

## PHARMACOKINETICS STUDY ON MESOPOROUS SILICA-CAPTAPRIL CONTROLLED RELEASE SYSTEMS.

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The design and development of new drug delivery systems with the aim of enhancing the efficacy of existing commercialized formulations represent an ongoing process in the pharmaceutical research. Captopril, an angiotensin converting enzyme inhibitor, was successfully loaded into unfunctionalized and functionalized mesoporous silica matrices. Pharmacokinetic evaluations were performed and UV-Vis and HPLC analysis were used in order to determine the plasma concentration of the released captopril. The following pharmacokinetic parameters were evaluated: time to the maximum concentration ( $T_{max}$ ), maximum value of plasma concentration,  $C_{max}$  ( $\mu\text{g/mL}$ ), half life ( $T_{1/2}$ , h), area under curve  $AUC_{0-72}$  ( $\mu\text{g h/mL}$ ). Pharmacokinetics experiments evidenced that temperature conditions for drug loading and chemical nature of silica surface are determining factors in the bioavailability of the loaded substance and directly influence the overall pharmacokinetics. Additionally, the toxicity of the obtained systems was evaluated using Kärber method. The tested silica-captopril formulations may be considered attractive controlled release systems, potentially offering better bioavailability and other benefits, compared to the commercial formulation.

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### 1. Introduction

In efforts to reduce the side effects triggered by fluctuating plasma drug concentrations and to enhance the compliance to treatments by reducing the frequency of drug administration, several attempts have been made to design controlled release formulations. Delivery of drugs by means of controlled release technology began in the 1970s and has continued to expand rapidly [1-3]. Controlled drug delivery system can be described as a system (matrix-drug) which controls the rate and period of drug delivery and targets specific tissues for action.

The design and development of new drug delivery systems with the intention of enhancing the efficacy of existing systems is an ongoing process in pharmaceutical research.

An ideal drug carrier system needs to demonstrate optimal drug uptake and release properties to the proper site in the body and to maintain the desired drug concentration in time. Also, an ideal carrier system must possess long shelf life and low toxicity, biocompatibility and high *in vivo* stability [4].

Considerable research efforts have been directed in recent years towards the development of silica mesoporous carriers (MSNs) as controlled drug delivery matrices. Their spectacular properties are controlled not only by the chemical composition, but also by their properties such as stable uniform porous structure, high surface area, tunable pore size and well-defined surface

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properties [5-7, 29]. It is widely known that MSNs possess vast amounts of nanopores which allow the inclusion of various kinds of drugs and release them in a more reproducible and predictable manner. In addition, their biocompatibility, high stability, low toxicity, high carrier capacity, capacity of incorporation in their structure of both hydrophilic and hydrophobic drugs, and feasibility of variable routes of administration, have improved their applicability [4]. Despite the considerable interest in the biomedical applications of mesoporous silica, only few studies regarding pharmacokinetics, biocompatibility and toxicity of the two most common types of mesoporous silica (MCM-41 and SBA-15) have been published [9-10].

Captopril is a representative member of the class of angiotensin converting enzyme inhibitors, which is in many countries the drug class of choice for the management of hypertension and congestive heart failure. Captopril falls under Biopharmaceutical Classification System (BCS) class III drug with high solubility and poor permeability [11]. Captopril effectively inhibits the conversion of angiotensin I to angiotensin II and bradykinin degradation. The resulting reduction in angiotensin II concentration confers blood pressure lowering effects [12-14]. After captopril oral administration of therapeutic dosage (12.5 to 100 mg), absorption occurs, with maximum plasma levels at about one to two hours and blood pressure reduction usually reaches its highest level later at about 60–180 min [15, 16]. Captopril contains a reactive thiol group, which is postulated to bind to the  $Zn^{2+}$  of the angiotensin-converting enzyme [17] and which forms disulfide linkages with thiol-containing residues of plasma proteins which are responsible for the extensive tissue binding of the drug [18].

In a previous paper we have studied the design of SBA-15 mesoporous silica-captopril systems and investigated the *in vitro* release behavior for both SBA-15 mesoporous silica and the functionalized SBA-15 mesoporous silica matrices loaded with captopril, showing that they could be used as effective controlled delivery systems for antihypertensive medications [19].

The objective of the present study is focused on the *in vivo* evaluation of the SBA-15 mesoporous silica-captopril systems.

## 2. Experimental

### 2.1 Reference formulation

Commercial Captopril 25 mg tablets from S.C.Terapia S.A. Cluj-Napoca, crushed as micronized powder, were used as reference formulation.

### 2.2 Animals

Pharmacokinetic study was performed on five groups (3 animals/group) Sprague-Dawley male rats (weighing 280–300 g) kindly provided by the Central Laboratory for Drug Testing, “Grigore T. Popa” University of Medicine and Pharmacy, Iasi. The rats were housed in a room with controlled temperature ( $24 \pm 2$  °C) and humidity (70–80%), and allowed to freely access standard rat food and tap water until experiments. All animals were allowed to adapt to laboratory conditions for at least 1 week before the studies.

All experiments were performed according to rules endorsed by European Union legislation (Directive of the EU Council no. 609 from 24 November 1986); IASP Committee on Ethical Issues: Guiding Principles in the Care and Use of Animals approved by the American Physiological Society and the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals; European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes; bioethical standards and protocols approved by the Committee of Animal Experimentation in Romania, in conformity with the Romanian Law 204/2004, for the animals protection and for the acceptable use of experimental animals. These guidelines and rules were included in standard protocols and approved by the Bioethics Committee of the “Gr. T. Popa” University of Medicine and Pharmacy Iasi.

### 2.2 Synthesis of Mesoporous Matrices

Synthesis of unfunctionalized matrix (labeled as SBA-15) and APTES functionalized silica matrices with 3-aminopropyl-triethoxysilane (labeled as SBA-15 - APTES) were described in detail in a previous paper [19-20].

## 2.4 Captopril Loading

Captopril loading was carried out by impregnation of drug on both unfunctionalized and functionalized SBA-15 matrices at room temperature (RT) and 80°C, using a ratio of 2g/100mL of 0.1M captopril solution. The drug loaded systems were denoted as SBA-15-captopril-RT, SBA-15-captopril-80°C, SBA-15-APTES-captopril-RT and SBA-15- APTES-captopril-80 C.

## 2.5. Characterization

BET (Brunauer, Emmett and Teller) specific surface areas ( $S_{\text{BET}}$  (m<sup>2</sup>/g) were obtained from the nitrogen adsorption experiments measured at -196°C after degassing the samples below 10<sup>-3</sup> Torr at 473 K for 2h on NOVA 2200e (Quantachrome Instruments, Boynton Beach, FL, USA). Pore size distribution was determined from the desorption branch of the isotherm using BJH (Barrett-Joyner-Halenda) method. The total pore volume (TPV, cm<sup>3</sup>/g) was calculated as the amount of nitrogen adsorbed at the relative pressure of approximately 0.99.

SEM images were obtained using the following devices: Quanta™ Scanning Electron Microscope (operating at an accelerating voltage of 30 kV); microscope Vega II LSH (with accelerating voltage of 30kV-Tescan Company).

Particles size distribution and mean pore diameter ( $D_p$ , nm) measurements were recorded using an optic measurement device SALD-7001 type Laser Diffraction Particle Size Analyzer (Shimadzu, Japan).

UV-Vis Captopril measurements were carried out using a Shimadzu-Japan spectrophotometer, in the range from 200 to 800 nm, at a maximum absorption wavelength of 209 nm.

Plasma Captopril concentration was determined using a Shimadzu Model-CTO-20A HPLC system with ZORBAX SB-C18 column (150 ×4.6 mm i.d.) under isocratic conditions using methanol/citrate buffer (45:55, v/v) pH 3.00 as mobile phase. Captopril concentration was determined with a calibration curve obtained with standard solutions of known Captopril concentrations in mobile phase in the range of 1–20 µg/mL, using HPLC software LC Solution Version 1.22 SP1 for integration and automatic determination of drug concentration in blood samples. All samples were analyzed at room temperature with 0.5 mL/min flow rate and an injection volume of 30 µL.

## 2.6. Pharmacokinetic Evaluation

*In vivo* evaluation was performed on five groups of male rats (weighing 280–300 g), each group having three rats. A single oral dose of 0.35 mg captopril/kgc was administered to rats. The experimental dose administered to each rat was calculated according to the weight of the rat, and dissolved into 10 mL of carboxymethyl cellulose (CMC). CMC was selected as dissolution medium because its high viscosity, non-toxic and non-allergenic properties [21]. After oral administration, rats were anesthetized for 1 min in a special box, containing diethyl-ether, and then blood samples were extracted from retro-orbital sinus with a Pasteur pipette. This procedure is relatively rapid because it allows blood collection from a large number of animals within a short period of time and the volume of blood obtainable per animal could vary from medium to large [22].

Blood samples (0.2 - 0.3 mL) were taken immediately after administration of the medication and at predetermined time points after administration, for up to 72h, and centrifuged at 10,000 rpm for 5 min. After centrifugation, plasma was isolated and kept at -20°C until analysis.

### 3. Results and discussion

#### 3.1. The Loading Captopril Capacity

Captopril loading on the calcined unfunctionalized and functionalized matrices was performed by impregnation method. Each experiment was performed in triplicate and the reported results were calculated as mean of the 3 measurements. The loading captopril capacity (LC), calculated used the following formula:

$$LC = (W_t - W_0) / W_0 \quad (\text{mg/g})$$

and determined as a function of time (Fig.1).

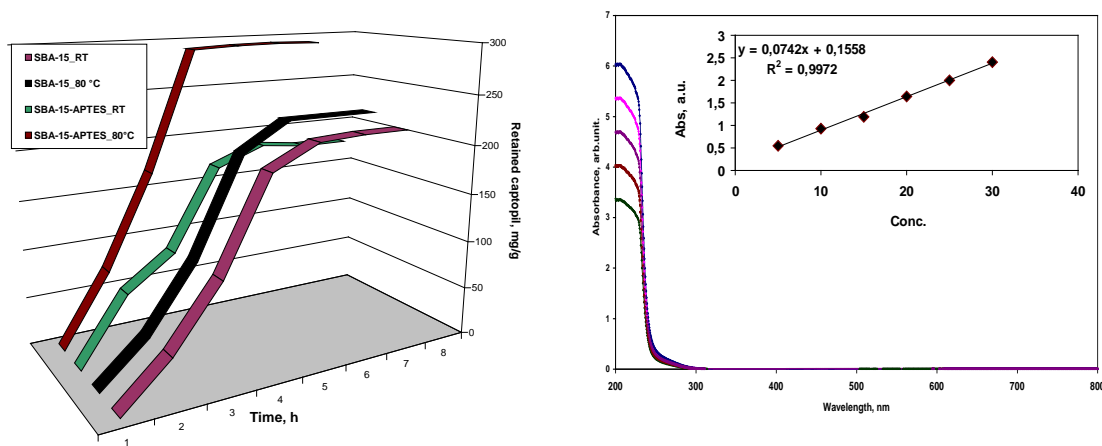


Fig. 1. The loading capacity as a function of time (A) and UV calibration curve obtained with standard solutions of captopril concentrations in water, in the range of 5-30  $\mu\text{g/mL}$ (B).

It is known that several factors might influence the loading profiles of guest molecules in mesoporous silica [23-24]. Our results states that the functionalized matrices showed an improved loading capacity for captopril, as a result of the influence of both size of mesoporous silica pores and chemical characteristics of mesoporous silica walls, which is consistent with a previous report [19].

The percentages of loaded captopril for each of the matrices evaluated were presented in tabel 1.

Tabel 1. Percentages of captopril loaded onto evaluated matrices

Matrix	Loading temperature	Loaded captopril (%)
SBA-15	Room temperature (RT)	200 mg captopril /g matrice (20%)
SBA-15-APTES	Room temperature (RT)	178 mg captopril /g matrice (17.8%)
SBA-15	80°C	225.9 mg captopril /g matrice (22.59 %)
SBA-15-APTES	80°C	260.1 mg captopril /g matrice (26.01 %)

SEM experiments performed for investigation of the morphological characteristics showed captopril presence on both functionalised and unfunctionalised matrices (Fig.2).

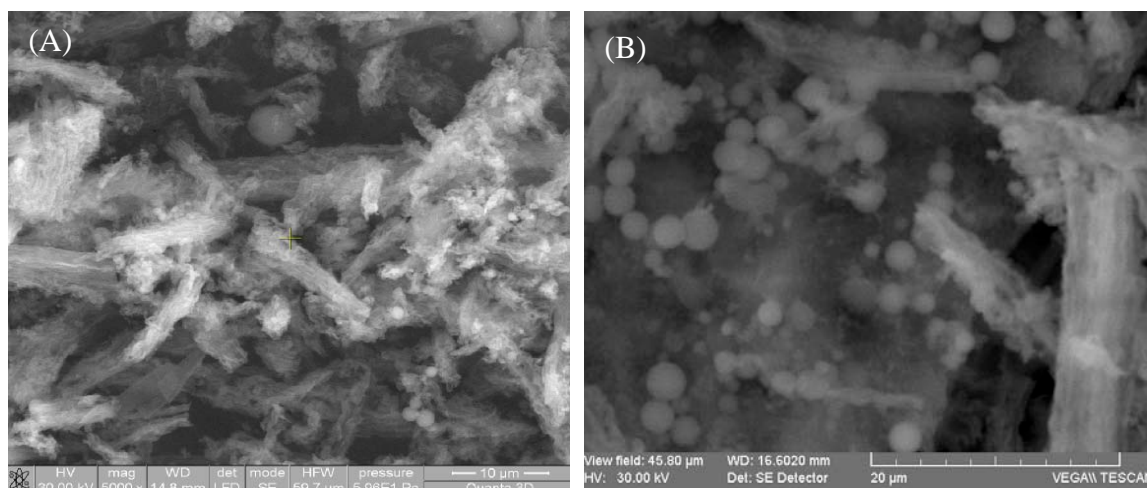


Fig. 2. SEM images of Captopril -SBA-15-80 °C sample (A) and Captopril - SBA-15 – APTES-80°C (B).

Compared to non-functionalized matrix SBA-15, pore size distribution of captopril-silica formulation showed that the mean pore diameter was reduced from 9.37 nm to 7.39 nm, BET surface area decreased from  $910 \text{ m}^2 \text{ g}^{-1}$  to  $592 \text{ m}^2 \text{ g}^{-1}$ , and pore volume decreased from  $1.2 \text{ cm}^3 \text{ g}^{-1}$  to  $0.98 \text{ cm}^3 \text{ g}^{-1}$  upon captopril loading. Decreasing of pore diameters, pore volumes and BET surface areas after drug loading indicated successful drug loading into mesoporous silica materials [19].

### 3.2. Toxicity results

While the lethal dose 50 ( $\text{LD}_{50}$ ) for captopril was evaluated at about 6590 mg/kg, the toxicity values of the synthesized materials were determined as 7410 mg/kg for SBA-15 and 6983 mg/kg for SBA-15-APTES. After captopril loading into mesoporous materials, the determined toxicity  $\text{LD}_{50}$  values were 7522 mg/kg for SBA-15- Captopril-RT, 7588 mg/kg for SBA-15-Captopril-80°C, 7160 mg/kg for SBA-15-APTES- Captopril-RT and 7396 mg/kg for SBA-15-APTES-Captopril-80°C. According to the Hodge and Sterner toxicity scale, the substances with  $\text{LD}_{50}$  between 5'000 – 15'000 mg/kg display a very low toxicity [25].

Evaluated by the Trimmed Spearman Karber method, SBA-15-Captopril and SBA-15-APTES- Captopril systems, both at room temperature and at 80°C, proved to have very low toxicity for rats (Fig. 3). The equivalent toxic dose in humans for such substances is estimated to be approximately one liter [26]

Studies performed in mice with captopril doses from 50 mg to 1350 mg/kg/day did not reveal any carcinogen effects [16]. In the present study it was assessed the quantity of the drug which after oral administration determines the death of 50% of the animals ( $\text{LD}_{50}$ ) and the reference value obtained for captopril was 6590 mg/kg.

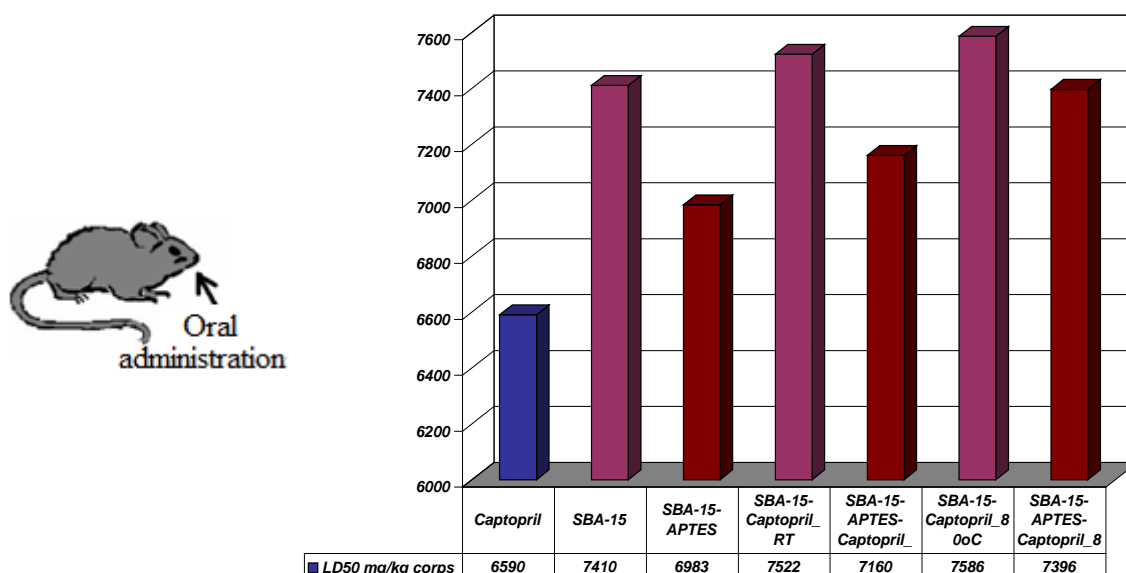


Fig. 3. Toxicity results obtained after oral administration of the samples in animals from the 7<sup>th</sup> -day.

The assessment of the matrices alone, not integrated into the system, indicated a lower toxicity compared to the reference value, the values being 7410 mg/kgc (for SBA-15), and 6983 mg/kgc for functionalised matrix. For the same type of matrices on which captopril as loaded, the values obtained were 7522 mg/kgc for SBA-15 when loading was performed at room temperature and 7586 mg/kgc when the unfunctionalised matrix was loaded at high temperature. For the functionalised matrix on which captopril was loaded at room temperature DL<sub>50</sub> was 7180 mg/kgc and 7396 mg/kgc, when the loading was performed at high temperature, respectively. The obtained results indicate that the matrices and systems prepared, are part of the materials with very low toxicity.

### 3.3. Plasma Captopril Results

In order to compare the *in vivo* behavior of captopril release from silica-captopril formulations with the release of captopril from commercial formulations, captopril plasma concentrations were investigated.

Measurements of captopril plasma concentration at predetermined time were followed after captopril serum extraction. Captopril extraction was performed after serum thawing as follows: 0.5 mL serum was mixed with 2 mL acetonitrile and vortexed for 15 min to assure complete serum deproteinization. After 5 min centrifugation at 4500 rpm the organic layer was passed through 0.45 µm syringe filter and directly injected into the HPLC system.

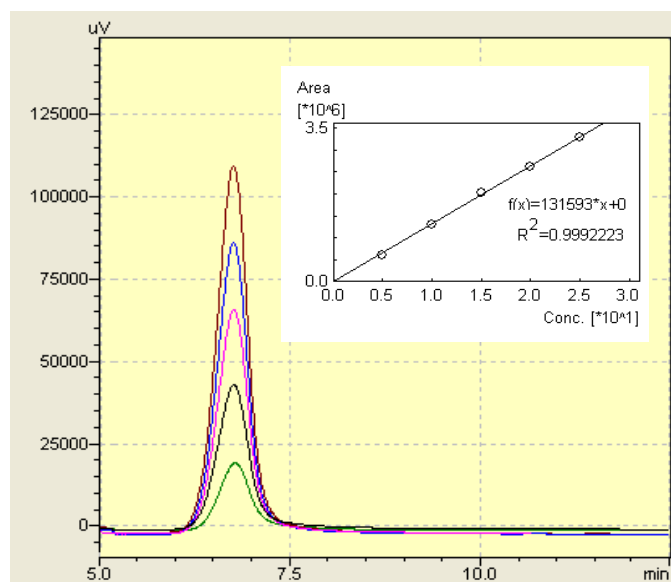


Fig. 4. Calibration curve obtained with standard solutions of known Captopril concentrations in mobile phase in the range of 5-25  $\mu\text{g}/\text{mL}$

Mean plasma concentration-time curves are depicted in Fig.5 and pharmacokinetic parameters are summarized in Table 1.

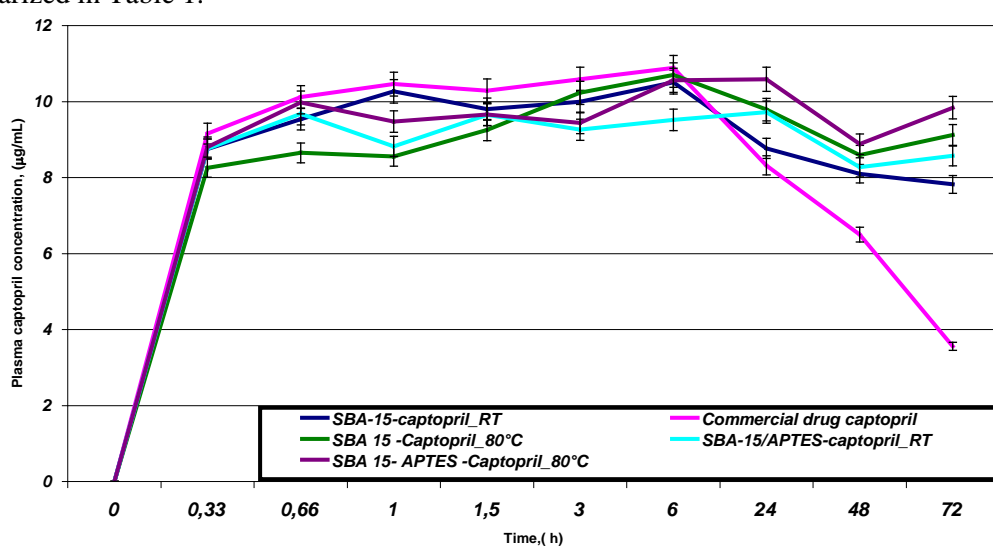


Fig.5. Mean plasma captopril concentration vs. time curves for the test formulations and reference commercial captopril tablet.

The area under the concentration vs. time curve from time zero to experimental time ( $\text{AUC}_{0-72}$ ) was calculated using the linear trapezoidal rule [27-28].

Using commercial captopril, captopril plasma level gradually increased to a maximum concentration  $C_{\text{max}}$  of 10.89  $\mu\text{g}/\text{mL}$  at  $T_{\text{max}} = 6$  h. Similar results were obtained in case of all non-functionalized silica-captopril formulations. Different results were obtained for functionalized silica-based matrices loaded captopril, which have induced values of maximum plasma concentration after a longer time interval, up to 24 hours, and revealed a specific tendency, observed after 48 h, related to the appearance of upward of captopril plasma level.

To elucidate this behavior we refer to the isotherms presented in Fig.6. The isotherm allure of SBA-15-APTES reveals a shorter contributions of micropores for  $\text{N}_2$  adsorption, compared with

pristine SBA-15, demonstrating the fact that APTES molecules are located inside of both the micropores and the mesopores.

Knowing that the attraction between the hydrophobic parts of APTES and captopril molecules in the micropores are stronger than that in mesopores, we explain the observed modifications of pharmacokinetic parameters as an expression of the delayed release of captopril molecule from micropores.

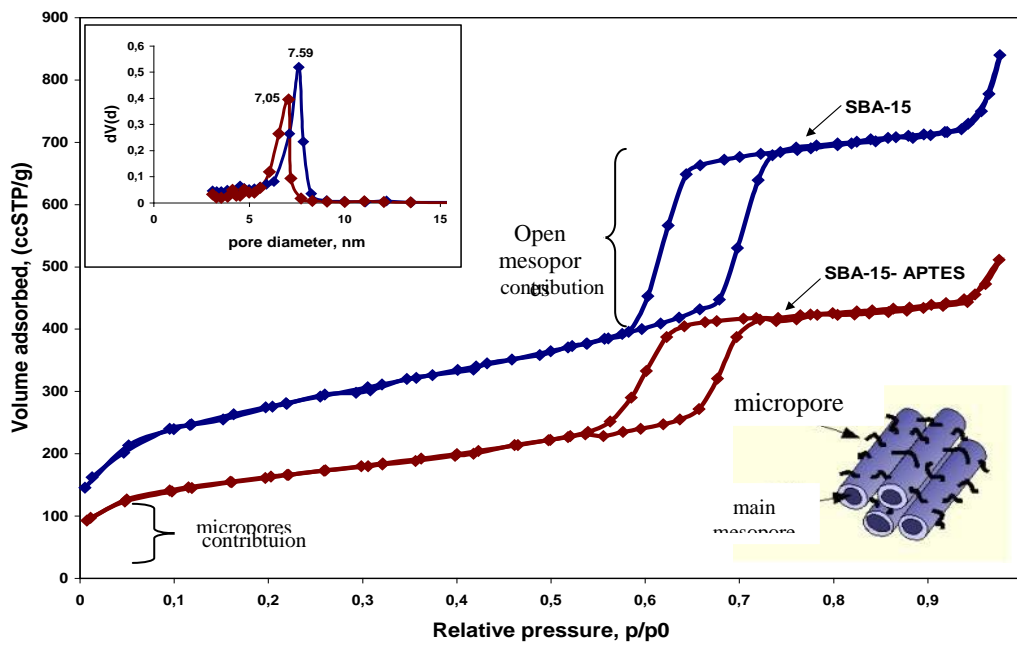


Figure 6. BET isotherms and pore distributions of studied matrices.

Table 1. *In vivo* pharmacokinetic Parameters

Sample	$C_{\max}$ ( $\mu\text{g}/\text{mL}$ )	$T_{\max}$ (h)	$AUC_{0-72}$ ( $\mu\text{g h}/\text{mL}$ )	Relative bioavailability, (%)
Commercial drug Captopril	10.89	6	502.51	Reference
SBA 15 -Captopril_RT	10.51	6	624.99	124.37
SBA 15 -Captopril_80°C	10.72	6	674.77	134.27
SBA 15/APTES-Captopril_RT	9.76	24	645.15	128.38
SBA 15/APTES-Captopril_80°C	10.59	24	705.22	140.33

Significant differences were indicated for the area under the plasma-time curves,  $AUC_{0-72}$  parameter, between all of test formulations compared to reference formulation.



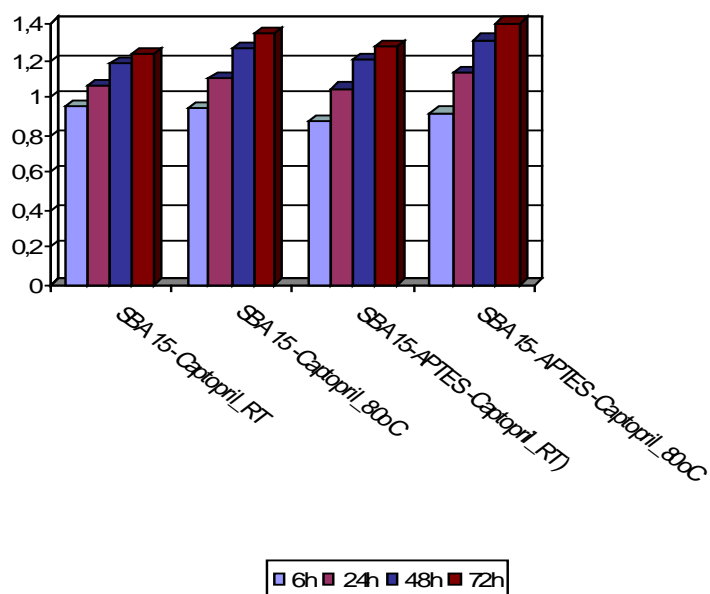


Figure 7. The relative  $AUC_{0-72}$  values for test formulations compared to commercial captopril.

The relative bioavailability was evaluated as the ratio, expressed as a percentage of the  $AUC_{0-72}$  value of the samples and the  $AUC_{0-72}$  value of commercial captopril:

$$\text{Bioavailability \%} = \frac{AUC_{\text{test } 0-72} (\text{mcg} \times \text{h/mL}) \times 100}{AUC_{0-72 \text{ captopril}} (\text{mcg} \times \text{h/mL})}$$

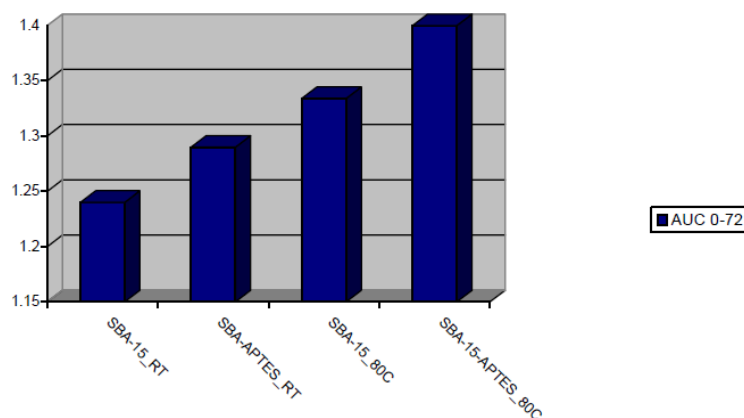


Figure 8. The relative bioavailability for Captopril-SBA-15 formulations compared to commercial formulation of captopril

Silica-captopril formulations obtained at 80°C showed the best biodisponibility values, due to better captopril loading capacity, compared to silica-captopril formulations performed at RT. It is also obvious that the both  $AUC_{0-72}$  value and the relative biodisponibility were higher for the functionalized silica-captopril formulations (Fig.8).

The obtained results showed higher  $AUC_{0-72}$  values for silica-captopril formulations compared with commercial captopril tablet and suggest that temperature loading conditions and chemical nature of silica surface are the main factors which influence the bioavailability of the loaded substance and its pharmacokinetic.

One of the possible explanations for this observation is linked to the active drug formulation and the way it is released and becomes available to the body. Although in this experiment all the animals received the same quantity of captopril per weight unit, in the

formulations in which the matrices were impregnated at high temperature, especially the functionalised one, the quantity of captopril was higher, as shown in table 1.

According to the Summary of Product Characteristics for reference substance (Captopril, Terapia S.A.) (16), captopril is well absorbed in intestine and approximately 25-30% binds to plasma proteins. Oral bioavailability is about 60% compared to the intravenous administration. The half time is 2-3 hours. Elimination of captopril is 95% via the renal route, out of which 40-50% unmodified. Elimination is delayed in renal insufficiency. Concomitant administration with food decreases the bioavailability with approximately 25-50%, but has no effect on the antihypertensive effect. Antiacids also decrease the bioavailability with 45%, increasing the  $T_{max}$  with 1.5h [16].

During the experiment in mice, the pharmacokinetic parameters calculated for captopril were different from the ones reported in human healthy volunteers, the time to maximum plasma concentration in this experiment was 6 hours, and the half time was above this value.

The new formulation of captopril evaluated, part of a controlled release system, significantly prolonged the plasma circulation time and consequently the duration of action of the drug. In our study, the half time for the formulation with functionalised matrix loaded at high temperature was more than 72h, compared to commercial formulation. The same quantity of pharmacologically active substance was administered to the animals in each experimental group, however, being released from the controlled delivery systems prepared, the metabolic activity is prolonged.

This might be explained by the limited quantity of free captopril in circulation when it is entrapped in the mesoporous matrix, keeping in mind that 40-50% of the administered captopril in commercial formulation is eliminated unmodified.

As the half time is a reference parameter in determining the number of drug administration per day, the controlled delivery systems evaluated offer the opportunity of developing optimized formulations with once per day administration or rarely.

The mesoporous silica nanoparticles used as matrices in this study open an encouraging perspective in the development of the controlled drug formulation, impacting the pharmacokinetics of the loaded substance. It was also demonstrated that the chemical properties of the matrix and synthesis and loading procedure are critical in defining the future pharmacokinetics of the system.

Our experiment indicated that mesoporous silica-captopril formulations offer multiple advantages over the current commercial ones, in maintaining an effective plasma level for the drug.

#### **4. Conclusions**

Captopril, an angiotensin converting enzyme inhibitor, was successfully loaded onto unfunctionalized and functionalized mesoporous silica matrices, in order to obtain new controlled release systems.

*In vivo* experiments showed that temperature loading parameter and chemical nature of silica surface are determinant factors in Captopril pharmacokinetic process.

Pharmacokinetic tests clearly indicated that mesoporous silica-captopril formulations offer highly advantages over commercial captopril in maintaining of an effective plasma captopril concentration.

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