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Water-induced phase separation of miconazole-poly (vinylpyrrolidone-co-vinyl acetate) amorphous solid dispersions: Insights with confocal fluorescence microscopy

Sugandha Saboo, Lynne S. Taylor*

Department of Industrial and Physical Pharmacy, College of Pharmacy, Purdue University, West Lafayette, IN 47907, United States

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ABSTRACT

The aim of this study was to evaluate the utility of confocal fluorescence microscopy (CFM) to study the water-induced phase separation of miconazole-poly (vinylpyrrolidone-co-vinyl acetate) (mico-PVPVA) amorphous solid dispersions (ASDs), induced during preparation, upon storage at high relative humidity (RH) and during dissolution. Different fluorescent dyes were added to drug-polymer films and the location of the dyes was evaluated using CFM. Orthogonal techniques, in particular atomic force microscopy (AFM) coupled with nanoscale infrared spectroscopy (AFM-nanoIR), were used to provide additional analysis of the drug-polymer blends. The initial miscibility of mico-PVPVA ASDs prepared under low humidity conditions was confirmed by AFM-nanoIR. CFM enabled rapid identification of drug-rich and polymer-rich phases in phase separated films prepared under high humidity conditions. The identity of drug- and polymer-rich domains was confirmed using AFM-nanoIR imaging and localized IR spectroscopy, together with Lorentz contact resonance (LCR) measurements. The CFM technique was then utilized successfully to further investigate phase separation in mico-PVPVA films exposed to high RH storage and to visualize phase separation dynamics following film immersion in buffer. CFM is thus a promising new approach to study the phase behavior of ASDs, utilizing drug and polymer specific dyes to visualize the evolution of heterogeneity in films exposed to water.

1. Introduction

Many of the new compounds emerging from pharmaceutical pipelines have low solubility and dissolution rates (Thayer, 2010). There are many solubility and dissolution enabling technologies available to address this issue (Williams et al., 2013). Amongst them, using the amorphous form of drug, molecularly dispersed in a polymeric matrix, producing a system commonly referred to as an amorphous solid dispersion (ASD), has proven to be a highly effective and viable formulation strategy to improve solubility and in turn, bioavailability of poorly soluble drug candidates (Leuner and Dressman, 2000; Newman et al., 2012).

The successful application of ASD is based on an important underlying assumption of homogeneity or miscibility of the drug and polymer at a molecular level in the ASD matrix. Lack of homogeneity in ASDs has been found to be of issue that can impact stability as well as the dissolution performance of ASDs. If the polymer is not homogeneously mixed with the drug, or phase separation has occurred resulting in drug-rich and drug-lean domains, the role of the polymer as a crystallization inhibitor is undermined (Alonzo et al., 2010; Ilevbare et al., 2012; Konno and Taylor, 2006). Phase separation in the solid ASD matrix can result from either crystallization of drug or demixing with both drug and polymer retaining their amorphous nature. The latter phenomenon is termed as amorphous–amorphous phase separation (AAPS). AAPS has been found to be a precursor to crystallization as the resultant drug-rich phase has increased propensity towards crystallization due to the lower local polymer concentration (Rumondor et al., 2009b). Crystallization, in turn, can be expected to negate the solubility advantage of the amorphous formulation (Yu, 2001). AAPS can occur during manufacturing (Qian et al., 2010), as a result of environmental moisture absorbed during storage (Marsac et al., 2010; Rumondor et al., 2009b) or in presence

Abbreviations: AAPS, amorphous amorphous phase separation; AFM, atomic force microscopy; ASD, amorphous solid dispersion; CFM, confocal fluorescence microscopy; DSC, differential scanning calorimetry; IR, infrared spectroscopy; LCR, Lorentz contact resonance; PVPVA, poly (vinylpyrrolidone-co-vinyl acetate); RH, relative humidity; XRD, X-ray diffraction.

* Corresponding author at: Department of Industrial & Physical Pharmacy, College of Pharmacy, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47907, United States.
E-mail address: lstaylor@purdue.edu (L.S. Taylor).

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of bulk water introduced into the matrix during the dissolution process (Purohit and Taylor, 2015b).

Conventional analytical techniques, such as differential scanning calorimetry (DSC) and X-ray diffraction (XRD), are useful for detecting crystallinity in the ASD matrix, but it may be challenging to detect AAPS using these approaches. DSC, for instance, can fail to detect phase separation due to its non-isothermal nature which can lead to remixing during heating (Qian et al., 2010), and is inherently limited for systems with a domain size of less than 30 nm (Newman et al., 2008). XRD is most suited for analyzing systems with long- range order, and research is still underway to extract interpretable information from complex amorphous systems such as ASDs. Microscopic and surface analysis techniques, such as atomic force microscopy and electron microscopies (scanning and transmission) have nano-scale spatial resolution but lack chemical specificity to differentiate drug and polymer (Karavas et al., 2007; Lauer et al., 2013). Infrared (IR) and Raman spectroscopy can provide useful chemical information about miscibility based on the presence of intermolecular interactions (Keratichewanun et al., 2015; Rumondor et al., 2009a) but data interpretation can be complex and not all systems are suitable to this type of analysis. Due to aforementioned challenges encountered with many conventional analytical methods, there continues to be interest in new approaches to evaluate ASD miscibility (Vogt, 2010).

An emerging analytical approach for miscibility evaluation involves fluorescence-based methods, both steady state fluorescence spectroscopy and fluorescence microscopy (Li and Taylor, 2016; Purohit and Taylor, 2015a,b; Tian et al., 2016). Both techniques require a fluorophore for successful application. ASDs of drugs which are autofluorescent have been investigated using this approach (Tian et al., 2016) while for non-fluorescent systems, extrinsic environment sensitive fluorescent probes have been used to monitor phase separation (Li and Taylor, 2016; Purohit and Taylor, 2015a, b). Fluorescence microscopy, utilizing external fluorescent probes, is a promising approach for visualizing phase separation in ASDs, but is an un-optimized technique. To date, most studies have focused on staining the drug-rich phase by incorporating a hydrophobic dye that preferentially interacts with the drug. However, in the biological sciences, it is common to employ multiple dyes, chosen to stain specific structures. This approach is also feasible for drug-polymer systems (Purohit et al., 2017), since the polymer is typically more hydrophilic than the drug. The objectives of this study were two-fold. First, to investigate confocal microscopy as a tool to probe miscibility in drug-polymer ASD films by employing fluorescent dyes with different affinities for drug and polymer. Second, to evaluate the evolution of microstructure in ASD films following water-induced AAPS occurring during preparation, storage at high relative humidity and during dissolution. The model drug- polymer system used was miconazole-poly (vinylpyrrolidone-co-vinyl acetate) (Mico-PVPVA); the miscibility of this model system has been found to vary depending on the preparation method used (spray drying versus hot melt extrusion) and stresses involved (compression pressure, dwell time, etc.) (Singh et al., 2015, 2014; Worku et al., 2014). It was hypothesized that the simultaneous contrast imaging of drug-rich and polymer-rich domains could be achieved by utilizing two fluorescent probes, added to the ASD matrix; a hydrophobic fluorescent probe which selectively partitions into the drug-rich phase, and a second hydrophilic probe which preferentially associates with polymer-rich phase. After screening several dyes, prodan and rhodamine-6G (R6G) were selected as drug-specific and polymer-specific dyes, respectively. An orthogonal technique, atomic force microscopy (AFM) coupled with infrared (IR) spectroscopy (AFM-nanoIR) and Lorentz contact resonance (LCR) measurements (AFM-LCR) was used to confirm the results obtained from confocal fluorescence microscopy (Li and Taylor, 2016).

2. Materials and methods

2.1. Materials

Miconazole (mico) was purchased from ChemShuttle (Jiangsu, China). Methanol and dichloromethane were procured from Macron chemicals (NJ, U.S.A.). Prodan was obtained from AnaSpec Inc. (CA, U.S.A.), 5-(4,6-dichlorotriazinyl) aminofluorescein (5-DTAF) and Alexa Fluor®488 were procured from ThermoFisher Scientific (CA, U.S.A.), while fluorescein, pyrene, Nile red, fluorescein isothiocyanate (FITC) and rhodamine 6G (R6G) were obtained from Sigma-Aldrich Co. (MO, U.S.A.). Kollidon VA 64 (PVPVA) was supplied by the BASF Corporation (Ludwigshafen, Germany). The chemical structures of model drug and polymer along with selected fluorescent probes are given in Fig. 1.

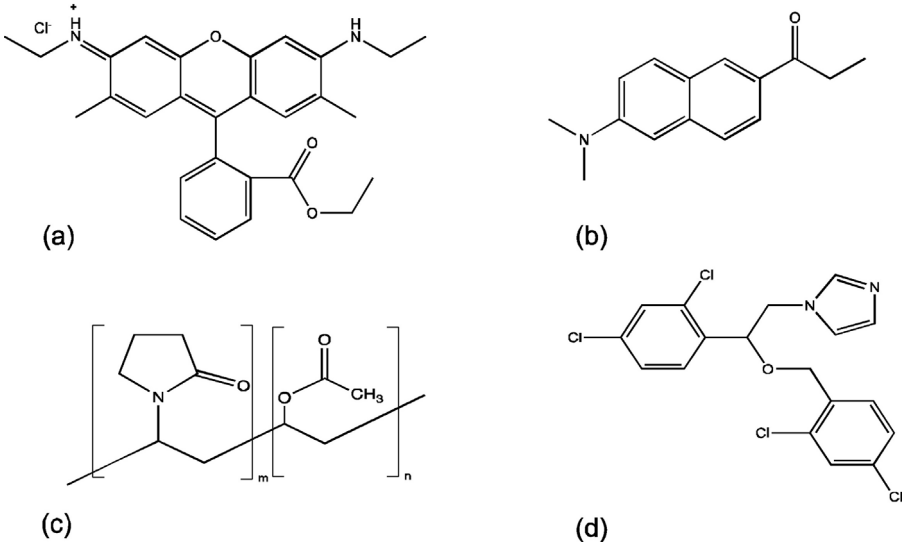

Fig. 1. Structures of the compounds used. Rhodamine-6G (R6G) (a), prodan (b), poly (1-vinylpyrrolidone-co-vinyl acetate) (PVPVA) (c) and miconazole (d).