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Vitamin delivery: Carriers based on nanoliposomes produced via ultrasonic irradiation

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ABSTRACT

In recent years much attention has been focused on using lipid carriers as potential delivery systems for bioactive molecules due to their favorable properties such as high biocompatibility, size and composition versatility. In this paper formulation, preparation and characterization of liposomes, a class of powerfully versatile lipidic carriers, produced by means of an innovative ultrasound-assisted approach based on the thin-film hydration method, are presented and discussed. The main aim of this study is to obtain nanostructures (Small Unilamellar Vesicles, SUVs), less than 100 nm in size, loaded with different vitamins (B12, tocopherol and ergocalciferol), starting from lipidic microstructures (Multilamellar Large Vesicles, MLVs). Suitable formulations, sonication protocols and nanoliposomes were pointed out. SUVs with diameter size ranging from 40 nm to 51 nm were achieved starting from MLVs with a diameter range of 2.9–5.7 μm. Starting from MLVs with higher encapsulation efficiency for all kind of vitamins, SUVs with an encapsulation efficiency of 56% for vitamin B12, 76% for α-tocopherol and 57% for ergocalciferol were obtained. Stability tests have shown that the used lipid composition allows to keep intact the nanovesicles and their content for more than 10 days if incubated at simulated extracellular environment conditions.

1. Introduction

In recent years, biocompatible materials for encapsulating health-enhancing ingredients and their controlled release have been used in numerous applications in food and pharmaceutical industries (Alexander, Acero Lopez, Fang, & Corredig, 2012; Burgain, Gaiani, Cailliez-Grimal, Jeandel, & Scher, 2013; Chau, Wu, & Yen, 2007; Laos, Lõugas, Mändmets, & Vokk, 2007; López-Rubio & Lagaron, 2012). The possibility of producing nanostructured materials for controlling the bioavailability of nutrient with strong biological activity has great industrial relevance for optimizing product formulation (food supplements, vitamin dosage systems) and processing thus contributing to people's health and well-being (Letícia Marques deAssis, Machado, da Motta, Costa, & de Souza-Soares, 2014; Letícia Marques deAssis, Zavareze, Prentice-Hernández, & de Souza-Soares, 2012; Emamifar, Kadivar, Shahedi, & Soleimanian-Zad, 2010; Lopez-Rubio, Gavara, & Lagaron, 2006; Sanguansri & Augustin, 2006; Soottitantawat et al., 2005).

The liposome-based drug delivery system is an innovative and promising technology for controlling the release of bioactive agents and assuring the efficient delivery of a large number of natural products with functional properties and drugs (Ulrich, 2002). Recently, much attention has been paid to using the nanoliposomes as carriers of bioactive ingredients in food and pharmaceutical dosage systems for encapsulating antioxidants (M. A. Azevedo, M. Â. P. R. Cerqueira, & A. A. Vicente, 2013; Gupta & Abu-Ghannam, 2011; He, Guo, Feng, & Mao, 2013; Isailović et al., 2013; Laouini, Andrieu, Vecellio, Fessi, & Charcosset, 2014; Liu, Ye, Liu, Liu, & Singh, 2013; Marsanasco, Márquez, Wagner, del V. Alonso, & Chiaramoni, 2011; Toniazzo et al., 2014). In fact using liposomes as antioxidant vitamin carriers represents a promising approach for developing new functional food formulations and solving the vitamin stability issues which strongly hinder their application (Letícia Marques deAssis et al., 2014; Fernández-García, Mínguez-

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Mosquera, & Pérez-Gálvez, 2007; Kwak, 2014; Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). Vitamins are particularly beneficial for the skin and correct pigmentation, they increase collagen synthesis, improve inflammatory skin conditions and protect against damage caused by UVA rays (He et al., 2013; Pandel, Poljšak, Godic, & Dahmane, 2013). In the nutraceutical field vitamins play an important role as dietary supplements which safeguard us from chronic diseases and antioxidant nutraceuticals are recommended for the prevention and treatment of CVD: it has been demonstrated that wine, which is rich in polyphenols, can reduce arterial disease (Rajasekaran, Sivagnanam, & Xavier, 2008).

Due to their low stability, it is essential to wrap vitamins in protective materials in order to prevent their deterioration during both food processes and their uptake in the organism. The main methods and technologies used for encapsulating vitamins are emulsions, solid-lipid nanoparticles, surfactant systems and polymer/lipid encapsulation. The latter includes liposome encapsulation which has recently drawn great interest due to its ability of prolonging shelf life and improving the bioavailability of vitamins (Liu et al., 2013) (Rovoli, Gortzi, Lalas, & Kontopidis, 2013) and of a wide variety of hydrophilic and hydrophobic molecules such as peptides, proteins, plasmic DNA, antisense oligonucleotides or ribozymes which are useful for pharmaceutical, cosmetic, biochemical and nutraceutical purposes (Ulrich, 2002).

As liposomal vectors are composed of biodegradable and sustainable material, they can increase food quality and safety (Reza Mozafari, Johnson, Hatziantoniou, & Demetzos, 2008). Under pharmaceutical point of view, the advantages of using liposomes rather than other delivery systems are their high level of biocompatibility, biodegradability, low intrinsic toxicity, immunogenicity and the flexibility to couple with site-specific ligands in order to achieve active targeting (Verma, Singh, Navneet, Mathur, & Valecha, 2010; Yu, Tai, Xue, Lee, & Lee, 2010).

The dimension of the liposomes is of great importance for their performance as drug carrier systems. It was observed that liposomes larger than 200–250 nm may not accumulate in the spleen by a filtration mechanism or be trapped in the lung capillaries (Šentjurc, Vrhovnik, & Kristl, 1999); liposomes with small (<50 nm diameter) size were found to resist deterioration in the blood and to circulate for several hours in human cancer patients (Sawant & Torchilin, 2012).

Various techniques can be used to produce loaded liposomes; all share the same basic steps (Bochicchio, Dalmoro, Barba, Grassi, & Lamberti, 2014; Samad, Sultana, & Aqil, 2007): 1) lipid hydration; 2) regulation of liposome size; 3) removal of non-encapsulated drug. The lipid hydration step is achieved by means of mechanical methods, methods based on organic solvents and methods based on detergent removal (Sipai Altaf Bhai., Vandana, & Prasanth, 2012). In particular by mechanical methods a thin lipid layer is formed when an organic solution evaporates and is then hydrated with an aqueous buffer solution under continuous stirring. The process generates multilamellar vesicles (MLVs), which can be sonicated or extruded through a porous membrane in order to produce small unilamellar vesicles (SUVs).

In this study the thin film hydration method was used to produce MLVs and duty cycle sonication was applied for reducing the size of the vesicles in order to generate SUVs. The size of the liposomes, which is determined during the production process, decreases due to the energy supplying. The energy, which is provided by ultrasonic radiation, is used to break the lipid bilayer into smaller pieces. These pieces enclose themselves in smaller spherical structures like the original ones (Barba, Bochicchio, Lamberti, & Dalmoro, 2014). The main advantages of using this ultrasound-assisted approach is that SUVs with predictable dimensions can be formed with diameters ranging from 50 to 100 nm depending on sonication rounds used; the main disadvantage is the poor control in size-uniformity of final vesicles.

Vitamin B12, α-tocopherol and ergocalciferol were used due to their great antioxidant potential and their different hydrophilic-lipophilic and solubility properties. Few are the information regarding the encapsulation of ergocalciferol and vitamin B12 in liposomes, a little bit more can be found in literature for α-tocopherol encapsulated in liposomes (Rovoli et al., 2013) but data are completely missing in relation to the production of liposomes smaller than 80–100 nm in size encapsulating vitamins.

Aim of this study was to produce nanoliposomal vectors (NLV) with high encapsulation efficiency of vitamins for a controlled and targeted release purposes. In this work, the ultrasound assisted process was combined with the thin film hydration method. The main challenge of this approach was to test the versatility of the developed preparative protocol to achieve tailored liposomes in terms of size and encapsulation efficiency. Starting from our previous study (Barba et al., 2014) production of 40–50 nm SUVs containing ergocalciferol, vitamin B12 and α-tocopherol was performed emphasizing that vitamins encapsulation efficiency for NLV smaller than 50 nm was never tested before.

2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (PC) from egg yolk (CAS n. 8002-43-5), Cholesterol (CHO) (CAS n. 57-88-5),L-αPhosphatidyl - DL glycerol sodium salt (PG) from egg yolk lecithin (over 99% pure), vitamin B12, B12, (CAS n. 68-19-9), α-tocopherol, TC (CAS n. 10191-41-0), ergocalciferol, D2, (CAS n. 50-14-6), potassium phosphate monobasic (CAS n.7778-77-0) and sodium hydroxide (CAS n. 1310-73-2) were purchased from Sigma Aldrich (Milan, Italy) as dried powders and used without further purification. All the other chemicals and reagents such as chloroform (CAS n. 67-66-3), ethanol (CAS n. 64-17-5), methanol (CAS n. 67-56-1) (Sigma Aldrich, Milan, Italy) used were of analytical grade.

2.2. Methods

2.2.1. Phosphate buffer solution preparation

A phosphate buffer solution (PBS) was prepared by dissolving potassium phosphate monobasic 0.2 mol/L and sodium hydroxide 0.2 mol/L in distilled water thus obtaining a pH 7.4 PBS final solution. This buffer solution was used for carrying out liposome production, vitamin load and stability tests.

2.2.2. Preparation of MLVs and SUVs loaded with B12 vitamin

Unilamellar liposome vesicles were prepared by means of the thin film hydration method (Bangham & Horne, 1964; Meure, Foster, & Dehghani, 2008) followed by sonication. In short, PC (40 mg), CHO (16 mg) and PG (4 mg) at 10:8:1 (mol:mol) ratio were dissolved in 2 mL of chloroform/methanol 2:1 (vol/vol). Part of the solvent was removed by evaporation at 50 °C in a rotary evaporator (Heidolph, Laborota 4002 Control, Bergamo, Italy) under reduced pressure until a lipid film was produced, subsequently any traces of the solvent were removed by evaporation under reduced pressure at 50 °C for 3 h a water bath. The dried lipid film was then hydrated at room temperature with 2 mL of pre-warmed PBS (pH 7.4) containing vitamin B12 (14.32 mg) and continuously stirred at 60 rpm for 30 min. The molar ratio of vitamin to the total amount of lipids was 1:10 (mol:mol).

The preparation containing MLVs was maintained at room temperature for 2 h. Part of this sample was used to assay morphology, size and encapsulation efficiency of MLVs. Most of the