International Journal of Pharmaceutics 433 (2012) 60–70

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A novel image analysis methodology for online monitoring of nucleation and crystal growth during solid state phase transformations

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ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form 26 April 2012
Accepted 27 April 2012
Available online 4 May 2012

Keywords:
Polarized light microscopy
Image analysis
Nucleation
Crystal growth
Solid dispersion
Antisolvent crystallization

ABSTRACT

This study focuses on the development of an automated image analysis method to extract information on nucleation and crystal growth from polarized light micrographs. Using the developed image analysis method, four parameters related to nucleation and crystal growth could be extracted from the images. These parameters were crystalline count (applied as a measure of nucleation), percentage area coverage, average equivalent diameter and average crystalline area (three last parameters applied as a measure for crystal growth). The developed image analysis method was used to investigate two pharmaceutically relevant case studies: first, nitrendipine antisolvent crystallization, and second, recrystallization of amorphous piroxicam solid dispersion in an aqueous environment. In both case studies, an amorphous-to-crystalline phase transformation were observed, which were successfully monitored using real-time Raman spectroscopy. For the both case studies, the parameters related to crystallization kinetics estimated by image analysis were in close agreement with the parameters estimated by Raman spectroscopy. The developed image analysis method proved to be a valuable tool for quantitative monitoring of nucleation and crystal growth with an obvious potential for high throughput screening.

1. Introduction

Characterization of solid state forms is an important part of the drug development process (Newman and Byrn, 2003). It is well known that most active pharmaceutical ingredients (APIs) can exist in a variety of solid state modifications, including both amorphous and various crystalline forms, between which conversions upon processing and storage may occur. Such solid state phase transformations can have a dramatic influence on API morphology, solubility, chemical stability as well as on processability (Heinz et al., 2009; Lee and Myerson, 2006). In solid state research, there is an increased focus on understanding of the amorphous API, due to its increased dissolution rate and solubility as compared to the respective crystalline forms with the potential of increasing bioavailability of many poorly water soluble APIs. However, the main concern related to an amorphous API formulation is the inherent physical instability of amorphous materials due to the increased free energy as compared to its crystalline counterpart (Karmwar et al., 2011), making it susceptible to recrystallization into a more thermodynamically stable form with the consequence of decreased solubility and bioavailability. Monitoring and the subsequent estimation of recrystallization kinetic parameters of solid state phase transformation of an amorphous API into its crystalline counterpart in both, the early development phase as well as the later production stage is thus crucial for the pharmaceutical industry.

Currently, a variety of techniques for monitoring and quantifying solid state phase transformations are available including thermal analytical techniques and spectroscopic techniques (Heinz et al., 2009), such as Raman (Qu et al., 2011; Savolainen et al., 2007), near infrared (NIR) (Zhou et al., 2006), and infrared (IR) spectroscopy (Taylor and Zografi, 1997), as well as wide angle X-ray diffraction (WAXD) (Andronis and Zografi, 2000). A common feature of the thermal analytical techniques is that they allow information related to thermodynamic properties of the sample to be derived, while for the spectroscopic techniques, information related to sample chemistry and molecular interactions can be gained (Reffner et al., 2005). In the solid state phase transformation process such as the amorphous-to-crystalline conversion, it has been shown that nucleation followed by crystal growth are the two important stages (Bhugra and Pikal, 2008). Both, nucleation phase (once the nuclei have reached a certain detectable size) and the following crystal growth can be visualized by the

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http://dx.doi.org/10.1016/j.ijpharm.2012.04.074

polarized light microscopy (PLM), allowing kinetic information related to solid state phase transformation to be derived (Andronis and Zografi, 2000).

Despite the potential underlying PLM, much of the studies involving PLM are of a qualitative character. By visually observing polarized light micrographs and by experience-based scoring of the extent of crystallinity, Eerdenbrugh and Taylor estimated the extent of inhibition of crystallization of drugs by different polymers (Van Eerdenbrugh and Taylor, 2010). Using the same scoring system, the authors characterized different APIs in terms of their crystallization rate from amorphous films (Van Eerdenbrugh et al., 2010). In another study by Andronis and Zografi, the authors used PLM to estimate nucleation and crystal growth rate of indomethacin by manually counting the nuclei and measuring the size of crystalline areas of individual polarized light micrographs (Andronis and Zografi, 2000). The above mentioned studies highlight that whilst PLM is a fast and convenient technique in observing nucleation and crystal growth, it is relying on labor intensive consistent counting and measuring of each polarized light micrograph (Andronis and Zografi, 2000). Using PLM and image analysis a study by De Anda et al. showed an example of using high speed camera in qualitative determination of nucleation onset and the presence of different crystal morphologies in batch crystallization (De Anda et al., 2005). An interesting study by Qu et al. demonstrated the ability of video microscopy, in monitoring nucleation and crystal growth in batch crystallization (Qu et al., 2006). However, the authors pointed out that the image analysis routine was complicated with many complex preprocessing steps, due to the complexity in the obtained images such as particulate out of focus problem. Caillet et al. demonstrated the potential of image analysis in online monitoring of crystal size distribution, but noticed that as the solid concentration exceeds a certain limit, their image analysis method gave unrealistic results (Caillet et al., 2007). Not only did these studies demonstrate the value of image analysis in understanding different facets of solid state phase transformation, but also highlight the need to validate the robust region from the image analytical method in which the obtained estimates can be trusted. The present study focuses on the method development and implementation of an automated image analysis routine that allows simultaneous determination of nucleation, crystal growth and overall recrystallization rate of amorphous-to-crystalline phase transformation from polarized light micrographs. The robustness of the developed image analysis routine was initially tested on images generated from computer simulated crystallization. In order to demonstrate the usefulness and broader applicability of the developed image analysis routine, two case studies were then performed.

The first case study is focusing on the determination of nucleation and crystal growth rate of nitrendipine during antisolvent crystallization (Xia et al., 2012), under the influence of increasing polymer concentrations in the aqueous phase. The second case study investigates the nucleation and crystal growth rate of amorphous piroxicam (PRX) formulated as solid dispersion using a solvent evaporation method. The recrystallization rate of PRX from the solid dispersions during a modified dissolution testing is investigated. For both studies, Raman spectroscopy is used as a reference method to follow the amorphous-to-crystalline solid state phase transformations.

2. Methods

2.1. Materials

Nitrendipine was obtained from the Nanjing Pharmaceutical Factory, China. Piroxicam anhydrate (PRX AH) was obtained from Chr. Olesen Pharmaceuticals, Denmark. Polyvinyl alcohol (PVA) with 30–70 kDA molecular weight and 88% alcoholysis was generously supplied by Shin-Etsu Chemical Ind. Co. Ltd., Japan. Polyethylene glycol 200 (PEG) was obtained from Sigma–Aldrich, USA. Polyvinylpyrrolidone (PVP) K90 was obtained from BASF, Germany. Methanol and acetone were obtained from LAB-SCAN analytical sciences, Poland.

2.2. Preparation of nitrendipine and piroxicam references

From earlier studies the nitrendipine supplied by the manufacture was identified as nitrendipine RS-mod. I (Burger et al., 1997; Xia et al., 2012). Amorphous nitrendipine was prepared by dissolving 30 mg/ml nitrendipine in 50% (v/v) PEG 200 and acetone. Two ml of the solution was transferred to 20 ml water kept in an ice-water bath. The precipitated sticky solid body immediately formed is transferred to an aluminum plate and Raman spectra of the sample are recorded.

Piroxicam monohydrate (PRX MH) was prepared in the same way as described in a previous work (Qu et al., 2011). The obtained PRX MH demonstrated identical X-ray powder diffraction pattern as those published in Cambridge Structural Database, CSD (CIDYAP01, Reck et al., 1988).

2.3. Nitrendipine antisolvent crystallization

Nitrendipine was dissolved in a mixture of PEG 200 and 50% (v/v) acetone, and the final drug concentration in the organic solution was 30 mg/ml. PVA was dissolved in aqueous solutions at different concentrations (0.1%, 0.5%, 1% and 2%, w/v), and kept in an ice-water bath. To precipitate the drug, 2 ml of the organic phase containing the drug was transferred to 20 ml of aqueous solution containing PVA under magnetic stirring. All experiments were performed in triplicate.

2.4. Piroxicam solid dispersion crystallization

Five hundred milligrams of PRX:PVP in ratio 1:1 was dissolved in 15 ml acetone and 3.5 ml methanol. 10 μl of solution was pipetted onto a microscope cover glass placed on a hotplate (Krüss G12, Germany) where temperature can be accurately controlled. The temperature on the glass surface was controlled at 30 and 50 °C, and was monitored with a thermo electrode. After complete solvent evaporation, the sample was immediately subjected to a PLM investigation. Samples for Raman spectroscopy were prepared by transferring 40 μl of solution onto an aluminum plate with temperature controlled at 30 and 50 °C on the surface of the plate. All experiments were performed in triplicate.

2.5. Polarized light microscopy

The suspended samples on a glass slide were covered with a 24 mm × 24 mm cover glass, and examined under cross polarized light (Axiolab, Carl Zeiss, Göttingen, Germany) using a 5× magnification and 0.12 numerical aperture objective. Using an attached digital camera (Deltapix, Måløv, Denmark), together with the Deltapix software (ver. 1.6 Deltapix, Måløv, Denmark), a video was captured in uncompressed Audio Video Interleave (AVI) format. Each image frame in the video has 240 ms exposure time and consists of three channels (red, green, blue), each an array of size 1024 × 1280. Particle number density per unit volume was calculated using the depth of field Eq. (1) (Andronis and Zografi, 2000):

$$D_f = \frac{\lambda(1 - NA^2)^{1/2}}{NA^2} \quad (1)$$