SISTEME CU ELIBERARE CONTROLATĂ- SILICE MEZOPOROASĂ - IRINOTECAN ȘI ULEI DE CIMBRU CU APLICAȚII BIOMEDICALE POTENȚIALE
MESOPOROUS SILICA-BASED DRUG DELIVERY SYSTEMS WITH IRINOTECAN AND THYME OIL WITH POTENTIAL BIOMEDICAL APPLICATIONS

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In order to obtain a drug delivery system used in tumoral treatment, this study presents the characterization of mesoporous silica /antitumor drug hybrid materials obtained in A NOVEL METHOD OF SYNTHESIS FOR NANOPOROUS SILICA MATERIALS [1]. There were synthesized eight hybrid materials, using two types of mesoporous silica as support material and irinotecan respectively thyme oil as active substances. Mesoporous silica was synthetized using two techniques, classical sol-gel and novel microwave-assisted hydrothermal method, resulting different structural characteristics for materials. Loading with irinotecan was realized in aqueous solution at neutral and acidic pH environment using impregnation method with drug solution. Other four materials were synthesized using thyme oil 3% and 5%.

In this study hybrid materials were characterized using different techniques such as X-ray diffraction, FT-IR, Thermal analysis, scanning electron microscopy which offered morpho-structural information. Others analysis performed were kinetics and in vitro cytotoxicity which established the potential use for biomedical applications showing the antitumoral activity.

În vederea obținerii unui sistem cu eliberare controlată folosit în tratamentul antitumoral, această lucrare prezintă un studiu comparativ al unor materiale hibride folosind ca substanțe active, irinotecanul, respectiv uleiul de cimbru și suport silicea mezoporoasă sintetizată în A NOVEL METHOD OF SYNTHESIS FOR NANOPOROUS SILICA MATERIALS[1]. Silicea mezoporoasă s-a sintetizat utilizând două tehnici, clasicul sol-gel și o metodă adaptată, metoda hidrotermală cuplată cu microunde, obținându-se diferite caracteristici structurale. Încărcarea cu irinotecan s-a realizat în mediul apos, la pH neutru și acid, utilizându-se metoda directă a impregnării suportului cu soluția de medicament. Alte patru materiale au fost obținute cu ulei de cimbru 3% și 5%.

În acest studiu s-au comparat caracteristicile structurale și proprietățile materialelor hibride folosind diferite tehnici precum difracția de raze X, FT-IR, analize termice, microscopie electronică de baleiaj, care au oferit informații morfo-structurale, despre încărcarea principiilor active, adsorbția acestora și microstructura. Cinetica, eliberarea principiilor active și potențialul de utilizare al materialelor în aplicații de sisteme cu eliberare controlată au fost evaluate cu ajutorul UV-Vis.

Keywords: *MCM, mesoporous silica, hexagonal mesostructure, drug delivery system, biomaterials, irinotecan, thyme oil, cancer treatment*

1. Introduction

Cancer is one of the most researched diseases in pharmaceutical field, being a frequent disease with complex therapeutic procedures. There are many types of cancer treatment, efficient therapy currently relaying on surgery for solid tumors, chemotherapy and radiation therapy. Drug delivery systems based on mesoporous silica are interesting cytostatic release therapeutic platforms. Mesoporous silica presents two important advantageous characteristics, biocompatibility and the possibility of controlling the morphology and pore size for absorption of high concentrations of drugs or biologically active substances. The unidimensional structure, uniform ordered in a hexagonal honeycomb recommend MCM-41 to be the most used mesoporous SiO_2 based material in medical field [2-8].

The first active substance used for mesoporous materials loading is irinotecan, with chemical formula $C_{33}H_{38}N_4O_6$, international known as CPT-11. Irinotecan, member of the class 1 '-[1,4'- bipyridine] carboxylic acid, is a semisynthetic derivative of natural alkaloid calprotectin (Calprotectin). It is a new cytostatic S-phase-specific agent with the following properties: 10 mg/ mL water solubility, white-yellow color. Irinotecan is a prodrug used for cancer treatment, mostly in terminal phases in combination with 5-fluorouracil and leucovorin. Its properties recommend irinotecan to be easily used as active substance in drug delivery systems with controlled release for cancer treatment. Currently this drug is administrated intravenously in shock doses in different concentrations depending on the regimen. This administration produces disastrous side effects such as myelosuppression, cardiotoxicity,

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alopecia, stomatitis, tissue damage, venous sclerosis [9]. Controlled release is a demonstrated method of reducing drug side effects.

Recognized since antiquity for their medicinal value, but often considered a relic of medieval medical practice by representatives of modern medicine, essential oils have recently received a completely new therapeutic interest. Their widespread use has been growing the interest of scientists in researching essential oils, in recent years their anti-microbial and anti-oxidant activity being investigated, as well as potential anti-cancer activity. There has recently been a significant increase in the use of natural products to replace the current treatment of patients who develop resistance to several drugs and major side effects. Many studies have highlighted the anticancer properties of plants which present fewer side effects, better patient tolerance. The first publications on the anti-cancer activity of essential oils date back to the 1960s. Their unique and complex structures cannot be easily obtained by chemical synthesis. The effects of essential oils have been investigated on glioblastoma, melanoma, leukemia, bone, breast, cervix, colon, kidney, liver, lung, ovary, pancreas, prostate and uterus. Due to their high heterogeneous compositions, it is difficult to define a unique mechanism of action for essential oils [10-15].

Thyme (Thymus L), belongs to the family Lamiaceae, and consists of about 215 to 350 species. The plant is native to the Mediterranean region, but can also be found in Asia, Southern Europe and North Africa. Aromatic plant species such as Thymus are important medicinal plants, which due to the major components – carvacrol an thymol - have a number of important therapeutic properties, used as: antirheumatic, antiseptic, antioxidant, antimicrobial, anti-inflammatory or antitumor [16, 17].

Even if mesoporous silica is widely used in drug delivery systems with irinotecan or essentials oils, the novelty of the study is the complex approach of the obtained systems: MCM-41/ irinotecan and MCM-41/thyme oil. This work is part of a comprehensive comparative study of antitumoral activity of thyme oil versus irinotecan drug delivery systems based on MCM-41. The study started with the development of improved MCM-41 synthesis method of MCM-41 in order to achieve better properties of the material for drug delivery system support use, data presented in *A NOVEL METHOD OF SYNTHESIS FOR NANOPOROUS SILICA MATERIALS [1]*. This work is the next step of the study and its purpose is the synthesis of mesoporous silica – irinotecan and mesoporous silica – thyme oil and characterization in order to be used as drug delivery system in cancer treatment.

2.Experimental

2.1.Materials

There are used for this purpose two types of mesoporous silica, with average particle size dimensions between 87-500 nm and a crystallized structure with symmetrical hexagonal pores with the average size of 4 nm [1]. The obtained MCM-41 support materials were loaded with irinotecan (Sigma-Aldrich) and thyme oil (Sigma-Aldrich, Carvacrol ≥98%) as chemotherapy.

2.2. Preparation of support materials

MCM-41 was synthetized using sol-gel method and microwave-assisted hydrothermal method as presented in *A NOVEL METHOD OF SYNTHESIS FOR NANOPOROUS SILICA MATERIALS* [1]. CTAB was solubilized in a ethanol solution (molar ratio H_2O:EtOH=7.7) followed by the addition of NH_4OH and TEOS with molar ratio CTAB:TEOS=0.43. For P1, reaction mixture was gelled for 12 hours at room temperature and dried at 95 °C after washing with purified water and ethyl alcohol. P2 was obtained using a modified hydrothermal treatment at 100 °C and 20 Bars for 1.5 h [1].

2.3.Preparation of MCM-41 – irinotecan hybrid materials

In order to obtain hybrid material substrate-drug, the direct method of substrate impregnation with a drug solution was used. Irinotecan is soluble in water, so it has a good stability in aqueous environment.

The impregnation procedure involved drying in vacuum 0.1 g from the two samples of mesoporous silica and magnetic stirring with a 6.65 mg/mL irinotecan solution. The maximum absorption time was 24 hours.

Because irinotecan has 2 shapes depending on its solution pH, to search into those influence to the release kinetic and the possibility of interaction with the substrate, there were evaluated at neutral pH and in acid environment, realized with hydrochloric acid [18].

There were synthesized four samples. Samples 1 and 2 were obtained by loading the mesoporous silica, obtained by sol-gel method (sample P1 HCl@irin – for the sample in acid environment and P1 H_2O@irin - for the sample with neutral pH), and the last two samples correspond to the hydrothermal method obtained silica and loaded in acidic and neutral pH (P2 HCl@irin and P2 H_2O@irin).

2.4.Preparation of MCM-41 – Thyme oil hybrid materials

MCM-41 materials were functionalized by impregnation in thyme oil, at room temperature,

Table 1

Materials code/ *Codurile materialelor*

Drug / Mesoporous silica	-	Irinotecan neutral PH	Irinotecan acidic pH	Thyme oil 3%	Thyme oil 5%
MCM - 41 sol-gel	P1	P1 H2O@irin	P1 HCl@irin	P1 3%@To	P1 5%@To
MCM - 41 microwave-assisted hydrothermal method	P2	P2 H2O@irin	P2 HCl@irin	P2 3%@To	P2 5%@To

under magnetic stirring until alcohol evaporation (used as dispersion environment). There were used different concentrations of thyme oil, 3% and 5%, in order to obtain four drug delivery systems based on MCM-41 synthesized by sol- gel method and microwave-assisted hydrothermal method, as presented in Table 1.

2.2. Characterization

The obtained MCM-41 – irinotecan and MCM-41 – thyme oil hybrid materials were characterized using different experimental techniques: X-ray diffraction (XRD), FT-IR and Raman microscopy, thermal analysis (DTA-TG), scanning electron microscopy (SEM) and UV spectrophotometry in order to obtain morpho-structural and kinetic information. Furthermore, for thyme oil-based materials antimicrobial effect and in vitro test were realized.

Diffraction techniques are the most used methods for structural properties powders, fibers and ceramics characterisation. X-ray diffraction shows information on cristalinity degree, phases structure and composition, cristalites dimensions, and for mesoporous materials informations about pores dimensions and order. Obtained powders dried at 95 °C were analyzed using X-ray diffraction at small angles using a PANanalytical Empyrean with CuKα radiation (λ= 1.541874A), equipped with hybrid monochromator. Electron microscopy is one of the traditional characterization techniques that allows direct observation of the morphology and size of micro and nanostructured systems. SEM images used in this paper were performed with a microscope FEI Inspect F50. The BET analysis was obtained on a Micrometrics Gemini V2 model 2380-surface area and pore size analyzer. The adsorption-desorption isotherms were obtained by measuring the amount of N_2 adsorbed across a wide range of relative pressures between 780 and 7.8 mmHg at a constant temperature of 77K and then measuring the gas removed as pressure was reduced. Raman spectra were realized with a LabRAM HR Evolution Horiba instrument, using a 633 nm laser and 50 x lens. The degree of loading and the amount of drug were evaluated by ATD- DSC thermal analysis, using a Shimadzu DTG-TA-50H equipment at 0-900^{o}C. Spectrophotometric analyses were realized in order to record the controlled drug release from the surface of mesoporous silica. It was used a UV-Vis spectrophotometer, the recording was performed using a wavelength of 274 nm, the maximum specific of irinotecan and 255 nm for thyme oil (carvacrol).

3. Results and discussion

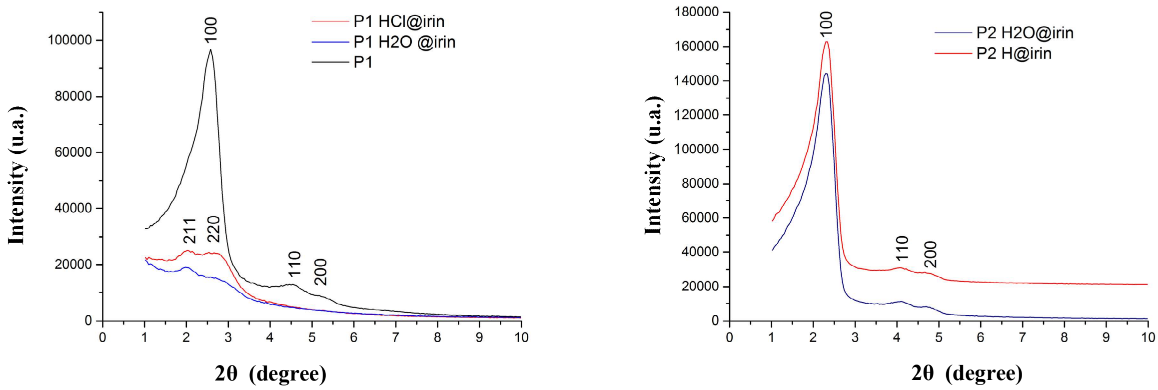
3.1. X ray diffraction

The obtained diffractograms are presented in Fig. 1 for MCM-41- irinotecan and Fig. 2. for MCM- 41 – thyme oil. As it can be observed, once the irinotecan is added in the first substrate, obtained by sol-gel method, P1, the diffractogram radically modifies. The modification on specific plane (100) in Figure 1 a can be explained by covering silica network with drug layer. For the hybrid materials synthesized by hydrothermal method, P2, after loading with irinotecan, there are no significant structural changes on diffractogram (Fig. 1 b), registering the same intensity of diffraction interreferences. However, there is a change for the specific interference plan (100) to smaller angles, specific for orientation given by synthesis procedure.

In the case of silica obtained by the sol-gel method and functionalized with thyme oil, no structural changes are observed, the pores remain arranged in the hexagonal shape characteristic of MCM-41 type silica. However, there is a displacement of the peak – specific plane (100) to smaller angles, which can be explained by filling the pores with oil. The oil deposited on the hydrothermally obtained silica does not influence the support, on the diffractogram presented in Figure 2 observing the overlap of the diffraction maxima of the loading support with the diffraction maxima specific to the simple support.

3.2. FT-IR microscopy

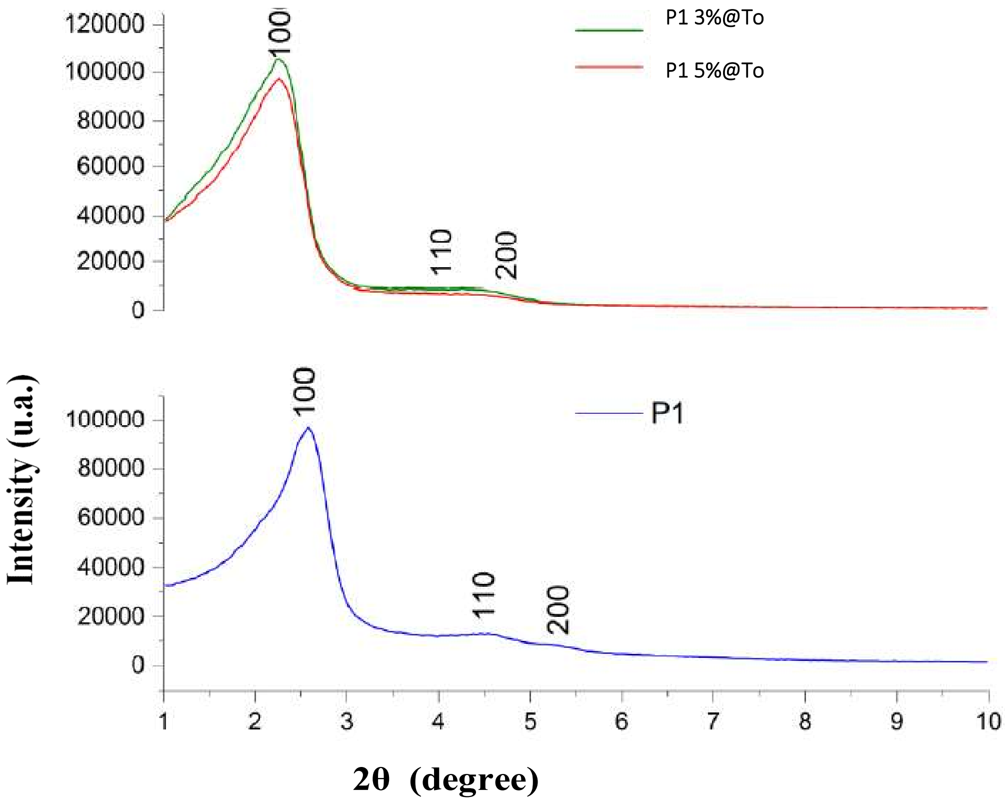
Figure 3 presents the irinotecan - P1 and irinotecan- P2 hybrid materials FT-IR spectra. In addition to the vibration bands characteristic of silica mesoporous supports (the very intense band from 1075 cm^{-1} and the medium intensity band from 805 cm^{-1} are attributed to the symmetrical and asymmetrical stretching vibrations of the O-Si-O groups from MCM 41, as well as the sharp band



a. b.

Fig.1 - a. XRD for P1 -irinotecan hybrid materials (P1, P1 H2O@irin, P1 HCl@irin) / *Difractograma de raze X pentru materialele hibride P1-irinotecan.*

b. XRD for P2 - irinotecan hybrid materials (P2, H2O@irin, P2 HCl@irin) / *Difractograma de raze X pentru materialele hibride P2 -irinotecan.*



a.

Fig. 2. - a. XRD for P1- thyme oil based hybrid materials (P1, P1 3%@To, P1 5%@To) / *Difractograma de raze X pentru materialele hibride cu ulei de cimbru și suport P1.*

from 972 cm^{-1} is attributed to the tensile vibrations of the Si-OH bond, whose intensity decreases depending on the mode of surface modification) can also be observed the appearance of vibration and tension bands of carbonyl units located in the range 1631- 1660 cm^{-1}, characteristic for the active molecule [18]. The decrease of intensity situated at 3424 cm^{-1} corresponding to hydroxyl groups vibration, present in hydrogen bond formation, also show the drugs absorption on mesoporous silica surface.

The spectra shown in Figure 4 highlight in this case, as for irinotecan-loaded samples, the specific bands of mesoporous silica represented by the very intense band from 1075 cm^{-1} and 972 cm^{-1}. Band located at 810 cm^{-1} can highlight the symmetrical and asymmetrical stretching vibrations of the O-Si-O groups from MCM 41 but

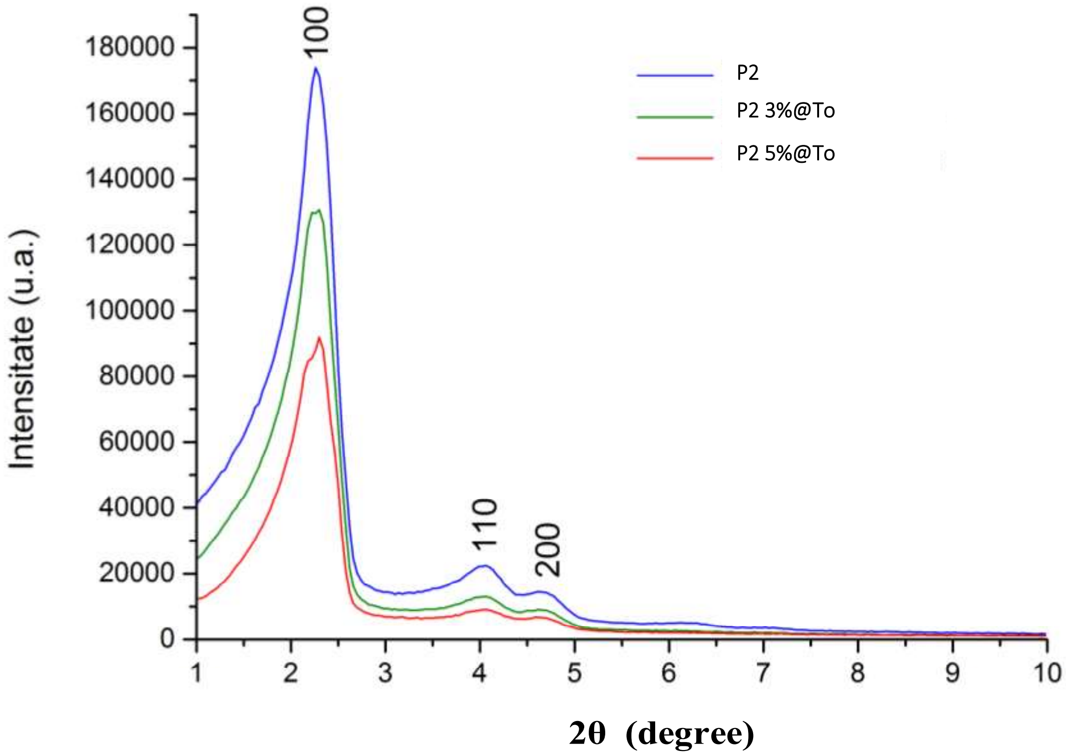


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Fig. 2 - b.XRD for P2- thyme oil based hybrid materials (P2, P2 3%@To, P2 5%@To) / *Difractograma de raze X pentru materialele hibride cu ulei de cimbru și suport P2*

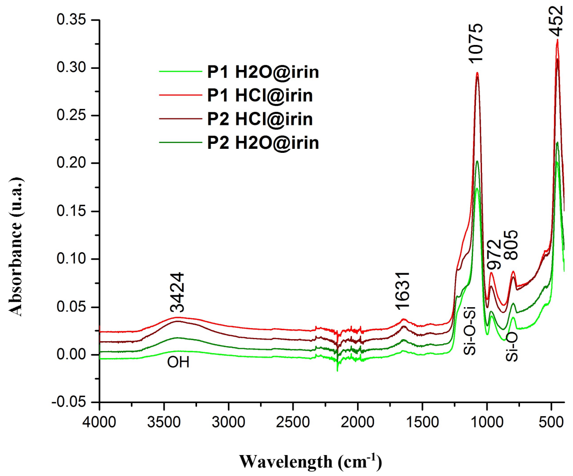


Fig.3 - FT-IR spectra obtained on mesoporous silica- irinotecan hybrid materials/ *FT-IR pentru materialele hibride obținute.*

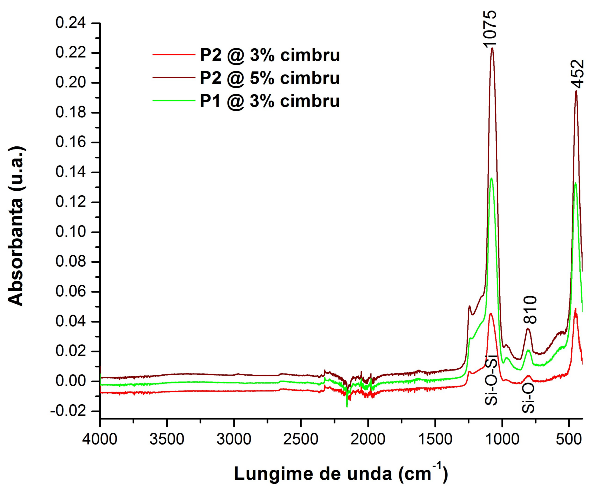
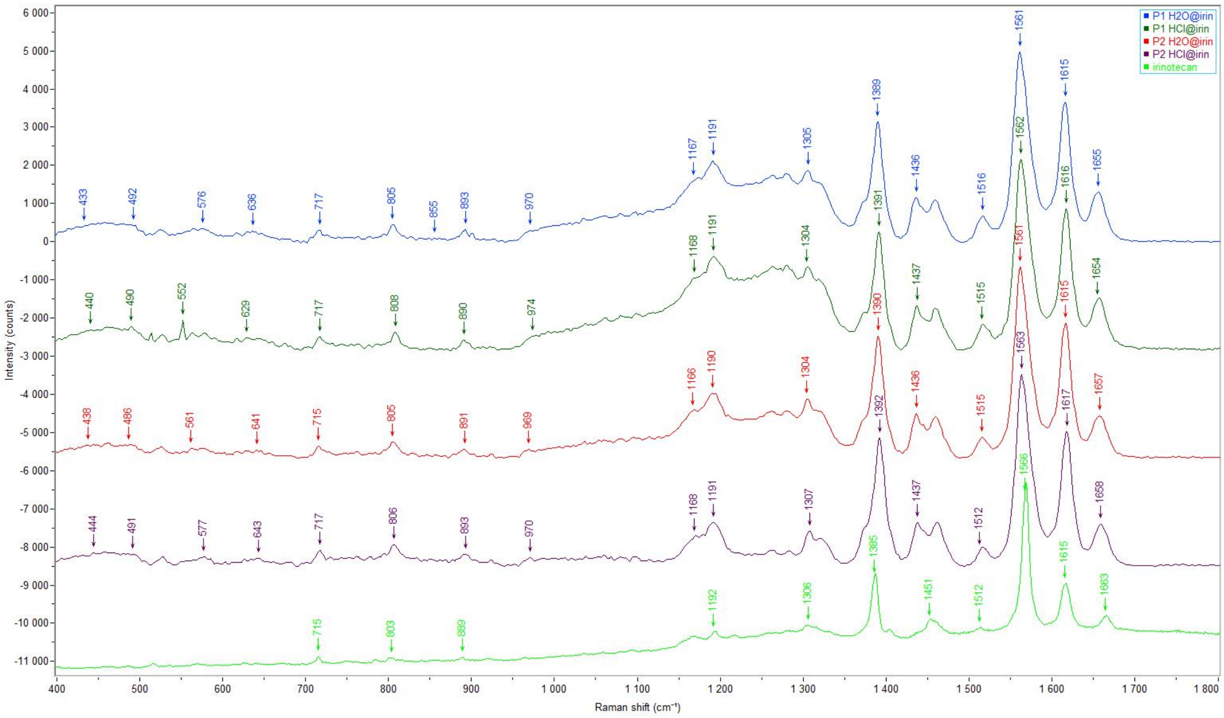


Fig.4 - FT-IR spectra obtained on mesoporous silica- thyme oil hybrid materials/ *FT-IR pentru materialele hibride obținute cu ulei de cimbru.*

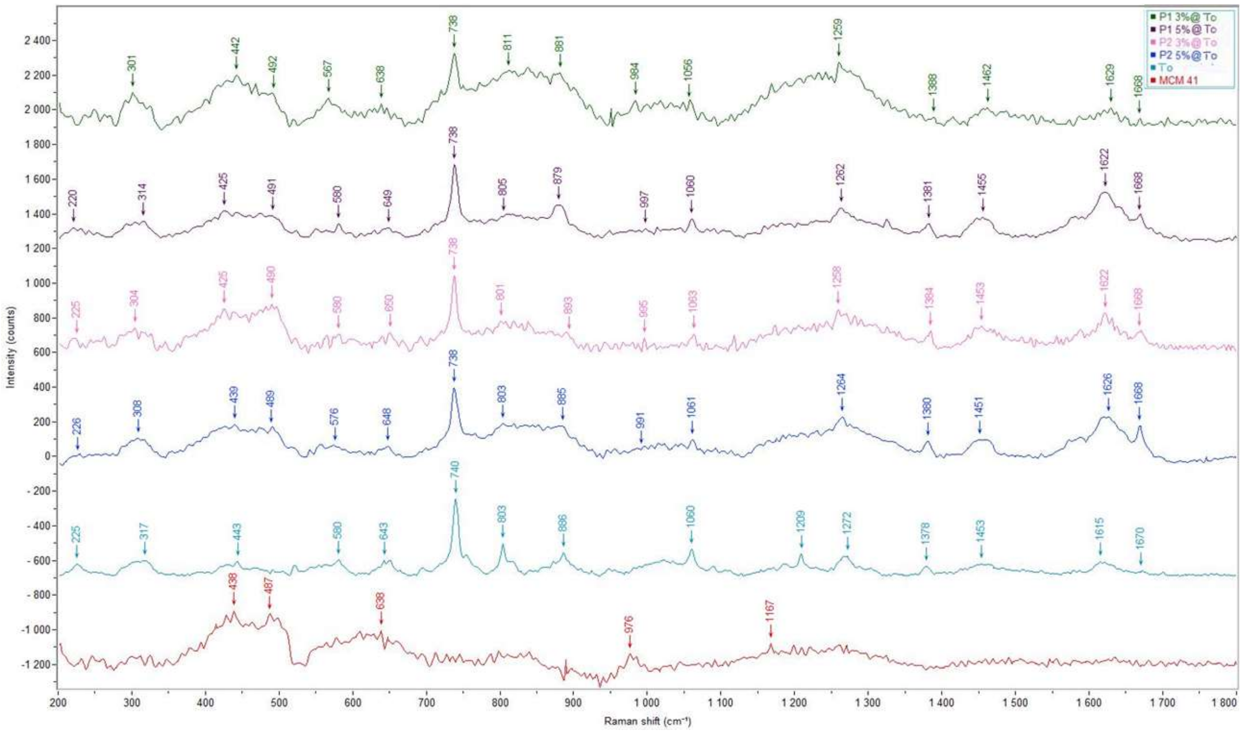
also the specific bands of carvacrol and thymol- the major components of thyme oil, attributed to the vibration of the extra-planar C-H bond, specific to certain types of substitution on the aromatic ring [19,20].

3.3. Raman microscopy

Figure 5 presents the Raman spectra for synthesized hybrid materials. All samples have the mesoporous silica specific bands especially asymmetric Si-O -Si bonds vibration between 438 cm^{-1} and 490 cm^{-1} and stretching vibration of Si-OH bond present in the region 970-990 cm^{-1} and specific interferences for irinotecan and respectively thyme oil. For irinotecan, bands situated at 1566cm^{-1} and 1663 cm^{-1} are assigned to the C=O bonds vibrations, and the stretching vibration of C=C bond appears at 1512cm^{-1}. Simple bond C-C has the vibration at 1615 cm^{-1} and 1385 cm^{-1}, where the C-N bond vibration is also present. The specific bands for C-H stretched bonds in the plan are between 1306-1190 cm^{-1},



a

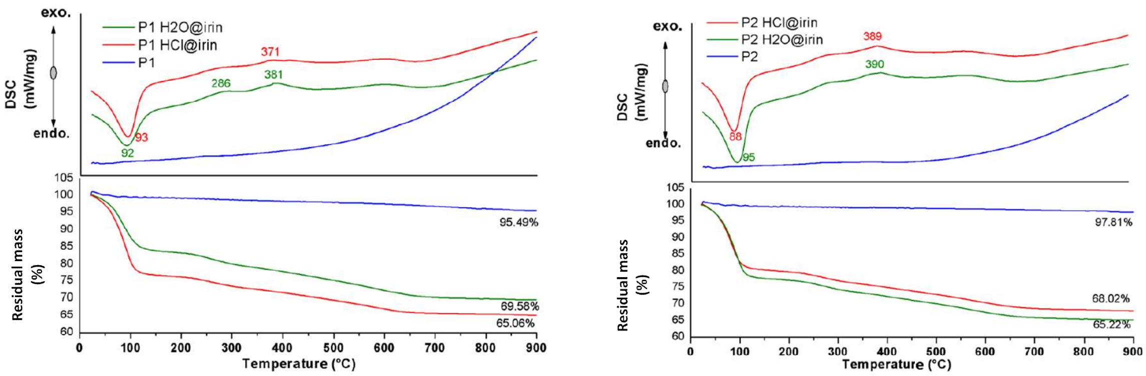


b

Fig.5 - a. Raman spectra obtained for mesoporous silica- irinotecan hybrid materials/ *Spectru Raman silice mezoporoasă- irinotecan*
b. Raman spectra obtained for mesoporous silica- thyme oil hybrid materials/ *Spectru Raman silice mezoporoasă- ulei de cimbru.*

and those outside the benzene ring plane, appear at 889 cm^{-1}. Also, it has been identified the stretching vibration of the C - C - C, which is located 715 - 717 cm^{-1} [20].

The Raman spectrum for thyme essential oil shows the presence of the most important bands at 1615, 1453, 1378, 1208, 1061, 803 and 740 cm^{-1}. The band from 1613 cm^{-1} is attributed to the stretching of the ring in the carvacrol structure, and the band from 1453 cm^{-1} is attributed to the deformation of the CH_3 / CH_2 bonds. At 1378 cm^{-1} appears the deformation band of the bonds from the methylene group bound to the aromatic nucleus. Most of the bands observed in the region



a. b.

Fig.6 - a. DSC analyze of P1- irinotecan hybrid material (silica synthesized by sol gel method), b. DSC analyze of P2- irinotecan hybrid material (silica synthesized by hydrothermal method), /*a. Analize termice pentru materialele hibride P1- irinotecan (silice sintetizată prin metoda sol-gel), b. Analize termice pentru materialele hibride P2- irinotecan (silice sintetizată prin metoda hidrotermală).*

Table 2

Weight loss(Δm) determined by thermal analysis for mesoporous silica loaded with irinotecan
Pierderile de masă (Δm) determinate prin analiză termică pentru silicea mezoporoasă încărcată cu irinotecan

Materials	**Temperature (°C)**			**Δm, %**
	0 - 120	120-700	700-900	0-900
P1 H2O@irn	14.94	14.79	0.67	30.42
P1 HCl@irin	22.49	11.78	0.58	34.94
P2 H2O@irin	21.41	12.56	0.73	34.68
P2 HCl@irin	19.01	12.24	0.72	31.98

1600–1000 cm^{-1} involve the vibration of the C-H bonds in the plane as well as the vibrations of the C = C bonds in the aromatic ring present at 1208 and 1061 cm^{-1}. Also, the bands present at 803 and 740 cm^{-1} are specific to the deformations within the ring present in the carvacrol structure [21].

3.4.DSC thermal analysis

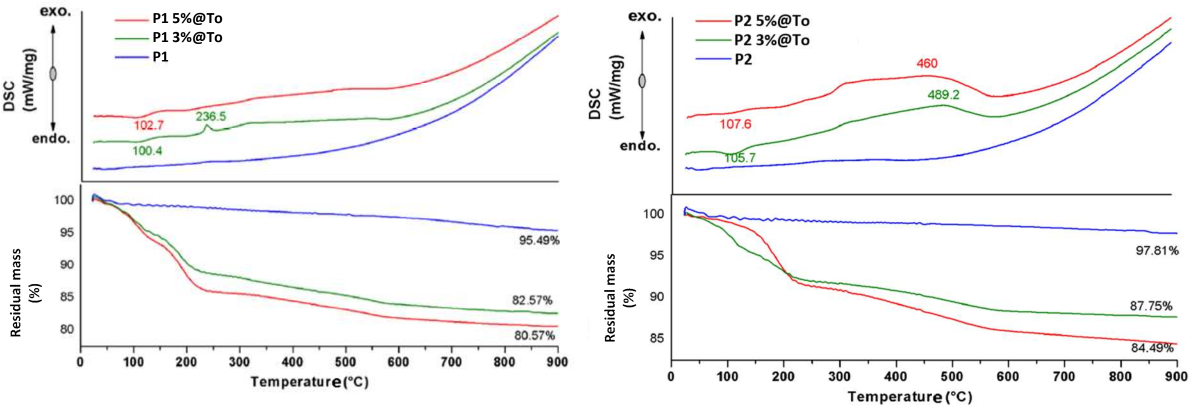
The degree of loading and the amount of irinotecan were evaluated in the results presented in Figure 6. Mesoporous silica- irinotecan hybrid materials total weight loss runs in 3 steps. Between 30-120ºC, total weight loss appears due to the evaporation of adsorbed water (the adsorbed drug, weakly bounded to the surface), for 200-700°C, total weight loss is attributed to drug decomposition and 700-900°C, total weight loss is due to the carbon burn, most likely with a low degree of crystallinity, obtained during drug decomposition.
Silica obtained by sol-gel method shows a better retention of irinotecan on the surface, but is influenced by the environment in which the loading was made. When the loading was carried out in an aqueous medium- neutral pH (P1 weight loss – 4.51%), the residual mass gets to 69.58%, which indicates the retention of a proportion of approximately 26% irinotecan. In case of the acid environment, it leads to retaining a higher percentage of the drug, approximately 30.5 %, corresponding to a 65.06% residual mass.

For silica obtained by microwave- assisted hydrothermal method, P2 (weight loss – 2.19%), the amount of irinotecan is higher than for P1. When the loading was carried out in an aqueous medium at neutral pH, the percentage of drug retained on the support was 32.59 %, but in the acidic environment dropped to 29.8 %.

The exothermic effects presented in DSC curves are attributed to the burning and elimination of the drug, for the silica synthesized by sol-gel and samples obtained by microwave- assisted hydrothermal method. It is observed that loading environment influences the combustion temperature, the acidic environment moving the interferences to the higher exothermic temperature. The drug is totally eliminated at 700°C.

The mesoporous silica supports, regardless of their method of synthesis, retain a significant amount of thyme oil. In the case of the mesoporous silica support P1 (weigh loss of 4.51%), the amount of oil it retains is dependent on the oil concentration. Thus, the absorption is 13% when the oil concentration at impregnation was lower (3%) and 15% when the oil concentration was higher (5%).

The total mass loss of thyme-drug hybrid materials occurs in three stages. In the temperature range 30-220ºC, the weight loss is due to the evaporation of the adsorbed water, in the temperature range 220-600ºC, the weight loss is due to the decomposition of the drug, and in the temperature range 600-900ºC, is attributed to combustion carbon, most likely with a low degree of crystallinity, resulting from the decomposition of



a. b.

Fig.7 - a. DSC analyze of mesoporous silica- thyme oil hybrid material (silica synthesized by sol gel method), b. DSC analyze of mesoporous silica- thyme oil hybrid material (silica synthesized by hydrothermal method), /*a. Analize termice pentru materialele hibride silice mezoporoasă- ulei de cimbru (silice sintetizată prin metoda sol-gel), b. Analize termice pentru materialele hibride silice mezoporoasă- ulei de cimbru (silice sintetizată prin metoda hidrotermală).*

Table 3

Mass loss(Δm) determined by thermal analysis for mesoporous silica functionalized with thyme oil
Pierderile de masă (Δm) determinate prin analiză termică pentru silicea mezoporoasă funcționalizată cu ulei de cimbru

Materials	Temperature (°C)			Δm, %
	0 - 220	220-600	600-900	0-900
P1 3%@To	10,68	5,28	1,48	17,43
P1 5%@To	13,15	4,93	1,30	19,43
P2 3%@To	7,74	4,05	0,71	12,25
P2 5%@To	7,78	6,11	1,54	15,51

organic components existing in thyme essential oil.

3.5. Scanning electron microscopy

The presence of irinotecan does not affect significantly, the particles morphology, its presence is observed in form of small interleaved platelets (Fig. 8 d.) as well as uniform coating of the particle. Thyme oil does not alter the morphology of the mesoporous silica samples presented in Figure 9. For the samples obtained by the sol-gel method (Fig. 9 a), b)), it can be observed that it covers the surface of the particles, a fact less visible in the case of silica samples made first with the microthermal method coupled with microwaves.

3.6.UV spectrophotometry

As it can be seen in Figure 10, controlled release of the drug with a different kinetic, depending of the impregnation medium pH, synthesis method of mesoporous silica support is obtained. Because P1 sample presents better retention of irinotecan, (as it can be observed in DSC analysis), the kinetic release is slower than the silica obtained by hydrothermal method. Neutral medium used in impregnation determines a larger amount of drug.

P1@To have a slower release kinetics than samples synthesized by the microwave-coupled hydrothermal method and the concentration of the oil with which they were loaded significantly influences this kinetics - the concentration of 5 % oil causes a much slower release compared to 3% which causes a faster release in the first 20 minutes (Figure 11).

3.7. Antimicrobial effect for MCM-41 thyme oil

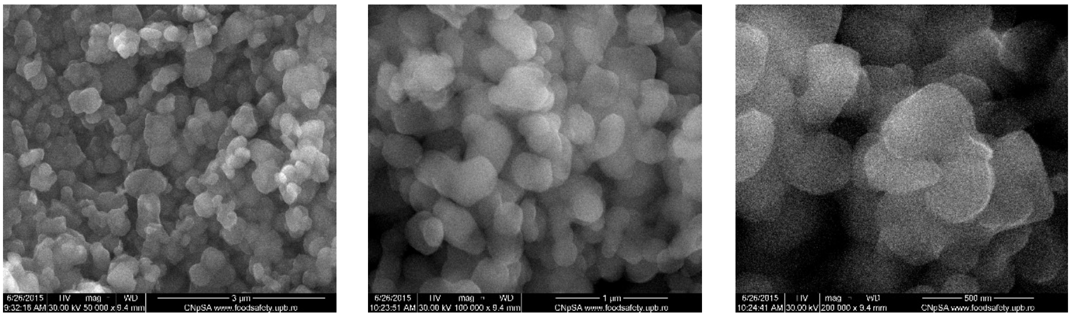
The antimicrobial effect was determined by two methods, establishing the minimum inhibitory concentration and biofilm - soluble / dispersible material - quantitative method.

3.7.1. Method 1 - minimum inhibitory concentration

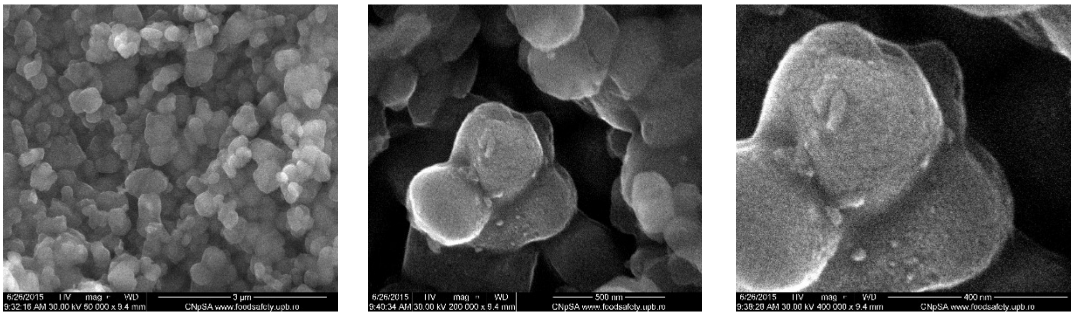
Using the minimum inhibitory concentrations method, it was observed that the nanomaterials tested have different values, depending on the type of microorganism, the nature of the material and the method of obtaining it. Mesoporous silica loaded with thyme oil had different antimicrobial effects, depending on the method of production, but also on the amount of thyme incorporated. Thus, for E. coli, the use of a 5% thyme concentration gave SiO_2 nanoparticles more effective antimicrobial effects, but the synthesis method did not influence the antimicrobial effect.

3.7.2. Method 2 - Biofilm - soluble / dispersible material - quantitative method

For obtained materials the effects of antibiofilm were also dependent on the type of microorganism, but the synthesis method did not significantly influence the results. It is observed



a)



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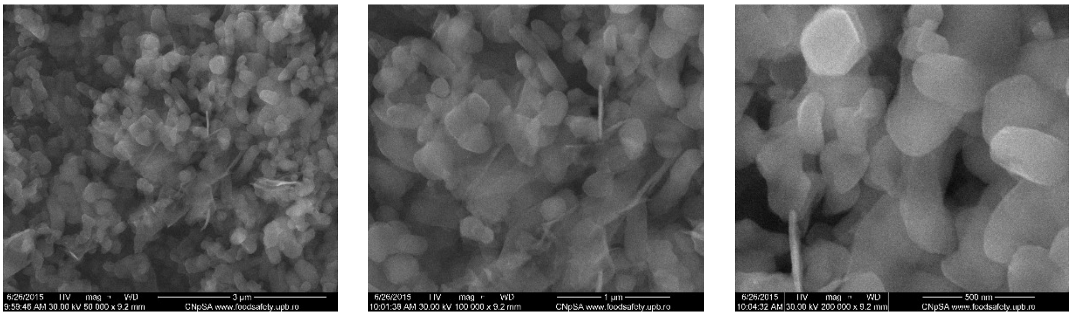

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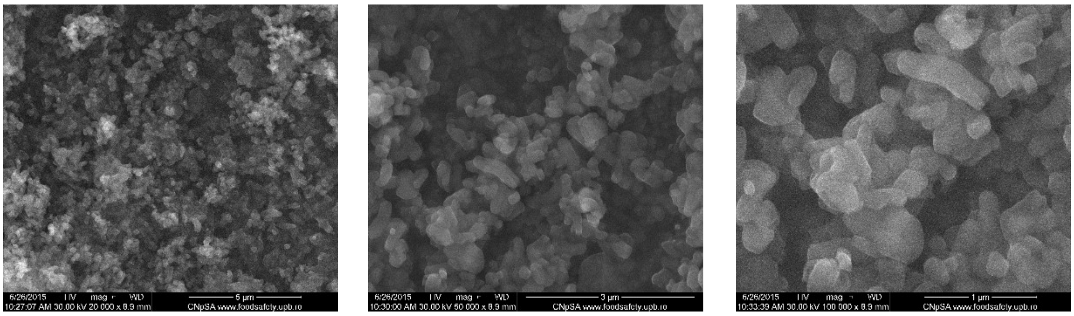


d)

Fig.8 - Scanning electron microscopy for mesoporous silica loaded with irinotecan
a)P1H2O@irin, b) P1 HCl@irin, c) P2 H_2O@irin, d)P2 HCl@irin
Microscopie electronică de baleiaj pentru silicea mezoporoasă încărcată cu irinotecan
a)P1H2O@irin, b) P1 HCl@irin, c) P2 H_2O@irin, d)P2 HCl@.

that, using concentrations higher than 1250 µg / mL all tested samples significantly inhibit the production of biofilms, the effects being more visible for the species S. aureus.

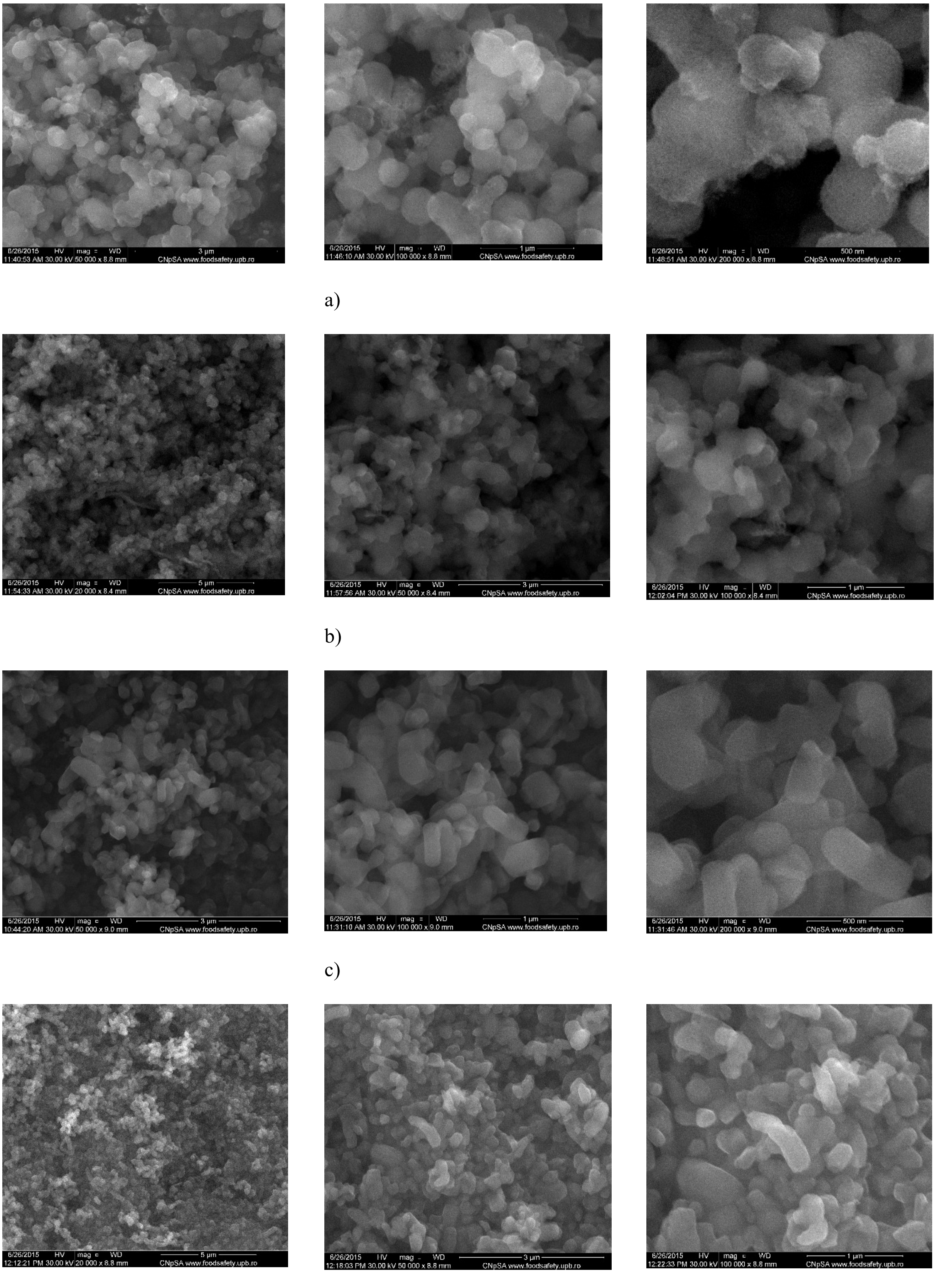


Fig.9 - Scanning electron microscopy for mesoporous silica loaded with thyme oil
a)P1 3%@To, b)P1 5%@To, c)P2 3%@To, d)P2 5%@To
Microscopie electronică de baleiaj pentru silicea mezoporoasă încărcată cu ulei de cimbru
a)P1 3%@To, b)P1 5%@To, c)P2 3%@To, d)P2 5%@To.

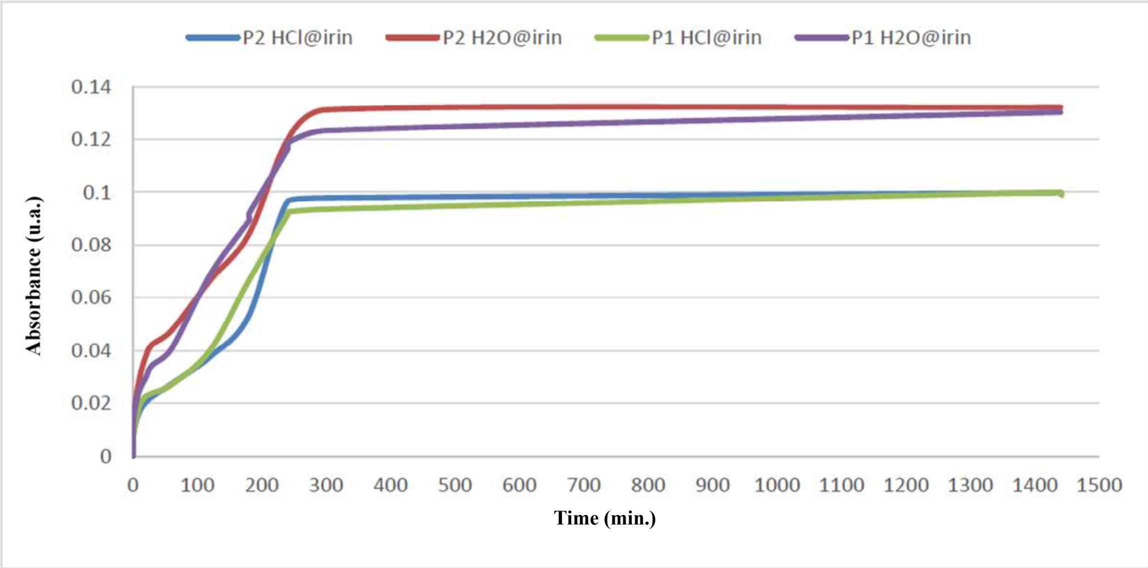


Fig.10 - In Vitro Controlled release of irinotecan from mesoporous silica support/ *Diagrama de eliberare controlată a irinotecanului din materialul suport, silice mezoporoasă (P1- obținut prin metoda sol-gel, P2 – obținut prin metoda hidrotermală cuplată cu microunde).*

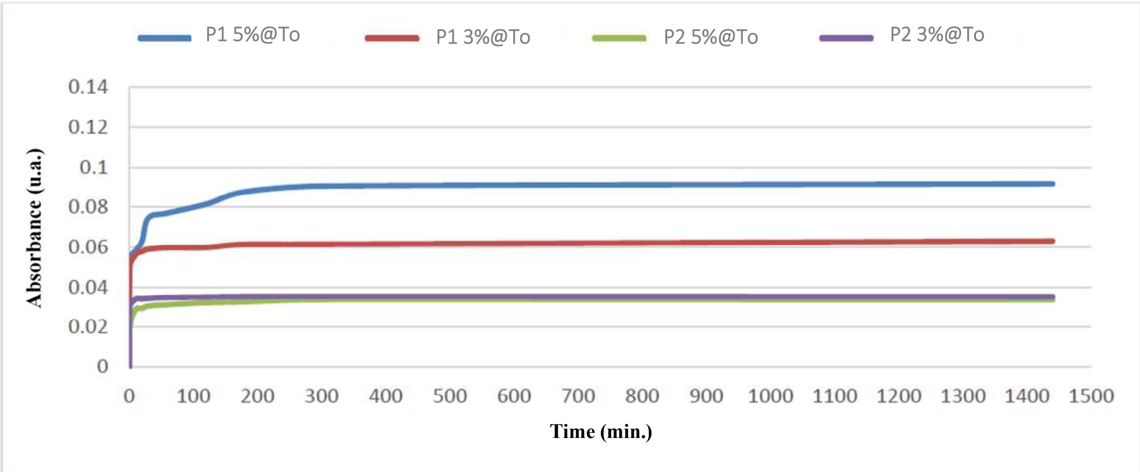


Fig.11 - In Vitro Controlled release of thyme oil(carvacrol) from mesoporous silica support/ *Diagrama de eliberare controlată a uleiului de cimbru (carvacrol) din materialul suport, silice mezoporoasă (P1- obținut prin metoda sol-gel, P2 – obținut prin metoda hidrotermală cuplată cu microunde).*

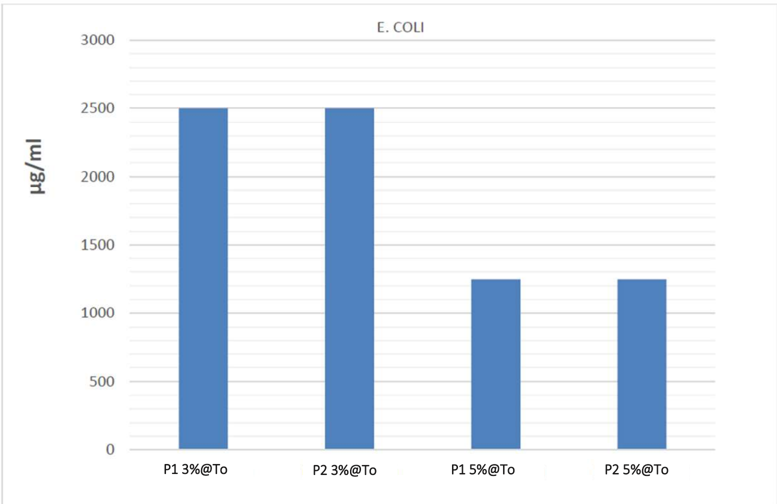
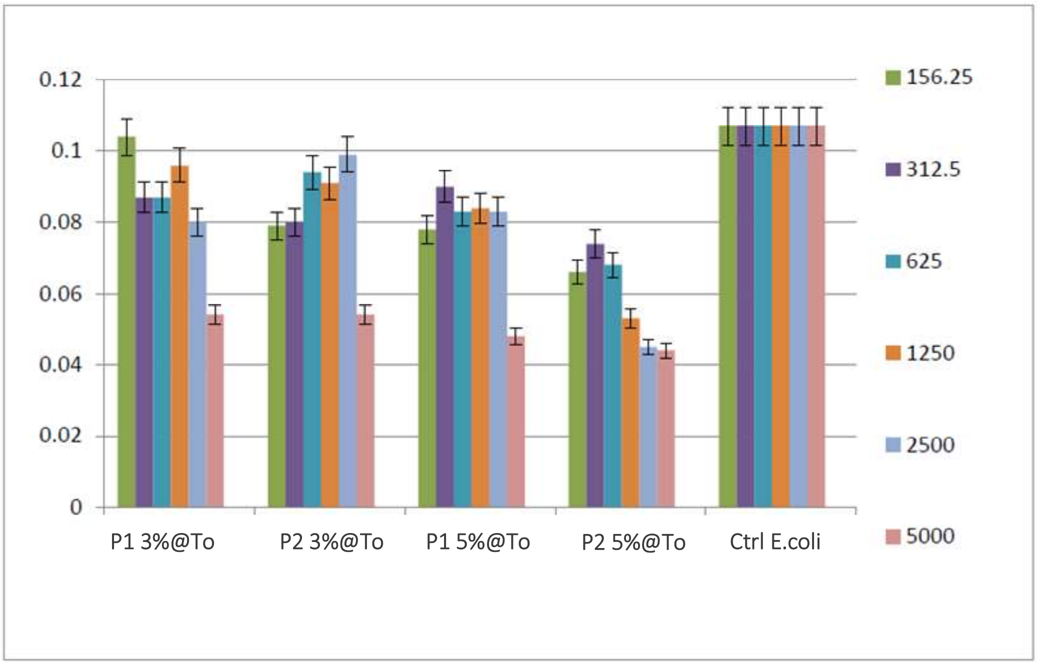
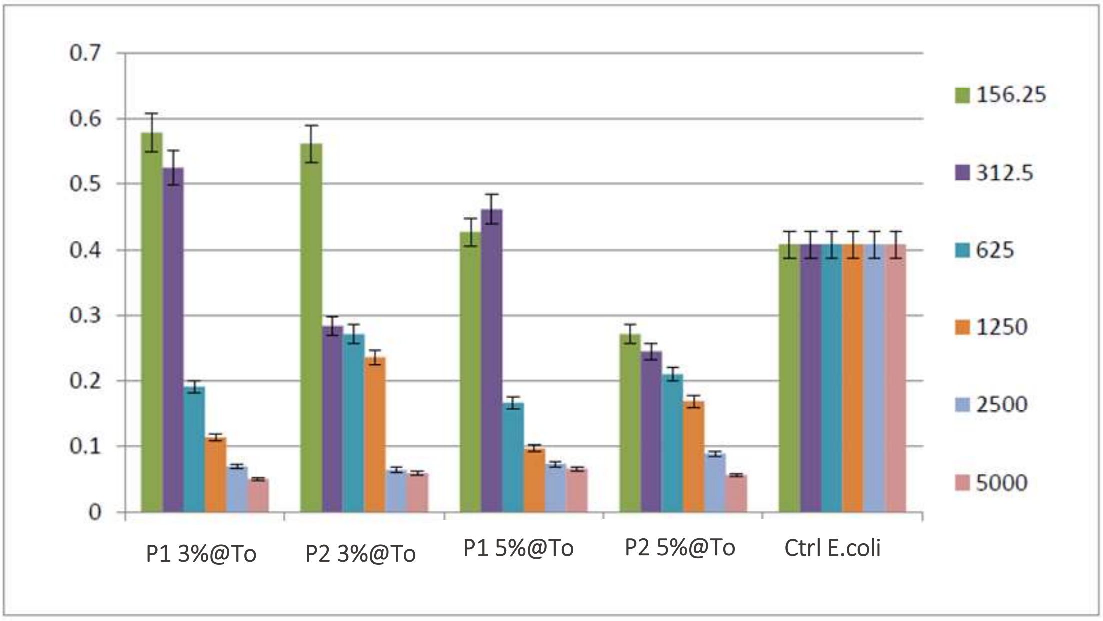


Fig.12 - Graphical representation of MIC values obtained from E. coli culture in the presence of tested nanomaterials / *Reprezentare grafică a valorilor CMI obținute în urma cultivării E. coli în prezența nanomaterialelor testate.*



a.



b.

Fig.13 - a. Graphical representation of absorbance values obtained from spectrophotometric analysis of E. coli biofilms / *a.Reprezentare grafică a valorilor absorbantelor obținute în urma analizei spectrofotometrice a biofilmelor de E. coli*
b. Graphical representation of absorbance values obtained from spectrophotometric analysis of S. aureus biofilms /*b.Reprezentare grafică a valorilor absorbantelor obținute în urma analizei spectrofotometrice a biofilmelor de S. aureus*

3.8. In vitro cellular test for MCM-41 thyme oil

ELISA biochemical tests have shown that thyme-loaded mesoporous silica nanomaterials have heterogeneous effects on cytokine secretion, depending on their type and method of synthesis. TNFα (Tumor necrosis factor alpha) is a cytokine involved in systemic inflammation belonging to the group of cytokines stimulated in the acute phase reaction. The primary role of TNFα is in relegating immune cells. Being an endogenous pyrogen causes fever, apoptotic cell death, cachexia, inflammation and inhibition of tumorigenesis, as well as viral replication, altering the production of IL1 and IL6.

The results showed that the production of TNFα is significantly inhibited by materials with a higher content of thyme oil. The most significant inhibition of TNFα secretion is given by silica obtained by the sol-gel method loaded with 3% thyme. (Fig. 14).

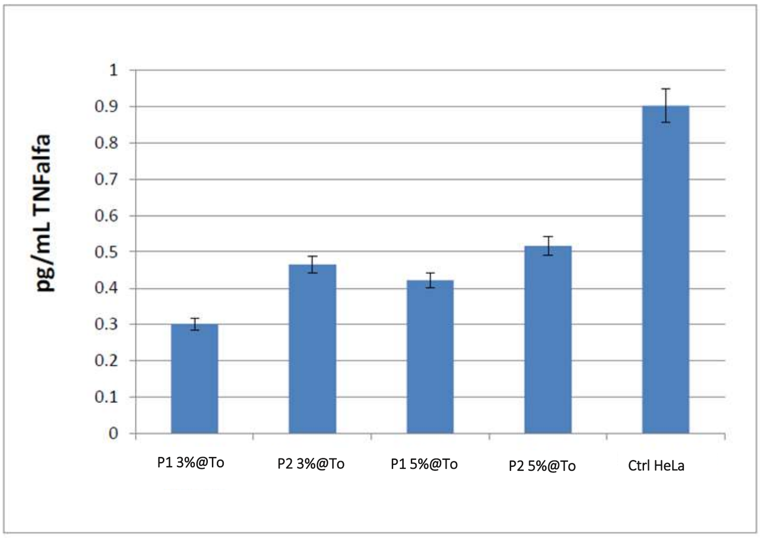


Fig. 14 - Comparative evaluation of TNFα secretion profiles in supernatants of HeLa cell cultures cultured in the presence of tested nanomaterials/ *Evaluarea comparativă a profilurilor de secreție ale TNFα în supernatantele de culturi de celule HeLa cultivate în prezența nanomaterialelor testate*

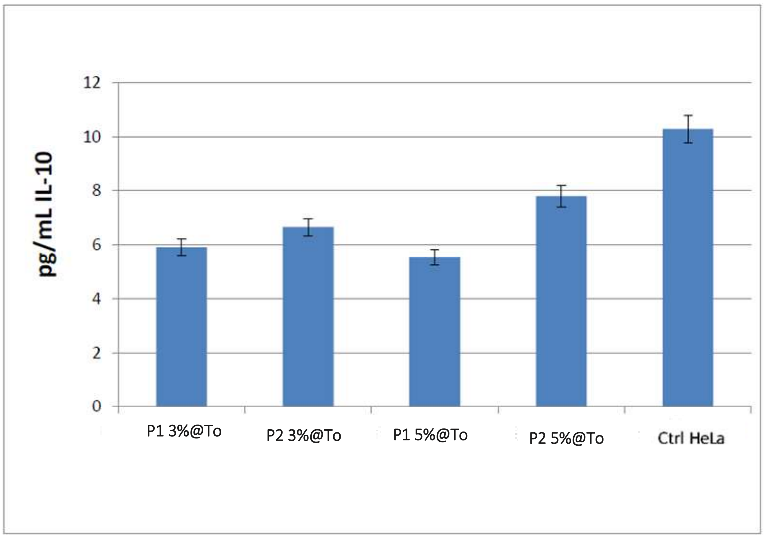


Fig.15 - Comparative evaluation of IL-10 secretion profiles in supernatants of HeLa cell cultures cultured in the presence of tested nanomaterials/ *Evaluarea comparativă a profilurilor de secreție ale IL-10 în supernatantele de culturi de celule HeLa cultivate în prezența nanomaterialelor testate*

IL-10 is an anti-inflammatory cytokine with pleiotropic effects in immunoregulation and inflammation. IL-10 can block NF-κB activity, intervene in apoptosis and play an important role in regulating the JAK-STAT signaling pathway. Biochemical results show that IL-10 secretion is inhibited by all nanomaterials used, to varying degrees. Significant values of inhibition of the production of this cytokine are given by mesoporous silica obtained by the sol-gel method and loaded with a quantity of 5% thyme (Fig. 15).

4.Conclusions

Due to its biocompatibility, morphology and specific surface, mesoporous silica is widely used as support for different drugs in drug delivery systems research [1-4]. Thus, the main focus of the present study is the preparation and characterization of MCM-41/active substances (drug and essential oil) with antitumoral and respectively antimicrobial/antoxidant/antitumoral activity.

Using two types of mesoporous silica, average particle size dimensions 87-500 nm and hexagonal symmetrical pores structure, average size of 4 nm [1] and irinotecan (commercial drug with the role of cytostatic) respectively biologically active substances in the composition of thyme oil (carvacrol and thymol) drug delivery systems with controlled release are obtained. According to XRD, FT-IR, RAMAN and thermal analyses applied to the two types of systems indicate good drug retention on the surface of mesoporous silica. Depending on environmental loading pH and the synthesis method of mesoporous silica support, the retention of active substances is different, the inorganic support prepared by hydrothermal method (P2) hosted higher concentration of irinotecan and lower concentration of thyme oil. Regardless of the amount of drug retained, morphology does not change the properties of the original carrier and the in vitro release of the drug and active substance presents the specific kinetics of controlled drug delivery systems.

All materials containing thyme oil showed important antimicrobial properties tested on E.Coli and S. Aureus species. Biochemical tests performed on HeLa cell cultures show that thyme-loaded mesoporous silica has effects on cytokine secretion, the most significant inhibitory effect, in the case of TNFα secretion, is given by P1 3% To, while for inhibition of cytokine IL-10 is given by P1 5% To.

In conclusion drug delivery systems based on MCM-41 as support and irinotecan/ thyme oil as active substance were obtained. Further analyzes will be performed for a comparative study in vivo efficacy of antitumoral activity regarding both types of active substances in the obtained systems.

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