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# Development and characterization of solid lipid nanoparticles (SLNs) made of cocoa butter: A factorial design study

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ABSTRACT

The present study is motivated by the development of solid lipid nanoparticles (SLNs) for food applications. A focus of the study is the use of a factorial design to optimize the preparation variables. SLNs were prepared by hot homogenization at 60 °C. Cocoa butter was used to form the lipid core and the surfactant blend used to emulsify and stabilize the system was a mixture of sodium stearoyl-2-lactylate (SSL) and mono- and diglycerides of fatty acids (MDG). The particle characteristics and stability of obtained SLN-suspensions were investigated. Moreover, the effect of various cooling conditions on the properties of SLNs and the storage stability during a period of three months were examined. Results proved that cocoa butter is suitable to prepare SLNs with a food-grade quality where the optimized preparation variables resulted in a particle size of 112.7 nm.



## 1. Introduction

Solid lipid nanoparticles (SLNs) were developed as an alternative carrier system showing many interesting advantages over the other colloidal carriers. In particular, they are prepared from physiological lipids (Aditya and Ko, 2015; Mehnert and Mäder, 2001) and show high chemical and physical stability, improved bioavailability, sustained release, and protection of sensitive incorporated molecules against environmental effects (Awad et al., 2008; Patidar et al., 2010). This makes the SLNs especially valuable to manufactures of functional foods as a novel way to introduce lipophilic but chemically sensitive bioactive compounds (Weiss et al., 2008). Due to the enhanced surface area of nanoparticles per mass unit, they are considered to show a higher biological activity compared to larger sized particles of the same chemical composition (Rai et al., 2015; Sozer and Kokini, 2009), which is of particular importance for food applications and offers promise of improving delivery properties, solubility, prolonged residence time in the gastrointestinal tract and efficient absorption of the bioactive compounds through cells (Ravichandran, 2010; Sozer and Kokini, 2009). Common disadvantages of solid lipid nanoparticles are the aggregation and gelation of the suspensions under production and/or storage conditions (Helgason et al., 2008).

Cocoa butter is a vegetable fat which constitutes the main lipid phase in chocolate (Garti and Widlak, 2012; Toro-Vazquez et al., 2005). Despite the simple composition, cocoa butter displays a very complex structural behavior resulting from the complex polymorphism of the representing triacylglycerides. The polymorphism of cocoa butter is well described in the literature and defined by six different polymorphic forms. These are ɣ(I), α(II), β′(III), β′(IV), β(V) and β(IV) in increasing order of melting points and stability (Garti and Widlak, 2012; Langevelde et al., 2001; Loisel et al., 1998). Cocoa butter either pure or blended with other lipids was used before as particle core in the preparation of SLNs. In the study by Kim et al. (2005), the formation of SLNs was achieved by changing the physical properties of curdlan by means of reducing the pH value of the system. This induced the solidification of curdlan around the cocoa butter droplets and thereby resulted in the forming of the SLNs. Qian et al. (2013) also investigated the preparation of SLNs using cocoa butter compared to hydrogenated palm oil or a mixture thereof using the hot homogenization. This study has focused on the effect of thermal treatment of the SLN samples after production on their physical stability and encapsulated bioactive compounds during storage.

SSL is an anionic, food grade surfactant, which forms ordered crystalline aggregates upon dispersion in water and, thus, naturally exhibits pickering properties (Kurukji et al., 2013). The emulsifying and stabilizing properties of SSL in O/W food emulsions were confirmed by several researchers (Flores et al., 2007; Zinoviadou

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et al., 2011). MDG is a nonionic, food grade surfactant, which is a mixture of mono- and diglyceryl esters of saturated and unsaturated long chain fatty acids (Food and Drug Administration (FDA), 2006). In addition to the good emulsifying properties of MDG (Hasenhuettl and Hartel, 2008), the presence of higher amount of mono- and diglycerides in the lipid phase (cocoa butter) used in the preparation of SLNs results in significantly smaller particles (Mehnert and Mäder, 2001) and causes, moreover, a disorganization of the lipid matrix. This enables the production of a particle core with more voids for a higher bioactive compounds entrapment (Gomes et al., 2013; Weiss et al., 2008). The aim of the present work was to investigate the influence of different preparation variables (i.e. the surfactant concentration, the cocoa butter concentration, the homogenization pressure and the number of homogenization cycles) on the physical properties of solid lipid nanoparticles made of cocoa butter as well as to optimize the experimental conditions in order to obtain a minimum droplet size and size distribution and a maximum zeta potential by using the experimental design methodology. Besides, the influence of different cooling temperatures and conditions were examined. Using a full factorial design the influence of preparation variables and their interactions on the properties of SLNs dispersion prepared by hot homogenization using cocoa butter as lipid core, lipophilic and hydrophilic surfactants and water for the continuous phase.

## 2. Materials and methods

### 2.1. Materials

Cocoa butter in small disc shaped chips was purchased from Pati-Versand.de (Herzlake, Germany). Sodium stearoyl-2-lactylate (SSL, E481) and mono- and diglycerides of fatty acids (MDG, E471), respectively, which were a kind gift from Palsgaard® (Juelsminde, Denmark). Tween® 20 and Tween® 80 were purchased from Carl Roth (Karlsruhe, Germany). Sunflower lecithin Lecistar S was obtained from Sternchemie (Hamburg, Germany).

### 2.2. Preparation of SLNs

Solid lipid nanoparticles were prepared using the hot homogenization method (Mehnert and Mäder, 2001; Sagis, 2015). Cocoa butter was initially treated by melting at 80 °C for 30 min and cooling to room temperature to delete any thermal memory (Malssen et al., 1996).

For the preparation of SLNs formulation, the appropriate amount of treated cocoa butter was melted at 60 °C using a water bath. Lipophilic surfactants were added to the melted cocoa butter. The required amount of water at the same temperature containing hydrophilic surfactants was added to the lipophilic phase. Next, the total mixture was stirred at a high speed at the equivalent melting temperature using an Ultra Turrax (Ystral, D-79282, Ballrechten, Dottingen, Germany) for 3 min at 19,000 rpm. Lastly, the obtained pre-emulsion was homogenized using a high pressure homogenizer (Niro Soavi S.P.A., NS100 1L 2K, S N.4810, Parma, Italy) at pressures between 500 and 1500 bar for 1 to a maximum of 10 homogenization cycles yielding a hot O/W nanoemulsion. The hot emulsion was cooled in order to solidify the core of obtained SLNs and the effect of different cooling conditions was investigated. All formulations were filled in standard test tubes with screw cap (12 ml sample volume) and stored in the dark at ~2 °C until the next day for analysis.

### 2.3. The effect of individual formulation variables

The effects of homogenization pressure and cycle number as well as different cooling conditions were first studied individually using the same compositions and concentrations of materials needed for the SLNs preparation. The performing of these one-factor-at-a-time (OFAT) experiments is useful in order to define the ranges of chosen variables to be investigated by the next experimental design methodology.

### 2.4. The experimental design

The main purpose of the experimental design is to establish a causal relationship between independent and dependent process variables and on the SLN dispersions properties as well as to minimize the required experiment number. I.e., all observations obtained are used for estimating the effects of each factor and the interactions between factors are estimated simultaneously.

A two levels full factorial design with three centerpoints was employed to investigate the influence of four variables in order to optimize the solid lipid nanoparticle preparation. Surfactant concentration, cocoa butter concentration, homogenization pressure and number of homogenization cycles were selected as process variables. Particle size, polydispersity index and zeta potential were considered as a function of investigated variables since they reflect the emulsion quality and physical stability. The required number of experiments for an experimental design with two levels is in general $2^k$, where $k$ is the number of variables. Four variables were used in the present study, therefore the necessary number of experiments was $2^4 = 16$. The particle size, PI and ZP values obtained from the 20 experiments including four experiments at the center points are illustrated in (Table 3-2).

### 2.5. Physicochemical measurements

The characteristic properties of SLNs determine their functions and usage for practical applications. In the following the main properties which need to be determined when colloidal systems are studied are described.

#### 2.5.1. Particle size, PDI and zeta potential

The mean particle diameter, PDI and zeta potential of obtained SLNs were measured using Zetasizer Nano-ZS (Malvern, USA) based on laser light scattering technique (refractive index 1.54, 0.8872 cp, at 25 °C). Samples were diluted with distilled water. Each sample was measured in triplicate and the mean value was calculated and used for the factorial design.

#### 2.5.2. Thermal measurements

Thermal properties of SLN dispersions were measured using differential scanning calorimetry (DSC Q2000, TA instruments, Germany). Samples of 5 mg for bulk cocoa butter as well as 10–15 mg for SLNs were placed in hermetic aluminum pans and sealed. A reference of a sealed empty hermetic pan was used and all samples were undergone a thermal program between 0 and 65 °C using a scan rate of 5 °C/min for both heating and cooling.

#### 2.5.3. Physical stability of SLNs

The stability study was carried out for SLNs dispersions filled in transparent test tubes as described above and stored in the dark at ~2 °C. During the investigated storage period of three months SLNs dispersions were checked for their stability by measuring their physicochemical properties (particle size, PDI and zeta potential) in time intervals of two weeks.

### 2.6. Data analysis

The results of the experimental design were analyzed using Design Expert 6.0.11 Software employing the statistical method: