A novel bottom-up process to prepare drug nanocrystals
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application of a 3-way nozzle to prevent premature crystallization for large scale production
3.1. Abstract

In a previous study we have developed a novel process to produce drug nanocrystals. This process, “controlled crystallization during freeze-drying” has shown to be a successful method to increase the dissolution rate of poorly water soluble drugs [Chapter 2]. This process consisted of two steps: a solution of a matrix material (mannitol) in water was mixed with a solution of a drug (fenofibrate) in TBA. This mixture was frozen and subsequently freeze-dried at relatively high temperature (-25 °C). Since the solution of matrix and drug in the water-TBA mixture is thermodynamically unstable, it had to be frozen immediately and fast after preparation to prevent premature crystallization of the drug resulting in the formation too large drug crystals. Therefore, small quantities were manually mixed in a vial and this vial was immersed in liquid nitrogen. To make this process ready for large scale production, the modification of this batch process to a semi-continuous process by the application of a 3-way nozzle was studied. With this nozzle, the aqueous and TBA-solutions were pumped into the nozzle via two separate channels and mixed just at the moment they left the nozzle. Thorough mixing was facilitated by the atomizing air, supplied via the third channel. Since the mixture was sprayed immediately into liquid nitrogen, premature crystallization was prevented. A further advantage was that the atomizing air generated small droplets which were directly immersed into liquid nitrogen. Consequently, the mixture was frozen even faster than in the batch process. This resulted in a reduced size of the drug crystals and hence a higher dissolution rate. Therefore, using the semi-continuous process does not only result in successfully making this process suitable for large scale production of the controlled crystallized dispersions, but it also results in a better product.
3.2. Introduction

Many new drugs evolving from drug discovery techniques like High Throughput Screening can be categorized according to the Biopharmaceutical Classification System as class II drugs [1]. These drugs have a poor oral bioavailability due to their low aqueous solubility. However, since they are easily absorbed throughout the gastro-intestinal membranes [2; 10] their bioavailability can be improved by increasing their dissolution rate [3; 19; 28].

The dissolution rate can be improved by decreasing their size. According to the Ostwald-Freundlich equation, the saturation concentration at the surface of small particles, especially in the nanorange, is higher than the saturation concentration at the surface of large particles [5]. In addition, by decreasing the particle size, the total surface area available for dissolution increases and consequently also the dissolution rate [6]. Therefore, the use of drug nanocrystals has been shown to be a suitable strategy to improve the dissolution rate [20; 28].

Recently, we have developed a novel bottom-up method to prepare such drug nanocrystals with improved dissolution behavior: “controlled crystallization during freeze-drying” [29]. In short, two solutions were prepared: one of a lipophilic drug in TBA, and another of a matrix material (i.e. mannitol) in water. Both solutions were mixed and subsequently immediately and quickly frozen to a temperature well below the Tg’, after which the solvents were removed by freeze-drying. This freeze-drying step was performed at a relatively high temperature (above the Tg’, but below the Tg) to allow the drug and matrix material to crystallize in the freeze-concentrated fraction. It was shown that the size of the drug crystals could be varied by adjusting several process parameters (such as freezing rate and water/TBA ratio).

Although the above described process is suitable for lab-scale production, technical problems will be encountered when this process is changed to a large scale production process. The most important problem is that the solution of drug and matrix material in a mixture of TBA and water is thermodynamically unstable [9]. To prevent premature crystallization, the mixture should be frozen immediately after mixing and quickly to a temperature well below the Tg’. Therefore, until now only small quantities of the two solutions were mixed in a glass vial and the vial was immediately frozen in liquid nitrogen. However, when this batch process is scaled-up to a large scale production it is difficult to freeze it immediately after mixing and to freeze it sufficiently fast.

The aim of this study was to evaluate whether the above mentioned problems can be circumvented by using a 3-way nozzle. This nozzle consists of three channels through which the aqueous solution, TBA solution, and an atomizing air flow separately. The nozzle is designed in such a way that the atomizing air mixes the two solutions just at the moment they leave the nozzle. When this mixture is sprayed directly into liquid nitrogen it is immediately frozen after mixing. In addition, due to the atomizing air, small droplets are formed, which are therefore frozen almost instantaneously.

To validate whether the 3-way nozzle can be used for scale-up of the controlled crystallization...
process from batch to semi-continuous, we first studied whether two liquids were mixed efficiently by using this 3-way nozzle. Secondly, the crystallinity and the dissolution rate of controlled crystallized dispersions prepared by the batch process and the semi-continuous process were compared. Fenofibrate was used as model drug, while mannitol was used as model matrix material.

3.3. Materials

Potassium iodate (KIO₃), sodium hydroxide (NaOH) and sulfuric acid (H₂SO₄) were obtained from Merck (Darmstadt, Germany). Boric acid (H₃BO₃) was supplied by Lamers & Indemans (’s Hertogenbosch, Netherlands). Potassium iodine (KI) was supplied by Genfarma B.V. (Maarssen, Netherlands). Sodium dodecyl sulphate (SDS) was provided by BUFA B.V. Uitgeest, The Netherlands. Fenofibrate and TBA were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). Mannitol was obtained from Roquette (France). Demineralized water was used in all experiments.

3.4. Methods

3.4.1. Mixing quality of the 3-way nozzle

To determine whether two liquids mix rapidly after they left the 3-way nozzle-tip, the adapted Villermaux/Dushman method was used [30]. This method has been originally developed to measure the mixing quality of microfluidic devices, by using two parallel reactions taking place when an acidic and a buffered iodine/iodate solution are mixed [31]. During mixing, either the acid is involved in the formation of triiodine (in case of slow mixing) or the acid is neutralized by the buffer by which no triiodine is formed (in case of fast mixing). Therefore the amount of triiodine formed can be used as a measure for the mixing quality, since a poor mixing quality will cause some of the acid to react with iodine and iodate to form triiodine, which can be detected spectrophotometrically.

For this purpose two solutions were prepared: one consisting of 0.00635 M KIO₃ and 0.0319 M KI in a 0.0909 M NaOH/H₃BO₃ buffer and a second solution of 0.030 M H₂SO₄ in water. Perfusion pumps (NE300, New Era Pump Systems Inc., Wantagh, NY, United States of America) were used to pump both solutions in a 1:1 v/v ratio separately through the 3-way nozzle (Büchi Labortechnik GmbH, the Netherlands). The nozzle consists of three separate channels: 1) the inner channel for the first liquid, 2) the middle channel for the second liquid, and 3) the outer channel for the atomizing air. After spraying the solutions, triiodine formation in the mixtures was measured spectrophotometrically at 286 nm (ThermoSpectronic, unicaam UV 500). The tested spray-settings were similar to the settings used during spray freeze-drying: an atomizing airflow of 500 L/hr (i.e. 500 L of air at 1 atm and 0 °C), a total liquid flow of 15 mL/min and a distance to the sprayed surface of 60 mm. As control, the two solutions were mixed without atomizing airflow.
3.4.2. Preparation of the controlled crystallized dispersions

To prepare the controlled crystallized dispersions, two solutions were prepared. Fenofibrate was dissolved in TBA and mannitol was dissolved in water (for compositions see Table 3-I). Both solutions were heated to approximately 60 °C before they were processed using the batch or semi-continuous process.

### Table 3.I. Composition of the different solutions used to prepare the controlled crystallized dispersions.

<table>
<thead>
<tr>
<th>Before lyophilization</th>
<th>After lyophilization</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>$C_{\text{mannitol/water}}$ (mg/mL)</td>
<td>$C_{\text{fenofibrate/TBA}}$ (mg/mL)</td>
</tr>
<tr>
<td>39</td>
<td>25</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>17</td>
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</table>

For the batch process, both solutions were mixed in a 20 ml glass vial. The volumes of the aqueous- and TBA-solutions used were 1.2 and 0.8 mL, respectively. Immediately after mixing, the vials containing the mixture were immersed in liquid nitrogen and subsequently lyophilized. For the semi-continuous process the heated solutions were pumped separately through a heated 3-way nozzle, with a flow of 9 and 6 mL/min for the aqueous- and TBA-solutions respectively, using perfusion pumps. The liquid (total liquid volumes of 55, 70, and 90 mL for the dispersions having a drug load of 30, 40, and 50% w/w respectively) was then sprayed, with an atomizing airflow of approximately 500 L/hr, directly into a metal tray filled with liquid nitrogen. Subsequently the frozen material was freeze-dried.

Freeze-drying was done in a Christ model Epsilon 2-4 lyophilizer (Salm en Kip, Breukelen, The Netherlands). First the temperature of the samples was equilibrated on a pre-cooled shelf (-50 °C) for 1.5 hours. Secondly, the temperature was increased to -25 °C to allow the drug and matrix material in the freeze-concentrated fraction to crystallize. To remove the solvents, the pressure was decreased to 0.220 mBar after three hours. After at least 10 hours the temperature was gradually increased to 20 °C. The samples were stored in a desiccator over silica gel at room temperature for at least 1 day before further processing.

Physical mixtures of fenofibrate ($x_{50} = 13 \mu$m; determined by laser diffraction) and mannitol ($x_{50} = 170 \mu$m; according to Roquette specifications) were prepared using a spatula and mortar to mix both components.

3.4.3. Tabletting

Round and flat shaped tablets with a mass of 100 mg and a diameter of 9 mm were compressed on an ESH compaction apparatus (Hydro Mooi, Appingedam, The Netherlands) with a compaction pressure of 5 kN and a compaction rate of 5 kN/s. The die was lubricated with magnesium stearate. The tablets were stored in a vacuum desiccator over silica gel at room temperature for at least 1 day before further processing.
3.4.4. Differential Scanning Calorimetry
DSC measurements were used to determine the degree of crystallinity of the components in the solid dispersions. The degree of crystallinity was calculated by dividing the heat of fusion of the component in the solid dispersion by the heat of fusion of the pure component as received, multiplied by the fraction of the component in the controlled crystallized dispersion. It was assumed that the supplied materials were fully crystalline. Samples of 2-10 mg were filled into hermetically closed aluminum pans. The samples were scanned at a heating rate of 2 °C/min, from -50 to 200 °C on a Q2000 calorimeter (TA Instruments, Ghent, Belgium).

3.4.5. X-Ray Powder Diffraction
XRPD measurements were performed by using an X’pert Pro MPD diffractometer (PANalaytical, Almelo, the Netherlands). CuKα radiation with a wavelength of 1.5405 Å at 40 kV and 40 mA was used. Samples were scanned from 4-60° 2θ with a step size of 0.008° and a time per step of 35 s. The powders were placed on a zero-background silicon holder of 32 mm in diameter and 2 mm thickness (PANalaytical, Almelo, the Netherlands).

3.4.6. Scanning Electron Microscopy
A JEOL JSM 6301-F Microscope (JEOL, Japan) was used to record SEM-pictures. The powder was dispersed on top of double-sided sticky carbon tape on metal disks and coated with a thin layer of gold/palladium in a Balzers 120B sputtering device (Balzers UNION, Liechtenstein).

3.4.7. Dissolution
Dissolution measurements were performed in 1 L 0.5% w/v sodium dodecylsulphate solutions at 37 °C, using a USP dissolution apparatus II (Rowa Techniek, Leiderdorp, The Netherlands). The paddle speed was set at 100 RPM. The fenofibrate concentration during dissolution was measured spectrophotometrically (Ultrospec III, Pharmacia LKB) at a wavelength of 290 nm.

3.5. Results and discussion

3.5.1. Mixing quality of the 3-way nozzle
The nozzle is designed to rapidly mix two liquids using the atomizing air, which results in the formation of homogeneous droplets. To test whether the two solutions were indeed effectively mixed, the degree of mixing of a solution prepared with an atomizing airflow of 500 L/hr (settings as used for the preparation of the controlled crystallized dispersions) was compared with a solution which was prepared without atomizing air, using the adapted Villermaux/Dushman method.

When the atomizing airflow of 500 L/hr was used, the average absorption was much lower (0.222 ± 0.06) compared to the solution prepared without atomizing air (1.163 ± 0.08). The lower absorption shows that less triiodine has been formed. Since the formation of triiodine is an indication for slow mixing, a lower absorption indicates faster and thus better mixing. Therefore the lower absorption shows that the two solutions were properly mixed by the
Fig. 3.1. A) Typical examples of DSC thermograms of a physical mixture (30% w/w) of fenofibrate and mannitol (top), and two controlled crystallized dispersions containing 30% w/w fenofibrate in mannitol: one prepared by the batch process (middle) and the other by the semi-continuous process (bottom).

B) Typical examples of X-ray diffraction patterns of fenofibrate as received, α-, β- and δ-mannitol and two controlled crystallized dispersions containing 30% w/w fenofibrate in mannitol: one prepared by the batch process (freeze-dried) and the other by the semi-continuous process (spray freeze-dried) (patterns of the mannitol polymorphs were taken from the ICDD-library).
atomizing air. Hence it can be expected that the use of this nozzle to mix the aqueous- and TBA-solution, necessary for the preparation of the controlled crystallized dispersion, results in a homogeneous mixture.

3.5.2. From batch to semi-continuous
DSC and XRPD measurements were performed to determine the crystallinity of fenofibrate and mannitol in the dispersions. The DSC thermograms (see Fig. 3.1A) show that the obtained dispersions (30% fenofibrate in mannitol prepared by the batch and the semi-continuous process) are indeed crystalline, as can be seen from the peaks in the DSC thermograms that correspond to the peaks at the same temperature as the peaks appearing in the thermogram of the physical mixture. Three crystalline anhydrous polymorphs of mannitol have been identified and their melting points differ only slightly [24]. Therefore also XRPD was used to identify which polymorphs were formed. Fig. 3.1B shows typical examples of XRPD-diffractograms of fenofibrate as received, α-, β- and δ-mannitol and two controlled crystallized dispersions containing 30% w/w fenofibrate in mannitol: one prepared by the batch process and the other by the semi-continuous process. The diffractograms of the samples clearly show that both dispersions contained crystalline fenofibrate and crystalline δ-mannitol. Since δ-mannitol was shown to be stable at ambient conditions for a few years, no shelf-life problems are to be expected [24].

![Figure 3.2](image-url)

**Fig. 3.2.** Dissolution profiles of tablets composed of a physical mixture (○) and controlled crystallized dispersions of 30% w/w fenofibrate in mannitol. Controlled crystallized dispersions were prepared by the batch (●) and semi-continuous process (●). (n=3-6; mean).
Dissolution rates of fenofibrate from tablets prepared from a physical mixture were compared to those of crystallized dispersions of 30% w/w fenofibrate in mannitol prepared by the batch and semi-continuous process (Fig. 3.2). The dissolution rate of fenofibrate from tablets composed of a physical mixture, was low (less than 50% dissolved after 2 hours). The dissolution rate of fenofibrate was clearly improved when the tablets consisted of the controlled crystallized dispersions prepared by the batch process, as was also shown in a previous study [29]. The tablets composed of the dispersion prepared by the semi-continuous process showed an almost identical dissolution profile. Therefore it can be concluded that the modification of the batch process to a semi-continuous process was successful.

The dissolution rate of the samples prepared by the semi-continuous process appeared to be even slightly higher than the samples prepared by the batch process which may indicate the formation of smaller drug crystals. As described earlier [29], a higher freezing rate results in the formation of smaller drug nanocrystals which can be explained by the fact that when the temperature of the samples during freeze-drying is above the $T_g$, but below the $T_e$, crystallization of both drug and matrix material in the freeze-concentrated fraction can occur. The growth of the crystals is limited by the size of the interstitial spaces between the solvent crystals. Since a higher freezing rate will result in smaller solvent crystals and consequently in smaller interstitial spaces, the final drug crystals will be smaller. With the semi-continuous process, droplets of the mixture were formed by atomizing the feed mixture which were directly immersed into

![Fig. 3.3. Scanning electron micrographs of controlled crystallized dispersions of 30% w/w fenofibrate in mannitol prepared by the batch and semi-continuous process (the pictures on the left have a magnification of 1500x and the pictures on the right a magnification of 10000x).](image-url)
liquid nitrogen (see Fig. 3.3 for a SEM picture of a freeze-dried droplet), while for the batch process 2 mL of liquid in glass vials was frozen in liquid nitrogen. Although the Leidenfrost effect could occur during spraying the mixture into liquid nitrogen [32-33], it has been shown that extremely rapid freezing rates could be achieved by spray-freezing into liquid nitrogen [34], because the volume of the individual droplets is smaller than the volume of the liquid in the glass vial. Therefore we assumed that the freezing rate for the semi-continuous process was higher than that of the batch process, and consequently smaller drug crystals were formed during the semi-continuous process.

3.5.3. High drugloads
In the previous paragraph it was shown that modification of the batch process to a semi-continuous process was successful. But it seemed to have an additional advantage. The dissolution rate of the samples prepared by the semi-continuous process was slightly higher than the dissolution rate of the samples prepared by the batch method. As described above, we speculated that the increased dissolution rate was the result of a smaller drug crystal size of fenofibrate in the controlled crystallized dispersions prepared by the semi-continuous process. However, the difference in dissolution rate between the samples prepared by the semi-continuous and the batch process was small. Obviously this is explained by the fact that the dissolution rate of the samples prepared by the small batch process was already quite high.

Fig. 3.4. Dissolution profiles of tablets composed of physical mixtures (open symbols) and controlled crystallized dispersions (closed symbols) containing 40% w/w (□, ■, ■) and 50% w/w (▽, ▼, ▼) fenofibrate in mannitol. Controlled crystallized dispersions were prepared either by the small batch (black symbols) or the semi-continuous (grey symbols) process. (n=3-6; mean).
Since it can be envisaged that the dissolution rate of fenofibrate from a tablet with a higher drug load would be lower, it was expected that differences in dissolution rate of samples prepared by the two methods would be more pronounced. Therefore, the dissolution rate of fenofibrate from tablets with higher drug loads of 40 and 50%, prepared both by the batch and the semi-continuous process, was determined.

Fig. 3.4 shows that by increasing the drug load from 30 to 40 or even 50% w/w, the dissolution rate decreases. This can be explained by the fact that the mass of all tablets is similar and that the tablets become therefore more lipophilic. Due to the higher lipophilicity, the wetting of the tablets will be slower, and hence the dissolution rate of the drug from the tablets will decrease. This effect was observed for the dispersions prepared by both the batch process and the semi-continuous process. Fig. 3.4 also shows that the difference in dissolution rate between the tablets composed of controlled crystallized dispersions prepared by the batch and the semi-continuous process is much larger than when a lower (30% w/w) drug load is used. Since this difference could be explained by differences in crystallinity or in differences in particle size, DSC and SEM measurements were performed.

DSC measurements indicated that controlled crystallized dispersions of both drug loads were highly crystalline and had a similar degree of crystallinity (the degree of crystallinity of fenofibrate was 86-93% and 82-86% for the samples prepared by the batch process and the
Therefore, the large difference in dissolution rate must be attributed to a smaller drug crystal size. SEM pictures show that the particles of both spray freeze-dried samples are much smaller than the particles of the freeze-dried samples (Fig. 3.5). This indicates that the crystal size in the spray freeze-dried samples is indeed smaller than in the freeze-dried samples. Furthermore, these SEM-pictures illustrate that the particle architecture consists of agglomerates of crystals and was similar for all three drug loads (see also Fig. 3.3). In addition, it can clearly be seen that the particles are smaller than 1 μm, i.e. that they are of nanoscale. Therefore it is concluded that using the 3-way nozzle not only allows scaling-up of the controlled crystallization process, but also results in smaller crystals with a higher dissolution rate.

3.6. Conclusions

In this study, it was demonstrated that the controlled crystallization process can be modified from a batch process to a semi-continuous process by using a 3-way nozzle. The adapted Villermaux/Dushman method indicated efficient and fast mixing of solutions immediately after they left the nozzle. Furthermore it was shown that the dissolution rate of controlled crystallized dispersions of 30, 40, and 50% w/w fenofibrate in mannitol prepared by the semi-continuous process were all high. In fact, the dissolution rate was higher than that of controlled crystallized dispersions prepared by the batch method. Since the degree of crystallinity is similar for the dispersions obtained from both processes, the differences in dissolution rate can be explained by the differences in crystal size. The freezing rate during the semi-continuous process was higher than during the batch process. The higher freezing rate resulted in smaller drug crystals and consequently a higher dissolution rate. Therefore we concluded that the controlled crystallization process can successfully be made ready for large scale production by applying a 3-way nozzle. In addition, controlled crystallized dispersions prepared by the semi-continuous process also showed a higher dissolution rate and therefore tablets with a higher drug load can be prepared. Therefore this process is not only ready for large scale production, but it also results in a better product.
APPLICATION OF A 3-WAY NOZZLE TO PREVENT PREMATURE CRYSTALLIZATION FOR LARGE SCALE PRODUCTION