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Autoclaving behavior of trimyristin nanoemulsions stabilized with different poloxamers

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Abstract

Lipid nanoemulsions are being investigated as carrier systems for the parenteral administration of poorly soluble drugs. Defined particle sizes, narrow particle size distributions, and sterility are prerequisites for the safe administration of such formulations to patients. In the current study, autoclaving of such formulations was performed to achieve both, sterility and a narrow particle size distribution. The high temperatures applied might, however, have an impact on emulsion stability. The influence of various types of poloxamer (Pol) on the formation of trimyristin (TM) nanoemulsions with around 100 nm (± 2 nm) mean particle size, as well as on the physical stability, particle size and particle size distribution changes during autoclaving of the emulsions was investigated. Higher homogenization pressures were required to achieve the target particle size in emulsion formulations stabilized with poloxamers of larger molecular size. A correlation between the autoclavability of the formulations and the cloud point of the respective poloxamer used for emulsion stabilization was observed. A PEO content of 70 % or above within the poloxamer molecule was needed to achieve stable nanoemulsions after autoclaving. In stable emulsions, Ostwald ripening occurred during autoclaving, indicated by particle size growth and narrowing of the particle size distribution, which was accompanied by changes in the melting behavior of the recrystallized emulsion droplets. Autoclaving of TM nanoemulsions stabilized with Pol 108, 188, 237, 238, 338 and 407 yielded systems with particularly well-defined particle sizes and narrow particle size distributions.

Keywords: lipid nanoemulsion, trimyristin, poloxamer, autoclaving, particle size, differential scanning calorimetry

1. Introduction

The development of colloidal drug carrier systems has attracted significant interest in pharmaceutical technology for many years. One option is using colloidal lipid emulsions, or lipid nanoemulsions, that are particularly interesting for the delivery of poorly water-soluble, lipophilic drugs (Floyd A. G., 1999; Hörmann and Zimmer, 2016). The mean droplet size of such emulsions is usually below 500 nm, which allows intravenous administration of the formulations.

One of the most common preparation methods for lipid nanoemulsions is high-pressure homogenization. In this process, a coarse pre-emulsion is first prepared from the aqueous emulsifier solution and the lipid phase, e.g. by rotor-stator treatment. For homogenization, the pre-emulsion is then forced through an narrow gap or an interaction chamber under pressure (Floyd A. G., 1999; Grumbach et al., 2022). Although the latter method (microfluidization), in particular, enables a relatively good control of the resulting mean particle size and the width of the particle size distribution, it is extremely difficult to achieve very narrow particle size distributions.

In the manufacturing process of parenteral lipid emulsions, the emulsions are usually autoclaved after homogenization to ensure sterility (Floyd A. G., 1999). For trimyristin (TM) nanoemulsions stabilized with Pol 188, a previous study demonstrated a positive effect of autoclaving on the uniformity of the particle size distribution (Göke et al., 2016). Subsequent research further explored this phenomenon to explain the underlying principle and mechanism. It was assumed that the effect was caused by accelerated Ostwald ripening during autoclaving with the formation of Pol 188 micelles and an increased solubility of TM during heat treatment playing a central role in the observed process (Göke et al., 2018).

A beneficial effect on particle size homogeneity was also observed upon autoclaving dispersions of supercooled smectic nanoparticles stabilized with Pol 188 (Kuntsche and Bunjes, 2007). Monoolein dispersions stabilized with Pol 407 did not only show a narrowing of the particle size distribution upon autoclaving but also a structural transformation: Monoolein particles that initially lacked a liquid crystalline cubic structure could be transformed into cubic phase particles by autoclaving (Wörle et al., 2006).

TM is a saturated triglyceride of myristic acid with a melting point of around 56 °C. As a special feature, it is known to form supercooled droplets when formulated in colloidal lipid nanoemulsions by melt-homogenization. This means that the emulsified lipid does not crystallize after cooling below the melting temperature of the bulk material (e.g., to room temperature in the case of TM), but remains in the liquid state (Bunjes et al., 1996).

Poloxamers are triblock copolymers consisting of a central hydrophobic poly(propylene oxide) (PPO) chain flanked by two hydrophilic poly(ethylene oxide) (PEO) chains. The molecular structure of these copolymers is denoted as PEO_a-PPO_b-PEO_c, indicating a flexible composition where the lengths of chains a, b, and c can vary significantly, depending on the poloxamer type. Due to their amphiphilic properties in aqueous solution – which are attributable to the water-soluble PEO and water-insoluble PPO segments – these copolymers can form micellar structures. Such structures are able to encapsulate hydrophobic drugs in their core, thereby enhancing the solubility of drugs that are poorly soluble in physiological media (Chiappetta and Sosnik, 2007).

Poloxamers are valued in pharmaceutical applications for their biocompatibility and generally low toxicity, which ensures safe use (Bodratti and Alexandridis, 2018).

In the monograph "Poloxamers", the European Pharmacopoeia describes five different types of poloxamers, namely poloxamer 124, 188, 237, 338 and 407 (European Pharmacopoeia, 10th ed.). Dosage forms that may contain poloxamers include tablets (Pol 188, 407), soft capsules, gels (Pol 124, 407), hard capsules, powders for suspension preparation, creams (Pol 188, 407), granules, solutions for oral and parenteral application (Pol 188), ocular application (Pol 188, 407) and suspensions for oral use (Pol 188) (Bodratti and Alexandridis, 2018).

For parenteral application, poloxamers are employed in various products, including Fluosol®, an emulsion of perfluorocarbon oxygen carriers used as a blood substitute containing 2.72 % (m/v) Pol 188 as emulsifier (Lowe, 2006). Other products available on the market that have been approved by the European Medicines Agency (EMA), such as the gene vector product Luxturna® (EMA. 2024g) offered as a concentrate and solvent for solution for injection and Zolgensma® (EMA. 2024d), provided as a solution for infusion, also contain Pol 188. Further, several peptide-/protein-containing products such as Sogroya® (EMA. 2024e) and Bemfola® (EMA. 2024a) solution for injection in prefilled pen as well as Orencia® (EMA. 2024f) and Mircera® (EMA. 2024b) solution for injection in prefilled syringe for subcutaneous application are formulated with Pol 188. In addition, the EMA has approved a prolonged-release suspension for injection of rilpivirine for the treatment of HIV infections which contains Pol 338 as a stabilizing agent (EMA. 2024c).

In the current study, a first goal was to develop TM nanoemulsions stabilized with different poloxamers with a target mean particle size of 100 nm (± 2 nm), focusing on the dependence of the particle size parameters on the average molecular weight of the poloxamers and the applied homogenization pressure. This involved adjusting the pressures employed during the homogenization process to achieve the target particle size for all formulations. After successful production of these nanoemulsions, the primary objective was to evaluate their physical stability under pharmacopeial reference conditions for steam sterilization (121 °C). Apart from observing the colloidal stability of the nanoemulsions, a further emphasis was placed on examining changes in particle size and particle size distribution after autoclaving. This comprehensive analysis aimed to provide insights into the suitability of various types of poloxamers for stabilizing TM nanoemulsions suitable for parenteral administration, with a major focus on the autoclavability of the emulsions.

2. Materials and methods

2.1. Materials

TM (Dynasan® 114, IOI Oleo GmbH, Hamburg, Germany) served as lipophilic phase. Pol 108, 124, 184, 188, 338, 407 (Pluronic F38, Kollisolv® P124, Pluracare® L64, Kolliphor® P188, Kolliphor® P338, Kolliphor® P407, BASF, Ludwigshafen, Germany), Pol 234, 235, 238, 333 (Adeka Nol P-84, Adeka Nol P-85, Adeka Nol F-88, Adeka Nol P-103, ADEKA Europe GmbH, Düsseldorf, Germany), Pol 237 (Synperonic PF/F 87-FL-(CQ), Croda Europe, Chocques, France), and Pol 403 (Pluronic® P123, Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) were used to stabilize the nanoemulsions (for more information on the different types of poloxamers see

table 1). TM and poloxamers were kind gifts by the respective manufacturer. For all nanoemulsions, sodium azide (Sigma-Aldrich Chemie, Steinheim, Germany) was used as preservative. Water was of bidistilled quality for the preparation of nanoemulsions and ultrapure water (EASYpureTM LF, Barnstead, Dubuque, IA, USA) for the dilution of nanoemulsions for particle size measurement. Cloud point experiments were carried out with sodium chloride (Carl Roth, Karlsruhe, Germany).

Table 1. Molecular weight, PEO content and PEO chain length of the investigated poloxamers (Alexandridis et al., 1994; Alexandridis, 1997; Russo and Villa, 2019)

Poloxamer	Average molecular weight [g/mol]	PEO content [%]	PEO chain length
124	2159	40	2 x 10
184	2634	40	2 x 13
234	4400	40	2 x 19
235	4600	50	2 x 26
108	4700	80	2 x 42
333	5000	30	2 x 17
403	5800	30	2 x 19
237	7700	70	2 x 67
188	8875	80	2 x 76
238	10900	80	2 x 103
407	12065	70	2 x 100
338	15497	80	2 x 132

2.2. Preparation of TM nanoemulsions and autoclaving

In all nanoemulsions, the aqueous phase contained 5.0 % poloxamer as emulsifier and 0.05 % sodium azide as preservative, both dissolved in bidistilled water. As lipid phase, 10.0 % TM was used. All quantities refer to the total dispersion (w/w) prior to homogenization. The aqueous and lipophilic phases were separately heated to 75 °C in a water bath, combined, and prehomogenized with an Ultra Turrax (T25 digital Ultra Turrax, IKA, Staufen, Germany) with 11000 rpm for 5 min. High-pressure homogenization (Microfluidizer M-110 P, Microfluidics, Newton, USA) was performed at different pressures (figure S1 in the supporting information) and at 75 °C for 10 cycles. Directly afterwards, the nanoemulsions were filtered through a non-sterile polyvinylidene fluoride (PVDF) syringe filter (ROTILABO®, Carl Roth GmbH, Karlsruhe, Germany) of 0.45 µm pore size and cooled down to room temperature. 2.5-3.0 ml of the nanoemulsions were filled into injection glass vials type 1 (Zscheile & Klinger GmbH, Hamburg, Germany), sealed by lyo-chlorobutyl stoppers (Zscheile & Klinger GmbH, Hamburg, Germany), crimped with an aluminum cap (Zscheile & Klinger GmbH, Hamburg, Germany) and autoclaved for 5, 10, 15 and 20 min, respectively, at 121 °C in a laboratory autoclave (Sanoclav TKL MCS 53, Adolf Wolf, Bad Überhingen, Germany). All non-autoclaved and autoclaved nanoemulsions were stored in glass vials at 20 °C (Binder KB 720, BINDER GmbH, Tuttlingen, Germany).

2.3. Visual characterization

Directly after preparation and autoclaving, the TM nanoemulsions were visually inspected for macroscopic appearance (homogeneity and/or destabilization).

2.4. Particle size analysis

The intensity-weighted mean diameter (z-average diameter) and polydispersity index (PdI) were measured using photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at an angle of 173°. The emulsions were diluted with ultrapure water (attenuator 6) before the measurements. The z-average and PdI values were calculated as mean of three subsequent runs at 25 °C, where each run lasted for 180 s, following an equilibration time of 300 s.

2.5. Rheology

The viscosity of different poloxamer solutions was measured using a rotational viscometer (HAAKE™ RheoStress 6000, Thermo Fischer Scientific, Karlsruhe, Germany) with a DG41 double gap Searle measurement geometry (gap height 5.1 mm). For each measurement, 10 ml of a 5 % poloxamer solution was placed in the external cylinder and equilibrated for 300 seconds after the inner cylinder had been brought into the measuring position. Each emulsifier solution was measured in triplicate at 25 °C. To determine the dynamic viscosity, the shear rate was increased from 0.1 to 500 s^{-1} (ascending curve) and then decreased again to 0.1 s^{-1} (descending curve). The data points were taken from HAAKE RheoWin Data Manager software.

2.6. Determination of cloud point

The cloud points of 1 % poloxamer solutions with different NaCl concentrations (1 M, 2 M, 3 M, 4 M) were determined with the water bath method (Göke et al., 2018). The temperature of the

solutions was measured with a temperature sensor (IKA® ETS-D5) during gradual heating (5 °C per minute) of the water bath. The solutions were stirred during the measurements, and the cloud point (defined as the occurrence of completely uniform turbidity of the emulsifier solution) was determined by macroscopic observation. The samples were each measured in triplicate, and the mean value (with standard deviations) was used for evaluation. The cloud point values obtained were plotted in dependence on NaCl concentration and the data points were linearly fitted. The cloud point of the NaCl-free solution could be read off from the intersection point of the linear fit of the data points with the y-axis (see figure S2 in the supporting information).

2.7. Determination of the concentration of free poloxamer in the aqueous phase

To determine the concentration of free poloxamer in the aqueous phase of the TM nanoemulsions, the aqueous phase was separated by centrifugal ultrafiltration and then analyzed refractometrically (Francke and Bunjes, 2021). Vivaspin® 6 concentrators with a cut-off of 300 kDa (Sartorius, Göttingen, Germany) were spun at 22 °C with 500 g in an Allegra™ 64R centrifuge (Beckman Coulter GmbH, Krefeld, Germany). Before use, the Vivaspin® 6 tubes were washed in three steps with bidistilled water to remove any residual glycerol and sodium azide that may interfere with subsequent analytical procedures from the filter membranes. In each washing step, 5 ml of bidistilled water was centrifuged for 10 minutes. Subsequently, to avoid dilution effects, a 10-min centrifugation step was performed under dry conditions to remove any remaining water from the membrane. Finally, 2 ml of TM nanoemulsion was added to the dry Vivaspins and centrifuged for 40 minutes to obtain approximately 300 µL of aqueous phase as ultrafiltrate. The concentration of free poloxamer in the filtrate was then determined refractometrically using an Abbemat-WR refractometer (Anton Paar, Ostfildern-Scharnhausen, Germany). The refractive index of aqueous phase was measured in triplicate at 25 °C and evaluated based on calibration lines (Francke and Bunjes, 2021).

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC 1 STAR^e system with FRS 5+ sensor calibrated with indium. About 20 mg of TM nanoemulsion was weighed into 40 µL aluminum pans (Mettler Toledo, Gießen, Germany) which were cold-welded. The nanoemulsion droplets were first crystallized by cooling from 25 °C to 0 °C with 2.5 K/min and then melted by heating from 0 °C to 85 °C at a scan rate of 2.5 K/min.

3. Results and discussion

3.1. Preparation conditions for TM nanoemulsions and rheological properties of poloxamer solutions

The first aim of this study was to obtain a target z-average mean particle size of 100 nm (± 2 nm) for the emulsions stabilized with different poloxamers. To achieve this goal, it was investigated whether the process parameters, such as the homogenization pressure, need to be adjusted during nanoemulsion preparation in order to obtain the desired mean particle size. For this purpose, different poloxamer-stabilized systems were first prepared at a homogenization pressure of 800 ±11 bar. For the Pol 108-, 333-, 403-, and 237-stabilized systems, mean particle sizes of 100 nm (± 2 nm) were directly obtained under these conditions (figure S1 A in the supporting information).

In order to achieve the target particle size within 10 cycles also for the remaining emulsion systems, the homogenization pressure was adjusted accordingly, preparing each TM nanoemulsion multiple times. The applied homogenization pressures and resulting mean particle sizes and PDI values are displayed in the supporting information (figure S1 B to I). Considering all 100 (± 2) nm-sized TM nanoemulsions stabilized with the different poloxamer types individually, the required homogenization pressure increased with the average molecular weight of the respective poloxamer (Figure 1).

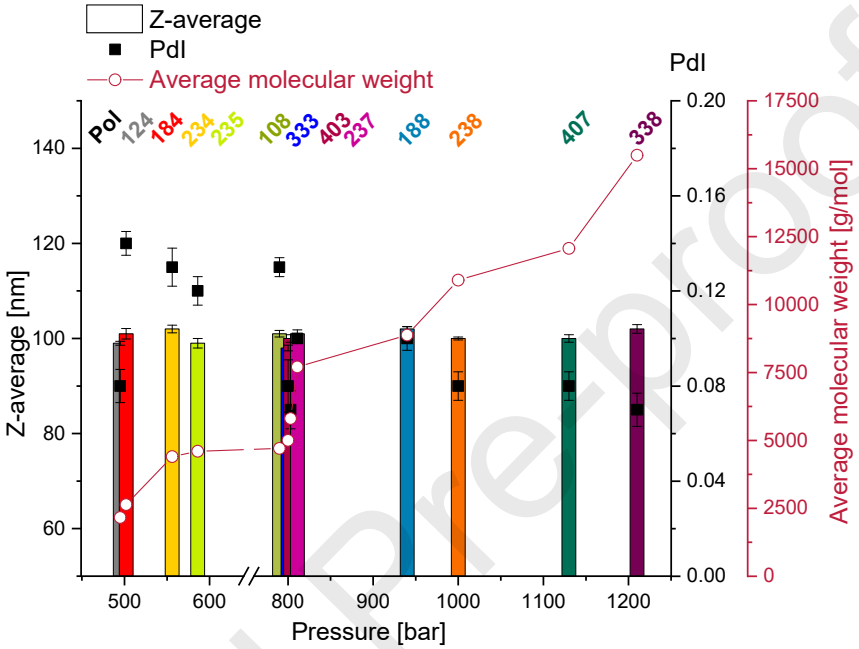


Figure 1. Influence of homogenization pressure on the mean particle size and PdI value of TM nanoemulsions (displaying the target mean particle size) stabilized with the different poloxamers. The line between data points is only drawn to guide the eye.

Regarding the PdI values obtained for these 100 nm-formulations, a general decrease was observed at higher homogenization pressures and average molecular weights, with only three formulations (Pol 124, 333 and 403) leading to smaller PdI values than expected from the general trend (Figure 1).

To evaluate whether the viscosity of the aqueous phase of the emulsion might affect the resulting mean particle size followed by the need to adjust the homogenization pressure, the viscosities of 5 % poloxamer solutions at 25 °C were determined. All poloxamer solutions exhibited Newtonian flow properties within the range of applied shear rates (figure S3 in the supporting information), i.e., the dynamic viscosity remained constant regardless of the shear rates.

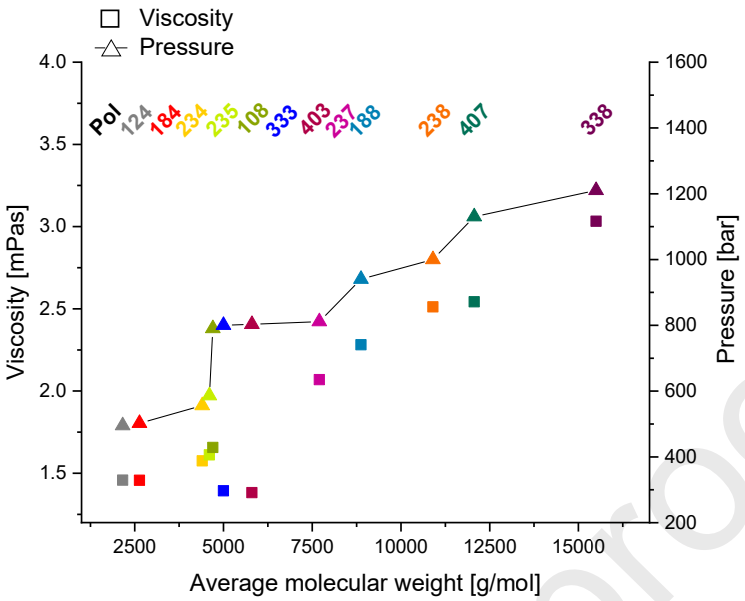


Figure 2. Effect of the average molecular weight of the poloxamers on the viscosity of the respective solutions with 5 % poloxamer and the homogenization pressure during emulsion processing needed to achieve 100 nm mean droplet sizes (the line for the viscosity data is only shown to guide the eye).

Poloxamers with higher average molecular weight led to much more viscous aqueous solutions than poloxamer types with lower average molecular weight (figure 2). The observed need to use higher pressures to achieve particle sizes of 100 nm (± 2 nm) during homogenization of emulsions containing poloxamers with a higher average molecular weight might thus be related to the fact that the aqueous phase of such emulsions is more viscous. An exception were the solutions prepared from Pol 333 (800 bar) and 403 (803 bar), which had lower viscosities, but this had no direct influence on the pressure required for emulsification.

The low viscosity observed in the solutions of Pol 333 and 403 was hypothesized to be associated with their micelle formation at relatively low temperatures. DSC measurements indicated that the critical micellization temperatures (CMT) for Pol 333 and 403 solutions (5% w/w) are notably below 25 °C, at 19 °C and 16 °C, respectively (Sukhbat et al., 2023). Pol 407 exhibited micelle formation at 24 °C (Sukhbat et al., 2023), suggesting a propensity for micelle formation across these compounds, albeit without directly correlating to the reduced viscosity observed in Pol 333 and 403.

Concerning the determined viscosity values of the 5 % poloxamer solutions at 25 °C, it is important to note that these conditions represent an approximation and do not fully reflect the circumstances during high-pressure homogenization at 75 °C. Other factors, such as the diffusion properties of the emulsifier molecules or the interfacial tension, can be assumed to also play a role for the resulting particle size and should not be disregarded.

3.2 General nanoemulsion stability upon autoclaving

The second aim of the study was to determine which of the poloxamers under investigation can stabilize the nanoemulsions during autoclaving at 121 °C for 5 up to 20 min. The overall physical stability of the nanoemulsions was evaluated in terms of their macroscopic appearance before and after autoclaving. Already after autoclaving for 5 min, the nanoemulsions stabilized with Pol 124, 184, 234, 235, 333 and 403 were broken (table 2; for an example see figure 3 B). In contrast, there were no changes in the macroscopic appearance of the systems stabilized with Pol 108, 188, 237, 238, 338 and 407, even after 20 minutes of autoclaving (table 2; exemplified in figure 3 A).

In a previous study, nanoemulsions stabilized with nonionic surfactants such as polysorbate 80, which also contains polyethylene oxide (PEO) chains in its structure and is suitable for parenteral applications, were destabilized upon autoclaving. In contrast, emulsions stabilized with Pol 188 remained stable after autoclaving. The authors hypothesized that the higher cloud point of Pol 188 was responsible for this stability (Jumaa and Müller, 1998). Further investigations were thus undertaken to elucidate the underlying factors contributing to the differences in stability observed upon autoclaving, with particular emphasis on the cloud point of the poloxamers studied here.

The cloud point is the temperature at which a clear emulsifier solution begins to get turbid due to a phase separation taking place. The cloud point is a characteristic feature of nonionic surfactants with PEO chains. These surfactants exhibit an inverse solubility behavior in water, meaning they become less soluble as the temperature increases. This leads to clouding or phase separation when hydrogen bonding between PEO and water molecules breaks down at higher temperatures increasing the hydrophobicity of the molecule (Khimani et al., 2014; Patel et al., 2007).

Table 2. Macroscopic appearance of the nanoemulsions before and after autoclaving

Emulsifier for TM nanoemulsion	Homogeneity	
	Before autoclaving	After autoclaving (maximum autoclaving time)

Pol 124	stable*	unstable** (5 min)
Pol 184	stable*	unstable** (5 min)
Pol 234	stable*	unstable** (5 min)
Pol 235	stable*	unstable** (5 min)
Pol 108	stable*	stable* (20 min)
Pol 333	stable*	unstable** (5 min)
Pol 403	stable*	unstable** (5 min)
Pol 237	stable*	stable* (20 min)
Pol 188	stable*	stable* (20 min)
Pol 238	stable*	stable* (20 min)

Pol 407	stable*	stable* (20 min)
Pol 338	stable*	stable* (20 min)

* Nanoemulsion with a milky white and homogeneous appearance

**Phase separation

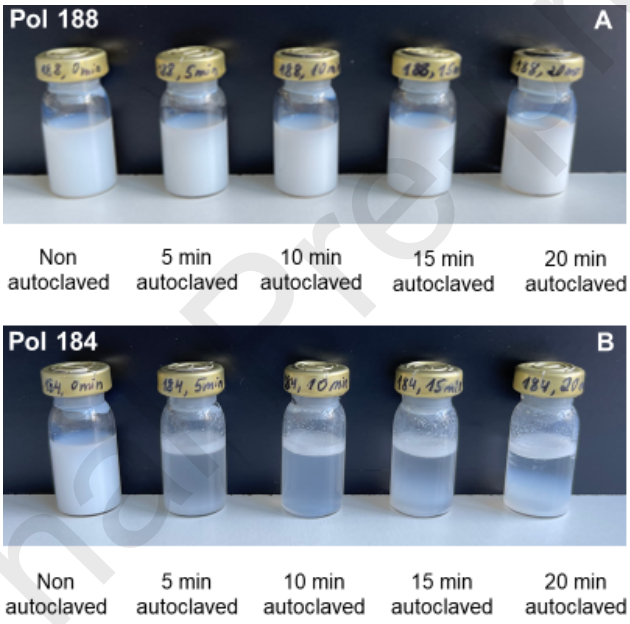


Figure 3. Examples of macroscopic appearance before and after autoclaving for a stable Pol 188-containing TM nanoemulsion (A) and a Pol 184-containing TM nanoemulsion (B) broken after autoclaving

3.3. Evaluation of the cloud points and their correspondence with emulsion stability

The TM nanoemulsions stabilized with Pol 124, 184, 234, 235, 333 and 403 significantly changed in optical appearance upon autoclaving. In contrast, the nanoemulsions stabilized with the other poloxamers (Pol 108, 188, 237, 238, 338 and 407) remained unaltered according to macroscopic investigation (table 2). It is important to emphasize that the poloxamers examined within this study

exhibit variations in their PEO content. The poloxamers used with the latter emulsions have a higher PEO content (table 1) which results in a higher hydrophilicity.

To evaluate if there is a correlation between the different types of poloxamer with various PEO contents and their cloud points, the cloud points were determined for all poloxamer solutions (figure 4; the data for the whole determination procedure are shown in figure S2). The solutions of Pol 108, 188, 237, 238, 338 and 407 (i.e. poloxamers with a PEO content of 70 or 80 %) displayed the highest cloud points (above 95 °C). The cloud points of the poloxamers with 30 - 50 % PEO content, such as Pol 124, 184, 234, 235, 333 and 403 were clearly lower (below 90 °C). These results align with literature data, e.g., by Khimani et al., who determined the clouding behavior of different poloxamers at various NaCl concentrations (Khimani et al., 2014). They showed that poloxamers with larger PEO content have higher cloud points than poloxamers with smaller PEO content (Khimani et al., 2014). Our observations are consistent with these findings. For poloxamers with a PEO content of at least 70 %, cloud points of 98.5 °C and above were determined. Only emulsions stabilized with these poloxamers remained stable upon autoclaving indicating a crucial importance of the cloud point for thermal stability.

The correlation between the cloud point of the copolymer and the thermal stability of the respective nanoemulsion can primarily be explained by the fact that a higher proportion of PEO leads to stronger hydration and thus a higher cloud point. As a result, the tendency toward phase separation at increasing temperatures is reduced, which keeps the system stable at high temperature. At the same time, longer PEO chains provide steric stabilization against droplet aggregation by surrounding the droplets in the nanoemulsion with a thick hydrophilic “protective layer”. This reduces the droplet aggregation at higher temperatures, thereby increasing thermal stability. When comparing the PEO chain lengths of the poloxamers used in this study (see table 1), it becomes evident that those with longer PEO chains lead to greater stability upon autoclaving than poloxamers with shorter PEO chains.

In contrast, the autoclaving time appeared to be irrelevant with regard to overall colloidal stability of the emulsions within the time periods under investigation.

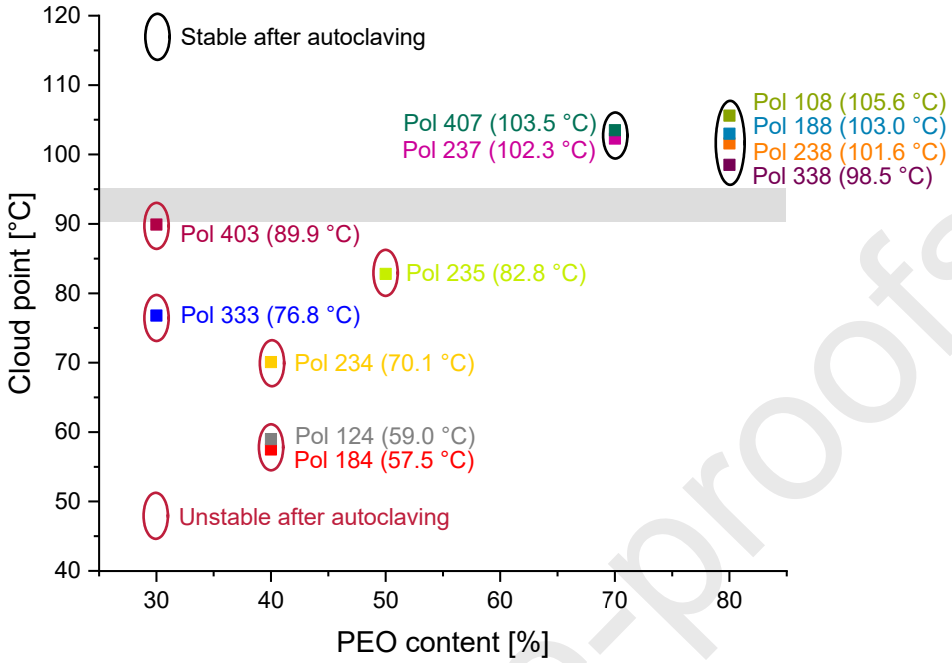


Figure 4. Correlation between cloud point and PEO content of poloxamer and their effect on nanoemulsion stability upon autoclaving

3. 4. Effect of autoclaving on particle size, particle size distribution and thermal behavior of the emulsion droplets

To further investigate the influence of the autoclaving process on the TM nanoemulsions, the z-average diameter and PdI of stable TM nanoemulsions were determined before and after autoclaving (figure 5).

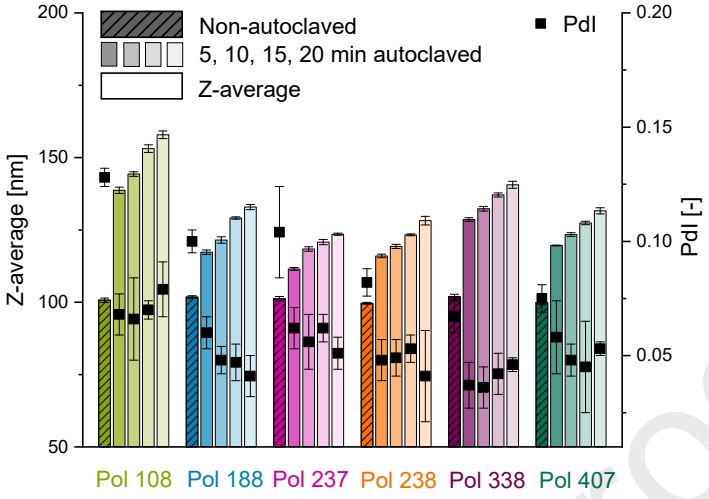


Figure 5. PCS results for TM nanoemulsions stabilized with different poloxamers before (0 min) and after autoclaving (5, 10, 15 and 20 min)

Before autoclaving, the particle sizes of the Pol 108-, 188-, 237-, 238-, 338- and 407-stabilized nanoemulsions ranged from 100 to 102 nm with PdIs between 0.07 and 0.13. Measurements after autoclaving indicated an increase in particle size and a decrease in size distribution width for all nanoemulsions at longer autoclaving times as previously described for TM nanoemulsions stabilized with Pol 188 (Göke et al., 2016). During autoclaving of the formulations, the particle sizes increased by approx. 11 – 57 % depending on the type of poloxamer used for emulsion stabilization. The most profound effect on the particle size parameters occurred during the first 5 min of autoclaving. Upon further heat treatment, all emulsions continuously increased slightly in particle size whereas there was no clear trend concerning the evolution of the PdI. The observed behavior is presumably due to Ostwald ripening. It was shown before that the autoclaving of triglyceride dispersions stabilized with Pol 188 resulted in accelerated Ostwald ripening leading to an increase in particle size and a decrease in PdI (Göke et al., 2018; Göke et al., 2016). As observed in the current study, this behavior obviously also occurs in emulsions stabilized with other types of poloxamer.

As reported previously for Pol 188-stabilized emulsions (Göke et al., 2016), the emulsions contain a considerable fraction of very small droplets before autoclaving as reflected in a relatively large PDI and a highly structured melting event of the recrystallized droplets (see below). The large changes in particle size distribution observed during the first 5 minutes of autoclaving can be attributed to both temperature-accelerated processes and an initially rapid Ostwald ripening due to the high gradient in trimyristin solubility between the many small and the larger droplets. The latter grow at the expense of the small droplets the fraction of which decreases rapidly over the initial phase of autoclaving. Thus, the system stabilizes with longer autoclaving times. Whether the small further increase in particle size is due to continued Ostwald ripening or to slow coalescence processes cannot be said with certainty since there is no clear trend in the PDI values.

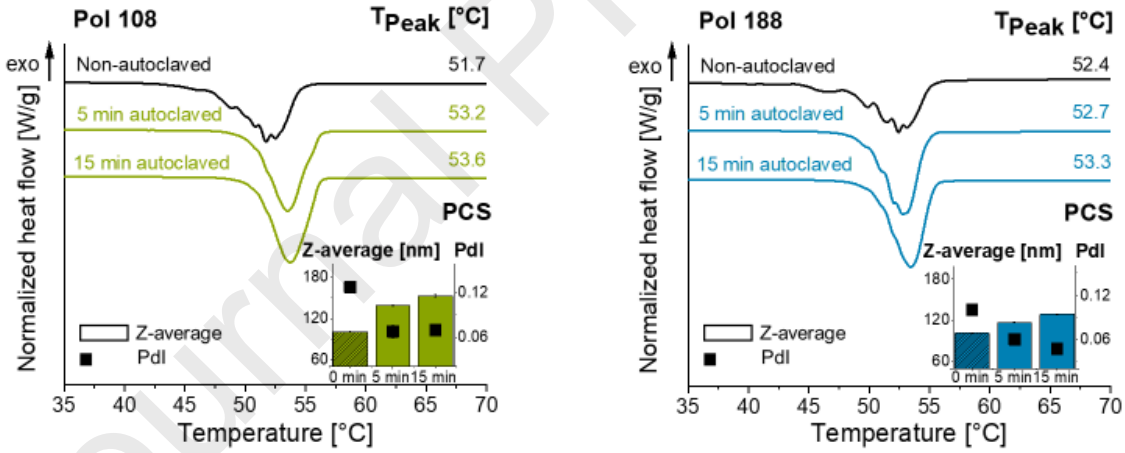
However, small alterations in the DSC melting patterns (see below) at longer autoclaving times point to a further disappearance of smaller particles at least for some of the emulsion systems (Figure 6).

Previous studies conducted with lipid nanoemulsions and -suspensions yielded similar results with regard to changes in particle size after autoclaving the formulations. Venkateswarlu et al. used Pol 188 as an emulsifier and examined various triglyceride formulations (such as trimyristin, tripalmitin and tristearin). In that study, however, the particle sizes of the formulations increased two to three times upon autoclaving. Unfortunately, potential changes in the polydispersity index (PdI) following autoclaving were not reported (Venkateswarlu and Manjunath, 2004). A direct comparison of the results of Venkateswarlu et al. with our findings is thus challenging, and drawing conclusions on the stability of the emulsions after autoclaving without data on the polydispersity index (PdI) is difficult.

Zhang et al. prepared soybean oil emulsions stabilized with Pol 188 and Pol 407. Both formulations remained stable after autoclaving, with an increase in particle sizes and smaller polydispersity indices (PdIs) (Zhang et al., 2017).

The emulsifier is not the only relevant factor for emulsion stability; the oil phase of the emulsion should also be considered. Previous work investigated the critical parameters for changes upon autoclaving Pol 188-stabilized lipid nanodispersions containing different fats and oils. Nanodispersions of triglycerides with esterified fatty acid chain lengths from C8 to C18 were autoclaved at different temperatures and durations. The length of the esterified fatty acid chains of the triglycerides was essential for the behavior upon autoclaving with short chains leading to breaking of the emulsions whereas the use of saturated triglycerides with longer chains resulted in at most small effects on particle size. This suggested an important contribution of the lipid polarity on the effects observed which was confirmed in further experiments with C18 triglycerides of different polarity (Göke et al., 2018).

To further confirm the changes in particle size distribution after autoclaving in the current study, DSC analyses were performed with the TM nanoemulsions (figure 6). After cooling below the crystallization temperature of the droplets, DSC heating curves of all non-autoclaved TM nanoemulsions displayed broad and jagged melting peaks. This peak broadening, combined with multiple separate melting events and a shift towards lower melting temperatures, indicates the presence of small particles in the respective TM nanoemulsions. Compared to the curves of non-autoclaved samples, the melting curves of the autoclaved formulations displayed a much sharper melting event the maximum of which had shifted to slightly higher temperature. These alterations in melting behavior indicate the loss of small particles (leading to a narrower particle size distribution) and an overall increase in particle size of the formulations (Göke et al., 2018; Göke et al., 2016). As observed for the particle size-related parameters, the most prominent changes in the melting behavior were observed already after 5 min of autoclaving and only minor alterations occurred at longer autoclaving times. Thus, the DSC data supported the PCS results, confirming a growth in particle size and a decrease in polydispersity during autoclaving.



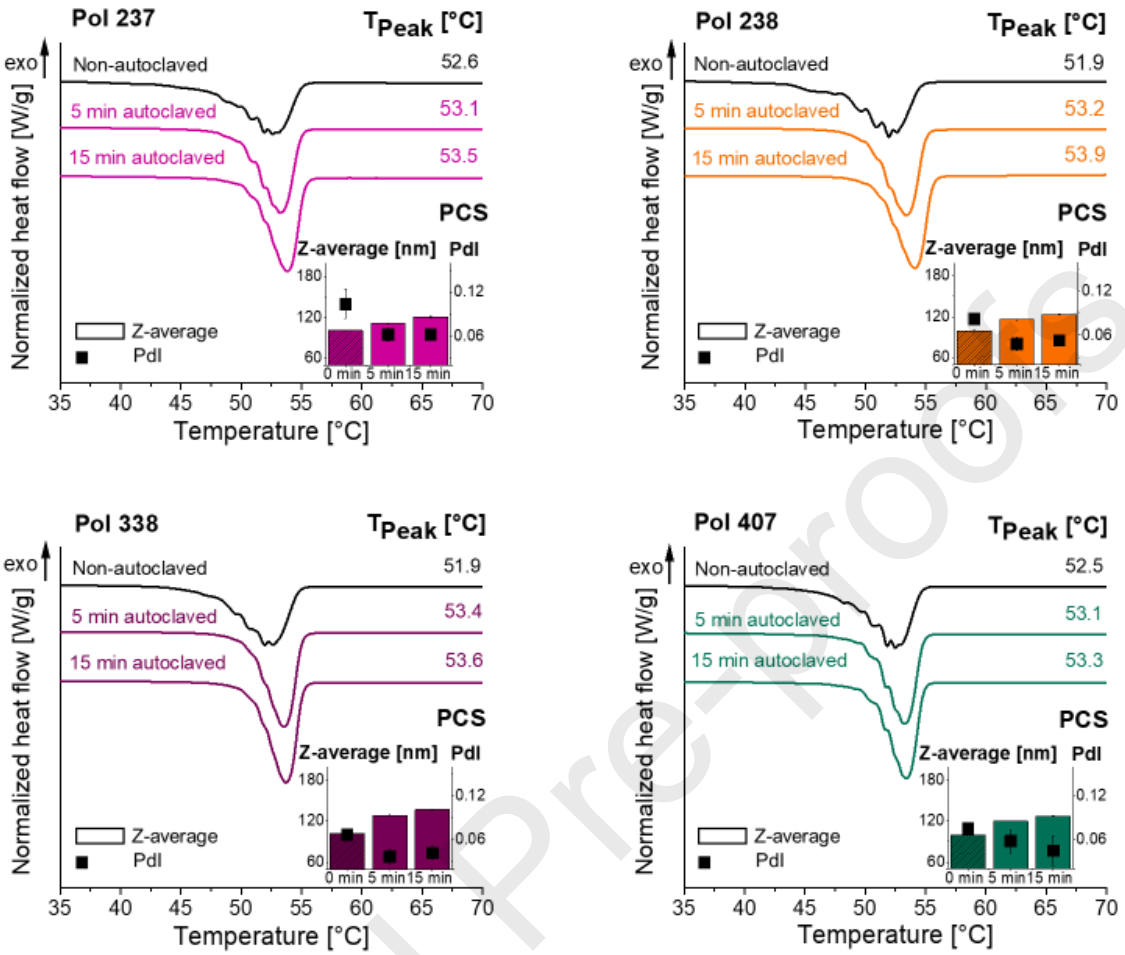


Figure 6. DSC heating curves (after recrystallization of the droplets) and PCS results for the non-autoclaved, 5 min and 15 min autoclaved Pol 108-, 188-, 237-, 238-, 338- and 407-stabilized TM nanoemulsions. The heating curves are normalized and shifted along the ordinate. The DSC curves of all samples obtained after the different times of autoclaving can be found in the supporting information (figure S4).

3.5. Effect of autoclaving on the concentration of free poloxamer in the aqueous phase and the crystallization temperature

As an oil-in-water emulsifier, poloxamer is soluble in water and accumulates at the interface between the water and the oil phase. To draw conclusions about the amount of poloxamer adsorbing to the droplet surfaces, the concentration of free poloxamer in the aqueous phase was quantified by measuring the refraction index in the ultrafiltrate of the emulsions. After autoclaving, the concentration of free poloxamer (Pol 108, 188, 237, 238, 338 and 407) had increased significantly (22 % to 185 %) with again the most prominent change occurring over the first 5 min of autoclaving (figure 7). As described above, the particles became larger upon autoclaving and the fraction of small particles disappeared, which resulted in a lower specific droplet surface area. This is associated with a lower demand for stabilizing emulsifier at the surface of the droplets.

Thus, excess emulsifier presumably detached from the particle surface, which led to an increased concentration of free emulsifier in the aqueous phase.

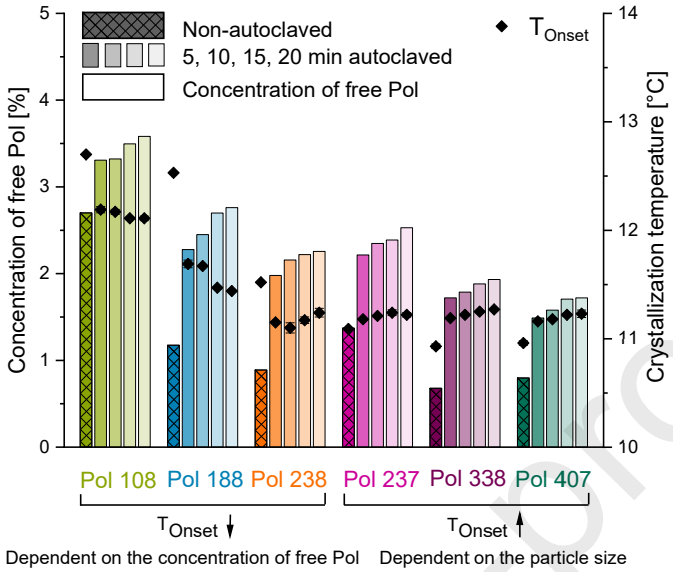


Figure 7. Influence of autoclaving on the concentration of free poloxamer in TM nanoemulsions and the crystallization temperature (T_{Onset}) of the droplets

Interesting effects on the crystallization behavior of the TM nanodroplets were observed in dependence on autoclaving and the type of poloxamer used for the stabilization of the respective emulsion (figure 7). The crystallization temperature of Pol 108-, 188- and 238-stabilized TM nanodroplets shifted to lower temperatures upon autoclaving the effect being particularly distinct after 5 min. In contrast, the use of Pol 237, 338 and 407 led to a slight increase in the crystallization temperature. The difference in the effect on the crystallization temperature is probably related to differences in the adsorption behavior of the poloxamers. Pol 108, 188 and 238 exhibit a distinctly concentration-dependent adsorption behavior (Roese and Bunjes, 2015; Sukhbat et al., 2022). Higher concentrations of free poloxamer in the aqueous phase are associated with an increased amount of poloxamer adsorbed to the droplet surface for such systems, which, in turn decreases the crystallization temperature of TM nanodroplets. Such behavior did not occur in TM nanoemulsions stabilized with Pol 237, 338 and 407, which display a much less concentration-dependent adsorption behavior (Roese and Bunjes, 2015; Sukhbat et al., 2022), in particular in the concentration range used for emulsion stabilization here. The slight increase in crystallization temperature observed for the corresponding formulations is probably related to the increase in droplet size upon autoclaving. As a further interesting observation the DSC crystallization peaks in the autoclaved samples were slightly sharper than in the non-autoclaved samples, which likely reflects the narrower particle size distribution (supporting information figure S5).

4. Conclusion

The various types of poloxamers were differently effective in producing emulsions with small droplets but by adjusting the homogenization pressures used in the preparation of the differently stabilized emulsions a mean particle size of 100 nm (± 2 nm) could be achieved for all

formulations. The observed impact of the type of poloxamer on nanoemulsion preparation through high-pressure homogenization is an important aspect yet to be addressed, highlighting the need for further investigation into how poloxamers influence the homogenization process and contribute to the final particle sizes. TM nanoemulsions stabilized with Pol 108, 188, 237, 238, 338 and 407 can be autoclaved under standard pharmacopeial conditions without affecting their colloidal stability. Since these Pol-stabilized TM nanoemulsions can be easily sterilized by autoclaving they can be considered for parenteral use in future projects provided that potential issues with chemical instability (Erlandsson, 2002; Marques et al., 2024; U.S. Food and Drug Administration, 2018) can be ruled out. The PEO content of the poloxamer molecule and its cloud point are important parameters for assessing whether a TM nanoemulsion is stable or not after autoclaving. A PEO content of at least 70 % seems to be required to ensure emulsion stability upon autoclaving. The resulting increase in particle size of the stable emulsions is most likely caused by Ostwald ripening as indicated by a simultaneous decrease in size distribution width. A corresponding decrease in specific surface area leads to a lower demand for emulsifier required for emulsion stabilization and thus to an increase in the concentration of free poloxamer after autoclaving.

CRediT authorship contribution statement

Oyunbileg Sukhbat: Writing – original draft, Methodology, Investigation, Visualization, Validation, Conceptualization. **Denise Steiner:** Writing – review and editing, Methodology, Supervision, Conceptualization. **Heike Bunjes:** Writing – review and editing, Methodology, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare no conflict of interest.

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Supporting Information

Additional results on the homogenization pressure, rheological properties, cloud point, and DSC determinations (PDF).

Data availability

The data are contained in the article.

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