Iranian Food Science and Technology Research Journal
Vol. 12, No. 2, June. July. 2016, p. 261- 275

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۲، شماره ۲، خرداد-تیر ۱۳۹۵ ص.۲۷۵-۲۶۱

Vitamin A palimitate-loaded nanoliposomes: study of particle size, zeta potential, efficiency and stability of encapsulation

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Received: 2014.08.17
Accepted: 2015.05.03

Introduction: The encapsulation of hydrophobic nutraceutical compounds such as fat soluble vitamins in nanoliposomes is a potentially effective way to protect them from from light, oxygen and chemical degradation during the maintenance. One of the potential benefits of liposomal structures is encapsulation of three water-soluble, fat-soluble and amphiphilic compounds and use of natural food ingredients such as lecithin with beneficial effects, in their production. In this study, the effect of lecithin-cholesterol concentrations on particle size, particle size distribution, encapsulation efficiency (EE) and physical stability of vitamin A palmitate loaded nanoliposome during the storage time were explored to get the optimized formulation

Materials and method:

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).

Methods:

Nanoliposomes were prepared from different concentrations of lecithin–cholesterol (60:0, 50:10, 40:20 and 30:30 mg) by thin-film hydration–sonication method. Lecithin and cholesterol were dissolved in absolute ethanol and then dried with vacuum evaporator. Prepared dried lipid film hydrated by aqueous phase. The resultant suspension was mixed for some time (Hydration-dehydration). Due to existence of water inside the lipid film, osmotic pressure runs the water into bilayer membrane and causes separation of lipid film and then liposomes were produced. In this method, mixture of Multilamellar Vesicles (MLVs) and Small Unilamellar Vesicles (SUVs) liposomes were produced. Reduction in particle sizes of prepared liposomes was done by ultra sound probe sonicator. The average diameter and span value of the particles were determined using particle size analyzer (Wing SALD 2101, Shimadzo, Japan), at 25°C and was calculated according to the DeBroukere mean in the Equation (1):

$$\overline{D}[4,3] = \frac{\sum n_i d_i^{\,4}}{\sum n_i d_i^{\,3}}$$

The span value is an index helpful to evaluate the particle size distribution and calculated applying the following Equation:

$$Span = \frac{D_{90\%} - D_{10\%}}{D_{50\%}}$$

Morphology of the nano-carriers was observed using trans- mission electron microscopy (Zeiss-Leo 906 TEM (Germany). To determine the zeta potential of nano liposomes loaded vitamin A, Zeta siyzer device (Nano-ZS -Malvern England) was used at 25°C temperature. Estimation of encapsulated vitamin in nanoliposomes (%EE) was carried out using HPLC (Knauer,Germany) equipped with a UV detector, C-18(10 mm 25mm_4.6 mm) column and acetonitrile– methanol (70:30%,v/v) as mobile phase and was calculated using the below equation

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$$\%EE = \frac{\text{Encapsulated Vitamin A}}{\text{Total Vitamin A}} \times 100$$

The stability of vitaminA loaded-nanoliposomes was assessed by determining the average particle size at 4 °C over storage time and studying the leak out of the vitamin from the nanoliposomes after one month(1,7, 15and 30days)of storage at 4 °C by the below equation

$$\%\text{Stability} = \frac{\text{Remained Vitamin A}}{\text{Initial encapsulated Vitamin A}} \times 100$$

Results and Discussion: Results showed use of sonication in completion thin-film hydration method, induced production of monomodular nanoliposomes with uniform distribution The particle size was in the range of 76-115nm and particle size distribution was monomodular (span= 0.6- 0.88). In agreement with particle size results, TEM image showed that the vesicles are in the form of small unilamellar vesicles by bilayer nature. In all concentrations of lecithin-cholesterol, obtained EE was low and by increasing the lecithin concentration, loading capacity of nano liposomes increased. By increasing the lecithin concentration, more vesicles are produced which causes increase in internal volume of liposomes and bio actives concentrate, consequently loading capacity of nano liposomes increased. By tightening of the membrane by cholesterol, entrapment efficiency of hydrophobic active compounds such as vitamin A palmitate reduces. Also probably existence of cholesterol in liposome membrane inhibits of rupture and changes in liposome membrane. Overall, increasing the ratio of cholesterol /lecithin had no significant effect on particle size but decreased encapsulation efficiency of vitamin A palmitate to 10.23%. Addition of cholesterol effected on stability of the particle size of nanoliposomes and also led to reduction encapsulation efficiency of vitamin A palmitate. Incorporation of cholesterol and vitamin A palmitate into the liposome structure was increased the zeta potential from -29 to -58 mv and improved electrostatic stability. 50-10 mg ratio of lecithin-cholesterol concentration was used for preparation of optimum formulation of nanoliposome by monomodular and small size distribution (76 nm, span=0.74) and encapsulation efficiency (15.8%). Stability of vitamin A in nano liposome with 50-10 mg lecithin-cholesterol, was almost low (32% reduction during storage time), may be due to increasing fluidity of membrane. Permeability of vitamin A into phospholipid chains causes reorientation of acyl chains which leads to fluidity of membrane and exit active compound from nano carrier and more its hydrolytic degradation and oxidation. While the use of thin film hydration method using ultrasonic waves, is successful way in producing nanoscale particles of vitamin A palmitate nanoliposomes that are stable and decrease over time, but due to low efficiency and low sustainability of encapsulation, use of other nanocarriers for encapsulating of vitamin A palmitate is recommended

Keywords: Nanoliposome, Vitamin A Palmitate, Encapsulation, Physical Stability.