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Communication

Preparation and Stabilization of Amorphous Piroxicam Using Co-milling Approach

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Abstract: Particle size reduction in order to obtain amorphous nanoparticulate systems has gained a lot of attention and one attractive approach is the co-milling of active pharmaceutical ingredient (API) together with polymers. In this study, piroxicam anhydrate form I (PRXAH) was used as a model API and PVP 25, PVP 90, and Soluplus® as model polymers. The physical stability of the obtained samples was analyzed using variable temperature X-ray powder diffractometry (VT-XRPD), XRPD and Raman. Furthermore, the dissolution performance was tested in the presence of simulated gastric fluid. Multivariate data analysis was applied to visualize the data. Co-milling of PRX-polymer mixtures is a suitable method for preparation of amorphous PRX-polymer systems. The stability of these co-milled samples was dependent on the PRXAH–polymer ratio. Dissolution of PRX from co-milled amorphous PRX-polymer solid dispersions was compared with the physical mixtures with the same polymers. Fast recrystallization of amorphous PRX was observed in the presence of simulated gastric fluid and it was already observed within the first minutes of testing. Both methods, at-line XRPD and on-line Raman spectroscopy, revealed that amorphous PRX crystallized as PRX monohydrate (PRXMH).

Keywords: co-milling; amorphous piroxicam; polymers; PVP; Soluplus®; dissolution behavior; on-line Raman spectroscopy; at-line XRPD.

1. Introduction

Nanoparticulate systems have gained a lot of attention during the last decade. Particle size reduction is considered to be one of the effective approaches in order to enhance the solubility of poorly soluble active pharmaceutical ingredients (APIs). Another one is the formation of amorphous form, it is well established that amorphous form can be a preferred solid state in the respect of enhanced apparent solubility, dissolution rate and bioavailability [1]. Several approaches have been introduced for obtaining amorphous state – milling under various conditions is one of the examples [1,2]. During milling, amorphous form is obtained through solid state transition as it leads to decreased crystallites size and increased disorder in crystals.

According to the revised ICH Q8 (R2) guideline comprehensive understanding of attributes affecting the drug substance properties are critical and those have to be linked to drug products performance [3]. Identifying and characterizing the factors having an impact on the final product performance is very much needed. It is important to identify whether the use of specifically designed physicochemical properties of an API can contribute to for example in an increase in the dissolution rate. Furthermore, formulation screening is necessary in order to develop a formulation stable throughout the products' shelf-life.

The aim of this study was to prepare stable amorphous API-polymer systems by screening three different polymers in four different weight ratios. The model API used was piroxicam (PRX) as it exhibits poor aqueous solubility and is known for its polymorphic behavior and solid state transformations during processing [4-9]. Furthermore, two preparation techniques, ball-milling at room and low temperature, and influence of storage conditions, at ambient and 4°C, were investigated. Based on the fact that solid dosage forms when administered orally have to pass through the stomach the dissolution behavior of prepared PRX-polymer systems was monitored.

2. Experimental Section

2.1. Materials

The model API, PRXAH form I (USP grade, 99.44%), was obtained from Lianyuangang Ruidong International Co., Ltd (China). Following polymers, polyvinylpyrrolidon (PVP) in two different grades PVP 25 and PVP 90 (BASF SE, Ludwigshafen, Germany) were used as obtained, whereas Soluplus® (BASF SE, Ludwigshafen, Germany) was pre-ball-milled for one hour in order to decrease the particle size.

Simulated gastric fluid used for dissolution and slurry experiments was prepared according to the USP NF 33 standards.

2.2. Methods

2.2.1. Preparation of Amorphous Formulations: Ball-milling

An oscillatory ball mill (Mixer Mill MM301, Retsch GmbH & Co., Haan, Germany) was used for co-milling of PRXAH mixtures with different polymers. A 25 ml stainless steel milling jar with one 12 mm diameter stainless steel ball was agitated at 28 Hz frequency for 180 min. Low temperature co-milling was carried out with the same equipment; however the milling jars were sealed with parafilm and immersed into liquid nitrogen for 2 min prior to milling. At 15 min intervals this procedure was repeated (in total for 13 times during 180 min milling).

The prepared samples were stored both at room and low temperature (4ºC) in order to study the stability and recrystallization behavior of the formulations.

2.2.2. Characterization Methods

2.2.2.1. X-ray Powder Diffractometry (XRPD)

XRPD analysis was performed using an X'Pert PRO MPD X-ray diffractometer (PANalytical B.V., Almelo, The Netherlands) and Ni filtered Cu $K\alpha_1$ radiation (λ=1.541Å). Samples were measured in Bragg Brentano reflection mode in the 2θ–range 2 - 40° using a PIXel detector (PANalytical B.V., Almelo, The Netherlands); step size 0.0334° 2θ. The operating current and voltage were 40 mA and 45 kV, respectively. Data were collected using X'Pert Data Collector (PANalytical B.V., Almeo, The Netherlands). Experimental results were compared to the theoretical patterns in the Cambridge Structural Database (CSD, Cambridge, UK) [10]. Refcodes BIYSEH [5], and CIDYAP01 [11] were used as reference crystal structures, for PRXAH, and PRXMH, respectively.

2.2.2.2. Raman Spectroscopy

Raman spectra were collected using a Raman spectrometer equipped with a T.E. cooled FFT-CCD detector (1024 x 64) and a two fiber coaxial optic probe (Control Development, Inc., South Bend, IN, USA). The laser source was a 300 mW diode laser system operating at 785 nm (Control Development, Inc., South Bend, IN, USA). The detection range was from 200 to 2200 cm^{-1}. A total of 4 scans per spectra with a 1.0 s integration time were acquired for each sample. CDI Spec32 software (Control Development, Inc., South Bend, IN, USA) was used for the collection of Raman spectra. The same equipment was used for on-line recrystallization monitoring.

2.2.2.3. Wet Slurry Experiments

On-line Raman spectroscopy and X-ray equipped with the A.P. stage were used to monitor the recrystallization of amorphous PRX from amorphous PRX-polmer systems as well as from physical mixtures of PRXAH and respective polymer in simulated gastric fluid.

2.2.2.4 Dissolution Testing

For dissolution testing the prepared amorphous formulations were weighed into hard gelatin capsules (n=3). The relative amount of PRX in each capsule was kept constant, 20 mg. The dissolution tests were carried out according to the USP 33 NF 28 monograph for PRX capsules; the time to complete dissolution testing was prolonged to 3 hours (Erweka DT 70, Erweka GmbH, Heusenstamm, Germany). Samples were withdrawn from the dissolution vessels every 3 minutes (replaced); and analyzed by using UV-Vis spectrophotometer (Evolution 300, Thermo Fisher Scientific, Waltham, MA) at the wavelength of 353 nm.

2.2.2.5. Multivariate Data Analysis

Principal component analysis (PCA) was used to visualize and analyze the XRPD and Raman spectroscopic data. All the data were preprocessed by using standard normal variate (SNV) correction and mean centering. Multivariate data analysis was carried out by using Simca-P+ software (v.12.0, Umetrics AB, Umeå, Sweden).

3. Results and Discussion

3.1. Formulation Screening

The physical stability of amorphous materials can be significantly different. Therefore the primary aim of the study was to look into different polymers in order to prepare stable amorphous formulations of crystalline PRXAH and polymer. Three different polymers were investigated: PVP 25, PVP 90, and Soluplus®; the API-polymer ratios analyzed were 1:0.5, 1:1, 1:2, and in case of Soluplus® 1:3 and 1:4 as ratios 1:1 and 1:2 did not result in amorphous systems, Fig. 1. Co-milling at room temperature with polymers, PVP 25 and PVP 90 in ratios 1:1 and 1:2, was sufficient to produce amorphous formulations. PRX co-milled together with both polymers and in both ratios stayed amorphous for at least one month at room and low temperature. Amorphous material was obtained faster, in less than 180 min, when PRXAH and polymer were ball-milled at low temperature.

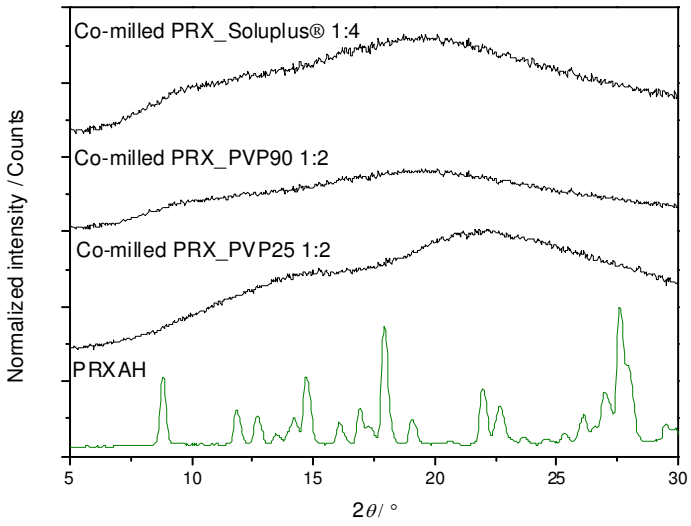

Figure 1. XRPD patterns of untreated crystalline PRXAH and co-milled amorphous samples with PVP 25 1:2; PVP 90 1:2 and Soluplus® 1:4. All patterns are normalized.

It was not possible to obtain amorphous formulations with Soluplus® at room temperature, and only ball-milling at low temperature was found to be suitable. Furthermore, because of the large particle size of Soluplus® it had to be pre-milled for one hour and was then sieved. In case of PRX-Soluplus® mixtures ratio 1:3 was first able to produce an amorphous system. The recrystallization of PRX-Soluplus® 1:3 mixture started after 48 h of storage at RT however the mixture 1:4 was stable up to three weeks, and the storage conditions (RT *vs* 4°C) did not affect the stability. In conclusion, the amount of polymer had a more significant role in case of PRX-Soluplus® formulations where both, the formation of amorphous systems as well as their stability, was dependent on the amount of polymer.

3.2. Recrystallization of Amorphous PRX-polymer Mixtures in Simulated Gastric Fluid

Administration of a solid oral dosage form includes the passage through the stomach where the API will be affected by gastric juice. With the intention of studying the influence of simulated gastric fluid on the stable PRX-polymer formulations slurry experiments were performed. The solid state transformations were monitored by on-line Raman spectroscopy and at-line XRPD. All co-milled PRX-polymer formulations converted to PRXMH in simulated gastric fluid, Fig. 2. Interestingly, in case of PVP 25 and Soluplus®, the transformations started within the first minute, whereas formulations prepared with PVP 90 showed the first signs of recrystallization within the first three minutes of testing, Figs. 2(a), 2(c) and 2(e). All the amorphous PRX-polymer systems transformed into PRXMH within 15 minutes and no other changes were detected up to 180 min of monitoring.

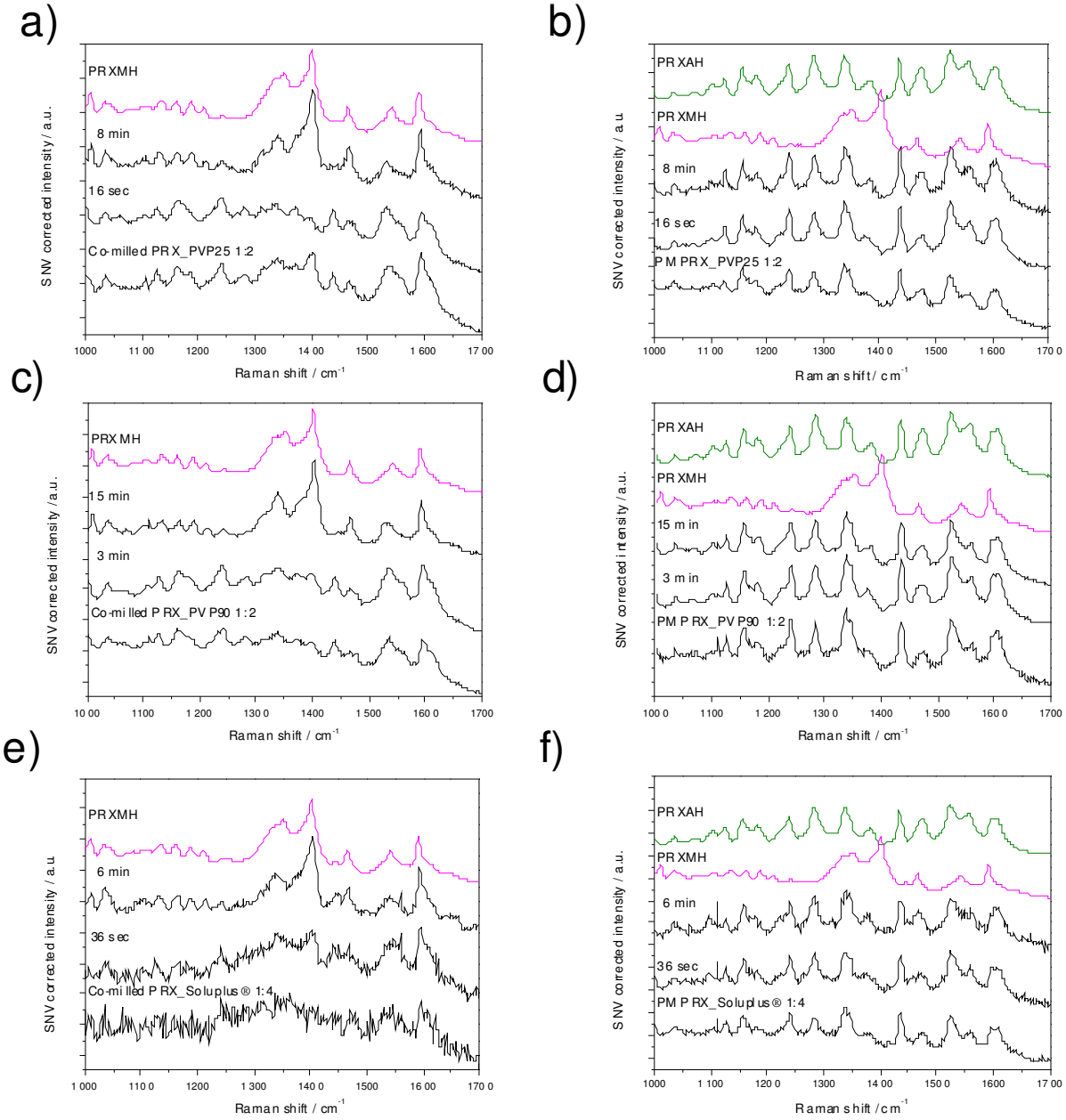

Figure 2. Recrystallization behavior of PRX in simulated gastric fluid from co-milled samples and physical mixtures (PM), monitored with on-line Raman spectroscopy (n=2) compared to crystalline PRXMH spectrum **(a)** PRX-PVP 25 (1:2), co-milled; **(b)** PRX-PVP 25 (1:2), physical mixture; **(c)** PRX-PVP 90 (1:2), co-milled; **(d)** PRX-PVP 90 (1:2), physical mixture; **(e)** PRX-Soluplus® (1:4), co-milled; and **(f)** PRX- Soluplus® (1:4), physical mixture. All the spectra were SNV corrected.

With the aim of further investigation how the physical mixtures of crystalline PRXAH and polymer would act in the presence of simulated gastric fluid another set of experiments was carried out. In these trials the crystalline PRXAH was mixed together with the polymer and simulated gastric fluid was added. All the conditions, including the ratio between the API and solution were kept the same as for the co-milled samples. Results of these trials showed, that no change in the solid state of the

PRX occurred in the early stage of dissolution, Figs. 2(b), 2(d), and 2(f). Explanation for a fast solvent mediated transformation in case of co-milled PRX-polymer samples compared to physical mixtures is supposedly the reduced particle size and fresh surfaces available.

3.3. Dissolution Studies

It is very important to confirm whether the use of amorphous form of an API in a mixture with a polymer can contribute to an improved dissolution rate and increased the total amount of drug substance dissolved. For this purpose the dissolution behavior of amorphous PRX-polymer co-milled solid dispersions was compared with the physical mixtures of crystalline PRXAH and the respective polymer using the same weight ratios.

The general trend seen with co-milled samples containing PRX-PVP 25/90 was that the dissolution rate of PRX was lowered compared to the crystalline PRXAH, Fig. 3. As mentioned in the previous chapter, a fast transformation of amorphous PRX to PRXMH appeared and this can be considered as a reason for lowered dissolution rate. Furthermore, the fact that the polymer is forming a protective layer around the drug particles can also prohibit the dissolution of the API. However, the total amount of drug in solution after two hours was higher when compared to the amount of crystalline material dissolved after the same time. Additionally, the differences in the molecular weight of PVPs (PVP 25 *vs.* PVP 90) and therefore also the swelling properties and viscosity were influencing the dissolution behavior of both investigated systems, Fig. 3(a) *vs.* Fig. 3(b), respectively.

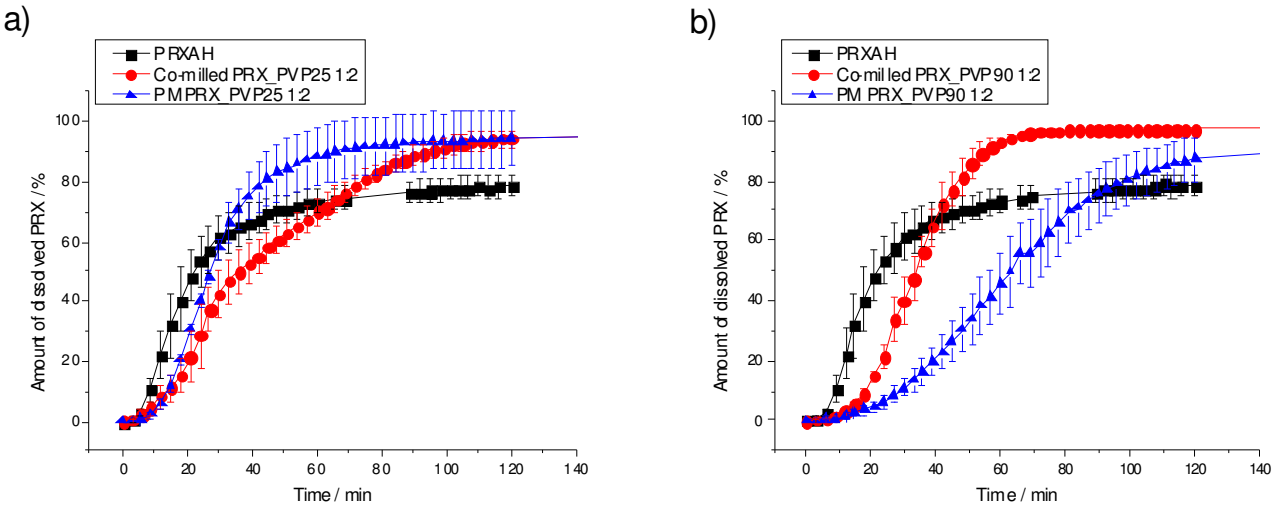

Figure 3. Dissolution behavior of PRX from co-milled samples and physical mixtures (PM) compared to crystalline PRXAH (n=3) **(a)** in the presence of PVP 25 in 1:2 ratio and **(b)** in the presence of PVP 90 in 1:2 ratio. Y-error bars indicate the standard deviation.

Interestingly, dissolution results of PRX-Soluplus® systems showed a significantly lower amount of PRX in solution when compared to crystalline PRXAH, Fig. 4, and formulations with PVPs, Fig. 3. Soluplus® is especially recommended for improving the solubility of poorly soluble drugs [12].

Based on the observations made during the dissolution studies it can now be emphasized that the water soluble characteristics of Soluplus® and formation of lumps impaired the dissolution of PRX from both samples, co-milled and physical mixture.

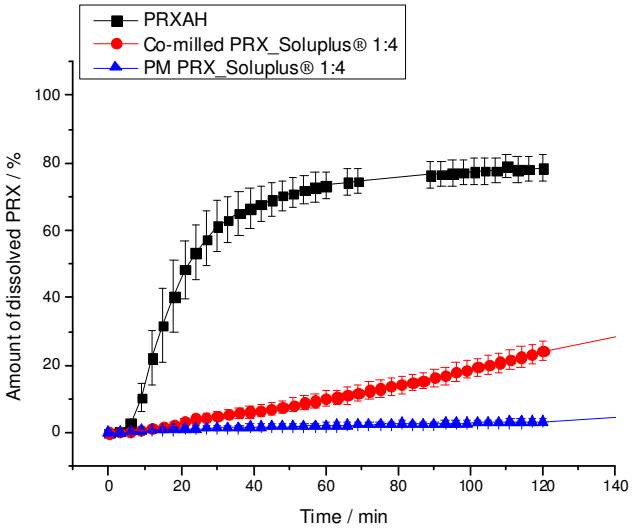

Figure 4. Dissolution behavior of PRX from PRX-Soluplus® co-milled samples and physical mixtures (PM) compared to crystalline PRXAH (n=3). Y-error bars indicate the standard deviation.

4. Conclusions

According to the findings co-milling can be considered as a suitable technique for obtaining stable and amorphous PRX-polymer mixtures. It was shown that the PRX-polymer ratio has an important role in developing stable formulations. Furthermore, low temperature ball-milling revealed to be more efficient compared to ball-milling at room temperature.

The main finding of this study was that the fast recystallization of the model amorphous API, PRX, in the simulated gastric fluid can negatively affect the overall dissolution rate. However, the gain of amorphous PRX-PVP 25/90 systems is significant, as the total amount of dissolved drug was increased. The major constraint for using Soluplus® for enhancing the dissolution of PRX in presented formulations was its tendency to form lumps in aqueous solutions. Through formulation screening studies it was possible to identify whether a sophisticated formulation approach should at all be considered.

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