

MAGNETIC POROUS SILICA-LIPID BILAYER HYBRID CARRIERS FOR TARGET DELIVERY OF CURCUMIN

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Abstract

Mesoporous silica nanoparticles with embedded magnetic particles and sizes around 80 nm were prepared. Curcumin loading in mesoporous silica was realized by a post-synthesis impregnation procedure. The loaded particles were additionally modified by surface coating with a phospholipid bilayer of a thickness of around 5 nm changing the surface charge of the carrier from negative to positive, which enables the efficient attachment to cells, increasing the probability of cellular uptake. The non-loaded, curcumin loaded and their surface-coated counterparts were characterized by XRD, N₂ physisorption, thermal analysis, TEM, and ATR-FTIR spectroscopy. Release experiments were performed and demonstrated that the phospholipid coating contributed greatly a sustained drug release to be achieved. A comparative study on the antiproliferative effect of curcumin loaded into the mesoporous carrier and lipid-coated vs. free drug was performed on a panel of three human cell lines. On the basis of the obtained results, it can be concluded that obtained hybrid magnetic silica-lipid particles are promising carriers of curcumin.

Key words: spherical mesoporous silica, curcumin, lipid coating

Introduction. Hybrid carriers for delivery of drugs, genes, protein, etc., are in the research focus of many interdisciplinary teams nowadays. New types of organic-inorganic (silica-polymer, silica-lipids, carbon-polymer, quantum dots-polymer), organic-organic (polymer-polymer, polymer-lipids), inorganic-inorganic

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(metallic particles-silica, magnetic oxides-silica, metallic particles-activated carbon) materials gives opportunity to combine benefits of more than one material and to obtain more efficient carriers [1]. Mesoporous silica is characterized by high loading capacity, chemical inertness, and an easy-to-modify surface, which makes silica nanoparticles perfect candidates for highly efficient drug carriers [2]. Nevertheless, the implementation of target delivery is a challenge, due to the lack of specific directing functional groups on the silica surface. A promising approach to overcome this drawback is the incorporation of magnetic nanoparticles into the silica, which will mediate the targeted delivery of the loaded biologically active molecules via applying an external magnetic field [3].

Natural flavonoids have attracted research interest due to their pleiotropic therapeutic potential [4]. Curcumin is one of the most commonly present in nature flavonoids, a strong antioxidant, and a major dietary flavonoid. However, the clinical realization of curcumin potential is greatly hindered by its unfavourable physicochemical and pharmacokinetic properties [5]. In order to overcome these shortcomings and to fully implement the pharmacological potential of curcumin, a suitable approach is to load it into carriers known for their abilities to enhance the application efficiency of bioactive substances, like magnetic porous silicates. This approach can not only solve the problems associated with its low solubility in water but also increase its stability in the systemic circulation and provide an opportunity for its targeted delivery to various tissue and even subcellular compartments.

Here, we report the design of a curcumin delivery system based on hybrid lipid-magnetic porous silica carriers. The delivery system was obtained in a step-wise manner and shows high antiproliferative activity against cancerous cells and enhanced dispersibility, making it promising for pharmacological application.

Materials and methods. Synthesis of MS. The composite iron oxide-silica particles were prepared as 180 mg freshly prepared iron oxide nanoparticles were dispersed by ultrasonication in distilled water and rapidly added to a solution of 2 g CTAB (cetyltrimethylammonium bromide) in 220 ml 2M NaOH. The mixture was heated at 80 °C for 2 h, after that 10 ml TEOS (tetraethyl orthosilicate) was added dropwise, and the mixture was stirred for 3 more hours. The obtained particles were collected and the template was removed by extraction in ethanol on reflux for 20 h. The obtained sample was named MMS (magnetic mesoporous silica).

Curcumin loading and phospholipid coating. Curcumin was loaded in as-prepared carriers by incipient wetness impregnation in a ratio of 1:2 [6]. As the next step curcumin-loaded silica nanoparticles were surface coated with a phospholipid layer composed of DPPC (dipalmitoylphosphatidylcholine) and pegylated DSPE-PEG2000 (dipalmitoylphosphatidylcholine) in molar ratio 95:5. Briefly, accurately weighed samples of 10 mg mesoporous silicate nanocarriers were placed in 50 ml round-bottomed flasks and 1 ml of a chloroform solution of

phospholipids, at a total lipid of 10 $\mu\text{mol/ml}$ was added. The flasks were then placed in a rotary evaporator and the organic solvent was completely evaporated at 37 °C under a vacuum. The resulting thin film on the walls of the flask was purged with inert gas and the flasks were placed in a vacuum oven for 12 h to completely remove traces of organic solvent. The dried coated sample was collected and assigned as MMS-Curc-LB.

Characterization of the nanoparticles. X-ray diffractograms were recorded by a Philips PW 1810/3710 diffractometer with Bregg–Brentano para-focusing geometry applying monochromatized $\text{CuK}\alpha$ ($\lambda = 0.15418 \text{ nm}$) radiation (40 kV, 35 mA), and proportional counter.

The specific surface area, pore volume and size of all samples were determined from N_2 physisorption isotherms collected at -196°C using AUTOSORB iQ-C-MP-AG-AG (Quantachrome Instruments (Anton Paar brand), USA). Samples were pretreated at 350 °C in vacuum.

The curcumin loading was determined by thermogravimetric measurements performed with a STA449F5 Jupiter type instrument of NETZSCH Gerätebau GmbH equipped with SiC furnace and sensor holders TG/TG-DSC/TG-TDA type S, temperature range RT up to 1600 °C. In a typical measurement, 20 mg of sample was placed in a microbalance ceramic crucible and heated in a flow of air ($100 \text{ cm}^3/\text{min}$) up to 500 °C at 5 °C/min and a final hold-up of 1 h.

The TEM images were taken on JEOL JEM 2100 TEM (200 kV). The samples were suspended in a small amount of ethanol and a drop of the suspension was deposited onto a copper grid covered by carbon supporting film and dried at ambient temperature.

ATR-FTIR spectra were recorded by means of IRAffinity-1 “Shimadzu” Fourier Transform Infrared (FTIR) spectrophotometer with MIRacle Attenuated Total Reflectance Attachment. The instrument is equipped with a temperature-controlled, high-sensitivity DLATGS detector and ATR attachment with KRS-5 prism. In general, 50 scans and 4 cm^{-1} resolution were applied. The spectral data were processed with an IRsolution software.

Determinations of nanoparticle size, polydispersity index, and zeta potential were performed using a Zetasizer (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). The samples were dispersed in distilled water and measured at a scattering angle of 90° and 25°.

In-vitro release study. An in vitro curcumin release study was performed in a phosphate buffered solution at pH 7 and 37 °C. The curcumin-loaded particles (2 mg) were incubated in 50 ml buffer under stirring (100 rpm). At appropriate time intervals, 3 ml samples were withdrawn from the release medium and analyzed with UV-Vis spectroscopy at a wavelength of 427 nm. The concentration of the released curcumin was calculated according to the standard curve ($r > 0.9993$).

Cell lines and cultured conditions. Human promyelocytic (HL-60), uri-

nary bladder cancer (Cal-29) and non-malignant human embryonal kidney (HEK-293) cells were cultivated in RPMI-1640 culture medium, with the addition of 2 mM L-glutamine and 10% fetal calf serum, and were kept in an incubator (BB 16-FunctionLine[®] Heraeus (Kendro, Hanau, Germany)) at 37 °C in a 5% CO₂ humidified atmosphere.

In vitro cytotoxicity. The cellular viability after exposure to free curcumin or its nanoporous magnetic silica formulations was assessed by the standard MTT-dye reduction assay as previously described [7] with few modifications [8]. The method is based on the ability of viable cells to transform the yellow tetrazolium salt MTT to a violet MTT-formazan by the mitochondrial succinate dehydrogenase. In brief, exponentially dividing cells were seeded in 96-well flat-bottomed microplates (100 µl/well) at a density of 1×10^5 cells per ml. By 24 h incubation at 37 °C the cells were treated with various concentrations of the tested curcumin formulations (corresponding from 1.5 to 7.5 µM of drug) or free curcumin (in the same concentration range), used as a reference antineoplastic agent for 72 h. For each concentration, a set of at least 8 wells was used. After the exposure period, 10 µl curcumin solution (10 mg/ml in PBS) aliquots were added to each well. The microplates were incubated for 4 h at 37 °C and the MTT-formazan crystals formed were dissolved through the addition of 100 µl/well of 2-propanol acidified with 5% formic acid. The MTT-formazan absorption was recorded using a Labexim LMR-1 microplate reader at 580 nm. Cell survival fractions were calculated as a percentage of the untreated control. In addition, IC₅₀ values were derived from the concentration-response curves.

Statistical analysis. The experiments were replicated three independent times and the data was presented as mean $\pm$ SEM. Statistical analysis was carried out using Student's *t*-test. Differences were considered statistically significant when *P*-value was less than 0.01.

Results and discussion. In the XRD patterns of the MMS (Fig. 1A) the characteristic reflections for two magnetic phases – magnetite (Fe₃O₄) and maghemite (Fe₂O₃) are observed. The observation has already been explained by us in a previous study [9], and it confirms the preservation of the magnetic properties of the mixed oxide phase after the embedding procedure. The crystallite size of the magnetic particles calculated by the Scherrer equation is 18 nm. The high crystalline nature of pure curcumin is confirmed by the sharp well-defined reflections at angles in the range of 8–32° 2 θ . The reflections in the diffractogram of the curcumin-loaded sample are characterized by much lower intensity, indicating that only a small amount of it can be found on the particles' surface or into interparticle voids, which means that curcumin is generally loaded into the pores of the support.

The parent and curcumin-loaded MMS particles were studied by N₂ physisorption, and the results show a decrease in the Brunauer–Emmett–Teller (BET) surface area from 820 to 444 m²/g, and the pore volume from 0.53 to

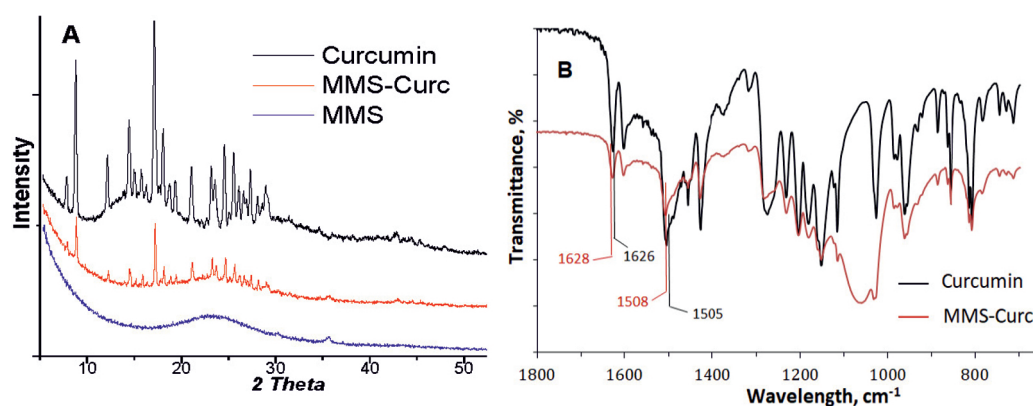


Fig. 1. XRD patterns of pure MMS, free curcumin and curcumin-loaded MMS (A) and IR spectrum of pure and MMS-loaded curcumin (B)

0.29 cm³/g, which is a clear indication for pore filling. Barrett–Joyner–Halenda (BJH) method was applied to determine the pore size distribution, and the average pore size was found to be 3.1 nm. The small size of the pores prevents the formation of big crystals of curcumin after its loading into the pores, which is a premise for improved solubility. The exact amount of the loaded bioactive compound was evaluated by thermogravimetric analysis (TGA), and it was found to be 30% (of 33% theoretically loaded). This result proved the efficiency of the applied loading technique and the high drug payload achieved. After coating of the loaded silica particles with the lipid bilayer, the observed weight loss was 38% which means that the coating was successful.

The interaction of the curcumin molecule with the surface of MMS was studied by infrared (IR) spectroscopy. The ATR-FTIR spectra of curcumin and the loaded particles are presented in the region of 1800–800 cm^{−1} (Fig. 1B). No bands can be seen in the typical spectral region of the carbonyl group (1800–1600 cm^{−1}), indicating that curcumin exists in the keto-enol tautomeric form. Curcumin displays characteristic bands at 1626 cm^{−1} due to stretching vibrations of C = C and C = O groups ($\nu(\text{C} = \text{C})$ and $\nu(\text{C} = \text{O})$) of the inter-ring chain and at 1605 cm^{−1} assigned to the C = C stretching ($\nu(\text{C} = \text{C})$) of aromatic rings. The most intense band at 1505 cm^{−1} is due to the C = O stretching ($\nu(\text{C} = \text{O})$) and CCC and CC = O bending vibrations ($\delta(\text{CCC})$ and $\delta(\text{CC} = \text{O})$) [10]. Due to the overlap with Si–O–Si stretching bands of mesoporous silica matrices in the region 1100–1000 cm^{−1}, only absorptions between 1800 and 1200 cm^{−1} are discussed. Upon drug loading small shifts of bands related to keto-enol moiety can be observed: from 1626 cm^{−1} to 1628 cm^{−1} ($\nu(\text{C} = \text{O}) + \nu(\text{C} = \text{C})$) and from 1505 cm^{−1} to 1508 cm^{−1} ($\nu(\text{CO}) + \delta(\text{CCC}) + \delta(\text{CC} = \text{O})$). These shifts imply weak interactions between the silica surface groups and curcumin, as well as preservation of the keto-enolic form.

Figure 2 shows transmitting electron microscopy (TEM) images of the as-prepared nanosized delivery systems. Particles are characterized by spherical

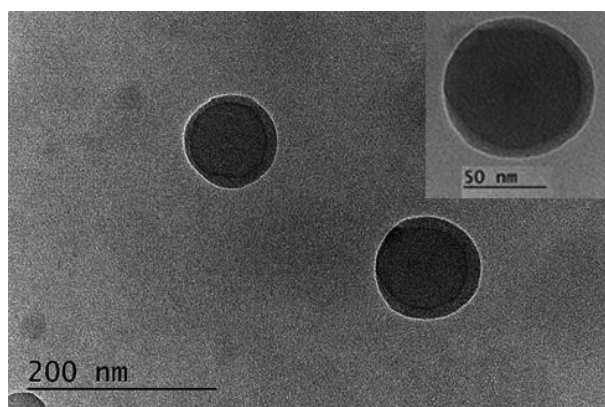


Fig. 2. TEM images of the obtained samples

shape and uniform size around 80–90 nm. It can be clearly seen that the particles are coated with thin phospholipid bilayer ~ 5 nm, which corresponds with the TGA results. The lipid bilayer contributes to dispersibility of the particles.

The release profiles of the two formulations were determined in a phosphate buffered solution at pH 7 and 37°C (Fig. 3). The MMS-Curc formulation shows faster curcumin release in comparison to MMS-Curc-LB. The determined amount of released curcumin corresponds very well to the loaded curcumin amount and it proves that there is no loss of curcumin during the coating procedure. The presence of a phospholipid film around the particles results in the formation of a membrane that hinders the free diffusion of the aqueous medium and therefore the transport of the curcumin molecules through the lipid layer is slowed down.

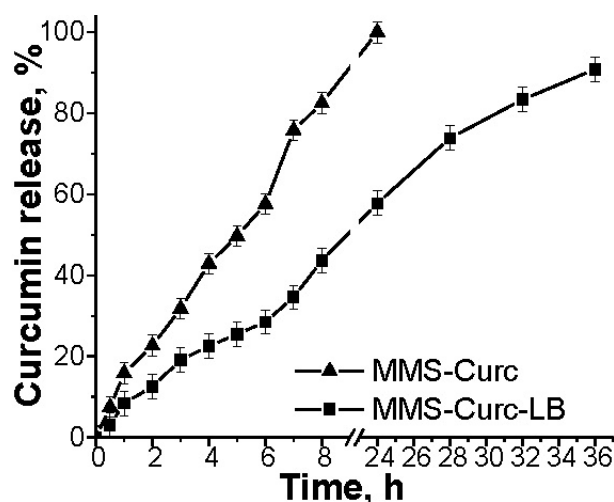


Fig. 3. Release profiles of curcumin loaded parent and lipid coated mesoporous silica

Thus, the deposition of phospholipid layer is an appropriate approach to achieving a sustained curcumin release. In addition to that, the encapsulation of the drug into phospholipid modified silica nanocomposites could increase its low chemical stability.

A comparative study of the cytotoxic effect of curcumin loaded into mesoporous silica vs. free curcumin (ethanol solution) on a panel of three human cell lines, namely non-malignant HEK-293 (human embryonal kidney) and malignant human HL-60 (promyelocytic leukemia), and CAL-29 (human bladder carcinoma) cells was performed. The growth inhibitory concentration-response curves are presented in Fig. 4 and the corresponding equieffective IC_{50} values are summarized in Table 1.

Free curcumin and its lipid coated mesoporous silica formulations evoked strong, concentration dependent inhibition of the growth of cultured tumour cells. The juxtaposition of the concentration-response curves clearly indicates that the encapsulation of curcumin into silica carriers was associated with a slight reduction

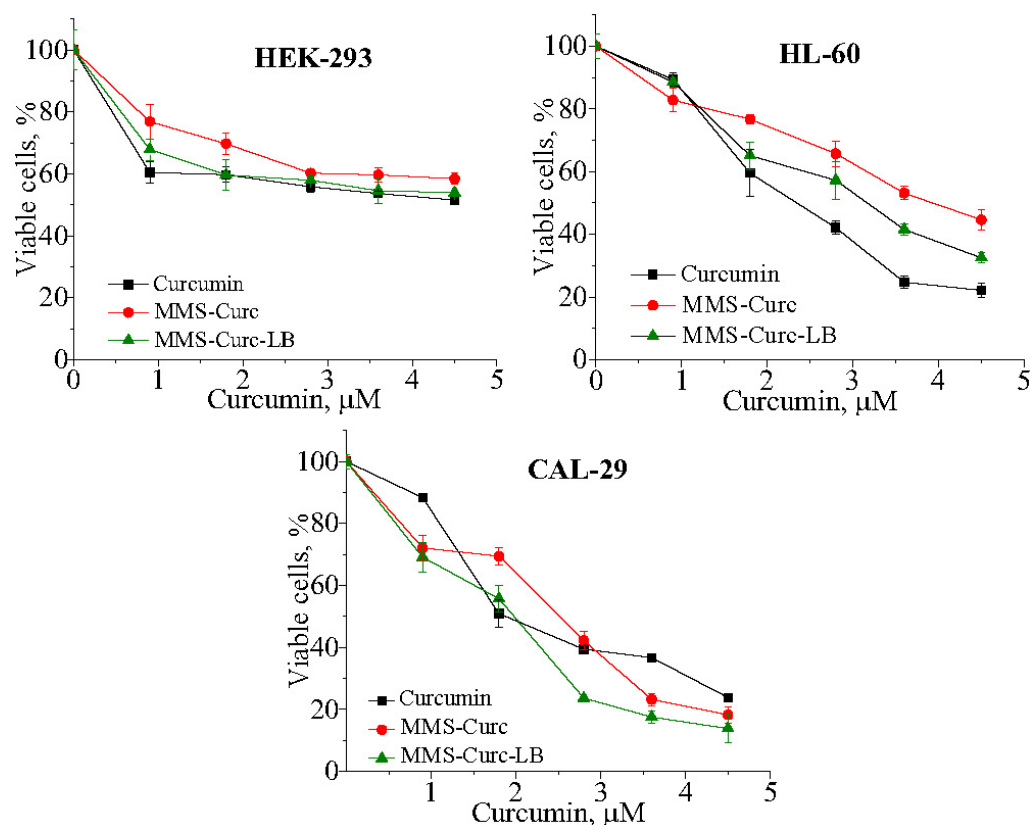


Fig. 4. The concentration-response curves determined by the MTT assay following 72 h continuous treatment at 37 °C. Each data point represents an average arithmetic value $\pm$ SD of at least 8 independent experiments

T a b l e 1

Equieffective concentrations of the tested non-loaded and curcumin containing magnetic mesoporous silica particles, vs. the free drug

Cell line	HEK-293	HL-60	CAL-29
Sample	IC ₅₀ (μM)		
MMS	n.d.	n.d.	n.d.
Curcumin	n.d.	2.3	0.18
MMS-Curc	n.d.	3.9	0.25
MMS-Curc-LB	n.d.	3.2	0.19

in the cytotoxic effect of loaded curcumin (shifting the curves to higher concentrations) due to its slower release from the carriers and hence, lower concentration of released curcumin to which the cells were exposed for a certain time of the treatment period as compared to the free agent administered as an ethanolic solution. More interesting, however, is that the coated carriers are characterized by lower IC₅₀ values and, respectively, higher antiproliferative activity of the drug compared to the uncoated formulations (Table 1). A possible explanation could be the interaction of the phospholipid layer of the coated silica particles with cell membranes and, respectively, facilitated cell internalization of curcumin.

In addition, the loading of curcumin into the developed silica nanocarriers does not compromise its inherited selectivity against malignant cells, as evidenced by the lack of the cytotoxicity against normal epithelial cells (HEK-293) in the tested concentration range. Unloaded carriers also did not show cytotoxicity at the concentrations studied.

The zeta potential of the non-coated MMS particles was negative (-12.5 ± 1.2 mV) whereas the positive value (9.2 ± 1.2 mV) was measured for particles coated by lipid layer. A positive charge of the obtained delivery system is preferred to enable efficient attachment to cells, increasing the probability of cellular uptake [11]. These observations proved the successful lipid coatings around the mesoporous silica nanoparticles. The drug loading did not influence the surface charge of the mesoporous silica nanoparticles, which was an indication for the predominant curcumin loading in the pores of MMS carrier. Determination of the size of curcumin loaded lipid-coated particles showed that a fraction of a size around 90 nm was about 70%, which was in a good accordance with TEM images.

Conclusions. Mesoporous silica particles of size around 80 nm with embedded magnetic nanoparticles were successfully prepared. Highly efficient curcumin loading in the silica support was achieved by post synthesis impregnation procedure. Lipid layer of 5 nm was formed around the silica nanoparticles changing the surface charge of the carrier from negative to positive favouring the interaction with the cells, and assisting particles dispersibility. The in vitro release profiles obtained show that the carrier is able to deliver curcumin in a sustained manner

for a period of 24 h. The presence of a phospholipid layer around the mesoporous nanoparticles appears to be a key factor that additionally slows down the rate of drug release. A comparative study on the antiproliferative effect of the loaded curcumin vs. free drug was performed on a panel of three human cell lines. The coated formulation displayed IC₅₀ values in the low micro molar range similar to those of the free drug, indicating good cellular internalization of the formulated curcumin. Further, loading of curcumin into the developed silica nanocarriers does not compromise its selectivity against malignant cells, as evidenced by the lack of the cytotoxicity against normal epithelial cells. On the basis of the obtained results it can be concluded that the coated silica particles are excellent carriers for curcumin delivery system with targeting function.

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