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Physicochemical Characterization of a Liposomal Formulation Based on Glucosamine and Vitamin D, Commercialized as a Nutritional Supplement

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GMR and RVZ designed the study and wrote the protocol. Author MCR managed the literature searches, performed the statistical analysis and wrote the first manuscript. Authors SSR and SCN collaborated with the literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

In the last years, the transport and release mechanism of bioactive compounds has been studied. The liposomes are multivesicular systems that enhance the absorption, stability, and transport of these compounds. The glucosamine is an amino-monosaccharide, it has been associated with many biological activities, but its bioavailability and oral stability are very low. For this reason, the glucosamine formulation in liposomal systems is a good alternative.

In this paper, the physicochemical characterization of a liposomal formulation based on glucosamine and vitamin D was realized, this formulation is commercialized as a diet supplement. The determination of pH, degrees Brix, refraction index and specific gravity was realized to

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characterize the formulation. On the other hand, the structure, size (diameter) and membrane thickness of the liposomes were measured by microscopic observation.
From the formulation analysis, it is possible to affirm that the liposomes are multivesicular structures. Also, the external membrane thickness is higher than the membrane thickness of the encapsulated liposomes, indicating the liposomes could be multilamellar structures. The multilamellar and multivesicular structures are related to a high stability, resulting in a beneficial aspect of the formulation. Additionally, the dimension and structure conformation are related with an efficient encapsulation process of the active components, which are glucosamine and vitamin D.

Keywords: Glucosamine; multilamellar liposomes; multivesicular liposomes; nutritional product; phosphatidylcholine; vitamin D.

1. INTRODUCTION

The liposomes have been widely studied in the last decades because of its function as transport systems. In food and pharmaceutical industry they are used by their ability to encapsulate, protect and transport bioactive components [1,2].

The loading systems conformation consists of one or several phospholipids bilayers that interact with each other forming a vesicle [1,3]. This membrane conformation provides the liposomes with some advantages and beneficial characteristics, such as biocompatibility and low toxicity [1,4]. Also, the liposomes enhance the bioavailability and allow the physicochemical modification of some active principle characteristics, which is related to an improvement in its biological activity [5].

Glucosamine is an essential amino-monosaccharide that conform the glycoproteins, proteoglycans, and glycosaminoglycans. It is the biochemical precursor of all amino-saccharides and is involved in the proteins and glycosylated lipids synthesis [6,7].

Considering the biochemical importance of glucosamine, it is associated with a high variety of biological activities, like anti-inflammatory and immune-modulators effects, antioxidant activity, cardioprotective effects, cognitive effects related with memory and learning, neuroprotection and also, antimicrobial activity [6,8].

It has been demonstrated that the glucosamine security profile is excellent. Only adverse effects such as gastric discomfort, nausea, and diarrhoea have been reported [6].

Liquid products to be administrated by oral route are useful alternatives to the administration of bioactive compounds, like glucosamine. The physicochemical properties, such as acidity, the percentage of soluble solids, conductivity, refraction index and relative density, are used to characterize the formulation and as quality control parameters [9,10].

Drinks that contain vitamins, minerals, amino acids, and fruit and vegetable ingredients in its formulation are known as functional beverages. These drinks had many health benefits, such as good heart health, improve immunity, digestion, healthy joints and energy and productivity-boosting [9].

The development of functional drinks has increased in the last years and more people prefer drinks healthier and lower in calories than sweet carbonated drinks due to its functionality and health benefits [9]. In this context, we pretend to promote the development and quality of functional foods. The objective of this work is to carry out the physicochemical characterization of a liposomal formulation based on glucosamine and vitamin D commercialized as a functional beverage. The obtained information could be used as the product quality control specifications. On the other hand, the evaluated parameters and the obtained information could be considered as a starting point to improve the formulation in terms of stability, organoleptic properties, and, customer acceptance.

2. MATERIALS AND METHODS

2.1 Liposomes Preparation

The liposomes preparation was carried out using phosphatidylcholine to which a sodium chloride solution was added slowly and with constant stirring. At the same time, specific amounts of water, potassium sorbate, potassium benzoate, glucosamine and vitamin D 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.

2.2 Physicochemical Characterization

2.2.1 Acidity and conductivity determination

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

2.2.2 Refraction index and degrees brix determination

The formulation diffraction index and Brix grades were measured on an automatic refractometer (Rudolph Research J57) at room temperature (≈ 25ºC). Measurements were performed in triplicate and the results were expressed as a mean ± standard deviation.

2.2.3 Specific gravity determination

The specific gravity was determined by picnometry. The assay was performed in triplicate and the results were expressed as a mean ± standard deviation.

2.2.4 Size and morphology

The liposomes size was quantified by light microscopy. 1 mL of the sample was placed with 100 µL of colourant red #40 to stain the liposomes. A small sample was placed on a slide for observation using an Olympus U-TV 0,63 XC light microscope. The liposomes diameter and the membrane thickness were measured using the computer program ImageJ, the results were expressed as a mean ± standard deviation.

3. RESULTS

Results of the product physicochemical analysis are described in Table 1, and the liposomes morphologic characterization are described in Table 2 and Fig. 1.

4. DISCUSSION

Actually, there is an increasing interest in the investigation of different strategies to improve the absorption and oral bioavailability of bioactive compounds, like glucosamine. Pharmacokinetic data of this amino-monosaccharide shows that its bioavailability is low and erratic. The oral administration only reach a 6,1 – 26% of the plasmatic concentrations obtained by intravenous administration, indicating the metabolism effect at gastrointestinal level and the pre-systemic metabolism [6-8,11].

Another factor that is related to the low bioavailability is the glucosamine absorption mechanism. It is a facilitated diffusion process through glucose transporters. However, despite this mechanism, the affinity between glucosamine and this transporter is very low, which reduce the absorption and entry to the cell [8].

The liposomal formulations are common alternatives used to enhance the oral bioavailability of different molecules [4]. For this reason, we have opted for the glucosamine encapsulation in liposomal aggregates, as described in this work.

However, due to the use of liposomal transporters is dependent of the system physical and chemical stability [12], it is necessary to characterize from a physicochemical point of view the formulations.

Table 1. Physicochemical characterization of a liposomal formulation based on glucosamine and vitamin D

Parameters	pH	Conductivity / µS cm^{-1}	Refraction index	Brix degrees / °Brix	Specific gravity
Mean ± Standard Deviation	3,18 ± 0,01	9280 ± 92	1,35525 ± 0,0	14,74 ± 0,0	1,0358 ± 0,0013

Table 2. Size and membrane thickness determination of liposomal carriers present in a formulation of a functional drink based on glucosamine and vitamin D

Multivesicular liposomes		Encapsulated liposomes	
Diameter	184 ± 103 µm	Diameter	8,4 ± 3,9 µm
Membrane thickness	3,72 ± 0,74 µm	Membrane thickness	0,72 ± 0,16 µm

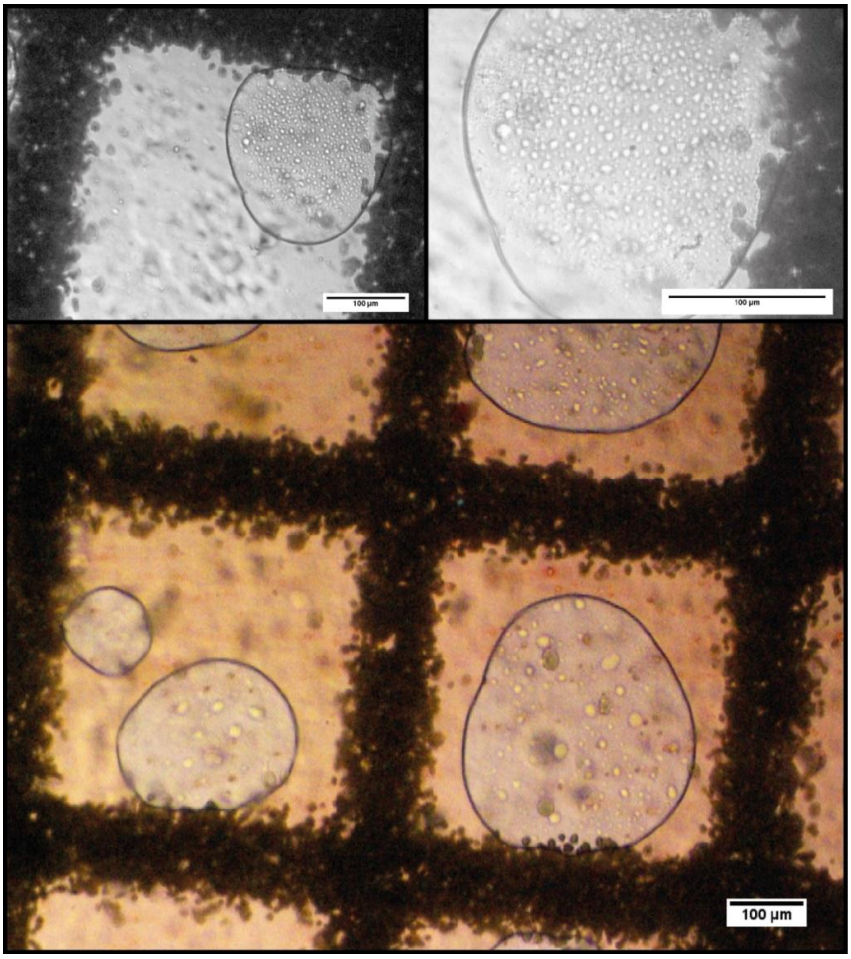

Fig. 1. Representative images of liposomal carriers present in a formulation of a functional drink based on glucosamine and vitamin D

The analyzed variables were size and morphology, acidity, conductivity, refraction index, degrees Brix and specific gravity. These variables are used as quality control tests and physicochemical stability indicators in food and pharmaceutical drinkable products.

From the results obtained in this work, it is possible to affirm that at pH and conductivity values obtained (Table 1), the liposomes remain stable during production, packaging, and storage.

The variations on pH values influence directly the liposomes stability and the liberation of the vesicles content. Is possible that changes in the system acidity compromise the stability of membranes that confirm the liposomes [13,14].

The acidity of these formulations is also related with the microbial growth, so formulations with low pH, like the studied formulation in this paper, have less susceptibility to suffer microbial contamination [9].

On the other hand, the degrees Brix, specific gravity and refraction index are physicochemical properties used for the characterization of drinkable products. The degrees Brix are used to know the soluble solids weight percentage, such as saccharose and sweeteners (Dongare, Buchade, & Shaligram, 2015) [15]. When comparing our results with the reports of several authors, it is possible to observe that drinkable products are within the range of 3,8 to 18,5ºBrix [9,10,16]

About the morphologic and structural characterization, in Fig. 1 is possible to see the liposomes, which are multivesicular systems. This conformation is characterized by the presence of an external membrane that

encapsulates liposomes with a lower size. The principal characteristic of this systems is the protection that offers the external membrane, which represents an advantage over simple liposomes [17].

With the liposomes size measure, shown in Table 2, was found that the multivesicular transporters had an average diameter of 184 ± 103 µm, while the membrane thickness is 3,72 ± 0,74 µm. Moreover, the diameter and membrane thickness of the encapsulated liposomes shows that the structures had a lower size and a lower membrane thickness. In this way, it is possible to relate the vesicular structure stability with their size, because a smaller size implies a greater stability of the liposomes [18].

About the membrane thickness, when comparing the obtained values, it was determined that the outer membrane is approximately five times larger than the encapsulated liposomes membrane. According to these results, we can suppose that the liposomes are multilamellar systems [19].

The thickness of an egg phosphatidylcholine bilayer is around 0,0069 µm [20]. It is an additional parameter to establish the hypothesis mentioned previously, and allow affirming that liposomes had a multilamellar structure because the membrane thickness in the vesicular systems is bigger than the reported value for only one phosphatidylcholine bilayer.
Multilamellar systems are related with a greater stability during production and storage process, as well as greater efficacy in terms of active components encapsulation, which is a technological advantage over other drugs-carrying systems [19,21].

The product physicochemical characterization and the liposomes morphological and structural analysis bring together all the necessary information to create an analysis certificate. This document guarantees the conformity of the product, which is directly related to its quality and stability. In addition, the obtained results are considered as a start point to improve and optimize the qualitative and quantitative formula for the product.

5. CONCLUSION

The physicochemical characterization realized in this work could be used as the product quality control parameters. In this way, it is intended that the parameters evaluated be maintained over time, evidencing the liposomal structures and the drinkable product stability.

Also, glucosamine encapsulation was realized satisfactory at pH = 3,18, forming liposomal structures with multivesicular and multilamellar conformation, which offer greater stability during production and storage. Likewise, the liposomes size and structure are closely linked with the active substances encapsulation efficacy, corresponding to glucosamine and vitamin D.

Finally, all the tests carried out and the evaluated parameters were used to create an analysis certificate for the functional drink. The collected data are critical parameters to the quality evaluation of the product. Additionally, the information obtained from all the tests are a starting point to improve and optimize the product, according to its stability and customer acceptance.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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