

Study on the influence of technological factors on drug loading of poorly water-soluble drug on MCM-41 mesoporous carrier

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Abstract

The present study explored solvent impregnation drug loading process of the poorly soluble non-steroid anti-inflammatory drug indomethacin on MCM-41 type mesoporous silica carrier. Different technological factors that can influence drug-loading process as time of reaction, temperature, use of non-solvent as well as different ratios between drug and MCM-41 were studied. TEM and DLS were used to characterize physicochemical properties of obtained particles. The influence of drug-loading rate on dissolution process were studied using in-vitro release tests. Our results established that changes in explored technological factors could lead to different indomethacin loading. Moreover, the in-vitro release tests proved that drug loading rate had a direct influence on indomethacin release from MCM-41 particles.

Our finding suggested that by tuning the main technological factors it would be possible different drug delivery systems with different drug loading rate to be obtained.

Keywords

drug loading, indomethacin, MCM-41, technological factors

Introduction

One of the biggest breakthroughs in the development of mesoporous silica materials was made in 1992 by Motor Oil Company. Researchers from the company for the first time used supramolecular structures, such as micellar aggregates as templates for synthesis of silica particles. This approach led to a production of new type of mesoporous silicate, which was the first of the M41S family of silica materials, including the most popular nowadays MCM-41 type. This material had a hexagonal mesopore structure, adjustable size and high loading capacity for biologically active substances. These properties allowed loading of larger drug molecules or enzymes increasing

the free volume of the particles, which in turn reflected in higher degree of drug loading. Moreover, MCM-41 also possessed particles with high chemical, physical and mechanical stability as well as varying size and mesopores, with the ability to modify the particle surface (Kresge et al. 1992; Ying et al. 1999; Huh et al. 2003; Suzuki et al. 2004; Trewyn et al. 2004; Hartmann 2005; Ying 2006).

The first exploration of MCM-41 as a drug delivery system was published by Vallet-Regi et al. (2001). MCM-41 particles with different pore sizes were studied as a carrier for non-steroidal anti-inflammatory drug (NSAID) ibuprofen. Ibuprofen release profiles showed different behavior, depending on the loading method used. Later, Yang et al. (2005) provided data on a new drug delivery system

for oral administration based on MCM-41 particles loaded with furosemide (substance with low solubility and permeability, predominantly absorbed in the stomach and proximal small intestine). The purpose of the obtained particles was to provide furosemide release in these GIT regions. Loading of furosemide into mesoporous silicate particles led to complete drug release within 90 minutes. Cavallaro et al. (2004) investigated MCM-41 mesoporous silica nanomaterials as drug delivery carriers for diflunisal, naproxen and ibuprofen. The release process was investigated at pH 1.2 and 6.8, simulating the pH in the gastrointestinal tract. The results showed that this carrier had a good potential as a controlled release system for diflunisal. Qu et al. (2006), disclosed a captopril-controlled release system using MCM-41 and SBA-15 silica mesopore systems as carriers and established higher captopril loading (up to 34%) into MCM-41 compare to acetylsalicylic loading.

In spite of the advantages offered by the MCM-41 material (large free volume, adjustable pore and particle size, possibility for functionalization, etc.), an inefficient loading with drug substances was often observed usually due to inappropriate loading conditions like: temperature, reaction time, concentration of drug substance in the reaction mixture (Balas et al. 2006; Katiyar et al. 2006; Trewyn et al. 2007; Manzano et al. 2008; Huang et al. 2010). Different loading conditions could lead to variations of loading rate (7 to over 50%). Yang et al. (2005) offered a method for developing pH-sensitive mesoporous silica nanoparticles, in which the load of the drug was performed over a period of 4 hours, whereas Zhao et al. (1998b) apply a method in which loading was over 72 hours. Differences were also observed in the other factors affecting drug loading like temperature, which could vary from 21 to over 70 °C. Unfortunately, a few scientific reports attempted to study the potential factors influencing the loading process and in practice, there was no complete clarification about the influence of the technological factors.

Because of all this, the aim of the study was to evaluate the influence of the main technological factors affecting drug loading during solvent-impregnation method. Indomethacin was chosen as a model drug because of its low water solubility. The factors studied were time of reaction, temperature, carrier/drug ratio and the use of non-solvent at the end of reaction time. The main physicochemical parameters of MCM-41 particles and drug release profiles were established via suitable methods.

Materials

The carrier – mesostructured MCM-41 with defined pore structure (hexagonal mesoscaled pores with mean diameter 3 nm and 1.09 cm³/g free volume) and the model drug – indomethacin were purchased from Sigma-Aldrich, St. Louis, USA. Disodium phosphate dihydrate, potassium phosphate (monobasic), hydrochloric acid and ethanol

were purchased from Merck, Darmstadt, Germany. Deionized water was prepared by ion exchange method.

Methods

Physicochemical characterization

The porous structure of the samples was characterized using transmission electron microscopy (JEOL JEM 2100 HR STEM – 200KV; point-resolution 0.23nm).

Determination of main physicochemical characteristics – particle size, index of polydispersity and zeta potential were performed using a Zetasizer Nano ZS (Malvern Instruments, UK). The samples were dispersed in distilled water and measured at a scattering angle of 90° and 25°. The measurements were made in triplicate.

Drug loading

The model drug substance was loaded into the pores of MCM-41 by using solvent impregnation method. Indomethacin (IND) was dissolved in 25 ml ethanol. Then, 100 mg mesoporous silica particles (MCM-41) preheated for 1h at 120 °C were added to indomethacin solution. After that, the mixture was incubated at predetermined temperatures under permanent stirring (180 rpm).

After 1, 3, 8, 15, 24 and 72 h, 50 ml of distilled water were added to the dispersion and ethanol was removed by vacuum distillation. The procedure continued with vacuum filtration (0.1 µm pore size), washing with distilled water and drying in a vacuum desiccator.

Drug loading rate determination

For determination of drug loading rate, 10 mg indomethacin-loaded particles were dispersed in 20 ml of methanol and stirred for 2 h at temperature 37 °C. Particles were then vacuum filtered using 0,1 µm membrane filters and re-dispersed in fresh methanol (20 ml). After a second filtration the supernatants were combine and the concentration of the indomethacin was determined by UV-vis spectrophotometry at a wavelength of 320 nm (Thermo Scientific Evolution 300). Standard curve ($r > 0.998$) was prepared in the concentration range 5–50 µg/ml.

In-vitro release tests

Drug loaded particles (10 mg) were incubated into 100 ml simulated gastric fluid (pH = 1.2) and phosphate buffer (pH = 6.8) at 37 °C under continuous stirring (100 rpm). At appropriate time intervals, 3 ml samples were taken and centrifuged at 15,000 rpm for 15 min. Concentration of the released indomethacin was determined by UV-vis spectrophotometry following the procedure, described in “Drug loading determination” section.

Results and discussions

Physicochemical characterization

Transmission electron microscopy was used for determination of the porous structure of non-loaded and indomethacin loaded particles (Fig. 1) in order to found potential changes in their mesostructures.

The unloaded MCM-41 particles (Fig. 1A) showed well-defined and homogeneous structure of the pores. The TEM images in Fig. 1B presented preserved porous structure after drug-loading procedures. The size of the particles that can be determined by the TEM technique correlated to DLS size-measurements.

Measurement of particle size, polydispersity index (PI) and zeta-potential, obtained by DLS assay showed mean size 370 nm (PI 0.45) and a slight tendency for increase of particles size and polydispersity index after drug loading (Fig. 2).

Measurements of zeta potential showed specific for the mesoporous silica materials negative value. Changes were observed in the surface charge of the loaded particles. Decrease of zeta-potential was observed for indomethacin-loaded particles because of the negatively charged carboxylic groups in the structure of indomethacin. The strongly negative value of drug-loaded samples could be considered as an indicator for high physical stability of the particles.

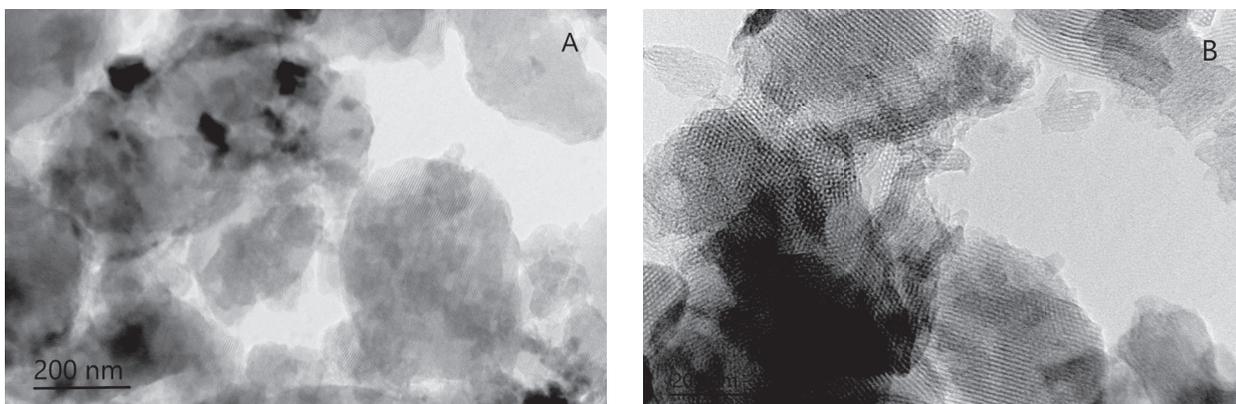


Figure 1. TEM images of empty (picture A) and indomethacin-loaded (picture B) particles.

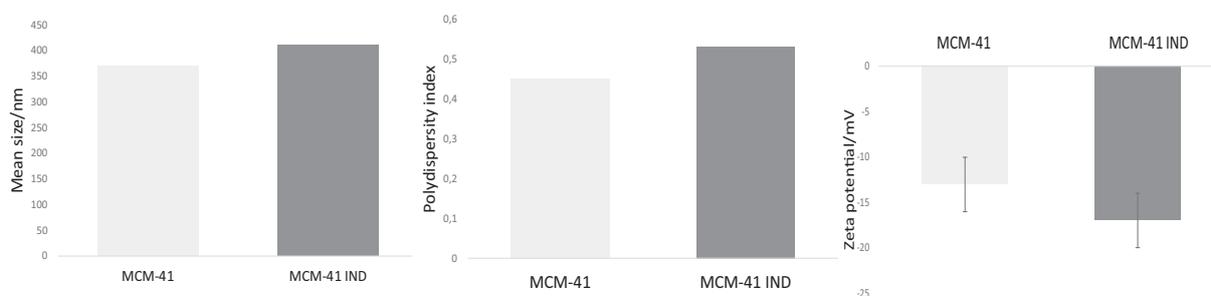


Figure 2. DLS determination of main physicochemical characteristics (size, polydispersity index and zeta-potential) of empty (MCM-41) and indomethacin-loaded particles (MCM-41 IND).

Study on technological factors, affecting drug loading

Reaction temperature

Reaction temperatures used during drug loading procedure were selected according to the literature data. Three limit values were used – 21, 37, 60 °C. Fig. 3 contained the results of the conducted research.

As it could be seen, there was a proportional dependency between the reaction temperature and the degree of drug loading. Our results showed that the difference in loading between particles, incubated at 21 °C and these, incubated at 37 °C was significant. In our opinion, the optimal temperature for indomethacin loading should be 37 °C, as the increase in the temperature to 60 °C led to a very small increase in the loading but would result in the inability of some thermally labile drug substances to be included in mesoporous silica nanoparticles.

Time of the reaction

Incubation time was another factor that had a direct impact on the loading rate. For the aim of experiment six times intervals (1, 3, 8, 15, 24, 72 hours) were used. As it could be seen from Fig. 4, the loading increased with increasing reaction time, as this trend was more pronounced up to 24 hours. The loading rate was significantly

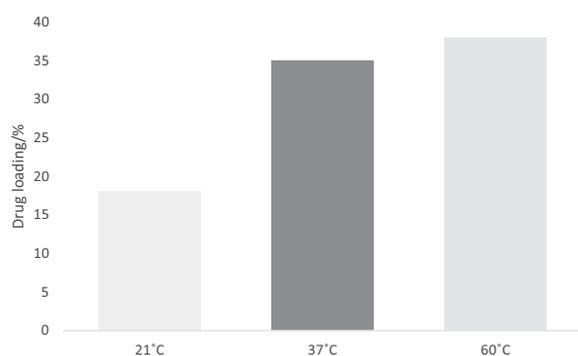


Figure 3. Drug loading of MCM-41 after incubation at different temperatures.

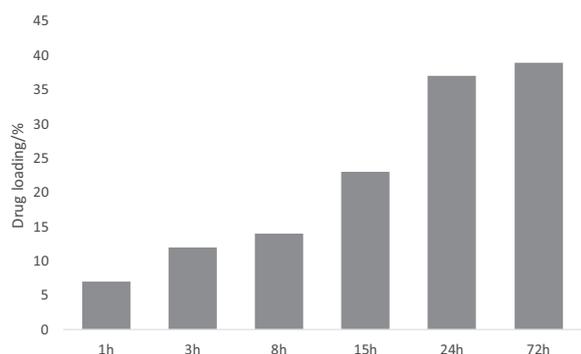


Figure 4. Drug loading degree (%) after different duration of the incubation stage.

increased, with 7% indomethacin loaded after one-hour incubation period, whereas after 24 hours incubation loading rate reached 36%. The additional three-fold increase in incubation time did not result in a significant increase in loading, so the optimum loading time should be set at 24 hours.

MCM-41/indomethacin ratio

The studies were conducted at three ratios between MCM-41 and indomethacin, namely 1:0.5, 1:1 and 1:2. Fig. 5 represented the results of drug loading as a function of carrier/drug ratio. As it could be seen, indomethacin

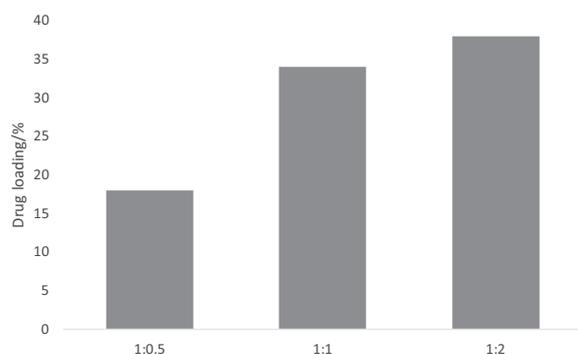


Figure 5. Drug loading rate (%) with using of different MCM-41/indomethacin ratios in the incubation stage.

loading increased with an increase in the concentration of the drug in the reaction mixture.

In the conditions of a continuous increase of indomethacin concentration, the loading increased, albeit to a lesser extent. Our studies showed that ratios above 1:1 are inappropriate because of potential loss of drug substance during the loading process (Table 1).

Table 1. Loss of indomethacin in the loading process, depending on the MCM-41/drug ratio.

Ratio	1:0.5	1:1	1:2
Losses (%)	28	30	62

Use of non-solvent at the final step of drug loading

The load process of unfunctionalized MCM-41 was mainly based on a diffusion process. Since diffusion occurs until equilibrium was reached, addition of a non-solvent and solvent removal at the final stage of the loading would result in a change in equilibrium towards the nanoparticle channels, which would theoretically increase loading and reduce the loss of active substance. Our studies (Fig. 6), where ethanol was used as a solvent and distilled water as non-solvent, showed a very high increase in indomethacin loading using a non-solvent.

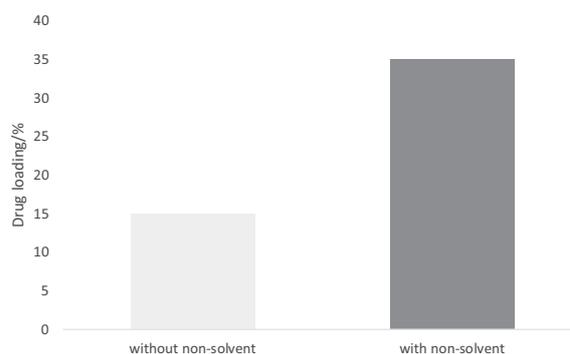


Figure 6. Drug loading degree (%) with and without using of non-solvent for indomethacin at the final stage of incubation process.

In vitro drug release

The influence of different drug loading rate on indomethacin release profiles was evaluated by in-vitro release tests. The tests were performed in two different media (pH 1.2 and pH 6.8) which are used to simulate the conditions in gastro-intestinal tract. Three different samples (respectively with 7, 15 and 35% drug loading) were used. The results are presented on Fig. 7.

Our results showed that all samples had burst release in the first 10 min with a tendency for faster release with decrease of the drug loading at both pH media. The second phase (from 10th min) showed different trend. The samples released loaded drug with different speed, depending

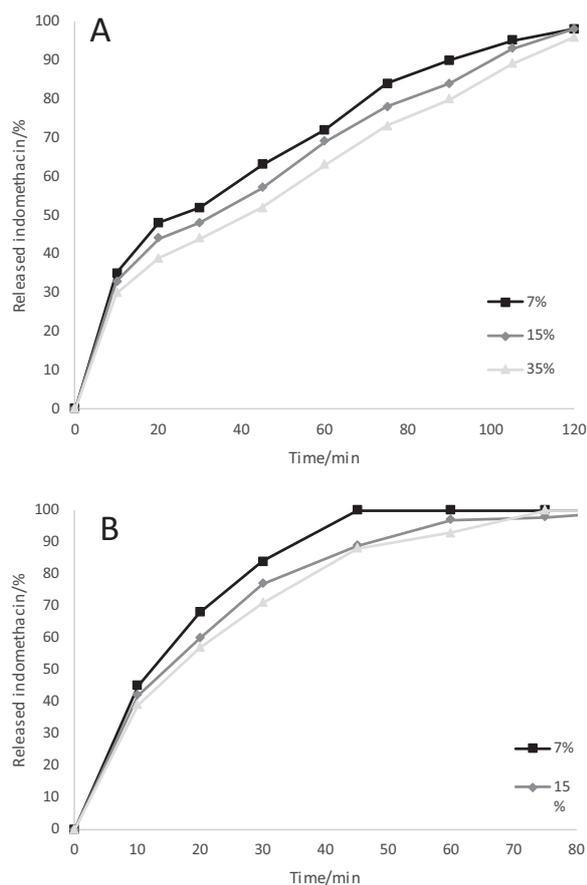


Figure 7. Release profiles of samples with different drug loading in simulated gastric fluid (A, pH = 1.2) and phosphate buffer (B, pH = 6.8).

on the loading rate. The particles with highest loading rate released indomethacin with slowest speed. Moreover, this tendency of indomethacin release in media with pH 6.8 was more pronounced (100% for 80 min).

This effect could be explained with the free spaces inside the mesoporous channels. Samples with low degree of drug loading had more free spaces in the channels. These spaces allowed more liquid to enter the pores which led to faster solubility of indomethacin. On the contrary, samples with higher drug loading possessed less free spaces which was a premise for minimal contact between drug and gastro-intestinal fluids resulting in slower drug release.

Conclusion

The main technological factors that could influence the drug loading process of indomethacin on mesostructured silica materials were explored. TEM and DLS measurements of MCM-41 particles with defined pore size and structure showed mean particle size of 370 nm, negative zeta-potential and preserved porous structure after drug-loading process. Our results showed that all the studied factors (time of reaction, temperature, use of non-solvent and different ratios between drug and MCM-41) could influence the drug loading. In-vitro release tests

conducted at pH 1.2 and 6.8 showed that the drug loading rate directly influenced indomethacin release from MCM-41 particles at both pH media.

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