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Crystallization of cephradine polymorphs and hydrates from mixed solvents of methanol and water

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Abstract

Cephradine monohydrate (CPH) crystallizes with a needle-like shape which leads to severe agglomeration and slow filtration. Effects of different operating conditions on the particle size and shape as well as the filterability of the crystal products are presented. The solubility and stability of one anhydrate of cephradine (CPA1) and CPH as a function of temperature and water activity was investigated in different methanol and water mixtures. A solvent-mediated transformation from CPH to CPA1 was observed at water mole fractions below 0.2-0.35 depending on the temperature. The crystallization behavior of CPH was studied by linear cooling and antisolvent crystallization. Lowering the cooling rate and addition of seeds led to an increase in particle size and improvement of filterability.

Keywords: Anhydrate/hydrate, Cephradine, Crystallization, Particulate properties, Solubility

1 Introduction

Crystallization is a technique used in many chemical industries and especially within the pharmaceutical industries, where 90% of all active pharmaceutical ingredients (APIs) are separated and purified by this processing step. The most common methods used for crystallization are by cooling, solvent evaporation and addition of an antisolvent. These methods are used to create supersaturation, after which desupersaturation will occur through nucleation and hence crystallization. There are several key properties of the solid API that are formed during the crystallization process, such as crystal size, shape, polymorphic form and/or solvates. Consequently, the goal of any crystallization process is to produce crystals with the desired properties. The above-mentioned properties determine not only the quality of the final medicine product but also the efficiency of the downstream operations, such as filtration, drying and pharmaceutical manufacturing processes e.g. tableting. The solubility and dissolution rate are key factors that may influence the bioavailability of the API [1-3]. A historical case, illustrating the importance of polymorphism was the peptidomimetic drug ritonavir. The drug was released in 1996, but in 1998 a more stable polymorphic form of ritonavir was formed, which was much less soluble than the form used in the original formulation and forced the removal of the drug from the market [4].

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historical example emphasizes the importance of having in-depth understanding of the crystallization of an API that is capable of forming different polymorphs and/or solvates. Furthermore, the particulate properties of the crystals determine the processability of the API, such as flowability and compressibility and may also influence the physical and chemical stability. These properties are mostly related to the further downstream processing (DSP) and formulation of the API but are equally important to consider [5–8].

It is well known that certain crystal habits are difficult to process, where needles and flakes are the worst due to poor filterability and flowability. The size and shape of the crystals depends on the nucleation rate and the growth rate of different crystal faces, which can be influenced by the various operation parameters of the crystallization process, such as supersaturation level, solvent composition, mixing condition, etc. Particle engineering conditions can be used to alter the particulate properties such as crystal size and shape, leading to better properties. The growth of one or several specific faces of a crystal may be disrupted by a solvent or an additive making it possible to do crystal habit modifications. Unfortunately, not all APIs are prone to crystal shape modification and this modification may therefore not be successful. An additional particulate modification to be considered is the particle size distribution (PSD). Ordinarily, the goal is to have a narrow unimodal PSD, which may be large or small depending on the API. During the crystallization process, the particles are built up from molecules of the API, and the size is ultimately determined by the relationship between nucleation, crystal growth, breakage, and agglomeration. In general, the degree of supersaturation determines the relative kinetics of nucleation and growth, where a low degree of supersaturation yields fewer larger particles and a high degree yields many small particles. Furthermore, in the processing of APIs agglomeration is a common phenomenon of particles adhering and growing to larger agglomerates. Formation of agglomerates often occurs as a result of cohesive properties of the API. The agglomerated particles may cause a larger apparent particle size and inconsistent PSD influencing e.g. dissolution [9]. The effect of the operating conditions on agglomeration has been studied in both model-free and model-based work [10–12]. The main contributors to agglomeration were found to be the degree of supersaturation, particle size, stirring rate and solid content in suspension.

In this work cephradine, a first-generation cephalosporin antibiotic, was selected as the model compound. Cephradine is reported to form at least 4 anhydrous polymorphs, one monohydrate, and one dihydrate [13,14]. The anhydrous forms of cephradine have been observed to be very unstable and oxidize to the closely related compound cephalexin, whereas the hydrated forms are stable towards oxidation and are therefore preferred. The molecular structure of the model compound, as well as cephalexin, are shown in Fig. 1. Only the monohydrate and one anhydrous form of cephradine were observed in this work.

Figure 1.1 and Figure 1.2

The influence of the water activity on the solubility and stability of cephradine anhydrate/monohydrate in methanol and water mixtures was investigated and operating conditions at which CPH is obtained are established. From preliminary cooling crystallization experiments, long needle-like CPH particles were crystallized out, which led to a high degree of agglomeration and poor filterability of the solid product. The influence of different process parameters, such as cooling rate, solvent composition, seeding, and crystallization method, on the particulate properties and filterability of the crystal product CPH was investigated with the objective of improving particulate properties and filterability.
2 Materials and methods

2.1 Chemicals and analytical methods

CPH was purchased from Xi’an Wharton Biological Technology Co. Ltd., Shananxi, China. A cephradine anhydrate standard is purchased from Sigma-Aldrich, further referred to as CPA2. This anhydrous form was used for comparison of Raman spectra and on a High-Performance Liquid Chromatography (HPLC). HPLC grade Methanol and acetonitrile were purchased from VWR Chemicals, Ultrapure water was from a Purelab® Chorus from ELGA Veolia Purelab® Ultrapure Water Purification Systems. The cephradine anhydrate form one (CPA1) was prepared by dehydration of the monohydrate in a simultaneous thermal analyzer (STA 449 F3 Jupiter®) from NETZSCH. CPH samples were heated from room temperature to just past the dehydration point at 142 °C in a ceramic crucible at a heating rate of 10 K/min.

Raman spectroscopy and X-ray powder diffraction (XRPD) were used in this work to confirm the anhydrate/hydrate form obtained during the crystallization processes, as shown in Fig. 2. A Bruker Senterra Dispersive Raman microscope with a 785 nm laser operating at 100 mW with a 10 s integration time and two scans were used to collect the Raman spectra. The XRPD measurements were made on a Rigaku MiniFlex600 benchtop X-ray diffractometer equipped with CuKβ X-ray source operating at 40 kV and 15 mA. A continuous scan was recorded for all samples from 5° to 35° 2θ with a step size of 0.02° 2θ and a scanning rate of 10° 2θ min⁻¹. Furthermore, the anhydrous form of cephradine bought from Sigma-Aldrich was found to be different from the one obtained from dehydration of the commercial CPH. Both anhydrous forms were analyzed by mass spectrometry and found to be the same compound, therefore the difference observed in the Raman spectra is due to the presence of two different polymorphs of cephradine. As shown in Fig. 2 there are differences between the CPH, CPA1, and CPA2. Only CPH and CPA1 were obtained in this work. The size and shape of the crystallized CPH was studied using a LEO 435 VP scanning electronic microscope.

2.2 Solubility measurement

The solubility of CPH/CPA1 was measured under isothermal conditions in various water and methanol mixtures at the following temperatures 15, 25, 35, 45, and 55 °C. A suspension with an excess amount of solute (CPH) was prepared in 8 mL glass vials with 3 mL solvent. The vials were sealed and maintained under stirring for 6 hours at a constant temperature. A period of 6 hours was chosen based on the stability issue of the compound cephradine transforming into cephalexin. From preliminary solubility experiments, no change in solubility was found at samples measured at 6-8 hours and 24 hours, indicating that the solid/liquid equilibrium could be reached at 6 hours. The saturated solution of cephradine was separated from the excess solid phase with a syringe and through a 0.2 μm PTFE filter. The samples were diluted and analyzed with HPLC to determine the concentration of cephradine. The experiments were performed in triplicates and solubility profiles were made using the average value. The solid separated from the suspension after solubility measurement was analyzed with Raman spectroscopy and XRPD to confirm whether the transformation of CPH to CPA1 has occurred.

2.3 Crystallization of cephradine

Nucleation kinetics of cephradine was investigated by performing linear cooling crystallizations. A Mettler Toledo Easymax 102 Advanced Synthesis Workstation with two 100 mL glass reactors, overhead stirrer, and a solid-state thermostat cooling/heating jacket was used to characterize the Meta Stable Zone Width (MSZW) of cephradine. Nucleation of cephradine was detected by monitoring the turbidity of the solution with a CrystalEyes turbidity probe from HEL, UK. Saturated solutions of CPH were prepared at 55 °C based on the measured solubility. Primarily, the saturated

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solution of cephradine was heated to 60°C to ensure complete dissolution. Secondly, the solution was filtered with a 0.2 μm PTFE filter to remove any non-dissolved impurities. Finally, the clear solution was transferred to the EasyMax and maintained at 60°C to dissolve any crystals that might have formed during the filtration and transfer of the solution. Following this, the cooling was started towards 15 °C with different cooling rates. At the end of the crystallization, crystals were separated with a vacuum filtration. The time for complete filtration was recorded and the filtration rate was defined by dividing the total yield with the recorded time. The filtrated crystals were dried at room temperature in fume hood, and were analyzed with Raman spectroscopy, XRPD, and scanning electron microscopy (SEM).

2.4 Effects of cooling crystallization parameters and crystallization methods

Effects of different operating parameters in cooling crystallization on the particulate properties as well as on filterability and agglomeration behavior of CPH were investigated. Different cooling rates have been tested, and the influence of the composition of solvent, seeding, and crystallization methods was explored. Measurements were repeated in triplicates for each parameter, except seeding, which was only repeated in a duplicate. The parameters investigated in the present work are summarized in Tab. 1. Seeding was performed with seeds that had a particle size of 71 – 125 μm. The seed crystals were prepared with sifting, where the particles between the sieves 71 μm and 125 μm were collected and used for seeding.

The effect of crystallization method was investigated by a 2-step cooling crystallization and by antisolvent crystallization. The 2-step cooling crystallization was performed like the previous cooling crystallization but was cooled to 35 °C, kept at this temperature for one hour and then the particles were filtered off and dried. The remaining liquid phase was further cooled to 15 °C, kept at this temperature for one hour and then the particles were filtered off and dried. Antisolvent crystallization was performed using water as the solvent and acetonitrile as the antisolvent. From preliminary experiments, the highest solubility was found at a mole fraction of water and acetonitrile being 0.75:0.25. The acetonitrile was added continuously to reach a final mole fraction of acetonitrile of 0.7.

Table 1

3 Results and discussion

3.1 Solubility of cephradine

The solubility of CPH measured at different temperatures and with different water and methanol mixtures is shown in Fig. 3. From the figure, the conditions where the highest solubility was obtained was found at a temperature of 55 °C with the solvent being pure water. A decrease in the solubility was observed as the methanol fraction was increased and the temperature lowered. Furthermore, a steeper increase in solubility with temperature was observed at solvent compositions of high water mole fractions.

Figure 3

When solubility of a hydrate/anhydrate system is measured in mixtures of water and an organic solvent, solvent-mediated transformation between the anhydrate and the hydrate might happen. Grant and Higushi (1990) [15] used the equilibrium constant K, to describe the equilibrium between a hydrate and an anhydrate. They describe how, at a given temperature, the relative stability of the anhydrate and the hydrate depends on the water activity in the surrounding medium. In our previous work [16,17], the solubility and relative stability of the anhydrous and dihydrate of carbamazepine in mixed solvents of water and ethanol was investigated. It was observed that the water activity at which the anhydrate and the dihydrate are in equilibrium increases with the temperature, and the dependence follows a nearly linear trend. In the current work, analysis by Raman spectroscopy of the
excess solid recovered from the solubility measurements showed that CPH transformed to CPA1 at water mole fractions below 0.2-0.35 depending on the temperature. This shows how the solvent mixture of water and methanol at which the anhydrate/monohydrate are in equilibrium changes with temperature.

When performing cooling crystallization, the crystal yield depends on the slope of the solubility curve; the steeper a slope the higher the yield. Consequently, it was chosen to conduct linear cooling crystallization using the solvent compositions corresponding to 1, 0.9, 0.7 and 0.5 mole fractions of water and methanol.

3.2 Influence of cooling rate

Primarily, the nucleation kinetics of cephradine was investigated by measurement of MSZW at different cooling rates. Raman analysis of the solid form yielded from all cooling crystallizations, confirmed the formation of CPH. The nucleation point was detected by turbidity measurement and visual observation. Exemplary results of cooling crystallization of the saturated solutions with cephradine at 55°C are shown in Fig. 4. Fig. 4A depicts a cooling rate of 1 K/min, 4B a cooling rate of 0.5 K/min, and 4C a cooling rate of 0.1 K/min, obtaining a MSZW of 39.5 °C, 20 °C and 4 °C respectively. Moreover, it was observed that the rate of crystallization was affected by the different cooling rates. Comparing the three graphs, the slowest increase in turbidity was observed in C indicating slower crystallization, and should, therefore, yield larger crystals, compared to the other cooling crystallization experiments.

Figure 4 A, b and C

All measured MSZWs are shown in Tab. 2, summarizing the effect of cooling rate and solvent composition. If only the cooling rate is regarded, a decrease in MSZW is observed with the decrease in cooling rate.

Table 2

The PSD could not be determined, due to the powder not being free-flowing after filtration and drying. Verification of the potentially larger crystals from a narrow MSZW, observed at the different cooling rates in pure water, was done by SEM and is shown in Fig. 5. Evidently, it is observed that with the decrease of cooling rate an increase of the width of needle-like particles is observed in Fig. 5 C.

Figure 5 A,B and C

The filtration rate measured by filtration in a Buchner funnel is shown in Tab. 3. It is apparent that the filtration rate increases with the decrease of MSZW and cooling rate. This is expected as there will be more growth of the crystals as the MSZW becomes narrow, indicating larger crystals and hence lower filter cake resistance.

Table 3

3.3 Influence of solvent composition

The solvent composition may also play a role in determining the crystal shape and size. The growth rates of the different faces of the particle may change due to interactions between the solvent and the surface [9,18]. Furthermore, the extent of agglomeration may also be affected by the physical and chemical characteristics of both solvent and solute [19]. Three different solvent compositions of water and methanol were investigated to establish the effect of methanol content on the particulate properties and filterability of CPH. In Tab. 2 the MSZW obtained at the different solvent compositions are shown and it was observed that there was an increase in MSZW with an increase in mole fraction of methanol.
As shown in the crystal images in Fig. 6 and the filtration rate in Tab. 4, no correlation could be drawn between the methanol content of the solvent and filtration rate or crystal morphology.

Figure 6 A,B and C

| Table 4 |

3.4 Influence of seeding

Addition of seed crystals within a predefined MSZW allows the growth of seeded crystals and hence larger crystals should be obtained leading to better filtration, but it also may induce secondary nucleation [1]. A set of experiments was carried out aiming at investigating the influence of addition of 5% seed crystals on crystal morphology and filtration rate. Pure water and a cooling rate of 0.1 K/min were used. SEM images of the solid obtained from seeding with 5 % seeds and a non-seeded experiment are shown in Fig. 7. It is clearly illustrated that larger crystals were obtained by seeding. Furthermore, a threefold increase of filtration rate was gained from the seeded experiment in Tab. 5, highlighting the effect of seeding on improving the filterability of the crystal product.

Figure 7 A and B

Table 5

4 Influence of crystallization method

During the cooling crystallization of cephradine, particles seeming like free-flowing in the suspension were observed until a certain point in the cooling profile, where after a thick white slurry was observed. Because of this, a 2-step cooling crystallization was performed with the objective of filtering off the particles while still being free-flowing in the suspension and hopefully gaining free-flowing particles after filtration. Nevertheless, these early formed particles agglomerated and cake formation happened during filtration and free flowing particles were not obtained after filtration.

Additionally, the effect of antisolvent crystallization was investigated on the particle size and shape as well as on filtration. As mentioned, acetonitrile was used as the antisolvent and added continuously. Two different addition rates were considered being 7.75 ml/min and 3.75 ml/min. A thick slurry was observed when all the antisolvent was added, and cake formation happened during the filtration indicating that there was a non-significant effect on the particulate properties from antisolvent crystallization.

5 Conclusion

Investigation of the crystallization of the needle-like morphology of CPH which leads to severe agglomeration and slow filtration was performed. Primarily, the solubility of cephradine was measured in different solvent compositions of water and methanol at different temperatures ranging from 15 °C to 55 °C. Raman spectroscopy revealed that the monohydrate was transformed into an anhydrous form at water mole fractions below 0.2-0.35 depending on the temperature. The solubility was observed to decrease with the increase of methanol content. Comparing the increase in solubility with temperature in the high water content- and low water content-composition, the increase is significantly steeper at a high water content. The effect of four different operating conditions on the particulate properties and filterability of CPH was investigated. The MSZW was observed to increase with increasing cooling rate and with increasing mole fraction of methanol. A narrow MSZW and a slow increase of the turbidity was observed at the low cooling rate compared to the other cooling rates. This indicates a slower crystallization yielding larger crystals which was confirmed by SEM images and an increase of the filtration rate. Seeding led to a threefold increase of filtration rate. Additionally, methanol content increased the MSZW, but with no direct correlation.
and effect on the filtration rate and crystal morphology. The influence of the crystallization method was also investigated but with no positive effect on the particle size and shape as well as on the agglomeration and filtration. It was observed that the particle size and shape could be altered by changing the operating conditions, but as agglomeration and cake formation was observed during filtration, optimization leading to free-flowing powder remains to be investigated.

**Acknowledgments**

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**Symbols used**

**Abbreviations**

API Active pharmaceutical ingredient  
CPA1 Obtained cephradine anhydrate from the commercial monohydrate  
CPA2 Standard cephradine anhydrate bought from Sigma-Aldrich  
CPH Cephradine monohydrate  
DFF Danish Council for Independent Research  
DSP Downstream processing  
HPLC High-Performance Liquid Chromatography  
MSZW Meta Stable Zone Width  
PSD Particle size distribution  
SEM Scanning electron microscope  
XRPD X-ray powder diffraction
References


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### Tables

**Table 1.** Overview of the cooling rate, solvent composition and seeding used in the investigation of the operating parameter on the particle size and shape. The investigated operating parameters 1, 2, 3 and 4 are the cooling rate, solvent composition, seeding, and crystallization method respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cooling rate [K/min]</th>
<th>Solvent composition [Water: Methanol mole fraction]</th>
<th>Seeding [% w/w]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>1:0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>1:0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
<td>1:0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.9:0.1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.7:0.3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.5:0.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>1:0</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>0.1 (2-step crystallization)</td>
<td>1:0</td>
<td>5 in both steps</td>
</tr>
<tr>
<td>4</td>
<td>Antisolvent</td>
<td>Water and acetonitrile (0.75:0.25 mole fraction)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2.** MSZW measurement of cephradine and effect of cooling rate and solvent composition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average ΔT saturation temperature to nucleation temperature [°C]</th>
<th>+/-</th>
</tr>
</thead>
<tbody>
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<td>Cooling rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 K/min</td>
<td>-38.75</td>
<td>2.3</td>
</tr>
<tr>
<td>0.5 K/min</td>
<td>-17</td>
<td>2.48</td>
</tr>
<tr>
<td>0.1 K/min</td>
<td>-2.5</td>
<td>1.14</td>
</tr>
<tr>
<td>Solvent composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 MeOH</td>
<td>-3.92</td>
<td>0.31</td>
</tr>
<tr>
<td>0.3 MeOH</td>
<td>-10.38</td>
<td>0.98</td>
</tr>
<tr>
<td>0.5 MeOH</td>
<td>-18.5</td>
<td>1.08</td>
</tr>
</tbody>
</table>
Table 3. Filtration rate obtained from the different cooling rates.

<table>
<thead>
<tr>
<th>Cooling rate [K/min]</th>
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<th>0.5</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration [g/min]</td>
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<td>0.2</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4. Filtration rate obtained from the different solvent compositions of methanol and water.

<table>
<thead>
<tr>
<th>Solvent composition [mole fraction MeOH]</th>
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<th>0.3</th>
<th>0.5</th>
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</thead>
<tbody>
<tr>
<td>Filtration [g/min]</td>
<td>1.44</td>
<td>1.39</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Table 5. Filtration rate obtained from a seeded experiment and a non-seeded experiment. The seeds used had a size of 71 – 125 μm.

<table>
<thead>
<tr>
<th>Seeding [% w/w]</th>
<th>5 %</th>
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<tbody>
<tr>
<td>Filtration [g/min]</td>
<td>9.99</td>
<td>2.72</td>
</tr>
</tbody>
</table>

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Figures

**Figure 1.** Molecular structure of cephradine and cephalexin. Cephradine is unstable and will in the anhydrous form transform into cephalexin.

**Figure 2.** Right Raman spectra and left XRPD profiles of the commercial cephradine monohydrate (CPH), obtained cephradine anhydrate from the commercial monohydrate (CPA1) and the standard cephradine anhydrate bought from Sigma-Aldrich (CPA2).

**Figure 3.** Solubility of cephradine in various water and methanol mixtures at different temperatures. Solid lines are drawn for visualization.
Figure 4. MSZW measurement of cephradine at a saturation temperature of 55 °C showing temperature and turbidity profiles. A) Cephradine in pure water and cooling rate of 1 K/min. B) Cephradine in pure water and cooling rate of 0.5 K/min C) Cephradine in pure water and cooling rate of 0.1 K/min. The solid curve represents the temperature and the blue represents the turbidity.
**Figure 5.** SEM images of the individual crystal morphology of the final crystal product, obtained from each cooling rate: A) 1 K/min B) 0.5 K/min and C) 0.1 K/min.
**Figure 6.** SEM images of the final crystal product, obtained from methanol-water solution with A) 0.1 mole fraction methanol B) 0.3 mole fraction methanol C) 0.5 mole fraction methanol.
Figure 7. SEM images of two identical cooling experiments (cooling rate of 0.1 K/min in pure water) with A) addition of 5% seeds and B) non-seeded.
Entry for the Table of Contents

Type of Article: The solubility and relative stability of cephradine anhydrate and monohydrate in water-methanol mixtures have been studied at different temperatures. Cephradine monohydrate was crystallized out from cooling crystallization from aqueous solutions, and it has proved that using slow cooling rate and seeding can improve the particulate properties as well as the filterability of obtained products.

Supporting information: No, there is no additional information.