Colloids and Surfaces B: Biointerfaces 140 (2016) 161–168

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Core–shell microcapsules of solid lipid nanoparticles and mesoporous silica for enhanced oral delivery of curcumin

Sanghoon Kim[a], Roudayna Diab[a,*], Olivier Joubert[b], Nadia Canilho[a], Andreea Pasc[a,*]

[a] SRSMC, UMR 7565, Université de Lorraine-CNRS, F-54506 Vandœuvre-lès-Nancy, France
[b] CITHEFOR, EA 3452, Université de Lorraine, F-54000 Nancy, France

ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form
26 November 2015
Accepted 20 December 2015
Available online 28 December 2015

Keywords:
Core–shell mesoporous silica
Curcumin
Caco-2 cells
Bioavailability

ABSTRACT

Newly designed microcapsules (MC) combining a core of solid lipid nanoparticle (SLN) and a mesoporous silica shell have been developed and explored as oral delivery system of curcumin (CU). CU-loaded MC (MC-CU) are 2 μm sized and have a mesoporous silica shell of 0.3 μm thickness with a wormlike structure as characterized by small angle X-ray scattering (SAXS), nitrogen adsorption/desorption and transmission electron microscopy (TEM) measurements. It was found that SLN acts as reservoir of curcumin while the mesoporous shell insures the protection and the controlled release of the drug. MC-CU displayed a pH-dependent *in vitro* release profile with marked drug retention at pH 2.8. Neutral red uptake assay together with confocal laser scanning microscopy (CLSM) showed a good cell tolerance to MC-CU at relatively high concentration of inert materials. Besides, the cell-uptake test revealed that fluorescent-MC were well internalized into Caco-2 cells, confirming the possibility to use MC for gut cells targeting. These findings suggest that organic core-silica shell microcapsules are promising drug delivery systems with enhanced bioavailability for poorly soluble drugs.

1. Introduction

Curcumin, a hydrophobic natural polyphenol derived from Curcuma longa, has been used over the centuries in traditional medicine as treatment for inflammatory diseases. While curcumin shows numerous pharmacological activities, including antioxidant and antimicrobial properties, within the past decades, the research has been focused on its anti-cancer activity due to the lack of serious side effects [1,2].

However, the medical applications of curcumin have been limited by its low solubility and bioavailability [3]. Indeed, curcumin has an extremely low solubility at pH values ranging from 1.2 to 7.4, physiological pH values of the gastrointestinal tract. Moreover, under alkaline conditions, curcumin undergoes a rapid hydrolysis, being decomposed into ferulic acid, its methyl ester and vanillin [4]. Curcumin is also susceptible to photochemical and thermal degradation that could be problematic for long-term storage [5]. Moreover, previous studies revealed that curcumin has a poor oral bioavailability and a short half-life, due to the rapid pre-systemic catabolism in the intestinal wall [6,7].

To overcome the aforementioned problems, various methods have been proposed, including encapsulation of curcumin into liposomes [8,9], polymers [10,11], micelles [12,13], or nanoparticles [14,15]. In addition to these drug delivery vectors, solid lipid nanoparticles (SLN) [16–18] and mesoporous silica nanoparticles [19–22] have also been proposed independently as carriers to improve the bioavailability of curcumin. Mesoporous silica exhibit many advantages over traditional drug carriers, such as facile synthesis, high surface areas, large pore volume, versatile surface modification and has already been approved by U.S. Food and Drug Administration (FDA) [23,24]. Recently, silica shell coated nanoparticles have been largely investigated [25,26] for applications in drug delivery, showing good biocompatibility as well as possible modifications of silica surface for designing stimuli-responsive drug vector as advanced drug delivery system [27,28]. Our group recently designed hybrid materials that combine those two candidates, solid lipid nanoparticles and mesoporous silica within the same material for drug delivery applications [29,30]. This could be achieved through a dual templating mechanism that consist in condensing the silica mesophase, formed by self-assembling, around the lipid particles loaded in a hydrophobic drug such as curcumin. Those materials showed that the drug release is highly dependent on the interaction between the 'guest' drug and the 'host' lipid, as well as the nature of the surfactant that imprints mesopores [31]. Moreover, this type of materials have also shown a poten-

* Corresponding authors.
E-mail addresses: roudayna.diab@univ-lorraine.fr (R. Diab), andreea.pasc@univ-lorraine.fr (A. Pasc).

http://dx.doi.org/10.1016/j.colsurfb.2015.12.040
0927-7765/

tial application to design supported biocatalysts [32] or iron oxide nanoparticles supported catalysts [33], due to enhanced diffusion rate provided by the hierarchical, meso-macroporous structure.

Mesoporous silica-based vectors used for oral delivery were previously reported [34]. Theses vectors enabled an enhanced intestinal absorption for drugs displaying poor solubility, such as fenofibrate [35], poor permeability, such as telmisartan [34], or for those undergoing cell efflux by P-glycoprotein pump, such as cyclosporine [36].

Herein, we report for the first time the design of curcumin loaded hybrid mesoporous silica microcapsules using solid lipid nanoparticles dispersions of N-hexadecylpalmitate (NHP) in a micellar phase of Tween 40. The curcumin loaded SLN and the hybrid silica capsule templated SLN were characterized by SAXS, nitrogen adsorption/desorption measurements, dynamic light scattering (DLS), nanoparticles tracking analysis (NTA) and TEM. To demonstrate the potential application of those novel hybrid silica capsule, *in vitro* release, cytotoxicity and cellular uptake into Caco-2 cells were carried out.

2. Experimental

2.1. Chemicals

Hexadecyltrimethylammonium bromide (CTAB), Tetramethylorthosilicate (TMOS), Curcumin and Nile Red (NR) were purchased from Sigma–Aldrich. *N*-hexadecylpalmitate was purchased from Acros. Tween 40 (polyoxyethylene (20) sorbitan monopalmitate) and ethanol were purchased from Alfa Aesar. Water was deionized and purified using a Milli-Q pack system (Millipore). All reagents were used without further purification. All the release media were prepared according to European Pharmacopoeia 8.0 guidelines—Section 5.17.1.

2.2. SLN preparation

Curcumin-loaded solid lipid nanoparticles were prepared by ultra-sonication of a hot emulsion obtained from a mixture of curcumin and a melted lipid dispersed in a micellar phase [31]. In a typical procedure, the mixture of 430 mg of NHP and 11 mg of CU were heated at 70 °C. The aqueous phase containing 1.6 wt% of Tween 40 was heated at the same temperature as the lipid phase. The oil phase was added to 20 mL of the Tween 40 micellar solution and the mixture was sonicated for 1.5 min with an ultrasonic device (Bandelin sonopuls, HD2200, 52% of output). The hot o/w emulsion was then rapidly cooled to room temperature under vigorous stirring to afford lipid solidification. The fluorescent, Nile Red labelled SLN were synthesized in a similar manner, by mixing the dye with the melted lipid.

2.3. Synthesis of hybrid SLN-silica microcapsules

510 mg of TMOS was added dropwise into 20 mL dispersion of CU-SLN. The surfactant/silica molar ratio (*R*) was 0.07. The mixture was stirred for 1 h and then transferred into sealed Teflon autoclaves and maintained at 40 °C for 48 h. Afterwards, the material was filtered, washed with water and dried at 40 °C in vacuo for 24 h. Nile Red labelled silica microcapsules were prepared in a similar manner.

2.4. Assay of the content of curcumin in hybrid SLN-silica microcapsules

5 mL of THF was added to 50 mg of hybrid SLN-silica microcapsules and then the sample was sonicated for 30 min in order to solubilize the lipid (NHP) and the curcumin into the organic phase. Afterward, 90 mL of ethanol was added and the sample was diluted 10-times with ethanol for UV–vis spectrometry analysis. The concentration was calculated using a standard curve of curcumin in ethanol.

2.5. In vitro *curcumin release experiments*

Release experiments were carried out in 3 different buffer solutions, pH 1.2, 2.8 and 7.4. Briefly, 50 mg of the hybrid silica microcapsule (0.31 mg of curcumin) was socked in 75 mL buffer solution containing 0.1 wt% of CTAB [37]. The system was maintained at 25 °C under magnetic stirring at 100 rpm. At regular intervals, 1.5 mL aliquots were withdrawn and replaced by an equal volume of receiving solution in order to maintain sink condition. The withdrawn samples were filtered on a 0.45 μm membrane filter, and then diluted 4-times. The curcumin release was analysed using a UV spectrometer at 423 nm and its concentration was calculated using a standard curve of curcumin established for each dissolution medium. The final cumulative release percentage was corrected using a dilution factor. All data reported are the mean value with standard deviation of at least three different experiments.

2.6. General characterization

Small-angle X-ray scattering measurements were carried out using SAXSee mc2 (Anton Paar) apparatus [38]. Transmission electron microscopy analysis was performed using a Philips CM200 microscope, operated at an accelerating voltage of 200 kV. Nitrogen adsorption/desorption isotherms were determined on a Micromeritics Tristar 3100 at −196 °C. The mesopore size distribution was calculated by the BJH (Barret, Joyner, Halenda) method. Dynamic light scattering experiments and zeta-potential values analyses were performed with a Malvern 3000HSA Zetasizer. Nanoparticle tracking analysis was performed with a Malvern NS300 Nanosight.

2.7. Cell culture

Caco-2 cells (ATCC, HTB-37TM, Manassas, VA, USA) were grown at 37 °C under a 5% CO_2 atmosphere in Eagle's minimum essential (EMEM) medium (Sigma–Aldrich, Saint-Quentin Fallavier, France) supplemented with 10% fetal bovine serum (SVF, Eurobio, Les Ulis, France), 1% non-essential amino acids (Sigma–Aldrich, Saint-Quentin Fallavier, France), 1% sodium pyruvate (Sigma–Aldrich, Saint-Quentin Fallavier, France), 1% antibiotics (100U/mL of penicillin, 0,1 mg/mL streptomycin, Sigma–Aldrich, Saint-Quentin Fallavier, France). Cells were trypsinized once a week (Trypsin-EDTA, Sigma–Aldrich, Saint-Quentin Fallavier, France), before counting and medium renewal. Passages 35–45 were used.

2.8. Cytotoxicity assay

Cells were seeded in 96-well plates at a 5×104 cells/cm^2 density. After incubation overnight, medium was removed by aspiration. Fresh medium was added and cells were exposed to blank or loaded MC at equivalent concentrations of encapsulated CU ranging from 5 to 75 mg/L during 24 h. Cell viability was then examined by Neutral Red assay according to Borenfreund et al. recommendations [39]. Briefly, after exposure to fluorescently-labelled silica microcapsules, medium was removed and cells were rinsed two times using Hank's balanced salt solution (HBSS) from Sigma Aldrich (Saint-Quentin Fallavier, France). Cells were then incubated with 50 mg/L Neutral Red in medium solution during 4 h. Working solution was then removed and the cells were rinsed two times with HBSS and lysis solution (50% water, 50% ethanol