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The relevance of co-amorphous formulations to develop supersaturated dosage forms: In-vitro, and ex-vivo investigation of Ritonavir-Lopinavir co-amorphous materials

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ABSTRACT

Ritonavir and Lopinavir have previously been demonstrated to decrease the maximum solubility advantage and flux in the presence of each other. The present study investigated the ability of Ritonavir and Lopinavir co-amorphous materials to generate a supersaturated state. Further, it explored the precipitation and flux behavior of co-amorphous materials. The co-amorphous materials of Ritonavir and Lopinavir were prepared by quench cool method and characterized in the solid state using XRPD, DSC, FTIR. The solubility studies were conducted in USP phosphate buffer (pH 6.8) for 12 h. The supersaturation potential and precipitation behavior were studied employing pH shift method. Further, the diffusion behavior was explored in vitro and ex-vivo using a semi-permeable membrane and intestinal everted sac method, respectively. The results showed that the co-amorphous materials have the potential to generate a supersaturated state. However, the reduction in the amorphous solubility was observed for both the drug(s) and the degree of reduction was found proportionate with the mole fraction of the compound in the co-amorphous material. Interestingly, the flux of both the drugs from co-amorphous material of 2:1 M ratio (Ritonavir 2: Lopinavir 1) was found exceeding the flux of the individual drugs in the amorphous form. The significant increase in the flux was attributed to the improved drug release properties due to precipitation of drug rich phase of nano/micro dimensions.

1. Introduction

Almost 75% of the developmental drug candidates suffer from the issue of limited aqueous solubility, and with ever-increasing specific targets like nuclear receptors, we can expect this percentage to go up in the near future (Di et al., 2012; Lipinski et al., 2001). As the aqueous solubility poses certain problems in the discovery and adds up to the significant cost in the developmental phase, there is a pressing need to address the issue. Many formulation approaches have been employed to overcome the challenge of poor solubility like co-solvents, surfactant-lipid blends, size reduction of API, complexation with cyclodextrin to name a few (Chaumeil, 1998; Khadka et al., 2014; Loftsson and Brewster, 1996; Tang et al., 2007). However, success with these techniques is usually marginal, as these techniques focus on increasing the crystalline solubility which further decreases the thermodynamic activity of the solute leading to the limited flux/diffusion (Barry et al., 1985; Indulkar et al., 2016). This led to the realization that increasing the solubility and thereby dissolution rate is often insufficient to improve the bioavailability. In this context, there is an increasing interest in the supersaturation drug delivery system, which presents a drug as a high energy amorphous material which upon dissolution generates supersaturated system. The solution is considered supersaturated when the thermodynamic activity of the solute exceeds that of the crystalline counterpart in a solution. In this way, supersaturated drug delivery system offers the unique advantage of simultaneous improvement of solubility and diffusion/flux (Kawakami, 2012; Miller et al., 2012; Raina et al., 2014). One of the interesting approaches (which has been used since 1961) to achieve the supersaturation is to disperse the drug

Abbreviations: RTV, Ritonavir; LPV, Lopinavir; RLQC 1:1, co-amorphous Ritonavir-Lopinavir prepared by quench cooling at 1:1 M ratio; RLQC 1:2, co-amorphous Ritonavir-Lopinavir prepared by quench cooling at 1:2 M ratio; RLQC 2:1, co-amorphous Ritonavir-Lopinavir prepared by quench cooling at 2:1 M ratio; LLPS, Liquid-Liquid Phase Separation; ASD, Amorphous Solid Dispersion

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in an inert carrier or matrix at solid state called as Amorphous Solid Dispersion (ASD) (Chiou and Riegelman, 1971). Polymers have been extensively employed to fabricate ASD, which stabilize the drug in high energy amorphous form and also act as a crystallization inhibitor in the solution form, thereby generating the supersaturated state (Baghel et al., 2016; Serajuddin, 1999). Recently there is renewed interest in replacing the polymers by small molecular weight compounds (either actives, excipients, and GRAS) and the materials fabricated by such method are (so) called as co-amorphous phases/formulations/systems (Dengale et al., 2016, 2015, 2014; Jensen et al., 2014; Laitinen et al., 2013; Teja et al., 2015).

Very few studies have been reported in the literature investigating the supersaturating ability of co-amorphous materials, which showed modest improvement in the supersaturation (Ojarinta et al., 2017; Shayanfar et al., 2013; Shayanfar and Jouyban, 2013; Craye et al., 2015; Heikkinen et al., 2015). Nevertheless, the phenomenon was not systematically and extensively studied so far. Trasi and Taylor (2015) studied the supersaturation behavior and membrane flux in the phosphate buffer for model compounds Ritonavir, Lopinavir, Felodipine, and diclofenac. They found that maximum flux across the semipermeable membrane is limited by the amorphous solubility of the drug and exceeding the amorphous solubility leads to a phenomenon called Liquid-Liquid Phase Separation (LLPS) without any further increase in the flux. Further, they reported that amorphous solubility of a poorly soluble hydrophobic compound is reduced by the presence of a second solute (Trasi and Taylor, 2015). These findings have implications for co-amorphous formulations, which consist of two or more amorphous drugs (solutes) which may result in a reduction of amorphous solubility of each component thereby reducing the maximum achievable supersaturation (Laitinen et al., 2017). This may lead to the decrease in the thermodynamic activity and hence reduction in the flux.

In the above context, the supersaturation and effect of another solute on maximum achievable supersaturation for poorly water-soluble drugs formulated into a single co-amorphous material was studied. The precipitation behavior and translation of achieved supersaturation of co-amorphous materials into the flux/diffusion (in-vitro, ex-vivo) were investigated. Further, the possibility of formation of drug rich phase for co-amorphous materials was also explored. Ritonavir (RTV) and Lopinavir (LPV) were chosen as a model drug to formulate co-amorphous materials, as first, they are marketed as a fixed dose combination (Cvetkovic and Goa, 2003; Murphy et al., 2008). Second, both these drugs are weak bases which would further enable us to study the precipitation at the higher pH and the possibility of LLPS/GLPS. Third, the phase behavior of these compounds had been studied in the presence of each other in the solution (Trasi and Taylor, 2015), so we thought to take the precedence from the literature and study the phase behavior and precipitation after formulating them into the co-amorphous materials.

2. Materials and methods

2.1. Materials

Ritonavir (RTV) and Lopinavir (LPV) were obtained from Lupin Pharmaceuticals, Pune, India as a gift sample. Their chemical structures are shown in Fig. 1. All drugs were used as received. HPLC grade solvents like Acetonitrile and Methanol were purchased from Merck Life Sciences Private Limited, Mumbai, India. Ultra-pure water was obtained from SIEMENS purification system (W3T197571, version 1.23, SIEMENS water technologies, Germany) installed in the lab.

2.2. Methods

2.2.1. Preparation of co-amorphous materials

A 500 mg mixture of RTV and LPV in the molar ratio of 1:1 (RLQC 1:1–267.04 mg of RTV and 232.96 mg of LPV), 1:2 (RLQC 1:2–182.16 mg of RTV and 317.84 mg of LPV), 2:1 (RLQC 2:1–348.14 mg of RTV and 151.90 mg of LPV) were taken in a porcelain dish and subjected to melting in a heating mantle at the rate of 10 °C/min. The heating mantle was allowed to heat with a linear increase in the temperature until the melting of both the components was achieved. The porcelain dish with the molten sample was then transferred to a glass desiccator containing dry ice. Further, the sealed desiccator was stored in an ultra-low freezer for 4.0 h at −80 °C. After 4.0 h the porcelain dish was taken out to scrap the sample. The scraped sample was then pulverized using ceramic mortar and pestle to get fine material of glassy (amorphous) form. The amorphous materials were stored under dry condition inside vacuum desiccator until further experiments.

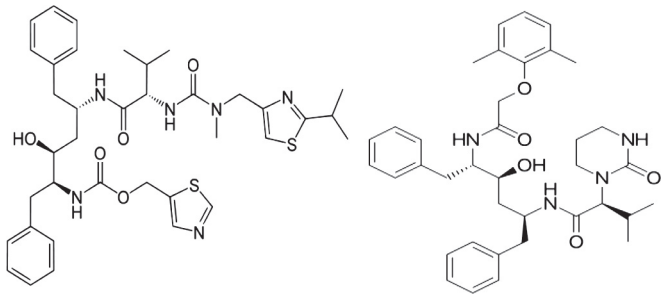

Fig. 1. Chemical structures of Ritonavir and Lopinavir.

2.2.2. Solid state characterization

X-ray powder diffractograms were recorded using Rigaku miniflex 600 X-ray diffractometer (Rigaku Co., Tokyo, Japan). The instrument was operated at 600 watts (X-ray tube), with a fixed tube current of 15 mA and a voltage of 40 kV. The diffracted X-ray beam was monochromated by a graphite monochromator, and a standard scintillation counter was used as the detector. Diffraction intensities were measured by fixed time step scanning method in the range of 5–40° (2Θ).

Differential Scanning Calorimetry (DSC) measurements were carried out by a Shimadzu DT-60 apparatus (Shimadzu Corporation, Kyoto, Japan). 3–5 mg of prepared co-amorphous material was placed in a flat-bottomed aluminum pan of 0.1 mm thickness and crimped with an aluminum lid. The samples were placed into sample holder and heated from 25 °C to 200 °C at the heating rate of 10 °C (or as required in the experiment) per minute under a nitrogen flow (10 cc/min).

FTIR spectra of the prepared samples were obtained with Shimadzu FTIR – 8300 systems (Kyoto, Japan) over a range of 4000–500 cm^{-1} (25 scans, resolution 4 cm^{-1}).

2.2.2.1. Gordon-Taylor equation. Gordon – Taylor equation was used for prediction of experimental glass transition temperature values of various prepared co-amorphous forms theoretically.

$$Tg_{mix} = \frac{W_1.\ Tg_1 + K.\ W_2.\ Tg_2}{W_1 + K.\ W_2} \quad (1)$$

where Tg (mix) is the glass transition temperature of prepared co-amorphous compounds, K is a constant and W_1, Tg_1 and W_2, Tg_2 are weight fractions and glass transitions of component 1 and 2, respectively.

The constant K can be further calculated as.

$$K = \frac{\rho 1.\ Tg1}{\rho 2.\ Tg2} \quad (2)$$

where ρ1 and ρ2 are the respective pycnometric densities of the components.