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Crystallized Miniemulsions: Influence of operating parameters during high pressure homogenization on size and shape of particles

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Abstract
Miniemulsions were produced by high pressure homogenization at 80 °C. Triglyceride oil, trimyristin, tripalmitin and propylene glycol monostearate were applied as dispersed phases. Tween 80 was used as emulsifier. Cooling down of tripalmitin and propylene glycol monostearate dispersions to room temperature induced crystallization of the lipid. Trimyristin dispersions required a cooling to 4-8 °C for crystallization of the lipid. Dispersed triacylglycerid oil stays liquid at any investigated temperature. Additionally nanostructured lipid carriers - which were two phase particles with liquid oil and solid lipid – were produced. This particles feature increase loading capacity compared to one phase particles for actives. The dispersions were characterized by dynamic light scattering and electron microscopy. Differences between solid and liquid particles are shown and discussed. The influence of the operating parameters homogenization pressure and residence time was studied. The dynamic viscosities of the oil and melts were measured in the same range. The interfacial tensions between triglyceride oil and the Tween 80 solution as well as trimyristin and Tween 80 solutions were measured.

Keywords: Emulsification, crystallization, dispersed systems, particle size, particle size distribution pharmaceutical nanoparticles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), miniemulsion

1. Introduction

Miniemulsions are also called nanoemulsions, or parenteral emulsions. They are - in contrast to microemulsions - kinetically stable systems while microemulsons are thermodynamically stable systems. They consist of particles a little larger than 100 nm.
Miniemulsions can be produced by high-pressure homogenization, ultrasonication, rotor-stator systems or membrane processes. The most important continuously operating emulsifying devices are rotor-stator systems (e.g. colloid mills) and high-pressure homogenizers. High-pressure homogenizers are particularly suitable for the production of finely dispersed emulsions [1,2].
Cystallization in miniemulsions is under intensive investigation [3-6] because it offers a route to large-scale production of small solid particles whose size, shape and size distribution may be extremely well controlled by the properties of the initial emulsion [7]. Aim of the present study was to investigate the operating parameters in the emulsification step and their influences on the particle size distribution and particle shape after crystallization.
At the beginning of the 1990s solid particles (so-called solid lipid nanoparticles (SLN)), became interesting for pharmaceutical applications to create alternative colloidal carrier systems [8]. These suspensions were designed to combine the advantages of polymeric particles [9], liposomes and emulsions [10] and to offer new drug carrier systems possessing good physical stability, protecting incorporated labile drugs from degradation, and controlling release and compatibility. At the turn of the millennium, an improved modification of SLN, the so-called nanostructered lipid carrier (NLC) was introduced in order to increase the loading of particles with cosmetic and pharmaceutical agents [11, 12]. The preparation is similar to that of emulsions and differs only in the physical state of the used lipid. In

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NLC a part of the lipid stays liquid at room temperature.
Beside drugs [8] and cosmetic agents [13] SLN and NLC technology is discussed for food applications [14, 15]. The technology for instance was used for water dispersible formulating the hydrophobic β-carotene [16].
For a sufficient residence time the achievable particle size in emulsions depends on:

- Adsorption kinetics of the emulsifier,
- Interfacial tension between dispersed and continuous phase,
- Viscosities of dispersed and continuous phases,
- Dispersed volume fraction,
- Mechanical energy input to deform and break up the droplets.

Energy density E_V [1] is the mechanical energy input per volume of the zone where the droplets are disrupted (dispersing volume). In high-pressure homogenizers the pressure difference over the nozzle corresponds to the energy density. When the stresses acting locally on the droplet exceed the retaining forces for a sufficiently long time, the droplet is disrupted. Then the result of disruption, expressed by a mean diameter x (e.g. the z-average diameter) can be described as a function of energy density by

$$x = C\, E_V^{-b} \qquad (1)$$

Here, b and C are constant. C depends i.a. on the efficiency of droplet disruption and b is affected i.a. by the flow conditions in the dispersing volume. The particle disruption in radial diffusers is predominantly due to inertial forces in turbulent flow. Disruption is also possible thru shear forces in turbulent flow and cavitation [1].
In case of crystallization inside the dispersed particles, the molecular conformation in the crystal lattice of the lipid solid particle has to be considered, due to the discrepancy from the spherical shape in the crystallized state. The systems investigated here contain 10% lipid dispersed as solid, liquid or solid and liquid particles in aqueous solution. Differences between solid and liquid particles are shown and discussed. Therefore, residence time and homogenizer pressure were varied. The interfacial tension between dispersed and aqueous phase was determined and the viscosity of the dispersed phase was measured.

2. Experimental

2.1. Materials

One phase systems were produced with middle chain triacylglyceride oil (MCT, Miglyol 812), trimyristin (MMM, Dynasan 114, Melting point T_m = 55-58 °C) and tripalmitin (PPP, Dynasan 116, Melting point T_m = 66 - 67 °C), from Sasol, Witten, Germany. Propylene glycol monostearate (PGMS) ALDO PGHMS® was from Lonza, New Milford, USA and used to produce one and two phase systems. PGMS (Melting point T_m = 42-43 °C) is a mixture of approximately 70% (w/w) propylene glycol mono- and distearates, about 29% (w/w) propylene glycol mono- and dipalmitates, and 1% (w/w) fatty acids (C14-C18). The major monoester component (about 90% of ALDO PGHMS) is a primary ester (1-PGMS), but it also contains secondary esters (2-PGMS). The two-phase particles were produced with PGMS and Lucarotin 10 SUN, which is a suspension of ß-carotene (10%) in oil. Lucarotin 10 SUN was provided by BASF Ludwigshafen, Germany. As emulsifiers sodium glycocholate and Tween 80 from Sigma Aldrich Chemicals, Seelze, Germany were used. As continous phase deionized water was used.

2.2. Methods

2.2.1. Preparation of dispersions

The emulsifiers were dispersed/dissolved in the aqueous phase and heated to 80 °C. The hot emulsifier solution was added to the molten lipid, which had been heated to the same temperature, and the mixture was predispersed by stirring (Ultra-Turrax T25, Janke&Kunkel, Staufen, Germany). The hot predispersion was passed through a heated (~80 °C) high-pressure homogenizer (EmulsiFlex-C5, Avestin, Ottawa, Canada) for the corresponding cycles and pressures. To investigate residence time and mechanical energy input the dispersions were prepared from 10% lipid and 4% Tween 80 for MCT, MMM or PPP and 2.5% Tween 80 for PGMS as dispersed lipid.
The MMM dispersions for freeze fracture TEM contained 3.2% Tween 80 and 0.8% sodium glycocholate in an aqueous phase and 10% lipid. The NLC dispersions were prepared from 6.5% PGMS 3.5% Lucarotin 10 SUN and 2.5% Tween 80. Samples were stored at room temperature (20 °C), trimyristin dispersions which were desired to crystallize were stored in the fridge (4-8 °C).

2.2.2. Dynamic viscosity

Dynamic viscosities of the molten lipids were calculated by the kinematic viscosity and the density. Measurements of the kinematic viscosity were carried out with an AVS 440, Schott-Geräte GmbH, Hofheim a. Ts., Germany. The density was measured with a DMA 58, Anton Paar, Graz, Austria.

2.2.3 Interfacial tension

The temperature dependent equilibrium interfacial tension between oil and the emulsifier solution as well as molten trimyristin and emulsifier solution were measured using a Pendant Drop OCA 20,

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DataPhysics, Filderstadt, Germany. This method measures interface tensions > 5 mN/m.

2.2.4. Particle size measurements

The intensity weighted mean hydrodynamic size (called z-average in this paper) and the polydispersity index (PdI) of the particles were measured by photon correlation spectroscopy (PCS; Zetasizer Nano Series (Nano ZS), Malvern, UK) at 25 °C in order to determine the size of the lipid particles. All samples were diluted with deionized particle-free water to an adequate scattering intensity prior to the measurement. A PdI from 0.03 to 0.06 is called monodispers. Values between 0.10 and 0.20 are narrow distributions and values between 0.25 and 0.50 are broad distributions [17].

2.2.5. Freeze fracture TEM

Small droplets were cryofixed by immersion into melting Freon 22 and freeze-fractured at -120 °C without etching with a BALZERS BA 360 M unit. Freeze-fractured specimens were replicated by application of Pt/C and C by electron-gun evaporation. The cleaned replicas were examined with a FEI Tecnai 10 transmission electron microscope operated at 80 kV. Cryoprotected samples were mixed with 30% glycerol before freezing.

3. Results and discussion

3.1. Dynamic viscosity

Dynamic viscosity of the dispersed phase influences droplet disruption in the used radial diffuser homogenizer [1]. Therefore, the melt viscosities of the used lipids were measured above their melting points up to the process temperature of 80°C (Fig. 1). The dynamic viscosities decrease with increasing temperature. This effect is due to size reduction of the lamellar units with increasing temperature [18]. At 80°C the viscosities are in the same range for all melts (between 6 and 13 mPas). Therefore, it can be assumed that the influence of dynamic viscosity on droplet disruption is if at all low.

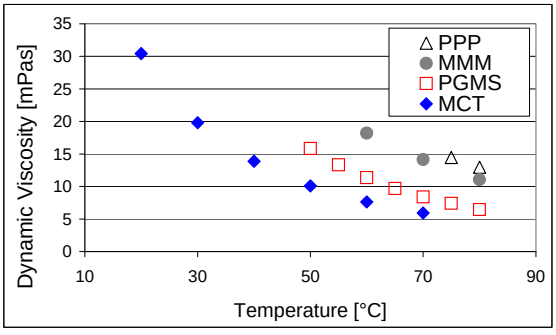

Fig. 1. Temperature dependent dynamic viscosity of the molten lipids and the oil.

3.2. Interfacial tension

The temperature dependent interfacial tension between oil and the emulsifier solution as well as oil and the emulsifier solution were measured (Fig.2). The content of the emulsifier in the aqueous phase was the same as used for preparing the miniemulsions.

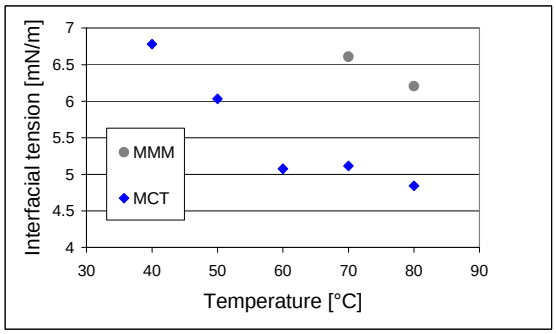

Fig. 2. Temperature dependent interfacial tension between the molten trimyristin (MMM) and the emulsifier solution as well as the triglyceride oil (MCT) oil and the emulsifier solution obtained by pendant drop measurement.

The interfacial tensions decrease with increasing temperature. For molten trimyristin (MMM) and oil (MCT) they are in the same range, with higher values for the component with the longer fatty acid chain.
With the method used only equilibrium interfacial tensions were measured. Beside the equilibrium value for the interfacial tension, in high-pressure homogenization the adsorption kinetic of the emulsifier at the newly created interface and the stabilisation properties against coalescence of the droplets is important. The measured interfacial tension can differ from the effective interfacial tension during high-pressure homogenization.

3.2. Residence time and mechanical energy input

The mean particle size z-average and the polydispersity index dependent on residence time are shown in Fig. 3. Residence time was adjusted by recycling the dispersions in the homogenizer for the appropriate passes. For one pass the mean residence time in the dispersion zone of a high-pressure homogenizer is approx. 0.001 – 0.01 s [19].
For a given pressure of 500 bar the particle size and the PdI stays constant after 3 passes. Residence time is sufficient for the given mechanical energy input. For tripalmitin and MCT-oil narrow particle size distributions are obtained. When PGMS as lipid was used monodisperse suspensions were revealed. PGMS itself is an oily soluble emulsifier with a hydrophilic-lipophilic balance (HLB) value of 3. Therefore, the interfacial tension between PGMS and water is lowered compared to triacylglycerides and emulsifying is eased. PGMS

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suspensions posses the highest mean particle diameter, the middle chain triacylglyceride oil MCT emulsions the lowest. The mean particle sizes of PPP suspensions are below that ones of PGMS suspensions. The solid platelets like particles consist of layers of two crystal lattices in lamellar units. The spacings for these lamellar layers were found as 5.9 nm for PGMS in α-modification [20] and 4 nm for PPP in ß-modification [21]. This means that the units from which the particles are composed of are bigger for PGMS than for PPP.
The emulsions possess the smallest z-average diameter compared to the suspensions, due to the method using the equivalent sphere theory. While the spherical emulsion droplets are measured correctly, the values for the platelet like particles in the suspensions are measured too high. This effect was shown for trimyristin dispersions. The z-average mean diameter was 0.142 µm for the emulsion and 0.163 µm for the suspension, respectively.

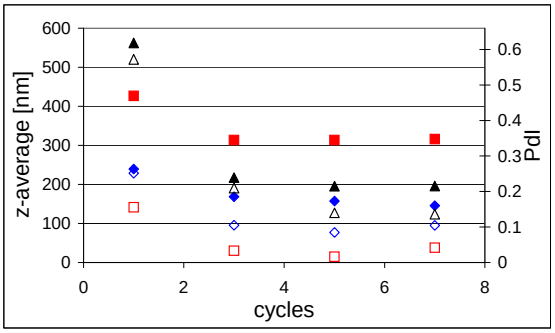

Fig. 3. Mean particle diameter (z-average - full symbols) and Polydispersity index (PdI - open symbols) of formulations homogenized at 500 bar with different residence times. ▲: tripalmitin (PPP) ■: propylene glycol monostearate (PGMS), ♦: middle chain triacylglyceride oil (MCT)

The z-average and the polydispersity index dependent on the homogenization pressure with constant number of cycles were investigated (Fig. 4). An energy density of 10^8 J/m^3 corresponds to a homogenizing pressure of 1000 bar.

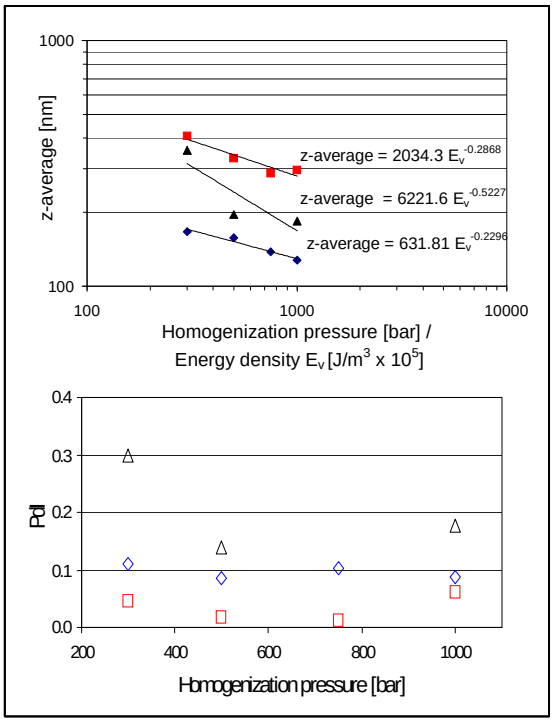

Fig. 4. Mean particle diameter (z-average - full symbols) and polydispersity index (PdI - open symbols) of formulations homogenized for 5 cycles at different pressures. ▲: tripalmitin ■: propylene glycol monostearate, ♦: middle chain triacylglyceride oil

As shown in Fig. 4 the mean diameter of MCT oil decreases from 167 nm to 127 nm as the energy density rises according to Eq. (1). The water soluble emulsifier stabilizes the droplets after disruption in the dispersing volume. Thus the time for adsorption of the emulsifier at the newly created interface determines the efficiency of the size reduction. Highly efficient droplet disruption can therefore be a problem when the emulsifier employed does not stabilize the droplets quickly enough after disruption with the result that the droplets can coalesce. It is also possible that droplet size remains constant or even increases as energy input rises [1,2]. Fig. 4 shows a constant particle size for PPP and PGMS particles at energy densities higher than 7.5 10^7 J/m^3.
The polydispersity index (Fig. 4) indicates for MCT-oil almost constant values for a narrow particle size distribution at all pressures. For the suspensions the values indicate a narrow particle size distribution except for tripalmitin at 300 bar. This can be explained by the process temperature of 80 °C, which is close to the melting point of tripalmitin (Tm = 66 - 67 °C). It can not be excluded that the lipid was - as least partly –not sufficiently molten or recrystallized during the production process. Polydispersity increased for suspensions homogenized at 1000 bar. This could be caused either because the adsorption of the emulsifier which was to slow or because the

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residence time which was not sufficient enough for the molten lipids.

3.3. Freeze fracture TEM

Electron micrographs of trimyristin dispersions were taken before (Fig. 5) and after (Fig. 6) crystallization. The crystallization temperature (Differential Scanning Calorimetry, data not shown) of the trimyristin dispersions is lowered, compared to the one-phase bulk, from 28 °C to 8 °C, due to a different crystallization mechanism. This means that formulations stored at room temperature are not crystallized, while refrigeration induced crystallization in the droplets.

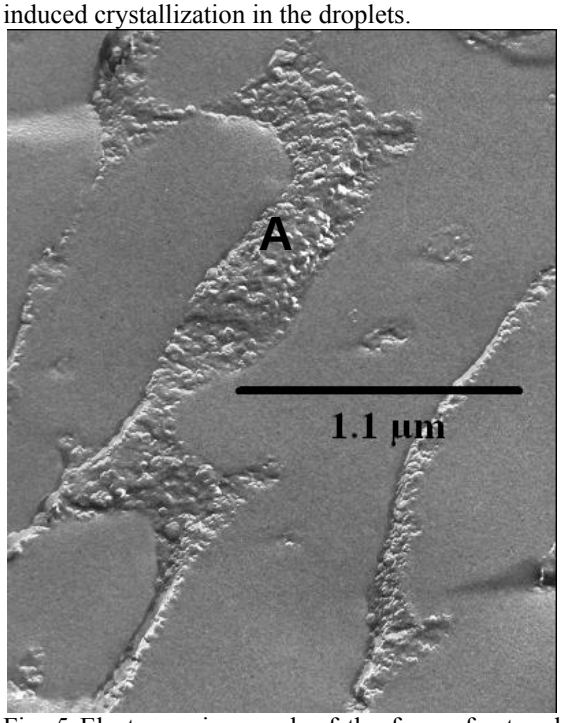

Fig. 5 Electron micrograph of the freeze-fractured trimyristin emulsion. A: Liquid oil.

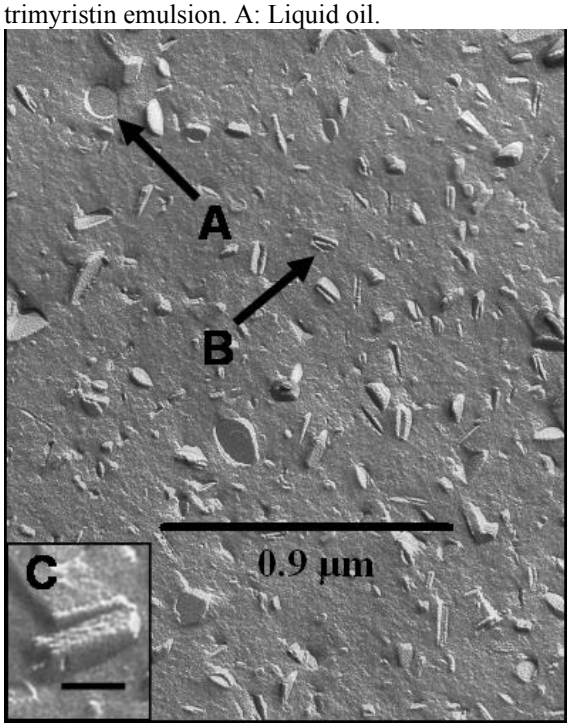

Fig. 6. Electron micrograph of the freeze-fractured cryoprotected trimyristin suspension. A: Particle in top view. B: Particle in side view. C: Internal structure (bar represents 0.1 µm).

The dispersions were passed through the homogenizer for 5 cycles at 800 bar. The trimyristin dispersions were homogeneous milky systems with z-average/polydispersity index of 0.142 µm/0.15 for the emulsion and 0.163 µm/0.21 for the suspension, respectively.

Although the particles in the not cryoprotected emulsion are compressed during freezing (Fig.5), the liquid state of the triglyceride is well-defined from the crystallized particles in the suspension as visible in Fig. 6. The solid particles are sharply edged nanocrystals with a planar layered internal structure, due to the ß-modification (verified by small angle X-ray scattering, data not shown). This corresponds well to literature [4].

The NLC dispersions were investigated with different operating parameters. Micrographs for low energy input of 500 bar homogenization pressure and one pass through the radial diffuser (Fig. 7) as well as for high energy input and a residence time of 5 passes were taken (Fig. 8).

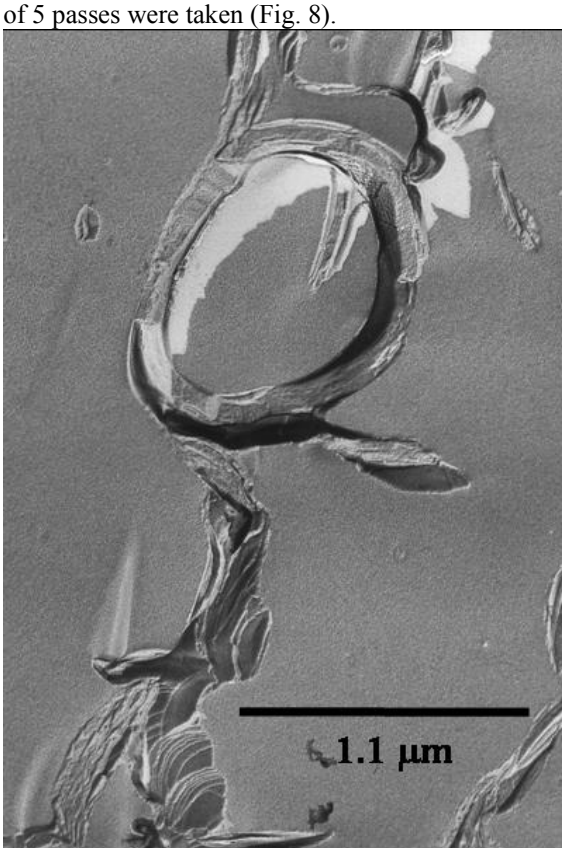

Fig. 7. Electron micrograph of the freeze-fractured NLC dispersion high-pressure homogenized at 500 bar for 1 cycle

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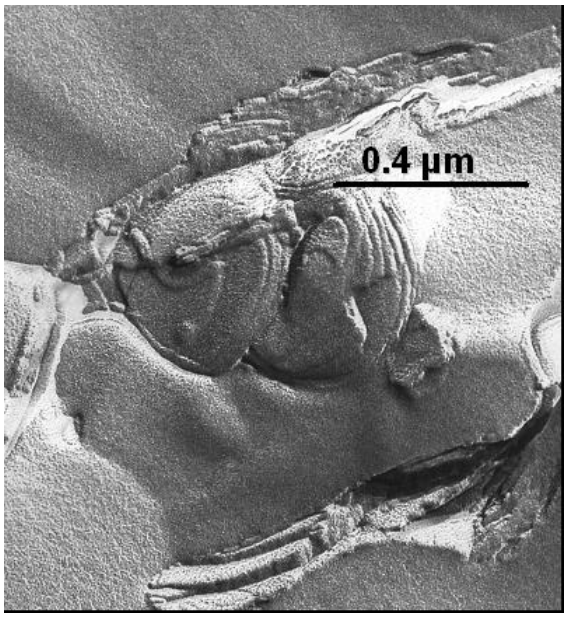

Fig. 8. Electron micrograph of the freeze-fractured NLC dispersion, high-pressure homogenized at 1500 bar for 5 cycles.

In the case of lower energy input and short residence time a structure with a liquid core and solid crystallized shell as well as platelet like structures was found (Fig. 7). The outer shell consisted of a planar layered structure. This capsule morphology could be of great interest if it could be produced systematically.
In the latter case of high energy input and long residence time the structures became smaller and a platelet structure occurred. In literature [12] nanostructured lipid carriers dispersions were made of glyceryl behenate and MCT. Homogenization at 500 bar for 3 cycles lead to thin platelets of solidified fat and liquid oil sticks to the solid lipid as a half drop, which was termed as a "nanospoon" structure.
Two phase so called nanostructured lipid carriers (NLC) can be regarded as hybrids between emulsion droplets and solid particles. They combine the advantage of high loading capacity for actives of emulsion droplets with the physical stability of solid particles.

4. Conclusion

Dispersions with narrow particle size distributions were obtained by confining the crystallization in miniemulsion droplets. High-pressure homogenization is an industrially feasible process to produce particles in a nanoscale range with the opportunity to control size and particle size distribution via the operating parameters residence time and homogenization pressure. The particle formation and formulating occurs in a single process. There were no additional steps required.
The emulsion droplets confine the volume for the following crystallization step. Nanoscale solid structures like the two phase NLC, require a minimum volume size, adjustable via the operating parameters. The particle size of MCT emulsions shows a steady decline with increasing mechanical energy input. The particle sizes of PPP and PGMS suspensions stay constant for higher mechanical energy inputs.

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