Al. I. Cuza University, Iasi, Romania
Department of Chemistry

Nanoporous materials – potential matrix for entrapping biologically active compounds

PhD Thesis Summary

Scientific coordinator:
Prof.univ.dr. Evelini Popovici

PhD student:
Alexa Iuliana Florentina

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PhD student Iuliana Florentina ALEXA

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KEYWORDS: controlled release, *in vivo*, *in vitro*, nanoporous matrix, mesoporous silica materials, layered double hydroxide, biologically active substances, captopril, aliskiren, methotrexate.

In the summary of the PhD thesis, the chapters, general conclusions, scientific activity and selective references are briefly presented. In editing, for chapters, subsections, figures, diagrams and tables the notations used in PhD thesis text have been preserved.

Justification of the chosen theme

"To succeed in transmitting science,
you need to be science creative yourself
or at least to try to be".

Costantin Neniţescu
(1902-1970)

The PhD thesis entitled ***"Nanoporous materials-potential matrix for entrapping biologically active compounds"*** is the result of some personal experimental results obtained after research made in the Laboratory of Materials Chemistry, Faculty of Chemistry at the Al. I. Cuza University.

Today, nanotechnology has proved its power to revolutionize the scientific world, by allowing manipulation of matter at atomic or molecular level, by using the interdisciplinary principles of physics, chemistry, engineering or biology [1]. Being a force of the present times and especially of future times, nanotechnology enriches every day both its intrinsic content and the range of applications for nanomaterials. The spectacular expansion of the nano-level science was driven by the incomparable beauty of the nanostructured materials science and the importance of the practical implications deriving from their use.

The research approached the modalities to influence pharmacokinetic parameters and the way they can be optimized in order to increase therapeutic efficiency of antihypertensive substances that are included in current therapy (captopril, aliskiren).

Research work conducted throughout this PhD thesis was aimed at improving efficiency of biologically active substances (captopril, aliskiren, methotrexate), by creating new drug delivery systems based on the use of nanostructures matrix with various morphologies and properties.

The general aim of the thesis was to study and experimental research the synthesis and characterization of nanoporous materials with remarkable properties and a vast area of applicability in medicine.

The thesis is structured in five chapters, containing a total of 234 pages, 101 figures, 33 tables, 18 formulas and 219 references, of which chapters 1 and 2 are allocated to the literature research part, which presents the current state of knowledge; the other three chapters exhibit the original research.

The thesis ends with references including professional personal publications in various journals and participation in the scientific manifestations.

PRESENT STATE OF KNOWLEDGE

Chapter I: General considerations on the importance of nanoporous materials

Chapter I describes the importance of nanoporous materials, factors which determine the increase of their performance, short summary of classification and their synthesis routes. Looking at the evolution of the number of publications on nanomaterials (Figure I.3.) we can specify that both now and in the future, improving the applications of nanoporous materials in medical science represent a true scientific challenge.

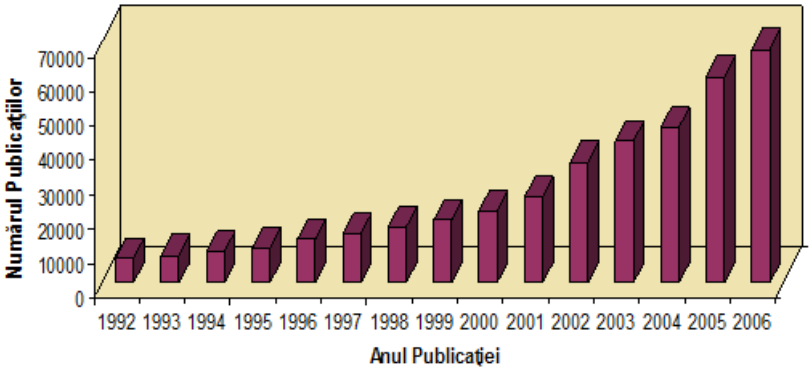


Figure I.3. Evolution of the publications on nanomaterials by number [11]

Because of their characteristics, nanomaterials enhance performance of drugs by improving their solubility and bioavailability, by increasing their *in vitro* stability, by increasing concentrations of bioactive compounds in cellular compartments and target cells, with the aim to achieve therapeutic efficiency [2].

Chapter II: Controlled release nanostructured systems

Chapter II describes the concept of drug delivery systems and classification of these systems, explaining the methods used for their investigation. This chapter also covers a significant bibliographic study of literature data specialties in drug delivery systems. An important aspect in this

new area of systems development is represented by the drug delivery systems that allow innovative therapeutic approaches, because of their small size which are able to carry active substances to a specific tissue or organ, across biological barriers, or biologically active substances to the intracellular space [72].

The toxicity and capacity to degrade of the biologically active substances are reduced when they are encapsulated in a non-toxic biocompatible nanoporous form, which exerts a modulatory effect on the diffusion of the biologically active substance after administration.

PERSONAL CONTRIBUTIONS PART

A perfect drug delivery system must demonstrate that it is able to assimilate the biologically active substance and maintain its concentration for a desired period of time. In order to obtain such systems, we studied the synthesis of mesoporous silica matrix and layered double hydroxides.

Chapter III: Synthesis and characterization of mesoporous silica matrix

The research purpose of this thesis was to obtain potential matrix for the entrapment of biologically active substances. Thus, Chapter III contains a detailed description of personal experimental research, results obtained and detailed conclusions regarding MCM-41, SBA-15 and MgO/SBA-15 mesoporous materials, subsequently used as matrix for entrapment of biologically active substances. These matrix syntheses has been chosen due to their characteristics, such as: pore sizes that can be easily modified, high structural ordering, ease of synthesis, synthesis by various economically advantageous methods, high thermal and hydrothermal stability, etc.

Characterization of the synthesized matrices (MCM-41, SBA-15, respectively MgO/SBA-15) is very important for their applications in biopharmaceuticals. In order to point out the differences between the three matrices, they were compared with each other using data obtained from XRD, N_2 sorption, particle size and TG-DTG (Table III.4.).

High values of specific surface area and pore volume are important properties in order to achieve a greater load of biologically active substances.

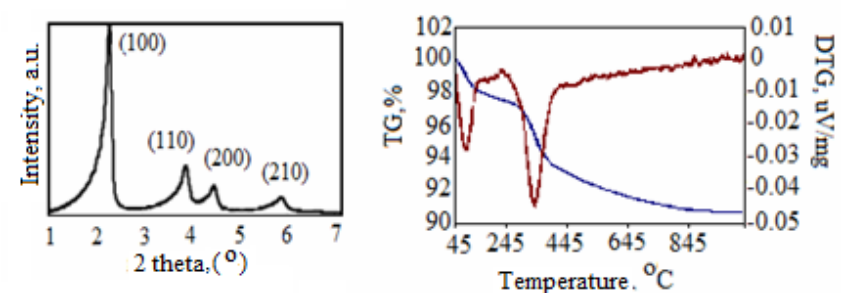


Figure III.2. X-ray diffractometry for MCM-41 matrix
Figure III.4. TG / DTG analysis for MCM-41 matrix

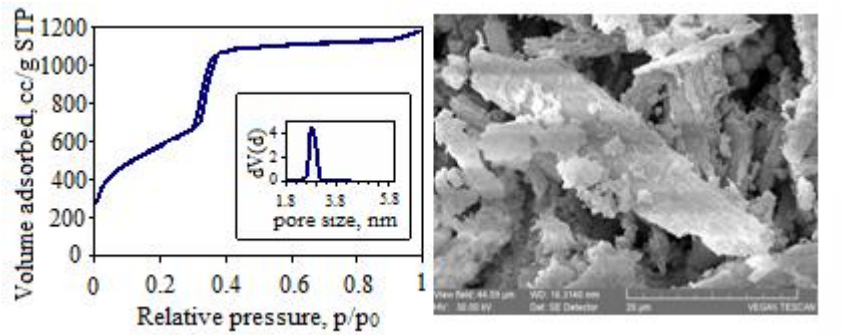


Figure III.5. N_2 adsorption and desorption isotherms and the corresponding pore size distributions of MCM-41 matrix
FigureIII.7. SEM images of MCM-41 matrix [129]

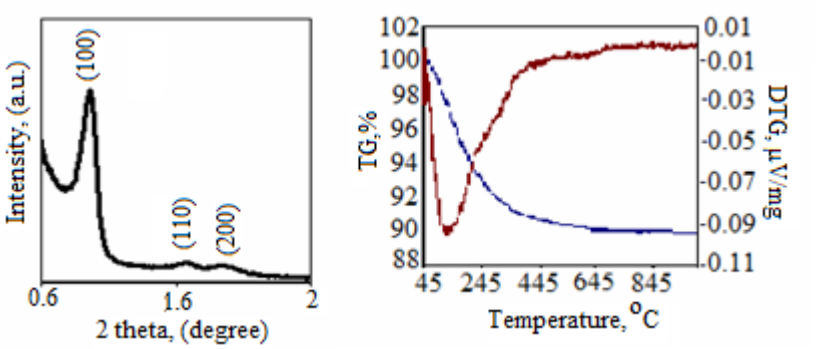


Figure III.9. X-ray diffractometry for SBA-15 matrix
Figure III.11. Analysis of TG / DTG for SBA-15 matrix

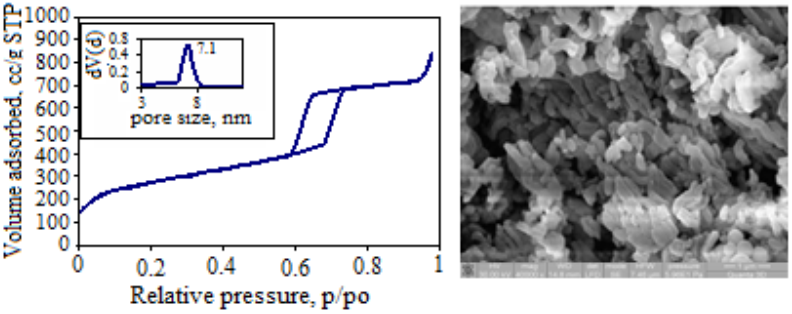


Figure III.12. N_2 adsorption and desorption isotherms and the corresponding pore size distributions of SBA-15 matrix
FigureIII.15. SEM images of SBA-15 matrix

III.3. Synthesis of MgO modified SBA-15 mesoporous matrix

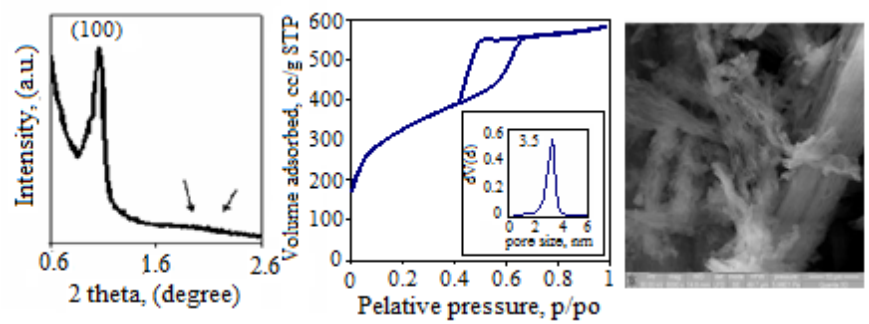


Figure III.16. X-ray diffractometry for MgO/SBA-15 matrix

Figure III.18. N_2 adsorption and desorption isotherms and the corresponding pore size distributions of MgO/SBA-15 matrix

Figure III.21. SEM images of MgO/SBA-15 matrix

Table III.4. Textural proprieties of the synthesized matrix

Parameters	**MCM-41**	**SBA-15**	**MgO/SBA-15**
d_{100}, (nm)	4.09	9.29	8.50
a_0, (nm)	4.72	10.73	9.82
Surface area, BET, (m^2/g)	1024.3	749.5	734.8
Pore diameter, BJH, (nm)	2.7	7.15	5.57
Micropore surface area t-Plot, (m^2/g)	-	647.9	536.1
External surface area t-Plot, (m^2/g)	-	102.3	198.7
Micropore volume t-Plot, (cm^3/g)	-	0.106	0.212
Pore wall thickness, t (nm)	2.02	3.58	4.25
Amount of external water losses, (%)	3.40	9.7	-
Amount of internal water losses, (%)	5.60	-	-
The total losses, (%)	9.00	9.7	-

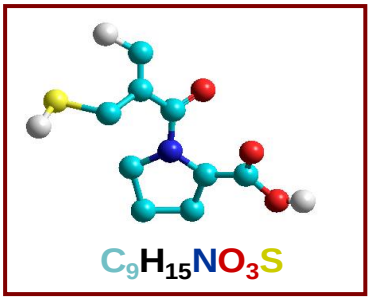
$2\mathbf{d_{100}}\sin\theta=n\lambda$; $\mathbf{a_0} = d_{100} \times 2 / \sqrt{3}$; $\mathbf{t= a_0} - Dp_{(BJH)}$;

IV.1. Synthesis and characterization of the mesoporous matrix – biologically active substance systems

The objective of this study consists in producing latest generation drug delivery systems, in which two antihypertensive substances (captopril and aliskiren) were introduced in the mesoporous matrix (SBA-15, MCM-41 and MgO/SBA-15), from where they can be released for various specific disorders [140], [149].

It was also intended to bring some personal contributions to the biomaterials quality of these materials, thus seeking to obtain systems that allow incorporation of biologically active substances and, at same time, to evaluate the *in vitro* release properties of the entrapped substances.

To obtain the mesoporous matrix-active substance systems, two pharmaceutical antihypertensive agents (captopril and aliskiren) were chosen.



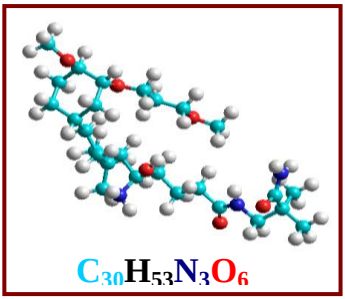


Figure IV.1. 3D structure of captopril [146]
Figure IV.2. 3D structure of aliskiren

IV.1.4. Synthesis of the mesoporous silica systems

Using impregnation method, the active component of the drug substance is dispersed on a support, through direct contact of the mesoporous solid with the solution containing the active component [156].

A typical procedure for loading [149] the antihypertensive drugs on SBA-15 and MgO/SBA-15 matrices involves mixing the components at a ratio of 1g matrix/50 mL of 0.1 M antihypertensive drug aqueous solution, at room temperature, followed by continuous stirring for 24h. Then, the antihypertensive drug - loaded samples were separated from the solution by filtration and dried at RT. The obtained samples were denoted as:

- **MCM-41-captopril , SBA-15-captopril, MgO/SBA-15-captopril**
- **MCM-41-Aliskiren, SBA-15-Aliskiren, MgO/SBA-15-Aliskiren**

Impregnation of antihypertensive substances was investigated using X-ray diffraction (XRD), adsorption/desorption of N_2 (BET- surface area, BJH – pore diameter, the pore wall thickness t-plot) and Scanning electron microscope (SEM) [149]. Textural characteristics of the obtained drug delivery systems are detailed in Table IV.2.

Table IV.2. Structural and textural characteristics of the studied samples

Sample	S_{BET}, m^2/g	D_{BJH}, nm	V_{tot}, cm^3/g	t-plot			t, (nm)
				Sμ, m^2/g	Sμ ext, m^2/g	Vμ, cm^3/g	
SBA-15	749.5	7.15	0.850	647.9	102.3	0.106	3.58
SBA-15-Aliskiren	538.4	6.83	0.726	438.5	99.9	0.085	4.19
SBA-15-Captopril	466.9	6.65	0.534	382.4	84.5	0.027	4.37
MgO/SBA-15	734.8	5.57	0.736	536.1	198.7	0.212	4.25
MgO/SBA-15-Aliskiren	645.3	5.02	0.573	471.7	173.6	0.135	4.83
MgO/SBA-15-Captopril	608.5	4.55	0.429	464.6	143.9	0.069	6.42
MCM-41	1024.3	2.70	0.915	-	-	-	2.02
MCM-41-Aliskiren	840.2	2.38	0.867	-	-	-	3.51
MCM-41-captopril	734.8	1.70	0.854	-	-	-	4.24

Comparing the structural and textural properties in Table IV.2, MgO–SBA-15 matrix shows a smaller pore diameter, a smaller surface area, but also a greater wall thickness to SBA-15 and MCM-41 matrix. This observation argues that MgO presented compared deposition mainly inside the SBA-15 pores. Meanwhile, analysis of data from table IV.2 highlights the positive effect of MgO on the greater quantities retention of drugs inside the pores. Analyzing the extra increase in of wall thickness, which is proportional to the quantities of the drug submitted, we can observe that captopril encapsulation is more convenient than aliskiren encapsulation [158].

IV.2. In vitro studies of availability of antihypertensive substances from siliceous mesoporous matrix

In order to allow a more detailed analysis of the systems obtained in this study, the release has been made in a solution that simulates the intestinal fluid (PBS) and a solution that simulates the human body plasma (SBF).

Table IV.4. Release test parameters of captopril and aliskiren for the extended release systems analyzed (PBS)

RELEASE PARAMETERS	
Instrument used	*HEIDOLPH, Magnetic Stirrer/ Hotplate, MR Hei Standard*
Dissolution medium	PBS (phosphate buffer, pH 7,4)
Volume	50 mL
Temperature	37± 2° C
Agitation	70 rpm
Sampling time	30, 60, 90, 120, 180, 240, 300, 360, 420, 480, 720, 1200, 1500, 1800 minute

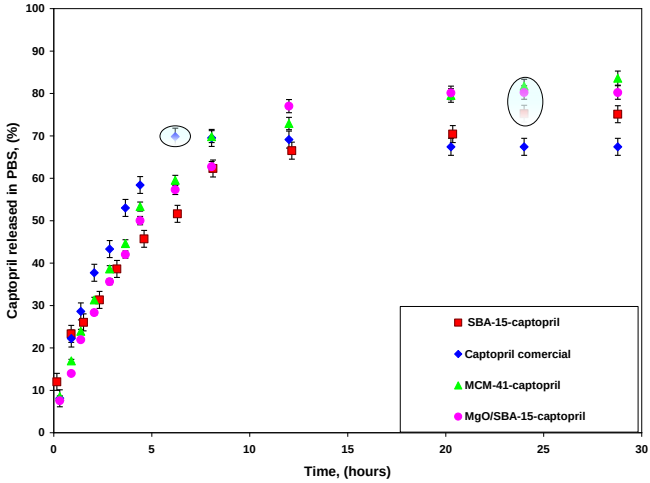


Figure IV.19. Captopril released in intestinal media (PBS).

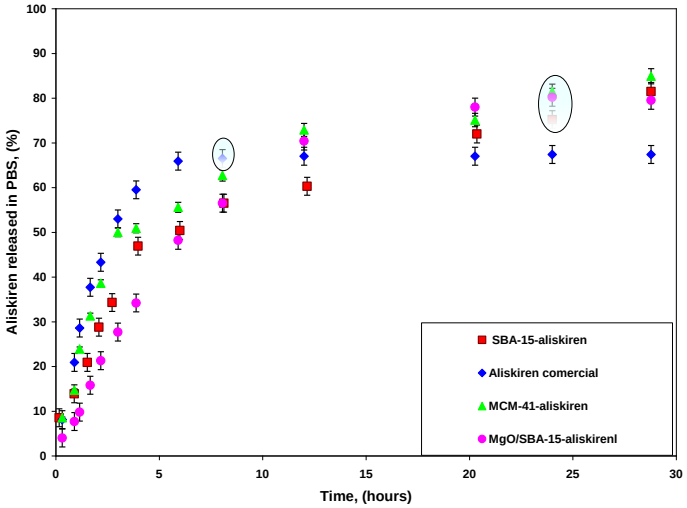


Figure IV.23. Aliskiren released in intestinal media (PBS).

The results obtained from in vitro release studies of antihypertensive substances have shown an increase of the quantity released from the studied mesoporous matrices.

Table IV.5. Release test parameters of captopril and aliskiren for the extended release systems analyzed (SBF)

RELEASE PARAMETERS	
Instrument used	*HEIDOLPH, Magnetic Stirrer/ Hotplate, MR Hei Standard*
Dissolution medium	SBF (standard buffer solution, pH 7,4)
Volume	50 mL
Temperature	37± 2° C
Agitation	85 rpm
Sampling time	30, 60, 90, 120, 180, 240, 300, 360, 420, 480, 720, 1200, 1500, 1800 minute

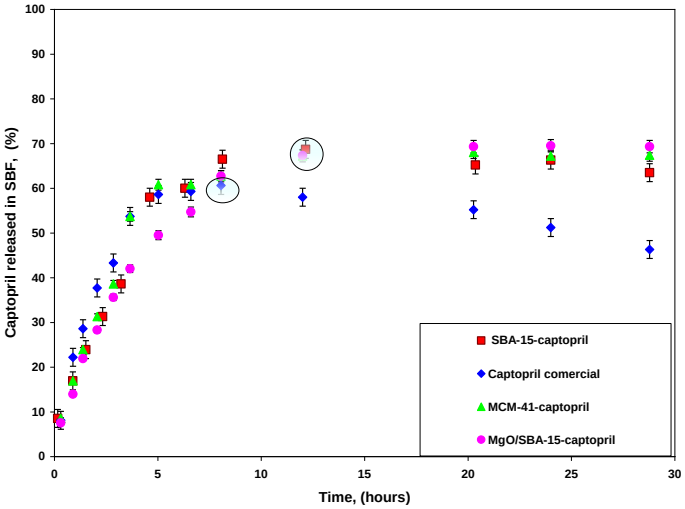


Figure IV.21. Captopril released in synthetic body fluid (SBF).

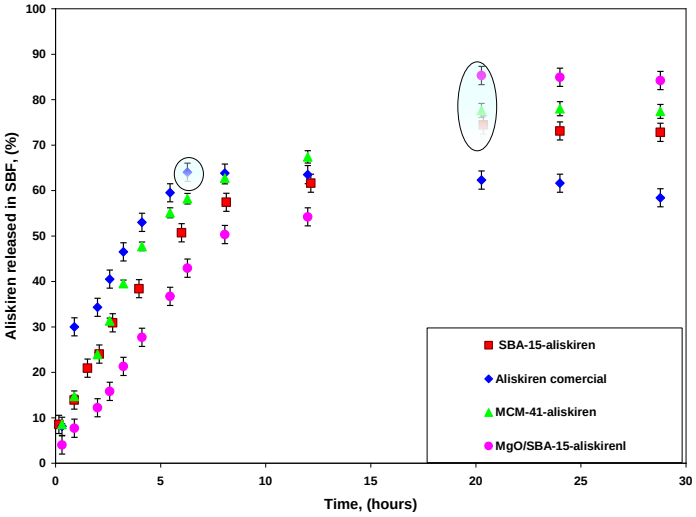


Figure IV.25. Aliskiren released in synthetic body fluid (SBF).

IV.3. Mathematical models for processes of controlled release of antihypertensive substances from the porous matrix

Korsmeyer Peppas model was described by the equation $M_t / M_\infty = kt^n$ [97] and Higuchi model was described by the equation $Q_t = K_H t^{0.5}$ [100].

Table IV.8. Release parameters for captopril and aliskiren sustained release tablets in PBS

Matrix	Higuchi		Korsmeyer – Peppas		
Parameters for PBS	R^2	K_H ($h^{-}_{1/2}$)	R^2	*exponent „n"*	$K(h^{-n})$
MCM-41-captopril	0.9098	15.31	0.9875	0.68	0.192
SBA-15-captopril	0.9138	14.61	0.9902	0.67	0.204
MgO/SBA-15-captopril	0.9265	15.73	0.9917	0.72	0.169
MCM-41-aliskiren	0.9126	13.86	0.9888	0.66	0.199
SBA-15-aliskiren	0.9141	16.80	0.989	0.65	0.198
MgO/SBA-15-aliskiren	0.9373	17.81	0.995	0.88	0.126

Table IV.9. Release parameters for captopril and aliskiren sustained release tablets in SBF

Matrix	Higuchi		Korsmeyer – Peppas		
Parameters for SBF	R^2	K_H ($h^{-}_{1/2}$)	R^2	*Exponent „n"*	$K(h^{-n})$
MCM-41-captopril	0.8078	12.13	0.9789	0.69	0.188
SBA-15-captopril	0.8482	14.78	0.9534	0.70	0.192
MgO/SBA-15-captopril	0.9221	13.70	0.9812	0.76	0.155
MCM-41-aliskiren	0.8488	13.81	0.9887	0.68	0.187
SBA-15-aliskiren	0.8350	15.50	0.9846	0.67	0.153
MgO/SBA-15-aliskiren	0.9744	18.43	0.9906	0.86	0.122

All studied systems showed release kinetics that best fitted the Korsmeyer-Peppas model, a model that shows the release is influenced by the antihypertensive substances diffusion phenomena and by the erosion of the studied mesoporous matrices.

Chapter V. Layered double hydroxides with applications in biopharmacy

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The general objective of Chapter V was to bring personal contributions on the use of layered double hydroxides as controlled release systems of biologically active substances, with applications in biopharmacy. Experimental research, results, applications and conclusions are presented.

Experimental research on synthesis and characterization of layered double hydroxide materials included selecting the synthesis method and studying the influence of Mg / Al molar ratio on the chemical and physical properties of these materials. The personal contributions have involved synthesis of three different systems using captopril and methotrexate as active substances in order to improve the efficiency of processes already used in practice, aiming at developing new products, testing their stability, monitoring and characterization processes.

Another objective was to determine the toxicity (biocompatibility) of the controlled release systems using *in vivo* technique and to determine the pharmacodynamic effect (bioavailability).

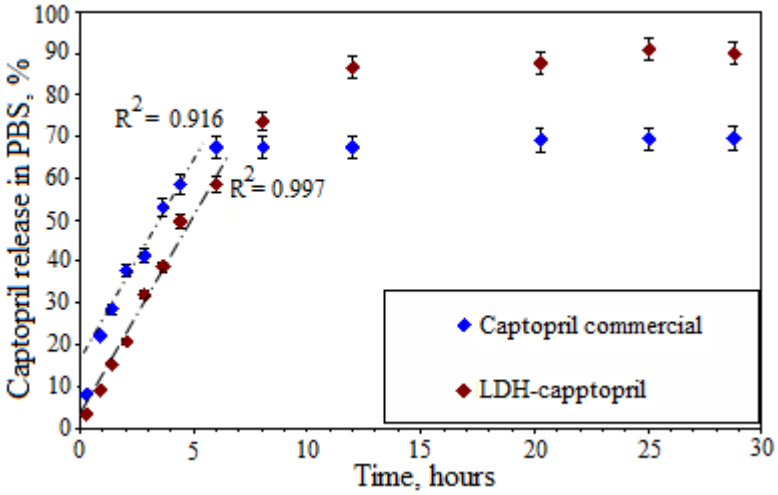
For structural and elemental characterization of layered double hydroxides X-ray diffraction, FTIR and UV-Vis spectroscopic methods were used, followed by morphology and texture analysis performed by SEM and BET.

Having characterized the two matrices and the systems obtained after intercalation of active substances, we chose to present a single system which achieved the most remarkable results.

V.3. Research on in vitro release of some bioactive substances from layered double hydroxides

In vitro tests were performed on samples two samples: LDH-captopril (Figure V.17.) and LDH-MTX (Figure V.18.) which were obtained by the reconstruction method, as has been shown to be the most advantageous method in terms of degree of incorporation.

Because of the high basicity of Mg_3Al-LDH, its use as drug delivery system in stomach media, where pH is 1-2, is debatable, because it can lead to partial dissolution of brucite layers. Thus release of active substances from Mg_3Al-LDH matrix was performed in a phosphate buffer solution (PBS) with a pH of 7.4 [161].



FigureV.20. Profiles of captopril release from LDH matrix and from the commercial tablet

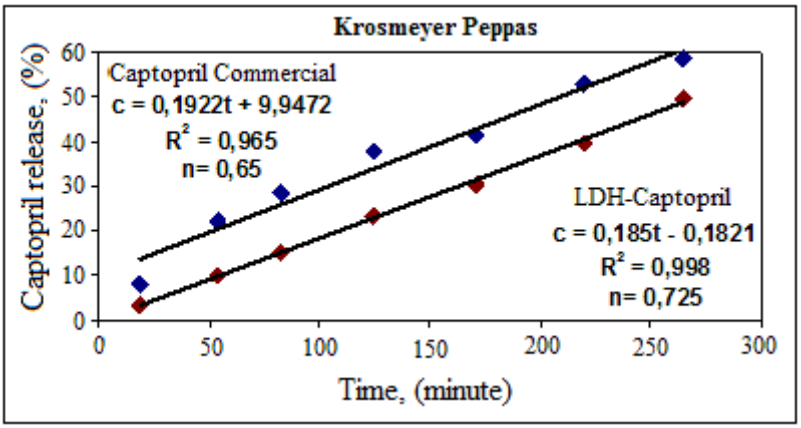
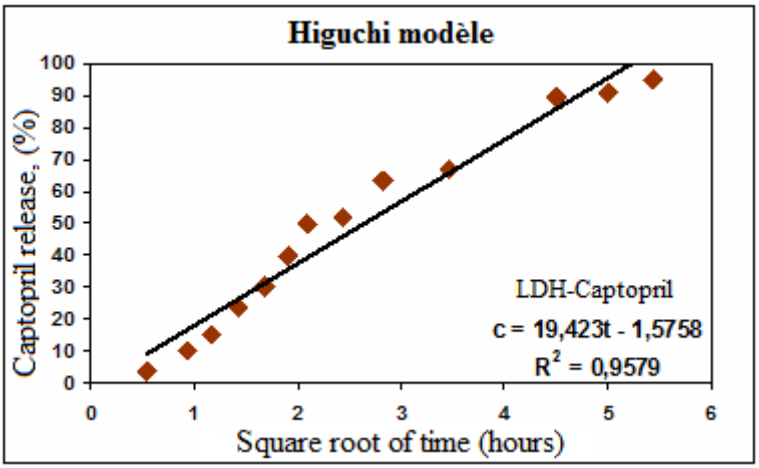


Figure V.22. Korsmeyer - Peppas model for 60% captopril release mechanism



FigureV.24. Higuchi model for the mechanism of captopril controlled release

The synthesised LDH-captopril system provided a sustained *in vitro* release of captopril for a period of 12 hours, compared to 6 hours for commercial captopril.

Also, the non-fickian release kinetics obtained using Korsmeyer - Peppas model, presented a much better linearity for LDH-captopril system compared with the tablets available on the market.

Thus these systems showed better therapeutic efficacy compared with the existing commercial tablet on the pharmaceutical market.

V.4. Contributions to the studies of in vivo release of captopril entrapped between the layers of layered double hydroxides

☞ To study the biocompatibility the short-term toxicity of the obtained matrix was defined and it was fitted in the appropriate class of toxicity. The most commonly used unit to define classes of toxicity is the median lethal dose (DL_{50}) [129], which is determined using statistical methods and which represents the amount of substance that causes death in 50% of animals in the experimental group [211].

☞ For the bioavailability study, two groups of Sprague-Dawley rats were used. Each group consisted of three rats, weighing between 280-300g. The rats were purchased from the Central Laboratory for Doping Control of the "Gr.T. Popa" University, Faculty of Medicine and Pharmacy. The working method used rats that received a single dose of 0.35 mg/ body kg substance. The dose for each rat was calculated based on its weight and was dissolved in 10 ml of carboxymethylcellulose (CMC). Carboxymethylcellulose was selected as dissolution medium because it has high viscosity grade and non-toxic and non-allergic properties [213].

V.4.3.1. Biocompatibility tests

According to the toxicity scale of Hodge and Sterner [216] the synthesized materials belong to the fifth group of toxicity with the degree "practically nontoxic". This degree corresponds to a single oral dose in the range of 5000 to 15000 mg / animal body kg.

V.4.3.2. Bioavailability tests

For determination of captopril from plasma, high performance liquid chromatography HPLC was used [71].

Table V.11. Operational parameters of the chromatographic method [71].

PARAMETERS	
Column chromatography	ZORBAX SB-C18 (150mm L × 4.6 mm).
Column temperature	30ºC
Injection volume	20 µL
Mobile phase composition	Solvent A: methanol, 45% Solvent B: buffer adjusted to pH=3, 55%
Debit	1ml/minute
Detection	UV
Wavelength	257,5

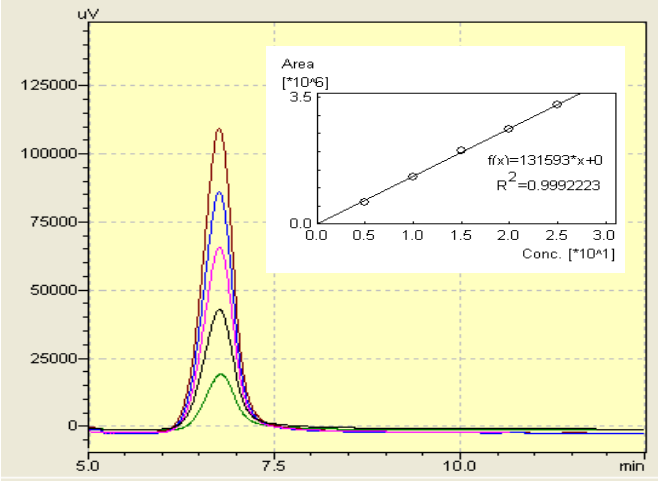


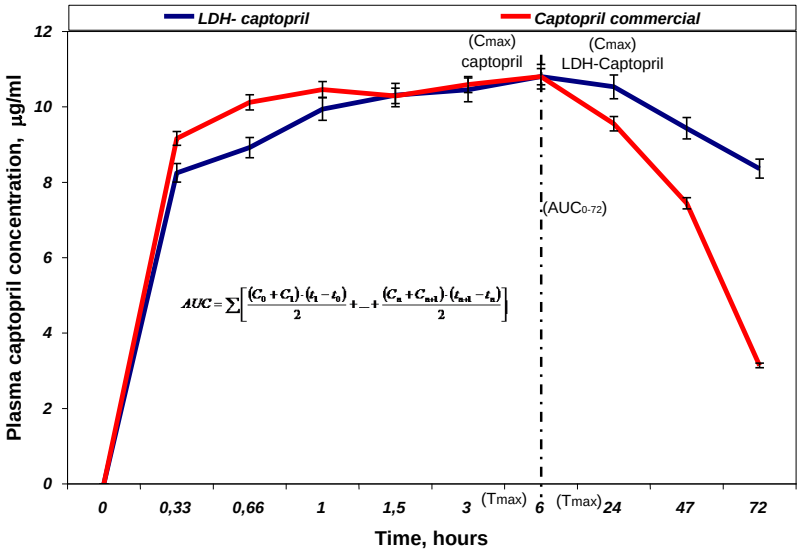
Figure V. 27. HPLC Chromatographic

The HPLC chromatogram (Figure V.27.) shows the retention times obtained in the mobile phase solutions with a known concentration of captopril in the range of 5-25 µg/ml.

The time when the substance elutes (leaves the column) is called "retention time"; under particular conditions, this is considered an unique

identifier for the given analyte, and in our case, captopril was obtained at the retention time of 6,6 min (Figure V.27). [71].

Mean plasma concentration-time curves are depicted in Figure V.28 and pharmacokinetic parameters are summarized in Table V.12.



FigureV.28. Release profile the captopril time based

Table V.12. Pharmacokinetic parameters [218]

Sample	T, °C	C_{max}, (µg/mL)	T_{max}, (h)	C_{12h}, (µg/mL)	AUC_{0-72}, (µg h/mL)	Bd_{rel}, (%)
Captopril Commercial	-	10.89	6	9.55	502.51	Reference
LDH-Captopril	20	10.89	6	10.53	614.56	124.37

In our study, the half-life for LDH-captopril system was originally three hours, but because after 12 hours the concentration decreases very little, it could be said that the half-life can considered at 6 hours.

General conclusions

Research conducted within this PhD thesis represent personal contributions on the synthesis of nanoporous materials, used as potential matrices for entrapping biologically active substances, with the aim of improving the efficiency of products that are already used in practice.

The theme selected is justified by the current interest in the use of new nanoporous materials in medicine, by storing biologically active substances with biopharmaceutical applications.

The general objective of this PhD thesis was focused on enriching both theoretical and practical knowledge concerning the synthesis of nanoporous materials and implementation of individual contributions, relating to improving and maintaining effective concentrations of biologically active substance over longer periods of time compared to existing forms on pharmaceutical market.

The study performed in the second part of the thesis presents original results, describing the synthesis and characterization of some mesoporous matrix: MCM-41, SBA-15, MgO/SBA-15 and double layered hydroxides. One overview of these results indicates the achievement of the proposed objectives. Physicochemical characterization methods used in analyzing the structural, morphological and textural properties of materials highlight the successful syntheses that were performed.

One of the contributions of the studies was performed using synthesized mesoporous silica matrix, which proved to have different structures, which is due to the synthesis conditions: MCM-41 material was synthesized by hydrothermal method in basic conditions and the SBA-15 and MgO/SBA-15 materials were synthesized by acidic sol-gel method.

The study described in Chapter IV. was focused on obtaining drug delivery systems. Therefore, two antihypertensive substances (captopril and aliskiren) were entrapped in SBA-15, MCM-41 and MgO/SBA-15 mesoporous matrices.

The antihypertensive substances (captopril and aliskiren) were incorporated using impregnation method, in the obtained silica matrix (Chapter III), studying the release kinetics of the active principle under conditions that simulate the biological environment. Both captopril and aliskiren were successfully obtained in the form of drug delivery systems.

The performed studies focused on the incorporation of the above biologically active substances and the analysis of the in vitro release properties of the entrapped substances.

Another scientific contribution was obtained from the study of the release of biologically active substances intercalated in the nanoporous matrices, achieving successful determination of the *in vitro* availability.

The results obtained from *in vitro* release studies of incorporated antihypertensive substances showed an increase in the amount transferred from the studied mesoporous matrices.

The most spectacular results were obtained using the MgO/SBA-15 mesoporous matrix, which represents an innovative drug delivery system.

The in vitro release kinetics study of antihypertensive substances obtained by applying two mathematical models: Krosmeyer-Peppas and Higuchi has shown that antihypertensive substances are disposed of formulations realized through a diffusion process, regardless of the experimentally used media.

All the studied systems showed release kinetics that best fitted the Krosmeyer-Peppas model, a model that shows the release is influenced by the antihypertensive substances diffusion phenomena and by the erosion of the studied mesoporous matrices.

A remarkable result was obtained after in vitro tests on MgO/SBA-15 matrix which have indicated that MgO layer significantly delayed the release rate of the antihypertensive substances, this being of great importance for the controlled release processes.

Through our research that is subject of the present PhD thesis, we have demonstrated that MCM-41, SBA-15 and MgO/SBA-15 mesoporous silica matrix can be successfully applied to obtain drug delivery systems.

In the international literature, there are no reported studies regarding controlled release systems based on mesoporous matrices entrapped with aliskiren. For the first time, we incorporated the aliskiren molecules into the pores of mesoporous silica matrices and studied the kinetics of release, achieving very promising results.

One practical contribution made by this study, described in Chapter V, relates to the improvement of efficiency of already used in the pharmaceutical market processes by realising both *in vitro* and *in vivo* study of layered double hydroxides entrapped with active substances.

For layered double hydroxides, the main monitored objective was to select the method for synthesis of the LDH matrix, which were prepared by coprecipitation in conditions of low supersaturation at constant pH, by investigating the influence of molar ratio Mg/Al during synthesis, kept in the range of 1-3, on the physicochemical properties of the matrices.

The best results were obtained when Mg_3Al-LDH matrices, which have shown basic properties and the largest surface area (195 m^2/g) and interbasal distance of 2.04 nm. These properties indicate the potential value of this matrix for adsorption or encapsulation of biologically active substances.

The performed study revealed the direct influence of the morphological characteristics of the materials on the loading of biologically active substances.

For layered double hydroxides materials, the studies were designed to determine the most advantageous method for the loading of biologically substances in the interbasal space, using an anticancer substance (methotrexate) and an antihypertensives substance (captopril).

Three methods of loading were used: Mg_3Al-LDH matrix obtained by coprecipitation in basic medium, reconstruction of Mg_3Al-LDH matrix based on memory effect and Mg_3Al-LDH matrix obtained by ion exchange with anions of captopril.

For the in vitro study, of all samples obtained only the samples that had a higher degree of active substance loading were chosen: LDH-captopril-3 and LDH-MTX-3, obtained using the reconstruction method.

The obtained new systems lead to a much better therapeutic efficacy compared to existing commercial tablet on pharmaceutical market and enjoy a large perspective of potential use.

At the same time, the results we obtained regarding the process of release of biologically active substances from LDH inorganic matrix brought new contributions to the development of some forms of drugs that facilitate a single administration per day of this dosing.

The *in vivo* study has demonstrated first, that the release dynamic can be controlled and sustained for synthesized systems compared to existing tablets on the pharmaceutical market.

An outstanding scientific contribution is represented by clearly proving that LDH-captopril system offers the advantage of maintaining an effective concentration of captopril over a longer period of time compared to commercial captopril.

It is worth mentioning that the studies were laborious and required collaboration from chemists, biologists and pharmacists. This collaboration has permitted a better understanding of the complex phenomena taking place, starting from the synthesis of nanosystems to the their potential application in the biological processes, facilitating multidisciplinary scientific approach to the theme by using a common language and setting suitable working strategies of such subjects.

Actuality and originality of research

Actuality

- The issue of incorporating biologically active substances in different types of matrices in order to obtain drug delivery systems based on the use of nanomaterials enjoys remarkable importance, being at the top of international research.

Originality

- The research has addressed the ways to influence the pharmacokinetics parameters and how they can be optimized in order to increase the therapeutic efficacy of some antihypertensive substances used in current therapy (captopril, aliskiren).
- Toxicity and degradation effect of biologically active substances are reduced when they are encapsulated in a non-toxic, biocompatible, nanoporous form and which exerts a modulator effect on the diffusion of the biological active substances after administration in the environment in which they are to exercise their action.
- The research conducted during this PhD thesis aimed to improve the efficiency of biologically active substances (captopril, aliskiren, methotrexate), by developing drug delivery systems based on the use of nanostructured matrices with different morphologies and properties.
- Given the obtained results, recognized by their publications in prestigious journals, it can be said that the PhD thesis has achieved and realized the proposed objectives, in accordance with the doctoral research program.

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