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Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Solubility is defined in quantitative terms as the concentration of the solute in a saturated solution at a definite temperature. Qualitatively, it may be defined as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. The solubility of a drug may be expressed as parts, percentage, molarity, molality, volume fraction, and mole fraction.

1.1 Drug Solubility

The drug solubility in saturated solution is a static property whereas the drug dissolution rate is a dynamic property that relates more closely to the bioavailability rate. Drug solubility is the maximum concentration of the drug solute dissolved in the solvent under specific condition of temperature, pH and pressure.

Almost 90% drugs available in market are administered orally. Drug absorption, sufficient and reproducible bioavailability and pharmacokinetic profile of orally administered drug substances are highly dependent on solubility of that compound in aqueous medium. More than 90% of drugs approved since 1995 have poor solubility. It is estimated that 40% of active new chemical entities (NCEs) identified in combinatorial screening programs employed by many pharmaceutical companies are poorly water soluble.

Orally administered drugs on the Model list of Essential Medicines of the World Health Organization (WHO) are assigned BCS classifications on the basis of data available in the public domain. Of the 130 orally administered drugs on the WHO list, 61 could be classified with certainty. 84% of these belong to class I (highly soluble, highly permeable), 17% to class II (poorly soluble, highly permeable), 24 (39%) to class III (highly soluble, poorly permeable) and 6 (10%) to class IV (poorly soluble, poorly permeable). The rate and extent of absorption of class II & class IV compounds is highly dependent on the bioavailability which ultimately depends on solubility. Due to this, solubility enhancement is one of the important parameters which should be considered in formulation development of orally administered drug with poor aqueous solubility. The pharmacopoeia lists solubility in terms of number of milliliters of solvent required to dissolve 1g of solute. If exact solubility is not known, the Pharmacopoeia provides general terms to describe a given range. These descriptive terms are listed in (Table1.1).
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Table 1.1: Expression for approximate solubility

<table>
<thead>
<tr>
<th>Descriptive terms</th>
<th>Relative amounts of solvents to dissolve 1 part of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very soluble</td>
<td>Less than 1</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>From 1-10</td>
</tr>
<tr>
<td>Soluble</td>
<td>From 10-30</td>
</tr>
<tr>
<td>Sparingly soluble</td>
<td>From 30-100</td>
</tr>
<tr>
<td>Slightly soluble</td>
<td>From 100-1000</td>
</tr>
<tr>
<td>Very slightly soluble</td>
<td>From 1000-10,000</td>
</tr>
<tr>
<td>Insoluble or practically insoluble</td>
<td>More than 10,000</td>
</tr>
</tbody>
</table>

1.2 Process of Solubilization
The process of solubilization involves the breaking of inter-ionic or intermolecular bonds in the solute, the separation of the molecules of the solvent to provide space in the solvent for the solute, interaction between the solvent and the solute molecule or ion (Fig. 1.1).

Step 1: Opening of spaces in the solvent

Step 2: Molecules of the solid breaks away from the bulk

Step 3: The freed solid molecule is integrated into the hole in the solvent

Figure 1.1: Mechanism of solubilization of solute in solvent
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When a drug dissolves, solid particles separate and mix molecule by molecule with the liquid and appear to become part of that liquid. Therefore, drug dissolution is the process by which drug molecules are liberated from a solid phase and enter into a solution phase. The use of poorly soluble drugs has a number of drawbacks such as increasing the dosage and its administration frequency along with the resultant occurrence of side effects. The rate-limiting step in the absorption process for poorly water-soluble drugs is the slow dissolution rate of such drugs in the gastro intestinal fluids. It is important to improve the oral bioavailability of poorly water soluble drugs by improving their dissolution rate and solubility. The solubility depends on the physical form of the solid, the nature and composition of solvent medium, temperature and pressure of system\(^6\) etc., some of these factors are represented in Fig. 1.2.

![Figure 1.2: Factors affecting solubility](image)

1.3 Need of Solubility Enhancement

Solubility of active pharmaceutical ingredients (APIs) has always been a concern for formulators, as the inadequate aqueous solubility may hamper development of products and limit bioavailability of oral products. Solubility plays an essential role in drug disposition, since the maximum rate of passive drug transport across a biological membrane, the main pathway for drug absorption, is the product of permeability and solubility\(^8\). Among the five key physicochemical screens in early compound screening, pKa, solubility, permeability, stability and lipophilicity, poor solubility tops the list of undesirable compound properties.
1.4 Methods of Solubility Enhancement

Compounds with insufficient solubility carry a higher risk of failure during discovery and development since insufficient solubility may compromise other properties, influence both pharmacokinetic and pharmacodynamic properties of the compound, and finally may affect the ability of the compound to develop as API. Currently only 8% of new drug candidates have both high solubility and permeability. Many strategies have been used to enhance the solubility of poorly soluble drugs and some commonly used solubility enhancement approaches are represented in Fig. 1.3.

Figure 1.3: Methods of solubility enhancement
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1.5 Nanotechnology and Solubility Enhancement

Amongst different approaches of solubility enhancement, nanotechnology is rapidly growing technique as it not only offers a means of providing novel formulations for already existing marketed drugs but also for the new drug candidates with poor water solubility. Nanotechnology based formulations shrink the particle size of the drug, increasing its surface area and enhancing the dissociation. When the size of material is reduced to less than 100 nm, the principles inherent in quantum physics increasingly apply and materials begin to demonstrate entirely new properties such as enhanced solubility which in turn may increase the oral bioavailability of poorly water-soluble drugs. This has led to a number of nano-based drug delivery systems being developed.

Table 1.2: Nanotechnology based approaches employed and commercialized by various pharmaceutical companies till 2009.

<table>
<thead>
<tr>
<th>Company</th>
<th>Nanotechnology-based formulation approach</th>
<th>Description and Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elan Pharma International</td>
<td>Nanocrystal drug particles (&lt;1000 nm) production by wet-milling and stabilized against agglomeration through surface adsorption of stabilizers, applicable to new molecular entities also for re-formulation of existing drugs e.g. sirolimus.</td>
<td>Nanocrystal drug particle</td>
</tr>
<tr>
<td>(Dublin, Ireland)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eurand Pharmaceuticals</td>
<td>Nanocrystal/amorphous drug production by physical breakdown of the crystal lattice and stabilized with biocompatible carriers (swellable microparticles or cyclodextrins).</td>
<td>Cyclodextrin nanoparticle</td>
</tr>
<tr>
<td>(Vandalia, Ohio USA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skye Pharma Plc,</td>
<td>Use of high shear, cavitations or impaction forming micro-nano micro particulate/droplet water insoluble drug core stabilized by phospholipids, applicable for insoluble drug delivery.</td>
<td>A polymer stabilizing nano reactor with the encapsulated drug core</td>
</tr>
<tr>
<td>(Piccadily, London, UK)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioSante Pharmaceuticals</td>
<td>Formation of MSNs that is calcium phosphate-based for improved oral bioavailability of hormones/proteins such as insulin; also as vaccine adjuvants.</td>
<td>Calcium phosphate MSNs</td>
</tr>
<tr>
<td>(Lincolnshire, Illinois, USA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Biosciences</td>
<td>Nanoparticle albumin-bound technology Injectable suspension of biocompatible Paclitaxel albumin MSNs</td>
<td></td>
</tr>
</tbody>
</table>
**Introduction**

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Description</th>
<th>Technology Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Blauvelt, NY, USA)</td>
<td>protein with drug improves solubility/removes need for toxic solvents; e.g. paclitaxel-albumin MSNs.</td>
<td>Nanoparticle self-assembly, self-assembly, or precipitation of drug molecules.</td>
</tr>
<tr>
<td>Baxter Pharmaceuticals (Deerfield, Illinois, USA)</td>
<td>Nano edge technology: drug particle size reduction to nano range by platforms including direct homogenization, micro-precipitation, lipid emulsions and other dispersed-phase technology.</td>
<td>Nano lipid emulsion</td>
</tr>
<tr>
<td>pSivida Ltd (Watertown, MA, USA)</td>
<td>Structuring of drug particles within the nano-width pores of biocompatible Bio-Silicon MSNs, membranes or fibres; which gives controlled release and improves the solubility and bioavailability of hydrophobic drugs.</td>
<td>Silicon MSNs</td>
</tr>
<tr>
<td>IMEDD Inc (Burlingame, CA, USA)</td>
<td>Use of silicon membrane with nano width pores (10–100 nm) used as part of an implantable system for drug delivery and bio-filtration.</td>
<td>Stretchable silicon nanomembrane</td>
</tr>
<tr>
<td>Pharma Sol GmbH (Berlin, Germany)</td>
<td>Nano structured lipid carriers: nano structured lipid particle dispersions with solid contents produced by high-pressure homogenization; lipid–drug conjugate MSNs provide high-loading capacity for hydrophilic drugs for oral delivery.</td>
<td>Drug encapsulated in lipid MSNs</td>
</tr>
</tbody>
</table>

The nanotechnology based solubility enhancement approaches can be viewed through synthesis, self-assembly, or by precipitation of drug molecules. Alternatively, Nanoparticles can be successfully generated using drug-fragmentation processes such as homogenization, microfluidation, or milling. Milling, most the process used in generating Elan’s NanoCrystal colloidal dispersions, is the most recognized approach in the area of nanoparticulate research. Elan Pharma Ltd (Dublin, Ireland), is one of the first companies to nanosize drugs, and currently boast four products on the market. Its first nanotechnology-based formulation of Wyeth’s Rapamune (sirolimus, an immune suppressant to prevent organ transplant rejection), was developed and approved by the US Food and Drug Administration in 2000 and has become the fastest selling drug in the transplant market. Some of the key nanotechnology-based approaches for the enhancement of drug solubility and oral bioavailability are highlighted in Table 1.2.
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Despite sophisticated new delivery systems, the development of satisfactory oral formulations has remained a challenge as adequate drug delivery for most formulations has not been attained. This is in part a consequence of low solubility presented by a good number of orally administered drugs. This low solubility implies a large variation in absorption/plasma levels as well as high manufacturing costs, both of which are unacceptable for drug development.

Although large numbers of approaches have been tried for solubility enhancement of poorly soluble drugs but there are some practical limitations with these techniques. The desired solubility and thus bioavailability enhancement may not always been achieved with these conventional approaches. Newer approaches are continuously being explored to enhance the solubility of poorly water soluble drugs. Another newly introduced approach of nanotechnology for solubility enhancement is the Mesoporous silica as potential drug carriers.

1.6 Mesoporous Silica
1.6.1 Classification

According to the IUPAC definition\textsuperscript{28}, solids that contain pores with pore diameter (i) \(>50\) nm are called as macroporous, (ii) between 2-50 nm are mesoporous and (iii) \(<2\) nm are microporous\textsuperscript{29}. Some of the examples of different porous materials are listed in the Table 1.3.

<table>
<thead>
<tr>
<th>Type of Material</th>
<th>Pore Size (nm)</th>
<th>Examples</th>
<th>Pore Size Range (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroporous</td>
<td>(&gt;50)</td>
<td>Porous glasses</td>
<td>(&gt;50)</td>
</tr>
<tr>
<td>Mesoporous</td>
<td>2-50</td>
<td>Pillared layered clays</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M41S</td>
<td>2-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SBA-15</td>
<td>8 - 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SBA-16</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatom biosilica</td>
<td>2-50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesoporous alumina</td>
<td>2</td>
</tr>
<tr>
<td>Microporous</td>
<td>(&lt;2)</td>
<td>Zeolites</td>
<td>(&lt;1.42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activated carbon</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZSM-5</td>
<td>0.45-0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zeolite A</td>
<td>0.3-0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta and Mordenite- Zeolites</td>
<td>0.6-0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Faujasite</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cloverite</td>
<td>0.6-0.132</td>
</tr>
</tbody>
</table>

Mesoporous silica nano-materials (MSNs) are gaining attention because of their emerging applications in drug delivery\textsuperscript{30}. Since their first appearance in materials science in the 1990s, these inorganic carriers have been successfully used
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In other research areas such as catalysis\textsuperscript{31}, purification\textsuperscript{32} and adsorption\textsuperscript{33}. The majority of ordered MSNs have two dimensionally ordered arrays of cylindrical pores of uniform size disposed parallel to each other and separated by thin walls\textsuperscript{34}.

1.6.2 Nomenclature of Mesoporous Materials

The MSNs are named after the company or research group introducing them or according to the structural characteristics of the developed material, some such MSNs are shown in Table 1.4.

Table 1.4: Different MSNs

<table>
<thead>
<tr>
<th>MSNs</th>
<th>Full name</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSU</td>
<td>Michigan State University</td>
</tr>
<tr>
<td>SBA</td>
<td>Santa Barbara Amorphous</td>
</tr>
<tr>
<td>MCM</td>
<td>Mobil Crystalline Matter/ Mobil Composite Matter</td>
</tr>
<tr>
<td>HMS</td>
<td>Hollow Mesoporous Silica</td>
</tr>
<tr>
<td>OMS</td>
<td>Ordered Mesoporous Silica</td>
</tr>
<tr>
<td>TUD</td>
<td>Technische Universiteit Delft</td>
</tr>
<tr>
<td>MCF</td>
<td>Meso Cellular Form</td>
</tr>
<tr>
<td>FSM</td>
<td>Folded Sheet Mesoporous</td>
</tr>
<tr>
<td>KIT</td>
<td>Korean Advanced Institute of Technology</td>
</tr>
<tr>
<td>AMS</td>
<td>Anionic Surfactant templated Mesoporous silica</td>
</tr>
</tbody>
</table>

1.6.3 Synthesis of Mesoporous Materials

The MSNs can be synthesized as silicas\textsuperscript{35}, transitional aluminas\textsuperscript{36} and pillard clays\textsuperscript{37}. In fact, in 1990, Yanagisawa and co-workers\textsuperscript{38}, described the preparation of mesoporous silicas with uniform pore size. However, the pores in these materials were generally irregularly spaced and broadly distributed in size. In 1992, a research team from Mobil Oil Company synthesized a new family of materials; the so-called M41S\textsuperscript{39-42} that presented ordered pore distributions, with homogeneous sizes ranging between 2 nm to 10 nm, including different materials like hexagonal MCM-41, cubic MCM-48, and lamellar MCM-50. These materials are characterized by regular arrays of uniform channels, whose dimensions can be tailored through the choice of surfactants, additives and synthesis conditions. These liquid crystal structures depend on the composition and chemical nature of the surfactant, and also on the solution mixture conditions, such as surfactant concentration, pH, temperature, the presence of additives, etc. In the final step of the synthetic process, once the silica source has condensed around the micelles, the surfactant is removed by thermal degradation or solvent extraction. This surfactant removal gives rise to a network of cavities within the silica framework that governs the physicochemical properties of the material.

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A series of inorganic mesostructures have been synthesized with the different surfactant and according to the structural requirement of the material. For example, hexagonal mesoporous silica (HMS) prepared using neutral amine as template possesses slightly disordered hexagonal structure and thicker walls, superior thermal stability upon calcination in air, and a smaller crystallite size, which affords complementary textural mesoporosity for improved access to the framework-confined mesopores.\(^3\). Michigan State University (MSU-1) synthesized MSNs by using polyethylene oxide (PEO) as a structure directing agent with a disordered channel structure.\(^4\) This material possesses large wall thickness and small particle size with considerable textural mesoporosity due to pores formed between the relatively small particles. Another widely used material is Santa Barbara Amorphous-15 (SBA-15), having highly ordered pores with thicker pore walls and two dimensional hexagonal structure which is synthesized by amphiphilic triblock-copolymer of poly (ethylene oxide) and poly (propylene oxide) (Pluronic P123) as the structure directing reagent in highly acidic media.\(^5\)

**Figure 1.4: General synthesis scheme for M41S mesoporous family**

The synthesis procedures are often modified to obtain the mesoporous structures according to the need of the application. For instance, MCM-41 and SBA-3 are typically synthesized in alkaline conditions, but the synthesis of SBA-1 involves acidic synthesis.\(^6\) The synthesis procedure for MCM-41 has been described by Beck et al.\(^2\) One fairly new member in the group of the porous silicas is TUD-1 (Technische Universiteit Delft), which was introduced by Jansen et al.\(^7\) in 2001. The major difference between TUD-1 and the other porous silicas is that the fabrication process for the TUD-1 silica is completely surfactant free. Instead of micelles or large organic compounds, the formation of pores is induced by aggregates of smaller molecules.\(^7\) This makes the process cost-effective. Briefly, a mixture containing organic template (e.g. tri-ethanolamine), silica source (TEOS), and water is aged and dried to form a homogenous gel, which is then
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transformed into mesostructured solid by calcination or by hydrothermal treatment.\textsuperscript{47}

Incorporation of hetero atoms such as Cu, Zn, Al, B, Ga, Fe, Cr, Ti, V, Sn etc. into mesoporous silica framework has also been investigated.\textsuperscript{48-66} Methodology to prepare mesoporous silica via the template synthesis is extended to preparation of some mesoporous metal oxides such as TiO\textsubscript{2}, Ta\textsubscript{2}O\textsubscript{5}, Nb\textsubscript{2}O\textsubscript{5}, ZrO\textsubscript{2}, Al\textsubscript{2}O\textsubscript{3}, V\textsubscript{2}O\textsubscript{5} etc. as well as synthesis of mesoporous aluminophosphate.\textsuperscript{81-84}

In spite of so many modifications, general diagrammatic scheme for synthesis of silica-based ordered MSNs can be given as in Fig. 1.4. Ordered porous structures have narrow pore size distributions and some of them have thick walls, which enhance their stability. They also show large surface areas and large pore volumes. The pore size and morphology of MSNs depend on the template. In the case of surfactants, the chain length is the most critical factor.\textsuperscript{85} On the other hand, the wall thickness and stability are determined by the interactions occurring between the silica precursor and the template.\textsuperscript{86} The mesoporous structure can be controlled by a sophisticated choice of templates (surfactants), adding auxiliary organic chemicals, and changing reaction parameters (e.g., temperature, pressure, crystallization time, water content, pH, compositions).

1.6.4 Mechanism of Formation of Mesoporous Silica

The MSNs are synthesized using a silica source and different organic structure directing agents, e.g., cationic surfactants containing long alkyl chain (8-16 carbons) quaternary ammonium compounds, often followed by addition of cosurfactants. The dependence of surfactant/silica molar ratio in a ternary synthesis system containing tetraethyl ortho-silicate (TEOS, silica source), water and cetyl tri-methyl ammonium (C\textsubscript{16}TMA\textsuperscript{+}) cations (surfactant) at 100 °C on appearance of different phases of MSNs is summarized in Table 1.5

<table>
<thead>
<tr>
<th>Ratio: Surfactant/silica</th>
<th>Different phases of M41S type materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.0</td>
<td>Hexagonal</td>
</tr>
<tr>
<td>1.0 – 1.5</td>
<td>Cubic</td>
</tr>
<tr>
<td>1.2 – 2.0</td>
<td>Thermally unstable materials</td>
</tr>
<tr>
<td>2.0</td>
<td>Cubic octamer</td>
</tr>
</tbody>
</table>

A number of models have been proposed to rationalize the mechanism of formation of MSNs by various synthesis routes. All these models are based on the role of surfactants in solution to direct the formation of silicate mesostructure. Different interactions between the surfactant and the inorganic precursor under
different synthesis conditions lead to different postulates for the mechanism of formation of MSNs, which are discussed briefly in following section.

**a) Liquid Crystal Templating (LCT) Mechanism**

The first mechanism proposed was a 'liquid crystal templating (LCT)mechanism' to explain the formation of M41S type MSNs.\textsuperscript{71, 72} The mesostructure formation depends on the hydrocarbon chain length of the surfactant tailgroup\textsuperscript{73}, the surfactant concentration and the presence of additional organic swelling agents. The lowest concentration at which surfactant molecules aggregate to form spherical isotropic micelles is called critical micelle concentration (CMC1). Further increase in the surfactant concentration initiates aggregation of spherical into cylindrical or rod-like micelles (CMC2).

There are three main liquid crystalline phases with hexagonal, cubic and lamellar structures (Fig. 1.5). The hexagonal phase is the result of hexagonal packing of cylindrical micelles, the lamellar phase corresponds to the formation of surfactant bi-layers and the cubic phase may be regarded as a bi-continuous structure.

Two synthesis mechanisms have been proposed.\textsuperscript{71,72} In the first route, the $\text{C}_n\text{H}_{2n+1}\text{(CH}_3)_3\text{N}^+$ surfactant species organize into lyotropic liquid crystal phase, which can serve as template for the formation of hexagonal MCM-41 structure.

![Figure 1.5: Mesophase structures of M41S: a) Hexagonal (MCM-41), b) Cubic (MCM-48) c) Lamellar (MCM-50)](image)

The surfactant micelles aggregate into a hexagonal array of rods, followed by interaction of silicate or aluminate anions present in the reaction mixture with the surfactant cationic head groups. There after condensation of the silicate species occurs, leading to the formation of an inorganic polymeric species. After
combusting off the surfactant template by calcination, hexagonally arranged inorganic hollow cylinders are produced (Fig. 1.6).

Figure 1.6: Liquid crystal templating (LCT) mechanism proposed for the formation of MCM-41; (A) liquid crystal phase initiated and (B) silicate anion initiated

In the second route, the hexagonal ordering is initiated by the presence of silicate species in the reaction mixture. Chen et al. explained that randomly distributed surfactant micelles with rod-like morphology form initially, and their interaction with silicate oligomers generate randomly oriented surfactant micelles surrounded by two or three silica mono layers. Further condensation between silicate species on adjacent rods occurs on heating, initiating the long-range hexagonal ordering (Fig. 1.7).

Figure 1.7: Silicate rod assembly proposed for the formation of MCM-41; and (2) random ordering of rod-like micelles and interaction with silicate species, (3) spontaneous packing of the rods, and (4) remaining condensation of silicate species on further heating

b) Charge Density Matching
The ‘charge density matching’ model proposed by Stucky et al., suggested that condensation occurs between initially formed silicate species by the electrostatic interaction between the anionic silicates and the cationic surfactant head groups.
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This reduces the charge density and therefore, curvature was introduced into the layers to maintain the charge density balance with the surfactant head groups, which leads to transformation of the lamellar mesostructure into the hexagonal one (Fig. 1.8A). Although this silica-initiated synthesis mechanism has been widely accepted, the presence of an intermediate lamellar species has been disputed.

![Figure 1.8: Transformation of surfactant-silicate systems from lamellar to hexagonal mesophases; (A) hexagonal mesophase obtained by charge density matching, and (B) folding of kanemite silicate sheets around intercalated surfactant molecules](image)

c) Folded Sheet Mechanism
The 'folded-sheet mechanism' postulated by Inagaki et al\(^77\) indicated the presence of intercalated silicate phases in the synthesis medium of the reaction products\(^78\) (Fig.1.8B & 1.9). The flexible silicate layers of kanemite fold around the surfactant cations, and cross-linking of the interlayer occurs by condensation of silanol groups on adjacent silicate sheets. On increase of pH, the amount of occluded CnH\(_{2n+1}\)(CH\(_3\))\(_3\)N\(^+\) cations in kanemite increases resulting in expansion of the kanemite inter layers to form another class of regular hexagonal structure called FSM-16.

![Figure 1.9: Model schematically representing “folded sheets” mechanism](image)

d) Silica Tropic Liquid Crystals
Firouziet al\(^79\) have developed a model based on cooperative organization of inorganic and organic molecular species into 3D structured arrays\(^80, 81\). According to this model, the physicochemical properties of a particular system were not
determined by the organic arrays having long range pre organized order, but by the dynamic interplay among ion-pair inorganic and organic species, so that different phases can readily be obtained through small variation of controllable synthesis parameters.

1.6.5 Generalized Liquid Crystal Templating Mechanism

a) Ionic Route (Electrostatic Interaction)
Huo et al\textsuperscript{82} proposed a generalized mechanism for the formation of mesostructures, which was based on specific types of electrostatic interaction between an inorganic precursor (I) and a surfactant head group (S)\textsuperscript{83}. In this concept, four different approaches were proposed to synthesize transition metal oxide mesostructures\textsuperscript{82}. The first route involves the charge density matching between surfactant cations and inorganic anions (will be referred to as $S^+I^-$ hereafter). The second route deals with the charge-reversed situation, i.e., anionic surfactant and cationic inorganic species ($S^-I^+$). Both the third and fourth routes are counter ion-mediated pathways. The third one demonstrates the assembly of cationic species via halide ions ($S^-X^+I^-$), while the fourth one depicts the assembly of anionic species via alkali metal ions ($S^-X^-I^+$) (Fig. 1.10). These synthesis strategies are acceptable for the formation of a wide variety of lamellar, hexagonal or cubic mesophases. However, a general problem negotiated very often is the poor stability of the inorganic framework, which frequently collapses after removal of the surfactant.

a) Neutral Templating Route (Hydrogen Bonding Interaction)
Tanev and Pinnavaia proposed another route to synthesize hexagonal mesoporous silicas (HMS) having thicker pore walls, high thermal stability and smaller crystallite size but, having higher amounts of interparticle mesoporosity and lower degree of long-range ordering of pores than MCM-41 materials\textsuperscript{84, 85}. This route is essentially based on hydrogen bonding between neutral primary amines and neutral inorganic precursors, where in hydrolysis of tetraethyl ortho silicate (TEOS) in an aqueous solution of dodecyl amine yields neutral inorganic precursor. Using the same approach, porous lamellar silicas with vesicular particle morphology have been synthesized with the aid of double headed alkyl amines linked by a hydrophobic alkyl chain\textsuperscript{85}.

a) Ligand-Assisted Templating Route (Covalent Interaction)
Antonelli and Ying\textsuperscript{86} have proposed a ligand-assisted templating mechanism for the synthesis of hexagonally packed mesoporous metal oxide completely stable to surfactant removal. In a typical synthesis, the surfactant was dissolved in the metal alkoxide precursor before addition of water to allow nitrogen–metal covalent bond formation between the surfactant head group and the metal alkoxide precursor. The existence of this covalent interaction was confirmed by $^{15}$N NMR spectroscopic studies. In this approach, the structure of the mesophases could be
controlled by adjustment of the metal/surfactant ratio, which led to a new class of mesoporous transition metal oxides analogous to M41S family.

Figure 1.10: Cooperative organization for the formation of silica tropic liquid crystal phase /silicate-surfactant mesophases; (A) organic and inorganic precursor solutions, (B) preliminary interaction of the two precursor solutions after mixing, and (C) multidentate interaction of the oligomeric silicate units with the surfactant molecules

1.6.6 Characterization
The synthesized material is characterized for its particle and pore morphology, structure and surface details using techniques such as low angle powder X-ray diffraction (XRD), FT-IR spectroscopy, nitrogen adsorption/desorption, scanning
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electron microscopy (SEM), transmission electron microscopy (TEM), and, differential scanning calorimetry (DSC). Adsorption analysis gives information about the porosity and surface area of the materials, while SEM gives particle size and morphology. Diffraction techniques, FTIR and TEM give insight to the degree of structural order and DSC measurements provide details regarding the drug loading in to the pores of MSNs.

1.6.6.1 Powder X-ray Diffraction
Despite long-range order, these materials are amorphous in nature, as demonstrated by high angle X-ray diffraction patterns. For this reason, X-ray diffraction analysis is generally measured at the low angle (0.5°–10°). The powder X-ray diffraction is the most rapid method to determine the nature and degree of pore order in the material. Generally, three peaks of varying intensity are observed, which can be indexed in a hexagonal pattern characteristic of MSNs. These peaks observed in the low-angle 2-theta region can be indexed as d100, d110, and d200 in case of MCM-41 type of material while in MSU type material; intense single peak is obtained at low 2 theta angles usually between 1° and 3° followed by two small peaks as observed with MCM-41.

1.6.6.2 Nitrogen Sorption Analysis
Nitrogen adsorption/desorption is an important aspect of material characterizing for the determination of pore size (diameter), pore volume, and surface area. Two calculation methods reported in the mid twentieth century facilitated the measurements of surface area and pore diameter/volume. Stephen Brunauer, P.H. Emmett, and Edward Teller have developed a method to calculate the surface area. This calculation, known as Brunauer-Emmett-Teller (BET), determines the surface area by measuring the adsorption of non-polar gases (N2, Ar).

The isotherms are categorized according to IUPAC as shown in Fig. 1.11. Type I is typical for microporous materials and show a plateau after the filling of the small pores at low relative pressures. Type II isotherms describe the formation of multi layers after the monolayer is completed at point B. This shape in combination with the complete reversibility upon desorption is typical for non-porous materials. Type III and V are rarely observed and reflect a low interaction energy between adsorbate and adsorbent. Type IV isotherms are typical for mesoporous samples. The original IUPAC classification defined the hysteresis as characteristic feature of MSNs. Type VI shows an example isotherm for samples with a stepwise multilayer absorption of a non-porous material. The calculation is modeled on the physical adsorption of gas on the surface of the material as a function of pressure.
Figure 1.11: Six isotherms defined by the IUPAC

Figure 1.12: Typical isotherm for a bulk sample of mesoporous MSNs

In Fig. 1.12, a typical isotherm for nanometer-sized MSNs (Type IV) with fields for the individual sorption steps is shown. The steps A to E can be attributed to the following processes: A: At low relative pressures a monolayer of adsorbate molecules is forming on the high inner surface of the material B: Multi layers are established C: Filling of the mesopores occurs (capillary condensation) D: The
remaining outer surface is covered (plateau) E: In samples with very small particles, the adsorbate condenses in the interparticle pores.

The most common sorption method is nitrogen sorption, because of a suitable heat of adsorption (5-25 kJ/mol) and a good access of nitrogen molecules (0.354 nm) into small pores. An evacuated sample cooled with liquid nitrogen is loaded with gaseous nitrogen. Due to the low temperature, the nitrogen is adsorbed on the sample surface, resulting in equilibrium between adsorbed film and gas phase at constant temperature. The isotherm shows the adsorbed amount of gas as a function of the pressure.

Surface area determinations involve creating the conditions required to adsorb an average monolayer of gas molecules onto a sample. As pressure increases, the amount of gas adsorbed quickly rises due to the capillary condensation in mesopores. This gas condensation occurring in the mesopores allows the fine porous structure of the sample to be evaluated. The pressure is increased until saturation is reached when all mesopores are filled with liquid. The pressure is reduced incrementally, evaporating the condensed gas from the system. Upon desorption, a hysteresis is commonly observed in MSNs. Hysteresis is the phenomenon in which the value of a physical property lags behind changes in the effect causing it. The hysteresis between the adsorption and desorption branches of the isotherm reveals information regarding pore size, volume, area, and shape. Hysteresis loops most likely arise from a combination of the thermodynamic and network effects. The thermodynamic hysteresis may be due to capillary condensation and capillary evaporation occurring at higher and lower pressures, respectively. The network effect may be caused by a decrease in pore diameter at the mouths of the pores, much like a wine bottle. The pore diameter and pore volume calculations were developed by Elliot P. Barrett, Leslie G. Joyner, and Paul P. Halenda. This calculation, known as BJH, assumes a similar theory to the BET of the adsorption/desorption process. The BJH is calculated when saturation is reached and all mesopores are filled by the adsorptive gas. The BJH calculates a pore diameter distribution, outputs a histogram, and an average pore size is reported. It applies only to the mesopores and small macro pore size range. This calculation assumes the approximate cylindrical pore geometry.

1.6.6.3 Scanning and Transmission Electron Microscopy
The scanning and transmission electron microscopy (SEM and TEM, respectively) are used to confirm the information obtained from low angel powder XRD technique. Scanning electron microscopy utilizes a lower voltage electron beam (<20 kV) and is useful for determining exterior particle morphology to about 50,000x magnification. The shape and size of the MSNs are readily observed by SEM. Transmission electron microscopy utilizes a stronger electron beam (about 300 kV), and allows for the visualization of pores. Transmission electron
**Introduction**

Microscopy is not a substitute for XRD, since only a small sampling is obtained. The magnification achievable by TEM is in the order of 300,000x. Transmission electron microscopy gives evidence in support of the powder XRD regarding pore structure and order.

1.6.6.4 FTIR Spectroscopy

Infrared spectroscopy is an elegant technique to determine the surface species and kinetics of surface reactions. Typically one can follow the changes at the surface by examining characteristic vibrations which appears or disappears. Fourier transform infrared (FTIR) spectroscopy deals with the vibration of chemical bonds in a molecule at various frequencies depending on the elements and types of bonds. After absorbing electromagnetic radiation the frequency of vibration of a bond increases leading to transition between ground state and several excited states. The energy corresponding to these transitions corresponds to the infrared region (4000–400 cm⁻¹) of the electromagnetic spectrum. The term Fourier transform refers to a recent development in the manner in which the data are collected and converted from an interference pattern to an infrared absorption spectrum that is like a molecular "fingerprint". In the case of porous silicates, the FTIR spectra in the 400–1300 cm⁻¹ region provides information about the structural details and silanol groups.

1.6.6.5 Differential Scanning Calorimetry

This thermal method provides data on thermodynamic effects by measuring the time-dependent heat flow between the sample and a reference. DSC is useful tool to check the drug loading in the pores of mesopores. The absence of melting peak of drug is observed when drug molecules get entrapped in pores of MSNs.

1.7 Mesoporous Materials and Drug

Mesoporous silica materials are potential drug carriers with the following features:

- An ordered nano sized pore network, which is very homogeneous in size and allows fine control of the drug load and release kinetics;
- A high pore volume to host the required amount of drug;
- A high surface area, which implies high potential for drug adsorption;
- A silanol-containing surface that can be functionalized to allow better control over drug loading and release.

Ordered mesoporous silica has stable mesoporous structure, large surface area, good biocompatibility and tailored size of mesopores, all these requisites exhibited promising application as immediate and controlled drug delivery system. Compared with amorphous colloidal and porous silica, mesoporous silicon exhibit higher loading of drugs and provide an immediate release and controlled drug release if modified by functionalisation. Different MSNs like MCM-41, SBA-15, TUD, MCM-50, HMS, MSU-H etc. have many important properties advantageous
Introduction to drug delivery applications. The small size of the pores confines the space of a drug and engages the effects of surface interactions of the drug molecules and the pore wall. The size of the pores and the surface chemistry of the pore walls may be easily changed and controlled.

The most important feature of these materials, from a practical point of view, is the feasibility to synthesize the mesoporous frameworks with different pore sizes and geometries. This fact opens a wide range of possibilities for hosting molecules larger than the ones exhibited in the traditional drug delivery systems. This is of great interest for designing materials which can be used as drug carriers, since the pore size of these materials are similar in magnitude to the molecular size of drugs. In view of all of these facts, MSNs have been developed as drug delivery systems since 2001. The importance of these materials as drug carriers is based on the ability of the silanol groups in the mesopore walls to adsorb molecules of pharmacological interest, followed by an immediate or controlled release of active molecules.

Due to their unique properties, ordered mesoporous silicates are very effective in terms of the enhancement of drug dissolution. Numerous recent studies have demonstrated that drugs lose their crystallinity when they are deposited in or onto the surface of mesoporous silicates, which enhances their dissolution rate. Mesoporous silicates have advantage over non-porous high surface area materials, as the deposited molecules are not just adsorbed onto the silica surface, but are also confined to pores that are only a few molecular diameters wide, which prevent the drug molecules from recrystallizing. Upon influx of water, the adsorbed drug is displaced from the silica surface and diffuses out of the pores. The pore channels of MSNs being able to change the crystalline state of a drug to an amorphous one, the pore channels also restrict drug recrystallization and reduce the particle size of the amorphous drug.

Silanol groups on the pore walls are also susceptible of undergoing a chemical modification with a large variety of organic groups through a functionalisation process. Indeed, the pore-wall modification would be performed depending on the functional groups of the drug molecules to be adsorbed. For example, sodium alendronate, a drug employed for osteoporosis treatments, has two phosphonate groups that would undergo stronger attracting interactions with amine groups than with silanols. Therefore, if the pore wall surface is covered by amine groups, there would be a larger alendronate loading than in unmodified materials. The results showed that drug loading increased from 14% (unmodified) to 37% (modified) for amine-grafted materials MCM-41.

The main functionalization procedures currently in use are the co-condensation procedure, in which the organic functional group is mixed with
the silica precursors and all the procedures are carried out in the same reaction vessel; and the post-synthesis grafting method\textsuperscript{111}, where the already formed inorganic silica matrix is reacted with the functionalizing agent in anhydrous conditions to yield organically modified silica mesopores.

![Diagram of Chemical composition: SiO\textsubscript{2}nH\textsubscript{2}O](image)

**Figure 1.13:** Scheme of the factors that affect to the properties of ordered mesoporous silica

Chemically grafting functional groups on the ordered mesoporous network change the adsorption characteristics of the silica surface and its polarity significantly. The chemical modification of the silanol groups at the pore walls has to be selected depending on the drug molecule to get the desired loading and release properties\textsuperscript{109}. Several factors influence the final adsorption properties of the mesoporous silicas, when intended for developing materials for drug delivery. These factors are schematically shown in Fig. 1.13.

1.8 The influence of textural properties on adsorption of drug molecules on/ into the pores of MSNs

1.8.1 Pore Size

The pore size is an important parameter when drug has to be incorporated in to a mesoporous matrix for this to be used as a drug delivery system (DDs). The drug incorporation is commonly carried out by soaking of the matrix in a highly concentrated drug solution with effective stirring and subsequent drying (Fig. 1.14). The process is mainly based on the adsorptive properties of MSNs. The pore size of MSNs determines the size of the molecule that can be adsorbed in to the mesopores. Thus, the adsorption of molecules in the mesoporous matrix is governed by size selectivity.

Ordinarily, the pore diameters slightly larger than the drug molecule dimensions (pore/drug size ratio>1) are enough to allow the adsorption of drug
inside the pores. One of the most important characteristics of MSNs is that the mesopores diameters can be tuned from 1.5 nm to several tens of nanometers by changing the chain length of the surfactant, employing polymeric structure-directing agents, or solubilizing auxiliary substances into micelles. These different tools allow mesoporous matrices to be tailored to host either small molecules or macromolecules such as proteins. MSNs exhibit molecular-sieve properties for relatively large molecules. Although the proposed system was not designed as a DDS, but considered the molecular-sieve properties of mesoporous silicas for the first time and the solvent polarity as fundamental factors for drug loading. However, when MSNs are designed as DDS, the pore size not only exerts a molecular-sieving function but also controls the drug-release rate. When MCM-41 was first tested as DDS, with two types of surfactant i.e. C12TAB and C16TAB as structure directing agents (C12TAB= dodecyl trimethyl ammonium bromide, C16TAB= hexadecyl trimethyl ammonium bromide). The MCM-41 with larger pore size, obtained using C16TAB, released 68% of the loaded ibuprofen (IBU) after 24h in simulated body fluid (SBF). In contrast, MCM-41 obtained with C12TAB released only 55% of the drug under similar conditions. Further studies confirmed the role of the pore size as drug-delivery controller. When IBU was incorporated into MCM-41 MSNs with pore diameters between 2.5 and 3.6 nm, an increase in release rate with pore size was evident (Fig. 1.15). The increased pore size facilitates the faster diffusion of the dissolution media in to the pores and causes rapid dissolution of the amorphous drug molecules present in pores. The concept of pore size as a kinetic-release controller is not only applicable.
to 2D hexagonal structures, such as MCM-41, but also to 3D cubic ones, such as MCM-48 MSNs.

![Figure 1.15: IBU fractions released from MCM-41 after 24h in SBF. The plot is represented as a function of pore diameter and the surfactants used are indicated for each case](image)

Qu et al\textsuperscript{11} reported that the release of captopril from several 2D hexagonal structures. They also pointed out that the pore-size effect can be evaluated only if the morphology is similar at the microstructure level. As an example, when the microstructure of the matrix consists of small spherical particles, the drug release is faster than that observed in bigger rod like particles, independent of the mesopore size.

### 1.8.2 Surface Area

The drug loading process is mainly based on the adsorptive properties of MSNs. Therefore, the surface becomes the most determining factor for the amount of adsorbed drug. In general terms, it is convenient to host large amounts of pharmaceuticals or, at least, to have the choice of incorporating high or low doses of a drug into the matrix. This challenge can be tackled by two different approaches: by increasing/reducing the surface area or by modifying the surface–drug affinity. The first approach involves the amount of surface available for the drug molecules. If the pore size allows the drug to get into the matrix, then higher surface area gives the higher amount of drug adsorbed. The final drug content can be very sensitive to the surface area $S_{\text{BET}}$. The fact was confirmed by preparing two MSNs, MCM-41 and SBA-15 (both 2D hexagonal structures) with $S_{\text{BET}}$ values of 1157 and 719 m$^2$/g, respectively. When both matrices were loaded with alendronate under the same conditions, the maximum loads of alendronate obtained were 139 and 83 mg/g for MCM-41 and SBA-15, respectively\textsuperscript{116}. Thus, the value of $S_{\text{BET}}$ is closely correlated with the maximum load of the matrix surface.
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1.8.3 Pore volume
Pore volume is an important factor when loading large volume molecules, such as proteins. This dependence on the space available to load large molecules was evidenced when up-taking BSA (transport protein) as a model protein into two mesoporous silica matrices with very different pore volumes; SBA-15 and mesocellular silica foams (MCF), with 1.1 and 1.9 cm$^3$/g respectively$^{117, 118}$. The behavior of these matrices was as expected, the higher is the pore volume, and the higher is the protein loading, which was 15% for SBA-15 and 24% for MCF. Another situation in which pore volume is a ruling factor in the amount of drug loaded is when high amounts of adsorbed molecules are required. That can be achieved by repeated and consecutive impregnations leading to the full pore volume filling. This experiment was carried out with ibuprofen and the successive impregnations promoted intermolecular drug-drug interactions, which increased the amount of drug loaded$^{96}$. The drug–mesopore interaction is a surface phenomenon; however, weak drug–drug interactions can result under loading conditions and could lead to the pore filling. In this case, the pore volume is a key factor in determining the amount of drug adsorbed. It was reported recently that several consecutive loadings of drug in ordered MSNs leads to larger filling of the mesopores, which was attributed to the increased drug intermolecular interactions within the pore voids, whereby larger pore volumes may result in greater drug loading$^{119}$.

1.8.4 Functionalisation
The keystone in the development of silica MSNs as DDSs is the modification or functionalization of the surface through organic groups$^{120-123}$ (Fig. 1.16).

![Figure 1.16: Pore geometry of MSNs](image)

This process provides numerous possibilities to control drug adsorption and release. Mesoporous silica shows a high density of silanol groups, which can be used to obtain functionalized surfaces by grafting organic silanes ((RO)3SiR’).
1.17 shows some of the most widely used functional groups along with the drugs with which the groups are used.

![Diagram showing pore wall functionalization in silica MSNs and structure of several drugs used in this system](image)

**Figure 1.17: Pore wall functionalization in silica MSNs and structure of several drugs used in this system**

The drug release from the MSNs can be effectively controlled by different methods and the preferred method is increasing the drug surface interaction. For this purpose the surface is functionalized with chemical groups that are able to link to the drug molecules through ionic bonds or through ester groups. One of the most studied cases is the adsorption of IBU on functionalized matrices. IBU was incorporated in these systems on the assumption that its carboxy group links the silanol groups at the surface (Fig. 1.18).

However, this situation is not exclusive in non-functionalized matrices, as drug–drug interactions are also present and lead to the formation of IBU dimers. This dimer configuration is formed by an intermolecular hydrogen bond through the carboxy groups. The IBU linkage is quite different when the surface is functionalized. Song et al., reported the functionalization of MCM-41 and SBA-15 with amino groups as an effective method to control IBU release. The ionic interaction between the carboxyl groups in IBU and the amino groups on the matrix surface allows the release rate of IBU from amino-functionalized MSNs to be effectively controlled.

Another recent strategy for effective control of drug release is functionalization of the surface with hydrophobic species. This does not necessarily increases the drug surface interaction, but the drug transport out of the matrix is seriously impeded as the aqueous medium cannot penetrate easily inside the pores. For example controlled release of erythromycin from SBA-15, was
modified with octyl and octa decyl moieties by treating the mesoporous matrix with tri-methoxy octyl silane and tri-methoxy octadecyl silane, respectively.

Figure 1.18: a) Non-functionalized matrix: IBU linked through weak hydrogen bonds between its carboxylic acid group and the silanol groups (left) and a physisorbed dimer molecule at the surface (right). b) Amino-functionalized matrix: IBU is linked to the pore wall through stronger ionic bonds between carboxylate and ammonium groups.

The octa decyl-functionalized sample exhibited a release rate one order of magnitude lower than that of non-functionalized SBA-15. Similar results were obtained with MSNs modified by silylation. Captopril\textsuperscript{127} and ibuprofen\textsuperscript{128} have been incorporated but both showed a lower drug loading when silylation is carried out. However, well-defined controlled drug release can be achieved by tailoring the surface properties of mesoporous silica materials by regulating the degree of silylation.

1.9 Drug Loading Confirmative Techniques
Once the MSNs have been selected, the next procedure is to confine the molecules into the mesopores. The host material is placed into a highly concentrated solution of the drug to be adsorbed with continuous magnetic stirring to favour the diffusion of the drug molecules into the mesopores (Fig. 1.19).

The solvent should be selected depending on the solubility of the drug. Thus, each type of drug presents its optimum solvent and concentration to obtain the highest possible amount of adsorbed drug. The drug loading can be confirmed
and quantified through different characterization techniques as are described below.

![Diagram of drug loading process](image)

**Figure 1.19:** Schematic representations of the factors affecting drug loading procedure

![X-ray diffraction and transmission electron micrographs](image)

**Figure 1.20:** X-ray diffraction (left) and transmission electron micrographs (right) before and after loading MCM-41 with ibuprofen. Mesostructural order is maintained after the loading process

The first step after the drug loading is to check that the mesostructure of the ordered host matrix has survived the loading process. This is normally confirmed by carrying out low angle X-ray diffraction (XRD) patterns before and after loading the drug. The same diffraction maxima of the corresponding mesostructure is observed in both cases to ensure that the uptake process has not destroyed the mesostructure. Transmission electron microscopy (TEM) can also be
employed to confirm the prevalence of the ordered mesostructure after the loading step (Fig. 1.20).

After confirmation that the mesostructure has not collapsed, it is necessary to check that whether the drug molecules confined inside the mesopores or they are just on the outer surface of the matrices.

Nitrogen adsorption analysis has been used to find out the inner morphology of the mesopores. The nitrogen adsorption isotherm can give the pore size distribution and the pore volume of the materials before and after the loading process. The surface area and pore volume normally decrease as a consequence of the host–guest interaction which suggests that the drug molecules are partially filling the mesopores, and are confined inside the pores. An interesting approach to ensure that the drug molecules are exclusively inside the pores and not on the external surface is the selective functionalisation of the external surface, leaving the internal pore volume available for drug molecule adsorption. Figure 1.21 shows a decrease in pore volume and pore diameter after loading; it means that the drug molecules are partially filling the mesopores.

When targeting these systems as drug delivery devices, it is necessary to confirm that the drug is inside the mesopores. For this, Fourier-transform infrared (FTIR) spectroscopy needs to be done before and after the loading.

**Figure 1.21: Nitrogen adsorption isotherms (left) and mesopores diameter (right) before and after loading MCM-41 with ibuprofen**

The vibration bands corresponding to the drug, which would depend on the adsorbed molecule, should be observed after the loading process (Fig. 1.22), thus confirming the presence of the drug in the matrix.
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Figure 1.22: Fourier-transformed infrared spectra of ibuprofen, MCM-41 and MCM-41 ibuprofen-loaded matrix

1.10 Release of Drug from the Pores
To study the release kinetics, the drug loaded MSNs are introduced into a simulated body fluid\textsuperscript{129} (SBF) at 37°C, emulating the physiological conditions (Fig. 1.23).

Figure 1.23: Schematic representation of the drug release procedure

Small quantities of the soaking solution are withdrawn at regular time intervals and the amount of drug released can be quantified using ultraviolet (UV) spectroscopy and high performance liquid chromatography (HPLC). The results are commonly presented in plots of released amount versus time. The release pattern of drug from the carrier matrix would determine the efficiency of the system. Although correlations of in vitro and in vivo drug release are uncommon,
in vitro drug release studies are essential for a drug delivery system investigation to predict the behavior of the carrier.

![Diagram: Loading possibilities of MSNs](image)

**Figure 1.24:** Loading possibilities of MSNs: Small-molecule, biomolecules, such as proteins (enzymes, antibodies) or nucleic acids can be either adsorbed or attached to the particle surface; and potentially loaded into the porous matrix, can be made bio-imageable for instance by attachment of fluorescent dyes or MRI-active complexes to either the particle surface and/or the pore walls for concurrent imaging of the drug delivery site/process.

Owing to their stable mesoporous structure and well-defined surface properties, MSNs seem ideal for encapsulation of pharmaceutical drug, proteins and other biogenic molecules. Some reports employing MSNs for hosting and delivering a variety of molecules of pharmaceutical interest have been appeared recently. A wide range of functional groups have successfully been introduced to MSNs using the co-condensation approach. Covalent attachments of both fluorophores and biologically active molecules onto these functional groups have been demonstrated. Often the fluorophore is pre-reacted with the amino-silane that is subsequently used in the co-condensation synthesis, yielding inherently fluorescent MSNs. This approach may also contribute positively when aiming at targeted delivery applications. Furthermore, it may also be beneficial to introduce...
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a surface layer or pore capping agents (e.g. ultra small MSNs consisting of some other material, “super molecules” or mechanically interlocked molecules, MIMs)\textsuperscript{131}, onto the outer particle surface to create gate-keeping properties, i.e. being able to keep the pores closed to prevent guest molecules from being released and then “open” upon an external stimulus, for example irradiation, application of a magnetic field or changes in temperature. General illustration of the bio-functionalization and cargo-loading possibilities is shown in Fig. 1.24.

It has been shown that both small and large molecular drugs can be entrapped within the mesopores by an impregnation process and release of these molecules can be controlled or enhance depending on type of MSNs used. A concise summary of different MSNs investigated for drug delivery shown in Table 1.6.

1.11 Mesoporous MSNs as Solubility Enhancers
The use of a mesoporous drug carrier may enhance the dissolution of API. The function is based on two factors: increasing the active surface area and reducing the crystallinity of the pharmaceutical substance. Decrease in crystallinity often leads to problems with stability, as the non-crystalline, disordered form has a high chemical potential which tends to transform to a crystalline form of a lower energy state. The porous carrier may, however, hinder or prevent these transformations by physically protecting the amorphous drug. Furthermore, loading of the drug substance into porous particles has been observed to enhance permeation\textsuperscript{132}.

Table 1.6: Different MSNs as a drug carrier

<table>
<thead>
<tr>
<th>MSNs</th>
<th>Drug</th>
<th>Category</th>
<th>Comment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCM-41</td>
<td>Ibuprofen, Piroxicam</td>
<td>NSAID</td>
<td>Faster release of drug and increase dissolution of drug</td>
<td>107</td>
</tr>
<tr>
<td>TUD-1</td>
<td>Ibuprofen</td>
<td>NSAID</td>
<td>Faster release of drug and increase dissolution of drug</td>
<td>97</td>
</tr>
<tr>
<td>MSNs</td>
<td>Gentamicin</td>
<td>Antibiotic</td>
<td>Controlled drug release</td>
<td>133</td>
</tr>
<tr>
<td>SBA-15, MCM-41</td>
<td>Ibuprofen</td>
<td>NSAID</td>
<td>Faster release of drug and increase dissolution of drug</td>
<td>102</td>
</tr>
<tr>
<td>MSNs</td>
<td>Camptothecin, Paclitaxel</td>
<td>Anticancer</td>
<td>Effectively targeted to human cancer cells</td>
<td>134</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MSN</strong>s</td>
<td>Hydralazine</td>
<td>Neuroprotector</td>
<td>Significant neuro protection to damage cells</td>
<td>135</td>
</tr>
<tr>
<td><strong>Mesostructure</strong>-cellular form</td>
<td>Ibuprofen</td>
<td>NSAID</td>
<td>Significantly increase dissolution rate</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>Antibiotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSNs</td>
<td>Gene</td>
<td>Protein</td>
<td>Effective penetration in cell membranes of animal and plant cells</td>
<td>137</td>
</tr>
<tr>
<td>SBA-15</td>
<td>Atenolol</td>
<td>Antihypertensive</td>
<td>Sustained delivery of drug</td>
<td>138</td>
</tr>
<tr>
<td>SBA-15, MCM-41</td>
<td>Sodium-alendronate</td>
<td>Anti-psoriatic</td>
<td>Enhanced release rate of drug</td>
<td>139</td>
</tr>
<tr>
<td>SBA-15, MCM-41</td>
<td>Amoxicillin</td>
<td>Antibiotic</td>
<td>Significantly increase dissolution rate</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
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<td></td>
<td></td>
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<tr>
<td>SBA-15</td>
<td>Itraconazole</td>
<td>Antifungal</td>
<td>Faster release of drug and increase dissolution of drug</td>
<td>141</td>
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<tr>
<td>MCM-41</td>
<td>Atenolol</td>
<td>Anti-hypertensive</td>
<td>Slow drug release</td>
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<tr>
<td>MCM-41</td>
<td>Ibuprofen</td>
<td>NSAID</td>
<td>Controlled release</td>
<td>143</td>
</tr>
<tr>
<td>Hybrid mesoporous silica</td>
<td>Folic acid, Cisplatin</td>
<td>Vitamin, Anticancer</td>
<td>Targeting drug delivery</td>
<td>144</td>
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<tr>
<td>Hollow mesoporous silica</td>
<td>Ibuprofen</td>
<td>NSAID</td>
<td>Sustained release</td>
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<td>MCM-48</td>
<td>Erythromycin</td>
<td>Antibiotic</td>
<td>Delayed release</td>
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<tr>
<td>SBA-15</td>
<td>Bovine serum</td>
<td>Protein</td>
<td>Controlled release</td>
<td>147</td>
</tr>
<tr>
<td>Mesoporous organosilica sphere</td>
<td>Tetracycline</td>
<td>Antibiotic</td>
<td>Sustained release</td>
<td>148</td>
</tr>
</tbody>
</table>

It has been reported that a small pore size is an important factor in the stabilization of the disordered drug\textsuperscript{149}. The pores are usually small enough to restrict the formation of an organized crystal structure inside them, and thus the loaded compound is forced to stay in the amorphous form and the phase
transitions upon storage are prevented. The structure of the carrier may also protect the loaded compound from external attacks by causing a steric hindrance. This kind of protection is especially needed for peptides which are vulnerable to enzymatic degradation in the body.

Dissolution of a drug that has been loaded into porous material is a bit more complicated process compared to that of the pure crystalline drug. If the carrier material is biodegradable, the dissolution of the drug is related to the decomposition of the carrier. Otherwise, the mass transfer from the pores determines the dissolution rate. If the drug–carrier interactions are strong, desorption may be the rate determining step in the dissolution process in exchange to the mass transfer. If rapid dissolution is aimed at, strong interactions are not favorable. In the case of certain carrier materials, different dissolution rates can be achieved depending on surface modification and porosity. This unique feature can be exploited for controlling the drug release; either sustained or accelerated release can be attained.

Surface pH of the porous material may also affect the dissolution of the pharmaceuticals that ionize within a definite pH-range. For example, calcium silicate and silica gel are known to create an alkaline local environment when moisture is adsorbed on them, and that has been observed to improve the solubility of ibuprofen, an acidic drug. The effect of surface pH might explain the reduction of the pH dependency of the dissolution obtained by loading the drug compound into microparticles.

The mesopores are usually small enough to provide satisfactory protection of the loaded drug, but adequate mass transfer rates can be, however, achieved, which is quite important in both dissolution and drug loading. As the diameter of the mesopores is typically several times bigger than the size of the drug molecule, the crystallization inside the pores is not totally impossible. Even though the drug typically is in its amorphous form, it may appear as small, nano sized crystals as well. The solubility of the nano crystals is much higher than that of the bulk material. Therefore, this form is also advantageous considering drug absorption, and the stability of the product is better than in the case of the amorphous drug. In order to obtain drug loading in a nano crystal form, extremely careful optimization and control of the loading process is required.

MSN in pharmaceutical applications, especially for targeted drug release for instance, are currently under intensive investigation, and probably some applications combining nanotechnology and porous materials will be seen in the future. A wide variety of porous carrier materials, both polymeric and inorganic, is available. The major advantage of the inorganic drug carriers over the polymeric ones is their high stability. Application of siliceous materials in drug delivery has
been intensively studied. Special attention has been paid to silica materials, especially on the ordered mesoporous silicas. Recently, porous silicon (PSi) has been suggested for various biomedical applications including the oral delivery of poorly soluble pharmaceuticals.

1.12 Biocompatibility and Toxicities of Mesoporous Silica

1.12.1 Chemistry
Chemically, mesoporous silica is an oxide of silicon, viz., silicon dioxide, and is generally colorless to white and insoluble in water. When associated with metals or minerals the family of silicates is formed. There are several water soluble forms of silica referred collectively to as silicic acid (ortho, meta, di, and tri-silicates), which are present in surface and well water in the range of 1-100 mg/L. Orthosilicic acid is the form predominantly absorbed by humans and is found in numerous tissues including bone, tendons, aorta, liver and kidney.

Silicon is a non-metallic element with an atomic weight of 28 and belongs to group IV of the periodic Table along with carbon, germanium, tin, and lead. It is tetravalent and the atom is structurally rigid. As expected, the chemistry of silicon is similar to carbon and it can form bonds with many of the same atoms such as silicon-silicon, silicon-oxygen, silicon-nitrogen, and silicon-carbon linkages. Silicon is not found freely in nature but exists as the oxide and as silicates which involves bonding of silicon dioxide with various metals. Silicon plays a potential role in structural organization of biomolecules such as mucopolysaccharides and collagen as a component of cell walls. It is thus likely that silica can exert biologically beneficial effects in vivo.

1.12.2 Role of Silica
Silica is omnipotent in nature making up 26% of the earth's crust by weight and is present in almost all of earth's minerals rocks, sands, and clays. The many forms of silica include quartz, emerald, feldspar, serpentine, mica, talc, clay, asbestos, and glass all of which have different uses. Silica has also been used as an ingredient in steel, in abrasives (silicon carbide), components of transistors (along with boron, gallium, arsenic, etc), solar cells, rectifiers, and other electronic solid-state devices. It has also found use as an ingredient of glass when derived from sand-based silica and in the production of computer chips. Silica is also a constituent of filler for paint and rubber ceramics, in lubricants, concrete and bricks, as well as being used for medical devices such as silicone implants.

Apart from industrial uses, silica has been used in a nutritional context as a food additive, i.e., anti-caking agent in foods, as a means to clarify beverages and control viscosity, as an antifoaming agent, dough modifier, and as an excipient in drugs and vitamins. Silica is used biologically by diatoms as a structural component of cell walls. Clearly, silica is omnipresent in the human environment and has a
diverse multitude of uses. Silica also appears in the food chain with concentrations tending to be much higher in plant-based foods, i.e., phytolithic, than animal foods. Beverages, however, are the major contributor to dietary silica, or silicon, and include water, beer (due to barley, hops, etc.), and coffee. Silica is prevalent in municipal water supplies but is particularly high in bottled spring and artesian waters depending on geological source. In fact, beverages alone contribute to 55% of total dietary intake of silicon as silica. Grains and grain products as part of food contribute around 14% and vegetables contribute 8%. It is noteworthy that refinement of grains removes silicon during the process but silica-derived food additives can replace the stripped silicon and increase the content.

1.12.3 Pharmacokinetics
Supplements and pharmaceuticals, crystalline gels and amorphous silica exhibit very low bioavailability. It would seem possible that solubility could be increased in the GI tract, but this seems to be limited. Cereals provide the greatest amount of silicon in the diet (30%) followed by fruit, beverages (hot, cold, and alcoholic combined), and vegetables. Collectively, these foods provide >75% of the daily silicon intake. When silicon is delivered as orthosilic acid as it exists in liquids, it is readily absorbed to >50% of the total ingested amount. Furthermore, silicic acid in foods is also readily absorbed with an approximate absorption of 40%. In one study, humans absorbed only about 1% of a large single dose of an aluminosilicate compound but over 70% was absorbed from a single dose of methylsilanetriol salicylate, a drug developed for the treatment of circulatory ischemia and osteoporosis. Proof of absorption of the forms of silicon as silica is verified by daily urinary silicon excretion, as magnesium orthosilicate, of >50% of daily silicon intake. In a bioavailability study, 48h after ingestion of silicon-32, 36% of the dose was excreted in the urine and elimination appeared to be complete.

Although absorption can vary a great deal, the delivery of dietary silica to potential tissue and organ target sites is critical for subsequent benefit although the biochemical function for silicon is unknown. Mono-meric silica penetrates all body liquids and tissues at concentrations less than its solubility and is readily excreted primarily in the urine. Findings that as much as 50% of dietary silicon is excreted in urine suggest some forms are well absorbed. Moreover, blood silicon levels are significantly higher in patients with kidney failure. Concentrations in body fluids approximate plasma levels indicating rapid whole body dissemination. It is noteworthy that normal human serum has a narrow range of silicon concentration (~50 ng/dl) similar to the concentrations of other dietary trace elements. Silicon is also widely distributed in tissues from plasma. For example, high levels are present in bone, as well as other tissues and organs including nails, tendons, walls of the aorta, RBC, liver, spleen, lung, and kidney. Moderate amounts are found in bone, skin, muscle, and testes. The fingernails contain the highest amount of silicic acid, which is 1500 mg/kg higher than levels in RBC or
serum (44 mg/kg and 20 mg/kg, respectively). Various connective tissues including the aorta, trachea, bone tendons, and skin contain most of the silicon in the body. Silicon, a surrogate for silica, is found throughout the body at numerous tissue and organ sites.

1.12.4 Silica Biocompatibility
Biocompatibility is the ability of a material to interface with a natural substance without provoking an unnatural response. The human body typically responds to contact with synthetic materials by depositing proteins and cells from body fluids at the surface of the materials. Materials that are tolerated by the human body are referred to as "Bio-inert". Application of mesoporous MSNs as drug delivery systems has to deal with the physiological environment when performing their functions during oral intake. Although silicon is known to be a biodegradable material, the use of silicon-based materials for medical applications is only possible if they are also biocompatible for the intended purpose. In this particular case, several factors need to be considered such as: (i) the different systemic pathway for removal and the toxicity of the degradation products; (ii) the mechanical integrity of the material during its degradation process; and (iii) the local interactions between the material and the surrounding tissues or cells. Furthermore, the degradation products of such biomaterials clearly need to have very low levels of toxicity and be readily removed from the body.

Silicon is essential in biological systems in small amounts as it affects both morphological development and metabolic processes. However, little is known about the biological processes that concern silicon at the molecular level. What is known is that silicon-induced toxicity may occur if a system is exposed to more silicon than is needed physiologically. Many studies have been carried out on the possible toxicity of implanted silicone, but there are few reports about the toxicity of silicon or silicon compositions. For mesoporous silicon materials there are just few biocompatibility tests published either, in particular concerning in-vivo tests. Canham was the first to demonstrate that a thick layer of high porosity silicon was completely dissolved away within a day of in vitro exposure to a simulated body fluid due to possible re-adsorption of silicon. This was later confirmed by an in-vivo study of both bulk and injected mesoporous nanoparticle at the subcutaneous site in a guinea pig model. An important issue for biomedical applications is the toxicity of the dissolved silica. In the human body, mesoporous silica degrades mainly into mono-meric silicic acid (Si(OH)₄), which is the most natural form of the Si in the environment. The average daily dietary intake of silicon in the Western world is about 20–50 mg/day and silica is an essential nutrient, which the human body needs. Human tests have revealed that the silicic acid concentration in the bloodstream rises only very briefly above typical values of about 1 mg/l. Urine excretion of silicic acid is also very efficient and expels all
the ingested silicon. Furthermore, degradation products of mesoporous silicon found in blood of normal healthy individuals were typically 10 μM\textsuperscript{162, 163}.

Another important aspect is the physicochemical properties of the degradation products of silicon in the body, in particular their pKa. The degradation product of porous silicon is orthosilicic acid (pKa = 9.5), which is the bioavailable form of dietary silicon that is readily excreted through the kidneys\textsuperscript{164}. However, when in body fluids (pH 7.4), only a small fraction of the silicic acid is deprotonated. Thus, this would have little effect, for example, on protein delivery, since the pH changes would not be relevant to induce loss of their biological activity. In addition, silicon is possibly important in human physiology by protecting against the toxic effects of aluminum. The in-vitro dissolution studies of MSNs confirm the fact that the silicic acid concentrations remain quite low and can be controlled with the porosity of MSNs\textsuperscript{165}. Beyond the simulated environments and in-vivo tests, MSNs has also been found to support living cultures of mammalian tissues\textsuperscript{166, 167}.

Little acute or chronic data exist on oral toxicity in humans generally due to the lack of any observed toxicity. Limited studies, however, have been conducted in rodents to determine a No Observed Adverse Effects Level (NOAEL). The NOAEL for dietary silica was determined to be 50,000 ppm (mg/L) demonstrating a huge margin of safety. In fact, this is equivalent to 2,500 mg/kg body weight/day for a rodent with the appropriately incorporated safety factors in the experimental design (100 fold). From this, the safe upper level for humans is calculated as 1,750 mg/day for a typical adult male (70 kg). In conclusion, many forms of silica exist in nature. Inhalation of crystalline silica is toxic, but consumption of water solubelc silica as orthosilicic acid is not toxic even at very high levels.

In general, foreign materials invade a living system through three pathways, namely the respiratory tract, the gastrointestinal tract, or by skin contact. The toxicity of airborne ultrafine particles and certain engineered nano-materials have been well documented\textsuperscript{168-174}, with colloidal silica generally being accepted as a nontoxic material or having low-toxicity\textsuperscript{172, 175, 176}. When living systems come into direct contact with amorphous silica nano-materials, a number of negative results may occur including chronic pulmonary changes during inflammation, generation of reactive oxygen species, and damage to intracellular DNA, RNA and proteins\textsuperscript{176-184}. However, when mesoporous silica employed as a drug delivery system, toxicity of this material is a critical concern for their applications in biological samples. As already discussed, the silica is generally treated as non toxic and is considered to be biocompatible and degradable in living tissues. It has been found that there are some parameters that govern the behavior...
of these materials when tested in vitro, such as concentration, particle size, shape, surface area, surface modification etc. (Fig. 1.25)

Figure 1.25: Factors governing cellular toxicity of MSNs

The size of the nano-material greatly influences its toxicity; particularly as the decrease in size of nano-material changes certain parameters. Many studies have shown that variations in the size of nano-materials account for the different toxicity levels between nano-sized and micrometer-sized materials. It is known that a reduction in size can increase the rate of uptake and translocation of silica nano-materials in vitro and in vivo, thereby inducing a more severe and transient toxicity. However, independently of the factors influencing the toxicity and biocompatibility commented on above, the administration route to the living body has been found as the governing factor in the toxicity of these bioceramics. It was found that when proceeding to subcutaneous injections of diverse mesoporous silicas, such as MCM-41, MCM-48 and MCF, at the static nerve in rats, and attending to histology, a good biocompatibility was observed at all time points. However, intra-peritoneal and intravenous injections in mice resulted in death or euthanasia.

Currently available information suggests that the shape of silica nano-materials can affect their toxicity in two ways. First, the shape has an effect on the rate of its cellular uptake; and second, it can affect the extent of nano-material aggregation, altering its cytotoxic properties. A recent in vitro toxicity study showed spherical nano-materials to be more toxic than rods. It was also shown to be more difficult for elliptical nano-materials to penetrate the skin layer than spherical nano-materials. The toxicity of surface-modified silica nano-materials
is largely determined by their surface functional groups. As an example, Kreuter reported that an apolipo protein coating on silica MSNs aided their endocytosis in brain capillaries through the LDL receptor\textsuperscript{197-200}.

Overall, silica nano-materials are low-toxicity materials, although their toxicity can be altered by surface modifications. Dose-dependent toxicity has frequently been observed in the study of nano-materials\textsuperscript{176-183}, with increasing doses of silica nano-materials invariably worsening their toxicity. Both, cell proliferation and viability were greatly hampered at higher doses observed in \textit{in-vitro} studies\textsuperscript{178, 180, 183}.

The toxicity is not only based on the amount or size of silica MSNs, but also on the type of cell line\textsuperscript{186}. Cancer cell lines (A 549, MKN-28) had a higher viability and resistance to silica MSNs than did normal cell lines\textsuperscript{178} (MRC-5, WS1 and CCD-966sk). Similarly, a previous study showed that A549 cells were more resistant to the treatment of silica MSNs than were macrophages\textsuperscript{179}. The dopants, dye molecules, in general, have little effect on the toxicity of MSNs because they are isolated from the environment. However, if the dopants are photo sensitizers or some other specialized molecules, they can have an effect on the toxicity of the MSNs. For example, when a hydrophobic photo sensitizer, PS HPPH [2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide], was doped inside organically modified silica MSNs\textsuperscript{200}, and the MSNs were delivered into cells (under irradiation), ROS were produced by the doped photo sensitizers and the cells were killed.

1.12.5 Health Implications
There a number of studies with compelling results suggesting the essentiality of silicon, delivered as silica, for humans. A functional role for silicon has yet to be identified but clearly it is feasible and likely. Silicon is known to be required by chicks and rats for growth and skeletal development. For example, inducing silicon deficiency produces profound results including deformities in skull and peripheral bones, poorly formed joints, reduced mineral contents of cartilage, collagen, and disruption of mineral balance in the femur and vertebrae. The obvious effect of silicon deficiency on bone supports the notion that it is critical for bone formation as in studies with chickens and rats\textsuperscript{201-204}. Chicks fed silicon-deficient diets also showed structural abnormalities in the skull and long bones such as the femur\textsuperscript{157}. More in depth studies using rats deprived of silicon showed decreased bone hydroxyproline levels and inhibited alkaline and acid phosphatases\textsuperscript{205}. Regarding the former, silicon has been shown to contribute to prolylhydrolase activity necessary for normal collagen formation\textsuperscript{157}. Silica enhances and maintains articular cartilage and connective tissue due to interaction of silicon with glycosaminoglycan formation, a structural building block of these tissues. Silicon is also a constituent of enzyme(s) involved in bone matrix formation suggesting a role in bone calcification. Clearly, silicon is localized in
sites of active bone growth supporting a role for dietary silica. Silicon has also been suggested to exert a protective role in atherosclerosis, in part, due to maintenance of blood vessels. For example, increased silica consumption reduces the incidence and severity of atherosclerosis presumably through its effects on blood vessel-associated glycosaminoglycan and collagen integrity and function. Silica is also thought to be beneficial in Alzheimer's disease because silicon can interact with aluminum and prevent aluminum toxicity often associated with Alzheimer's disease. This protective effect has also been noted in humans where dietary silicon protected against aluminum accumulation and presumably neurodegenerative effects. Collectively, evidence supports a protective role for silicon in maintaining bone health, cartilage and connective tissue structure, prevention of toxicity to the brain, and maintenance of blood vessel integrity.
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References:

7. Shinde A. Solubilization of poorly water soluble drugs. Pharmainfo.net 2007; 8: 1-5.
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41 Beck JS, Chu CT, Johnson ID, Kresge CT, Leonowicz ME, Roth WJ, Vartuli JC, US
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</thead>
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92 Jacobs PA, Martier WY, Zeolites 1982; 2: 226.
Introduction


Thomas MJK, Slipper I, Walunj A, Jain A, Favretto ME, Kallinteri P, Douroumis D.


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177 Chang JS, Chang KLB, Hwang DF, Kong ZL. In vitro cytotoxicity of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. Environ Sci. Technol 2007; 41: 2064–2068.


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