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Book of Abstracts

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Ethosomes: an approach for bioactive plant extract preservation envisaging cosmetic applications

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The present work is focused on upgrading the commercial potential of berry crop by-products by encapsulating them into liposomes to preserve their bioactivity. The extracts have been obtained with an ethanol-water mixture using ultrasound-assisted extraction, and the most promising ones were encapsulated in ethosome system. To achieve this goal, ethosomes were prepared using the cold method. Ethosomal suspensions were characterized concerning particle size distribution by laser dispersion, differential scanning calorimetry, infrared Fourier-transform spectroscopy, encapsulation efficiency, and morphological analysis using optical, scanning electron, and transmission electron microscopy. These results indicate that ethosomes are an appropriate method to encapsulate hydroethanolic bioactive plant bioresidue extracts and a good option to preserve them for further use in industrial applications, such as cosmetics. Future work will include optimizing the process and proof of concept by developing a cosmetic application.

Introduction

Returning to ancient cosmetic raw materials is a trend. The use of natural extracts has been growing since the beginning of the XXI century, mostly with those plants that have evolved various photo-adaptive mechanisms, including the production of antioxidants and UV-absorbing compounds, by being exposed to intense radiation in their environmental conditions [1]. The waste biomass derived from berry crops is a new focus of study, since producers are increasingly interested in its valorization, namely through the production of high-added-value products. In this context, the leaves and other aerial parts are good examples of waste biomass that can be exploited for several applications in cosmetic formulations since these residues possess interesting chemical compositions and consequent bioactive properties. On the other hand, plant extracts are not stable for useful periods without adding preservatives, and European regulations are very restrictive for those types of cosmetic formulation additives. Studies showed that encapsulation results in the preservation of the plant's extract, like hyssop, also concluding that this process is an effective way to increase the antioxidant activity of the extract and, if added to edible oils with natural antioxidants, increasing their shelf-life [2]. The ethosome system has several advantages, including greater drug permeability, increased drug entrapment, and better drug delivery [3], [4]. Ethosomes are simple to make, stable, and safe to use. This kind of ultradeformable vesicles has already proved their potential to transmit medicinal chemicals via the skin without negative effects two decades after their development. Examples like antiaging and hyperpigmentation, hair tonics for hair growth, or topical cellulite cream, have been on the cosmetic market. The use of ethosomes with appropriate vehicles such as creams, gels, and patches improve skin permeability and therapeutic benefits [5].

Objectives

The main objectives of this work was to encapsulate hydroethanolic extracts previously obtained from berry plant cropping waste through innovative technologies, that are expected to provide more stable ethosomes, preserve the bioactive and antioxidant properties of the extracts, while enhancing skin permeation, improve drug delivery, and increase drug entrapment efficiency.

Methods

Classical liposomes have been used as an option to encapsulate lipophilic compounds. In the present work, the used extracts are a mixture of compounds with a predominantly hydrophilic profile, so ethosomes were used as an alternative. The cold process of encapsulation was employed. Briefly, the aqueous extract solution is added while stirring to the mixture of organic ethanol and soy phosphatidylcholine once full dissolution has been achieved [6]. The study started with producing five distinct ethosomal suspensions, with different ethanol contents, from 20 to 40%, and miglyol replacing the extract. The ethosomes were analyzed by optical microscopy and laser dispersion to determine their shape and particle size distribution. Following, the extract was encapsulated using the formulation that best suited the intended use, using 35% of ethanol in the organic phase. Laser dispersion, differential scanning calorimetry, infrared Fourier-transform spectroscopy, encapsulation efficiency, and morphology using optical, scanning electron, and transmission electron microscopy were used to characterize the extract-loaded ethosomal suspension.

Results

Since the used extract had a significant amount of hydrophilic components, it was possible to dissolve in the aqueous phase.

Irregular surface morphology and the three-dimensional nature of ethosomes were further confirmed by SEM, suggesting the

vesicular ultra-deformability characteristics possessed by these novel kinds of carriers [7] (Figure 1). TEM morphology analysis techniques showed us the formation of a lipidic shell with an aqueous core, in which both fractions were encapsulated [8]. Osmium tetroxide is fixed by the phospholipidic layer, revealing a darker tone. It is used to give contrast to lipidic chains. It is also possible to observe the particles' irregular shape (Figure 1).

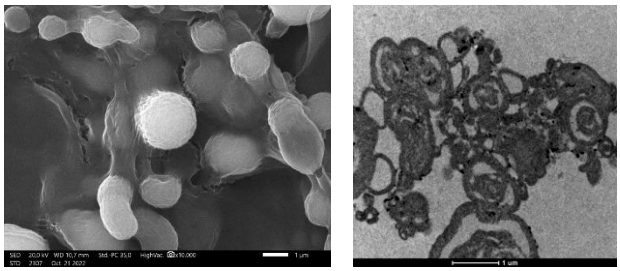

Figure1. SEM and TEM (scale= 1µm).

The particle size obtained for extract-loaded ethosomes is above the desired for the intended application (Figure 2). Overall, the mygliol-loaded ethosomes' size in volume increased with an increase in the ethanol fraction. The size distributions of blueberry extract-loaded ethosomes also increase compared with mygliol-loaded ethosomes (see data in Table 1).

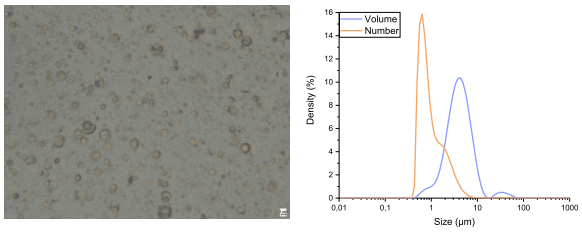

Figure 2. OM analysis (400x magnification, scale= 1µm), size distributions blueberry extract loaded ethosomes (35% in ethanol).

Conclusions

It is feasible to encapsulate hydrophilic extracts using the current approach. With ethosomes, the achieved particle size was greater than expected for the intended use. Generally, the size and volume of mygliol-loaded ethosomes increase with the ethanol content in the organic-phase mixture. The size distributions of blueberry extract-loaded ethosomes increase as compared to mygliol-loaded ethosomes.

Table 1. Statistical analysis (D10, D50, and D90) of size distributions (in number and volume), blueberry extract (BB), and mygliol (Mg) loaded ethosomes

Variable	Sample	D10 Size (nm)	(±) std	D50 Size (nm)	(±) std	D90 Size (nm)	(±) std	SPAN Text	D4:3 Size (nm)	(±) std
in number	Et_35_BB	529	0,44	802	1,77	2350	2,41	2,27	-	
	Et_35_Mg	634	7,66	1040	6,69	2130	5,84	1,44	-	
in volume	Et_35_BB	1760	9,03	3960	24,00	8240	109,00	1,64	5210	94,97
	Et_35_Mg	1240	0,99	2560	0,52	4910	2,34	1,43	2860	0,00

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