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# Mesoporous Silica-Based Supports for the Controlled and Targeted Release of Bioactive Molecules in the Gastrointestinal Tract

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**Short version of title:** Smart delivery systems based on MSPs

**Keywords:** controlled delivery, targeted delivery, porous silica, molecular gates, gastrointestinal tract

**Abstract**

Mesoporous silica particles (MSPs) have attracted increasing interest as supports in the design of controlled delivery materials. Besides their excellent properties as loading supports (i.e. large surface area and pore volume), the modification of their external surface with molecular/supramolecular ensembles allows the design of gated MSPs. Delivery systems based on gated MSPs show "zero delivery" until an adequate stimulus is present and triggers gate opening and the cargo is released. Encapsulation of bioactive molecules in gated MSPs may improve biological stability, facilitate component handling, mask unpleasant sensorial properties and modulate the bioaccessibility of target molecules along the gastrointestinal tract. These properties make gated MSPs excellent candidates for encapsulating bioactive molecules and their subsequent utilization in the formulation of functional foods. This text highlights the most significant endogenous triggering stimuli that might be applied to design these site-specific delivery systems, as well as the strategies to develop them. Given the novelty of using MSPs in the food sector, the benefits and current potential limitations of employing MSPs in human food have been identified and discussed.

## 1. **Mesoporous silica particles as encapsulation supports**

Mesoporous silica particles (MSPs) are structures of silicon dioxide ($SiO_2$) which are arranged so that they create pores of 2-50 nm (Zhao 2006). The first described porous silica with a uniform pore size, called folded sheet mesoporous material (FSM-16), was reported by Kuroda and co-workers in 1990 (Yanagisawa and others 1990). A few years later, in 1992, researchers of the Mobil Company reported the synthesis of a family of mesoporous silica materials called M41S (Beck and others 1992), which include hexagonal MCM-41, cubic MCM-48 and lamellar MCM-50.

Since its discovery, applications of MSPs have grown exponentially as a result of their unique properties. Specifically, MSPs have demonstrated to have huge applications in the food sector, where they could be employed as catalysts in the synthesis of nutrients and bioactive molecules (Márquez-Ávarez and others 2004), in sensor technology (Climent and others 2009) and also as carriers in the design of smart delivery systems (Bernardos and others 2008, Pérez-Esteve and others 2015). Of these applications, the design of smart delivery systems is viewed as challenging given the possibility of improving the handling and utilization of different bioactive molecules or functional ingredients, and the subsequent formulation of functional food (Bernardos and Kourimská 2013).

Although there is neither a regulatory nor a standard definition of “functional foods” (Aryee and Boye 2015), this term refers to the foods and food components that may offer health benefits beyond basic nutrition (Bech-Larsen and Grunert 2003). The terms food components and bioactive ingredients with beneficial biological activity include basic nutrients (i.e. carbohydrates, proteins, lipids, vitamins, minerals, etc.), bioactive components (i.e., omega-3 fatty acids, amino acids and peptides, and phytochemicals), sensory appeal compounds (i.e. organic acids, flavors and pigments), as well as pre- and probiotics, healthy oils, spices and herbs (Fang and Bhandari 2012).

Despite the increase in functional products in markets and the scientific literature, the incorporation of these functional ingredients into existing food formulations is still viewed as challenging. On the one hand, most studies on the functionality of food compounds have been done *in vitro*, which thus excludes studying changes in potential active compounds during food processing, storage, ingestion and interaction with gut microflora. On the other hand, some bioactive components are most complicated to be handled or are not compatible with the food matrix in terms of solubility (lipophilic compounds), sensorial properties (i.e. fish oils or garlic extracts), or are very susceptible to degradation (vitamins, antioxidants). The desire to overcome these limitations has increased the interest in the encapsulation of bioactive components because after encapsulation, they could be released in a particular site-of-action of the digestive tract and/or be absorbed in their native form, which thus avoids problems related to instability or to unpleasant sensory properties (McClements 2012).

Typically, food applicable encapsulating systems are based on carbohydrates, proteins or lipids (Fathi and others 2012; Wang and others 2012; Fathi and others 2014). However, these systems exhibit low structure stability while food is processed and stored, a poor capability to control the release rate or to provide a targeted delivery, and a very poor effect on the protection of the encapsulated substance while it passes through the stomach. Some of these

problems could be avoided if mesoporous silica particles (MSPs) are used as encapsulating supports. Compared to other organic polymer-based carriers, MSPs are more stable, rigid and biocompatible. They also better resist the harsh conditions of the stomach and microbial attack. MSPs are also able to protect entrapped guest molecules against enzymatic degradation or denaturation induced by pH or temperature (Arcos and Vallet-Regí 2013).

This review critically assesses the possible use of mesoporous silica materials to design site-specific smart delivery systems capable of encapsulating, protecting, transporting and releasing bioactive molecules in a controlled fashion in the gastrointestinal tract (GIT).

## 2. *Fabrication of gated MSPs*

### *2.1 Synthesis and features of the inorganic support*

MSPs are synthesized using two main elements: a) a template whose function is to direct the construction of the high ordered (crystalline) porous net; b) a polymeric precursor which self-organizes around the template and, upon polymerization, builds up the final rigid structure. Synthesis starts with the polymerization, in an aqueous solution, of the inorganic siliceous precursor (i.e. tetraethyl orthosilicate) around surfactant micelles (i.e. *N*-cetyltrimethylammonium bromide -CTAB-). The mesoporous inorganic scaffold obtained under these conditions presents cylindrical unidirectional empty channels of approximately 3 nm in diameter (when CTAB is used as a surfactant), arranged in a hexagonal distribution. Mesoporous materials are obtained by the subsequent removal of the surfactant by extraction with adequate solvents, or by aerobic high temperature calcination (500-600ºC) (Hoffman and others 2006). Figure 1 schematically represents the complete synthesis procedure.

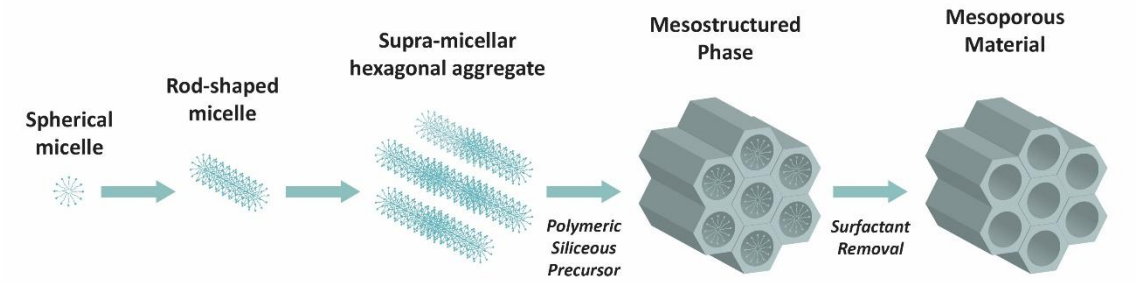



**Figure 1.** Schematic representation of the synthesis of mesoporous silica particles by structure-directing agents

Minor changes in the synthesis route make it possible to modify final key features in the solid to produce other types of mesoporous silica, such as hexagonal mesoporous silica (HMS) (Tanev and Pinnavaia 1995), Michigan State University material (MSU) (Bagshaw and others 1995), Santa Barbara Amorphous Silica (i.e. SBA-15) (Zhao and others 1998 a,b), Technische Universiteit Delft material (i.e. TUD-1) (Jansen and others 2001), Universidad Valencia Material (i.e. UVM-7) (el Haskouri and others 2002), and a wide variety of hollow silica spheres (Li and others 2004; Zhang and others 2009; Cao and others 2013). TEM and FESEM pictures of some of these particles are provided in Figure 2.

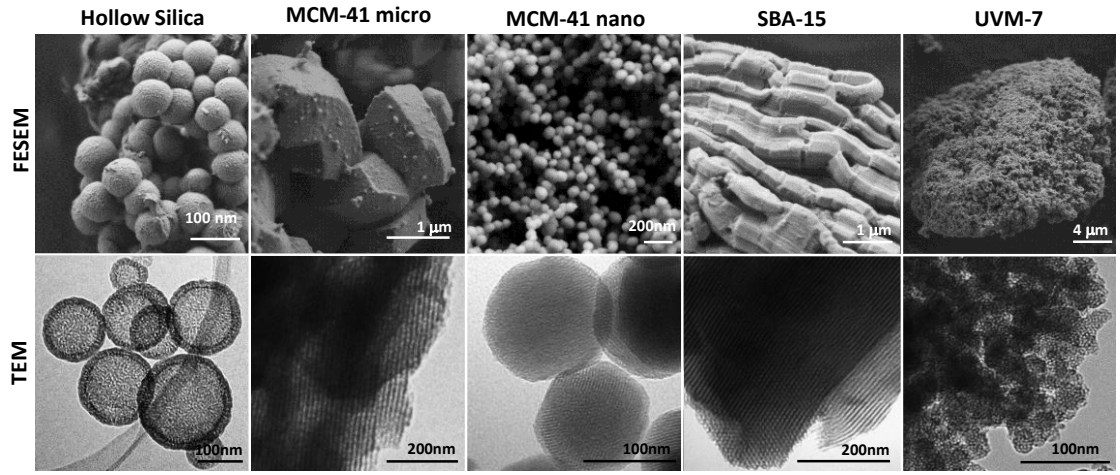



**Figure 2**. TEM and FESEM images of different mesoporous silica particles.

Given the potential application of MSPs to develop oral controlled delivery systems, different attempts to synthesize MSPs from food-like precursors have been successfully made. On the one hand, rice husk ashes have been employed as a silica source for the synthesis of different mesoporous silicas (Jang and others 2009; Bhagiyalakshmi 2010). On the other hand, polyglycerol esters of fatty acids, myristic acid ester of pentaglycerol and oleic acid have also been employed as food grade structures directing agents (Kapoor and others 2010; Han and others 2011; Ishii and others 2012).

In any case, different MSPs share their composition, which is based on a $SiO_2$-network, an ordered mesostructure and the presence of silanol groups on the particle surface. Some differ from others in size, shape, porous size and volume, specific surface area and density of silanol groups on the surface to provide different surface charges (Pérez-Esteve and others 2014). The morphology and porosity of different MSPs are determined by processing parameters: type of surfactant template, silica source, pH, temperature, aging time, additives, and solvents (Kierys and others 2010). The textural properties of different MSPs have been previously revised and compared in different publications (Wang and others 2011; Wright 2008).

In general, MSPs stand out for being supports that can be synthesized with a controlled size from 50 nm to a few microns. This range in size is important in scope. While small MSPs can cross epitheliums and can be distributed in the body to be non specifically internalized by certain cells, oversized particles cannot easily cross physical membranes in the body. As particle size has been demonstrated to play a key role in the distribution and behavior of particles in living systems, large particle sizes are preferred for developing orally administrated controlled release devices (Arcos and Vallet-Regí 2013).

MSPs can also be synthesized with uniform tunable porosity. Pore size can be tailored between 2-10 nm (Aznar and others 2009a). The presence of a mesoporous network provides large surface areas (700-1000 $m^2g^{-1}$) and a great loading capacity compared to large pore volumes (0.6-1 $cm^3g^{-1}$) (Colilla and others 2013). Pore size, pore volume and a proper surface charge are essential for encapsulating a sufficiently large amount of a certain bioactive component and for efficiently retaining it during storage. Adsorption of bioactive molecules into mesoporous

140 silica is governed by size and charge selectivity. Only the molecules with a size smaller than the
141 porous size of the silica support can be entrapped by the porous structure (Arcos and others
142 2013). Other factors that determine adsorption and the release kinetics of a bioactive
143 compound in a certain media are pore length and pore ordering (Izquierdo-Barba and others
144 2009a; Burguete and others 2012), particle morphology (Manzano and others 2008), surface
145 area (Balas and others 2006), macroscopic form (Izquierdo-Barba, 2009b) and modification or
146 functionalization of the silica surface with functional groups (Nieto and others 2008).

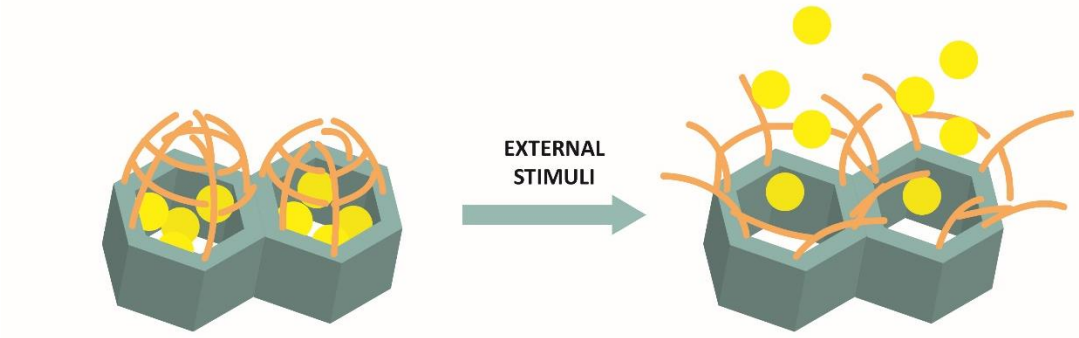
147 Finally, the surface of MSPs can be easily functionalized with molecular/supramolecular
148 ensembles to develop gated MSPs that show "zero delivery" and are capable of releasing their
149 cargo on-command in response to specifically designed external stimuli (Mondragón and
150 others 2014). These unique features of MSPs make them excellent candidates for developing
151 smart delivery systems.

152

153 ***2.2 Functionalization of MSPs to develop triggered delivery systems***

154 The surface of MSPs presents a high concentration of structural defects in the form of silanol
155 (Si-OH) groups that can easily react with trialkoxysilane derivatives ((R'O)3-Si-R) and allow the
156 possibility of generating organic–inorganic hybrid materials (Vinu and others 2005).

157 In this area, one appealing concept is the development of "molecular gates". Molecular or
158 supramolecular gates are defined as nanoscopic supramolecular-based devices that are
159 attached to certain solid supports, in which mass transport can be triggered by a target
160 external stimulus that can control the state of the gate (closed or open) at will (Aznar and
161 others 2009). In particular, and depending on the type of stimulus applied, it is possible to
162 modify the properties of anchored molecules (i.e. polarity, conformation, size, interaction with
163 other species, bond hydrolysis etc.) which, in turn, results in controlled delivery (Coll and
164 others 2007, Casasús and others 2008, Aznar and others 2009b, Bernardos and others 2012). A
165 schematic representation of a gate-like superstructure is shown in **Figure 3**.




166
167 **Figure 3**. Schematic representation of the operation principle of a molecular gate in a
168 mesoporous support. Molecular gates (orange lines) hinder the release of a guest molecule
169 (yellow spheres) entrapped in the mesoporous supports (gray container) since a suitable
170 external stimulus changes the structure/size of the gate and the guest can be delivered.

171

As observed, smart delivery systems based on gated MSPs contain two components: a suitable inorganic support which acts as a nanocontainer (for loading the cargo); a switchable "gate-like" ensemble capable of being opened or closed when certain external stimuli are applied. Both components are important, and their selection determines the controlled release performance of the hybrid support (Bernardos and others 2010; Burguete and others 2012).

The first example of a molecular gate was reported by Fujiwara and co-workers in 2003 (Mal and others 2003). Since then, a number of gated systems that have used mesoporous silica supports which respond to a wide variety of stimuli have been described (Aznar and others 2009a; Coll and others 2013; Arcos and Vallet-Regí 2013).

## 3. Design of site-specific delivery systems that act along the gastrointestinal tract through gated MSPs

As previously stated, the encapsulation and later administration of bioactive molecules at a particular site-of-action of the digestive tract (mouth, stomach, intestine or colon) offer huge possibilities to develop new functional foods or medical therapies. Hence the design of systems capable of controlling the release of basic nutrients, bioactive components, sensory appeal compounds, and pre- and probiotics, and even drugs, is a very challenging strategy that can be easily achieved by using capped MSPs.

When designing a site-specific delivery system based on hybrid organic-inorganic supports, there are two factors that should be taken into account. On the one hand, the porous system of the inorganic support should be able to entrap the target molecule. On the other hand, the capping molecule should be responsive to a triggering stimulus, and is present in a particular cavity of the gastrointestinal tract. Moreover, it must remain unchanged in the cavities that proceed. An overview of these stimuli is provided in Figure 4.

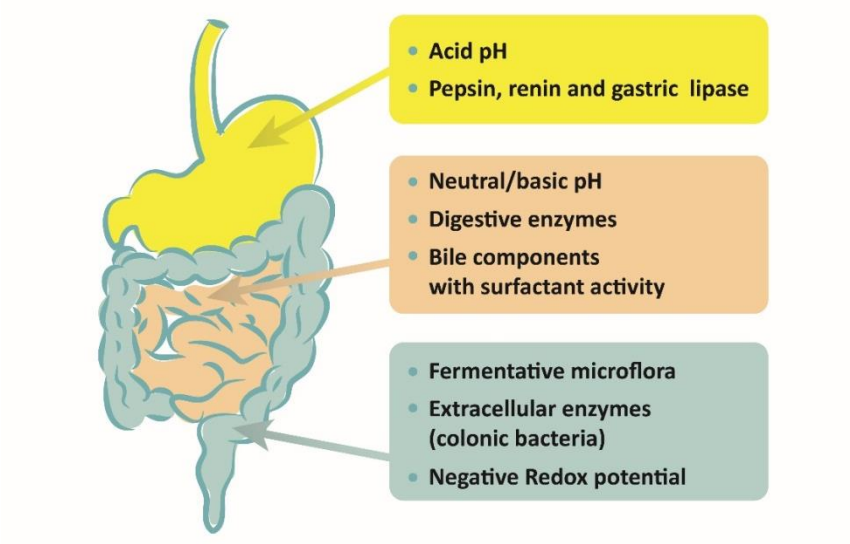



**Figure 4**. Summary of the chemical and biological stimuli able to trigger capped-MSPs during digestion.

This section describes the suitable stimuli found along the gastrointestinal tract that could be employed in developing site-specific delivery systems and all the approaches developed to date to design molecular gates responsive to these stimuli.

## 3.1 A brief physicochemical description of the digestive system

### 3.1.1 Mouth

Gastrointestinal tract activity begins in the mouth where the ingested food is chewed and mixed with saliva to allow bolus formation and to enhance taste (Humphrey and Williamson 2001; Chen 2009). Saliva is a complex heterogeneous clear fluid (pH 5.6-7.6) that consists in roughly 98% water and 2% organic and inorganic substances, including electrolytes, mucus, glycoproteins, proteins, antibacterial compounds, enzymes, and others (Levine and others 1987).

Of all the enzymes contained in saliva, α-amylase is the most important. The interaction of amylase with starch-based ingredients produces a breakdown of starch into simpler sugars (i.e. maltose and dextrins), which can be further broken down in the small intestine. Despite this enzymatic action of saliva, it should be stated that salivary $\alpha$-amylase is most active at its optimum pH of 7.4, and is inactivated in the stomach because of gastric acid. Thus even though enzyme interaction begins almost immediately after food ingestion, its contribution to full starch breakdown is relatively insignificant. Most starch digestion results from pancreatic amylase rather than from salivary amylase (Chen 2009). Salivary glands also secrete salivary lipase that starts the degradation of dietary triglycerides into fatty acids and diglycerides that start with fat digestion. However, salivary lipase does not play a digestive role in adult humans. Recent studies have suggested that it plays only a role in fat taste and texture perception (Drewnowski and Almiron-Roig 1997).

The residence time in the oral cavity is short, and varies by 2-5 min seconds depending on saliva swallowing and water intake. Thus the main suitable triggering stimuli in the buccal cavity are pH (neutral) and presence of α-amylase and salivary lipase. However, due to the short residence time and low enzyme activity, the influence of the mouth on the action of molecular gates could be considered negligible.

### 3.1.2 Stomach

Once food is swallowed, it passes into the stomach. In the stomach, food stuffs find gastric juice secretion. Gastric juice provides a harsh environment characterized by a very acid media (pH 1-2) that is rich in electrolytes, proteases (pepsin, renin and gastric lipase) and lipases (Chiras 2015). Microflora in the stomach is predominantly Gram-positive and aerobic, and the bacterial concentration is usually $<10^3$ colony-forming units CFU/mL (Campieri and Gionchetti, 1999). The redox potential in the stomach is +150 mV (Friend 1992). The residence time of food in the stomach depends on the digestibility of meals; while light meals based on carbohydrates may be ready to pass into the small intestine through the pyloric valve in 2 h, heavy meals that contain proteins and fats may require up to 6 h to perform the same action. After this period, proteins are transformed into large polypeptides, and about 10-30% of

dietary fat has been hydrolyzed (Krohn and others 2008). The digestion process is thus completed in the small intestine.

### 3.1.3 Small intestine

In the small intestine, the hydrolysis of all the majority food structures and macronutrients occurs by the combined action of small intestine and accessory organs (pancreas and liver) secretions.

Once the chyme arrives to the duodenum, the pancreas secretes pancreatic juice. Pancreatic juice is a liquid that contains water, sodium chloride, sodium bicarbonate and a number of digestive enzymes (i.e. amylases, lipases, proteases, ribonucleases and deoxyribonucleases) that help finish the digestive process that started in the stomach. Sodium bicarbonate neutralizes the high acidity of the chyme. In this manner, the duodenum pH is 6.0 (within the 5.7-6.2 range) and gradually increases through the small intestine to pH 7.5 (within the 7.3-7.7 range) (Fallingborg 1999). This difference with the stomach pH allows the design of pH-responsive devices. The enzymatic profile of pancreatic juice is completed by enzymes of microvilli that constitute the brush border (i.e. saccharidases, peptidases and nucleases). Working together, both types of enzymes are able to hydrolyze almost all large molecules into absorbable food components.

The duodenum also receives a fluid though the bile duct which is produced in the liver and stored in the gallbladder, and is known as bile. Bile is composed of water, cholesterol, lecithin (a phospholipid), bile pigments (with no digestive function), bile salts (sodium glycocholate and sodium taurocholate) and bicarbonate ions. The powerful surfactant activity of bile components helps with the digestion and adsorption of lipophilic components.

Regarding microflora, the proximal small bowel is similar to that of the stomach. The bacterial concentration is $10^3$-$10^4$ CFU/mL. However, the distal ileum is able to support anaerobic bacterial flora. Consequently, the concentration of microorganisms increases in the distal ileum to levels of $10^5$-$10^9$ CFU/mL and the redox potential in the small intestine lowers from -50 mV in the duodenum or jejunum to -150 mV in the ileum (Friend 1992; Campieri and Bionchetti 1999).

After this complete digestive process, which lasts between 2-5 h, most food structures have been disintegrated into absorbable molecules. Undigested food remains pass through the ileocaecal valve to the large intestine.

### 3.1.4 Large intestine

The large intestine, which comprises the caecum, colon and rectum, is the last part of the digestive tract. Its main objectives are to absorb the water and electrolytes that escape from absorption in the small intestine, and to store and remove feces during defecation. Understanding the last part of the GIT offers different possibilities to design triggered responsive MSPs for controlled release in the large intestine.

The large intestine pH varies according to the food ingested. In general, the pH in the ascending colon is 6-7.1 due to fermentation processes, and varies along the large intestine

length. The transverse colon exhibits a pH of 7.4, descending colon, pH 7.5, sigmoidal colon, pH 7.4, and rectum, pH 7.2. The shallow pH gradient between the small intestine and the colon does not allow the design of colonic delivery drug carriers based on pH changes (Milabuer and others 2010).

However, the large intestine is the natural habitat for a huge microbial community. The colon contains $10^{11}$ to $10^{12}$ CFU/mL. Predominant species include *Bacteroides*, *Bifidobacterium* and *Eubacterium*. Anaerobic gram-positive cocci, as well as *Clostridium*, enterococci, and various species of *Enterobacteriaceae* are also present. It allows us to talk about a final digestion stage carried out by a wide variety of metabolic processes, including fermentation, enzyme-mediated reactions, and the reduction of a wide range of organic functional groups. Among the different extracellular enzymes produced by colonic bacteria, azoreductases, oxidoreductases, ureases, dextranases and a number of saccharidases capable of breaking indigestible carbohydrates, stand out.

The total metabolic and bacterial activity in the large intestine generates a characteristic redox potential (-200 mV) that can be used as a highly selective mechanism for targeting in the colon (Friend 1992; Chourasia and Jain 2003). The residence time in the large intestine ranges from 2–72 h. In most individuals, mouth-to-anus transit times are usually longer than 24 h. More detailed information is provided in Table 1.

296 **Table 1**. Summary of suitable digestive stimuli for designing triggered MSPs-based delivery systems

| | Chemical | Enzymatic | | |
|---|---|---|---|---|
| | | Enzyme | Substrate | Origin |
| Mouth | Neutral pH | α-amylase (ptyalin) | Starch | Salivary glandules |
| | | Salivary lipase | Triacylglicerids | Salivary glandules |
| Stomach | Acid pH | Gastric lipase | Triacylglicerids | Gastric chief cells |
| | | Pepsin | Proteins and polipeptids | Gastric chief cells |
| | | Renin | Casein | Gastric chief cells |
| Small intestine | Neutral/basic pH | Chymotrypsin (endopeptidase) | Proteins (endopeptidase) | Pancreas |
| | Bile acids (cholic and deoxycholic acid) | Carboxypeptidase A & B (exopeptidase) | Proteins | Pancreas |
| | Phospholipids | Cholesterol esterase | Cholesterol esters | Pancreas |
| | | Colipase | Favours the action of the lipase | Pancreas |
| | | Deoxyribonuclease | *Deoxyribonucleic acid* (*DNA*) | *Pancreas* |
| | | Elastase | Elastin fibres | Pancreas |
| | | β–fructofuranosidase (Sucrase or Isomaltase) | *Sucrose* | *Brush border* |
| | | Pancreatic α-amylase | Starch | Pancreas |
| | | Pancreatic lipase | Fat and triglycerides | Pancreas |
| | | Phospholipase A2 | Phospholipids | Pancreas |
| | | Ribonuclease | Ribonucleic acid (RNA) | Pancreas |
| | | Trypsin *(endopeptidase)* | *Proteins* | *Pancreas* |
| | | β-1-4 galactosidase (Lactase) | *Lactose* | *Brush border* |
| | | α-glucosidase (Maltase) | *Maltose* | *Brush border* |
| | | α-limit dextrinase | *Limit dextrines* | *Brush border* |
| | | Nucleosidase | *Nucleosides* | *Brush border* |
| | | Peptidases | Small peptides | *Brush border and mucosal cells* |

| | | | | |
|---|---|---|---|---|
| Large intestine | Basic pH | α-L-arabinosidase | α-L-arabinofuranosides, arabinoxylans and arabinogalactans | *Colonic bacteria* |
| | Redox potential | Azoreductases | *Azo (N=N) bonds* | *Colonic bacteria* |
| | | Dextranase | Dextran | *Colonic bacteria* |
| | | β-D-galactosidase | β-D-galactosides (i.e. galactooligosaccharides) | *Colonic bacteria* |
| | | β-D-glucosidase | β-glucosides (i.e. cellulose and hemicellulose) | *Colonic bacteria* |
| | | β-glucuronidase | β-D-glucuronic acid residues | *Colonic bacteria* |
| | | Oxidoreductase | Transfer of electrons (i.e. pyruvate oxidation) | *Colonic bacteria* |
| | | Polysaccharidases | *Indigestible polysaccharides (i.e. amylose, chitosan, dextrans…)* | *Colonic bacteria* |
| | | Urease | Urea | *Colonic bacteria* |
| | | β-D-xylosidase | β-D-xylans, xylobiose | *Colonic bacteria* |

### 3.2 Strategies to develop site-specific smart delivery devices

After discussing the most significant digestive stimuli that could be used to design capped MSPs for controlled release purposes in the gastrointestinal tract, the current MSP-based systems that can be opened using these triggering principles are presented in this section.

#### *3.2.1 pH-responsive molecular gates*

The first strategy to develop pH-responsive gated materials was based on using ionizable simple molecules anchored to the material surface, which undergo conformational and/or solubility changes in response to environmental pH variation, which modifies its conformation. Based on this approach, Martínez-Máñez and co-workers developed the first pH-driven molecular gate in 2004 (Casasús and others 2004). Their mechanism was based on the protonation/deprotonation processes of polyamines grafted onto the pore outlets of the mesoporous inorganic scaffolds. At an acid pH, the columbic repulsions between the protonated amino groups hinders pore access (gate closed), while at a neutral pH, unprotonated amines tend to interact with each other, which favors pore access (gate open). Figure 5 illustrates the action mechanism of this reversible smart delivery system. Bearing in mind all these concepts, Bernardos and others (2008), developed the first controlled release system mediated by a gastrointestinal stimulus. Given the objective of protecting riboflavin from acidic stomach conditions and of releasing the load in the intestine, these authors encapsulated vitamin riboflavin in an MCM-41 type support and functionalized its surface with the described pH-controlled gate-like scaffolding. They found a zero release under the stomach-like conditions (acid pH, gate closed) and a time-modulated delivery under the intestine-like conditions (neutral pH, gate open).

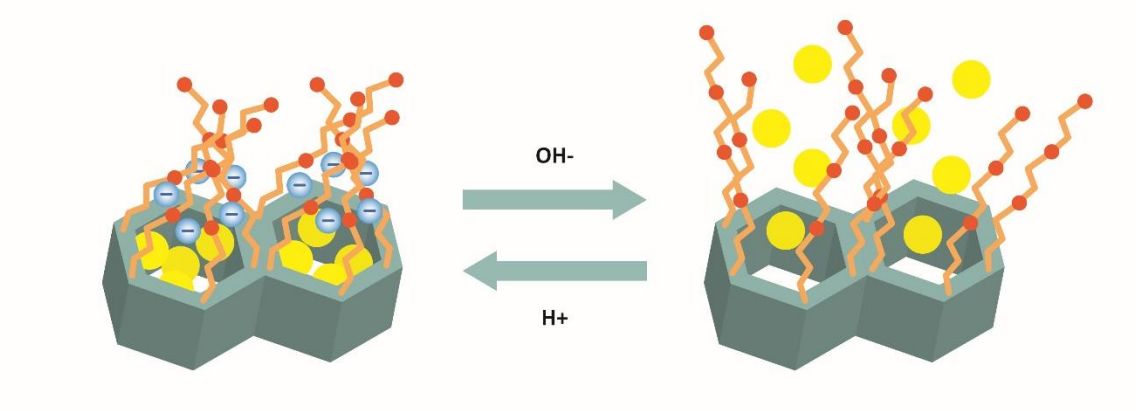



**Figure 5**. Schematic representation of a pH-driven molecular gate-like material based on the use of polyamines. Amines (orange lines) are protonated at a low pH. Deprotonation favors coloumbic repulsions among different chains and coordination with anionic species (blue dots) than block pores. Under this condition, the guest molecule (yellow spheres) cannot escape from the porous support (gray container). At a neutral, pH amines are unprotonated, which allows cargo delivery.

A second strategy involved modifying the chemical interactions among the molecules covalently anchored to the surface of the mesoporous silica as a result of changes in pH. Following this approach, Lee and others (2008) described the use of mesoporous silica

nanoparticles loaded with sulfasalazine (an anti-inflammatory prodrug used for bowel disease) functionalized with trimethylammonium functional groups via the direct co-condensation of a trimethylammonium silane. Undert acidic conditions, the cargo remained inside the voids of the porous support. However under neutral conditions, the deprotonation of the silanol groups generated a strong electrostatic repulsion, which triggered the sustained release of the loaded molecules.

The third strategy comprised the design of devices capped with molecules anchored with acid-sensitive bonds, whose cleavage enabled the release of cargo molecules. By bearing this principle in mind, Zhao and others (2010) developed a pH-responsive nanoparticle capable of being opened under acid conditions. The design strategy involved using mesoporous silica nanoparticles loaded with rhodamine B and functionalized with β-cyclodextrins through imine double bonds. The β-cyclodextrin rings on the surface of nanoparticles served as gates to store cargo molecules (i.e., rhodamine B) inside the nanopores of nanoparticles under neutral conditions. At an acidic pH the cleavable imine bonds that attached β-cyclodextrines to the particle's surface were hydrolyzed and the cargo was released.

Besides polyamines, trymetylammonium groups and cyclodextrins, other capping molecules (such as polymers, peptides, proteins and DNA) have been used as gatekeepers in pH-triggered capped materials based on mesoporous silica (see Table 2).

347 **Table 2**. Selected examples of gated materials responsive to changes in pH.

| Gating molecule or system | Closed | Opened | Cargo | Suitable delivery location | Reference |
|---|---|---|---|---|---|
| Carboxylic acid | Neutral | Acid | Vancomycin | Stomach | Yang and others 2005 |
| Chitosan | Neutral | Acid | Ibuprofen | Stomach | Popat and others 2012a |
| $\alpha$-cyclodextrine | Neutral | Acid | Propidium iodide | Stomach | Du and others 2009 |
| $\beta$-cyclodextrine | Neutral | Acid | Rhodamine B | Stomach | Guo and others 2010 |
| Peptide $K_8$ | Neutral | Acid | Doxorubicin | Stomach | Luo and others 2013 |
| Polydopamine | Neutral | Acid | Doxorubicin | Stomach | Zheng and others 2014 |
| Poly(4-vinyl pyridine) | Neutral | Acid | Tris(bipyridine)ruthenium(II) chloride | Stomach | Liu et al 2011 |
| 3-aminopropyltrimethoxysilane and 4-sulfophenyl isothiocyanate | Acid | Neutral | Ibuprofen | Small Intestine | Cauda and others 2010 |
| β-lactoglobulin | Acid | Neutral | Ibuprofen | Small Intestine | Guillet-Nicolas and others 2013 |
| Bovine serum albumin conjugated with lactobionic acid | Acid | Neutral | Doxorubicin | Small Intestine | Luo and others 2012 |
| Hydroxypropyl methylcellulose phthalate | Acid | Neutral | Famotidine | Small Intestine | Xu and others 2009 |
| Lysozyme | Acid | Neutral | Rhodamine B | Small Intestine | Xue and others 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Oligonucleotide | Acid | Neutral/Basic | Rhodamine B | Small Intestine | Chen and others 2011 |
| Poly(acrylic acid) | Acid | Neutral/Basic | Salidroside | Small Intestine | Peng and others 2013 |
| Polyamines | Acid | Neutral | Squaraine | Small Intestine | Casasús and others 2004 |
| | | | Tris(bipyridine)ruthenium(II) chloride | | Casasús and others 2008 |
| | | | Riboflavine | | Bernardos and others 2008 |
| | | | Folic acid | | Pérez-Esteve and others 2015 |
| Trimethylammonium groups | Acid | Neutral | Sulfasalazine | Small Intestine | Lee and others 2008 |
| | | | | | Cheng and others 2011 |

### *3.2.2 Redox-responsive molecular gates*

As occurred with changes in pH, the evolution of the redox potential along the gastrointestinal tract might allow the design of redox-driven gated mesoporous materials, especially for colon-targeted delivery. To date, no specific system based on naturally-occurring changes in redox potential changes along the GI to modulate the delivery of bioactive molecules has been provided. However, there are a number of approaches that could be the basis for future developments.

Lai and others (2003) prepared a controlled delivery system to encapsulate several pharmaceutical drug molecules and neurotransmitters inside an organically functionalized mesoporous silica framework. In particular, this nano-device was prepared using MCM-41-type mesoporous silica nanospheres as an inorganic support and cadmium sulfide (CdS) nanocrystals as chemically removable caps. Addition of disulfide-reducing molecules, such as dithiothreitol (DTT) and mercaptoethanol (ME), to the aqueous suspension of the particles triggered a rapid release of the mesopore-entrapped cargo by breaking the chemically labile disulfide linkages between the MSP and CdS nanoparticles. Also based on disulfide linkages, Liu and others (2008) prepared a calcined MCM-41 solid support loaded with dye molecules, with the surface functionalized by the grafting of a poly(*N*-acryloxysuccinimide). The openings of the resulting hybrid material remained blocked due to the cross-linked reaction between the *N*-oxysuccimide groups along the polymer chain and the cystamine of the media. In contrast, the presence of disulfide-reducing agents, such as (DTT) cleavage of the disulfide bond of cystamine, induced pore opening and controlled dye release.

A different approach was published by Hernandez and others (2004). These authors described the use of an MCM-41 mesoporous scaffold loaded with an iridium complex dye and functionalized with a 1,5-dioxynaphtalene derivative (DNPD) as a redox-responsive delivery system. The addition of cyclobis-(paraquat-*p*-phenylene) ($CBPQT^{4+}$) induced the formation of a pseudorotaxane on the external surface of the solid. This new non covalent supramolecular ensemble blocked pores and prevented dye delivery. When a reductive agent was added to the mixture (cyanoborohydride in this case), the reduction in DPND started a spontaneous dethreading of the $CBPQT^{4+}$ ring to allow guest release. The evolution of that gated system was the achievement of a total reversible hybrid material capable of being open or closed on command in a reversible manner. In this case, Nguyen and others (2005) firstly synthesized a [2]rotaxane-containing DNPD and a tetrathiafulvalene moiety (TTF) as a redox centre to link each other through a oliogoethylenglycol chainRotaxane was completed by the presence of a rigid spacer and a $CBPQT^{4+}$ as the movable molecule. Preference for CBPQT4+ for TTF or DNPD groups as a result of the oxidation state of TTF (dependent on the addition of oxidant or reducing species) caused gate movement, which changed from a closed to an open conformation.

### ***3.2.3 Surfactant-responsive molecular gates***

The surfactant-induced molecular gates concept was introduced by Giménez and others (2014). This new material consisted of nanoparticles of MCM-41 functionalized on the external

surface with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). The presence of DOPC created a lipid bilayer around pore outlets that inhibited cargo release. However, the system released its cargo after the addition of dodecyltrimethylammonium bromide (DTAB), a single-chain cationic surfactant whose activity is similar to phosphocholine (lecithin).

### *3.2.4 Enzyme-responsive molecular gates*

The wide variety of enzymes present along the gastrointestinal tract, and their selective location (stomach, brush border, colon,) allowed the design of very specific site release systems. One of the first examples of gated MSPs capable of delivering an entrapped cargo in the presence of saccharases was described by Bernardos and others (2009). These authors designed a mesoporous silica particle capped with a covalently anchored lactose derivative. Cargo delivery from aqueous suspensions was negligible because the formation of a dense network of lactose groups linked through the hydrogen-bonding interaction around pore outlets. The addition of β-D-galactosidase enzyme (lactase) induced progressive cargo release, which was clearly related to the enzymatic hydrolysis of the glycosidic bond in disaccharide lactose. This is a clear example of the potential use of an enzyme-responsive molecular gate to hinder cargo release during food processing, storage and the first part of the digestion in the stomach, and one that is able to release the guest molecule in the small intestine in the presence of enzymes of brush border mucosa.

In line with this, the same authors functionalized the surface of a loaded MCM-41 support with three different commercially available hydrolyzed starches (Glucidex 47, 39 and 29) via the derivatization of starch with an alkoxysilane. Cargo release was achieved by enzymatic hydrolysis in the presence of pancreatin (an enzyme cocktail that contains pancreatic amylase), which showed different release kinetics according to the the degree of starch hydrolysis (Figure 6). The lower the hydrolysis rate of starch, the lower the delivery rate (Bernardos and others 2010).

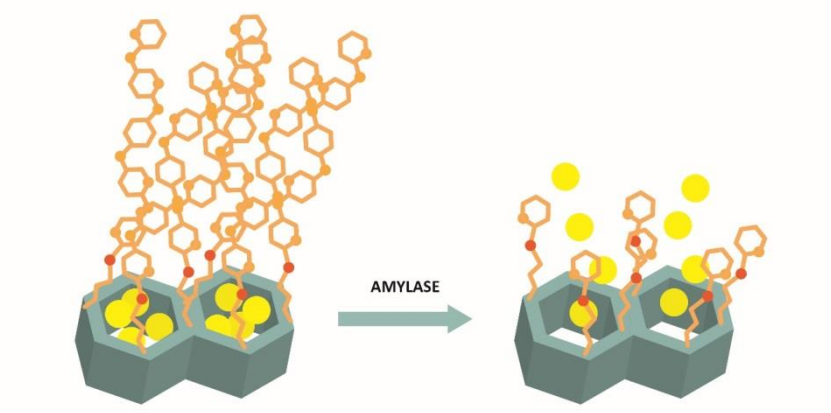



**Figure 6.** Schematic representation of an enzyme-driven molecular gate-like material functionalized with hydrolyzed starch. In the absence of pancreatin, starch derivatives (orange chains) hinder the release of the guest molecule (yellow spheres) from the porous support (gray container) by steric hindrance. In the presence of amylases, starch is hydrolyzed, which allows cargo delivery.

Bein and co-workers prepared the first molecular gate opened by the presence of a protease (Schlossbauer and others 2009). Capping systems consisted in attaching avidin to a biotinylated MSP. The addition of protease trypsin induced the hydrolysis of the attached avidin and cargo release. Along the same lines, Coll and others (2011) employed a click chemistry reaction to functionalize the external surface of an MSP with a peptide to develop a nanodevice capable of hampering cargo release. Delivery was observed in the presence of proteases (Figure 7).

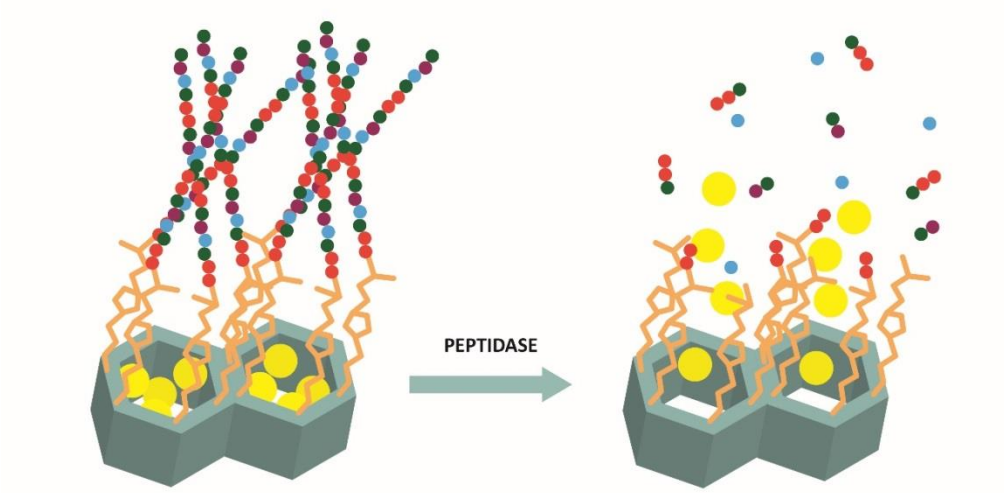



**Figure 7**. Schematic representation of an enzyme-driven molecular gate-like material capped with a peptide. In the absence of proteases, peptidic chains (dot chains) hinder the release of the guest molecules (yellow spheres) from the porous support (gray container) by steric hindrance. In the presence of peptidases, peptides are hydrolyzed and payload is delivered.

Some examples of deoxyribonuclease-triggered delivery systems have also been reported. Zhu and coworkers presented an oligodeoxynucleotide-capped material using hollow MSPs that was opened in the presence of DNase I (Zhu and others 2011a). Zhang and others (2014) reported the use of a porous material loaded with the drug colchicine and capped with oligodeoxynucleotides that was able to be uncapped also when DNase I was used.

The possibility of using enzymes secreted from colonic microflora to design smart delivery systems has been previously reported. Agostini and others (2012a) described an ethylene glycol-capped hybrid material for the controlled release of a certain cargo in the presence of esterase. In the absence of an esterase enzyme, the steric hindrance imposed by bulk ester glycol moieties inhibited cargo release. Upon the addition of the esterase enzyme, cargo delivery occurred due to the hydrolysis of the ester bond, which reduced the of the glycol derivative. In another work, the same authors prepared MSPs loaded with Rhodamine B and functionalized with an alkylgluconamine derivative of a galacto-oligosaccharide (GOS) capable of delivering its cargo in the presence of β-galactosidase (Agostini and others 2012b). Mas and others (2013) reported the synthesis of a hybrid material capped with an azopyridine derivative. This material was designed to show "zero delivery" in the absence of enzymes and

to display cargo release in the presence of azo-reductases, which are usually present in the colon.

More examples of enzyme-responsive gated materials are shown in Table 3. The profound analysis of all the reported examples allowed a conclusion to be drawn that the most extended enzymes used as triggering stimuli are amylases, proteases, peptidases and deoxyribonucleases (which can be used for delivery in the small intestine) and reductases, esterases and ureases (which can be used for controlled cargo delivery in the colon). However, the real development of enzyme-responsive gated materials with applications in the design of site-specific delivery systems that act along the gastrointestinal tract is still in its incipient steps.

460 **Table 3.** Selected examples of gated materials responsive to the presence of target enzymes.

| Gating molecule or system | Closed | Opened | Cargo | Suitable delivery location | Reference |
|---|---|---|---|---|---|
| Avidin–biotin complex | Absence of trypsin | Presence of trypsin | Fluorescein | Small intestine | Schlossbauer and others 2009 |
| Bioactive peptide shell | Absence of thermolysin and elastase | Presence of thermolysin and elastase | Fluorescein isothiocyanate-labelled dextran | Small intestine | Thornton and Heise, 2010 |
| β-cyclodextrin | Absence of α-amylase and lipase | Presence of α-amylase and lipase | Calcein | Small intestine | Park and others2009 |
| Hydrolysed starch | Absence of pancreatine | Presence of pancreatine (amylases and β-D-galactosidase) | Tris(bipyridine)ruthenium(II) chloride | Small intestine | Bernardos and others 2010 |
| Lactose | Absence lactase (β-D-galactosidase) | Presence lactase (β-D-galactosidase) | Tris(bipyridine)ruthenium(II) chloride | Small Intestine | Bernardos and others 2009 |
| Oligodeoxynucleotide | Absence of deoxyribonuclease | Presence of deoxyribonuclease | Fluorescein | Small intestine | Zhu and others 2011a |
| Peptide sequence | Absence of peptidases or acid pH | Presence of peptidases and neutral pH | Tris(bipyridine)ruthenium(II) chloride | Small Intestine | Coll and others 2011 |
| Poly(L-lysine) | Absence of α-chymotrypsin | Presence of α-chymotrypsin | Fluorescein | Small intestine | Zhu and others2011b |

| | | | | | |
|---|---|---|---|---|---|
| Protamine | Absence of trypsin | Presence of trypsin | Diclofenac | Small intestine | Radhakrishnan and others 2014 |
| Single-stranded DNA | Absence of deoxyribonuclease | Presence of deoxyribonuclease | Colchicine | Small intestine | Zhang and others 2014 |
| α-cyclodextrin included onto a polyethyleneglycol fragment | Absence of esterase | Presence of bacterial esterases | Rhodamine B | Colon | Patel and others2008 |
| Azobenzene-4,4'-dicarboxylic acid | Absence of azo-reductase | Presence of bacterial azo-reductase | Ibuprofen | Colon | Li and others 2014 |
| Azopyridine derivative | Absence of azo-reductases and esterases | Presence of bacterial azo-reductases and esterases | Rhodamine B | Colon | Mas and others 2013 |
| Choline-sulfonatocalix[4]arene [2]pseudorotaxane | Absence of urease | Presence of bacterial ureases | Rhodamine B | Colon | Sun and others 2013 |
| Ethylene glycol | Absence of esterase | Presence of bacterial esterases | Tris(bipyridine)ruthenium(II) chloride | Colon | Agostini and others 2012a |
| Galacto-oligosaccharide (GOS) | Absence of β-galactosidase | Presence of β-galactosidase | Rhodamine B | Colon | Agostini and others 2012b |
| Sulfasalazine | Absence of bacterial azo-reductase | Presence of bacterial azo-reductase | Sulfasalazine | Colon | Popat and others 2012b |

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### *3.2.5 Dual stimuli-controlled release*

One step forward in the design of gated mesoporous supports is the possibility of preparing gated materials that could be opened by using two different stimuli. For instance, Casasús and others (2008) studied pH- and anion-responsive gated-like ensembles in anion complex formation terms with polyamines. This study came to the conclusion that larger anions pushed tethered polyamines toward pore openings and reduced the pore aperture. More recently, Popat and others (2014) reported the use of silica nanoparticles that were responsive to multiple digestive stimuli (pH and enzymes). Their system consisted of an MCM-48-type structure loaded with sulfasalazine, and functionalized with amino groups coated with a succinylated soy protein isolate (SSPI). The resultant delivery system showed both pH and enzyme responsiveness, depending on the location of the nanoparticles in the GIT. In both the stomach and duodenum, the low environmental pH (pH 1.2 and ca. 5, respectively) restricted the release of sulfasalazine due to the capping effect of the SSPI. In contrast, when the delivery system reached the small intestine (pH 7.4) the change in pH induced the hydrolyzate destabilization, which favors protein hydrolysis by the pancreatin enzyme. The result was a controlled, slow and sustained drug release in the small intestine.

## 4. Benefits and potential current limitations of MSPs for their use in human food

As previous proved, delivery systems based on hybrid organic-inorganic MSPs show most of the desired properties for a smart delivery system: high loading capacity, controlled release rate of a bioactive molecule at a particular site in response to a particular trigger, good biocompatibility, low-cost fabrication given its composition and easy handling, etc. Yet given its novelty, some limitations (toxicological, technological, semantic, legal and sociological) still need to be overcome, which should be solved before starting to use MSP-based smart delivery systems in food and nutrition.

### *4.1 Toxicological: lack of conclusive studies*

Despite silica not being considered harmful for humans, it is known that engineered nanomaterials are not governed by the same laws as larger particles (Pérez-Esteve and others 2013). If we bear in mind that change in size affects the functionality of particles, it could also affect people exposed to newly developed particles. In this context, in recent years, several studies have addressed the toxicological and biocompatibility properties of MSPs.

The impact of nanoparticles general lydepends on certain properties, such as particle size, size distribution, shape, solubility, reactivity, mass, chemical composition, surface properties (area and charge) and aggregation state (Chau and others 2007; Athinarayanan and others 2014).

He and others (2009) studied the effect of particle size (nano- and microparticles), concentration, biodegradation products, and residual surfactant on the cytotoxicity of human breast-cancer cell lines (MDA-MB-468) and African green monkey kidney cell lines (COS-7). These authors observed that 190 nm and 420 nm particles showed significant cytotoxicity at

concentrations above 25 mg/mL, while microscale particles of 1220 nm showed only slight cytotoxicity due to reduced endocytosis. In line with this in an *in vivo* study with male nude mice, Souris and others (2010) confirmed that after oral administration, silica nanoparticles located in the liver could be excreted into the intestine by the hepatobiliary excretion process. Later, Fu and others (2013) demonstrated with female ICR mice that silica nanoparticles (110 nm in size) are absorbed into the body at 24 h of oral administration. Yet once absorbed, particles are transported via the portal vein to the liver and are then eliminated during a 7-day period by fecal excretion, and also through urine, without changing the kidney microstructure. These results agree with the studies done into tissue distribution and excretion kinetics of orally administered silica nanoparticles in rats carried out by Lee and others (2014). These authors reported that after ingestion, particles are distributed to kidneys, liver, lungs and spleen. However, silica particles are easily decomposed and eliminated via urinary and fecal excretion after oral exposure. The smaller the particles, the more rapidly they are secreted, presumably because they are more easily decomposed.

As well as particle size, particle shape seems important when talking about potential toxicology. Tao and others (2008) evaluated the effect of two types of mesoporous silica particles on mitochondrial $O_2$ consumption. For this purpose, the effect of SBA-15 (irregular rods of ca. 1000 nm in length and aspect ratio of 1:5) and MCM-41 (spheres of 300-1000 nm in diameter) on mitochondrial $O_2$ consumption (respiration) was evaluated in HL-60 (myeloid) cells, Jurkat (lymphoid) cells, and isolated mitochondria. These authors observed that while SBA-15 inhibited cellular respiration at 25-500 μg/mL, MCM-41 had no noticeable effect on the respiration rate.

Finally, surface properties also seem relevant for potential toxicology (Tang and others 2012). Specifically, van Schooneveld and others (2008) reported the improved biocompatibility and pharmacokinetics of silica nanoparticles by means of a lipid coating. In their extensive study on bare and lipid-coated silica nanoparticles in mice, these authors concluded that coating porous silica with organic molecules can increase the biocompatibility and half-lives of cells by more than 10-fold compared to bare silica mesoporous supports.

Thus despite adverse effects having been observed in some cells or animals treated with different concentrations of some MSPs, other *in vitro* and *in vivo* studies have suggested that certain particles are well tolerated by both cells and superior animals. Therefore, it is hard to draw conclusive conclusions about the biocompatibility and toxicity of MSPs as a unique concept. In any case, the use of mesoporous silica microparticles functionalized on their surface with biocompatible organic molecules seems a good strategy to minimize the risks associated with using MSPs as supports to develop smart delivery systems.

### *4.2 Technological problems: mass production and impact of MSPS-based delivery systems on the food matrix*

There is no doubt that the application of MSP-based delivery systems to the formulation of novel functional foods opens up new strategies for the food industry. However, before

launching foods that contain MSPs to the market, some technological problems should be solved.

First, one problem is related with the mass production of MSPs. To date, processes for the synthesis, loading and functionalization of MSPs are being developed on a laboratory scale. As a result, production costs are high and mass production is practically underdeveloped.

The second technological problem is related to the compatibility of these devices with the food matrix. Generally, introducing new ingredients or additives to a food matrix can affect the physico-chemical and sensory properties of the product. However, it is considered that a delivery system suitable for a particular application should be compatible with the food or beverage matrix that it is to be incorporated into, and should cause no adverse effects on product appearance, flavor, texture, mouth feel or shelf life.

Despite the importance of this aspect, as far as we know, there is only one publication that has dealt with determining the influence of MSPs on physical properties of the food matrix to which they could be included (Pérez-Esteve and others 2014). However, since MSPs have a high load capacity and bioactive compounds exhibit their functional properties at very low concentrations, it is assumed that the amount of support needed to release an adequate concentration of the component is very low. Thus it is foreseeable that the physicochemical features of the matrix that is to incorporate these supports should not be affected by the presence of encapsulating systems.

### *4.3 Semantic: Disharmonized and changing and denominations*

As previously described, the MSPs concept involves structures of silicon dioxide ($SiO_2$) arranged in such a way that they are able to create pores of 2-50 nm. This structure on the nanoscale is the key to design molecular or supramolecular capped materials. Its design, fabrication, manipulation and characterization are possible thanks to nanotechnology. Therefore, should MSPs be considered nanomaterials? It is clear that mesoporous silica nanoparticles are nanomaterials. But what happens with mesoporous silica microparticles? By taking into account only European recommendations and regulations, denominations are disharmonized and have changed over the years.

Regulation (EC) No. 1169/2011, on the provision of food information to consumers, defined the engineered nanomaterial concept as intentionally produced materials that have one dimension or more in the order of 100 nm, or less, or is composed of discrete functional parts, either internally or on the surface, many of which have one dimension or more in the order of 100 nm, or less, including structures, agglomerates or aggregates, whose size above the order may be 100 nm, but retain characteristic properties of the nanoscale. Characteristic of the nanoscale includes: (i) those related to the large specific surface area of the materials considered; and/or (ii) the specific physico-chemical properties that differ from those of the non nanoform of the same material. According to this definition, and regardless of size, MSPs can be considered nanomaterials as they are intentionally produced to modify their physico-

chemical properties and to create nanoporous structures to increase their specific surface area.

In the same year, the European Commission defined nanomaterials as natural, incidental or manufactured material that contains particles, in an unbound state, or as an aggregate or agglomerate, where for > 50% of the particles in the number size distribution, one external dimension or more falls within the 1 nm-100 nm size range (EU 2011). This definition is in line with the opinion of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), included the size distribution of a material as a defining element, and excludes other types of nanostructured materials, such as nanoporous or nanocomposite materials, since there is not enough evidence to guide what materials should be included.

These definitions, apart from being technical, affect regulatory aspects and food labeling. Thus, they are vital for the future of these systems. The NanoDefine Project (FP7) is expected to deliver an implementable test scheme for regulatory purposes to distinguish nano from non nanomaterials by 2017.

### *4.4 Legal: Lack of specific regulations*

According to their composition ($SiO_2$), MSPs should be authorized for use in food. $SiO_2$ is "Generally Recognized as Safe" (GRAS) by FDA regulations. It is also an authorized additive in Europe and achieves the E-551 classification (Contado and others 2013). In the food industry, synthetic amorphous silica has been used for many years to clear beers and wines, as an anti-caking agent to maintain the flow properties of powder products, and as a carrier agent for flavorings and aromas, and to thicken pastes.

However when we consider their physical features, MSPs could be classified as novel food ingredients based on engineered nanomaterials. Thus in order to place a specific MSP as a food ingredient in the Community market, the applicant should submit a request to the Member State in which the product would be placed (Regulation (EC) No. 258/97). If approved, the presence of the engineered nanomaterial should be clearly indicated in the list of ingredients by writing the word "nano" in brackets (Regulation (EC) No. 1169/2011).

### *4.5 Sociological: in the face of the unknown, the precautionary principle*

The uncertainty in purely semantic aspects and in conclusive toxicological studies has not only consequences at a regulatory level, but also influences consumers' risk perception and acceptance. Although very little research has been conducted in developing countries on consumer attitudes toward foods that contain nanostructured ingredients, recent studies point out that lack of information about the impact of nanotechnology on environmental and health consequences leads consumers to apply the precautionary principle and, therefore, to reject such products (Chau and others 2007).

For novel foods to be accepted, consumers must perceive that any potential benefits outweigh potential risks or negative effects (for example, potential for a negative impact on the

environment, human and animal health, or ethical concerns, such as animal welfare or social equity) (Frewer and Fischer 2010).

For this to happen, information about the potential benefits and potential risks should not only be accurate, but also very clear. This entails properly regulating the use of nanotechnologies in food and publishing conclusive studies about the potential risks of each type of MSP by considering all the variables that can affect their toxicity. Until this time comes, generalizations, doubts or risk perceptions will outweigh the real benefits.

## 5. Conclusions

Gated MSPs have the potential to encapsulate bioactive molecules and, consequently, to protect them from the environment during production, storage and digestion, to mask their odor and taste, to improve their compatibility with the food matrix, and to amend their bioaccessibility along the GIT. This review reports the most recent research into the design of gated mesoporous siliceous materials for controlled release along the GIT using physiologic stimuli. It also highlights the possibilities of naturally-occurring stimulus along the GIT that could be used to develop new gated systems. Applications for these capped materials can be found in the design of novel functional foods. Nevertheless, given their novelty, the incorporation of gated-MSPs into food still poses major challenges (i.e. technological, toxicological, legal, sociological, etc.) that need to be overcome by researchers and regulatory bodies. Researchers have the task of evaluating the potential hazards of MSPs-gated systems in human health and the environment, and to design specifically designed systems to be triggered in the gastrointestinal tract. Regulatory bodies should provide specific regulations and criteria to be followed when evaluating the safety of this new smart delivery system to be used in food applications. Collaborative work from those groups will be essential in forthcoming years to generate confidence in industry and consumers. Only then will functional foods developed by this new technology be available in the food chain.

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