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Influence of co-surfactants on crystallization and stability of solid lipid nanoparticles

Hanna Salminen [a,*], Thrandur Helgason [a], Susanne Aulbach [a], Bjarki Kristinsson [b], Kristberg Kristbergsson [b], Jochen Weiss [a]

[a] Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 21/25, 70599 Stuttgart, Germany
[b] Faculty of Food Science and Nutrition, University of Iceland, Eiriksgötu 2, 101 Reykjavik, Iceland

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ABSTRACT

Hypothesis: The purpose of this study was to find a suitable co-surfactant to replace non-food grade bile salts in solid lipid nanoparticle (SLN) formulations. The hypothesis was that the molecular structure and physical properties of co-surfactant modulate the stabilization of SLNs upon polymorphic transition.
Experiments: Tristearin SLNs were prepared by using two main surfactants: saturated high-melting lecithin, and unsaturated low-melting lecithin. As co-surfactants we used sodium taurodeoxycholate (i.e. bile salt), Pluronic F68, Tween 60 and 80, and amino acids tyrosine, tryptophan, and phenylalanine. The influence of co-surfactants on crystallization behavior and physical stability of SLNs was investigated by differential scanning calorimetry and static light scattering, respectively.
Findings: The results showed that the aromatic amino acids had optimal structures and properties to act as effective co-surfactants in SLNs. Our study suggests that ideal co-surfactants are amphiphilic with pronounced hydrophobic areas, but highly water soluble so that they can have a reservoir of molecules readily available for interfacial stabilization. They adsorb fast to the interfaces, but without inducing polymorphic transition. This work demonstrates how the right structure can facilitate the desired function.

1. Introduction

Solid lipid nanoparticles (SLNs) have emerged as a delivery system for bioactive ingredients due to several advantages over other colloidal carrier systems. First, they can be composed of physiological lipids, which reduce to risk for acute and chronic toxicity [1,2]. Second, SLNs can be manufactured economically on a large-scale continuous process using high-pressure homogenization without the use of solvents [2]. Third, as SLNs are crystallized nanoemulsions, which are composed of a lipophilic bioactive incorporated in a carrier lipid that is solid at room and body temperature [3], the major advantage of SLNs is the entrapment of the bioactive ingredients inside the solidified lipid matrix [3–5]. This prevents the diffusion of the bioactive ingredients to the surface of the emulsion droplet, where they are prone to oxidative reactions [6–8].

On the other hand, SLNs are prone to instabilities due to their complex crystallization behavior based on polymorphic transition [9]. Upon polymorphic transition, the initially spherical emulsion droplets containing α-subcell crystals will polymorph into platelet-shaped β-subcell crystals. This shape change increases the surface area of the particles, which leads to uncovered hydrophobic patches that will aggregate and may eventually gel. Moreover, polymorphic transition from α- to β-subcell crystals reduces the bioactive loading capacity in SLNs due to increased ordering and packing density of the lipid crystals, thus leading to expulsion of the bioactives from the crystal matrix [10]. Therefore, for SLNs to function as a good delivery system for bioactive ingredients, the crucial point is to control the polymorphic transition.

Abbreviations: SLN, solid lipid nanoparticle; SSS, tristearin; 80H, high-melting lecithin Phospholipon 80H; PC75, low-melting lecithin Alcolec PC75; Phe, phenylalanine; Trp, tryptophan; Tyr, tyrosine; Tween 60, polyoxyethylene sorbitan monostearate; Tween 80, polyoxyethylene sorbitan monooleate; DSC, differential scanning calorimetry; SD, standard deviation; HLB-value, hydrophilic–lipophilic balance; $T_{m(\alpha)onset}$, onset melting temperature of α-subcell crystals; $T_{m(\alpha)\ Peak}$, mean melting temperature of α-subcell crystals; T_{Conset}, onset crystallization temperature; $T_{C\ Peak}$, mean crystallization temperature; ΔH_{α}, enthalpy change of α-subcell crystals; ΔH_{total}, total enthalpy change; cmc, critical micelle concentration.

* Corresponding author. Fax: +49 711 459 24446.
E-mail addresses: hanna.salminen@uni-hohenheim.de (H. Salminen), helgason01@gmail.com (T. Helgason), Susanne.Aulbach@gmx.de (S. Aulbach), bhk1@hi.is (B. Kristinsson), kk@hi.is (K. Kristbergsson), j.weiss@uni-hohenheim.de (J. Weiss).

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Aggregation and polymorphic transition can be prevented by adding enough surfactant that will quickly adsorb to the newly formed surfaces upon polymorphic transition [11]. However, this usually requires the use of high concentrations (5–6%) of surfactants [11], which in turn is undesirable due to restrictions in food applications or pharmaceutical products as well as high-cost. Therefore, a better solution is to modulate the crystallization behavior by using a lower concentration of specific surfactant(s) that can interact with the lipid droplets.

Previous studies have shown that saturated and unsaturated phospholipids can be used to impact the polymorphic transition and thus physical stability of SLNs [6,7,12,13]. However, phospholipids alone are not sufficient at forming or stabilizing nanoemulsions. This is because phosphatidylcholine has a geometrical structure of a truncated cone due to its large head group and double lipid chains [14]. Therefore, a co-surfactant is required to increase the curvature of the interfacial layer. In addition, phospholipids tend to form vesicles [5,14]. This reduces their mobility, and thus they are too slow to cover newly created surfaces during storage when there is no external power input to disrupt the bilayers [5].

Bile salts (e.g., sodium glycocholate, sodium taurodeoxycholate), however, are effective co-surfactants in SLN stabilized with phospholipids [5–7,12,13,15]. This is because bile salts can disrupt the phospholipid vesicles, and rapidly adsorb to uncovered interfacial regions upon polymorphic transition [5]. Nevertheless, bile salts are relatively expensive and have a bitter taste [16]. Therefore, the aim of this study was to find a suitable co-surfactant to replace bile salts, and form stable SLNs. Co-surfactants are surface active amphiphilic molecules with a small-sized polar head group, and are thus usually ineffective in stabilizing emulsions themselves.

We hypothesized that an effective co-surfactant will be an amphiphilic molecule that contains: (i) a hydrophilic part, and (ii) an aromatic ring structure or lipophilic tail as hydrophobic part, or a solid structure that does not increase the mobility of the crystallized lipid at the interface. We expected that an effective co-surfactant will help to stabilize the emulsion droplets during homogenization and upon polymorphic transition during storage. In this study, two different main surfactants, high and low-melting lecithins, were used to test the functionality of the following co-surfactants with varying melting points: aromatic amino acids tryptophan, tyrosine, and phenylalanine, non-ionic Tween 60 and 80, non-ionic polyoxyethylene–polyoxypropylene–polyoxyethylene block co-polymer Pluronic F68, and bile salt (i.e. taurodeoxycholate). As a reference, SLNs containing only main surfactants were also produced.

2. Materials and methods

2.1. Materials

Tristearin (Dynasan 118) was a gift from Sasol (Witten, Germany). The high-melting (T_m = 52 °C) lecithin Phospholipon 80H (⩾60.0% hydrogenated phosphatidylcholine, ⩽10% hydrogenated lysophoshatidylcholine, 85% stearic acid, 15% palmitic acid, and no DL-α-tocopherol detected), and the low-melting (T_m < 0 °C) lecithin Alcolec PC75 (68.0–73.0% phosphatidylcoline, 7.0–10.0% phosphatidylethanolamine, ⩽3% lysophosphatidylcholine, 17–20% palmitic acid, 2–5% stearic acid, 8–12% oleic acid, 58–65% linoleic acid, 4–6% linolenic acid, and 0.1–0.2% DL-α-tocopherol) were both donated by Lipoid GmbH (Ludwigshafen, Germany). Sodium taurodeoxycholatehydrate (bile salt, purity ⩾ 97%, T_m = 163 °C), Tween 80 (T_m = -21 °C), Pluronic F68 (T_m = 52 °C, HLB-value > 24), phenylalanine (T_m = 270–275 °C), phosphate monobasic (purity ⩾ 99.0%), phosphate dibasic (purity ⩾ 99.0%), and sodium azide (purity ⩾ 99.0%) were all obtained from Sigma–Aldrich (Steinheim, Germany). Tween 60 (T_m = 55–57 °C) was obtained from Fluka (Buchs, Switzerland). Tryptophan (T_m = 280–285 °C) was purchased from Carl Roth GmbH + Co KG (Karlsruhe, Germany), and tyrosine (T_m = 297–298 °C) was obtained from Merck (Darmstadt, Germany). All materials were used without further purification. Distilled, deionized water was used throughout the study.

2.2. Solution preparation

A buffer solution (pH 7) was prepared by dissolving 4.23 mM sodium phosphate (monobasic) and 5.77 mM sodium diphosphate (dibasic) in distilled water. A buffer solution (pH 9.5) was prepared by dissolving 10 mM sodium diphosphate in distilled water, and adjusted with 1.0 N sodium hydroxide to pH 9.5. Sodium azide (0.02%) was added to prevent microbial growth. The surfactant (1.2%) and co-surfactant concentrations (0.3%) were chosen according to previous research that had found phospholipids and bile salt to perform well in forming solid lipid nanoparticles [5–7,12,13]. Aqueous surfactant solutions were prepared into sodium phosphate buffer at appropriate pH in the following combinations: (i) 1.2% (w/w) lecithin (either Phospholipon 80H or Alcolec PC75) at pH 7, (ii) 1.2% (w/w) lecithin (80H or PC75) and 0.3% (w/w) co-surfactant, namely taurodeoxycholate, Pluronic F68, Tween 60, or Tween 80, at pH 7, and (iii) 1.2% (w/w) lecithin (80H or PC75) and 0.3% (w/w) co-surfacntant, namely Phe, Trp, or Tyr, at pH 9.5. Thus, this created 16 compositionally different surfactant solutions.

2.3. SLN preparation

SLNs were prepared by using hot high-pressure homogenization method. Tristearin was fully melted at 90 °C in a water bath. A course pre-emulsion was formed by mixing the hot tristearin (5% w/w) with the aqueous surfactant solution (95% w/w) held at 90 °C using an ultra-turrax (Labworld, Staufen, Germany) at high speed (24000 min^{-1}) for one min followed by five passes through a microfluidizer (Microfluidizer Processor M-110EH-30 equipped with H-interaction chamber H10Z (diameter: 100 μm), Microfluidics Corporation, Newton, MA, USA) at 10,000 psi. The microfluidizer was heated up prior to homogenizing by cycling hot water (90 °C) through the machine to prevent crystallization during the homogenization procedure. The five passes through the microfluidizer took less than 5 min. The emulsion was sealed and let to cool down to 20 °C. The samples were stored at 20 °C.

2.4. Particle size determination

The particle size distribution of the lipid nanoparticles was determined by a laser diffraction instrument (LS230 Small Volume Module Plus, Beckmann Coulter Inc., Miami, FL). In laser diffraction, particle size distributions are calculated by comparing the sample's scattering pattern with an appropriate optical model using a scattering theory (i.e. Mie theory). Results are given as surface area mean diameter: $d_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$ and volume mean diameter: $d_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$, where n_i is the number of droplets of diameter d_i. Refractive indexes of 1.51 and 1.33 for solid tristearin and phosphate buffer, respectively, were used to calculate particle size distributions.

2.5. Differential scanning calorimetry

The melting and crystallization behavior of the lipid nanoparticles was analyzed by using differential scanning calorimetry (DSC