CONTROLLED RELEASE STUDY OF CEFTRIAXONE FROM MCM-41-NH_2 MESOPOROUS MATERIAL

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The present article focuses on the synthesis of the Ceftriaxone loaded MCM-41-NH_2 based materials and the determination of the release profile of Ceftriaxone from these materials. The optimum amount was assessed through progressive loading of the MCM-41 material. A calibration curve was used for the determination of the released quantity. We also assessed the best temperature for the functionalization reaction.. The obtained release profile is almost linear for lower concentrations of cetriaxone deposited in the porous channels.

Keywords: Ceftriaxone, MCM-41-NH_2, mesoporous, controlled release

1. Introduction

Reducing the dosage required for the desired inhibitory effect is of outmost importance in the field of chemistry and in the field of medicine due to the toxic effect caused by the excess of antibiotic in the human body [1]. Controlled release drug delivery systems based on MCM-41 are widely used, with different types and techniques of surface modification [2-8], in order to accommodate them for various applications[2-3,5-6], such as delivery of non-steroids anti-inflammatory drugs – NSAID, antibiotics, antihypertensive drugs, cytostatics, etc. [9].

Beside the obvious antibacterial purpose, in the treatment of various infections, such as sexually transmitted diseases like Syphilis [10] and Neisseria gonorrhoeae [11], food poisoning diseases like the ones with Salmonella [12] and Enterococcus faecalis [13], and other bacterial infections, like Streptococcus pneumoniae [14], the strains causing bacterial meningitis [15], ceftriaxone treatment can also be used for other medical purposes, like the ease of symptoms of withdrawal induced in cocain dependent pacients [16], intense alcohol abusers [17, 18], and non-medical purposes like corosion inhibitor [19].

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There were previous experiments of controlled release for ceftriaxone, from PMMA (polymethylmethacrylate) beads [20] and from porous hidroxyapatite scafolds [21], but to our knowledge it was not tried so far on MCM-41 mesoporous materials or on $–NH_2$ modified mesoporous materials. MCM-41 type materials have a very good biocompatibility, high surface area and large pore volume, and are good candidates for controlled drug release.

Silica materials used for controlled release provide several advantages, among which are the possibility of using both hydrophilic and hydrophobic antibiotics [22] and the fact that it ensures physical and chemical protection of the entrapped species [22] and a stable and slow release profile, and in some cases (silica fibers) a very long release time (1-2 weeks) [22].

2. Materials and methods

Ceftriaxone disodium salt was purchased from Sigma Aldrich and was used as such without further purification. Cetyl trimethyl ammonium bromide (CTAB) was purchased from FLUKA, reagent grade, Tetraethylorthosilicate (TEOS), (3-Aminopropyl)trimethoxysilane (APTMS), Ethylic alcohol, Methylic alcohol, Ammonium hydroxide solution 25%, were purchased from Sigma Aldrich, reagent grade.

0.5g CTAB were dissolved in 96 mL of distilled water, under heavy magnetic stirring. After the solution turned clear, 34 mL of ethylic alcohol and 10 mL of ammonium hydroxide solution were added. After 5 minutes of intense stirring, 2 mL of TEOS were added. The reaction was left stirring for 3 hours at room temperature. The final product was filtered, dried overnight at room temperature, then heated at 550°C with 5°C/min, 11 hours hold time.

The method for the synthesis of MCM-41 is the one used by H.I. Melendez-Ortiz [23] with minor modifications. After the white powder has cooled, it was characterized by IR spectroscopy and Transmission Electron Microscopy - TEM in order to confirm that the MCM-41 hexagonal mesoporous structure was obtained.

For the functionalization reaction between MCM-41 and APTMS, 100 mg of MCM-41 were placed in a 4 mL quartz reaction vessel with PTFE lining. The lid was chose so that it would withstand the pressure created inside the reaction vessel. The water molecules are competing with the reagent in this type of reaction, so a closed vessel with no exposure to atmospheric conditions is the viable option in this case. On top are added 1,5 mL of APTMS, the excess of reagent assuring that the water molecules do not get to compete for the MCM-41 active sites.

A study was performed in order to assess the best conditions for the reaction, and the temperature was the parameter of choice. We used 4 reaction

vessels, each exposed to a different reaction temperature for the same amount of time. The temperatures of choice were 60, 80, 100 and 120 ^oC. The reaction time (12 hours) was maintained the same.

After complete cooling, the reaction mass was washed thoroughly with dried methanol, and was left to dry at room temperature overnight. The obtained product was characterized through TEM microscopy and FTIR spectroscopy and named MCM-41-NH_2 for future references.

100 mg of MCM-41-NH_2 together with 30 mg of Ceftriaxone were grinded to a fine powder, and afterwards 10 mL of ethanol were added on top in order to facilitate the diffusion of the antibiotic. The alcohol was removed overnight through evaporation.

For release profile determinations, 50 mg of loaded material were placed inside a container made from filter paper and the release profile was recorded in distilled water. For the release profile the quaternary pump and the UV-Vis detector from an Agilent 1100 chromatographic system was used.

The optimum amount of Ceftriaxone which can be loaded in the mesopores for a desired type of release profile is very important, hence we decided to asses this amount by means of successive loading and recording of the release profile. We used several loadings of Ceftriaxone, on only one of the functionalized materials, the one that showed the most stable release curve, in the range 10-40 mg of ceftriaxone (e.g. 10 mg ceftriaxone/100mg MCM-41-NH_2).

In order to determine the amount of Ceftriaxone released, a calibration curve was recorded using different quantities of pure compound (10-50 mg). The parameters for the calibration curve ($y = b0 + b1*x$) are r^2=0.9956, b_0=243.61 and b_1=31.123.

All FT-IR spectra were recorded using a Thermo Nicolet 6700 series spectrometer fitted with ZnSe crystal/microATR accessory. The samples were grinded to a very fine powder before recording. TEM measurements were made using a Tecnai™ G2 F30 S-TWIN type microscope, equipped with a STEM/HAADF detector.

3. Results and discussion

From the high resolution TEM images can be noticed the hexagonal network of mesopores specific to the MCM-41 type material (**Fig. 1**). The mean pore size ranges between 2.8 and 3.2 nm, size which classifies the material in the mesoporous region according to IUPAC [24]. From the HRTEM images can be seen that the structure remains unaffected by the organic functionalization process, and so does the pore size (**Fig. 1**).

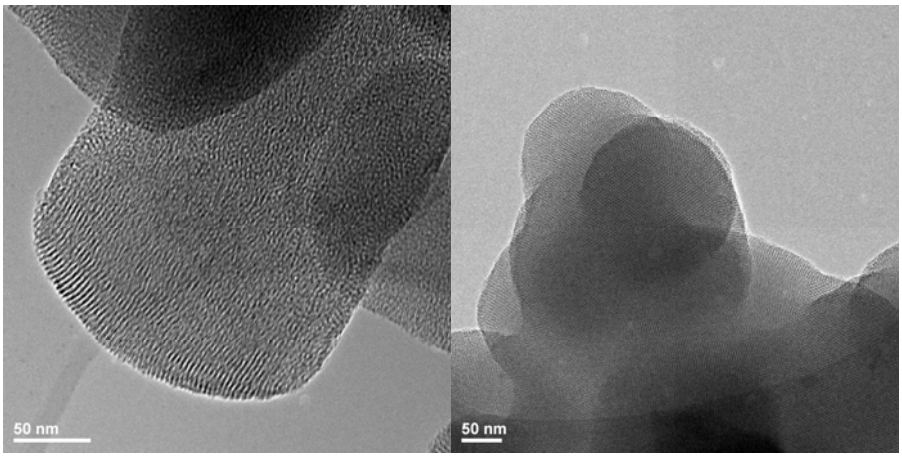

Fig. 1 HRTEM images of unmodified MCM-41 material (left) and $–NH_2$ modified MCM-41 (right)

The FTIR spectra in Fig. 2 clearly show that the functionalization reaction took place successfully for all the temperatures taken into account.

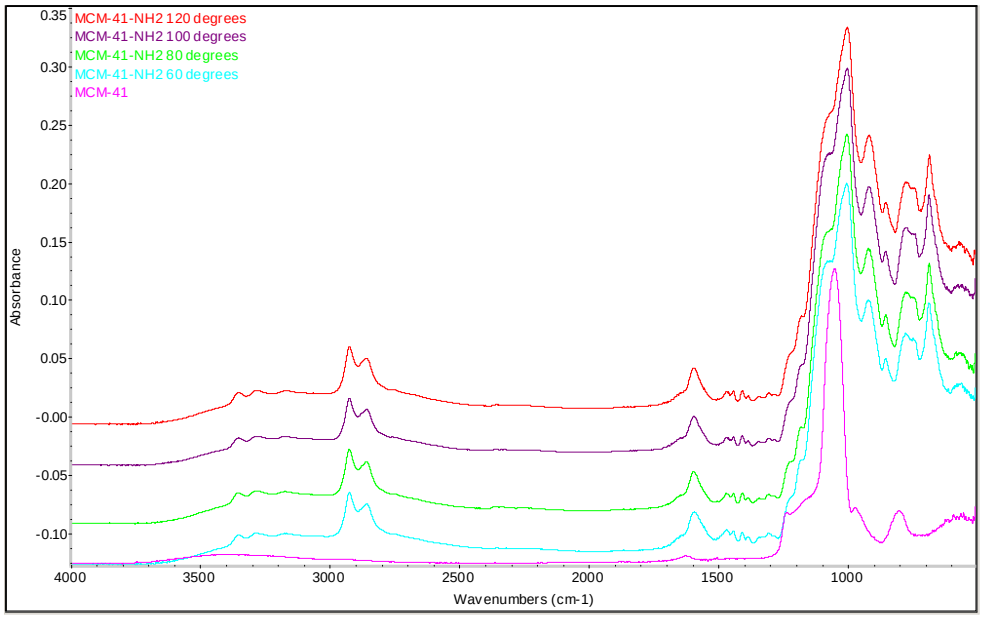

Fig. 2 FTIR spectra of MCM-41-NH_2 material for all the tested temperatures for the functionalization reaction

The presence in the FTIR spectra of the bands at 2926 and 2859 cm^{-1}, characteristic to the $–CH_2-$ bond, and the bands at 3354 and 3286 cm^{-1}, characteristic to the $–NH_2$ bond, and the modification of the fingerprint region of

the deformation vibrations (2000-500 cm^{-1}), clearly proves the functionalization of MCM-41. The shift in wavelenght corresponding to the Si-O absorbtion maximum from 1058 cm^{-1} in the MCM-41 matrix towards smaller values, 1004 cm^{-1}, in the functionalised material, is a proof of the reaction, through covalent binding, of the aminopropylsilil linker from APTMS.

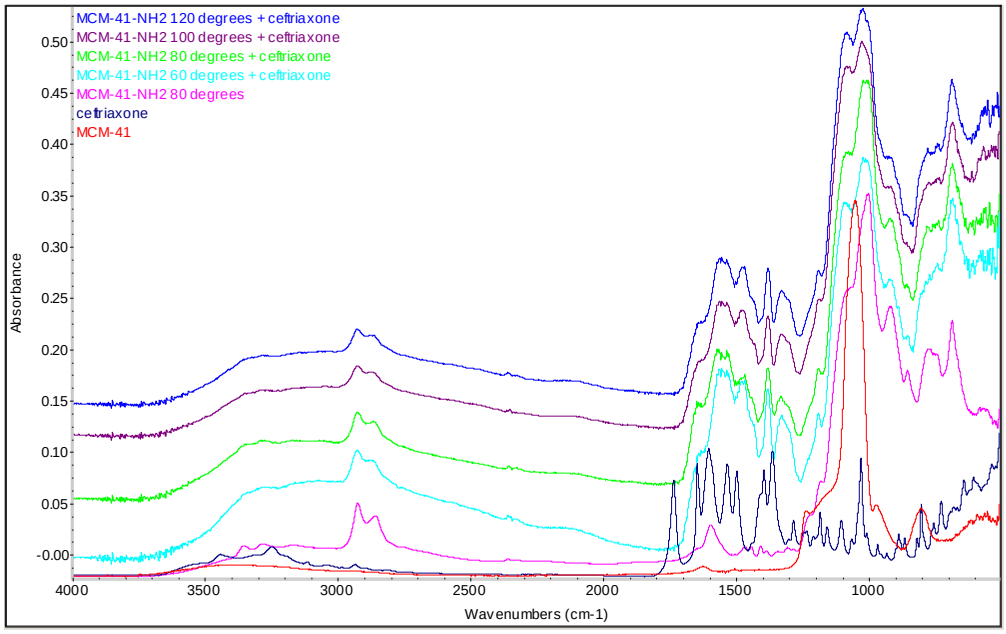

Fig. 3 FTIR spectra for the MCM-41-NH_2 materials loaded with ceftriaxone for all the tested temperatures for the functionalization reaction

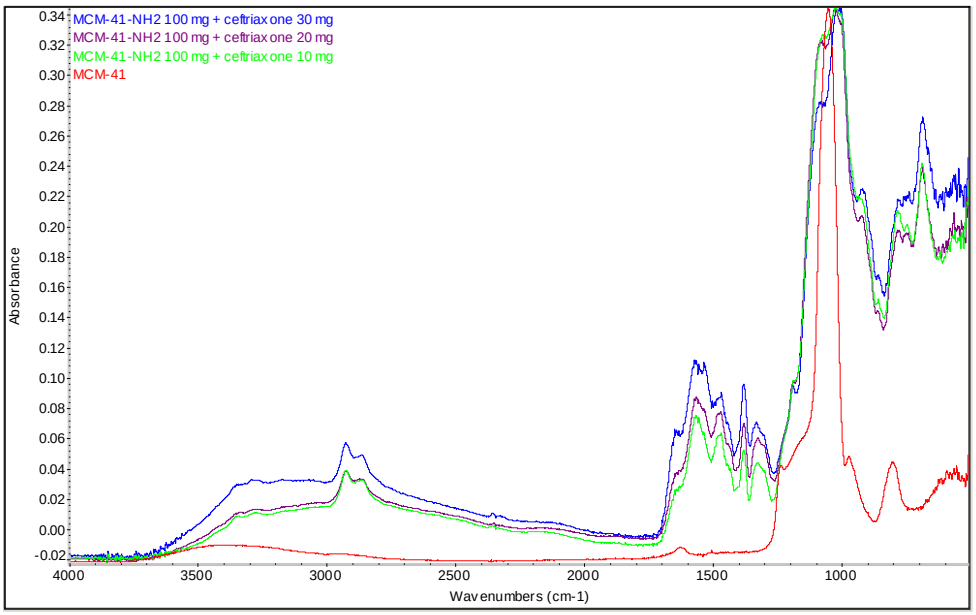

Fig. 4 FTIR spectra for the progressive loading with ceftriaxone, 10, 20, 30 and 40 mg ceftriaxone

For all the temperatures taken into consideration, the FTIR spectra in Fig.3 show that the loading process was successful, as the characteristic peaks for the ceftriaxone are present at their respective wavelengths. In the case of the progressive loading with ceftriaxone, a corresponding increase is observed in the measured absorbance in Fig.4.

The analysis of the difference in the release profiles in **Fig. 5** leads to some observations. All profiles correspond to a slow release (around 10% of total quantity absorbed), the amino groups functionalization being a good method to slow down the release curve for ceftriaxone. For all the profiles, the change in slope happens in the first 15-35 minutes. The slope change is a natural phenomenon, being caused by the amount deposited on the surface which has an accelerated release compared to the amount deposited in the porous channels. The profiles were grouped in two types of behavior, a group being formed by 60 ^{o}C and 80^{o}C reaction temperature profiles, and the other by the 100 ^{o}C and 120^{o}C reaction temperature profiles. This change can be assumed to come from unwanted dendritic ramifications between the linkers in the porous system, which can cause a partial blockage of the pores. Even in the case of the dendritic ramification, the slow release profile is maintained due to the high surface area. For the sample obtained through reaction at 120^{o}C a significant change in the slope can be noticed, change which can be due to a significant amount of blockage.

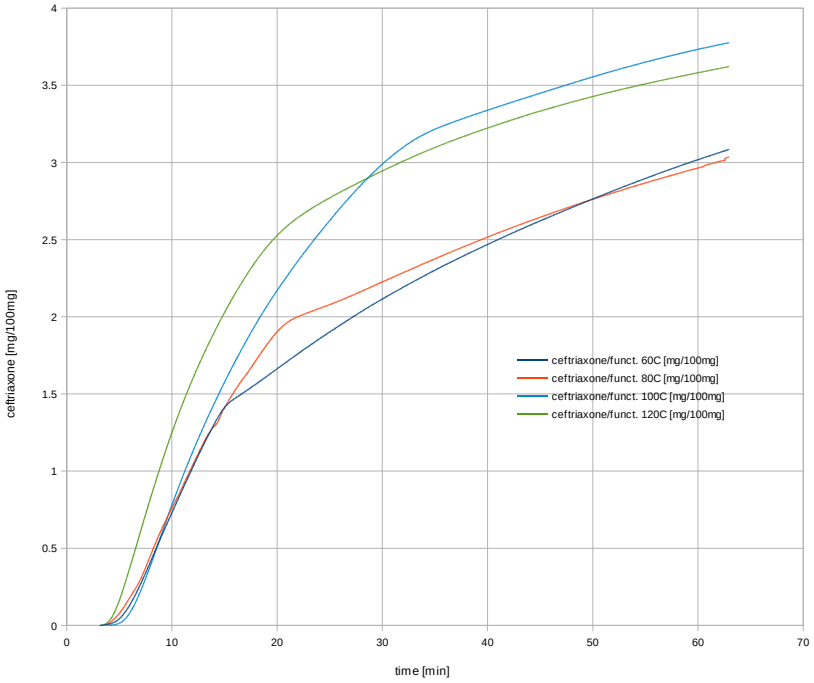

Fig. 5 Release profiles for ceftriaxone from MCM-41-NH_2 material, different reaction temperatures

The 80^{o}C sample has a practical advantage, from the fact that the change in slope occurs at higher concentration than in the case of sample functionalized at 60^{o}C, followed by a much slower slope. To conclude, the material functionalized at 80^{o}C was chosen for the progressive loading experiments.

In Fig. 6 an ascending profile is observed for the sample obtained at 80^{o}C, increasing according to the increase in amount of ceftriaxone. The position of the point in which the slope changes, shifts towards longer times with the increase of the amount of ceftriaxone. This is a normal behavior, after the maximum loading has been achieved, the amount of ceftriaxone which is easily available through diffusion growing with the excess of ceftriaxone deposited on the surface. The release rate is almost linear at lower concentrations. Depending on the aimed scope, a concentration can be chosen. In treating infections it is usual to give a higher dosage to start the inhibition process, than a lower dosage to maintain it. If that is desirable, than the amount of 40 mg/100 mg is a better alternative. If the release has to be constant, than the lower amount, 10 mg/100 mg is better suited.

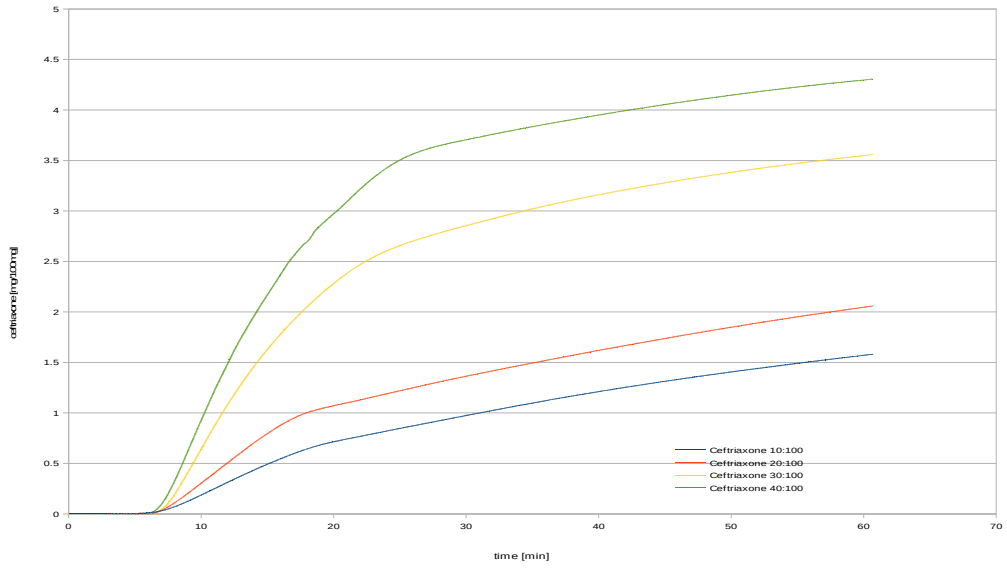

Fig. 6 Release profile from MCM-41-NH_2 material functionalized at 80°C for progressive loadings with ceftriaxone, (10, 20, 30, 40 mg)

4. Conclusions

The results reccomend the amino-functionalised MCM-41 material as a good candidate for controlled releases. Depending on the desired effect, the release time can be controlled through the means of the loading concentration and the functionalistation temperature. This tunability of the release profile is important because some application require a very slow release profile (several weeks, in the case of implants [22]), other require a slow profile (oral antibiotics, from 8hours to a full day [21]), ans some others require a slightly faster release (2-4 hours, oral intake) but require protection from the action of the gastric acid. This tunability of the release profile is of outmost importance and can be exploited for further biologically active substances in the future.

Acknowledgments

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