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Characterisation and stability evaluation of bixin nanocapsules

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ABSTRACT

The aim of this study was to produce bixin nanocapsules by the interfacial deposition of preformed poly-ε-caprolactone (PCL). PCL (250 mg), capric/caprylic triglyceride (400 μL), sorbitan monostearate (95 mg) and bixin were dissolved in a mixture of acetone (60 mL) and ethanol (7.5 mL) under stirring (40 °C). This organic solution was added to the aqueous solution (130 mL) containing Tween 80 (195 mg). The size distributions in the formulations with bixin concentration from 11 to 100 μg/mL were evaluated periodically during 3 weeks of storage at ambient temperature. The optimal formulation (bixin concentration of 16.92 ± 0.16 μg/mL) was characterised in terms of particle size distribution, zeta potential, bixin content and encapsulation efficiency, and showed a volume-weighted mean diameter ($D_{4,3}$) of 195 ± 27 nm, around 100% of encapsulation efficiency and the nanocapsules were considered physically stable during 119 days of storage at ambient temperature.

1. Introduction

Annatto is a natural colourant that is mostly used in food products because of its low cost and high-quality sensorial characteristics, such as cheeses, ice creams, butters and meats (Cardarelli, Benassi, & Mercadante, 2008). The primary colouring component found in annatto seeds is bixin, a carotenoid formed by nine conjugated double bonds and two carboxylic groups (Fig. 1). The structure of bixin is responsible not only for its light absorption and antioxidant activity but also for its poor water-solubility, which impairs its use in low-fat foods (Rodriguez-Amaya, 2001).

Like other carotenoids, bixin is an efficient quencher of singlet oxygen and a scavenger of reactive species of oxygen and nitrogen (Chisté et al., 2011; Rios, Antunes, & Bianchi, 2009; Rios, Mercadante, & Borsarelli, 2007). Bixin is considered to be unstable in the presence of oxygen, heat and light. However, some studies showed that the techniques of complexation and encapsulation decrease the degradation rate of bixin caused by light, air, ozone, oxygen and high temperature (Barbosa, Borsarelli, & Mercadante, 2005; Lyng, Passos, & Fontana, 2005; Marcolino, Zanin, Durrant, Benassi, & Matioli, 2011; Parize et al., 2008).

In general, encapsulation improves the stability, solubility and bioavailability of encapsulated species and promotes its controlled release (Paese et al., 2009; Shaikh, Ankola, Beniwal, Singh, & Ravi Kumar, 2009; Zuidam & Shimoni, 2010). Nanoencapsulation is a process by which one compound is covered by another, producing particulate dispersions or solid particles, with sizes ranging from 10 nm to 1 μm. Depending upon the method of preparation of nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the bioactive compound is soluble in the core, confined to a cavity surrounded by a polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed (Mohanraj & Chen, 2006).

Nanocapsule systems are used for the delivery of drugs, peptides, proteins, genes, etc., and several compounds have been encapsulated (Couvreur, Barratt, Fattal, Legrand, & Vauthier, 2002). In the literature a number of methos are cited; most nanoparticles have been mainly prepared by dispersion of preformed polymers, polymerisation of monomers and ionic gelation or coacervation of hydrophilic polymers (Mohanraj & Chen, 2006).

For carotenoids, most research has been dedicated primarily to the encapsulation of β-carotene. Qian, Decker, Xiao, and McClements (2012) studied the effects of adding ascorbic acid, vitamin E acetate, coenzyme Q10 and ethylenediametetraacetic acid (EDTA) on the inhibition of β-carotene degradation in oil-in-water nanoemulsions. Silva et al. (2011) produced nanoemulsions of β-carotene using a high-energy emulsification-evaporation technique, studied the effect of processing variables (homogenisation time, shear rate and number of cycles), and evaluated the stability during storage.

The bixin encapsulation has been studied by Parize et al. (2008) and Barbosa et al. (2005). Parize et al. produced, characterised and evaluated the thermal stability of the urucum pigment (containing

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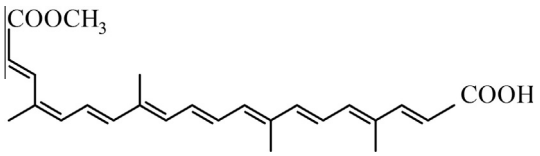

Fig. 1. Structure of *cis*-bixin (methyl hydrogen 9′-*cis*-6,6′-diapocarotene-6,6′-dioate).

bixin) microcapsules prepared by the technique of spray drying using chitosan as encapsulating agent in different solutions (acetic acid 5%, lactic acid 5% and citric acid 5%), and Barbosa, Borsarelli, and Mercadante (2005) evaluated the light stability of spray-dried bixin encapsulated with gum arabic or maltodextrin. However, to date, no studies have been published on the production and stability evaluation of bixin nanocapsules.

Indications that bixin may be important to human health and bixin's prevalence in the food industry as a colourant and antioxidant motivates the study of nanoencapsulation as a suitable technique for increasing the solubility of bixin in aqueous media. Therefore, the aim of this work was to prepare and characterise bixin nanocapsules and to evaluate their stability during storage.

2. Materials and methods

2.1. Materials

The polymer poly-ε-caprolactone (PCL) (Mw = 80,000) and sorbitan monostearate (Span 60) were obtained from Sigma (St. Louis, MO, USA). The capric/caprylic triglycerides (CCT) and polysorbate 80 (Tween 80) were purchased from Delaware (Porto Alegre, Brazil). Annatto seeds were obtained from the local market in Porto Alegre, Brazil. All other chemicals and solvents were of analytical or pharmaceutical grade.

2.2. Bixin standard

A bixin standard was prepared in triplicate according to the method of Rios and Mercadante (2004). This method consisted in the production of a bixin standard extracted from annatto seeds. Annatto seeds (25 g) were twice washed with hexane (100 mL). The solvent was discarded and the seeds were washed twice with methanol (100 mL). Methanol was also discarded and bixin was extracted from the seeds with ethyl acetate (100 mL). Each wash or extraction was carried out under magnetic stirring during 15 min. The extract was filtered and concentrated under reduced pressure in a rotary evaporator (Fisatom, model 801/802, São Paulo, SP, Brazil). After concentration, the recipient containing the extract was placed in a cold bath and dichloromethane (5 mL) was added slowly to this extract. After the addition of dichloromethane, ethanol (99.7%) was added slowly (20 mL). This solution was held at −18 °C during 12 h for crystallisation. The crystals formed in the bottom of the recipient were filtered, washed with 50 mL of ethanol (99.7%) and dried under reduced pressure ($T < 30$ °C). The purity of the standard was evaluated by high performance liquid chromatography (HPLC).

2.3. Bixin nanocapsules

Bixin nanocapsules were prepared by the technique of interfacial deposition of preformed polymers according to the method of Venturini et al. (2011). The polymer (PCL) (250 mg), triglycerides (CCT) (400 μL), span 60 (95 mg) and bixin were dissolved in a mixture of acetone (60 mL) and ethanol (7.5 mL) under magnetic stirring at 40 °C. After the solubilisation of PCL, CCT and Span 60, the standard of bixin (98.7%) was added and remained under magnetic stirring for 10 min (40 °C). This organic phase was added into an aqueous phase (130 mL) containing Tween 80 (195 mg) and remained under stirring for 10 min. The dispersion was concentrated under reduced pressure until it reached a final volume of 25 mL. In this method, acetone and ethanol (a water-miscible solvent) were used to solubilise PCL and Span 60. As the solvents migrate to the aqueous phase, an interfacial turbulence is created due to the spontaneous diffusion between the phases leading to the spontaneous formation of nanocapsules. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved (Mohanraj & Chen, 2006).

In preliminary tests, the bixin concentrations tested in the bixin nanocapsule formulations were 100, 58, 37, 16 and 11 μg/mL; these were stored under ambient conditions (25 ± 1 °C) in amber glasses, and the parameter of size distribution was evaluated periodically during three weeks.

Based on the nanocapsules stability, an optimal formulation was prepared in triplicate and was characterised in terms of viscosity, bixin content, encapsulation efficiency, pH, diameter, zeta-potential and colour. Moreover, the stability of the optimum formulation was studied during storage at ambient temperature. The pH, diameter and bixin concentration were evaluated weekly for 9 weeks; after this period, the evaluation was performed every 2 weeks up to 119 days of storage.

2.4. Viscosity

The viscosity of the bixin nanocapsule suspension was measured immediately after preparation using a Brookfield rotational viscometer (model DV-II + Pro, spindle LV2, Brookfield Engineering, USA) at 25 °C. Data were analysed using Brookfield Rheocalc 32 software.

2.5. Colorimetric analysis

The bixin nanocapsule suspension (optimal formulation) (10 mL) and a free bixin solution (10 mL) were analysed using a portable colorimeter (Konica Minolta model CR 400, Singapore). Both samples were prepared in triplicate in the same bixin concentration (16.92 μg/mL). The free bixin was solubilised in ethanol:water (2:8) due to the low solubility of bixin in pure water. The colorimetric parameters were obtained according to the Comission Internationale de l'Eclairage (CIELAB system); the coordinates were L^* (lightness), and the colour coordinates a^* (red-green component) and b^* (yellow-blue component), which were measured using the illuminant D_{65} and an angle of viewing of 0°.

2.6. Encapsulation efficiency

The total content of bixin was determined through the extraction of bixin from the bixin nanocapsule suspension. This method consisted of the extraction from an aliquot of 250 μL of formulation with acetonitrile (4.75 mL). This extract was sonicated by ultrasound (30 min) and centrifuged (15 min at 2820×*g*). The supernatant was injected in the HPLC. The bixin content in the aqueous phase of the bixin nanocapsule suspension was determined through the injection of the filtrate in the HPLC. The filtrate was obtained after the ultrafiltration/centrifugation of an aliquot of bixin nanocapsule suspension (400 μL) using a Ultrafree-MC® (10,000 MW, Millipore, Bedford, USA) in a centrifuge (15 min at 1690×*g*). The encapsulation efficiency was determined according to the method of Venturini et al. (2011), by dividing the difference between the total concentration of bixin and the concentration of bixin in the aqueous phase by the total concentration and multiplying the results by 100.