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Optimization of α-tocopherol loaded solid lipid nanoparticles by central composite design

Sabrina Matos de Carvalho[a,*], Carolina Montanheiro Noronha[a], Caroline Louise Floriani[a], Renata Calegari Lino[a], Gabriela Rocha[a], Ismael Casagrande Bellettini[b], Paulo José Ogliari[a,c], Pedro Luiz Manique Barreto[a]

[a] Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Santa Catarina, 88034-001 Florianopolis, Brazil
[b] Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianopolis, Brazil
[c] Departmento de Informática e Estatística, Universidade Federal de Santa Catarina, 88040-900 Florianopolis, Brazil

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ABSTRACT

Solid lipid nanoparticle (SLN) systems were developed using response surface methodology to optimize the mean particle size, α-tocopherol recovery rate and zeta potential of SLNs containing α-tocopherol. The optimization of α-tocopherol-loaded SLNs was characterized by X-ray diffraction analysis, differential scanning calorimetry, and the analysis of morphology and physical stability. The optimal conditions for an α-tocopherol-loaded SLN preparation were a particle size, α-tocopherol recovery rate and zeta potential of 214.5 nm, 75.4% and −41.9 mV, respectively; this preparation was stable during storage at 6 °C for 21 days. Furthermore, Compritol® 888 CG ATO changed its crystalline nature from the β′ polymorphic form in the pure formulation to the α and β′ polymorphic forms in SLNs.

1. Introduction

Oxidation processes are the major cause of deterioration in lipid-based foods, especially those containing polyunsaturated fats. This process decreases food quality by increasing the development of rancidity, off-flavor compounds, polymerization, reversion and other reactions that reduce the shelf life, nutritive value and sensory quality of the food product. In addition, oxidation is implicated in the pathogenesis of numerous chronic diseases. A method of protection against oxidation is the addition of antioxidants (Brewer, 2011; Ndhlala et al., 2010; Shahidi and Zhong, 2010; Mozafari et al., 2006), either as direct additives or indirectly through diffusion from packaging material (van Aardt et al., 2004). Antioxidants play a major role in preventing or delaying oxidation of a substrate when present in low concentrations compared with an oxidizable substrate. Both synthetic and natural antioxidants are widely used in food products; however, some synthetic antioxidants have become controversial due to their potential adverse effects on human health. Natural sources of antioxidants, such as tocopherols and other compounds, have been extensively applied in the food industry (Shahidi and Zhong, 2010; Mozafari et al., 2006).

Vitamin E is a nutrient that occurs naturally in vegetable oils or in deodorizer distillates (Shahidi and Zhong, 2010; Evans et al., 2002). This fat-soluble antioxidant can effectively scavenge lipid peroxyl radicals and act as a synergist with many other antioxidants (Byun et al., 2011; Ndhlala et al., 2010; Shahidi and Zhong, 2010; Yenílmez and Yazan, 2010). Vitamin E has several isomers, including α-tocopherol, which is the most abundant form in nature (Byun et al., 2011; Gonnet et al., 2010; Hatanaka et al., 2010). This form is widely used in vitamin supplementation and as an antioxidant in the food, cosmetic, and pharmaceutical industries (Gonnet et al., 2010; Hatanaka et al., 2010; Yoo et al., 2006). However, the fact that these compounds are insoluble in water has made their use problematic in food formulation (Weiss et al., 2008; Yoo et al., 2006; Tan and Nakajima, 2005); vitamin E is often present in food at levels below the therapeutic threshold and in forms with reduced or insufficient bioavailability (Cheong et al., 2008).

To overcome this limitation, nanotechnology offers the food industry the potential to significantly improve the solubility and bioavailability of many functional ingredients, including carotenoids, polyunsaturated fatty acids and numerous other compounds, such as α-tocopherol (Cheong et al., 2008; Mozafari et al., 2006; Weiss et al., 2008; Dingler et al., 1999).

Solid lipid nanoparticle (SLN) systems, introduced in 1991, are one of the most promising encapsulation technologies employed in the rapidly developing field of nanotechnology (Mozafari et al., 2006) and represent an alternative carrier system to traditional

* Corresponding author at: Rodovia Admar Gonzaga, 1346, Florianopolis, Santa Catarina 88034-001, Brazil. Tel.: +55 48 37215368; fax: +55 48 37219943.
E-mail addresses: sabrinamcar@gmail.com, sabrinacarvalho13@yahoo.com.br (S.M. de Carvalho).

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colloidal carriers, such as emulsions, liposomes, and polymeric micro- and nano-particles (Freitas and Müller, 1999).

The physicochemical properties of SLNs are controlled by many factors, such as the nature and relative amount of lipids and surfactants and the ratio of solid lipids to drugs in the formulation. Because the ingredients significantly affect the physicochemical properties and drug-release profiles of the nanoparticles, the design of an optimized formulation is an important aspect of SLN production (Mehnert and Mäder, 2012; Hao et al., 2011; Karn-orachai et al., 2012).

In these situations, where several variables may influence the system properties, response surface methodology (RSM) is an appropriate technique to evaluate the relationships between the response and independent variables and to optimize the processes or products (Calado and Montgomery, 2003; Baş and Boyacı, 2007); RSM requires less experimentation and provides estimates of the relative significance of different variables (Hao et al., 2011).

The purpose of this research was to determine the optimal conditions for α-tocopherol-loaded SLNs produced by the HPH. Central composite design (CCD) was used to investigate the influence of the surfactant concentrations and α-tocopherol amount on the mean particle size, zeta potential, and α-tocopherol recovery rate. Additionally, the optimized α-tocopherol-loaded SLNs and the control formulation (α-tocopherol-free SLN) were evaluated by differential scanning calorimetry (DSC), X-ray diffraction, transmission electron microscopy (TEM) analysis, and analysis of the physical stability to characterize the systems. The α-tocopherol entrapment efficiency was determined for the optimized α-tocopherol-loaded SLN formulation.

2. Materials and methods

2.1. Materials

The solid lipid glyceryl behenate (Compritol® 888 CG ATO, melting point ~73 °C) was obtained from Gateffossé (Saint-Priest, France). α-Tocopherol (98% purity HPLC grade) and methanol (HPLC grade) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Soya lecithin (LIPOID S 75, 68–73% phosphatidylcholine) was supplied by Lipoid GmbH (Ludwigshafen, Germany). Poloxamer 188 (Pluronic F68) was kindly donated by BASF AG (Ludwigshafen, Germany). All others chemical reagents and solvents were of analytical grade and were used as received.

2.2. Preparation of α-tocopherol-loaded SLNs

α-Tocopherol-loaded SLNs (α-TC-loaded SLNs) were prepared by the hot high homogenization technique (Müller et al., 2000) with a high-pressure homogenizer (APLAB-10, Artepeças, Brazil) at 500 bar. Compritol® 888 CG ATO was heated to 80–85 °C, and α-tocopherol and soya lecithin were dispersed in the molten lipid to form the lipid phase (LP). The LP was dispersed in the hot surfactant (Pluronic F68) solution of distilled water and water phase (WP) using an Ultra-Turrax T25 (IKA, Germany) at 9000 rpm for 1 min. This pre-emulsion underwent seven high-pressure homogenization cycles. The dispersion obtained was cooled to 20 °C, filtered through an 8 μm filter paper, and stored in dark bottles. The homogenization circuit was maintained at 85 °C by a water bath coupled to the machine, and the duration of the seven cycles was approximately 5 min. The different formulations produced are shown in Table 1.

2.3. Experimental design optimization

A resolution III $2^{(7-1)}$ fractional factorial design was previously employed to select the most important independent variables (factors) that can influence the physicochemical properties of the SLNs. Next, response surface methodology using central composite design (CCD) with three independent variables was applied to determine the optimal levels for the mean particle size (Y_1), α-tocopherol recovery rate (Y_2), and zeta potential (Y_3).

The selected factors were the active compound concentration (α-tocopherol, %, X_1), lipophilic surfactant concentration (soya lecithin, %, X_2), and hydrophilic surfactant concentration (poloxamer 188, %, X_3), which were studied at five different concentration levels: one central point (X_1: 0.75%, X_2 and X_3: 1.0%), level 1 (X_1: 1.0%, X_2 and X_3: 1.5%), level −1 (X_1, X_2 and X_3: 0.50%), level α (X_1: 1.17%, X_2 and X_3: 1.84%), and level $-\alpha$ (X_1: 0.33%, X_2 and X_3: 0.16%). The α value (1.682) was obtained from the equation $\pm\sqrt{3}$ for $k = 3$ (three independent variables).

According to the CCD generated by the STATISTICA software (version 7.0, 2004 StatSoft, Inc., EUA), a total of 18 experiments, including 8 factorial points (levels −1 and +1), 6 axial points (levels

Table 1
Central composite design parameters obtained for various runs and experimental values of particle size, α-tocopherol recovery rate, and zeta potential of α-tocopherol-loaded SLNs.

Run no.	Block	Assigned independent variables			Real independent variables			Responses		
		X1	*X2*	*X3*	*α-Tocopherol concentration (%)*	*Soya lecithin concentration (%)*	*Poloxamer 188 concentration (%)*	*Particle size (nm)*	*α-Tocopherol recovery rate (%)*	*Zeta potential*
1	Day 1	−1	−1	−1	0.5	0.5	0.5	459	74.2	−37.4
2	Day 1	−1	−1	1	0.5	0.5	1.5	190	70.7	−36.3
3	Day 1	−1	1	−1	0.5	1.5	0.5	324	73.4	−53.3
4	Day 1	−1	1	1	0.5	1.5	1.5	213	74.2	−47.6
5	Day 1	1	−1	−1	1.0	0.5	0.5	326	75.8	−38.7
6	Day 1	1	−1	1	1.0	0.5	1.5	171	82.1	−31.4
7	Day 1	1	1	−1	1.0	1.5	0.5	292	76	−51.9
8	Day 1	1	1	1	1.0	1.5	1.5	224	76.4	−46.1
9	Day 1	0	0	0	0.75	1.0	1.0	205	72.1	−45
10	Day 1	0	0	0	0.75	1.0	1.0	215	74.5	−46.5
11	Day 2	−1.682	0	0	0.33	1.0	1.0	230	69.2	−47
12	Day 2	1.682	0	0	1.17	1.0	1.0	217	76.7	−45.6
13	Day 2	0	−1.682	0	0.75	0.16	1.0	211	73.2	−30.2
14	Day 2	0	1.682	0	0.75	1.84	1.0	268	58.5	−54.3
15	Day 2	0	0	−1.682	0.75	1.0	0.16	615	71.3	−52.6
16	Day 2	0	0	1.682	0.75	1.0	1.84	196	71.1	−40.6
17	Day 2	0	0	0	0.75	1.0	1.0	217	74.2	−46.15
18	Day 2	0	0	0	0.75	1.0	1.0	204	74	−47.8