation, which

do not influence the mean size of particles, since they all have similar diameters. In the fluorinated sample the particles obtained were too small to see the pore system in TEM. The reason for this could be that the micelles are very stable, which leads to many points of nucleation of small size, i.e., due to its stability there is no aggregation. In order to increase the diameter, a solution of NaCl 4.8 M was added to the synthesis. The addition of salt decreases the colloidal stability due to the charge screening at the micelle surface (at higher ionic strength).[9] This sample was named of 1-MSNs-HFDePC-NaCl (Figure 3). The mean diameter obtained was 61 ± 10 nm.

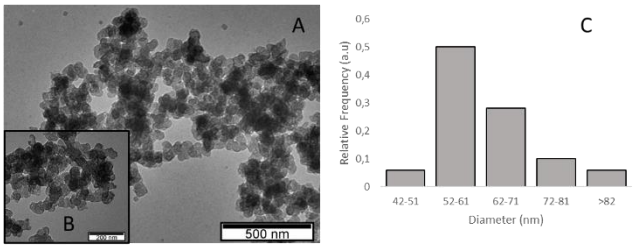

Figure 3: TEM images of sample 1-MSNs-HFDePC-NaCl: A - 500 nm scale; B-200 nm scale, and respective size distribution (C). Mean diameter: 61 ± 10 nm.

After the addition of salt, larger particles were obtained, as expected, with the diameter increasing from 21 nm to 61 nm. Compared with the 1-MSNs-HFDePC sample, we observe that the shape of the MSNs of the 1-MSNs-HFDePC-NaCl sample is not completely spherical, and that the pore system is not as organized as in the 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr samples. Also, the pores of these nanoparticles do not appear to be hexagonally arranged (Figure 3).

A synthesis was also made with the HFDePC template with a higher concentration - 7.2 mM. The CMC of HFDePC is higher than the CMC of the other surfactants, which are around 1mM, so in order to obtain approximately the same quantity of micelles we increase the concentration of the fluorinated surfactant in the synthesis. The initial concentration (5.7 mM) was selected to be 4.7mM higher than the CMC of CTAB, Met and Pyr, therefore we used a concentration of 7.2 mM of fluorinated surfactant (also 4.7 mM higher than its CMC). The resulting nanoparticles are shown in Figure 4. The particles obtained are not spherical: there are particles with an oblong shape and some larger agglomerates. Therefore, it is not possible to determine the diameter of these NPs. However, it is possible to see that the particles are porous.

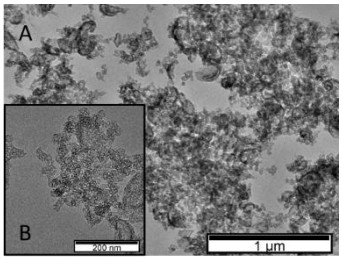

Figure 4: TEM images of sample 1-MSNs-HFDePC (7.2 mM): A - 1 µm scale; B-200 nm scale.

According to the literature, the HFDePC surfactant leads to different particle shapes and pore arrangements when different ratios of TEOS and fluorinated surfactant concentrations are used. The larger the ratio between the concentration of TEOS and HFDePC, the rounder the particles will be and the pore structure will have more order. Therefore, a synthesis was performed using a quantity of TEOS 10 times higher for the same concentration of fluorinated surfactant (5.7 mM) which increased the concentration ratio by 10 times. [23,24] As expected, we obtained more particles, due to the fact that it was used much more surfactant and, therefore, it was formed more micelles. Because of this there are more active centers for TEOS-template interaction and so more particles can be formed. The results for this sample, 1-MSNs-HFDePC(+TEOS) are shown in Figure 5. The mean diameter obtained was 17 ± 3 nm.

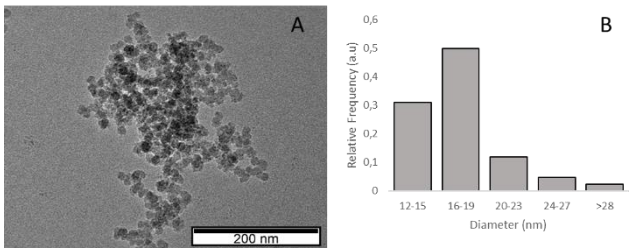

Figure 5: TEM images of sample 1-MSNs-HFDePC(+TEOS): A - 200 nm scale and respective size distribution (B). Mean diameter: 17 ± 3 nm.

As in the 1-MSNs-HFDePC sample (Figure 2) the NPs are too small to see if the pores are ordered or even if there are pores. Therefore, the same approach as before was used, an addition of a 4.8 M NaCl solution during the synthesis, to increase the particle size, allowing the visualization of the pores. The resulting NPs (1-MSNs-HFDePC(+TEOS)-NaCl) are shown in Figure 6.

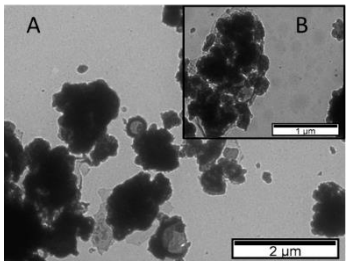

Figure 6: TEM images of sample 1-MSNs-HFDePC(+TEOS)-NaCl: A - 2 µm scale; B- 1 µm scale.

As we can see, very large agglomerates of particles were obtained with some vesicle-like hollow silica particles. It is possible to observe in Figure 6B that the particles are more elongated. This could mean that too much NaCl was used or that the TEOS/HFDePC concentration ratio was too low. In the literature[23], it is reported that samples with a TEOS/HFDePC concentration ratio between 30 and 170 have vesicle-like particles and are more elongated, so since our sample have a ratio of 82, these results are in agreement. In order to obtain rounder particles, the concentration ratio should be increased. As the particles are very agglomerated, it is not possible to determine their diameter or their size distribution.

The mean diameter of each sample obtained by TEM is present in Table 2. We can see that 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr are all the same size. In these three templates, the only difference is the cation since they have the same aliphatic chain, so we can conclude that the cation has no influence on the size of the nanoparticles. When the hydrophobic part is not the same, we can see that the diameter changes, i.e., when using a fluorinated surfactant (1-MSNs-HFDePC) the micelles formed are very stable which leads to many points of nucleation of small size, i.e., due to its stability there is less micelle aggregation with smaller micelle bundles, leading to smaller particles. It is possible to conclude that when adding NaCl, the colloidal stability decreases due to charge screening at the surface of the micelles, therefore the nanoparticles were obtained with a bigger diameter. Finally, when using more TEOS, more nanoparticles are formed but because the micelles are too stable, like the initial sample 1-MSNs-HFDePC, they have a small size too.

Table 2: Particles' mean diameter of each sample obtained by TEM and respective standard deviation.

Sample	D_{TEM} (nm)
1-MSNs-C	69 ± 6
1-MSNs-Met	73 ± 10
1-MSNs-Pyr	64 ± 10
1-MSNs-HFDePC	21 ± 2
1-MSNs-HFDePC-NaCl	61 ± 10
1-MSNs-HFDePC(+TEOS)	17±3

2.1.2. Template Removal and Porosity Characterization

The main goal is to be able to remove the templates selectively, i.e., solvents must be found that remove one template but not the other. One of the most important factors for an effective template removal is its solubility in the solvent used, since it facilitates its removal from the pores. Therefore we tested the solubility of the four surfactants, CTAB, Met, Pyr e HFDePC, in various solvents.[13,14] The solvents were chosen in order to comprise most of the solvent families, i.e., ethers, alkanes, chlorinated chains, alcohols, etc. Thus, four solvents were tested, THF, hexane, dichloromethane (DCM) and EtOH. The surfactants are all non-soluble in hexane, so this one is excluded. In THF, DCM and EtOH may have selectivity in removing templates since there is no solubility or insolubility in all of them. Thus, the removal of the four surfactants was tested with acidified EtOH, refluxing THF and refluxing DCM. Removal by calcination was also carried, where all template is removed and can be used as control to evaluate the extractions efficiency with the different solvents.

Empirically, it can be seen if there was template removal if the mass obtained after the extraction is lower when compared with the initial mass. However, the precise data must be obtained by nitrogen adsorption. In all of the studies were used 500 mg of each set of particles as initial mass, except in calcination and in the extraction of 1-MSNs-HFDePC-NaCl with EtOH/HCl, where we used 300 mg. The results are shown in Table 3 and Table 4.

Table 3: Weight loss after template removal by THF, DCM and acidified EtOH.

Solvent	THF	DCM	EtOH/HCl
Weight Loss (%)			
1-MSNs-C	20	14	62
1-MSNs-Met	18	12	72
1-MSNs-Pyr	27	13	67
1-MSNs-HFDePC	27	24	43
1-MSNs-HFDePC-NaCl	--------	--------	39

Table 4: Initial and final masses of NPs after template removal by calcination.

	Calcination		
	Initial Mass (mg)	**Final Mass (mg)**	**Weight Loss (%)**
1-MSNs-C	500	241	52
1-MSNs-Met	416	200	52
1-MSNs-Pyr	430	240	44
1-MSNs-HFDePC	278	180	35
1-MSNs-HFDePC-NaCl	500	290	42

It is known that surfactant removal by calcination is the most efficient process since it removes all organic components from the silica material.[11,13,25] The results obtain by this method will be used as control for comparison, in order to check the washing with EtOH/HCl extraction, since this method is known to also remove almost all of the existing surfactant.[25] As we can see in Table 3, comparing with the results obtained in Table 5, with THF in reflux there is a partial removal of all surfactants, so this solvent cannot be used for selective removal. With DCM extraction, as in THF extraction, there is a partial removal of all surfactants but less pronounced, i.e., it removes less and therefore this solvent cannot be used for selective removal. With acidified EtOH we can see that there is a similar removal for all surfactants, which means that it cannot perform a selective extraction. This method has a greater mass loss in relation to calcination. Since the mass loss values cannot be higher than those of calcination, we can conclude that there were losses during the procedure, probably during centrifuging and washing. The efficiency of extraction using acidified ethanol has to do with the fact that the H^+ in HCl is such a small ion that it can penetrate the pores of the NPs and therefore destabilize the ionic bond between the

surfactants and the surface anionic groups of silica. With the same reasoning, one way to improve the extraction with THF is to use a salt, in this case a lithium salt, in which the Li^+ ion will play the same role as the H^+ ion. Template removal with THF and lithium bromide (LiBr, 0.5 M) was made in 1-MSNs-C, in reflux and at room temperature. The results are shown in Table 5.

Table 5: Final masses of 1-MSN-C NPs after template removal by THF with LiBr at different temperatures.

1-MSN-C NPs Extraction by THF with LiBr

	Reflux	Room Temperature
Initial Mass (mg)	250	268
Final Mass (mg)	161	182
Weight Loss (%)	36	32

As expected, the weight loss increases when a salt is used, which confirms the previous hypothesis. However, the values obtained did not come close to the mass loss values obtained in the extraction with EtOH/HCl.

The results obtained between different surfactants extracted with the same method, were compared using the number of moles of surfactant lost per gram of particle used in the extraction (Table 6).

Table 6: Surfactant Loss (mmol)/g particles after template removal by THF, DCM and acidified EtOH.

Solvent	THF	DCM	EtOH/HCl
Surfactant Loss (mol)/g particles			
1-MSNs-C	0.55	0.40	1.70
1-MSNs-Met	0.53	0.34	2.10
1-MSNs-Pyr	0.79	0.37	1.98
1-MSNs-HFDePC	0.48	0.42	0.77
1-MSNs-HFDePC-NaCl	--------	--------	0.69

When using THF, we can see that all the surfactants are similarly removed. However, 1-MSNs-Pyr shows larger removal than the others, which may suggest a selective extraction method. It is, however not very efficient due to the difference not being very accentuated. In the extraction with DCM, we can conclude that the removal of each surfactant is very similar, so there is no selective removal of any surfactant. With EtOH/HCl, the removal is more efficient in 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr, as it removes more than twice as much as in 1-MSNs-HFDePC, and, therefore, it can be considered a selective method to this surfactant (again, it may not be very efficient since the variation is not very large). It should be noted that the HFDePC surfactant is the least removed with EtOH/HCl, which may suggest that it is not as easy to remove as the remaining surfactants, even with the most effective method.

To confirm the results obtained above, it is necessary to compare them with the results obtained by nitrogen adsorption, that is, by the BET (Brunauer-Emmett-Teller) and BJH (Barrett-Joyer- Halenda) method, in which the surface area is obtained in the first, and the pore volume and pore diameter in the second. Both in BET and in BJH methods, nitrogen is usually used because of its high purity and strong interaction with most solids.

The materials prepared in this project typically present mesopores, so the most likely isotherm will be of type IV. Nitrogen adsorptions were performed for 1-MSNs-C, 1-MSNs-Met, 1-MSNs-Pyr and 1-MSN-HFDePC, extracted with EtOH/HCl, THF and calcinated, to obtain the specific surface area (S_{BET}), the pore volume (V_p) and the pore diameter (D_p) (Figure 7, Figure 8, Table 7 and Table 8).

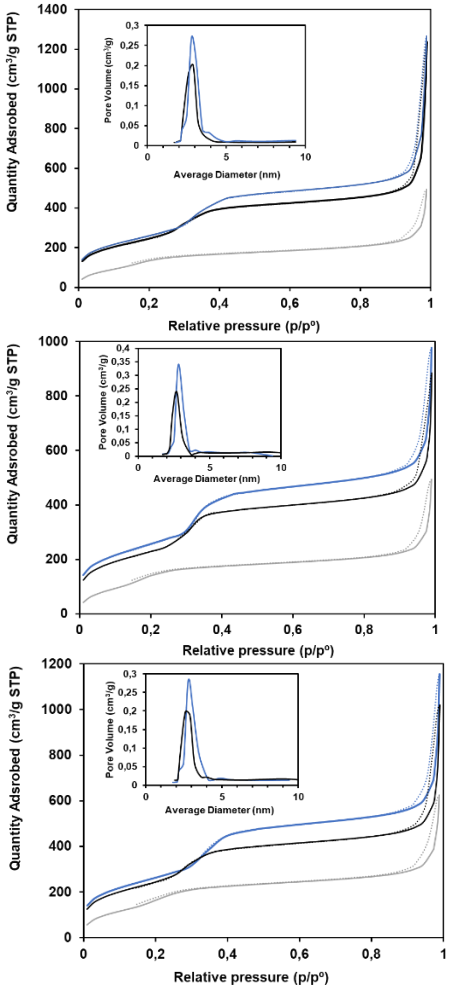

Figure 7: Nitrogen adsorption (solid line)-desorption (dotted line) isotherms for 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr calcinated (black), extracted with EtOH/HCl (blue) and with THF (grey) and corresponding pore size distribution (inset).

Table 7: Results obtained for 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr by nitrogen adsorption.

	S_{BET} (m^2/g)	V_p (cm^3/g)	D_p (nm)
1-MSNs-C Calcination	900	0.68	2.9
1-MSNs-C EtOH/HCl	950	0.77	2.8
1-MSNs-C THF	570	0.31	2.3
1-MSNs-Met Calcination	840	0.62	2.7
1-MSNs-Met EtOH/HCl	940	0.75	2.8
1-MSNs-Met THF	600	0.32	2.5
1-MSNs-Pyr Calcination	900	0.75	2.7
1-MSNs-Pyr EtOH/HCl	970	0.80	2.8
1-MSNs-Pyr THF	650	0.42	2.6

As we can see in Figure 7, as expected both extractions with EtOH/HCl and calcination yield type IV isotherms in all samples, which means that practically all surfactant are removed. However, the THF extraction it is a type IV isotherm but very little accentuated also in all samples, which suggests that not all surfactant was removed. These conclusions are supported with the results shown in Table 7 since the BET surface area, the pore volume and the pore diameter are significantly higher in both calcination and extraction with EtOH/HCl than in extraction with THF. The extraction with THF is therefore less efficient than extraction with EtOH/HCl or calcination, as concluded earlier by the study of mass loss before the extraction. That said, it is possible to confirm that the smaller the mass loss, the less surfactant was extracted by the method. The different values of calcination and extraction with EtOH/HCl derive from the condensation and contraction of the silica structure when submitted to high temperatures.

Finally, the results for 1-MSNs-HFDePC are shown in Figure 8 and Table 8.

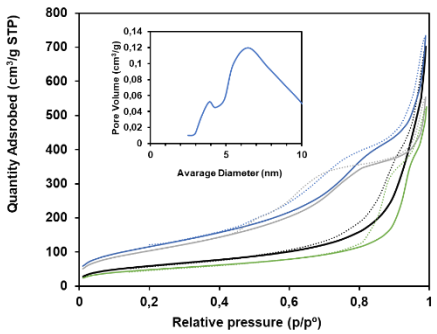

Figure 8: Nitrogen adsorption (solid line)-desorption (dotted line) isotherms for 1-MSNs-HFDePC calcinated (black), extracted with EtOH/HCl (blue), with THF (grey) and for 1-MSNs-HFDePC-NaCl calcinated (green) and corresponding pore size distribution (inset).

Table 8: Results obtained for 1-MSNs-HFDePC by nitrogen adsorption.

	S_{BET} (m^2/g)	V_p (cm^3/g)	D_p (nm)
1-MSNs-HFDePC Calcination	220	0.72	220
1-MSNs-HFDePC-NaCl Calcination	175	0.70	175
1-MSNs-HFDePC EtOH/HCl	430	0.71	430
1-MSNs-HFDePC THF	390	0.62	390

As we can see, in Figure 8, the isotherms are a little different from the isotherms of the previous particles, most notably in the fact that the adsorption and desorption isotherms are not very coincident. The extractions with EtOH/HCl and THF are type IV isotherms. However, for 1-MSNs-HFDePC and 1-MSNs-HFDePC-NaCl calcinations, the isotherms are a type IV but very little accentuated They are almost a type II isotherm, which suggests that not all surfactant was removed and that the pore structure collapsed, probably due to the high temperatures during calcination. These conclusions are supported with the results shown in Table 8, since the BET surface area and the pore volume are significantly higher in both EtOH/HCl and THF extractions. It can be confirmed that calcinations, when using this template, are not the best extraction method since the values obtained in Table 8 are very low. Comparing the THF extraction and EtOH/HCl extraction results to the mass loss study, since these methods were the only ones that did not collapse, the second method removed more surfactant in both studies.

As all the results of the nitrogen adsorptions are in agreement with the empirical results of the mass loss study after the extraction, it can be concluded that the extractions with DCM would have results very similar to those of THF, that is, smaller surface areas and smaller pore volume, so it is an inefficient extraction method for all surfactants, which means that with this solvent it is not possible to do a selective extraction of templates. In the extraction with THF with LiBr (mass loss analysis) we know that there was higher removal when compared with pure THF, so we would expect that the results of the adsorption of this method would be better, that is, the nanoparticles would have a greater surface area and a larger pore volume, making it a more efficient method. We can also conclude that none of the studied extraction methods can selectively remove one of the surfactants efficiently, since there are no significant differences in the different nanoparticles extracted by the same method.

2.2. Synthesis and Characterization of Double Template MSNs (2-MSNs)

Two syntheses were performed with a dual system of templates, HFDePC and CTAB, since these are the ones that differ the most in their molecular structure among the studied surfactants and, therefore, they have the largest

probability of not forming mixed micelles and also of being selectively extracted. The syntheses were done with the same amounts of all components as the 1-MSNs. The only difference is the use of two surfactants, which are added with the same mass as in 1-MSNs. The only difference between the experiments was the addition of a NaCl solution in one of them (2-MSNs-C-HFDePC-NaCl), to evaluate the effect of this salt in syntheses with a double pore system. These experiments were named 2-MSNs-C-HFDePC and 2-MSNs-C-HFDePC-NaCl.

The results obtained for the sample 2-MSNs-C-HFDePC and 2-MSNs-C-HFDePC-NaCl are shown in Figure 9.

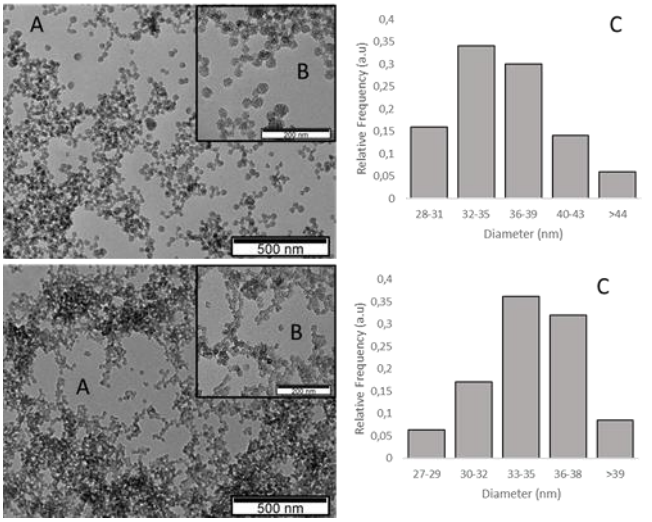

Figure 9: TEM images of samples 2-MSNs-C-HFDePC and 2-MSNs-C-HFDePC-NaC, respectively: A - 500 nm scale; B- 200 nm scale, and respective size distribution (C). Mean diameter: 35 ± 4 nm and 34 ± 3nm, respectively.

The mean diameter of each sample obtained by TEM is present in 35 ± 4 for 2-MSNs-C-HFDePC and 34 ± 3 for 2-MSNs-C-HFDePC-NaCl. That said, the particle diameters of the two samples are the same, which may suggest that the quantity of NaCl used was too small. However, analyzing Figure 9 it is possible to see some differences. The particles in the sample 2-MSNs-C-HFDePC are rounder and appear to have more cylindrical, visible pores (with what appear to be larger-than-normal pores, visible in Figure 9B). The particles from sample 2-MSNs-C-HFDePC-NaCl appear to be less round and with a more disorganized pore system. All this can be explained by the fact that the salt (NaCl) causes electrostatic screening of the micelles surface charge, i.e., there will be a decrease in intermicellar repulsion, which affects packing of the micelles, leading to a different pore morphology in the final nanoparticles.[9]

3. Conclusions

The aim of this project was to develop a novel hybrid nanocontainer, based on mesoporous silica nanoparticles with a double pore system for selective release control. To make this possible, four surfactants, CTAB, Met, Pyr and HFDePC, were tested in the synthesis of mesoporous silica nanoparticles with a single pore system, and then their removal was evaluated by different methods: calcination, extraction with EtOH/HCl, with pure THF, with THF and LIBr and with DCM, in order to select a pair of templates for the preparation of MSNs with a double pore system.

The nanoparticles (1-MSNs-C, 1-MSNs-Met, 1-MSNs-Pyr and 1-MSNs-HFDePC) were all synthetized under the same conditions, 5.7 mM of surfactant, by the modified Stöber method and were analyzed by TEM. For 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr the mean diameter was very similar, 69 ± 6 nm, 73 ± 10 nm and 64 ± 9 nm, respectively, as well as the pore diameters obtained by BET. For 1-MSNs-HFDePC, the mean diameter was very small when compared to the others, 21 ± 2 nm, from which it is concluded that the micelles formed are very stable which leads to many points of nucleation of small size, i.e., due to its stability there is no aggregation, leading to a smaller sized particle. In order to increase the size of these particles several hypotheses were tested: addition of NaCl during synthesis (1-MSNs-HFDePC-NaCl), increasing HFDePC concentration to 7.2 mM (1-MSNs-HFDePC(7.2 mM)), addition of more quantity of TEOS during synthesis (1-MSNs-HFDePC(+TEOS)) and finally, addition of more TEOS and NaCl during synthesis (1-MSNs-HFDePC(+TEOS)-NaCl). In 1-MSNs-HFDePC(7.2 mM) the particles obtained were not spherical and were formed larger agglomerates, however the particles are porous and so, it was not possible to determine the diameter. The expected increase in size was obtained in 1-MSNs-HFDePC-NaCl, with a diameter of 61 ± 10 nm. From this we can conclude that, since in the 1-MSNs-C, 1-MSNs-Met and 1-MSNs-Pyr samples have the same aliphatic chain but with different cations, the cation does not influence the size of the nanoparticles, as opposed to the change in the aliphatic chain, in the fluorinated surfactant, which influences the final size.

The extraction processes demonstrate that none of the methods can selectively remove one of the surfactants efficiently, since in none of the studies there were a significantly difference between results. However, two methods stand out, and may be promising if studied further: THF removed more template in 1-MSNs-Pyr than in other nanoparticles, and EtOH/HCl removed less template in 1-MSNs-HFDePc when compared to other nanoparticles.

In order to test particles with a double pore system, MSNs with both CTAB and HFDePC were prepared, either with no NaCl (2-MSNs-C-HFDePC) and with NaCl (2-MSNs-C-HFDePC-NaCl). The diameters obtained were very similar, 35 ± 4 for the sample without salt and 34 ± 3 for the sample with salt.

Overall, our results are very promising for developing novel nanoparticles with a double pore system for selective cargo release.

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