

Chemical Stability of α -Tocopherol in Colloidal Lipid Particles with Various Morphologies


Anja Schröder,* Joris Sprakel, Karin Schroën, and Claire C. Berton-Carabin

Colloidal lipid particles (CLPs) are promising encapsulation systems for lipophilic bioactives, such as oil-soluble antioxidants that are applied in food and pharmaceutical formulations. Currently, there is no clear consensus regarding the relation between particle structure and the chemical stability of such bioactives. Using α -tocopherol as a model antioxidant, it is shown that emulsifier type (Tween 20 or 40, or sodium caseinate) and lipid composition (tripalmitin, tricaprylin, or combinations thereof) modulated particle morphology and antioxidant stability. The emulsifier affects particle shape, with the polysorbates facilitating tripalmitin crystallization into highly ordered lath-like particles, and sodium caseinate resulting in less ordered spherical particles. The fastest degradation of α -tocopherol is observed in tripalmitin-based CLPs, which may be attributed to its expulsion to the particle surface induced by lipid crystallization. This effect is stronger in CLPs stabilized by Tween 40, which may act as a template for crystallization. This work not only shows how the architecture of CLPs can be controlled through the type of lipid and emulsifier used, but also gives evidence that lipid crystallization does not necessarily protect entrapped lipophilic bioactives, which is an important clue for encapsulation system design.

Practical Applications: Interest in enriching food and pharmaceutical products with lipophilic bioactives such as antioxidants through encapsulation in lipid particles is growing rapidly. This research suggests that for efficient encapsulation, the particle architecture plays an important role; to tailor this, the contribution of both the lipid carrier and the emulsifier needs to be considered.

Dr. A. Schröder, Prof. K. Schroën, Dr. C. C. Berton-Carabin
Laboratory of Food Process Engineering
Wageningen University and Research
Bornse Weiland 9, Wageningen 6708 WG, The Netherlands
E-mail: anja.schroder@wur.nl; claire.ber-ton-carabin@wur.nl

Dr. A. Schröder, Prof. J. Sprakel
Laboratory of Physical Chemistry and Soft Matter
Wageningen University and Research
Stippeneng 4, Wageningen 6708 WE, The Netherlands

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/ejlt.202000012>

© 2020 The Authors. *European Journal of Lipid Science and Technology*
Published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. This is
an open access article under the terms of the Creative Commons
Attribution License, which permits use, distribution and reproduction in
any medium, provided the original work is properly cited.

DOI: 10.1002/ejlt.202000012

1. Introduction

The interest in enriching food and pharmaceutical products with lipophilic bioactives (for example, vitamins, flavors, pigments, antioxidants) is growing rapidly, and often involves encapsulation to protect these bioactives against chemical degradation, to enhance their solubility, activity, or absorption, and to control their delivery.^[1–3] Sub-micron and partly solid lipid droplets that may be referred to as colloidal lipid particles (CLPs) have emerged as potential encapsulation systems for lipophilic bioactive compounds, and have been extensively studied lately.^[4–9]

It may be useful to clarify the related terminology: the term “solid lipid nanoparticle” (SLN) has been extensively used, but is not always appropriate, because i) the lipid phase may not be fully solid, and ii) the prefix “nano” applies to “natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate of which, for 50% or more of the particles in the number size distribution, one or more external dimensions is between 1–100 nm” (Bleeker et al.^[10]). This classification does not hold for all particles termed SLNs (Bleeker et al.,^[10,11]). This is why “solid lipid particle” (SLP) should be preferred^[12]; or, if the lipid phase is not fully crystallized, “(nano)structured lipid carrier” may be more accurate,^[13] or “colloidal lipid particle”, which is more generic, and will be used in the present work.

CLP dispersions can be manufactured easily at large-scale using high pressure homogenization, which also allows elevated temperatures when using high melting point (HMP) fats.^[14] Biocompatible lipids can be used to make a lipid matrix with minimal toxicity, and relatively high encapsulation efficiency for lipophilic components compared to, for example, liposomes.^[3,15–17] Lipid materials that are partly or fully crystallized have been suggested to protect labile lipophilic molecules against chemical degradation (often, oxidation) by preventing accumulation at the interface^[11,18,19] and by limiting diffusion of molecules involved in the oxidative reaction throughout the lipid phase.^[12] A solid lipid matrix has also been suggested to increase control over bioactive release, for example, under

digestive conditions,^[12,13,20] to improve absorption into the lymph and blood,^[21,22] and to delay lipid digestion.^[12]

The design of CLPs entrapping a lipophilic bioactive brings along challenges since the functionality depends on the lipid matrix structure.^[23] Lipids with a sufficiently high melting point may crystallize directly after CLP preparation in a polymorphic form that will depend on the lipid purity, presence of other ingredients (in particular, emulsifiers), and cooling rate. CLPs may also re-crystallize during storage into more stable polymorphic forms. Lipids with a high purity can form highly ordered crystalline structures, which can result in the expulsion of the encapsulated lipophilic compound from the crystalline matrix.^[19] This is especially the case when CLPs are manufactured with HMP alkanes or triacylglycerols with a narrow melting range.^[2,24,25] This may lead to enhanced chemical degradation of the encapsulated compound by aqueous phase reactants,^[25,26] although in some applications slow release (i.e., a certain degree of expulsion) may be desirable, for example, when the activity of an antioxidant should be maintained for prolonged periods.^[5]

To counteract such an often undesirable expulsion and to increase the lipophilic compound-loading capacity, the use of blended lipids has been proposed.^[13,20] This leads to a lipid phase with a broader melting range compared to a pure lipid, resulting in a more disordered crystalline structure that is less prone to polymorphic transitions.^[2] Alternatively, fat crystallization can be influenced by the molecular structure of emulsifier used to stabilize the CLPs.^[6,18,27] For instance, surfactants with a high melting point alkyl chain can induce surface-templated crystallization of the lipid phase, promoting crystal growth from the interface therewith initiating particle structure and morphology,^[28–30] which promoted the stability of labile lipophilic molecules.^[3,29]

We selected α -tocopherol (vitamin E), as model lipophilic bioactive to be encapsulated in CLPs. Being a naturally occurring chain-breaking antioxidant, it is relevant to a broad range of food and biobased applications, and poses challenges due to its chemical instability and poor solubility in water.^[5,7,31,32] A few studies have attempted at encapsulating α -tocopherol in related systems; Dingler et al.^[33] showed that SLNs protected it better against chemical degradation than an oil-in-water emulsion.^[33] Oehlke et al.^[5] found that tocopherol-containing SLNs had good physical stability and showed a gradual release of tocopherol, which is important for systems in which long term radical scavenging activity is desired.^[5] For oil-in-water emulsions, it was found that up to 95% of tocopherol was located in the emulsifier layer rather than the core of the droplets.^[34] Although these findings are very relevant, general guidelines that link the choice of lipids and emulsifiers to CLP structure, and to bioactive stability, are not available yet.

In the present work, we therefore systematically investigated the effect of lipid (tripalmitin, tricaprylin, or combinations thereof) and emulsifier (Tween 20 or Tween 40, i.e., surfactants with alkyl chains of low or high melting point, respectively; or sodium caseinate, i.e., a disordered protein) on CLP morphology, crystalline structure, and the stability of α -tocopherol. Understanding these aspects, and how they are linked, can be used as the starting point for the rational design of CLPs as delivery systems for lipophilic bioactives such as oil-soluble antioxidants.

2. Experimental Section

2.1. Materials

Tripalmitin (#T8127, purity > 99%), tricaprylin (#91 040, purity > 99.6%), Tween 20 (#P1379), Tween 40 (#P1504), sodium phosphate monobasic (#S9638), sodium phosphate dibasic (#S9763), sodium chloride (#S9888, purity 99%), iron(II) sulfate heptahydrate (#F8633), ethylenediaminetetraacetic acid disodium salt dihydrate (#E6635), and α -tocopherol (#T3251) were purchased from Sigma-Aldrich (Saint Louis, USA). Methanol (#813 012 802), chloroform (#803 010 802), and hexane (#808 023 502) were obtained from Actua-All Chemicals (Oss, the Netherlands). Sodium caseinate (#41 610, spray dried, protein content 91.0%) was supplied by DMV International (Veghel, the Netherlands). Ultrapure water (18 M Ω) was prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA) and was used for all the experiments. All other chemicals used were of analytical grade. The chemicals were used without further purification.

2.2. Methods

2.2.1. Preparation of Colloidal Lipid Particles (CLPs)

An aqueous phase (95% w/w) containing 1% w/w sodium caseinate or 2% w/w Tween 20 or 40 in phosphate buffer (10 mM, pH 7.0) was heated to 80 °C in a water bath and added it to a melted fat phase (5% w/w) (tripalmitin, tricaprylin, or tripalmitin mixed with tricaprylin in a mass ratio 4:1) preheated at the same temperature, which had previously been spiked with 100 μ L methanolic solution of α -tocopherol (200 mg mL⁻¹). Final α -tocopherol concentration was 4 mg g⁻¹ of fat. A coarse emulsion was prepared by high speed stirring the mixture at 11 000 rpm for 1 min using a preheated rotor-stator homogenizer (Ultra-turrax IKA T18 basic, Germany). We then homogenized this coarse emulsion using a high pressure homogenizer (Microfluidizer Processor MF 110Y equipped with a Y-shaped interaction chamber (F12Y; minimum internal dimension: 75 μ m); Microfluidics, Newton, Massachusetts, USA) at 800 bar (five cycles) and 80 °C to obtain submicron-sized droplets, which were left to cool at refrigerated temperature (4 °C) over \approx 2 h, inducing crystallization, except for the CLPs made with pure tricaprylin as the oil phase, which were fully liquid at the temperatures used in this work.

2.2.2. Characterization of CLPs

The particle size distribution and average diameters ($d_{3,2}$) were determined by static light scattering (Malvern Mastersizer 3000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). The following optical properties were used: refractive indices of 1.540 (lipid phase) and 1.330 (water) with an absorption index of 0.01. Particle size distributions of CLPs after 14 days incubation under oxidative conditions are reported in Figure S1, Supporting Information.

Particle surface charge was evaluated by ζ -potential measured with a dynamic light scattering instrument (Zetasizer Nano ZS,

Malvern Instruments, UK) at 25 °C. Emulsions were diluted ≈ 500 times in phosphate buffer (10 mM, pH 7.0) to prevent multiple scattering.

Transmission electron microscopy (TEM) was performed on CLPs (dispersions were diluted ≈ 100 -fold) deposited onto a freshly glow discharged carbonized copper grid (200 mesh). The excess solvent was blotted using standard filter paper. The particles were stained with a 1% w/w phosphotungstic acid solution (PTA). Images were recorded on a JEOL JEM 1400 plus transmission electron microscope (Peabody, USA) operating at 120 kV in combination with a JEOL CCD camera Ruby (8 M pixel).

The melting and crystallization behavior of CLPs was investigated using a differential scanning calorimeter (Discovery Series DSC 250, TA Instruments, Zellik, Belgium). CLP dispersion (≈ 25 mg) was placed in a T zero pan closed with a hermetic lid, and was heated from -10 to 80 °C at 1 °C min^{-1} , then cooled down to -10 °C at 1 °C min^{-1} followed by two identical heating/cooling cycles. An empty pan was used as a reference. The thermograms were evaluated using the TRIOS software; melting and crystallization enthalpies are reported in Table S2, Supporting Information.

2.2.3. Incubation of CLP Dispersions

An oxidation initiator system consisting of an equimolar mixture of FeSO_4 and EDTA was prepared by mixing equivalent volumes of separately dissolved FeSO_4 and EDTA (8.4 mM) in phosphate buffer under moderate stirring in the dark for one hour.^[35] Aliquots of CLP dispersion (2 g) were distributed in 15-mL polypropylene centrifuge tubes, and the oxidation initiator solution (100 μL) was added to obtain a final concentration of 200 μM of both iron and EDTA. The tubes were rotated in the dark at 2 rpm at 25 °C for 72 h (SB3 rotator, Stuart, Staffordshire, UK).

2.2.4. Extraction of α -Tocopherol

α -Tocopherol was extracted from the CLPs by adding 4 mL of chloroform, 3 mL of methanol, and 1 mL of saturated sodium chloride solution to 2 mL CLP dispersion in a 15-mL polypropylene centrifuge tube, which was vortexed followed by centrifugation at $2000 \times g$ for 8 min. The clear chloroform phase was then collected by cautiously boring a hole in the bottom of the centrifugation tube.

2.2.5. Quantification of α -Tocopherol by HPLC Analysis

The obtained extracts were analyzed on a UltiMate 3000 liquid chromatography system (Thermo Scientific, Sunnyvale, CA, USA) using a carotenoid C30 reversed phase column, 3 μm , 150 mm \times 4.6 mm (YMC, Dinslaken, Germany). Extracts were eluted at 1 mL min^{-1} at 30 °C using a mobile phase with a linear gradient going from 81% methanol, 14% methyl t-butyl ether (MTBE), and 4% ultrapure water to 74% methanol, 22% methyl t-butyl ether, and 4% ultrapure water within 8 min, and going back to its initial composition in 2 min. α -Tocopherol was detected

with a UV-vis detector at 292 nm (Dionex UltiMate 3000 Variable Wavelength Detector), and contents were calculated using a calibration curve that was linear in the range from 5 $\mu\text{g mL}^{-1}$ to 5000 $\mu\text{g mL}^{-1}$. When studying the chemical degradation of α -tocopherol during incubation of CLP dispersions, the results were expressed as normalized α -tocopherol amount (%), taking as a reference the α -tocopherol concentration in the respective CLP suspension just after production.

2.2.6. Experimental Design

All CLPs were prepared and characterized as at least independent duplicates. Size and ζ -potential measurements were performed in triplicate, and extractions and HPLC analyses were performed in duplicate. All results are reported as the mean and standard deviation of all measurements.

3. Results and Discussion

3.1. Physical Characteristics of CLPs

CLPs were produced by high pressure homogenization of an aqueous phase containing Tween 20, Tween 40, or sodium caseinate with either melted tripalmitin, or tripalmitin mixed with tricaprylin in a mass ratio 4:1, or tricaprylin, followed by cooling. The particle size distribution of all CLPs just after preparation was unimodal, with a mean diameter ($d_{3,2}$) of 90–110 nm for surfactant-stabilized CLPs, or 120–150 nm for sodium caseinate-stabilized CLPs (Figure 1A–C). The particle size was thus slightly dependent on the type of emulsifier, which can be explained by the fact that surfactants lower the interfacial tension more compared to proteins, which facilitates droplet break-up during homogenization.^[36] For a given emulsifier, increasing the liquid fat content slightly decreased the droplet size, which can be attributed to the lower viscosity of these oil phases, which, again, facilitates droplet break-up.^[37] Fat composition also affected the surface charge of CLPs stabilized by Tween 20 and Tween 40, with more negative ζ -potential for tripalmitin particles (Figure 1D). This could be caused by the crystallinity of the fat in the CLPs, promoting ion binding at the particle surface.^[38] CLPs stabilized by Tween 20 and Tween 40 showed a lower net ζ -potential (-5 to -15 mV) compared to sodium caseinate-stabilized CLPs (-30 to -35 mV): polysorbate surfactants are non-ionic,^[39] whereas sodium caseinate contains ionizable groups and has an isoelectric point around 4.6,^[40] making it strongly negatively charged at neutral pH.

We visualized the CLPs with transmission electron microscopy, and found clear morphological differences (Figure 2). Pure tripalmitin-based CLPs stabilized with Tween 20 or Tween 40 had a lath-like morphology with a high aspect ratio. When they contained a fraction of tricaprylin, more platelet-like particles with a much lower aspect ratio were obtained. Conversely, pure tripalmitin-based CLPs stabilized with sodium caseinate were nearly spherical. When these sodium caseinate-stabilized CLPs contained a fraction of tricaprylin, their morphology also showed a low aspect ratio, but were more irregular, compared to tripalmitin-based ones. The morphological differences of the

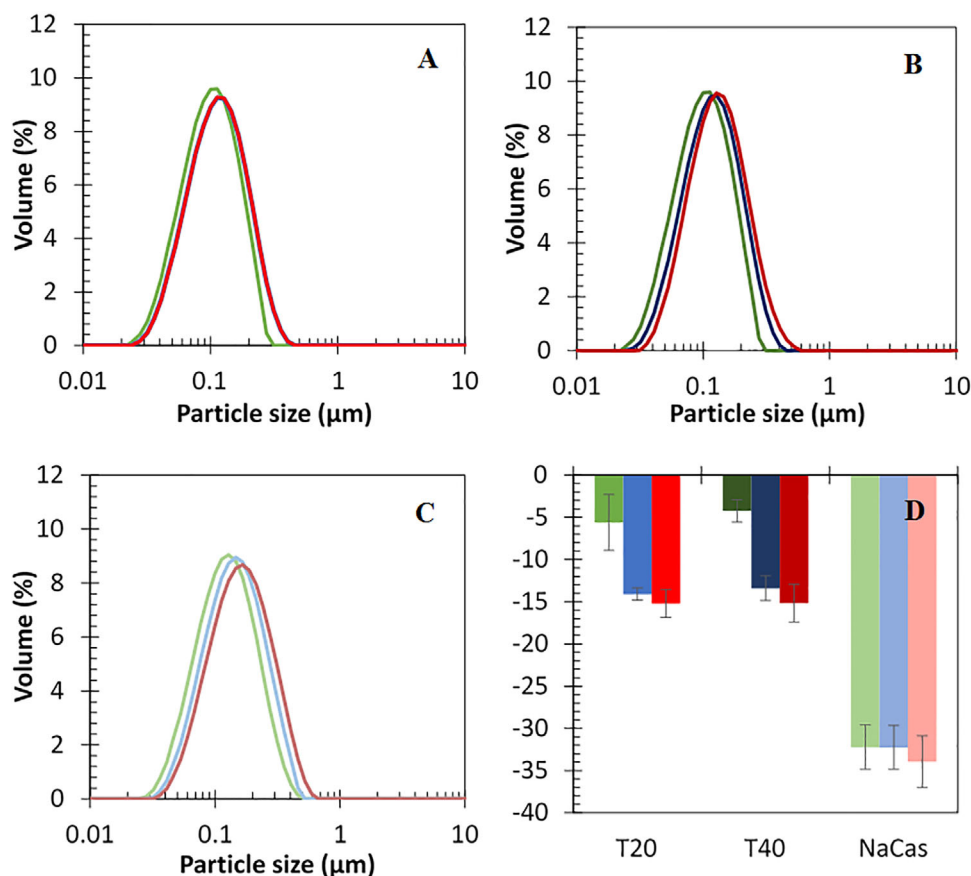


Figure 1. Particle size distributions of A) Tween 20-, B) Tween 40-, and C) sodium caseinate- stabilized CLPs containing tripalmitin (red), tripalmitin:tricaprylin 4:1 (blue), or tricaprylin (green), and their D) ζ -potential. On panel D, T20, T40 and NaCas stand for Tween 20, Tween 40, and sodium caseinate, respectively.

particles may be related to their crystalline microstructure, which was further investigated by differential scanning calorimetry (Figure 3).

Differential scanning calorimetry (DSC) melting thermograms of all pure tripalmitin-based CLPs showed a sequence of melting peaks that point to a complex layered structure, which would melt in multiple identifiable events from the interface.^[30,41,42] The melting thermograms of sodium caseinate-stabilized CLPs also showed a small exothermic peak that indicates crystal reorganization and recrystallization into a more stable polymorphic form.^[43,44] The cooling thermograms of pure tripalmitin-based CLPs showed a crystallization onset at about 23, 29, and 32 °C for Tween 20-, Tween 40-, and sodium caseinate-stabilized CLPs, respectively (Figure 3A,C,E), which was much lower than for bulk tripalmitin (about 43 °C), indicative of a strong supercooling effect in all CLPs.^[44–46]

The cooling thermograms of Tween 20-stabilized tripalmitin CLPs showed a main crystallization peak with a small shoulder, which probably corresponds to a dominant β -subcell fraction and a small fraction of β' -subcell crystals.^[3,18,30] Tween 40-stabilized tripalmitin CLPs showed two distinct crystallization peaks, of which the main one corresponds to β -subcell crystals that were formed in the core of the CLP, and the other one most probably to α -subcell crystals that were formed by Tween 40-induced

surfactant-templated crystallization,^[8,17,47] as reported for high melting lecithin-induced crystallization.^[3] Tween 40 primarily contains palmitic acid (C16:0) as alkyl chain, which can align with alike alkyl chains of tripalmitin at the interface, promoting crystallization at the interface. Conversely, sodium caseinate-stabilized tripalmitin CLPs showed a single crystallization peak, indicating that the tripalmitin crystallized into one polymorphic form, most likely corresponding to α -subcell crystals.^[43]

The melting thermograms of CLPs made with tripalmitin-tricaprylin blends showed a sequence of melting peaks indicative of multiple melting events, although these events were less distinct, and showed lower enthalpies than in pure tripalmitin particles (Table S2, Supporting Information). Tween 20-stabilized CLPs showed one crystallization peak most likely corresponding to β -subcell crystals, whereas for Tween 40-stabilized CLPs α -subcell crystallization was found corresponding to surfactant-templated interfacial crystallization, followed by two overlapping β' and β -subcell crystallization peaks. Last, sodium caseinate-stabilized CLPs showed one main α -subcell crystallization peak followed by a small β' or β -subcell crystallization peak.^[3,18] In general, the CLPs made with tripalmitin-tricaprylin blends showed lower crystallization enthalpy and melting temperatures compared to tripalmitin CLPs, which can be attributed to less ordered crystals.^[29,48]

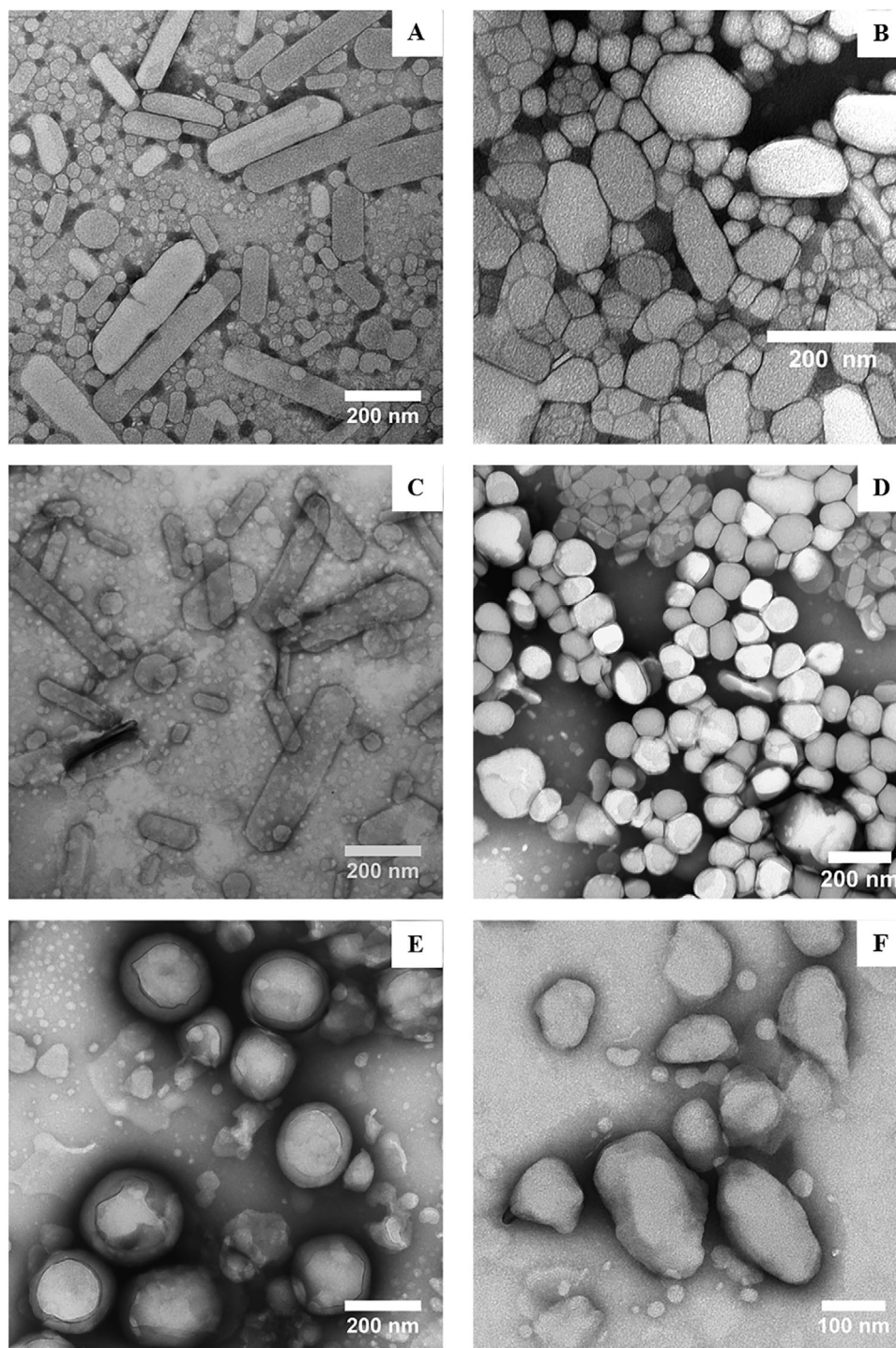


Figure 2. TEM images of CLPs made of tripalmitin (left), or tripalmitin/tricaprylin 4:1 w/w blend (right), stabilized by A,B) Tween 20, C,D) Tween 40, or E,F) sodium caseinate.

3.2. Chemical Stability of α -Tocopherol

The amounts of α -tocopherol recovered in the CLPs immediately after production were between 77.0% and 90.1%, with no significant differences between the particles (Table S3, Supporting Information). These initial losses can be caused by the high

temperature, pressure, and presence of oxygen during homogenization. To further study the chemical stability of α -tocopherol upon storage of the CLP suspensions in accelerated ageing conditions, we incubated the samples with 200 μ M FeSO₄/EDTA at 25°C, and measured the concentration of α -tocopherol in time.

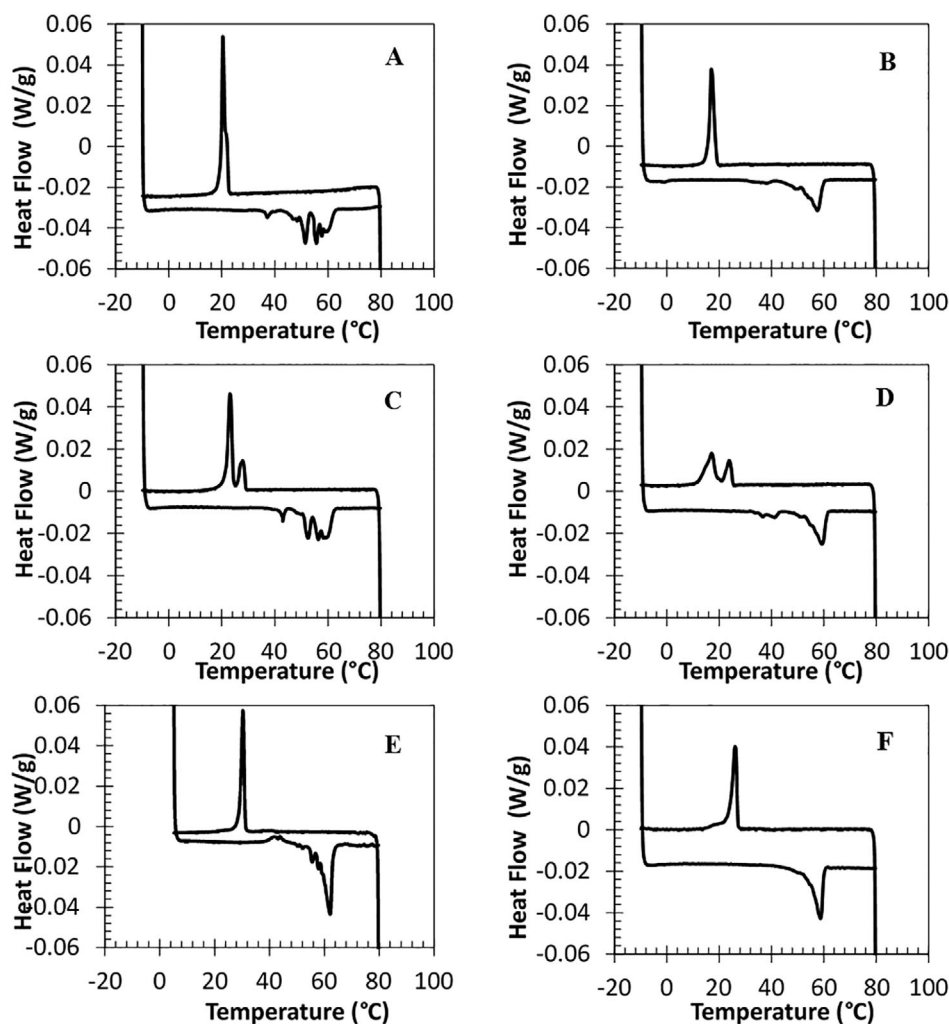


Figure 3. DSC crystallization and melting thermograms of CLPs made of tripalmitin (left) or tripalmitin/tricaprylin 4:1 w/w blend (right), and stabilized by A,B) Tween 20, C,D) Tween 40, or E,F) sodium caseinate.

The chemical stability of α -tocopherol was considerably lower in tripalmitin-based CLPs than in pure tricaprylin-based ones (Figure 4). After 14 days storage, pure tripalmitin CLPs showed remaining amounts of $\approx 85\%$, 60% , and 80% when stabilized by Tween 20, Tween 40, or sodium caseinate, respectively, compared to $\approx 95\%$ in tricaprylin CLPs. Increasing the fraction of liquid tricaprylin in the CLPs increased the chemical stability of α -tocopherol when using Tween 40 and sodium caseinate as emulsifiers, but not when using Tween 20. With regard to the effect of the emulsifier type, the chemical stability of α -tocopherol was the lowest for Tween 40-stabilized CLPs, compared to Tween 20- or sodium caseinate-stabilized CLPs.

We found a high α -tocopherol stability in tricaprylin CLPs irrespective of the emulsifier used. Tripalmitin or tricaprylin-tripalmitin blends resulted in crystallized CLPs with various morphologies, in which the chemical stability of α -tocopherol was lower compared to that in tricaprylin-based CLPs. Although immobilization of lipophilic bioactives within a solid lipid matrix has sometimes been suggested as protective,^[8,49,50] the location of the bioactive and the structure of the solid lipid ma-

trix also need to be taken into account.^[3,24] For example Berton-Carabin et al.^[26] demonstrated that the location and mobility of small lipophilic molecules were largely dependent on their structure and on the physical state of lipids in emulsion systems, which could substantially affect their chemical stability. For molecules structurally close to α -tocopherol, fat crystallization promoted their localization and immobilization at the interface, but did not change their chemical reactivity with aqueous phase reactants.^[26]

Physical exclusion of the bioactive compound can take place during crystallization, resulting in closed compartments loaded with the compound, which leads to an enhanced chemical stability when in the core of the droplet.^[13,17,19,50] If these compartments are close to the particle surface, degradation may be promoted, since they may come into contact with aqueous oxidant species,^[2,13] and leach out due to partitioning. Jenning & Gohla^[4] hypothesized that bioactives with a melting point lower than that of the lipids used for encapsulation may be pushed toward the particle surface due to lipid crystallization in the lipid core prior to the bioactive. This could have been the case in our

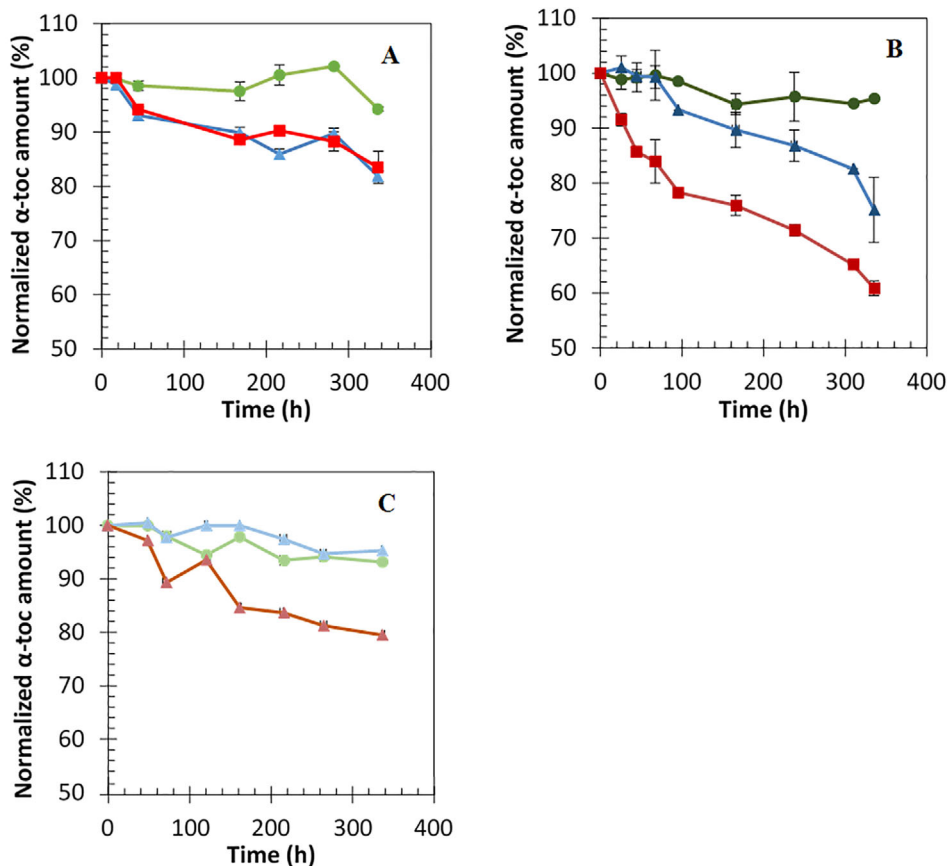


Figure 4. Chemical stability of α -tocopherol, expressed as normalized α -tocopherol amount (%), in CLPs stabilized by A) Tween 20, B) Tween 40, or C) sodium caseinate (NaCas), and prepared with tripalmitin (TP100, red), tripalmitin:tricaprylin 4:1 blend (TP80, blue), or tripalmitin:tricaprylin (TPO, green), during storage under oxidative conditions.

research, resulting in lower chemical stability of α -tocopherol in solid or semi-solid CLPs, compared to liquid ones.

In literature, it was suggested that high melting point surfactants form a shell by surface templating that limits diffusion of prooxidants or oxygen to the oxidizable components.^[17,19] Such a shell let tripalmitin crystallize in a loosely packed crystal form (α or β^1 -subcell), in which lipophilic compounds can be encapsulated better than in tightly packed β -subcell crystals.^[8,17,47] Therefore, it was expected that Tween 40 would limit α -tocopherol degradation more compared to Tween 20 or sodium caseinate, but the opposite was observed. We expect that highly ordered β -subcell crystals were formed in the core of Tween 40-stabilized tripalmitin CLPs, as revealed by DSC, which resulted in needle-shaped CLPs with a high surface area. α -Tocopherol molecules could have been pushed out from the particle core by the growing crystals, and thus present close to the particle surface, where they would be prone to chemical degradation by aqueous phase oxidants.^[25] Tween 40-stabilized CLPs with mixed tripalmitin-tricaprylin contained less β -subcell crystals compared to pure tripalmitin-based CLPs, had less surface area, and could contain some liquid lipid patches in the core of the CLP.^[32] All these effects may have kept α -tocopherol more buried within the particles, leading to higher chemical stability. Tween 20-stabilized CLPs contained mostly β -subcell crystals, which are

expected to induce migration of α -tocopherol from the core to the surface of the CLPs, where it would eventually have been degraded. Compared to Tween 40, less α -tocopherol could have been initially present at the interface, leading to slower degradation. Sodium caseinate-stabilized CLPs were able to inhibit degradation of α -tocopherol to a larger extent compared to both surfactant-stabilized CLPs; for the tripalmitin-tricaprylin blend, the stability was even as good as for tripalmitin. Although sodium caseinate is known to be a metal chelator that effectively prevent lipids from oxidation when present in large excess in the aqueous phase,^[35,51] this seems an unlikely explanation for our results, as only low concentrations of excess proteins were present ($< 1 \text{ g L}^{-1}$). More likely, the less ordered crystalline structure compared to Tween 20 and Tween 40 CLPs allows for α -tocopherol immobilization, or its inclusion in liquid patches within the core of the CLPs. This would lead to slow release of α -tocopherol, which is important for products in which antioxidant activity needs to be maintained for an extended period.

4. Conclusions

In this work, we investigated colloidal lipid particles (CLPs) made with tripalmitin, tripalmitin, or a blend thereof, as encapsulation

systems for α -tocopherol. We showed that emulsifier type and lipid composition modulate the morphology and crystalline structure of the particles, which consequently affected the chemical stability of α -tocopherol. Tween 20 and Tween 40 allowed tripalmitin to crystallize in highly ordered structures with lath-like morphology, from which α -tocopherol was probably expelled. In Tween 40-stabilized CLPs, α -tocopherol probably accumulated at the surface of the particles, where it was prone to chemical degradation by aqueous pro-oxidants. In future work, it would be interesting to validate this by in situ measurements in particle dispersions, for example, by front-surface fluorescence spectroscopy or by electron spin resonance. Sodium caseinate tripalmitin CLPs crystallized in a nearly spherical shape with less ordered crystalline structure in which the α -tocopherol stability was high. When a lipid blend was used, the chemical stability of α -tocopherol increased in most cases, possibly due to the formation of liquid patches that would keep the bioactive buried within the particle core. Tricaprylin CLPs had highest chemical stability of α -tocopherol irrespective of the emulsifier used, showing that lipid crystallization does not necessarily protect lipophilic bioactives. From these results it is clear that for efficient encapsulation of lipophilic bioactives, the particle architecture plays an important role; to tailor this, both the lipid and the emulsifier need to be considered.

Abbreviations

CLP, colloidal lipid particle; DSC, differential scanning calorimetry; EDTA, ethylenediaminetetraacetic; HMP, high melting point; NaCas, sodium caseinate; TEM, transmission electron microscopy; SLN, solid lipid nanoparticle; T20, Tween 20; T40, Tween 40

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Research presented in this publication was financially supported by the Graduate School VLAG (Voeding, Levensmiddelentechnologie, Agrobiotechnologie en Gezondheid). The authors wish to thank Rutger Visser for performing preliminary experiments.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors conceived the experiments. A.S. conducted the majority of experiments, with Rutger Visser contributing. A.S. wrote the manuscript, and revisions were made by all other authors.

Keywords

encapsulation, lipid crystallization, lipophilic bioactives, solid lipid nanoparticles, vitamin E

Received: January 14, 2020

Revised: April 1, 2020

Published online:

- [1] D. J. McClements, *Adv. Colloid Interface Sci.* **2012**, *174*, 1.
- [2] C. Qian, E. A. Decker, H. Xiao, D. J. McClements, *Food Res. Int.* **2013**, *52*, 342.
- [3] H. Salminen, T. Helgason, B. Kristinsson, K. Kristbergsson, J. Weiss, *Food Chem.* **2013**, *141*, 2934.
- [4] V. Jenning, S. H. Gohla, *J. Microencapsulation* **2001**, *18*, 149.
- [5] K. Oehlke, D. Behnsnlian, E. Mayer-miebach, P. G. Weidler, R. Greiner, *Edible solid lipid nanoparticles (SLN) as carrier system for antioxidants of different lipophilicity.* **2017**, 1–18.
- [6] H. Salminen, T. Helgason, S. Aulbach, B. Kristinsson, K. Kristbergsson, J. Weiss, *J. Colloid Interface Sci.* **2014**, *426*, 256.
- [7] S. Trombino, R. Cassano, R. Muzzalupo, A. Pingitore, E. Cione, N. Picci, *Colloids Surf., B* **2009**, *72*, 181.
- [8] N. Yadav, S. Khatak, U. Vir, S. Sara, *Int. J. Appl. Pharm.* **2013**, *5*, 8.
- [9] A. zur Mühlen, C. Schwarz, W. Mehnert, *Eur. J. Pharm. Biopharm.* **1998**, *45*, 149.
- [10] C. Bleeker, Geertsma, Jong, D., Heugens, Koers-Jacquemijns, ... Environment, D. N. I. for P. H. and the. **2012** RIVM Letter Report 601358001/2012. Retrieved from <http://www.rivm.nl/dsresource?objectid=rivmp:181801&type=org&disposition=inline>.
- [11] D. J. McClements, *Nanoemulsions versus microemulsions: terminology, differences, and similarities.* **2012**, 1719.
- [12] D. J. McClements, Y. Li, *Adv. Colloid Interface Sci.* **2010**, *159*, 213.
- [13] N. Dan, *J. Food Eng.* **2016**, *171*, 37.
- [14] N. P. Aditya, S. Ko, *RSC Adv.* **2015**, *5*, 30902.
- [15] S. Mukherjee, S. Ray, R. S. Thakur, *Indian J. Pharm. Sci.* **2009**.
- [16] G. Suresh, K. Manjunath, V. Venkateswarlu, V. Satyanarayana, *AAPS PharmSciTech* **2007**, *8*, E162.
- [17] H. Thrundur, T. S. Awad, K. Kristberg, A. D. Eric, J. M. A. David, W. Jochen, *J. Agric. Food Chem.* **2009**, *57*, 8033.
- [18] T. Helgason, T. S. Awad, K. Kristbergsson, D. J. McClements, J. Weiss, *J. Colloid Interface Sci.* **2009**, *334*, 75.
- [19] H. Salminen, C. Gömmel, B. H. Leuenberger, J. Weiss, *Food Chem.* **2016**, *190*, 928.
- [20] N. Dan, *Langmuir* **2014**, *30*, 13809.
- [21] A. Bargoni, R. Cavalli, O. Caputo, A. Fundarò, M. R. Gasco, G. P. Zara, *Pharm. Res.* **1998**, *15*, 745.
- [22] A. Miglietta, R. Cavalli, C. Bocca, L. Gabriel, M. Rosa Gasco, *Int. J. Pharm.* **2000**, *210*, 61.
- [23] H. Bunjes, K. Westesen, M. H. J. Koch, *Int. J. Pharm.* **1996**, *129*, 159.
- [24] R. V. Tikekar, N. Nitin, *Soft Matter* **2011**, *7*, 8149.
- [25] U. Yucel, R. J. Elias, J. N. Coupland, *J. Colloid Interface Sci.* **2012**, *377*, 105.
- [26] C. C. Berton-Carabin, J. N. Coupland, R. J. Elias, *Colloids Surf. A* **2013**, *431*, 9.
- [27] S. Arima, T. Ueji, S. Ueno, A. Ogawa, K. Sato, *Colloids Surf., B* **2007**, *55*, 98.
- [28] D. Rousseau, *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 283.
- [29] H. Salminen, S. Aulbach, B. H. Leuenberger, C. Tedeschi, J. Weiss, *Colloids Surf., B* **2014**, *122*, 46.
- [30] A. Schröder, J. Sprakel, K. Schroën, C. Berton-Carabin, *Soft Matter* **2017**, *13*, 3190.
- [31] S. Dima, C. Dima, G. Iordachescu, *Food Eng. Rev.* **2015**, *7*, 417.

- [32] V. Saez, I. D. L. Souza, C. R. E. Mansur, *Int. J. Cosmet. Sci.* **2018**, *40*, 103.
- [33] A. Dingler, R. P. Blum, H. Niehus, R. H. Müller, S. Gohla, *J. Microencapsulation* **1999**, *16*, 751.
- [34] V. Sánchez-Paz, M. J. Pastoriza-Gallego, S. Losada-Barreiro, C. Bravo-Díaz, K. Gunaseelan, L. S. Romsted, *J. Colloid Interface Sci.* **2008**, *320*, 1.
- [35] C. Berton, M. H. Ropers, M. Viau, C. Genot, *J. Agric. Food Chem.* **2011**, *59*, 5052.
- [36] M. A. Bos, T. van Vliet, *Adv. Colloid Interface Sci.* **2001**, *91*, 437.
- [37] P. Walstra, *Chem. Eng. Sci.* **1993**, *48*, 333.
- [38] C. Freitas, R. H. Müller, *Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions.* **1998**, *168*, 221–229.
- [39] A. Teo, K. K. T. Goh, J. Wen, I. Oey, S. Ko, H. Kwak, S. Je, *Food Chem.* **2016**, *197*, 297.
- [40] A. L. M. Braga, M. Menossi, R. L. Cunha, *Int. Dairy J.* **2006**, *16*, 389.
- [41] H. Bunjes, M. H. J. Koch, K. Westesen, *Langmuir* **2000**, *16*, 5234.
- [42] J. N. Coupland, *Curr. Opin. Colloid Interface Sci.* **2002**, *7*, 445.
- [43] T. S. Awad, T. Helgason, J. Weiss, E. A. Decker, D. J. McClements, *Effect of Omega-3 Fatty Acids on Crystallization, Polymorphic Transformation and Stability of Tripalmitin Solid Lipid Nanoparticle Suspensions & DESIGN* **2009**, *2009*, 12.
- [44] A. Schröder, J. Sprakel, K. Schroën, J. Spaen, C. C. Berton-Carabin, *J. Food Eng.* **2018**, *234*, 63.
- [45] S. Abramov, P. Ruppik, H. P. Schuchmann, *Crystallization in Emulsions: A Thermo-Optical Method to Determine Single Crystallization Events in Droplet Clusters*, **2016**.
- [46] K. Westesen, H. Bunjes, *Int. J. Pharm.* **1995**, *115*, 129.
- [47] H. Salminen, T. Helgason, B. Kristinsson, K. Kristbergsson, J. Weiss, *J. Colloid Interface Sci.* **2017**, *490*, 207.
- [48] F. D. Gunstone, J. L. Harwood, A. J. Dijkstra, *The Lipid Handbook with CD-ROM. The Lipid Handbook* **2007**, *1*.
- [49] V. Jenning, M. Schäfer-Korting, S. Gohla, *Vitamin A-loaded solid-lipid-nanoparticles for topical use: drug release properties.* **2000**, *66*, 115–126.
- [50] P. Relkin, J. M. Yung, D. Kalnin, M. Ollivon, *Food Biophysics* **2008**, *3*, 163.
- [51] H. Faraji, D. J. McClements, E. A. Decker, *J. Agric. Food Chem.* **2004**, *52*, 4558.