MCM-41 ŞI NANOTUBURI DE SILICE NANOSTRUCTURATE : O COMPARAȚIE A ABILITĂȚILOR DE ELIBERARE CONTROLATĂ

MCM-41 AND NANOSTRUCTURED SILICA NANOTUBES: A COMPARISON OF DRUG DELIVERY ABILITIES

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In this work it have been investigated the difference of the encapsulation efficiency and the release behavior of silica nanotubes and MCM-41 by using Ibuprofen as drug model. The highest encapsulation efficiency was in MCM-41, while the highest loaded Ibuprofen amount was in silica nanotubes. According to their larger size folded, silica nanotubes release percentage was around 80% which is higher than MCM-41 which was around 26%. It is worth mentioning that TEOS was used as silica precursor, and CTAB as a surfactant for the synthesis of both the nanostructured materials alike.

In această lucrare au fost investigate diferențele între eficiența de încapsulare şi comportarea la eliberarea controlată a unor nanotuburi de silice şi respectiv MCM-41 utilizând Ibuprofen ca model. Cea mai mare eficiență de încapsulare a fost obținută pentru MCM-41 în timp ce nanotuburile de silice au încapsulat cea mai mare cantitate de Ibuprofen datorită spațiului interior. Experimentele de eliberare favorizează MCM-41, 26% în 50 min., în timp ce nanotuburile eliberează 80% din medicament. Materialele investigate au fost sintetizate prin proceduri sol-gel raportate pornind de la TEOS şi diferiți agenți de direcționare a structurii şi morfologia lor a fost investigată prin microscopie SEM şi TEM.

Keywords: *MCM-41, silica nanotubes, Ibuprofen, Encapsulation efficiency, C-Silica, A-Sol-gel*

1. Introduction

The use of silica materials for drug delivery systems and controlled release of drug molecules have been reported as early as 1983, while nanostructured silica materials were widely employed as drug delivery system in about the last ten years. The beginning of using silica materials was mainly in the form of xerogels loaded with different bioactive molecules. For example, this xerogels have been utilized as implantable carrier for controlled release drugs. Generally, the properties of silica nanostructured materials such as their biocompatibility, biodegradability and easy formation with drugs, attract the attention for using them for different biomedical and pharmaceutical applications especially as drug carrier [1].

MCM-41-type silica shows a hexagonal array with cylindrical shaped pores which have size around (2–4 nm) with a high pore volume. It is worth mentioning this materials have one-dimension channel with uniform pores. In addition, the internal surface area is very large approaching to 1000 m^2 g^{-1} which is the above features make this MCM-41-type silica suitable as carrier substance. Many molecules can be loaded into the matrix of this materials such as catalysts molecules, sensors, optical materials and drug molecules [2 -4]. This type of silica nanomaterial has attracted the attention of control drug delivery due to its tuneable porosity, thus facilitating the control of the kinetics of drug release. Prolonged release through the pores leads to keep the drug release in the therapeutic level, moreover extending the time of release, thus increasing the therapeutic period [5].

Due to their unique features, small dimensions, tuneable properties, reduced toxicity, "needle-like" form silica nanotubes have also gotten the attention of the researchers for medical and pharmaceutical uses. The first synthesis of nanotubes was by using carbon as building material, which open the way to utilize other compounds, inorganic grid or structure directing agents (SDA) [6]. The most notable advantage of silica nanotubes, beside the existence of inner channels, with an increase volume for encapsulation, is the possibility to be easily functionalized both inside the cavity and on the outer shell allowing specific encapsulation or targeted delivery including magnetically modulation [7-9]. Encapsulating bioactive molecules such as drugs into nanotubes can protect those molecules from the physiological conditions and reduce their toxicity. Based on the above, many biological applications of the silica nanotubes have been reported in the literature such as separation of oligonucleotide, recognition of protein and immobilization of enzyme catalysts [9]. Various types of guest materials such as photosensitizers

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for photodynamic therapy, drugs with small molecules, peptides, proteins, DNAs and RNAs encapsulated into the silica nanotubes present enhanced properties [10] for example as therapeutics for targeting several diseases, heart, cancer and Parkinson's disease, etc.

In this study, we compared two types of encapsulation materials, MCM-41 and silica nanotubes in terms of efficiency of encapsulation and kinetics of drug release . For this reason we have selected a model drug, Ibuprofen in order to gain information about the behavior of these systems which may be extrapolated to other drug molecules. The choice of Ibuprofen as drug model was according to its availability, stability, low cost and ease monitoring and identification by UV and IR respectively.

Ibuprofen which is non-steroidal drug with functional carboxylic weakly acidic group able to form strong bonds with many functional groups via acid–base interactions. Among the delivery systems, and according to the literature, unmodified mesoporous silica nanoparticles considered as the best immobilization system and they introduced the highest capacity of Ibuprofen [11, 12]. In general, drug molecules are entrapped in silica molecules through weak non-covalent interactions, such as physical adsorption, electrostatic forces and/or hydrogen bonds. Ibuprofen can be loaded with non-modified surface of silica nanoparticles by hydrogen bond between silanol group of silica, and carboxylic acid group of the Ibuprofen [13, 14].

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2. Experimental

2.1. Materials and Method

The chemicals, tetraethoxysilane (TEOS), cetyl trimethylamonium bromide (CTAB), ∝-methyl benzyl amine (MBA) , Ibuprofen ((*RS*)-2-(4-(2-methylpropyl)phenyl)propanoic acid) as sodium salt, ethanol and 25% ammonia aqueous solution were obtained from Sigma-Aldrich and used as received without any purification. 1,3 :2,4-bis-O-(*p*-Nitrobenzylidene)-D-sorbitol (NO_2- DBS) was prepared as previously reported [15]. Demineralized water type 2 (~10MΩ) obtained from a Millipore Elix5 device was used in all the experiments.

2.2. Material characterization

UV-Vis spectra were recorded on Thermo scientific evolution 220, FTIR spectra were recorded on Thermo Nicolet 6700. SEM analysis was performed applied on HITACHI S2600N scanning electron microscope with EDX, in primary electrons fascicle, on samples covered with silver thin layer. TEM analysis was performed on TecnaiTM G2 F30 S-TWIN type microscope, equipped with STEM/HAADF as detector. The release experiments were performed using the pump and the UV-detector of a HPLC Agilent 1100 system.

2.3. Synthesis of nanostructured silica nanotubes

Silica nanotubes were prepared according to reported procedure [16]. In a typical experiment to a solution of 1% organogelator in 3mL absolute ethanol, 30 mg (1 equiv) of organogelator and 5-7 mg (0.25 equiv.) CTAB, were dissolved with heating and occasionally stirring. To the clear solution 40 mg (2 equiv.) of MBA were added and the mixture was omogenized for 5 min. by sonication. To the obtained viscous solution 0.27 mL (16 equiv) TEOS partially hydrolyzed by previous stirring for 15 min with 0.1 mL (7,5 equiv) water were added and the stirring was continued for 5 min. The sample was sealed in a glass tube and left for ageing for 7 days (~170 h.). Subsequently the sample was heated at 100^{o}C for 6 h, then at 200˚C for 2 h, under vacuum (~10 mm Hg). The solid residue was then calcinated for 6 h at 650^{o}C under atmospheric conditions, heating rate 5^{o}/min.

2.4. Synthesis of MCM-41

MCM-41 was prepared according to previously described procedure [17]. A solution of 0.5 g of CTAB in 96 mL water a solution of 2 mL of TEOS in 34 mL of ethanol was added followed by dropwise addition with stirring of 10 ml of 25% ammonia aqueous solution. The mixture was stirred for an additional 3h and left overnight. The resulted material was separated by filtration and silicagel was calcinated at 550 ˚C for 3 h., heating rate 5^{o}/min, in order to perfect the reaction and for decomposition of the template.

2.5. Drug loading

Drug loading was performed by mixing for 24 h 50 mg of nanostructured silica materials with 10 mL aqueous solutions of Ibuprofen of selected concentrations in order to achieve an initial concentration of 0.36, 0.54 and 0.72 mg/ mg of silica material respectively. The loaded materials were filtered, washed with 10 mL of water and dried .The initial filtrate and the washing waters were used to determine the encapsulation efficiency.

2.6. Encapsulation efficiency was determined spectrophotometrically by measuring the drug concentration for every material in the combined filtrate and washing waters normalized at 25 mL, using a calibration curve.

2.7. Calibration curves

This calibration have been prepared by dissolving specific amounts of ibuprofen each amount in 50 ml water and measuring the UV-vis absorbance at 266 nm. The results and the correlation coefficient are presented in (Figure 1).

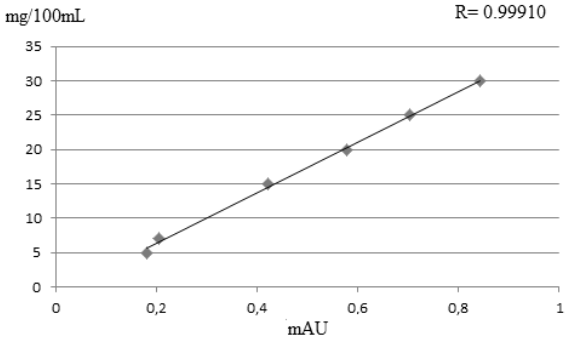


Fig. 1 - Calibration curve at 266 nm for the determination of encapsulation efficiency / *Curba de calibrare la 266 nm pentru determinarea eficienței de încapsulare.*

2.8. Drug release

Drug release experiments were conducted at room temperature as follows: a sample of 0.5mg drug loaded material was placed in filtration paper with dimensions 5*5 cm shaped as envelope and immersed in 150 mL water as release medium continuously stirred at 400 rpm. Aliquots of the release medium were continuously collected using a HPLC pump and analyzed though UV-detector of the system as previously reported in the literature [18]. Continuous loop was achieved by placing the inlet and the outlet of this system in the same release which containing the release solution with stirring by magnetic stirrer. A supplementary calibration curve was established due to the characteristics of the HPLC UV detector with wave length 300 nm (Figure 2).

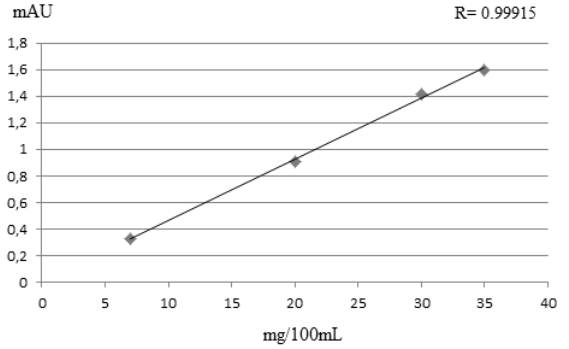


Fig. 2 - Calibration curve at 300 nm used for release study / *Curba de calibrare la 300 nm utilizată pentru studiile de eliberare*.

3. Results and discussion

3.1. Silica nanotubes synthesis

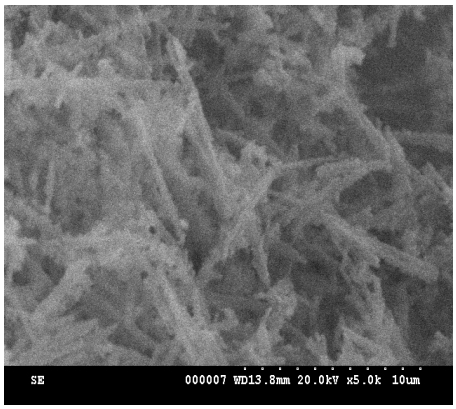
Silica nanotubes were prepared using a templated sol-gel synthesis using TEOS as silica precursor and a mixture of organogelator (NO_2-DBS) and a surfactant (CTAB) in the presence of an organic amine (MBA) as catalyst following the procedure previously reported [16]. The morphology of the resulted material was investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Hollow tubes of 5- 10μm and 300-500 nm in diameter were obtained as presented in Figure 3a. TEM micrograph Figure 3b) revealed an continuous inner tube of about 80-100 nm in diameter.

3.2. MCM-41 Synthesis

The mechanism of the synthesis of mesoporous silica nanoparticles generally proposed that the nucleation of MCM-41 nanoparticles starts with the configuration of rod form of CTAB micelles surrounded by silica. The silica-surfactant micelles accumulate as untidy spherical nanoparticles followed by internal re-arrangement then the micelles of silica-surfactant suffers cylindrical expansion [19]. The determination of the morphology of MCM-41 it had been done by transmission electron microscopy (TEM) analysis. Small sized and coordinated nanopores can be recognized on the surface of the silica (Figure 4). According to the TEM analysis the pore size in average was around 3 nm.

3.3. Encapsulation of the drug

The silica nanotubes and MCM-41 have been loaded with Ibuprofen by soaking these materials separately into the Ibuprofen solutions. The loading inside the silica nanostructured materials have been proved through FTIR spectroscopy (Figure 5). Characteristic bands of the model drug were identified as follows: the stretching vibration of both aromatic and aliphatic C-H bond between 2800-3010 cm^{-1}. The stretching vibration of C=O from the carboxylate group of ibuprofen at



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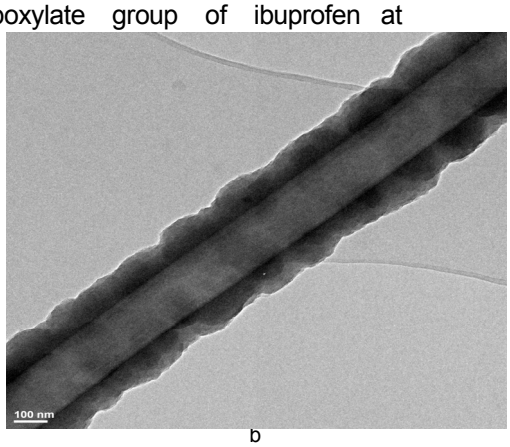

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Fig. 3- a- a- SEM micrograph of silica nanotubes, b- TEM micrograph for hollow silica nanotube
a- Micrografie SEM a nanotuburilor de silice, b- Micrografie TEM a tuburilor goale de silice.

1547cm^{-1}, while this peak is slightly shifted to 1545 cm^{-1} after loading the drug into silica matrix [20]. This shifting might refer to the formation of H-bonding between the carboxylic groups of ibuprofen and the silanol groups of the pore wall in silica matrix as previously reported [21]. The characteristic Si-O-Si stretching broad band at 1061 cm^{-1} of the silica nanostructured material overlapped with ibuprofen FTIR spectra to appear in the silica loaded ibuprofen at 1096 cm^{-1} belonging to both the silica supports. This proves that the ibuprofen have been successfully loaded into the pores of the silica matrix.

Encapsulation experiments were conducted at different concentrations and eficiency of encapsulation was determined spectrometrically. The results are presented for both materials in (Figure 6).

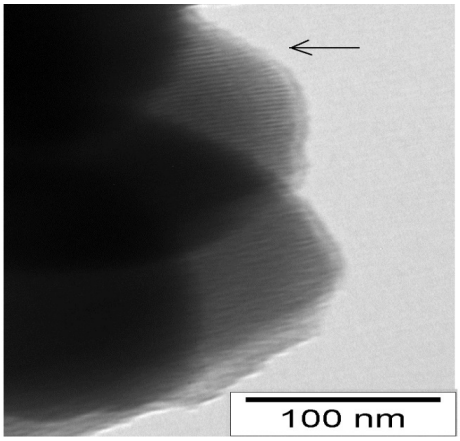

Fig. 4 - TEM analysis for MCM-41 shows the nanopores structure / *Micrografie TEM a MCM-41 punând în evidență structura nanoporilor.*

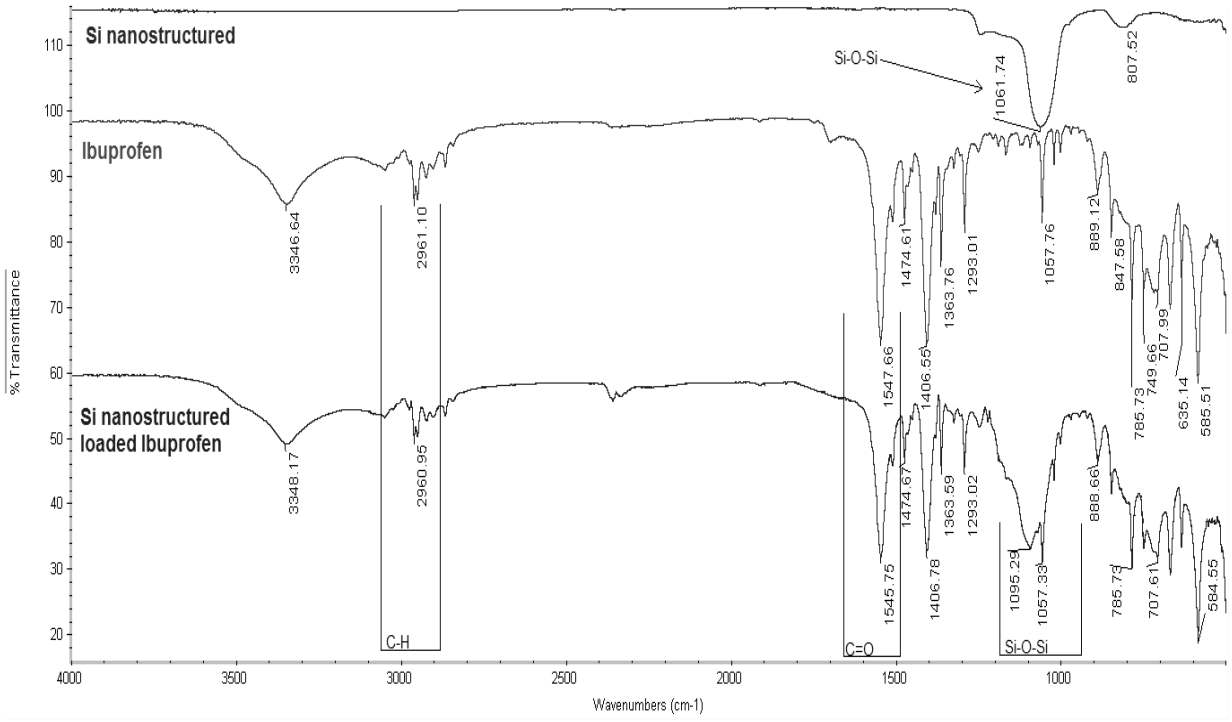

Fig.5 - FTIR spectra for the nanostructured silica before and after loading with Ibuprofen
Spectre FTIR pentru silice nanostrcturată, Ibuprofen şi material încărcat cu medicament.

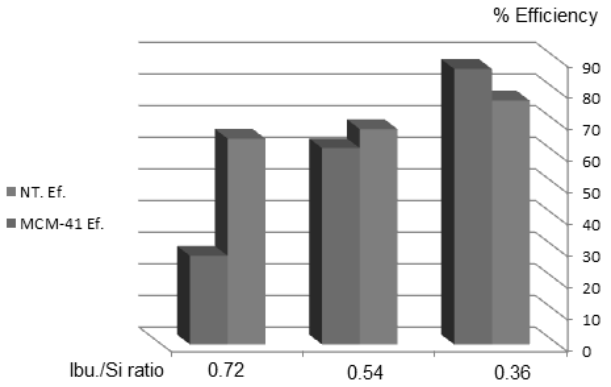

Fig. 6 - The relation between encapsulation efficiency and Ibuprofen/Si ratio / *Relația între eficiența de încapsulare şi raportul Ibuprofen/silice*.

From the results of encapsulation efficiency it can be recognized that this efficiency increases with decrease the drug: material ratio for both silica nanotubes and MCM-41 alike**.** This may happened according to the repletion of the inner surfaces for the silica nanostructures by the drug molecules then leaching these molecules after certain drug ratio and time. According to their smaller nanoporous size, MCM-41 has the highest efficiency 87% with the lowest Ibuprofen to MCM-41 ratio. The small size of nanoporous MCM-41 leads to absorb certain amounts of the drug, in the same time the encapsulation is tight which leads to non-leaching of the drug molecules in the low Ibuprofen ratio. On the other hand, at high ratio of Ibuprofen to MCM-41 leads to the lowest

encapsulation efficiency (28%) according to the same reason above. The highest amount of Ibuprofen can be found in the silica nanotubes. The encapsulation efficiency was 65% when a ratio of Ibuprofen to silica nanotubes of 0.72 was employed. The ability for physical adsorption of the silica nanotubes towards the drug molecules is superior due to the existence of their inner channels so the loaded amount is higher because the drug is kept both in the pores of the tubes wall (similar to MCM-41) and inside the large hollow tubes

3.4. Drug release

The release study have been done to the sample which gave the highest encapsulation efficiency at Ibuprofen to silica ratio of 0.36 for both silica nanotubes (77%) and MCM-41 (87%) (Figure 7). Generally as drug delivery system, mesoporous silica materials shows high dependency on the nature of the pores in term of shape, size and connectivity of the mesoporous structure and their geometry [22]. Silica nanotubes show the highest release percentage reaches to 80% of the Ibuprofen after 50 min. This behavior can be accounted by the larger diameter of the silica nanotubes which gives the opportunity for the Ibuprofen molecules to be released at an increase rate. The release was fast because Ibuprofen molecule is very small and the physical forces that determine the adsorption are not so strong. We suppose that for a guest molecule with a significant larger volume and multiple functionalities the encapsulation inside the inner channel of the nanotubes will be an advantage and the release will be slowed to the desired kinetics. On the other hand, for MCM-41, even a small volume drug, as the model Ibuprofen employed in this experiment, is tightly retained in the pores of the material so, during 1 h just about 26% have been released. As previously reported In this case we consider that MCM -41 is suitable for bioactive molecules that requires a slow release during an extended period of time at small concentrations taking into account that the actual quantity of encapsulated drug was moderate.

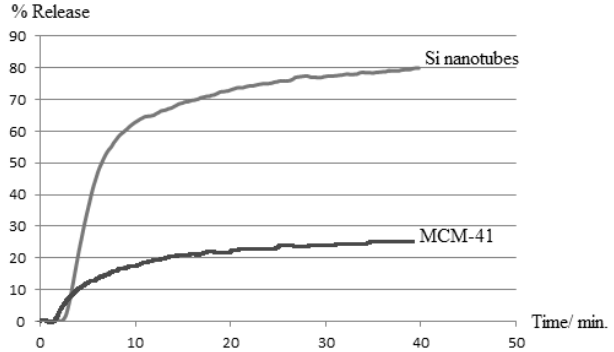

Fig.7 - Cumulative drug release of Ibuprofen from silica nanotubes and MCM-41 / *Curba de eliberare cumulativă a Ibuprofenului pentru nanotuburi de silice şi MCM-41.*

According to the small size pores of MCM-41 which does not allow to the drug to release out of the silica matrix, the cumulative drug release of the Ibuprofen is much less comparing with the silica nanotubes. In their recent research Gonzalez G. et al. clarified that the mesoporouse silica materials have different performance as drug delivery system, meanwhile it shows dependency on their pore size and the size of the guest drug molecules. Taking into consideration that ibuprofen is a rather small molecule with van der Waals surface of 1.3 × 0.6 nm^2, it is easily accommodated in the pores of the nanostructured silica materials and immobilized via hydrogen bonding between the carboxyl group of the ibuprofen and silanol group of the silica matrix, as proved by FT-IR spectra [23]. We can account that the association via hydrogen bonding is more efficient in the confined space of the MCM-41 pores of about 3 nm diameter than in a larger inner channels of the silica nanotubes of about 100 nm diameter. Thus we can account for the fast release of the drug in the case of nanotubes as compared to MCM-41 which is reported as prolonged for 3 days until complete all the loaded amount [21].

However, targeting the affected site inside the human body needs selective drug carrier and protection of this drug from the body conditions until delivery. Due to their specific morphology the silica nanotubes can perform as nano-scale needles inside the human body which makes them more selective with a suitable outer shell functionalization together with the ability to encapsulate large quantity of voluminous drugs.

4. Conclusion

Silica nanotubes and MCM-41 have been successfully prepared by using TEOS as silica precursor. Surface morphology of the silica nanotubes and MCM-41, is important for encapsulation and release properties which has been investigated by SEM and TEM respectively. Ibuprofen was used as model drug for estimation of encapsulation efficiency and the release profile. The decrease of Ibuprofen to silica ratio is leading to increase the encapsulation efficiency. The highest encapsulation efficiency, 87% was obtained for MCM-41, while the cumulative drug release after 1 h was moderate. On the other hand, silica nanotubes provided the highest amount of loaded bioactive molecule. The release of the Ibuprofen from silica nanotubes was fast, due to the small volume of the drug molecule opening the possibility to have better results in the case of larger molecules which may present a saturation phenomenon when encapsulated in MCM-41.

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