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MULTI-VESICULAR PHOSPHATIDYLCHOLINE LIPOSOMES AS VEHICLES FOR CURCUMIN IN A NUTRITIOUS DRINKABLE FORMULATION

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

[1]Laboratory of Biopharmacy and Pharmacokinetics (LABIOFAR), Institute of Pharmaceutic Investigations (INIFAR), Faculty of Pharmacy, University of Costa Rica, San José, Costa Rica.
[2]School of Pharmacy, Universidad Latina de Costa Rica, San José, Costa Rica.
[3]Investigative Direction, Universidad Latina de Costa Rica, San José, Costa Rica.

***Corresponding Author: Madrigal-Redondo G.**
Laboratory of Biopharmacy and Pharmacokinetics (INIFAR), Institute of Pharmaceutic Investigations (LABIOFAR), Faculty of Pharmacy, University of Costa Rica, San José, Costa Rica.

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ABSTRACT
Liposomal vesicles are used as a strategy to enhance the stability and bioavailability of its bioactive components. Large part of its characteristics can be attributed to the likeness between the liposomal and biological membranes, given both are formed by amphiphilic molecules. Since they have a vesicular shape, liposomes have the particularity to encapsulate bioactive molecules. This way certain physicochemical and pharma-kinetic characteristics can be enhanced and optimized. Curcumin is a polyphenolic compound with a high therapeutic potential. However, its bioavailability, stability and solubility are relatively low. Due to this, alternatives have been explored, such as liposomes to enhance the aforementioned properties. This investigation yielded a characterization of the physicochemical properties of a nutritious product using curcumin as a bioactive component. Brix levels, pH levels, specific gravity, refraction index and conductivity were measured. Additionally, liposomal vesicles were observed through optical microscopy to recognize structure, size (diameter) and membrane width. Physicochemical characterization indicates that the formula is stable. Also, they can be utilized as quality control for the product and represent a starting point to accomplish possible formula enhancements and optimization. The microscopic observation indicates that liposomes are multi-vesicular and multi-layered structures. This fact indicates that liposomal vesicles are more stable which results favorable for successful formulation.

KEYWORDS: curcumin, Phosphatidylcholine, multi-vesicular liposomes, multi-layered liposomes, nutritious product.

1. INTRODUCTION
Since the decade of 1970, liposomes have been studied as transport and delivery mechanisms for bioactive compounds (Giansanti et al., 2016). Liposomes are spherical vesicles with an aqueous nuclei surrounded by one or several bi-layers of phospholipids (Alavi, Haeri, & Dadashzadeh, 2017). Phospholipids are the same molecules forming biological membranes. This way there is high biocompatibility between the liposomal vesicle membranes and the cell membranes. (Mirafzali, Thompson, & Tallua, 2014).

Liposome use is rather broad, and it is possible to find applications in the pharmaceutical, cosmetic and agricultural field (Liu, Wei, Ye, Tian, & Han, 2017) and in the food industry (Frenzel & Steffen-heins, 2015; Liu et al., 2015, 2017). One of the main advantages or characteristics liposomes share are its high biocompatibility, efficiency and capacity to encapsulate both hydrophilic and hydrophobic components, which in turn enhances their pharmacokinetic and pharmacodymanic profiles (Giansanti et al., 2016).

The food industry mostly uses liposomes to respond to needs related to the control of liberation of active components, such as antioxidants, enzymes, proteins and vitamin (Liu et al., 2017). Nonetheless, maintaining the stability and efficiency for these components implies big challenges. High potency active ingredients can be unstable, have low bioavailability or be negatively directed to the gastrointestinal tract. As such, the use of liposomes for encapsulating biologically active molecules pretends to increase the drug bioavailability, favor its bio-distribution (Alavi, Haeri, & Dadashzadeh, 2017) while enhancing the stability by providing protection from degradation phenomena. (Alavi et al., 2017).

Such is the case of curcumin, the main component found in curcuma, obtained from the rhizomes of the plant *Curcuma longa*. Curcumin is a polyphenolic compound traditionally used in Asia as a nutritious supplement and natural medicine (Catalan-Latorre et al., 2016). The therapeutic potential of the curcumin is related to its antioxidant, anti-inflammatory and anti-mutagenic activity (Gómez-Mascaraque, Casagrande, Gaziola, & López-Rubio, 2017; Peng et al., 2017) while also referred to because of their effect on cardiovascular and neurodegenerative afflictions. (Catalan-Latorre et al., 2016).

Regardless of its important biological activity, the curcumin possesses a compromised bioavailability, low aqueous solubility, low absorption, rapid metabolism and systemic elimination (Karewicz et al., 2013; Peng et al., 2017). Due to this reason, the use of vectors has been recurred to optimize the absorption, stability, solubility and effect of the compound (Catalan-Latorre et al., 2016). Emulsions, liposomes, micelles, hydrogels and nanoparticles have been used to attempt enhancement on the aforementioned characteristics. (Peng et al., 2017).

In this investigation, the liposomal transporters were used to encapsulate the curcuma and in this way be able to yield a drinkable nutritious product. The objective of this investigation is to form a physicochemical characterization of the liposomal formulation using curcumin as the active component.

2. MATERIALS AND METHODS

2.1 Liposome preparation

Liposome preparation was done by using phosphatidylcholine to which a sodium chloride solution was added (slowly and with constant stirring). Simultaneously, quantities of water, potassium sorbate, potassium benzoate and curcumin are mixed and stirred; always guaranteeing the complete dissolution of each of the mentioned components. Later, this solution was incorporated on the phosphatidylcholine and the sodium chloride firmly stirring to avoid and eliminate any aggregated.

2.2 Physicochemical characterization

2.2.1 Acidity and conductivity determination

The pH determination and conductivity of the formulation was done by using a pHmeter-conductimeter (Thermo Scientific Orion 3 Star) at room temperature (≈ 25°C).

2.2.2 Determination of refraction index and Brix levels

The refraction index and Brix levels were measure in an automatic refractometer (Rudolph Research J57) at room temperature (≈ 25°C).

2.2.3 Determination of specific gravity

Specific gravity was determined by picnometry.

2.2.4 Size and morphology

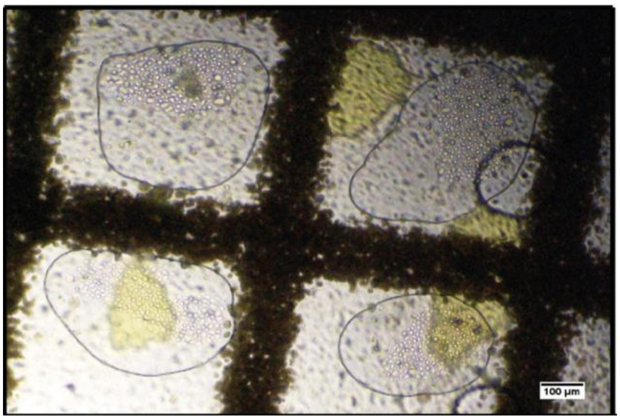
The size of the liposomes was quantified through optical microscopy. 1mL of sample was placed with 100 µL of Red No. 40 to dye the liposomes. A small sample was placed in an object holder to observe using a light microscope model Olympus U-TV 0,63XC.

The diameter of the liposomes, and the width of the membrane in question was quantified using the computing program Image J.

3. RESULTS

Table: 1. Physicochemical characterization of the liposomal formulation using curcumin as base.

Sample	pH	Conductivity µS cm^{-1}	n_D^{23}	Brix Levels / °B	Specific gravity
1	4,62	1439	1,34926	10,97	1,00378
2	4,62	1428	1,34944	10,97	1,00375
3	4,62	1436	1,34957	11,15	1,00474
Average	4,62	1434,3	1,3494233	11,03	1,00409
Standard Deviation	0,00	5,7	0,0001557	0,10	0,00057



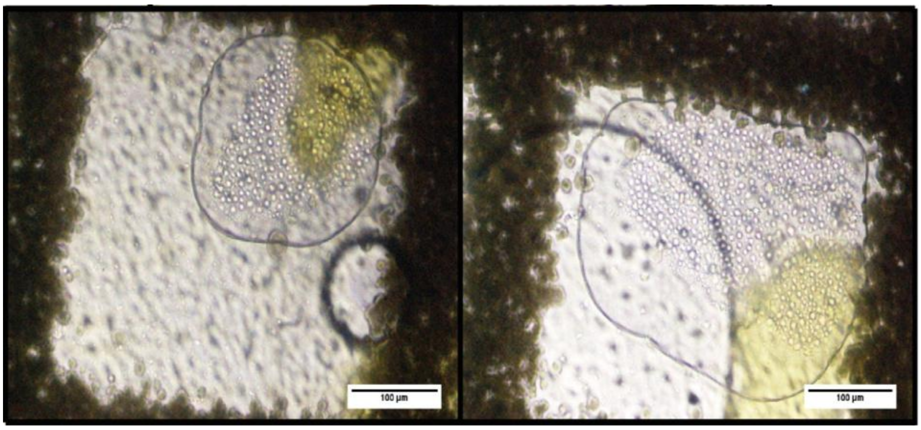


Fig 1. Images representative of the multi-vesicular liposomes present in the curcumin-based nutritious product.

Table: 2. Size and width determination of the liposomal transporter membrane present in the formulation of a curcumin-based nutritious product.

Multi-vesicular Liposomes		**Encapsulated Liposomes**	
Diameter	343 ± 83 μm	Diameter	4,59 ± 0,84 μm
Membrane Width	4,10 ± 0,94 μm	Membrane Width	0,93 ± 0,35 μm

4. DISCUSSION

Curcumin is a polyphenolic compound used in traditional Chinese medicine. It possesses a grand variety of associated biological activities (Karewicz et al., 2013; Peng et al., 2017). However, its aqueous solubility and stability are low, implying a low oral and topic bioavailability. Thus, the use of this product in the pharmaceutical and food industry is rather limited (Karewicz et al., 2013).

The design of carrying vehicles facilitates transport and delivery of curcumin in biological systems, which opens up the possibility of exploiting diverse pharmaceutical and food-oriented applications (Karewicz et al., 2013). Liposomes are commonly used due to their capacity to encapsulate, protect and transport bioactive components (Koynova & Tihova, 2010; Tai et al., 2017).

In this investigation, phosphatidylcholine was used for the extraction of liposomes. This molecule is a commonly employed phospholipid for the formation of liposomal vesicles due to its similarity ad high compatibility with biological membranes (Huang & Mason, 1978).

Obtained liposomes are characterized by being formed by small liposomal vesicles conformed of small non-centrical liposomal vesicles. This kind of conformation has been called multi-vesicular liposomes (Chen, Han, Zhang, Yuan, & Tang, 2010; Mirafzali et al., 2014). These systems have a higher degree of stability that the systems conformed of simple vesicles, which represents an advantage for its formulation (Chen et al., 2010; Joo et al., 2013).

The membrane width of multi-vesicular liposomes is 4,10 ± 0,94 μm, while the width of the encapsulated vesicles membrane is 0,93 ± 0,35 μm. Hence, the width of the external membrane is approximately 4 times the width of the membrane for the smaller liposome. As such, the possibility exists to be working with a transporting system confirmed of multiple layers of phospholipids, which are known as multi-layered systems (Mirafzali et al., 2014).

Multi-layered liposomes are characterized for possessing a more stable, resistant membrane; while also providing more efficiency for encapsulation. Both aspects represent an advantage in terms of production for these types of liposomes. (Prévoteau & Faure, 2012).

In terms of the physicochemical parameters, various properties were analyzed, amongst them the pH value, Brix levels, conductivity, specific gravity and refraction index. The objective was to characterize the formulation from a physicochemical point of view. Apart from these results, alternatives can be searched for optimization and enhancement of the formula in terms of stability and organoleptic properties.

The values presented on Table 1 can be used as the the formula quality control, as it has been shown that with the manufacturing method used, the values are kept constant during both the production and storing phases, implying the stability of the liposomes under the mentioned conditions.

According to the measurements taken, it is possible to observe that the formulation has a slightly acidic pH. This characteristic can be linked to be less susceptible to suffer a microbial growth (Shittu, 2013). Additionally, the acidic medium has great influence on the stability of the membranes that make up the liposomes (Ju et al., 2017; Liu et al., 2017) and, as consequence, on the stability of the formulation.

The Brix level measurement has, as an objective, to recognize the percentage of weight on soluble solids in the formulation, such as sucrose (Dongare, Buchade, & Shaligram, 2015).

Drinkable formulations within the range of the Brix levels are very broad an these are between 3,8 a 18,5 °Brix (Kim et al., 2014; King et al., 2007; Shittu, 2013). It is possible to observe in the current formulation that the obtained Brix levels are found under the commonly used values in these types of products.

5. CONCLUSIONS

The liposomes morphological and structural analysis indicates that the formulation in question is composed of multi-vesicular and multi-layered structures. This conformation provides great stability to the vesicles, which positively favors formulation.

In respect with the measurement of physicochemical properties, it is possible to affirm that parting from the obtained results these can be optimized an enhanced for the product to serve a better purpose, this being accomplishable by the modification of the specified properties. On the other hand, physicochemical characterization is also related to the quality control of the product, which can guarantee a level of stability.

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