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Research paper

Dissolution behavior of co-amorphous amino acid-indomethacin mixtures: The ability of amino acids to stabilize the supersaturated state of indomethacin

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ABSTRACT

Arginine, phenylalanine, and tryptophan have been previously shown to improve the solid-state stability of amorphous indomethacin. The present study investigates the ability of these amino acids to prolong the supersaturation of indomethacin in both aqueous and biorelevant conditions either when freely in solution or when formulated as co-amorphous mixtures.

The co-amorphous amino acid-indomethacin mixtures (molar ratio 1:1) and amorphous indomethacin were prepared by cryomilling. Dissolution and precipitation tests were performed in buffer solutions (pH 5 and 6.5) and in Fed and Fasted State Simulated Intestinal Fluids (FeSSIF and FaSSIF, respectively). Precipitation tests were conducted with the solvent shift method. The supersaturation stability of indomethacin and the precipitation inhibitory effect of amino acids were evaluated by calculating the supersaturation factor and the excipient gain factor, respectively.

Biorelevant media exerted a significant effect on indomethacin solubility but had little effect on the supersaturation stability. Arginine had the most significant impact on the dissolution properties of indomethacin, but also phenylalanine and tryptophan stabilized supersaturation in some media when formulated as co-amorphous mixtures with indomethacin. Only arginine stabilized supersaturation without co-amorphization, an effect only observed in media of pH 6.5. The unique behavior of the arginine-indomethacin mixture was further demonstrated by the abrupt formation of a precipitate, when an excess physical mixture of arginine and indomethacin was added to FeSSIF (pH 6.5). The solid-state investigation of this precipitate indicated that it probably consisted of crystalline arginine-indomethacin salt with possibly some residual crystalline starting materials.

1. Introduction

Orally administered drugs must have sufficient water solubility to ensure their bioavailability and pharmacological activity [1]. However, modern drug development often is seeking to achieve higher pharmacological potency by increasing the drug's molecular weight and lipophilicity [2,3]. In these cases, the limited water solubility may become a major challenge for successful formulation. Takagi et al. investigated the solubilities of the 200 most popular drugs in four developed countries; approximately 40% of these drugs were considered as being practically insoluble [4]. In particular, the impact of higher lipophilicity was noteworthy among newly discovered and developed drugs.

Aqueous solubility may not provide a sufficiently comprehensive perspective of the dissolution behavior of a drug in the gastrointestinal tract since there are numerous factors, such as differences in pH and the presence of naturally occurring surfactants and food components, which may influence the dissolution process [5]. To overcome this challenge, fluids that simulate gastric

Abbreviations: AA, amino acid; ACN, acetonitrile; ARG, arginine; AUC, area under the curve; CA, co-amorphous; DMSO, dimethyl sulfoxide; DS, degree of supersaturation; DSC, differential scanning calorimetry; EGF, excipient gain factor; FaSSIF, fasted state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; IND, indomethacin; PHE, phenylalanine; PM, physical mixture; SF, supersaturation factor; ssNMR, solid-state nuclear magnetic resonance spectroscopy; TFA, trifluoro acetic acid; T_g, glass transition temperature; T_m, melting temperature; TRP, tryptophan; XRPD, X-ray powder Diffractometry.

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or intestinal fluids, and are easy to prepare in the laboratory, have been introduced [5,6]. These include for example fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) which have been found to predict drug solubility in actual human intestinal fluid with sufficient accuracy in the early stages of drug development [6].

If a drug is highly permeable but poorly soluble (Biopharmaceutical Classification System class II), dissolution may limit the rate of absorption; hence manipulating solubility or dissolution properties may significantly improve its bioavailability [7,8]. Converting a crystalline material into an amorphous form is one of the most promising ways to improve apparent solubility, since the maximum concentrations in solution achieved with the amorphous form may be significantly higher due to its higher internal energy than with its crystalline counterpart [9–12]. Furthermore, the increased apparent solubility does not decrease the drug's permeability through intestinal wall [13–15]. Unfortunately, the greater internal energy and molecular movement of the amorphous form may also cause the material to convert spontaneously back to its crystalline form during processing, storage or dissolution.

Amorphous solid dispersions with polymers have been extensively studied and shown to stabilize both the drug supersaturation in solution and the amorphous form in the solid state [16,17]. In a solution, an amorphous form of a drug can be described as a spring that triggers the high initial concentration whereas the polymer acts as a parachute to prevent the recrystallization and precipitation of the drug [18]. However, the use of polymers as stabilizers of the amorphous drugs has some limitations, such as the hygroscopicity of many polymers, and the limited miscibility of drugs to polymers, which results to large polymer consumption [19]. To overcome these formulation challenges, co-amorphous formulations have been introduced and shown to stabilize the amorphous form in the solid state [12,19]. By the definition of Dengale et al., these systems contain two or more small molecular weight compounds that are homogenously mixed to form an amorphous single phase system [19]. For example, two active compounds have been combined to produce co-amorphous mixtures [20,21], but finding compatible drug-drug pairs may, however, be challenging, which has increased the interest in combining a pharmacologically active molecule with an inactive low molecular weight excipient, such as an amino acid (AA) [22–24].

Löbmann et al. studied the ability of carbamazepine and indomethacin (IND) to form co-amorphous mixtures with arginine (ARG), tyrosine, phenylalanine (PHE) and tryptophan (TRP) [24]. They noted that both drugs formed co-amorphous blends with suitable amino acids. In addition, their study demonstrated that co-amorphous blends were more stable and had higher intrinsic dissolution rates than the amorphous drugs alone. The improved solid-state stability was explained by the increased glass transition temperature (T_g) of co-amorphous blends, by the presence of stabilizing intermolecular interactions, and by the AAs acting as "impurities" at the molecular level. The improvement in the intrinsic dissolution rate was especially significant with respect to the ARG-IND co-amorphous mixtures; this was attributable to salt formation. The increased solid-state stability or intrinsic dissolution rate, however, provided no information on the ability of AAs to stabilize drug supersaturation in solution. Therefore, producing dissolution profiles that provide information on how significant and how long-lasting supersaturation may be achieved with a particular AA-drug combination, would be very useful when evaluating the actual dissolution properties of co-amorphous formulations.

The present study examined in detail the dissolution properties of ARG-IND, PHE-IND and TRP-IND co-amorphous mixtures, which were prepared using similar methods as described by Löbmann et al. [24,25]. The aim was to investigate whether the AAs would be able to act as molecular parachutes and prolong the supersaturated state of amorphous IND in simple buffer solutions (pH 5.0 and 6.5) as well as in biorelevant solutions (FaSSIF (pH 6.5) and FeSSIF (pH 5.0), which have rarely been applied in dissolution studies with (co-)amorphous formulations [26]. Precipitation tests were conducted by the solvent shift method in buffers and biorelevant media to gather relevant information on the ability of the amino acids to maintain supersaturation when they are present freely in solution.

Interestingly, in preliminary studies (where also FaSSIF of pH 5.0 and FeSSIF of pH 6.5 were used) a precipitate was formed when the physical mixture of ARG and IND was dissolved in FeSSIF of pH 6.5. Although these media were otherwise not investigated in this study, since the effect of pH could be demonstrated with buffer solutions and these media did not resemble any physiological state, the characterization of the precipitate is included in this report.

2. Materials and methods

2.1. Materials

IND (γ-form) was purchased from Hangzhou Dayanchem (Hangzhou, China). ARG, PHE, and TRP were all supplied by Sigma-Aldrich Co. (St. Louis, USA).

Solvents used were acetonitrile (ACN, VWR Chemicals, Fontenay-sous-Bois, France), ultra-purified water (Elga Purelab Ultra, Model ULXXXANM2, Snr: ULT00002345), methanol (Mallinckrodt Baker B.V., Netherlands), and dimethyl sulfoxide (DMSO, Fisher Chemical, Loughborough, UK). Trifluoro acetic acid (TFA) was purchased from Alfa Aesar GmbH & co (Germany).

FeSSIF and FaSSIF were prepared by adding a commercially available SIF powder® (SIF Powder Original®, biorelevant.com, Surrey, UK) to a blank buffer (FeSSIF blank, an acetate buffer with a pH of 5.0, or FaSSIF blank, a phosphate buffer with a pH of 6.5) according to the recommendations of the SIF-powder® manufacturer (SIF Powder Original® How To Use 1.4, 2013). Sodium hydroxide (NaOH) was obtained from both Oy FF-Chemicals AB (Finland) and Mallinckrodt Baker B.V. (Netherlands), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{*}H_2O$) from Merck (Germany), sodium chloride (NaCl) from Mallinckrodt Baker B.V. (Netherlands) and glacial acetic acid (CH3COOH) from both VWR (France) and Riedel de Haën (Germany). The pHs of the buffer solutions were adjusted with 1 M or 5 M hydrochloric acid (HCl) or NaOH solutions (pH meter: Metrohm 744, Herisau, Switzerland; electrode: Metrohm Ag 9191 Herisau, Switzerland).

2.2. Preparation of materials for dissolution tests

Physical mixtures of AAs and IND (AA-IND PM) were mixed by hand in a mortar. The components were mixed in a molar ratio of 1:1, which corresponds to a mass ratio of 0.49:1 for ARG-IND; 0.46:1 for PHE-IND; and 0.57:1 for TRP-IND.

Co-amorphous AA-IND (AA-IND CA) mixtures were prepared by cryomilling (MM400, Retsch GmbH, Haan, Germany). In addition, pure IND was milled to produce amorphous IND. Subsequently, 500 mg of AA-IND PM or pure IND were milled in 25 ml milling chambers containing two 12 mm stainless steel balls at 30 Hz for 60 min. The milling chambers were placed every 10 min in liquid nitrogen for 2 min to prevent unwanted solid-state transformations or degradation caused by heat. AA-IND CAs and amorphous IND were stored in refrigerator (approximately 5 °C) at 0% relative humidity (RH) (phosphorus pentoxide).