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Influence of process and formulation parameters on the preparation of solid lipid nanoparticles by dual centrifugation

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ABSTRACT

A promising strategy to formulate poorly water-soluble active pharmaceutical ingredients (APIs) is the application of these substances in solid lipid nanoparticles. These drug carrier systems are commonly prepared by high-pressure homogenization above the melting temperature of the utilized lipid. While being very useful for large-scale production this method is quite resource-consuming and does not allow simultaneous processing of multiple samples, e.g. for screening purposes. For this reason, an alternative manufacturing process, dual centrifugation, is introduced to prepare solid lipid nanoparticles. The ingredients of the dispersions were directly weighed into 2 mL vessels at room temperature without the need to prepare a pre-mix emulsion. Due to an additional rotation of the samples in the heated centrifuge as well as the addition of grinding media an intensive stressing of the samples was achieved. The emulsification process was finished within 10 min with sample temperatures of up to 90 °C being obtained. Dependent on the process set-up like grinding media size, filling ratio or process temperature and the composition of the lipid formulation, the achieved particles sizes were below 200 nm and had a narrow, monomodal size distribution.

1. Introduction

Innovative drug discovery programs find promising new active pharmaceutical ingredients (APIs) but studies indicated that most of these substances are poorly water-soluble as a result of high lipophilicity and/or a highly stable crystal lattice (reflected in a high melting temperature) (Bergström et al., 2016). This often leads to a low bioavailability of the substances and may result in a rejection of the respective API at a very early development stage (Lipp, 2013; Lesson, 2016). To overcome these limitations, an appropriate formulation is essential. A promising approach is the formulation of the poorly water-soluble APIs in colloidal lipid dispersions especially when the substances are lipophilic (Bunjes, 2010). Lipids are biocompatible excipients and enable many different routes of administration like e.g. peroral, parenteral or transdermal (Mehnert and Mäder, 2012; Lim et al., 2012).

Lipid emulsions have been in medical use for over 50 years for the parenteral nutrition of critically ill patients who cannot be fed orally (Driscoll, 2006). They can also be employed for the solubilization of poorly water-soluble APIs by the lipid droplets in order to overcome the solubility challenge (Hörmann and Zimmer, 2016). Dependent on their molecular properties the APIs may be distributed in the liquid core, in the aqueous phase or in the droplet interface (Berton-Carabin et al., 2013; Kupetz and Bunjes, 2014). For interface-localizing API molecules the loading capacity of emulsion droplets is rather limited since, for geometric reasons, the specific surface area of the spherical droplets is quite low. Thus, solid lipid nanoparticles are an interesting alternative. (Tri)glyceride nanoparticles, for example, can crystallize in a platelet-like shape (Illing and Unruh, 2004; Jores et al., 2004; Petersen et al., 2011), providing a higher capacity for the adsorption of the API molecules because of their increased specific surface area. For some APIs like, e.g., amphotericin B and curcumin, higher drug loads could be realized when solid trimyristin nanoparticles where chosen as carrier system instead of trimyristin nanoemulsions (Kupetz and Bunjes, 2014).

In an early stage of the formulation development process when the amount of available API is very limited, an appropriate method for formulation screening is essential. A robust process for the preparation of different formulations of solid lipid nanoparticles in parallel within a short time period and with a very small batch size would be of great advantage in this regard. A promising screening tool for the preparation of lipid dispersions could be dual centrifugation. Introduced in the

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1970s as a technology for the rapid mixing of viscous components, the area of application has been extended in the last years. Nowadays, the method is also used for the preparation of API nanosuspensions or lipid nanodispersions likes liposomes and emulsions (Massing et al., 2008; Hagedorn et al., 2017; Tenambergen et al., 2013; Hagedorn et al., 2019; Meier et al., 2015). The technological advancement of the dual centrifuge compared to a conventional centrifuge is based on the superimposition of two movements in the batch vessels by implementing a second rotation wheel. As a result, the orientation of the samples continuously changes during the process (see Fig. 1). The two rotations introduce high stress intensities in the formulations which can be further increased by using grinding media in the batch vessels.

Studies by Hagedorn et al. demonstrated that dual centrifugation is suitable for the nanomilling of poorly water-soluble APIs with ceramic grinding beads in 2 mL plastic vials. They achieved particle sizes of less than 200 nm under controlled temperature conditions (Hagedorn et al., 2017). The results were comparable to those achieved with larger scale agitator-mills (Hagedorn et al., 2019). Liposomes could be reproducibly prepared in small batch vessels under optimized process conditions using glass beads to increase the stress intensities. However, the results indicated a wide particle size distribution for the processed liposomes, reflected by high polydispersity indices (PdIs) (Massing et al., 2008). The preparation of soybean oil emulsions by dual centrifugation was described by Tenambergen et al. Without the use of grinding media in the batch vessels, droplet sizes of approx. 800 nm were achieved in three consecutive mixing steps. The experiments were performed at a sample temperature of 55 °C with the single components being separately heated up and combined in the vessel (Tenambergen et al., 2013). In the outlined studies, the dual centrifuge was operated at room temperature or below and the processed lipids were either liquid at room temperature or were pre-heated before processing.

In the current study, the possibility of manufacturing nanoemulsions and nanosuspensions from solid triglycerides in a dual centrifuge was investigated. To enable processing of the lipids above their melting point, the dual centrifuge was equipped with an additional heating device. The aim of this study was to evaluate if high quality lipid nanoemulsions and solid lipid nanoparticles can be prepared from lipids with a melting temperature above 50 °C with this heatable dual centrifuge. Thus, it was to be evaluated if the technique could be prospectively used as a screening tool for this type of lipid formulations. The manufacturing process would be considered suitable when particle sizes below 200 nm with a narrow particle size distribution could be achieved. The influence of formulation (triglycerides with different melting points, different triglyceride and emulsifier concentrations) and process parameters (e.g., pre-heating temperature in the process chamber, amount and size of grinding media) on the success of the process was also a point of interest as were potential effects of this unconventional manufacturing technique on the short-term stability in comparison to formulations manufactured by high-pressure homogenization.

2. Material and methods

2.1. Materials

The triglycerides trimyristin (Dynasan® 114, T_m = 56 °C), tripalmitin (Dynasan® 116, T_m = 66 °C) and tristearin (Dynasan® 118, T_m = 73 °C) from Hüls AG/Cremer Oleo (Witten, Germany) were kind gifts from the manufacturer. For the stabilization of the dispersions, poloxamer 188 (P188, Kolliphor® P188, BASF, Ludwigshafen, Germany; kind gift from manufacturer) was used. Sodium azide (Roth, Karlsruhe, Germany) was used as preservative. For all formulations bidistilled water was used.

The formulations were prepared in 2 mL DC-Twist-Top-vials from Andreas Hettich GmbH & Co KG (Tuttlingen, Germany; kind gift from the manufacturer). Yttrium stabilized zirconium dioxide beads were used in four different sizes (d_{GM} = 0.1–0.2 mm, 0.3–0.4 mm, 0.5–0.7 mm and 0.8–1.0 mm) from Sigmund Lindner GmbH (Warmensteinach, Germany). The beads had a spherical shape and a material density of ρ_{GM} = 6.05 kg dm^{-3}.

2.2. Sample preparation by dual centrifugation

The experiments were performed with the modified ZentriMix 380 R as shown in Fig. 1 (Andreas Hettich GmbH & Co KG, Tuttlingen, Germany). The installation of a heating coil at the bottom of the centrifuge enables operation of the dual centrifuge at temperatures above room temperature. Thus, a temperature range from T_P = −20 °C to T_P = 60 °C could be realized in the process chamber. The actual temperature in the

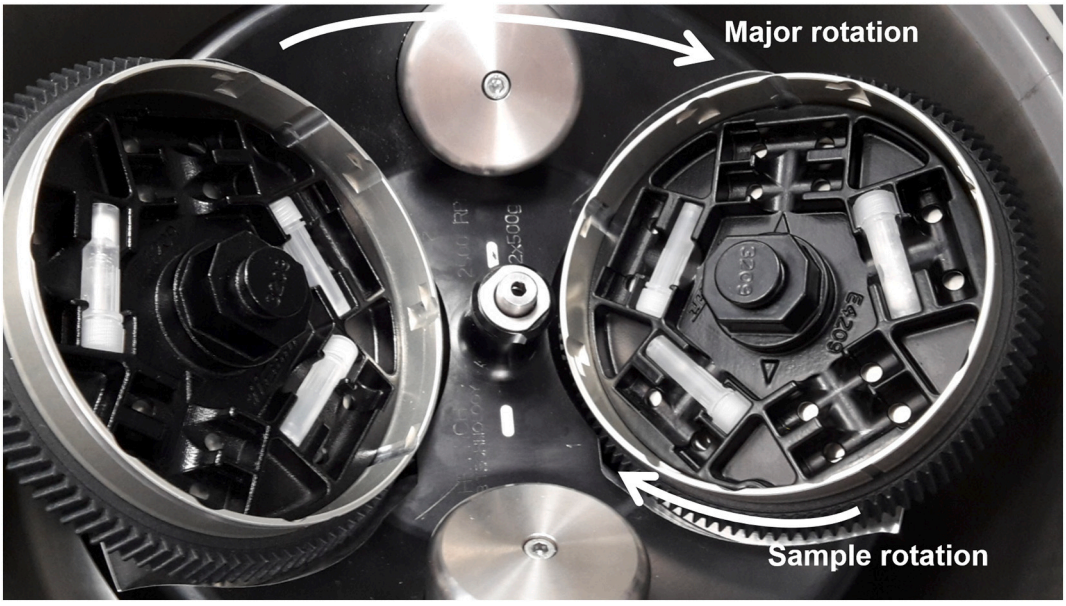

Fig. 1. Operation principle of the dual centrifuge ZentriMix 380 R: superimposition of the major rotation of the centrifuge and the rotation of the sample holder (speed ratio 3:1). In this set-up, ten 2 mL vials can be placed in each sample holder. An additional adapter can be positioned on top of the sample holders to enable simultaneous processing of up to 40 samples.

ZentriMix was measured with a thermometer located at the bottom of the centrifuge. In order to avoid a temperature gradient in the centrifuge during the process, the equipment was pre-heated at the requested temperature and rotor speed for 30 min before the samples were placed in the ZentriMix for emulsification. The process temperature was varied between $T_P = 48$ °C and 60 °C and the rotor speed was set between $v_{ZM} = 1700$ and 2350 rpm (revolutions per minute).

The investigated formulations contained a triglyceride concentration between $c_{lipid} = 5\%$ and 20% (all concentrations given in this study are *w*/w). The lipid droplets were stabilized with additives dispersed in the water phase. Unless stated otherwise, the stabilizer fraction in the aqueous phase referred to the lipid concentration in the formulation and was between $c_{add} = 0.1$ and 2.0. Sodium azide ($c_{preserv} = 0.0005$, referred to the total sample weight) was added as preservative to the formulations intended to be included in the short-term stability study.

Samples for dual centrifugation were usually prepared by weighing all components directly into the vials at room temperature. First, the 2 mL batch vials were filled with the grinding beads. The filling ratio of the grinding media, φ_{GM}, referred to the bulk density of the beads and to the volume of the used 2 mL vials. The filling ratio was varied between $\varphi_{GM} = 0.2$ and $\varphi_{GM} = 0.5$. On top of the beads, the unmolten lipid as well as a solution of the stabilization additive in water was given. The total amount of formulation in a vial was 1 g for all experiments. When investigations were performed using a pre-mix emulsion, trimyristin and the water phase (containing dissolved P188) were separately heated to 60 °C. Both liquids were combined and mixed for 2 min with 13,000 rpm (T25 digital ULTRA TURRAX®, IKA, Staufen, Germany). 1 g of the pre-mix emulsion with a temperature of approx. 60 °C was afterwards filled into the 2 mL batch vial.

The process time of the samples was varied between $t = 2.5$ and 15 min. After the emulsification process, the emulsions were cooled down to room temperature and characterized. When solid lipid nanoparticle dispersions were to be prepared, the dispersions were cooled at 5 °C for 120 min to ensure lipid crystallization. Afterwards, the formulations were stored at room temperature.

To record the temperature of the emulsions at the end of the process time in the dual centrifuge, the samples were directly measured after their preparation. For this purpose, a temperature sensor (PT 1000 in combination with RCT basic, IKA, Staufen, Germany) was inserted into the formulation and the temperature was read. It can be assumed that the temperature of the samples at the end of the process time was slightly higher than measured due to the short delay caused by the deceleration of the centrifuge and the removal of the vials from the sample holder.

2.3. Sample preparation by high-pressure homogenization

For comparison, a formulation containing 10% trimyristin, the emulsifier P188 ($c_{P188} = 1.2$, referred to the lipid content in the formulation) and sodium azide ($c_{preserv} = 0.0005$, referred to the total sample weight) as preservative was manufactured by high-pressure homogenization. First, a pre-mix emulsion was prepared by separately pre-heating the lipid phase as well as the emulsifier solution (P188 dissolved in water) to 65 °C and mixing them together for 4 min at 13,000 rpm (T25 digital ULTRA TURRAX®, IKA, Staufen, Germany). The pre-mix emulsion was high-pressure homogenized using a Microfluidizer M110-P (Microfluidics, Westwood/Massachusetts, USA) at 350 bar in 10 cycles. After homogenization, the preservative was added and the formulation was split in two parts: one part was cooled down to room temperature and stored as nanoemulsion, the other part was cooled in an ice bath for 30 min in order to crystallize the lipid droplets to achieve solid lipid nanoparticles. Both formulations were afterwards stored at room temperature.

2.4. Short-term stability study

A short-term stability study was performed over a time period of 4 weeks at 40 °C. 2 mL of the prepared emulsions or suspensions were filled in 5 mL glass vials which were closed with a plastic lid. The vials were stored at 40 °C in a Heratherm Incubator with a temperature stability of ±0.2 °C (Thermo Scientific, Waltham/Massachusetss, USA). Samples were taken after 2 and 4 weeks and further characterized.

2.5. Particle size analysis

Photon correlation spectroscopy (PCS) was employed to determine the intensity weighted mean diameter (z-average) and the polydispersity index (PdI) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom) at an angle of 173°. Three measurements of 60 s each were performed at 25 °C after an equilibration time of 120 s. The mean and the standard deviation of these three measurements were calculated for the z-average and the PdI. The particle size distribution of the emulsions and suspensions was measured with a laser light diffractometer (LD) with polarization intensity differential scattering technology (LS 13320, Beckman-Coulter, Krefeld, Germany). Each sample was measured three times for 90 s and the volume distribution was calculated according to the Mie theory-based evaluation model for the samples. Independent of the analytical method, all formulations were diluted with purified water before the measurement to achieve an appropriate particle concentration for the analysis. For the lipid nanoparticles a refractive index of 1.46 and an absorption index of 0.01 was assumed. The refractive index of the water was set to 1.33.

2.6. Differential scanning calorimetry

Melting events of the lipids were investigated with differential scanning calorimetry (DSC). The measurements were performed with a Mettler Toledo DSC 1 STARe system with FRS5 sensor (Mettler Toledo, Gießen, Germany). 18 µL of the formulation was weighed into 40 µL aluminium crucibles which were cold welded. During the measurement, the samples were heated from 25 °C to 85 °C with a heating rate of 5 K min^{-1}.

2.7. Viscosity measurement

The dynamic viscosity of the emulsifier solutions was measured with the rotational viscometer HAAKE™ RheoStress 6000 (Thermo Fischer Scientific, Waltham/Massachusetts, USA) using the double gap Searle measurement system DG41. The measurements were performed with 10 mL fluid at shear rates between 0.1 s^{-1} and 1000 s^{-1} and with a gap height of 5.1 mm. The formulations were measured at temperatures between 25 °C and 70 °C. All formulations displayed Newtonian flow properties for the applied shear rates, thus, the dynamic viscosity is given independently of the shear rates.

3. Results and discussion

3.1. Progress of the emulsification process using dual centrifugation

Dual centrifugation has already been used for the nanomilling of APIs, preparation of nanoemulsions or liposomes (Massing et al., 2008; Tenambergen et al., 2013; Hagedorn et al., 2019). While these studies could be performed at room temperature or below, the manufacturing of solid lipid nanoparticle dispersions takes place above the melting temperature of the respective lipid. In order to examine the course of emulsification in the modified dual centrifuge in general, a well-characterized trimyristin formulation (10% lipid and P188 $c_{P188} = 1.2$) was chosen (Göke et al., 2016). Trimyristin nanodispersions are known to form supercooled droplets. The emulsion droplets crystallize far below the crystallization temperature of their bulk material and thus,

liquid droplets remain in the formulation when it is cooled down to room temperature after preparation (Bunjes et al., 1996). Once the trimyristin droplets have been crystallized at temperatures below approx. 10 °C, the nanoparticles stay solid when they are heated up to room temperature. Thus, trimyristin nanoparticles can exist in a liquid or a solid state at room temperature.

The progress of the emulsification process in the dual centrifuge (preheated to 60 °C) was evaluated regarding the particle size distributions as well as PdIs of a corresponding pair of trimyristin emulsion and suspension. There was a clear decrease of the z-average and PdI over the process time (Fig. 2, left). The smallest particles, which were achieved after 10 min, had a size of 175 nm for the lipid emulsion and 193 nm for the lipid suspension with PdIs of 0.16 and 0.19, respectively. Besides the characterization of the particle sizes using PCS, the particle size distributions were determined by LD. A nearly monomodal particle size distribution could be confirmed for the lipid emulsion, exemplarily shown in Fig. 2 (right, bottom), as well as for the solid lipid nanoparticles after 10 min emulsification in the dual centrifuge. However, when shorter process times were chosen, a multimodal size distribution of the trimyristin emulsions (see Fig. 2, right, top) and suspensions was obtained. Small differences in the particle sizes as determined with PCS and LD are not uncommon because of the different physical principles the methods are based on. Although no significant improvement in particle fineness occurred when the formulations were processed for additional 2.5 min after an emulsification period of 7.5 min, clear differences in the PdIs as well as the particle size distributions were detected. After the additional 2.5 min the formulations were more homogeneous and their quality had increased. The PdIs had decreased from 0.23 to 0.16 for the emulsion and from 0.25 to 0.18 for the suspension. Although no completely monomodal distribution could be achieved after 10 min, all particles were in the submicron range. This manufacturing method seems to be suitable for, e.g., screening studies, when many formulations need to be prepared with limited material availability but a very narrow particle size distribution (PdI < 0.10) is not absolutely necessary. In this study, PdIs of 0.20 and below were aimed for.

The z-average values for the liquid lipid droplets were typically smaller than those of the solid lipid particles. While the shape of the lipid emulsion droplets is spherical the trimyristin particles crystallize in the β-polymorph and form platelet-like structures (Petersen et al., 2011) what results in a higher z-average when measured with PCS. The particle size difference between the lipid dispersion types was higher for shorter process times, when particles or droplets were larger. This is caused by the high volume of the bigger lipid droplets that form larger platelet-like structures. For better comparison the particle size of the lipid emulsions is discussed in the following unless stated otherwise.

The temperature profile of the formulation during the emulsification process indicates that after only 2.5 min a sample temperature of 65 °C could be reached. As a result of pre-heating of the process chamber (T_P = 60 °C) and the intensive stressing of the formulation by the grinding beads, the sample is heated up from room temperature at $t = 0$ min to the melting temperature of the trimyristin within a very short time period. With longer process times the sample temperature further increased, caused by the stress induced in the vials by the grinding media. After a process time of 10 min a sample temperature of approx. 90 °C was measured. However, this led to a strong pressure increase in the vials and emulsification was no longer possible because at higher temperatures the polymer vials started to burst. In this study, the experiments were terminated when sample temperatures of 90 °C or above were reached.

To verify that all the solid lipid in the vial could be melted during processing with the dual centrifuge the trimyristin emulsion was heated in the DSC and the results were compared to the melting process of a trimyristin suspension. A clear melting event with a specific melting enthalpy of 16.4 J/g was detected for the suspension with a maximum heat flow at 53.8 °C (see Fig. S1, supporting information). Integrating the DSC curve of the emulsion over the same temperature section as for the suspension, a barely visible heat flow could be measured (melting enthalpy of 0.3 J/g). This is probably due to the not fully monomodal particle size distribution of the emulsion after a processing time of 10 min (Fig. 2, right, bottom). Larger particles have a less pronounced supercooling tendency and may recrystallize at room temperature thus being detectable as melting event in the DSC heating run. Nevertheless, it is highly unlikely that any solid lipid remains in the vial during processing in the dual centrifuge as a result of the high energy input and high temperatures as well as intensive mixing during the process.

Tests to evaluate the reproducibility of the experiments were performed with the same formulation and process parameters as given in Fig. 2 (T_P = 60 °C, d_{GM} = 0.5–0.7 mm, φ_{GM} = 0.3 and v_{ZM} = 2350 min^{-1}). The experiments were reproduced independently five times using a process time of 10 min. All particle sizes achieved for the trimyristin emulsions in these experiments were between 170 nm and 178 nm (see Fig. S2, supporting information) leading to an average particle size of 175 nm with a standard deviation of 3 nm. With PdIs between

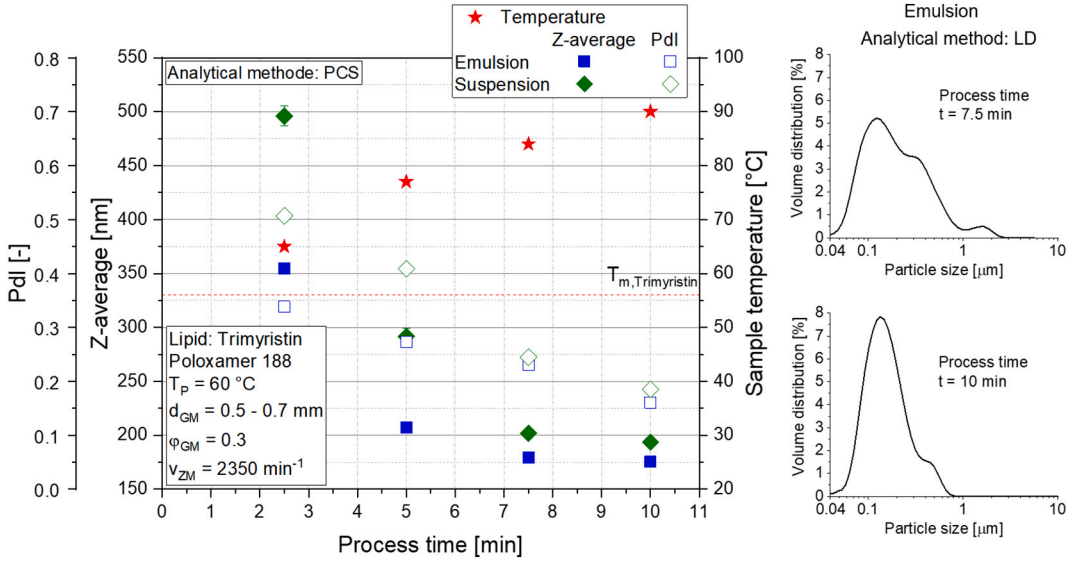

Fig. 2. Progress of the emulsification process, indicated by the particle sizes, PdI values and sample temperatures of a 10% trimyristin (c_{P188} = 1.2) emulsion and suspension (left) and particle size distributions of emulsions for t = 7.5 min and t = 10 min (right).

0.15 and 0.17, the size distributions of all emulsions were below the set quality level of 0.20. Similar experiments with the same lipid formulation but with larger grinding media (d_{GM} = 0.8–1.0 mm) confirmed the good reproducibility of the experiments with an average particle size of 174 nm (standard deviation 3 nm) and PdIs between 0.18 and 0.19 after a processing time of 10 min. Based on these results and if not stated otherwise, all following experiments were performed as $n = 1$.

One of the advantages of manufacturing solid lipid nanoparticles with the dual centrifuge is that the samples can be simply prepared for processing at room temperature. Each component can be directly weighed into the vial, while for high-pressure homogenization a pre-mix emulsion is usually needed. To confirm that the preparation process does not significantly influence the results of emulsification with the dual centrifuge, a pre-mix emulsion with a mean particle size of approx. 20 µm was prepared for comparison. The pre-mix emulsion was filled in the vial with a sample temperature of 60 °C and further emulsified in the dual centrifuge. The resulting particle sizes and sample temperatures of the emulsions were compared to the formulation weighed in as physical mixture at room temperature.

The largest difference in particle size was obtained for the shortest process time (t = 2.5 min; Fig. 3). While for the physical mixture the lipid had to melt in the vial during the process, in the pre-mix emulsion the lipid was already molten and pre-dispersed that further homogenization could start right from the beginning, resulting in lower particle sizes and higher sample temperatures. Process times of t = 5 min or longer yielded only slight differences between the particle sizes and sample temperatures of the emulsions. After 10 min, both formulations reached the termination temperature of 90 °C. By preparing a pre-mix emulsion prior the emulsification process an emulsion with 20 nm smaller droplets and a PdI of 0.15 instead of 0.16 was achieved. This demonstrates that especially for formulation screenings, the time-saving use of physical mixtures which are weighed in the vial at room temperature does not lead to significant disadvantages regarding the resulting particle sizes or size distributions compared to the pre-mix emulsion.

3.2. *Influence of manufacturing process on product characteristics*

It was shown above that the preparation of solid lipid nanoparticles as well as nanoemulsions from trimyristin with a melting temperature of T_m = 56 °C is possible by using dual centrifugation. Nevertheless, this manufacturing process differs significantly from established methods such as high-pressure homogenization. In dual centrifugation, the formulation is continuously stressed by the grinding media during the entire process time; the temperature of the formulation rises steadily and reaches 90 °C and above within 10 min. During high-pressure homogenization, a reduction in droplet sizes is achieved by forcing the formulation through a narrow gap at high pressures. This process is repeated until the final droplet fineness is attained. The stress on the formulation is non-permanent with this method and due to the relaxation phases of the emulsion between the single passages, the temperature of the emulsions remains almost constant during the entire process, in particular, when thermostatic measures are taken in between cycles. It can, however, be assumed that the mechanical stressing of the formulation in the process chamber leads to a short-lasting increase in its temperature.

A short-term stability study of the trimyristin emulsions and suspensions was performed in order to uncover possible degradation effects of the components in the formulations caused by the high stress intensities and sample temperatures above 90 °C during processing with the dual centrifuge. Such effects might have an influence on the stabilization against agglomeration or coalescence of the dispersions. Therefore, particle size distributions of the trimyristin emulsions and suspensions manufactured with both methods were analyzed after storage of 2 and 4 weeks at 40 °C. Due to the different manufacturing processes, the particle sizes as well as the PdIs of the starting emulsion and suspension prepared by high-pressure homogenization were smaller than those of the dispersions manufactured by dual centrifugation.

Over storage time, the particle sizes (z-average, see Table S1 of supporting information) did not exhibit noticeable changes, neither for the emulsions nor the suspensions independent of the manufacturing process. Only a marginal change in the PdIs was detected for the emulsions and suspensions manufactured by dual centrifugation. Thus, it can be concluded that for the formulations under investigation the manufacturing process did not significantly influence the evolution of the z-average as well as the PdI values of the dispersions over storage time.

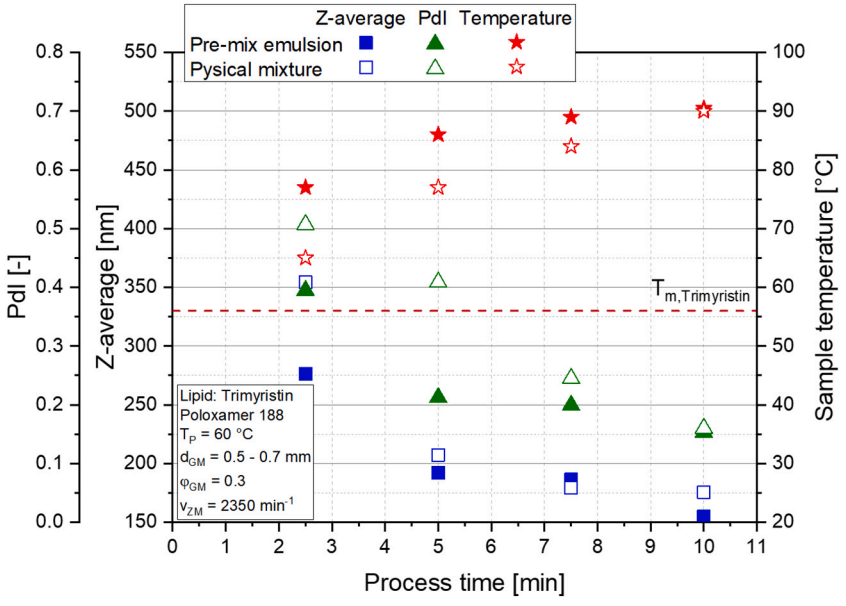

Fig. 3. Influence of the sample preparation on the particle size and the sample temperature: comparing emulsification progress of a pre-mix emulsion with a start temperature of 60 °C (particle size 20 µm) to a formulation prepared as physical mixture, where components were weighed in at room temperature.

3.3. Influence of the process parameters on the lipid formulations

This section deals with the influence of several process parameters (grinding media filling ratio, grinding media size, process temperature) on the resulting particle sizes as well as sample temperatures. The investigations were performed with a 10% trimyristin formulation stabilized with P188, $c_{P188} = 1.2$. Changes in the filling ratio of the grinding media influence the number of grinding beads in the vial and thus the number of stress events on the lipid droplets. The experiments with a variation of the bead filling ratio ($\varphi_{GM} = 0.2$ to $\varphi_{GM} = 0.5$) indicate that the influence of the amount of beads in the vial on the droplet fineness is quite low for process times of 7.5 min and longer (Fig. 4, left). Noticeable differences were only observed for the formulations processed with the highest filling ratio $\varphi_{GM} = 0.5$ at process times of 5 min and shorter. This phenomenon can be attributed to the restricted mobility of the grinding media in the vial and the resulting shorter path length of the beads in the vial during emulsification. With longer process times, this effect does not seem to negatively influence the particle sizes of the emulsion.

The sample temperatures showed a higher dependence on the number of grinding beads in the vials. While after 10 min the temperatures of the samples processed with $\varphi_{GM} = 0.4$ and 0.5 were clearly higher than 90 °C, they decreased to 90 °C for $\varphi_{GM} = 0.3$ and 87 °C for $\varphi_{GM} = 0.2$. This is caused by the lower number of stress events resulting in less heat production during the process. Another characteristic that is influenced by the total number of stress events is the PdI. The lowest PdI, 0.10, was achieved for the formulation manufactured with the highest filling degree with grinding beads $\varphi_{GM} = 0.5$. With decreasing grinding media filling ratio, the PdI increased to a value of 0.24 for $\varphi_{GM} = 0.2$ indicating a distinctly broader particle size distribution for this emulsion. Due to the fact that the termination criterium (sample temperature $\geq$ 90 °C) was not reached for the formulation $\varphi_{GM} = 0.2$ within 10 min, the process time was extended to 15 min. Although the droplets did not become smaller within the additional 5 min, the PdI could be slightly reduced to 0.23 until the termination temperature of 90 °C was also reached for this formulation after 15 min.

The grinding media size is also supposed to influence the fineness of the emulsion. Regarding the emulsification behavior of the trimyristin formulation with four different bead sizes between 0.1 and 0.2 mm and 0.8–1.0 mm (Fig. 4, right) at a constant filling ratio ($\varphi_{GM} = 0.3$), a faster emulsification progress could be achieved in 5 min with the two largest bead fractions. Regarding the emulsification kinetics for the various bead sizes, former studies indicated that for a constant relative velocity, higher shear rates can be expected in a fluid with larger beads (Schmidt et al., 2013; Brenner, 1961). Due to the high shear rates induced in the formulation with the largest beads ($d_{GM} = 0.8$–1.0 mm) droplet sizes of 275 nm were achieved within only 2.5 min. With increasing process time, the differences in the droplet fineness obtained with the different bead sizes decreased. After a process time of 10 min the same sizes of approx. 170 nm were observed for all samples independent of the applied grinding media. The PdIs of the emulsions, as an indication for the width of the size distribution, differed, however, for these lipid emulsions. The narrowest distribution (PdI of 0.11) was obtained for the formulation prepared with the smallest beads $d_{GM} = 0.1$–0.2 mm. The PdI steadily increased with the size of the beads reaching a value of 0.19 for $d_{GM} = 0.8$–1.0 mm.

Similar results regarding the droplet sizes as well as the width of the size distributions were observed for the emulsification of dodecane in water with a stirred media mill and grinding beads sizes of 100 µm and 400 µm, respectively (Schmidt et al., 2013). The smallest dodecane droplets were obtained with bead sizes of 100 µm for process times of 2 h and more. The authors concluded that beyond a certain point the high stress intensity induced by the larger beads increase the coalescence tendency of the droplets which superimposes the droplet breakup. Also for the present study it may be assumed that increasing coalescence of the droplets is the cause of decreasing emulsification progress for the formulations processed with the larger grinding media. However, the increased number of stress events, caused by the higher number of grinding beads for, e.g., $d_{GM} = 0.1$–0.2 mm at a constant filling ratio, should not be neglected and might also result in higher droplet finenesses for process times longer than 10 min. Due to the increase in sample temperature to above 90 °C (independent of bead size) such experiments could, however, not be performed. In order to finally identify the mechanism that causes this phenomenon further experiments with an adjusted process set-up will have to be performed. Oils, for example, could be used for these experiments because they can be processed at room temperature what results in lower sample temperatures (see Fig. 5) and enables longer process times.

The influence of the temperature in the chamber of the centrifuge on the particle fineness and the sample temperature after a process time of 10 min was evaluated for temperatures between $T_P = 48$ °C and 60 °C (Fig. 5). Temperatures of 55 °C and above did not seem to have a significant influence on the z-average of the formulations, but the lowest PdI was achieved for the highest process temperature, $T_P = 60$ °C. When the process temperature in the centrifuge was decreased below 55 °C, both, the particle sizes and PdIs increased steadily. A closer look at the temperature profile of the formulation processed at the lowest temperature $T_P = 48$ °C revealed that the increase in temperature was much slower for this emulsion than for the formulation processed at $T_P = 60$ °C (data not shown). However, the investigations showed that the sample temperatures of all formulations were above the melting temperature of the lipid after $t = 2.5$ min. Overall, a temperature difference of 15 °C was

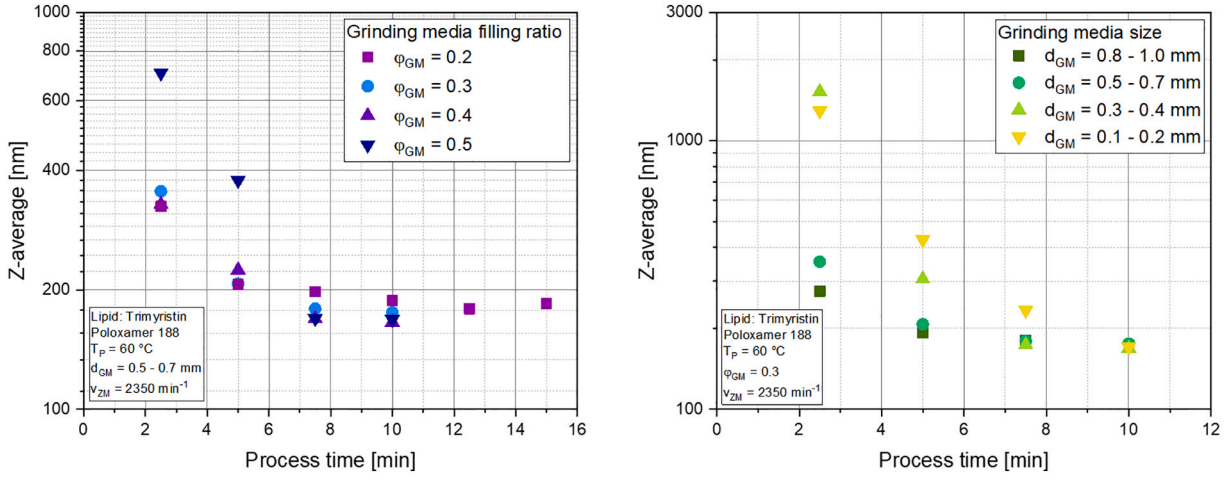

Fig. 4. Influence of the grinding media filling ratio (left) and the grinding media size (right) on the particle fineness of trimyristin emulsions.

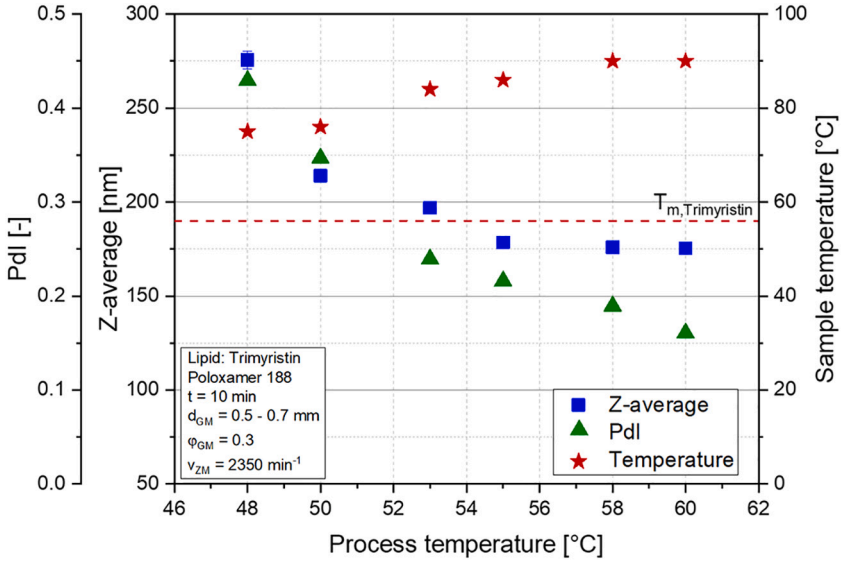

Fig. 5. Dependence of particle size, PdI and sample temperature of trimyristin emulsions (processed for 10 min) on the process temperature of the dual centrifuge.

determined between emulsions prepared at the lowest and highest process temperatures after 10 min what approximately reflects the difference in the pre-heating temperature of the centrifuge. The positive effect of higher process temperatures on the droplet sizes is assumed to be caused by the temperature dependent decrease of the trimyristin viscosity (Seekkuarachchi et al., 2006; Gupta et al., 2016), potentially in combination with an effect on the viscosity of the continuous phase (see below).

3.4. *Influence of formulation parameters on the characteristics of the dispersions*

Besides the process parameters, the formulation has a significant influence on the resulting lipid nanodispersions. In this study, the influence of the emulsifier concentration and the lipid content in the emulsion was investigated. Moreover, two additional lipids, tripalmitin (T_m = 66 °C) and tristearin (T_m = 73 °C) were processed in order to investigate if an efficient emulsification could also be performed for lipids with higher melting temperatures.

3.4.1. *Emulsifier concentration*

All investigations presented above were performed with a P188 content c_{P188} = 1.2 (referring to the lipid content), which is synonymous to a total emulsifier content of c_{tP188} = 0.12 in the formulation. In order to study the effect of the emulsifier concentration, the total amount of P188 in trimyristin emulsions was varied between c_{tP188} = 0.05 and c_{tP188} = 0.20. Based on the results of former studies, it was assumed that even with the lowest emulsifier concentration of c_{tP188} = 0.05 stabilization of the formulation against agglomeration and coalescence is possible (Göke et al., 2018).

The results obtained by dual centrifugation revealed a clear dependence of the droplet size distribution on the emulsifier content (Fig. 6). An increased emulsifier content resulted in a pronounced decrease in particle size and narrowing of the particle size distribution. The size distribution of the emulsion droplets changed from rather heterogeneous (PdI 0.45) with a large z-average diameter of 293 nm for c_{P188} = 0.05 to a clearly monomodal distribution with a mean droplet size of 117 nm (PdI 0.11) for c_{tP188} = 0.20.

It appears unlikely that this large effect is caused by an influence of emulsifier concentration on the interfacial tension. The critical micellization temperature (CMT) of a 5.0% (*w/v*) aqueous P188 solution has

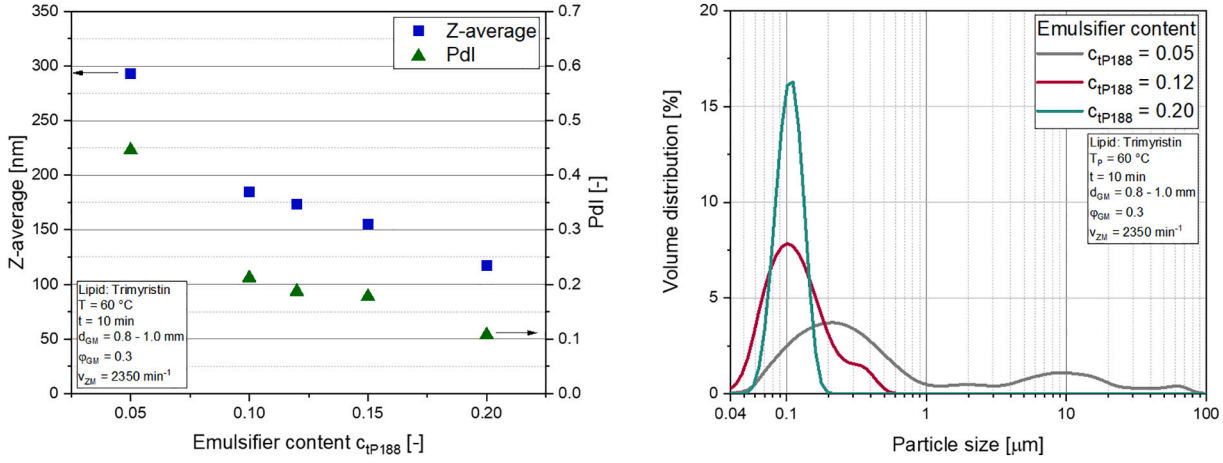

Fig. 6. Influence of emulsifier content c_{tP188} on the particle size and PdI of the trimyristin emulsions (c_{lipid} = 0.10; left), and the particle size distribution for selected formulations (c_{tP188} = 0.05, 0.12 and 0.20; right). Emulsions were processed at 60 °C for *t* = 10 min with v_{ZM} = 2350 $min^{-1}$and grinding media d_{GM} = 0.8–1.0 mm with a filling ratio φ_{GM} = 0.3.

been reported as 40 °C decreasing with increasing P188 concentration (Alexandridis et al., 1994). Under the conditions employed during emulsification, the emulsifier concentration should thus be above the critical micelle concentration (CMC) for all formulations.

Instead, an effect of viscosity is assumed to be the major cause for the alterations in droplet size distribution. During the emulsification experiments a clear influence of the different P188 concentrations on the viscosity of the continuous phase of the emulsion was observed with higher emulsifier concentrations leading to higher viscosities.

Different groups have worked on the prediction of droplet sizes that can be achieved during emulsification. While in 1934 Taylor analyzed the deformation of single droplets in macroemulsions in a laminar flow field (Taylor, 1934), Hinze followed in 1955 and described droplet formation under turbulent flow conditions (Hinze, 1955). In order to predict the droplet sizes of nanoemulsions manufactured e.g. by high-pressure homogenization Gupta et al. adjusted the model of Hinze in 2016 (Gupta et al., 2016). Independent of the flow conditions and the size of the droplets, all models indicated that the viscosity or the density of the continuous phase have a significant influence on the resulting fineness of the emulsion. In principle, the droplet size is inversely proportional to the viscosity or to the density of the continuous phase. The validity of these models has been confirmed by different groups in recent years for oil-in-water nanoemulsions prepared by high-pressure homogenization. With an increase in the viscosity of the continuous phase and thus, with a higher disruptive shear stress in the formulation, smaller droplet sizes were achieved (Seekkuarachchi et al., 2006; Wooster et al., 2008; Qian and McClements, 2011; Finke et al., 2014).

For the emulsifier solutions used in the present study, the viscosity varied with P188 concentration and temperature, as shown in Fig. 7. The lowest viscosity was measured for the solution $c_{tP188} = 0.05$ for which the viscosity continuously decreased with increasing temperature. This is presumably due to the decreasing viscosity of water at higher temperatures (Korson et al., 1969). The viscosities of the formulations with higher P188 concentrations, $c_{tP188} = 0.12–0.24$, passed through a minimum at approx. 30–50 °C and increased again at higher temperatures. It is assumed that this strong temperature dependence and nonlinear effect is caused by two opposing phenomena. On the one hand, the viscosity of water and simple aqueous solutions decreases with increasing temperature. On the other hand, P188 in the formulations starts to form micelles above a certain temperature in a concentration dependent manner as outlined above. An increasing number of micelles in the formulation with higher temperatures results in a so called "thermoreversible gelation" and leads to higher solution viscosities (Alexandridis et al., 1994).

3.4.2. Lipid concentration

In order to evaluate a potential influence of the lipid concentration on the emulsification results, the trimyristin content was varied between $c_{lipid} = 0.05$ and 0.20. To ensure sufficient stabilization of the lipid droplets for all formulations, the P188 content was kept constant at $c_{P188} = 1.2$ (referred to the lipid concentration). This results in different total emulsifier concentrations in the formulations and thus, viscosities of the continuous phases (see Fig. 7). To enable a better comparison of the resulting particle sizes and PdIs for the different emulsions, the total P188 concentration (c_{tP188}) in the formulation is additionally given in Fig. 8 for the various lipid concentrations. Two different grinding media sizes $d_{GM} = 0.5–0.7$ mm and $d_{GM} = 0.8–1.0$ mm were used in these experiments and all samples were emulsified for 10 min.

In general, smaller droplets as well as lower PdIs were achieved for formulations with a higher lipid content (Fig. 8). Up to a lipid concentration of 15% there were no significant differences in the droplet sizes for formulations processed with the different grinding media sizes $d_{GM} = 0.5–0.7$ mm or $d_{GM} = 0.8–1.0$ mm indicating that the stress intensities induced by the grinding media were high enough in these samples to enable a droplet breakup in the emulsions independent of the formulation viscosities. The noticeable decrease of the particle sizes for a lipid content of 15% compared to 10% is assumed to be caused by the nonlinear increase of the viscosity of the continuous phase from 6.3 mPas ($c_{lipid} = 0.10$) to 16.5 mPas ($c_{lipid} = 0.15$) ($T = 70$ °C; see Fig. 7).

Comparing the emulsion with a lipid concentration of 5% and a total P188 content $c_{tP188} = 0.06$ with the formulation obtained with a slightly lower total P188 concentration ($c_{tP188} = 0.05$) but twice the amount of the lipid ($c_{lipid} = 0.10$, see Fig. 6), a higher fineness is achieved when less lipid is in the formulation. This indicates that a higher number of stress events referred to the total number of droplets in the emulsion positively influences the resulting sizes when the filling ratio of the grinding beads

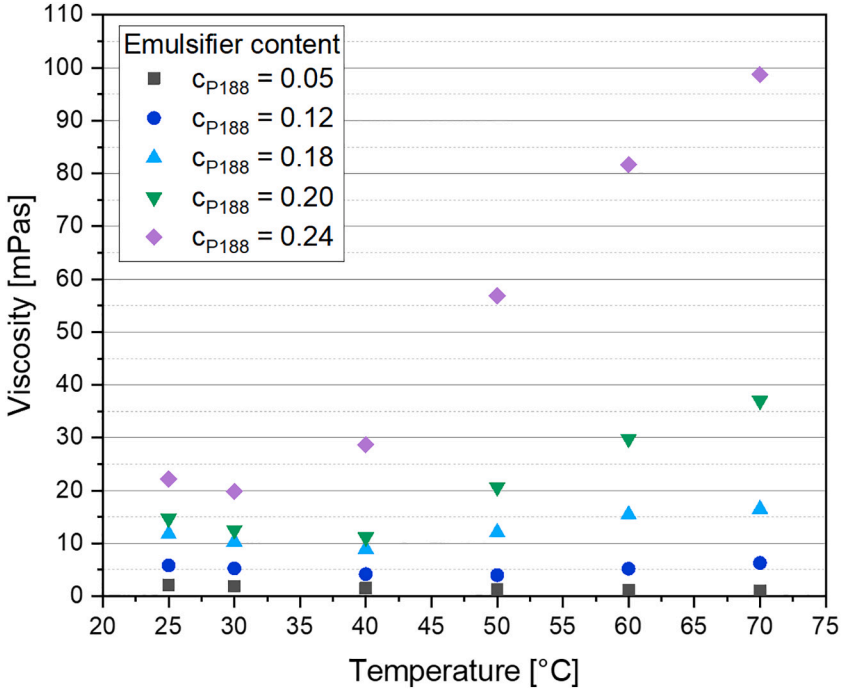

Fig. 7. Viscosity of the aqueous emulsifier solution at different P188 contents ($c_{tP188} = 0.05–0.24$) and temperatures.

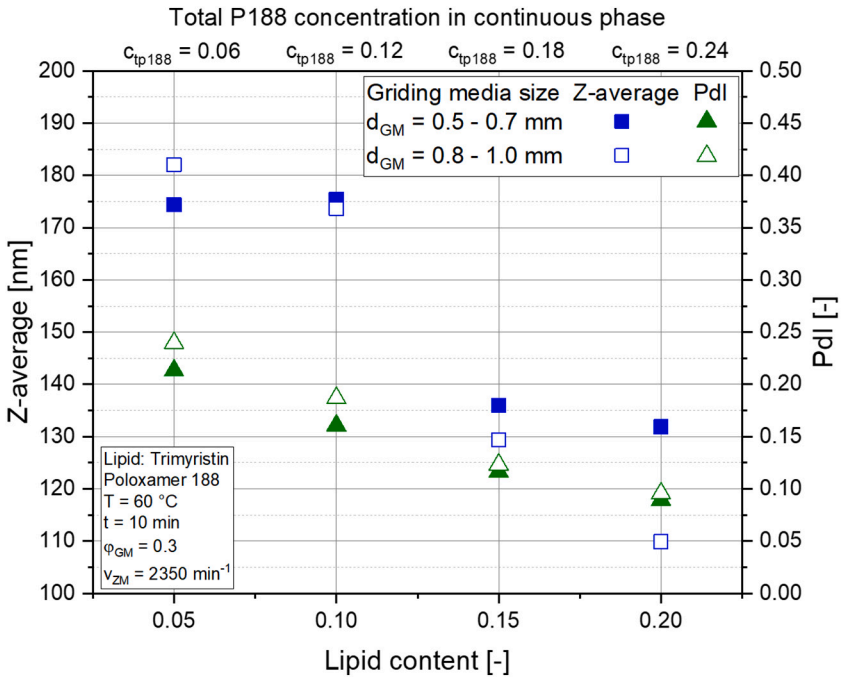

Fig. 8. Particle sizes and PdIs after a process time of $t = 10$ min for emulsions with different lipid (trimyristin) concentrations processed with two different grinding media sizes (0.5–0.7 mm and 0.8–1.0 mm).

was kept constant in these studies.

A difference in the droplet size of the formulations processed with different bead sizes occurred when the lipid concentration was 20%. With a total P188 content of 0.24, the viscosity of the continuous phase increased significantly due to the linear behavior at higher formulation temperatures (shown in Fig. 7). This resulted in only slightly smaller droplet sizes for the emulsion prepared with grinding media between d_{GM} = 0.5–0.7 mm compared to a lipid concentration of 15% in the emulsion. But when the larger grinding beads (d_{GM} = 0.8–1.0 mm) were used for the emulsification, a further decrease in the droplet size was obtained. Due to the higher shear stresses induced in the emulsion by the larger grinding media the influence of the sample viscosity on the droplet breakup was smaller and resulted in a higher droplet fineness.

The PdI, reflecting the width of the particle size distribution, seemed to be rather independent of the grinding media size and thus, the induced stress intensities. With PdIs of less than 0.10 for the emulsions with 20% trimyristin a very narrow size distribution could be achieved for these formulations with the dual centrifuge.

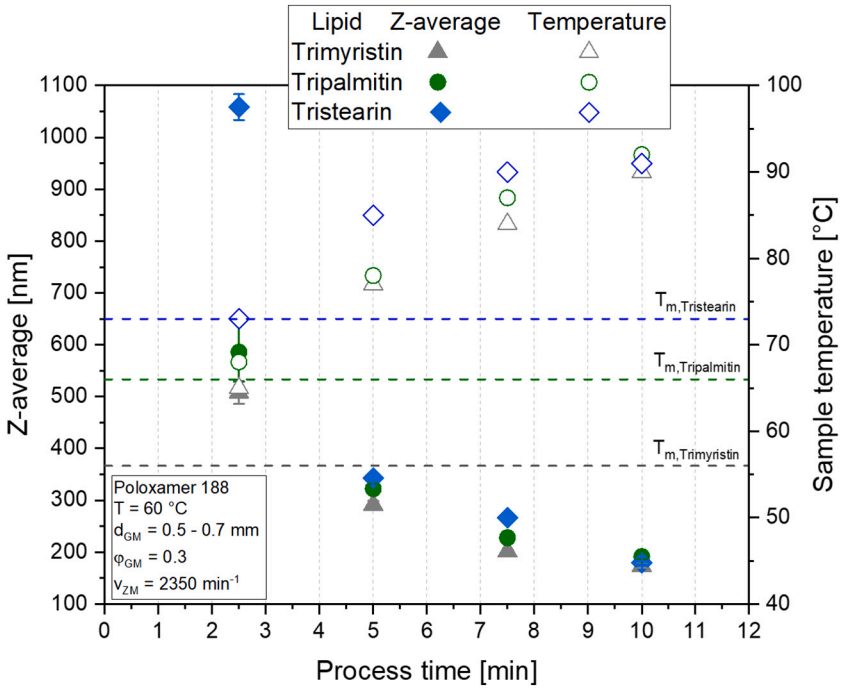

Fig. 9. Particle size characteristics of solid lipid nanoparticles prepared from three different triglycerides (trimyristin, tripalmitin and tristearin) with melting temperatures between 56 °C and 73 °C.

3.4.3. Type of lipid

The experiments described above for trimyristin dispersions indicated that sample temperatures of up to 90 °C were achieved within a process time of 10 min after pre-heating the centrifuge to $T_P = 60$ °C. Thus, it was investigated if the higher-melting triglycerides tripalmitin, $T_m = 66$ °C, and tristearin, $T_m = 73$ °C, could also be processed with the dual centrifuge under these conditions. Tripalmitin and tristearin nanoparticles do not form supercooled melts at room temperature (Bunjes et al., 1996); to achieve comparable conditions all emulsions were cooled to 5 °C after emulsification and the sizes of the resulting solid lipid nanoparticles were determined.

Within the first 2.5 min of processing, all formulations reached temperatures between 65 °C and 73 °C (Fig. 9). The melting temperatures of trimyristin and tripalmitin were already exceeded at this point and droplet sizes of less than 600 nm were achieved in these samples. The higher melting temperature of tristearin ($T_m = 73$ °C), had probably not been reached after the first 2.5 min giving little opportunity for effective emulsification thus leading to particle sizes larger than 1 µm. With longer process times, the melting temperatures of all lipids could be clearly exceeded in the samples and after a process time of 10 min all PdIs were between 0.14 and 0.18 and thus below the set quality criteria of 0.20. After a process time of 10 min all formulations reached the termination temperature of 90 °C or above and the emulsification was stopped. Even though it is assumed that there are small differences in the viscosity of the molten lipids, these did not strongly influence the emulsification progress as long as the process time was 10 min.

4. Conclusion

With the modified dual centrifuge ZentriMix 380 R it was possible to manufacture solid lipid nanoparticles with sizes below 200 nm from different triglycerides (melting temperatures between 56 °C and 73 °C). Prior to centrifugation, the matrix lipids of the particles as well as the emulsifier solution could simply be filled in the 2 mL vials at room temperature while no pre-mix emulsion or pre-heating of the lipid was necessary. Neither the size of the grinding beads nor their filling ratio significantly influenced the size of the resulting trimyristin droplets as long as the process times were 7.5 min or longer. Nevertheless, a positive influence of higher process temperatures on the particle sizes and the width of the size distributions was detected. The experiments indicated that – apart from the process time – the composition of the formulation and in particular the emulsifier content which significantly influenced the viscosity of the continuous phase had the strongest influence on the resulting droplet sizes. Thus, with a lipid concentration of 20% (trimyristin) and a total emulsifier concentration of 24% (P188) in the formulation droplets with a z-average of 110 nm and a PdI 0.09 could be achieved.

This study illustrated that dual centrifugation is an appropriate method to manufacture solid lipid nanoparticles at a small batch scale within a few minutes. Up to 40 samples can be processed in parallel and thus, the manufacturing method is highly useful for, e.g., screening studies at an early stage in the development process when the amount of available API is very limited.

Declaration of Competing Interest

None.

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References

Alexandridis, P., Holzwarth, J.F., Hatton, T.A., 1994. Micellization of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers in aqueous solutions: thermodynamics of copolymer association. Macromolecules 27, 2414–2425.
Bergström, C.A.S., Charman, W.N., Porter, C.J.H., 2016. Computational prediction of formulation strategies for beyond-rule-of-5 compounds. Adv. Drug Deliv. Rev. 101, 6–21.
Berton-Carabin, C.C., Coupland, J.N., Elias, R.J., 2013. Effect of the lipophilicity of model ingredients on their location and reactivity in emulsions and solid lipid nanoparticles. Coll. Surf. A Physicochem. Eng. Asp. 431, 9–17.
Brenner, H., 1961. The slow motion of a sphere through a viscous fluid towards a plane surface. Chem. Eng. Sci. 16, 242–251.
Bunjes, H., 2010. Lipid nanoparticles for the delivery of poorly water-soluble drugs. J. Pharm. Pharmacol. 62, 1637–1645.
Bunjes, H., Westesen, K., Koch, M.H.J., 1996. Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. Int. J. Pharm. 129, 159–173.
Driscoll, D.F., 2006. Lipid injectable emulsions: pharmacopeial and safety issues. Pharm. Res. 23, 1959–1969.
Finke, J.H., Niemann, S., Richter, C., Gothsch, T., Kwade, A., Büttgenbach, S., Müller-Goymann, C.C., 2014. Multiple orifices in customized microsystem high-pressure emulsification: the impact of design and counter pressure on homogenization efficiency. Chem. Eng. J. 248, 107–121.
Göke, K., Roese, E., Arnold, A., Kuntsche, J., Bunjes, H., 2016. Control over particle size distribution by autoclaving poloxamer-stabilized trimyristin nanodispersions. Mol. Pharm. 13, 3187–3195.
Göke, K., Roese, E., Bunjes, H., 2018. Heat treatment of poloxamer-stabilized triglyceride nanodispersions: effects and underlying mechanism. Mol. Pharm. 15, 3111–3120.
Gupta, A., Eral, H.B., Hatton, T.A., Doyle, P.S., 2016. Controlling and predicting droplet size of nanoemulsions: scaling relations with experimental validation. Royal Soc. Chem. 12, 1452–1458.
Hagedorn, M., Bögershausen, A., Rischer, M., Schubert, R., Massing, U., 2017. Dual centrifugation - a new technique for nanomilling of poorly soluble drugs and formulation screening by an DoE-approach. Int. J. Pharm. 530, 79–88.
Hagedorn, M., Liebich, L., Bögershausen, A., Massing, U., Hoffmann, S., Mende, S., Rischer, M., 2019. Rapid development of API nano-formulations from screening to production combining dual centrifugation and wet agitator bead milling. Int. J. Pharm. 565, 187–198.
Hinze, J.O., 1955. Fundamentals of the hydrodynamic mechanism of splitting in dispersion processes. AICHE J. 1, 289–295.
Hörmann, K., Zimmer, A., 2016. Drug delivery and drug targeting with parenteral lipid nanoemulsions - a review. J. Control. Release 223, 85–98.
Illing, A., Unruh, T., 2004. Investigation on the flow behavior of dispersions of solid triglyceride nanoparticles. Int. J. Pharm. 284, 123–131.
Jores, K., Mehnert, W., Drechsler, M., Bunjes, H., Johann, C., Mäder, K., 2004. Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J. Control. Release 95, 217–227.
Korson, L., Drost-Hansen, W., Millero, F.J., 1969. Viscosity of water at various temperatures. J. Phys. Chem. 73, 34–39.
Kupetz, E., Bunjes, H., 2014. Lipid nanoparticles: drug localization is substance-specific and achievable load depends on the size and physical state of the particles. J. Control. Release 189, 54–64.
Lesson, P.D., 2016. Molecular inflation, attrition and the rule of five. Adv. Drug Deliv. Rev. 101, 22–33.
Lim, S.B., Banerjee, A., Önyüksel, H., 2012. Improvement of drug safety by the use of lipid-based nanocarriers. J. Control. Release 163, 34–45.
Lipp, R., 2013. The innovator pipeline: bioavailability challenges and advanced oral drug delivery opportunities. Am. Pharm. Rev. 16, 10–16.
Massing, U., Cicko, S., Ziroli, V., 2008. Dual asymmetric centrifugation (DAC) - a new technique for liposome preparation. J. Control. Release 125, 16–24.
Mehnert, W., Mäder, K., 2012. Solid lipid nanoparticles. Prod. Characteriz. Appl. Adv. Drug Deliv. Rev. 64, 83–101.
Meier, S., Pütz, G., Massing, U., Hagemeyer, C.E., von Elverfeldt, D., Meißner, M., Ardipradja, K., Barnert, S., Peter, K., Bode, C., Schubert, R., von Zur Muhlen, C., 2015. Immuno-magnetoliposomes targeting activated platelets as a potentially human-compatible MRI contrast agent for targeting atherothrombosis. Biomaterials 53, 137–148.
Petersen, S., Steiniger, F., Fischer, D., Fahr, A., Bunjes, H., 2011. The physical state of lipid nanoparticles influences their effect of in vitro cell viability. Eur. J. Pharm. Biopharm. 79, 150–161.
Qian, C., McClements, D.J., 2011. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size. Food Hydrocoll. 25, 1000–1008.
Schmidt, J., Damm, C., Romeis, S., Peukert, W., 2013. Formation of nanoemulsions in stirred media mills. Chem. Eng. Sci. 102, 300–308.
Seekkuarachchi, I.N., Tanaka, K., Kumazawa, H., 2006. Formation and characterization of submicron oil-in-water (O/W) emulsions, using high-energy emulsification. Ind. Eng. Chem. Res. 45, 372–390.

Taylor, G.I., 1934. The formation of emulsions in definable fields of flow. Proc. R. Soc. A Math. Phys. Eng. Sci. 146, 501–523.
Tenambergen, F., Maruiama, C.H., Mäder, K., 2013. Dual asymmetric centrifugation as an alternative preparation method for parenteral fat emulsions in preformulation development. Int. J. Pharm. 447, 31–37.
Wooster, T.J., Golding, M., Sanguansri, P., 2008. Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir 24, 12758–12765.