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Regular Article

Tuning of shell thickness of solid lipid particles impacts the chemical stability of encapsulated ω-3 fish oil

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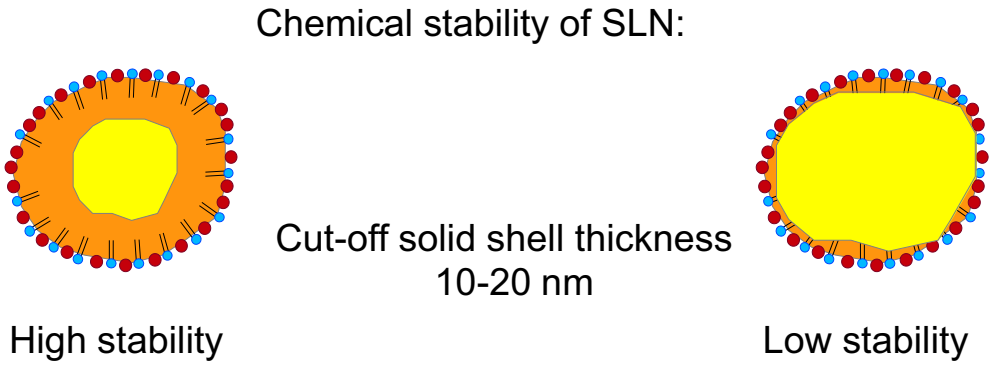
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: This study demonstrates that tuning the shell thickness of lipid particles can modulate their oxidative stability. We hypothesized that a thick crystallized shell around the incorporated fish oil would improve the oxidative stability due to the reduced diffusion of prooxidants and oxygen.
Experiments: We prepared solid lipid nanoparticles (5% w/w lipid phase, 1.5% w/w surfactant, pH 7) by using different ratios of tristearin as carrier lipid and ω-3 fish oil as incorporated liquid lipid stabilized by high- or low-melting lecithin. The physical, polymorphic and oxidative stability of the lipid particles was assessed.
Findings: The high-melting lecithin was the key in inducing the formation of a solidified tristearin shell around the lipid particles by interfacial heterogeneous nucleation. Lipid particles containing a higher ratio of tristearin showed a better oxidative stability. The results revealed that a crystallized tristearin layer above 10 nm was required to inhibit oxidation of the incorporated fish oil. This cut-off was shown for lipid particles containing 50–60% fish oil. This research gives important insights into understanding the relation between the thickness of the crystallized shell around the lipid particles and their chemical stability.

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Abbreviations: d_i, diameter; d_{32}, surface area mean diameter; d_{43}, volume mean diameter; ΔH_C, crystallization enthalpy; $\Delta H_{m(\alpha)}$, melting enthalpy of the α-subcell crystals; DSC, differential scanning calorimetry; *m*, mass; n_i, the number of droplets; LM, low-melting; PDI, polydispersity index; PUFA, polyunsaturated fatty acids; *r*, particle radius; R^2, regression coefficient; r_{shell}, thickness of the shell; SLN, solid lipid nanoparticles; SSS, tristearin; T_C, crystallization temperature; T_m, melting temperature, HM, high-melting; V, volume; *w*, mass fraction.

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1. Introduction

Oxidation of lipids is a major problem in the food, feed, cosmetic and pharmaceutical industries leading to chemical degradation of various products. Lipid oxidation is an extremely complex phenomenon that involves formation and decomposition of various oxidation products into numerous volatile and non-volatile compounds which influence the product quality by affecting its flavor and texture profile, color, and nutritional value [1,2]. In addition, changes in macromolecule functionality and potential formation of harmful compounds caused by oxidation reactions is of great concern for the safe use of products [1,2].

The majority of food and non-food applications comprise lipids formulated into dispersions or emulsions. An example of such disperse system is an oil-in-water emulsion that is stabilized by surface active emulsifier(s) [3]. In these dispersions, the oxidation reactions occur predominantly at the lipid droplet interfaces because many prooxidants are also surface active, and are therefore preferably located at the interfaces. Transition metals such as iron have been suggested to catalyze the decomposition of lipid hydroperoxides, both ubiquitous in food ingredients and biological tissues, into highly reactive alkoxyl and peroxyl radicals that can induce scission of unsaturated fatty acids and yield numerous volatile compounds that are responsible for the product deterioration [4].

In solid lipid particles, i.e. in the crystallized counterpart of oil-in-water emulsions, the oxidation reactions may be reduced because of the limited mass transport of molecules through the crystallized lipid phase [5]. Such reduction in oxidation of bioactive ingredients entrapped in crystallized lipid particles has been illustrated in previous studies [6–9]. Nevertheless, successful inhibition of oxidation reactions in solid matrices requires the control of crystallization behavior of the carrier lipids. This is because the lipid particles are prone to instabilities due to polymorphic transition. Polymorphism of lipids is the ability of the lipid molecules to adapt different crystal subcell structures with diverse spatial arrangements and molecular packings [10]. Which of these subcell crystal structures the lipids will reveal, depends on the laws of thermodynamics. In general, the crystallized lipid molecules always prefer the lowest energy state possible, and therefore it is thermodynamically favorable for the lipid molecules to transition from the most disordered crystals structure to the most densely packed configuration. In saturated triacylglycerides, the polymorphic transition occurs according to Ostwald's step rule: from α-subcell crystals to β'- to β-subcell crystals [11].

The physical and polymorphic stability can be manipulated, for example, by appropriate selection of lipids and surfactants as well as by controlling the particle size and temperature [12]. In this study, we focused on using two types of surfactants to modulate the crystal structure of the solid lipid nanoparticles (SLN): a high- and low-melting lecithin. The impact of high- and low melting phospholipids on the polymorphic stability of solid lipid nanoparticles have been reported in earlier studies [6–9,13,14].

The purpose of this study was to investigate how the ratio of the crystallized carrier lipid to the incorporated liquid lipid impacted the physical, polymorphic and oxidative stability of the lipid nanoparticles. As carrier lipid, we chose tristearin, a saturated triacylglyceride, and as liquid oil we selected a fish oil rich in ω-3 fatty acids. Our particular interest was in evaluating the thickness of the crystallized layer and its subsequent impact on the oxidation rate. We hypothesized that the thicker the crystallized shell around the incorporated fish oil is, the better the oxidative stability due to the reduced diffusion of prooxidants and oxygen. We also expected that the different lecithins would show distinctly different oxidation kinetics. Our hypothesis was that the lipid particles emulsified with high-melting lecithin will show a significantly better oxidative stability compared to the lipid particles stabilized with low-melting lecithin as long as the shell thickness is enough to prevent oxidation of the fish oil. This is based on the fact that the saturated high-melting lecithin can induce crystallization of the tristearin and form a solid layer around the liquid fish oil, whereas the unsaturated low-melting lecithin remains fluid and leads to random crystallization of the carrier lipid [7–9]. Therefore, we expected that the oxidation will proceed more linearly with decreasing solid lipid content in the lipid particles emulsified with low-melting lecithin. On the other hand, when the shell reaches a critical thickness, the tristearin shell will not prevent either the diffusion of ω-3 fatty acids towards the aqueous phase from the core of the particles or diffusion of prooxidants towards the fish oil molecules, upon which oxidation will progress rapidly.

2. Materials and methods

2.1. Materials

Tristearin (Dynasan 118) (T_m = 70–73 °C) was obtained from Sasol (Witten, Germany). Omega-3 fish oil was donated by Lysi (Reykjavik, Iceland). It contained 49% polyunsaturated fatty acids (PUFA) with 18% eicosapentaenoic acid and 12% docosahexaenoic acid, 24% monounsaturated fatty acids, 27% saturated fatty acids, 0.25% free fatty acids, peroxide value ⩽5.0 mequivalent of peroxides/kg of oil, and anisidine value ⩽30, according to the specifications of the manufacturer. The fish oil was stored in a freezer at −75 °C until use. The high-melting (T_m = 52 °C) lecithin Phospholipon 80H (60% hydrogenated phosphatidylcholine, 10% hydrogenated lysophosphatidylcholine; fatty acid composition: 85% stearic acid, 15% palmitic acid; no DL-α-tocopherol detected), and the low-melting (T_m < 0 °C) lecithin Alcolec PC75 (70% phosphatidylcholine, 8.5% phosphatidylethanolamine, and 2.2% lysophasphatidylcholine; fatty acid composition: 17–20% palmitic acid, 2–5% stearic acid, 8–12% oleic acid, 58–65% linoleic acid, and 4–6% linolenic acid; 0.1–0.2% DL-α-tocopherol) were from Lipoid GmbH (Ludwigshafen, Germany). Sodium taurodeoxycholatehydrate (bile salt, purity ⩾97%), phosphate monobasic (purity ⩾99.0%), phosphate dibasic (purity ⩾99.0%), sodium azide (purity ⩾99.0%), ammonium thiocyanate (purity ⩾97.5%), barium chloride dihydrate (purity ⩾99.0%), ferrous sulfate heptahydrate (purity ⩾99.0%), cumene hydroperoxide (purity 80%), propanal (purity 97%), and hexanal (purity 98%) were all obtained from Sigma-Aldrich (Steinheim, Germany). All solvents (1-butanol, isooctane, 2-propanal, and methanol) were of analytical grade from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Double distilled, deionized water was used throughout the study.

2.2. Preparation of solutions

A 10 mM sodium phosphate buffer solution (pH 7) was prepared by dissolving 4 mM sodium dihydrogen phosphate (monobasic) and 6 mM disodium hydrogen phosphate (dibasic) in water. The pH was adjusted if necessary by 0.1 M sodium hydroxide or hydrochloric acid. Sodium azide (0.02%) was added to inhibit microbial growth. Aqueous surfactant solutions were prepared by dispersing 1.2% (w/w) lecithin and 0.3% (w/w) sodium taurodeoxycholatehydrate as co-surfactant. The highly hydrophilic taurodeoxycholate with a fast diffusion rate is essential in stabilizing newly formed solid lipid particle surfaces upon recrystallization [15]. Bile salts are generally recognized as safe (GRAS) and permitted for specific food use [16].

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