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Nanodispersions of taxifolin: Impact of solid-state properties on dissolution behavior

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ABSTRACT

Nanosizing is an advanced formulation approach to address the issues of poor aqueous solubility of active pharmaceutical ingredients. Here we present a procedure to prepare a nanoparticulate formulation with the objective to enhance dissolution kinetics of taxifolin dihydrate, a naturally occurring flavonoid with antioxidant, anti-inflammatory, and hepatoprotective activities. Polyvinylpirrolidone was selected as a carrier and the solid nanodispersions of varying compositions were prepared by a co-precipitation technique followed by lyophilization. The formulation technology reported herein resulted in aggregate-free, spherical particles with the mean size of about 150 nm, as observed by scanning electron microscopy and measured by photon correlation spectroscopy. Furthermore, the co-precipitation process caused taxifolin dihydrate to convert into an amorphous form as verified by X-ray powder diffraction, differential scanning calorimetry, hot stage microscopy and Raman spectroscopy. Finally, *in vitro* dissolution behavior of the nanodispersion of taxifolin was shown to be superior to that of either pure drug or a drug–polymer physical mixture, reaching 90% of taxifolin released after 30 min. Such enhanced drug release kinetics from the nanodispersion was attributed to both the reduced particle size and the loss of crystallinity.

1. Introduction

The poor solubility of active pharmaceutical ingredients (APIs) in water and their low dissolution rate in the aqueous gastro-intestinal fluids often leads to insufficient bioavailability and is one of the most difficult and non-solved problems in pharmaceutical technology. The common approaches used to address the issues of poor aqueous solubility include, for instance, salt formation (Stahl and Wermuth, 2002), particle size reduction (Merisko-Liversidge et al., 2003), and solid dispersion formulations (Chiou and Riegelman, 1971; Pozharitskaya et al., 1999; Leuner and Dressman, 2000), whereby an API is homogeneously dispersed within a carrier.

Solid dispersions are particularly regaining interest within the pharmaceutical industry as they enable to obtain a physically stable formulation of an API in amorphous state (Van den Mooter et al., 2000; Forster et al., 2002; Miller et al., 2008; Tong and Zografi, 2001). Within the polymeric matrix, the API can be present in two forms: solid solution (molecularly dispersed) or nanodispersed with particle sizes preferably lower than 500 nm (amorphous/crystalline material) (Karavas et al., 2007). Polyvinylpyrrolidone (PVP; Fig. 1B), a freely water soluble amorphous polymer, has been most widely used as a carrier for such solid dispersion systems.

Flavonoids are naturally occurring substances possessing some positive effects on human health (Harborne, 1994). These substances with variable polyphenolic structures are found in numerous food products, such as fruit, vegetables, nuts and beverages (coffee, tea, red wine), as well as in different parts of herbs (Hollman and Katan, 1997; Nijeveldt et al., 2001). Flavonoid taxifolin (2β(R), 3α(S)- 3′,4′,5,7-tetrahydroxyflavanonol) (Fig. 1A) is widely distributed in the rind of Siberian and Dahurian larchs (*Larix sibirica* Leder. and *L. gmelini* Rupr. (Rupr.), syn. *L. Dahurica* Turoz) (Teselkin et al., 2000). Taxifolin significantly dilates blood vessel, improves microcirculation, increases cerebral blood flow, and inhibits platelet aggregation activity. It has been widely used in the treatment of cerebral infarction and sequela, cerebral thrombus, coronary heart disease and angina pectoris (Landolfi et al., 1984; Tzen et al., 1991). In recent years taxifolin also has shown anti-inflammatory, antioxidant, and hepatoprotective activities (Plotnikov et al., 2005).

Flavonoids are slightly soluble in water and show a slow dissolution rate from solid oral dosage forms, restricting their clinical use. Hence, the purpose of this study was to enhance dissolution rate of

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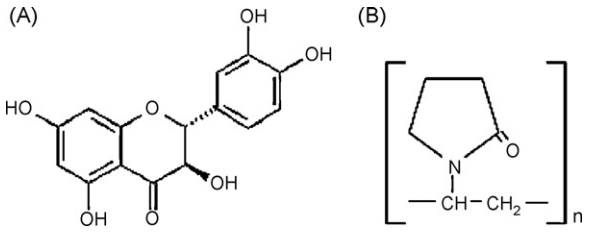

Fig. 1. Molecular structures of taxifolin (A) and PVP (B).

a model flavonoid taxifolin via nanodispersion formulations with PVP. The nanodispersions were prepared by co-precipitation followed by lyophilization and their solid-state properties were then probed by means of X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), hot stage microscopy (HSM), Raman spectroscopy, photon correlation spectroscopy (PCS) and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

Taxifolin was of pharmaceutical purity grade and was provided by INPF Wood Chemistry Ltd. (Irkutsk, Russia). Amorphous taxifolin used as a reference in this study was prepared in situ in the DSC experiment by heating the sample up to 250 °C followed by a fast cooling to −10 °C. PVP (Kollidone 17PF) was from BASF (Ludwigshafen, Germany), while ultrapure water was prepared using a Milli-Q system (Millipore, MA, USA).

2.2. Methods

2.2.1. Preparation of nanodispersions

Taxifolin–PVP nanodispersions over a range of compositions (1:7, 1:8, 1:9, 1:10, 1:12, w/w) were prepared using a co-precipitation technique. Varying amounts (1.4, 1.6, 1.8, 2.0 or 2.4 g) of taxifolin were dissolved in water (1 part of solid in 10 parts of solvent) in a glass vessel and heated in a water bath with mild stirring. Thereafter a taxifolin solution was mixed with an appropriate amount of a 20% (w/v) PVP solution under stirring, cooled and lyophilized (Shikov et al., 2008). Finally, the solids were pulverized, passed through a 200 μm sieve, and stored in a desiccator over a silica gel at room temperature (21 ± 2 °C) before analysis.

2.2.2. Physical mixtures

Physical mixtures (1:10) were prepared by gently mixing taxifolin with PVP using a mortar and pestle. The mixture was passed through a 200 μm sieve before further experiments.

2.2.3. Characterization of nanoparticle morphology and size

The surface morphology (roundness, formation of aggregates) and size of the nanoparticles were studied by scanning electron microscopy (SEM). A drop of particle suspension was deposited on a metal plate, let to dry and sputtered for 20 s with platinum (Agar Sputter Coater, Agar Scientific Ltd., Essex, UK) and, finally, analyzed with a SEM (DSM 962, Zeiss, Jena, Germany). Size distribution of the nanoparticles was determined with Malvern Zetasizer 3000HS (Malvern, Worcestershire, UK). Particle sizing was based on photon correlation spectroscopy (PCS); the results were analyzed by CONTIN algorithm and the sizes presented based on the intensity distributions.

2.2.4. XRPD

The XRPD studies were performed with a theta–theta diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany) in a symmetrical reflection mode using Cu Kα radiation (1.54 Å) at 40 mA and 40 kV and Göbel Mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. Data points were collected between 5 and 30° 2θ with steps of 0.05° and a measuring time of 1 s/step at ambient conditions.

2.2.5. DSC

Thermal behavior of the materials was determined using DSC822^e differential scanning calorimeter (Mettler Toledo, Columbus, USA). Approximately 10 mg of sample was accurately weighed into aluminum pans and crimped by aluminum caps with a pinhole. The samples were initially cooled down to 0 °C and then heated at a rate of 10 °C/min up to 260 °C under nitrogen purge. Data analysis was performed using STARe software (Mettler Toledo).

2.2.6. HSM

A hot stage (FP900, Mettler Toledo GmbH, Greifensee, Switzerland) mounted on an optical microscope (Leica Microscopie und Systeme GmbH, Wetzlar, Germany) was used for thermomicroscopic investigations. Measurements were performed from 25 to 260 °C/min at a heating rate of 5 °C/min.

2.2.7. Raman spectroscopy

Raman spectra were collected between 200 and 1900 cm^{-1} using a Kaiser PhAT system with a 785-nm excitation wavelength probehead (Kaiser Optical Systems, Inc., Ann Arbor, MI, USA).

2.2.8. Dissolution studies

The release rate of taxifolin as a pure substance and from the physical mixture and nanodispersions was measured in a dissolution apparatus type Erweka (Erweka DT 600, Germany), using the basket method (USP I method). The test was performed at 37 ± 1 °C with a rotation speed of 100 rpm. Samples equivalent to 100 mg of taxifolin were placed in each dissolution vessel. The dissolution medium was 900 ml of water. At predetermined time intervals, samples of 3 ml were withdrawn from the dissolution medium and replaced with the same volume of water. The samples were filtered through 0.45 μm membranes and the amount of taxifolin in the filtrate was analyzed by HPLC (Pozharitskaya et al., 2009). HPLC analyses were performed at room temperature using the Shimadzu HPLC system (Kyoto, Japan), consisted by two LC20AD pumps, DGU-20A3 degasses, and SPD-M20A diode array detector. Separation was achieved using a Luna C18 (4.6 i.d. × 150 μm, 5 μm) column (Phenomenex, USA) and a Security Guard pre-column (2.0 mm) with the same sorbent (Phenomenex, USA). The data analysis system consisted of the LC Solution PC software (Shimadzu, Kyoto, Japan). The mobile phase for gradient elution consisted of two solvent systems: solvent A, 0.03% (v/v) water solution of trifluoroacetic acid; solvent B, acetonitrile. A gradient elution was carried out as follows: 20% (v/v) solvent B was used in the first minute, then solvent B percentage was linearly increased to 40% during 10 min, then to 100% during next 10 min and reverse to initial condition during next 5 min. The flow rate was 1 ml/min. UV detection was at 290 nm at room temperature. The drug release was calculated as the percent dissolved drug with respect to the initial drug loading. It should be noted that PVP had no absorption at these conditions.

3. Results and discussion

3.1. Solid-state characterization of taxifolin and PVP

Preliminary solid-state characterization of taxifolin revealed that it might exist in at least four solid-state forms, including the *dihydrate, monohydrate, anhydrate* and *amorphous* forms. Physicochemical properties of all these solid forms will be reported in

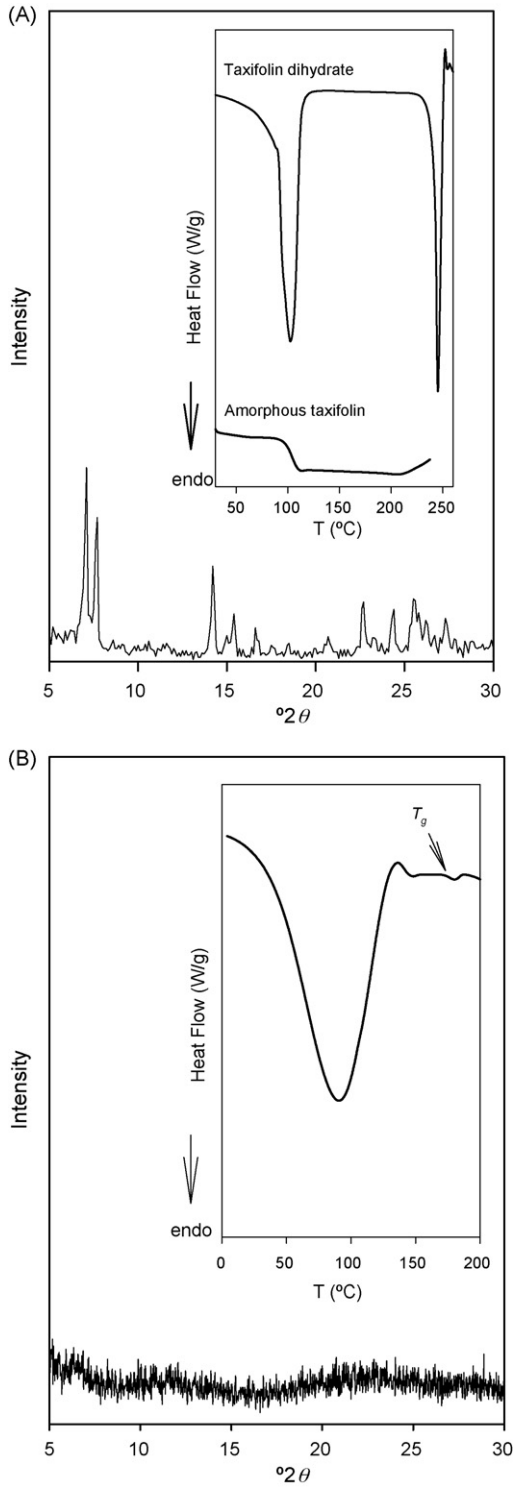

Fig. 2. XRPD patterns with DSC thermograms shown as insets of (A) commercial taxifolin dihydrate and (B) PVP.

a subsequent publication. Commercial taxifolin used as a starting material in the present study was the dihydrate form, as verified by XRPD, DSC and HSM. The most intense diffraction peaks of taxifolin dihydrate were observed at 7.0, 7.7, and 14.2° 2θ (Fig. 2A). Dehydration of taxifolin dihydrate occurred between 60 and 120 °C and was seen as a single endotherm (T_{max} = 102.7 °C) on the DSC thermogram (see inset in Fig. 2A). The total weight loss during dehydration was about 14%, which is in reasonable agreement with the theoretical water content of 11.8% for taxifolin dihydrate. The discrepancy is likely attributable to the capability of taxifolin dihydrate to adsorb slightly more water than the theoretical stoichiometric ratio. Such phenomenon has been previously reported for several other pharmaceutical hydrates (Stephenson et al., 1998; Morris, 1999; Stephenson and Diseroad, 2000) and it has been assumed that the additional water molecules might be localised either at intestinal sites or on some amorphous phase around the crystals (Authelin, 2005). The anhydrous taxifolin produced upon dehydration of the dihydrate melted at around 240 °C. Amorphous taxifolin prepared in situ by melting method yielded a diffused background XRPD pattern (not shown) and showed a glass transition temperature (T_g) at around 103 °C on the DSC thermogram (inset in Fig. 2A).

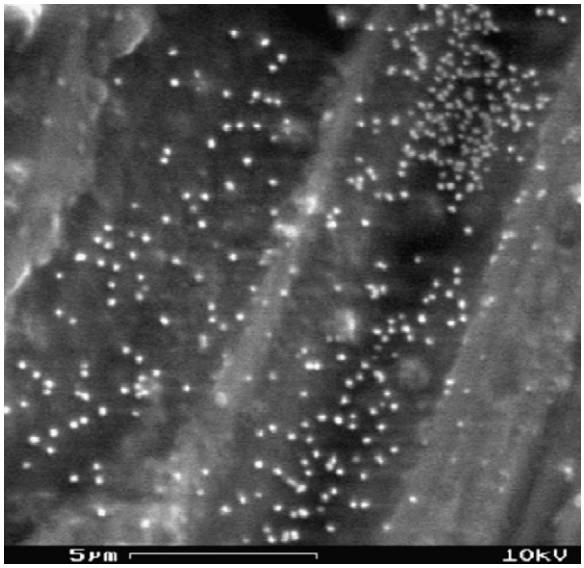

Fig. 3. SEM image of taxifolin:PVP (1:10) nanoparticles (scale bar 5 µm).

The XRPD pattern of PVP exhibited diffraction halos with the two maxima at around 12 and 22° 2θ (Fig. 2B), which is characteristic of amorphous material and is in accordance with the previous reports (Novoa et al., 2005). Using the combination of DSC with HSM, the T_g of PVP was detected at around 168 °C, coinciding with the previously published values (Nyamweya and Hoag, 2000; Buckton et al., 2006), and was preceded by a broad endotherm between 50 and 150 °C due to the release of adsorbed moisture (see inset in Fig. 2B).

3.2. Morphology and size of nanodispersion formulations

Mean sizes for the taxifolin nanodispersion formulations were around 150 nm, indicating that the formulation technology employed in this study caused efficient, down to nanoscale, particle size reduction. Among the compositions studied, round and smooth "high-quality" nanoparticles were formed with the 1:10 taxifolin loadings (Fig. 3). Higher (1:7; 1:8; 1:9) and lower (1:12) drug loadings were, however, also investigated to gain a better understanding of effect of the polymer on physical state of the drug during the co-precipitation process.

3.3. Physicochemical characterization of taxifolin–PVP physical mixture and nanodispersions

The physicochemical properties of taxifolin in the physical mixture and nanodispersions investigated by means of XRPD, DSC, HSM and Raman spectroscopy are compared in Fig. 4. The diffraction peaks characteristic of taxifolin dihydrate were clearly seen in the XRPD pattern of the physical mixture, while the XRPD

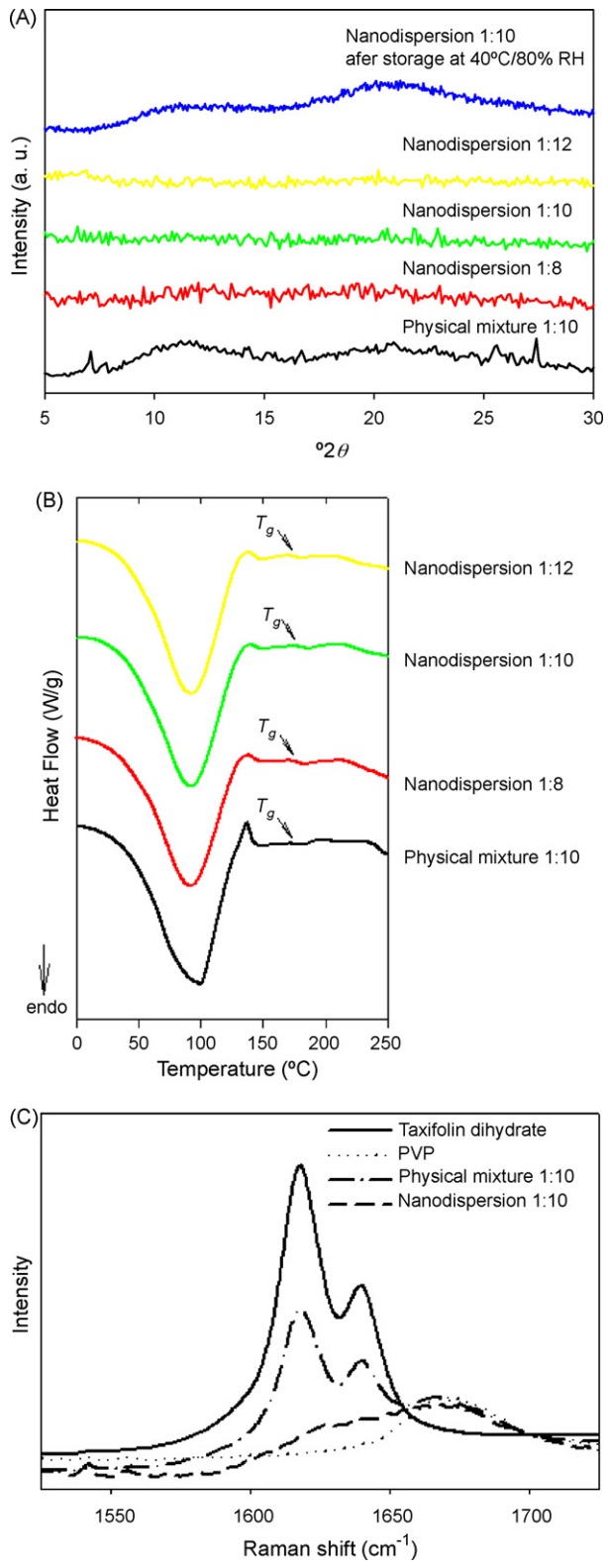

Fig. 4. Comparison of physicochemical properties of taxifolin–PVP nanodispersion and their physical mixture (1:10). XRPD patterns (A); DSC thermograms (B), and Raman spectra (C) in the C=O stretching region are shown.

patterns of the nanodispersions displayed no sharp diffraction peaks attributable to the crystalline drug (Fig. 4A). However, owing to the small particle size of the nanodispersions, the absence of any distinct diffraction peaks in their XPRD patterns does not necessarily indicate the lack of crystallinity in the samples. For this reason, a multitude of complementary analytical techniques was employed to verify the physical state of the drug in the formulations.

In particular, the DSC thermograms of the nanodispersions (Fig. 4B) yielded no melting endotherm attributable to the crystalline drug, while a T_g at around 165 °C was observed. The latter thermal event was verified by HSM (data not shown), as the endotherm at around 90 °C due to the loss of residual moisture in the samples could hinder the second T_g.

The single glass transition observed in the nanodispersions suggests taxifolin to be miscible with the carrier polymer and assumes specific interactions between the drug and polymer. Furthermore, the theoretical T_g value for the 1:10 nanodispersion calculated by applying the Gordon–Taylor equation (Gordon and Taylor, 1952) was found to be 161 °C, which is slightly lower than the experimental one. This inconsistency supports the assumption that specific interactions take place in the formulations, which was further verified by analyzing the samples with Raman spectroscopy.

The comparison of the Raman spectra of the nanodispersions and the starting materials showed the spectra to clearly differ in the C=O stretching region (Fig. 4C). Specifically, the 1:10 nanodispersion yielded the Raman spectra with the characteristic peaks of taxifolin dihydrate (1618 cm^{-1}) and PVP (1669 cm^{-1}) shifted to 1627 and 1666 cm^{-1}, respectively, signifying the formation of H-bonds between the drug and polymer. In addition, the intensity of the peaks characteristic of taxifolin was notably decreased. It should also be noted that the Raman spectrum of the physical mixture was similar to that of taxifolin dihydrate, in agreement with XRPD results.

Finally, the following storage stability studies revealed that all nanodispersions remained XRPD-amorphous after being stored at either 40 °C and 80% relative humidity for 2 weeks (Fig. 4A) or ambient conditions for 10 months, thus demonstrating high physical stability of the formulations.

3.4. Effect of formulation on dissolution rate of taxifolin

Dissolution profiles of taxifolin dihydrate, the physical mixture, and nanodispersion are shown in Fig. 5. Among the samples studied, taxifolin dihydrate demonstrated the slowest dissolution rate, with less than 19% of the drug being dissolved within 45 min. The dissolution rate of taxifolin from the physical mixture (1:10) was significantly faster (about 60% within 45 min). The increased drug release rate observed for the physical mixture can be attributed to the reduced interfacial tension between the drug particles and the dissolution medium due to the presence of the polymer. The drug release kinetics from the nanodispersion was superior (91% within 30 min and 95% within 45 min) to that of both taxifolin dihydrate

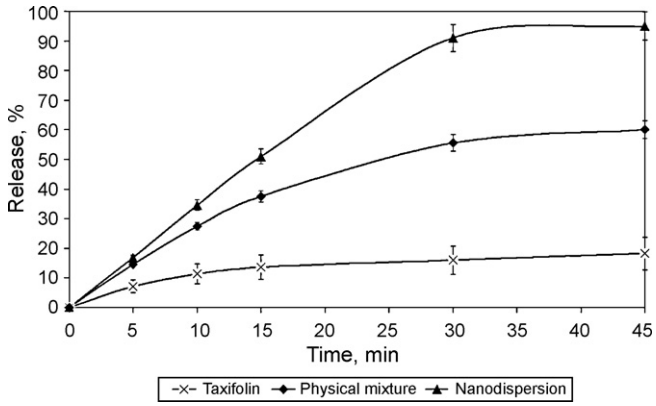

Fig. 5. Comparative dissolution profiles of different formulations of taxifolin in water at 37 °C. Each point refers to mean ± SD (n = 3).

and physical mixture. Several mechanisms, including particle size reduction of a drug, decreased crystallinity, and increased wettability, have been proposed to account for the increased dissolution rate of drugs from solid dispersions (Craig, 2002; Ruan et al., 2005). The results of the present study show that all these factors are likely to be responsible for the enhanced release kinetics of taxifolin from the nanodispersions obtained.

4. Conclusion

Co-precipitation followed by lyophilization proved to be an effective technique for the preparation of nanodispersions of taxifolin in PVP. Combination of X-ray powder diffractometry, thermal analysis, and Raman spectroscopy was used to elucidate the effect of the polymer and processing on the physical state of taxifolin in the formulations. During the co-precipitation process, taxifolin dihydrate was shown to convert into an amorphous form and to be stabilized within the carrier polymer via non-specific intermolecular interactions. The drug release kinetics from the nanodispersion was superior to that of either taxifolin dihydrate or the drug–PVP physical mixture, which was attributed to the simultaneous particle size reduction, the loss of crystallinity, and increased wettability due to the presence of the hydrophilic polymer.

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