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RESEARCH ARTICLE

QUALITY CONTROL PERFORMED ON LIPOSOMAL FORMULATIONS BASED ON GUARANA EXTRACT, VITAMINS A, C, E, B1, B2, B5, B6, B12, BIOTIN AND FOLIC ACID, USED AS NUTRITIONAL PRODUCTS

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ABSTRACT

The study of different strategies to improve the stability and bioavailability of bioactive components has increased in the last decades. One of the mechanisms that has acquired great relevance is to formulate using liposomal vesicles. Liposomes are structures that enhance the absorption, stability and transport of active compounds, which is reflected by an increase in the bioactivity of the encapsulated molecules. The guarana extract has proven to be rich in methylxanthines and phenolic compounds. These metabolites are associatedto a wide variety of pharmacological properties, and exert a stimulating effect. For this reason, it has become popular in nutritional products. In this workit was characterized physicochemically a nutritional product based on guarana, vitamins and folic acid. In this product the active components were encapsulated in liposomal vesicles, which were analyzed to know their structure, size (diameter) and membrane thickness. Results and analysis indicate that liposomes are multivesicular and multilamellar structures. This structural conformation is related to a greater stability of the vesicles, which represents a favorable aspect for the formulation. On the other hand, the data obtained from the physicochemical characterization can be used as part of the quality control performed on the formulation. Additionally, they represent a starting point for improvements and optimization of this nutritional product.

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INTRODUCTION

In the last decade there has been a boom in the development of functional foods. However, the bioavailability of molecules with biological activity is generally limited (Liu *et al*., 2015). On the other hand, lack of efficacy is attributed to the instability of the molecules during the manufacturing and storage stages, interactions with other components of the formulation, rapid degradation after ingestion and low absorption (Li, Arranz, Guri, and Corredig, 2017). Encapsulation techniques offer an alternative to protect the active ingredients, which favors their bioactivity (Koynova and Tihova, 2010). Liposomes are spherical vesicles composed of one or more phospholipid bilayers with amphiphilic characteristics.

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These systems function as carriers of molecules of lipophilic and hydrophilic character (Li *et al*., 2017; Liu *et al*., 2015). The use of liposomes is very common in the pharmaceutical, cosmetic and food industries since it represents an improvement in the stability, protection, absorption and release of the active components (Frenzel and Steffen-heins, 2015, Li *et al*., 2017, Tan, Feng, Zhang, Xia, and Xia, 2016). The walls of the gastrointestinal tract are composed of epithelial cells covered by a layer of gastric mucus. Both barriers regulate the diffusion, absorption and therefore, bioavailability of the particles. In order to improve the bioavailability of active compounds, it is possible to modify their physicochemical properties using transport systems, such as liposomes, which have the capacity to be absorbed directly by the enterocytes (Li *et al*., 2017). This is attributed to the similarity between the biological membranes and the phospholipid bilayers that they are composed of (Koynova and Tihova, 2010; Li *et al*., 2017).
The plant *Paullinia cupana*, known as guarana, is native to Brazil (Matsuura *et al*., 2014; Portella *et al*., 2013). Recently, it

has become an important source of raw material for the cosmetic and food industry because of its stimulating, aphrodisiac and healing properties (Heard, Johnson, Moss, and Thomas, 2006; Hertz *et al*., 2015; Marques Medeiros *et al*.., 2016). The main metabolites of *Paullinia cupana* are derived from methylxanthines, such as caffeine, theophylline and theobromine, which have important stimulating effects on the central nervous system (Heard *et al*., 2006; Heckman, Weil, and Gonzalez, 2010; Marques Medeiros *Et al*., 2016). The proportion of caffeine is approximately 2.1 - 6%, while the proportion of theophylline and theobromine is less than 0.2%. Methylxanthines block adenosine receptors and inhibit phosphodiesterase, increasing norepinephrine activity, due to they are marketed as providing a stimulating effect and facilitating weight loss (Heard *et al*., 2006). On the other hand, a high proportion of antioxidant phenolic compounds, mainly catechins and epicatechins, has also been reported (Antonelli Ushirobira *et al*., 2007; Hertz *et al*., 2015). A wide variety of pharmacological properties related to the presence of the above mentioned secondary metabolites have been described. These include anticarcinogenic, cytoprotective, antiproliferative, antimicrobial, antioxidant, thermogenic and anxiolytic activity(Marques Medeiros *et al*., 2016, Matsuura *et al*., 2014, Portella *et al*., 2013). The objective of this study was to perform a physicochemical characterization of a liposomal formulation of guarana that is used as a nutritional product.

MATERIALS AND METHODS

Preparation of liposomes

The preparation of the liposomes was carried out using phosphatidylcholine to which a solution of sodium chloride was added slowly and with constant stirring. At the same time, specific amounts of water, potassium sorbate, potassium benzoate, guarana extract and vitamins were mixed in another container, always guaranteeing the complete dissolution of each 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.

Physico-chemical characterization

Determination of acidity and conductivity: The determination of pH and conductivity of the formulation was performed using a pH meter - conductivity meter (Thermo Scientific Orion 3 Star) at room temperature (≈ 25 ° C). Three measurements were performed; the results are expressed as the mean ± standard deviation.

Determination of the refractive index and Brix degrees: The diffraction index and Brix grades of the formulation were measured on an automatic refractometer (Rudolph Research J57) at room temperature (≈ 25 ° C). Measurements were performed in triplicate and the results are expressed as the mean ± standard deviation.

Determination of specific gravity: Specific gravity was determined by pycnometry. The assay was performed in triplicate and the results are expressed as the mean ± standard deviation.

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

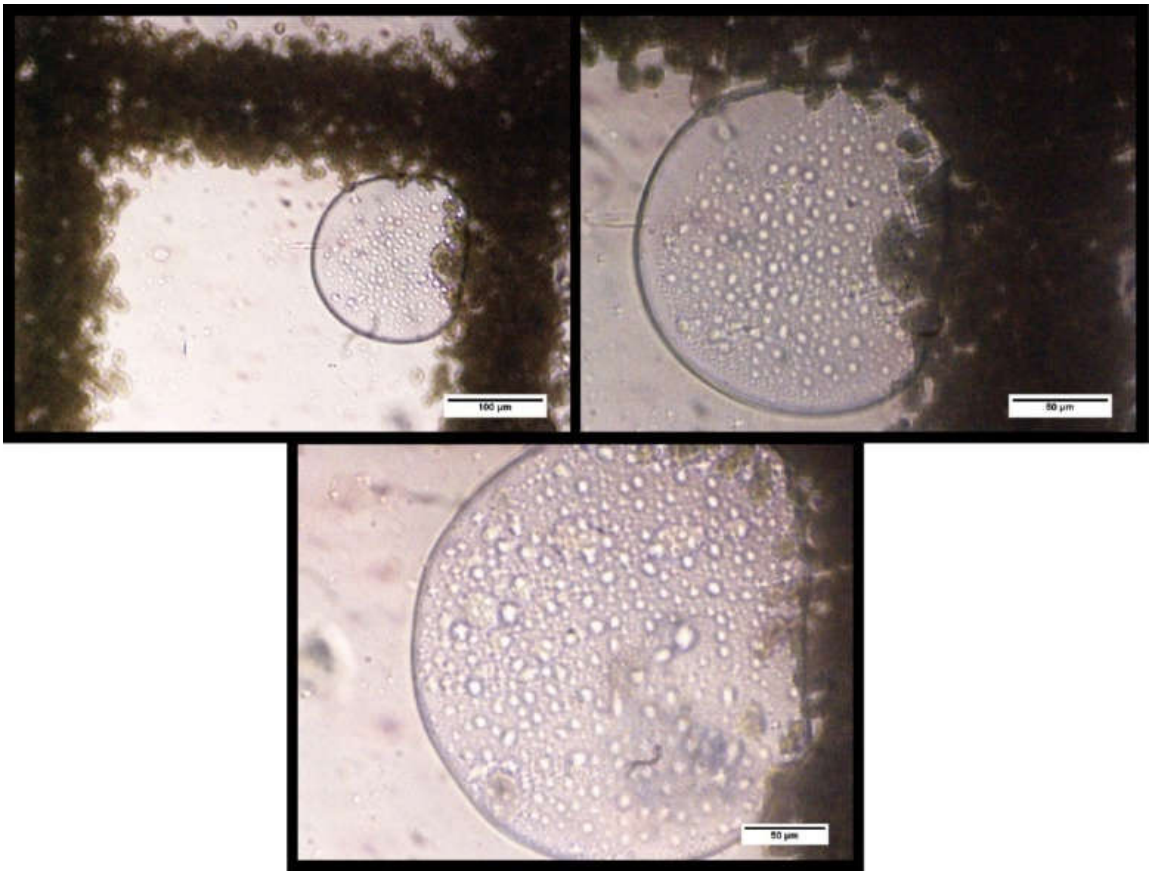

Fig. 1. Representative images of liposomal carriers present in a formulation of a nutritional product based on *Paullinia cupana*

the membrane thickness were quantified using the computer program ImageJ, the results are expressed as the mean ± standard deviation.

RESULTS

Table 1. Physicochemical characterization of a liposomal formulation based on *Paullinia cupana*

Sample	pH	Conductivity µS cm^{-1}	n_D^{23}	Degrees Brix / ° B	Specific Gravity
1	4,06	1452,0	1,34825	10,300	1,00717
2	4,02	1466,0	1,34825	10,400	1,00704
3	4,05	1453,0	1,34826	10,300	1,00822
Average	4,04	1457,0	1,348253	10,330	1,00748
Standard deviation	4,02	7,8	0,000006	0,058	0,00065

Table 2. Determination of the size and membrane thickness of liposomal carriers present in a formulation of a nutritional product based on *Paullinia cupana*

Multivesicular Liposomes		Encapsulated Liposomes	
Diameter	214 ± 64 µm	Diameter	8.9 ± 4.1 µm
membrane thickness	5.03 ± 0.91 µm	membrane thickness	2.32 ± 0.63 µm

DISCUSSION

In this study the physicochemical characterization of a liposomal formulation was performed. Liposomes are highly effective transport systems with the function of encapsulating and protecting the active components of a formula, which represents an improvement in their pharmacodynamic and pharmacokinetic profiles (Giansanti *et al*., 2016; Tai *et al*., 2017). From this principle, the objective of encapsulating in liposomal vesicles *Paullinia cupana* extract, vitamins and folic acid is to promote the bioavailability and stability of these components. Phosphatidylcholine was used to obtain the liposomes. This is a phospholipid commonly used in the formation of liposomal vesicles because of its similarity and compatibility with biological membranes. Phosphatidylcholine has been used to study systems formed by lipid bilayers, mainly their physical properties (Huang and Mason, 1978). The liposomes obtained in this work have a multivesicular conformation. In this way, liposomes with an average size of 8.9 ± 4.1 µm are encapsulated in a non-concentric array by a larger and thicker membrane (Chen, Han, Zhang, Yuan, andTang, 2010; Mirafzali, Thompson, and Tallua, 2014). These systems are characterized by providing greater stability compared to simple vesicles (Chen *et al*., 2010; Joo *et al*., 2013). On the other hand, according to the observations made, the membrane thickness of an outer vesicle correspondsto approximately 2 times the membrane thickness of a small liposome, therefore, it is possible that it is a multi-layered conveyor system of phospholipids, also known as multilamellarliposomes (Mirafzali *et al*., 2014).

Multilamellar liposomes have higher encapsulation efficiency than unilamellarliposomes, which represents a significant production advantage. In the same way, the stability is greater in the vesicles whose membrane is conformed by several lipid bilayers (Prévoteau and Faure, 2012). In addition to the morphological and structural characterization of the liposomes, several physicochemical properties were analyzed. The pH value, Brix grades, conductivity, specific gravity and refractive index were determined in order to characterize the formulation. The pH of the formulation has a slightly acid value, which is related to the organoleptic characteristics of the formulation and to the microbial growth (Elvira, Durán, Urrejola, Montero, and Espinosa, 2014). Also, Brix grades provide a measure of soluble solids in the formulation, such as sucrose and other sweeteners, which also influence organoleptic characteristics (Dongare, Buchade, and Shaligram, 2015). Organoleptic characteristics are related to consumer acceptability, which represents a highly relevant aspect in oral administration formulations (Elnaggar, Talaat, Bahey-El-Din, and Abdallah, 2016). When comparing the results of pH, Brix degrees and density with others reported of several authors, it is possible to observe that our measurements are within the usual ranges for these parameters in drinking formulations (Elvira *et al*., 2014; Kim *et al*. 2014). On the other hand, the objective of determining the physicochemical properties that characterize the formulation, consist in using such results as a parameter to be considered in the quality control of the product, which will be an indication of stability, as well as the morphological and structural characterization of liposomal vesicles. In the same way, the characterization performed in this work also has the function of establishing a starting point to improve the formulation. This is to optimize the organoleptic properties, to provide greater stability, to modify specific characteristics, such as viscosity, acidity, proportion of specific components, among other aspects.

Conclusion

The present investigation provides information about the physicochemical properties of a liposomal formulation based on *Paullinia cupana*. The morphological and structural analysis performed on the vesicles suggests that they are multivesicular and multilamellar liposomes. Regarding the physicochemical characterization performed, the data obtained provide specific values that are intended to be used as quality control parameters of the formulation. In addition, considering the results obtained, the formulation can be optimized and improved by establishing the measurements taken as a starting point.

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