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Performance comparison between crystalline and co-amorphous salts of indomethacin-lysine

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ABSTRACT

The introduction of a highly water soluble amino acid as co-amorphous co-former has previously been shown to significantly improve the dissolution rate of poorly water soluble drugs. In this work, dry ball milling (DBM) and liquid assisted grinding (LAG) were used to prepare different physical forms of salts of indomethacin (IND) with the amino acid lysine (LYS), allowing the direct comparison of their solid-state properties to their *in vitro* performance. X-ray powder diffraction and Fourier-transformed infrared spectroscopy showed that DBM experiments led to the formation of a fully co-amorphous salt, while LAG resulted in a crystalline salt. Differential scanning calorimetry showed that the samples prepared by DBM had a single glass transition temperature (T_g) of approx. 100 °C for the co-amorphous salt, while a new melting point (223 °C) was obtained for the crystalline salt prepared by LAG. Intrinsic dissolution and powder dissolution studies demonstrated an increased dissolution rate of the drug in the co-amorphous salt compared to pure amorphous IND and also the crystalline drug-LYS salt. Furthermore, the co-amorphous IND-LYS salt presented long term physical stability in dry conditions at 25 °C and 40 °C. Overall, it has been shown that the co-amorphous form of a salt has a superior performance in comparison to a crystalline salt.

1. Introduction

It is estimated that more than 90% of the new active pharmaceutical ingredients (APIs) under development are poorly water soluble (Grohganz et al., 2014; Rumondor et al., 2016). This is a characteristic of drugs classified as class II or IV in the Biopharmaceutics Classification System (BCS) (Amidon et al., 1995), having solubility as a limiting step for oral absorption and consequently bioavailability (Savjani et al., 2012). For this reason, improving solubility is one of the most important areas within pharmaceutical drug development. Amongst several strategies to improve drug solubility, salification, or salt formation, is the most commonly applied technique to improve solubility and dissolution rate of poorly water soluble APIs (Serajuddin, 2007; Elder et al., 2013). Crystalline salts are defined as multicomponent crystals, *i.e.*, crystals composed by two or more components − atoms, ions or molecules − associated through ionic interactions (Childs et al., 2006). It is generally accepted that a salt between an acidic and a basic molecule can be obtained when the ΔpK_a is greater than 2 to 3 (Stahl and Wermuth, 2002) and that an enhancement in dissolution rate and solubility in water can be expected due to the potential formation of ionizable species, which will be more soluble in aqueous biological fluids (Tilborg et al., 2014).

Amorphous pharmaceuticals can also be used to enhance solubility, dissolution rate, and potentially bioavailability, of poorly water soluble APIs (Hancock and Zografi, 1997). Laitinen et al., (2013) provide a thorough review on polymeric solid dispersions, mesoporous silica carriers and co-amorphous blends, as means to physically stabilize drugs in the amorphous form. Among these, the co-amorphous formulation approach has been gaining interest due to a potentially lower bulk volume and hygroscopicity, compared to amorphous polymeric dispersions (Dengale et al., 2016). In this sense, co-amorphous formulations benefit from the introduction of a second low molecular weight component to form one single amorphous phase with the desired fast dissolution, high solubility and physical stability properties (Chieng et al., 2009; Laitinen et al., 2014; Jensen et al., 2015). Co-amorphous formulations between relevant drug–drug combinations (Chieng et al., 2009; Löbmann et al., 2011) and drug-excipients (Löbmann et al., 2013a; Jensen et al., 2015) have been reported, and recently, Jensen et al. (2016) have shown that some amino acids (AAs) can be used as co-amorphous salt formers in combination with a feasible drug, resulting in co-amorphous salts. For example, the co-amorphous indomethacin-arginine salt shows a 200-fold increase in dissolution rate compared to pure crystalline IND, and was physically stable for > 10 months when stored under dry conditions. Huang et al.

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(2017) showed comparable results for the co-amorphous valsartan-arginine salt. However, so far there has been no comparison of crystalline and co-amorphous salts and it remained unclear whether the dissolution increase of the co-amorphous salts was a result of (i) the amorphization of the drug itself, (ii) the salt formation between the drug and the AAs, or (iii) of both processes in parallel.

In this work, a co-amorphous salt comprising the drug indomethacin (IND) and the AA lysine (LYS) was prepared and compared to its crystalline salt, and the respective crystalline and amorphous forms of IND. The preparation of the co-amorphous and crystalline IND-LYS salts was investigated through mechanical activation using vibrational ball milling. Mechanical activation, or simply 'milling', is commonly employed as a pharmaceutical process aiming to decrease API particle size, but its energetic input can lead to several solid-state transitions, such as crystalline to amorphous (Hancock and Zografi, 1997) or amorphous to crystalline transformations (Trask et al., 2005), polymorphic interconversions (Trask et al., 2004), co-crystal formation (Rehder et al., 2011) as well as co-amorphization (Löbmann et al., 2013a) and salt formation (Jensen et al., 2016). Thus, the influence of the milling process on the solid state of IND-LYS has been investigated using two milling principles, namely 'dry ball milling' (DBM) and 'liquid assisted grinding' (LAG). In the former, a physical mixture of IND and LYS has been subjected to the milling procedure under dry conditions, whereas in the latter, a small amount of solvent, a solvent drop, is added to the milling process. The small amount of solvent can act as medium, increasing molecular diffusion, in order to facilitate a potential reaction between the components in the milling chambers. In this study, the feasibility of preparing co-amorphous and crystalline salts between the acidic drug IND and the basic AA LYS, using DBM and LAG, in order to obtain drug-amino acid salt products with different physical characteristics was investigated. Their physico-chemical properties were investigated by X-ray powder diffraction, differential scanning calorimetry and infrared spectroscopy. Furthermore, their performance with regard to physical stability, intrinsic dissolution and supersaturation potential were tested and compared to pure crystalline and amorphous IND.

2. Materials and methods

2.1. Materials

Indomethacin (IND, γ-polymorph, M_W = 357.78 g mol^{-1}, $pK_{a(IND)}$ = 4.45) was purchased from Hawkins Pharmaceutical group (Minnesota, USA) and L-lysine (LYS, M_W = 146.2 g mol^{-1}, $pK_{a(LYS)}$ = 10.5) was purchased from Sigma Aldrich (St. Louis, USA). Their chemical structures are shown in Fig. 1. Potassium dihydrogen phosphate and phosphorus pentoxide (P_2O_5) were purchased from Sigma-Aldrich, and di-sodium hydrogen phosphate heptahydrate was kindly donated by Merck (Darmstadt, Germany). All substances were of reagent grade and used as received.

2.2. Methods

2.2.1. Preparation of salts between drug and AA

Dry ball milling (DBM) was used to prepare the co-amorphous blend between IND and LYS. In brief, 1000 mg of powder containing the drug and the amino acid at an equimolar ratio was filled into 25 mL milling jars containing two stainless steel balls with a diameter of 12 mm each.

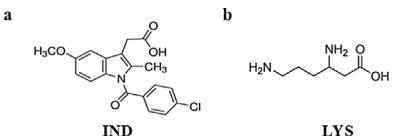

Fig. 1. Chemical structures of (a) the acidic drug IND and (b) the basic amino acid LYS.

The samples were subsequently milled at a frequency of 30 Hz for 60 min using a vibrational ball mill (Mixer mill MM400, Retsch GmbH & Co., Haan, Germany) located in a cold room (+ 6 °C). Furthermore, 1000 mg of pure IND and pure LYS were milled for comparison, using the same dry ball milling conditions.

In order to prepare the crystalline salt, 50 µL of ultrapure water was added to the powder mixture using the same milling conditions as described above, resulting in a liquid assisted grinding (LAG) process. In order to remove the residual solvent after the milling process, the samples were allowed to dry over P_2O_5 overnight in a desiccator before further analyses.

In addition, solid-state transitions during the milling process were monitored at different times by XRPD. For that, at the beginning of the process, 5 min of mixing in the milling jars but without the stainless steel balls was carried so that a physical mixture of the crystalline materials could be collected ('0 min' milling sample). The stainless steel balls were then added and the milling process started. At predetermined times (1, 5, 15, 30 and 60 min) the milling process was stopped, and approximately 10 mg powder samples were collected and analyzed.

2.2.2. X-ray powder diffraction (XRPD)

XRPD measurements were performed using an ẊPert PRO X-ray diffractometer (PANalytical, Almelo, The Netherlands) with CuKα radiation (1.5418 Å), acceleration voltage of 45 kV and current of 40 mA. The samples were scanned in reflectance mode between 5 and 35° 2θ in zero background plates, with a scan rate of 0.0625° 2θ/s and a step size of 0.026°. The data was collected and analyzed using ẊPert Data Collector software (PANalytical, Almelo, The Netherlands).

2.2.3. Thermal analysis

Differential scanning calorimetry (DSC) was performed using a DSC Discovery (TA Instruments, New Castle, USA), under nitrogen flow of 50 mL/min. All powder samples of approximately 1–6 mg were weighed in Tzero aluminum pans and closed with Tzero lids. The analyses of the co-amorphous blends after 60 min of milling were conducted in the modulated temperature mode, with a heating rate of 2 °C/min, amplitude of 0.212 °C and period of 40 s. After an isothermal step of 5 min at −10 °C, the samples were heated until 180 °C. The experimental glass transition temperatures (T_g, midpoint) were determined from the reversing heat flow signal of three replicates using Trios v3.3.0.4055 software (TA Instruments, New Castle, USA). The experimental T_g obtained for IND was used to calculate the theoretical T_g of the co-amorphous IND-LYS DBM formulation by using the Gordon-Taylor equation, as discussed elsewhere (Löbmann et al., 2013a). In addition, the physical mixture, IND-LYS LAG crystalline salt and the pure crystalline components were analyzed by DSC in standard temperature mode, with an applied heating rate of 10 °C/min, from 25 to 240 °C. In these cases, the melting points (T_m) were recorded as the onset temperatures of the endothermic event of three replicates using Trios software.

2.2.4. Fourier-transformed infrared spectroscopy (FTIR)

The FTIR analyses were carried out using a Nicolet 380 Fourier-transformed infrared spectrometer (Thermo Scientific, Wisconsin, USA) and an attenuated total reflectance accessory with a diamond plate (ATR, Smart iTR, Thermo Scientific, Madison, USA). The infrared spectra were collected with OMNIC software (Thermo Scientific version 8.1.11) between 4000 and 600 cm^{-1}, and calculated as a mean of 32 spectra with a resolution of 4 cm^{-1}.

2.2.5. Physical stability studies upon storage

The samples of amorphous and co-amorphous blends were maintained at three storage conditions as depicted in Table 1. Dry conditions were obtained in dessicators at 25 °C using P_2O_5 and at 40 °C using silica gel. Humid conditions at 25 °C were obtained using a saturated sodium chloride solution, and the humidity levels were continuously