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Research paper

Amino acids as co-amorphous stabilizers for poorly water-soluble drugs – Part 2: Molecular interactions

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ABSTRACT

The formation of co-amorphous drug–drug mixtures has proved to be a powerful approach to stabilize the amorphous form and at the same time increase the dissolution of poorly water-soluble drugs. Molecular interactions in these co-amorphous formulations can play a crucial role in stabilization and dissolution enhancement. In this regard, Fourier-transform infrared spectroscopy (FTIR) is a valuable tool to analyze the molecular near range order of the compounds in the co-amorphous mixtures. In this study, several co-amorphous drugs – low molecular weight excipient blends – have been analyzed with FTIR spectroscopy. Molecular interactions of the drugs carbamazepine and indomethacin with the amino acids arginine, phenylalanine, and tryptophan were investigated. The amino acids were chosen from the biological target site of both drugs and prepared as co-amorphous formulations together with the drugs by vibrational ball milling. A detailed analysis of the FTIR spectra of these formulations revealed specific peak shifts in the vibrational modes of functional groups of drug and amino acid, as long as one amino acid from the biological target site was present in the blends. These peak shifts indicate that the drugs formed specific molecular interactions (hydrogen bonding and π–π interactions) with the amino acids. In the drug–amino acid mixtures that contained amino acids which were not present at the biological target site, no such interactions were identified. This study shows the potential of amino acids as small molecular weight excipients in co-amorphous formulations to stabilize the amorphous form of a poorly water-soluble drug through strong and specific molecular interactions with the drug.

1. Introduction

In the first part of this study, the formation of highly stable co-amorphous drug–amino acid combinations with improved dissolution rates was reported [1]. The amino acids in this study were chosen from the receptor binding site of the drugs because these amino acids have specific interactions with the drug in the body and thus could potentially also interact with the drug in a solid-state co-amorphous formulation. The amino acids from the biological active site were arginine (ARG) and tyrosine (TYR) in the cyclooxygenase II for indomethacin (IND) [2], and phenylalanine (PHE) and tryptophan (TRP) in neuronal Na+ channels for carbamazepine (CBZ) [3]. It was possible to prepare several co-amorphous mixtures with receptor but also non-receptor amino acids. However, TYR showed poor amorphization properties, and no co-amorphous blend could be prepared with this amino acid. All the obtained co-amorphous blends containing either one of the two drugs CBZ or IND, and the amino acids ARG, PHE, and TRP showed a single glass transition temperature indicating that they were homogeneous single phase systems, where the components in the mixture were fully dissolved in each other.

The co-amorphous mixtures further showed a significant improvement in dissolution over the individual drugs and remained stable at 40 °C under dry conditions for several months. The improved stability of these samples was explained by the T_gs of these blends, which were markedly higher than those of the individual drugs, and the molecular level mixing obtained through the vibrational ball milling preparation method.

In previous studies on co-amorphous drug–drug mixtures containing two low molecular weight drugs, improved dissolution kinetics and physical stability over that of the individual drug compounds could be related to strong molecular interactions between the drugs in the co-amorphous blend [4]. These specific interactions prevented both drugs in the co-amorphous mixtures from crystallizing. It is thus crucial to identify the interactions in the co-amorphous drug–amino acid blends presented in part I of this study.

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Vibrational spectroscopy is a valuable tool for identifying molecular interactions and providing insight into the molecular arrangement in amorphous and co-amorphous systems. As spectroscopic techniques probe the molecular level, solid-state changes in the molecular near range order, such as, e.g., hydrogen bonding or π–π interactions, can be detected. In general, these changes are reflected in peak shifts in the vibrations of functional groups of a given compound [5–8]. Thus, molecular interactions between substances in a mixture are indicated in specific peak shifts in the vibrations of functional groups involved in these interactions [6,7,9].

In this study, a detailed analysis on the molecular near range order in co-amorphous drug–amino acid mixtures investigated in part I of this study was conducted with the use of FTIR spectroscopy. The main aim was to find out if the components in the co-amorphous blends interact with each other on a molecular level. In addition, the possible influence of using amino acids from the biological active receptors of the drugs, i.e., phenylalanine and tryptophan for carbamazepine and arginine for indomethacin, on the nature drug–amino acid interactions in the co-amorphous mixtures was investigated.

2. Materials and methods

2.1. Materials

The two drugs carbamazepine (CBZ, M = 236.27 g/mol) and indomethacin (IND; M = 357.79 g/mol) were both purchased from Hawkins, Inc. Pharmaceutical Group (Minneapolis, USA). The amino acids L-arginine (ARG, M = 174.20 g/mol), L-phenylalanine (PHE, M = 165.19 g/mol), and L-tryptophan (TRP, M = 204.23 g/mol) were obtained from Sigma–Aldrich (St. Louis, USA). All compounds were used as received. The molecular structures of the compounds are shown in Fig. 1.

2.2. Methods

2.2.1. Preparation of the amorphous materials

The amorphous samples were prepared by vibrational ball milling as described in part 1 [1]. Briefly, 500 mg of crystalline powder (pure substance, or mixtures at the molar ratios 1:1 or 1:1:1) was milled in 25 ml milling jars with two 12 mm stainless steel balls at +6 °C with an oscillatory ball mill (Mixer Mill MM400, Retch GmbH & Co, Haan, Germany) for 90 min at 30 Hz. For the molecular interaction analysis, only the samples that resulted in co-amorphous mixtures (IND-ARG, IND-PHE, IND-TRP, and CBZ-TRP, as well as the ternary mixtures IND-ARG-PHE, IND-PHE-TRP, CBZ-PHE-TRP, and CBZ-ARG-TRP) were investigated (see [1]).

2.2.2. Fourier-transform infrared spectroscopy (FTIR)

Infrared spectra were obtained using a Nicolet 380 FT-IR (Thermo Scientific, Madison, USA) attached with an attenuated total reflectance accessory with diamond plate (ATR, Smart iTR, Thermo Scientific, Madison, USA). Spectra were collected with Thermo Scientific OMNIC software (version 8.1.11) over a range of 4000–400 cm^{-1} (64 scans, resolution 4 cm^{-1}). For the analysis, the spectral region of 1000–1800 cm^{-1} was chosen and the spectra were normalized for better visualization.

3. Results and discussion

The various co-amorphous mixtures were analyzed with respect to peak shifts of functional groups. In general, the peaks in the co-amorphous spectra were slightly broader and lower in intensity than those in the respective amorphous and crystalline single drug or amino acid spectra.

3.1. Interactions between IND and its receptor amino acid ARG in co-amorphous binary IND-ARG mixtures

The IR spectra of co-amorphous IND-ARG mixtures showed various peak shifts when compared to amorphous IND and crystalline ARG (Fig. 2). A calculated addition spectrum of both is also included for the ease of interpretation. Unfortunately, an amorphous reference spectrum of ARG was not available for direct comparison with the co-amorphous IND-ARG because vibrational ball milling of pure ARG did not result in amorphization and quench cooling as an alternative method failed because of degradation during melting due to the high melting point of the amino acids. The same problem was observed for the other two amino acids (PHE and TRP).

The most significant change, when comparing the spectra in Fig. 2, occurs in the region from 1500 to 1750 cm^{-1}, where the vibrational modes for the carbonyl groups and carboxylic acid groups of both substances, and the amino and guanidyl groups of ARG were found. Upon co-amorphization, the peak structure of

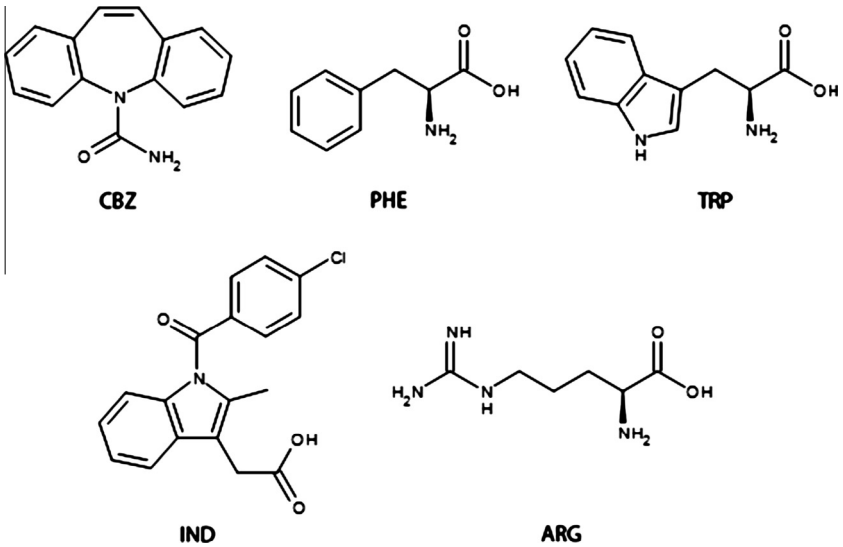

Fig. 1. Chemical structures of the drugs carbamazepine (CBZ) and indomethacin (IND), and the amino acids phenylalanine (PHE), tryptophan (TRP), and arginine (ARG).