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Vitamin A palmitate-bearing nanoliposomes: Preparation and characterization

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ABSTRACT

The encapsulation of fat soluble vitamins in nanoliposomes seems to be an effective method for protecting them from light, oxygen and chemical degradation. In this study, nanoliposomes containing vitamin A palmitate were prepared from different concentrations of lecithin–cholesterol (60:0, 50:10, 40:20 and 30:30 mg) by thin-film hydration–sonication method. Fourier transform infrared spectra (FTIR) were utilized to study the possible bioactive–lipid complex formation and the results indicated that the complex between vitamin A and liposomes were formed by physical interaction. Particle size, morphology, encapsulation efficiency and physical stability tests were carried out to determine the physicochemical properties of the resulted vitamin A-bearing liposomes. The size of particles were in the range of 76–115 nm and the particle size distributions were monomodular (span=0.6–0.88). The results showed that using the highest cholesterol concentration for preparing of liposomes containing vitamin A palmitate induces lower encapsulation efficiency and 50/10 mg lecithin–cholesterol concentration was used for preparation of optimum formulation of vitamin A palmitate-loaded nanoliposomes with mean size of about 76 nm and monomodular size distribution (span=0.74) and the encapsulation efficiency was 15.8%.

1. Introduction

The desirable reduction of fat in a healthy diet creates difficulties in providing the required amounts of essential hydrophobic nutraceuticals, like fat-soluble vitamins, antioxidants, phytosterols and polyunsaturated fatty acids. The enrichment of foods with hydrophobic bioactive ingredients and development of new functional foods are promising strategies for solving this problem and promoting health of wide populations. Having in mind the low solubility of fat soluble vitamins such as vitamin A in aqueous phase, they would have unfavorable effects on the flavor, odor and transparency of beverages. In addition, their instability in the presence of light, free radicals and heavy metals are main problems in fortification of foods with this type of vitamins (Fathi, Mozafari, & Mohebbi, 2012; Gonnet, Lethuaut, & Boury, 2010). Encapsulation of this vitamin has been reported as an appropriate solution for these problems. In recent years, scientists have focused on the using of nano-particle as a carrier in order to protect bioactive compounds from oxidation and isomerization reactions and to improve their bio availability, shelf life, disperse ability, stability and controlled release in specific doses and in specific sites (Rao & McClements, 2012; Yang, Marshall-Breton, Leser, Sher, & McClements, 2012). Lipid-based nano-carriers in food industry comprise nano-spheres, nanoliposomes, nanoemulsions, niosomes, phythosomes and lipid nano-particles with solid structures (Das & Chaudhury, 2011; Fathi et al., 2012; Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). One of the most common lipid based nano-carriers is nano-liposome which is colloidal carrier with vesicular structure of an aqueous core enclosed by a hydrophobic lipid bilayer, which is basically created by the phospholipids (Bouarab et al., 2014; Fang & Bhandari, 2010; Mozafari et al., 2006). Phospholipids are components of cell membranes containing a hydrophilic head and one or more hydrophobic tails and they have antioxidant activity. This structure enables them to encapsulate both hydrophilic and lipophilic bioactive materials. The possibility of production in industrial scale, lower gravitational separation

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and relatively high encapsulation efficiency are other advantages of liposomes in comparison to some other delivery systems. In the food industry, for a given industrial application, membrane stability and structure are two important factors when designing liposomes. Cholesterol addition to phospholipids increases liposome stability by reducing the permeability of the lipid membrane and stiffening the membrane.

Food-grade nanoliposomes are being increasingly used in food and beverage industries to encapsulate and deliver hydrophilic or lipophilic functional components such as vitamin E and vitamin C (Marsanasco, Márquez, Wagner, Alonso, & Chiaramoni, 2011), ascorbic acid (Alexander, Lopez, Fang, & Corredig, 2012) nutraceuticals (Yao, McClements, & Xiao, 2015), essential ω_3 fatty acids (Eckert et al., 2011; Rasti, Jinap, Mozafari, & Yazid, 2012), medium chain fatty acids- vitamin C (Li et al., 2015), cinnamon oil (Wu et al., 2015), polyphenols including catechins (Gadkari & Balaraman, 2015; Lu, Li, & Jiang, 2011), curcumin (Hasan et al., 2014); resveratrol (Mignet, Seguin, & Chabot, 2013), hibiscus extract (Gibis, Zeeb, & Weiss, 2014).

Ko and Lee (2013) showed that film hydration method can supply retinol loaded nanoliposomes with encapsulation efficiency of more than 99%. Hasan et al. (2014) studied the encapsulation of curcumin in nanoliposomes based on soy, salmon and rapeseed lecithin to achieve an improved bioavailability of a poorly absorbed hydrophobic compound. Bouarab et al. (2014) also investigated the effect of type and composition of phospholipids on the production of liposomes based on soy lecithin, salmon and docosahexaenoic acid phospholipids (PL-DHA) in order to encapsulate cinamic acid. Researches about nano-liposomes show that they are appropriate carrier for fortification of food stuffs with hydrophobic bioactive compounds.

The principal aim of this study was to prepare vitamin A encapsulated nano-liposome formulations as a mean to improve its aqueous dispensability and to study the effect of lecithin–cholesterol concentrations on the particle size, encapsulation efficiency (EE) and physical stability of vitamin A loaded nanoliposomes to get the optimized formulation. According to our knowledge, there is not any study about producing food-grade liposomes which makes this study superior to others. In dead, the production of nanoliposomes to design functional food products requires the use of food-grade ingredients which was the main advantages of this research over others.

2. Materials and methods

2.1. Materials

Phospholipid (L-α-granular Lecithin) with purity of 99% was obtained from Across (USA). Cholesterol with 95% purity was supplied by Merck (Germany). Other chemicals were analytical grade and procured from Sigma (Merck Chemical Co. Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of vitamin A palmitate loaded nano-liposome

Vitamin A palmitate encapsulated nano-liposomes were prepared by thin layer hydration method with slight modification and appropriate concentrations of lecithin–cholesterol (Jesorka & Orwar, 2008). Lecithin and cholesterol were dissolved in absolute ethanol and then they were dried with vacuum evaporator. Prepared dried lipid films were hydrated by aqueous phase (deionized water) and resultant suspension was mixed for some time. Due to the existence of water inside the lipid film, osmotic pressure runs the water into the bilayer membrane and causes separation of lipid film and in this way liposomes are produced. In this method, mixture of multilamellar vesicles (MLVs) and small unilamellar vesicles (SUVs) liposomes were produced. Reduction in the particle size of prepared liposomes was done by ultra sound probe sonicator. Sonication method is widely used in producing SUV liposomes, but it may cause non uniform particles. Producing high energy during sonication process is one of the main disadvantages of ultrasound probe sonication. Hence, in the size reduction step, prepared liposomes were putted in an ice bath in order to protect the structure of liposomes from destruction. In using probe sonication, 1 min time breaks have been applied between sonication times in order to inhibit membrane fraction. The place of the sonicator probe inside the solution is another important parameter in the size reduction. Therefore, probe was placed near the surface of solution because in this way all particles can receive ultrasound waves equally in order to reach nano-scale particles. This procedure causes homogenous and monomodular particle size distribution. In this study, optimal formulation for producing vitamin A-loaded nano-liposome was investigated by changing the concentration of the lecithin/cholesterol as listed in Table 1.

Table 1
Composition of vitamin A palmitate-loaded nanoliposomes.

Formulation	Lecithin (mgr)	Cholestrol (mgr)	Water (ml)
F1	60	0	12
F2	50	10	12
F3	40	20	12
F4	30	30	12

2.2.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to determine any active material–excipient interaction at functional groups. The infrared spectra were scanned on a FTIR spectrophotometer (Shimadzo, Japan), at 4 cm^{-1} resolution in frequency range between 4000 and 400 cm^{-1} using KBr Pellet method with sample to KBr ratio of 10:100.

2.2.3. Particle size and size distribution

The average diameter and span value of the particles were determined using particle size analyzer (Wing SALD 2101, Shimadzo, Japan), at 25 °C. The dispersion of nanoliposomes was diluted with distilled water until suitable obscuration to prevent multiple scattering phenomena because of inters particle interaction. The average particle size was calculated according to the average volume diameter or DeBroukere mean in the Eq (1):

$$\bar{D}[4,3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

The span value is an index helpful to evaluate the particle size distribution and calculated applying the following Eq. (2):

$$\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (2)$$

D(90%): describes diameter where 90% of the distribution has a smaller particle size and 10% has a larger particle size.

D(10%): describes diameter where 10% of the distribution has a smaller particle size and 90% has a larger particle size.

D(50%): describes diameter where 50% of the distribution has a smaller particle size and 50% has a larger particle size (Hamishehkar et al., 2009).

2.2.4. Morphology characterization

Morphology of the nano-carriers was observed using transmission electron microscopy (Zeiss-Leo 906 TEM (Germany)).