UNIVERSITY POLITEHNICA OF BUCHAREST

Applied Chemistry and Materials Science Doctoral School

Composites based on bioactive substances for medical applications

PHD THESIS ABSTRACT

SUPERVISOR

Prof. Dr. Eng. Daniela BERGER

AUTHOR

Eng. Mihaela DEACONU

Keywords: mesoporous silica, colloidal silica, silica degradation, drug delivery systems, release kinetics, mathematical models, antibiotics, doxycycline, lomefloxacin, norfloxacin

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Introduction

Ordered mesoporous silica was prepared for the first time by Mobil researchers in 1992 and applied in catalysis [1], and nearly a decade later it was used as carrier for drug delivery systems [2]. This was the starting point of a new research field, the development of drug delivery systems based on mesoporous silica, due to their advantageous features. Most common mesoporous silica materials are MCM-41, SBA-15 and MCM-48, first two presenting a hexagonal pore array geometry, and the latter showing a cubic pore network with interconnected pores. The high specific surface area and pore volume values of the mesoporous silica nanoparticles (MSN) determines the accommodation of high amounts of biologically active molecules, and through surface functionalization, these materials offer the possibility to control the release of the therapeutic agent adjusting the interactions between drug molecules and carrier.

The studies performed for this PhD thesis are focused on the synthesis and characterization of colloidal silica nanoparticles, with particles diameter below 100 nm and favorable textural properties for drug delivery applications, and their degradation in simulated biological fluids. Also, new silica-antibiotic composites were obtained by tailoring the interactions between silica carrier and antibiotic molecules, thus the antibiotic release kinetics. Mesoporous silica carriers were functionalized with organic groups or modified with magnesium ions to tailor the drug-support interactions, in order to slow down the antibiotic release kinetics. Furthermore, new composites containing antibiotic based on mesoporous silica supports with various pore sizes were designed to enhance the solubility of poorly water-soluble antibiotics.

The PhD thesis comprises two main parts: the first part consists in the state of art in literature throughout eight chapters, and the second part presents the original contributions along six chapters. The literature survey consists of an introduction about mesoporous silica, followed by the next two chapters describing the synthesis and functionalization methods of the mesoporous silica nanoparticles. In the third chapter an analysis of the literature data regarding the influence of the synthesis parameters on the morphology and structure of MSN is presented, along with data concerning the biocompatibility of MSN depending on particle size, shape or surface properties, and their degradation depending on the medium or particles morphology. In chapter seven, antibiotic-based composites are summarized for which the release kinetics was tailored, or the antibiotic water solubility was enhanced. This chapter also contained a short description of the antibiotics used as model drug in the experimental studies, doxycycline and lomefloxacin, which are water-soluble antibiotics, and norfloxacin, a poorly water-soluble antimicrobial agent. In the last chapter, the mathematical models used for fitting the drug release experimental data are listed. These models enable the assessment of the release kinetics of the therapeutic agent in terms of transport mechanism or drug diffusion rates.

In the second part of the thesis, *Original contributions*, is described the justification of topic choice, followed by the chapters in which the synthesis, characterization, and behavior of silica nanoparticles in simulated biological fluids is described. In chapters four and five, composites based on water-soluble antibiotics (doxycycline and lomefloxacin), and poorly water-soluble antibiotics (norfloxacin) are presented, along with the analysis of each antibiotic release kinetics from the mesoporous silica-type carriers.

The results obtained for this PhD thesis were disseminated in 5 ISI papers, a book chapter and 7 contributions at conferences, where 6 were oral presentations and 1 poster.

II.1. Justification of topic choice

In the PhD thesis, *Composites based on bioactive substances for medical applications*, chemical engineering concepts were applied in medicine, especially for bacterial infections treatment.

The aim of this thesis was to prepare colloidal silica nanoparticles and mesostructured silica materials for the development of antibiotic-based composites containing pristine and functionalized mesoporous silica. The new antibiotic delivery systems were used to tailor water-soluble drug release kinetics, e.g., doxycycline from the tetracyclines class, lomefloxacin from the fluoroquinolones class, both antibiotics being used for the treatment of the urinary tract infections. Also, for the solubility enhancement of poorly water-soluble antibiotics, like norfloxacin, drug delivery systems were designed. The objectives of the PhD thesis were:

- ✓ Synthesis and characterization of pristine and functionalized colloidal silica nanoparticles;
- ✓ Study of colloidal silica nanoparticles degradation in simulated biological fluids;
- ✓ Synthesis and characterization of mesostructured pristine MCM-41 and SBA-15-type silica;
- ✓ Tailoring the surface properties of nanostructured silica;
- ✓ Antibiotic adsorption into the pristine and functionalized mesoporous silica carriers in order to obtain antibiotic delivery systems;
- ✓ Study of the antibacterial agent release kinetics from silica-type carriers in simulated biological fluids;
- ✓ *In vitro* analysis of the antibacterial activity for the developed drug delivery systems.

II.2. Characterization methods for colloidal silica nanoparticles and their degradation study

The pristine and functionalized colloidal silica samples were characterized by physicochemical methods and their biocompatibility was evaluated on healthy and tumoral cell lines. The characterization methods were FTIR spectroscopy, adsorption-desorption isotherms, TEM microscopy, small-angle X-ray diffraction and inductively coupled plasma-optical emission spectroscopy (ICP-OES).

II.3. Colloidal mesoporous silica nanoparticles

II.3.1. Synthesis and characterization of pristine and functionalized colloidal silica nanoparticles

In this chapter a new synthesis method was developed for the preparation of colloidal silica nanoparticles (NP) with the particle diameter below 100 nm and high porosity, NP that could be employed as carriers for biologically active molecules. Also, functionalized colloidal silica NP were obtained by co-condensation approach.

II.3.2. Behavior of pristine and functionalized colloidal silica nanoparticles in biological fluids

The stability of the colloidal silica NP pore array was assessed over time using two phosphate buffer solutions with different pH as degradation medium: pH 7.4 and pH 5.7 to simulate a healthy cellular environment and a tumoral cellular medium, respectively.

II.3.3. Cellular biocompatibility of colloidal silica nanoparticles

II.3.4. Conclusions regarding synthesis and degradation of colloidal silica nanoparticles

A new method was developed for the preparation of mesoporous silica NP with colloidal stability. The influence of the synthesis parameters like the structure directing agent, the use of a stabilizer, or thermal treatment conditions was assessed. The presence of polyethylene glycol had a favorable effect on the particles morphology, avoiding their agglomeration, but an increased ratio of stabilizer/TEOS led to a decrease of the pore network ordering. Although a slight improvement of the pore framework ordering is noticed, thermal treatments led to NP agglomeration and widening of the pore size distribution curve.

Silica NP were functionalized with different hydrophilic and hydrophobic organic groups through co-condensation method to obtain a uniform distribution of the organic groups on the internal and external surface of the silica particles. Functionalized silica NP presented

specific surface area values ranging from 675 m^2/g to 1157 m^2/g, spherical shape, and a diameter depending on the nature of the organic groups grafted on the silica surface.

The degradation of pristine and functionalized silica NP was studied in phosphate buffer solutions (PBS) pH 7.4 and pH 5.7, which simulate the cellular environment for healthy and tumor cells, respectively. The study showed a different behavior depending on either pH or surface modification with functional groups. In PBS pH 5.7, the hydrolysis of functionalized NPs is slower compared to pristine ones, and the hexagonal mesopore network is more stable, the pore diameter being generally closer to that of the initial material. In pH 7.4, silica hydrolysis is fast, the difference between the amount of silica hydrolyzed after 6 h and 24 h being very small, for both pristine and functionalized silica NPs. After 24 h in PBS pH 7.4, the pore diameter increases considerably, and the pore size distribution curves are wider than those of the degraded samples in PBS pH 5.7. Also, in PBS pH 7.4, a reorganization of the pore framework is observed in the small-angles X-ray diffraction patterns, illustrated by the appearance of a second intense diffraction peak, before (100) Bragg reflection, at lower q values, which indicates higher distances between the planes of the pore array.

The TEM investigation showed that silica nanoparticles are degraded on the surface and inside the mesopore channels. The presence of hydrophobic groups, such as methyl or vinyl, on the inner surface of the pore walls did not alter the behavior of functionalized silica compared to pristine samples, while the presence of aminopropyl groups causes a prominent hydrolysis in both media and a significant degradation of the pore network, probably due to strong electrostatic interactions between amine groups, which are protonated in both fluids, and phosphate ions. Particle size did not seem to influence the silica hydrolysis, nor the stability of the hexagonal pore array.

Biocompatibility of pristine and functionalized mesoporous silica nanoparticles was assessed by cellular viability (MTT method) on two cell lines, healthy HaCaT epithelial cells and MelJuso melanoma cells, using concentrations in the range of 50-250 μg/mL. The results indicated a concentration-dependent cell viability. The best biocompatibility was noticed for MSN-CH_3 and MSN-CN samples, and the lowest cell viability was obtained for MSN-SH and MSN-$CH{=}CH_2$ samples, which, however, prevented the proliferation of tumor cells.

II.4. Preparation and characterization of the drug-loaded composites

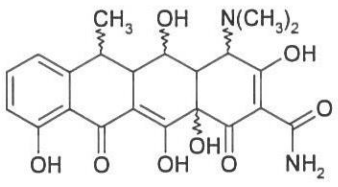
Mesoporous support-antibiotic composites were obtained by incipient wetness impregnation method. A concentrated antibiotic solution was prepared, close to the drug solubility limit, added to the mesoporous silica, followed by vacuum removal of the solvent. All antibiotic composites were prepared with 20 % (wt.) drug content.

In vitro release profiles were determined at 37°C, under magnetic stirring (150 rpm), in simulated biological fluids, pH 7.4 or pH 5.7 phosphate buffer solution (PBS).

II.5. Composites based on water-soluble antibiotics and mesoporous silica as carriers

II.5.1. Doxycycline composites

Data presented in this chapter were published in the paper *Tailored doxycycline delivery from MCM-41-type silica carriers* in Chemical Papers [3].



Scheme 1. Doxycycline chemical structure

Doxycycline (Doxy), an antibiotic from the tetracyclines class with a broad spectrum of activity [4], was the first drug model used in this PhD thesis to prepare drug delivery systems based on pristine and functionalized mesostructured silica supports. *The aim in this chapter was to tailor the interactions between the silica carrier and doxycycline molecules, thus functionalized MCM-41-type carriers were employed as carriers, as well as a magnesium-modified MCM-41 support.*

In this chapter, six MCM-41-type materials with tailored textural and surface properties were synthesized and used as carriers: two pristine silica of different average pore size synthesized with different structure directing agents, three MCM-41 materials functionalized with phenyl, mercaptopropyl and propylsulfonic moieties, and a magnesium-modified MCM-41 carrier.

Small-angle XRD patterns illustrated an ordered mesophase for all carriers (Fig. 1), while wide-angle XRD analysis (Fig. 2, insert) demonstrated that no crystalline MgO phase was obtained on the silica particles surface.

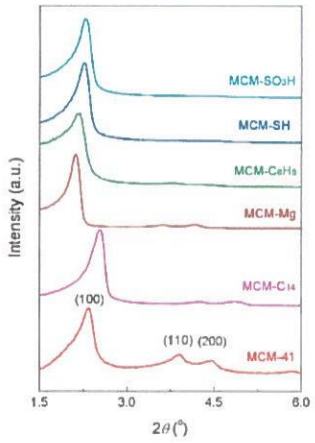


Fig. 1. Small-angle XRD patterns of MCM-41-type carriers

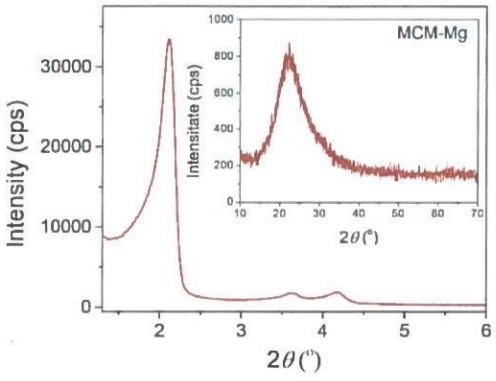


Fig. 2. Small-angle XRD and wide-angle XRD (insert) of MCM-Mg carrier

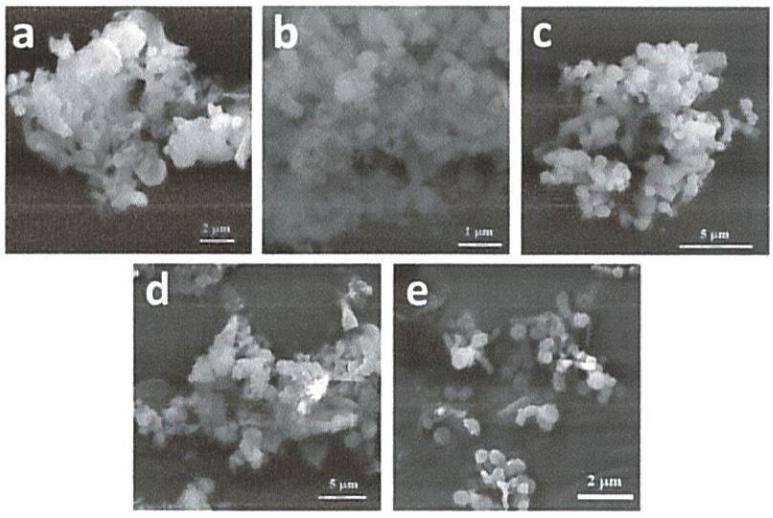


Fig. 3. SEM micrographs of: MCM-C_{14}(a), MCM-41(b), MCM-Mg(c) MCM-C_6H_5(d) and MCM-SO_3H (e)

The morphology of MCM-41 materials was evaluated by SEM and TEM analyses. Generally, the synthesized MCM-41 carriers consist of spherical particles with a diameter in the range of 200-400 nm, with a slight tendency to agglomerate (Fig. 3). The TEM investigation

of selected carriers proved the formation of an ordered pore array with long cylindrical nanochannels for both MCM-41 (Fig. 4a) and MCM-Mg (Fig. 4b).

The presence of doxycycline molecules into the carrier mesopores was evidenced by FTIR spectroscopy (Fig. 5). The absence of diffraction peaks associated with crystalline phase of the drug in wide-angle XRD patterns (Fig. 6) demonstrates the adsorption of the drug into the support mesopores in amorphous state.

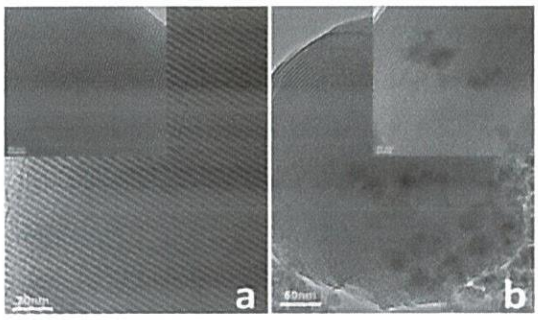


Fig. 4. TEM images of MCM-41 (a) and MCM-Mg (b)

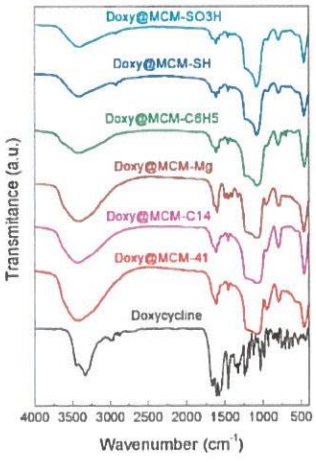


Fig. 5. FTIR spectra of doxycycline composites

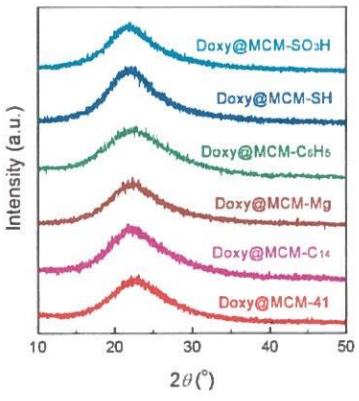


Fig. 6. Wide-angle XRD patterns of doxycycline composites

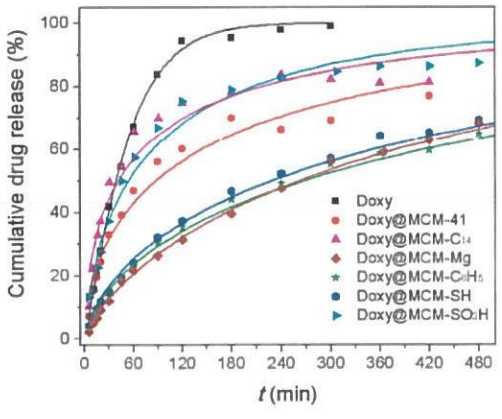


Fig. 7. Cumulative drug release data fitted with Weibull model

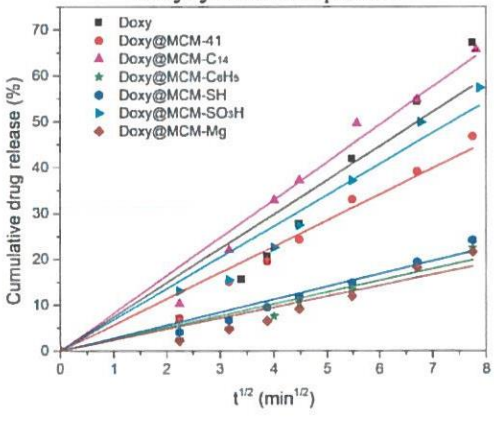


Fig. 8. Cumulative drug release data fitted with Higuchi model

Doxycycline exhibited a slower release kinetics from all carriers compared to the doxycycline dissolution (Fig. 7). For all doxycycline composites, the *b* parameter values of the Weibull function are lower than 0.75, which indicates a Fickian diffusion of the drug molecules

through the carrier mesopores. The presence of magnesium oxide nanoparticles on silica pore walls surface in MCM-Mg support slowed down the sustained release of doxycycline, because of the enhanced basicity as result of the magnesium oxide particles.

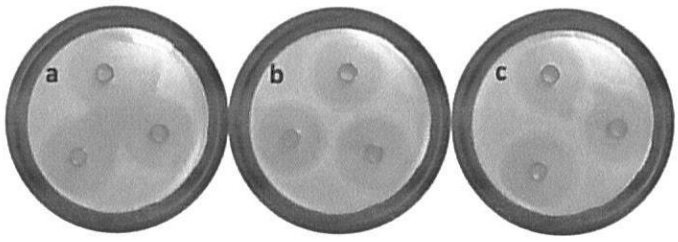


Fig. 9. Antibacterial activity of doxycycline (a), Doxy@MCM-41 (b) and Doxy@MCM-SH (c) performed in triplicate

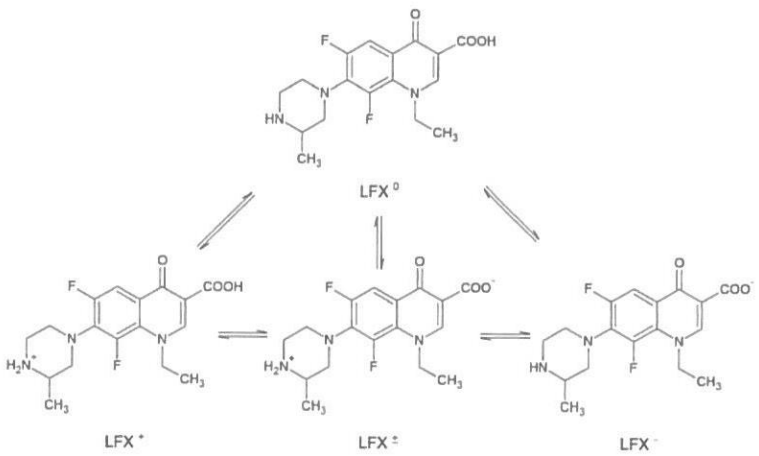
Conclusions regarding the silica-doxycycline composites

Mesostructured MCM-41-type materials with different textural, structural, and surface properties were successfully prepared and applied as carriers for doxycycline. It was proved that the drug release kinetics can be tailored through the carrier surface functionalization. A lower drug release rate was observed when hydrophobic groups were grafted on the silica pore surface or by the magnesium introduction into the MCM-41 carrier. It was also demonstrated that the doxycycline release rate can be controlled by tailoring the MCM-41 silica surface properties without altering its antibacterial activity. The antibacterial activity of doxycycline-loaded samples was tested against *Klebsiella pneumoniae* ATCC 10031 (Fig. 9) and it is similar to that of the antibiotic alone.

II.5.2. Lomefloxacin composites

The results presented in this chapter are found in *Exploiting the zwitterionic properties of lomefloxacin to tailor its delivery from functionalized MCM-41 silica* and *Heteroatom modified MCM-41-silica carriers for Lomefloxacin delivery systems*, both papers published in *Microporous and Mesoporous Materials* [5,6].

Lomefloxacin (LFX) has two ionizable functional groups, carboxylic and piperazinyl moieties, so the drug can exist in four forms (Scheme 2). After administration, the drug exists in zwitterionic ($LFX^{\pm}$) and neutral (LFX^{0}) forms [7], the ratio between the two forms influencing the antibiotic biodistribution and bioavailability.



Scheme 2. Lomefloxacin chemical structure.

Herein are presented *lomefloxacin delivery systems based on pristine and magnesium-modified mesoporous silica*, which demonstrated that the presence of magnesium oxide on the

inner walls of the silica slows down the release rate of LFX. However, to exploit the zwitterionic nature of lomefloxacin and to simplify the systems, *new drug delivery systems were designed based on MCM-41 silica functionalized with different acidic properties for tailoring the antibiotic release kinetics*, avoiding the introduction of heteroatoms.

The structural properties of functionalized silica carriers were assessed through small-angle XRD analysis (Fig. 10). FTIR spectroscopy was employed to demonstrate the presence of the organic groups grafted on the silica surface, and the textural features were determined from the nitrogen adsorption-desorption isotherms (Fig. 11 and Table 1).

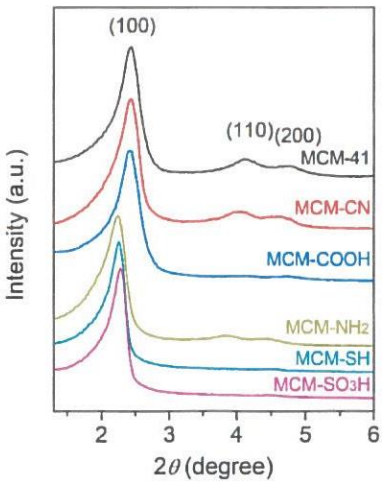


Fig. 10. Small-angle XRD patterns of functionalized MCM-41 carriers

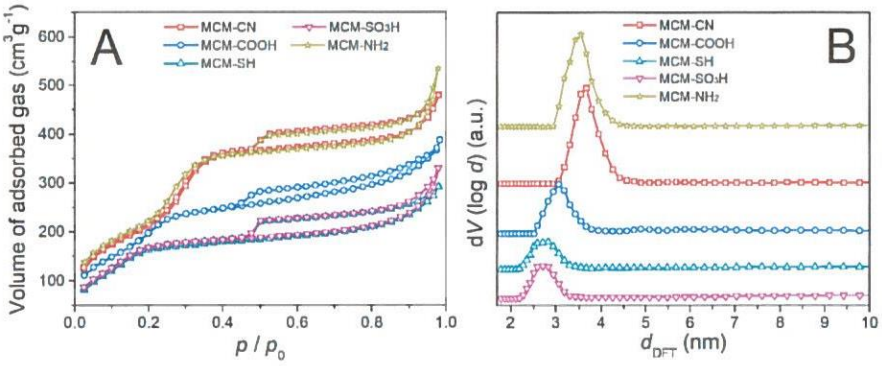


Fig. 11. Nitrogen adsorption-desorption isotherms (A) and pore size distribution curves computed with DFT model (B) of functionalized silica carriers

Table 1. Textural parameters of mesoporous carriers and LFX-loaded materials

Support	d_{BJH} (nm)	d_{DFT} (nm)	S_{BET} (m^2/g)	V_p (cm^3/g)	FG:SiO_2 raport molar	LFX@support			
						d_{BJH} (nm)	d_{DFT} (nm)	S_{BET} (m^2/g)	V_p (cm^3/g)
MCM-CN	2.52	3.66	793	0.71	0.087	2.39	3.54	330	0.35
MCM-COOH	2.13	3.06	678	0.57	0.055	2.00	3.06	131	0.18
MCM-NH_2	2.38	3.54	830	0.78	0.105	2.26	3.42	69	0.07
MCM-SH	1.75	2.82	730	0.43	0.219	-	-	20	0.05
MCM-SO_3H	1.75	2.68	664	0.49	0.169	-	-	87	0.12
MCM-Mg	2.52	3.66	551	0.42	-	-	-	51	0.12
MCM-41	2.82	3.93	920	0.92	-	-	-	31	-

FG – functional group, d_{BJH} – average pore diameter; S_{BET} – specific surface area; V_p – total pore volume;

Commercial MCM-41: d_{BJH} = 2.67 nm; d_{DFT} = 3.66 nm; S_{BET} = 976 m^2/g; V_p = 0.74 cm^3/g.

Lomefloxacin composites were characterized by wide-angle XRD analysis (Fig. 12), FTIR spectroscopy (Fig. 13), and nitrogen adsorption-desorption isotherms (Fig. 14) to evaluate the drug adsorption into the carrier mesopores.

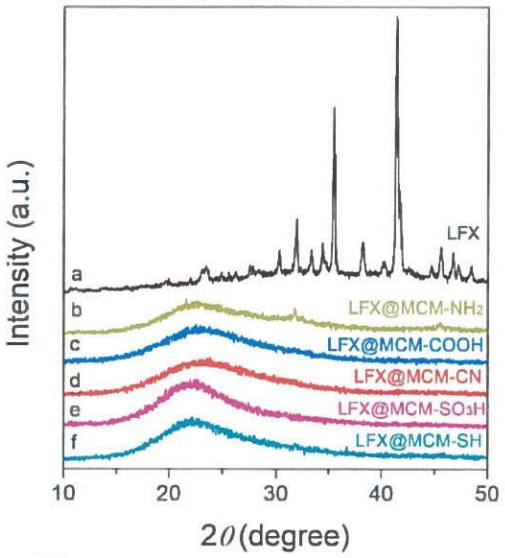


Fig. 12. Wide-angle XRD patterns of lomefloxacin composites

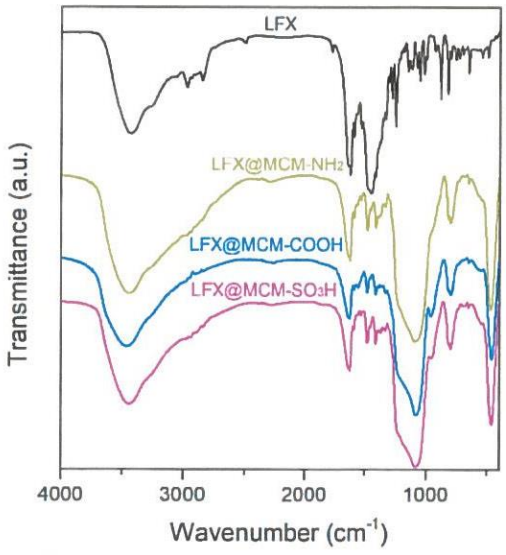


Fig. 13. FTIR spectra of lomefloxacin composites

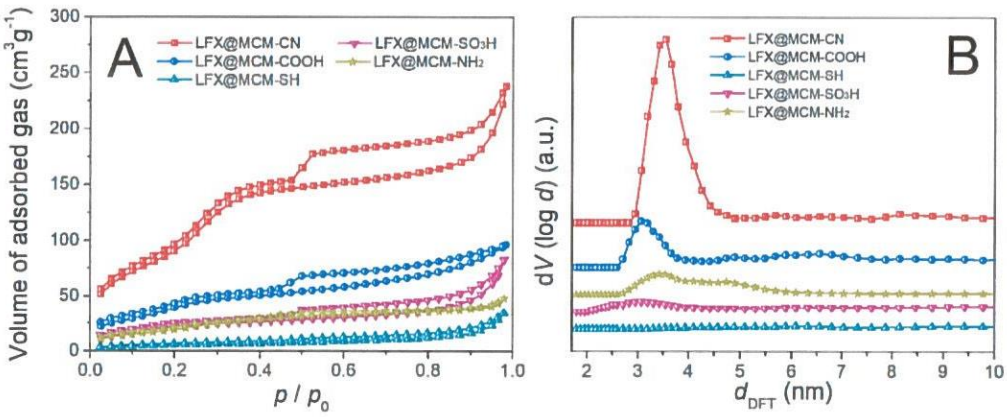


Fig. 14. Nitrogen adsorption-desorption isotherms (A) and pore size distribution curves computed with DFT model (B) of lomefloxacin composites

The antibiotic experimental release data were fitted with several mathematical models that could offer a better understanding of the drug transport and release process: the three-parameter [8] (Fig. 15), Weibull [9] (Fig. 17 and Fig. 18) and Higuchi models [10] (Fig. 19 and Fig. 20), good correlation coefficients were obtained for all models. The slowest kinetics was obtained for MCM-CN carrier, which has the most basic organic groups grafted on silica surface, though its average pore diameter is larger than for MCM-SH and MCM-SO_3H. This means that the pore diameter had no significant influence on the antibiotic delivery kinetics. The release behavior of lomefloxacin from functionalized MCM-41-type carriers depended on the pK_a values of the functional groups grafted on the silica pore walls surface (Fig. 16).

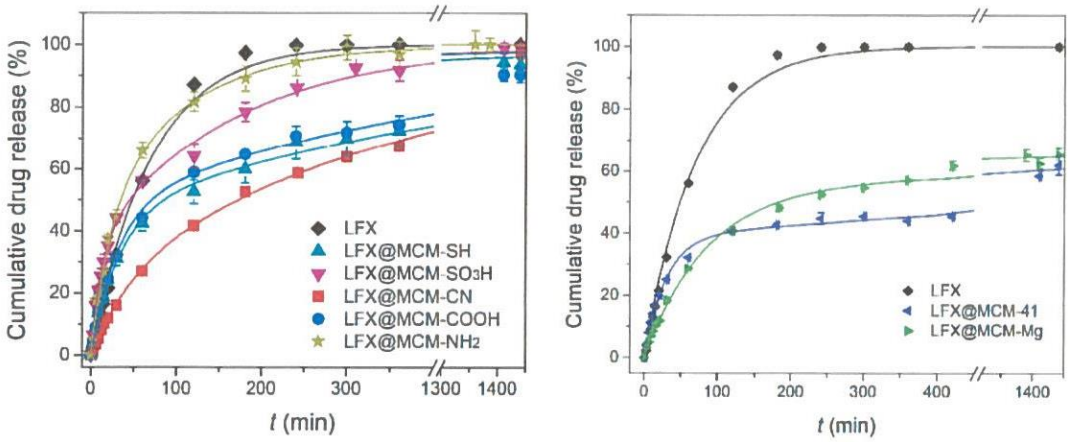


Fig. 15. Lomefloxacin experimental release data fitted with three-parameter model.

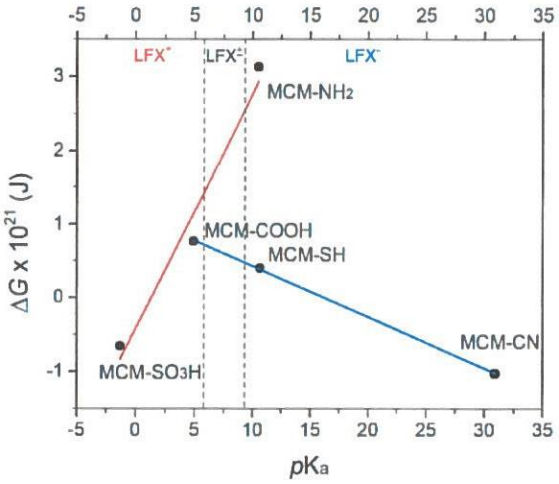


Fig. 16. Dependence of ΔG parameter on pK_a value of the organic groups grafted on MCM-41 support.

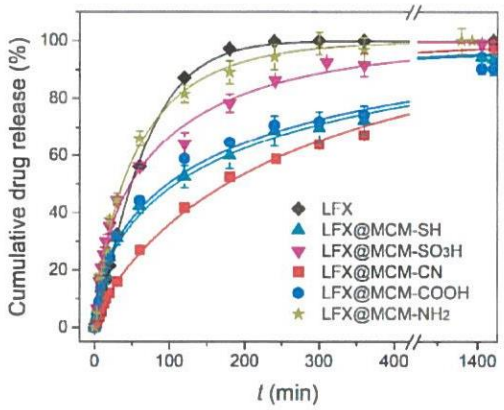


Fig. 17. Lomefloxacin experimental release data fitted with Weibull model

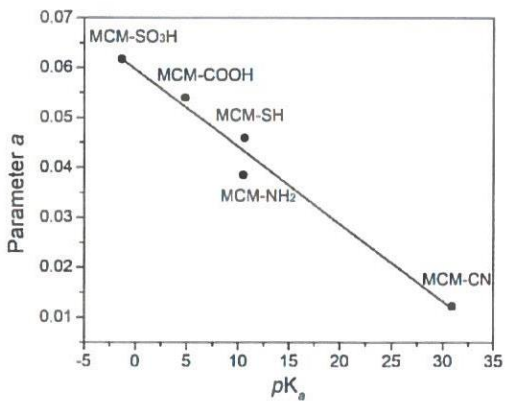


Fig. 18. Dependence of a parameter on pK_a value of the organic groups grafted on MCM-41 support

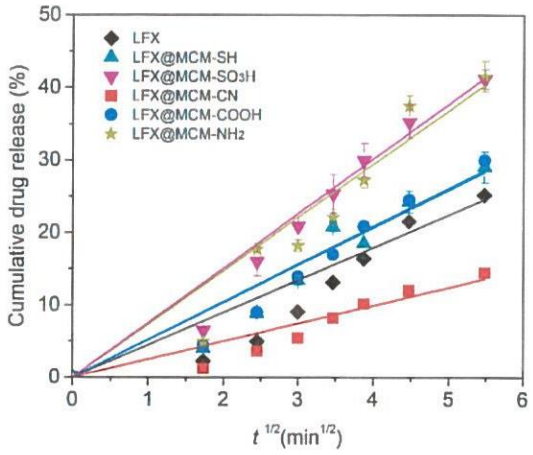


Fig. 19. Lomefloxacin experimental release data for functionalized MCM-41 supports fitted with Higuchi model

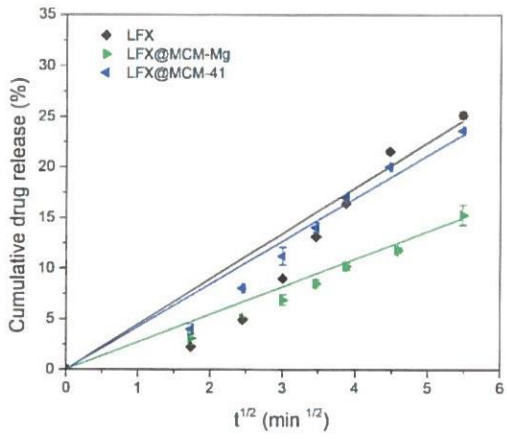


Fig. 20. Lomefloxacin experimental release data for MCM-41 and MCM-Mg supports fitted with Higuchi model

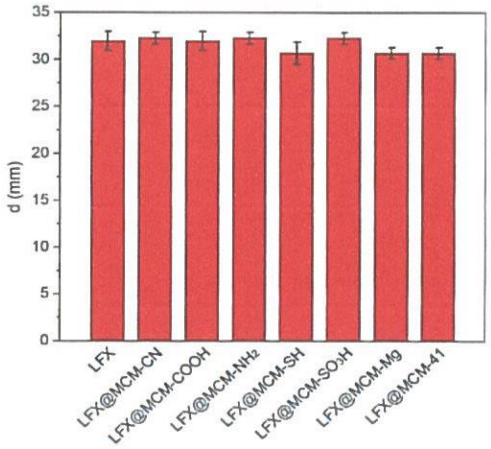


Fig. 21. Diameters of growth inhibition zone of lomefloxacin-loaded carriers in comparison with that of the antibiotic alone

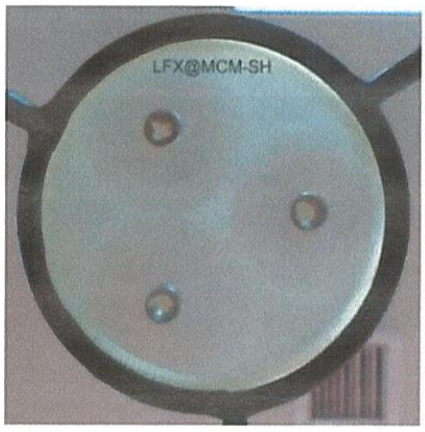


Fig. 22. Antibacterial activity of LFX@MCM-SH composite against *E.coli*

Conclusions regarding silica-lomefloxacin composites

Five functionalized mesoporous silica supports (aminopropyl, propionitrile, propionic acid, mercaptopropyl and propylsulfonic acid), a pristine silica carrier and a magnesium-modified MCM-41 were employed as carriers for the preparation of lomefloxacin delivery systems. All MCM-41-type supports exhibited ordered hexagonal pore array and high porosity.

The antibiotic molecules were loaded into support mesopores as sodium salt by incipient wetness impregnation method having an antibiotic content of 20 % (wt.). The drug molecules were adsorbed into mesopores of functionalized silica in amorphous state, except in the case of MCM-NH_2 material, which contained also crystalline drug phase, as wide-angle XRD proved. The MCM-SO_3H and MCM-NH_2 carriers interacted with lomefloxacin through piperazinyl moieties, while MCM-CN and MCM-SH by carboxylate groups. Only MCM-COOH support could interact with both functional groups of the drug.

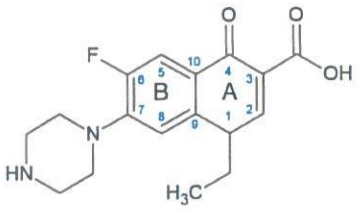
The experimental drug release data were fitted with several mathematical models to better understand the lomefloxacin release kinetics. The acidity of functional organic groups grafted on the inner pore walls of silica surface determined the drug-support interactions and thus, influenced the lomefloxacin release profiles. The transport of antibiotic molecules through

meso-channels of silica-type supports is a Fickian diffusion for carriers functionalized with acidic groups and a more complex process for supports grafted with base moieties. The overall drug delivery rate obtained from the Weibull model decreased with the increase of functional groups pK_a value. It was demonstrated that due to zwitterionic nature of lomefloxacin at physiological *p*H, very base organic moieties should be linked on silica pore walls surface to ensure strong drug-carrier interactions and therefore, slow down the delivery process in both burst and sustained release stages. The same behavior was noticed for the magnesium-modified support, which shows higher basicity due to presence of magnesium oxide on the silica pore walls surface.

All lomefloxacin-loaded functionalized MCM-41 materials show good antibacterial activity against *Escherichia coli* ATCC 8739 strain (Fig. 21 and Fig. 22), their diameters of inhibition growth zone being similar when compared to that of the antibiotic alone.

II.6. Composites based on poorly water-soluble antibiotics and mesoporous silica as carriers

Norfloxacin (NFX) (Scheme 3) is slightly soluble in ethanol and acetone and practically insoluble in water [11,12], thus NFX exhibits a limited bioavailability [13]. Norfloxacin was synthesized at the National Institute for Chemical-Pharmaceutical Research and Development according to the procedure previously reported [14] and it was used in drug release experiments after recrystallization in *N,N*-dimethylformamide.



Scheme 3. Norfloxacin chemical structure

II.6.1. Norfloxacin-SBA-15 composites

The experimental data from this chapter were published in UPB Scientific Bulletin B, article *Solubility enhancement of poorly water-soluble norfloxacin by encapsulation into SBA-15-type silica carriers* [15].

Herein is reported the employment of *pristine and functionalized mesoporous SBA-15 silica matrices for the dissolution rate enhancement of norfloxacin*, a poorly water-soluble antibiotic. It is based on the concept that nanoconfinement of a poorly water-soluble drug in amorphous state into silica mesopores increases the drug solubility in aqueous medium.

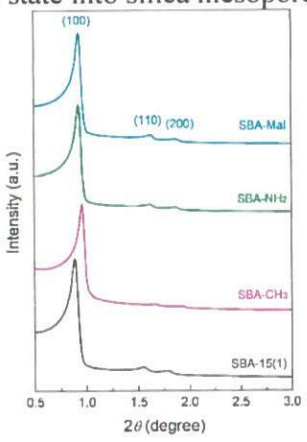


Fig. 23. Small-angle XRD of SBA-15-type carriers

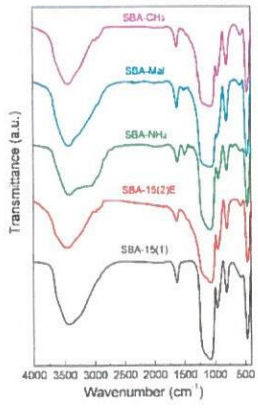


Fig. 24. FTIR spectra of SBA-15-type carriers

All SBA-15 materials exhibited a highly ordered hexagonal pore array evidenced in their small-angle XRD patterns by the presence of the intense (100) diffraction peak and the two smaller (110) and (200) Bragg reflections (Fig. 23). The carriers functionalization was demonstrated by FTIR spectroscopy (Fig. 24), and the textural parameters are listed in Table 2. In Fig. 25 one can observe the SEM images of SBA-15-type carriers.

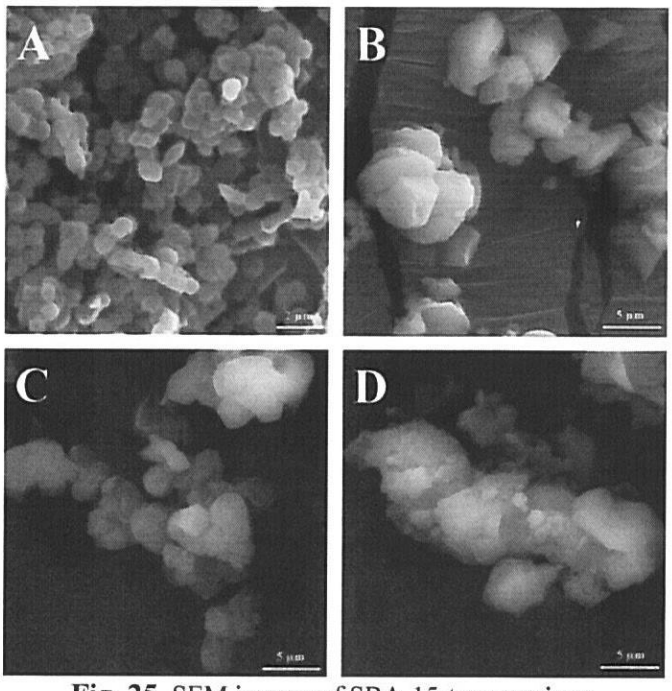


Fig. 25. SEM images of SBA-15-type carriers:
(A) SBA-15(1), (B) SBA-15(2)E, (C) SBA-Mal, (D) SBA-CH_3

Table 2. Structural and textural parameters of carriers

Sample	S_{BET} (m^2/g)	V_p (cm^3/g)	d_{BJH} (nm)
SBA-15(1)	748	1.11	5.82
SBA-15(2)E	506	0.58	3.94
SBA-15(2)	712	0.81	3.94
SBA-Mal	595	0.94	5.42
SBA-CH_3	631	0.96	4.74

A higher drug content of norfloxacin-loaded pristine SBA-15 (30% wt.) determined a partial crystallization of the antibiotic evidenced in the wide-angle XRD pattern (Fig. 26) Also, the presence of the functional groups bonded on silica pore walls surface determined the partial crystallization of the antibiotic. NFX characteristic vibration bands [16] were observed in all FTIR spectra of the drug-loaded composites (Fig. 27).

The drug release experiments (Fig. 28) were performed in intestinal simulated fluid pH 7.4. The results revealed that all NFX-loaded carriers showed an increased solubility exhibiting a type I release profile as classified by Ye *et al.* [17]. When pristine carriers were employed, 90% of the loaded drug content was solubilized in the first 15-20 min. For SBA-15 silica functionalized with amide groups support (obtained from the chemical reaction between silica modified with aminopropyl moieties and malonic acid), SBA-Mal, almost instant

solubilization of the antibiotic was observed, where in 3 minutes 95% of the total drug content was released.

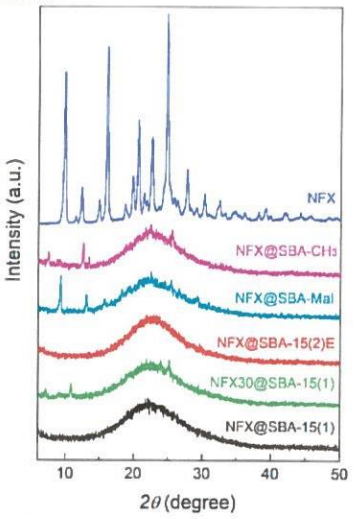


Fig. 26. Wide-angle XRD patterns of norfloxacin composites

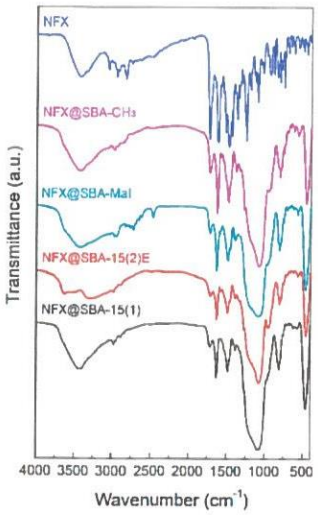


Fig. 27. FTIR spectra of norfloxacin composites

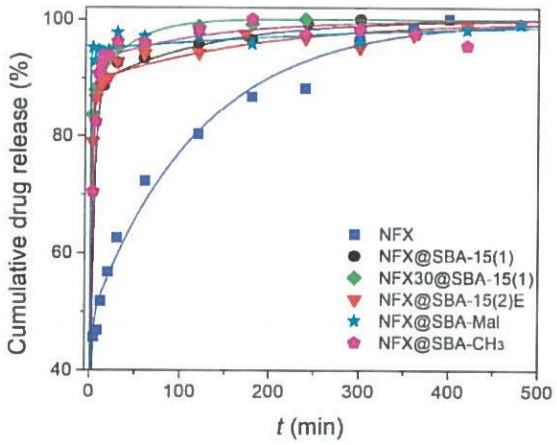


Fig. 28. Drug release profiles of NFX from SBA-15-type carriers

II.6.2. Norfloxacin-MCM composites

In the previous chapter the increase of dissolution rate of norfloxacin adsorbed onto pristine and functionalized SBA-15 silica supports was demonstrated, however the antibiotic was partially crystalline in these composites. Therefore, this work was focused further on the total amorphization of the antibiotic by decreasing the carrier pore diameter, along with the reduction of the particles size that could improve the cellular uptake. Five mesoporous silica supports were used as carriers for norfloxacin: a MCM-41 support with cylindrical pores, two types of pristine spherical nanoparticles, along with vinyl-functionalized ones, and a colloidal MCM-48 support. The results regarding the norfloxacin solubility enhancement based on MCM-type composites are presented in *Norfloxacin delivery systems based on MCM-type silica carriers designed for the treatment of severe infections* in *Materials Chemistry and Physics* [18].

FTIR analysis was used to check the presence of organic groups on the silica materials and the surfactants removal (Fig. 29). In agreement with small-angle XRD patterns (Fig. 30), TEM images of MCM-41 material (Fig. 30a) prove a highly ordered pore framework consisting of long parallel channels, while the materials prepared in the presence of PEG (MSN-E and

MCM-vinyl), presented small, very well-defined spherical nanoparticles with uniform-sized pores forming short channels (Fig. 30 b and c).

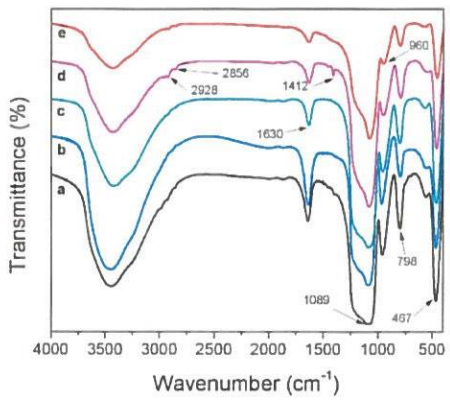


Fig. 29. FTIR spectra of mesoporous silica carriers: (a)MCM-41; (b) MSN-E; (c) MSN, (d) MSN-vinyl; (e) MCM-48

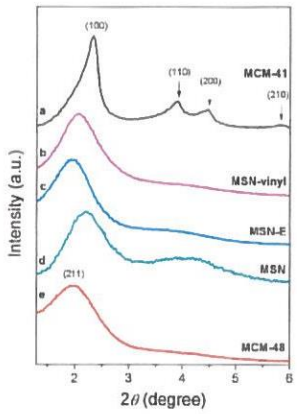


Fig. 30. Small-angle XRD patterns of mesoporous carriers: (a)MCM-41; (b) MSN-vinyl; (c) MSN-E, (d) MSN; (e) MCM-48

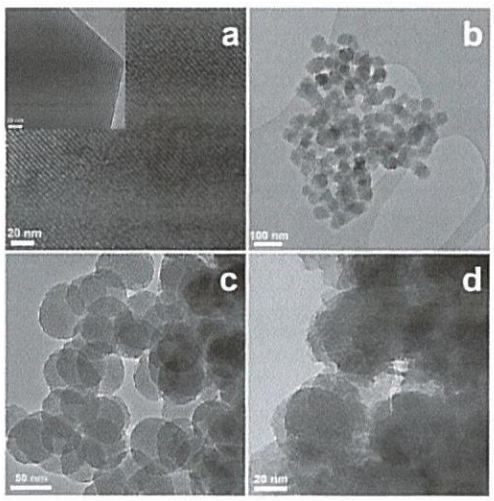


Fig. 31. TEM images of mesoporous silica carriers: (a)MCM-41; (b) MSN-E (58,6 ± 8,1 nm); (c) MSN-vinyl (58,8 ± 8,3 nm); (d) MCM-48 (46,1 ± 2,3 nm)

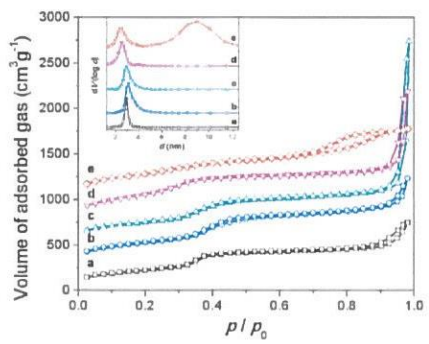


Fig. 32. N_2 adsorption-desorption isotherms and their corresponding pore size distribution curves (inset) of carriers: (a)MCM-41; (b)MSN-E; (c)MSN, (d)MSN-vinyl; (e)MCM-48

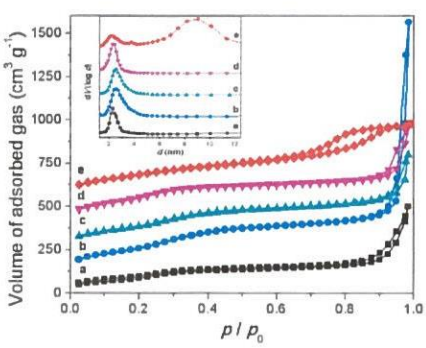


Fig. 33. Nitrogen adsorption-desorption and pore size distribution curves of NFX-loaded materials: (a)NFX@MCM-41; (b) NFX@MSN-E; (c) NFX@MSN, (d) NFX@MSN-vinyl; (e) NFX@MCM-48

MCM-48 sample has a bimodal pore size distribution curve (Fig. 32e - insert), specific for this type of material having interconnected pores forming a cubic pore array with pore sizes of 2.5 nm and 9 nm (Table 3). The presence of norfloxacin into the carrier mesopores was proved by infrared spectroscopy (Fig. 34). The antibiotic-loaded samples were characterized by wide-angle XRD (Fig. 35). All prepared composites containing NFX exhibited only the broad peak specific to an amorphous sample and no NFX diffraction peak associated to the crystalline phase was noticed.

Table 3. Textural and structural parameters of mesoporous silica carriers and NFX-loaded materials

Sample	S_{BET} m^2/g	$V_{p\ d<10nm}$ cm^3/g	d_{BJH} nm	a_0 nm	wt nm
MCM-48	1115	1.11	2.50 and 9.0	10.53	0.93
MSN-E	992	0.97	3.14	5.24	2.10
MSN	917	0.75	2.97	4.94	1.97
MSN-vinyl	1222	0.83	2.53	5.02	2.49
MCM-41	824	0.71	2.98	4.42	1.44
NFX@MCM-48	483	0.60	2.15 and 9.0	-	-
NFX@MSN-E	583	0.42	2.38	-	-
NFX@MSN	495	0.43	2.53	-	-
NFX@MSN-vinyl	528	0.39	2.25	-	-
NFX@MCM-41	301	0.22	2.25	-	-

S_{BET} – specific surface area; d_{BJH} – pore diameter; V_p – total pore volume; $V_{p\,d<10\,nm}$ – pore volume determined for pores with diameter up to 10 nm; a_0 – unit cell parameter (for MCM-48: $a_0 = \sqrt{6}\,d_{211}$; for MCM-41 silica samples: $a_0 = 2\,d_{100}/\sqrt{3}$); wt – wall thickness (for MCM-48: $wt = \left(1 - \frac{V_p \cdot \rho}{1 + V_p \cdot \rho}\right) \cdot \frac{a_0}{x_0}$; for MCM-41 carriers: $wt = a_0 - d_{BJH}$); ρ – pore walls density (2.2 g/cm^3); x_0 – constant (3.0919)

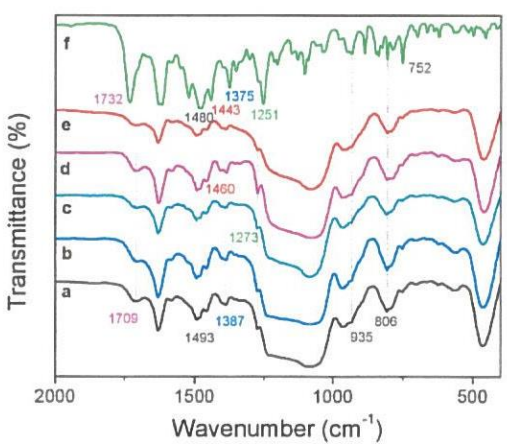


Fig. 34. FTIR spectra of NFX-loaded mesoporous silica carriers: (a)NFX@MCM-41; (b) NFX@MSN-E; (c) NFX@MSN, (d) NFX@MSN-vinyl; (e) NFX@MCM-48; (f) NFX

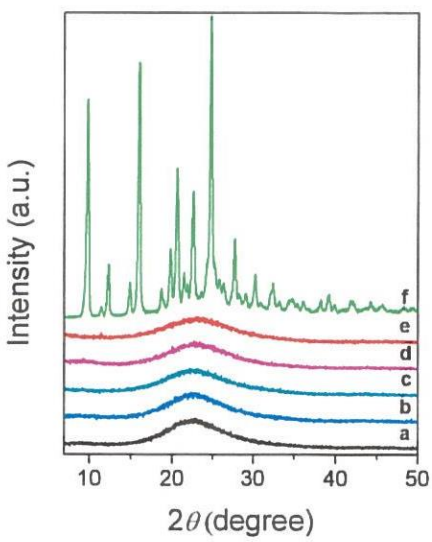


Fig. 35. Wide-angle XRD patterns of NFX-loaded carriers: (a)NFX@MCM-41; (b) NFX@MSN-E; (c) NFX@MSN, (d) NFX@MSN-vinyl; (e) NFX@MCM-48; (f) NFX

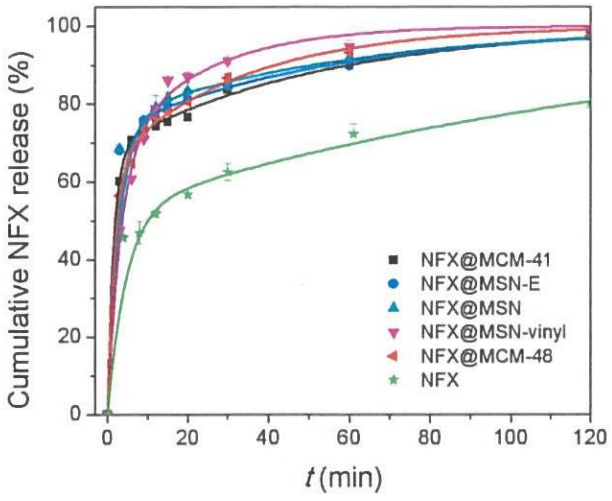


Fig. 36. Norfloxacin release profiles from MCM-type supports

All experimental data showed an enhanced dissolution rate of NFX after adsorption into MCM-type silica mesopores (Fig. 36). After 20 minutes, the cumulative norfloxacin release exceeded 75% (wt) of the total antibiotic amount, in all cases, while the dissolution rate of the crystalline drug, in the same conditions, reached only 57% drug solubilized. The results are also in agreement with experimental data obtained by Huang *et al.* on pristine TiO_2 nanotubes [19] or Fan *et al.* on nanoporous calcium phosphate [20].

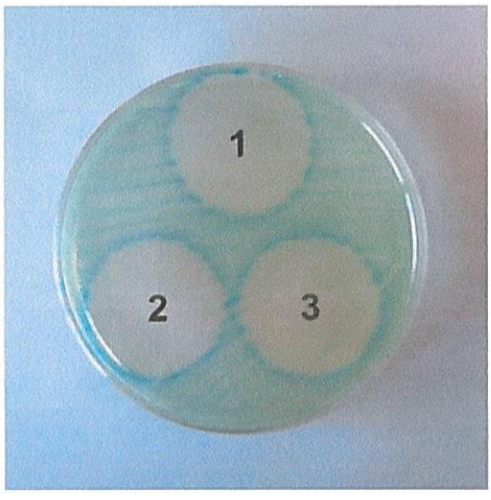


Fig. 37. Diameters of microbial growth inhibition of:
(1) NFX – 33 mm, (2) NFX@MCM-48 – 32 mm, (3) NFX@MSN – 33 mm

II.6.3. Conclusions regarding silica-norfloxacin composites

Mesostructured SBA-15-type silica materials were used for the solubility enhancement of norfloxacin, a poorly water-soluble antibiotic with low bioavailability. Pristine and functionalized SBA-15 materials were prepared and employed as carriers for norfloxacin. The adsorption of norfloxacin mainly in amorphous state into all studied SBA-15-type materials improved its solubility. In the burst stage of the release process, a slightly higher NFX release rate was observed when functionalized SBA-15 silica-type carriers were used, with an almost instant solubilization in the case of amide-functionalized material (SBA-Mal). It was

demonstrated that the release kinetics was not significantly influenced by the high drug content, but in the case of 30 % (wt.) antibiotic content in the composites, a partial crystallization of the drug was noticed.

An original procedure was developed for the preparation of small, spherical pristine and vinyl functionalized silica nanoparticles of about 60 nm with good colloidal stability, which were further applied as carriers for norfloxacin and compared with a highly ordered MCM-41 and MCM-48 materials. The presence of antibiotic molecules inside the carrier mesopores in amorphous state was evidenced through wide-angle XRD analyses and by significantly decrease of carriers porosity, as well as average pore diameters of NFX-loaded samples in comparison with the corresponding support.

The NFX delivery from MCM-type supports demonstrated an improved dissolution rate of poorly-water soluble fluroquinolone when compared with the drug dissolution in the same conditions. The main advantage of the employment of mesoporous silica-type carriers is the improvement of the drug solubility in aqueous environment, which does not require drug-support chemical bonds, since the solubility enhancement is based only on the lattice energy decrease of the amorphous drug. Furthermore, the antibacterial activity assessment indicated a good inhibition activity against *E. coli* ATCC 25922 similar to NFX alone (Fig. 37). Due to the enhanced solubility of NFX, the drug delivery systems based on MCM-type materials could be employed in applications where high initial concentration of drug is required immediately after administration, such as sepsis and septic shock.

II.7. Final conclusions and perspectives

In the doctoral thesis *Composites based on bioactive substances for medical applications* pristine and functionalized colloidal silica nanoparticles were prepared through a new synthesis procedure, and pristine and functionalized mesostructured materials were also synthesized that were employed in the development of antibiotic delivery systems.

Pristine colloidal silica nanoparticles were prepared using an original synthesis method consisting of spherical particles, with diameter below 100 nm. The synthesis was performed in the presence of triethanolamine, which provided the base nature of the reaction mixture necessary for the hydrolysis and condensation reactions of the silica precursor, and also prevented the growth of nanoparticles size. Along with triethanolamine, a low molecular weight polymer, polyethylene glycol, was used to hinder the particles agglomeration. The pore array of MSN was formed around the micelles of surfactant, cetyltrimethylammonium bromide. Besides the reduced size of the nanoparticles, a pseudo-hexagonal pore framework was formed, with specific surface area values and volume of pores below 10 nm ranging from 867 to 1272 m^2/g and from 0.52 to 0.90 cm^3/g, respectively. The functionalized colloidal silica nanoparticles were obtained by co-condensation to ensure a uniform distribution of the functional groups in the pore walls and on the particles surface. Functionalized mesoporous silica with mercaptopropyl, methyl or vinyl moieties of 50-60 nm were obtained.

Considering the large amount of studies regarding the use of mesoporous silica materials in medicine, especially as carriers for various therapeutic agents, the safety use in human treatments is still under debate, as their metabolization / elimination after administration is still not elucidated. Therefore, in this PhD thesis the behavior of pristine and functionalized with different organic groups (aminopropyl, mercaptopropyl, methyl, vinyl and cyanoethyl) mesoporous silica particles was studied in phosphate buffer solutions pH 7.4 and pH 5.7, that simulates healthy tissue cytoplasm and tumor cells environment, respectively. The original contribution of this study was the assessment of the degradation of pristine and functionalized silica nanoparticles over time by small-angles X-ray diffraction that highlighted the pore array transformation at different time intervals (3, 6, 16 and 24 h) depending on the pH of the medium in which the experiments were performed. The results showed that the colloidal silica

nanoparticles had a different degradation mechanism depending on pH: in PBS pH 7.4 one can notice a reorganization of the pore framework with larger interplanar distances, while in PBS pH 5.7, MSN almost completely lost their porosity.

In this PhD thesis antibiotic delivery systems were developed, based on water-soluble antibiotics, doxycycline and lomefloxacin, and a poorly water-soluble antibiotic, norfloxacin.

For the water-soluble antibiotics, the surface of the mesoporous silica carriers was modified either by functionalization with organic moieties, or by ionic change with magnesium ions, both methods allowed the tailoring of the antibiotic-carrier interactions. Functionalized mesoporous silica materials exhibited an ordered pore array, either the functional groups were grafted by co-condensation or post-synthesis approach, and lower textural parameter values than pristine silica carriers.

For the poorly water-soluble antibiotic, norfloxacin, mesoporous silica carriers with hexagonal pore array, pristine and functionalized SBA-15 and MCM-41 supports, and carriers with cubic pore framework, MCM-48-type colloidal silica nanoparticles, were employed. A method for improving aqueous solubility of a drug is the amorphization of the antibiotic by using mesoporous silica-type supports, resulting in a reduction of the lattice energy due to drug crystallization hindering by nanoconfinement. Among the silica supports with hexagonal pore array, MCM-41-type carriers are a better choice, because they allow the accommodation of the antibiotic molecules in amorphous state due to their small pore size, unlike SBA-15 materials, where the antibiotic has a tendency to form crystalline phases.

In this PhD thesis all composites based on antimicrobial agents were prepared by adsorption of the therapeutic agent molecules inside the pores through incipient wetness impregnation method. This method enabled the adsorption of the drugs in amorphous state, evidenced by the wide-angles X-ray diffraction analysis.

The presence of antimicrobial agents in the carriers mesopores was proved by FTIR spectroscopy, where one could notice the characteristic vibration bands of each antibiotic, and also by the decrease of the carriers porosity after the adsorption of the antibiotic molecules into the mesopores.

In vitro release profiles were determined experimentally in simulated biological fluid, PBS pH 7.4, and the experimental data were fitted with different mathematical models, with high correlation coefficients ($R^2 > 0.90$). The release process of the therapeutic agents from the mesoporous silica-type supports was assessed using Higuchi, Weibull and three-parameter models on experimental data. The results described the diffusion mechanism of the antibiotics through the pores, and also the interaction between the drug molecules and functional groups linked on the silica surface.

Both water-soluble antibiotics that belong to different classes, doxycycline from the tetracyclines class and lomefloxacin from the fluoroquinolones class, showed a different release kinetics from MCM-41-type silica materials. The data obtained in the "burst" stage for both antibiotics, based on the Higuchi equation, indicated that the release rate is high for the support functionalized with sulfonic acid groups. The experimental results fitted with the Weibull model indicated a Fickian transport for doxycycline molecules from all supports used in this study, evidenced by the *b* parameter values, while for lomefloxacin, only in the case of some supports a Fickian diffusion was observed, the others showing a more complex transport. Lomefloxacin, which is a zwitterion, determined strong interactions between drug molecules and functional groups linked on silica, which were described using the three-parameter model. The release mechanism of lomefloxacin molecules is determined by the pK_a value of the functional groups grafted on the surface of silica support.

The water solubility of norfloxacin after its adsorption into the pores of the mesoporous carrier was higher than that of the drug alone. In the first 20 minutes, 78% of the total amount of norfloxacin from MCM-48 support was solubilized, more than 80% in the case of MCM-41-type supports and above 90% in the case of SBA-15-type materials. The experimental data of

the norfloxacin delivery profiles were fitted using the three-parameter model. The rate constants for the solubilization of norfloxacin from SBA-15 supports are higher by an order of magnitude than those for MCM-41 supports, due to the larger pore diameter of SBA-15 carriers. MCM-48 colloidal nanoparticles determined a lower diffusion rate due to the interconnected pores.

The adsorption of the antimicrobial agents into the pores of the mesostructured or the colloidal silica supports did not alter the antimicrobial properties of therapeutic agents. The tested doxycycline-based composites, as well as those based on norfloxacin or lomefloxacin, showed an antimicrobial activity similar to that of the pure antibiotic against the tested bacteria, *Klebsiella pneumoniae* and *Escherichia coli*. The obtained results were similar in the case of both disk diffusion method and micro-dilutions method.

The use of the drug delivery systems for the administration of an antibiotic influences their efficiency against bacterial pathogens and allows the possibility to control the release profile or to target a certain tissue. Furthermore, an antibiotic delivery system could reduce the administered dose and may hinder antibacterial resistance.

Main original contributions

The main original contributions presented in this PhD thesis are:

(i) A new original method was established for the preparation of colloidal mesoporous silica nanoparticles, with a diameter below 100 nm that enables their use in drug delivery applications where cellular uptake is critical.

(ii) An *in vitro* degradation study of the colloidal silica nanoparticles was performed in two simulated biological fluids with different pH, one simulating the healthy cells cytoplasm (pH=7.4) and one simulating the tumoral cells medium (pH=5.7). The influence of the pH against the mesophase stability of the pristine and functionalized colloidal silica nanoparticles was assessed through small-angle X-ray diffraction analysis.

(iii) Another element of originality of this PhD thesis was the development of norfloxacin delivery systems for enhancement of the drug solubility for the severe infections treatment. Also, a comparation between mesostructured silica carries *versus* colloidal silica nanoparticles was made.

(iv) Pristine and functionalized mesoporous silica-type carriers were employed for water-soluble antibiotics to develop new drug delivery systems with tailored properties. Two antibiotics were used for this purpose, doxycycline from the tetracyclines class, and lomefloxacin from the fluoroquinolones class, both antibiotics being used in the treatment of urinary tract infections and other infections-types. Therefore, the surface properties of the mesostructured silica carriers were modified, and then they were employed as carriers to tailor the antibiotics release kinetics.

(*v*) Exploring the zwitterionic nature of lomefloxacin, new drug delivery systems were developed based on pristine and functionalized MCM-41-type silica carriers. The release kinetics was controlled by tailoring the interactions between the antibiotic molecules and the carrier. The surface functionalization with groups having strong base nature led to strong interactions between lomefloxacin and carrier in PBS pH 7.4, thus the release kinetics was slowed down either during the burst stage or steady state release stage.

Perspectives

- ✓ Further study of degradation of colloidal functionalized silica nanoparticles prepared by post-synthesis method in order to obtain nanoparticles with similar particle size;
- ✓ Degradation study of colloidal silica nanoparticle functionalized with acidic moieties in simulated biological fluids;
- ✓ Biocompatibility assessment of pristine and functionalized silica nanoparticles on other healthy and tumoral cell lines;

✓ Development of new antibiotic delivery systems with improved antibacterial activity against resistant bacteria strains.

Publications list

1. **M. Deaconu**, A.-M. Brezoiu, R.-A. Mitran, I. Nicu, B. Manolescu, C. Matei, D. Berger, *Exploiting the zwitterionic properties of lomefloxacin to tailor its delivery from functionalized MCM-41 silica*, Microporous and Mesoporous Materials 305, **2020**, 110323. doi: 10.1016/j.micromeso.2020.110323
   IF = 4,551
2. A.-M. Brezoiu, **M. Deaconu**, I. Nicu, E. Vasile, R.-A. Mitran, C. Matei, D. Berger, *Heteroatom modified MCM-41-silica carriers for Lomefloxacin delivery systems*, Microporous and Mesoporous Materials 275, **2019**, 214-222. doi: 10.1016/j.micromeso.2018.08.031
   IF = 4,551
3. **M. Deaconu**, L. Pintilie, E. Vasile, R.A. Mitran, G. Pîrcălăbioru-Grădișteanu, C. Matei, M.C. Chifiriuc, D. Berger, *Norfloxacin delivery systems based on MCM-type silica carriers designed for the treatment of severe infections*, Materials Chemistry and Physics 238, **2019**, 121886. doi: 10.1016/j.matchemphys.2019.121886
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4. **M. Deaconu**, L. Pintilie, R.A. Mitran, C, Matei, D. Berger, *Solubility enhancement of poorly water-soluble norfloxacin by encapsulation into SBA-15-type silica carriers*, UPB Scientific Bulletin B 80(4), **2018**, 3-16. ISSN: 1454-2331
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2. **M. Deaconu**, D. Constantin, C. Goldmann, C. Matei, D. Berger, *Behaviour of small pristine and functionalized mesoporous silica nanoparticles in biological fluids* (oral presentation), Young Researchers' International Conference on Chemistry and Chemical Engineering – YRICCCE 2nd edition, 3rd - 5th of May 2018, Budapest, Hungary; Abstracts volume ISBN 978-963-9970-78-6, pg. 23;
3. **M. Deaconu**, R.A. Mitran, I. Nicu, C. Matei, D. Berger, *Influence of organic groups grafted on MCM-41-silica on the lomefloxacin release kinetics* (oral presentation), Romanian International Conference on Chemistry and Chemical Engineering – RICCCE 20th edition, 6th - 9th of September 2017, Poiana Brașov, Romania;
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