Sonochemical Development of Magnetic Nanoporous Therapeutic Systems as Carriers for 5-Fluorouracil

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Abstract: Therapeutic nanosystems based on magnetic mesoporous silica nanoparticles are successfully obtained by a facile, reproducible and time-saving sonochemical method. Hydrophilic citrate-capped magnetite nanoparticles of about 20 nm are firstly prepared by ultrasound-assisted chemical precipitation. Secondly, freshly-dried magnetite nanoparticles are coated with mesoporous silica shell by sonochemically-modified Ströber method. The applied procedure provides easily-separable, stable core-shell nanoparticles consisting of superparamagnetic Fe_3O_4 cores and mesoporous silica shell. SEM micrographs showed that core-shell nanoparticles are smaller than 400 nm, a prerequisite for biomedical applications by intravenous administrations. Further, these sonochemically prepared magnetic nanoparticles are employed as biocompatible matrices to host and deliver 5-fluorouracil, a highly-toxic low-molecular antimetabolite chemotherapeutic drug. For this, drug molecules are confined into unmodified and amino-modified mesopores of the silica shell by adsorption from alcoholic solutions. A detailed study was performed using XRD, N_2-sorption measurements, SEM and FTIR spectroscopy with the primary goal of investigating possible structural, textural and morphological modifications aroused after pore-functionalization and drug nanoconfinement. Magnetic behavior of prepared therapeutic nanosystems is visualized using vibrating sample magnetometry (VSM). Finally, the release profile of 5-fluorouracil from the unmodified and amino-modified nanoparticulate magnetic matrices in PBS solution (pH 7.4) is followed by means of liquid chromatographic measurements. The HPLC method used for determination of 5-fluoruracil in releasing media was fully validated in house, in terms of specificity, linearity, precision, LOD and LOQ establishment.

Keywords: Chemotherapeutic agents, drug nanoconfinement, controlled release, 5-fluorouracil, magnetic mesoporous silica, targeted delivery, ultrasonic irradiation.

INTRODUCTION

Nowadays, control of cancer is considered to be a major public health issue. Conventional chemotherapy is commonly used in the clinical management of many forms of cancer but with severe side effects registered due to agent's cytotoxicity or to genetic heterogeneity of the tumors. Thus, the worldwide scientists are making huge efforts in finding new possibilities for early diagnosis and treatment of this life-taking disease. Despite of these intensive research efforts, in the last few decades, cancer remains one of the leading causes of death in the world.

A shining light is brought by nanotechnology which has generated tremendous hopes towards the design of advanced multifunctional nanodevices dedicated to cancer-related biomedicine: assisting in human body imaging, delivering therapeutic cargo in a targeted manner, encapsulation of toxic therapeutic agents to enhance tolerance etc. Recent research trends indicate that controlled targeting at the site of action and reduced time of exposure at non-targeting tissues increases the efficacy of treatments and reduce toxicity and side effects, thus improving patient compliance and convenience [1, 2]. In this respect, nanosized materials such as magnetic iron oxide nanoparticles and mesoporous silica display indeed fascinating mesoscopic properties that, if tuned properly, can be exploited to design new bio-diagnostic and therapeutic strategies as well as innovative biotechnology methodologies [1-4].

Due to their erratic bioavailability, most chemotherapeutic drugs are currently in clinical use as intravenous administrations. Until early 1970's it was considered impossible to administer pharmaceutical suspensions (dispersion of solid particles in a liquid) intravenously, due to the risks of embolism. Today, the development of nanoparticle suspensions which contain medicines has made it possible to increase the therapeutic index of many components (activity enhancing, toxicity reduction) by selectively directing them towards the sick organ. The shift in size from tens of micrometers to hundreds of nanometers has been a significant technological and medical breakthrough [5]. The general rule for nanoparticles in drug delivery for intravenous administration is that the carrier is non-toxic, nonimmunogenic and of sizes that avoids

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embolization of capillary ducts. The larger particles are, the shorter their plasma half-life period is [6]. Another key parameter influencing the use of nanoparticles in biomedical applications is related to their surface chemistry. It is essential to avoid the action of the reticuloendothelial system (RES) which is part of the immune system and increase the half-life in blood stream.

Thus, coating of magnetic nanoparticles with proper inert matrix that offer at the same time protection of the magnetic cores against corrosive environment of body fluids and hosting capacity for large amounts of bioactive molecules is highly desirable, increasing the circulatory half-life from minutes to hours or days [7].

In this work we present the steps followed for sonochemical development of new therapeutic systems as carriers for 5-fluorouracil. 5-Fluorouracil (5-FU) is an effectual chemotherapy option available for the treatment for colorectal cancer, stomach cancer, breast cancer, brain tumor, liver cancer, pancreatic cancers and lung cancer [8-10]. It is a pyrimidine analog that inhibits the biosynthesis of deoxyribonucleotides for DNA replication by inhibiting thymidylate synthase activity, leading to thymidine depletion, incorporation of deoxyuridine triphosphate into DNA and cell death [11, 12]. An additional mechanism of cytotoxicity is the incorporation of uridine triphosphate into RNA, which disrupts RNA synthesis and processing [13]. However, 5-FU has limitations such as short biological half-life due to rapid metabolism, incomplete and non-uniform oral absorption due to metabolism by dihydropyrimidine dehydrogenase [14], toxic side effects on bone marrow and gastrointestinal tract (GI) and non-selective action against healthy cells [15]. For successful cancer treatment, overcoming the toxic side effects on bone marrow or GI track is enormously required, which might possibly be achieved by the control release of the drug by intercalation of into a nanocarrier that helps protecting the healthy tissues and organs, offering at the same time the huge advantages of targeting and “on-site” delivery with minimum lose along its way to the sick organ.

The therapeutic nanosystems developed herein, consist of core-shell nanoparticles in which the core is formed by magnetite nanoparticles coated with a shell of mesoporous silica. This way, due to its superparamagnetic properties, the magnetite core will act as guiding entity, while the mesoporous silica shell plays the role of host for large amounts of 5-fluorouracil. On the other hand we have investigated the adsorption and release of 5-FU on magnetic nanoparticles coated with mesoporous silica shell having different surface properties: silica with its surface consisting on silanol groups (MSN) and silica with amino groups grafted on the mesopore surface (MSN (F)). The development of these new therapeutic nanosystems imply the completion of few very precise steps as follows: (i) the synthesis of magnetite nanoparticles with diameters close to 20 nm; (ii) coating magnetite nanoparticles with mesoporous silica shell, resulting in formation of core-shell nanoparticles with homogeneous particle sizes smaller than 500 nm; (iii) immobilization of drug molecules within unmodified and amino-modified mesopores of the silica shell by adsorption from alcoholic solutions. The novelty of this research study relies on use of ultrasonic irradiation during each step of the development of these therapeutic nanosystems. This way, the time necessary for the formation of mesoporous silica shell was considerably reduced from 24 h (under magnetic stirring) to only 2 h, while only 2 h were sufficient to achieve equilibrium during drug immobilization steps [16].

The research work consisted also of XRD, N_2-sorption measurements, SEM and FTIR spectroscopic analysis after each step of the development was completed, with the primary goal of investigating possible structural, textural and morphological modifications aroused during nanoparticles preparation, pore-functionalization and drug nanoconfinement. The magnetic behavior of prepared therapeutic nanosystems was evaluated using vibrating sample magnetometry (VSM). The amount of drug immobilized on core-shell nanoparticles was assessed by means of UV-spectroscopy. Finally, the resulted core-shell nanoparticles are employed as modified drug delivery systems and the release profile of 5-fluorouracil from the unmodified and amino-modified nanoparticulate magnetic matrices in phosphate buffer solution (PBS) (pH 7.4) is followed by means of liquid chromatographic measurements.

MATERIALS AND METHODS

Materials

Tetraethyl orthosilicate (TEOS, 98%) was received from Merck; 5-Fluorouracil (5-FU, 99%), cetyl-trimethyl-ammonium bromide (CTAB, 99%) and 3-Aminopropyltriethoxi silane (APTES) were purchased from Sigma Aldrich; aqueous ammonia (25% NH_3) and ethanol (EtOH, 99%) were acquired from Chemical Company S.A. (Romania); All chemicals were used as

received without further purification. Deionized water used throughout the experiments was prepared with an ELGA purelab water system.

Preparation of Core-Shell Magnetic Mesoporous Silica Nanoparticles

The synthesis of core-shell magnetic silica NPs was performed by modifying Ströber method *via* hydrolysis of tetraethyl orthosilicate (TEOS) in the presence of magnetite nanoparticles, as described earlier [16]. The chemical composition of the reaction mixture was: TEOS: 0.08 Fe_3O_4: 0.3 CTAB: 95 EtOH: 15 NH_3: 246 H_2O. The synthesis was performed under ultrasonic irradiation for 2 h in a pulsed mode 3 s on / 1 s off. A light-brown abundant precipitate was obtained and washed several times with hot deionized water. The as-prepared core-shell magnetic silica NPs were collected magnetically and dried at 60 °C overnight. Template removal was performed by calcination in two steps: at 120 °C for 3 h, followed by temperature rise to 550 °C with 1 °/min and a plateau for 8 h on this final temperature. Final product, representing core-shell magnetic mesoporous silica nanoparticles, was deposited in tight containers, at room temperature, protected from heat and humidity until further use in characterization and drug immobilization steps.

Throughout this paper this sample is encoded as MSN.

Modification of the Silica Mesopore Surface with Amino Groups

Modification of the mesoporous silica surface with amino functional groups was carried out by post-synthesis procedure, using 1 g of calcined MSNs dispersed in ethanol-diluted APTES (5 mM) under vigorous stirring at room temperature for 8 h. The mixture was then carefully heated to evaporate the solvent.

Throughout this paper this sample is encoded as MSN (F)

Drug Immobilization

The procedure applied for the nanoconfinement of the antineoplastic drug into magnetic mesoporous matrices involved mixing the components at a ratio of 20 mg matrix/10 mL of an alcoholic solution of 5-Fluorouracil, with a concentration of 1 mg/ml. Nanoconfinement was performed sonochemically for 2 h, using an ultrasonic generator SONICS VIBRA Cell™ Model CV 33 (1.13 cm diameter Ti horn) with 750 W power. To avoid rapid increase of temperature in the reaction medium, the generated ultrasound is triggered following a cycled periodic pulse. Duration of the ultrasound was 3 s, and the resting time was 1 s. We have extensively investigated this procedure for immobilization of 5-FU within magnetic silica nanoparticles taking into account some parameters that can influence the uptake procedure. The results are presented in details elsewhere [16]. The drug-loaded nanosystems were collected from solution by magnetic decantation and several centrifugations, while dried at 60 °C overnight. The amount of 5-FU loaded on mesoporous matrix was assessed by means of decrease of 5-FU concentration from the initial solution, using UV-vis spectrophotometry. All solutions were measured at a fixed wavelength of 266 nm. The UV-vis spectra were recorded in the wavelength range of 200 - 800 nm.

Throughout this paper these samples are encoded as: FU-MSN, representing the nanosystem with 5-FU confined within the unmodified silica mesopores, respectively as FU-MSN (F) representing the nanosystem with 5-FU confined within the amino-modified silica mesopores.

Characterization

The XRD measurements were carried out on Panalytical X'Pert Pro MPD diffractometer using CuK_α radiation (λ = 1.54059 Å). The samples were analyzed in the range of 2θ = 1.5 ^{o} to 80 ^{o} with scanning angle rate of 0.02 ^{o} and 1 ^{o}/min count time. *Nitrogen sorption isotherms* were recorded on a Quantachrome Nova 2200 Instrument & Pore Size Surface Area Analyzer at – 196 °C. Before measurements, the magnetic matrices were outgassed under high vacuum at room temperature for 4 h. The BET specific surface area (S_{BET} (m^2/g)) was calculated from the linear part of the BET plot. Micropore area (S_μ (m^2/g)) was determined by t-plot method. The average pore diameter (Dp (nm)) was estimated using the desorption branch of the isotherm and the Barrett–Joyner–Halenda (BJH) method. The volume of liquid nitrogen adsorbed P/P_0 = 0.95 was used to assess the total pore volume (V_p (cm^3/g)). The *FIB/SEM micrographs* were recorded on a Focused Ion Beam-Scanning Electron Microscope Carl Zeiss CrossBeam NEON 40 EsB. *Scanning electron microscopy (SEM)* investigations were performed using a Vega Tescan electron microscope. *Magnetic measurements* were performed on a vibrating sample magnetometer (VSM) Princeton Instruments Micromag™ 3600 at room temperature under an

applied field of 10,000 Oe. Contributions of the holder and silica matrix were subtracted from the recorded magnetic data. *FTIR spectra* were collected on a Bruker Tensor 27 Spectrometer equipped with a DigiTectTM detector, in the spectral range 4000 – 400 cm^{-1}, with a resolution of 2 cm^{-1} and a wavenumber accuracy of 0.01 cm^{-1}. All samples have been measured as KBr pellets using 5 mg of probe sample. *Drug loading* on magnetic mesoporous nanoparticles was assessed on the base of disappearance of 5-FU from solution after being in contact with the host matrix for 2 h under ultrasonic irradiation. The UV-vis spectra of the 5-FU solution before and after adsorption tests were recorded using a Shimadzu, Japan UV – 2401PC spectrophotometer. Spectra were recorded on the entire spectral range of 200 to 800 nm. Collected solutions were measured at a fixed wavelength of 266 nm, typical for analysis of solutions containing 5-fluorouracil.

The concentrations of 5-FU in the release media were assessed by means of HPLC analysis using a high performance liquid chromatography Surveyor Plus system provided by Thermo Fisher Scientific – USA, equipped with Surveyor LC – Pump, 400 bar maximum pressure, a Surveyor Autosampler, sample tray compartment, thermostated column and a UV-VIS, 650 photo-sensible diode array detector. The chromatographic method applied for *in-vitro* release tests was developed and completely validated in terms of system suitability, method linearity, precision and accuracy, establishing limits of detection and limit of quantification. *The detection limit* was found to be 0.0203 mg/ml 5-FU and the *quantification limit* was found to be 0.0677 mg/ml 5-FU.

In Vitro Dissolution Test and Data Analysis

In vitro dissolution tests have been performed using a *SR 8 Plus Series* (AB & L Jasco) device, according to the following experimental protocol: *dissolution medium: 150* mL of PBS solution (pH = 7.4); *Apparatus 2 (paddles); bath temperature* 37 ± 0.5 ºC; *rotation speed*: 50 rpm. The experimental protocol was set as follows: 500 mg nanoparticulated sample, accurately weighted, were introduced in a dialysis membrane containing 10 ml of PBS solution. Then, the dialysis membrane sack containing the sample is immersed in 150 ml of PBS solution with pH = 7.4; the sampling interval was set at 15 min during the first 60 min of the test, and to 1 h for the next 24 h. Aliquots of 1 ml were withdrawn according to sampling schedule and subjected to HPLC analysis in order to determine the amount of 5-FU released; after every sampling, these aliquots were replaced with the same medium volume at 37 ºC. All the dissolution tests were made in triplicate, with the mean values reported in graphics (relative standard deviation, RSD < 5%).

RESULTS AND DISCUSSIONS

In this work core-shell magnetic silica nanoparticles have been prepared by coating magnetite nanoparticles with mesoporous silica by under ultrasonic irradiation. The formation of the silica layer was controlled by careful tune of experimental parameters to control the competition between nucleation and growth of mesoporous silica. The use of ultrasonic irradiation made possible the formation of core-shell nanoparticles with well-defined particle shape in only 2 h due to the remarkable reaction conditions which lead to unique properties. Thus, the developed core-shell magnetic silica nanoparticles showing properties best suited for modified drug delivery were further employed as carriers for 5-fluorouracil anticancer agent. In the following, results obtained on full characterization of the therapeutic host matrix (core-shell magnetic silica nanoparticles) and of the final therapeutic systems carrying 5-FU, as well as *in-vitro* release profiles, are presented and discussed in details.

Morphological Investigation of the Therapeutic Host Matrix

Representative SEM micrograph recorded on magnetic silica nanoparticles, before surface modification and drug immobilization, is presented in Figure **1**, showing that the nanoparticles are spherical in shape, well formed, with sizes mostly in the range of 500 nm. One the other hand, it looks like some of these nanoparticles are glued together, forming particle agglomerates. In Figure **1** right is displayed the FIB-SEM micrograph recorded on a particle agglomerate, proofing core-shell nature of the therapeutic host matrix. It is clearly observed that the core consists of multiple small magnetite nanoparticles (determined as 20 nm by SEM micrograph recorded on uncoated sample (data reported elsewhere)) covered by a thick silica shell.

Magnetic Behavior of the Therapeutic Host Matrix

Magnetic behavior of core-shell nanoparticles was investigated using VSM technique in applied magnetic field at room temperature. The field dependence of isothermal magnetization for MSN sample, shown in

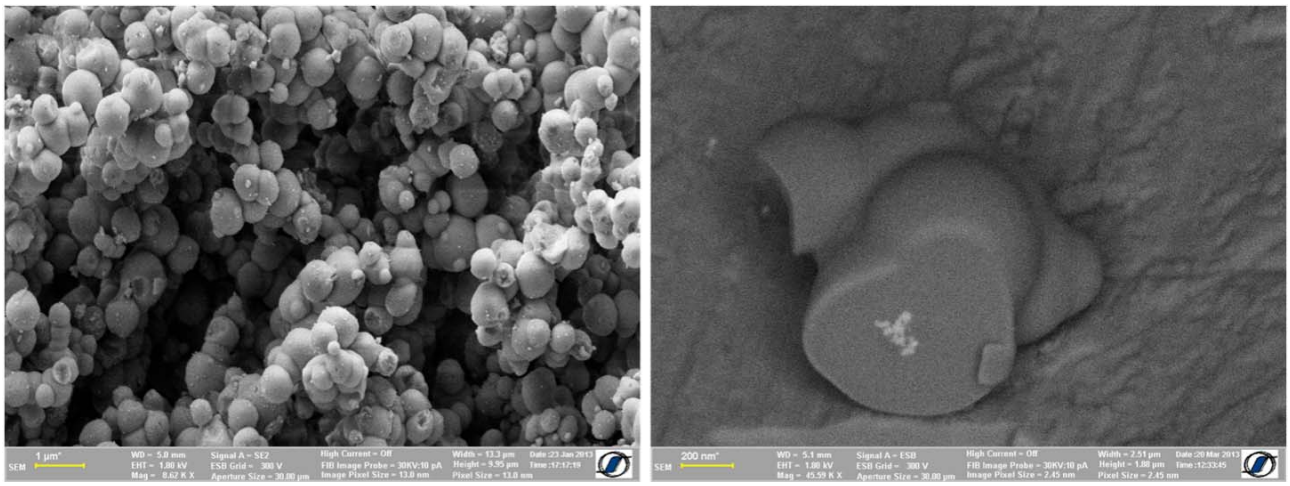

Figure 1: SEM (left) and FIB-SEM (right) micrographs for prepared magnetic silica nanoparticles.

Figure **2**, reveals superparamagnetic behavior. The coercive field (*Hc*) was found to be 15 Oe and saturation magnetization (Ms) 1.5 emu/g for core-shell MSNs, while remanent magnetization (Mr) is 0.1 emu/g suggesting a multidomain superparamagnetic behavior (Mr/Ms < 0.1).

To prove the capacity of final therapeutic nanosystems to respond to magnetic field, two vials were loaded with 50 mg of newly developed therapeutic nanosystem in 3 ml of PBS (100.0 mM, pH 7.4). As illustrated in the inset of Figure **2**, without the presence of magnetic field, the drug-loaded nanoparticles are homogeneously dispersed in PBS solution. After applying extern magnetic field, most MSNs were attracted to one side of the vial, where the magnet is fixed, in 30 minutes. By removing the magnet, the nanoparticles revert to dispersed state.

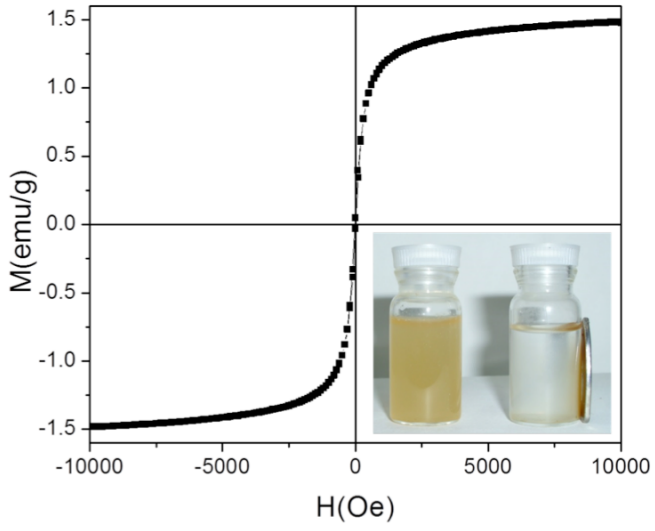

Figure 2: Magnetization curve of developed core-shell MSNs; the inset shows photograph of drug-containing MSNs in PBS solution in the presence or absence of magnetic field.

Structure Investigations Using Powder X-Ray Diffraction

Powder X-ray diffraction technique (XRD) was used for the phase identification and crystallinity assessment within prepared nanosystems. The measurements were carried out in 2θ range from 1.5 to 80°. In the small-angle range (Figure **3** left), the XRD pattern of non-functionalized nanoparticles displays a very intense diffraction peak at 2θ = 2.52° and one broad diffraction peak at 2θ values ranging from 3.75° to 5.75°, suggesting that the silica shell possess MCM-41-type hexagonal arrangement of cylindrical mesopores. Adsorption of 5-FU onto unmodified MSN resulted in slight decrease of the intensity of the main peak at 2θ = 2.52°, while no modification of the broad diffraction peak at 2θ values ranging from 3.75° to 5.75° was observed. This fact demonstrates that 5-FU was indeed localized within the mesopores of silica shell without modifying its structure. As expected, after surface modification, intensity of the main peak at 2.52° decreases more significantly, while the broad peak at 3.75 – 5.75° disappears. This does not necessary mean loss of mesoporous ordering, but rather the filling of the mesopores with APTES molecules. Further, after adsorption of 5-FU within the mesopores of amino-modified MSNs the intensity of the main diffraction peak decreases but it does not completely disappears, showing that the hexagonal arrangement of the mesopores is maintained within silica shell even after supplementary treatments as functionalization and drug immobilization are applied. These results are confirmed by N_2-sorption analysis.

The nature of magnetic iron oxide core was investigated by recording XRD patterns in the 2θ ranges of 15 to 80° (Figure **3** right). The presence of

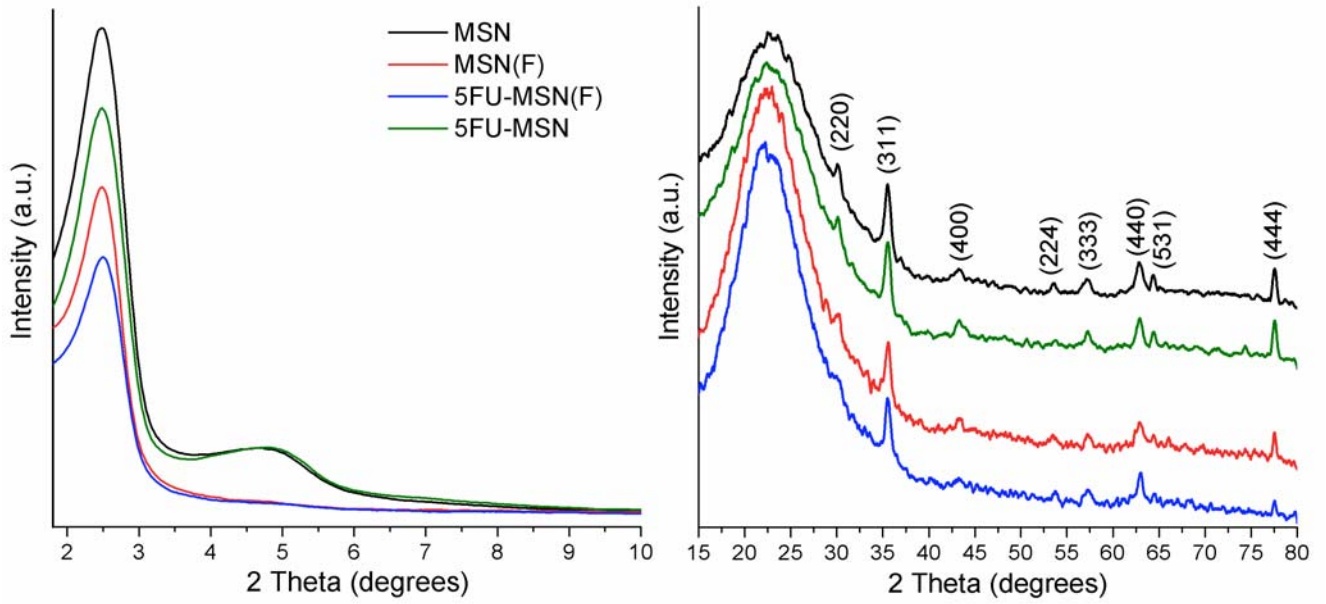

Figure 3: Small angle (left) and wide-angle (right) XRD patterns of the magnetic matrices and final therapeutic nanosystems.

diffraction peaks at 29.8, 35.5, 42.7, 52.9, 56.2, 62.7, 64.3 and 77.6° corresponding to (220), (311), (400), (224), (333), (440), (531) and (444) wide-angle reflections of magnetite face-centered cubic spinel structure (space group Fd3m - Powder Diffraction Files PDF 4, Ref. no 98-004-1482), The broad peak at 2θ ~ 22° is typically assigned to amorphous silica. The significant reduced intensity of the magnetite characteristic peaks in the recorded diffractograms suggests that agglomeration of magnetite nanoparticles is prevented by silica coating, thus they remain very small in size. Immobilization of drug molecules and mesopores surface modification with APTES molecules does not have any significant influence on the size and crystallization of magnetite core nanoparticles. Thus, these magnetite nanoparticles are well protected by the silica shell against further chemical and/or thermal treatments. Just slightly increase in the intensity of the amorphous silica-characteristic peak is observed. This is correlated with the slight decrease of the peaks intensity observed in low-angle range measurements.

Textural Properties of the Therapeutic Nanosystems

The surface area, pore volume and pore diameter are important textural features of the mesoporous silica shell that contribute significantly to the uptake and release of drug molecules. Nitrogen physisorption analysis is the best suited method to investigate any modification of porous properties of the silica shell occurred after APTES-functionalization and/or drug immobilization. Using BET (Brunauer – Emmet – Teller) equation we have calculated the specific surface area of magnetic silica nanoparticles before and after drug adsorption and surface modification. The calculated porous characteristics are summarized in Table **1**.

As clearly observed, before drug adsorption and surface modification, the MSN sample shows high surface area and pore volume, with a pore diameter of 2.60 nm. Slight decrease of the calculated specific surface area, pore volume and pore diameter is registered after drug adsorption, proving its presence

Table 1: Textural Characteristics of the Magnetic Matrices and Final Therapeutic Nanosystems

Sample	S^a_{BET} (m^2/g)	S^b_{μ} (m^2/g)	TPV[c] (cm^3/g)	P^d_d (nm)	Loaded drug[e] (mg 5FU/g matrix)
MSN	1420	-	0.944	2.60	-
FU-MSN	1350	-	0.853	2.36	58
MSN(F)	573	454	0.357	1.75	-
FU-MSN(F)	429	340	0.275	1.52	124

[a]BET surface area, [b]Micropore surface area, [c]Total pore volume, [d]Average pore diameter (nm), [e]Amount of drug immobilized on host matrix, determined by UV-vis spectroscopy.
See *Characterization methods* section for calculations.

within the mesopores. On the other hand, after modification of mesopore surface with amino groups, the surface area and the pore volume decrease significantly. At this point, the specific surface of the pores in the microporous range could be calculated using the t-plot analysis. Further, the adsorption of drug molecules on the amino-modified magnetic matrix leads to supplementary pore narrowing and diminishing of the surface area and pore volume.

Structural Proof of Drug Immobilization

Although the results from N_2-sorption analysis clearly prove the localization of drug molecules within the mesopores and the successful amino-functionalization of pore surface further investigations were necessary in order to investigate the interactions occurred between the host and the bioactive molecules. Therefore, we have employed FTIR Spectroscopy, which is a powerful tool used to investigate the drug-matrix interactions, based on the fact that each molecule has specific functional groups characterized by specific rotational and vibrational frequencies, corresponding to discrete energy levels [17]. We subjected to FTIR measurements unmodified core-shell magnetic silica nanoparticles (MSN), drug containing MSN (5FU-MSN), drug free APTES-modified MSNs (MSN (F)) and drug containing APTES-modified MSNs (5FU-MSN (F)). The recorded spectra are shown in Figure **4**.

No obvious modifications are identified in the wavenumber range 400 – 1250 cm^{-1}, where the IR absorption bands characteristic to 5-FU are overlapped by the very intense adsorption bands at 450, 810, 950, 1081 and 1250 cm^{-1} corresponding to Si – O – Si vibrational modes of silica material [18]. However, valuable information is obtained in the IR spectral range from 1350 to 1800 cm^{-1}, where proof of the grafting of amino-propyl groups on the pore surface is gained due to the IR band registered at 1639 cm^{-1}, seconded by low-intensity band at 1515 cm^{-1}. Typically these bands are assigned to asymmetric and symmetric deformation modes of NH_2 group in adsorbed APTES located near a silicate surface [19]. Usually the IR spectrum of 5-FU shows adsorption bands located at 1725 cm^{-1} (weak) and 1662 cm^{-1} (strong), 1429 cm^{-1} (strong), 1350 cm^{-1} (weak) [20].

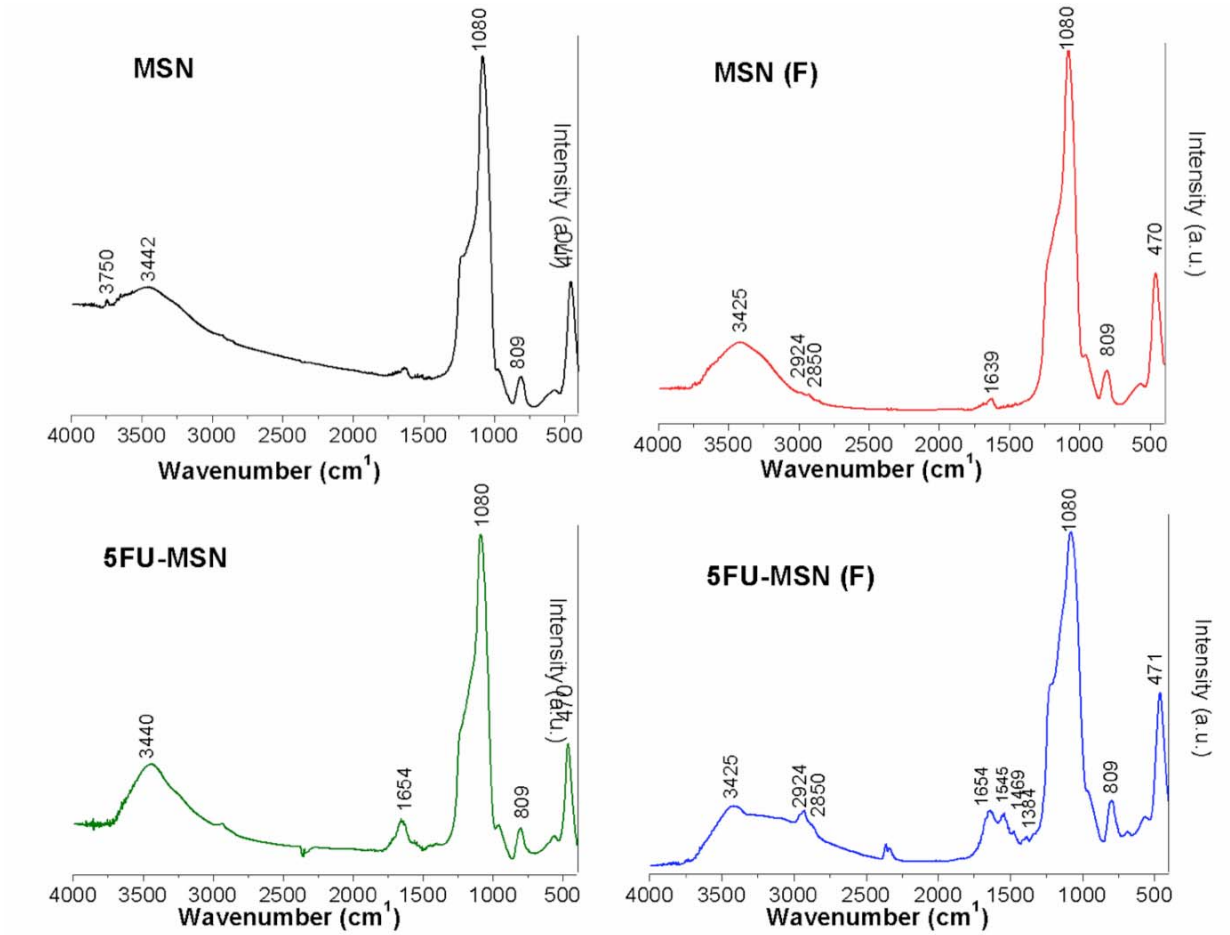

Figure 4: FTIR spectra recorded on the magnetic matrices and final therapeutic nanosystems.

Thus, in the FTIR spectra of 5FU-MSN and 5FU-MSN(F) samples, the presence of the band at 1468 cm^{-1} and that at 1386 cm^{-1} due to pyrimidine ring vibrations in 5-FU prove successful incorporation of the drug within the matrix. More, the strong band observed at 1660 cm^{-1} is characteristic to amide I band. At high wavenumbers, the MSN sample show a very weak adsorption peak at 3745 cm^{-1} assigned to isolated hydroxyl groups on the silica source which disappears completely after functionalization and drug immobilization. Strong, broad adsorption band is observed on all samples at 3000 – 3500 cm^{-1} attributed to vibrational modes of adsorbed water. For functionalized nanoparticles, this band completely overlaps the bands characteristic to asymmetric and symmetric stretch modes from amino groups of APTES molecules, typically positioned at 3250 and 3350 cm^{-1}. Functionalized sample containing 5-FU molecules (5FU-MSN (F)) give bands characteristic to – C (=O) – NH – C vibrational modes from FU molecule at 3300 and 3085 cm^{-1}, a wide shoulder from 3100 to 3300 cm^{-1} probably due to adsorbed water, hiding R – NH_2 stretching characteristic bands and two IR adsorptions at 2927 and 2870 cm^{-1} assigned to stretching modes of methylene groups [17] in APTES molecules.

UV-Adsorption Spectra

Figure **5** shows the UV-vis spectra recorded on 5-FU solution after its immobilization within the unmodified and amino-modified magnetic host matrices. The spectrum of the initial solution of 5FU is compared with the UV-vis spectra recorded on the solutions after being in contact with the host materials for 2h under ultrasonic irradiation. The hypothesis that 5-FU molecules have been retained within unmodified and amino-modified mesopores of the magnetic matrices is sustained by the diminishing of 5-FU concentration in solution, indicated by the decrease in absorbance. Applying the Lambert-Beer law and the equation obtained when calibration curve was drawn, we have calculated the amount of drug immobilized through adsorption on each magnetic matrix. The results are presented in Table **1**. It is clearly seen that significantly larger amount of 5-FU was loaded on amino-modified magnetic matrix in comparison with the unmodified one. This is due, on one hand, to the smaller pore diameter in amino-modified matrix closer to the molecular size of 5-FU and on the other hand to more favorable hydrogen bonding between 5-FU and the amino modified surface of its carrier [16].

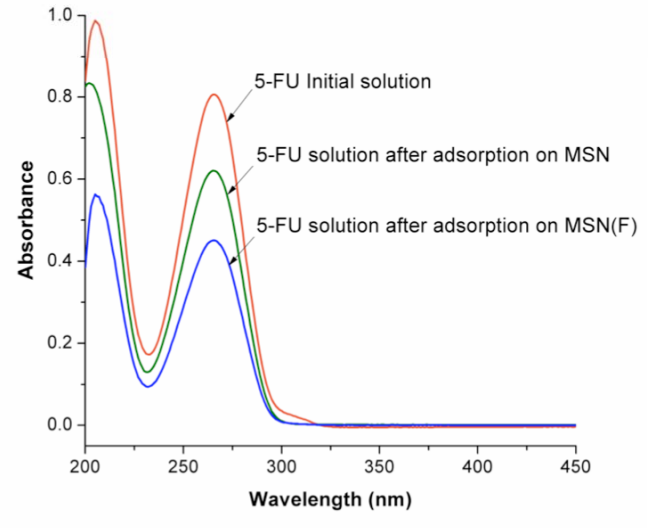

Figure 5: UV-vis spectra recorded on the solution of 5-FU before and after immobilization through adsorption within magnetic matrices.

Drug Release Results

Figure **6** shows the release of 5-FU from the unmodified and amino-modified magnetic therapeutic nanosystems in close comparison with its release from commercially available product Adrucil (50 mg/ml). The release experiments were performed at 37°C in PBS solution with a pH of 7.4. All samples show a quicker release in the beginning and a slower release in the late releasing process. However, as clearly observed, 97 % of 5-FU is released from the commercially available product in only 3h, while delayed release profile are registered for newly developed therapeutic nanosystems. Particularly, only 42 % of 5-FU was release by amino-modified MSN in the first 3h. Then, the release of 5-FU is significantly slowed down and the MSN (F) sample released 54 % of its cargo in 24 h. On the other hand, the release of 5-FU is even more

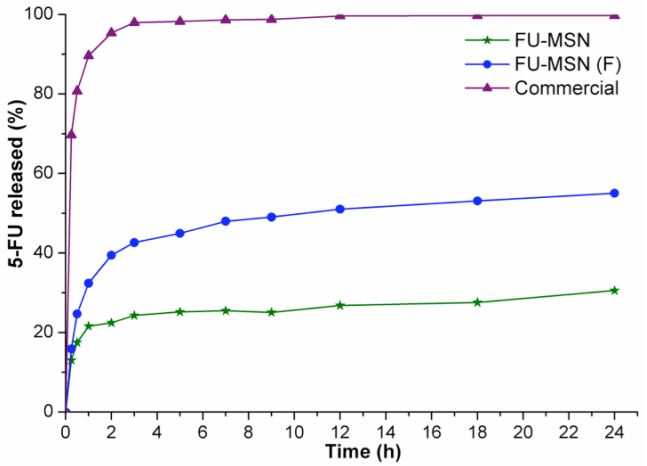

Figure 6: Release of 5-FU from developed magnetic therapeutic nanosystems and commercially available product (Adrucil 50 mg/ml).

delayed from unmodified MSN, reaching a total release of 30% after 24 h.

The slower release property of these new therapeutic nanosystems is not only related to the difference in concentration in each sample, but rather to the nature of interactions between the drug and its carrier. In the amino-modified therapeutic nanosystems (FU-MSN (F)), the interaction between the drug and its carrier relies on quite strong hydrogen bonding between the atoms (O, N and F) with strong negativity in 5-FU and the hydrogen of – NH_2 groups present on the mesopore surface. Thus delayed release profile is obtained. In unmodified magnetic therapeutic nanosystems (FU-MSN) apparently, the much stronger electrostatic attraction between the silanolate groups (≡Si-O^-) present on the silica surface at this pH values and positively charged 5-FU molecules, prevents the easy-release 5-FU, dictating its significantly delayed release.

These results show that the uptake and the release rate of a drug from a modified drug delivery system based on core-shell magnetic mesoporous silica nanoparticles can be controlled by careful tuning of the surface properties.

CONCLUSIONS

New therapeutic nanosystems based on core-shell magnetic mesoporous silica nanoparticles as carriers for 5-fluorouracil chemotherapeutic agents were successfully prepared in sonochemical conditions. The proposed method is feasible, easily reproducible and time-saving. It implies completion of the following steps: (i) the synthesis of magnetite nanoparticles with diameters close to 20 nm; (ii) coating magnetite nanoparticles with mesoporous silica shell, resulting in formation of core-shell nanoparticles with homogeneous particle sizes smaller than 500 nm; (iii) immobilization of drug molecules within unmodified and amino-modified mesopores of the silica shell by adsorption from alcoholic solutions. After detailed investigation by means of SEM, FIB-SEM, N_2 sorption, XRD, FTIR and VSM analysis, one can conclude that the newly developed magnetic core-shell nanoparticles possess proper morphological, textural, structural and magnetic properties to be employed as carriers for drug molecules. Further, the results of the *in vitro* dissolution tests show that pore diameter and more significantly pore surface chemistry play important roles in adsorption and desorption of low molecular chemotherapeutic agents such as 5-fluorouracil.

Thus, drug carriers with targeting properties and modified release functionalities can be easily prepared by coating magnetite nanoparticles with mesoporous silica shell under ultrasonic irradiation.

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