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Solid-state transformations of ribavirin as a result of high-shear mechanical processing

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ABSTRACT

Ribavirin ($C_8H_{12}N_4O_5$; anti-viral agent) was crystallized as two unique, phase-pure polymorphs (R-I and R-II). Calorimetrically determined isobaric heat capacities and heat of transition data were utilized to determine the solid-state transition temperature (T_{tr}), confirming enantiotropism, while R-I was determined to be kinetically stable at ambient temperature. Unprocessed samples of the low T_m polymorph, R-II, did not convert into R-I when held isothermally well above T_{tr} for 7 days. In contrast milled R-II completely transformed to R-I after 15 min at the same storage conditions, indicating that defects sustained during processing reduced the energy barrier for transformation, allowing it to occur. R-II was subjected to both cryogenic milling and impact milling at ambient temperature for various durations. Cryomilling resulted in an *in situ* progressive reduction of crystallinity, with complete conversion to amorphous ribavirin after 2 h. Limited molecular mobility attributable to the low milling temperature ($T_{exp} = -196\,^{\circ}C$) likely inhibited recrystallization, allowing the amorphous solid to persist. In contrast, continuous impact milling at ambient temperature resulted in complete *in situ* conversion from R-II to R-I after 3 h. The data suggested rapid conversion to R-I from highly disordered regions during extended milling, facilitated by localized heat buildup that likely exceeded T_g and/or T_{tr}.

1. Introduction

Ribavirin ($C_8H_{12}N_4O_5$; *1-β-D-*Ribofuranosyl-1H-1,2,4-triazole-3-carboxamide), an anti-viral drug, is a synthetic nucleoside analog structurally related to guanine. The combined use of ribavirin and pegylated interferon-α is approved by the US Food and Drugs Administration (FDA) for treatment of hepatitis C in normal and HIV-infected individuals (Ghany et al., 2009). According to the Essential Medicines List of the World Health Organization, ribavirin can also be used to treat viral hemorrhagic infections (World Health Organization, 2015), such as Lassa fever and Crimean-Congo fever. Further, it is also used to treat respiratory syncytial virus infections in moderately to severely immunocompromised patients (Marcelin et al., 2014).

Ribavirin is predominantly administered via solid oral dosage forms. Some marketed products include Copegus® (200 mg film coated tablets; Hoffman La-Roche), Rebetol® (200 mg hard capsules and 40 mg/mL oral solution; Merck), Ribasphere® (200, 400 and 600 mg hard capsules; Three Rivers pharmaceuticals), and Moderiba™ (200, 400, 600 mg film coated tablets, AbbVie) (Goodarzi et al., 2016). Additionally, it is also given as a powder for inhalation solution (Virazole® 6 g vials; Valeant Pharmaceuticals). Regardless of the delivery system, processing of solid ribavirin requires some degree of high-shear mechanical processing, including milling, granulation and compaction, making unanticipated solid-state transformations, including crystal-to-crystal and crystal-to-glassy solids, a possibility during manufacturing.

Over the last few decades, numerous examples of process-induced phase changes of pharmaceutically relevant materials have been reported (Airaksinen et al., 2005; Alshahrani et al., 2015; Feng et al., 2008; Hadžović et al., 2010; Wildfong, 2009; Zhang et al., 2004). Some estimates suggest that approximately 30% of active pharmaceutical ingredients (API) are susceptible to solid form changes during mechanical processing (Wildfong, 2009). Since high-shear processing does not automatically result in solid form changes, however, case-by-case identification of process-induced transformation potential, followed by thorough characterization, elucidation of underlying mechanisms, and identification of risk to eventual drug products is consistent with the materials understanding initiatives at the heart of worldwide regulatory guidances.

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Ribavirin was previously reported to solidify as one of two unique crystalline polymorphs (Prusiner and Sundaralingam, 1973; Witkowski et al., 1972), conventionally named R-I and R-II, which respectively represent the high-melting and low-melting temperature enantiotropes (Tong et al., 2005). Careful selection of a consistent solid form during pre-formulation, which persists in the final drug product, is, therefore, warranted. In addition, the impalpable nature of ribavirin powder, accompanied with low and variable tapped densities (reported range 0.32–0.45 g/mL; Liebowitz et al., 2002) pose considerable challenges to material handling. While flow properties and processability of ribavirin can be improved through secondary manufacturing procedures, such as pelletization (Kerrish et al., 2004), roller compaction (Liebowitz et al., 2002) and wet granulation (Matharu and Patel, 2002); the potential for concurrent solid-state transformations during manufacturing has not been investigated. Should these occur, it is likely that, consistent with other small molecule crystalline materials, unanticipated partial or complete phase conversions can alter the mechanical properties, processability, and performance attributes of the drug substance in the final drug product, posing an unidentified risk associated with exposure of this material to conventional manufacturing environments.

Solid form conversions of pharmaceutically relevant materials are a known potential consequence of milling (Crowley and Zografi, 2002; Feng et al., 2008; Matsumoto et al., 1988; Otsuka et al., 1986). Application of high-shear stress, accompanied by heat transfer from processing equipment, can elicit direct structural changes to the crystal, or may proceed rapidly following introduction of lattice defects. Moreover, accumulated defects that persist following processing can contribute to altered chemical (Lin et al., 2010) and physical (Feng et al., 2008) stability of drug substances, particularly with respect to temperature excursions occurring during storage and shipping of process intermediates or the final drug product. While empirical models of mechanical activation potential exist (Morris et al., 2001; Wildfong et al., 2006; Lin et al., 2009), their utility is restricted to specific experimental conditions such as temperature, equipment dimensions, and sample size, making universal predictions elusive. Experimental observation of mechanically induced transformations, therefore, remains important, especially if a range of temperatures, processing stresses and other conditions are expected.

In the present work, the influence of thermal and mechanical stresses on ribavirin was investigated as they pertained to the potential occurrence of solid-state phase transformations during and after high-shear processing. Following the observation that the kinetics of a temperature-mediated enantiotropic conversion was prohibitive for unprocessed ribavirin, it was hypothesized that transitions between polymorphs could be observed only after defects resulting from pulverization enabled more rapid transformations at elevated temperatures. To test the hypothesis, phase pure samples of each ribavirin enantiotrope were generated, and the enantiotropic transition temperature (T_{tr}) was determined. Reversibility of the polymorphic transition (R-I → R-II) was also investigated by room temperature exposure of the phase-pure metastable polymorph to varied conditions of relative humidity. Samples were milled, with and without temperature control, and the outcomes of mechanical and/or thermal stimuli were characterized and recorded. Particle size reduction, commensurate with increased specific surface area, and the extent of crystal damage were measured to identify which of these factors predominate in the observed phase transformations. This additional effort was expected to provide better understanding of the likelihood of phase conversions during other manufacturing steps (such as compaction, which also involves particle fracture and crystal damage). Ultimately, these observations serve as a reference for materials that may be similarly susceptible to mechanically-induced phase changes.

2. Materials and methods

Ribavirin was purchased from Jinan Jiaquan Chemicals Co. Ltd. (Jinan, China, Lot number 02110330). Samples were stored in a desiccator containing phosphorous pentoxide (P_2O_5) at ambient temperature prior to use.

2.1. Recrystallization of ribavirin polymorphs

Phase-pure samples of ribavirin polymorphs were prepared by recrystallization from solvents, using previously reported methods with slight modification (Prusiner and Sundaralingam, 1973; Witkowski et al., 1972). To obtain pure R-II, a slight excess of vendor supplied ribavirin (R-RW) was dissolved in water at 80 °C to form a supersaturated solution. The suspension was filtered and left to cool slowly to room temperature. The resulting crystals were vacuum-dried at 25 °C overnight and stored for further characterization.

Pure R-I was prepared by fractional recrystallization, where a supersaturated solution of R-RW was formed in methanol (>99% HPLC grade, Fisher Scientific) at 60 °C and filtered. The filtrate was dried under a current of air, which facilitated rapid crystallization, owing to rapid evaporation of organic solvent, accompanied by a temperature drop. Upon appearance of the first few crystals, the mother liquor was re-filtered to harvest pure R-I; phase purity was confirmed by rapid differential scanning calorimetry (DSC) and peak-by-peak comparison of powder X-ray diffraction (PXRD) patterns with the known crystal structure (CSD refcode: VIRAZL). Experimentation demonstrated that the re-filtration to harvest R-I crystals was required to prevent recrystallization of a mixture of phases. Isolated R-I crystals were dried in a vacuum oven, and used as seeds for producing subsequent batches (0.5–1.0 g) of pure R-I.

2.2. Generation of amorphous ribavirin

Amorphous ribavirin was prepared *in situ* during DSC experiments. An accurately weighed sample of R-II was heated to $T_m + 10$ °C at a rate of 5 °C/min, and held isothermally for 10 min. The molten sample was rapidly cooled at 40 °C/min, with no observation of recrystallization, and the T_g was determined during the subsequent DSC heating cycle.

2.3. Isothermal heating experiments

Conversion to the high-temperature stable enantiotrope, R-I, was attempted using a method similar to that reported for chlorpropamide enantiotropes (Wildfong et al., 2007). Approximately 500 mg of R-II powder was placed in glass petri dishes, which were held isothermally at 150 °C ($T < T_m$ for either polymorph) in an Isotemp 13-246-506GA gravity convection oven (Fisher Scientific, Dubuque, IA). Thermal exposure was maintained as long as the sample did not exhibit signs of degradation. Samples were periodically characterized to identify the emergence of the R-I phase, by comparison of experimental diffraction patterns with the calculated PXRD patterns of each enantiotrope.

2.4. Milling experiments

R-II was milled at frequency of 30 Hz in a vibratory impact ball mill (Mixer Mill MM200, Retsch GmbH & Co., Germany) for durations of 60, 180 and 270 min. Each 0.5 g powder sample was placed in a 25 mL volume stainless steel jar containing one 12 mm diameter stainless steel ball. Milled samples were immediately