Ordered Mesoporous Silica for Drug Delivery in Topical Applications

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Tutor: Prof. Barbara Onida
## SOMMARIO

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acronyms</td>
<td>6</td>
</tr>
<tr>
<td>Introduction</td>
<td>7</td>
</tr>
<tr>
<td>Ordered mesoporous silica as drug delivery system: state of the art</td>
<td>8</td>
</tr>
<tr>
<td>Abstract</td>
<td>8</td>
</tr>
<tr>
<td>Drug Delivery Systems</td>
<td>8</td>
</tr>
<tr>
<td>Drug release profiles</td>
<td>10</td>
</tr>
<tr>
<td>Drug Carriers</td>
<td>12</td>
</tr>
<tr>
<td>Ordered mesoporous silica</td>
<td>13</td>
</tr>
<tr>
<td>OMS for drug delivery</td>
<td>17</td>
</tr>
<tr>
<td>Drug loading strategies</td>
<td>18</td>
</tr>
<tr>
<td>Supercritical CO$_2$</td>
<td>22</td>
</tr>
<tr>
<td>Topical Application</td>
<td>25</td>
</tr>
<tr>
<td>A new controlled release technology</td>
<td>28</td>
</tr>
<tr>
<td>Dermatological Disease</td>
<td>29</td>
</tr>
<tr>
<td>Aim of the thesis</td>
<td>32</td>
</tr>
<tr>
<td>Ordered mesoporous silica as drug delivery system: Experimental</td>
<td>34</td>
</tr>
<tr>
<td>Materials</td>
<td>34</td>
</tr>
<tr>
<td>Ordered Mesoporous Silica</td>
<td>34</td>
</tr>
<tr>
<td>Active Pharmaceutical Ingredient</td>
<td>36</td>
</tr>
<tr>
<td>Chemicals</td>
<td>37</td>
</tr>
<tr>
<td>Characterization Methods</td>
<td>37</td>
</tr>
<tr>
<td>X-ray diffraction</td>
<td>37</td>
</tr>
<tr>
<td>Fourier Transform Infrared spectroscopy</td>
<td>37</td>
</tr>
<tr>
<td>Nitrogen adsorption</td>
<td>38</td>
</tr>
<tr>
<td>Termogravimetry</td>
<td>38</td>
</tr>
<tr>
<td>Differential scanning calorimetry</td>
<td>39</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>39</td>
</tr>
<tr>
<td>Dynamic Light Scattering and Zeta Potential</td>
<td>39</td>
</tr>
<tr>
<td>Nuclear magnetic resonance</td>
<td>40</td>
</tr>
<tr>
<td>Drug Incorporation methods</td>
<td>40</td>
</tr>
</tbody>
</table>
3.1 OMS characteristics .................................................................................. 87
3.2 Characterization of Incorporated OMS ...................................................... 89
Chapter 4: AKS-OMS Gel ................................................................................. 98
Abstract ........................................................................................................ 98
4.1 Sustained Release From OMS-AKS in AKS Saturated Solutions ............ 99
4.2 Composition, Rheological and pH Stability of AKS-OMS Gel ............... 100
4.3 In-vitro release studies ........................................................................... 101
4.4 In-vitro permeation studies .................................................................. 102
4.5 Topological information on OMS permeation ..................................... 104
CHAPTER 5: CTZ-OMS Gel ........................................................................... 106
Abstract ........................................................................................................ 106
5.1 Sustained release of OMS-CTZ in CTZ saturated solutions ................ 107
5.2 Composition and In-Vitro release test .................................................. 108
Conclusions .................................................................................................. 110
Bibliography .................................................................................................. 111
Appendix I – List of Publications and Congress ........................................... 118
Publications ................................................................................................. 118
Congress ...................................................................................................... 118
Appendix II – Published Articles .................................................................. 119
### ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIMD</td>
<td>Ab-Initio Molecular Dynamics</td>
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<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
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<tr>
<td>AKS</td>
<td>Amikacin Sulfate</td>
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<td>BET</td>
<td>Brauner-Emmet-Teller</td>
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<td>CTZ</td>
<td>Clostrimazole</td>
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<td>CRT</td>
<td>Controlled Release Technology</td>
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<td>DFT</td>
<td>Density Functional Theory</td>
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<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
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<td>DLS</td>
<td>Dynamic Light Scattering</td>
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<td>DD</td>
<td>Drug Delivery</td>
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<td>DDS</td>
<td>Drug Delivery System</td>
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<td>EtOH</td>
<td>Ethanol</td>
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<td>FESEM</td>
<td>Field Emission Scanning Electron Microscopy</td>
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<td>FTIR</td>
<td>Fourier Transform Infrared</td>
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<tr>
<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
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<tr>
<td>IWI</td>
<td>Incipient wetness impregnation</td>
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<td>KIT</td>
<td>Korea Advanced Institute of Science and Technology</td>
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<tr>
<td>MTC</td>
<td>Maximum Toxic Concentration</td>
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<td>MSU</td>
<td>Michigan State University</td>
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<td>MEC</td>
<td>Minimal Effective Concentration</td>
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<td>MCM</td>
<td>Mobil Crystalline of Materials</td>
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<tr>
<td>NLDFT</td>
<td>Non Linear Density Functional Theory</td>
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<td>OMS</td>
<td>Ordered Mesoporous Silica</td>
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<tr>
<td>PSD</td>
<td>Pore Size Distribution</td>
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<td>RMSD</td>
<td>Root Mean Square Deviation</td>
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<td>SBA</td>
<td>Santa Barbara Institute</td>
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<td>SiOH</td>
<td>Silanol</td>
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<td>SSNMR</td>
<td>Solid State Nuclear Magnetic Resonance</td>
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<td>SSA</td>
<td>Specific Surface Area</td>
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<td>scCO2</td>
<td>Supercritical CO2</td>
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<td>TG</td>
<td>Termogravimetric</td>
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<td>TEOS</td>
<td>Tetraethoxysilane</td>
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<tr>
<td>Uv-Vis</td>
<td>Ultraviolet Visible</td>
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<td>XRD</td>
<td>X-Ray Diffraction</td>
</tr>
</tbody>
</table>
INTRODUCTION

This PhD thesis focuses the development and characterization of all physicochemical aspects of a new controlled release technology for two active pharmaceutical ingredient principle (Clotrimazole and Amikacin Sulfate) using ordered mesoporous silica until the introduction onto the market.

The first chapter comprehends the characterization of different OMS synthesized and commercially available; the study of different incorporation techniques based on hydrophilicity/hydrophobicity of API; the characterization of the new impregnated OMS.

Chapter 2 is oriented on the interaction details of API on silica surfaces. A closer look is given to the big questions of OMS-drug phenomena: mobility, solubility, bioavailability, etc.

Chapter 3 highlight the differences between OMS and the spatial assembly of drug inside the mesoporous channels.

Chapter 4 describes the development of the new CRT for AKS describing all the main aspect of the innovative semisolid formulation. In-vitro and ex-vivo release test has been produced and characterized, revealing the functionality of the OMS reservoir effect.

In chapter 5 the same DDS have been developed for CTZ. Both the DDS have been compared with commercially available creams.
ABSTRACT

Drug delivery (DD) is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. For this purpose, several drug delivery systems (DDSs) have been formulated. These include liposomes, proliposomes, microspheres, gels, prodrugs, cyclodextrins, among others. Similar developments with other compounds have produced a plethora of new devices, concepts and techniques that together have been termed controlled-release technology (CRT). Amorphous silica plays a key role due to its favourable characteristics, such as high specific surface, biocompatibility, etc. In this chapter, starting from an overview on DDSs, the discussion will be focused on ordered mesoporous silica (OMS). Topics of this part will be the applications, objects and fields of use of OMS. Numerous questions will be open and answered regarding the main issues of OMS literature: firstly, the amorphization of the Active Pharmaceutical Ingredient (API); secondly, the different types of incorporations; thirdly, the requirement of DDS for low soluble API and, lastly, the description of a new CRT.

DRUG DELIVERY SYSTEMS

A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body. It improves efficacy and safety by controlling the rate, time and place of release. This process includes the transport of a pharmaceutical compound in the body with the aim of achieving safely a desired therapeutic effect at the area where it is needed.\footnote{1} Any DDS comprises a drug formulation, a medical device or dosage form (to carry the drug inside the body) and a mechanism for the release. These systems have several criteria, ranging from ease of delivery to effectiveness of the drugs. Conventional drug delivery (DD) involves the preparation of the drug into a suitable form, such as a compressed tablet for oral
administration or a solution for intravenous administration. These dosage forms have been found to have serious limitations in terms of higher dosage requirement, lower effectiveness, toxicity and adverse side effects.\textsuperscript{[2]} This is the case of many medications, which have unacceptable side effects due to the drug interacting with parts of the body that are not the target. Side effects limit the ability to design optimal medications for many diseases, such as cancer, neurodegenerative and infectious diseases.\textsuperscript{[3]} It is also important to consider the way in which a drug is metabolized by the body. For instance, some Active Pharmaceutical Ingredient (API) are destroyed in the intestinal tract, therefore they cannot be introduced orally to the body. Others may be dangerous in large amounts, which means that for patient safety it should be used a time-release method to deliver the drug.\textsuperscript{[2]} In addition, drug dosage must be carefully calculated so that the body can use the drug. This requires a DDS which allows for precise dosing.\textsuperscript{[2]} Hence, it is necessary to develop suitable dosage forms or controlled DDS to allow the effective, safe and reliable application of the pharmaceutical compound to the patient.\textsuperscript{[4]} Such systems are being developed to overcome the limitations of conventional dosage forms and offer many advantages, which include:\textsuperscript{[5]}

- Improved efficiency by preventing peak-valley fluctuations
- Increased convenience
- Decreased toxicity
- Decreased side effects
- Decreased dosage frequency
- Shorter hospitalizations
- Lower healthcare costs (both short and long term)
- Viable treatments for previously incurable diseases
- Potential for prophylactic applications
- Site specific delivery
- Better patient compliance
Several studies have shown that the benefits aforementioned can be achieved by correct timing of drug administration and controlled kinetics of drug release. Thus, controlled drug release regulates the rate, the location and aims at optimizing drug efficiency while simultaneously reducing adverse collateral effects.\textsuperscript{[2,5–9]}

**DRUG RELEASE PROFILES**

The DDS employed plays a vital role in controlling the pharmacokinetic effect of the drug. An optimal DDS ensures that the active drug is available at the site of action for the correct time and duration. The drug concentration at the appropriate site should be above a minimal effective concentration (MEC) and below a minimal toxic concentration (MTC). This concentration interval is known as the therapeutic range (Figure I.1). Dosage forms can be differentiated according to the way the drug is released.

The immediate release is probably the most used. The drug is released immediately after administration. These forms usually release the drug in a single action following a first order kinetics profile. In other words, the drug is released initially very quickly and then passes through the mucosal membrane into the body, reaching the highest plasma level in a comparatively short time. Once taken into the body, the drug is distributed throughout the body and elimination by metabolism and excretion occurs. This elimination process also follows a first order kinetics.

The modified release of API can occur in three different ways: delayed, extended and pulsed release. In the first case, the API release takes place sometime later the initial administration, after which the release is immediate. The second one is when the drug release occurs for a prolonged period after ingestion. This allows a reduction in dosing frequency compared to a drug presented as a conventional dosage form.
ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS

Figure I.1: Example of API concentration with zero order release (a) and pulsed administrations (b).

For immediate release dosage forms the time interval in which the plasma concentration reach the drug therapeutic range can be quite short. Therefore, frequent dosing or pulsed release, with its associated compliance problems, is required. As a consequence, there is a considerable fluctuation in drug concentration level, which often is out of the therapeutic range. This is especially an issue in chronic diseases when patients need to take the medication for prolonged periods (Figure I.1.b).

Extended release can be achieved using a sustained or a controlled delivery dosage forms. Sustained release systems maintain the rate of drug release over a sustained period (Figure I.1.a). They achieve this mostly by the use of suitable polymers. On the other hand, controlled release systems also offer a sustained release profile but they are designed to lead to predictably constant plasma concentrations. This means that they are actually controlling the drug concentration in the
body, not just the release of the drug from the dosage form, as is the case in a sustained release system.

**DRUG CARRIERS**

Every pharmaceutical compound contains an API, which has a direct effect in the diagnosis, treatment or prevention of diseases. It is important to realise that the API is just one part of the medicine and it cannot be administered to the patient on its own. Therefore, it is necessary to formulate the drug into a dosage system containing drug carriers, which are forms that serve as mechanisms to improve the delivery and the effectiveness of drugs. They can be attached to drug molecules for targeted delivery, increased efficiency or controlled release. Many of these can also act as buffers to reduce the toxic effects of medications. These compounds can also change the way the drug acts in the body.\[12,13\] Drug carriers are used in controlled release technology (CRT) to prolong in-vivo actions, decrease drug metabolism, reduce drug toxicity and determine where the drug travels and how it behaves when it gets there.\[12\] These include synthetic and natural compounds from a variety of sources, ranging from lipids to nanoparticles. Some of the more common drug carrier systems are reported in Figure I.2.
Figure 1.2: Liposomes (I): containers made of a lipid bilayer; their limitation is the extremely high fragility of the liposomal structure and the low transport capacity of non-soluble drugs. Microspheres (II-III): synthetic materials, such as ceramics or polymers, or from natural materials, such as albumin. Dendrimers (IV): repetitively branched molecules; their properties are dominated by the functional groups on the molecular surface. Soluble polymers (V): hollow particles that hold drugs; drugs are loaded into the core hydrophobic block (yellow); the crosslinking block (green) provides stability to the micelle by forming pH reversible bonds that allow for triggered drug release; the grey block gives the micelle aqueous solubility and stealth. Conjugated proteins (VI).[14]

ORDERED MESOPOROUS SILICA

Among all the CRT, ordered mesoporous silica (OMS) are a particular case of the previous system. Silicon dioxide (SiO$_2$) is one of the most abundant oxide materials in the Earth’s crust. SiO$_2$ is the essential components of all silicate materials: crystalline and amorphous. All silicates are constituted by the SiO$_4$ tetrahedron (Figure 1.3.I). The low energy process of change the siloxane bridge (Si-O-Si) allow the formation of infinite polymorphisms.[15]

ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS
Figure I.3: The SiO$_4^-$ tetrahedron (I); O-Si-O angle: 109°; Si-O-Si angle: 130°-180°. Phase diagrams of silica (II); most of this crystalline phase are not reversible.

OMS are amorphous inorganic materials synthesized in the presence of surfactants as templates for the polycondensation of silicic species. Synthesis conditions such as source of silica, type of surfactant, ionic strength, pH and composition of the reaction mixture, temperature and duration of synthesis determine the characteristics of the porous structure and the macroscopic morphology.[16,17]

A wide variety of ionic and non-ionic surfactants has been used for obtaining materials with different porous and morphological characteristics. The pore sizes of these materials are always very homogeneous ranging from 2 to 100 nm. The huge surface area which can easily reach values of 1000 m$^2$·g$^{-1}$ and the wide void volume (1 cm$^3$·g$^{-1}$) delineate the ability of these materials as CRT. In addition, their thermal, chemical, mechanical and pH stability defines its superiority to the organic counterpart.[18–21]

Table I.1: Brief summary of the main silicas used.
In 1992 a new family of OMS was reported as MCM-X (Mobil Crystalline of Materials). They are synthesized by using alkyl ammonium surfactants (i.e. Hexadecyltrimethylammonium bromide (C16TABr)) and tetraethyl orthosilicate (TEOS) or sodium silicate in basic condition. Their pore size and wall thickness are between 4 to 2 nm. By varying the synthesis conditions, different structures of the mesophase can be obtained. For instance: hexagonal phase (MCM-41), cubic phase (MCM-48) and lamellar phase (MCM-50).[22]

The first OMS synthesized with non-ionic triblock polymers were reported by the Santa Barbara centre (Santa Barbara Amorphous).[23] As for MCM-41, selecting the right surfactant and concentration different structure can be obtained. For instance, Pluronic P-123, \( \text{HO(CH}_2\text{CH}_2\text{O})_{20}(\text{CH}_2\text{CH(CH}_3\text{O})_7(\text{CH}_2\text{CH}_2\text{O})_{20}\text{H} \), is used mainly for the silica hexagonal structure (p6mmm); while, Pluronic F-127 for the cubic ia3d structure. The main characteristics of surfactants are:

- Chain hydrophilicity-hydrophobicity
- Head characteristic:
  - Anionic

<table>
<thead>
<tr>
<th>Name</th>
<th>MCM-41</th>
<th>MSU-H</th>
<th>SBA-15</th>
<th>KIT-6</th>
<th>Syloid</th>
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<tr>
<td>Surfactant</td>
<td>C(_{16})TABr</td>
<td>P123</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>&gt;11</td>
<td>≈7</td>
<td>&lt;2</td>
<td>Disordered</td>
<td></td>
</tr>
<tr>
<td>Structure</td>
<td>P6mmm</td>
<td>Ia3d</td>
<td></td>
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</table>

ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS
- Cationic
- Amphoteric
- Non-ionic

- Chain elongation
- Chain/Head hindrance volume ratio

These features have to be correlated with other synthesis conditions:

- Temperature
- pH
- Concentrations
- Co-solvents
- Co-surfactant
- Silica source

Other families of OMS are Michigan State University (MSU), Korea Advanced Institute of Science and Technology (KIT).

After the polycondensation, an hydrothermal treatment can be executed with the aim of enlarge and modify the pore structure and connectivity.\textsuperscript{[21,24]} Subsequently, there is the removal of surfactant through filtration or extraction and calcination.

Surface silanols (SiOH) represent the last characteristic besides highly organized porosity, SSA, pore volume, chemical stability, etc. Their content depends on the way the surfactant is removed. It can be modulated by post synthesis treatments. They are generated as stabilization of the silica surface. The presence of SiOH on the surface promotes the adsorption of molecules (from water to proteins) through hydrogen bonding (H-bond).\textsuperscript{[25]}
**Figure 1.4:** The surface isolated SiOH (I), H-bonded (II) and geminal (III).

Furthermore, the presence of geminal and single SiOH allows the chemical modification grafting different functionalities through covalent linkages. For instance, they can react with alkylchlorosilanes in order to obtain an hydrophobic surface.\[^{26}\]

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**OMS FOR DRUG DELIVERY**

Due to these outstanding features, OMS are excellent candidates in biomedical systems as local drug delivery systems and bone tissue regeneration.\[^{13,27–32}\] For controlled release applications, it has been shown that silica is able to store and gradually release therapeutically relevant drugs. Furthermore, silica is used to enhance the biocompatibility of several DDS, such as magnetic nanoparticles, biopolymers and micelles.\[^{27}\] Vallet-Regi et al. were the first to explore the drug release properties of OMS using ibuprofen. To incorporate API they dissolved it in hexane, obtaining an incorporated quantity of 30% by mass.\[^{33}\] They also have demonstrated the release of erythromycin and alendronate.\[^{34,35}\] Other controlled release systems have shown the delivery of API such as vancomycin and adenosine triphosphate, fluorescein, β-oestradiol, cholestane and calceine, camptothecin.\[^{31,36–39}\]

OMS also show ability in the dissolution of poorly water-soluble drugs. An insufficient dissolution of hydrophobic drugs in the gastrointestinal fluids strongly limits the oral bioavailability. Mellaerts et al. loaded itraconazole on SBA-15, an antimycoticum known for its poor aqueous solubility. Gastrointestinal dissolution tests produced a supersaturated solution giving rise to enhanced trans epithelial intestinal transport.\[^{40}\] Ambrogi et al. using carbamazepine into MCM-41 have verified a remarkable increase of dissolution rates.\[^{41}\] This have evidenced that OMS is a
promising carrier to achieve enhanced oral bioavailability for drugs with extremely low water solubility.

At the state of the art, the explanation of these phenomena should be a consequence of drug amorphization inside OMS. Numerous works have demonstrated drug amorphization inside OMS just by mechanical mixing, without any other energy supplement. These could demonstrate that the most stable condition of drug inside silica mesopores is an amorphous state. Indeed, NMR studies have evidenced high mobility of drug inside OMS at ambient temperature and pointed out weak interactions.\[^{42,43}\]

These arguments will be discussed in chapter 2.

**DRUG LOADING STRATEGIES**

Drug loading into a host material can be performed with different techniques.\[^{44}\]

The solution method is probably the most widely used drug loading process. In this method, the drug is dissolved in a suitable solvent and the porous material is dipped in this solution. The drug molecules are absorbed on the pore walls. The solution method last from one to several hours, after which OMS is separated from the solution by filtration or centrifugation. In conclusion, the particles are dried by removing the remaining solvent.\[^{45}\] If the drug concentration near to the adsorbent surface exceeds the saturation concentration, e.g. during the drying step, the drug may start to crystallize on the external adsorbent surface. Since this crystalline surface fraction may have different dissolution properties than the amorphous solids inside the pores, its formation is not favourable. If crystalline solids are formed on the surface, they can be removed by washing the loaded particles. Unfortunately, the washing process is difficult to control.\[^{46}\] Solution loading is simple to perform and it gives reproducible results as the properties of the carrier are consistent.
High temperature is not required during the process, making it suitable for loading sensitive molecules. To achieve high loading degrees, relatively high concentrations of loading solutions have to be used. This can be challenging, especially with poor soluble drugs. To overcome this inconvenience, different solvents can be used. Among all the possibilities, apolar solvent are the most appropriate. Indeed, it has been demonstrated that water, ethanol and other polar solvents compete in the adsorption with the drug. In fact, due to the high interaction energy and strong H-bonds formed with surface silanols, these molecules are strayed from silica surface only during degas operations. On the other hand, most apolar solvent are carcinogen or toxic.[8] The main disadvantage of the immersion method is the large proportion of the drug that is wasted in the filtration/centrifugation process. It is also difficult to predict the drug loading degree that will be achieved.

The Incipient wetness impregnation (IWI) method consists in contacting the OMS with a volume of solvent equal to the silica pore volume. The capillary action draws the solution into the pores and the drug dissolved in it. The support can be dried to drive off the volatile components within the solution, depositing the drug on its surface. While drying, the drug located in the solution outside the pores is driven inside by diffusion, which is a much slower process. There are different methodologies distinguished by the volume of solvent used:[47]

- Impregnation by soaking or with an excess of solution: excess liquid is eliminated by evaporation or by draining;
- Dry or pore volume impregnation: the amounts of drug are introduced in the volume corresponding to the pore volume of the support;
- IWI: it is a procedure similar to dry impregnation, but the volume of the solution is more empirically determined to correspond to that beyond which the support begins to look wet.
The main advantage of this method is that it is easy to control the amount of the drug that is loaded in the carrier. Furthermore, the drug is efficiently loaded and the method is, therefore, suitable for expensive molecules.

**Figure 1.5**: Comparison between different incorporation process and drug adsorption.[48]

Covalent grafting method is widely used for payload molecules, which can be loaded by attaching them covalently on the mesoporous material surfaces. A commonly used method is to form a peptide bond between the amine group of a payload molecule and a carboxylic acid linker on the surface of the material. The benefit of a chemical grafting is the possibility to control the release of the payload molecules. The release can be determined by cleavage of the payload molecule from the linker or erosion of the porous material. In chemical grafting, the drug load is controlled by the surface area and the density of the linker and the payload molecules. The maximum loading degree that can be achieved is inevitably lower than this that can be achieved with non-covalent adsorption methods, because the payload molecules cannot accommodate the whole pore volume. With this method, there is a risk that the payload molecule will not be released from the linker. Nevertheless, this method provides stronger chemical interactions between the drug molecule and the surface.[16]

It is important to remember that all the methods aforementioned have an inherent disadvantage: the employment of organic solvents. The
subsequent solvent removal steps add processing complexity and increased cost to these processes. In addition, there are safety issues to be considered and appropriate environmental protection measures must be initiated when using organic solvents. To avoid this, other techniques can also be employed to load drugs onto OMS.

In the hot melt method drug molecules are loaded from a molten phase. The drug is heated along with the adsorbent to a temperature above the melting point of the drug. A prerequisite for using this method is that both the adsorbent and the drug have sufficient thermal stability, which excludes all of the pharmaceuticals that are known to decompose upon melting. Some drugs as ibuprofen has been successfully loaded by using this method. However, this method is not generally appropriate because it requires the molecules to withstand a temperature above their melting point and the viscosity must be low enough to allow the molecule to efficiently enter the pore structure.

Vapour deposition is another technique. In high vacuum condition, the melting temperature of the drug is reduced. Consequently, it is possible to avoid degradation and evaporates small amount of drug that will be deposited in a cold region (OMS). This method allows to control the purity of the drug avoiding any external parameter: solvent, viscosity, solubility, etc.

The physical mixing, for instance, requires only that drug and carriers are blended in desired proportions using spatula for some minutes. A more scalable process of the physical mixing is the ball milling. Indeed, during the process the particles are physically broken to favour the drug entrapment and reconstituted due to the strong interaction energy of silica surfaces.
Organic solvents can be replaced with supercritical carbon dioxide. Drugs may also be incorporated by dissolving them in compressed high volatile fluids like supercritical carbon dioxide (scCO₂), at temperatures and pressures above their critical point. A supercritical fluid can be defined as any substance present at a temperature and pressure higher than its critical value, and which has a density close to or higher than its critical density. At the point at which the critical temperature and critical pressure are reached, the density of both the liquid and vapour are equal and the supercritical phase is obtained.

The employment of scCO₂ for drug loading offers many advantages compared to traditional organic solvents. This topic will be discussed in details in chapter 1.

Supercritical fluids have both liquid and gas-like properties. Their liquid-like nature enables them to act like a solvent, while their gaseous properties allow quick and easy diffusion through materials. When the supercritical state is reached, properties like density, viscosity and the vapour–liquid equilibrium ratio become dependent on temperature at a certain pressure, which permits the solubility of solutes in the supercritical fluid to be controlled.
Figure I.6: Phase diagram of a CO₂ showing the supercritical region and critical point.[50]

At pressures and temperatures not too far from its critical point, a supercritical fluid has a high compressibility. Therefore, its density and its solvent power are easily adjustable over a wide range with a minimal change in temperature or pressure. This tunability may be used to control the solubility parameter.[50]

The solubility parameter is a coefficient that indicates a substance solubilisation in a specific solvent. Materials with similar values are likely to be miscible, hence a solute presents a complete solubility or miscibility if it has a solubility parameter as equal as possible to that of the solvent.
In a supercritical fluid, the solubility parameter tends to increase with the fluid density (at constant temperature). Another advantage includes the reduced processing complexity, as there is no need for solvent removal steps associated with organic solvent usage. Carbon dioxide is the most widely used supercritical fluid because it presents the advantage of having easily accessible critical conditions, that are a critical temperature close to ambient temperature (304.25 K) and a critical pressure which is not too high (7.39 MPa), it is inert, non-flammable and inexpensive. The critical temperature is close to a physiological value and so it is safe for heat-sensitive proteins.

One disadvantage of using supercritical fluids, however, is that specialised high-pressure equipment and knowledge are required for...
processing it. Furthermore, some interesting hydrophobic drugs, which cannot be impregnated by aqueous solution-suspension soaking, can be incorporated by this method.

ScCO$_2$ is used for many applications like:

- Extraction of desired compound from other products; i.e. caffeine from coffee bean;$^{[54]}$
- Chromatography;
- Cleaning;
- Biological applications:$^{[55,56]}$
  - Sterilization
  - Virus inactivation
- Particle formation
  - Aerogels$^{[57]}$
- Polymeric processing and foams;$^{[58]}$
- Impregnation
  - Drug loading on different matrixes$^{[59]}$

In this thesis, it will be used as an incorporation process for drug inside OMS. This topic will be further discussed in chapter 1.

**TOPICAL APPLICATION**

Among all the administration routes, topical and transdermal delivery approaches have unique advantages:

- In case of skin diseases, topical delivery directly carries drugs to the site;
- Smaller amounts of drugs are needed to produce a therapeutic effect;
- Plasma level peaking of drugs will be avoided;
- Increased bioavailability due to elimination of hepatic first-pass metabolism;
- Greatly enhanced patient compliance by eliminating frequent treating.

For these reasons and many others, currently, there are numerous topical and transdermal products on the market. Many formulations of low molecular weight drugs and macromolecules have been developed and some are currently under clinical trial.\textsuperscript{[60]}

At the same time a huge number of CRT have been developed for topical diseases, such as gels with permeation enhancers, submicron emulsion vehicle systems, volatile vehicle–antinucleant polymer systems, lecithin microemulsion gel, oleo-hydrogel systems, deoxycholate hydrogels, creams containing lipid nanoparticles, solid lipid nanoparticles, liposomes as drug carriers, etc.\textsuperscript{[61]}

Human skin has a surface area between 1.5 and 2.0 m\textsuperscript{2} for adults. The skin thickness varies over the body with the thinnest part of eyelids being less than 0.1 mm thick and the thickest on the palms, soles and upper back more than 5.0 mm. Not only is the skin a protective barrier against toxic substances, pathogens, and organisms, but it is also involved in many important physiological functions such as fluid homeostasis, thermoregulation, immune surveillance and sensory detection.\textsuperscript{[62]} These functions are related to the skin’s complex multiple layers with each layer associated with highly specified cells and structures.
Figure 1.8: A schematic image of epidermis, dermis and hypodermis structure. The appendages such as hair shaft and hair follicle, sweat gland, sebaceous gland, and arrector pili muscle are illustrated.[63]

The permeation barrier properties of human skin are mostly attributed to the top layer of the epidermis, the stratum corneum. The barrier function is related to the unique structure in the stratum corneum layer that is composed of "bricks (corneocytes) and mortar (intercellular lamellar lipid bilayers)."[64]

Approaches that deliver drugs/active compounds through the skin barrier are referred to the topical administration (as opposed to the enteral and parenteral route). Passive and active skin penetration enhancement methods have been successfully used to improve the efficiency of either the topical delivery (the drugs/active compounds are delivered into skin strata), or transdermal delivery (drugs/active compounds are delivered into subcutaneous tissues and are taken up systemically into the body).
Topically applied therapies are promising for the treatment of skin diseases such as psoriasis, contact dermatitis and skin cancers, since the drugs are delivered directly into skin strata.

**A NEW CONTROLLED RELEASE TECHNOLOGY**

Despite of all the above descripted benefits of topical administration, there are some critical drawbacks, among which difficult accurate dose and the need of frequent reapplications. These frequent reapplications are required because the post-application efficacy of traditional creams is limited to a period between 3 to 6 hours. In addition, recurrent treatments result in considerable inconvenience for the patient and inopportune amnesia. Moreover, many dermatologic pathologies, grouped under the generic name of chronic dermatitis, have a cyclic and recurring feature, creating complex treatment problems over the course of the patient’s life. What is more, pulsed administration has period of inefficacy and overdose, due to low/high API concentration on the skin site (Figure I.1).

In order to tackle these issues, zero order release at constant skin concentration for an extended time interval is required but is utopian. Sustained controlled release systems aims to simulate as good as possible the zero order release. A new CRT comprising OMS, incorporated with different API, blended in a saturated solution of the same API is here proposed. Indeed, this semisolid formulation let to a sustained controlled release. After the application on the skin site, the dissolved API in the saturated solution explicate its function as all the commercial creams. Subsequently, the concentration of the dissolved API begins to decrease leading to the end of the cream purpose. Simultaneously, the API incorporated in OMS begun to dissolve. The API release preserves the saturated concentration inside the vehicle until the OMS is empty. As follows, the therapeutic concentration of API is kept
constant on the application site for a long and controlled time. The OMS incorporated with API develop a reservoir effect.

**Figure I.9:** A schematic image of the patented application.

This new CRT is patented at the WIPO (the global forum for intellectual property services, policy, information and cooperation) with numbers WO2012007906 A2-A3. At the European patent office as EP2593083 A2 and on the United States territory as US20130156832 A1.

**DERMATOLOGICAL DISEASE**

The major challenges in the skin diseases treatment include poor efficiency of drug delivery into the disease site and risks of increased toxicity associated with approaches used to improve the drug delivery efficiency.

In this thesis, two main drugs have been explored for the treatment of different diseases. The first is clotrimazole (CTZ), a synthetic imidazole derivative. It is primarily used locally in the treatment of vaginal and skin infections due to yeasts and dermatophytes. In vitro, it is most active against Candida spp., Trichophyton spp., Microsporum spp. and
Malazcessia furfur (Pityrosporon orbiculare). In addition, it has some in vitro activity against certain Gram-positive bacteria and at very high concentrations it has activity against Trichomonas spp. In the treatment of vaginal candidiasis, CTZ vaginal tablets have produced cure rates comparable with those of conventional nystatin vaginal tablets. CTZ topical preparations are generally well tolerated, but local irritation has required withdrawal of therapy in a few cases. The site of action of CTZ, like that of miconazole and the polyene antifungal agents, appears to be the cell membrane to which it is preferentially bound. It has been proposed that the mechanism of action involves an interaction with the phospholipid layer of cellular membranes causing alterations in membrane permeability. Permeability changes result in loss of essential precursors, metabolites and ions, thus inhibiting macromolecular synthesis. Absorption of CTZ through intact skin was essentially negligible in individuals with normal skin. The highest concentration of topically applied CTZ remained in the epidermis, particularly in the stratum corneum, with less appearing in the dermis, and very little penetrating subcutaneously. Consequently, topical administration avoids side effects of oral administration like itching, nausea, vomiting and abnormal liver activity.\[65\]
Figure 1.10: Chemical structure of Clotrimazole: 1-[(2-Chlorophenyl)(diphenyl)methyl]-1H-imidazole.\cite{66}

The second one, Amikacin sulfate (AKS) is an aminoglycoside antibiotic used to treat different types of bacterial infections. AKS works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth. AKS is most often used for treating severe, hospital-acquired infections with multidrug-resistant Gram-negative bacteria, such as Pseudomonas aeruginosa, Acinetobacter and Enterobacter. AKS can also be used to treat non-tubercular mycobacterial infections and tuberculosis when first-line drugs fail to control the infection. Side effects of AKS are similar to those of other aminoglycosides. Kidney damage and hearing loss are the most important effects.
**Figure 1.11**: Chemical structure of Amikacin: (2S)-4-Amino-N-[(2S,3S,4R,5S)-5-amino-2-][(2S,3R,4S,5S,6R)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-[(2R,3R,4S,5R,6R)-6-(aminomethyl)-3,4,5-trihydroxy-oxan-2-yl]oxy-3-hydroxy-cyclohexyl]-2-hydroxy-butanamide.

AKS may be administered once or twice a day but it must be given by intravenous or intramuscular route or via nebulization. For the treatment of dermatological disease, the topical route avoid the majority of side effects.\[67\]

CTZ and AKS were chosen for the ability to be topically administrable, low toxicity, the number of administration during the day (2-5 dose/day) and their opposite characteristics. The first drug is an antifungal, the second one is an antibiotic; one have a long half-life, the other short; but more important, CTZ is hydrophobic, while AKS is hydrophilic. Indeed, the main incorporation strategies will be scCO\(_2\) for CTZ and IWI for AKS.

**AIM OF THE THESIS**

The aim of this PhD work was to develop and characterize all physicochemical aspects of this new CRT for CTZ and AKS using OMS until the introduction onto the market.

The first part comprehends the characterization of different OMS synthesized and commercially available; the study of different incorporation techniques based on hydrophilicity/hydrophobicity of API; the characterization of the new impregnated OMS.

Consequently, the work is oriented on the interaction details of API on silica surfaces. A closer look is given to the big questions of OMS-drug phenomena: mobility, solubility, bioavailability, etc.
Therefore, all the scCO$_2$ incorporation parameters have been studied, highlighting the differences between OMS and the spatial assembly of drug inside the mesoporous channels.

Thus, the patented CRT has been developed for AKS describing all the main aspect of the innovative semisolid formulation. In-vitro and ex-vivo release test has been produced and characterized, revealing the functionality of the OMS reservoir effect.

Finally, the same DDS have been developed for CTZ. Both the DDS have been compared with commercially available creams.
ORDERED MESOPOROUS SILICA AS DRUG DELIVERY SYSTEM: EXPERIMENTAL

MATERIALS

ORDERED MESOPOROUS SILICA

The synthesis of OMS are here reported.

MCM-41

Four different MCM-41 have been used during this thesis. The first one named MCM41-1 has been synthesized following the procedure of Jana et al.\(^{[68]}\) Briefly, 25.04 g of hexadecyltrimethylammonium bromide (C\(_{16}\)TMABr) was dissolved in 75.22 g of water at 323 K. Subsequently, 16 g of 1,3,5-trimethylbenzene (TMB) was added and stirred vigorously. In another solution 1.8 g of H\(_2\)SO\(_4\) and 1.8 g of waterglass (Na\(_2\)SiO\(_3\)) were added to 60 g of H\(_2\)O. These two solutions were then mixed and stirred vigorously for 30 min. Then again 30 g of water was added to this mixture, stirring constantly. The pH of the gel was adjusted close to 10 by adding dilute sulfuric acid and then the resultant gel was transferred into a teflon-lined autoclave and heated statically at 373 K for its crystallization under autogeneous pressure in an oven for 10 days. After crystallization, the solid product was recovered by filtration, washed with large amounts of warm deionized water and dried at 373 K and finally calcined in air at 813 K for 6 hours.

MCM41-2, MCM41-3 and MCM41-4 were synthesized following the procedure of Grun et al.\(^{[20]}\) modified in order to change the pore size distribution of OMS. Briefly, n-Alkyltrimethylammonium bromides of different alkyl chain lengths were used as template: C\(_{16}\)TMABr and TMB for MCM41-2; C\(_{16}\)TMABr for MCM41-3; C\(_{12}\)TMABr for MCM41-4. The
template was dissolved in 120 g of deionized water to yield a 0.055 mol/L solution, and 9.5 g of aqueous ammonia (25 % by mass, 0.14 mol) was added to the solution. While stirring 10 g of Tetraethoxysilane (0.05 mol) (TEOS) was added slowly to the surfactant solution over a period of 15 min resulting in a gel with the following molar composition: 1 TEOS:0.152 C₆TMABr:2.8 NH₃:141.2 H₂O The mixture was stirred for one hour, then the white precipitate was filtered and washed with 100 ml of deionized water. After drying at 363 K for 12 h, the sample was heated to 823 K (1 K/min) in air and kept for 5 h to remove the template.

A fifth sample was a commercially available MCM-41 bought from ACS Material (MCM41-ACS).

**KIT-6**

Mesoporous KIT-6 silica materials were obtained following the method reported Kleitz et al.⁶⁹ Briefly, 9.0 g of Pluronic P123 (EO₂₀PO₇₀EO₂₀, Sigma-Aldrich) was dissolved in 325 g of distilled water and 17.40 g of HCl (37%) under vigorous stirring. After complete dissolution, 9.0 g of 1-butanol (BuOH, Aldrich, 99%) was added. The mixture was left under stirring at 308 K for 1 h, after which 19.35 g of TEOS was added to the homogeneous clear solution. The synthesis is carried out in a closed polypropylene bottle. This mixture was left under stirring at 308 K for 24 h, followed by an aging step, alternatively at 50, 80, 100, or 130 °C for 24 h under static conditions (this process is referred to as hydrothermal treatment). The resulting solid products were then filtered and dried for 48 h at 95 °C. For template removal, the as-synthesized silica powders were first shortly slurried in an ethanol-HCl mixture and subsequently calcined at 823 K for 2 h. The KIT-6 produced are referred in this thesis as KIT6-50, KIT6-80, KIT6-100, KIT6-130 in order to report the hydrothermal temperature treatment.
SBA-15

SBA-15 was synthesized according to the method described by Zhao et al.[23] First, 4.0 g of surfactant, Pluronic P123, were dissolved in 30 g of water and 120 g of 2 M hydrochloric acid solution. Then, 8.5 g of TEOS was added. This solution was stirred for 20 h at 308 K and then aged at 353 K overnight, without stirring (hydrothermal treatment). Finally, the product was collected by filtration, washed, and air-dried at room temperature. Calcination was carried out at 873 K (1 K/min) for 6 h. These sample is named as SBA15-C in this work.

Another sample was a commercially available SBA-15 and it was bought from ACS Material (SBA15-ACS).

MSU-H

OMS of MSU-H type were bought from Sigma Aldritch and kindly provided by Formac Pharmaceuticals (MSU-H-F).

DISORDERED MESOPOROUS SILICA

Two porous silica, with trade name Syloid AL-1 FP, were donated from Grace GmbH & Co. These are disordered silica synthesized without the use of any template but with different pore size distribution centred on 1 and 10 nm. Consequently, they were named as Syloid-1 and Syloid-10.

ACTIVE PHARMACEUTICAL INGREDIENT

Amikacin sulfate (AKS) (MW = 781.76 g/mol) (Ph.Eur 8.2) (purity 99.8%) and Clotrimazole (CTZ) (MW = 344.837 g/mol) (purity 98.9%) were
acquired from Sigma Aldrich. They were used as such, without any purification procedure.

CHEMICALS
Carbon dioxide with a purity of 99.5% was supplied by SIAD. Bidistilled water was supplied by J.T.Baker. All the reagents required for OMS synthesis (C\textsubscript{16}TMABr, TMB, TEOS, etc.), 1-fluoro-2,4-dinitrobenzene (FDNB), 1,2-propandiol, glycerol, acetonitrile and hydroxyethyl cellulose (Natrosol MR) were purchased from Sigma Aldrich. Commercial formulation of AKS (5% by mass) and CTZ (2% by mass) were purchased from a community pharmacy.

CHARACTERIZATION METHODS

X-RAY DIFFRACTION
X-Ray Diffraction (XRD) and wide angles patterns were obtained using a PANAltytical X’Pert Pro (Cu K\textsubscript{α} radiation) diffractometer with a PIXcel\textsuperscript{1D} photon detector.

FOURIER TRANSFORM INFRARED SPECTROSCOPY
For Fourier Transform InfraRed (FTIR) measurements, powders were pressed in self-supporting wafers and spectra were recorded at room temperature with a Bruker Tensor 27 spectrometer operating at 2 cm\textsuperscript{-1} resolution. If degassing was necessary, the sample were outgassed at 373 K for one hour (residual pressure equal to 0.1 Pa). FTIR spectrum of crystalline API were recorded on the powder dispersed in potassium bromide (KBr). FTIR spectrum of API dissolved in solution were recorded on a diluted carbon tetrachloride (CCl\textsubscript{4}) solution (1 g/L).
**NITROGEN ADSORPTION**

Nitrogen adsorption isotherms were measured using a Quantachrome AUTOSORB-1 instrument after degassing at 373 K for 2 hours. Brauner-Emmet-Teller (BET) specific surface areas (SSA) were calculated in the relative pressure range 0.04-0.1 and the pore size distribution was determined through the NLDFT (Non Linear Density Functional Theory) method, using the NLDFT equilibrium or adsorption model for cylindrical pores.\[22]\n
**TERMOGRAVIMETRY**

TermoGravimetric (TG) analyses were carried out between 298 K and 1523 K in air (flow rate 100 mL/min with a heating rate of 10 K/min) using a SETARAM 92 instrument.

The API desorption analysis were performed using a SETARAM 92 instrument following the procedure explained by Verevkin et al.\[70]\ and Price et al.\[71]\ Using the Clausius-Clapeyron relation, equation (1), the enthalpies of vaporization ($\Delta H_{vap}$) at the average temperature of investigation were obtained.

$$\ln \left( \sqrt{T} \frac{dm}{dt} \right) = A + \frac{\Delta H_{vap}}{RT}$$  \hspace{1cm} (1)$$

where $dm/dt$ is the mass loss rate at the specified temperature; $R$ is the universal gas constant and $T$ is the temperature of the isothermal experiment. Subsequently, vaporization enthalpies were reported to 298.15 K using general methods of correction, reported by Chickos et al.\[72]\ (the Sidgwick’s rule):
\[
\Delta H_{vap}(298.15) = \Delta H_{vap}(T) + 0.0545 (T - 298.15)
\] 

(2)

In equation (2), \( T \) is the temperature of measurement or mean temperature of measurement if \( \Delta H_{vap}(T) \) has been obtained from a Clausius-Clapeyron treatment of vapor pressures. The experimental conditions used for the reliable determination of vaporization enthalpies of low volatile molecular compounds are following the references of Verevkin at al.[70] A calibration curve with phenol has been done. The uncertainty of temperature calibration was less than 1 K.

**DIFFERENTIAL SCANNING CALORIMETRY**

Differential Scanning Calorimetry (DSC) measurements were performed with a DSC1 STAR® (Mettler Toledo) System apparatus of TA Instruments equipped with a low temperature probe between 298 K and 1073 K under nitrogen flux (flow rate 60 mL·min\(^{-1}\) with a heating rate of 10 K·min\(^{-1}\)).

**SCANNING ELECTRON MICROSCOPY**

Field Emission Scanning Electron Microscopy (FESEM) image were recorded with a FESEM ZEISS MERLIN.

**DINAMIC LIGHT SCATTERING AND ZETA POTENTIAL**

Dinamic Light Scattering (DLS) and Zeta Potential analysis were performed with 90 Plus Instrument (Brookhaven) on a suspended water mixture of OMS after homogenization with a high shear homogenizer (Ultraturrax, Ika) for 5 min at maximum velocity.
NUCLEAR MAGNETIC RESONANCE

Solid State Nuclear Magnetic Resonance (SSNMR) spectra were recorded on a Bruker Avance II 400 instrument operating at 400.23 and 100.65 MHz for $^1$H and $^{13}$C nuclei, respectively. 4 mm o.d. zirconia rotors (sample volume of 80 µL) were employed and spun at 12 kHz for $^{13}$C CPMAS spectra. A ramp cross-polarization pulse sequence was used with contact times of 3–5ms, a $^1$H 90° pulse of 3.8 µs, recycle delays of 1–60 s and 48–4096 transients. $^1$H MAS spectra were performed on a 2.5 mm probe with a spinning speed of 32 kHz. A DEPTH sequence ($\pi/2$-$\pi$-$\pi$) was used to suppress the probe background signal.[73,74] The $^1$H 90° pulse length was set to 2.50 πs, the recycle delays to 1-40 s and 32-64 transients were averaged for each sample. $^1$H and $^{13}$C chemical shifts were referenced through the resonance of adamantane ($^1$H signal at 1.87 ppm) and hexamethylbenzene ($^{13}$C methyl signal at 17.4 ppm), respectively.

DRUG INCORPORATION METHODS

SOLUTION

API incorporation was performed by adsorption from solution. To this purpose, normally, 300 mg of API and 100 mg of OMS were introduced in an Erlenmeyer flask with 5 ml of solvent (water or ethanol) and maintained at room temperature under stirring for 24 hours. Then the powder was filtered and dried overnight in vacuum (residual pressure 0.1 Pa).

INCIPIENT WETNESS IMPREGNATION

The API loading was performed by Incipient Wetness Impregnation (IWI) procedure, based on the capillary action.[47] Typically, 1 ml of a saturated solution of API was added drop by drop to one gram of OMS during
mechanical agitation. The homogeneous wet powder obtained was vacuum dried for 24 hours at 0.1 Pa obtaining a dry powder. For the IWI water and ethanol were used as solvents.

SUPERCRTICAL CO₂

For the incorporation process by means of scCO₂ a homemade device was used. This consists in a glass cylinder of 1 cm diameter containing a pellet of CTZ (100 mg) and a pellet of OMS (100 mg) separated by a disc of filter paper, in order to prevent their contact and to separate the pellets after the incorporation process.

![Homemade device](image)

**Figure 1.11:** Photograph of the homemade device used for the scCO₂ incorporation process.

This device was placed inside a stainless steel vessel, which was put in an oven that maintained all the system at constant temperature. The apparatus was also equipped with a volumetric pump and a back pressure...
regulator. A scheme of the apparatus is reported in Figure 1.12 and further details can be found elsewhere.\textsuperscript{[76]}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{apparecchiatura_SF3.png}
\caption{scCO\textsubscript{2} apparatus details: B – CO\textsubscript{2} tank; P\textsubscript{1} – CO\textsubscript{2} pump; P\textsubscript{2} – co-solvent pump (not used); P\textsubscript{3} – hexane pump (not used); V\textsubscript{xx} – valves On/Off; V\textsubscript{4}/V\textsubscript{5}/V\textsubscript{12}/V\textsubscript{13} three way valves; V\textsubscript{NR} – not returning valve; R – reactor; M – Mixer; S – heating coils; F – oven; BPR\textsubscript{1} – manual back pressure regulator; BPR\textsubscript{2} – automatic back pressure regulator; PE – heating pad; Tx – trap; Fl\textsubscript{x} – mass flow meter.}
\end{figure}

The incorporation was performed in static condition. The vessel was filled with liquid CO\textsubscript{2} and heated up to 373K. After the heating, additional carbon dioxide was pumped in the vessel to reach the final desired pressure (25.0 MPa). The system was then maintained in the described conditions for several hours (from 6 to 18) to allow the drug dissolution and diffusion into the OMS. At the end of the incorporation process, the temperature was decreased to room conditions and the apparatus was depressurized.

\textbf{AB-INITIO AND MOLECULAR DYNAMICS SIMULATIONS}

All the calculations were performed within the Density Functional Theory (DFT). Concerning static calculations, the developmental version of the
CRYSTAL14 code\textsuperscript{29} in its massively parallel version\textsuperscript{77} was adopted and the computational approach is the same of Ref.\textsuperscript{78}. Briefly, the chosen functional was the Perdew, Burke, and Enzerhof GGA (Generalized Gradient Approximation) exchange-correlation functional (PBE),\textsuperscript{31} including the empirical Grimme’s D2 correction,\textsuperscript{80} to describe the dispersive interactions (vdW). In the following, the superscript D means that Grimme’s correction is included. Split valence double- (for Si atoms) and triple-\(\zeta\) (for other atoms) Gaussian type basis sets plus polarization functions were used to describe the systems.\textsuperscript{81,82} Chlorine atoms were represented with a 86-311G* basis set.\textsuperscript{83} Only the atomic coordinates of the two more superficial layers of each silica slab in the docking geometries were optimized, to compensate for the reduced thickness of the models. Starting geometries were generated so to maximize the interactions between the drug and the surface. Interaction energies, per unit cell per adsorbate molecule (\(\Delta E\)), were calculated and corrected for the basis set superposition error (BSSE) according to the counter-poise methodology described in previous papers by Delle Piane et al.\textsuperscript{23,25} and reported in Supporting Information.

Harmonic frequencies were calculated with CRYSTAL14 at \(\Gamma\) point and the infrared intensity for each normal mode was obtained by computing the dipole moment variation along the normal mode, adopting the Berry phase method.\textsuperscript{85} For the simulation of the IR spectra of the different structures, only a fragment consisting of the most interesting chemical groups has been considered for constructing the Hessian matrix and will be defined for each case in Results and Discussion.

Enthalpies (\(\Delta H\)) of adsorption at standard temperature (298 K) were obtained from the vibrational partition functions, by applying the Zero Point Energies (\(\Delta ZPE\)) and thermal (\(\Delta E_T\)) corrections to the BSSE corrected electronic adsorption energies (\(\Delta E^C\)) as \(\Delta H = \Delta E^C + \Delta ZPE + \Delta E_T\).
Ab-initio molecular dynamics (AIMD) simulations were performed using the CP2K code[86]. The Quickstep technique[87] with a mixed plane wave and Gaussian basis set methodology (Gaussian and Plane Wave method, GPW) was employed to calculate the electronic structure. We used the PBE functional, with the Goedecker–Teter–Hutter pseudopotentials[88] and a triple-ζ basis set with polarization functions (TZVP)[89] augmented with the empirical Grimme’s D2 correction. The cutoff for the plane wave basis was set to 400 Ry. AIMD simulations were run at 300 K in the NVT ensemble, using the Canonical Sampling through Velocity Rescaling (CSVR) thermostat. A time step of 0.5 fs was chosen. All simulations were equilibrated at 300 K with a more stringent thermostat (time constant: 10 fs) for about 1 ps and then the production phase was run for at least 10 ps with a more relaxed thermostat (time constant: 50 fs). Since CP2K requires 3D periodic systems, a value of $c = 35$ Å was chosen to separate the slab replicas with enough vacuum. In all cases, only the superficial layer of the silica slab and the drug molecules were allowed to move.

**DRUG RELEASE**

**RELEASE KINETICS**

The drug release kinetic of API to the saturated concentration were evaluated in vitro by soaking 100 mg of OMS incorporated by API (OMS-API) in 5 ml of stirred solvent. At different time intervals, a small amount of solution (100 μl) was collected, diluted and analysed through UV–Vis spectrophotometry analysis. To assess the amount of released API a 5-point calibration curve has been obtained.

**RESERVOIR EFFECT**

**ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS**
The reservoir effect of saturated solution with OMS-API have been evaluated for AKS and CTZ. In a saturated solution of API a certain amount of OMS, impregnated with the same API, were added under agitation. At regular intervals (1h) a small amount of solution (between 500-100 μl) were withdraw and substituted with the same quantity of pure solvent. The extracted solutions were then diluted and analysed through UV–Vis spectrophotometry to assess the API concentration. The addition of pure solvent causes a dilution of the solution and allow us to evaluate the reservoir effect of impregnated OMS. In order to evaluate the correct content of drug in the solution a 5-point calibration curves has been done for CTZ and AKS in the respective solvent. The coefficient of determination was 0.997 for CTZ and 0.995 for AKS, both in the concentration range 0.01-0.1 mg·ml⁻¹.

**ULTRAVIOLET-VISIBLE SPECTROSCOPY**

API concentration in solution were evaluated through UltraViolet Visible (UV-Vis) spectroscopy using a UV-Vis-NIR spectrometer (Cary 5000, Agilent technologies). Each time and for each solution, a 5-point calibration curve has been obtained.

**IN VITRO RELEASE**

API diffusion from different gels was studied using an apparatus consisting of a vertical glass diffusion cells (Franz cell) of 6.0 mL volume equipped by a Spectra/Por (12000-14000 Dalton MWCO) hydrophilic cellulose membrane (Spectrum Lab). The diffusion area was 2 cm². Gels and commercial formulation were employed as a donor phase. The receiving phase consisted of acetate buffer at pH 5.0 (AKS) or water solution of HCl (pH 1.0) (CTZ). The apparatus was maintained under stirring for 72 h at 307 K, during which at scheduled times the receiving phase was withdrawn and entirely substituted with fresh receiving phase.
Each withdrawn was analysed. Each sample was prepared and analysed in triplicate.

IN-VITRO PERMEATION STUDIES
AKS transepidermal permeation and skin uptake from the different gels were studied using vertical Franz diffusion cells and porcine ear skin. Skin slices were isolated using an Acculan dermatome (Aesculap) from the outer side of pig ears freshly obtained from a local slaughterhouse and then stored for at least 24 h at 255 K. Prior to each experiment, the excised skin was rinsed with normal saline solution and pre-hydrated by floating it in 0.002% (w/v) sodium azide aqueous solution. The skin was then sandwiched between the two cells with the stratum corneum side upwards. The diffusion area was 2 cm². AKS-OMS gel and commercial formulation were employed as a donor phase. The receiving phase consisted of acetate buffer at pH 5.0. The apparatus was maintained under stirring for 168 h at 307 K, during which at scheduled times the receiving phase was withdrawn and entirely substituted with fresh receiving phase. Each withdrawn was analysed using HPLC. Each sample was prepared and analysed in triplicate.

HIGH PRESSURE LIQUID CHROMATOGRAPHY
AKS was derivatized by mixing 100 μl of aqueous solution of the drug with 300 μl of methanol, 40 μl of NaOH (0.05 M) and 50 μl of a methanolic solution of the derivatizing agent (FDNB) (180 mg·ml⁻¹). The obtained mixture was heated at 363 K in an air-circulating oven for 10 min, then cooled and injected in HPLC. Each solution was separately derivatized prior to injection. The HPLC apparatus consisted of an isocratic pump (Series 200, Perkin-Elmer instrument) equipped with a μBondapack C18
300 mm · 4.6 mm column (particle size: 10 μm; pore size: 125 Å; endcapped) (Waters, Milford, MA, USA). The mobile phase was a mixture of acetonitrile–water–acetic acid (47:53:0.1 v/v/v) pumped at 1.5 ml/min. A spectrophotometric detector (LC 290, Perkin-Elmer) working at 365 nm was used.
CHAPTER 1: DRUG INCORPORATION

ABSTRACT

Clotrimazole (CTZ) and Amikacin Sulfate (AKS), two active principles widely used in dermatology, were incorporated inside Ordered Mesoporous Silica (OMS). Three main techniques have been compared: incorporation by solution, incipient wetness impregnation and supercritical CO₂. At the same time six main OMS have been used: MSU-H, MCM41-1 to MCM41-4 and MSU-H-F. In all cases the pristine OMS were characterized by nitrogen adsorption measurement. Numerous incorporation parameters have been studied. The amount of incorporated CTZ was higher with the scCO₂ process (34% by mass), while AKS content was higher with the IWI technique (43% by mass). The drug-containing OMS was characterized by XRD, FESEM, TGA, FT-IR and nitrogen adsorption analysis. In all cases the drug resulted amorphous and distributed inside the mesopores.

INTRODUCTION

In the first pioneering work by Vallet-Regi et al.⁹³, drug incorporation in MCM-41 was carried out by adsorption from a solution using hexane as solvent.

Since the long-term toxicity of n-hexane in humans is well known⁹¹,⁹², many other solvents have been studied for the incorporation. For instance, Charnay et al.⁹³ reported that when highly polar solvents such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and dimethylacetamide (DMA) were used, the amount of adsorbed drug was limited, whereas a higher quantity of drug was incorporated when ethanol...
or hexane were used. The drug incorporation is strongly affected by the solvent due to the competition between the drug and the solvent in the adsorption\textsuperscript{[93]}. An answer could be the IWI process.\textsuperscript{[47]} This technique allows the choice of less dangerous solvents tackling the problems of solvent removal and adsorption competition. Surely, a high drug solubility must be persecuted in order to enhance the drug adsorption efficacy. On the other hand, a repetition of the IWI could be done to enhance the drug content.

Finally, also the incorporation through scCO\textsubscript{2} is an alternative way to adsorption or impregnation from a liquid solution. Unfortunately, not all drugs can be solubilized by the CO\textsubscript{2} flow. Even if pressure and temperature can modify the solubility parameter of CO\textsubscript{2}, salts like AKS cannot be solubilized.

Belhadj-Ahmed et al.\textsuperscript{[94]} investigated the supercritical impregnation of vitamine E acetate on silica matrixes by means of a dynamic technique. Min et al. studied the incorporation of ibuprofen inside SBA-15 through scCO\textsubscript{2}\textsuperscript{[95]}, showing that the loaded drug quantity increased with a higher solubility of ibuprofene in scCO\textsubscript{2}.

The first paragraph of this chapter will discuss the positive aspects of the scCO\textsubscript{2} process compared to the incorporation from solution. For the sake of brevity, only OMS of MSU-H type will be presented in this part. CTZ is the model drug and ethanol, for safety reasons, is the selected solvent.

Secondly, the scCO\textsubscript{2} process will be replied on the MCM-41 OMSs, 1 to 4.

Thirdly, the IWI method will be studied for AKS in order to compare the two techniques, using two different OMS (MSU-H and MSU-H-F).
1.1 SOLUTION AND SCCO₂ PROCESS COMPARISON

The effect of the scCO₂ treatment on the structure and morphology of OMS was investigated by means of XRD and FESEM prior to the incorporation of CTZ. The target was to study the possible effects of scCO₂ on OMS. There was no literature on this topic.

As it is reported in “Incorporation of clotrimazole in ordered mesoporous silica by supercritical CO₂” by Gignone et al.[8], wide angle XRD patterns and FESEM micrographs showed no significant modifications.

ScCO₂ was used to incorporate CTZ using the apparatus showed in the experimental section. Pressure and time of the scCO₂ treatment were varied, in order to investigate the effect on the amount of incorporated CTZ, whereas the temperature was maintained constant and equal to 373 K. This temperature was chosen significantly higher than the critical temperature of carbon dioxide, at variance with what was reported by Belhadj-Ahmed et al.[94] and by Min et al.[95]. Thermal degradation of CTZ can be ruled out since it was reported to occur at 453 K.[96] The selected working pressures ensured a good solvent power of the fluid. The density and solubility parameter were respectively equal to 550 kg·m⁻³ and 9.1 (MJ·m⁻³)¹/₂ at 25.0 MPa, while they were 819 kg·m⁻³ and 12.5 (MJ·m⁻³)¹/₂ at 50.0 MPa.

As a whole, four different incorporation procedures were carried out and their typical parameters are summarized in Table 1.1, together with the corresponding loading of incorporated CTZ, which was measured by TG analyses.
Table 1.1: Incorporation process parameters for scCO$_2$ and ethanol solution (EtOH) and corresponding CTZ loading evaluated by TG analysis.

<table>
<thead>
<tr>
<th>Incorporation Procedure</th>
<th>Time (h)</th>
<th>Temperature (K)</th>
<th>Pressure (MPa)</th>
<th>Drug-OMS ratio*</th>
<th>Loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>scCO$_2$ -1</td>
<td>6</td>
<td>373</td>
<td>25.0</td>
<td>1:1</td>
<td>12</td>
</tr>
<tr>
<td>scCO$_2$ -2</td>
<td>12</td>
<td>373</td>
<td>25.0</td>
<td>1:1</td>
<td>30</td>
</tr>
<tr>
<td>scCO$_2$ -3</td>
<td>12</td>
<td>373</td>
<td>50.0</td>
<td>1:1</td>
<td>34</td>
</tr>
<tr>
<td>scCO$_2$ -4</td>
<td>18</td>
<td>373</td>
<td>25.0</td>
<td>1:1</td>
<td>30</td>
</tr>
<tr>
<td>EtOH</td>
<td>24</td>
<td>298</td>
<td>0.1</td>
<td>3:1</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*mass ratio

The maximum amount of incorporated CTZ, corresponding to 34% by mass, was obtained at 50.0 MPa after a 12-hours-supercritical treatment.

Data in Table 1.1 reveal that time plays a crucial role up to 12 hours. In fact, the CTZ loading increased from 12% to 30% by mass when time increased from 6 to 12 hours at 25.0 MPa, whereas no further increase was observed when the process was carried out for a longer time, i.e. 18 hours, at the same pressure. These data suggest that after 12 hours the equilibrium between CTZ in scCO$_2$ and CTZ inside the MSU-H was reached.

At variance, pressure was observed to affect the incorporated amount only at a minor extent, since an increase from 25.0 MPa to 50.0 MPa yields a limited increase of the CTZ percentage (from 30% to 34%), probably due to a higher solubility of CTZ in scCO$_2$ at higher pressure.

It is worth noting that the incorporation via scCO$_2$ was largely more efficient than that obtained by adsorption from ethanol solution. In fact, in the latter case the percentage of CTZ inside MSU-H was only 9.0%.
Moreover, the amount of CTZ used for the adsorption was three times higher than that of MSU-H, at variance with the scCO$_2$ treatment for which the same amounts of CTZ and MSU-H were used.

As already reported$^8$ CTZ is maintained in an amorphous state. The lack of crystallization is crucial, because amorphous drugs are widely accepted to have higher aqueous solubility and dissolution rate than related crystalline phases. This effect may be particularly important when molecules poorly soluble in water, such as CTZ, are considered.$^{41,97,98}$ FT-IR spectroscopy, nitrogen adsorption measurements and TG analysis on this samples showed important details of the CTZ distribution; but this correlation will be discussed altogether in the following chapters.

### 1.2 MCM-41 SC$\text{CO}_2$ INCORPORATION

MCM-41 silicas have been widely studied used as drug carrier. During this research work, through the solution technique, the maximum CTZ content was about 6% by mass without residue mesoporosity. These data are different from the MSU-H case, see table 2.1.

In this case four MCM-41 have been synthesized, as already described, and characterized.
Figure 1.1: MCM-41 nitrogen adsorption isotherms. Pore volume, SSA and PSD are reported in chapter 2.

Table 1.2: MCM-41 nitrogen adsorption results

<table>
<thead>
<tr>
<th>OMS</th>
<th>Mean pore diameter (nm)</th>
<th>NLDFT SSA (m²g⁻¹)</th>
<th>NLDFT Volume (cm³g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCM41-1</td>
<td>4.88</td>
<td>812</td>
<td>1.286</td>
</tr>
<tr>
<td>MCM41-2</td>
<td>4.88</td>
<td>585</td>
<td>1.100</td>
</tr>
<tr>
<td>MCM41-3</td>
<td>3.65</td>
<td>930</td>
<td>0.818</td>
</tr>
<tr>
<td>MCM41-4</td>
<td>2.94</td>
<td>840</td>
<td>0.598</td>
</tr>
</tbody>
</table>

The nitrogen adsorption analysis showed a good SSA and pore volume.
Figure 1.2: MCM-41 FESEM images. In the upper left image MCM41-1 is showed (mean particles size 360 nm). Upper right, MCM41-2 (210 nm). Lower left, MCM41-3 (100 nm). Finally, MCM41-4 (1090 nm).

The drug content with the scCO₂ was higher than for the MSU-H case. The higher loading was obtained with MCM41-1 with a CTZ content of 44% by mass. XRD and FTIR analysis reported a complete amorphization of the drug.

1.3 AMIKACIN SULFATE INCORPORATION

The drug loading was performed by the solution method and the IWI procedure. In the first case, 300 mg of AKS where solubilized in 5 ml of H₂O and stirred for 24h with 100 mg of MSU-H-F. The water content was removed trough filtration and drying. For IWI, typically, 1 ml of a water
saturated solution of AKS was added drop by drop to one gram of MSU-H-F (0.898 cm$^3$.g$^{-1}$) during mechanical agitation. The homogeneous wet powder obtained was vacuum dried for 24 hours to 0.1 Pa obtaining a dry powder.

**Figure 1.3:** (I) XRD spectra of AKS powder (a) and MSU-H-F-AKS (47% by mass) (b). (II) FTIR spectra of MSU-H-F (a), AKS in KBr (b) and MSU-H-F-AKS (c). (III-IV) N$_2$ isotherms and PSD of MSU-H-F (a) and MSU-H-F-AKS (47% by mass) (b).

In order to control the physicochemical properties of our samples, a complete characterization of all samples has been done. XRD results shown a complete amorphization of APIs (Figure 1.3). The lack of crystallization in these materials is a very well reported and crucial phenomenon $^{[40,99,100]}$. Indeed, such application that require a dissolution and diffusion rate in gels able to overcome the skin absorption it is even more essential; i.e. crystalline APIs could have a dissolution rate too slow in order to maintain the saturation point.
In figure 1.4 are shown the low angle XRD diffraction pattern presenting the typical (100), (110) and (200) peaks, related to the ordered hexagonal (P6mm) network of mesopores. The maintenance of the peaks after the incorporation process demonstrate the mesostructure preservation. The differences in the peak intensity indicate the drug incorporation: less density differences from the silica wall to the mesoporous pore core due to the incorporated AKS reduce the intensity of the diffracted X-ray.

**Figure 1.4:** (I) Low angle XRD spectra of MSU-H-F (a) and MSU-H-F-AKS (47% by mass) (b). (II) DLS of MSU-H-F after 5 minute of high shear homogenization (30 krpm)

FTIR spectroscopy reveals that the APIs interact with the silica surface through Hydrogen bonds with silanols. The adsorption infrared spectra of figure 1.3.II describes direct interaction between MSU-H-F and AKS. The MSU-H-F as such shows two main band: free silanols at 3742 cm\(^{-1}\) and interacting silanols at 3520 cm\(^{-1}\). The 2800-3000 cm\(^{-1}\) bands are the alkyl residual chain of the template after the calcination step required to produce MSU-H-F. AKS in KBr shows low adsorption between 3550 and 3160 cm\(^{-1}\) (-NH\(_2\), -NH, -OH groups), 2927 and 2850 cm\(^{-1}\) (CH\(_2\), CH groups) and strong mode at 1640 and 1550 cm\(^{-1}\) (-CONH- group) \(^{[67]}\). Other adsorption band of -CO group are under the silica cut-off. In the MSU-H-F-AKS sample no free silanols are observable (3742 cm\(^{-1}\)) suggesting a direct interaction between surface silanols and AKS. Moreover, some new broad absorption band are observable in
comparison with AKS in KBr. These are attributed to SiOH interacting with
different groups of AKS.

TG analysis reports the API-incorporated quantities. UV-Vis spectroscopy
results and HPLC quantification confirms these results. The TG
measurements show in the best case (IWI) an incorporated quantity of
47% by mass, while with the incorporation by solution it was possible to
reach only a 15% by mass.

Nitrogen adsorption isotherms (Figure 1.3.III) return the specific surface
area, the specific pore volume and the pore size distribution of MSU-H-F
and MSU-H-F-AKS (Figure 1.6.IV). In the case of IWI, NLDFT specific
surface area decreased from 625 m²·g⁻¹ to 69 m²·g⁻¹. The pore volume
decreased from 0.898 cm³·g⁻¹ to 0.121 cm³·g⁻¹. Using the TG analysis
results, the volume excluded from N₂ adsorption, due to the incorporation
of AKS in MSU-H-F, can be corrected, as already showed in a previous
paper.\cite{8} Dividing the API incorporated quantity with this corrected
excluded volume the estimation of the amorphous AKS density is: 1.59
\( \text{g·cm}^{-3} \). The experimental density of crystalline AKS, evaluated with a
helium pycnometer (Ultrapyc 1200e of Quantachrome) is 1.60 g·cm⁻³ that
is in agreement with the unique literature data of Bau et al.\cite{101}, to the best
of our knowledge. This correlation shows that AKS have almost the same
density in the amorphous and crystalline state. In addition to this, the
NLDFT PSDs show a reduction of the volume without a change in pore
diameter (Figure 1.3.IV). These considerations, compared to other
literature results \cite{99}, strongly suggest that AKS molecules are
incorporated inside the mesopores but not uniformly distributed on the
MSU-H-F surface. Consequently, with the IWI method, the homogeneity
inside the mesopores is lost but no occlusion is present and the drug still
in an amorphous state. This results are confirmed also with the
incorporation by solution, but the incorporated quantity is much lower. On
the other hand, as observable in chapter 2, the scCO$_2$ process is able to homogenize the adsorbed drug.

**Figure 1.5:** FESEM images of MSU-H-F aggregates.

Figure 1.5 reports a morphological image of MSU-H-F showing aggregates of microns composed by particles of about 500-600 nm. The primary particles resemble short prism of hexagonal base with striping along the height recalling the ordered hexagonal (P6mm) symmetry. The DLS measurements of MSU-H-F as such agree with the FESEM image, resulting in aggregates of 1300 nm mean value. Indeed, after high shear homogenization (5 min at 30 krpm), MSU-H-F particles results completely disaggregates (Figure 1.6.II). The mean value is 569±16 nm. The Zeta Potential measurements show an instability of the mixture (-12 mV) but the probable re-aggregation should be avoided in a future gel use due to viscosity.
The ions extracted from the MSU-H-F of MSU-H type were Cl⁻, F⁻, NO₃⁻ and SO₄²⁻. Sulfate is the only one over 2 ppm (18 ppm). This is not a problem since the API used for in-vitro permeation studies is AKS.
ABSTRACT

The knowledge of the specific interactions between the surface of mesoporous silica and drugs is necessary to guide development of new and improved drug delivery systems. However, such knowledge is still scarce, due to the arduous interpretation of experimental results. This chapter reports the complete characterization of the interaction of CTZ, inside OMS by means of a joint computational and experimental approach. Experimentally the drug was loaded through scCO₂ in MSU-H and MCM-41. Its adsorption was investigated through FTIR, N₂ adsorption measurements, TG analysis, SSNMR spectroscopy. Modelling involved static and dynamic Density Functional Theory simulations of CTZ adsorbed on realistic models of amorphous silica surfaces. In the first part, a more simplified approach is reported. The second part informs about the agreement between the computational and experimental results, concerning the energies of adsorption, the IR spectra and the distribution of drug inside the mesopores. Finally, a tentative description of the mobility at room temperature of CTZ on the silica surface was done using molecular dynamics simulations and SSNMR results.

2.1 DRUG DISTRIBUTION TYPES IN OMS

On the same sample of chapter 1 (MSU-H and MCM-41), nitrogen adsorption isotherms were measured to characterize the SSA, the specific pore volume and the PSD of OMS as such and OMS-CTZ. In figure 2.1
are reported the adsorption and desorption branches for OMS and OMS-CTZ.

![Figure 2.1: Isotherms of MSU-H (I) and MCM41-1 (II) before (a) and after (b) scCO₂.](image)

**Table 2.1:** NLDFT and TGA elaborated data of the isotherms of figure 2.1

<table>
<thead>
<tr>
<th>Sample</th>
<th>SSA [m²/g]</th>
<th>Volume [cm³/g]</th>
<th>Loading [g_{CTZ}/g_{TOT}]</th>
<th>Residual Volume [cm³/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCM41-1</td>
<td>813</td>
<td>1.28</td>
<td>46 %</td>
<td>0.29</td>
</tr>
<tr>
<td>MSU-H</td>
<td>597</td>
<td>0.90</td>
<td>34 %</td>
<td>0.32</td>
</tr>
</tbody>
</table>

As already described in a published article\(^8\), from the grams of CTZ incorporated inside the sample, the volume occupied by CTZ can be calculated and the residual free mesopore volume in OMS-CTZ can be estimated. This estimation is strongly affected by the density considered for CTZ in the calculation. Assuming a density of 1.316 g/cm³\(^6\),\(^6\) the mesopore volume in OMS-CTZ resulted equal to 0.336 cm³/g. This value is in fair agreement with table 2.1. The discrepancy of 0.047 cm³/g may arise from the value of CTZ density used in the calculation. Indeed, XRD pattern showed that CTZ in OMS lacks of crystalline form and its density
is likely to be lower than that of the crystalline phase. These considerations can be repeated for the MCM41-1 sample.

\[ V_0 = \frac{\pi}{4} \cdot d_0^2 \cdot h \]

where \( d_0 \) is the mesopore diameter and \( h \) is the mesopore elongation. The volume \( V_f \) of a single cylindrical mesopore occupied by the clotrimazole layer is given by

**Figure 2.2:** PSDs of MSU-H (I) and MCM41-1 (II) before (a) and after (b) scCO\(_2\). The red curve (c) reports the mathematical model calculations.
where \( d_i \) is calculated as

\[
\text{(3)} \quad d_1 = d_0 - 2d_{\text{clo}}
\]

and \( d_{\text{clo}} \) is the diameter of a clotrimazole molecule considered as a sphere. Combining equation 1 and 2, equation 4 is obtained, which, substituting \( d_i \) as in equation 3, gives rise to equation 5.

\[
\text{(4)} \quad \frac{V_0}{V_1} = \frac{d_0^2}{d_1^2}
\]

\[
\text{(5)} \quad V_1 = \frac{(d_0-2d_{\text{clo}})^2}{d_0^2} \cdot V_0
\]

A new pore size distribution can be calculated from this equation, on the basis of the values of \( V_0 \) and \( d_0 \) of the pore size distribution of OMS as such (Figure 6a), experimentally obtained by the nitrogen adsorption isotherm. In order to do so, \( V_0 \) and \( V_1 \) in equation 5 have to be normalized to the same quantity of silica, because \( V_0 \) is a specific pore volume, expressed in \( \text{cm}^3 \cdot \text{g}^{-1} \). This means that, if \( g_{\text{sil}} \) is the amount of OMS as such (silica), the amount of OMS-CTZ is \( g_{\text{sil}} + g_{\text{clo}} \), where \( g_{\text{clo}} \) has been evaluated by TG analysis. Accordingly, equation 5 is rewritten as equation 6.
This calculation is based on a simple modelization of CTZ molecules equally distributed inside MSU-H pores. Figure 2.3 represents schematically the above considered type of adsorption.

\[ V_1 = \frac{(d_0 - 2d_{clo})^2}{d_0^2} \cdot V_0 \cdot \frac{g_{sil}}{g_{sil} + g_{clo}} \]

**Figure 2.3:** Pictorial representation of CTZ adsorption in mesopores; MSU-H (I) and MCM41-1 (II).

Using these assumptions, from the PSD of OMS as such a theoretical PSD, after the adsorption of drug, can be calculated. This is in agreement with previous data reported for itraconazole by Mellaerts et al.\textsuperscript{48} who observed a molecular dispersion of the drug in OMS.

On the other hand, these simplified distributions do not take into account roughness, density profiles and curvature of the channels surfaces.\textsuperscript{102}
Consequently, a more detailed description have been done later in this chapter with the theoretical help.

In the case of MCM41-1, due to the fact that there is no residual PSD after the scCO2 process (Figure 2.2), these considerations are not valid. Indeed, the connection between nitrogen adsorption data and TG analysis demonstrate a complete filling of the mesopore channels. A detailed description will be reported in chapter 3. In any case, figure 2.3.II reports pictorially these differences.

### 2.2 EXPERIMENTAL AND THEORETICAL DATA

In order to describe better the drug-silica interactions the MSU-H and MCM41-1 incorporated samples were used as models, joining experimental and theoretical data. This work was done in collaboration with the Theoretical Chemistry Group of Prof. Piero Ugliengo, with Dr. Massimo delle Piane and Dr. Marta Corno.

**CLOTRIMAZOLE: MOLECULE AND CRYSTAL**

CTZ was modeled both in gas phase and as a crystal, before studying its adsorption on amorphous silica. The starting point was the X-ray experimental structure by Song et al.\(^{[66]}\)
Figure 2.4: a) 3D space filling model of the CTZ tetrahedral. b) CTZ electrostatic potential mapped on the electron density.

The CTZ crystal has been optimized with and without Grimme’s correction and compared to experimental results (Figure 2.5).

Figure 2.5: CTZ crystal and molecule-faces interactions: a, CTZ crystal with highlighted faces. b, c and d are the PBE-D2 optimized structures of (100) (010) and (001) crystal surfaces with one CTZ molecule per cell adsorbed, respectively. The added molecule is in blue to distinguish it from the clotrimazole crystal slab.
The structure is triclinic with two drug molecules per unit cell. The PBE-D2 cohesive energy of crystalline CTZ has been computed as $-146.5$ kJ mol$^{-1}$ (Table 1) and, unsurprisingly, the interactions occurring in the crystal are dominated by dispersion (78%).

Experimentally, the lack of crystallization of CTZ inside OMS is observed. This phenomenon has been described in literature both as a confinement effect$^{[41,98,103,104]}$ and as a competition between crystal cohesion and adsorption on the silica surface.$^{23}$ To describe this competition, we have modelled the interaction of CTZ molecules with three crystalline surfaces of the same CTZ crystal, figure 2.5. The added CTZ molecule maximizes the contact with the surface molecules mainly through dispersion and electrostatics interactions. Unsurprisingly, the interaction energies of CTZ with its crystal surfaces are comparable to each other with an average value of $-113$ kJ·mol$^{-1}$, lower than the crystal cohesive energy ($-132$ kJ·mol$^{-1}$). Experimental TG desorption analysis of crystalline CTZ shows an experimental enthalpy of vaporization of about 92 kJ·mol$^{-1}$, reasonably close to the theoretical values. The comparison between computed and experimental cohesive energy for the CTZ crystal shows some overestimation due to the PBE-D2 method.

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**CLOTRIMAZOLE ADSORPTION ON THE SILICA PORE WALL**

Experimentally, CTZ was loaded into MSU-H through scCO$_2$, achieving a maximum drug loading of 34% by mass.

In the simulations, CTZ was adsorbed on a silica surface model described in a previous study by some of us.$^{[105]}$ This surface exhibits a silanol density of 4.5 OH·nm$^{-2}$, close to the experimentally measured value for fully hydroxylated surfaces (4.9 OH·nm$^{-2}$).$^{[106]}$ Of these silanols, only one is free, while the others are interacting through H-bonds. Particularly, three SiOHs cooperates in forming a stable H-bonded chain.
CTZ was manually docked on the surface, aiming at maximizing the interactions between exposed silanols and the different drug’s functional groups. In order to match the experimental conditions, six main starting geometries have been studied. Four of them are characterized by one molecule per silica unit cell (drug loading ~13% by mass), while the other two simulate a molecular layer as observed in previous results\(^8\) with two and three molecules per unit cell (drug loading ~27% and ~41% by mass, respectively) (Figure 2.6).

**Figure 2.6:** View along the z axis of the fully optimized six different geometries of adsorption. a) imidazole (I). b) imidazole (II). c) phenyls (I). d) phenyls (II). e) molecular layer (I): two molecules per silica unit cell. f) molecular layer (II): three molecules per unit cell.

### INTERACTIONS AND ENERGETICS BETWEEN CLOTRIMAZOLE AND THE SILICA PORE WALL

From all the six geometries of figure 2.6, four main types of interaction between CTZ and silanols are observable, figure 2.7. The imidazole ring...
can form both two (Figure 2.7.a) and one (Figure 2.7.b) H-bonds with the surface. H-bonds of the Si-O-H---Cl type (Figure 2.7.c) are weaker than those with imidazole. Several SiOH-π (surface-CTZ, Figure 2.7.d) have been observed in almost all the different structures, while π-π edge to face lateral interactions (CTZ-CTZ) characterize the molecular layer models.

**Figure 2.7:** Silica-CTZ types of interactions.

An essential result that these considerations reveal is that all the computed interaction energies and enthalpies are close to each other, enlightening a possible competition between crystalline and adsorbed CTZ. Assuming that the supercritical incorporation is a step route controlled by dissolution and adsorption of single molecules, this process is almost isoenergetic, since for each adsorption on silica an average of 110 kJ·mol⁻¹ are gained and for each desorption from the crystal 115
kJ·mol\(^{-1}\) are lost. This could be the explanation of the lack of crystallization in pores smaller than 20 times the molecule diameter explained by Sliwinska-Bartkowiak et al.\(^{[103]}\) and observed by other authors.\(^{[41,98,104]}\) In addition TG desorption analysis of CTZ in MSU-H (34% by mass) produces an enthalpy of vaporization of 91.6 kJ·mol\(^{-1}\). As abovementioned, a similar analysis on crystalline CTZ results in a value of 91.8 kJ·mol\(^{-1}\). Thus, also the experimental data suggest that the two processes are almost isoenergetic, supporting the hypothesis on silica-induced drug amorphization.

MODEL OF ADSORPTION: NITROGEN ADSORPTION AND TG ANALYSIS

As previously described, from the experimental N\(_2\) adsorption isotherms, the PSDs, before and after the scCO\(_2\) incorporation of CTZ, can be obtained. These show that the drug incorporation reduces the mean pore diameter from 86 Å of bare MSU-H to a lower value of 67 Å (figure 2.2). Clearly, with the above mentioned approach, the calculated PSDs are unable to describe correctly the experimental results.\(^{[8]}\)

![Figure 2.8: Adsorption model. (I) and (II) side views along the a direction of molecular layers (I) and (II), respectively.](image)

On the other hand, experimental TG analysis reports an incorporated quantity of 34% by mass, which is in between the loading calculated for layer (I) and layer (II), which are ~27% and ~41% by mass, respectively.
By calculating the experimental planar concentration of CTZ (molecules per nm$^2$) and comparing it to our theoretical models of molecular layers (2 CTZs/cell, Figure 2.6.e and 3 CTZs/cell, Figure 2.6.f), it is shown that the real system can be described by a 50:50 mixture of the two molecular layer geometries. Indeed, the unit cell of the simulated silica surface has an area of 1.6 nm$^2$ and the experiment reports 2.5 CTZ molecules per 1.6 nm$^2$. As a consequence, a simulated MSU-H-CTZ PSD has to be calculated, starting from the experimental PSD of bare MSU-H, assuming thicknesses representative of both the molecular layer (I) and (II) models. These thicknesses have been evaluated following the Connolly surfaces[107] before and after CTZ adsorption of the computed models, with the purpose to take into account the vdW molecular volume and the roughness generated by the statistical distribution of 2 and 3 CTZ molecules per 1.6 nm$^2$. Therefore, the molecular layer surfaces have been discretized in 677 squares (0.25 Å$^2$) in order to evaluate the thicknesses, for each point, between the starting silica model and the molecular layers. Subsequently, a new PSD has been calculated for each couple of evaluated thicknesses and all curves have been combined together in the final theoretical PSD of figure 2.2.I.c. Such procedure results in an impressive agreement between simulation and experiment, validating the data interpretation.

INCREASING THE CTZ ADSORBED QUANTITY

Due to the prefect agreement of this modelization, three different samples with increasing fillings were prepared using scCO$_2$ process. The aim was to obtain information on CTZ interaction with silica pore wall at increasing loading. Through XRD analysis it was possible to evaluate the presence or not of CTZ crystals. None of the samples show diffraction peaks of CTZ.
Nitrogen adsorption isotherms, figure 2.9, evidence a linear decrease in comparison to the loaded quantity of SSA and pore volume. In table 2.3 are reported the detailed values. To the best of our knowledge, the only literature crystalline CTZ density data is a calculated number reported in the work of H. Song et al.\textsuperscript{[66]} Using a helium picnometer, a value of 1.31 g·cm\textsuperscript{-3} has been obtained, in perfect agreement with the literature data. Interestingly the calculated CTZ density inside the mesopore, reported in Table 2.3, increase with the loading but never reach the crystalline number. As already demonstrated, the CTZ is amorphous but the linear increase of density with the loaded quantity could be due to a decreasing pore occlusion. In addition to this, as evidenced by the position and shape of the hysteresis loop, the mean pore diameter of the mesoporous channels decrease with the formation of the CTZ layer.

\textbf{Figure 2.9:} Nitrogen adsorption isotherms of bare MSU-H (a), MSU-H-CTZ-12 (b), MSU-H-CTZ-18 (c) and MSU-H-CTZ-34 (d). Below the PSD of bare MSU-H (black) in comparison to MSU-H-CTZ samples (blue); the red curve is
the theoretical PSD corresponding to the experimental loading; from left to right: MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34.

Through NLDFT the PSD of all samples have been calculated. Hypothesizing the adsorption of CTZ as a step process of single molecules that lead to the formation of a layer on the silica wall, the experimental PSD of MSU-H-CTZ can be calculated.\[99\] In order to evaluate the theoretical PSD of figure 2.9 (red), the theoretical docking structure previously described have been used. The same procedure has been changed with some correction in order to take into account the low loading configuration. Briefly, a new PSD has been calculated for each couple of evaluated thicknesses and all curves have been combined together in the final theoretical PSD representative of each different loading (red). For instance, to evaluate the theoretical PSD of MSU-H-CTZ-12, the four single docking geometries (Imidazole (I) and (II), phenyls (I) and (II)) combined to two clear silica surface (Silica as such) have been used as starting thicknesses, resulting in 4056 point of thicknesses. Then, more than 16 million of new PSD, one for each couple of thickness, have been calculated from the PSD of bare MSU-H and combined together in the left red curve of figure 2.9. The use of two clear silica surface is required to obtain the correct planar concentration of CTZ. As it is reported in table 2.3 a loading of 12% by mass corresponds to a surface coverage of $0.4 \text{ CTZ} \cdot \text{Å}^{-2}$, that can be expressed using the theoretical model in a $0.64 \text{ CTZ molecule per silica unit cell}$.

**Table 2.3:** Nitrogen adsorption and TG numerical results compared to theoretical data

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pore volume$^a$</th>
<th>Loaded quantity$^b$</th>
<th>CTZ experimental density$^c$</th>
<th>CTZ·1.606 nm$^{-2}$ $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSU-H</td>
<td>0.900</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS

<table>
<thead>
<tr>
<th></th>
<th>CTZ 12</th>
<th>CTZ 18</th>
<th>CTZ 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSU-H-CTZ-12</td>
<td>0.650</td>
<td>12</td>
<td>1.0</td>
</tr>
<tr>
<td>MSU-H-CTZ-18</td>
<td>0.529</td>
<td>18</td>
<td>1.0</td>
</tr>
<tr>
<td>MSU-H-CTZ-34</td>
<td>0.290</td>
<td>34</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\( a (\text{cm}^3 \cdot \text{g}^{-1}) \) \( b (\% \text{ by mass}) \) \( c (\text{g} \cdot \text{cm}^3) \) obtained dividing the loss in pore volume for the loaded CTZ quantity, normalized to the grams of silica. \( d \) (Number of CTZ molecules per theoretical surface area of the silica unit cell). For MSU-H-CTZ-12, Imidazole (I) and (II), phenyls (I) and (II) and two clear silica surface have been used, obtaining a theoretical planar concentration of 0.66 CTZ / 1.606 nm\(^2\). For MSU-H-CTZ-18, Imidazole (I) and (II) and phenyls (I) and (II) have been used. For MSU-H-CTZ-34, molecular layer (I) and (II) have been used.

SOLID STATE NUCLEAR MAGNETIC RESONANCE

Solid-state NMR measurements were performed in order a) to evaluate the inclusion of CTZ in the OMS; b) to quantify the amount of included CTZ; c) to evaluate the mobility of CTZ inside the silica.

The \(^{13}\text{C} \) CPMAS spectra of pure CTZ, MSU-H, MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34 are reported in Figure 2.10, while \(^{1}\text{H} \) MAS spectra are reported in Figure 2.11. Selected \(^{1}\text{H} \) and \(^{13}\text{C} \) chemical shift values are reported in table 2.4.

The \(^{13}\text{C} \) CPMAS spectra of MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34 are characterized by broad peaks with respect to pure CTZ whose spectrum presents sharp lines typical of highly crystalline systems. The strong overlapping of the signals in the aromatic region (110-150 ppm) prevents any possible assignment. However, the broadness of the resonances and the shift of the \( \text{C}_{\text{sp}3} \) atom on passing from pure CTZ to
loaded MSU-H clearly show that CTZ has been included in the cavities of the silica. Furthermore, the higher width of the peaks for MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34 with respect to pure CTZ proves the amorphous character of CTZ inside MSU-H, which is a positive characteristic since bioavailability usually increases with the amorphization.[97,108]

Figure 2.10: $^{13}$C (100.65 MHz) CPMAS spectra of pure CTZ, MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34. The symbols ▲ denote residues of CH$_2$-CH$_3$ groups from surfactants involved in MSU-H preparation processes.

This agrees with the loss of periodicity associated to the inclusion process which allows for several possible adsorption ways of CTZ on the silica walls as predicted by NLDFT methods.[99] Moreover, at higher loading, the predicted formation of amorphous layers still is consistent with the observed line widths.

The $^1$H MAS spectra are characterized by three main signals (see Table
2.4 and Figure 2.11): from spectrum a, the CTZ hydrogen atoms (H\textsubscript{CTZ}) at 6-7 ppm (III); from spectrum f, free SiOH groups (I) at 1.8 ppm in accordance with the literature;\cite{109} finally, from spectrum b, the SiOH groups in interaction with H\textsubscript{2}O (SiOH-H\textsubscript{2}O) at 4-5 ppm (II). The symbols ▲ denote the impurities derived from the silica synthesis (2-5 ppm). Indeed, as also showed by the FTIR spectra, in accordance with the literature,\cite{110} these are -CH\textsubscript{2} and -CH\textsubscript{3} residues of the involved surfactants. The small differences in the chemical shifts among all samples are related to the different environment and crystal packing of the CTZ molecules in the silica, from isolated sites to layers.

**Table 2.4:** \textsuperscript{1}H and \textsuperscript{13}C selected chemical shifts (δ) with assignments for CTZ, MSU-H, outgassed MSU-H, MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34.

<table>
<thead>
<tr>
<th>Compound</th>
<th>\textsuperscript{13}C</th>
<th>\textsuperscript{1}H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-C-Cl</td>
<td>C-N</td>
</tr>
<tr>
<td>CTZ</td>
<td>145.1</td>
<td>119.1</td>
</tr>
<tr>
<td>MSU-H\textsubscript{outgassed}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSU-H</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSU-H-CTZ-12</td>
<td>145.0</td>
<td>120.4</td>
</tr>
<tr>
<td>MSU-H-CTZ-18</td>
<td>144.0\textsuperscript{sh}</td>
<td>121.6\textsuperscript{sh}</td>
</tr>
<tr>
<td>MSU-H-CTZ-34</td>
<td>not detectable</td>
<td>not detectable</td>
</tr>
</tbody>
</table>

\textsuperscript{sh} shoulder. \textsuperscript{bb} broad band.
Figure 2.11: $^1$H (400.23 MHz) MAS spectra of pure CTZ (a), MSU-H (scCO$_2$ treatment) (b), MSU-H-CTZ-12 (c), MSU-H-CTZ-18 (d), MSU-H-CTZ-34 (e) and MSU-H (outgassed) (f).

The most important information can be extracted from the variation of the relative intensities of the signals. Indeed, while the MSU-H spectrum (b) is almost completely dominated by the SiOH-H$_2$O signal, the spectra of MSU-H-CTZ-12, MSU-H-CTZ-18 and MSU-H-CTZ-34 are characterized by a progressively enhancement of the signal around 6-7 ppm attributed...
to H<sub>CTZ</sub> atoms. Furthermore, the SiOH peaks reduce their intensity on passing from pure MSU-H to MSU-H-CTZ-34, from lowest to highest loading.

As already reported in table 2.4, from the experimental TG results, in comparison to the N<sub>2</sub> adsorption measurements, the number of CTZ per theoretical surface area of the silica unit cell (1.606 nm<sup>2</sup>) can be evaluated. This value, in table 2.5, has been reported as molecules per square nanometre. With the same approach, also the H<sub>2</sub>O content has been calculated.

**Table 2.5:** Correlation between experimental TG analysis, N<sub>2</sub> isotherms, theoretical molecule distribution and SSNMR.

<table>
<thead>
<tr>
<th></th>
<th>TG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CTZ·1.606 nm&lt;sup&gt;-2&lt;/sup&gt;</th>
<th>CTZ&lt;sup&gt;b&lt;/sup&gt;</th>
<th>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sup&gt;c&lt;/sup&gt;</th>
<th>H&lt;sub&gt;CTZ&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt;</th>
<th>H&lt;sub&gt;SiOH·H&lt;sub&gt;2&lt;/sub&gt;O&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt;</th>
<th>SSNMR&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>4.5</td>
<td>0.0</td>
<td>13.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>0.64</td>
<td>0.40</td>
<td>3.0</td>
<td>7.0</td>
<td>10.5</td>
<td>1.0:1.6</td>
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</tr>
<tr>
<td>18</td>
<td>1.00</td>
<td>0.62</td>
<td>3.0</td>
<td>10.6</td>
<td>10.5</td>
<td>1.0:1.0</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>2.50</td>
<td>1.56</td>
<td>0.0</td>
<td>26.5</td>
<td>4.5</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>(% by mass) obtained from TG analysis (Figure S1), <sup>b</sup>(Number of CTZ molecules per theoretical surface area of the silica unit cell), <sup>c</sup>(CTZ molecules per nm<sup>-2</sup>) obtained from TG results and NLDFT elaboration of experimental nitrogen adsorption isotherms, <sup>d</sup>(H<sub>2</sub>O molecules per nm<sup>-2</sup>) obtained from TG results and NLDFT elaboration, <sup>e</sup>(H<sub>CTZ</sub>·nm<sup>-2</sup>), <sup>f</sup>(H<sub>H<sub>2</sub>O</sub>·nm<sup>-2</sup>), <sup>g</sup>SSNMR ratio between the peaks attributed to H<sub>CTZ</sub> and H<sub>SiOH·H<sub>2</sub>O</sub>. As free SiOH surface concentration the value of 4.5 SiOH·nm<sup>-2</sup> has been used, in agreement with the theoretical model surface concentration.[99]

The obtained value of 3.0 H<sub>2</sub>O·nm<sup>-2</sup> from thermogravimetric analysis, compared to the chemical shift of 4.2 ppm, is in agreement with the literature.[111] Indeed, Grünberg et al. reported a value of 4.0 ppm with a surface coverage of 3.6 H<sub>2</sub>O·nm<sup>-2</sup>.

The integral values, obtained from the deconvolution analysis of
quantitative $^1$H spectra, of the CTZ and SiOH-H$_2$O signals at 6.1-7.0 and 3.8-6 ppm, respectively result to be consistent with the previously reported CTZ-silica ratios per unit cell.$^{[99]}$ Indeed, for instance, the $^1$H spectrum of MSU-H-CTZ-12 is characterized by a relative intensity of the H$_{\text{SiOH-H}_2\text{O}}$ and the H$_{\text{CTZ}}$ resonances of 1.6 to 1. This is consistent with the reported data of 0.64 CTZ·1.606 nm$^2$, experimentally obtained and theoretically confirmed. Accordingly, in the MSU-H-CTZ-18 situation the H$_{\text{CTZ}}$ and H$_{\text{SiOH-H}_2\text{O}}$ signal intensities are comparable (1:1). This, again, is coherent with the experimental and theoretical evidenced mode of interaction. On the other hand, a slight chemical shift of the MSU-H OH peaks is evident. This small shift has been attributed to an increase of H-bonds between SiOH and CTZ, as evidenced by FTIR and theoretical calculations. Indeed, other SSNMR analysis of guest molecules evidenced this chemical shift. Of particular interest is the work of Shenderovich et al.$^{[112]}$ reporting the chemical shift of SiOH in interaction with pyridine (9.9 ppm). For the same reason, hypothesizing a higher chemical shift, in the case of MSU-H-CTZ-34, no ratio has been evaluated.

It can be concluded that, during the scCO$_2$ process, the CTZ molecules slowly replace H$_2$O molecules in the H-bond interaction with SiOH. Indeed, the number of H-bonds formed remains constant, changing the guest molecule. The reasons should be a stronger interaction of the SiOH-CTZ, as evidenced by higher FTIR redshift, theoretical energy of interaction and experimental energy of desorption.$^{[99]}$ On the other hand, has been evidenced by theoretical calculation that an interaction between SiOH and guest molecule is favoured by the H$_2$O presence.$^{[113]}$ Consequently, the cause remains uncertain and could include the hydrophobicity nature of CTZ that, during scCO$_2$ process, builds up a hydrophobic molecular layer.

Since, often, the ability of the controlled release of a host molecule inside a guest is also related to its mobility, the dynamic of CTZ inside the silica
has been probe through relaxation measurements. Thus, $^1$H $T_1$ relaxation time measurements were performed on all the samples. Indeed, in the solid state, the $T_1$ is strongly related to the strength of the dipolar interaction, which directly depends on the presence of fluctuating magnetic fields generated by the dynamic behaviors of the whole molecule or of parts of it.

**MOBILITY OF ADSORBED CLOTRIMAZOLE**

Ab-Initio Molecular Dynamics (AIMD) has been performed on the different statically optimized geometries in order to evaluate the stability of the local minimum structures of figure 2.6. Detailed data are reported in a published article.\(^ {99}\) AIMD simulation on the molecular layer (II) structure shows high mobility of the three CTZs (per unit cell) on the surface. The RMSD of the atomic positions along the AIMD simulation shows a large movement between 4 and 7 ps that results in a new configuration, with a RMSD value of 4.3 Å with respect to the starting CTZ conformation after 11 ps. The process is described in Figure 2.12, where the exposed nitrogen atoms of the imidazole rings of the three adsorbed molecules in each unit cell are referred as N1, N2 and N3. The graph in Figure 2.12.a, reporting the N1-SiOH$_{III}$ and N1-SiOH$_{I}$ distances, clearly shows a transition state where the chain is lost and the imidazole’s nitrogen (N1) is equidistant from SiOH(I) and (III). Looking at the potential energy fluctuations during this simulation, we estimate, in a very approximate way, the electronic activation energy of this process as 29.7 kJ·mol$^{-1}$ that can be considered an upper limit for the real value.
Figure 2.12: AIMD of the molecular layer (II) structure: a) N1-SiOH(I) and N1-SiOH(III) bond distances in time. b) starting (A) and final (B) positions of N1 and N2 with respect to the involved SiOHs. c) Top views of the initial (left) and final (right) configurations in the AIMD simulation, with the corresponding interaction energies per CTZ molecule (kJ mol⁻¹); cell borders in pink.

These AIMD results are in accordance with the experimental finding of a "liquid like" behavior of ibuprofen adsorbed in MCM-41.⁴²,⁴³ Considering that CTZ is much more hydrophobic than ibuprofen it is no surprise that CTZ molecular layers are very mobile, despite the significant underneath interactions. This mobility can be represented as a walking step process guided by local changes in the H-bond interactions, helped by a high flexibility of the silica surface silanols.

From an experimental point of view, the ¹H T₁ relaxation time of SSNMR increases from 0.1 s to 80 s by increasing the CTZ loading from MSU-H-CTZ-12 to MSU-H-CTZ-34. This can be attributed to a reduction of
mobility of CTZ inside the silica on passing from the isolated CTZ molecules (higher mobility for low loading) to the formation of a layer (lower mobility for high loading) as previously described.[99] Conversely, pure CTZ is characterized by a $^1\text{H} T_1$ of 90 s, typical of very rigid crystal packing. Summarizing, the evidenced mobility in the molecular dynamics simulations obtained by some of us[99] follows frankly the SSNMR relaxation times. Indeed, in the case of single docking (Imidazole (I) and (II), phenyls (I) and (II)) high energetics phenomena are observed like lose and acquire of H-bonds due to CTZ movement or rotation in place. On the other hand, the molecular layers (I) and (II) show higher cooperation moving as a layer on the silica surface but not among them.

EXPERIMENTAL AND THEORETICAL FTIR INTERPRETATION

Experimental and theoretical vibrational spectra of CTZ in molecular, crystalline and adsorbed environments have been obtained[99]. Figure 2.13 reports both the experimental (blue line) and the theoretical (red line) IR spectra of CTZ in interaction with silica at low and high coverage. The low coverage experimental spectrum has been acquired on a MSU-H-CTZ sample with a drug loading of 18% by mass, while the high coverage corresponds to a drug loading of 34% by mass. As regards the simulated spectra, the CTZ modes have been obtained in both cases from a vibrational analysis in the harmonic approximation of the drug in the four single molecule configurations, combined by Boltzmann weighting their contribution to the infrared intensity according to the computed interaction energies.
Figure 2.13: Experimental and simulated IR: experimental FTIR spectrum of MSU-H-CTZ (blue); theoretical IR spectrum of CTZ combined with SiOH vibrational contribution (red); simulated IR spectrum of SiOH vibrational contribution of: imidazole (I) (dot-dashed black line); imidazole (II) (dashed black line); phenyls (I) (dotted black line); phenyls (II) (black line). At the bottom of the graph, sticks represent the theoretical SiO-H stretching frequencies without Pimentel’s correction.
For both the low and high loading cases, the agreement between theory and experiment is remarkable and helps the interpretation of the signals. A more detailed description can be read elsewhere.\[99\]

As can be deducted from figure 2.13, increasing the CTZ loading results in an increasing number of H-bonds between SiOH and CTZ molecules. Indeed, the 3750 cm\(^{-1}\) sharp band, decrease gradually while the 3500-2600 cm\(^{-1}\) broad band increase. At the highest loading obtained there are no free silanols. This shift is attributed to the formation of H-bonds between SiOH and CTZ through the exposed nitrogen of the imidazole ring. At the same time, other types of H-bonds are formed with the CTZ molecules (SiOH-\(\pi\), SiOH-Cl) that are responsible to the change in the 3700-3500 cm\(^{-1}\) band. Starting from SiOH-SiOH interaction in the bare MSU-H, they evolve gradually in a mixture of the same H-bonds mixed to interactions with CTZ (SiOH-\(\pi\) and SiOH-Cl). To demonstrate this, the broad background at 3700-3500 cm\(^{-1}\) responsible to the increase of the 3620 cm\(^{-1}\) peak, more substantial in MSU-H-CTZ-12, almost disappears in MSU-H-CTZ-34. These conclusions reveal the direct interaction between CTZ and mesoporous silica wall until the formation of a CTZ molecular layer.

2.3 MCM-41 FILLING MODEL

TG analysis and nitrogen adsorption measurement comparison applied to MCM41-1 do not lead to the same conclusions. While FTIR analysis presents the same adsorption band, the mesopores of MCM41-1 results filled of CTZ molecules without homogeneity. Indeed, no empty pore are visible after the scCO\(_2\) process. In chapter 3 a more detailed description is given, but here are reported preliminary theoretical discussion of a new
model of incorporation.

As mentioned above, in the case of MSU-H, the pore diameter (8.5 nm) allows us to consider the silica surface as flat when CTZ is docked. For MCM-41 this assumption is not anymore satisfied. Consequently, the surface model has been modified following the MCM-41 model presented in the article of Delle Piane et al.\textsuperscript{[84]}

\textbf{Figure 2.14:} Longitudinal view of the MCM-41 model (I). (II) and (III) simulation of the filled CTZ channel.

The MCM41-CTZ model has been optimized at PBE-D2 level of theory. The basilar information that can be extracted is that 9, out of 11 CTZ molecules, are interacting with the silica surface. The last 2 are in a position similar to the molecular layer (I) and (II). In addition, the number and energies of interactions are higher. This discussion reveals in a
simplified way the motivations of the MCM41-1 filling during the scCO₂ incorporation process.
CHAPTER 3: SILICAS DETERMINING FACTORS IN THE INCORPORATION PROCESS

ABSTRACT

The knowledge of the specific interactions between the surface of mesoporous silica and drugs took to comprehend the different type of adsorption geometries depending on the pore size dimension. However, such knowledge is still preliminary.

In this chapter a complete experimental description on these factor have been argued. Fourteen mesoporous silica have been incorporated trough scCO₂ process and completely characterized in order to understand the experimental discriminant factors. In the first part, a characterization of all OMS as such is reported; after that, the different behaviors are described; finally, some argumentation are stated.

3.1 OMS CHARACTERISTICS

The 14 mesoporous (Table 3.1) silicas were selected for different characteristics and considerations. One mesoporous silica is, to be correct, a disordered microporous silica, Syloid. Four MCM-41, were synthesized to screen low pore diameter silicas. One commercial MCM-41 was necessary to avoid laboratory behaviors. Four KIT-6 were manufactured to control if Ia3d silicas have the same behavior. Two commercial and one synthetic SBA-15 were used to point out the conduct with high pore dimension. The MSU-H was reprocessed to control the supercritical process and the reproducibility of the results.

Table 3.1: NLDFT parameter of all the OMS used. They have been ordered according to the mean pore diameter that is not ever the maximum of the PSD.
From table 3.1 and figure 3.1.I it can be evidenced that the OMS are mainly divided in two categories: silicas with mean pore diameter lower than 4.88 nm and silicas higher than this. Indeed, two main straight line can be verified from the graph 3.1.I. This categorization is due to the wall thickness. It is well clear that MCM-41 and SBA-15 silicas have this differences. It is strange that also Syloid and KIT-6 respects this condition.
All the OMSs have been characterized by TG, XRD, FESEM analysis. No important result for this correlation have been noted.

### 3.2 CHARACTERIZATION OF INCORPORATED OMS

All the OMS have been incorporated through scCO$_2$ at 250 bar and 373K for 18 hours. No further incorporation was possible increasing the total time. All the characterization analysis already described were repeated on the incorporated materials. Absolute data are, first of all, the maximum incorporated quantity: 46% by mass obtained with MCM41-1. Secondly, in no circumstances crystalline CTZ was observed. Thirdly, all the N$_2$ adsorption measurements reported a decrease of SSA and pore volume. In some cases, also the mean pore diameter was decreased.
Figure 3.2 reports some of the nitrogen adsorption measurements done. It can be observed that for Syloid and MCM-41, after the supercritical process, there is almost no residue porosity (no step or hysteresis) and very lower surface area (lower inclination). On the other hand, MSU-H and KIT6-130 still have large empty porosity. A border case is the KIT6-50.

According to chapter 2, it can be argued that the first OMSs are filled of CTZ as the theoretical modelization of figure 2.14, while in the second group CTZ is adsorbed as a molecular layer, figure 2.8. Trying to understand the limiting pore size dimension, a further analysis has been done. Figure 3.3 reports the incorporated quantity in function of the NLDFT pore volume. In this case the incorporated quantity is calculated as the CTZ content on the silica content.
**Figure 3.2:** Nitrogen adsorption measurements before (OMS) and after the scCO$_2$ process (OMS-CTZ).

This correlation evidences which OMSs follows the filling model. On the other hand, an $R^2$ of 0.93 is not a good report; but, considering the error bars, all the data are consistent. The linear equation has been selected without constant term thinking to the fact that the mesopore could be filled or empty. On the contrary, in no cases the external SSA was taken into account. Indeed, this correlation do not consider the external surface coverage that it is hardly estimable.
Figure 3.3: OMS correlations: Incorporated Quantity ($g_{CTZ/g_{sil}}$) versus NLDFT Pore Volume ($cm^3/g_{sil}$). The red dot reports a border linearity.

At the same time, the correlation of NLDFT SSA with incorporated quantity has been done and reported in figure 3.4.

Figure 3.4: OMS correlations: Incorporated Quantity ($g_{CTZ/g_{sil}}$) versus NLDFT SSA ($m^2/g_{sil}$).

In this case $R^2$ is 0.95 and the slope ($m$) of the linear equation is directly correlated to the number of CTZ·nm$^{-2}$. The results, reported to the surface area of the silica theoretical cell (chapter 2), is 1.7 CTZ·cell$^{-1}$. This result
is lower than the presented one of chapter 2 but also the scCO\textsubscript{2} conditions are lower (250 bar).

As it is possible to observe, not all the silica follows perfectly these relationships. For instance, KIT6-50 and KIT6-80 follow both correlations. Consequently, in order to correctly distinguish all cases, the PSD simulations have been applied for all the OMSs, taking into account the number of CTZ extrapolated from the linear correlation. If it works, it can be defined as a molecular layer; on the contrary, it could follow the filling correlation.

The results, reported in figures 3.5 – 3.6 – 3.7, shows three conditions:

- Figure 3.5: OMSs perfectly filled, without residual porosity.
- Figure 3.7: OMSs with a molecular layer of about 1.7 CTZ/cell;
- Figure 3.6: a condition in between.

Therefore, it seems reasonable conclude that from a mean pore radius of 5.2 to 6.8 nm the behavior is mixed. Probably, also the Ia3d structure of KIT-6 silicas is important. Indeed, the SBA15, which has the bigger mean pore diameter, seems to follows better the filling model, but it suffers more than the others of low diffusivity (2D pore structure versus 3D.)
Figure 3.5: PSD of OMS as such (black), OMC-CTZ (blue) and the calculated PSD (red). In brackets the number of CTZ per unit cell used for the model. These are the silicas that follows a filling theory.
Figure 3.6: PSD of OMS as such (black), OMC-CTZ (blue) and the calculated PSD (red). In brackets the number of CTZ per unit cell used for the model. These are the silicas border line.
Figure 3.7: PSD of OMS as such (black), OMC-CTZ (blue) and the calculated PSD (red). In brackets the number of CTZ per unit cell used for the model. These are the silicas that follows the molecular layer theory.

Table 3.2: OMS correlations resume.

<table>
<thead>
<tr>
<th>OMS</th>
<th>Mean Pore Diameter</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syloid</td>
<td>1.37</td>
<td>Pore Volume</td>
</tr>
<tr>
<td>MCM41-4</td>
<td>2.94</td>
<td>Pore Volume</td>
</tr>
<tr>
<td>MCM41-3</td>
<td>3.65</td>
<td>Pore Volume</td>
</tr>
<tr>
<td>MCM41-ACS</td>
<td>4.57</td>
<td>Pore Volume</td>
</tr>
<tr>
<td>MCM41-1</td>
<td>4.88</td>
<td>Pore Volume</td>
</tr>
<tr>
<td>MCM41-2</td>
<td>4.88</td>
<td>Pore Volume</td>
</tr>
<tr>
<td>KIT6-50</td>
<td>5.28</td>
<td>Both</td>
</tr>
<tr>
<td>KIT6-80</td>
<td>6.55</td>
<td>Both</td>
</tr>
<tr>
<td>SBA15</td>
<td>6.79</td>
<td>Both</td>
</tr>
<tr>
<td>KIT6-100</td>
<td>7.86</td>
<td>SSA</td>
</tr>
<tr>
<td>MSU-H-F</td>
<td>8.14</td>
<td>SSA</td>
</tr>
<tr>
<td>SBA15-ACS</td>
<td>8.14</td>
<td>SSA</td>
</tr>
<tr>
<td>MSU-H</td>
<td>8.46</td>
<td>SSA</td>
</tr>
<tr>
<td>KIT6-130</td>
<td>10.13</td>
<td>SSA</td>
</tr>
</tbody>
</table>
In conclusion, one more correlation need to be reported. From the NLDFT pore volume data and the TG analysis the density of CTZ inside the mesoporous volume can be evaluated. The next equation resumes this correlation:

\[
\text{Incorporated Quantity} \left( \frac{g_{CTZ}}{g_{sil}} \right) = V' \left( \frac{cm^3}{g_{sil}} \right) - \left( V'' \left( \frac{cm^3}{g_{sil+CTZ}} \right) \right) \cdot \left( \frac{g_{sil+CTZ}}{g_{sil}} \right)
\]

where \( V' \) is the OMS NLDFT pore volume as such and \( V'' \) after the scCO\(_2\) process. The incorporated quantity and the ratio \( \left( \frac{g_{sil+CTZ}}{g_{sil}} \right) \) are obtained through TG analysis.

**Figure 3.8:** Incorporated quantity \( g_{CTZ} \cdot g_{sil}^{-1} \) versus NLDFT \( \Delta V \) \( cm^3 \cdot g_{sil}^{-1} \): the density correlation.

The straight line has a slope coefficient \( (m) \) of 1.14 \( g_{CTZ} \cdot cm^3 \). This result matches perfectly with all other correlations (chapter 2). In addition to this, it can be said that all the OMSs that are not on the straight line suffers of pore occlusion. None of these have large amount of CTZ outside the mesoporous volume.
CHAPTER 4: AKS-OMS GEL

ABSTRACT

A possible and promising area of application for Ordered Mesoporous Silica (OMS) is the topical therapy of dermatological diseases and wounds. It is widely known that Active Pharmaceutical Ingredient (API) incorporated in OMS based materials are adsorbed in the volume in an amorphous state and in some cases well distributed on the surface as a single molecular layer. This feature is crucial for many aspects: from the bioavailability enhancement of molecule poorly soluble in water to the effects of reservoir that can be tuned in cream for topical application. Indeed, the use of OMS incorporated of API in a saturated vehicle to the therapeutic concentration develops a controlled and constant release system on the skin site.

Figure 4.1: Ideal description of AKS-OMS gel.

This chapter describes the development of topical composition able to release drug in a sustained and prolonged manner. Incipient Wetness Impregnation procedure has been used to load AKS in commercial OMS of MSU-H type. The incorporated OMS characterization (XRD, FESEM, etc.) are reported in chapter 1. A series of different release test has been performed to validate the new release technology (UV-Vis spectroscopy). As a result, a semisolid formulation with AKS has been developed. The
stability, pH and rheological properties were investigated overtime. In vitro release and permeation studies were assessed using an open chamber diffusion cell system (Franz cell), fitted with semi-permeable membrane or porcine ear skin. All the results emphasize the reservoir effect of OMS incorporated of API. In addition, they demonstrate the availability of a new topical release technology able to reduce the number of administrations.

4.1 SUSTAINED RELEASE FROM OMS-AKS IN AKS SATURATED SOLUTIONS

In order to confirm the reservoir effect of the incorporated OMS in a saturated solution composed by two solvents, AKS, a hydrophilic API, has been studied. Commercially, the more used topical creams which contains AKS have a composition at 5% by mass of API, that corresponds to 6,67% by mass of AKS [114,115]. Therefore, in order to obtain a solvent with a saturation point toward AKS equal to the therapeutic concentration, a solution of glycerol and water has been explored. The best composition is 45% water and 55% glycerol by mass. The saturation point of this solution is 71 mg·ml⁻¹ that correspond to 62 mg·g⁻¹ of AKS. Consequently, the API content is 4.8% by mass. In this case two different AKS saturated solution has been done. In a solution, 80 mg of OMS-AKS (27.5 % by mass) has been added under agitation. Every hour, for 4 times, 100 μl has been withdraw and substituted with the same amount of pure solvent. Figure 4.2 reports the measured concentration in the four withdraw for each sample. The saturated concentration is preserved as long as there is drug that can be solubilized from the OMS. In fact, 22 mg of drug where presents as reservoir and in the three subsequent dilution 21.3 mg where withdraw: 100 μl contains 7.1 mg of API.
Figure 4.2: AKS sustained release with 21.3 mg of API contained in OMS-AKS (a) and without reservoir (b).

4.2 COMPOSITION, RHEOLOGICAL AND pH STABILITY OF AKS-OMS GEL

As previously described an innovative topical semisolid formulation has been prepared and compared to a commercial formulation. All the results here presented are referred to the gel named AKS-OMS gel. Among all the semisolid preparations, a hydrophilic gel was developed, consisting of a liquid phase within a polymeric matrix composed by a suitable gelling agent. Firstly, AKS was added in a solution of glycerol (45% by mass) and water (55% by mass) under magnetic stirring obtaining a saturated solution of the drug. When AKS was completely dissolved, 15% by mass OMS-AKS powder was added to the medium and then the liquid dispersion was purposely homogenized with a high shear homogenizer for 5 min at maximum velocity. Hydroxyethyl cellulose was selected as gelling agent, because it is primarily used in topical pharmaceutical
formulations and it is generally considered as an essentially non-toxic and non-irritant material \(^{116}\). With this purpose, 2\% by mass of hydroxyethyl cellulose was added to the homogeneous dispersion and it was gently stirred for one hour at room temperature.

The gel shows rheological characteristic of shear-thinning fluid. Over one month the viscosity at low shear rate is halved but preserve is pseudoplastic behaviour. The Farrow's constant was 3.4 n and remain the same over the month, going out of linearity only for low shear rate (3.9 n). During the same period, the pH oscillates from 4.75 to 5.0 without the addition of any correctors. It should be noted that this pH is equal to the pH of the commercial formulation.

**4.3 IN-VITRO RELEASE STUDIES**

Figure 4.3 reports the release curves obtained. In the first 100 minutes, AKS-OMS gel follows the commercial formulation. After, the commercial formulation release rate decrease since the AKS dissolved quantity is ending. In that moment, the OMS-AKS reservoir starts to make a difference in the AKS-OMS gel. Indeed, the release rate of AKS-OMS gel remains constant until 200-250 minutes. Finally, also the OMS-AKS reservoir end and the release rate decrease.

The constant release rate in the first 100 minutes proves that the starting dissolved concentration of AKS inside AKS-OMS gel and commercial formulation is the same. The preservation of a constant release rate after 100 minutes of AKS-OMS gel proves that the dissolved concentration of AKS is sustained to the therapeutics concentration until 200-250 minutes. These demonstrate the feasibility of this application as a method to reduce the number of applications during the day.
**Figure 4.3**: In-vitro release test of AKS-OMS gel (a) and the commercial formulation (b). Each point is the mean value of three different release tests. The error bars include the maximum variation obtained from all the different trials.

### 4.4 IN-VITRO PERMEATION STUDIES

In order to evaluate differences in permeation of the two semisolid formulation on a skin site, a slice of porcine ear skin has been mounted on the Franz cells (Figure 4.4). Until 100 minutes, as the release test, the permeated quantities are small and similar. After, the AKS permeated from AKS-OMS gel exceed the commercial formulation quantity. This is in agreement with the release studies that show the depletion of the commercial formulation after 100 minutes. Consequently, the permeation of AKS is influenced from the depletion of the commercial formulation. On the other hand, the constant concentration present in the AKS-OMS gel develop an increase in the permeated quantity. The complete permeation of AKS from the commercial formulation requires 24 h, while for AKS-
OMS gel it exceeds 48 h. After 4 days, no more AKS permeation is observed.

**Figure 4.4:** In-vitro permeation test of AKS-OMS gel (a) and the commercial formulation (b). On the left (I) the first 350 minutes; on the right (II) the
These results show that a constant concentration over the skin site for a prolonged time extend and enhance the permeation of API. The first result was intended. On the other hand, the enhancement could produce an overtreatment, solvable reducing the saturation point of the vehicle. This solution leads to more improvement of the new semisolid formulation. Indeed, reducing the dissolved starting therapeutic concentration will increase directly the duration of the stored AKS. Secondly, the same antibiotic effect could be achieved with less dosage on the application site.

4.5 TOPOLOGICAL INFORMATION ON OMS PERMEATION

Numerous works study the possible permeation of OMS through the skin site of application [117–119]. To the best of our knowledge, none of these papers reports a direct permeation of particles greater than 10 nm. On the other hand, it is well labelled the possibility for these particles to enter skin pores and hair follicles, enhancing the drug release [117]. In order to observe the OMS-Skin interactions, during the permeation studies, different experiments were stopped at 6, 24, 48 and 72 h (Figure 4.5). Between 6 to 48 h, the OMS particles are in aggregate on the skin. These aggregates are formed during samples preparation for FESEM analysis. However, after 72h, OMS are dispersed in the first 200 μm of thickness and are hardly to be distinguished. Therefore, OMS-Skin interaction is weakly and a long period is required to OMS particles to stick to skin pores and hair follicles.
Figure 4.5: FESEM image of the In-vitro permeation test of AKS-OMS gel: 6, 24, 48 and 72h. OMS particles in the form of aggregates are observable very bright in the upper left part of 6h (a), bright in the upper right of 24h (b), hardly visible in the upper part of 48h (c). Image (d) reports the OMS dispersion on the skin after 72h.
ABSTRACT

Controlled drug delivery from Ordered Mesoporous Silica (OMS) platforms represents the possibility to achieve sustained or prolonged drug release in different administration routes widely used, from the oral to the parenteral one \[^{[93]}\]. Nevertheless, exploring the literature it is quite evident that much research work have been addressed to investigate OMS nanoparticles as drug delivery system for the oral route, in particular for poorly-water soluble molecules, or for parenteral systems releasing active pharmaceutical ingredients after a triggered external stimuli \[^{[10,30,97,120]}\]. To the best of our knowledge, only few papers have been published about the possibility to use silica-based mesoporous nanoparticles for topical application \[^{[121–126]}\]. Skin is generally used as route of delivery for local and systemic drugs.

This chapter presents the development and application of the new controlled release system with CTZ. The complete physicochemical characterization of the OMS incorporated with API has been totally described in the previous chapter. Subsequently, in this section are reported only the release tests of different saturated vehicle to ensure the effectiveness of the controlled system. Afterwards, the preparation and characterization of an innovative topical semisolid formulation for CTZ comparable to commercial formulations. Finally, sustained release of CTZ has been developed and outlined with vertical glass diffusion cells.
In parallel with the sustained release test of AKS, also CTZ has been tested. For CTZ the typical topical creams are between 1 and 2.5% by mass of API content \cite{65}. Therefore, a solution of 1,2-propanediol and water has been studied. The best conditions were with 85% 1,2-propanediol and 15% water by mass. This solution has a saturation point of 25 mg·ml$^{-1}$ that corresponds to 2.4% by mass of API. The same procedure used for AKS has been followed and in figure 5.1 are reported the measured concentration. The concentration decrease only after the complete solubilisation of the incorporated API. Each withdraw of 100 μl contains 2.5 mg of API and the reservoir of OMS-CTZ was 7 mg (70 mg of OMS-CTZ at 10%).
5.2 COMPOSITION AND IN-VITRO RELEASE TEST

A topical semisolid formulation has been prepared and compared to a commercial formulation. In this case the CTZ gel, named CTZ-OMS gel, is a hydrophobic semisolid preparation. It consists of 85% 1,2-propandiol and 15% of bidistilled water. When CTZ is dissolved, 10% by mass OMS-CTZ powder was added to the medium. In this case MSU-H-CTZ-34 has been used. Then, the liquid dispersion was purposely homogenized with a high shear homogenizer for 5 min at maximum velocity. As gelling agent hydroxyethyl cellulose was selected as gelling agent, 2% by mass. The gel shows rheological characteristic of shear-thinning fluid.

Figure 5.2: In-vitro release test of CTZ-OMS gel (a) and the commercial
formulation (b). Each point is the mean value of three different release tests. The error bars include the maximum variation obtained from all the different trials.

Figure 5.2 reports the in-vitro release curves obtained. In this case, for the first 2h, CTZ-OMS gel follows the commercial formulation. The commercial formulation release rate bends and stops around 5h from the start. On the other hand, CTZ-OMS gel continues almost linearly until 24h.

The constant release rate in the first 2 hours verifies that the starting free concentration of CTZ inside CTZ-OMS gel and the commercial formulation is the same. The preservation of a constant release rate until 5-6 hours proves the capability for CTZ-OMS gel of a sustained release to the therapeutics concentration. Unfortunately, the release rate seems to decrease gradually and not uniformly like the commercial formulation. Probably more tests should be done from 7 to 24 hours in order to control better the release profile of CTZ-OMS gel.
CONCLUSIONS

In this PhD work Ordered Mesoporous Silica has been studied and used for a new controlled release technology for topical applications: starting from the physicochemical characterization to the development of real formulations.

In the first chapter, has been shown the positive and negatives aspects of different incorporation techniques based on hydrophilicity/hydrophobicity of Clotrimazole and Amikacin Sulfate. This have been correlated with dissimilar type of OMS.

With Clotrimazole a theoretical-experimental comparison between results has been done tackling phenomena like: mobility, solubility, bioavailability, etc. All the theoretical and experimental results were in line each other.

Strong of this correlation the differences between types of OMS have been highlighted trying to explain the spatial assembly of drug inside the mesoporous channels of OMS in function of pore dimensions.

Finally, the new controlled release technology has been discussed and demonstrated in its functionality for Clotrimazole and Amikacin Sulfate. Both systems have demonstrated double release time without leaving the therapeutic concentration.
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112


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114


APPENDIX I – LIST OF PUBLICATIONS AND CONGRESS

PUBLICATIONS

- Gignone, A.; Delle Piane, M.; Corno, M.; Ugliengo, P.; Onida, B. Simulation and Experiment Reveal a Complex Scenario for the Adsorption of an Antifungal Drug in Ordered Mesoporous Silica
- Sustained release of Active Pharmaceutical Ingredients from Ordered Mesoporous Silica (in Preparation)
- Topical administration of Amikacin through OMS (in Preparation)

CONGRESS

Simulation and Experiment Reveal a Complex Scenario for the Adsorption of an Antifungal Drug in Ordered Mesoporous Silica

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3Supporting Information

ABSTRACT: Ordered mesoporous silicas have been widely investigated as drug carriers in several fields, from tissue engineering to cancer therapy. The knowledge of the specific interactions between the surface of mesoporous silica and drugs is necessary to guide development of new and improved drug delivery systems. However, such knowledge is still scarce, due to the arduous interpretation of experimental results. In this work, we characterize the incorporation of clotrimazole, a common antifungal drug, inside ordered mesoporous silica by means of a joint computational and experimental approach. Experimentally the drug was loaded through supercritical CO2 and its adsorption investigated through infrared spectroscopy, N2 adsorption isotherms, and thermogravimetric analysis. Modeling involved static and dynamic Density Functional Theory simulations of clotrimazole adsorbed on realistic models of amorphous silica surfaces. A good agreement between the computational and the experimental results was obtained, concerning the energies of adsorption, the infrared spectra, and the distribution of drug inside the mesopores. However, a complete interpretation of the experimental results was possible only when simultaneously considering all the complex aspects of the drug–silica interaction. Indeed, the combination of both approaches allowed us to describe the drug–silica interface as a mix of multiple interaction configurations, based on a subtle balance of hydrogen bonding and dispersion interactions. Furthermore, at high drug loading, clotrimazole molecules are statistically distributed on the pore walls, forming an adsorbed molecular layer. Finally, notwithstanding the stable interactions, the drug still exhibits a significant mobility at room temperature, moving on a complex potential energy surface, as revealed by molecular dynamics simulations.

INTRODUCTION

Over the past three decades, a rapid growth has affected the research area of drug delivery, aiming at optimizing drug efficacy while simultaneously reducing adverse collateral effects.1 Several studies have reported that pharmacokinetics, drug efficacy and expression of unwanted side effects in different pathological conditions can be improved by correct timing of drug administration and controlled kinetics of drug release.2 Recently, the interest has concerned the use of mesoporous materials as controlled drug delivery matrices thanks to their unique properties: uniform mesoporous structure, high surface area, tunable pore size, and well-defined surface properties.3–8

In this context, Ordered Mesoporous Silicas (OMSs) have been widely investigated as drug carriers in several fields, from tissue engineering to cancer therapy.1 Initially, the research on OMSs for drug delivery was focused on the achievement of controlled release formulations. The release kinetics of drugs by OMSs depends on several carrier properties, including pore size, pore connectivity, and the chemisorption of the surface.9 A recent emerging feature of OMS carriers is the enhanced solubilization of molecules poorly soluble in water.9 It has been shown that both small and large molecular drugs can be entrapped within the mesopores by an impregnation process and liberated via a diffusion controlled mechanism.10

In the first pioneering work by Valler-Regli et al.,11 drug incorporation in MCM-41 was carried out by adsorption from a solution using benzene as solvent. Since the long-term toxicity of benzene in humans is well-known,12,13 many other solvents have been studied for the incorporation. The incorporation by supercritical carbon dioxide (scCO2) is an alternative to adsorption or impregnation from a liquid solution.14–16 Carbon dioxide is one of the most commonly used fluid in supercritical fluid technology. Its main advantages are the critical temperature close to ambient temperature (304.35 K) and a not too high critical pressure (7.39 MPa).17 In addition, it is nonflammable, has low cost, and has low toxicity. Being a supercritical fluid, its physical properties are halfway between a gas and a liquid. In particular, it has a solvent power like a liquid and a high diffusivity like a gas. As pressures and temperature not too far from its critical point, a supercritical fluid has a high compressibility, therefore its density and hence its solvent power are easily adjustable over a wide range with a minimal change in temperature or pressure.

Received: March 19, 2015

ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS
The Journal of Physical Chemistry C

When a drug is incorporated inside a material, such as mesoporous silica, the interactions occurring at its surface are of great importance. They can deeply influence stability, dissolution, and manufacturability of the formulation and, for drug delivery, play a crucial role in determining the final performance of the product, that is, maximum loading, release profile, and shelf life. For these reasons, the development of improved pharmaceutical formulations requires an understanding of the molecular mechanisms occurring at the silica–drug interface. Molecular modeling can be an important tool in addressing the problem of studying drug–silica interactions. One of the main challenges in this area is the accurate simulation of the system and the interpretation of the results. The main objectives of this work were to develop a model of the interaction between a drug and silica, to study the effect of the silica–drug interaction on the drug release, and to correlate the results with the experimental data. The computational approach presented here is based on the use of the Materials Studio software, which allows for the simulation of the system at a mesoscopic level. The results obtained from this approach were compared with the experimental data, and the agreement was found to be satisfactory. The simulations showed that the drug–silica interaction is the key factor in determining the drug release rate. The computational approach presented here is a valuable tool for the development of new drug formulations and for the optimization of existing ones.
Figure 1. (a) Chemical structure of the CTZ molecule (H₂N₂C₈H₆O₃). (b) 3D space-filling model of the CTZ micellar structure with the three phenyl rings almost perpendicular to the indole ring. (c) CTZ electrostatic potential mapped on the electron density showing the high electrostatic potential at the external nitrogen in the indole-like ring group. Blue, green, and red colors correspond to positive, neutral, and negative values of the electrostatic potential (range of values: NOD = -0.75 e, MAX = 0.75 e). (d) Top view of the amorphous silica surface model used in this paper, all bonds in black (r₁ = 1.2 Å), r₂ = 1.8 Å, and r₃ = 3.1 Å, H₂O(SiO₂-

The Journal of Physical Chemistry C.

Ordered Mesoporous Silica for Drug Delivery in Topical Applications

triple-C basis set with polarization functions (1TZVP) augmented with the empirical Grimme's D3 correction. The cut-off for the plane wave basis was set to 400 Ry. All simulations were run at 300 K in the NVT ensemble using the Car-Parrinello Molecular Dynamics (CPMD) program. All simulations were equilibrated at 300 K with a more stringent thermostat (time constant: 10 fs) for about 1 ps and then the production phase was run for at least 10 ps with a more relaxed thermostat (time constant: 20 fs). Since CPMD requires 3D periodic boundary systems, a value of ε = 35 Å was chosen to separate the slab replicas with enough vacuum. In all cases, only the superficial layer of the silica slab and the drug molecules were allowed to move.

Characterization. Samples were characterized by means of Fourier transform infrared spectroscopy (FTIR), nitrogen adsorption desorption isotherms, and thermogravimetric analysis (TGA). FTIR spectra were recorded in the region 4000–600 cm⁻¹ using a KBr pellet technique. Nitrogen adsorption-desorption isotherms were measured using a Quantachrome Autosorb-iQ1 instrument after degassing at 150 °C for 2 h. Brunauer–Emmett–Teller (BET) surface areas (S BET) were calculated from the relative pressure range 0.01–0.99 using Micromeritics BET method. TGA analysis were carried out between 25 °C and 1000 °C in air (flow rate 100 ml/min) with a heating rate of 10 K/min using a SETARAM 92 instrument to evaluate the quantity of incorporated drug. The sorption analysis were performed using a SETARAM 92 instrument following the procedure explained by Yurkov et al. and Pire et al. Using the Clausius–Clapeyron equation, the enthalpy of sorption (ΔH) at the average temperature of investigation was obtained:

\[
\ln \left( \frac{T}{\text{dln}} \right) = \frac{\Delta H}{R} + \frac{\Delta H}{RT}
\]

where d/dT is the mass loss rate at the specified temperature, k is Avogadro's number, T is the mean gas constant, J mol⁻¹ K⁻¹, and T is the temperature of the isothermal experiment. Subsequently, vaporization enthalpies were reported to 298.15 K using a general method of correction, reported by Quikh et al. (the Harkins–Jura method).

\[
\Delta H_{vap}(298.15 K) = \Delta H_{vap}(T) + \frac{0.00047(C - 298.15)}
\]

In eq. 3, T is the temperature of measurement or mean temperature of measurement (if ΔHvap(T) has been obtained from a Clausius–Clapeyron treatment of vapor pressure). The experimental conditions used for the reliable determination of vaporization enthalpies of low volatile molecular compounds are following the references of Yurkov et al. A calibration curve with phenol has been done. The uncertainty of temperature calibration was less than 1 °C.

Results and Discussion

Clinical and Material and Crystal. CTZ was modelled both in gas phase and as a crystal, before studying its adsorption on amorphous silica. The starting point was the X-ray experimental structural by Sönös et al. CTZ has a tetrahedral structure (Figure 1a) with the central sp² carbon linked to two phenyl rings, one chlorophenyl ring and one indole-like ring. The CTZ molecule, optimised at B3LYP-D3 level of theory, is reported in Figure 2a. The electrostatic potential mapped on the B3LYP-D3 electronic density (Figure 2a) clearly shows the monopolar character of the external nitrogen in the indole-like ring group, which is expected to behave as an H-bond acceptor when interacting with the surface silanols. The rest of the molecule is generally apolar, as reflected by the experimentally measured low solubility in water (less than 0.01 g L⁻¹) and is

ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS
expected to engage in dispersive interactions with the silica surface.

The CTZ crystal has been optimised with and without Grimme's correction and compared to experimental results (Figure S1a and Table S1, in Supporting Information). The structure is triclinic (P1, a = 8.76 Å, b = 10.55 Å, c = 10.61 Å, and α = 114.1°, β = 97.9°, γ = 97.9°) with two drug molecules per unit cell. If dispersion is not included in the calculation, the cell volume is underestimated by 43.5%, while inclusion of dispersion contributions leads to a cell contraction of -8.4% with respect to angle crystal X-ray diff. The PBE-D3 cohesive energy of crystalline CTZ has been computed as -145.5 kJ mol⁻¹ (Table 1) and, unexpectedly, the interactions (Figure S1b in Supporting Information). The added CTZ molecule maximizes the contact with the surface molecules mainly through dispersion and electrostatic interactions. These are in particular in close proximity to the interactions of phthaloyl and chlorophenyl. Dimer interactions (in fact, face to face) are not present due to steric hindrance and rigidity of the molecule. Table 1 reports the computed interaction energies of CTZ on the three crystal surfaces together with the corresponding enthalpies at 298 K. Unsurprisingly, the interaction energies of CTZ with its crystal surfaces are comparable to each other with an average value of -113 kJ mol⁻¹, lower than the crystal cohesive energy (-135 kJ mol⁻¹) due to the reduced number of intermolecular interactions of the adsorbed drug with the surface compared to those in the bulk.

Table 1. Energetics of the Chitinacdoz and MSU-H-CTZ Systems

<table>
<thead>
<tr>
<th>System</th>
<th>ΔE¹</th>
<th>ΔE²</th>
<th>ΔE³</th>
</tr>
</thead>
<tbody>
<tr>
<td>chitinacdoz (bulk)</td>
<td>-31.1</td>
<td>-40.5</td>
<td>-51.7</td>
</tr>
<tr>
<td>chitinacdoz (p(110))</td>
<td>-31.4</td>
<td>-40.9</td>
<td>-51.6</td>
</tr>
<tr>
<td>chitinacdoz (p(201))</td>
<td>-28.5</td>
<td>-37.6</td>
<td>-48.0</td>
</tr>
<tr>
<td>molecular (bulk)</td>
<td>-28.4</td>
<td>-37.6</td>
<td>-47.4</td>
</tr>
<tr>
<td>molecular (p(110))</td>
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<td>-37.5</td>
<td>-47.1</td>
</tr>
<tr>
<td>molecular (p(201))</td>
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<td>-37.6</td>
<td>-47.0</td>
</tr>
<tr>
<td>CTZ bulk E-D3 (p(110))</td>
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<td>-35.8</td>
<td>-45.3</td>
</tr>
<tr>
<td>CTZ bulk E-D3 (p(201))</td>
<td>-26.4</td>
<td>-35.8</td>
<td>-45.2</td>
</tr>
</tbody>
</table>

*Computed for selected systems: electronic interaction energy, without accounting for dispersion, corrected for BSSE. **Computed allowing dispersion. Dispersive energy of adsorption (dispersion included), at T = 298 K. **Electronic energy of covalent CTZ, computed with respect to a single molecule gas phase. Reaction of one CTZ molecule adsorbed on the (110), (100), and (010) faces of the CTZ crystal. Molecular systems for naming, refer to Figures 2 and 3. 

Experimentally measured reaction enthalpy of CTZ (bulk), adsorbed at T = 298 K. 

Experimental measured reaction enthalpy of CTZ (bulk), adsorbed at T = 298 K. 

CTZ adsorption on the silica pore wall. Experimentally, CTZ was loaded into MSU-H through iH-CO₂, achieving a minimum drug loading of 3% by mass (side insets).

In the simulations, CTZ was adsorbed on a silica surface model described in a previous study by some of us and already employed to simulate the adsorption of disperse dye. 

The use of a flat surface in the present work, in variance with an explicit model of MSU-H, is justified by the curvature of the MSU-H pore, whose diameter (8.3 nm) is much larger than the CTZ molecule. Indeed, a flat surface model represents a very good approximation of what the drug is "seeing" inside the pores. This surface is represented in Figure 1d and exhibits a titular density of 4.5 O atoms n⁻², close to the experimentally measured value for fully hydrated surfaces (4.0 O atoms n⁻²). The silica surface model contains 111 atoms in the unit cell (a = 12.6 Å, b = 12.8 Å, and c = 11.4 Å), cell composition H₂O₅Si₂O₈. The surface exposes eight silanols per unit cell. Of these, only one is free, while the others are interacting through H-bonds. Particularly, three SiOHs cooperate in forming a stable H-bonded chain. 

CTZ was manually docked on the surface, aiming at maximizing the interactions between exposed silanols and the different drug's functional groups. In order to match the experimental conditions, six main starting geometries have been studied. Four of them are characterized by one monomer per silica unit cell (drug loading ~13% by mass), while the other two simulate a molecular layer adsorbed on silica with two and three molecules per unit cell (drug loading ~27% and ~41% by mass, respectively). All models are shown in top view in Figure 2: (a) chitinacdoz (b) and (c) phthaloyl (d) and (e) chlorophenyl (f) are structures with CTZ interacting through its imidazole ring, while in (g) phthaloyl (h) and (i) phthaloyl (j) the molecule is adsorbed through its phthaloyl part. (c) molecular layer (1) and (f) molecular layer (2) are the highest loading structures. The 13% leading models (a-d) have been optimized both with and
ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS
ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS
ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS

The Journal of Physical Chemistry C.

Figure 3. Silica (SiO₂) and clotrimazole (CTZ) B3LYP (c and d) with respect to the initial structure for all AIMS simulations. For the silica surface, only the atoms free to move in the simulation have been considered. (a) trilayer (1), (b) bilayer (2), (c) monolayer (3), and (d) molecular layer (4). All five MD simulations have been performed with the same force field parameters. The experimental free energy of adsorption (ΔG°) was calculated from the following equation:

ΔG° = -RT ln(K)

where K is the equilibrium constant and R is the gas constant. The simulations showed that the adsorption of CTZ on the silica surface was favored due to the hydrogen bonding and hydrophobic interactions between the drug molecules and the silica surface. The simulations also indicated that the adsorption of CTZ on the silica surface was reversible, and the drug molecules could desorb from the surface under certain conditions.

Model of Adsorption: Nitrogen Adsorption and TG Analysis. Experimental N₂, adsorption isotherms (Figure 5a in Supporting Information) were used to determine the porous structure of the silica sample. The results were compared with the theoretical model of pore size distribution (PSD) obtained from the adsorption isotherms. The PSDs were used to calculate the surface area and average pore diameter of the silica sample. The silica sample showed a high surface area and a narrow pore size distribution, indicating that it was a suitable material for drug delivery applications.

Analysis of the Adsorption Data. The adsorption data were analyzed using the Langmuir isotherm model, which assumes that the adsorption takes place on a homogeneous surface. The Langmuir isotherm equation is given by:

Q = QmKc/(1 + KC)

where Q is the amount of drug adsorbed per unit mass of silica, Qm is the maximum amount of drug that can be adsorbed, K is the adsorption constant, and C is the concentration of drug in the solution. The Langmuir isotherm model was used to fit the experimental adsorption data, and the parameters were determined using nonlinear regression analysis. The results showed that the Langmuir isotherm model was a good fit for the adsorption data, and the parameters Qm and K were determined for different concentrations of drug in the solution.

Mobility of Adsorbed Clotrimazole. The mobility of adsorbed clotrimazole was measured using the electrophoretic mobility technique. The results showed that the mobility of adsorbed clotrimazole was lower than that of free drug, indicating that the adsorption of clotrimazole on the silica surface resulted in a decrease in its mobility. This property is important for the topical delivery of clotrimazole, as it allows the drug to penetrate into the skin and reach the target site.
Figure 6: AIMD of phenyl (c) and (b): (a) exposed nitrogen–SiOH distance over time; phenol (d) in red; phenyl (b) blue. (b) 3D space filling models of phenol (f) at 0 and 11 ps. (c) 3D space filling model of phenyl (b) at 0 and 11 ps. The nitrogen–SiOH distance plotted in the graph is highlighted in green.

Figure 7: AIMD of the molecular layer (b) structure: (a) N1-SiOH(II) and N1-SiOH(III) bond distances in time. (b) Starting (A) and final (B) positions of N1 and N2 with respect to the involved SiOHs. (c) Top view of the initial (left) and final (right) configurations in the AIMD simulation, with the corresponding interaction energies per CTZ molecule (kJ/mol), cell borders in pink.

of the local minimum structures of Figure 2. In Figure 5 the Root Mean Square Deviations (RMSD) of the atomic positions, during the production, with respect to the first frame, are reported for the four AIMD simulations (imidazole (b), phenyl (c), phenol (d), and molecular layer (e)). RMSD are separated in the CTZ and silica contributions. In all cases, the mobility of the adsorbed drug is higher than that of the silica surface. The latter seems equilibrated during all simulations.

The imidazole (b) structure, which is the most stable configuration of CTZ on silica according to Table 1, shows a general stability of the adsorbed molecule during the 11 ps.
Figure 6. AIMD of phenyl (I) and (II): (a) extended nitrogen-SiOH distance over time; phenyl (I) in red, phenyl (II) in black. (b) 3D space filling models of phenyl (I) at 9 and 11 ps. (c) 3D space filling model of phenyl (II) at 6 and 11 ps. The nitrogen-SiOH distance graphed in the graph is highlighted in green.

Figure 7. AIMD of the molecular layer (B) structure: (a) N1-SiOH (I) and N1-SiOH (II) local distances in time. (b) Starting (A) and final (B) positions of N1 and N2 with respect to the involved SiOH. (c) Top view of the initial (left) and final (right) configurations in the AIMD simulation, with the corresponding interaction energies per CTZ molecule (ΔE/CTZ); cell borders in pink.

of the local minimum structures of Figure 2. In Figure 5 the Root Mean Square Deviations (RMSD) of the atomic positions, during the production, with respect to the first frame, are reported for the four AIMD simulations (imidazole (I), phenyl (II), phenyl (III), and molecular layer (II)). RMSDs are reported in the CTE and silica contributions. In all cases, the mobility of the adsorbed drugs is higher than that of the silica surface. The latter seems equilibrated during all simulations.

The imidazole (I) structure, which is the most stable configuration of CTZ on silica according to Table 1, shows a great stability of the adsorbed molecule during the 11 ps
ORDERED MESOPOROUS SILICA FOR DRUG DELIVERY IN TOPICAL APPLICATIONS

The Journal of Physical Chemistry C

Figure 8. Experimental and simulated IR: experimental FTIR spectra of MCM-41 (CTZ at 10% by mass (Max)) and simulated IR spectra of CTZ combined with SO3H vibrational contribution (red). The dashed black lines represent the theoretical SO3H stretching frequencies without experiment's correction.

Figure 9. Experimental and simulated IR: experimental FTIR spectra of MCM-41-CTZ at 5% by mass (Max), after (Max) and simulated IR spectra of CTZ combined with SO3H vibrational contribution (red). The dashed black lines represent the theoretical SO3H stretching frequencies without experiment's correction.

Panell's empirical rule to account for the broadening effect due to H-bonding. The uncorrected computed SO3H frequencies have been reported in the lower part of both Figures 8 and 9 as vertical lines. The final theoretical spectra (red lines) of Figures 8 and 9 are the combination of these SO3H stretching modes and the CTZ signals. Figure 8 also includes the theoretical spectra computed for the four lower loading concentrations, limited to the SO3H stretching modes.

For both the low and high loading cases, the agreement between theory and experiment is remarkable and helps the interpretation of the signals. Considering CTZ modes, the 3150–3100 cm⁻¹ experimental bands are due to the ensemble of the C–H symmetric stretching of SO3H–H interacting aromatic rings. Peaks between 3000 and 2800 cm⁻¹ are due to alkyl infrared in the silica sample. As regards SO3H signal, the steep peak at 1750 cm⁻¹ (only in the low loading case) corresponds to isolated ions. The broad band between 1700 and 1550 cm⁻¹ corresponds to the silanol signals of SO3H–H interactions. All silanol signals at lower wavenumbers (1400–2500 cm⁻¹) are due to SO3H–silanol interactions. As expected, the SO3H stretching frequency is highly affected by this interaction, with broadening of bands up to more than a thousand wavenumbers, as shown by the individual signals reported in both figures (stick lines) and particularly for the high loading case. The higher intensity of this broad band in both experimental spectra of Figures 8 and 9 with respect to the simulation due to Panell's approximation which cannot account for all the subtle features due to anharmonic coupling and intensity scaling between modes in H-bond interactions.

A first effect of increasing the drug loading is the loss of the isolated silanol peak, suggesting that all silanols become involved in interactions (e- spectra of Figures 8 and 9). A second result is an intensification of the SO3H–silanol signals with respect to the 1400–1500 cm⁻¹ ones, suggesting that a decreasing amount of silanols is able to interact with the phenyl groups and an increasing amount of SO3H–mesoporous H-bonds is formed. Such behavior seems consistent with respect to the energetics of Table 1: computed DFT suggest that by increasing drug loading more stable configurations (mesoporous-driven) should be populated earlier than less stable ones (phenyl-driven), while FTIR shows that SO3H–phenyl signals are more prominent in the low loading case than the high-loading one. This apparent disagreement can be resolved by taking into account the complexity of CTZ adsorption on the silica pore walls. Comparisons between computed and experimental evolution of adsorption (Table 1) suggest that at least some energetically very stable silanol-driven interactions (such as that of Figure 5a) are quite rare in a real sample as they depend on the presence of specific surface sites. Moreover, given the structure of the CTZ molecule, it adsorbs through its imidazole ring; more silanols are influenced by its phenyl and chlorophenyl moieties than by the SO3H–N interaction, which can be deduced from the individual simulated spectra of Figure 8, in which signals corresponding to SO3H–N are present in all four configurations, independently of what drives the interactions. Furthermore, the strong bathochromic shift caused by the SO3H–N bonds corresponds to a significant signal broadening, so that at low loading these modes are smeared out over the whole spectrum. Finally, AM1 simulations proved that some phenyl-driven adsorptions, although based on relatively weak interactions, are indeed stable at room temperature; given the bulky conformation of CTZ (Figure 1c), such configurations might be highly probable during mesoporation, also at low loadings.

CONCLUSIONS

We have reported the concurrent experimental and theoretical (both static and dynamic) characterization of a new antifungal drug, clotrimazole (CTZ), incorporated into ordered mesoporous silica of the MCM-41 type. Comparison between experimental and computed interaction energies, peak size distributions, and vibrational spectra resulted in a very good agreement. This suggests that DFT simulation of drug adsorption on realistic amorphous silica surface models can be used to predict the behavior of drugs inside the pores of mesoporous silica materials, provided that a good sampling of the different interactions is achieved.

As regards the MCM-41-CTZ system, results shown here reveal that CTZ is adsorbed, over the incorporation with silica, following a homogeneous and statistical distribution on the silica surface. This picture is explained only considering...
REFERENCES


The Journal of Physical Chemistry C

Ordered Mesoporous Silica for Drug Delivery in Topical Applications


Ordered mesoporous silica (OMS) is a class of materials with highly ordered, three-dimensional, mesoscopic structures. These materials are of significant interest as potential candidates for drug delivery systems due to their tunable properties, such as pore size and surface chemistry, which can be tailored for specific applications. The use of OMS in topical applications allows for the precise delivery of drugs to the skin or superficial tissues without the need for systemic absorption, which can reduce side effects and improve therapeutic efficacy.

The key advantages of using OMS for drug delivery in topical applications include:

1. Controlled Release: The pore size and surface chemistry of OMS can be tailored to control the release rate of the drug, allowing for sustained delivery over an extended period.
2. Site-Specific Delivery: OMS can be designed to target specific areas of the skin, improving the efficiency of drug delivery.
3. Enhanced Permeation: The high surface area and porosity of OMS can enhance the permeation of drugs through the skin, facilitating more effective treatment.
4. Reduced Systemic Absorption: By delivering drugs directly to the skin, systemic absorption is minimized, reducing potential side effects.
5. Biocompatibility: OMS materials are biocompatible and can be safely used in contact with the skin, making them suitable for topical applications.

Several studies have explored the use of OMS in topical drug delivery, demonstrating its potential advantages over conventional delivery methods. Further research is needed to optimize the design and properties of OMS for specific applications, ensuring their safe and effective use in clinical settings.

References:


Incorporation of clotrimazole in Ordered Mesoporous Silica by supercritical CO₂

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ARTICLE INFO

Abstract

Clotrimazole, a poorly water soluble antifungal active principle widely used in dermatology, was incorporated inside Ordered Mesoporous Silica (OMS) of the MCM-41 type, using supercritical CO₂ (scCO₂), at sublim. The pristine OMS was characterized by a specific surface area of about 600 m²g⁻¹, a total specific pore volume of 0.900 cm³g⁻¹ and an average pore size of 8.5 nm. The incorporation process was carried out in a high pressure stainless steel reactor; 22.5 MPa and 320 MPa, at 373 K. The time of incorporation process varied from 6 to 10 h. The amount of incorporated clotrimazole was observed to increase with increasing incorporation time up to 7.5 h. Longer times (10 h) did not affect the incorporated amount. The obtained drug loading was 9.8% w/w, three times higher than that obtained by adsorption from ethanol solution (0.5% w/w).

The obtained composite (OMS) was characterized by X-ray diffraction, field emission scanning electron microscopy, thermal gravimetry, infrared spectroscopy and nitrogen adsorption. The clotrimazole molecular transformation and mesoporous structure inside the mesopores, a model of mesoporous acylation by clotrimazole molecules in the form of a molecular layer, is proposed.

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1. Introduction

Controlled drug release aims at optimizing drug efficiency while simultaneously reducing adverse side effects. Several studies have shown that pharmacokinetics, drug efficiency and suppression of undesired side effects in different pathological conditions can be improved by controlled drug administration and controlled release formulations. The release kinetics of drugs by OMSs depends on several carrier properties, including pore size, pore connectivity [4] and the chemical composition of the surface [5]. A recent emerging feature of OMS carriers is the enhanced oral bioavailability of molecules that are poorly soluble in water [6].

In the first pioneering work by Vallet-Regí et al. [7], drug incorporation in MCM-41 was carried out by adsorption from a solution using heptane as the solvent.

Since the long-term toxicity of antifungals in humans is well known [8,9], many other solvents have been studied for the incorporation. For instance, Chatay et al. [10] reported that when highly polar solvents such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and N,N-dimethylacetamide (DMA) were used, the amount of loaded drug was limited, whereas a higher quantity of drug was incorporated when heptane or heptane was used. This drug incorporation is strongly affected by the solvent due to the competition between the drug and the solvent in the adsorption [10,11].

The incorporation through supercritical carbon dioxide (scCO₂) is an alternative to adsorption or impregnation in a liquid solution.

Carbon dioxide is one of the most commonly used fluids in supercritical fluid technology [12]. It presents the advantage of having a critical temperature close to ambient temperature (304 K) and a critical pressure which is not too high (7.39 MPa). In addition, it is non-flammable, it has low cost and low toxicity. As all supercritical fluids, supercritical carbon dioxide has physical properties in between those of a gas and a liquid; in particular, it has a solvent power like a liquid and a high diffusivity like a gas. At pressures and temperatures not too far from its...
critical point, supercritical fluid has a high compressibility, therefore its density and hence its solvent power are easily adjustable over a wide range with minimal change in temperature or pressure. This tunability may be used to control the phase behavior in separation processes.

The incorporation of drugs in polymers [13,14] as well as in aerogels [15] using scCO2 has been widely reported. On the other hand, only a few examples of using active principles in Ordered Mesoporous Silica by using scCO2 [16-18] can be found. Furthermore, in one of these cases acetone (acetone or methanol) was used [18].

Beck and coworkers [14] investigated the supramolecular impregnation of vitamin E acetate on silica matrices by means of a dynamic technique. MIE et al. studied the incorporation of ibuprofen into SBA-15 through scCO2 [17], showing that the loaded drug quantity increased with the higher solubility of ibuprofen in scCO2.

The aim of this work is to study the incorporation of crocin (1:10 (w/w)) in a random copolymer (PLA/g-out-layer(STMA) poly(styrene-co-butylmethacrylate) microspheres by using sodium silicate as a silica source and the high yield polymerization, see Table 1. The framework structure of SBA-15 is analogous to that of SBA-15 and consists of large ordered pores connected by mesopores in the pore walls. Shape and size of SBA-15 differ from that of SBA-15 is that SBA-15 mesopores are usually assembled into larger and more monodisperse particles [16].

Crocin is an active principle ingredient that is poorly soluble in water and is present in traditional topical medications commonly used in the treatment of fungal infections (both human and animal) [3]. It is commonly available as an over-the-counter drug in various forms including creams.

The formulation of eudismic compositions containing OMS, which shows a constant concentration of the active principle ingredient on the skin, is essential. Traditionally, the skin is a complex organ and the most common method of drug release is through the skin. The incorporation of crocin in silica (silica) nanosilica from aqueous solution has been previously reported [27]. Instead, at the best of our knowledge the incorporation of crocin in OMS inside OMS particles is herein reported for the first time. In particular, on effects of incorporation of this molecule into a porous and hydrophilic matrix, such as scCO2 is available in the literature.

The incorporation of crocin in silica (silica) nanosilica from aqueous solution has been previously reported. Instead, at the best of our knowledge the incorporation of crocin in OMS inside OMS particles is herein reported for the first time. In particular, on effects of incorporation of this molecule into a porous and hydrophilic matrix, such as scCO2 is available in the literature.

2. Experimental

Crocin (C15H24O6), and Ordered Mesoporous Silica (MSU-11 type) were purchased from Sigma-Aldrich. Carbon dioxide with a purity of 99.5% was supplied by SAD.

2.1. Drug loading

A homemade device was used to perform the incorporation of the drug by means of scCO2. This consists in a glass cylinder of 1 cm diameter containing a pellet of crocin (100 mg) and a pellet of OMS (100 mg) separated by a disc of filter paper, in order to prevent their contact and allow their separation at the end of the incorporation process. The approach differs from those previously reported by Baret-Naude et al. [18] and by MIE et al. [17], where the non-polymerized OMS powder was used. This device was placed inside a stainless steel vessel, which was put in an oven that maintained all the system at constant temperature. The apparatus was also equipped with a volumetric pump and a back pressure regulator. Further details on the apparatus can be found elsewhere [24,25].

The incorporation was performed in static condition. The vessel was filled with liquid carbon dioxide and heated up to 132 K. After the heating, additional carbon dioxide was pumped in the vessel to reach the desired pressure (250 MPa) or 50 MPa. The system was then maintained in these conditions for several hours (from 6 to 18) to allow the drug dissolution and diffusion into the OMS.

At the end of the incorporation process, the apparatus was depressurized and the temperature was decreased to room conditions.

In order to characterize the effects of the scCO2 on the OMS, an additional experiment (250 MPa, 773 K, 12 h) was performed on a solid pellet of OMS, i.e., without the pellet of crocin in the glass cylinder.

For comparison, incorporation of crocin was also performed by adsorption from ethanol solution. To this purpose, 300 mg of crocin and 50 mg of OMS were introduced in a centrifuge tube with 5 mL of ethanol (96%, Sigma-Aldrich) and maintained at room temperature under stirring for 24 h. Then the powder was filtered and dried overnight in vacuum (residual pressure 0.1 Pa).

2.2. Characterization

Samples were characterized by means of X-ray diffraction (XRD), thermogravimetric analysis (TGA), infrared spectroscopy (FT-IR), nitrogen adsorption isotherms and field emission scanning microscopy (FESEM).

XRD patterns were obtained using a Panalytical XPert Powder (Cu Kα radiation) diffractometer. XRD patterns were recorded between 20° and 70° 2θ at a scan rate of 3° s⁻¹ using a step size of 0.02° 2θ.

FT-IR spectra were recorded between 200 and 4000 cm⁻¹ on a Perkin Elmer Spectrum 400 spectrometer operating at 2 cm⁻¹ resolution, after outgassing the sample at 373 K for 1 h (residual pressure equal to 0.1 Pa).

FT-IR spectrum of crystalline crocin was recorded on the powder dispersed in potassium bromide. FT-IR spectrum of crocin in solution was recorded on a diluted methanol solution (1:10).

1. Results and discussion

The effect of the scCO2 treatment on the structure and morphology of OMS was investigated by means of XRD and FESEM prior to
the incorporation of clonazepam because no study of the effects of 
scCO2 on OMS is reported in literature.

Fig. 1 reports the wide angle XRD patterns of the sample before (Curve 1) and after (Curve 2) the scCO2 treatment, which was carried out at 25.0 MPa and 373 K for 12 h without clonazepam.

No significant changes are observed in the pattern, which is typical of the MFI hexagonal structure [19].

Fig. 2 reports the SEM micrographs of the same sample before (1) and after (2) the scCO2 treatment. As for the XRD data, negligible variations are observed. Both XRD and SEM results show that the OMS precursor did not affect significantly the structure and the morphology of the OMS used in the study.

scCO2 was used to incorporate clonazepam (Scheme 1) inside the OMS. Pressure and time of the scCO2 treatment were varied, in order to investigate the effect on the amount of incorporated clonazepam, whereas the temperature was maintained constant and equal to 373 K. This temperature was chosen significantly higher than the critical temperature of carbon dioxide, at variance with what was reported by Bethadyya-Arreb et al. [20] and by Min et al. [21]. Thermal degradation of clonazepam can be noted only if it was reported to occur at 453 K [22]. The selected working pressure ensured a good solvation power of the fluid. The density and solubility parameter were respectively equal to 550 kg m⁻³ and 5.1 (MPa)¹/² at 25.0 MPa, while they were 419 kg m⁻³ and 3.9 (MPa)¹/² at 56.8 MPa.

As a whole, different incorporation procedures were carried out and their typical parameters are summarized in Table 1, together with the corresponding loading of incorporated clonazepam, which was measured by TG analysis. The maximum amount of incorporated clonazepam, corresponding to 34% (w/w) (percentage by weight), was obtained at 56.8 MPa after a 12-h supercritical treatment.

Data in Table 1 reveal that slight changes up to 12 h. In fact, the clonazepam loading increased from 12% (w/w) to 38% (w/w) when time increased from 0 h to 12 h at 25.0 MPa, whereas no further increase was observed when the process was carried out for a longer time, i.e., 18 h, at the same pressure. These data suggest that after 12 h the equilibrium between clonazepam in scCO2 and clonazepam inside the OMS was reached. This is the result obtained by Bethadyya-Arreb et al. [20], who observed no change in the active principle incorporated amount for periods of time longer than 1 h. This discrepancy is probably due to the different experimental procedure.

At variance, pressure was observed to affect the incorporated amount only at a minor extent, since an increase from 250 MPa to 56.8 MPa yields a limited increase of the clonazepam percent-

age (from 36% to 38%), probably due to a higher solubility of clonazepam in scCO2 at higher pressures.

It is worth noting that the incorporation via scCO2 was largely more efficient than that obtained by adsorption from ethanol solution. In fact, at the latter case the percentage of clonazepam inside OMS was only 9.6%. Moreover, the amount of clonazepam used for the adsorption was three times higher than that of OMS, at variance with the scCO2 treatment for which the same amounts of clonazepam and OMS were used.
Table 1: Incorporation process parameters for SC1 and ethaned solution (E2H) and corresponding crystalline loading (% w/w) evaluated by TGA analysis.

<table>
<thead>
<tr>
<th>Incorporation process</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Pressure (MPa)</th>
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<th>Crystallinity (% w/w)</th>
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</table>

- % w/w: Weight percentage

Fig. 3: XRD patterns of (1) crystalline chitromazine, (2) crystalline-containing OMS (SC1, SC1), (3) crystalline-containing OMS (SC1, SC1), (4) crystalline-containing OMS (SC1, SC1), (5) crystalline-containing OMS (SC1, SC1).

Fig. 4: Representative (1) OMS, (2) crystalline-containing OMS (SC1, SC1), (3) crystalline-containing OMS (SC1, SC1), (4) crystalline-containing OMS (SC1, SC1).

Fig. 5: Representative (1) OMS, (2) crystalline-containing OMS (SC1, SC1), (3) crystalline-containing OMS (SC1, SC1), (4) crystalline-containing OMS (SC1, SC1).

Fig. 6: Representative (1) OMS, (2) crystalline-containing OMS (SC1, SC1), (3) crystalline-containing OMS (SC1, SC1), (4) crystalline-containing OMS (SC1, SC1).

The table shows the incorporation process parameters for SC1 and ethaned solution (E2H) along with the corresponding crystalline loading evaluated by TGA analysis. The XRD patterns and representative OMS and crystalline-containing OMS samples are also presented in Figs. 3-6.
Fig. 6a reports the pore size distributions obtained from the nitrogen sorption isotherms in Fig. 5. For OMS as such (Curve 1), a main family of pores with an average diameter of 8.5 nm is observed. In the case of clenbuterol-containing OMS (Curve 2), this family of pores is not present, whereas new families of pores (apparently two) with smaller diameters, in the range 3-7 nm, appear. The presence of smaller mesopores in the clenbuterol-containing OMS is in agreement with the coverage of the internal mesopores surface by clenbuterol molecules, as revealed by FTIR data.

For the first time, an attempt has been made to model the occupation of mesopores by drug molecules. In fact, in the previous works [16-18], no calculation about the drug distribution was presented.

On the basis of the above results, a model corresponding to a layer of clenbuterol molecules on the surface of mesopores was chosen.

Fig. 7 is a pictorial representation of empty mesopores, i.e., those present in OMS as such (as the left), together with mesopores occupied by the clenbuterol molecules layer, i.e., those assumed for clenbuterol-containing OMS (as the right).

Starting from the pore size distribution of OMS as such [19, 6a, Curve 1], the pore size distribution for clenbuterol-containing OMS can be calculated. The volume $V_0$ of a single cylindrical empty mesopore is given by:

$$V_0 = \frac{\pi}{4} d_0^2 h$$

where $d_0$ is the mesopore diameter and $h$ is the mesopore elongation.

The volume $V_1$ of a single cylindrical mesopore occupied by the clenbuterol layer is given by:

$$V_1 = \frac{\pi}{4} d_1^2 h$$

where $d_1$ is calculated as:

$$d_1 = d_0 - 2\sigma$$

and $\sigma$ is the diameter of a clenbuterol molecule considered as a sphere (1.3 nm) calculated by MOLMOL molecular graphics [11].

Combining Eqs. (1) and (2) $V_1$ is obtained, whereby substituting $d_1$ as in Eq. (3), given in Eq. (5).
Therefore it can be concluded that clonazepam was incorporated inside the OMS mainly in the form of a molecular layer.

References