ORIGINAL ARTICLE

ASSESSMENT OF THE *IN VITRO* RELEASE OF ALENDRONATE SODIUM FROM MESOPOROUS SILICA PARTICLES

LACRAMIOARA OCHIUZ[1#], MIHAELA CATALINA LUCA[2#], IULIAN STOLERIU[3#], MIHAELA MOSCALU[2#], DANIEL TIMOFTE[2,4#], GLADIOLA TANTARU[1*#], ALINA STEFANACHE[1#]

[1] *"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, 16 Universitatii Street, 700115, Iasi, Romania*
[2] *"Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Faculty of Medicine, University street, no 16, 700115, Iasi, Romania*
[3] *"Al. I. Cuza" University, Faculty of Mathematics, 11 Bvd. Carol I, 700506, Iasi, Romania*
[4] *"Sf. Spiridon" Hospital, Surgery Department, 1 Piata Independentei, 700111, Iasi, Romania*

**corresponding author: gtantaru2@yahoo.com; gladiola.tantaru@umfiasi.ro*
[#] *All authors had equal contribution to this research*

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Abstract

The objective of this study was to assess the influence of pore structure on the process of loading and releasing alendronate (AL) from modified release particles based on mesoporous silica. Two types of systems were prepared: AL@MCM41, using Mobile Composition of Matter no 41 (MCM 41) silica, with cylindrical pores arranged in a hexagonal structure, and AL@MCM48, based on Mobile Composition of Matter no 48 (MCM 48) silica with cubic structure and cylindrical radial pores diverging in three directions. After preparing the silicate matrices, AL was immobilized in the two mesoporous silica using the adsorption technique. For each type of silica particles we performed the *in vitro* dissolution test with simulating gastrointestinal fluids. Subsequently, the release profile of AL from the two types of mesoporous silica was obtained and the AL pharmacokinetics was analysed by fitting on mathematical models. The results obtained showed that pore structure of silicate materials have a key role in both loading capacity of silica with AL and rate of release of this drug substance.

Rezumat

Obiectivul acestui studiu a fost cercetarea influenței structurii porilor asupra procesului de încărcare și eliberare a alendronatului (AL) din particule ce au cedare modificată pe bază de silice mezoporoasă. Au fost preparate două tipuri de sisteme poroase: AL@MCM41 prin utilizarea materialului compozit silicat numărul 41 (MCM 41), cu pori cilindrici și aranjament hexagonal și AL@MCM48 pe bază de material compozit silicat numărul 48 (MCM48) care prezintă pori cilindrici ramificați în trei direcții și aranjament cubic. După prepararea matricelor silicate gazdă, AL a fost imobilizat în cele două sisteme mezoporoase prin tehnica adsorbției. Pentru fiecare tip de particule silicate am realizate testul de dizolvare *in vitro* în medii de simulare a fluidelor gastrointestinale. A fost obținut profilul de cedare a AL din cele două tipuri de sisteme mezoporoase și a fost analizată cinetica de eliberare al AL prin fitare pe modele matematice. Rezultatele obținute au evidențiat că structura și arhitectura porilor materialelor silicate au un rol esențial, atât în capacitatea de încărcare a silicei cu AL, cât și asupra vitezei de eliberare a acestei substanțe active.

Keywords: alendronate, mesoporous silica, modified drug release

Introduction

In recent years, interest in mesoporous silica as a system of drug delivery has increased rapidly. MCM (Mobile Composition of Matter no 41, 48) (MCM 41 and MCM 48) belong to the category of hydroxylated amorphous silicates with large pores that are generated from a so-called liquid crystal arrangement of the surfactant molecules, formed by self-assembling [1, 2]. These two types of silica are different in pore nature and architecture. MCM 41 shows the so-called "honeycomb" architecture resulting from the hexagonal arrangement of the cylindrical pores, while MCM 48 is formed by the Ia3d cubic wrapping of an enantiomeric pair of tridimensional pore systems.

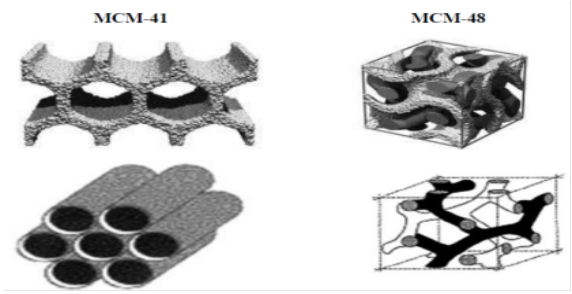

Figure 1.
Pore architecture of mesoporous silicas [2]

Basically, MCM 41 shows a one-dimensional system of cylindrical pores aligned hexagonally, while in MCM 48 the pores form cylindrical channels diverging in three directions and interconnecting only within the same channel system (Figure 1).

Alendronate (AL) is one of the main bisphosphonates orally administered in the treatment of demineralisation bone diseases. The reduced oral bioavailability of AL (0.1 to 1%) is the main limiting factor in the therapeutic efficacy of this active substance. The causes of its low oral bioavailability are: the fact it belongs to the 3rd class in the BCS (Biopharmaceutical Classification System), $T_{½}$ = 0.5 - 2 h, Ca^{2+} chelation with the formation of non-absorbable complexes [3, 4]. Aiming at increasing the oral bioavailability of AL, we loaded it into two biocompatible mesoporous silica (MCM 41 and MCM 48), thus obtaining AL modified-release drug delivery systems (AL@MCM41 and AL@MCM48) intended for oral administration. The research results on the compatibility and stability of AL in the two silicate materials were previously published [5]. The main objective of this study is to investigate the influence of mesoporous silica type on the uptake and pharmacokinetics of AL.

Materials and Methods

Materials

Alendronate sodium tryhidrate was supplied by Apotex Pharmaceutics Inc. (Canada). For the synthesis of the mesoporous silica matrices, tetraethyl orthosilicate (TEOS, 98%), cetyltrimethylammonium bromide (CTAB, 99%) and NaOH were purchased from Merck (Germany). Ethanol (99%), ammonia (25%) and hydrochloric acid (32%) were purchased from MedChim (Romania). All reagents were used without further purification.

Methods

Synthesis of the MCM 41 silica matrix was performed by modifying the Ströber method *via* the hydrolysis of tetraethylorthosillicate (TEOS), under ultrasonic irradiation [6]. In a typical synthesis procedure of MCM 41 nanoparticles, the chemical composition of the reaction mixture was TEOS : CTAB : EtOH : H_2O = 0.3 : 95 : 15 : 246. The formed gel was sonicated for 2 h, in pulsed mode on/off 3s/1s. The white solid was recovered by centrifugation and decantation, washed with water several times with hot deionized water and dried at 60°C overnight. Template removal was performed by calcination at 550°C for 8 h.

Synthesis of the MCM 48 silica matrix was performed by hydrothermal treatment in alkaline media, at 100°C for 72 h. TEOS was used as silica source, CTAB as templating agent and sodium hydroxide as mineralizing agent The composition of the initial reaction mixture was TEOS : CTAB : NaOH : H_2O = 0.2 : 0.48 : 0.48 : 55. The obtained white, abundant precipitate was washed several times with deionized water, filtered and dried at 60°C overnight. Template removal was performed by calcination at 550°C for 6 h, with a temperature increasing rate of 1°C/min.

The process of d*rug immobilization* involved mixing the components at a ratio of 50 mg MCM 41 matrix/50 mL of AL solution (1 mg/mL). The tests were performed sonochemically for 120 min, using an ultrasonic generator SONICS VIBRA CellTM Model CV 33 (1.13 cm diameter Ti horn) with 750 W power, by applying a periodic pulse cycle of 3 s ultrasound irradiation / 1 s resting time. The amount of AL loaded on MCM 41 and MCM 48 was determined by UV-VIS spectrometry using a complexation method with 9-FMOC (Fluorenyl-methyloxycarbonyl chloride), developed and validated in house according to USP 32 [7].

In vitro dissolution test. The release profile of AL from AL@MCM41 and AL@MCM48 particles was obtained using a *SR 8 Plus Series* (AB & L Jasco) device, according to the following experimental protocole: *dissolution medium:* 500 mL of simulated gastric fluid (pH 1.2, 0.1 N HCl) for the first 2 hours and 500 mL simulated intestinal fluid (pH 6.8 - phosphate buffer) for the next 22 hours; *Apparatus 2 (paddles); bath temperature* 37ºC ± 0.5ºC; *rotation speed*: 50 rpm; the sampling interval was set at every hour for 24 hours. 3 mL of sample were taken each hour and they were replaced with the same volume of media. One sample of aliquot was subjected to the derivation and quantitative determination described in USP monograph for HPLC analysis of Sodium Alendronate. All the experiments were performed in triplicate.

The analysis of the difference factor (f_1) and the similarity factor (f_2)

In order to emphasize the similarities and differences on the dissolution profile of AL from the studied silica matrices (AL@MCM41 and AL@MCM48), the results of the *in vitro* tests were used to calculate the difference factor (f1) and the similarity factor (f2) according to equations 1 and 2:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100 \quad (1)$$

$$f_2 = 50 \log_{10} \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

where: n = the number of sampling time points, R_t = released AL percentage of the reference formula at time point t, T_t = released AL percentage of the test at time point t and $\log_{10}x$ represents the logarithm of x to the base 10 [8].

Evaluation of AL pharmacokinetics - experimental data obtained from the *in vitro* dissolution test were analysed by fitting on Higuchi model, equation 3, and Korsmeyer-Peppas model, equation 4:

$$M = K_H t^{1/2} \quad (3)$$

in which: M - the amount of drug released at time t; K_H - Higuchi release constant.

$$M_t / M_\infty = K_P t^n \quad (4)$$

in which: M_t / M_∞ - the ratio between the amount of drug released at time t; K_P - Peppas-Korsmeyer constant of release rate; n - diffusion coefficient.

Data fitting was performed by linear and nonlinear regression using Matlab 7.1 [9-11]. Data were presented as mean ± standard deviation and were considered statistically significant at $p < 0.05$.

Results and Discussion

Obtained data from AL loading in the silica matrices showed that AL adsorption in host matrices is a quick process and that adsorption equilibrium is reached in less than 2 hours (Figure 2). Additionally, it was revealed that MCM 41 has a higher alendronate adsorption capacity than MCM 48.

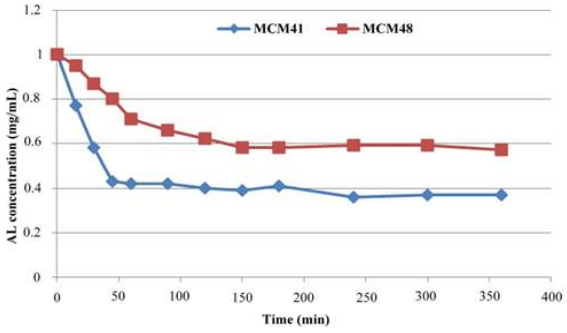

Figure 2.
Influence of reaction time on AL immobilization on MCM41 and MCM48 mesoporous silica

The final loading degree was 125 mg AL/g matrix for AL@MCM41 and only 85 mg AL/g matrix for AL@MCM48. In addition, adsorption equilibrium was reached harder in MCM 48 than in MCM 41, where adsorption was slower. This observation points to the importance of the silica pore architecture. MCM 41 exhibits a one-dimensional system with cylindrical pores of 3 nm diameter, arranged hexagonally (honeycomb), whereas MCM 48 shows a three-dimensional system of cylindrical pores with 2.45 nm diameter cubically arranged. This feature is due to the fact that pore length in MCM 48 is significantly higher than in MCM 41 and justifies the low AL uptake and the slower loading rate. The results are consistent with other data reported in the literature for MCM 48 and MCM 41 mesoporous silica encapsulation of active substances with similar characteristics to AL [12-15]. The results of the *in vitro* dissolution tests showed major differences in the release profile of AL, from the two silicate matrices. The cubic arrangement of the mesopores in the AL@MCM48 matrix structure restricts the AL release process which occurs slowly; consequently, only 82.49% of the encapsulated AL quantity was released after 24 hours. The one-dimensional AL@MCM41 matrix with cylindrical pores generates a "burst effect" within the first two hours of the test by releasing 45.55% AL, after which the release rate becomes increasingly slower (Figure 3).

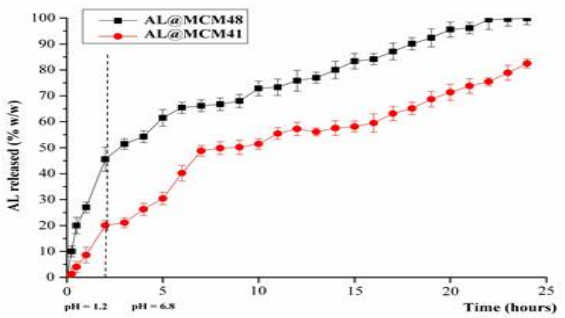

Figure 3.
In vitro dissolution release of AL from silica matrices

The influence of the "burst effect" phenomenon is also observed in the values from the release profile of AL from silicate matrices, although AL is released from both systems by Fickian diffusion due to fitting on model Korsmeyer-Peppas (Table I and Table II) [16, 17].

Table I
The values of the difference factor f1 and the similarity factor f_2

Reference formula (Rt)	*Factor*	*Test formula (Tt)*
AL@MCM41	*f1 = 30.8132*	AL@MCM48
	f2 = 32.9173	

The difference factor is 30.81, which points to major differences in the release profile of AL in the studied systems [18].

Table II
Parameter values of the kinetic release

Silica matrix	*Higuchi model*		Korsmeyer-Peppas model		
	K_H ($h^{-0.5}$)	R^2	Kp (h^{-n})	n	R^2
AL@MCM41	21.9163	0.9432	28.6757	0.4	*0.9836*
AL@MCM48	15.8649	0.9737	13.8656	0.55	*0.9784*

Conclusions

The aim of this study was to analyse the influence of pore structure on the process of AL release from modified release particulate systems based on MCM 48 and MCM 41 mesoporous silica. The pore architecture of the host silicate material has a crucial role both in the adsorption capacity of AL and in its release characteristics. Obtained results showed that MCM41, with cylindrical unidirectional disposed pores, exhibits a superior AL adsorption capacity compared to the MCM 48 silica, which has a three-dimensional system of cylindrical pores and cubic arrangement. The unidirectional disposition of the MCM 41 silica pores favours the emergence of a "burst effect" phenomenon in releasing AL from the AL@MCM41 system. Release kinetics analysis showed that AL is released by Fickian diffusion from both AL@MCM41 and AL@MCM48. These results indicate that pore structure and architecture influence the release rate of AL from the porous matrix, but they do not interfere by chemical phenomena in the diffusion of AL towards the exterior of the matrix.

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