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Research article

Liposomal carrier systems used to encapsulate garcinia extract, green coffee, and carnitine in a drinkable product

Madrigal-Redondo G[1,2*], Vargas-Zúñiga R[1,2], Chavarría-Rojas M[2], Sibaja-Rodríguez S[2], Chaves-Noguera S.[3]

[1]*Laboratorio de Biofarmaciay Farmacocinética (LABIOFAR), Instituto de Investigaciones Farmacéuticas (INIFAR), Facultad de Farmacia, Universidad de Costa Rica, SanJosé, Costa Rica.*
[2] *School of Pharmacy, Universidad Latina de Costa Rica, San José, Costa Rica.*
[3] *Research Directorate, Universidad Latina de Costa Rica, San José, Costa Rica.*

Key words: Carnitine, Phosphatidylcholine, Garcinia, Multivesicular Liposomes, Multilamellar Liposomes, Nutritional Product.

***Corresponding Author: Madrigal-Redondo G.,** Laboratorio de Biofarmaciay Farmacocinética (LABIOFAR), Instituto de Investigaciones Farmacéuticas (INIFAR), Facultad de Farmacia, Universidad de Costa Rica, SanJosé, Costa Rica.

Abstract

In the last decades, the study and development of nutritional products have increased. Garcinia plants, green coffee, and carnitine are natural products that have been investigated due to its beneficial effects on health and pharmacological properties. Liposomes are a very common formulation option to improve the physicochemical properties and increase the bioavailability and stability of natural products.
In this study, a drinkable product with garcinia extract, green coffee, and carnitine encapsulated in liposomal vesicles was characterized. The liposomes morphology and structure were analyzed by light microscopy to determine its shape, size (diameter), and membrane thickness. The formulation was characterized measuring the following parameters: pH, degrees Brix, conductivity, refraction index, and specific gravity.
The obtained results and the execute analysis indicate that liposomes are multivesicular and multilamellar structures, a conformation with higher stability than simple liposomes. The physicochemical parameters measured can be used as quality control tests and as a start point to get better and optimize the formulation in terms of stability and organoleptic properties.

Introduction

Liposomal vesicles are spherical structures with an aqueous core surrounded by one or more phospholipids bilayers [1]. Phospholipids are amphipathic molecules oriented in bilayers that compound the cell membranes. An advantage related to liposomes is its high biocompatibility due to the similarity between biological and liposomal membranes [2].
Liposomes applications comprise different areas, like cosmetic, pharmaceutic and food industries [3-4]. Pharmaceutical and food fields have study the liposomes as transport and delivery mechanisms of bioactive compounds. They are used due to its ability to encapsulate, protect and transport molecules with hydrophilic and hydrophobic character in addition to its high biocompatibility [5–7]. In this way, using liposomes it is possible to modify and optimize the physicochemical characteristics of active compounds, which could be observed as variations in pharmacokinetics and pharmacodynamics profiles [7].
In last years the use and development of functional foods have become popular. However, a common issue is the low oral bioavailability of bioactive molecules [8]. On the other hand, the instability of these molecules during production and storage phases is related to interactions between components, degradation and low absorption [9]. Encapsulation techniques, such as liposomal vesicles, are an alternative to bring protection and improve the activity of bioactive compounds [5]. In this work, a drinkable product was formulated using liposomal vesicles to encapsulate garcinia extract, green coffee,andcarnitine.
The plants of *Garcinia* genus are found mainly in the rainforests of India, Indonesia, Africa and Brazil, they include more than 300 species and its fruits, flowers, leaves, barks, and stems are used as traditional medicine[10]. *Garcinia* plants are related to pharmacological effects, such as antiulcerogenic, antioxidant, hypoglycemic, cytotoxic, hepatoprotective, erythropoietic and diuretic effect [11].
The presence of bioactive metabolites in *Garcinia* plants has been demonstrated. Compounds like tannins, saponins, flavonoids, terpenoids, alkaloids, benzophenones and benzoquinones are related with the mentioned effects [12-13]. Garcinol is a benzophenone derivateand is the principal compound isolated from *Garcinia* fruits. *In vivo* and *in vitro* assays have demonstrated antioxidant, anti-inflammatory and anticancer properties of garcinol [10].
On the other hand, the popularity and use of green coffee have increased recently due to its rich composition in

antioxidants, mainly polyphenols. Caffeine and phenolic acids, such as chlorogenic and caffeic acids are the main compounds of green coffee [14]. They exhibit anticarcinogenic, antimutagenic and antioxidant activities, and have been linked to antihypertensive effects, inflammatory inhibition, modulation of glucose metabolism and show a tendency to reduce visceral fat and body weight[14–17]. Due to the presence of phenolic compounds and its potential health benefits, the food and nutritional supplement industries have developed many products with the above-mentioned phenolics [14].

Carnitine is a hydrophilic quaternary amine, its main function in the transfer of long-chain fatty acids to mitochondria for subsequent β-oxidation. Carnitine can be obtained from diet or endogenous biosynthesis [18]. It is considered a nutraceutical product, facilitates body weight loss and improves glucose tolerance. In consequence, it is used in food, pharmaceutical and cosmetic industries [19].

The objective of this study was to carry out a physicochemical characterization of a liposomal formulation with garcinia, green coffee, and carnitine, commercialized in Costa Rica as a nutritional product.

Experimental

Materials and Methods

Materials

Garcinia and green coffee extracts were standardized extracts supplied by Syder S.A Poas de Alajuela San Jose Costa Rica,. Carnitine, sodium chloride, potassium sorbate, potassium benzoate and phosphatidylcholine were purchased from Sigma-Aldrich Company. St. Louis, Missouri USA.

Preparation of liposomes

The Liposomes were obtained using phosphatidylcholine. Phosphatidylcholine was contained in a flask and a solution of sodium chloride was added slowly and with constant stirring. At the same time, specific amounts of water, potassium sorbate, potassium benzoate, garcinia extract, green coffee, and carnitine were mixed in another container, always guaranteeing the complete dissolution of the components. Subsequently, this solution was incorporated into the mixture of phosphatidylcholine and sodium chloride with strong shaking to avoid and eliminate any aggregate.

Physicochemical characterization

Determination of acidity and conductivity

The determination of pH and conductivity of the formulation was determined using a pH meter - conductivity meter (Thermo Scientific Orion 3 Star) at room temperature (≈ 25 ° C). The results are expressed as the mean ± standard deviation of three parallel measurements.

Determination of the refractive index and degrees Brix

The refractive index and degrees Brix of the formulation were measured on an automatic refractometer (Rudolph Research J57) at room temperature (≈ 25 ° C). The results are expressed as the mean ± standard deviation of three measurements.

Determination of specific gravity

Specific gravity was determined by pycnometry. The results are expressed as the mean ± standard deviation of three measurements.

Size and morphology

The size of the liposomes was quantified by light microscopy. 1 mL of sample was placed with 100 µL of red 40 to stain the liposomes. A small sample was placed on a slide for observation using an Olympus U-TV 0.63XC light microscope.

The diameter of the liposomes and the membrane thickness were quantified using the computer program Image J, the results are expressed as the mean ± standard deviation.

Statistical analysis

The collected data mean values and standard deviation of the mean were analyzed and evaluated using the software Minitab version 16 (Minitab Inc.).

Results and Discussion

Liposomes are auto-assembled spherical structures formed by a phospholipid bilayer with an aqueous nucleus. Liposome vesicles are highly used as a delivery mechanism of bioactive compounds. Due to the membrane configuration, liposomes are characterized by its biocompatibility, low toxicity, low immunogenicity, and biodegradability [5-6]. Liposomal structures encapsulate active compounds, which bring protection during packing, transport and storage stages [6].

In this study, phosphatidylcholine was used to form the liposomal vesicles. This phospholipid is used as the prime component of liposomal membranes due to its similarity with biologic membranes phospholipids, which is related to the high biocompatibility that characterized liposomal carriers [20].

Using phosphatidylcholine, multivesicular liposomes (MVL) were obtained, which are composed of non-concentric and closed-packed lipid vesicles (Figure 1). The main advantage of MVL is its high stability compared with simple liposomes [2, 21-22].

The thickness difference between liposomal membranes and the encapsulated vesicles membranes (Table 2) could be attributed to the formation of multilamellar liposomes (MLL). The MLL are formed by two or more phospholipid bilayers, which improves the vesicle stability and increases the encapsulation efficiency [2,23].
In addition to the morphological and structural analysis, the liposomal formulations were also physicochemically characterized. The evaluated properties were pH, conductivity, degrees Brix, specific gravity and refraction index (Table 1).
The obtained results show that formulation is slightly acid. This pH value, that characterized the formulation, is related to the liposomes membrane stability. Some environmental factors, such as pH, temperature, oxygen and the presence of enzymes, can damage the structural integrity of liposomes and alter the surface properties [3]. For this reason, the pH value is considered a formulation quality parameter.
Degrees Brix, refraction index, and specific gravity are also quality control parameters used in drinkable products. The results obtained in this study are accordingly with the values usually recommended for the above-mentioned products [24-25].
Technically, the degrees Brix means the percentage by weight of all soluble solids in solution, usually sugar solids like sucrose and other sweeteners agents [26]. Therefore, degrees Brix value is a parameter directly related to the organoleptic characteristics of drinkable products, and in consequence with the consumers' acceptance.
The results of physicochemical parameters studied can be used as quality control test of the product. The main objective is to guarantee the formulation stability, and ensure that liposomal vesicles maintain integrity during production, storage, distribution and commercialization phases. On the other hand, the obtained results are a start point to get better the formulation in terms of stability, physicochemical and organoleptic properties, and consumer acceptance.

Table 1. Physicochemical characterization of a liposomal formulation based ongarcinia extract, green coffee, and carnitine.

Sample	pH	Conductivity µS cm^{-1}	n_D^{23}	Degrees Brix / °B	Specific gravity
Mean ±Standard Deviation	4.350 ±0.017	2089.7 ±7.3	1.3502 ±0.0	11.5 ±0.0	1.03921 ±0.00059

Table 2. Size and membrane thickness determination of liposomal carriers present in a formulation of a nutritional product based on garcinia extract, green coffee, and carnitine.

Multilamellar Liposomes		Encapsulated Liposomes	
Diameter	207.0 ± 5.1µm	Diameter	7.1 ± 10.4µm
Membrane thickness	5.1 ± 1.3µm	Membrane thickness	1.48 ± 0.51µm

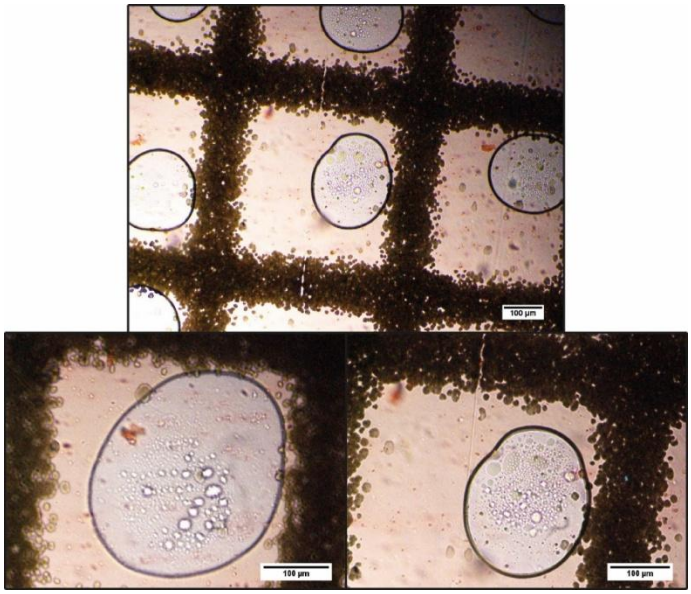

Figure 1. Representative images of liposomal carriers present in a formulation of a nutritional product based on garcinia extract, green coffee, and carnitine. All the liposomes contain and encapsulate the mixture of garcinia extract, green coffee, and carnitine.

Conclusions

In the present study, the liposomes obtained are multivesicular and multilamellar structures, a conformational arrangement that brings higher stability than simple vesicles. It is a beneficial and favorable aspect of the formulation.
The physicochemical characteristics evaluated, and the morphologic and structure observations can be considered as formulation quality control parameters that maintain its stability and represent a start point to optimize and get better the product formula.

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