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Characterization of Physicochemical Properties of Ferulic Acid

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Ferulic acid (3-methoxy, 4-hydroxy cinnamic acid) is a flavoid component possessing antioxidant property. The compound is currently under development as a new drug candidate for the treatment of the dementia. The objective of this preformulation study was to determine the physicochemical properties of ferulic acid. The *n*-octanol to water partition coefficients of ferulic acid were 0.375 and 0.489 at the pHs of 3 and 10, respectively. Accelerated stability study for ferulic acid indicated that the *t* 90 value for the drug was estimated to be 459 days at 25°C. Ferulic acid was also found to be unstable under the relative humidity of more than 76%, probably because of the hygroscopic nature of the drug. In order to study compatibility of ferulic acid with typical excipients, potential change in differential scanning calorimetry spectrum was studied in 1: 1 binary mixtures of ferulic acid and typical pharmaceutical excipients (e.g., Aerosil, Avicel, CMC, Eudragit, lactose, PEG, PVP, starch and talc). Avicel, CMC, PVP and starch were found to be incompatible with ferulic acid, indicating the addition of these excipients may complicate the manufacturing of the formulation for the drug. Particle size distribution of ferulic acid powder was in the size range of 10-190 μm with the mean particle size of 61 μm. The flowability of ferulic acid was apparently inadequate, indicating the granulation may be necessary for the processing of the drug to solid dosage forms. Two polymorphic forms were obtained by recrystallization from various solvents used in formulation. New polymorphic form of ferulic acid, Form II, was obtained by recrystallization from 1,4-dioxane. The equilibrium solubility for Form I was approximately twice of that for Form II. The dissolution rate of Form II was higher than that of Form I in the early phase (<6 min). Therefore, these physicochemical information has to be taken in the consideration for the formulation of ferulic acid.

Key words: Ferulic acid, Physicochemical properties, Preformulation, Polymorphism, Solubility, Stability

INTRODUCTION

Ferulic acid (3-methoxy, 4-hydroxy cinnamic acid), a flavonoid component, is an antioxidant (Fig. 1). This compound is currently under development as a new drug candidate for the treatment of the dementia.

Prior to the development of dosage form(s) for a new drug candidate, it is essential that certain fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information will dictate a number of the subsequent events and possible approaches in formulation development. This first, learning phase about a drug is known as preformulation (Aulton, 2002; Lachman *et al.*, 1986). These studies should focus on the characterization of physicochemical properties of the new compound that could affect drug performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification. Important physicochemical properties for a new drug candidate that has to be characterized include assessments for the stability and solubility, potential incompatibility with typical pharmaceutical excipients and identification of crystalline polymorphism. Since ferulic acid is a new drug

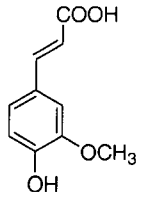

Fig. 1. Chemical structure of ferulic acid (4-hydroxy-3-methoxycinnamic acid)

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candidate, the physicochemical properties have not been systematically characterized. The objective of this study, therefore, is to characterize the physicochemical properties for the drug. We were particularly interested in the assessment of stability and solubility, the incompatibility and crystalline polymorphism for ferulic acid.

MATERIALS AND METHODS

Materials

Ferulic acid was supplied by the Scigenic Co. Ltd. Other chemicals and solvents were of analytical reagent grade or HPLC grade and were used without further purification.

The powder X-ray crystallography diffraction (PXRD) patterns were measured with a Rigaku DMA S-A (Rigaku, Japan) under the following conditions: Ni-filtered Cu-K radiation (1.542 Å) with the voltage of 30 Kv at the current of 20 mA. The differential scanning calorimetry (DSC) analysis was carried out using a Mettler DSC 12E thermal analyzer (Mettler, Swiss) using samples of approximately 2 mg in weight on pierced aluminum pans. The heating rate on the instrument was 10°C/min and the temperature ranged from 20 to 300°C. In this study, UV/VIS spectrophotometry was carried out with Beckman Du650 Spectrophotometer (Beckman, U.S.A). Particle size distribution of ferulic acid powder was characterized using a laser particle size analyzer, Mastersizer 2000 (Malvern, UK). The dissolution rate of ferulic acid was measured by paddle method of USP XXIII using dissolution tester (Duksan pure chemical Co., Korea).

Analytical method

Initially, the UV spectrum of ferulic acid in 0.1 mg/mL aqueous solution was studied in the range from 200 to 350 nm. The absorption maximum for the drug was obtained at 216 nm. To construct a calibration curve for ferulic acid, known amounts of the prepared samples were dissolved in water and the absorption determined for the standard solutions. When it was necessary to determine the concentration of ferulic acid, the absorption of the sample was measured at 216 nm and the expected concentration calculated from the predetermined calibration curves. To validate the analytical procedure, six sets of aqueous standard solutions of ferulic acid were prepared twice a day in three-day period. The absorbance values of these solutions were measured at 216 nm. Analytical parameters such as linearity, precision and accuracy were, then, evaluated.

Determination of apparent partition coefficients for ferulic acid

N-octanol (25 mL) was added to 25 mL of aqueous solution of different pH values (Rivera *et al.,* 2002). The mixture was shaken vigorously for 30 minutes in a separating funnel. After standing for 24 h, the phase was separated. Then, two phases were separately collected in 50 mL tube. This procedure was to saturate the aqueous and organic phase with other phases.

A 50 mg amount of ferulic acid was added to 5 mL of the aqueous phase. The resulting solution was then magnetically stirred for 3 h and, subsequently, centrifuged for 4 minutes at 4000 rpm. The supernatant was, then, filtered through 0.45 μm membrane filter. The filtrate was then serially diluted and the concentration (C1) determined spectrophotometrically.

Aliquot (4.5 mL) of the aqueous phase containing known concentration of the drug was added to a fresh tube and an equal volume of *n*-octanol was added. The mixture was agitated by a magnetic stirrer, and centrifuged. The mixture was, then, stood for separation and the concentration of ferulic acid in aqueous solution was determined spectrophotometrically (C2).

The concentration of ferulic acid in the organic solution was determined by the difference between the initial and the final concentration in aqueous solutions (viz, C1-C2). Apparent partition coefficients were determined from the ratio between the organic and aqueous concentration of the drug.

Determination of hygroscopicity for ferulic acid

Six glass desiccators, approximately 22 cm in diameter and 18 cm in height, were used to determine sorption isotherms by the traditional desiccator method. Saturated salt solutions at 25°C were prepared from analytical grade salts and purified water. The salts used were dibasic phosphate, ammonium sulfate, sodium acetate, sodium nitrite, zinc nitrate, and lithium chloride. Saturated salt solutions in a small beaker was placed alongside the exposed drug sample, and stored in a larger sealed container.

In this study, six desiccators with 95%, 81%, 76%, 66%, 42% and 15% of relative humidity were used. An amount of 200 mg of dehydrated ferulic acid was weighed and exposed to the desired relative humidity. The samples were kept in open plastic petri dishes at 24°C for 1 week at each of the humidity described, and the gain in weight of ferulic acid was then determined up to the saturation humidity.

Determination of particle size and flowability for ferulic acid powder

Particle size distribution of ferulic acid powder was estimated using a laser particle size analyzer, Mastersizer 2000 (Malvern, UK). In this study, triplicate measurement was carried out and expressed as mean of the measurements. Flowability was estimated using a Flowability

Tester (Erweka, Germany), by measuring the time of powder flowing through a hopper orifice.

Accelerated stability testing for ferulic acid

In this study, the accelerated stability of ferulic acid in solid was evaluated. For the assessment of stability of ferulic acid in solid, 2 mg ferulic acid was placed in brown vials and stored at 5, 40 and 60°C for 1 to 7-month period. When it was necessary to determine the remaining content, an aliquot of the sample was dissolved in water and the concentration determined spectrophotomerically. Then, the content remaining was calculated from the concentration.

Compatibility of ferulic acid with excipients

Thermal analysis was used to study compatibility of ferulic acid with excipients (Botha, *et al.,* 1989; Holgado, *et al.,* 1995; Sohn *et al.,* 1999; Sohn *et al.,* 2003). Thus, 1: 1 binary mixture of ferulic acid with typical pharmaceutical excipients (e.g., Aerosil, Avicel, CMC, Eudragit, lactose, PEG, PVP, starch and talc) was prepared and the differential scanning calorimetry spectrum in the mixtures assessed.

Polymorphism

Recrystallization was performed to study the potential crystalline polymorphism for ferulic acid. In this study, methanol, ethanol, acetonitrile, acetone, chloroform, dichloromethane, dichloroethane, dimethylformamide, isopropylalcohol, isoamylalcohol, ethylacetate, buthanol, dioxane, heptane, tetrahydrofuran, trichloroethane, tetrachloroethane and tetrachlorocarbon, solvents used in typical formulation of drugs, were used as re-crystallization solvents. Each solvent was saturated with ferulic acid powder, then the saturated solvent was stood at -72°C, -30°C, -10°C, 5°C, or room temperature.

In order to study whether the alteration in the crystal morphology lead to a polymorphic transition, X-ray powder diffraction analysis was used (Haleblian *et al.,* 1969; Sohn *et al.,* 2000; Sohn *et al.,* 2002).

Dissolution of ferulic acid

The dissolution rate of ferulic acid was measured by paddle method of USP XXIII using dissolution tester (Duksan pure chemical Co., Korea). The dissolution vessels contained 900 mL of filtered distilled water at 37°C and a paddle rotation speed of 100 rpm was used throughout the study. At pre-determined time intervals, an aliquot (1 mL) was withdrawn and immediately filtered through a 1.45 μm syringe filter, 1 mL of water at the same temperature was replaced in the vessel. The concentration of the drug in the various samples was determined by the UV assay for ferulic acid.

RESULTS AND DISCUSSION

Determination of ferulic acid concentration

The concentration of ferulic acid was readily determined in the concentration range of 0.04 to 0.15 mg/mL. The equation of the fitted model was: Absorbance=0.02379+ 0.12734×concentration. The calibration curve was used throughout the study to determine the concentration of ferulic acid.

Apparent partition coefficient for ferulic acid

n-Octanol to water apparent partition coefficient for ferulic acid was 0.3753 at pH 3 and 0.489 at pH 10. Since the partition coefficient is less than 1, the drug is relatively hydrophilic as suggested by the chemical structure of the drug (Fig. 1).

Hygroscopicity analysis

The percentage of saturation humidity was studied as a function of the relative humidity. The moisture content up to 66% was remained unchanged at 0%. In contrast, the content was increased to approximately 25% at the relative humidity of 76%, indicating that ferulic acid is not physically stable at the relative humidity over 76% at 25°C. Therefore, these observations indicated that ferulic acid should be stored below 76% relative humidity to prevent the adsorption of water vapor, which may lead to a potential instability.

Particle size distribution and flow property for ferulic acid powder

Mean particle size and the distribution were studied for ferulic acid powder. The distribution of frequencies was found to be close to the normal distribution (Fig. 2) with the mean particle size of 61 μm with the size range of 10-190 μm.

In this study, flow property of ferulic acid was also assessed. Therefore, 30 g of powder flowed for 10 seconds through a hopper orifice. And flow angle of ferulic acid was 72 deg 7 min. Free flowing particle is likely to have a high flow rate and low flow angle. It is generally accepted that the flow angle above 50 deg would indicate that the powder

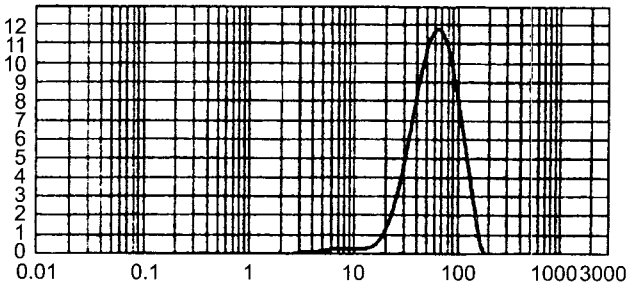

Fig. 2. Frequency-particle size distribution curve of ferulic acid.

flows only with great difficulty, if at all. Since the observed flow angle of ferulic acid is over 70 deg, the flow characteristic is clearly inadequate. During the manufacture of solid dosage forms, flowability is an important factor since content uniformity may determine by the physicochemical property. Therefore, granulation of ferulic acid may be necessary to improve the flowability for a successful formulation of ferulic acid into solid dosage forms (e.g., tablet and/or hard gelatin capsule).

Accelerated stability testing for ferulic acid in solid

The method of accelerated testing of pharmaceutical products based on the Arrhenius Equation. According to this technique, elapsed time to 90% remaining at elevated temperatures (40, 60°C) were obtained and plotted against the reciprocals of the absolute temperature. Using the relationship, the expected stability (i.e., 90% remaining) at room temperature may be extrapolated to 25°C. In this study, stability in elevated temperature was studied in solid (Fig. 3). The time elapsed to 90% remaining from the original strength was evaluated by the linear regression and calculated for elevated temperatures in solid. The time to 90% of the original potency was then plotted against 1/ temperature in solid (Fig. 3). The decomposition data revealed that a t 90 value (elapsed time to 90% of the original potency) at 25°C was 459 days for ferulic acid powder.

Compatibility with excipients

In this study, compatibility of ferulic acid with typical excipients was studied. The selected excipients represent components that are generally added during typical formulation processes. The typical thermogram of ferulic acid is shown in Fig. 4A with the estimated melting point

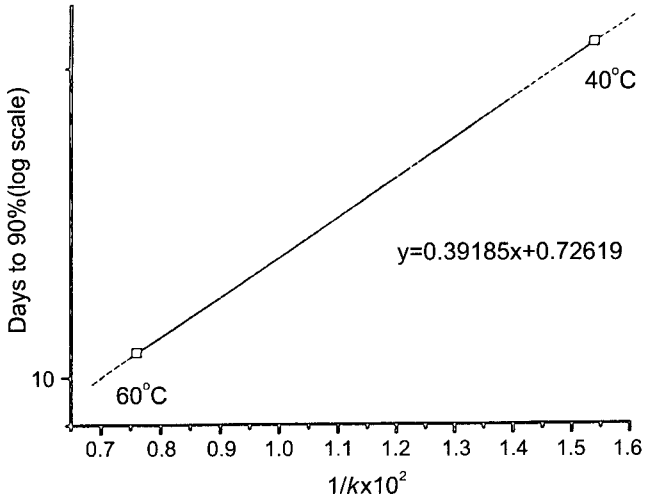

Fig. 3. Time in days required for drug potency to fall to 90% of original value at solid state.

of 175°C. No additional endotherm was observed from this thermogram with a sharp peak, indicating that ferulic acid has a well defined crystalline phase. Sum of DSC curve for the drug and Aerosil was almost superimposable for that (Fig. 4B) obtained from 1:1 binary mixture of the drug and Aerosil, indicating that there was no appreciable interaction between the drug and Aerosil. Similar DSC characteristics were obtained from 1:1 binary mixture of the drug and Eudragit (Fig. 4C), lactose (Fig. 4D), PEG (Fig. 4E) and talc (Fig. 4F). These observations indicated that Eudragit, lactose, PEG and talc are compatible with ferulic acid and, thus, the excipients may be added in the formulation of the drug.

In contrast, there appeared to be a significant interaction between ferulic acid and excipients such as Avicel (Fig. 4G), CMC (Fig. 4H), starch (Fig. 4I), PVP (Fig. 4J). In general, DSC thermogram of ferulic acid in the presence of the excipients indicates an observable change in the peak shape and area. For example, the peak was broaden at the point of 175°C (i.e., the melting point of the drug). Furthermore additional endothermic peak were detected at 135 (Fig. 4G) for Avicel, 160°C (Fig. 4H) for CMC and 140°C (Fig. 4I) for starch. Therefore, a strong interaction of ferulic acid with Avicel, CMC, starch and PVP may occur and, thus, the excipients may not be added during the formulation of the drug.

Crystalline polymorphism for ferulic acid

Form is the ferulic acid standard and donated one, which is identical to solid phase of recrystallization from methanol, ethanol, acetonitrile, acetone, chloroform, dichloromethane, dichloroethane, dimethylformamide, isopropylalcohol, isoamylalcohol, ethylacetate, buthanol, heptane, tetrahydrofuran, trichloroethane, tetrachloroethane and tetrachlorocarbon. Form was obtained by dissolving in 1,4-dioxane at 25°C and subsequent recrystalline process. The 1,4-dioxane solution of ferulic acid was prepared at 25°C, which was then cooled at 5°C for 2 weeks. The formed crystals were, then, separated by filtering under reduced pressure and dried over silica gel in a desiccator at 25°C.

The XRD patterns of the two crystal forms of ferulic acid are shown in Fig. 5-A (Form I) and B (Form II). The XRD patterns of the two crystal forms are clearly different. The DSC curves of the two crystal forms of ferulic acid are also shown in Fig. 5-C (Form I) and D (Form II). DSC profiles obtained for Form I (Fig. 5-C) showed sharp endotherm peaks corresponding to the melting of ferulic acid at 175, consistent with the previous observation, and, the heats of fusion for Form I was found to be 40.2 cal/g. Form II had three endothermic peaks (Fig. 5-D) at 105°C, 148°C and 182°C and their corresponding heats of fusion were 9.3 cal/g, 12.8 cal/g and 4.9 cal/g, respectively.

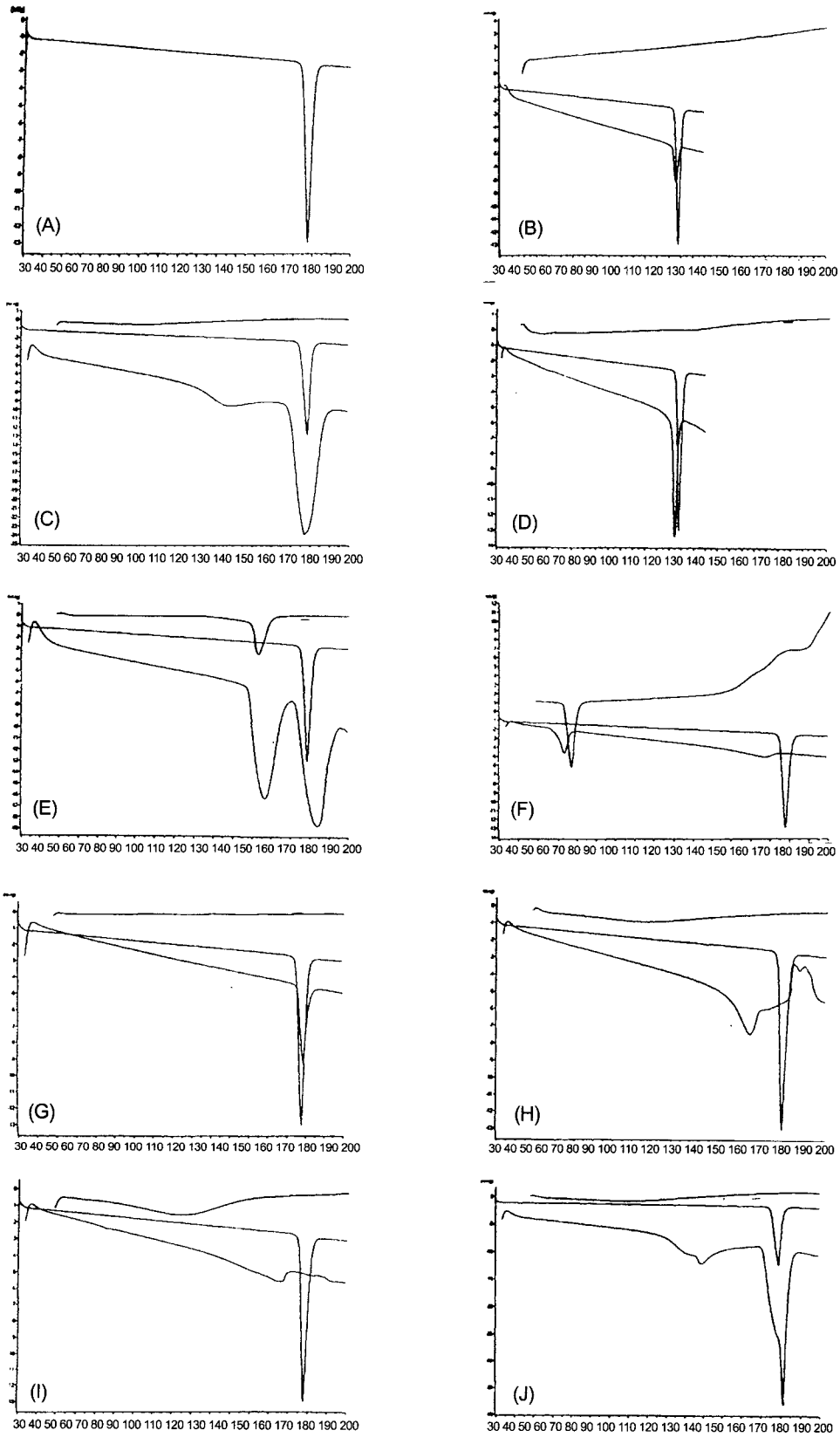

Fig. 4. DSC thermogram of ferulic acid, typical excipients and 1:1 binary mixture of the drug and excipients. Panel A: DSC curve of ferulic acid alone. From panel B to I: DSC curves of ferulic acid, the excipients, and the mixture with the drug and the corresponding excipients. Panel B: Aerosil. Panel C: Avicel. Panel D: Eudragit. Panel E: lactose. Panel F: PEG. Panel G: talc. Panel H: CMC. Panel I: PVP. Panel J: starch.

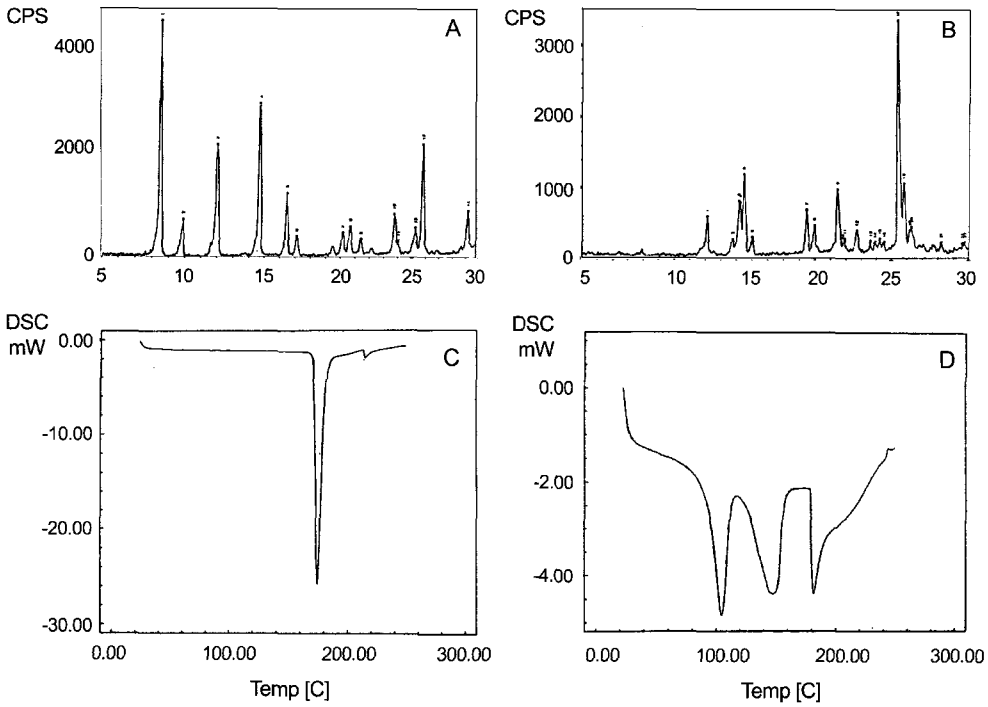

Fig. 5. XRD and DSC patterns for two types of ferulic acid crystals. Panel A: XRD pattern for Form I. Panel B: XRD pattern for Form II. Panel C: DSC pattern for Form I. Panel D: DSC pattern for Form II.

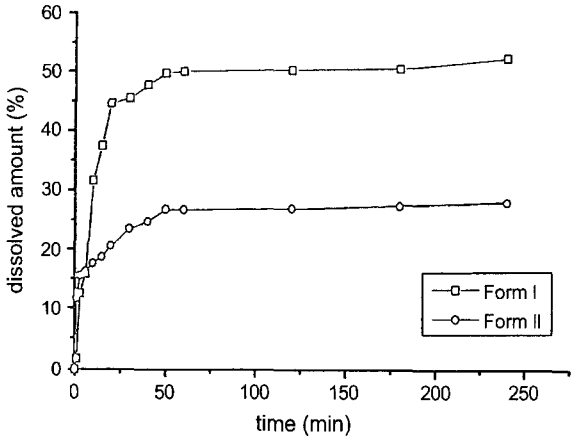

Fig. 6. Dissolution patterns of two crystal forms of ferulic acid in distilled water at 37°C.

Dissolution of ferulic acid

Fig. 6 shows dissolution profiles of Form I and Form II in distilled water at 37±0.5. The solubility of Form II was 50% of that of Form I after 4 h, indicating that Form I is the more soluble form of the two. The rate of dissolution for Form II, however, was apparently higher than that for Form I in early phase (<6 min) of the study. Underlying mechanism for the equilibrium solubility and the dissolution rate, however, is not directly investigated in this study.

In summary, the preformulation study was carried out for ferulic acid. The drug is apparently hydrophilic as evidenced by the fact that the apparent partition coefficient to n-octanol was below the unity at the pHs tested. The moisture content of the drug was significantly elevated when the drug was stored at the relative humidity of more than 76%, indicating that the storage of the drug at a high relative humidty may lead to a physical instability. The powder of ferulic acid had an average particle size of approximately 61 μm with a potential flow problem, indicating that granulation is necessary for the processing into solid dosage forms. The drug is apparent-ly stable in the room temperature. In addition, potential incompatibility for ferulic acid was found for Avicel, CMC, starch and PVP. The drug is apparently polymorphic; Form I appears to be more soluble in water than Form II. Therefore, the physicochemical properties of ferulic acid have to be taken into consideration for the future formulation of the drug.

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