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Design and characterization of astaxanthin-loaded nanostructured lipid carriers

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ABSTRACT

This study aimed to select proper lipids for designing astaxanthin-loaded nanostructured lipid carriers (astaxanthin-NLC), and then to evaluate the influence of formulation composition on astaxanthin-NLC characteristics and optimize the formulation by response surface methodology. Tween 80 and lecithin were employed as emulsifier, and oleic acid and glyceryl behenate were selected as appropriate lipids. Astaxanthin-NLC (with 5% lipid phase) were prepared by melt emulsification–sonication technique and stored at ≈19 °C for 25 days. The lipid phase to Tween 80 ratio (LTR) and oleic acid content of the lipid mixture (OCL), as independent variables, had significant effects on physical characteristics of fresh formulations and their storage stability. The optimum formulation of astaxanthin-NLC (with OCL: 22.4% and LTR: 1.8) had greater values of particle size, polydispersity index and ζ-potential than the astaxanthin-free formulation. X-ray diffraction and thermal analyses exhibited a new crystalline lattice with lower crystallinity for the optimum formulation compared to glyceryl behenate.
Industrial relevance: Evidence that carotenoids have many valuable physiological functions in human body persuades the manufacturers to insert them into foods and beverages. However, fortification of aqueous-based foods with carotenoids is currently limited due to their poor water-solubility, low bioavailability, and chemical instability. NLC are O/W nanoemulsions in which a major portion of the lipid phase is constituted by solid lipid, yield high encapsulation efficiency by effective-immobilization of the encapsulated lipophilic compounds, and can therefore improve their utilization, bioavailability and stability in fat-free and low-fat foods and transparent/opaque beverages. In order to design an appropriate formulation of astaxanthin-NLC, we selected a suitable lipid mixture, and investigated the effect of contents of oil and surfactant on characteristics of astaxanthin-NLC. These results provide useful information for designing proper NLC for delivery of astaxanthin and other lipophilic nutraceuticals into foods, transparent beverages and pharmaceutical products.

1. Introduction

With growing consumer demands for functional foods, manufacturers are increasingly trying to enrich foods with health-promoting compounds. Astaxanthin, a type of carotenoid, exhibits a stronger antioxidant activity than vitamin E and β-carotene (Naguib, 2000), and as a nutraceutical possesses many valuable physiological functions, such as general enhancement of immune responses, and prevention of oxidative stress, cardiovascular diseases, certain forms of cancer, cataract development, macular degeneration, inflammation, and *Helicobacter pylori* infection (Higuera-Ciapara, Félix-Valenzuela, & Goycoolea, 2006). However, the utilization of astaxanthin in food, beverage, and pharmaceutical products is currently limited due to its low bioavailability, poor water-solubility, high melting point, and instability under adverse conditions (e.g., acidic environments, heat, light, transition metal ions, singlet oxygen, and free radicals).

To improve the utilization or bioavailability of astaxanthin, different strategies have been investigated, including structural modification (Nakao, Sumida, Katano, & Fukami, 2008), molecular inclusion (Yuana, Jinb, & Xu, 2012), nanocrystallization (Anarjan & Tan, 2013), microencapsulation (Bustos-Garza, Yáñez-Fernández, & Barragán-Huerta, 2013), and incorporation into liposomes (Peng, Chang, Peng, & Chyau, 2010) and O/W emulsions (Ribeiro, Rico, Badolato, & Schubert, 2005) or nanoemulsions (Meor Mohd Affandi, Julianto, & Majeed, 2011).

At the beginning of the 1990s, solid lipid nanoparticles (SLN) were introduced and designed to combine the advantages of polymeric particles, liposomes, and emulsions (Hentschel, Gramdorf, Müller, & Kurz, 2008). SLN are O/W nano-/micro-emulsions in which the lipid phase is fully crystallized and has a highly-ordered crystalline structure at room/body temperature. SLN immobilize the incorporated active compound within the fat lattice (not always), and may increase its stability; they provide the possibility of controlled release, and have high encapsulation

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efficiency (Mehnert & Mäder, 2012). However, main deficiencies of SLN are low drug loading capacity, and drug expulsion after polymorphic transition of the lipid core (i.e., α-crystal → β′-crystal → β-crystal) during storage (Mehnert & Mäder, 2012; Müller, Radtke, & Wissing, 2002), that make SLN improper for many food applications. Nanostructured lipid carriers (NLC) or oil loaded-SLN are modified SLN in which the lipid phase consists of a biocompatible mixture of solid and liquid lipids (Müller et al., 2002), were designed to dispel the limitations of SLN by R. H. Müller et al. at the end of the 1990s. NLC have an amorphous solid structure or a less-ordered crystalline structure which isn't fully crystallized. The incorporation of oil into the core of a solid lipid leads to a higher loading capacity and controlled drug release as the drug is dissolved in the oil and simultaneously encapsulated in the solid lipid (Varshosaz, Eskandari, & Tabakhian, 2010). With respect to the nanoscale of dispersed phase, SLN and NLC (as well as nanoemulsions) scatter light weakly and so can be incorporated into optically transparent products, have high *Brownian motion* and may therefore be very stable to particle aggregation and gravitational separation, and they may increase the bioavailability of incorporated lipophilic compounds.

From the above, it can be concluded that NLC have some advantages in certain circumstances compared to other colloidal carriers. NLC are widely used to encapsulate lipophilic drugs in pharmaceutical researches. NLC can be produced from food-grade or GRAS ingredients on industrial scale, and hot homogenization technique is the most feasible method for production of these nanoparticles (Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). Currently, NLC containing active compounds are increasingly introduced as ingredients for food applications (Fathi, Varshosaz, Mohebbi, & Shahidi, 2013; Hejri, Khosravi, Gharanjig, & Hejazi, 2013; Hentschel et al., 2008; Liu, Wang, & Xia, 2012; Liu & Wu, 2010). Medium chain triglycerides and oleic acid (as liquid lipids), stearic acid, glycerol monostearate, glyceryl palmitostearate and glyceryl behenate (as solid lipids), and Tween 80 and lecithin (as emulsifiers), are the most commonly used food-grade ingredients in NLC formulation (Tamjidi et al., 2013). Since the lipid phase has an important effect on NLC features (e.g., loading capacity and physical stability), the first aim of this study was to select an appropriate lipid mixture for entrapment of astaxanthin in NLC. Moreover, no information is available concerning the influence of formulation composition on physicochemical characteristics and storage stability of astaxanthin-loaded NLC (astaxanthin-NLC). This point is of particular interest, since changes in physicochemical properties of astaxanthin-NLC may affect the utilization of this active ingredient in food formulations, as well as the biological fate of the nanoparticles. Therefore, the second aim of this study was to apply response surface methodology (RSM) to investigate the influence of concentrations of oil and surfactant on physical and chemical characteristics of astaxanthin-NLC, and to develop an optimum formulation of astaxanthin-NLC. Finally, the characteristics of the optimum formulation were further investigated by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). These nanoparticles will be suitable for fortification of clear beverages, as well as other non-fat or low fat drinks and foods.

2. Materials and methods

2.1. Materials

An astaxanthin oleoresin (astaxanthin content: 40%), extracted from the alga *Haematococcus pluvialis*, was purchased from Wuhan Ereli Import & Export Co. Ltd. (Wuhan, China). Soybean oil (Naz Vegetable Oil Co., Isfahan, Iran), corn oil (Zareentalia, Kasisuri Co. Ltd., Thailand) and refined olive oil (Zafrin Co., Manjil, Iran) were purchased from a local supermarket. Compritol® 888 ATO (glyceryl behenate; GB) and glycerol monostearate (GMS) were purchased from Gattefossé Co. (Saint-Priest, France) and Condea Co. (Hamburg, Germany) respectively. Sodium phosphate monobasic, sodium phosphate dibasic, stearic acid (SA), oleic acid (OA), dichloromethane, methanol, Tween 20 and Tween 80 were purchased from Merck Co. (Darmstadt, Germany). Sodium azide and Lecithin (L-α-phosphatidylcholine, Type IV-S ≥ 30%, TLC) were purchased from Sigma Co. (St. Louis, MO, USA). Deionized double-distilled water was used.

2.2. Methods

2.2.1. Lipid screening

2.2.1.1. Solubility of astaxanthin in liquid lipids. To investigate the solubility of astaxanthin in liquid lipids, a weight of ≈15 mg of astaxanthin was mixed with 1000 μL of the tested oil within a glass vial, and 3 mL of dichloromethane was added until a homogeneous mixture was obtained. After dichloromethane evaporation with N_2 flushing, the suspensions were stored at room temperature (RT; 19 ± 2 °C) and darkness for 24 h to equilibrium. Samples were then centrifuged (4000 rpm, 15 min), and 250 μL of the supernatant was diluted with dichloromethane to 25 mL. The amount of astaxanthin oleoresin in this solution was then measured using a UV–visible spectrometer (Secomam, Alès, France) at 484 nm. The liquid oil that dissolved the highest amount of astaxanthin was the only one selected for use in the test of "miscibility".

2.2.1.2. Miscibility of liquid lipid with solid lipid. To determine the miscibility of the selected liquid lipid with solid lipids, binary mixtures of liquid and solid lipids in ratio of 3:7 were weighed into glass vials, and heated up to 85 °C. The mixtures were checked visually after 24 h solidification at RT. Those which created a single phase without oil droplets were the only ones selected for use in the test of "physical stability of NLC".

2.2.1.3. Physical stability of NLC. NLC formulations consisting of 1 wt.% lipid phase (1.0 mg lecithin + 59.7 mg liquid lipid + 139.3 mg solid lipid) and 99 wt.% aqueous phase (0.396 g Tween 80 + 19.404 g PBS (phosphate buffer solution (5 mM; pH = 7) containing 0.02 wt.% sodium azide)) were produced as the procedure described in Section 2.2.2. The NLC formulations were checked during storage at RT, and only the solid lipid(s) which yielded transparent NLC and stable nanoparticles were selected for preparation of astaxanthin-NLC.

2.2.2. Preparation of astaxanthin-NLC

Based on the results of lipid screening (Table 1), the OA–GB mixture was selected as an appropriate lipid blend for designing astaxanthin-NLC. Fixed amounts of lipid phase, astaxanthin and lecithin were used for preparation of astaxanthin-NLC formulations, and lipid phase to Tween 80 ratio (LTR) and OA content of the lipid mixture (OCL) were selected as independent variables. Astaxanthin-NLC formulations (Table 2), consisting of 5 wt.% lipid phase (5 mg lecithin + 20 mg astaxanthin + 975 mg lipids) and 95 wt.% aqueous phase (a solution of Tween 80 in PBS; 19 g), were prepared by melt-emulsification and ultra-sonication techniques. Briefly, a volume of 2 mL dichloromethane was added to the lipid phase within a capped flask and warmed to 35 °C until a clear homogeneous phase was obtained. Then, the organic solvent was removed under N_2 flushing, and both lipid and aqueous phases were heated to 78 ± 1 °C, simultaneously. Subsequently, the aqueous phase was added to the lipid phase, and the mixture was stirred at 2000 rpm for 3 min at the same temperature. The mixture was then transferred into a bath sonicator (Powersonic 505; Hwashin Technology, Seoul, Korea) with 25 °C, for 15 min. The suspension was warmed to 78 °C again, and homogenized further using a probe-type sonicator (Bandelin, Berlin, Germany; amplitude: 50%; power: 100 W; probe: TT13) for 4 min with a 2-s pulse-on period and a 2-s pulse-off period. Finally, the hot O/W nanoemulsion was cooled down to 10 °C in an ice bath under continuous stirring. All samples were stored in capped glass tubes in the dark at RT, and analysis of particle size (PS), polydispersity index (PDI), ζ-potential and astaxanthin content was performed after 0 and 25 days.