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Impact of Surfactant Properties on Oxidative Stability of β-Carotene Encapsulated within Solid Lipid Nanoparticles

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The impact of surfactant type on the physical and chemical stability of solid lipid nanoparticle (SLN) suspensions containing encapsulated β-carotene was investigated. Oil-in-water emulsions were formed by homogenizing 10% w/w lipid phase (1 mg/g β-carotene in carrier lipid) and 90% w/w aqueous phase (surfactant + cosurfactant) at pH 7 and 75 °C and then cooling to 20 °C. The impact of surfactant type was investigated using aqueous phases containing different water-soluble surfactants [2.4% w/w high-melting (HM) lecithin, 2.4% w/w low-melting (LM) lecithin, and 1.4% w/w Tween 60 or 1.4% w/w Tween 80] and a cosurfactant (0.6% taurodeoxycholate). The impact of the physical state of the carrier lipid was investigated by using either a high melting point lipid (tripalmitin) to form solid particles or a low melting point lipid (medium chain triglycerides, MCT) to form liquid droplets. A higher fraction of α-crystals was detected in solid particles prepared with high-melting surfactants (HM-lecithin and Tween 60) than with low-melting surfactants (LM-lecithin and Tween 80). With the exception of the HM-lecithin-coated solid particles, the suspensions were stable to particle aggregation during 21 days of storage. β-Carotene degradation after 21 days of storage was 11, 97, 100, and 91% in the solid particles (tripalmitin) and 16, 21, 95, and 90% in the liquid droplets (MCT) for HM-lecithin, LM-lecithin, Tween 80, and Tween 60, respectively. These results suggest that β-carotene may be stabilized by (1) LM- or HM-lecithin when liquid carrier lipids are used and (2) HM-lecithin when solid carrier lipids are used. The origin of this latter effect is attributed to the impact of the surfactant tails on the generation of a crystal structure better suited to maintain the chemical stability of the encapsulated bioactive.

KEYWORDS: Solid lipid nanoparticles; interfacial nucleation; polymorphic transition; emulsions β-carotene

INTRODUCTION

Solid lipid nanoparticles (SLNs) are colloidal carrier systems that have been developed to encapsulate, protect, and deliver lipophilic functional components, such as bioactive lipids and drugs (*1*−*5*). SLNs are typically manufactured using the hot homogenization process where a liquid lipid phase (carrier lipid melt and/or lipophilic functional ingredient) and an aqueous surfactant solution are homogenized at a temperature above the melting temperature of the lipids to produce an oil-in-water nanoemulsion. This hot nanoemulsion is then cooled to a temperature below the crystallization temperature of the carrier lipid, leading to the formation of solid particles (*6*).

Numerous studies have suggested that ingestion of appropriate amounts of carotenoids may reduce the risk of chronic diseases such as cancer and cardiovascular disease (*7*−*9*). Unfortunately, the bioavailability of many carotenoids is very low, and their oxidative stability decreases rapidly during processing due to the superimposition of chemical, mechanical, and thermal stresses (*10*−*13*). Incorporation of carotenes into SLNs may increase their bioavailability since absorption of carotenes is greater when they are consumed in a mixture with other lipids (*10*, *14*−*16*). Moreover, because oxidation of these materials usually takes place at the droplet surface (*17*, *18*), the inclusion of carotenoids within a solid core could provide additional protection against their oxidative attack by reducing the mobility of reactants. For example, Hentschel et al. found that SLNs could provide some limited protection against β-carotene breakdown, but additional antioxidants were needed to achieve a sufficient stabilization during storage (*19*).

The reason for the only relatively minor increase in β-carotene stability after incorporation into SLNs reported by Hentschel and co-workers could be because of an initial suboptimal structural arrangement of the triacylglycerol molecules within the crystallized particles or a recrystallization of the SLN into less suitable crystal structures during storage (*5*). This hypothesis is supported

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by studies that have shown that drug molecules may be expelled from the crystallized lipid matrix of SLNs during storage due to polymorphic transitions from α- to β'- to β-subcell structures (*6*, *20*). Moreover, we and others previously reported on polymorphic transitions resulting in morphological changes in SLNs, causing particles to assume platelet- or needle-shaped configurations, thereby increasing the particle surface area (*20*−*25*). An increase in particle surface area in combination with an increased concentration of expelled bioactive molecules at the particle surface may lead to rapid degradation of any chemically labile bioactives, such as carotenoids due to their exposure to lipid hydroperoxides located at the droplet surface and transition metals in the aqueous phase (*26*). On the basis of previous studies, we therefore believe that a key to the protection of carotenoids from breakdown in SLNs is (i) to carefully control the initial crystallization process so as to create optimum crystal matrices within SLNs to trap bioactive components within the particle interior and (ii) to reduce any polymorphic transitions during storage so as to maintain a dispersion of the bioactive ingredient throughout the solid lipid core thereby preventing its accumulation at the particle surface.

In a number of studies, Bunjes and co-workers have reported some success in slowing polymorphic transitions in SLNs. The authors manufactured SLNs of tristearin and high-melting lecithin with SLNs consisting of almost exclusively α-subcell crystals, and more than 70% of the α-subcell structure remained after 6 months of storage (*27*). The authors speculated that the high-melting lecithin crystallized prior to the lipid carrier oil, thereby surface-initiating the crystallization of the lipid matrix through a heterogeneous nucleation process (*27*, *28*).

While Bunjes and coauthors investigated the formation of stable SLN and modifying morphologies of SLNs, the focus of this study was to evaluate the functionality of SLNs in terms of their ability to stabilize chemically labile lipophilic ingredients. We used high-melting and low-melting surfactants to manufacture a series of SLN dispersions that contained β-carotene. The objective of this study was to test the hypothesis that the stability of bioactive ingredients in SLN dispersions depends on the initial subcell structure and the long-term stability of the lipid crystals, which may be regulated by the choice of surfactants. Furthermore, we hypothesized that the usage of lecithin as a surfactant for SLNs may result in chemical stabilization of the incorporated compound since lecithin has been suggested to act as an antioxidant (*29*−*32*). Ultimately, we intended to elucidate the conditions required to create a delivery system capable of better protecting highly unstable lipophilic bioactive compounds such as carotenoids.

MATERIALS AND METHODS

Materials. Tripalmitin (#92903) was purchased from Fluka (Buchs, Switzerland). Miglyol 812 was a gift from Sasol (Witten, Germany). Sodium phosphate monobasic (#7558-80-7) and sodium phosphate dibasic (#7558-79-4) were purchased from Fisher Scientific (St. Clair Shores, MI). Sodium azide (#S2002), polyethylene glycol sorbitan monostearate (Tween 60, #P1629), and polyethylene glycol sorbitan monooleate (Tween 80, #P1754) were purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO). Alcolec PC 75 (80% purity) and Phospholipon 80H (70% purity) (**Table 1**) were a gift from Lipoid LLC (Newark, NJ). All materials were used without further purification.

Solution Preparation. A buffer solution (pH 7) was prepared by dissolving 4 mM sodium phosphate (monobasic) and 6 mM sodium diphosphate (dibasic) in distilled water. Sodium azide (0.02% w/w) was added to prevent microbial growth. An aqueous surfactant solution was prepared by dispersing either 2.4% (w/w) lecithin and 0.6% taurodeoxycholate as a cosurfactant or 1.4% Tween 60 or Tween 80 with 0.6% taurodeoxycholate as a cosurfactant (**Table 2**). Taurodeoxycholate was used as a cosurfactant because studies have found that a highly water-soluble surfactant with fast diffusion rates is needed to cover newly formed surfaces upon recrystallization (*23*). Because the Tween surfactants are more effective at reducing interfacial tension and stabilizing o/w interfaces, a lower amount was required to yield particles with similar sizes (**Table 3**).

Table 1. Chemical Composition of Low- (Alcolec PC 75) and High-Melting (Phospholipon 80H) Lecithins

composition	LM: Alcolec PC 75	HM: Phospholipon 80H
phospholipid	70% phosphatidylcholine	60% hydrogenated phosphatidylcholine
	8.5% phosphatidylethanolamine	10% hydrogenated lysophosphatidylcholine
	2.2% lysophosphatidylcholine	
fatty acid	17−20% palmitic acid	85% stearic acid
	2−5% stearic acid	15% palmitic acid
	8−12% oleic acid	
	58−65% linoleic acid	
	4−6% linolenic acid	
DL-α-tocopherol	0.1−0.2%	not detected

SLN Preparation. SLNs were prepared using the hot high-pressure homogenization method (*25*). A lipid phase [10% (w/w) tripalmitin] was fully melted by heating to 70−80 °C and 1 mg β-carotene per gram tripalmitin was dissolved in the melt. The melt was flushed with nitrogen, sealed, and kept in the dark while stirring (200 rpm) for 60 min to allow the β-carotene to fully dissolve at 70−80 °C. The lipid phase was then mixed with the aqueous surfactant solution held at 70−80 °C using a hand-held high-speed blender at 30% power for 1 min (model SDT-1810, EN shaft, Tekmar Co., Cincinnati, OH) to form a coarse emulsion premix. The hot emulsion premix was homogenized with five cycles at 12000 bar using a thermostatted microfluidizer (Microfluidics, Newton, MA) at 70−80 °C to prevent solidification during the homogenization procedure. The five passes through the microfluidizer took less than 5 min. The particle size of the fabricated particles prior to solidification is shown in **Table 3**. The emulsion (200 mL) was sealed and placed in a temperature-controlled incubator at 20 °C to crystallize the lipid particles (average cooling speeds ∼1−2 °C/min). The sample was stored in a closed opaque plastic bottle.

***β*-Carotene Breakdown.** The absorbance of β-carotene containing emulsions and solid particles was used to determine β-carotene stability. The absorbance at 466 nm was measured with a Shimadzu UV-21001 PC UV−visible scanning spectrophotometer equipped with an integrating sphere assembly (Shimadzu, Kyoto, Japan). Utilization of an integrating sphere allowed for assessment of the absorbance of β-carotene in concentrated emulsions and suspensions, therefore eliminating the need for solvent extraction (*18*). The β-carotene content in samples was determined using a standard curve created by serially diluting the β-carotene-containing emulsions with emulsions without β-carotene. The β-carotene content was expressed as relative β-carotene breakdown C in percent:

$$C = c_0/c(t) \quad (1)$$

where $c(t)$ is the β-carotene content after storage for a period t and c_0 is the β-carotene content at the time of preparation.

Particle Size Determination. Dynamic light scattering was performed using a dynamic light scattering instrument (Nano ZS, Malvern Instruments, Malvern, United Kingdom). Samples were diluted 1:100 using a 10 mM phosphate buffer solution at pH 7 to prevent multiple scattering effects. The instrument reports the mean particle diameter (z average) and the polydispersity index (PDI) ranging from 0 (monodisperse) to 0.50 (very broad distribution). The particle diameters of the respective emulsion or suspension are shown in **Table 3**.

To investigate the influence of crystallization on the shape of the lipid particles, samples were melted at 75 °C and then placed in the measurement chamber, and the particle diameter was measured during cooling from 45 to 5 at 2 °C intervals. Because the hydrodynamic radius (z average size) of a disk-shaped particle is higher than that of spherical particle of the same volume, it can be used to evaluate the shape of the particles (*25*, *33*). For further explanation, see below.

Table 2. Concentrations and Type of Surfactants and Lipids Used in the Formation of Lipid Nanoparticles

particle system	main surfactant (w/w)	cosurfactant (w/w)	lipid (w/w)
high-melting lecithin (PPP)	2.4% Phospholipon 80H	0.6% taurodeoxycholate	10% tripalmitin
low-melting lecithin (PPP)	2.4% Alcolec PC 75	0.6% taurodeoxycholate	10% tripalmitin
Tween 80 (PPP)	1.4% Tween 80	0.6% taurodeoxycholate	10% tripalmitin
Tween 60 (PPP)	1.4% Tween 60	0.6% taurodeoxycholate	10% tripalmitin
high-melting lecithin (Miglyol)	2.4% Phospholipon 80H	0.6% taurodeoxycholate	10% Miglyol
low-melting lecithin (Miglyol)	2.4% Alcolec PC 75	0.6% taurodeoxycholate	10% Miglyol
Tween 80 (Miglyol)	1.4% Tween 80	0.6% taurodeoxycholate	10% Miglyol
Tween 60 (Miglyol)	1.4% Tween 60	0.6% taurodeoxycholate	10% Miglyol

Table 3. Diameter and Polydispersity Index of Solid and Liquid Particles[a]

SLN system	diameter (nm) solid	PDI solid	diameter (nm) liquid	PDI liquid
high-melting lecithin (PPP)	154.0	0.159	140.7	0.124
low-melting lecithin (PPP)	173.4	0.185	140.6	0.120
Tween 80 (PPP)	159.1	0.111	136.5	0.109
Tween 60 (PPP)	150.7	0.110	133.4	0.108
high-melting lecithin (Miglyol)	X	X	117.6	0.122
low-melting lecithin (Miglyol)	X	X	99.01	0.134
Tween 80 (Miglyol)	X	X	110.7	0.095
Tween 60 (Miglyol)	X	X	95.9	0.112

[a] X indicates the formation of a gelled particle network where determination of a particle diameter and polydispersity index was not possible.

Static Light Scattering. The particle size distribution was also assessed by static light scattering using a Mastersizer X (Malvern, Malvern Instruments Ltd., Westborough, MA). Here, measurements are reported as the volume−surface mean diameter, $d_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$, and volume fraction−length mean diameter, $d_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$, where n_i is the number of particles of diameter d_i in the population. The instrument finds the particle size distribution that gives the best fit between the experimental measurements and the predictions made using light scattering theory (Mie theory). Refractive indices of 1.54, 1.43, and 1.33 were used for solid tripalmitin (*28*), MCT, and water, respectively, to calculate the particle size distributions.

Differential Scanning Calorimetry (DSC). The physical state of the emulsified tripalmitin (e.g., solid or liquid) and crystal structures of solid lipids were determined by DSC (Q1000, TA Instruments, New Castle, DE). Emulsions (8−10 mg) were placed in aluminum pans and hermetically sealed. Empty pans were used as a reference for the emulsion samples. Each sample was heated from 20 at 10 °C/min to 75 °C and then cooled down to 5 at 10 °C/min. Initially, the crystallization phenomenon was investigated at different cooling rates, but no differences in properties of generated particle were observed (data not shown). Consequently, a cooling rate of 10 °C/min was used for all samples.

Statistical Analysis. All measurements were repeated three times using triplicate samples. Means and standard deviations were calculated using Excel.

RESULTS AND DISCUSSION

Surfactant Characteristics. The objective of this study was to investigate the impact of surfactant type on crystal structure, particle stability, and oxidative stability of encapsulated β-carotene. SLNs were therefore prepared using four surfactants with different molecular characteristics (head and tail groups) and physicochemical properties (melting points): LM-lecithin, HM-lecithin, Tween 60, and Tween 80 (*27*). These surfactants were selected because previous studies suggest that the physical state of the surfactants plays a significant role in the particle crystallization process (*33*). Tweens are nonionic surfactants with a polyoxyethylene headgroup and a single hydrocarbon tail. Tween 60 has a stearic acid (C18:0) tail with a relatively high melting point (≈57 °C) and is solid at room temperature, whereas Tween 80 has an oleic acid (C18:1) tail with a relatively low melting point

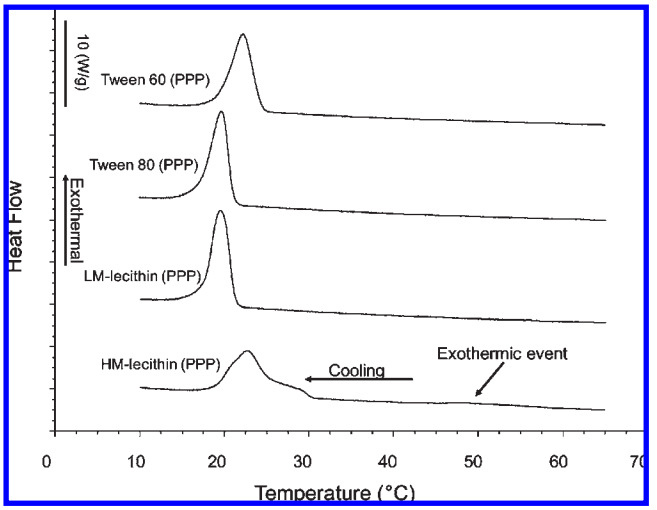

Figure 1. DSC thermographs during the cooling of different SLN systems.

(−20.5 °C) and is liquid at room temperature (*34*). The lecithins are anionic surfactants (at the pH used in this study) with two hydrocarbon tails. The HM-lecithin (Phospholipon 80H) has a saturated nonpolar tail with a high melting temperature (≈52 °C) and is solid at room temperature, whereas LM-lecithin (Alcolec PC 75) has an unsaturated nonpolar tail with a low melting point (< 0 °C) and is liquid at room temperature (*27*, *28*). The composition of the two lecithin samples is summarized in **Table 1**. This choice of surfactants allowed us to investigate the impact of the tail group type on the crystal structure and physical stability of SLN as well as on the chemical stability of encapsulated β-carotene.

Influence of Surfactant Type on Particle Crystallization Temperature. The purpose of these studies was to determine the influence of surfactant characteristics on the crystallization temperature of the emulsified lipids in tripalmitin oil-in-water emulsions containing encapsulated β-carotene. The temperature at which crystallization is first observed provides useful information about the nucleation mechanism of emulsified lipids, for example, homogeneous, surface-heterogeneous, or volume-heterogeneous. DSC thermographs of liquid tripalmitin oil-in-water emulsions during a cooling cycle showed that lipid carrier crystallization occurred at higher temperatures for the emulsions stabilized by high-melting surfactants (HM-lecithin and Tween 60) than those stabilized by low-melting surfactants (LM-lecithin and Tween 80) (**Figure 1**). The observed increase in crystallization temperature of the lipid matrix for the high-melting surfactants suggests that tripalmitin crystallization may have been promoted by surface-heterogeneous nucleation initiated by the surfactant (*35*). This type of nucleation has been suggested to be promoted by solidification of surfactant molecules absorbed at droplet surfaces (*28*, *35*−*41*). The emulsion droplets coated by high-melting surfactants crystallized at significantly higher

temperatures than those stabilized by low-melting surfactants: 30.3 ± 0.1 °C for HM-lecithin, 24.4 ± 0.1 °C for Tween 60, 21.2 ± 0.1 °C for LM-lecithin, and 21.0 ± 0.1 °C for Tween 80.

A small exothermic peak was observed in the DSC thermograms around 50 °C for the HM-lecithin-coated droplets (**Figure 1**), which suggested that the surfactant layer at the droplet surface may have crystallized before the carrier lipid within the droplet interior. This exothermic event was not seen when tripalmitin was replaced with MCT nor when HM-lecithin in water was scanned in the absence of carrier oil (data not shown). These observations suggest that the crystallization of HM-lecithin depended on its physical location (bulk vs surface) and its chemical environment (MCT vs tripalmitin). Other researchers have observed similar events and suggested that only a small fraction of the tripalmitin molecules are incorporated into phospholipid membranes and crystallize at higher temperatures (*27*, *28*). The underlying mechanism of this phenomenon is currently not fully clear and a more thorough investigation is required. Nevertheless, the data suggest that a surface-initiated nucleation is involved due to the fact that samples with the high-melting surfactants had substantially higher crystallization temperatures than samples stabilized by low-melting surfactants. Differences between the high-melting surfactants may be explained by the fact that in surface-initiated crystallization, the initiation takes place in confined spaces and kinetic effects may play a role.

After crystallization, the SLN suspensions were stored at 20 °C and their melting profiles and particle size distributions were recorded over time. The melting profiles of SLN suspensions stored for 1 day are shown in **Figure 2**. SLN suspensions that had been prepared using low-melting surfactants (LM-lecithin and Tween 80) did not contain any α-subcell crystals (the least stable polymorphic form). Instead, they consisted of the more thermodynamically stable β'- and β-subcell crystals. Conversely, SLN suspensions produced using high-melting surfactants (HM-lecithin and Tween 60) contained relatively high amounts of α-subcell crystals, as evidenced by the endothermic transitions at 42.1 ± 0.3 and 40.9 ± 0.5 °C for the Tween 60 and HM-lecithin systems, respectively (**Figure 2**). Apparently, surface heterogeneous nucleation promoted by high-melting surfactants promoted the crystallization of the carrier lipid within the droplets into the α-subcell crystal form, a fact that has also been observed by other researchers (*27*, *28*, *42*). Previously, this has been attributed to the ability of the crystalline surfactant tail layer acting as a template for nucleation of the lipid within the droplets (*25*).

During storage, the α-subcell crystal content decreased in systems stabilized with HM-lecithin and Tween 60 (**Figure 3**), indicating that the α-subcell structure was unstable. Nevertheless, during the course of the experiment, a small amount of α-subcell structure persisted, suggesting that some of the tripalmitin, most likely the molecules in direct contact with the crystallized surfactant layer at the interface, resisted the polymorphic transition (**Figure 3**). This assumption is supported by previous studies that have shown that particles coated with high-melting surfactants had α-subcell structures close to the interface but β-subcell structure in the core (*27*).

Effect of Surfactant Type on Physical Stability of SLN. The physical instability of many SLN preparations has been a key problem that has limited their widespread industrial use. After preparation, the lipid phase of SLN suspensions may undergo polymorphic transitions (α- to β-subcell structure) that cause the particle shape to change from spherical to either disklike or needlelike. As a consequence of these shape changes, there is an increase in the surface area of the particles exposed to the surrounding aqueous phase. If there is insufficient surfactant present in the aqueous phase to cover all of the hydrophobic patches formed on the particle surfaces, then extensive particle flocculation occurs, leading to the formation of a three-dimensional network that gives the suspension gel-like characteristics (*20*, *21*, *33*). It is for this reason that sodium taurodeoxycholate was used in the present study as a cosurfactant because previous work has found that it rapidly adsorbs to SLN particle surfaces, preventing their subsequent aggregation (*23*).

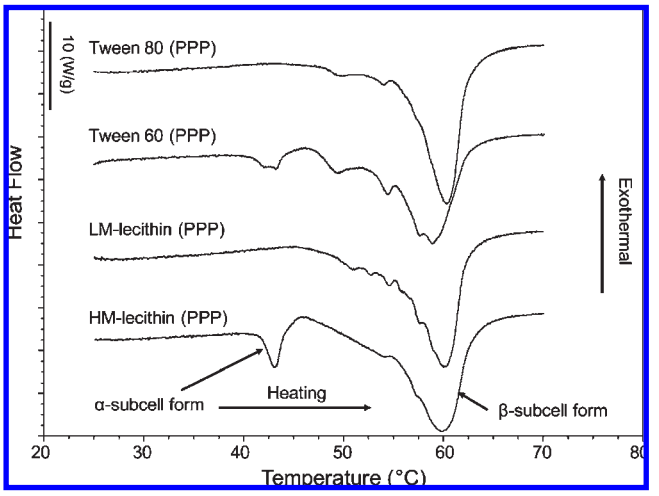

Figure 2. Melting thermographs of SLN containing tripalmitin (PPP) as the lipid matrix after 1 day of storage at 20 °C for different surfactant systems.

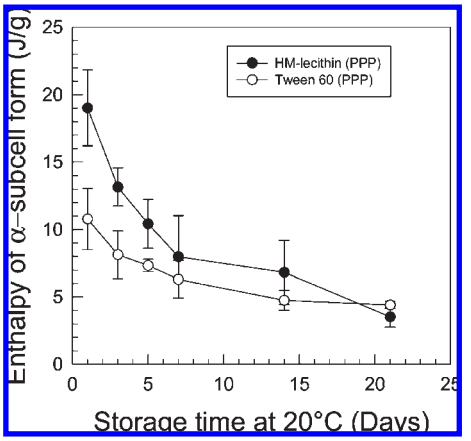

Figure 3. Enthalpy of α-crystal melting for high-melting lecithin and Tween 60 containing tripalmitin (PPP) as the lipid matrix.

The mean particle diameter of the SLNs did not change significantly during 1 day of storage independent of surfactant type (d_{32} = 0.21 ± 0.02 μm), indicating that all surfactants produced suspensions with relatively good short-term stability. The long-term stability of the SLN suspensions was measured after 21 days of storage (**Table 4**). All of the SLN suspensions were relatively stable to droplet aggregation during storage, except the one stabilized by HM-lecithin, which showed an appreciable increase in mean particle diameter after 3 weeks of storage. The mean particle diameter of emulsions prepared using the same surfactants, but using a liquid carried oil (MCT), was also measured (**Table 4**). All of these emulsions were stable to particle aggregation during storage, which indicated that it was the combination of solidified lipid phase and surfactant type that led to instability in the SLN suspension stabilized by HM-lecithin. Because the HM-lecithin-stabilized system was physically stable

Table 4. Mean Diameters d_{32} and d_{43} Calculated from the Particle Size Distribution of Dispersions Measured by Static Light Scattering

	day 1				day 21			
SLN system	d_{32} (μm)	SD	d_{43} (μm)	SD	d_{32} (μm)	SD	d_{43} (μm)	SD
high-melting lecithin (PPP)	0.20	0.01	0.26	0.01	30.20	1.39	36.09	1.57
low-melting lecithin (PPP)	0.24	0.02	0.27	0.01	0.24	0.00	1.71	1.08
Tween 80 (PPP)	0.19	0.01	0.24	0.01	0.14	0.01	2.11	1.10
Tween 60 (PPP)	0.20	0.01	0.25	0.01	0.17	0.01	0.37	0.10
high-melting lecithin (Miglyol)	0.17	0.00	0.22	0.01	0.17	0.00	0.22	0.01
low-melting lecithin (Miglyol)	0.16	0.00	0.21	0.00	0.16	0.00	0.21	0.00
Tween 80 (Miglyol)	0.16	0.01	0.21	0.01	0.16	0.01	0.20	0.00
Tween 60 (Miglyol)	0.16	0.01	0.20	0.01	0.16	0.01	0.21	0.01

after 1 day of storage but became unstable after 21 days of storage, it is possible that the instability problem is associated with long-term changes in the polymorphic form of the crystals in the SLN particles (**Figure 3**). It is clear that further work is required to form physically stable SLN using this type of surfactant.

Influence of Surfactant Type on Particle Shape. Previous studies have investigated the shape of SLN using electron microscopy, dynamic light scattering, and field flow fractionation and found that the particles formed changed shape from spherical to disk-shaped or needle-shaped particles after undergoing a polymorphic transition. Disk-shaped or needle-shaped particles have a larger hydrodynamic diameter than spherical particles of the same mass (*20*), and hence, polymorphic transformations that induce changes in particle shape can be monitored using dynamic light scattering (*25*, *33*, *43*−*45*).

SLN suspensions containing solidified tripalmitin particles were melted by heating to 75 °C, and then, their hydrodynamic diameter was measured during cooling from 45 to 5 °C. The mean particle diameters before crystallization (45 to 35 °C) were 141, 141, 137, and 133 nm for SLN coated by HM-lecithin, LM-lecithin, Tween 80, and Tween 60, respectively (**Figure 4**). When the particles were cooled below about 25 °C, an increase in hydrodynamic diameter was recorded, which can be attributed to a change in particle shape. The most dramatic increase in hydrodynamic diameter was recorded for LM-lecithin-stabilized SLN with 33 ± 2% increase in the hydrodynamic diameter. In comparison, the hydrodynamic radius of Tween 80- and Tween 60-stabilized SLN increased by 22 ± 2 and 19 ± 1%, respectively (**Figure 4**). Remarkably, there was almost no increase in the particle diameter of the SLN-stabilized by HM-lecithin (2.7 ± 0.5%). As mentioned earlier, the particle diameter changed more significantly after 1 day of storage, where the mean hydrodynamic diameter of HM-lecithin-coated SLNs increased to 154 nm (**Table 3**). In addition, the SLNs coated with HM-lecithin exhibited extensive aggregation after 21 days of storage. Apparently, the shape change does take place but happens over the course of hours or days as compared to minutes as observed with the other systems. This suggests that the HM-lecithin layer itself, or its impact on the crystal structure of the lipid carrier matrix, retards the polymorphic transitions and shape change of the particles. Future studies should focus on determining the impact of particle size and lipid types on these processes to find optimal conditions for the manufacturing of physically stable crystal structures.

Influence of Surfactant Type on β-Carotene Breakdown. In this series of experiments, we examined the impact of surfactant type on the chemical degradation of encapsulated β-carotene (**Figure 5**). SLN suspensions manufactured using HM-lecithin showed a remarkable ability to inhibit β-carotene breakdown with only 11 ± 4% of the β-carotene broken down after 21 days. In contrast, β-carotene encapsulated within SLN stabilized by LM-lecithin degraded rapidly with 97 ± 3% breakdown after

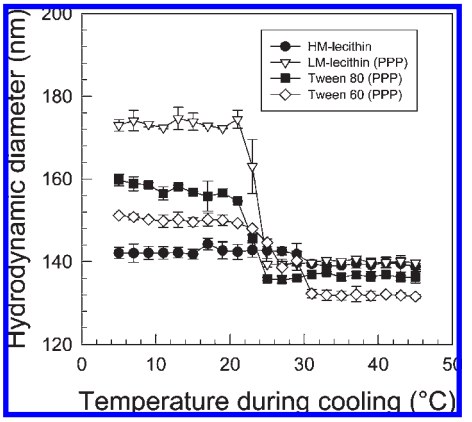

Figure 4. Size increase of all SLN systems containing tripalmitin (PPP) as the lipid matrix during cooling from 45 to 5 °C.

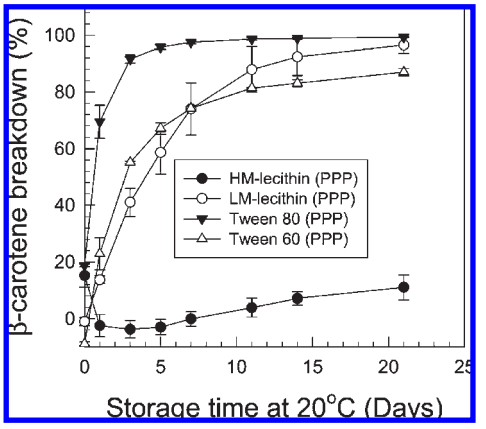

Figure 5. β-Carotene breakdown as a function of storage time at 20 °C for SLN containing tripalmitin (PPP) as the lipid matrix.

21 days (**Figure 5**). The β-carotene also degraded rapidly in both Tween-stabilized systems. After 3 days, the β-carotene content in the low-melting Tween 80 system decreased by 92 ± 2% and in the high-melting Tween 60 system by 55.2 ± 0.4% (**Figure 5**).

The SLN suspensions stabilized by high-melting surfactants (HM-lecithin and Tween 60) had appreciably better β-carotene stability than those stabilized by low-melting surfactants (LM-lecithin and Tween 80), with the HM-lecithin system showing the best protection. A possible explanation for this phenomenon is that the tripalmitin crystal structure was modified by the presence of high-melting surfactants at the droplet surface. For example, if nucleation begins at the droplet surface (rather than in the droplet interior), then a solid shell is formed that protects the encapsulated β-carotene from pro-oxidants in the aqueous phase. On the other hand, if nucleation begins within the droplet interior (rather

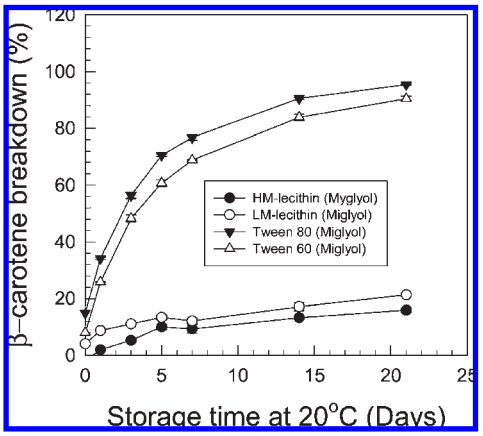

Figure 6. β-Carotene breakdown as a function of storage time at 20 °C for SLN manufactured with Miglyol as a lipid matrix.

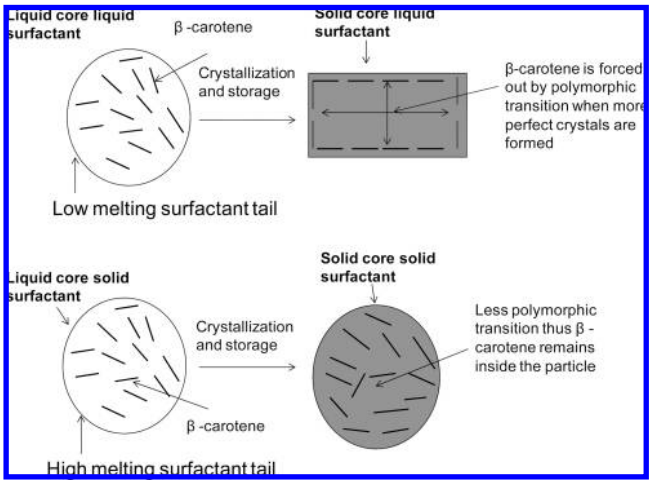

Figure 7. Schematic drawing of the β-carotene breakdown mechanism.

than at the droplet surface), then the β-carotene may be expelled to the droplet surface where it is in closer proximity to prooxidants in the aqueous phase. Previous research has shown that highly lipophilic components are expelled more easily from highly ordered β-subcell structures than from less perfect α-subcell structures. Thus, polymorphic transitions in SLN may have dramatic consequences for the stability of encapsulated bioactives. Not only will the polymorphic transformation increase the surface area available for oxidative processes, but it may also expel the vulnerable bioactive molecules to the interface. This has been previously observed with the oxidation of methyl linolenate in oil-in-water emulsions (*26*).

It should be noted that some types of lecithin have been reported to act as effective antioxidants in colloidal systems (*46*); therefore, part of the observed differences between the chemical degradation of β-carotene in lecithin- and Tween-stabilized systems may be due to a chemical effect, rather than physical alterations in crystal structure.

Further insights into the origin of the impact of surfactant type on the chemical degradation of β-carotene were obtained using emulsions manufactured from liquid carrier oil (MCT) rather than solid carrier oil (tripalmitin). In this case, the β-carotene is not trapped within a solid particle matrix, and the interfacial crystallization of the surfactant layer may be inhibited. β-Carotene degradation after 21 days in MCT oil-in-water emulsions was ≈16, 21, 95, and 90%, respectively, for HM-lecithin, LM-lecithin, Tween 60, and Tween 80, respectively (**Figure 6**). The increased stability of lecithin-stabilized liquid dispersions as compared to that of Tween can be explained by the antioxidant properties of lecithin mentioned above (*46*). Phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine are effective antioxidants (*29*) and are the major phospholipids within the two lecithin ingredients used in this study (**Table 1**). The mechanism of lecithin antioxidant activity in colloidal systems is however not yet fully understood with explanations ranging from the formation of Maillard reaction products (*29*), metal scavenging (*30*, *31*), and formation of oxygen barriers (*32*) to the unsaturation of the phospholipids (*47*). If the particle changes shape, bile salts will diffuse to the interface, thus increasing the ratio of bile salts at the interface as compared to lecithin. It is possible that this might result in less of an oxygen barrier. This may partly explain why the β-carotene breaks down faster in samples where shape change occurs. Nevertheless, further studies are needed to prove or disprove that hypothesis.

Proposed Physicochemical Mechanism for Surfactant and Carrier Lipid Effects. To explain the underlying mechanism for the chemical and physical stability of the SLN, the following three cases may be distinguished, which are schematically illustrated in **Figure 7**:

(i) Solid core and "liquid" surfactants: When low-melting ("liquid") surfactants are used to stabilize particles, the tripalmitin crystalline core may rapidly recrystallize during storage into a more stable polymorphic form (α to β). In the process, the particle not only changes shape but also expels incorporated bioactive ingredient(s) (**Figure 7**). This is because the more stable crystal structure has less capability to hold the loaded bioactive. Thus, if the crystal structure is highly ordered, most β-carotene can be expected to be located at the particle surface.

(ii) Solid core and "solid" surfactants: When high-melting ("solid") surfactants are used to stabilize the particles, the tripalmitin crystallizes in a less ideal and less tightly packed crystal form (e.g., α or β') that is better capable of accommodating β-carotene molecules (**Figure 7**). Recrystallization processes in these particles are retarded. Such a formulation can be expected to provide the highest chemical stability of β-carotene, particularly if the surfactant exhibits additional antioxidant activities.

(iii) Liquid core and "solid" or "liquid" surfactants: If "liquid" or "solid" surfactants are used in combination with a liquid carrier lipid (e.g., MCT), the particles are liquid droplets and remain spherical and β-carotene is not expelled to their surface. This will lead to slower β-carotene breakdown in emulsions than in nonideal SLN particles (i.e., solid core and "liquid" surfactant) but a more rapid degradation than in ideal SLN particles (i.e., solid core and "solid" surfactant) due to the higher mobility of the β-carotene in the liquid matrix (**Figure 7**).

In conclusion, this study has shown that utilization of high-melting surfactants can change the crystallization behavior of SLNs, yielding more stable α-subcell polymorphs. SLNs formed by surface heterogeneous nucleation initiated by a suitable high-melting surfactant better protected encapsulated β-carotene against chemical degradation. This is because in these particles, predominantly α-crystals are formed that are able to accommodate the encapsulated bioactives inside the less perfect crystalline matrix. The good chemical stability achieved using this purely

structural approach is particularly remarkable considering that additional chemical antioxidants were not used.

Once an appropriate crystal structure has been formed, it is then essential that the crystal form is maintained since polymorphic transition from α- to β′- to β-subcell crystals can lead to expulsion of incorporated bioactive molecules, rendering them more vulnerable to pro-oxidants at the interface or in the surrounding aqueous phase. The surfactant thus has a double role, inducing the formation of appropriate crystals structures and maintaining the characteristics of the formed system.

Finally, it should be noted that the SLN size prior to crystallization may play a key role in this mechanism. If particles are too large, then surface-initiated crystallization becomes less important, regardless of what surfactant is present at the particle surface. The exact relationship between size, surfactant type, and lipid matrix still remains to be established. Moreover, process conditions (cooling speeds and target temperatures) are additional parameters that may influence the outcome of the solidification process. Manufacturers thus need to consider a large amount of interrelated parameters to successfully produce the appropriate structures to encapsulate chemically labile bioactive molecules using SLN technology.

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