Development and evaluation of a novel membrane mimic system (PC/CHOL liposome- β-Lg formulation) for vitamin E delivery

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Introduction

Vitamin E (VE) or α-tocopherol (a-toc) is the major fat soluble antioxidant in the human body, since it protects cellular membranes and other lipids against oxidative damage as it reduces the formation of hydroperoxides (1). VE is easily oxidized in the air molecule, so must be protected from pro-oxidant elements which could affect its chemical integrity and decrease its physiological benefits (2)(3). Encapsulation constitutes a promising approach to preserve VE native properties over time and increase its concentration in aqueous media. Microencapsulation reduces reactivity with the environment (water, oxygen, light), decreases the evaporation or the transfer rate to the outside environment, promotes handling ability, masks taste and enhances dilution to achieve a uniform distribution in the final product when used in very small amounts(4).Liposomes have been studied as sustained delivery systems. β-lactoglobulin (β-Lg) has been studied extensively and has shown to bind a variety of hydrophobic molecules including fat soluble vitamins in vitro (5). The aim of this study was the preparation and characterization of β-lg-liposome formulation and the determination of VE encapsulation efficiency, in order to develop a new more efficient carrier for VE in aqueous media.

Materials and Methods

DRV (Dehydrated Rehydrated Vehicles) liposomes encapsulating β-Lg and a-toc were prepared. Mixtures of lipids (PC/Chol in molar ratio 2/1) and a-toc (1 mg) were dissolved in absolute ethanol which then was evaporated until a thin film formed. The film was hydrated with TBS (pH 7.40). Next, SUV (Small Unilamellar Vehicles) liposomes were prepared by probe sonication and then 5 ml of SUV dispersion was mixed with 5 ml of a β-Lg solution (1 mg/ml). The mixture was freeze-dried and then rehydrated with a controlled stepwise protocol. The final dispersion was freeze-dried again and stored in -40°C until analyzed.

Encapsulation Efficiency:

HPLC Analysis. HPLC provides a convenient method for quantification and characterization purposes of a-toc. The analysis was carried out as reported previously by Lalas et al. (6).

SDS polyacrylamide gel electrophoresis. The presence of β-LG in the liposome fractions was evaluated by SDS-PAGE electrophoresis.

Evaluation of liposome's stability during storage. The samples were diluted in ddH_2O and their stability was evaluated after 24, 48 and 72 h by measuring the turbidity by fluorescence spectroscopy. A Shimadzu 1501 spectrofluorimeter equipped with a thermostatic cuvette holder was used with an excitation and emission wavelength of 450 nm in a 1 cm cuvette at room temperature 25°C.

Observation of liposomes. The liposome suspension was diluted in ddH_2O and the membrane-reorganizing effect of β-Lg and a-toc was visualized by phase contrast microscopy. The pictures were taken in oil immersion with an optical magnification of 100x.

Results and Discussion

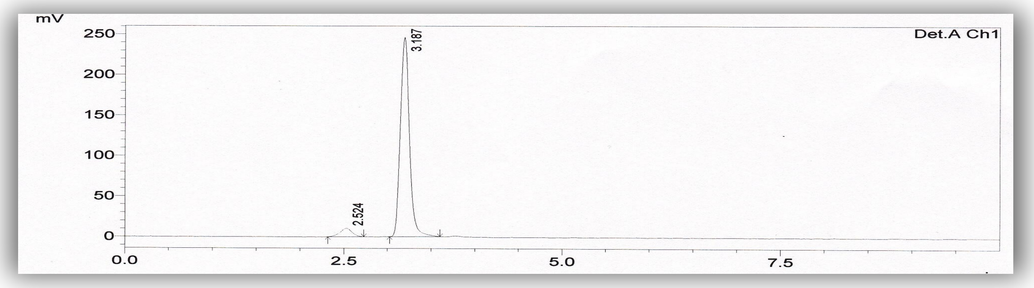
Encapsulation efficiency (EE)

Trapping efficiencies for a-toc in SUV liposomes with PC/Cholesterol and PC/Cholesterol/β-Lg was determined by HPLC (Fig.1). The results are recorded in the Table 1.

Table 1

Sample	Encapsulation %
Liposomes with a-toc	59.42 ± 2.31
Liposomes with a-toc + β-Lg	96.59 ± 1.52

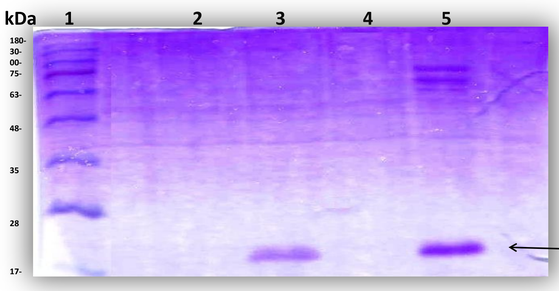
Figure 1



The % EE of a-toc in liposomes in absence and presence of β-Lg was 59.42% (± 2.31%) and 96,59% (± 1.52%), respectively. The percentage of encapsulation of a-toc into liposomes/β-Lg was higher compared to the percentage of a-toc into liposomes. This may be explained by the ability of β-Lg to act as an intracellular transporter of lipids (fatty acids) (7), since their transportation is enabled by the interaction between lipids binding proteins and lipid vesicles via either a diffusion controlled mechanism or a direct collision of the proteins with the membrane (8).

The presence of β-Lg into liposomes was evaluated by SDS-PAGE electrophoresis after disrupting the liposomes with methanol (Fig 2).

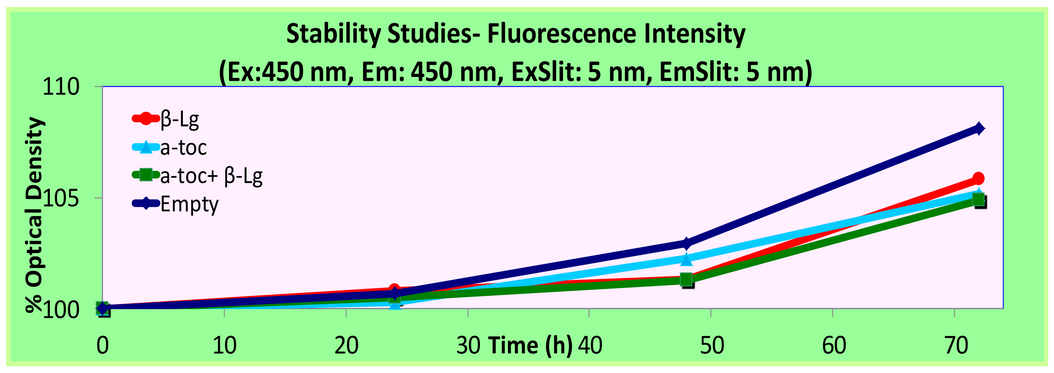
Figure 2



Evaluation of liposome's stability after freeze-drying

Turbidity measurements are often used as a tool to determine the stability of the scattering particles. In the case of liposomes, an increase in turbidity is interpreted as an increase in the aggregation of the vesicles due to the decrease of their stability. Studies have shown that a-toc has played an important role in the morphology and structure of the PC membrane (9).

Figure 3

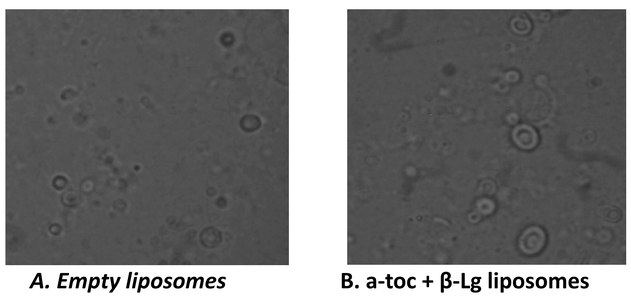


The results (Fig 3) showed that the liposomes contained a-toc and β-Lg had an improved stability behavior comparing with the empty liposomes or the liposome samples contained only β-Lg or a-toc, respectively.

Observation of liposomes

Most of the initial liposomes were unilamellar and exhibited internal structure (Fig. 4-A). In the presence of b-Lg and VE the liposomes appeared bigger and irregularly shaped (Fig. 4-B). An expanded view of SUV in the presence of β-Lg and VE is presented in Fig. 3-B. The edges of the liposomes appeared as relatively thick dark bands because of the difference in the refractive indices of the internal and the external media.

Figure 4



A. Empty liposomes B. a-toc + β-Lg liposomes

Conclusions

The aim of this study was the preparation and characterization of β-lactoglobulin-liposome formulation and the determination of VE encapsulation efficiency, in order to develop a new more efficient carrier for VE in aqueous media.

- A high encapsulation percentage of a-toc was observed in liposomes + β-Lg
- A very promising stability behavior of the liposomes containing β-Lg + a-toc was observed.

The newly synthesized membrane mimic system showed a very promising behaviour and further investigations should be carried out.

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