

*Review*

## **Designing advanced functional periodic mesoporous organosilicas for biomedical applications**

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**Abstract:** Periodic mesoporous organosilicas (PMOs), reported for the first time in 1999, constitute a new branch of organic-inorganic hybrid materials with high-ordered structures, uniform pore size and homogenous distribution of organic bridges into a silica framework. Unlike conventional mesoporous silicas, these materials offer the possibility to adjust the surface (hydrophilicity/hydrophobicity) and physical properties (morphology, porosity) as well as their mechanical stability through the incorporation of different functional organic moieties in their pore walls. A broad variety of PMOs has been designed for their subsequent application in many fields. More recently, PMOs have attracted growing interest in emerging areas as biology and biomedicine. This review provides a comprehensive overview of the most recent breakthroughs achieved for PMOs in biological and biomedical applications.

**Keywords:** Mesoporous silicas; adsorption; immobilization; enzymes; drug carriers; nanoparticles

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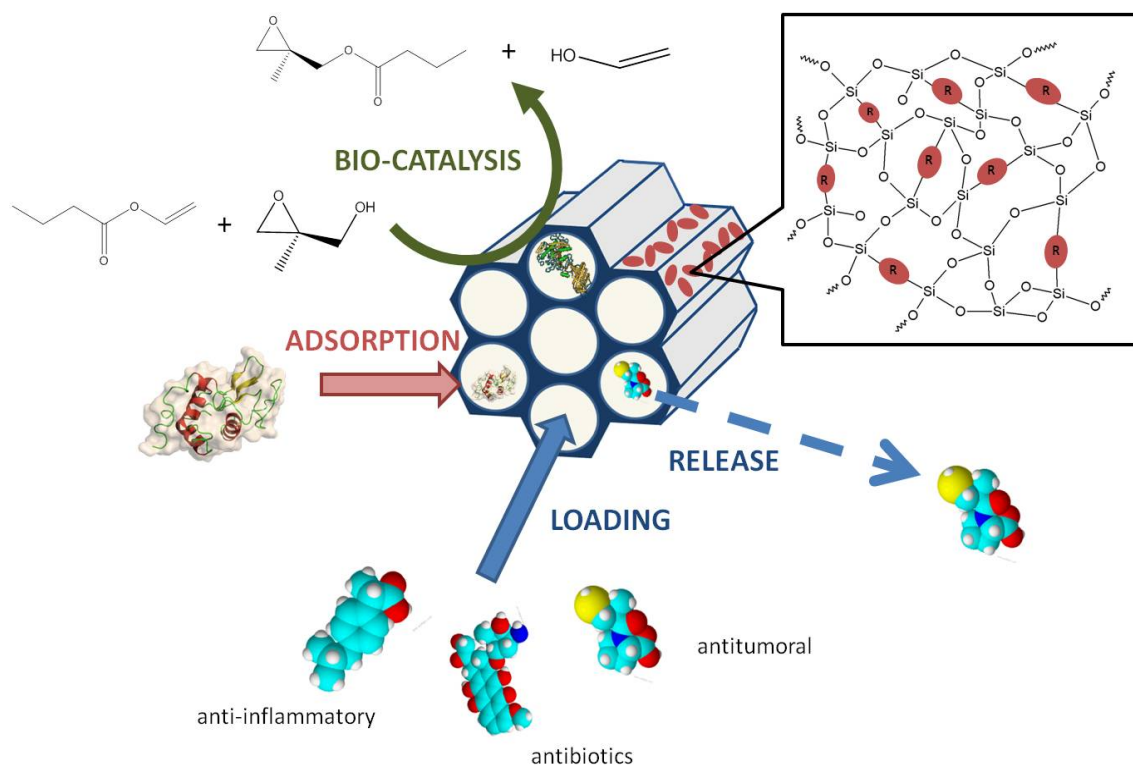
## 1. Introduction

Since the development of the first inorganic mesoporous materials by Kresge et al.[1] in 1992, numerous advances have been reported in this field producing a vast variety of mesoporous silica-based materials through template-directed synthesis. Periodic mesoporous silicas (PMS) with well-ordered structures, large surface areas, high pore volumes and well-defined pore size (2–50 nm) have attracted tremendous research interest in different areas. However, their surface functionalization was required in order to extent and to improve their application as adsorbents, catalysts, trapping agents, sensors, etc. The surface hydrophobization of mesoporous silicas has been accomplished by post-synthetic (“grafting”) or in-situ (“co-condensation”) processes using organoalkoxysilanes bearing terminal organic groups [2,3].

Periodic mesoporous organosilicas, commonly named as “PMOs”, are a new class of ordered organic-inorganic hybrid materials in which the organic units are homogenously distributed into the silica framework [4–6]. They are synthesized by silsesquioxane precursors of the type  $Z_3Si-R-SiZ_3$ , being Z a hydrolysable group (normally ethoxy or methoxy groups) and R the organic bridging group, in the presence of surfactants as structure-directing agents. Up to date, numerous organic moieties, from simple units (methylene, ethylene, ethenylene, phenylene) to more complex ones bearing different functionalities (thiol [7], chiral groups [8], metal complexes [9], heterocyclic compounds [10], etc.), have been successfully incorporated within the pore walls.

PMOs preserve the characteristic properties of mesoporous silicas — high surface areas and pore volume, tunable pore size, highly ordered mesostructures — but additionally exhibit some advantages as high hydrothermal and mechanical stability over their silica counterparts owing to the incorporation of high loading of organic moieties into their framework [11]. Moreover, unlike organo-functionalized mesoporous silicas, functionalities are embedded into the pore walls which overcome those drawbacks associated with grafting and co-condensation processes.

Nowadays, PMOs are considered a class of promising nanomaterials for potential applications, such as catalysis [12], chromatography [13], electronics [14], metal adsorption [15], immobilization or encapsulation of biomolecules and biomedicine. Herein, we will focus on the use of PMOs within the fields of biology and biomedicine. The objective of this review is to show the recent advances accomplished in the design of functional PMOs for their subsequent application as suitable supports/carriers of different biomolecules, biocatalysts and drugs. Figure 1 shows an overview of those application areas that will be deal with throughout this review.



**Figure 1. Application areas—biocatalysis, biomolecule adsorption and drug delivery systems—of PMOs.**

## 2. PMOs As Supports For Biomolecule Immobilization

The immobilization of biomolecules — proteins, enzymes, peptides and so on— onto solid supports has attracted a great deal of attention for practical applications. Common problems associated with the lack of stability of enzymes under extreme conditions and their reusability are clearly overcome after their immobilization, providing additionally several advantages as the easy separation from the reaction media, modification of the catalytic properties and prevention of protein contamination among others [16]. The immobilization of proteins onto porous hosts is accomplished by three common methods: physical adsorption, covalent attachment and encapsulation/entrapment[17]. Among them, physical adsorption is considered the most cost-effective and simple approach to immobilize proteins.

Since its discovery, mesoporous silicas have been extensively studied as supports for the immobilization of bioactive molecules [18,19]. The principal driving forces in the adsorption of biomolecules onto porous hosts are electrostatic, hydrophobic (weak van der Waals forces) and hydrogen-bonding. The main influencing factors may include the experimental conditions, such as the temperature, pH of the buffer solutions, ionic strength and the material properties, such as nanopore size, composition, mesostructure and morphology. Undoubtedly, another critical and determining factor in bioadsorption processes is the surface functionalization of mesoporous silicas.

Numerous functional groups have been anchored on silica surfaces in order to enhance their interactions with the protein surfaces.

The periodic mesoporous organosilicas (PMOs) with organic groups homogeneously distributed inside the channel wall provide new opportunities for controlling the chemical, physical and mechanical properties of these materials through incorporation of different kinds of bridging organic units in the mesoporous walls. Despite high participation of mesoporous silicas in bioadsorption processes, is not until 2005 when Hudson et al.[20] reported for the first time the use of PMOs as supports for protein/enzyme immobilization. They described a systematic methodology to study each influencing factor involved in the adsorption of proteins onto mesoporous silicates. For that, they carried out the immobilization of cytochrome *c* (cyt *c*) onto two adsorbents—SBA-15 and ethane-PMOs—with similar physical properties but different chemical composition. Adsorption measurements showed that electrostatic interactions dominated in SBA-15 while weak hydrophobic forces were the most prominent in ethane-PMO. These results were subsequently supported by Qiao et al. [21] who performed the cyt *c* immobilization on a highly ordered large-pore PMO with a rodlike morphology. They found that its adsorption capacity was not much higher than that of a pure silica with identical morphology and pore structure because the bioadsorption of this protein was mainly controlled by electrostatic forces and not hydrophobic ones. Different results were obtained by this research group in the immobilization of lysozyme (Lys), an antimicrobial protein, on an ethane-PMO with similar structural properties. In this particular case, electrostatic and hydrophobic interactions as well as the cohesive attraction and the repulsion between lysine and the amino acids present in the lysozyme molecules were determinant in the bioadsorption process [22,23].

More recent studies have examined the immobilization of these proteins, cyt *c* and Lys, onto PMOs with different hydrophobicity. Ha et al. [24] synthesized large pore PMOs with dipropylamine, phenylene and biphenylene bridging groups for the adsorption of lysozyme. Adsorption kinetics of benzene- and biphenylene-PMO for lysozyme were faster than that of SBA-15 at pH near the isoelectric point (pI) of the lysozyme because under these conditions the hydrophobic interactions were predominant. Comparing both hydrophobic organic silicas, biphenylene-PMO had a higher adsorption capacity owing to its greater hydrophobic character. These researchers also compared the bio-adsorptive properties of two PMOs with different hydrophobicity—benzene and biphenyl-PMO—as well as SBA-15 in the adsorption of cytochrome *c*. Although SBA-15 showed a higher adsorption capacity, as reported previously, in terms of hydrophobic forces these were greater on biphenyl-PMO than benzene-PMO [25].

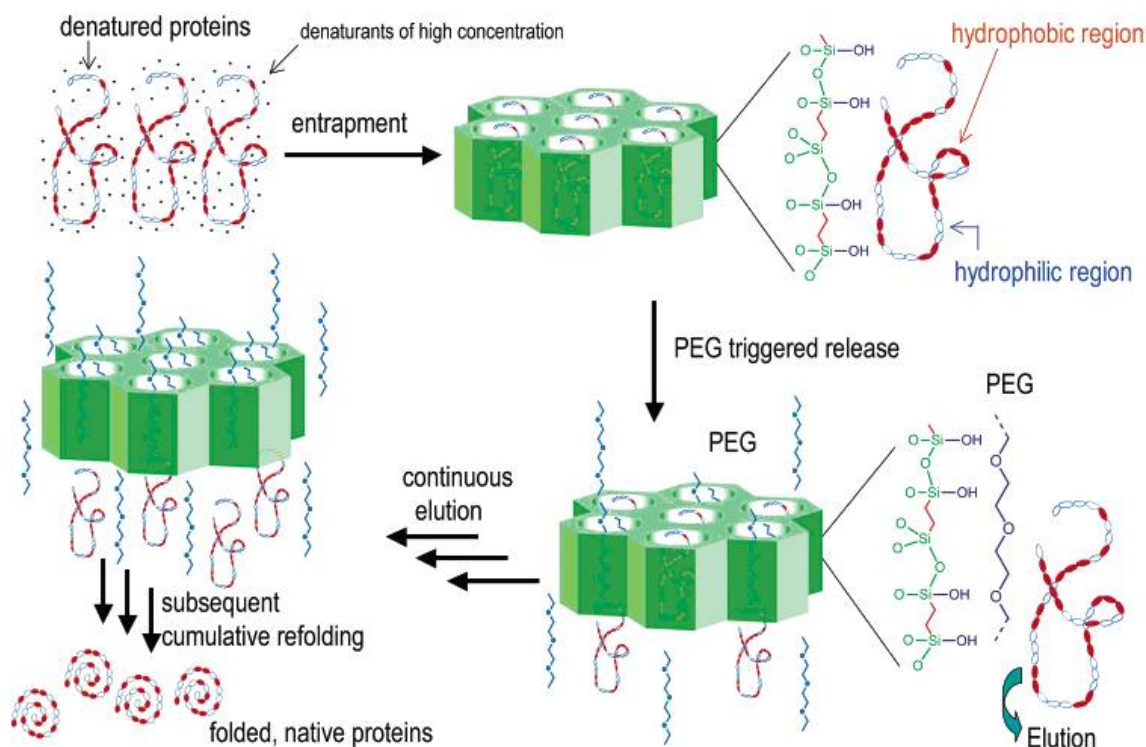
Further efforts on fabricating more hydrophobic walls to adsorb biomolecules involved the use of the so-called bifunctional PMOs. These materials composed of bridging organic units in the mesoporous wall and terminal organic groups protruding into the pore channels or by combination of various bridging organic units in the silica framework allow obtaining mesoporous materials with versatile surface properties. For instance, Yang et al. [26] synthesized different bifunctional PMOs with 1,4-diethylenebenzene and ethane bridging groups and used them as sorbents to investigate the

adsorption of lysozyme. A higher adsorption capacity on those materials was observed as the amount of 1,4-diethylenebenzene in the framework increased due to the fact that this aromatic derivative can form stronger hydrophobic and hydrogen-bonding interactions with Lys than the ethane groups. More recently, Zhang et al. [27] synthesized other bifunctional PMOs by post-grafting of amine- or carboxylic acid functionalized trialkoxysilanes on highly ordered ethane-PMO surface. These functionalized PMOs with different hydrophobicity and net charge were employed to selectively adsorb and purify three proteins—bovine serum albumin (BSA, pI = 4.8), hemoglobin (Hb, pI = 6.8) and lysozyme (Lys, pI = 11.0)—with different shapes and isoelectric points. These materials showed a high affinity to BSA than to Hb, while they were unsuitable for Lys adsorption.

Although protein immobilization has been extensively studied on PMOs, its role as biologic supports has not been limited to this type of biomolecules. For instance, Ha et al. [28] investigated the adsorption behavior of several amino acids on PMOs. In this case, three different functional PMOs—dipropylamino, benzene and biphenyl PMO—were synthesized and employed in this study. The experimental results showed that the isoelectric point and the hydrophobicity of the PMO as well as the hydrophobicity of the amino acid were the most important factors governing the bioadsorption process.

### **3. Refolding And Enrichment Of Biologic Molecules**

Besides their use as supports of biomolecules, PMOs have also found application in relevant processes for the bioengineering industry. For instance, the protein refolding process to obtain biologically active proteins from inclusion bodies. Although many proteins can be refolded properly without any external assistance at low concentrations, when protein and denaturant concentrations are high, it is necessary to find an effective procedure that can compete with the formation of inactive protein aggregates. Wu et al. [29] proposed a new method for lysozyme refolding using ethylene-bridged periodic mesoporous organosilicas. Owing to its unique surface and structural properties, this material could entrap individual denatured proteins inside their mesopores, minimizing the formation of protein aggregates, and subsequently exhibiting a stimuli-responsive controlled release of encapsulated proteins into the refolding buffer (Figure 2).



**Figure 2. Protein refolding assisted by PMOs. Adapted with permission from Ref.[29]. Copyright (2007) American Chemical Society.**

PMOs can also act as hosts for peptide enrichment. This accurate and efficient process is essential to detect and identify a diverse variety of peptides of significant interest in life sciences. The first attempts in this field were carried out by Yang et al. [30]. They synthesized bifunctional PMOs with ethane groups as bridging units and phosphonic acid groups grafted on the pore walls. The capability of coordination of these phosphonic groups with metal ions as  $Zr^{+4}$  and  $Fe^{+3}$  made them effective as potential IMAC (*immobilized metal affinity chromatography*) adsorbent for the selective capture of phosphopeptides. Later, Yu et al. [31] achieved the detection of 36 and 28 peptides from the bovine serum albumin digestion in presence of ethane-bridged PMO and amine-functionalized PMO, respectively. In comparison with pure silica materials with similar mesostructure, both materials showed higher and more selective peptide enrichment. Firstly, as mentioned above, PMOs with homogeneously distributed hydrophobic organic groups in their framework facilitated the peptide adsorption. Secondly, and more important, the opposite charge surface of both PMOs allowed to manipulate the electrostatic interactions between peptides and adsorbents, leading to porous materials with selective affinity for positively and negatively charged peptides, respectively. In order to improve the peptide enrichment capacity of PMOs, these researchers extended their studies to the detection of the peptide E7 from biological systems by designing ethane-PMO with spherical morphology and tunable pore size from 2.6 to 7.3 nm [32,33].

More recently, taking advantage of the amphiphilic character of PMOs, Liu et al. [34] reported a new approach for identification of membrane proteins from mouse liver. The presence of hydrophobic (-CH<sub>2</sub>-CH<sub>2</sub>-) and hydrophilic (-Si-OH) groups in the same material allowed its participation in multiple tasks of substrate dissolution, enrichment and digestion. In a first step, ethane-PMO was perfectly dispersed on a methanol solution to concentrate the dissolved membrane proteins. Next, the resultant protein-loaded PMO was redispersed in an aqueous solution containing hydrolytic enzymes, thus proceeding rapidly to the protein digestion.

#### 4. PMOs As Enzymatic Supports “Biocatalysts”

The use of enzymes as biocatalyst has been the subject of an increased interest for chemical and pharmaceutical industries in the production of a wide range of natural products. Lipases are considered one of the most important enzymes in biocatalysis owing to their availability, stability and capability of resolving a wide variety of different substrates. The first attempts to immobilize this biocatalyst onto PMOs were performed by Shakeri et al. [35] in 2008. Ethane-PMO exhibited a higher adsorption capacity than SBA-15 toward *Rhizopus oryzae* lipase (ROL) adsorption, due to the co-existence of electrostatic and hydrophobic interactions for the immobilization of the enzyme. In this particular case, the specific surface characteristics of lipase, more particularly, the presence of hydrophobic domains on its external surface generated stronger hydrophobic interactions than electrostatic interactions with the hydrophobic PMO surface. After its immobilization onto PMOs, factors as the hydrophobicity, the lid movement of the ROL and easy access of the substrate to the active sites led to a higher reaction activity versus that immobilized onto SBA-15 or even the free ROL.

Although the hydrophobization of hosts is undoubtedly essential in the immobilization of this kind of biocatalysts, the structural characteristics and porous topology can also play a decisive role during the bioadsorption/biocatalysis processes. Blanco et al. [36,37] carried out a comparative study of an ethylene-bridged PMO and several organosilicas with tunable hydrophobicity for lipase immobilization. The immobilization yield achieved by PMO was much higher than that of a non-functionalized SBA-15 (91 versus 44 mg/g) for lipase from *Candida Antarctica* with identical pore size (around 7–8 nm) due to the combination of electrostatic-hydrophobic forces in the adsorption. Moreover, this adsorption capacity also exceeded that of a methyl-functionalized SBA-15 having similar morphology, pore diameter and particle size owing to the weak hydrophobicity provided by the methyl groups in comparison with the ethane groups embedded in the pore walls. Nevertheless, the maximum enzyme loading capacity was exhibited by amorphous silica grafted with octyl groups; its higher pore size (23 nm) facilitated the continuous diffusional access throughout the pores. In terms of catalytic efficiency—catalytic activity per milligram of immobilized enzyme—PMO showed the highest activity among the studied supports. Although those octyl groups grafted on amorphous silica showed the highest enzyme loading and a negligible leaching, at the same time, its

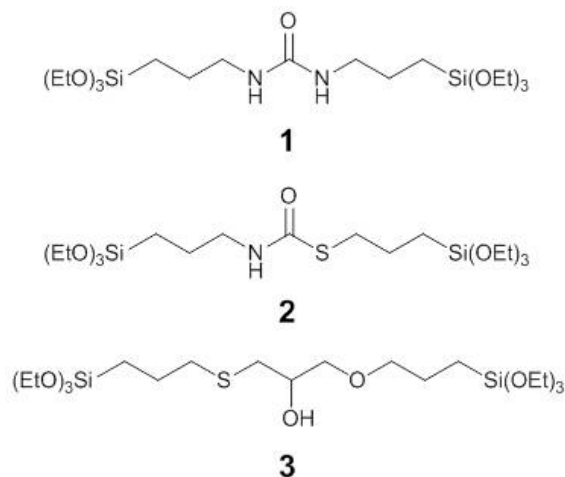
stronger interaction with lipase caused a higher distortion of the tertiary structure of the enzyme and therefore a lower activity.

Hartmann et al. [38,39] evaluated the immobilization of lipase from *Thermomyces lanuginosus* and its catalytic activity onto several large cage-like PMOs with ethylene, ethenylene and phenylene bridging groups. The largest adsorption efficiency was exhibited by a phenylene-bridged PMO due to its higher hydrophobicity. This surface property was also responsible for its higher relative activity in transesterification and hydrolysis reactions. As previously found for hydrophobic carriers, the PMO facilitated the fast diffusion of the organic substrates to the enzyme and promoted the interfacial activation through stabilization of its active open conformation.

Despite the wide interest to immobilize lipases, also other types of enzymes have been immobilized on PMOs to participate as biocatalysts of relevant industrial processes.[40-42] For instance, Hudson et al. [43] reported the synthesis of different PMO materials by co-condensation of TEOS with bis [3-trimethoxysilyl]propyl] amine to be used as supports of chloroperoxidase (CPO) (ca. 6.2 nm), a heme peroxidase. This enzyme was successfully immobilized at pH = 6 in those materials with pore entrances large enough to allow the enzyme to enter the pores. Although the immobilized CPO showed lower activity than the free enzyme, it could be reused 20 times with only a small loss in activity.

More recently, another heme enzyme, the horseradish peroxidase (HRP) (3.7 nm × 4.3 nm × 6.4 nm) was immobilized on functionalized periodic mesoporous silicas. Zhu et al. [44] reported the synthesis of PMOs containing urea and carbamothioic units into the silica framework. They used mixtures of precursors (1) and (2) (see Figure 3) in the presence of P123 to produce materials with different composition, morphology and structural and surface properties. These characteristics were clearly decisive in HRP adsorption. Thus, the material with the highest C and N content adsorbed more HRP (40 mg/g) due to the combination of hydrophobic interactions between the propyl groups of the support and the hydrophobic domain of the enzyme, and hydrogen bonds between the secondary amine groups and the carboxyl groups present in the enzyme. Moreover, the pore size and the morphology of these PMOs also affected the adsorption process. Both the catalytic activity and the stability of the immobilized HRP were dramatically improved in comparison with the free enzyme. Later, these researchers extended their HRP adsorption studies to a different class of periodic mesoporous organosilicas with hydrophilic bridging units containing –OH, –O– and –S– groups (3) into the pore walls [45].

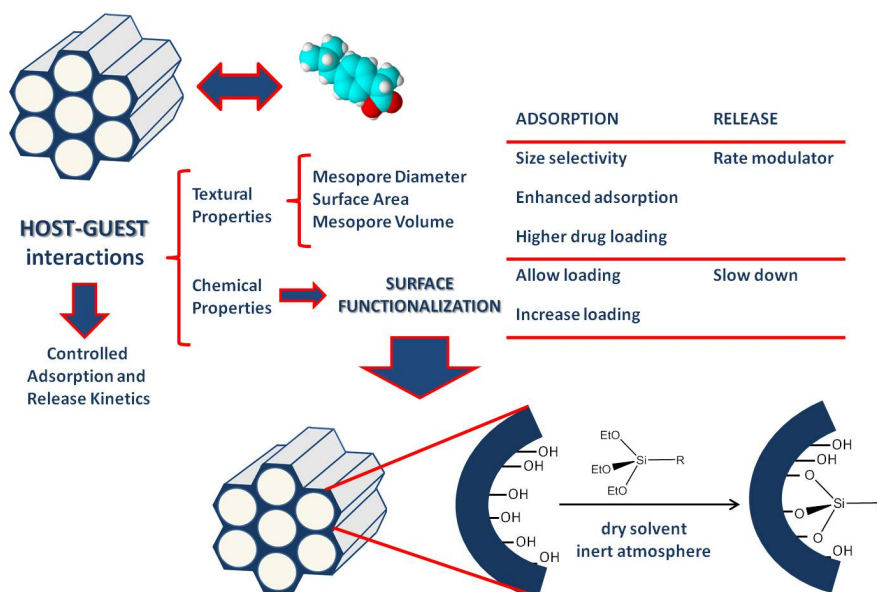




**Figure 3. PMO precursors for horseradish peroxidase immobilization.**

## 5. PMOs as Potential Drug Delivery Systems

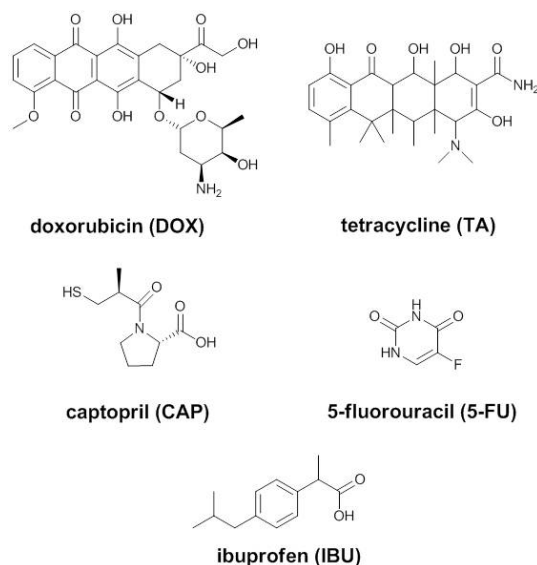
In the last few years, numerous efforts have been devoted by the scientific biomedical community to the search of new kinds of host materials as controllable drug delivery systems (DDS). These DDS should have the capability to transport the therapeutic drugs without any loss to the targeted cells or tissues and, once reached its destination, to release the cargo in a controlled manner. A wide variety of materials, such as hydroxyapatite [46], biodegradable polymers [47], hydrogels [48] or mesoporous silicas [49] have been employed in controlled drug delivery systems. Among them, silica-based mesoporous materials have been proven to be promising candidates to confine drugs or biologically active species in their mesopores. Numerous model drugs, such as ibuprofen, amoxicillin, gentamicin, erythromycin, naproxen, aspirin and alendronate, have been incorporated into ordered mesoporous silicas. Their increased participation as host matrices is due to several factors, such as uniform and tunable pore size, large surface areas, high pore volumes, nontoxic nature and good biocompatibility. However, in some cases, the hydrophilic silica walls need to be functionalized with organic groups to allow the loading of hydrophobic drugs. This functionalization seems to be the main controlling factor on the drug adsorption/desorption processes, leading to higher drug loading capacities and slower release kinetics, avoiding the well-known “burst release” effect. Excellent reviews on this matter have been published by Vallet-Regí et al. [50,51].



**Figure 4. Parameters that govern the loading and release rate of drug molecules in silica-based ordered mesoporous materials. Reproduced from Ref.[51]**

As depicted in Figure 4, functionalization or post-modification by silylation with functional alkoxy silanes leads unavoidably to pore narrowing or even pore blocking. Nevertheless, PMOs with well ordered structures and high loading of organic groups embedded into the pore walls show a lower steric hindrance to the access to drug molecules into their mesopore channels.

Controlled drug release systems based on PMOs were reported for the first time in 2009 [52]. Since then, PMOs have been explored as host matrices for different kinds of drugs -anti-inflammatory, antibiotics or antitumoral- (see Figure 5).



**Figure 5. Model drugs employed in adsorption/release processes onto periodic mesoporous organosilicas.**

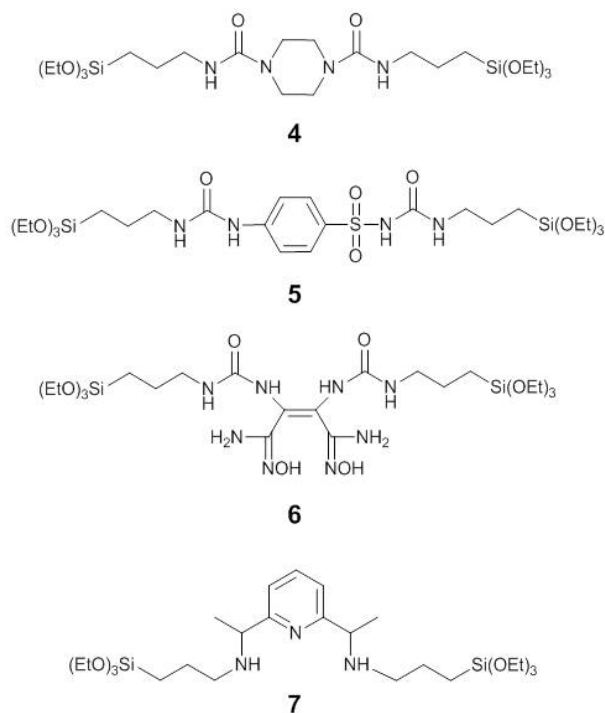
Solid and hollow spheres of ethylene-bridged PMOs were reported as drug delivery systems, where tetracycline (TA)—a broad spectrum antibiotics—was chosen as model drug to study the adsorption and release processes. Higher adsorption capacities on those PMO hosts than on solid spheres of pure silicas owing to the stronger hydrophobic interactions between the pore walls and tetracycline were reported. These forces were also determinant in drug release process, leading to lower release rates in the former materials. Although the wall composition had a great influence, the morphology also played an effective role in the adsorption of guest molecules. Thus, the hollow PMO, with higher pore volume and surface area, exhibited better adsorption (loading) and release performances for tetracycline.

Recently, Kao et al. [53,54] achieved to synthesize functionalized PMOs with ethylene- or phenylene bridges embedded into the pore walls and a high loading of protruding carboxylic acid groups. The carboxylic acid group (COOH) is well-known in organic chemistry for its ability to be easily deprotonated under neutral and basic conditions, giving rise to negatively charged groups. These bifunctional PMOs with negative COOH and hydrophobic ( $-\text{CH}_2-\text{CH}_2-$  or  $-\text{C}_6\text{H}_4-$ ) groups showed an excellent adsorption capacity for a positively charged, hydrophobic anticancer drug, doxorubicin (DOX). In fact, the stronger electrostatic interactions between the negatively and positively charged host and guest molecules, respectively, in combination with hydrophobic forces, led to lower release rates in those materials with higher  $-\text{COOH}$  functionality.

PMOs with specific functional groups can also be effective carriers for hydrophilic drugs (see Figure 6). For instance, Ha et al. [55] prepared PMO materials with a long functional chain by condensation of the bis-silane precursor (4), that contains ureylene ( $-\text{NHCO}-\text{N}-$ ) and a heterocyclic ring (piperazine), and TEOS. The resulting materials were tested in vitro in the loading and release of two hydrophilic drugs, i.e. CAP (highly hydrophilic) and 5-FU (weakly hydrophilic), at pH 7.4. On the one hand, that material with a higher content of the ureylene moiety showed a higher adsorption capacity of CAP (25 %) and 5-FU (22 %). The incorporation of this drug into PMO materials was mainly driven by hydrogen bonding interactions between the organic functionalities (imine or carbonyl) and the drug molecule. On the other hand, even though all materials exhibited similar and sustained release kinetics, the slowest release rate was observed on that host material with the smallest pores. Similar observations were reported for the adsorption/desorption of CAP and 5-FU onto PMOs containing urea (NHCONH) and sulphonamide functionalities (5) [56].

Additionally, on the basis of these facts, Ha's group took this research a step further by introducing an amphoteric ligand *-amidoxime-* as bridging group in the framework of PMOs through co-condensation reactions of (6) with TEOS.[57] These materials with ureylene ( $-\text{NHCO}-\text{N}-$ ) and amidoxime ( $\text{H}_2\text{N}-\text{C}=\text{N}-\text{OH}$ ) moieties were examined as drug loading/release carriers for both hydrophobic (IBU) and hydrophilic (5-FU) drugs in a phosphate buffer solution at different pH values. As for other cases, they observed that the drug loading capacity of both drugs into that material increased as the content of organic bridges increased. Moreover, all materials had a higher affinity for IBU than for 5-FU due to the existence of more favorable hydrophobic/hydrogen bonding

interactions between the host and IBU than those for the hydrophilic 5-FU.



**Figure 6. Functionalized bridged precursors used in the synthesis of PMOs for controlled drug delivery.**

## 6. PMO Nanoparticles For Medical Applications

The growing interest in the field of nanobiotechnology has promoted the development of novel and biocompatible nanomaterials. Particle morphology and size are decisive factors in the biocompatibility of these materials. In recent years, the use of nanoparticles in biomedicine has exponentially increased in applications such as drug delivery, biomedical imaging and sensing and cell tracking. Among those different kinds of nanoparticles employed in biomedicine, MSNs (mesoporous silicas nanoparticles) have been extensively reported owing to their controllable sol-gel synthesis, easy post-functionalization, high biocompatibility and low toxicity. Unlike the silica mesoporous nanoparticles, PMO nanoparticles (PMO NPs) not only have the already-mentioned advantages of bulk PMOs (hydrothermal and mechanical stabilities, hydrophobicity and homogenous distribution of organic groups), but also some additional properties related to their nanometric size such as fast mass transport, effective adhesion to substrates and good dispersity.

One of the earliest studies in the preparation of nanometer-sized mesoporous organosilica particles was described by Lu et al. [58] in 2006. They obtained hollow spheres of ethylene-bridged PMOs with highly ordered hexagonal mesostructure and tunable particle size (300–500 nm). In the following years, numerous efforts have been devoted to the development of PMOs with smaller

particle size (< 300 nm) and ordered mesostructures. However, most of the synthetic approaches were unsuccessful, resulting nanomaterials of suitable particle size and worm-like mesostructures [59-62].

In the last two years, great advances have been accomplished in the synthesis of adequate PMO nanoparticles for biomedical applications. Huo et al. [63] reported a new route to synthesize a variety of highly ordered PMO NPs with methylene-, ethylene-, ethenylene- and phenylene- bridging groups using cetyltrimethylammonium bromide (CTAB) under basic conditions. The ammonia concentration and co-solvent content in the reaction mixture influenced significantly the particle size. Once confirmed their high thermal stability and good dispersion in organic solvents, they studied the internalization of methylene-bridged PMO into HeLa living cells. They observed that the PMO nanoparticles could enter into the living cells and accumulate preferably in the perinuclear region. Moreover, cell viability measurements in the presence of 4–125 µg/ml PMO nanoparticle for 24 h revealed that a maximum of 25 % of the HeLa cells died at the highest concentration. These values, similar to those obtained with mesoporous silica nanoparticles, confirmed the low toxicity of PMO NPs and, therefore, their biocompatibility.

In a recent study published in 2013, novel functional PMO NPs containing pyridine units into the pore walls were synthesized by co-condensation of (7) with TEOS [64]. The spatial arrangement of the pyridine ring and two close N–H groups endowed this PMO with unique properties to participate in a wide range of applications such as chemosensor for nucleobases or drug nanocarrier, among others. In the former case, this chemosensor allowed to selectively recognize thymidine — a nucleobase — over other competitive nucleobases (adenosine, guanine and cytidine) due to the existence of strong intermolecular three-point hydrogen-bonding interactions between the organic moieties and thymidine. In the latter one, these materials were evaluated as suitable nanocarriers for the loading and release of 5-FU in cancer therapy. In this sense, the pyridine-containing PMO exhibited a high loading capacity of 5-FU (128 mg/g), whose release was controlled by pH, giving rise to a stimuli-response carrier. Finally, *in vitro* cytotoxicity studies confirmed the effectiveness of 5-FU loaded PMO for cancer-cell treatment as well as its excellent biocompatibility.

## 7. Future Prospects

In this review, a comprehensive overview of the incipient applications of periodic mesoporous silicas in biology and biomedicine has been reported. The most important developments in the use of PMOs as supports for the immobilization of active biomolecules—protein, aminoacids and enzymes—have been summarized. In most cases, periodic mesoporous organosilicas showed higher adsorption capacity than silica-based mesoporous materials due to the existence of hydrophobic interactions between the organic groups embedded in the pore walls and the guest molecules. Additionally, PMOs presented a lower steric hindrance to the access of bulky molecules inside the pores than functionalized silica materials where pore narrowing was unavoidably observed. The possibility of

using PMOs as controlled drug delivery systems into simulated body fluids has been also outlined. PMOs provide great possibilities for designing suitable host-matrices for this application through the careful choice of suitable bridges able to interact with functional groups of the drug. In this sense, these tailor-made materials could enable to control the drug loading and their subsequent release. Finally, the most recent advances aimed at developing nanometer-size PMOs for their application in bionanotechnology have been presented. Although the studies on this field are really scarce, their good biocompatibility and low cytotoxicity open challenging opportunities for the use of PMOs as biomaterials in the future.

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