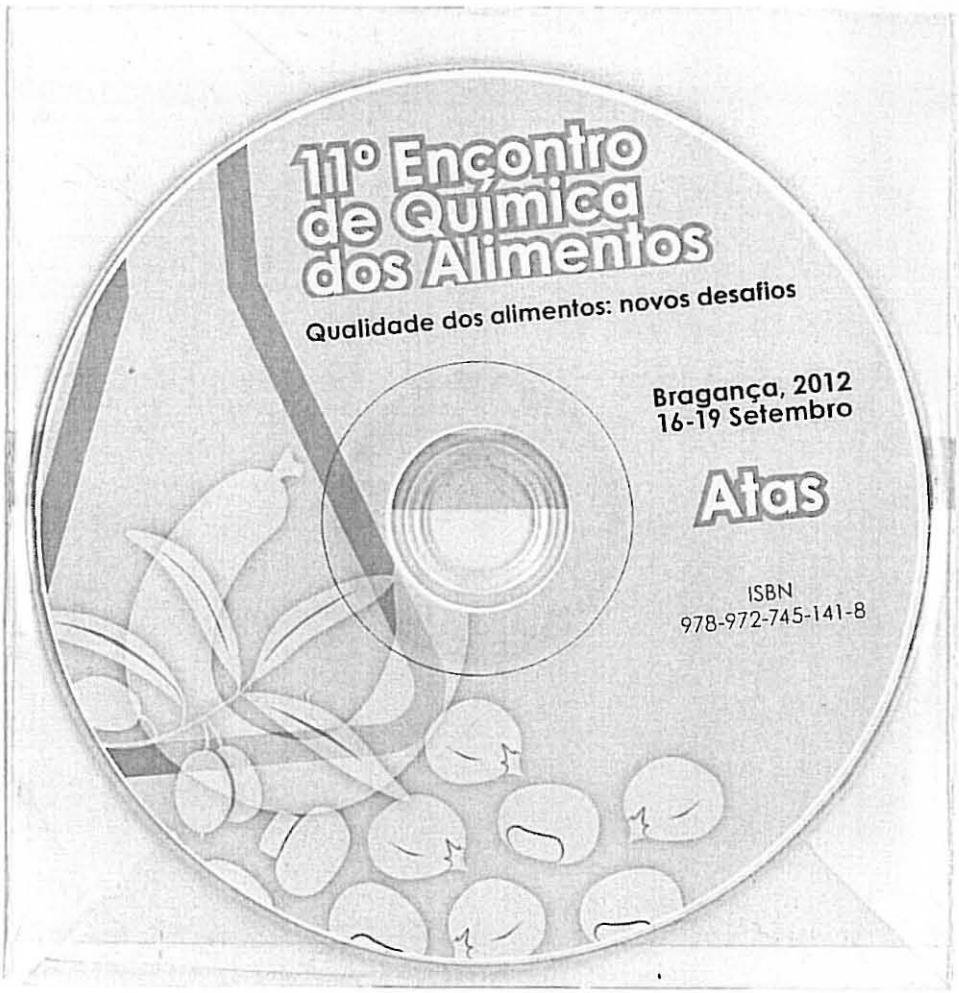
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α-Tocopherol microencapsulation using chitosan and alginate: swelling behaviour under different pH

Andreia Ribeiro[a,#], *Dalila Vieira*[a,#], *Isabel P. Fernandes*[b], *Joana S. Amaral*[a,c], *Filomena Barreiro*[b,*]

[a]ESTiG/Polytechnic Institute of Bragança, Bragança, Portugal
[b] LSRE, Polytechnic Institute of Bragança, Bragança, Portugal
[c]REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal
* barreiro@ipb.pt; # both authors contributed equally in this work

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ABSTRACT

Vitamin E is known for its antioxidant activity, particularly for its effective protection against lipid oxidation. Currently, the intake of this important class of bioactive compounds is associated with health benefits. Moreover, due to its antioxidant capacity, vitamin E is frequently added to several foods in order to extend shelf life, mainly by inhibiting lipid oxidation. Vitamin E comprises a set of eight compounds (α-, β-, γ-, δ-tocopherols and tocotrienols), of which α-tocopherol is considered, in vitro, to present the highest capacity of free radicals uptake. Due to α-tocopherol instability and sensitivity towards temperature, oxygen and light, vitamin E supplements are generally administered in its most stable form (acetate or succinate derivatives). However, these forms are considered to have a lower intestinal absorption. In this perspective, microencapsulation can be a viable alternative to preserve α-tocopherol thus maintaining its bioavailability in foods and other target products.

In this study, microencapsulation of α-tocopherol was tested using two polymeric matrices (chitosan and alginate). The obtained microspheres were submitted to acidic, neutral and basic media in order to evaluate its behaviour under pH conditions similar to those of the gastrointestinal tract (acid and basic for stomach and intestine, respectively) and under storage conditions (neutral).

1. 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 vitamins, 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, olive oil, tree nuts, seeds, cereals, green vegetables, among others. Owing to its antioxidant capacity by acting as an important chain-breaking radical scavenger, it is frequently added in the food industry sector 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 (acetate or succinate). However, these forms are considered to have a lower intestinal absorption compared to α-tocopherol. In this way, microencapsulation could be an interesting approach, protecting the compound from its surrounding environment, avoiding its degradation, and allowing the administration/incorporation of α-tocopherol in its natural form in supplements or foods. In this study, microencapsulation of α-tocopherol was tested using two polymeric matrices (chitosan and alginate). These polymers were chosen since both are biocompatible, biodegradable and non-toxic. Chitosan can be obtained by the partial *N*-deacetylation of chitin, which is the second most abundant polysaccharide in nature, after cellulose. Chitosan is a weak base insoluble in water and organic solvents but soluble in dilute acidic aqueous solutions (pH < 6.5), which are capable of converting the glucosamine moieties into its soluble form ($R\text{-}NH_3^+$) [2]. By the contrary, alginate, a linear polysaccharide consisting of β-1,4-mannuronic acid and α-1,4-glucoronic, can be obtained from brown algae. It presents a high stability at acidic pH, being easily swollen under mild alkali conditions [3]. Previous works reported the use either of chitosan or alginate gels to produce microcapsules/microspheres aiming at achieve protection and controlled release.
In this work, microspheres have been produced using a NISCO Var J30 unit. Firstly, the process was optimized with the chitosan-based system. In a second step the alginate-based one was tested and tuned, and thereafter α-tocopherol was microencapsulated using both systems. As a last step microspheres behaviour towards acid, neutral and basic pH environments was evaluated using optical microscopy (OM)

2. MATERIALS AND METHODS

2.1 Chitosan microspheres preparation

Chitosan solution (3%, w/v) was prepared by dissolving chitosan in 3% (v/v) diluted acetic acid and stirring overnight (200 rpm at 50ºC). The solution was filtered through a 5µm pore membrane and then used to prepare the oil-in-water (o/w) emulsion as follows: 10 mL of chitosan 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

completed with another 20 mL of the chitosan solution. The prepared mixture was homogenized at 11000 rpm during 15 min using a CAT Unidrive ultraturrax resulting in a fine divided emulsion that was then submitted to atomization using a NISCO Var J30 unit. The system uses a syringe pump to dispense the emulsion through a nozzle (0.3 mL/min), where the spray is formed under pressurized nitrogen (0.4 bar). The obtained microspheres were consolidated upon contact with a coagulation solution of NaOH (250 mL, 8%, w/v) during 20 h. After the consolidation period, microspheres were recovered by decantation, washed with deionised water and analysed by optical microscopy (OM).

2.2 Alginate microspheres preparation

Sodium Alginate solution (3%, w/v) was prepared by dissolving alginate in deionised water and stirring overnight (200 rpm at 50ºC). The o/w emulsion was prepared as described for chitosan (section 2.1) and the obtained emulsion atomized with the NISCO unit, using in this case a pressure of 0.1 bar. The consolidation bath was constituted by 250 mL of a $CaCl_2$ (4%, w/v) coagulation solution during 20h. After consolidation, microspheres were recovered by decantation, washed with deionised water and observed by OM.

2.3 Microspheres swelling behaviour

The obtained chitosan and alginate microspheres (0.2 g) were put into glass test tubes containing 5mL of solutions with different pH, namely 2, 7 and 10 (prepared by the addition of HCl 1M or NaOH 0.5 M). Samples of each tube were analysed immediately after contact with the solution and after regular periods of time (1-5 hours at regular intervals of 1 hour, 2, 3 and 7 days) by OM. The size of the swelled microspheres was estimated using the available microscope software module.

3. RESULTS AND DISCUSSION

To accomplish the microspheres production process several variables have been optimized starting with the chitosan-base system. For the o/w preparation the following have been tested: type and concentration of emulsifier, stirring speed used in the homogenization step and the volume ratio o/w. The emulsifier was added to the oily phase. In what concerns the atomization conditions, the emulsion was delivered to the atomization nozzle at a flow rate of 0.3 mL/min and only the atomization pressure was tested. Figure 1 shows, both chitosan and alginate microspheres, produced with the optimized experimental conditions, as summarized in the experimental section.

In general, the produced microspheres have a spherical shape with a size ranged from 8.4 μm to 67.4 μm. The alginate ones correspond to isolated particles whereas for the ones based on chitosan aggregation was detected. This is possibly due to the intrinsic adhesive properties of chitosan that makes difficult the achievement of individual particles. Nevertheless it is well perceptible that this effect is less pronounced after the washing step, where the microspheres are stored at neutral pH.

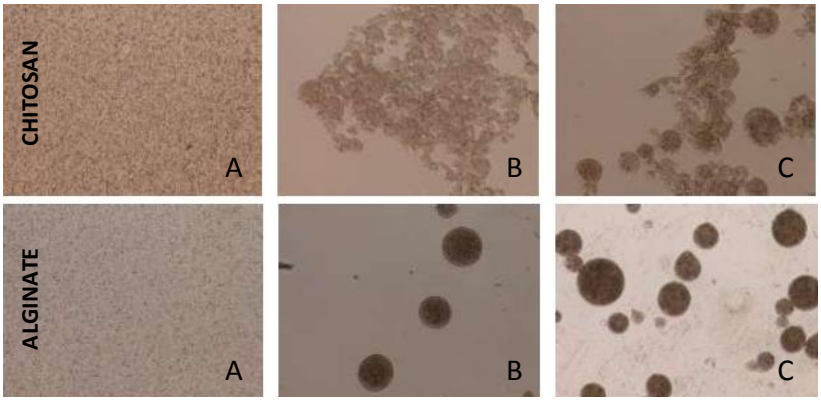

Figure 1. Chitosan and alginate microspheres (magnification 0f 100X): Emulsion visualization (A), and Microspheres visualization after consolidation (C) and after washing (D).

As expected for the swelling tests, it was found that both systems maintain their integrity at neutral pH. The microspheres based on chitosan resist basic pH conditions and swell and even disintegrate at acidic pH. In opposition, alginate microspheres tolerate the acidic pH conditions being highly swelled in basic medium, however without being destroyed. Figure 2 reports the microspheres aspect after a 7 days period in acidic and basic medium, for chitosan and alginate, respectively.

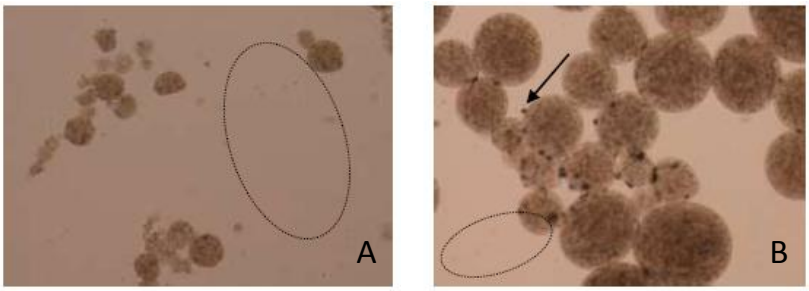

Figure 2. Microspheres observation after 7 days (magnification 0f 100X): Chitosan at pH=2 (A) and Alginate at pH=10 (B). Dashed circles and arrow put in evidence the liberated oil.

4. CONCLUSIONS

Face to the obtained results the developed microencapsulation process constitute an interesting solution to protect α-tocopherol when controlled release in acidic (stomach) or basic (intestinal tract) is desired, respectively if chitosan and alginate are used. The work will proceed to further optimize the process and access the release profiles.

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