Use of mesoporous silicate nanoparticles as drug carrier for mefenamic acid

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Abstract. The aim of the study is to evaluate the use of mesoporous silicate nanoparticles for the loading and release of the non-steroidal anti-inflammatory drug, mefenamic acid. Nanoparticles of the mesoporous silicate materials, MCM-41 and SBA-16 were synthesized and characterized by XRD, SEM, FT-IR, TGA and BET surface area techniques. Both silicate systems were loaded with mefenamic acid with loading capacities of 18.6% and 11.6%, respectively. The in vitro release of mefenamic acid into simulated body fluid (pH = 7.4) at 37°C was investigated. The percent release was non-linearly regressed against time (t) according to the first order kinetic release model; Higuchi’s first burst model and Kopcha’s empirical model. The overall %release was obtained for both silicate systems and was found to be about 60%. Analysis of results show the rate of drug release is more rapid from SBA-16 (the more interconnected porous network) than from MCM-41. It also show that drug release from either mesoporous silicate is a diffusion controlled process.

1. Introduction
The 2D hexagonal mesoporous material MCM-41 was first synthesized by Mobil Corporation scientists [1]. Following that, many other silicate mesoporous materials have been synthesized such as SBA-series. The SBA-16 system (Santa Barbara Amorphous No.16) exhibits a 3D cubic structure and characterized by thick walls [2]. Due to their ordered porous structure, thermal stability, tuneable pore sizes and ability to be functionalized, mesoporous silicate materials have been employed in several applications, including water treatment [3], gas sensing [4], catalysis [5] and drug delivery [6].

Recently, mesoporous silicate nanoparticles have been investigated extensively as drug carriers, due to their high permeability and good biocompatibility [7]. Other favourable features of nanoparticles of interest are: tunable particle size, tunable pore diameter and high surface area. Nanoparticles of MCM-41 were first prepared by two different research groups [8-9] and the first use of mesoporous silicate nanoparticles in drug delivery was by Lai et al. [10]. Herein, we report on the use of nanoparticles of MCM-41 and SBA-16 for loading and release of mefenamic acid (Figure 1), a non-steroidal anti-inflammatory drug (NSAID) [11]. It is a poorly soluble in aqueous media with a solubility in water = 4.18 mg/L at 37°C [12]. Enhancing the solubility of mefenamic acid have been investigated by many research groups via using solid dispersions [13-16], encapsulation by polymeric nanoparticles [17], inclusion into β-cyclodextrin [18-19], nanostructured lipid carrier [20] and microspheres [21-23].

2. Experimental Details

2.1. Materials
Tetraethyl orthosilicate (Si(OE2H5)4), cetyltrimethylammonium bromide CH3(CH2)15N-(CH3)3Br (CTAB) and pluronic 127 triblock copolymer, poly(ethyleneoxide)-block-poly(propylene oxide)-
block-poly(ethylene oxide) (EO106PO70EO106) with average molecular weight of ~12,500 Daltons were obtained from Sigma Aldrich. Mefenamic acid was kindly donated by Pharma International- Amman. The simulated body fluid was prepared, as described by Oyane et al. [25].

2.2. Instrumentation

The morphology and particle size estimates of synthesized mesoporous silicate materials were obtained by scanning electron microscopy (SEM) using Inspect F50 FEI SEM, Netherlands. XRD spectra were obtained using XRD-7000 Shimadzu diffractometer. XRD spectra of SBA-16 were obtained using the low angle instrument PW-1840 Philips diffractometer. Percent loading and release were estimated using UV-Visible spectrophotometry (Varian Cary 100 Bio UV-Visible spectrophotometer). BET surface areas, and pore diameters were evaluated through BET and BJH modeling using a nitrogen adsorption/desorption instrument (Nova 2200e). Thermal gravimetric analysis (TGA) was conducted using the NetzschSta409 PC instrument (NETZSCH-Ger) operating from room temperature up to 1000°C at rate of 20°C/min. FT-IR spectra were recorded using a Thermo Nicolet NEXUS 670 FT-IR spectrometer (4000 to 400 cm⁻¹, 4 cm⁻¹ spectral resolution, KBr pellets).

2.3. Synthesis of mesoporous silicate nanoparticles

Nanoparticles of MCM-41 [9] and nanoparticles of SBA-16 [25] were prepared following literature methods with slight modifications.

2.4. Loading of mefenamic acid

A saturated solution of the drug was prepared by dissolving 1.6 g of mefenamic acid in 10 mL tetrahydrofuran (THF) and placed in a Schlenk flask with an O-ring cap. A 0.80 g sample of the mesoporous material was added to the solution and the mixture was stirred at room temperature for 24 hours. It was then centrifuged at 5000 rpm for 5 minutes and the solid material was separated, washed with propan-2-ol (2 mL) and dried at 50°C in vacuum for 3 hours. The percent loading was determined spectrophotometrically using the following procedure: Accurately weighed about 15 mg of the loaded mesoporous silicate were soaked in 100.0 ml of aqueous 0.1 M NaOH and the mixture was stirred for 24 hours. The mixture was filtered using a 0.2 µm nylon filter. A blank solution was prepared by dissolving 15 mg of the unloaded mesoporous material in 100.0 ml 0.1 M NaOH. With suitable dilution for calibration curve, the UV-absorbance was measured against the appropriately diluted blank at λ=285 nm.

2.5. In vitro release of mefenamic acid

An accurately weighed disc about 0.3 g of the drug-loaded nanoparticles were soaked in 250.0 mL of SBF at pH 7.4 and 37°C, in a thermostatic bath shaker. Out of the resulting solution, 5.00 mL samples were withdrawn at predetermined time intervals. The withdrawn samples were readily replaced with the same volume of fresh SBF. The withdrawn samples were filtered using a 0.2 µm nylon filter. The concentration of the drug released was estimated from the corresponding absorbance measured at 285 nm against a calibration curve of the drug in SBF at pH 7.4 and 37°C.

3. Result and Discussion

3.1. Characterization of mesoporous silicate nanoparticles

The synthesized samples of mesoporous silicates, MCM-41 and SBA-16, were characterized by SEM, XRD, FT-IR, N₂ adsorption/desorption isotherms and TGA. The SEM images for MCM-41 and SBA-
16 are shown in Figure 2. Both SEM micrographs of MCM-41 and SBA-16 show nanoparticles with spherical morphology [9,25] and with average diameter of about 31 nm. The XRD spectrum of MCM-41 shows a pattern with a strong peak at 20 of about 2.7 degrees and low intensity peaks in the 20 range of 4-6 degrees. The pattern is comparable with that reported by Cai et al. [9]. The XRD pattern of SBA-16 shows a peak at 20 of 0.7 degrees in agreement with values reported in literature [25].

![Figure 2. SEM images of (a) MCM-41, and (b) SBA-16.](image)

The FT-IR spectra of both systems show characteristic bands that are consistent with literature reported data [26]. The corresponding bands assignment were hydrogen-bonded Si-O-H and adsorbed water at 3437cm$^{-1}$, Si-O-Si asymmetric stretching (external) at 1250cm$^{-1}$, Si-O stretching symmetric (external) at 965cm$^{-1}$ and H-O-H bending at 1635cm$^{-1}$.

Nitrogen adsorption-desorption isotherms were used to estimate the specific surface area ($m^2/g$) and the average pore diameter (nm) for MCM-41 and SBA-16. The adsorption branch was used to estimate the specific surface area using the Brunauer-Emmett-Teller (BET) theory, while the desorption branch was used to estimate the average pore diameter using the Barrett-Joyner-Halenda (BJH) theory. Both systems show isotherms (Figure 3) characteristic of type IV. The increase in the adsorption curve at relative pressure $P/P_o$ (0.3 for MCM-41 and 0.58 for SBA-16) corresponds to capillary condensation within a uniform mesoporous silicate material [25]. The surface area and the pore size for MCM-41 and SBA-16 were estimated at 615 $m^2/g$, 3.05 nm and 957 $m^2/g$, 3.04nm, respectively.

![Figure 3. Nitrogen adsorption/desorption isotherms for (a) MCM-41 and (b) SBA-16.](image)
The thermal gravimetric analysis for MCM-41 were measured in the temperature range between room temperature to 1000°C. The mass loss in the temperature range, 25 -300°C is due to desorption of water molecules within the pores in addition to the water molecules that are hydrogen-bonded to the surface silanol groups, while the mass loss in the range (300-1000°C) is due to the condensation of silanol groups [9,27]. The densities of surface silanol groups that were extracted from TGA thermogravimetric curves of unloaded MCM-41 and SBA-16 are 1.25 and 1.28 N Si-OH/nm², respectively. The very close values of surface silanol group densities in both mesoporous silicate nanoparticles would suggest similar behaviour in loading and release.

### 3.2. Loading and release of mefenamic acid

As previously mentioned, the dimensions of mefenamic acid (Figure 1) are smaller than pore dimensions of MCM-41 or SBA-16 silicate systems; so it can be encapsulated into their pores. In the loading procedure, a saturated solution of mefenamic acid in THF was used. THF was chosen because of its high capacity to dissolve mefenamic acid, as compared with other solvents (acetone, ethanol, and aqueous ethanol). The loading capacity of mefenamic acid into the mesoporous materials was determined by UV absorption spectrophotometry. The results of %loading was 18.6 in MCM-41 and 11.6 in SBA-16. Loading of the drug into the silicate system was confirmed by IR spectrometry. The IR- spectra of the loaded samples show the presence of mefenamic acid by the appearance of the corresponding characteristic vibrational bands at 1651 cm⁻¹, 1576 cm⁻¹, 1507 cm⁻¹, 1447 cm⁻¹, which denote the carbonyl, and aromatic carbon-carbon stretching vibrations [28].

The in vitro release of mefenamic acid from the mesoporous silicates MCM-41 and SBA-16 were measured to explore possible factors affecting the release process. The mefenamic acid concentration in the release medium was analyzed using UV absorption spectrophotometry, by measuring the absorbance at 285 nm using a blank sample for base line correction. The concentration of mefenamic acid was estimated with reference to a calibration curve of mefenamic acid in SBF at the same conditions according to the linear equation (1):

\[ A_{285\text{ nm}} = 36.697 C \text{ (mg/ml)} + 0.0194 \]  

(1)

The release experiments were conducted in triplicate, and the results for the three experimental runs were averaged and analyzed for %release kinetics and kinetic parameters.

The measured concentrations of mefenamic acid (C, in mg/mL) released into the SBF buffer solution, at different time intervals t, were used to calculate the mass of drug (Q, in mg) released into the medium of volume V in mL according to \[ Q_t = C_t \times V. \]  

This was subsequently corrected for concentration depletion due sampling to obtain the cumulative drug release (equation 2)

\[ Q_{corr} = Q_t + \left(\frac{V}{V}\right) Q_{t-1} = Q_t + \left(\frac{V}{V}\right) \sum_{1}^{t-1} Q_t \]  

(2)

where \( Q_t \) and \( Q_{corr} \) are both given in mg, the sample volume is typically 5.00 mL, V is the volume of the SBF release medium which was kept constant (typically 250 mL) withdrawn samples with same volumes from the SBF buffer. The corrected drug release \( Q_{corr} \) was divided by the drug load (\( W_0 \)) to obtain the percent drug release, according to: % Release = \( \left(\frac{Q_{corr}}{W_0}\right) \times 100\% \). The percent release was nonlinearly regressed against time t according to the first order kinetic release model [29] according to equation (3):

\[ \frac{Q_{corr}}{W_0} = 1 - e^{-kt} \]  

(3)

where the best fit estimate of the first order kinetic release rate constant (k) is obtained. Further modeling of drug release against time was carried out using Higuchi’s first burst model [28], \( Q_{corr}/W_0 = k_H t^{1/2} + c \) (4)

and Kopcha’s empirical model [30], \( Q_{corr}/W_0 = A t^{1/2} + B t \) (5)

In the Higuchi’s first burst model, \( k_H (h^{-1/2}) \) is the Higuchi first burst constant, and c is a constant characteristic of the drug and host being formulated. It is based on Fickian diffusion, applied to the initial first burst of drug release. This model assumes that the % drug release \( (Q_t/W_0) \) varies with the square root of time \( t^{1/2} \), which typically occurs relatively fast, compared with
subsequent release. In contrast, Kopcha’s model yields estimates of the individual contributions of drug diffusion (A) and erosion (B) to %Release. The first order release profiles for mefenamic acid loaded into the mesoporous carriers MCM-41 and SBA-16 are shown in Figure 4, which depicts plots of cumulative drug quantity released against time (solid data points).

![Figure 4. Release profile of (a) mefenamic acid from MCM-41 and (b) from SBA-16](image)

A summary of the loading and release parameters for mefenamic acid are listed in Table 1, below.

| Carrier | % Loading | W_D^a (mg) | % Release | k^c (h^-1) | k_H^d (h^{-1/2}) | A/B^e |
|---------|-----------|------------|-----------|------------|-----------------|-------|
| MCM-41  | 18.6      | 37.8       | 59.6%     | 0.0202     | 0.044           | 0.036 / 0.002 |
| SBA-16  | 11.6      | 23.9       | 61.6%     | 0.0457     | 0.090           | 0.075 / 0        |

^aW_D = drug load in mg.  
^b % Release = 100 \times \frac{Q_{corr}}{W_D}  
^c k = First order kinetic release rate constant (1st order model ⇒ % Release = 1 – e^{-kt})  
^d k_H = Higuchi first burst release model (Higuchi’s model: % Release = k_H \times t^{1/2} + constant )  
^e A & B are factors denoting the relative contributions of diffusion and erosion processes to drug release (Kopcha’s empirical release model ⇒ % Release = A \times t^{1/2} + B \times t ).

It is observed that %release of mefenamic acid from both MCM-41 and SBA-16 are close to each other. Further, the rate constants are approximately doubled in SBA-16. In addition, Kopcha’s constant A is much higher than constant B, thus indicating that release of both drugs from either mesoporous silicate is a diffusion controlled process.

4. Conclusions

The results of the investigation of loading into, and %release, of mefenamic acid from the MCM-41 and SBA-16 reveals that: Mefenamic acid in the SBF does exist as a carboxylate anion (pK_a = 4.55), which interacts with silanol groups that are mostly neutral. Although both systems have the same %loading, MCM-41 exhibits half as much the rate for SBA-16. This could be due to the
interconnected porous network of SBA-16. The pores of MCM-41 are one dimensional and thus imposing more restriction to free drug diffusion into the release buffer medium, and hence a lower rate of release.

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