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Preparation and characterization of co-amorphous Ritonavir–Indomethacin systems by solvent evaporation technique: Improved dissolution behavior and physical stability without evidence of intermolecular interactions

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ABSTRACT

The aim of this study was to stabilize the amorphous form of Ritonavir (RTV) a BCS class-II drug with known amorphous stabilizing small molecule Indomethacin (IND) by co-amorphous technology. The co-amorphous samples were prepared by solvent evaporation technique in the molar ratios RTV:IND (2:1), RTV:IND (1:1), RTV:IND (1:2) and their amorphous nature was confirmed by XRPD, DSC and FT-IR. Physical stability studies were carried out at temp 25 °C and 40 °C for maximum up to 90 days under dry conditions. Solubility and dissolution testing were carried out to investigate the dissolution advantage of prepared co-amorphous systems. The amorphous mixtures of all tested molar ratios were found to become amorphous after solvent evaporation. The same was confirmed by detecting halo pattern in diffractograms of co-amorphous mixtures. The *Tg* values of all three systems were found to be more than 40 °C, the highest being 51.88 °C for RTV:IND (2:1) system. Theoretical *Tg* values were calculated by Gordon–Taylor equation. Insignificant deviation of theoretical *Tg* values from that of practical one, corroborated by FT-IR studies showed no evidence of intermolecular interactions between RTV and IND. Almost 3-folds increase in the solubility for both amorphous RTV and IND was found as compared to their respective crystalline counterparts. The study demonstrated significant increase in the dissolution rate as well as increase in the total amount of drug dissolved for amorphous RTV, however it failed to demonstrate any significant improvement in the dissolution behavior of IND.

1. Introduction

The major part of 50 most sold drug products (84%) worldwide is administered orally. Significant drug absorption and appropriate drug delivery are prerequisites for successful oral treatment of diseases. Low aqueous solubility is indeed one of the major reasons behind failures in the development of oral drug delivery system (Brahamsson and Lennernas, 2002). There is a significant increase in insoluble new chemical entities (NCE) in the research portfolio, in recent past. According to Di et al. (2012) recent studies showed that 75% of the drug development candidates had low solubility and belonged to biopharmaceutical classification system (BCS) classes II and IV. A number of novel approaches for enhancing low aqueous solubility of drugs have been attempted and continued to evolve over a period. Reduction in particle size (nano-drug delivery) and increased surface area, the use of alternative salt forms, solubilization of drug in co-solvents or micellar solutions, complexation with cyclodextrins or the use of lipid based vehicles for the delivery of lipophilic drugs to name few. Although some of these techniques have been effective at enhancing oral bioavailability for specific compounds, success is usually marginal and highly dependent on the physicochemical properties of the drug. Since these compounds are not specifically lipophilic either, lipid and surfactant based drug delivery systems do not constitute a universal development approach for poorly water soluble drugs (Kawakami, 2012). Amorphous system is among the most promising formulation approach for such drugs which offers unique

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opportunity in dealing with and manipulating inherent lipophilicity.

Amorphous state appears to be the most convincing formulation approach because it possesses the advantage of improved aqueous solubility over its crystalline counterpart (Hancock and Zografi, 1997), which can be expected to enhance dissolution behavior and thereby bioavailability of poorly water soluble drugs. The advantages of amorphous state come at expense of inherent poor physical stability and thereby increased risk of conversion of metastable amorphous state into stable polymorphic form (Hancock and Parks, 2000). Also, the dissolution behavior of amorphous solids can be negated by the crystallization of the amorphous solid on contact with the dissolution medium (Alonzo et al., 2010). The most extensive approach to stabilize amorphous form is solid dispersion technology, in which drug is incorporated into amorphous polymer, which increases glass transition temperature (*Tg*) of the system and provides stability to it (Vasconcelos et al., 2007). However there are several disadvantages of solid dispersion technology, discussed in an excellent review by Riikka Laitinen et al. First, drug–polymer systems are often hygroscopic, and in that case, the absorbed moisture reduces the glass transition temperature (*Tg*) of the system, leading to phase separation and recrystallization. Second, due to the limited miscibility of some drugs in the polymer, large quantities of polymer are often required for sufficient drug loading, leading to large bulk volumes of the final dosage forms (Laitinen et al., 2013). Thus, the incremental innovative and alternative method to amorphous polymer dispersion is required to efficiently stabilize amorphous form of drugs.

In order to counter many of the disadvantages of solid dispersion technology, the concept of co-amorphous systems has been introduced. In these systems, a combination of two small molecules (drugs or excipients) is used instead of drug–polymer mixtures. These systems have been found to provide high stability and enhanced dissolution rates for the drugs. The primary reason for the enhanced stability and dissolution has been found to be solid-state interactions between the two drugs present in the system (Laitinen et al., 2013). The first report of usage of small molecules instead of large molecular weight polymers to stabilize drug into their amorphous form dates back to 1989 when Fakuoka et al. (1989) reported formation of stabilized amorphous form of salicilin along with phenobarbital with improved physicochemical properties. In early 1990s, with advent of solid dispersion technology, the technique of binary amorphous drugs in spite of having many advantages, was sidelined. It was only after the year 2002 the problems concerning solid dispersion technology began surfacing which shifted researcher's attention toward binary amorphous system, which has been evident by several publications after 2002 on area of binary amorphous systems. Yamamura et al. combined two drug molecules and found that cimetidine formed an amorphous binary mixture with the nonsteroidal anti-inflammatory drugs (NSAIDs) naproxen (Yamamura et al., 1996), indomethacin (Yamamura et al., 2000) and diflunisal (Yamamura et al., 2002) upon precipitation from ethanol solution. Allesø et al. (2009) reported binary mixtures of naproxen and cimetidine which were found to become amorphous upon ball milling for 60 min at 4 °C. Löbmann et al. (2012) reported improvement in stability and solubility of Glipizide while combined with Simvastatin. Chieng et al. (2009) showed that crystalline indomethacin can be made amorphous and stabilized by co-milling with crystalline ranitidine hydrochloride.

RTV is the protease inhibitor with very low bioavailability due to its limited aqueous solubility (BCS Class-II member). Several attempts have been made to stabilize amorphous form of RTV, some reports claiming successful preparation of solid dispersion of RTV with poly(ethyleneglycol) 8000 (Law et al., 2001) and polyvinylpyrolidone (PVP) (Poddar et al., 2011). United States Food and Drug Administration (US-FDA) approved Abbott's Kaletra tablets (Lopinavir/Ritonavir 200/50 mg) and Norvir (Ritonavir 100 mg) in the year 2005 and 2010 respectively, containing amorphous solid dispersion prepared by melt extrusion techniques. In the current study, we attempted to stabilize RTV with proven glass forming small molecule Indomethacin (IND), as there are many reports of co-amorphous systems involving IND as one of the small molecules (Yamamura et al., 2000; Chieng et al., 2009; Lobmann et al., 2011). The combination of RTV and IND offers same set of advantages as discussed by Alleso et al. In case of Naproxen–Cimetidine binary system, first, the combination of two drugs can provide therapeutic advantage as the side effects of RTV which includes back pain and fever (Medical reviews submitted to US-FDA Centre for Drug Evaluation and Research, 2013), can be countered by IND which is a potent NSAID. It can be seen as a potential way of preparing candidates for new formulations intended for combination therapy.

2. Materials and methods

2.1. Materials

Ritonavir (M = 720.9 g/mole) and Indomethacin (M = 357.7 g/mole) were obtained from Lupin pharmaceuticals, Ltd. Pune (India) and Hetero drugs, Ltd. Hyderabad (India), respectively as gift samples. Their chemical structures are shown in Fig. 1. Both drugs were used as received.

2.2. Methods

2.2.1. Preparation of amorphous precipitate of RTV–IND binary system

Coamorphous system of RTV and IND were prepared by solvent evaporation technique using methanol (ICH Class-2, Solvents to be limited) as a solvent. A total of 1000 mg in molar ratios of 1:1 (668.4 mg of RTV and 331.6 mg of IND), 1:2 (501.9 mg of RTV and 498.1 mg IND), 2:1 (801.2 mg of RTV and 198.8 mg of IND) were mixed homogeneously and then dissolved in 50 ml methanol. The solvent was evaporated under reduced pressure at 40 °C. The residual solvent left after evaporation was then removed completely by placing the sample under vacuum for 2 days inside desiccator containing $CaCO_3$. The precipitates were stored in desiccator until its use in the experiment.

2.2.2. Preparation of amorphous Ritonavir and Indomethacin by quench cooling

It was observed during experiment that individual RTV and IND cannot be made amorphous by solvent evaporation technique. As the prerequisite for calculation of factor K in Gordon Taylor equation, RTV and IND were made amorphous by quench cooling technique. Sample powder (2–3 mg) was crimped in an aluminum pan and melted upon heating in DSC. The pan containing melted sample was carefully removed from DSC furnace, then immersed in dry ice (because unavailability of in situ cooling facility in instrument) and kept there for several minutes. DSC was cooled to 20 °C and quench cooled sample then heated in DSC up to 200 °C at 10 °C min^{-1} in order to establish the glass transition temperature (*Tg*) of an amorphous phase.

2.2.3. Theoretical Tg values (Gordon Taylor equation)

The experimental glass transition values of various solvent evaporated amorphous precipitates were compared with the predicted *Tg* values calculated from Gordon–Taylor equation

$$Tg\ mix = \frac{W1 \cdot Tg1 + K \cdot W2 \cdot Tg2}{W1 + K \cdot W2} \quad (1)$$