Food Chemistry 244 (2018) 83–92

Contents lists available at ScienceDirect

Food Chemistry

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

Formulation and characterization of novel nanostructured lipid carriers made from beeswax, propolis wax and pomegranate seed oil

Yasamin Soleimanian[a], Sayed Amir Hossein Goli[a,*], Jaleh Varshosaz[b], Sayed Mohammad Sahafi[a]

[a] Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Department of Pharmaceutics, Faculty of Pharmacy and Novel Drug Delivery Systems Research Center, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran

ARTICLE INFO

Keywords:
Nanostructured lipid carriers
Propolis wax
Beeswax
Pomegranate seed oil

ABSTRACT

The objective of this study was to develop functional nanostructured lipid carriers (NLCs) using beeswax (BW), propolis wax (PW) and pomegranate seed oil (PSO). NLCs were prepared by a melt emulsification-ultra sonication technique. The influences of solid lipid composition, surfactant blend concentration (2, 4, and 6% of formulation) and PSO content (10, 30 and 50% of total lipid phase) were investigated. Statistical evaluations revealed that the formulation variables had significant effects on physical properties of NLC. The developed nanocarriers presented particle sizes ranging from 71 to 366 nm, leading to excellent physical stability. The optimum formulations with minimum particle size and high zeta potential value were PW and BW + glycerol behenate samples, containing 10% oil and 6% surfactant. DSC and XRD studies indicated that the addition of oil to the lipid phase could disturb the crystalline order and form lattice defects. TEM observations exhibited spherical morphology of the NLCs.

1. Introduction

Despite having noteworthy health benefits, lipophilic bioactive compounds pose a real challenge to food and pharmaceutical industries due to their poor water solubility and insufficient bio-accessibility which limits the development of formulations deliverable by oral and parenteral routes. Among different strategies to overcome the solubility issue and other problems, such as high melting point, instability, undesired organoleptic properties and interactions with food molecules associated with these components, lipid-based colloidal dispersions have attracted increasing attention (Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013).

Although several lipid colloidal carriers, including emulsions, liposomes and solid lipid nanoparticles (SLNs), have been studied extensively for the past few decades (Muller, Mäder, & Gohla, 2000), a great interest has focussed on nanostructured lipid carriers (NLCs). NLC, consisting of a binary mixture of a solid lipid and a distinct liquid lipid, is known as a delivery system with less-ordered crystalline structure, developed for overcoming the limitations of SLNs. The incorporation of oil into the core of a solid lipid offers several advantages, including higher efficiency and loading capacity, better physical and chemical stability, as well as controlled release after encapsulation of active components (Tamjidi et al., 2013). Yang, Corona, Schubert, Reeder, and Henson (2014) reported that considerably less surfactant was required to produce stable NLC compared to SLN which was attributable to both reduced particle shape change and increased mobility of surfactant molecules.

Lipids are the main ingredients of lipid nanoparticles that influence stability, drug loading capacity and sustained release behaviour of the formulations. Although lipid nanoparticle dispersions, based on a variety of lipid materials, including fatty acids, triglycerides or partial glycerides, have been broadly investigated, quite less attention has been paid to wax-based NLCs. Waxes are defined as simple esters of fatty acids with long chain alcohols (Jenning & Gohla, 2000). Due to differences in chemical composition, glycerides and wax display striking dissimilarities in physical properties and crystal order. Glycerides crystallize in three main subcell packings: hexagonal (α crystal), orthorhombic (β′ crystal) and triclinic (β crystal) and exhibit marked polymorphic transitions. β prime is the dominant polymorph in waxes and the polymorphic transition rate is very low due to the longer chains of fatty acids (Jenning & Gohla, 2000; Wong, Li, Bendayan, Rauth, & Wu, 2007). It has been reported that wax-based lipid nanoparticles could be physically more stable and show excellent particle size distribution (Jenning & Gohla, 2000). Beeswax is commercially the most important natural wax and contains a mixture of several compounds, mainly palmitate, palmitoleate, hydroxypalmitate and oleate

* Corresponding author.
E-mail address: amirgoli@cc.iut.ac.ir (S.A.H. Goli).

http://dx.doi.org/10.1016/j.foodchem.2017.10.010
Received 6 May 2017; Received in revised form 18 September 2017; Accepted 4 October 2017
Available online 05 October 2017

esters of long chain (C30–C32) aliphatic alcohols. However, the composition of beeswax is very variable and depends on the genetic characteristics of the bees (Negri, Marcucci, Salatino, & Salatino, 2000a).

Propolis (bee glue) is a natural resinous substance produced by bees from plant-derived products (resins and gums). Propolis is well known for its antibacterial and antifungal properties. Bees use propolis mainly to repair the cracks of their hives and also as an antibiotic against hive colonization with diseases. In general, propolis is composed of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other different substances, including organic debris (Tosi, Re, Ortega, & Cazzoli, 2007).

In order to provide more therapeutic benefits and improve delivery properties, different healthful oils such as corn oil (Liu & Wu, 2010), olive oil (Lacatusu et al., 2012), fish oil (Lacatusu et al., 2013) and grape seed oil (Lacatusu, Badea, Ovidiu, Bojin, & Meghea, 2012) have been successfully utilized as liquid lipid for NLC production.

Pomegranate seed oil (PSO) is an attractive nutraceutical ingredient possessing an enriched phytochemical composition with high punicic acid and antioxidant activity due to the presence of phenolic compounds, especially ortho-diphenols. Punicic acid has many outstanding functions related to human health, such as anti-carcinogenic, anti-diabetes, anti-hyperlipidemia, anti-obesity and anti-atherosclerotic properties (Cao, Wang, He, Zhang, & Wang, 2014).

Thus, we thought it was worthy of interest to formulate a novel nanostructured lipid carrier based on natural waxes (propolis wax and beeswax) as solid lipid and PSO as liquid oils for oral delivery of food bioactive components. The effects of PSO ratio in the lipid mixture, surfactant concentration, and solid lipid composition were investigated on the particle size and size distribution, surface charge and encapsulation efficiency of the resulting delivery systems. Eventually, thermal, morphological and antioxidant properties, as well as crystalline structure of the optimized NLC formulation, were evaluated.

2. Materials and methods

2.1. Materials

Propolis sample and beeswax (BW, melting point of 65–67 °C) were obtained from Espadana Mokamel Co. (Isfahan, Iran). Propolis wax (PW, melting point of 62–64 °C) was extracted using a Soxhlet apparatus and petroleum ether as solvent. PSO (with fatty acid composition of 78.1 ± 0.16% punicic acid, 7.6 ± 0.04% linoleic acid, 7.12 ± 0.04% oleic acid, 3 ± 0.04% palmitic acid and 2.52 ± 0.02% stearic acid) was provided by a local supplier. Compritol® 888 ATO US/NF (COMP, glyceryl behenate (GB), a mixture of ~15% monoglycerides, 50% diglycerides and 35% triglycerides of behenic acid, melting point of 71–74 °C), was kindly donated by Gattefosse (Saint-Priest, France). Lecithin (L-α-phosphatidylcholine) was purchased from Daejung Co. (Korea). All other chemicals were of analytical grade and supplied by the Merck Co. (Darmstadt, Germany).

2.2. NLC preparation

Nanostructured lipid carriers were formulated and prepared by melt-emulsification, coupled with a ultrasonication technique (Zhu, Zhuang, Luan, Sun, & Cao, 2015). Total lipid phase concentration, consisting of solid fat and liquid oil, was kept constant at 10%. The effects of solid lipid composition [BW, PW and their binary mixture (1:1) with GB], PSO content (10, 30 or 50% of total lipid phase) and concentration of surfactant mixture (2, 4 and 6%) composed of Tween 80 and lecithin (1:0.25 w/w) on NLCs formation and their properties, were investigated. For NLC production, a certain amount of solid lipid phase, PSO and lecithin was melted at 80–85 °C, to form a homogeneous and clear oil phase. The aqueous phase [a solution of Tween 80 in phosphate buffer solution (SPB, 10 mM; pH = 7) containing 0.02% sodium azide], at the same temperature was then added to the molten lipid phase with gentle stirring with a magnetic stirrer (IKA RH Basic 2, Germany) at 300 rpm. The mixture was further dispersed with a high-speed mixer (Ultra-Turrax T25 basic, IKAStaufen, Germany) at 14,000 rpm and 85 °C for 10 min to produce the hot primary emulsion. It was then immediately treated by a probe-type sonicator (Adeeco, Iran, power: 250 W), for 8 min (on for 2 s at intervals of 2 s, 250 W) while maintaining the temperature around the melting point of the lipids. The attained emulsion was cooled in an ice bath for 30 min to recrystallize lipid and form NLC. Analysis of particle size (PS), polydispersity index (PDI), zeta potential and encapsulation efficiency was performed for all samples. Formulations showing the best properties were selected for stability study and further investigations. The freshly prepared NLC formulations were frozen at −80 °C for 24 h, and then lyophilized (ALPHA 2–4, Martin Christ Inc., Osterode, Germany) at −70 °C and 0.001 bar for 48 h for evaluation of thermal, crystalline structure and antioxidant properties.

The formulations of SLNs used in the differential scanning calorimetry (DSC) and X-ray diffraction (XRD) experiments were similar to the optimal composition of NLC except that the liquid oil (PSO) was replaced by respective solid lipids. SLNs were prepared by the same procedure as described previously.

2.3. Determination of PS, PDI and zeta potential

The analysis of PS (as Z-average) and PDI of NLC formulations was performed by photon correlation spectroscopy (PCS), using a Zetasizer (NanoSizer 3000, Malvern Instruments, Malvern, UK) at an angle of 90° in 0.01 m width cells at 25.0 ± 0.1 °C. The electrical charge (zeta potential) of lipid nanoparticles was determined in a capillary cell, using the same instrument which utilized the Helmholtz–Smoluchowski equation to convert the measured particle electrophoretic mobility into zeta potential. Prior to analysis, NLCs were diluted 1:100 with the same buffer solution to avoid multiple scattering effects.

2.4. Determination of entrapment efficiency for PSO

Entrapment efficiency (EE) was determined to assess the extent of PSO incorporation in the nanoparticles, using a centrifugation method. Briefly, a 0.5 ml of NLC dispersion was placed in a centrifugal filter tube with a cut-off of 10 kDa (Millipore, Bedford, MA, USA) and ultracentrifuged at 10,000 rpm for 10 min. The NLCs along with the encapsulated PSO remained in the outer chamber whereas the aqueous dispersion medium, containing the free unloaded oil, moved to the sample recovery chamber through the filter membrane. After separation, the amount of free oil was estimated by measuring the concentration of punicic acid (the most important bioactive compound in pomegranate seed oil) in the dispersion medium by gas chromatography. An agilent model 6890N gas chromatograph, equipped with flam ionization detector (FID) and HP-88 capillary column (100 m × 250 μm), was used. Fatty acid methyl esters were prepared by methylation with sodium methoxide (0.5 N). Injection (2 μl) was performed in the splitless mode. Nitrogen was the carrier gas with a flow rate of 1.1 ml/min. The column temperature was programmed to be increased from 150 °C (held for 1 min) to 190 °C at 5 °C min^{-1} held for 2 min, up to 240 °C at 5 °C min^{-1}, and held for 8 min. Injector and detector temperature were 150 °C and 250 °C, respectively.

The PSO entrapment efficiency was subsequently calculated from the following equations:

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of PSO entrapped in NLCs}}{\text{Theoretical total amount of PSO added to NLCs}} \times 100$$

The amount of PSO entrapped was calculated by subtracting the amount of free PSO from the theoretical amount of PSO added to NLCs (Ali, El-Sayed, Sylvester, & Nazzal, 2010).