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Encapsulation of eugenol rich clove extract in solid lipid carriers

Diego F. Cortés-Rojas, Claudia R.F. Souza, Wanderley P. Oliveira *

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Av. do Café s/n, Ribeirão Preto, SP 14040-903, Brazil

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ABSTRACT

Clove (*Syzygium aromaticum*) is one of the richest sources of natural phenols. In this study, lipid formulations containing clove extract were spray dried to encapsulate the volatile and poor water soluble compounds, eugenol and eugenyl acetate, aiming to obtain solid redispersible powders. Five formulations were prepared to test two different solid lipids, two surfactants, and three drying carriers. The dried product was characterized by the eugenol and eugenyl acetate retention, *in vitro* antioxidant activity, and by relevant physical properties. The formulation containing glyceryl behenate, Poloxamer 188, and Maltodextrin DE10 presented better retention of bioactive compounds and good antioxidant activity. Therefore, this formulation was selected to study the influence of the dispersion method (high-shear mixing ultraTurrax, ultrasonication and high-pressure homogenization) and the drying technique (spray- and freeze-drying). The freeze-dried samples presented significantly higher retention of eugenol and eugenyl acetate than the spray-dried ones.

1. Introduction

Plants are important sources of nutritional and medicinal compounds. However, it is necessary to develop effective dosage forms that allow their proper utilization. Natural antioxidants have gained great interest due to their effectiveness in preventing and combating health problems caused by oxidative stress (Somogyi et al., 2007). Moreover, natural antioxidants could avoid the secondary effects of synthetic antioxidants that are employed as food additives (Krishnaiah et al., 2011).

Clove (*Syzygium aromaticum*) is an important medicinal plant that has been employed for centuries as food preservative and pain reliever. It is also claimed to be the richest source of phenolic antioxidants (Pérez-Jiménez et al., 2010; Shan et al., 2005). Eugenol and eugenyl acetate, which are poorly water soluble molecules, are the main compounds of clove associated with their medicinal and nutritional benefits (Ohkubo and Shibata, 1997; Park et al., 2007; Pérez-Conesa et al., 2006; Rana et al., 2011).

Buriti oil is obtained from the fruits of palm tree *Mauritia flexuosa*, native of the Amazon region in Brazil. It was employed in this study as an adjuvant in lipid formulations and also because it is a rich source of carotenoids (1706 ± 54 μg of total carotenoids/g), oleic acid (60.3%), α-tocopherol and other unsaturated fatty acids that are of great interest for nutraceutical applications (De Rosso and Mercadante, 2007; Ribeiro et al., 2012; Zanatta et al., 2010).

The development of formulations containing instable, volatile and poorly water soluble compounds is a challenging task, and several strategies have been employed to achieve this objective, such as the development of emulsions, liposomes, and polymeric micro- and nanoparticles. Recently, lipid nanoparticles have gained special attention, for the formulation of oral, topical and parenteral pharmaceutical products due to the high encapsulation efficiency, stability and modification of solubility (Joshi and Müller, 2009). The first generation of lipid particles were named as solid lipid nanoparticles (SLN) and consisted of the dispersion of the active compound in a melted solid lipid or a blend of solid lipids, then the lipidic matrix is cooled incorporating the active molecules between fatty acid chains or lipid lamellae (Xia et al., 2007). Depending on the physicochemical properties of the lipids and their purity degree, perfect crystalline lattice could form reducing the incorporation of active molecules (Guimarães and Ré, 2011). A second generation of lipid particles known as nanostructured lipid carriers (NLC) consists of a combination of solid and liquid lipids, creating crystals with many imperfections that provide an additional space for the loading of active molecules, improving in this way the amount of the actives compounds incorporated and reducing the probability of expulsion from the lipid phase (Bourezg et al., 2012; Varshosaz et al., 2012; Xia et al., 2007).

Factors as the kind of lipid, surfactant, emulsification process and the drying/cooling conditions could influence the properties of the particles obtained. For this reason it is important to examine each step of the production process and study the mechanism of formation of the particles to achieve the desired characteristics of the final product (Guimarães and Ré, 2011).

Different methods are employed to produce lipid nanoparticles, however, high pressure homogenization and ultrasound are the most common methods in pharmaceutical and food industries

* Corresponding author. Tel.: +55 16 36024185.
E-mail address: wpoliv@fcfrp.usp.br (W.P. Oliveira).

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Table 1
Composition of formulations (% w/w).

Component	Function	Formulation				
		F1	F2	F3	F4	F5
Clove extract	Antioxidants source	63.0	63.0	63.0	63.0	63.0
Glyceryl dibehenate[a]	Solid lipid	8.1	8.1	8.1	–	8.1
Stearic acid	Solid lipid	–	–	–	8.1	–
Buriti oil	Liquid lipid and antioxidants source	0.9	0.9	0.9	0.9	0.9
Polysorbate 80	Surfactant	0.9	0.9	0.9	0.9	–
Poloxamer 188[b]	Surfactant	–	–	–	–	0.9
Lactose	Drying carrier	–	18.0	–	–	–
Maltodextrin	Drying carrier	18.0	–	9.0	18.0	18.0
Arabic gum	Drying carrier	–	–	9.0	–	–
Water	Solvent	9.0	9.0	9.0	9.0	9.0

[a] Compritol® 888 ATO.
[b] Kolliphor® P 188.

for the formulation of drugs and dietary supplements due to the simplicity and facility of scaling up (Guimarães and Ré, 2011).

A new alternative employed to increase the stability of the lipid nanoparticles dispersions was reported by Bourezg et al. (2012). Redispersible powders from lipid nanoparticles were obtained by three drying methods, spray drying, freeze drying and fluidized bed drying comparing the advantages and disadvantages of each process for the production of a pediatric formulation of spironolactone.

There are several reports of the production of NLC employing plant-derived compounds such as curcumin (Puglia et al., 2012), baicalein (Tsai et al., 2012), eugenol (Pokharkar et al., 2011), lutein (Mitri et al., 2011), β-carotene (Hentschel et al., 2008; Hung et al., 2011) and quercetin (Chen-yu et al., 2012), among others. The combination of clove extract which is rich in polyphenols with buriti oil, which is rich in carotenoids was never done before. The drying of the lipid formulations obtained also represents a new tendency especially for oral applications since solid forms are more stable and could be easily redispersible when needed.

The aim of this work was to produce powdered dried particles of NLC containing natural antioxidants comparing the influence of the solid lipid, surfactant, drying carrier and drying methods on the physicochemical and antioxidant properties of the powders.

2. Materials and methods

2.1. Chemicals

Dried clove buds were acquired from the region of Valença, BA, Brazil in collaboration with the Agronomic Institute CEPLAC. Eugenol 99%, eugenyl acetate, ⩾98% FCC, 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) [ABTS] were bought from Sigma–Aldrich (Brazil). Compritol® 888 ATO (Gattefosse, France) was acquired from Brasquim (São Paulo, Brazil), buriti oil was a gift of Beraca (Ananindeua, PA, Brazil), stearic acid (Viafarma, São Paulo, Brazil), Tween 80 (Polysorbate 80) and potassium persulfate were acquired from LabSynth (Diadema, SP, Brazil), Kolliphor P 188 Micro was kindly donated by BASF (Brazil). Other materials employed were, Lactose M-200 (Natural Pharma, São Paulo, Brazil), Maltodextrin DE10 (MOR-REX 1910 – Corn Products of Brasil®, Mogi Guaçu, SP, Brazil), Arabic gum – Encapcia (NEXIRA, São Paulo, Brazil).

2.2. Clove extract preparation

The clove extract was prepared by dynamic maceration employing milled dried clove buds (knife-mill model MA-680, Marconi, Piracicaba, SP, Brazil) placed in jacked glass containers coupled with a thermostatic bath (Marconi MA-184, Piracicaba, SP, Brazil) set at 50 °C. Ethanol at 70% (v/v) was employed as extraction solvent, with a plant to solvent ratio of 1/10(w/v). The extraction was performed for 30 min. The extract was filtered through filter paper (80 g/m^2, 14 μm pore diameter, and thickness of 205 mm; J. Prolab, S. José dos Pinhais, PR, Brazil) using a Buchner funnel (No. 4, diameter of 150 mm) connected to a vacuum pump (model 131, Prismatec, São Paulo, Brazil) set at a vacuum pressure of 500 mmHg. The extractive solution was concentrated in a rotary evaporator using a vacuum pressure of 600 mmHg at a maximum temperature of 55 °C. The concentrated extract had a final solid concentration of 7.75 ± 0.46% (w/w), measured in a moisture analyzer (Sartorius MA35 Goettingen, Germany).

2.3. Preparation of formulations

Lipid formulations were prepared according to the compositions described in Table 1 by the ultrasonication method. The conditions used for the preparation of lipid formulations containing the concentrated clove extract were adapted from previous works (Das et al., 2012; Liu and Wu, 2010; Puglia et al., 2012). The solid lipid was melted in a water bath at 10 °C above its melting point and mixed with the liquid lipid (Buriti oil). The surfactant was dissolved in hot water, mixed with the clove extract and then heated to the same temperature of the lipid phase. The aqueous phase was homogeneously dispersed into the lipid phase by using a high-speed stirrer (UltraTurrax T18, IKA-Wilmington, NC, USA) at 18.000 rpm/min for 5 min. The o/w emulsion formed was sonicated in a ultrasonic sonicator VCX-750 (SONICS Vibracell, Newtown, USA), with aid of 13 mm probe at frequency of 20 kHz, intensity of 70% for 3 min. Finally, the solid drying carrier was added, and the composition was spray dried. Solid concentration of compositions was standardized at 33% (w/w) (including the solids concentration of the concentrated clove extract which was 7.75% (w/w)).

2.4. Comparison of homogenization and drying technique

The formulation 5, composed by Compritol®, Poloxamer 188 and Maltodextrin DE10 was selected to compare the homogenization process (high shear mixing, ultrasound and high pressure homogenization) and the drying methods (spray drying and freeze drying). High pressure homogenization was performed in a Panda Plus (GEA Niro Soavi, Parma, Italy) applying three cycles at 500 bar. High shear mixing was performed in a UltraTurrax (T18, IKA-Wilmington, NC, USA) at 18,000 rpm/min for 5 min. For the sonication process it was employed a ultrasonic sonicator VCX-750 (SONICS Vibracell,