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Research paper

Development of a pharmaceutical cocrystal with solution crystallization technology: Preparation, characterization, and evaluation of myricetin-proline cocrystals

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2,4-Dinitro-fluorobenzene (PubChem CID: 6264)
Potassium phosphate monobasic (PubChem CID: 516951)
Sodium bicarbonate (PubChem CID: 516892)
N,N-dimethylformamide (PubChem CID: 6228)
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Sodium acetate (PubChem CID: 517045)
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ABSTRACT

Myricetin shows low oral bioavailability (<10%) in rats due to poor aqueous solubility, although it has demonstrated various pharmacological activities such as those related to anticancer, anti-diabetes, and hepatic protection. To overcome this issue, in this study, pharmaceutical cocrystals were designed to efficiently deliver myricetin by oral administration. A 1:2 stoichiometric cocrystal of myricetin with proline was prepared successfully by solution crystallization based on the ternary phase diagram (TPD) principle, and it is presented as a new sphericity-like crystalline phase characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM). The formation of myricetin-proline cocrystals was a spontaneous and exothermic process, probably due to the supramolecular interactions between themselves, which were determined by Fourier transform-infrared spectroscopy (FT-IR). Consequently, the dissolution efficiency of myricetin from cocrystals was increased 7.69-fold compared with that of coarse myricetin, and the oral bioavailability of myricetin cocrystals in rats was enhanced by approximately 3.03 times compared with that of pure myricetin. The present study provides useful information for the potential application of cocrystal technology for water-insoluble drugs, especially flavonoid compounds.

1. Introduction

Pharmaceutical cocrystals are multi-component crystals with a stoichiometric ratio of active pharmaceutical ingredients (APIs) and cocrystal coformers (CCFs) that are assembled by noncovalent interactions such as hydrogen bonds, π-π packing, and van der Waals forces [1–3]. In the recent years, pharmaceutical cocrystals have attracted increasing attention to improve the physicochemical properties of APIs without changing their chemical structure, such as the solubility, dissolution rate, melting point, stability, and bioavailability. For example, the solubility of quercetin in the quercetin-caffeine cocrystal was enhanced 14-fold compared with that in quercetin dehydrate; simultaneously, its oral bioavailability was improved up to 2.57-fold [4]; under accelerated stability conditions, the temozolomide-succinic acid cocrystal retained its

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white color for over 6 months, while temozolomide changed to tan-brown discoloration in less than 1 month, indicating that the shelf life of the cocrystal is much longer than that of the coarse drug [5]. Obviously, pharmaceutical cocrystals are especially beneficial for compounds that have intrinsic barriers to drug delivery.

Myricetin, 3,3′,4′,5′,5,7-hexahydroxy flavone (Fig. 1A), widely exists in many herbs, such as *Myrica rubra* Sieb. et Zucc., *Vitis vinifera* Linn., and *Dioscorea bulbifera* L., [6–8] and also in several foods, including onions, berries, grapes, and red wine [9,10]. As a naturally occurring phytochemical, myricetin is not only potent in antioxidant, anti-inflammatory, hepatic protection, and antimicrobial properties [9,11,12] but also has protective effects against cancer and diabetes [13,14]. Unfortunately, raw myricetin exhibited poor water solubility (16.6 μg/mL) [15] and an unfavorable bioavailability (the absolute bioavailability in rats was only 9.62%) [16], providing an obstacle for orally delivering myricetin in its medical application. However, myricetin is a rather weak acid with a p*Ka* of 6.63 [15] and possesses many competitive hydrogen bonding sites in its chemical structure, *i.e.*, donors and acceptors (6 hydroxyls and 1 carbonyl), providing the possibility for the formation of myricetin cocrystals with the proper CCFs. Therefore, the present work attempted to design a myricetin cocrystal to efficiently deliver myricetin by oral administration.

Amino acids, the building blocks of proteins and polypeptides, have important functions in both nutrition and health, including protein synthesis, osmolyte synthesis, regulation of hormone secretion, gene expression, and cell signaling [17]. As Generally Regarded as Safe (GRAS) compounds, they widely exist in food products such as wheat, rice, corn, and sorghum [18]. Interestingly, the amino and carboxylic groups of amino acids are donor and acceptor groups [19] that have a high tendency to form potential hydrogen bonds with other groups such as hydroxyl, carboxyl, pyridyl, amidogen, and phenolic hydroxyl groups [20,21]. In addition, amino acids are known to show low toxicity. Obviously, they seem to be a suitable choice for CCFs. Until now, some studies have been focused on forming pharmaceutical cocrystals with amino acids as CCFs. For instance, a very simple enantiomeric resolution method for the separation of DL-arginine by cocrystallization with fumaric acid was achieved that exploited the connection between the industrial and pharmaceutical needs for chiral organic substances and cocrystal formation [22], and in powder dissolution experiments of ezetimibe-proline cocrystals, the concentration of ezetimibe from the cocrystals was five times higher than that from pure ezetimibe within the first minute [23]. In this paper, the amino acid proline (Fig. 1B) was selected as a CCF to prepare myricetin cocrystals and is anticipated to enrich further the application of amino acids in the pharmaceutical cocrystal field.

To date, many formation methods were applied to produce pharmaceutical cocrystals, such as solution crystallization [24], grinding methods [25,26], and melt recrystallization [27,28]. Among the above mentioned methods, solution crystallization exhibits many advantages, including easy scale-up for manufacture, a relatively low cost for preparation, and simple confirmation of cocrystal formation, compared with other methods [29,30]. For example, adefovir dipivoxil-saccharin cocrystals were prepared using the solution crystallization approach, which was scaled up to 30 g for subsequent studies [31], and cocrystals of caffeine with 2-hydroxy-1-naphthoic acid were discovered *via* a screening method based on solution-mediated phase transformation, which maximized the screening efficiency and could be performed easily, while other screening and crystallizing methods failed [32].

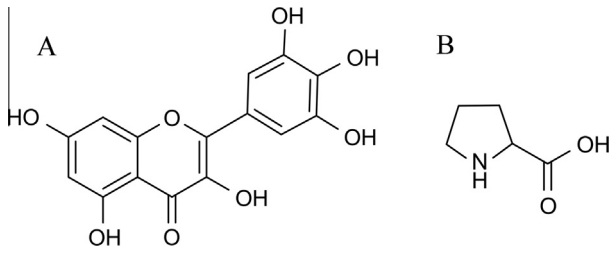

Fig. 1. Chemical structures of myricetin (A) and proline (B).

In this paper, pharmaceutical cocrystals of myricetin-proline were prepared using solution crystallization. Specifically, the phase solubility diagrams (PSDs) of myricetin and proline in ethanol were constructed, and the effect of temperature on the formation of myricetin-proline cocrystals was presented. The obtained cocrystals were characterized by differential scanning calorimetry (DSC), Fourier transform-infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM). Moreover, the *in vitro* and *in vivo* evaluations of myricetin cocrystals, including the solubility, dissolution rate, and oral bioavailability, were also investigated. The present study will provide some references for designing the effective delivery system of flavonoid compounds by cocrystal technology.

2. Materials and methods

2.1. Materials

Myricetin was purchased from Shanghai DND Pharm-Technology Co., Inc. (Shanghai, China). Proline was obtained from Sigma Aldrich Co. LLC (Shanghai, China). 2,4-Dinitrofluorobenzene (DNFB) was obtained from Zhengzhou Alfachem Co., Ltd. (Zhengzhou, China). Potassium phosphate monobasic, sodium bicarbonate, N,N-dimethylformamide, acetonitrile, and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were used as received without further purification.

2.2. Phase solubility diagrams (PSDs)

PSDs were determined by adding excess myricetin to different concentrations of proline ethanol solutions while adding excess proline to myricetin. The resulting suspensions were allowed to reach equilibrium after stirring at 298 K, 310 K, and 323 K for 36 h. Aliquots of solutions were withdrawn and centrifuged (13,000 rpm, 10 min), and the concentration of myricetin [15] and proline (see Appendix) in the supernatant was separately determined by high-performance liquid chromatographic (HPLC).

2.3. Preparation of myricetin-proline cocrystals

Myricetin-proline cocrystals were prepared by adding myricetin (210.0 mg, 3.699% (w/w)) to nearly saturated solutions of proline (42.0 mg, 0.740% (w/w)) in ethanol (7 mL, 95.561% (w/w)), and the mixture was sonicated for 15 min and equilibrated overnight at 37 °C. The precipitate was obtained by suction filtration after it precipitated completely and was dried totally in a fume hood. A physical mixture was also prepared by simply mixing myricetin and proline at the same ratio with myricetin-proline cocrystals.

2.4. Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra were acquired on a Bruker Avance III 600 MHz spectrometer equipped with a 5-mm PABBO probe (Bruker Corporation, Fällanden, Switzerland) at 25 °C. Typically, approximately 2.0 mg of pure myricetin and myricetin-proline cocrystals was dissolved in a glass vial in 0.5 mL of methanol-d_4, pure proline was