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Influence of lecithin–lipid composition on physico-chemical properties of nanoliposomes loaded with a hydrophobic molecule

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ABSTRACT

In this work, we studied the effect of nanoliposome composition based on phospholipids of docosahexaenoic acid (PL-DHA), salmon and soya lecithin, on physico-chemical characterization of vector. Cinnamic acid was encapsulated as a hydrophobic molecule in nanoliposomes made of three different lipid sources. The aim was to evaluate the influence of membrane lipid structure and composition on entrapment efficiency and membrane permeability of cinnamic acid. These properties are important for active molecule delivery. In addition, size, electrophoretic mobility, phase transition temperature, elasticity and membrane fluidity were measured before and after encapsulation.

The results showed a correlation between the size of the nanoliposome and the entrapment. The entrapment efficiency of cinnamic acid was found to be the highest in liposomes prepared from salmon lecithin. The nanoliposomes composed of salmon lecithin presented higher capabilities as a carrier for cinnamic acid encapsulation.

These vesicles also showed a high stability which in turn increases the membrane rigidity of nanoliposome as evaluated by their elastic properties, membrane fluidity and phase transition temperature.

1. Introduction

Among different techniques of encapsulation, nanoliposomes have become very versatile tools in biology, biochemistry and medicine because of their enormous diversity of structure and composition.

The main constituents of liposomes are phospholipids, which are amphiphilic molecules containing water soluble, hydrophilic head section and a lipid-soluble, hydrophobic tail section. This property of phospholipids gives liposomes unique properties, such as self-sealing, in aqueous media and make them an ideal carrier system with applications in different fields including food, cosmetics, pharmaceutics, and tissue engineering [1].

Liposomes were first made synthetically in England in 1961 by Alec D. Bangham, who found that phospholipids combined with water form a sphere because of their unique properties. Liposomes are spherical, closed structures, composed of curved lipid bilayers, which enclose part of the surrounding solvent into their interior [2]. Due to their biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs, liposomes have been investigated as parenteral drug carrier systems and more recently as transdermal drug delivery systems [2].

The drug delivery properties of liposomes are largely affected by the physico-chemical characteristics of the lipid bilayer, which are determined by factors such as the lipid composition, the particle size and the drug loading [3].

The preparation method of nanoliposomes has some control over the size range (as narrow as possible) and, polydispersity index (as low as possible). By considering these parameters, the extrusion technique was chosen to prepare liposomes. Extrusion is a common method for nanoliposomes production in a laboratory scale and there are numerous reports on liposome preparation with this technique to obtain small particle size [4,5].

Currently, *in vitro* and animal studies indicate that n-3 PUFAs suppress carcinogenesis. Several studies present a new insight on effectiveness of marine phospholipids for suppression of colon carcinogenesis to investigate growth inhibition and apoptosis inducing effects of n-3 PUFA in the form of marine phosphatidylcholine (PC) on chemically induced (1,2-dimethylhydrazine) colon cancer in rats [6].

Marine lecithin from salmon (*Salmosalar*) contains a high percentage of polyunsaturated fatty acids (PUFAs), especially

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eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), those are critical to several physiological processes [7].

Soya lecithins also consist mainly of contain esterified mono- and poly-unsaturated fatty acids such as oleic (C18:1 n-9), linoleic (C18:2 n-6), and linolenic acids (C18:n-3). The linoleic and linolenic acids are categorized as essential fatty acids and are important to human health [8].

The cinnamic acid and its derivatives are well known for their biological and pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, and antitumoral activity [9]. Some cinnamic acid derivatives represent secondary metabolites in plants and they have been the subjects of a great number of chemical, biological, agricultural and medical studies, the most important category being the hydroxy-cinnamic acids. In fact these biophenols are very effective peroxyl radical scavengers and protect low-density lipoproteins from oxidative modification [10].

Cinnamic (*trans*-3-phenyl-2-propenoic) acid is widely used in food, cosmetics, and pharmaceuticals fields. Recent studies, devoted to the use of *trans*-cinnamic acid in cosmetics, revealed its importance as a nonphotosynthetic pigment in photoprotection [11].

Cinnamic acid is an effective anticancer constituent of traditional Chinese herbal medicines. The molecular mechanisms of anticancer effects of this constituent and its target have long been unknown. As a product of a potential tumor suppressor gene, cinnamic acid participates in the regulation of cell growth, proliferation, and cell differentiation [12].

Due to their interesting properties we encapsulated cinnamic acid into liposomal systems as vectors containing the phospholipids to introduce a double functionalization with therapeutic use.

They are suitable for low molecular weight drugs, imaging agents, peptides, proteins, and nucleic acids, therefore suitable vectors for the cinnamic acid which has a molecular weight of 148.16 g/mol [5]. Particle size, phase transition temperature and fluidity of membranes are important in the manufacture and application of liposomes. Controlling the above factors is important in using liposomes as drug carrier systems. In addition, entrapment efficiency of liposomes is an important factor in their practical use [5].

In this work, we compared the effect of three different compositions on physico-chemical properties of cinnamic acid encapsulated liposome such as size, electrophoretic mobility, phase transition temperature and fluidity. We used one natural lecithin extracted by enzymatic process and two commercial lecithins. The encapsulation efficiency of cinnamic acid was determined by using HPLC after physical separation of entrapped and non-entrapped cinnamic acid using ultracentrifugation process. Furthermore to characterize the resulting liposomes, a variety of techniques were used to investigate the rheological properties of systems. This study allowed us to explain the correlation between the lipid composition and the physico-chemical properties of nanoliposome and liposomal carrier. In addition we studied the influence of lipid composition on acid cinnamic encapsulation.

2. Materials and methods

Trans-cinnamic acid (3-phenylpropenoic acid) (CA) and sodium thiosulfate pentahydrate were purchased from Merck (Germany), Trifluoroacetic acid about 100% from VWR Prolabo (Belgium), methanol for HPLC from group CARLO ERBA reagents (France), BF_3 (boron trifluoride)/methanol (purity = 99%) and hexane (purity = 97%) used for gas chromatography (GC) were purchased from Sigma–Aldrich (France) and Fisher (France).

TMA-DPH (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatrienep toluenesulfonate) from Invitrogen (City, State).

PHOSPHOLIPON 85G (phospholipon with 89.5% soya phosphatidylcholine) from Lipoid PHOSPHOLIPID GmbH (Germany), PL-DHA (phospholipids of docosahexaenoic acid (DHA) from POLARIS (France), and salmon lecithin was extracted by use of a low temperature enzymatic process in the absence of organic solvent [13].

2.1. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared as described by Ackman [14]. Separation of FAMEs was carried out on a Shimadzu GC-2010 Plus gas chromatograph, equipped with a flame-ionization detector. A fused silica capillary column was used (60 m × 0.25 mm × 0.25 μm film thicknesses SP ™-2380), purchased from Supelco (USA). Injector and detector temperatures were set at 250 °C. A temperature program of column initially set at 120 °C for 2 min, then rising to 220 °C at a rate of 3 °C/min and held at 220 °C for 25 min was used. Standard mixtures (PUFA1 from marine source and PUFA2 from vegetable source; Supelco, Sigma–Aldrich, Bellefonte, PA, USA) were used to identify fatty acids. The results were presented as triplicate analyses.

2.2. Lipid classes

The lipid classes of the different fractions were determined by Iatroscan MK-5 TLC-FID (Iatron Laboratories Inc., Tokyo, Japan).

Each sample was spotted on ten Chromarod S-III silica coated quartz rods held in a frame. The rods were developed over 20 min in hexane/diethyl ether/formic acid (80:20:0.2, v:v:v), then oven dried for 1 min at 100 °C and finally scanned in the Iatroscan analyzer.

The Iatroscan was operated under the following conditions: flow rate of hydrogen, 160 mL min^{-1}; flow rate of air, 2 L/min. A second migration using a polar eluent of chloroform, methanol, and ammoniac (65:35:5, v:v:v) made it possible to identify polar lipids [15].

The FID results were expressed as the mean value of ten separate samples. All standards were purchased from Sigma (Sigma–Aldrich Chemie GmbH, Germany). The recording and integration of the peaks were provided by the ChromStar internal software.

2.3. Cinnamic acid solubility

To determine the maximum solubility of cinnamic acid in each lipid phase, excess powder of cinnamic acid was added to known volumes of lecithins aqueous solutions ($[\text{cinnamic acid}]_{final}$ = 5 mg/mL).

The mixtures were then incubated at 37 °C with gentle stirring for more than 48 h. Finally, the solutions were centrifuged at 200,000 × *g* for 10 min and aliquots of the supernatant saturated solution were diluted and analyzed by HPLC [16].

The supernatant of each sample was pipetted and then 20 μl of supernatant was diluted 1000-fold with methanol. Cinnamic acid in methanol with concentration of 20 μg/mL was used as standard suspension. HPLC measurements were performed by a HPLC system (Shimadzu, Japan) equipped with a quaternary pump (LC-20AD), an auto-injector (SIL-20AC HT), a UV–vis photodiode array detector (UV–vis PDA, SPD-M20A), a column oven (Zorbax – 15 cm-C18) and Labsolution data software.

All suspension was analyzed using isocratic mode of methanol (v/v, 75%) and (H_2O–0.1% trifluoroacetic acid) TFA at pH 3 (v/v, 25%) at a flow rate of 0.4 mL min^{-1}. The suspensions (20 μl) were injected onto a Alltima™ [HP C18, 3 μm (150 × 3 mm i.d.) column (GRACE, Deerfield, IL, USA)] protected by a 2.1 mm × 7.5 mm Alltech guard