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Research paper

Enhancement of dissolution rate through eutectic mixture and solid solution of posaconazole and benznidazole

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ABSTRACT

Benznidazole (BNZ), the only commercialized antichagasic drug, and the antifungal compound posaconazole (PCZ) have shown synergistic action in the therapy of Chagas disease, however both active pharmaceutical ingredients (APIs) exhibit low aqueous solubility potentially limiting their bioavailability and therapeutic efficacy. In this paper, we report for the first time the formation of a eutectic mixture as well as an amorphous solid solution of PCZ and BNZ (at the same characteristic ratio of 80:20 wt%), which provided enhanced solubility and dissolution rate for both APIs. This eutectic system was characterized by DSC and the melting points obtained were used for the construction of a phase diagram. The preservation of the characteristic PXRD patterns and the IR spectra of the parent APIs, and the visualization of a characteristic eutectic lamellar crystalline microstructure using Confocal Raman Microscopy confirm this system as a true eutectic mixture. The PXRD result also confirms the amorphous nature of the prepared solid solution. Theoretical chemical analyses indicate the predominance of π-stacking interactions in the amorphous solid solution, whereas an electrostatic interaction between the APIs is responsible for maintaining the alternating lamellar crystalline microstructure in the eutectic mixture. Both the eutectic mixture and the amorphous solid solution happen to have a characteristic PCZ to BNZ ratio similar to that of their pharmacological doses for treating Chagas disease, thus providing a unique therapeutic combination dose with enhanced apparent solubility and dissolution rate.

1. Introduction

Chagas disease or American trypanosomiasis is a zoonotic infectious disease caused by the protozoan flagellate *Trypanosoma cruzi* (Chagas, 1909). The current therapy for Chagas disease relies on the active pharmaceutical ingredient (API) with antiprotozoal activity benznidazole (BNZ) (DNDi, 2016).

During the acute phase of the disease, BNZ can promote healing in approximately 100% of children and 60% of adults (De Andrade et al., 1996). However, in the chronic phase, when irreversible damages to the heart, esophagus and colon have occurred, there is no satisfactory treatment (Sosa-Estani et al., 2009). Typically, the treatment period is over, 30–60 days in the acute phase and more than 5 months in the chronic phase. The long duration of treatment and the serious side effects associated with the use of BNZ have reduced patient compliance and caused temporary discontinuation in many cases.

BNZ is a poorly soluble API (solubility in water: 0.4 mg/mL) (Kasim et al., 2004), available only in a tablet dosage form of 100 mg for adults and 12.5 mg for children and newborn (DNDi, 2017), which has low and variable oral bioavailability (Mosqueira et al., 2015). Thus, it is observed that the activity of this API is limited by its low solubility in water and the use of a suitable pharmaceutical system to enhance the apparent solubility and dissolution rate of the API would be highly desirable. The increased bioavailability of this molecule should allow the API to achieve optimal concentrations at therapeutic targets with greater exposure in the affected tissue, thereby also decreasing the

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required dose and the associated toxicological effects. However, the restrictive biological action of BNZ during the chronic phase of the Chagas disease is still a limitation.

The antifungal agent posaconazole (PCZ) has also been examined against *T. cruzi*. This API has demonstrated *in vitro* and *in vivo* efficacy during the acute and chronic phase of the disease, acting as an inhibitor for ergosterol biosynthesis, a necessary process for the survival, development and growth of this parasite (Da Silva et al., 2012; Ferraz, 2005). The antitrypanosomal activity of PCZ is also described in studies conducted in patients with chronic phase of Chagas disease at a dose of 400 mg and 100 mg (Molina et al., 2014). Most importantly, combined therapy of PCZ with BNZ on *T. cruzi* infection has been envisioned as an ideal approach since it may improve treatment efficacy whilst decreasing toxicity and the likelihood of resistance development. This therapeutical combination was shown to be more efficacious in reducing parasitemia levels than the APIs given alone, which indicates synergistic activity of PCZ and BNZ (Diniz et al., 2013).

PCZ has a low solubility of less than 1 μg/mL in water but can be partially solubilized in a strongly acidic medium (Fang et al., 2011). Due to this API's extremely low aqueous solubility and the promising activity against *T. cruzi*, mainly in the chronic phase of the Chagas disease for which there is no satisfactory treatment available, it would be very desirable to deliver PCZ together with BNZ in a pharmaceutical system capable of increasing the solubility and bioavailability of both APIs, thus providing a combination therapy for promoting greater antichagasic action in acute and chronic phases.

Although there are many reported studies and drug formulations containing PCZ and BNZ separately, a suitable pharmaceutical composition capable of delivering these two APIs together in a single system with enhanced dissolution is still lacking. The objective of this study is to investigate methods of preparation of binary pharmaceutical compositions of PCZ and BNZ in order to identify whether the formation of either a eutectic mixture or an amorphous solid solution can lead to enhance solubility and dissolution rate for both APIs. The results so obtained should provide insights regarding conditions and governing parameters favoring the formation of such binary compositions potentially useful as therapeutic combination dose with enhanced apparent solubility and dissolution rate.

2. Material and methods

2.1. Material

Posaconazole (PCZ, batch No. PO-20150201-01) was purchased from ScinoPharm Shangai Biochemical Technoloy, Ltd. (Shanghai, China). Benznidazole (BNZ, batch No. 301045) was kindly provided by Nortec Química (Xerém Duque de Caxias – Rio de Janeiro, Brazil). All other chemicals were reagent grade obtained commercially and used as received.

2.2. Preparation of PCZ/BNZ mixture samples

The eutectic mixture is classically defined as the mixture of two or more components exhibiting the lowest melting point than either of the components (Cherukuvada and Row, 2014). This feature arises as a result of its heterogeneous crystalline arrangements consisting of an alternating lamellar microstructure (each lamella represents a crystalline solid solution rich in a particular component/API) held by weak interphase interactions, which in turn are responsible for its high thermodynamic functions such as free energy, enthalpy and entropy, and thus the characteristic lower melting point and dissolution advantages (Cherukuvada and Nangia, 2014).

In contrast, the amorphous solid solution is defined when two components are totally miscible and soluble, stabilized through specific molecular interactions between them. In this system, the two components are molecularly dispersed forming a homogeneous solution with no crystalline structure. The interaction energy between the two components is sufficiently high resulting in a true solution that is homogeneous at the molecular level; therefore only one single phase is present (Vasconcelos et al., 2007). This system is usually classified as a type of solid dispersion, however in the present work it was achieved without the use of a polymer, thus it is treated as a drug–drug amorphous solid solution.

Three methods for the preparation of these binary pharmaceutical compositions of PCZ and BNZ were tested:

2.2.1. Evaporative crystallization method

Crystalline PCZ/BNZ mixtures were prepared by a solvent evaporation method. Different proportions of PCZ and BNZ (96:04, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90 w/w%, respectively) were mixed and dissolved in absolute ethyl alcohol. The solvent was evaporated at 50 °C for around 1 h resulting in a white/off-white crystalline powder. The obtained eutectic crystalline PCZ/BNZ mixtures had their particle size further reduced by grinding and the resulting powder with particle size between (75–150 μm) collected using a minisieve for further characterization. For initial screening, mixtures of different composition ratios of PCZ/BNZ were evaluated by Differential Scanning Calorimetry (DSC). Melting peaks so obtained were used to construct the phase diagram and to identify both the eutectic composition ad their particle size further reduced by grinding and the resulting powder with particle size between (75–150 μm) collected using a minisieve for further characterization. For initial screening, mixtures of different composition ratios of PCZ/BNZ were evaluated by Differential Scanning Calorimetry (DSC). Melting peaks so obtained were used to construct the phase diagram and to identify both the eutectic composition (PCZ/BNZ, 80:20 wt%; see Results section) and the eutectic melting temperature.

2.2.2. Hot melt and cooling method

Amorphous PCZ/BNZ mixtures were prepared by melting the PCZ/BNZ powder mixtures, at the composition ratios described above, in a temperature-controlled oil bath. The mixture was constantly stirred and heated until a molten liquid is resulted (around 138 °C) with a distinctive color change: from white (or off-white) to an orange (or orange-yellow) melt in different shades. Mostly amorphous PCZ/BNZ mixtures were obtained when the homogeneous melt was cooled at room temperature to form an intense orange (or orange-yellow) powder. The obtained PCZ/BNZ mixtures had their particle size further reduced by grinding and the resulting powder with particle size between (75–150 μm) collected using a minisieve for further characterization. These PCZ/BNZ mixtures (10:90, 40:60, 60:40, 80:20 and 90:10, w/w%) were evaluated for the presence of amorphous halo in the Powder X-ray Diffraction (PXRD) spectra, which confirms the amorphous nature of these mixtures (amorphous solid solution).

2.2.3. Alternative method for generating crystalline eutectic mixture

The crystalline eutectic mixture can also be obtained from further treating samples prepared by the hot melt and cooling method. In this case, PCZ seed crystals (around 0.1 wt% in the total amount) were added to the molten orange colored amorphous PCZ/BNZ mixture at the eutectic composition (PCZ/BNZ, 80:20 wt %) (as obtained in Sec. 2.2.2) during the cooling step in order to induce crystallization of the system. Then the mixture was re-heated until 138 °C on a hot stage microscope (Nikon