The preparation of prednicarbate nanoemulsions – a comparison of three homogenizers

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ABSTRACT: It is a fact, that approximately 40 % of the new chemical entities are poor water soluble, which limits the bioavailability. The combination of the cook, here the scientist, the meal, thus the drug, and the kitchen, a laboratory, does not fit. So, what should be done to solve the problem? Where there’s a will, there’s a way. Creativity is a possible solution. Both can be realized with nanonization by different methods like chemical reactions, bottom up and top down techniques. The main focus in this article is on particle size reduction and stability of an emulsion containing prednicarbate as the drug by high pressure homogenization with two different homogenization types (microfluidization and piston-gap homogenizers) and three different homogenizer models (Microfluidizer M-110Y and the two piston-gap models EmulsiFlex C5 and APV Gaulin LAB 40). There is not much of a difference between the homogenizers according to their capacity to reduce the particle size of the emulsion. The performance of the homogenizers was investigated by measuring the particle size and size distribution of the nanoemulsion and their stability after storage. © Global Scientific Publishers 2013

KEYWORDS: Nanoemulsion, high pressure homogenization, piston-gap homogenizer, microfluidizer, prednicarbate.

1. Introduction

Forty percent (40%) of the drugs are in the development and 60 % of the newly synthesized drugs are poorly soluble [1]. An alternative way has to be created to make these drugs suitable for drug research. Regarding the increasing number of poorly soluble drugs, some techniques were created for the nanonization development. Three different methods, including chemical reactions, bottom-up and top-down techniques, are available for the production of nanoparticles.

Chemical reactions are usually not used for manufacturing nanoparticles containing pure active pharmaceutical ingredient (API), but used for the production of pharmaceutical coating materials, e.g. latex dispersions. By using this technique polymeric nanoparticles made of a matrix forming polymer can be obtained.

The beginning of the bottom-up process is preparing drug molecules in solution. The drug molecule precipitates in larger formation when the conditions of the solution are changed. The classical precipitation starts with solving the drug in water and mixing organic solvent. Mixing this drug solution with an aqueous phase induces precipitation, referred as “solvent/antisolvent” approach. These processes were made several times [2, 3]. The evaporation precipitation into aqueous solution (EPAS) is known as one of these processes [4]. Unlike the usual method, the API is dissolved here in an organic solvent, which is not water-miscible. Afterwards this solution is sprayed into heated water which is leading to an immediate evaporation of the organic solvent and forming the nanoparticles. Alternative methods were developed, known as the spray-freezing into liquid (SFL) [5] and the ultra-rapid freezing (URF) [6]. Academic research groups and companies also developed nanonization techniques using supercritical fluid technologies [7, 8]. The most important supercritical fluid is carbon dioxide, which shows dual behavior above its supercritical point by having a low density, but acting as a solvent. That dual behavior is used for nanoparticle production. Two different principles can be used, where the supercritical fluid acts as solvent or antisolvent. The rapid expansion of supercritical solutions (RESS) is explained in the literature, when the API is soluble in supercritical fluids like carbon dioxide [9]. When the supercritical drug solution expands into an expansion chamber, the phase changes extremely fast from supercritical to gas-like state. The API leaves the expansion nozzle, looses solvent power and precipitates. That fast precipitation leads to very porous structured and usually amorphous API particles. Being typically solvent-free is the big advantage of the RESS technology. When the API is dissolved in an organic solvent
the supercritical antisolvent (SAS) method is referred. Using a special nozzle this solution is mixed with a supercritical fluid. The API precipitates, because it is not soluble in the supercritical fluid, and is collected as a fine powder [10].

Top down techniques are contrast to the bottom-up technology. They consist of the comminuting of large particles to nanoparticles by different disintegration methods like pearl / ball milling [11, 12] and high pressure homogenization. High pressure homogenization can be divided in piston-gap or microfluidization.

The wet ball milling method is generally used for the production of nanocrystalline dispersions, where a milling chamber contains milling media like zirconium dioxide beads, silicium nitride beads or polystyrene beads, aqueous stabilizer / surfactant solution and micronized API. High shear forces are caused by moving the milling media, which leads to attrition of the drug particles [13]. In order to produce at large scale, the dispersion can be pumped continuously through the milling chamber. The drug particles can be separated from the milling media by using a separate gap or a filter cartridge. Due to the fact that this technology shows the importance of particle size reduction by wet ball milling, five products are manufactured and marketing on the drug store.

The nanoparticles with a piston-gap homogenizer were the first technology that was developed based on high pressure homogenization (HPH) [14, 15, 16]. The coarse dispersion is compelled through a very tiny homogenization gap in the process. Cavitation forces, shear forces and particle collision are causing mainly the particle size reduction. HPH can be also performed in non-aqueous and in water-reduced media [17].

Furthermore, the nanoparticles can also be produced by using jet stream homogenizers and the high shear technology, known as microfluidization. Two fluids streams are colliding under pressures of up to 1700 bar, leading to particle diminution by collision, shear and cavitation forces. The need of 50 to 100 passes for a sufficient particle size reduction is time consuming, a major disadvantage of this technique [18, 19].

To the best of our knowledge there is no report in the literature about the comparison of these three different homogenizers, although the comparison of two different homogenizers are investigated, e.g. LAB 40 and Avestin B3 [20], and LAB 40 and Avestin C 50 [21].

In this study nanoemulsions (NEs) containing the poor water soluble drug prednicarbate (PC) were produced using the above three different homogenizers: EmulsiFlex C5 (C5), Microfluidizer M-110Y (M-110Y) and APV Gaulin LAB 40 (LAB 40). The aim of this study was to evaluate if one of these homogenizers is superior to the others according to its capacity producing stable NEs. For that purpose the particle characteristics like the particle size, the size distribution and the zeta potential of the NEs were investigated after storage.

### Table 1: Composition of the nanoemulsion

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oil phase</strong></td>
<td></td>
</tr>
<tr>
<td>Prednicarbate</td>
<td>0.25</td>
</tr>
<tr>
<td>Eutanol G</td>
<td>20</td>
</tr>
<tr>
<td>Phytosphingosine</td>
<td>0.6</td>
</tr>
<tr>
<td>Lipoid E 80</td>
<td>2</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Water phase</strong></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>2</td>
</tr>
<tr>
<td>Potassium sorbate</td>
<td>0.1</td>
</tr>
<tr>
<td>Water</td>
<td>ad 100</td>
</tr>
</tbody>
</table>

### 2. Materials and Methods

#### 2.1 Materials

Prednicarbate was a gift from mibe GmbH (Brehna, Germany) and phytosphingosine (2S-amino-1,3S,4R-octadecanetriol) was obtained from Degussa (Essen, Germany). Polysorbate 80 (Tween® 80, Uniquema, Everberg, Belgium and Merck, Germany, respectively) and a less purified egg lecithin (Lipoid E 80, Lipoid KG, Ludwigshafen, Germany) were chosen as emulsifiers because of their known stabilizing effects for NEs [22]. The liquid wax Eutanol® G (octyldodecanol, Caesar and Lorenz GmbH, Hilden, Germany) was used as oil phase, because results in primary studies showed that it was a suitable lipophilic compound for solubilizing phytosphingosine (PS) [23]. Potassium sorbate (Caesar and Lorenz GmbH) and α-tocopherol (Synopharm, Barsbüttel, Germany and DSM, Basel, Switzerland, respectively) were used as preservative and as antioxidant, respectively. All other chemicals used were of pharmaceutical grade and follow the specifications of the European Pharmacopeia.

#### 2.2 Methods

##### 2.2.1. Production of the nanoemulsions

The NEs (Table1) were produced by high pressure homogenization using an EmulsiFlex C5 (Avestin, Ottawa, Canada), Microfluidizer M110Y (Microfluidics, Newton, MA, USA) and LAB 40 (APV Deutschland GmbH, Unna, Germany). The oil phase and the water phase were prepared separately before homogenization. The oil phase consisted of Lipoid E80, α-tocopherol and Eutanol G as lipid base, PC as the drug, PS for the positive charge. PS was added to the heated Eutanol G (105–110 °C). The dispersion was kept at 105–110 °C and stirred with a magnetic stirrer until PS was dissolved completely. Before adding and solving the more heat sensitive com-
pounds α-tocopherol and Lipoid E80, the solution was cooled down to 75 °C. After that, the oil phase was cooled down to 50 °C and PC was added and dissolved in the oil phase. The water phase was obtained by dissolving Tween 80 and the preservative potassium sorbate in water, which was heated up to 50 °C. This water phase was added to the oil phase, adjusted to the homogenization temperature of 50 °C and the pre-emulsion was obtained by using the high speed stirrer Ultra-Turrax T25 (Janke and Kunkel GmbH, Staufen, Germany) and DI 25 basic, respectively, with 8000 rpm for 3 min at 50 °C and subjected afterwards to high pressure homogenization. After that the pH of this NE was adjusted to 5.5 ± 0.1 using diluted 0.1 N hydrochloric acid (HCl) to cause a positive charge (pKB of PS is approx. 9). At that point the used homogenizers C5, M110Y and LAB40 should be illustrated more precise.

2.2.2 High pressure homogenization

For the production of the positively charged PC containing NEs (see Table 1), three different homogenizers including two piston-gap homogenizers [EmulsiFlex C5 (C5) and APV Gaulin LAB 40 (LAB 40)] and one microfluidizer [Microfluidizer M-110Y (M-110Y)] were used. The properties of these three homogenizers are listed in Table 2.

2.2.2.1 EmulsiFlex C5

The high pressure homogenizer EmulsiFlex C5 has a capacity of 1-5 L/h, depending on the selected homogenization pressure. The C5 is working with a sample volume of 7 mL, at least, and can be processed with a hold back volume of less than 1 mL. The pneumatically controlled pressure is adjustable between 30 and 2000 bar (500 - 30000 psi). The C5 has an air/gas driven single-acting high-pressure pump. Due to a specially designed pump motor pilot valve the C5 facilitates a quiet operation. No “O”-rings and no gaskets are comprised in the entire product path. All face seals are precision-machined metal to metal or metal to ceramic. The C5 provides stainless steel heat exchangers to control the inlet and the outlet temperatures. For temperature controlling the entire C5 can be immersed in a water bath.

The C5 can be equipped with a high-pressure filter/extruder for the extrusion of emulsions or liposomes through membranes and sterile filtration at high pressures. The C5 is equipped with a dynamic homogenizing valve, which can be easily disassembled for cleaning or inspection. The entire C5 is suitable for Steam-In-Place (SIP) sterilization, for clean room and GMP manufacturing. Moreover all wetted parts are autocleavable. The application of C5 includes pharmaceuticals, chemicals, cosmetics, coatings, inks, food and more.

2.2.2.2 LAB 40

In the piston-gap homogenizer, the dispersion is forced with an extremely high velocity through a tiny homogenization gap, typically in the range of 5-20 µm (depending on the pressure applied and the viscosity of the dispersion medium). Using a Micron LAB 40, the formulation is supplied from a metal cylinder by a piston, and the cylinder diameter is approximately 3 cm. The formulation is moved by the piston having an applied pressure between 100 and 1500 bar. The piston-gap homogenizer corresponds to a tube system in which the tube diameter narrows from 5 to 20 µm. A self-made temperature control jacket was used for the production at elevated temperatures.

2.2.2.3 Microfluidizer M-110Y

The Microfluidizer M-110Y is equipped with the following major components:

- Intensifier Pump
- Interaction Chamber
- Auxiliary Processing Module

The M-110Y has an air powered and explosion-proof intensifier pump supplying the desired operating pressure from 206 - 1586 bar (3000 - 23000 psi), enabling the processing of a wide variety of products. Inside the air motor, air pressure pushes a large piston which, in turn, pushes a smaller (product) chrome-plated plunger in the intensifier pump. When the large piston is transferred to the reduced area of the smaller product plunger, the air pressure over the area of the large piston is greatly intensified. An isolator separates the air motor and the pump. Therefore the product cannot be contaminated from the air supply or carried over into the air motor.

The product stream enters the interaction chamber and passes, with a constant flow-rate range of 100-500 ml/min through the unique wear-resistant ceramic-geometrically fixed micro channels, causing it to accelerate to very high velocities. The product is acted upon by two primary forces, which bring about the desired results:

- Shear - deformation of the product stream; occurring from contact with channel walls at high velocity.
- Impact - collision; occurring when the high velocity product stream impinges upon the discharge wall and/or itself.

The system is easy to operate and is designed for clean-in-place without disassembly.

All wetted metal parts are made of 300 series stainless steel, 17-4 PH stainless steel, or Nitronic 60. The product path is immersible in cooling or heating bath and an open jacket cooling or heating coil with a feed temperature range of -25°C to 75°C is possible. The sample size volume of 60 mL can be performed in a discontinuous way.
Table 2: Comparison of the homogenizers

<table>
<thead>
<tr>
<th>Properties</th>
<th>Emulsi Flex C5</th>
<th>M-110Y</th>
<th>LAB 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working</td>
<td>continuous-discontinuous</td>
<td>continuous-discontinuous</td>
<td>discontinuous</td>
</tr>
<tr>
<td>Pressure [bar; psi]</td>
<td>30 - 2000 bar (500 - 30000 psi)</td>
<td>206 - 1586 bar 3000 - 23000 psi</td>
<td>100 - 1600 bar</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1-5 L/h</td>
<td>100 - 500 mL/min</td>
<td>-</td>
</tr>
<tr>
<td>Volume [mL]</td>
<td>&gt; 7</td>
<td>60</td>
<td>20 - 40</td>
</tr>
<tr>
<td>Temperature range</td>
<td>-25°C to 75°C</td>
<td>-25°C to 75°C</td>
<td>depends on the unit</td>
</tr>
<tr>
<td>Advantage</td>
<td>small volume</td>
<td>batch size of up to 5 L</td>
<td>small dead volume</td>
</tr>
<tr>
<td>Disadvantage</td>
<td>sensitive valve</td>
<td>high dead volume</td>
<td>small batch of only 40 mL</td>
</tr>
</tbody>
</table>

2.3 Characterization

2.3.1 Particle size analysis

The particle size of the NEs was measured directly after their production (day 0) and on the days 7, 14 and 28 after storage at 25 ± 2 °C and 40 ± 2 °C. For measuring the mean particle size with dynamic light scattering (DLS) a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) was used. The DLS technique yields a light intensity weighted mean diameter (z-average) and the polydispersity index (PDI) as a degree for the width of the size distribution. The PDI below 0.2 indicates a narrow size distribution. DLS has an upper detection limit of approximately 3 μm. For excluding the existence or the occurrence of larger particles (emulsion droplets), low angle static light scattering (laser diffraction, LD, LS 230, Beckmann-Coulter, Krefeld, Germany and Mastersizer 3000, Malvern, UK) was used. Mie theory with the optical parameters 1.456 (real refractive index) and 0.01 (imaginary refractive index) were used for analyzing the LD90 results. LD90 means that 90 % of the measured particles are smaller than the given value.

2.3.2 Zeta potential

The surface charge of the NEs, expressed as zeta potential, was determined by measuring the electrophoretic mobility. 20 μL of the sample was added to 40 mL purified water and measured. A pH of 5.5 ± 0.1 is required for all measurements and is adjusted, if necessary, with diluted HCl. Prior to the measurement, the mixture was adjusted to a conductivity of 50 μS/cm using % 0.9 sodium chloride (NaCl) solution. The zeta potential was calculated by applying the Helmholtz–Smoluchowski equation using the Zetasizer Nano ZS [24, 25].

3. Results and Discussions

3.1 Influence of production parameters

The formulation of the NE (Table 1) was homogenized with the three different homogenizer types C5, M110Y and LAB 40. There are reports in the literature about the comparison of two homogenizers like LAB 40 and Avestin B3 (Liedtke 2000) and LAB 40 and Avestin C 50 (Shegokar 2011), but to the best of our knowledge this is the first time that three homogenizers were compared. For investigating the performance of the homogenizers the homogenization time of 1, 2, 3, 4 and 5 minutes (C5 and M110Y) and the number of homogenization cycles of 3, 5, 8 and 10 (LAB 40), respectively, were examined according to the particle size, PDI and LD size. For that purpose two different aspects should be considered, the tendencies of the particle characteristics (Table 2) and the comparison of these characteristics to each other.

Although the process parameter homogenization pressure and homogenization temperature are very important for optimizing a formulation, they were only tested exemplary with LAB 40. The homogenization pressure of 500 bar and the homogenization temperature of 50 °C were kept constantly. The particle size of the NE produced with C5 did not change significantly with values from 169 nm after 1 min to 166 nm after 5 minutes (Fig. 1). The particle size of the NEs decreased only slightly from 224 nm (1 minute) to 207 nm (5 minutes) for M110Y (Fig. 1) and from 212 nm (1 minute) to 186 nm
(5 minutes) for the LAB 40 (Fig. 2). This result accords with other reports about the particle size reduction of NEs with increasing number of homogenization cycles (Yılmaz and Borchert 2005). Regarding the final particle size of the NEs with 166 nm (C5), 207 nm (M110Y) and 186 nm (LAB 40) reveals that the homogenizers do not differ significantly to each other (Figs. 1-2). The PDI of the NE homogenized with C5 and LAB 40, respectively, did not changed significantly with increasing homogenization time and decreased only slightly from 0.11 to 0.08 (C5) (Fig. 1) and from 0.1 to 0.09 (LAB 40) (Fig. 2), respectively. A clear reduction of the PDI from 0.21 to 0.07 could be achieved only when homogenized with M110Y (Fig.1). Again, the final PDI of the NEs produced with the different homogenizers are equal.

As expected, with an increased duration of homogenization and number of homogenization cycles, respectively, the LD size decreases for all three homogenizers from 280 nm to 221 nm (C5), from 206 nm to 161 nm (M110Y) (Fig. 3) and from 254 nm to 221 nm (LAB 40) (Fig. 2). At that point it is interesting that the LD size of the NE with 206 nm after a homogenization time of only 1 minute with M110Y is decreased compared to 221 nm after5 minutes for C5 and LAB 40. According to the LD size the M110Y has apparently an advantage over C5 (Fig. 3) and LAB 40 (Fig. 2). The zeta potential was only measured for the NEs homogenized with C5 and M110Y and revealed that although the zeta potential of the NE produced with the M110Y (64 mV) is much higher compared to C5 (43 mV) after 5 minutes (Fig. 3), both zeta potential values over 30 mV are generally regarded as physically stable [24, 25].

3.2 Stability of the nanoemulsions

3.2.1 Particle size analysis

The mean particle size of the NE produced with C5 was 166 nm at the day of production (day 0), 162 nm and 157 nm after storage of 28 days at 25 ± 2 °C and 40 ± 2 °C, respectively (Fig. 4). On day 0 the mean particle size of the NE homogenized with M110Y was 207 nm, 237 nm and 269 nm after storage of 28 days at 25 ± 2 °C and at 40 ± 2 °C and for the NE produced with LAB 40 a mean particle size of 186 nm on day 0 and 188 nm after storage at 25 °C was obtained (Fig. 6). The PDI and the LD size of C5 and LAB 40 are extreme stable and not significantly changing after storage (Figs. 4-6), while the LD size of the NE produced with M110Y is slightly increasing from 161 nm to 213 nm and to 231 nm after storage of 28 days at 25 ± 2 °C and 40 ± 2 °C, respectively. The PDI increased from 0.07 to 0.12 and to 0.23 after storage of 28 days at 25 ± 2 °C and 40 ± 2 °C.

3.2.2 Zeta potential

The zeta potential of both NEs decreased from 43 mV to 35 mV (C5) and from 64 mV to 52 mV (M110Y) after storage of 28 days at 40 ± 2 °C (Fig. 5).

A formulation with a zeta potential value over 30 mV is generally regarded as physically stable [24, 25]. Following that rule, our NEs are meeting that criteria with zeta potentials of greater than 30 mV, even the zeta potential values for both homogenizers decreased after storage at elevated temperatures.
Table 3: The tendencies of the NE characteristics produced with different homogenizers

<table>
<thead>
<tr>
<th></th>
<th>C5</th>
<th>M110Y</th>
<th>LAB40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time or number of cycles</td>
<td>↓</td>
<td>↓↓</td>
<td>→</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

→ = no significant change; (↓) = slight decrease; ↓ = decrease; ↓↓ = strong decrease

![Figure 3](image1.png)

Figure 3: The LD size and the zeta potential of the NE produced with C5 and M110Y as a function of the homogenization time.

![Figure 4](image2.png)

Figure 4: The particle size and the polydispersity index of the NE produced with C5 and M110Y after storage of 28 days at 25 ± 2 °C and 40 ± 2 °C.

![Figure 5](image3.png)

Figure 5: The LD size and the zeta potential of the NE produced with C5 and M110Y after storage of 28 days at 25 ± 2 °C and 40 ± 2 °C.

![Figure 6](image4.png)

Figure 6: The particle size, LD size and the polydispersity index of the NE produced with LAB 40 after storage of 28 days at 25 ± 2 °C.

4. Conclusions

Regarding the impact of the different homogenizers reveals that all three homogenizer types are capable to reduce the particle size of the formulation effectively, with a narrow size distribution and a sufficient zeta potential of greater than 30 mV (only for C5 and M110Y). The smallest particles of approximately 160 nm with a narrow size distribution (PDI of 0.07) could be achieved with the C5. The obtained LD size results of approximately 220-
230 nm were similar for all homogenizer types. Although the NE produced with M110Y showed the highest zeta potential of 52 mV, the C5 resulted also in an acceptable formulation. Each type has appropriate advantages and disadvantages according to the properties like the handling, volume, particle characteristics etc. of the resulting formulation (table 2). To sum up: for a target-oriented production of nanoparticles, the one or the other homogenizer can be preferred.

References