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Study of the encapsulation efficiency and release profiles of alginate microspheres containing α-tocopherol

P-BE14

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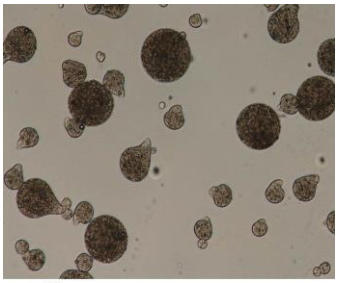

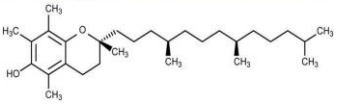

Vitamin E, a lipophilic natural antioxidant comprising different vitamers (α, β, γ and δ-tocopherols and tocotrianols), is frequently used in food supplements and added in different products, such as foodstuffs and cosmetics, to prevent lipid oxidation processes. Due to solubility and stability issues, α-tocopherol is generally administrated as succinate or acetate derivatives, which have lower bioavailability. In this work, alginate microspheres containing α-tocopherol were produced and evaluated for encapsulation efficiency and release profiles at different pH conditions during 24h. In vitro release tests showed that alginate microspheres maintain its integrity under simulated gastric conditions. By the contrary, under simulated intestinal conditions, an almost complete release is achieved during 24h, with a major portion (70%) being released after 2h.

Introduction

Vitamin E is a known lipophilic natural antioxidant since it has the capacity of scavenging free radicals, being particularly important in the prevention of lipid oxidation processes. Vitamin E encompasses different compounds presenting a chromanol ring and a saturated (α-, β-, γ- and δ-tocopherols) or unsaturated (α-, β-, γ- and δ-tocotrienols) phytol side chain. These compounds are believed to be involved in a diversity of physiological and biochemical functions, mainly due to their action as antioxidants but also because they can act as membrane stabilizers, thus generating different health benefits. The recommended ingestion of Vitamin E varies among the countries and according to criteria such as sex and age. In the USA, the recommended daily allowance (RDA) for an adult is 15 mg/day, whereas in Europe it is 4-15 and 3-12 mg α-tocopherol/day for man and women, respectively. Among the referred compounds, much attention has been given to α-tocopherol since it is considered as the one presenting the highest capacity of free radicals uptake [1]. α-Tocopherol is naturally present in several foods, such as vegetable oils, tree nuts, seeds, among others. Owing to its antioxidant capacity it is frequently included in supplements and used in the food industry being added to several foodstuffs in order to extend its shelf-life. Nevertheless, due to α-tocopherol instability and sensitivity towards oxygen and light, vitamin E, either present in supplements or added in foods, is generally administered in the most stable form of α-tocopherol, namely as acetate or succinate. However, these forms are considered to have a lower intestinal absorption compared to α-tocopherol [2]. In this way, microencapsulation could be an interesting approach, protecting the compound from its surrounding environment, avoiding its degradation, and allowing the incorporation of α-tocopherol in its natural form.

In this study, microencapsulation of α-tocopherol was tested using alginate as a polymeric matrix. This polymer, a linear polysaccharide obtained from brown algae consisting of β-mannuronic acid and α-guluronic acid units, was chosen due to its biocompatibility, biodegradability and non-toxicity. Moreover, it presents a high stability at acidic pH, being easily swollen under mild alkali conditions [3]. In this work, alginate microspheres (ME) loaded with α-tocopherol were produced using a NISCO Var J30 atomization unit, following a previously optimized methodology [4]. The produced microspheres were then evaluated for encapsulation efficiency and active principle release profile at different pH values (pH=1.2 and pH=7.4) simulating stomach and intestinal conditions.

Materials and Methods

Alginate microspheres preparation

Sodium alginate solution (3%, w/v) was prepared by dissolving alginate in deionised water and stirring overnight (200 rpm at 50ºC). Subsequently, an oil-in-water (o/w) emulsion was prepared as follows: 10 mL of alginate solution were measured into a Falcon conic test tube, added with 0.2 g of α-tocopherol, 0.25 mL of an emulsifier solution (Tween 20 (HLB=16.7) at 2%,

v/v) and additional 20 mL of the alginate solution. The prepared mixture was homogenized at 11400 rpm during 15 min using a CAT Unidrive ultraturrax resulting in a fine divided emulsion which was then atomized using a NISCO Var J30 unit operating at a pressure of 0.1 bar and a flow rate of 0.3 mL/min. The obtained ME were consolidated upon contact with 250 mL of a $CaCl_2$ (4%, w/v) coagulation solution during 20h. After consolidation, ME were recovered by filtration, washed with deionised water and observed by optical microscopy (OM).

Encapsulation Efficiency

The encapsulation efficiency (E.E.) was determined by the indirect method, based on the quantification of the nonencapsulated α-tocopherol present in the coagulation solution and in the wash solution. α-Tocopherol was quantified by spectrophotometry by measuring the absorbance at 297 nm. The amount of encapsulated α-tocopherol was calculated by difference, considering the maximum theoretical amount of α-tocopherol in the atomized emulsion volume.

Release profiles of α-tocopherol

α-Tocopherol release into simulated gastric (pH 1.2) and intestinal (pH 7.4) media [5,6] was determined by mixing 1 g of ME into 50 mL release media. The suspension was stirred at 100 rpm to ensure good contact between microspheres and media, and samples were periodically collected during a total of 24h. Each sample (5 mL) was centrifuged at 2000 rpm for 1 min and the amount of α-tocopherol in the supernatant was determined by spectrophotometry. After reading the absorbance, the sample was gently homogenized and reintroduced in the flask containing the ME suspension.

Results and Discussion

The produced ME were individualized and had a spherical shape when visualized by MO (data not shown). Table 1 presents the encapsulation efficiency results obtained for different assays**.**

Table 1. Encapsulation efficiency (E.E.) of alginate microspheres (ME).

Assay	Atomized Emulsion (mL)	ME (g)	Non-encapsulated Tocopherol (mg)	E.E. (%)
I	26	6.44	0,42	99.79
II	25	7.67	0,20	99.90
III	18	5.39	0,24	99.88

The results showed that a very high E.E. was attained, probably due to the high hydrophobicity of the encapsulated substance. Release profiles of α-tocopherol, showed that within 24h a maximum of 2% was released (data not shown) while under intestinal simulated conditions a complete release (100%) was attained. As can be observed in Fig.1 after two hours, approximately 70% of the active substance was released.

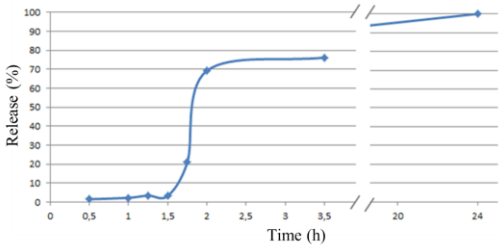

Figure 1. Release profile under simulated intestinal conditions (pH = 7.4)

Conclusion

The proposed ME process allowed achieving a very high encapsulation yield of α-tocopherol. As expected, the release of the active substance from the ME was very low at acidic pH and much higher at pH 7.4, attaining almost a complete release under simulated intestinal conditions. Thus, the proposed microencapsulation process can constitute an interesting solution to protect α-tocopherol, allowing its release under gastrointestinal conditions.

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