ASSESSMENT OF SBA-16 ADSORPTION CAPACITY TOWARDS ACTIVE SUBSTANCES WITH DIFFERENT CHEMICAL STRUCTURES

MARIUS NICULAUA, BOGDAN I. CIOROIU,[*] ALINA M. TOMOIAGĂ,[**]
MONA E. CIOROIU[***] and MIHAI I. LAZAR[*]

"Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, 3, Mihai Sadoveanu Alley, Iasi, 700490, Romania
[*]*Department of Drug Analysis, "Gr. T. Popa" University of Medicine and Pharmacy, 16, Universitatii Str., 700115, Iasi, Romania*
[**]*Department of Chemistry, "Alexandru Ioan Cuza" University from Iasi, 11, Carol I Bvd., 700506, Iasi, Romania*
[***]*Department of Clinical Biochemistry, Clinical Hospital of Pulmonary Disease, 30, Dr. I. Cihac Str., 700115, Romania*
✉ *Corresponding author: B. I. Cioroiu, bogdan.cioroiu@gmail.com*

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Mesoporous silica was used for the determination of the encapsulating capacity of different active pharmaceutical substances. The type of support was SBA-16, which has the particularity of having spherical morphology and 3D cubic arrangement of pores. The matrix showed high throughput for the encapsulation of piroxicam and two types of glucosaminoglycans (chondroitin and glucosamine sulfate). The type of intermolecular forces was hydrogen bonding, which permitted the use of the material without any supplementary functionalization. The capacity of the material was demonstrated to range from 197 mg for piroxicam to 375 mg for chondroitin sulfate and 473 mg for glucosamine sulfate. The process of encapsulation was affected by temperature and the optimum contact time was 60 minutes. Structural evaluation was performed using SEM, TGA and FTIR procedures and the evaluation of intake was determined by HPLC methods. Piroxicam stability was evaluated by mass spectrometry testing.

Keywords: glucosaminoglycans, analytical function, adhesive bonding, mesoporous materials, silica particles

INTRODUCTION

In the last decade, mesoporous silica materials have been extensively used due to their capacity to include different types of active pharmaceutical substances, from small molecules and to compounds with high molecular masses, such as proteins, peptides or other biological systems.[1] This capacity is due to their uniform and ordered pore system, which gives a high surface area available for contact and inclusion. The major advantages that recommend mesoporous silica as very promising materials to host and deliver drugs to specific sites are: (i) biocompatibility and biodegradability; (ii) high surface area and large pore volume to host high amounts of drugs with large molecules; (iii) flexibility for surface modification in order to give new functionalities; (iv) possibility to combine it with various agents in order to enhance targeting and/or labelling capabilities. For example, mesoporous silica nanoparticles (MSNs) are proven as an ideal platform for the cell marker through the co-condensation process. Dye (FITC) functionalized hexagonal crystal-like mesoporous silica nanoparticles were synthesized with high yield and the cell labelling capability was demonstrated.[2,3] These nanoparticles are about 30-300 nm in size and appear to have no apparent cytotoxic effects. Also, their application as cell markers in normal, cancer, and stem cells was demonstrated. Magnetic resonance contrast agents are formed using functionalized MSNs (Gd-EDTA and Fe_2O_3) and used to track cells.[4,5] These nanoparticles showed high cellular uptake efficiency and can be used in tracking the distribution of stem cells. The most important application is targeting, and this is enhanced by their capacity to incorporate various biologically important groups. For example, by modification

with suitable antibodies or short chain peptides, MSNs were used to target specific cells.[6-8] Similarly, MSNs were demonstrated as an ideal platform for drug- or enzyme-release systems.[9-12] The active properties of the substrate are determined by the surface functionalized nanoparticles, which are capable of intermolecular forces (hydrogen bonding, ion-induced dipole forces, dipole-dipole forces).[13]

Since the early '90s, different types of materials have been developed, such as MCMs,[14] HMSs[15] and SBAs,[16] possessing different pore sizes and architectures. In particular, MCM41, which belongs to the class of MCM materials discovered by the researchers at Mobil Oil Corporation in 1992,[16,17] consists of a regular arrangement of cylindrical pores closing down in a 1-D hexagonal pore system.[16,18] The class of HMS (Hexagonal Mesoporous Silicas) materials is represented by a series of hexagonal pore system-based silica synthetized using dodecyl amine and tetraethyl orthosilicate as starting materials.[19]

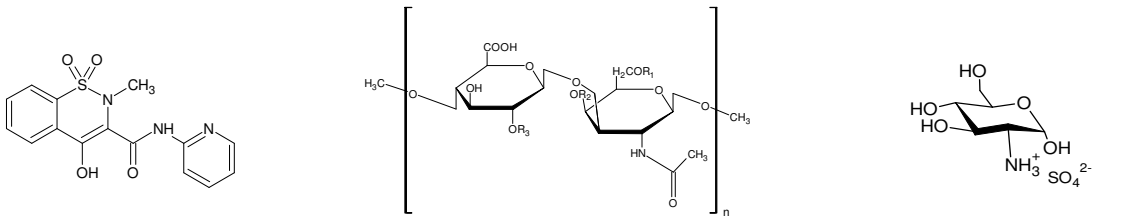
From the class of SBA (Santa Barbara) mesoporous silica, SBA 15, having a 2-D hexagonal arrangement of mesopores with microporous walls, was previously considered an optimized form with crystallographic morphology and pore structure of MCM-41,[20,21] more suitable for hosting bulk branched organic molecules. However, SBA-16 with spherical morphology offers many advantages to be considered as hosting matrix for bulk active substances. Comparatively with SBA-15, which has long cylindrical pores, SBA-16 can offer the major advantage of slow drug release, due to its shorter mesopores with cubic arrangement, corresponding to Im3m crystallographic group.[22]

In this work, we have employed SBA-16 mesoporous silica as hosting matrix for active pharmaceutical substances with different functionalities (chemical properties) and structural geometries. The selected molecules are: piroxicam, chondroitin sulfate and glucosamine sulfate, and their structures are shown in Figure 1.

Piroxicam is widely used for semi-topical and oral formulations, principally as an anti-inflamator non-steroidal drug. Piroxicam acts as an anti-rheumatic with high efficacy and with durable effect and it is produced by the inhibition of cyclooxygenase and the reduction of the leucocyte chemotaxis.[23]

Chondroitin sulfate is a glucosaminoglycan composed of a chain of alternating sugars (N-acetyl-galactosamine and glucuronic acid). Glucosamine sulfate is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. For medical formulations, glucosamine occurs in combination with other supplements, such as chondroitin sulfate.[24] One important aspect is that both glucosamine and chondroitin sulfate are considered inefficient for the treatment of symptomatic osteoarthritis of the knee because of their low bioavailability.[25] In order to produce a modification of the behaviour of glucosaminoglycans, several studies have been carried out to verify the suitability of the compounds for hydrogel synthesis.[26,27]

The purpose of the present study has been to investigate the adsorption rates of these substances in matrices of mesoporous silica.



Piroxicam Chondroitin sulphate Glucosamine sulfate

Figure 1: Structure of target compounds

EXPERIMENTAL

Materials

The study was performed using the following reagents: tetraethyl orthosilicate (TEOS, 98%) as source of silica, Pluronic F127 (poly(ethylene-oxide)-block-poly(propylene-oxide)-block-poly(ethylene-oxide), M_{av} = 12600 used as co-surfactant was purchased from Sigma-Aldrich, USA. Buthanol and hydrochloric acid were purchased from Merck Chemicals GmBH, Germany. Formic acid, sodium decansulphonate, sodium hydroxide (99%), hydrochloric acid (38%) were also purchased from Sigma-Aldrich, USA. All reagents were used without further purification. Chondroitin sulfate (CHND) and glucosamine sulfate (GLC) were purchased from Sigma-Aldrich, USA, and Piroxicam (PRX) was a European Pharmacopoeia reference standard. Deionized water was prepared with an ELGA Purelab water system and used throughout the experiments.

Synthesis of SBA mesoporous silica matrices

Synthesis was performed according to literature.[28] SBA-16 mesoporous silica material was synthesized using Pluronic F127, as a structure directing agent, which was reacted at room temperature. For this preparation, 3.0 g of Pluronic P127 was dissolved in 144 mL of water and 13.9 mL of HCl 38% solution with constant stirring at 25 °C. After 30 min, 11 mL of the co-surfactant butanol was added to achieve a 1:3 (F127:BuOH) mass ratio in the ternary system. Next, 15.3 mL of TEOS was added to the solution with stirring at 45 °C for 24 h, under reflux. After aging at 100 °C for 24 h also under reflux, the solid was collected by vacuum filtration and dried at 60 °C overnight. The surfactant was removed by calcination, which was carried out under open atmosphere by increasing the temperature to 550 °C gradually with 1 °C/min, and a plateau of 8 h.

Drug uptake

For drug immobilization tests, 50 mg of silica matrix was suspended in 50 mL of active compound PRX solution (1 mg/mL) or in 50 mL of solution containing CHND and GCL (1 mg/mL), and vigorously mixed by magnetic stirring. The contact time was optimized and evaluated using separate solutions, in order to avoid loss of material and variation of concentration of active compounds. The resulted complexes were recovered by centrifugation, followed by decantation and filtration. The solids were dried overnight at 60 °C. The amount of active compounds, PRX and the association of CHND and GCL, loaded on the inorganic matrices were assessed by means of the decrease of their concentration in the remaining solution, using chromatographic methods. The method was fully validated for linearity, accuracy, precision and determination of the detection limit and of the quantification limit.

Characterization methods

The ESEM studies were performed on samples fixed on copper supports. The surface was examined by using an Environmental Scanning Electron Microscope (ESEM) type Quanta 200, operating at 20 kV with secondary electrons in low vacuum mode (LFD detector).

TGA experiments were conducted on a STA 449 F1 Jupiter device (Netzsch, Germany). Samples were heated in alumina crucibles in nitrogen atmosphere at a flow rate of 50 mL/min. A heating rate of 10 °C/min was applied.

Fourier Transform Infrared Spectra (FTIR) were recorded on a Bruker Tensor 27 Spectrometer equipped with a DigiTectTM detector, in the spectral range of 4000-400 cm^{-1}, with a resolution of 2 cm^{-1} and a wavenumber accuracy of 0.01 cm^{-1}. All samples were measured as KBr pellets using 5 mg of probe sample.

Chromatographic determinations

The immobilization of active substances on the inorganic nanoporous matrices was monitored by chromatographic methods. Analysis was carried out using an Ultimate 3000 chromatographic system with diode array detector set at 300 nm for PRX. The mobile phase consisted in a mixture of sodium sulfate (pH 3.5): acetonitrile and methanol 65:15:20.[29] Chondroitin and glucosamine sulfate were determined by a derivatization reaction with sodium nitrite in acid media at 330 nm.[30] CHND and GLC used ion pair reagent sodium decansulphonate 0.005 mM and the flow rate for both methods was 0.5 mL/min.

Also, both methods used reversed phase C18 column, Thermo Fisher Hypersil Gold150×4.6 mm, 5 µm particle size.

The HPLC methods were fully validated, including specific parameters determined according to EP.[31] We applied precision repeatability, accuracy range and limits of detection and quantification. The parameters are included in Table 1.

Mass spectrometry studies were carried out on a triple quadrupole mass spectrometer Thermo TSQ Access Max equipped with HESI interface. Global parameters were: ion spray source of 3.5 kV, ion source temperature of 375 °C, sheath gas pressure of 10 psi and collision energy of 10 eV.

RESULTS AND DISCUSSION

Generally, when mesoporous materials are involved in the adsorption of drug molecules, the drug intake rate and immobilization are governed by the size and geometry of the mesopores within the host matrix, because steric effects may impair the inclusion process. Another factor is the pore surface chemistry, since the immobilization is dependent on weak forces, which are created in interaction with the target molecule. In this work,

SBA-16 mesoporous silica with cubic arrangement of the mesopores is employed as host matrix for bulk molecules of pharmaceutically active substances, such as piroxicam, chondroitin sulphate and glucosamine sulfate. The silica surface is covered with free OH groups, which act as adsorption active sites. Due to their different geometries and chemical properties, the three active agents are expected to be differently adsorbed within the mesopores of the silica matrix. To understand their features, the prepared samples were investigated using SEM, TGA and FTIR.

Scanning electron microscopy

Representative SEM micrographs recorded on the SBA16 system, before drug adsorption are presented in Figure 2. Using different levels of magnitude, there can be observed the surface of particles and the shape of the nanostructured surface with planar conformation, high exposure contact with the target analyte. The average size of particles is of 5 μm.[32]

Thermogravimetric analysis

The TGA shows an initial 5% weight loss due to evaporation of water confined in the pore. Piroxicam has a decomposition temperature of 200 °C. The TGA diagram shows only one inflection point from 150 to 350 °C. The difference is of 200 °C and the mass loss is of approximately 10% (Fig. 3a). The TGA of synthesized silica is also given. Chondroitin sulfate has a melting temperature of 190-194 °C and glucosamine sulfate of 150 °C. The diagram shows several mass losses as the temperature increases up to the decomposition of silica nanoparticles. A loss from 124.48 °C up to 298.6 °C is registered, which is associated with the loss of the glucosamine sulfate. The inflection point from 298 °C to 450 °C is associated with chondroitin sulfate (Fig. 3(b)). The total loss is up to 80%, which can be correlated with the drug intake determined by chromatographic methods.[33]

Table 1
Method validation parameters

Parameter		PRX	CHND	GLC
Repeatability	Retention time	0.31	0.13	0.99
Within day precision, RSD%		2.38	1.46	0.69
Intra-day precision, RSD%		3.05	4.08	2.65
Peak purity		0.997	0.996	0.997
Linearity	Slope	1381072.1	25852525.9	4319277.4
	Intercept	-99495.5	-1001120.2	28451.1
	Correlation coefficient	0.998	0.999	0.991
Accuracy	Average recovery	103.18	100.47	100.46
	Skewness O_x%	0.85	0.31	0.42
Limit of qualification, mg/mL		0.22	0.14	0.41
Confidence level of slope, 95%		1248474-1513670	24246511-27458540	3544005-5094549

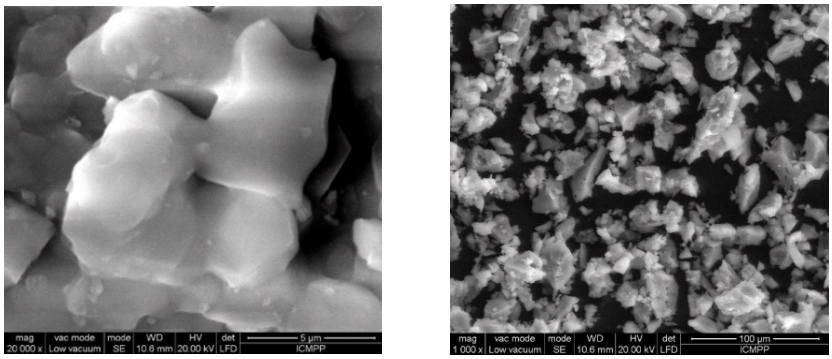


Figure 2: SEM micrographs of SBA-16-type mesoporous silica-based matrices

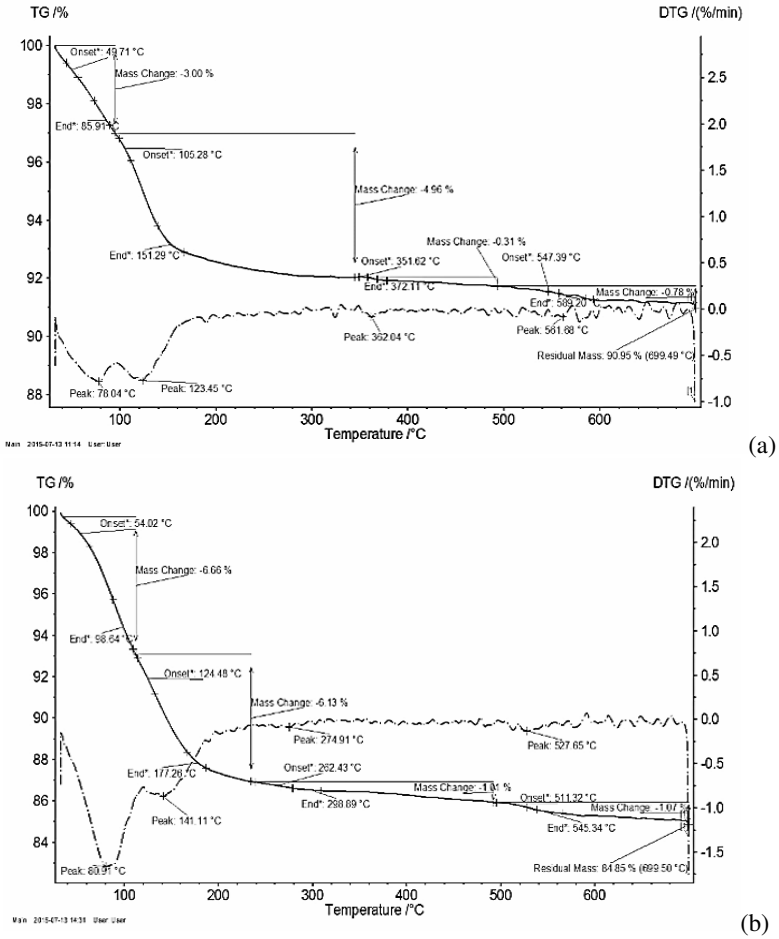


Figure 3: TGA spectra of SBA-16-type mesoporous silica-based matrices, (a) piroxicam and (b) chondroitin and glucosamine sulfate

FTIR analysis

The applications of infrared spectroscopy are of fundamental importance and utility in the physico-chemical analysis portfolio in the pharmaceutical field. The spectroscopic studies performed in the infrared range with Fourier transform application are useful mostly for compound identification, quantitative estimation of the content of an active substance or excipient, as well as for formulated pharmaceutical products.[34]

The FTIR spectra of siliceous SBA-16 substrate before and after drug intake are presented in Figure 4. For parent SBA-16, the bands observed at 1234.3 cm^{-1} and 1041.2 cm^{-1} are characteristic peaks. The peak at 456.1 cm^{-1} is characteristic of Si-O-Si bending. The broad band around 3500 cm^{-1} may be due to surface silanols and adsorbed water molecules, which indicates the silica framework is hydrophilic.[35,36]

The presence of chondroitin and glucosamine sulfate may be determined by the increase of the broad band at 3472 cm^{-1}, since the active substances have a high number of peripheral OH groups, and also the bands from 520 cm^{-1} are characteristic of primary amine functional groups in the molecules of active substances.

The presence of piroxicam is revealed on the FTIR spectra considering the maxima at 1631, 1530, 1435 and 1351 cm^{-1}. All these bands and spectral lines were obtained using a higher concentration of substrate in the FTIR analysis and the correspondence with the reference standard assured the presence of PRX along with the substrate.

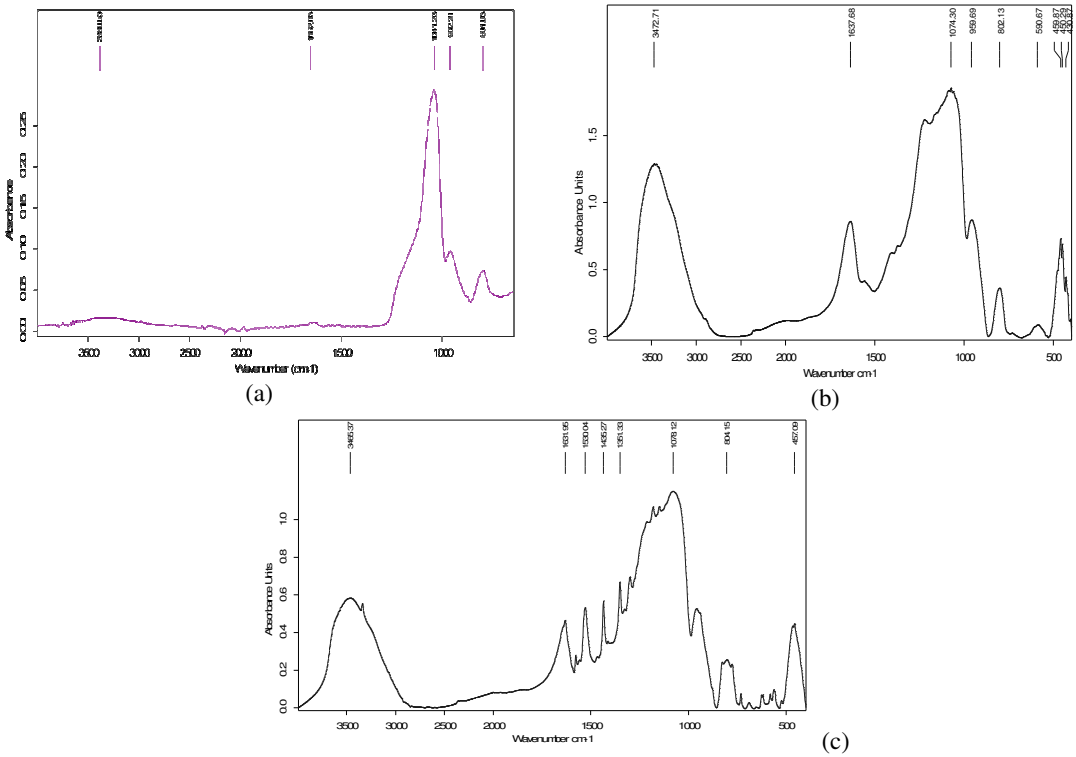


Figure 4: FTIR recordings; a) MIR spectra of SBA-16; b) MIR spectra of complex SBa-16-CHND-GLC, and c) MIR spectra of SBA-16-PRX

Study on drug immobilization

High performance liquid chromatography has the capability to separate the constituents of highly complex mixtures, which are included in a high variety of concentrations (Fig. 5).

The aim of the study was to monitor the inorganic substrate behaviour at the immobilization of different active compounds with different structures. PRX has in its molecule benzothiazine carboxamide and also pyrymidyl groups, while chondroitin has up to 6 chondroitin sulfate monomers and glucosamine has only the glyosidic chain. Under these conditions, the behaviour may be different upon the interactions with these compounds.

This study was performed using stock solutions of PRX in ethanol of 1 mg/mL and a common solution of glucosamine and chondroitin sulfate in water. During the adsorption study, we collected aliquots from the solution, at given time intervals, until a plateau was observed.

For the first step, we have monitored the optimum absorption time by using the stock solutions and a quantity of 50 mg matrix suspended. Sampling was done after 15, 30, 60, 120 and 180 minutes.

The analysis of the remaining amount of active substances in the media indicated that adsorption equilibria were reached after 1 h for SBA matrices. This condition is fulfilled for every compound in the study (Fig. 6). After the interval of 60 minutes, in every case, there was a desorption process because the weak inflections of the hydrogen bond tend to decrease and so the concentration of the substances tends to increase in the stock solutions. A similar and comparable behaviour was considered in the literature.[37] Also, a principal role of the decrease of concentration is the thermic effect produced by the mechanical homogenization. The hydrogen bonds tend to break on the increase of the thermic effect.[38]

The amounts determined by the decrease of chromatographic signals lie between 197 mg for PRX, 375 mg for CNDR and 473 mg GLC. So, the highest amount of drug was loaded on non-functionalized SBA-16 type silica matrix, due to the presence of these hydroxyl functional groups that inherently favour the binding with the substrate.

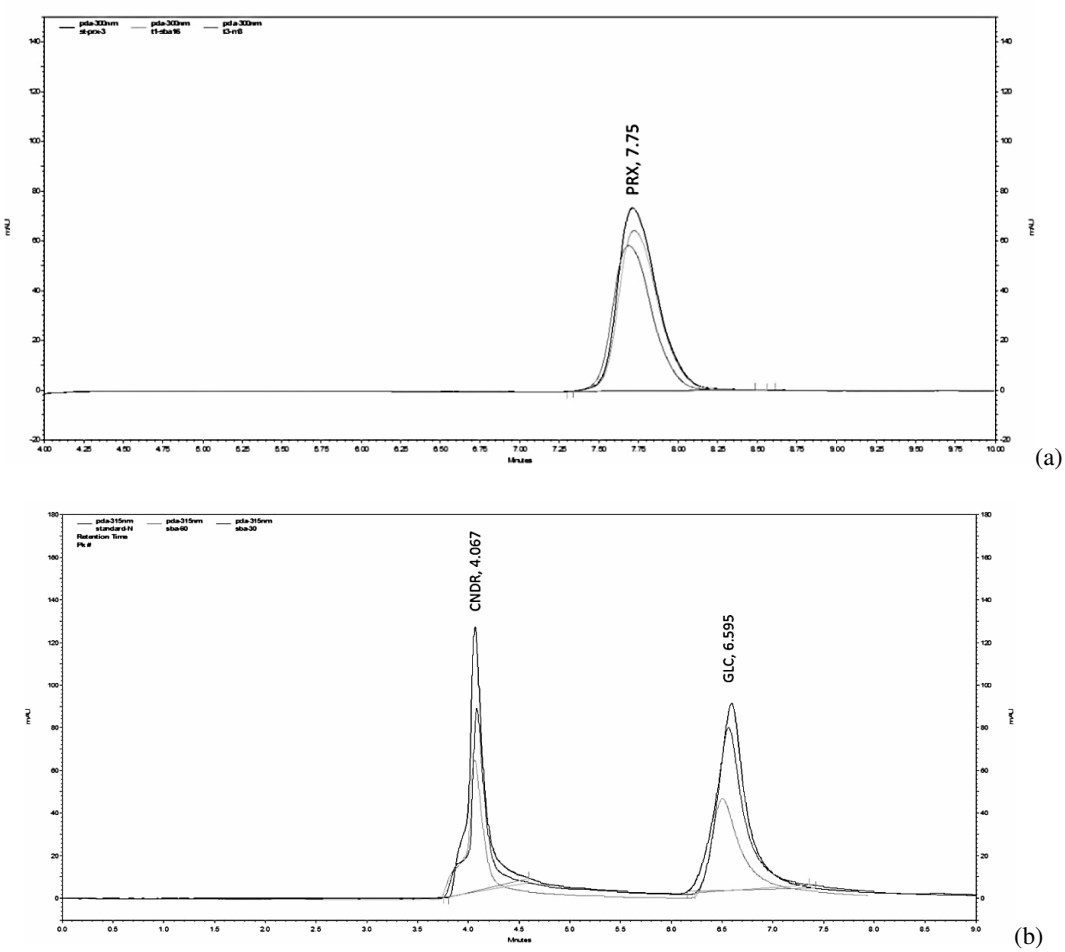
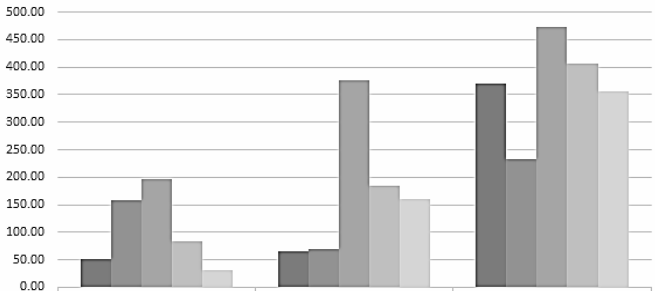
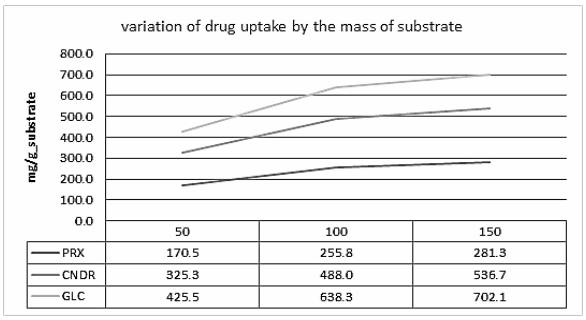


Figure 5: HPLC chromatograms of piroxicam (a) with standard (1 mg/mL), sample at 30 min and 60 min; (b) Common solution of CHND and GCL standard (1 mg/mL), sample at 30 min and 60 min

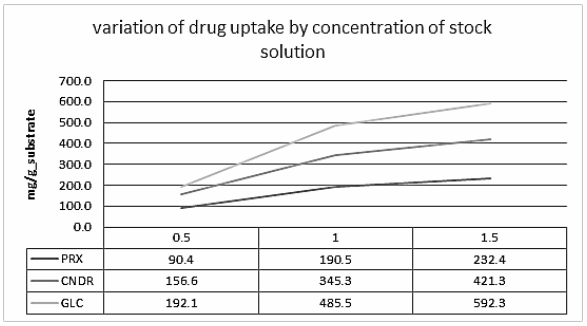


	PRX (mg/g_substr)	CNDR (mg/g_substr)	GLC (mg/g_substr)
15	52.05	64.39	369.92
30	158.50	68.88	233.42
60	197.09	375.33	473.34
120	84.27	183.83	406.70
180	30.00	160.37	356.02

Figure 6: Immobilization on inorganic nanoporous matrices by adsorption from solution



(a)



(b)

Figure 7: Drug uptake by inorganic nanoporous matrices as function of drug concentration (a) – – variation as a function of mass of substrate and b)- variation of a function of stock solution concentration

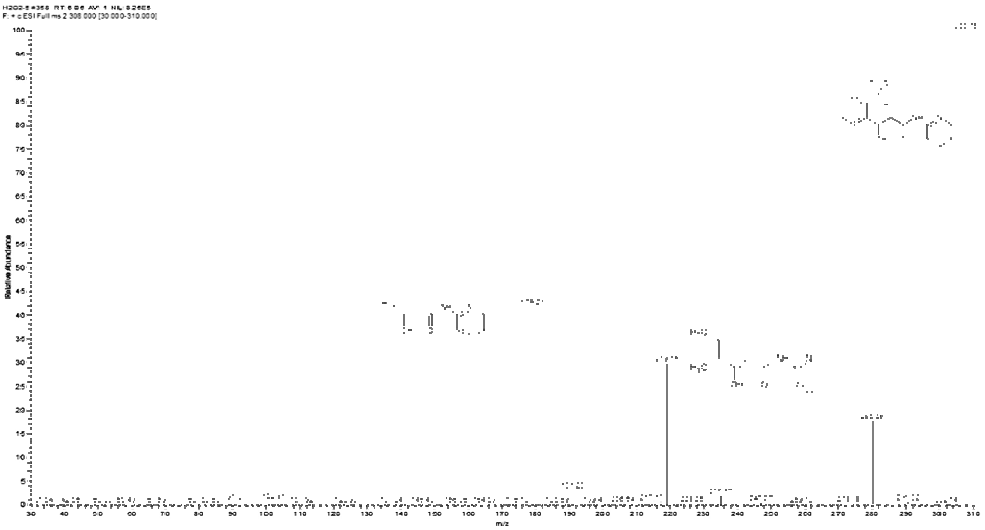


Figure 8: Degradation product of piroxicam

Mass spectrometry analysis

Recently, mass spectrometry has been employed to determine the compounds based on their ability to develop molecular ions in heated electrospray ionization. Multiple reaction monitoring produces fragments starting from 1 precursor in order to produce a structure confirmation.

Analysis on full scan and also scan dependent fragmentation revealed the presence of impurity 1, which is based on the retrosynthetic disconnection in two steps N-3-C2, with the addition of OH group of the potassium hydroxide in the degradation media of piroxicam. The disubstituted benzene favours one more disconnection at N3-S4 with the elimination of hydroxy-N-methylmethanamine. The resulting compound, impurity 1 was (2Z)-3-[2-(dioxo-l6-sulfanyl)phenyl]-3-hydroxy-N-(pyridin-2-yl)prop-2-enamide. The molecular ion has a theoretical molecular weight of 306.4 (δ=0.75) (Fig. 8).

Among the influential factors of the loading process, the polarity of the solvent is very important. In our study, MCL was dissolved in water, due to the high drug solubility. Attempts to use ethanol showed negligible drug loading for all three levels of concentration studied.

CONCLUSION

Inorganic mesoporous silica has been employed to determine its encapsulation capacity towards various active substances with different functional groups and different conformational structures.

It was demonstrated that the process of encapsulation is more efficient when the mass of substrate is varied. Also, a variation of concentration of active substances showed an increased influence on the process.

Inorganic nanoporous matrix SBA-16 is suitable for adsorbing molecules with molecular masses ranging from 300 up to 678 Dalton (chondroitin-6-sulfate), and also at the surface level for chondroitin, which has a chain structure with up to 100 individual sugar units.

Another aspect related to the structure of the substance was the presence of free OH groups, which produced intermolecular interactions by the hydrogen bonding. Under these conditions, the hydroxyl groups on inner surfaces of silica produced the interactions and there was no need of functionalization with other groups in order to enhance the absorption.

It was found that all used substrates were efficient to encapsulate the drug. They had enough efficiency for every compound. Thus, the high amount of glucosamine was easily accommodated by the selected matrices (varying from 151 to 472 mg drug/g matrix). The drug uptake capacity decreased in the following order: glucosamine, chondroitin and piroxicam. It must be mentioned that the limited capacity of the materials to intake the drug substances was determined by the thermodynamic equilibria.

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