Colloids and Surfaces B: Biointerfaces 148 (2016) 1–11

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Solid lipid nanoparticles coated with cross-linked polymeric double layer for oral delivery of curcumin

Taoran Wang[a], Xiaoyu Ma[b], Yu Lei[b,c], Yangchao Luo[a,*]

[a] Department of Nutritional Sciences, University of Connecticut, Storrs, CT, 06269, USA
[b] Department of Biomedical Engineering, University of Connecticut, Storrs, CT, 06269, USA
[c] Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, CT, 06269, USA

ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form 3 August 2016
Accepted 26 August 2016
Available online 27 August 2016

Keywords:
Solid lipid nanoparticles
Sodium caseinate
Pectin
Cross-linking
Coating
Curcumin
Encapsulation
Controlled release
Nano spray drying
Antioxidant

ABSTRACT

Solid lipid nanoparticles (SLNs) are regarded as promising carriers to improve the safety and effectiveness of delivery for drugs and nutrients, however, the clinic applications for oral administration are limited by their poor stability in gastrointestinal conditions. In this study, surface modification was explored to confer new physicochemical properties to SLNs and thus achieve enhanced functionalities. Novel SLNs with biopolymeric double layer (DL) coating using two natural biopolymers, i.e. caseinate (NaCas) and pectin, were prepared to encapsulate and deliver curcumin, a lipophilic bioactive compound studied as a model drug/nutrient. The DL coating was chemically cross-linked by creating covalent bonds between NaCas and pectin, using two different cross-linkers, i.e. glutaraldehyde (GA) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-Hydroxysuccinimide (EDC/NHS). Prior to cross-linking, the mean particle size, polydispersity index and zeta potential of DL-SLNs were 300–330 nm, 0.25–0.30, −45–40 mV, respectively. It was found that cross-linking with GA had a more prominent effect on particle size and polydispersity index than EDC/NHS. The cross-linking process significantly improved physicochemical properties of DL-SLNs, resulting in higher encapsulation efficiency and loading capacity, better stability and slower release profile in simulated gastrointestinal conditions. Particularly, an optimal zero-order release kinetic was observed for EDC/NHS crosslinked DL-SLNs. The electron microscopy revealed that both cross-linked DL-SLNs exhibited spherical shape with homogeneous size and smooth surface. Encapsulation of curcumin in SLNs dramatically enhanced its antioxidant activity in aqueous condition. The cross-linking process further helped spray drying of SLNs by forming homogenous powder particles. These results indicated that coating with cross-linked polymers could significantly improve the physicochemical properties of SLNs and expand their potentials as oral delivery systems for lipophilic nutrients and drugs.

1. Introduction

Since first report of solid lipid nanoparticles (SLNs) in 1991, they have been suggested to be one of the most promising nanoscale delivery systems for drugs that are highly lipophilic with limited bioavailability and biological efficacy [1]. SLNs are usually prepared with physiological saturated lipids or fatty acids that are biodegradable in vivo, such as triglycerides, and thus are well known for their biocompatibility and have recently received great interest to encapsulate and deliver nutrients for applications in food industry [2]. For instance, SLNs have been studied to improve physical and chemical stability of β-carotene during storage [3,4], to increase skin permeation rate of caffeine [5], as well as to enhance cellular uptake and oral bioavailability of curcumin [6,7]. The clinical applications of SLNs, however, are limited by two major hurdles [8]. First, the SLNs will easily aggregate to bulky structure under strong acidic condition, i.e. stomach, due to the protonation of carboxyl groups and neutralization of surface charge. Second, drying process (lyophilization and spray drying) usually induces severe agglomeration of lipid nanoparticles into large aggregates, owing to the significant change of lipid physical state by water removal.

In the last decade, increasing interest has been drawn to design surface-modified SLNs with various coatings, such as polymers [9], poly (ethylene glycol) (PEG) [10], and small molecular surfactants (ionic and non-ionic) [11]. Such surface modifications are

* Corresponding author at: Department of Nutritional Sciences, University of Connecticut, 3624 Horsebarn Road Extension, U-4017, Storrs, CT 06269-4017, USA.
E-mail address: yangchao.luo@uconn.edu (Y. Luo).

http://dx.doi.org/10.1016/j.colsurfb.2016.08.047
0927-7765/

considered to confer new physicochemical properties to SLNs and have been shown to bring enhanced functionalities. For instance, chitosan coated SLNs demonstrated great potential to deliver both hydrophobic [12–14] and hydrophilic drugs, including peptides [15,16], insulin [17,18], and quercetin [9,19], by enhancing the encapsulation efficiency and intestinal absorption as well as avoiding phagocytosis by mononuclear phagocytic system after intestinal uptake. On the other hand, the pharmacokinetic and tissue distribution study of PEG-coated SLNs indicated that the stealth effects of PEG significantly prolonged blood circulation time and topical concentration in brain when administered intravenous injection [20]. Furthermore, different synthetic surfactants may result in different physicochemical properties and loading efficacy of coated SLNs [21]. Nevertheless, there are often potential toxicities associated with synthetic coatings or emulsifiers used in the preparation of SLNs [8].

Coating SLNs with natural biopolymers (carbohydrates and proteins) have been rarely explored so far, which is probably due to the weak association between natural biopolymers and solid lipids at the colloidal interface. Sodium caseinate (NaCas) and pectin are the natural biopolymers from food sources, i.e. major protein fraction from milk and polysaccharide from plant cells, respectively. Owing to its amphiphilic property, NaCas is well known for its emulsification property by adsorbing to oil/water interface [22], while pectin is widely studied for its capability to stabilize NaCas particles at acidic conditions by forming complex particles [23]. The complexation between NaCas and pectin is due to the facile electro-deposition process induced by adjusting pH and subsequent thermal treatment, and the resultant complex particles can be either nanoscale [24] or microscale [25], depending upon the concentration of two biopolymers.

We have previously demonstrated that NaCas, together with lecithin, was able to emulsify solid lipids in aqueous phase to form SLNs and the subsequent coating of pectin was realized via electro-deposition process [26]. The as-prepared SLNs were characterized to have a layer-by-layer coated structure and showed excellent stability during spray drying process, after which ultra-fine spherical particles with smooth surface were obtained. In this work, we aimed to explore the drug/nutrient delivery potentials of SLNs with biopolymeric double layer (DL) coatings, consisting of NaCas and pectin, with curcumin studied as a model compound. To further improve the encapsulation efficiency, physical stability and sustained release in simulated gastrointestinal conditions, the polymeric coatings (NaCas and pectin layers) were chemically cross-linked by different cross-linkers, i.e. glutaraldehyde (GA) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-Hydroxysuccinimide (EDC/NHS) and the effects of cross-linking were compressively investigated herein.

2. Materials and methods

2.1. Materials

Compritol ATO 888® (Glyceryl behenate) was a gift sample from Gattefossé Corporation (Paramus, NJ, USA). Sodium caseinate from bovine milk and pectin (galacturonic acid content ≥74%) from citrus peel were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). Glutaraldehyde (GA) with concentration of 25%, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-Hydroxysuccinimide (NHS), acetone, ethanol, hydrochloric acid, and sodium hydroxide were obtained from Fisher Scientific Co. (Pittsburgh, PA, USA). Unless noted otherwise, all reagents and solvents were of analytical grade and used without further purification.

2.2. Preparation of single-layer and double-layer solid lipid nanoparticles

Biopolymeric single layer (SL) curcumin-loaded SLNs were prepared by combining the solvent-diffusion and hot homogenization technique, as detailed elsewhere [13]. Briefly, 20 mg of ATO 888 was completely dissolved in the organic phase (preheated to 80 °C in a water bath), consisting of equal volume of acetone (0.5 mL) and ethanol (0.5 mL), to give a final lipid concentration of 20 mg/mL. To this organic phase, different amount (80, 200, or 400 μL) of curcumin solution (2.5 mg/mL in ethanol) was added at the same temperature. The obtained solution was then sheared into 20 mL of aqueous phase containing NaCas at 1.2 mg/mL under homogenization at 13,5000 rpm for 3 min using a dispenser (IKA T18 digital ULTRA-TURRAX®, Germany), followed by 3 min sonication using a sonicator (Misonix Sonicator® 3000, USA). Then the sample was cooled down in ice bath with magnetic stirring at 400 rpm until reaching room temperature. To coat NaCas-emulsified SLNs with pectin and generate biopolymeric double layer (DL) SLNs, pectin, which was pre-hydrated overnight and pH adjusted to 6.8, was added into the aqueous phase. To induce electro-deposition of pectin onto the surface of NaCas layer, the mixture was then adjusted to pH 5 before heating at 80 °C for 30 min to reinforce the coating structure. After that, samples were rapidly cooled down to room temperature in ice bath to solidify SLNs. The final sample was then filtrated through a 0.45 μm syringe filter and centrifuged (8,000 × *g*, 30 min) to remove insoluble free curcumin.

2.3. Cross-linking of polymeric coatings

DL-SLNs were cross-linked with two different cross-linkers: GA and EDC/NHS. Briefly, 0.25 mL of GA (25%) or EDC/NHS (1 mg/mL, EDC: NHS = 1:1, w/w) solution was added into 5 mL sample to initiate chemical cross-linking process under continuous stirring for 1 h. After that, the excess cross-linker and unloaded free curcumin were removed through dialysis (10 kDa molecular cutoff) for about 12 h, with water changed every 6 h. The obtained samples were hereafter labelled as G-DL-SLNs (GA cross-linked) and E-DL-SLNs (EDC/NHS cross-linked) and stored in the dark at 4 °C.

2.4. Characterization of SLNs

The particle size, polydispersity index (PDI), and zeta potential of SLNs were measured by Zetasizer Nano ZS at 25 °C (Malvern Instruments Ltd, Worcestershire, UK). Particle size was determined by dynamic light scattering (DLS) at a scattering angel of 173°. Samples were diluted 10 times with ultrapure water to avoid any effects of multiple scattering. PDI, which is a parameter to evaluate the size distribution and homogeneity of the nanoparticles in the dispersion, was recorded together with particle size measurement. Zeta potential was calculated from the electrophoretic mobility of the sample and used to determine the stability of nanoparticles.

For Fourier transform infrared (FTIR) spectrum analysis, liquid samples were cast-dried on aluminum pan in fume hood and then stored in a vacuum desiccator to minimize air exposure. The cast-dried samples were measured by Nicolet iS5 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA). The infrared spectra were collected from the wavenumber of 500–4000 cm^{-1} at a resolution of 4 cm^{-1} and analyzed using OMNIC software version 8.0.

The stability of SLNs in simulated gastric and intestinal conditions was investigated. Briefly, 1 mL of SLNs was added to 9 mL of simulated gastric fluid (pH 2 or 4, with 1 mg/mL pepsin) and the mixture was incubated at 37 °C for 2 h. After that, 1 mL of the above mixture was mixed with 9 mL of simulated intestinal fluid (pH 7.5 with 10 mg/mL pancreatin) followed by incubation at 37 °C for 4 h.