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Solid lipid nanoparticles for encapsulation of hydrophilic drugs by an organic solvent free double emulsion technique

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ABSTRACT

Encapsulation of hydrophilic compounds for drug delivery systems with high loading efficiency is not easily feasible and remains a challenge, mainly due to the leaking of the drug to the outer aqueous phase during nanoparticle production. Usually, encapsulation of hydrophilic drugs is achieved by using double emulsion or inverse miniemulsion systems that often require the use of organic solvents, which may generate toxicological issues arising from solvent residues. Herein, we present the preparation of solid lipid nanoparticles loaded with a hydrophilic compound by a novel organic solvent free double emulsion/melt dispersion technique. The main objective of this study was to investigate the influence of important process and formulation variables, such as lipid composition, surfactant type, sonication parameters and lipid solidification conditions over physicochemical characteristics of SLN dispersion. Particle size and dispersity, as well as dispersion stability were used as responses. SLN dispersions with average size ranging from 277 to 550 nm were obtained, showing stability for over 60 days at 4 °C depending on the chosen emulsifying system. Entrapment efficiency of fluorescent dyes used as model markers was assessed by fluorescence microscopy and UV–vis spectrophotometry and results suggest that the obtained lipid based nanoparticles could be potentially applied as a delivery system of water soluble drugs.

1. Introduction

Following the development of liposomes and polymer-based nanoparticles, solid lipid nanoparticles (SLN) were introduced in the early 1990s as an efficient and non-toxic drug carrier system made up of natural lipids that are solid at body temperature [1–3]. Physiological lipids and biocompatible surfactants are commonly used to prepare SLN dispersions, which makes SLN well tolerated in living systems and therefore, no acute toxic effects are expected from SLN degradation [1,4,5].

Due to the lipophilic characteristic of the lipid matrix, SLN are well suitable systems for encapsulation of hydrophobic compounds. Hydrophilic drugs, on the other hand, as a result of the lack of affinity between drug and lipid, are expected to be poorly encapsulated as they have a strong tendency to partition into the outer aqueous phase during the preparation process [3]. Nevertheless, under optimized conditions, both lipophilic and hydrophilic drugs can be successfully incorporated.

Under hydrophilic drug encapsulation perspective, double emulsion/solvent evaporation technique is widely used to reach satisfactory loading efficiency in both solid lipid and polymer nanoparticles [3,6–10]. Nevertheless, along with organic solvents usage, toxicological problems may eventually arise from solvent residues within nanoparticles dispersion [5]. One possible way to overcome this problem is by combining melt dispersion technique with double emulsion, wherein a lipid melt is used rather than a lipid solution in an organic solvent. This technique has been successfully used by Bodmeier et al. [11] to prepare pseudophedrine HCL-loaded wax microparticles and by Reithmeier et al. [12] to prepare peptides-loaded glyceryl tripalmitate microparticles with high encapsulation efficiency and controlled release patterns. Little is known, however, about the impact of formulation variables on the production of solid lipid particles in the submicron size range by double emulsion/melt dispersion.

The aim of this work is to adapt the double emulsion/melt dispersion technique described by Bodmeier et al. [11] and Reithmeier et al. [12] targeting the production of solid lipid nanoparticles rather than microparticles for encapsulation of both hydrophilic

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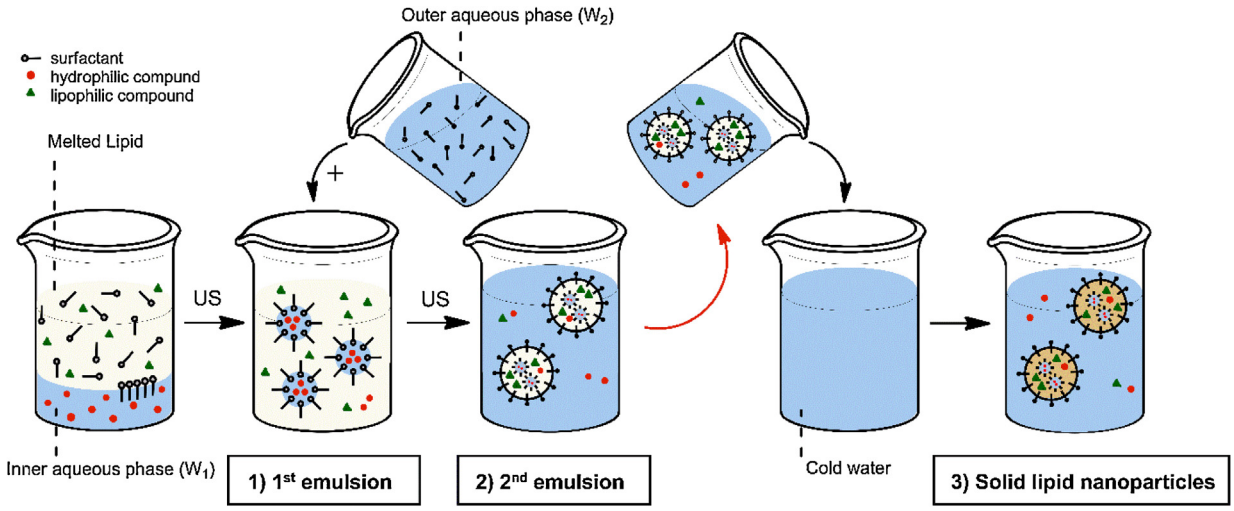

Scheme 1. Schematic representation of the production process of SLNs by double emulsion/melting dispersion.

and lipophilic drugs. Several surfactant combinations were tested in respect of their suitability for preparing lipid nanoparticles by double emulsion. Additionally, the effect of other experimental variables such as sonication parameters, cold water volume and lipid composition were investigated. Particle size, size distribution and dispersion stability were used as responses. Stearic acid and capric/caprylic triglycerides were used as solid and liquid lipid, respectively, both being considered non-toxic and safe under Generally Recognized as Safe (GRAS) status approved by Food and Drug Administration (FDA) [2].

2. Experimental

2.1. Material

All chemicals and materials were used as received. Capric/caprylic acid (Crodamol, GTCC, Chemical Alpha) and stearic acid (SA, Vetec) were used as lipids. As water soluble surfactants, polyoxiethylene-20-sorbitan monooleate (Tween 80, Vetec) and Poloxamer 407 (Pluronic F127, Sigma–Aldrich). As liposoluble surfactants, sorbitan monooleate (Span 80, Oxiteno), polyglycerol polyricinoleate (PGPR, Dhaymers) and soy lecithin (Alfa Aesar). And as hydrophilic and hydrophobic fluorescent model markers, Sulforhodamine 101 (SR-101, Sigma–Aldrich) and Coumarin-6 (Sigma–Aldrich) respectively. Water was distilled before use.

2.2. Preparation of solid lipid nanoparticles (SLN)

SLNs were prepared by an organic solvent free method that combines the principles of double emulsion and melt dispersion techniques adapted from elsewhere [11,12] aiming to produce solid lipid particles in the submicron range (Scheme 1). Each batch was prepared in triplicate in 15 mL test tubes. In a typical experiment, 0.6 g of stearic acid, or a mixture of stearic acid and crodamol, was heated above its melting temperature and mixed with lecithin, or other surfactant with a low hydrophilic-lipophilic balance (HLB), under magneticstirring. 0.2 mL of hot water was added and the mixture was sonicated (Fischer Scientific, Sonic Dismenbrator Model 500, 1/8" tip) during 15 s at 45% amplitude (20 W) to form the first emulsion (W_1/O). Then, 6.0 mL of a warm Tween 80 aqueous solution, or other high HLB surfactant, was added to the so prepared first emulsion and the mixture was homogenized by ultrasound at 45% amplitude (20 W) in a pulse regime (10 s on, 5 s off) during 60 s, forming the second emulsion ($W_1/O/W_2$). This double emulsion was poured into 90.0 mL of cooled water (2–5 °C) under mild magnetic stirring for 5 min to promote solidification of lipid nanoparticles. When used, Sulforhodamine 101 was added as the internal aqueous solution (216 μg/mL), replacing the internal blank aqueous solution (W_1), and Coumarin-6 was dissolved in crodamol (145 μg/mL) and mixed with stearic acid. The amount of ingredients used in each formulation is summarized in Table 1.

2.3. Characterization

2.3.1. Particle size analysis and zeta potential measurements

The intensity average size (Dp) and dispersity (PDI) of SLNs were measured by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS 3600 (Malvern Instruments), equipped with a detector at 173° (backscatter detection). Measurements were performed at 20 °C without dilution. The zeta potential (ZP) of diluted dispersions (0.1 wt%, distilled water as dispersive phase) was assessed by Laser Doppler Micro-electrophoresis at 25 °C using disposable folded capillary cells in a Zetasizer Nano ZS 3600 (Malvern Instruments). The zeta potential of the SLN dispersions was determined by measurement of electrophoretic mobility, which is converted to zeta potential by Henry equation:

$$U_E = \frac{2\varepsilon(ZP)}{3\eta} \times f(\kappa a) \quad (1)$$

where U_E is the electrophoretic mobility, ε the dielectric constant, ZP the zeta potential, η the viscosity of the solvent medium and $f(\kappa a)$ the Henry's function. For all measurements, the Smoluchowski approximation was used ($f(\kappa a) = 1.5$).

2.3.2. Morphology

The morphology of the nanoparticles was assessed by transmission electron microscopy (TEM) using a Jeol JSM-1011 with an accelerating voltage of 80 kV. For measurements, one drop of diluted SLN dispersion was placed onto a 300 mesh copper grid coated with a parlodium film and allowed to dry under ambient conditions. The samples were stained with 5% (wt/v) uranyl acetate solution to enhance microscopy contrast and subsequently coated with a carbon film to avoid degradation under the electron beam.

2.3.3. Entrapment efficiency

Entrapment efficiency of the fluorescent markers was indirectly determined by measuring its unloaded fraction in the