



Continuous contact- and contamination-free ultrasonic emulsification—a useful tool for pharmaceutical development and production

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Abstract

A novel concept was developed here for the continuous, contact- and contamination-free treatment of fluid mixtures with ultrasound. It is based on exciting a steel jacket with an ultrasonic transducer, which transmitted the sound waves via pressurised water to a glass tube installed inside the jacket. Thus, no metallic particles can be emitted into the sonicated fluid, which is a common problem when a sonotrode and a fluid are in direct contact. Moreover, contamination of the fluid from the environment can be avoided, making the novel ultrasonic flow-through cell highly suitable for aseptic production of pharmaceutical preparations. As a model system, vegetable oil-in-water emulsions, fed into the cell as coarse pre-emulsions, were studied. The mean droplet diameter was decreased by two orders of magnitude yielding Sauter diameters of 0.5 μm and below with good repeatability. Increasing the residence time in the ultrasonic field and the sonication power both decreased the emulsion mean diameter. Furthermore, the ultrasonic flow-through cell was found to be well suited for the production of nanoparticles of biodegradable polymers by the emulsion-solvent extraction/ evaporation method. Here, perfectly spherical particles of a volume mean diameter of less than 0.5 μm could be prepared. In conclusion, this novel technology offers a pharmaceutically interesting platform for nanodroplet and nanoparticle production and is well suited for aseptic continuous processing.

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1. Introduction

Emulsification and homogenisation are common unit operations in the pharmaceutical, cosmetic, food, chemical and other industries. A number of mechanical processes are employed to produce emulsions, among them stirring, toothed disc dispersing (often referred to as homogenising or rotor–stator dispersing), colloid mill-

ing and high-pressure homogenisation [1]. Ultrasonic emulsification has been studied for many decades [2,3] and gathered increasing interest recently [4–6]. Studies comparing ultrasonic emulsification with rotor–stator dispersing [6,7] found ultrasound to be competitive or even superior in terms of droplet size and energetic efficiency. Microfluidisation (a type of high-pressure homogenisation) was found to be more efficient than ultrasound, but less practicable with respect to equipment contamination and aseptic processing [6].

A straightforward way to produce an emulsion by ultrasound is by immersion of a sonotrode either into

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the mixture of all components or into the continuous phase and adding gradually the phase to be dispersed during sonication. This procedure works well for small batches, but scale-up is difficult. As the intensity of ultrasound in a liquid decreases rapidly with the distance to the sonotrode, larger volumes may not be well homogenised. Results may be improved by stirring the volume or moving around the sonotrode [6]. However, continuous systems forcing the fluids to be sonicated through a small volume in the vicinity of the sonotrode are preferred. Such systems may consist of a flow-through beaker [8], a flow channel with ultrasonic transducers and reflectors installed in the walls [9] or a flow channel into which one or more sonotrodes protrude [4].

Still, existing ultrasonic processing systems are not well suited for the production of, e.g., pharmaceutical emulsions for the delivery of therapeutic agents, fat emulsions for parenteral nutrition or liposomes. Such pharmaceutical products require manufacturing equipment that can be readily cleaned and sterilised, and which offers the possibility of aseptic production. Furthermore, as ultrasonic emulsification is mainly driven by cavitation, ions or particles are emitted into the product by cavitation abrasion of the sonotrode. Frequently, such sonotrodes consist of metallic alloys, leading to critical product contamination.

The objective of this study was to evaluate a novel ultrasonication concept for the production of pharmaceutical dispersions using different vegetable oils in water as model system. The equipment basically consists of a glass tube, through which the fluid mixture is pumped, surrounded by a jacket filled with pressurised water for conduction of the sound waves. Ultrasound is transmitted to the system by a sonotrode attached to the jacket. In addition to the preparation of oil-in-water emulsions, the system was also found useful for the preparation of biodegradable nanoparticles using the solvent extraction/evaporation method. The novel methodology is highly advantageous for continuous,

contact-free emulsification and homogenisation. The process can be run in a closed system to prevent any environmental contamination of the product, and thus provides an opportunity for aseptic manufacturing. Future work will implement this process in the preparation of drug-loaded biodegradable microspheres, completing our group's portfolio of aseptic microencapsulation technologies [10,11].

2. Materials and methods

2.1. Design of the ultrasonic flow-through cell and experimental set-up

The ultrasonic flow-through cell consisted of a cylindrical steel jacket, in which a glass tube of 2 mm inner diameter for conveying the emulsion was installed (Fig. 1). A sonotrode fixed to a piezoelectric transducer (24 kHz, UIP250, Dr. Hielscher, Teltow, Germany) was welded to the outside of the steel jacket to provide ultrasonic vibration. Through the space between the glass tube and the jacket, pressurised water was passed for sound conduction [12]. For the experiments described here, water pressure was maintained between 4.5 and 5.5 bar.

The continuous phase, fed by a double-piston pump (L-6000, Hitachi-Merck, Darmstadt, Germany), and the disperse phase, fed by a syringe pump (syritol 12, Heinzerling Medizintechnik, Rotenburg a.d. Fulda, Germany), were pre-mixed in a 3 ml glass cell by means of a cross-shaped magnetic stirrer (Fig. 2). For the oil-in-water emulsions, two such mixing cells were connected in series, while for nanoparticle production, a single cell was found sufficient. The pre-mixed coarse emulsion was transported to the ultrasonic flow-through cell, where it was further homogenised.

Sonication power was controlled by the amplitude of the transducer's oscillation. To quantify the power con-

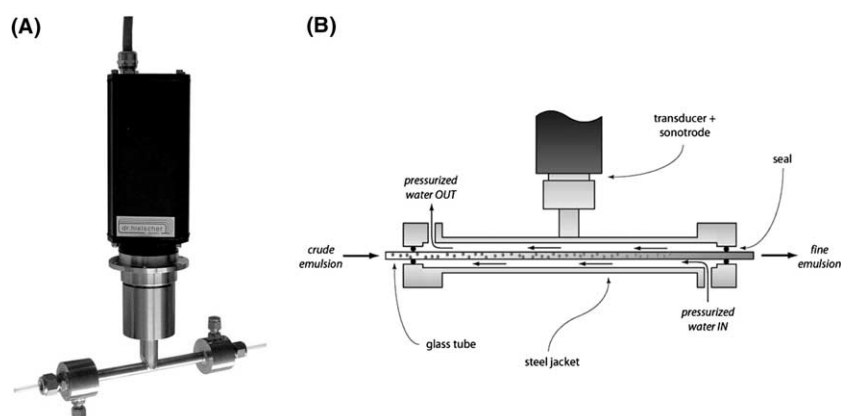


Fig. 1. Photograph (A) and design (B) of the ultrasonic flow-through cell.

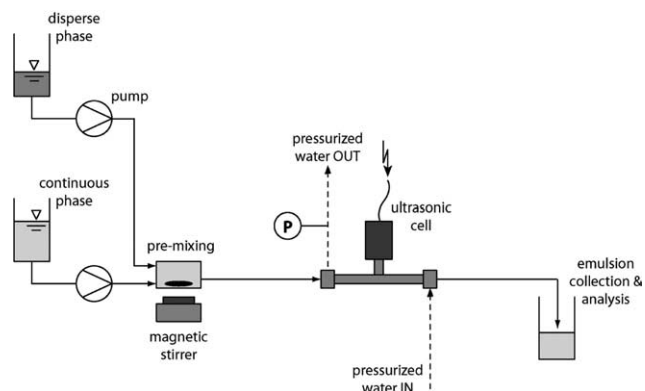


Fig. 2. Experimental set-up used for the production of oil-in-water emulsions.

sumed for emulsification, the power intake of the high frequency generator driving the transducer was recorded using a standard household power monitor (PowerMonitor pro, Conrad Electronic, Hirschau, Germany). For 100%, 80% and 60% of the maximum amplitude, the power intake amounted to 32, 25 and 17 W, respectively. Assessment of the actual power transferred to the sonicated emulsion is usually done by measuring the heat taken up by the emulsion, which for the present ultrasonic flow-through cell would have been difficult to do with reasonable accuracy. Still, it is reasonable to assume that the power consumption by the generator should be proportional to that delivered to the emulsion [13,14].

The residence time of the emulsion in the ultrasonic field, t_R , was calculated from the dead volume of the flow-through cell (0.53 ml) divided by the respective emulsion flow rate. Residence times were in the range of 7–50 s.

2.2. Preparation of oil-in-water emulsions

Olive and linseed oil (Ph.Eur./Ph.H.VIII grade) were obtained from Hanseler (Herisau, Switzerland), soybean oil from Sigma Chemie (Buchs, Switzerland) and Tween 40, used as emulsifier, from Fluka (Buchs, Switzerland). Water was of NANOpure-quality (Barnstead, Dubuque, USA).

The three vegetable oils were emulsified in water containing 3% (w/w) Tween-40 (HLB 15.6). After pre-filling the experimental equipment with the aqueous solution, the magnetic stirrer of the pre-mixing cell and the ultrasonic flow-through cell were activated. Thereafter, oil was injected into the pre-mixer. The product of the first 10 min of processing was discarded to let the process reach steady-state. During product collection, a sample of 0.5 ml was taken directly from the US cell outlet for droplet size analysis. For each set of parameters, production was repeated three times.

The dynamic viscosity of the oils was determined by means of a cone/plate rotational viscosimeter (VT 550/

PK 100, Haake, Karlsruhe, Germany) using a 1° cone. The interfacial tension between the different oils and the aqueous surfactant solution was measured using a droplet volume tensiometer (DVT30, Krüss, Hamburg, Germany).

2.3. Preparation of PL(G)A nanoparticles

End-group capped poly(lactic acid) (PLA) of approx. 0.2 dl/g inherent viscosity (Resomer® R202) and end-group uncapped 50/50 poly(lactic-co-glycolic) acid (PLGA) of approx. 0.38 dl/g inherent viscosity (Resomer® RG503H) were purchased from Boehringer-Ingelheim (Ingelheim, Germany). Synthesis grade dichloromethane (DCM) was from EGT Chemie (Tae-gerig, Switzerland). Poly(vinylalcohol) (PVA, Mowiol® 4–88), used as dispersion stabiliser, was kindly donated by Kuraray Specialities (Frankfurt/M., Germany). Water was of NANOpure-quality.

Nanoparticles were produced using a modified solvent extraction/evaporation process [15]. PLA or PLGA was each dissolved in DCM at 2% and 5% concentrations. Water containing 0.5% (w/w) PVA was used as continuous phase. After pre-filling the equipment with continuous phase, the magnetic stirrer of the pre-mixing cell and the ultrasonic flow-through cell were activated, and polymer solution was injected into the pre-mixing cell. The flow rates of the polymer solution and continuous phase were set at a 1:8 ratio for all experiments. The product of the first 5 min of processing was discarded to let the process reach steady-state. Thereafter, the dispersion of nascent nanoparticles was collected for 30 min in a beaker pre-filled with 500 ml of continuous phase and gently stirred for a further 60 min to extract and evaporate the polymer solvent. For each set of parameters, two nanoparticle batches were prepared.

Particle collection for subsequent SEM analysis was done by centrifuging the nanoparticle dispersion at 5000 rpm for 5 min. The resulting pellet was re-dispersed twice in purified water and centrifuged. Finally, the washed nanoparticles were re-dispersed in 500 µl of purified water and freeze-dried.

2.4. Size measurement of oil droplets and PL(G)A nanoparticles

The size distribution of the oil-in-water emulsions and the nanoparticle dispersions was determined by laser light scattering (Mastersizer X, Malvern, Worcestershire, UK) using a Mie diffraction model taking into account the refractive indices of the oils, PLGA and water. All size distributions are presented in the volume-weighted mode. Following the common usage in the literature, the oil-in-water emulsions were characterised by the *surface*-moment average of the size distribu-

tion, $D[3,2]$, also called Sauter diameter, while for the nanoparticles, the diameter calculated from the *volume-moment* average of the size distribution, $D[4,3]$, was chosen as characteristic mean diameter.

3. Results

3.1. Oil-in-water emulsions

The coarse emulsions produced by the two serial pre-mixers were compared with the emulsions further processed in the ultrasonic flow-through cell by light microscopy. The pre-emulsions exhibited oil droplets measuring mostly from 50 to 200 μm (Fig. 3A and B) and strongly tending to coalesce. After processing in the ultrasonic cell, the droplet size was reduced by a factor of approximately 100, yielding mean diameters of less than 1 μm (Fig. 4). While occasional oil droplets of 5–10 μm could be observed in emulsions processed at an ultrasonic power of 25 W (Fig. 3C, arrows), practically no droplets were microscopically visible at 32 W, i.e. at full power (Fig. 3D).

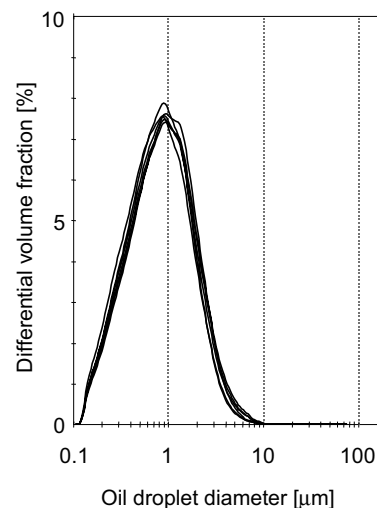


Fig. 4. Droplet size distribution for repeated production of a 20% (v/v) olive oil in water emulsion. Emulsion production was repeated six times under identical conditions ($t_R = 13$ s, 32 W sonication power).

The repeatability of the oil-in-water emulsification at full sonication power (32 W) was generally good irre-

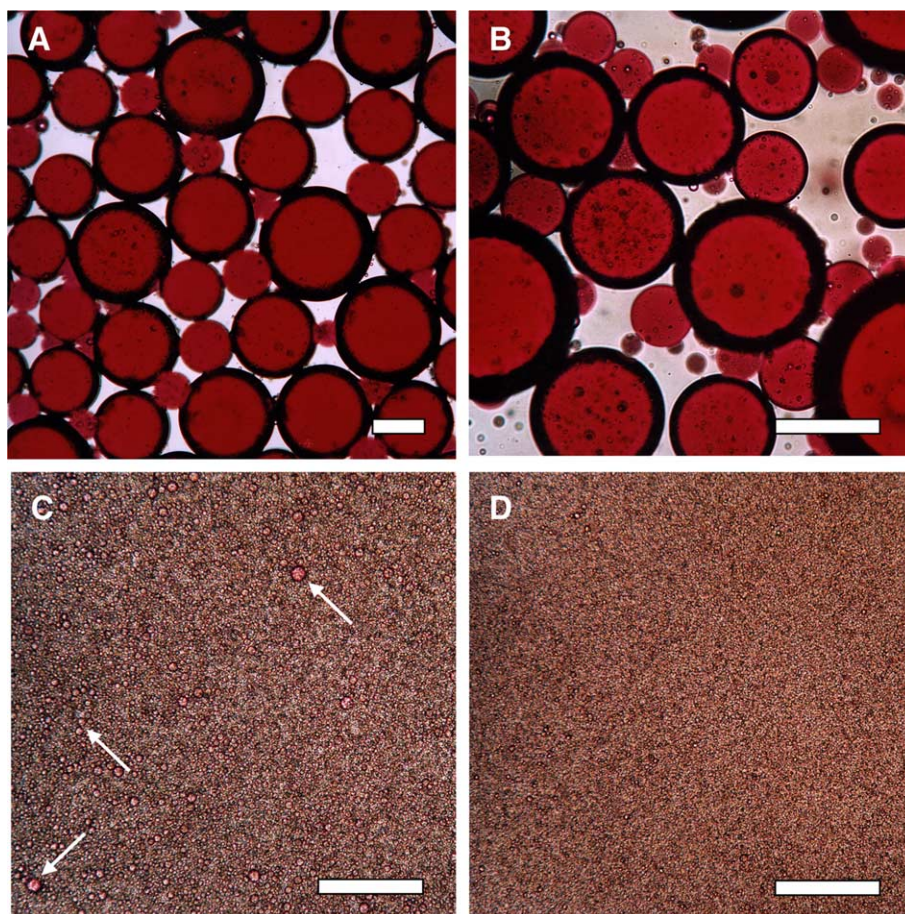


Fig. 3. Light microscope micrographs of coarse, pre-mixed (A,B) and post-ultrasonication (C,D) emulsions. (C) and (D) show emulsions processed at 25 W and 32 W sonication power, respectively. Arrows in (C) point at single larger droplets. Emulsions were prepared with 20% (v/v) olive oil (stained with Fat Red 7B) in water; residence time $t_R = 13$ s. Bars represent 100 μm .

spective of the residence time, the oil-to-water ratio and oil type. As an example, for six batches of olive oil in water emulsions prepared under identical conditions at maximum power virtually superimposed, mono-modal distributions were obtained (Fig. 4).

When sonication power was decreased from 32 W to 25 W, the Sauter diameter increased from 0.54 to 0.73 μm at likewise slightly increased batch-to-batch variability (Fig. 5). The larger droplet mean diameter resulted from an increased span rather than a shift of the size distribution. At 32 W, the 10 and 90% percentiles of the droplet size distribution amounted to 0.26 and 1.88 μm whereas at 25 W values of 0.30 and 3.69 μm were obtained. A further decrease of the sonication power to 17 W yielded a very inhomogeneous emulsion

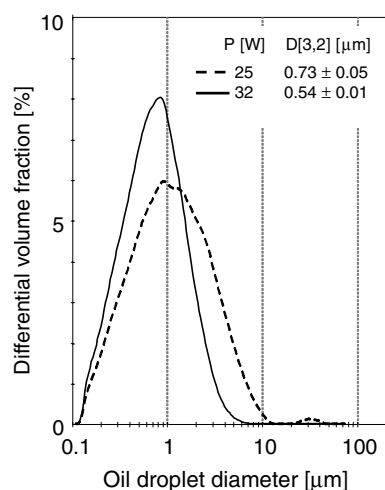


Fig. 5. Change of the droplet size distribution of a 20% olive oil in water emulsion with sonication power: 25 W (- - -) and 32 W (—). Residence time $t_R = 25$ s. For both power levels, the resulting emulsion Sauter diameter $D[3,2]$ is given as mean of three batches \pm standard deviation.

with a large number of macroscopically visible droplets of >1 mm and therefore, this experiment was not repeated. Sonication below 17 W did not afford proper emulsification.

The residence time of the emulsions in the ultrasonic flow-through cell was controlled by proportionally varying the flow rate of both the oil and water. The oil content of the emulsion was altered by varying the oil flow rate and keeping the water flow rate constant. Irrespective of the oil content, it was observed that the mean droplet size decreased with longer residence time of the emulsion in the sonic field (Fig. 6, left panel). The droplet size reduction occurred in a degressive manner, i.e. increasingly longer residence times were required to reduce the mean droplet diameter by a given decrement. For none of the curves, the diameter could be reduced below that displayed for the longest respective residence time; a more prolonged sonication did not further improve results. Therefore, 0.65, 0.50 and 0.47 μm represent the limiting Sauter diameters achievable for 33%, 20% and 11% olive oil-in-water emulsions with the present ultrasonic flow-through cell. The variability for repeated production was generally low as reflected by standard deviations of generally below 0.03 μm . For a fixed residence time, the emulsion mean droplet diameter increased with the oil content. In agreement with the findings for varied sonication power, the decrease of the mean droplet size with increasing residence time is caused by a narrowing span of the size distribution. For an 11% olive oil in water emulsion and residence times of 7, 14 and 28 s, size ranges of 0.30–2.98, 0.24–1.67 and 0.23–1.39 μm (10–90% percentiles) were obtained (Fig. 6, right panel). All droplet size distributions were mono-modal. For the 33% olive oil emulsions and the most shortly sonicated 20% emulsion, very few macroscopically visible oil droplets of >1 mm in diameter were observed. These droplets were not detectable by

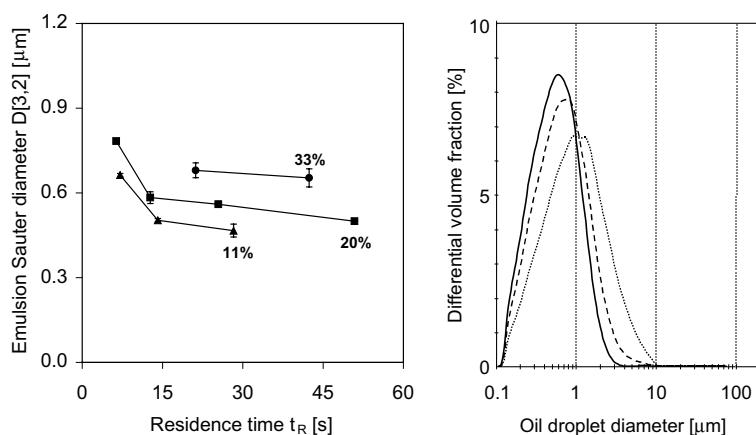


Fig. 6. Left panel: Sauter diameter as a function of the residence time in the ultrasonic field for 11% (▲), 20% (■) and 33% (v/v) (●) emulsions of olive oil in water. Error bars are for repeated productions ($n = 3$). Right panel: Influence of the residence time on the droplet size distribution of 11% (v/v) olive oil in water emulsions: 28 s (—), 14 s (- - -) and 7 s (· · ·). Sonication power was set to 32 W for all experiments.

laser light scattering. Sonicating the 33% emulsion for only 11 s resulted in an increased occurrence of large droplet of >1 μm ; therefore, this experiment was excluded from droplet size determination.

Finally, vegetable oils of different viscosity and interfacial tension towards the water phase were compared. When olive oil with the highest viscosity and interfacial tension was exchanged for soybean oil, which has slightly lower viscosity and interfacial tension, almost no reduction in the droplet size was observed (Table 1). Linseed oil, whose viscosity and interfacial tension are much lower than those of olive oil, yielded a clearly decreased Sauter diameter of 0.47 μm compared to 0.62 μm for olive oil.

3.2. PL(G)A nanoparticles

Nanoparticles with a mean diameter of 0.49 μm were readily prepared from a 2% PLGA solution in DCM at 32 W sonication power (Table 2). The size distribution was mono-modal with a slight tailing (Fig. 7A). Nanoparticle sizes extended from 0.18 to 0.76 μm according to the 10 and 90% percentiles. Repeatability of the production process was good for all preparations examined, as reflected by superimposed size distributions (not shown) and only minor variability in the mean particle diameter (Table 2). Lowering the emulsion's residence time in the sonic field from 14 to 7 s had only a minor impact on the nanoparticle size. A reduction of the sonication power from 32 to 25 W, however, resulted in a significant increase of the mean particle size from 0.49 to 0.7 μm , caused by a more pronounced tailing of the size distribution curve (Fig. 7A). A less prominent,

though significant increase in the mean particle size from 0.49 to 0.6 μm was found when using a 5% instead of a 2% PLGA solution. Finally, the more hydrophilic PLGA was exchanged for the more hydrophobic and lower molecular weight PLA without noticeable changes in particle mean size and size distribution.

No differences could be observed in the morphology of the different particles prepared from 2% polymer solutions. They all exhibited perfectly spherical shapes and smooth surfaces (Fig. 7B). The particles made from the 5% PLGA solution, however, were less spherical, showed slightly wrinkly surfaces, and fusions of two or—less frequently—more particles were observed (Fig. 7C).

When particles were produced from a 2% PLGA solution using water saturated in DCM, no difference could be seen in terms of morphology and particle size compared to particles made using non-saturated water (not shown).

4. Discussion

4.1. Working principle of the ultrasonic flow-through cell

The key innovation of the ultrasonic flow-through cell examined in this study was the transmission of sonic waves from an ultrasound emitting source (sonotrode) to the liquid mixture to be emulsified via a pressurised transmission fluid. The transmission fluid surrounded a glass tube through which the emulsion flowed. Using this set-up, direct contact between the sonotrode and the emulsion was prevented, avoiding

Table 1
Influence of the oil type on the droplet size of an oil-in-water emulsion

Oil type	Viscosity, η [mPas]	Interfacial tension ^a , $\sigma_{o/w}$ [mN/m]	Sauter diameter, D [3,2] [μm]
Linseed oil	43.9	1.32	0.47 \pm 0.01
Soybean oil	59.3	2.20	0.60 \pm 0.01
Olive oil	72.3	2.65	0.62 \pm 0.01

Emulsions were prepared at 32 W sonication power, a residence time of $t_R = 13$ s and an oil content of 20%. Data are given as mean of three production runs \pm SD.

^a Interfacial tension between the vegetable oil droplets and an aqueous solution of 3% Tween 40.

Table 2
Mean diameter of PLGA50:50 and PLA nanoparticles prepared under different conditions

Polymer type ^a	Polymer concentration [% (w/w)]	Sonication power [W]	Residence time [s]	Mean particle diameter, D [4,3] [μm]
PLGA 50:50	2	32	14	0.49 \pm 0.02
PLGA 50:50	2	32	7	0.50 \pm 0.02
PLGA 50:50	2	25	14	0.70 \pm 0.02
PLGA 50:50	5	32	14	0.60 \pm 0.01
PLA	2	32	14	0.48 \pm 0.00

Data are given as mean of two nanoparticle batches \pm absolute deviation.

^a PLGA 50:50 was Resomer[®] RG 503H; PLA was Resomer[®] R202

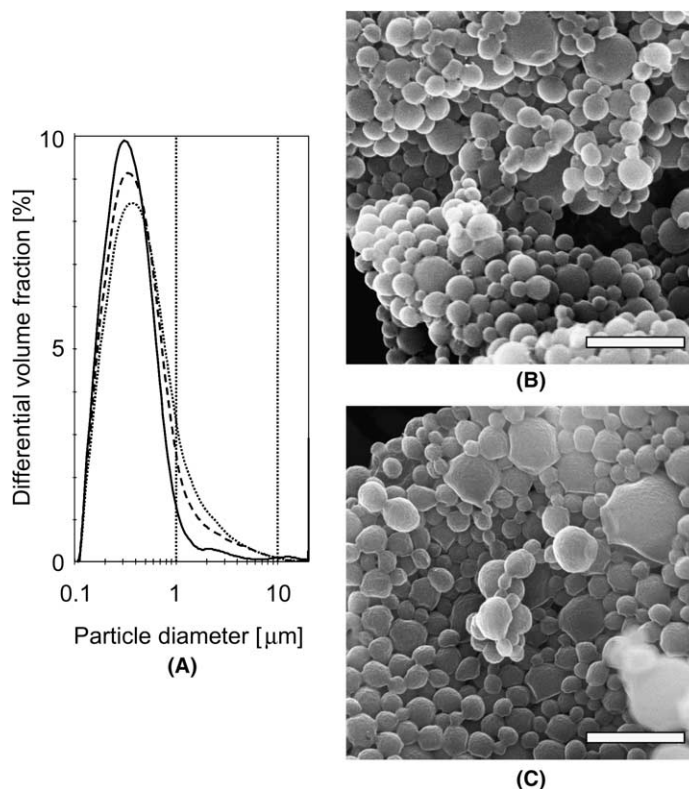


Fig. 7. PLGA nanoparticles. (A): Size distribution of particles prepared at a polymer concentration/sonication power of 2%/32 W (—), 5%/32 W (- - -), and 2%/25 W% (· · ·); residence time $t_R = 14$ s. (B) and (C): SEM pictures of particles prepared of 2% and 5% polymer solutions, respectively. Residence time $t_R = 14$ s; sonication power was set to 32 W. Bars represent 1 μ m.

contamination of the emulsion with ions or particles eroded from the metallic sonotrode by cavitation. Furthermore, as the glass tube is the only part of the ultrasonic cell coming into contact with the emulsion, cleaning and aseptic production is facilitated. Prior to production, the tube can be sterilised by any suitable means and easily installed into the cell under aseptic conditions. At the end of production, the tube can be removed from the cell and either cleaned or exchanged for a new one.

Cavitation is a well described phenomenon in the interaction of high-intensity ultrasound with liquids [16]. Very briefly, above a specific sound intensity, microbubbles form in the liquid during the low-pressure phase of the sonic oscillation. After oscillating for several pressure cycles accompanied by an overall bubble growth, these microbubbles will collapse resulting in a shock wave with local pressure and temperature of up to 1000 bar and several thousand Kelvin [16]. However, when a liquid is pressurised above a specific pressure threshold, which depends on temperature and the physicochemical characteristics of the fluid, cavitation will not occur. For the experiments accomplished in this study, pressures of above 4.5 bar were found to be sufficient to suppress cavitation in the transmitting water. Thus, acoustic energy was efficiently transferred via

the glass tube into the emulsion. As the latter was not pressurised, cavitation occurred, resulting in break-up of the oil droplets.

Ultrasonic emulsification was described as a two-step process [17,18]. First, instable interfacial waves form at the oil-water interface, which results in the eruption of rather large oil droplets (approx. 50–100 μ m) into the water phase. Second, the shock waves of cavitation events in the close vicinity of the coarse oil droplets will cause their disruption into much finer droplets. In viscous fluids, the formation of instable interfacial waves is compromised. Thus, a pre-mixing step producing a coarse emulsion, which can be readily broken up further by cavitation, is beneficial for such substances. In our experiments with the ultrasonic flow-through cell, emulsions of vegetable oils in water could not be prepared without pre-mixing. Pre-mixing is indeed crucial when using the flow-through cell, as compared to a sonotrode-beaker set-up, as the latter will provide streaming and, thus, macroscopic mixing which does not occur in the flow-through cell.

4.2. Oil-in-water emulsions

Optical micrographs of the emulsions prepared at 25 and 32 W power output (Fig. 3C and D) mirrored well

the droplet size distributions obtained by laser light scattering (Fig. 5). Larger droplets of 5–10 μm were observed microscopically and by light scattering analysis for the emulsions prepared at 25 W sonication power, but not for the preparation treated with 32 W.

The emulsion droplet mean size decreased both with increasing sonication power and residence time, in agreement with findings in the literature [6,7,19]. Both measures increase the energy transferred to the emulsion by enhancing the absolute number and, for increased power, also the intensity of the cavitation events, resulting in a more effective droplet size reduction. However, an optimal power input exists above which coalescence will become predominant resulting in a re-increase of the droplet mean diameter [16]. The narrowing of the droplet size distribution observed with both increased sonication power and residence time results from the susceptibility of larger droplets to be broken up by cavitation, while smaller droplets are more resistant or even stable. Enhancing the number of cavitation events by either increased power or residence time predominantly affects larger oil droplets and leads to an accumulation of fine droplets (given that coalescence is comparatively slow).

An increased oil content in the emulsion yielded, at fixed sonication power and residence time, larger oil droplets, which is agreement with earlier studies [7]. As cavitation occurs predominantly in the continuous aqueous phase [16], a larger fraction of oil requires more cavitation events per unit volume emulsion. Therefore, more concentrated oil-in-water emulsions require longer residence time to yield a desired mean droplet size. Nonetheless, the limiting droplet diameter increased with the oil content even under prolonged sonication. This is probably due to intensified coalescence in the concentrated emulsions as well as to changes in the sound conduction and cavitation properties of the liquid mixture. Moreover, higher oil contents would possibly have required increasing amounts of surfactant for stabilisation.

In emulsions with high oil content, few macroscopically visible oil droplets survived the sonication process. As these droplets were considerably larger than those produced by the pre-mixer, they must have formed by accumulation and coalescence of coarse pre-emulsion droplets in the inlet tube or the nodes of the ultrasonic cell. Repeatedly passing the emulsion a second or more times through the cell abolished this problem.

When different vegetable oils were compared, as could be expected from literature [6,16], the least viscous linseed oil yielded a reduced Sauter diameter. The break-up of low viscosity droplets will require less vigorous cavitation shock waves than needed for more viscous ones, promoting break-up. However, a decrease in the emulsion droplet mean diameter was not observed when olive oil was exchanged for soybean oil, which has an

intermediate viscosity. While the interfacial tension between the aqueous surfactant solution and the olive ($2.65 \pm 0.23 \text{ mN/m}$) and soybean oils ($2.20 \pm 0.18 \text{ mN/m}$) was found to be similar, that of linseed oil, however, was significantly lower ($1.32 \pm 0.20 \text{ mN/m}$). Thus, for linseed oil, reduced interfacial tension and viscosity may have added to result in simplified droplet disruption, while for soybean oil these parameters were not sufficiently different from those of olive oil to exert a significant effect.

The energy density, i.e. the energy input per unit volume of emulsion, was estimated from the power dissipated per unit volume of emulsion divided by the emulsion flow rate. In the production of a 20% vegetable oil-in-water emulsion, energy densities of around 10^8 J m^{-3} were required to yield Sauter diameters of approximately 0.4–0.5 μm [19]. For the ultrasonic flow-through cell examined here, the power consumption of the high frequency generator was measured instead of the power dissipated in the emulsion. An energy consumption of approx. 10^9 J m^{-3} was found to yield a Sauter diameter of 0.5 μm . Relating this energy consumption with that required for emulsification, an energetic efficiency of approx. 10% was obtained. Therefore, the energy transfer in the flow through cell is less efficient than in a classical system of a sonotrode installed in a beaker (26–75%) [13,14]. The lower efficiency of the ultrasonic cell could be expected from the more complex mechanism of energy transfer. In the beaker set-up, the sound emitting sonotrode is in direct contact with the emulsion, while in the flow-through cell, the sonotrode excites the steel jacket, which in turn transfers the energy to the pressurised water and the glass tube, which then finally excites the emulsion. Moreover, the mode of excitation is different in both systems. In the beaker system, the sonotrode basically performs a longitudinal vibration, whereas the steel jacket of the flow-through cell transforms the longitudinal oscillation of the attached sonotrode into a transversal oscillation. Finally, as the volume sonicated in the present cell is very small (0.53 ml), the energy expended for exciting the apparatus itself largely outweighs the energy expended for emulsification. With slightly to moderately larger diameter glass tubes or a manifold of parallel small tubes installed in the cell's steel jacket, the sonicated volume would increase, probably without much impact on the overall energy consumption.

4.3. *PL(G)A nanoparticles*

Solvent extraction/evaporation employing ultrasonic emulsification is a common process for the preparation of biodegradable PLA/PLGA micro- and nanoparticles [15,20]. Briefly, it consists of forming an emulsion of an organic solution of the polymer in usually an aque-

ous continuous extraction phase. A drug to be encapsulated may be co-dissolved or dispersed in the polymer solution. The organic solvent is then either extracted by the continuous phase (solvent extraction), diffuses into the same and evaporates to the environment (solvent evaporation), or is removed by a combination of both. The size of the resulting particles is mainly determined by the emulsification conditions, but other factors like polymer concentration, viscosity, the ratio of dispersed to continuous phase do also play a role. Although ultrasound is frequently used to generate emulsions for nanoparticle preparation, high-pressure homogenisation or rotor–stator dispersion are equally employed.

In the present study, we demonstrate the feasibility of nanoparticle preparation in the ultrasonic flow-through cell, opening the road to nanoparticle production under aseptic conditions. For PLA/PLGA nanoparticles, emulsion formation in the ultrasonic flow-through cell was fast, as the reduction of the residence time from 14 to 7 s did not markedly increase the particle diameter. In analogy to the oil-in-water emulsions, particle sizes increased at decreased sonication power, though the differences in size distribution were less pronounced than observed for the oil emulsions. By increasing the polymer solution concentration, and thereby its viscosity, larger particles were produced. Interestingly, no impact on the particle size was observed when PLGA was substituted by PLA having roughly half the inherent viscosity, while in the literature a correlation between polymer inherent viscosity and nanoparticles size was reported [15]. Obviously, viscosity is not the only physicochemical parameter governing emulsification, as already noted for the oil-in-water emulsions. Factors like interfacial tension and suitability of the employed surfactants are equally important, especially with respect to droplet coalescence.

The deformed and partially fused nanoparticles observed for the 5% PLGA solution probably resulted from rapid solvent deprivation and particle solidification, reducing the time available for droplet break-up (larger and fused particles) and shape rearrangement.

5. Conclusions

In the present study, a novel technology for the continuous treatment of fluid mixtures with ultrasound was characterised using vegetable oil-in-water emulsions as a model system. The flow-through equipment consisted of a glass tube for the conduction of the sonicated fluid, which was installed in a steel jacket excited by a transducer and filled with pressurised water for the transmission of the sound waves. Its design makes this apparatus well suited for aseptic processing, an important issue in pharmaceutical development and production. The mean

droplet diameter of oil-in-water emulsions could be decreased by two orders of magnitude starting from coarse pre-emulsions and yielding Sauter diameters of 0.5 μm and below. The reduced efficiency of sound energy transfer compared to directly contacting sonotrode and fluid might be improved by scaling-up the cell. Furthermore, the ultrasonic flow-through cell was found to be well suited for emulsion-solvent extraction/evaporation based production of biodegradable polymeric nanoparticles. Future research will be directed towards scaling-up the process and increasing the power input to yield even finer emulsions. In addition, the suitability of the cell for the preparation of water-in-oil emulsions, e.g. for further processing into drug-loaded microspheres, will be studied.

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