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Technological stability of solid lipid nanoparticles loaded with phenolic compounds: Drying process and stability along storage

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ABSTRACT

Solid lipid nanoparticles (SLNs) have been widely studied and tested as vehicles for natural compounds. Recently, Witepsol and Carnauba SLNs were shown to be effective systems for the entrapment of rosmarinic acid (RA) and herbal extracts. In the present work, the improvement of stability and bioactivity of these systems was studied. Thus, the freeze-drying of SLNs produced with Witepsol and Carnauba waxes loaded with RA and herbal extracts (sage and savory) were tested. The use of three different cryoprotectants (glucose, mannitol and trehalose) at two different concentrations (5 and 10%, w/v) were evaluated. Furthermore, the prepared SLNs were stored under different conditions (atmosphere, temperature, absence or presence of light) and in different packaging materials, over 365 days. The effect on the SLNs physical stability and bioactivity was assessed. The most suitable cryoprotectant was mannitol at 10% (w/v) for all formulations tested. The solid state of SLNs, with storage at room temperature, in glass flasks, protected from light and under N_2 controlled atmosphere were the best storage conditions in which the SLNs bioactivity was maintained during 365 days.

1. Introduction

Rosmarinic acid (RA) is an important bioactive polyphenolic compound with several biological activities, such as antioxidant, anti-inflammatory, anti-mutagenic, anti-bacterial, antiviral, among others (Nunes et al., 2015). *Salvia* sp. and *Satureja montana* (sage and savory, respectively) medicinal herbs are rich in RA, besides other phenolic compounds present in minor quantities (e.g. caffeic acid, rutin). The use of natural extracts obtained from these herbs lowers the production costs at industrial scale and facilitates the dealing with regulatory issues.

Nanotechnology enables the development of nanometer carriers to the delivery of these type of compounds (Paulino et al., 2011). Nevertheless, some difficulties are encountered in the industrial manufacturing due to the poor long-term physical and chemical stability, as well as the choice of pharmaceutical and food grade materials (Blasi et al., 2007). Lipid NPs seem to possess favorable features to overcome the mentioned issues (Blasi et al., 2007; Wissing et al., 2004). Lipid NPs produced with lipids that remain solid at the body temperature (known as solid lipid nanoparticles, SLN®) generally possess higher stability than most emulsions due to their solid state. However, particle stability depends on a number of different features, such as particle size, polydispersity, particle shape, surface charge, and storage conditions, among others. In an aqueous medium, the poor stability of these systems is a barrier for industrial application, but the reduction of water content in aqueous samples could improve SLNs stability (Abdelwahed et al., 2006b; Chacon et al., 1999).

Freeze-drying process allows the dehydration of samples, which may improve and increase stability throughout storage (Abdelwahed et al., 2006b). Furthermore, the production of a powder can facilitate the processing and storage. But, the crystallization of ice may induce mechanical stress on SLNs, which may possibly lead to their destabilization. Hence, in order to enable better handling and stability of certain samples it is necessary to add special excipients, thus contributing to the protection of these

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fragile systems (Abdelwahed et al., 2006b). Cryoprotectants can be used to protect from the stress of freezing, and to preserve the native structure of SLNs (Abdelwahed et al., 2006b). The most popular cryoprotectants are sugars such as trehalose, sucrose and glucose, or the sugar-alcohol mannitol, which, at concentrations of 5–15%, prevent the aggregation of SLNs and protect them against the mechanical injury induced by this process (Franks, 2013; Pikal, 1999).

The aim of this work was to assess the long-term physical and chemical stability of two lipid-based SLN types loaded with RA or with sage and savory extracts in fresh and dehydrated (by freeze-drying) states.

2. Materials and methods

2.1. Materials

The lipid matrices tested were Witepsol H15 (Sasol, Hamburg, Germany) and Carnauba yellow no. 1 wax (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA). The surfactant Tween 80 (polysorbate), rosmarinic acid (RA) and the three cryoprotectants tested (glucose, trehalose and mannitol) were purchased from Sigma-Aldrich Chemistry (St. Louis, Missouri, USA). The extracts of sage and savory were produced as described by Campos et al. (2015). Peptone (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA), Plate Count Agar (PCA) (Merck, Darmstadt, Germany), Rose Bengal agar (RBA) and chloramphenicol supplement (Lab M, Lancashire, UK) were used for the microbiological analyses. Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), sodium carbonate and gallic acid (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA) were used for the evaluation of total phenolic compounds.

2.2. Production of solid lipid nanoparticles

The Witepsol SLNs were produced in duplicate, according to Campos et al. (2014) and the Carnauba wax SLNs were produced according to Madureira et al. (2015). Briefly, SLNs were prepared with RA at the final concentration of 0.15 mg/mL by the hot melt ultrasonication method. Lipid matrices were used at 1% (w/v) and the surfactant at 2% (v/v).

2.3. Evaluation of the freeze-drying ideal cryoprotectant

In order to optimize the SLNs freeze-drying process, studies were performed in duplicate only for RA-SLNs. Three different cryoprotectants, namely glucose, mannitol and trehalose were studied at two different concentrations, i.e. 5 and 10% (v/v). The freeze-drying process was performed using a vacuum freeze drier (Model FT33, Armefield, UK), under a vacuum pressure of 100 militorr; the temperature in the freezing chamber was −46 °C and the temperature in the sample chamber was 15 °C. Samples without cryorprotectant were also processed as control. Freeze-dried SLNs were evaluated for their visual appearance, viscosity and free powder, as well as, physical and chemical stability.

2.3.1. Particle size and zeta potential analyses

The physical properties of the two types of SLNs were assessed. In the case of the freeze-dried RA-SLNs, these were first reconstituted with phosphate buffer (PBS; 0.1 M; pH 7.0) at a final concentration of 70 mg/mL. The particle size (PS), polydispersity index (PI) and zeta potential (ZP) were measured using a dynamic light scattering (DLS) from ZetaPALS, Zeta Potential Analyzer (Holtsville, New York, USA). All analyses were carried out in triplicate with an angle of 90° at 25 °C.

2.3.2. Evaluation of thermal properties

The thermodynamic behavior of the SLNs was determined by using Differential Scanning Calorimetry (DSC-60, Shimadzu, Columbia, USA). Briefly, 3 mg of SLN were placed on an aluminum pan. Thermal behavior was determined in the range 20–100 °C at a heating rate of 10 °C/min. Enthalpy values and optimal melting temperatures were calculated by the equipment software (ta60 version 2.10, DSC software, Shimadzu, Columbia, USA). The crystallinity indexes (CI%) of SLNs were calculated according to (Kheradmandnia et al. (2010)) using the following equation:

$$CI\% = \frac{\text{Melting enthalpy (SLN dispersion)(J/g)}}{\text{Melting enthalpy (bulk material without RA)(J/g). Concentration of lipid phase(\%)}} \times 100 \quad (1)$$

All raw materials used in the formulations were evaluated individually in triplicate and in combination.

2.4. Assessment of solid lipid nanoparticles long-term stability

Evaluation of long-term stability studies was performed on SLNs loaded with RA and herbal extracts (sage and savory). A design study to measure the chemical and thermodynamic stability in different physical states was performed. Thus, during 1 year, samples were taken at 0, 15, 30, 90, 180 and 365 days, however only the time points where the major differences were found are shown. In Table 1 are described the different conditions tested for SLNs storage, which generated three studies (A, B and C). Different features were evaluated such as, antioxidant capacities, percentage of encapsulated RA, RA release percentage, SLN powder color, water activity and microbiological analyses to evaluate the occurrence of eventual contaminations.

2.4.1. SLNs color

SLNs color was evaluated with a portable CR-400 Chroma Meter (Minolta, Osaka, Japan). The CIELab color scale was used to determine the lightness (L), redness ($+a^*$)/greenness ($-a^*$) and yellowness ($+b^*$)/blueness ($-b^*$) of the samples. SLNs samples were

Table 1
Experimental design of the processing parameters applied to SLNs.

Storage conditions		Physical state		
		A (Liquid)	B (Solid)	C (Solid)
Packaging material	Plastic	✓	✓	
	Glass			✓
Temperature	±25 °C (Room)		✓	✓
	±4 °C (Refrigerated)	✓		
Light	Presence		✓	
	Absence	✓		✓
Atmosphere	Room	✓	✓	
	Nitrogen			✓