

## Research Article

# Comparison of Spray Freeze Drying and the Solvent Evaporation Method for Preparing Solid Dispersions of Baicalein with Pluronic F68 to Improve Dissolution and Oral Bioavailability

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**Abstract.** The objective of this study was to prepare solid dispersions consisting of baicalein and a carrier with a low glass transition/melting point (Pluronic F68) by spray freeze drying (SFD). We compared these powders to those produced from the conventional solvent evaporation method. In the SFD process, a feeding solution was atomized above the surface of liquid nitrogen following lyophilization, which resulted in instantaneously frozen microparticles. However, solid dispersions prepared by the solvent evaporation method formed a sticky layer on the glass flask with crystalline baicalein separated out from the carrier. The powder samples were characterized by scanning electron microscopy (SEM), powder X-ray diffraction (PXRD), surface area measurement, differential scanning calorimetry, and Fourier transform infrared spectrometry. SEM and PXRD results suggested that the majority of baicalein in the SFD-processed solid dispersion was in the amorphous state, which has a higher specific surface area than pure baicalein. However, the majority of baicalein was recrystallized in the solid dispersion at the same composition prepared by the solvent evaporation method, which showed a similar dissolution rate to the physical mixture. SFD product was physically and chemically stable after being stored at 40°C with low humidity for 6 months. After enzyme hydrolysis, baicalein in the SFD product displayed a significantly shorter  $T_{\max}$  and higher  $C_{\max}$  than pure baicalein after oral dosing. The relative bioavailability of the SFD product *versus* pure baicalein determined by comparing the  $AUC_{0-12}$  was 233%, which demonstrated the significantly improved oral bioavailability of baicalein produced by the SFD technique.

**KEY WORDS:** baicalein; bioavailability; solid dispersion; solvent evaporation (SE); spray freeze drying (SFD).

## INTRODUCTION

It has been reported that nearly 40% of the new chemical entities currently being discovered are poorly water-soluble active pharmaceutical ingredients (APIs), which show poor and variable oral bioavailability *in vivo* (1). Various techniques have been developed to solve the dissolution and absorption problems for these sparingly soluble APIs, including salt formation, solid dispersion, complexation, and micronization. Among them, solid dispersion has proved to be the most successful method in industrial production. Melting, kneading, or solvent evaporation methods are commonly used techniques for the preparation of solid dispersions. However, heating processes cannot be avoided no matter which method is used. To avoid heating during the preparation of thermosensitive drugs, spray freeze drying (SFD) has been successfully developed to prepare solid dispersions at ambient temperature, which was made

significant development by the research work of William III (2–4). SFD technology involves the atomization of a feed liquid containing poorly water-soluble or insoluble APIs and excipients directly into a cryogenic liquid at ambient temperature to produce a frozen micronized powder that is subsequently dried. This process offers a variety of advantages compared to traditional technologies for solid dispersions, including amorphous structure and high surface area (2–4).

Pluronic F68, also known as Poloxamer 188, is a hydrophilic nonionic polyoxyethylene–polyoxypropylene block copolymer. It is widely used in pharmaceutical formulations as an emulsifying or solubilizing agent. Some reports demonstrated that Pluronic F68 performs better than other carriers (e.g., polyvinylpyrrolidone (PVP) and hydroxypropylmethylcellulose (HPMC)) in terms of dissolution rate enhancement because it has double roles in the solid dispersion formula, one as a polymeric carrier and the other as a surface active agent (5). Unlike other carriers including PVP ( $T_g \sim 185^\circ\text{C}$ ) (5) or HPMC ( $T_g \sim 172^\circ\text{C}$ ) (6), Pluronic F68 has a relatively low glass transition temperature and low melting point (e.g.,  $T_g \sim -63.4^\circ\text{C}$  and  $T_m \sim 54^\circ\text{C}$ ) (7,8). Our preliminary experiments showed that during spray drying above its  $T_g$  and  $T_m$ , Pluronic F68 exists as a sticky material that is not suitable to be produced by spray drying in large scale, and the yield of the powder was relatively low. Therefore, SFD and the conventional rotary evaporation method were selected in the

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subsequent study to remove organic solvents during the preparation of solid dispersions with Pluronic F68.

Baicalein (5, 6, 7-trihydroxyflavone), one of the major flavones isolated from the root of *Scutellaria baicalensis*, has been demonstrated to have numerous pharmacological activities including anti-inflammatory (9,10), antioxidative (11), anticancer (12), anti-allergic (13), and antiviral (14) effects. It is a class II (i.e., poorly water soluble and highly permeable, with  $P_{app}=1.7 \times 10^{-5}$  cm/s (15)) API according to the Biopharmaceutics Classification System (BCS) (16). Its poor water solubility (~0.13 mg/mL), inadequate dissolution, and extensive first-pass metabolism (17–20) result in its low oral bioavailability (18) and limit its use in the pharmaceutical field. As only a few formulation approaches have been performed to improve its oral absorption by formation of a complex with HP- $\beta$ -CD or preparation of solid dispersions with PVP by the solvent evaporation method (21,22), there is still space for other formulation approaches.

Whereas considerable efforts have been made to develop SFD technology in the preparation of solid dispersions for BCS class II drugs, there have been few comparisons between SFD technology and the conventional solvent evaporation method using a low glass transition/melting point carrier to improve the dissolution rate and oral bioavailability of BCS class II drugs. Using baicalein as a model BCS class II API and Pluronic F68 as a model carrier with a low  $T_g$  and  $T_m$ , the objectives of the present study were as follows: (1) to explore the feasibility of using SFD and the solvent evaporation method with Pluronic F68 for developing a stable solid dispersion formulation with an improved dissolution rate for oral administration, (2) to characterize the prepared solid dispersion (in terms of morphology, crystallinity, dissolution rate, surface area, thermal behavior, and stability) *in vitro*, and (3) to evaluate the relative oral bioavailability of the developed baicalein solid dispersion *versus* the unformulated drug in rats.

## MATERIALS AND METHODS

### Materials

Baicalein (purity  $\geq 98\%$ ) was obtained as a micronized powder from Dongfangyuan Bio-Technology Co., Ltd. (Mianyang, China). Formononetin (purity  $\geq 98\%$ ) was purchased from Shanghai Winherb Medical S&T Development Co., Ltd. (Shanghai, China). Polyvinylpyrrolidone K40 (PVP K40), pharmacopoeial grade Pluronic F68, and  $\beta$ -glucuronidase (G0751; CAS number 9001-45-0) were purchased from Sigma (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) glacial acetic acid was obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Methanol for HPLC analysis was obtained from Merck, Germany Industries Inc. (Darmstadt, Germany). Acetonitrile was purchased from Tedia Company, Inc. (Fairfield, CT, USA). All other reagents were commercially available and used as received. Purified water was obtained from the Milli-Q water purification system of the Millipore Corporation (Bedford, MA, USA).

### Preparation of Solid Dispersions by SFD and the Solvent Evaporation Method

In SFD, baicalein was dissolved in cosolvents, which consisted of ethanol and *n*-butyl alcohol with the ratio of

20:30 (v/v) at a fixed concentration of 3 mg/mL. The carrier, Pluronic F68 or PVP K40, was dissolved in water at 3, 6, 12, or 27 mg/mL, respectively. Ethanol was used to make *n*-butyl alcohol miscible with water. The above solutions were mixed at a cosolvent/water ratio of 50:50 (v/v) to obtain various drug/carrier ratios of 1:1, 1:2, 1:4, or 1:9 (w/w). Apparatuses used in this study were described in our previous publication (23). In brief, the solutions prepared above appeared as clear yellow solutions, which were used as feeding solutions and sprayed via a twin-fluid nozzle from a Mini Spray Dryer B290 (Buchi, Switzerland) at ambient temperature. In this study, spray freezing above liquid nitrogen was used, and a minimum distance of 4 cm was kept between the nozzle and liquid nitrogen surface to prevent the nozzle from clogging. The flow rate of the feed solution was 20 mL/min, and the atomization pressure of nitrogen gas was 4 bar. After the completion of the spray drying step, the content was transferred into a freeze dryer (CoolSafe™, ScanLaf A/S Company, Denmark) and kept at room temperature under 45.3 Pa for 72 h. For there were some samples splashing during the feed solution spraying to the surface of liquid nitrogen, the average product yield in the SFD process was only about 75%. In the solvent evaporation method, baicalein and the carrier were dissolved in a minimum volume of acetone, and the solvent was removed under vacuum in a rotavapor at 40°C and 45 rpm until no visible solvent was left. Then the samples were kept in silica gel for 24 h to completely remove organic solvent, followed by grinding and going through an 80-mesh sieve. All samples were stored with silica gel until further physicochemical characterization.

### Preparation of Physical Mixtures

Because the particle size of the Pluronic F68 is relatively large, it was ground using a mortar and pestle for 5 min and then mixed with the appropriate amount of baicalein to obtain the physical mixture.

### HPLC Analysis

An Agilent 1200 series HPLC system (Santa Clara, CA, USA) equipped with a 250×4.6-mm Agilent 5- $\mu$ m Zorbax SB-C<sub>18</sub> column and photodiode array detector was used for *in vitro* sample analysis. The baicalein peak was eluted at 5 min with the mobile phase (buffer solution (formic acid 0.1%, pH 2.0)/acetonitrile at 50:50 ratio, v/v) running at 1 mL/min. The detection wavelength was 276 nm with an injection volume of 20  $\mu$ L. A calibration curve based on the area of baicalein (0.1–100  $\mu$ g/mL) had excellent linearity with drug concentration ( $R^2 > 0.999$ ). The relative standard deviations (RSDs) of intra- and inter-day precision and accuracy for baicalein at three concentrations (1, 10, and 50  $\mu$ g/mL) were less than 5% ( $n=5$ ).

### Dissolution Studies

The dissolution of powders was determined using a standard USP dissolution tester (DT 706 1000LH, Erweka, Germany). Samples containing approximately 20 mg baicalein were added to 900 mL water at 37±0.5°C with 100 rpm rotation rate. A 5-mL sample was taken automatically by

autosampler at 5, 10, 20, 30, 45, 60, and 120 min and filtered through a 0.45- $\mu\text{m}$  filter, which was further diluted two times by the mixed solution of acetonitrile and formic acid (0.1%, pH 2.0) comprising 1% ascorbic acid (50:50, v/v), and the sample was then analyzed by HPLC. An equal volume of corresponding dissolution medium was added back into the dissolution media to maintain a constant volume. All experiments were repeated three times.

### Sample Characterizations

#### Scanning Electron Microscopy

A JSM-6330F cold field emission scanning electron microscope (SEM; JEOL Inc., Japan) was used to examine the surface morphology of each sample powder at  $\times 300$ – $\times 5,000$  magnification. The samples were fixed to an SEM stage with double-sided adhesive tape and gold sputter coated. The operating voltage was 15 kV.

#### Powder X-ray Diffraction

Powder X-ray diffraction (PXRD) was conducted using Cu  $\text{K}\alpha_1$  radiation with a wavelength of 1.54056 Å at 3 kV and 20 mA on a RIGAKU X-ray diffractometer (Tokyo, Japan). The sample powders were placed into sample holders and were scanned from 2° to 50° (2 $\theta$ ) at a step size of 0.02°/s, with a counting time of 2 s.

#### Surface Area Measurement

A Flow Sorb III 2310 surface area analyzer (Mercuritics Corporation, USA) was used to determine  $\text{N}_2$  sorption at 17°C. A known amount of powder (~100 mg) was loaded into a sample cell and degassed for 30 min prior to analysis. The specific surface area was calculated by surface area/sample weight.

#### Differential Scanning Calorimetry

Thermal investigations were performed using a differential scanning calorimetry (DSC) instrument (PerkinElmer, USA). Indium ( $T_m=156.6^\circ\text{C}$ ;  $\Delta H_f=28.45$  J/g) was used for calibration. Samples, containing about 2.0 mg baicalein, physical mixtures, or solid dispersion, were placed in hermetically sealed aluminum pans and scanned at 10°C/min from 35°C to 300°C under nitrogen purge. The nitrogen flow rate was 40 mL/min.

#### Fourier Transform Infrared Spectroscopy

KBr tablets were prepared by gently mixing ~1 mg powder (Pluronic F68, baicalein, physical mixture, or SFD) with 200 mg dried KBr. Fourier transform infrared spectra (FTIR; 450–4,000  $\text{cm}^{-1}$ ) were obtained on a PerkinElmer Spectrum100 FTIR apparatus (Buckinghamshire, UK) with a resolution of 1  $\text{cm}^{-1}$ .

### Stability Studies

An accelerated 6-month stability study was conducted on SFD-processed baicalein samples. The samples (~200 mg), packed in glass vials with silica gel desiccant, were stored in a drug stability test chamber at 40°C and analyzed initially and after 1 to 6 months for baicalein content and dissolution by HPLC analysis. Because the solid dispersions are sensitive to humidity and were aggregated when stored under a high humidity environment in our preliminary experiment, the relative humidity in these stability studies was controlled to be 0.

### Pharmacokinetics Analysis in Rats

#### Animal Study

Male Sprague–Dawley rats with body weights of ~250 g were supplied by Guangdong Medical Laboratorial Animal Center (Guangzhou, China). The animal experiment was approved by the internal animal ethics committee at the Institute of Chinese Medical Sciences, University of Macau (Macau SAR, China). Animal experiments are conducted in full compliance with national regulatory principles. The rats were maintained in an air-conditioned animal quarter with alternating 12-h light/dark cycles at a room temperature of  $22\pm 2^\circ\text{C}$  and a relative humidity of  $50\pm 10\%$ . The rats were given a commercial rat chow and water *ad libitum*. The animals were fasted overnight (~12 h) and had free access to water throughout the experimental period. The rats were randomly divided into two equal groups of ten rats per group. Groups of rats were given a single dose of 75 mg/kg baicalein by intragastric intubation (one group received raw material, and the other received solid dispersions). The blood samples (~300  $\mu\text{L}$ ) were taken from the jugular vein into tubes pre-treated by heparin before dosing and subsequently at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12 h post-dosing. Following the blood sampling, the blood samples were centrifuged at  $3,000\times g$  for 10 min at 4°C to give duplicate 100- $\mu\text{L}$  plasma samples. One hundred microliters of 1% ascorbic acid was added to each sample and immediately stored at  $-80^\circ\text{C}$  until analysis.

#### Plasma Sample Preparation

For the assay of the free form of baicalein, the thawed plasma samples (100  $\mu\text{L}$ ) were mixed with 10  $\mu\text{L}$  internal standard (IS) spiking solution (formononetin, 100  $\mu\text{g}/\text{mL}$ ) and 10  $\mu\text{L}$  1% ascorbic acid. Then, the mixture was acidified with 10  $\mu\text{L}$  0.1% formic acid and vortex-mixed with 1,000  $\mu\text{L}$  ethyl acetate for 3 min followed by centrifugation at  $15,700\times g$  for 15 min. The ethyl acetate extract (1,000  $\mu\text{L}$ ) was reduced to dryness under nitrogen at 30°C. The residue was reconstituted in 100  $\mu\text{L}$  water/acetonitrile (60:40, v/v, containing 0.1% formic acid). After centrifugation at  $15,700\times g$  for 1 min, 100  $\mu\text{L}$  supernatant was loaded into the sample vial for HPLC analysis. The concentration of conjugated metabolites of baicalein in plasma was determined after  $\beta$ -glucuronidase/sulfatase treatment. For enzymolysis, 100  $\mu\text{L}$  plasma was mixed with 100  $\mu\text{L}$   $\beta$ -glucuronidase/sulfatase treatment (5,000 U/mL in pH 4.5 acetate buffers) and incubated at 37°C for 2 h. After hydrolysis, the plasma sample was subjected to the process described above.

### HPLC Analysis for Plasma Samples

An Agilent 1200 series HPLC system (Santa Clara, CA, USA) equipped with 250×4.6-mm Agilent 5 μm Zorbax SB-C<sub>18</sub> column and photodiode array detector was used for sample analysis. The baicalein peak was eluted at 8.5 min and that of IS was eluted at 11.5 min with the mobile phase (buffer solution (formic acid 0.1%, pH 2.0)/acetonitrile at 60:40 ratio, *v/v*) running at 1 mL/min. The detection wavelength was 276 nm with an injection volume of 20 μL. The assay was fully validated according to the US FDA guidance on bioanalytical method validation (24). The calibration curve was prepared by determining the best fit of the peak area ratios of analyte to IS (*Y*) versus concentrations (*X*, nanograms per milliliter), and the typical regression equation for baicalein was  $Y=0.0008X-0.0073$ . The average correlation coefficient ( $n=5$ ) was >0.99. The validation of the analytical method for baicalein showed that the chosen method was precise and accurate over the range of 50–10,000 ng/mL with a correlation coefficient >0.99. The lower limit of quantification was 50 ng/mL. The method also showed acceptable precision and accuracy within 15% RSD. The intra-day and inter-day variation at three concentrations (100, 500, and 2,000 ng/mL) was 7–12% and less than 10%, respectively. The baicalein absolute recovery from the plasma at three concentrations (100, 500, and 2,000 ng/mL) was 76.9–86.7%.

### Data Analysis

The plasma concentration–time data for baicalein in rats were analyzed by WinNolin 5.2.1 software (Pharsight, Mountain View, CA, USA), employing a non-compartmental model. The area under the plasma concentration–time curve from zero to the last measurable plasma concentration point ( $AUC_{0-t}$ ) was calculated by the linear trapezoidal method. Unpaired Student's *t* tests were used for the statistical comparison of pharmacokinetic parameters between oral dosing of pure baicalein and solid dispersion.

## RESULTS AND DISCUSSION

### The Comparison of Solid Dispersions Prepared by SFD and the Solvent Evaporation Method

In the SFD process, the feeding solution that contained baicalein and the carrier appeared transparent and yellow. The lyophilized products were fluffy and cotton-like, with a yellow color. No crystals of baicalein could be observed with the naked eye (Fig. 1a). In the solvent evaporation method, the yellow powders firmly adhered to the glass flask wall with some shiny yellow crystals at the bottom of the flask (Fig. 1b), suggesting that baicalein may have recrystallized in the prepared solid dispersion, which needed confirmation by the following PXRD test. The powders prepared by the solvent evaporation method after drying in silica gel overnight are shown in Fig. 1c.

### Dissolution Studies

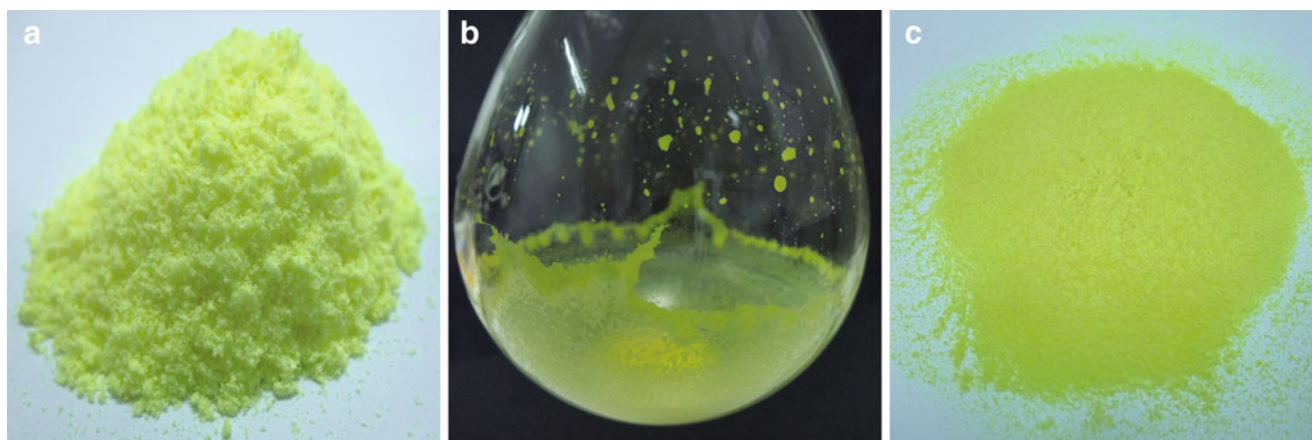
The dissolution profiles of the solid dispersions for baicalein prepared by two different methods, SFD and the solvent evaporation method, are shown in Fig. 2. The

dissolution rate of powders prepared by the solvent evaporation method containing baicalein–Pluronic F68 (1:4, *w/w*) was very low; in 120 min, only ~11% of the baicalein was released in water, which was similar to the physical mixture (Fig. 3). The dissolution result indicates that the majority of baicalein may be crystallized during the solvent evaporation process and could not be dissolved into water, which needed to be confirmed by the following PXRD test. Figure 2 illustrates the dissolution profiles of the SFD powders for baicalein prepared with different excipients, PVP K40 and Pluronic F68. The initial dissolution rate of SFD containing baicalein–PVP K40 (1:1, *w/w*) was slow, and only ~19% of the baicalein was released in 120 min. With the increased weight ratio of carrier from 1:1 to 4:1 (PVP K40/baicalein, *w/w*), the dissolution increased to ~57% within 120 min. The SFD powders with a carrier of Pluronic F68 (baicalein–Pluronic F68 (1:4, *w/w*)) displayed much faster initial dissolution rates (with a larger slope at the initial linear curve range) and ~82% of the drug was dissolved within 120 min in water. The above results suggested that Pluronic F68 performed much better than PVP K40 for dissolution enhancement, and it was selected as the carrier for preparing solid dispersion for the subsequent studies. Moreover, as shown in Fig. 2, nearly identical amounts of baicalein (~90%) were released within 120 min from the solid dispersion of baicalein–Pluronic F68 or baicalein–PVP K40 prepared by SFD techniques in 1% SDS (used as a surfactant), confirming that Pluronic F68 in solid dispersion formulations has double roles, one as a polymeric carrier and the other as a surface active agent (5), which could be used to explain its better performance than PVP K40 in enhancing the dissolution of poorly soluble baicalein in water.

Using Pluronic F68 as a carrier, dissolution profiles of SFD powders prepared with various drug/carrier ratios are presented in Fig. 3. The physical mixture exhibited significantly faster initial dissolution rates than the pure baicalein, which may be attributed to an improvement of wetting and to local solubilization by the carrier in the diffusion layer. With the increased ratio of baicalein to Pluronic F68 (i.e., 1:1, 1:2, and 1:4), the dissolution within 120 min increased to 57%, 73%, and 81%, respectively, all of which were much higher than the pure baicalein (~2.4%). The increased dissolution rate of SFD powders may be attributed to factors including the amorphous nature of the baicalein, the increased surface area, and intimate dispersion of the drug and hydrophilic excipient Pluronic F68 of the solid dispersion, which needed to be verified in the subsequent characterizations. Moreover, the dissolution of baicalein–Pluronic F68 (1:9, *w/w*) was 80% in 120 min, which is similar to that of the ratio at 1:4. To obtain a higher drug loading and reduce the carrier used in preparation, the drug/carrier ratio at a 1:4 weight was finally determined and was used for the subsequent solid characterization, stability, and *in vivo* bioavailability studies.

### Solid-State Characterizations

SEM was used to determine the surface morphologies of pure baicalein, Pluronic F68, and SFD samples. As shown in Fig. 4a, pure baicalein powders exist as crystalline particles with a cubic shape. A large cubic baicalein crystal with a small attached irregular Pluronic F68 crystal on its surface could be



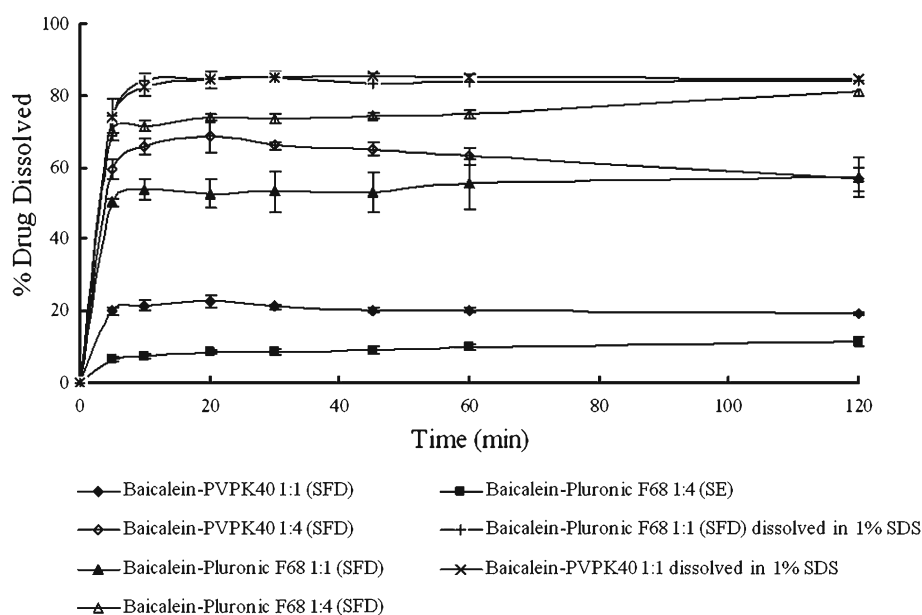
**Fig. 1.** Macroscopic appearance of solid dispersions prepared by SFD method, baicalein–Pluronic F68 1:4 (a); solvent method, baicalein–Pluronic F68 1:4 before desiccation (b); and solvent method, baicalein–Pluronic F68 1:4 after desiccation (c)

observed from the SEM micrograph of the physical mixture (Fig. 4b), indicating no interaction between baicalein and Pluronic F68 during the blending. In contrast, the SFD particles had a reduced geometric diameter at a range of several micrometers compared to the raw baicalein material (Fig. 4c). This may be due to the rapid freezing rate after the instant atomization and the prolonged drying process in vacuum conditions.

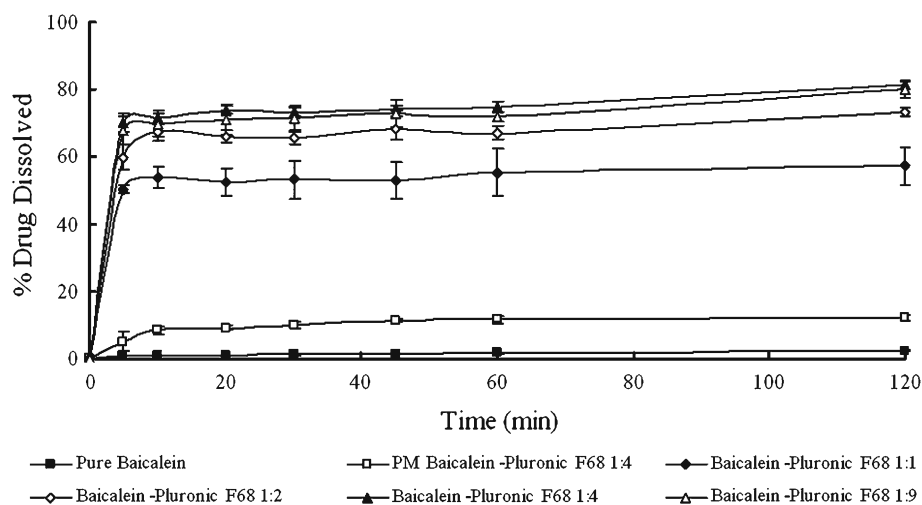
The surface area of the SFD baicalein–Pluronic F68 1:4 powder was 27.64 m<sup>2</sup>/g, which was about two times larger than that of pure baicalein (10.91 m<sup>2</sup>/g). According to the Noyes–Whitney equation (25), the amount of solute dissolved per unit time,  $dm/dt$ , is proportional to the surface area,  $A$ , and the difference between the concentration at time  $t$ ,  $C_t$ , and the solubility,  $C_s$  (i.e.,  $C_s - C_t$ ). Under sink conditions, i.e., when  $C_s \gg C_t$ ,  $dm/dt = KAC_s$  where  $K$  is a constant incorporating the diffusion coefficient ( $D$ ), solution volume ( $V$ ), and diffusion thickness ( $h$ ) terms, assuming that dissolution is diffusion-controlled. In this study, the formation of a solid dispersion of baicalein in Pluronic F68

will lead to an increase in surface area (as depicted by the  $A$  term in the equation) and hence an increase in dissolution rate.

PXRD patterns of pure baicalein, Pluronic F68 with or without SFD process, PM of baicalein with Pluronic F68, SFD-processed powders with baicalein, and powders produced by solvent evaporation method are presented in Fig. 5. The pure baicalein displayed numerous distinctive peaks at 10.1°, 11.3°, 13.2°, 15.3°, and 26.3° ( $2\theta$ ), indicating that the raw material of baicalein has a high degree of crystallinity. Pluronic F68, as shown in Fig. 5, was also in the crystalline form, having two distinct peaks at 19.1° and 23.3° and a relative broad peak with low intensity between 26° to 27° ( $2\theta$ ). After SFD processing, the characteristic peak of Pluronic F68 remained unchanged, but the intensity was significantly reduced. Some of the distinctive peaks of baicalein (i.e., 10.1°, 11.3°, 13.2°, and 15.3°) or Pluronic F68 (i.e., 18.4°, 23.1°, and 26.3°) were still detectable in their physical mixture but with lower intensity. No new peaks could be observed, suggesting the absence of interaction between



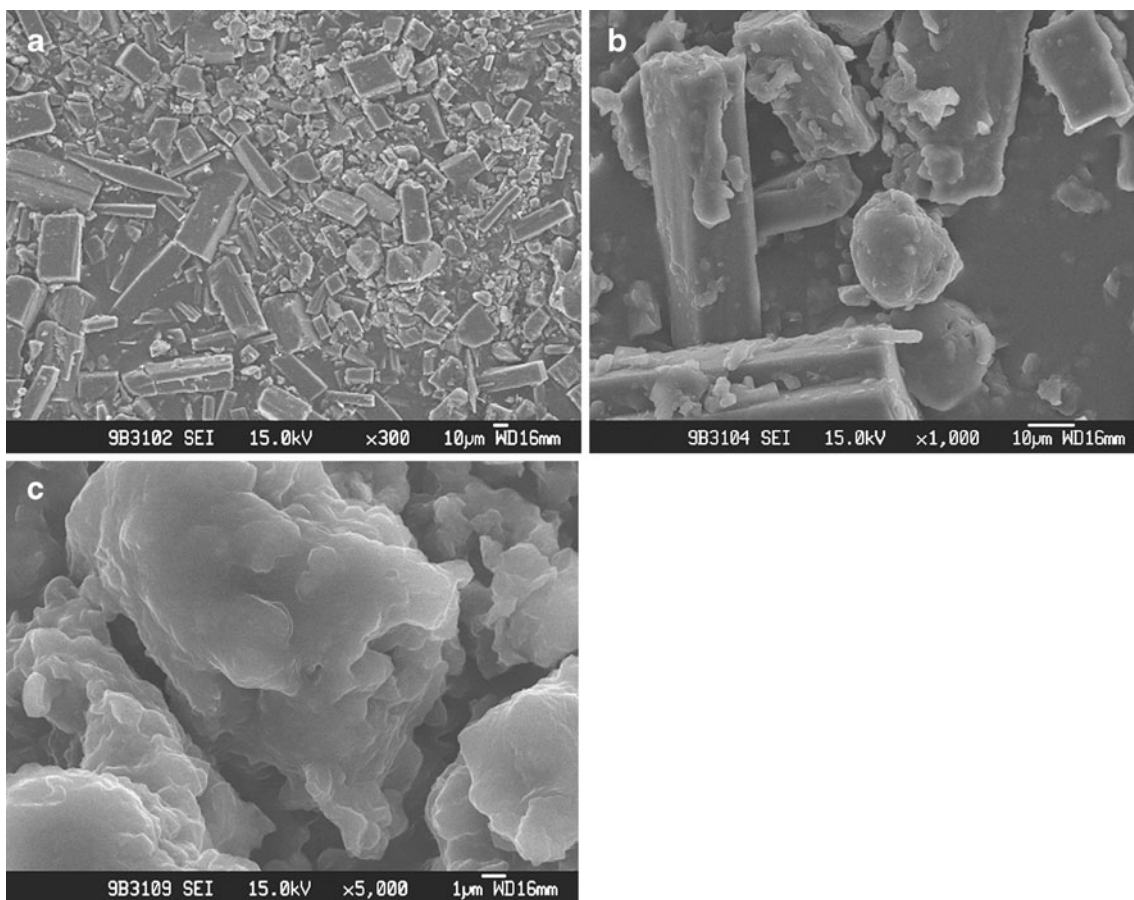
**Fig. 2.** Dissolution profiles of SFD baicalein–PVP K40 and SFD baicalein–Pluronic F68 with various drug/carrier ratios in water (mean  $\pm$  SD,  $n=3$ )



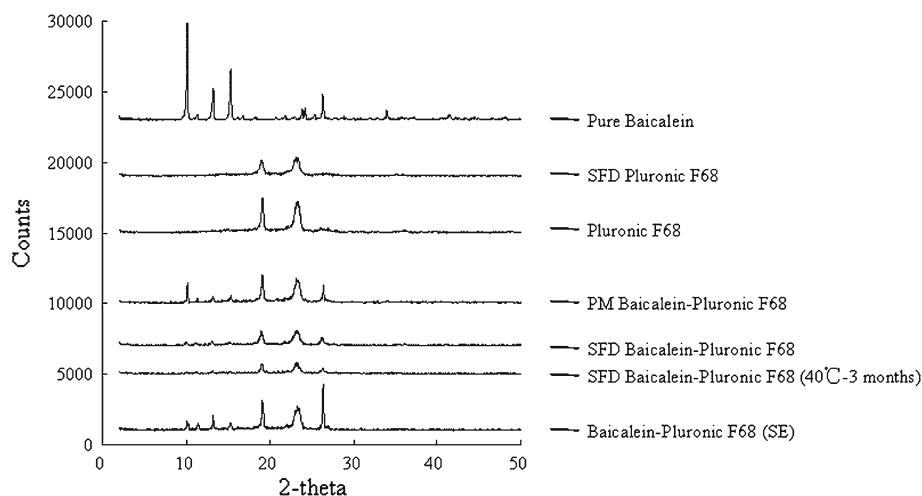
**Fig. 3.** Dissolution profiles of SFD baicalein-Pluronic F68 with various drug/carrier ratios in water (mean  $\pm$  SD,  $n=3$ )

the drug and the carrier. However, PXRD of the SFD baicalein-Pluronic F68 1:4 powder containing 20% ( $w/w$ ) baicalein exhibited a significant reduction in peak intensity for baicalein compared to the physical mixture, and only the three characteristic peaks of Pluronic F68 at 18.4°, 23.1°, and 26.3° could be obviously observed, although the peaks of baicalein did not completely disappear. These results may suggest that the majority of crystalline baicalein was con-

verted to the amorphous state in the solid dispersion produced by SFD. More optimization is necessary to completely convert crystalline baicalein into the amorphous baicalein in future studies. No energy is required to break up the crystal lattice of baicalein in the amorphous state during the dissolution process, and drug solubility and wettability may be increased by surrounding hydrophilic carriers (26), all of which would lead to the faster and more



**Fig. 4.** SEM micrographs of **a** pure baicalein (magnification  $\times 300$ ), **b** PM of baicalein with Pluronic F68 (magnification  $\times 1,000$ ), and **c** SFD baicalein powders (magnification  $\times 5,000$ )



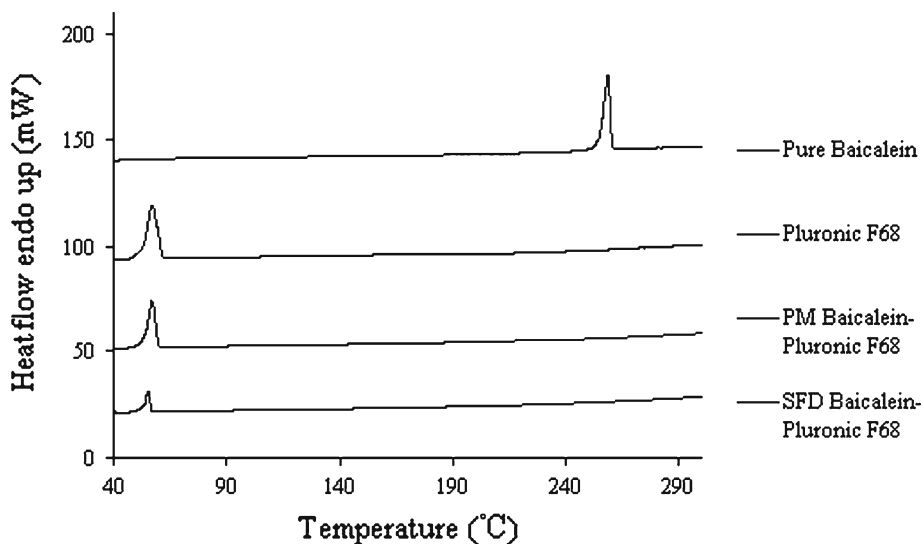
**Fig. 5.** Powder X-ray diffraction patterns of pure baicalein, Pluronic F68, PM of baicalein with Pluronic F68, SFD and solvent evaporation prepared powders of baicalein

complete dissolution of baicalein from the solid dispersion (Fig. 3). On the contrary, most of the distinctive peaks of baicalein (i.e.,  $10.1^\circ$ ,  $11.3^\circ$ ,  $13.2^\circ$ , and  $15.3^\circ$ ) could be obviously observed from the PXRD of solid dispersion produced by the solvent evaporation method, indicating that the majority of baicalein still existed as the crystalline form and did not convert into the amorphous form. No matter which method was used, the diffraction peaks of Pluronic F68 at  $18.4^\circ$ ,  $23.1^\circ$ , and  $26.3^\circ$  could still be obviously observed, suggesting that Pluronic F68 was still present in the crystalline form in the two prepared solid dispersions. During the solvent evaporation process, Pluronic F68 will be recrystallized into its crystalline form without changing its crystal forms (evidenced by the similar distinctive peaks) shown in Fig. 5.

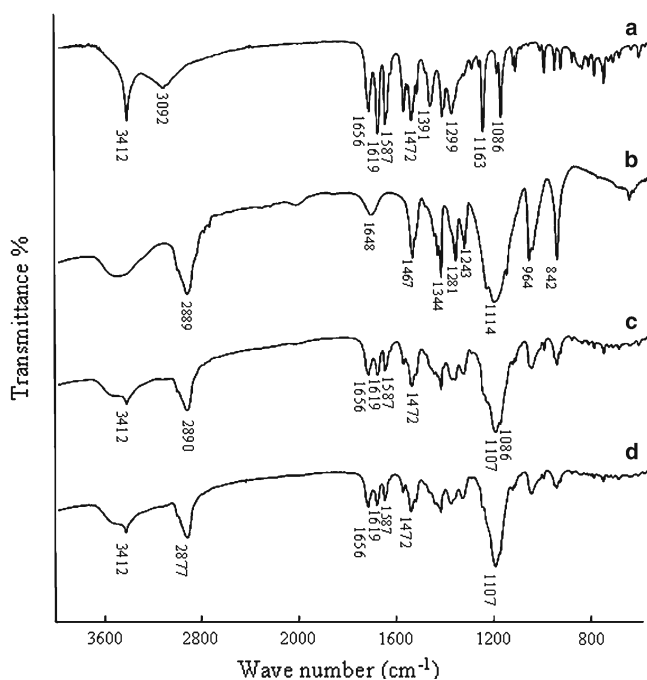
Figure 6 shows the DSC thermograms of pure baicalein, Pluronic F68, PM of baicalein with Pluronic F68, and SFD baicalein powders. The pure baicalein or Pluronic F68 exhibited a single sharp endothermic peak at around  $260.7^\circ\text{C}$  and  $53.6^\circ\text{C}$ , respectively, corresponding to their melting

points. The physical mixture and the SFD-processed solid dispersion exhibited a sharp endotherm at  $53.6^\circ\text{C}$ , corresponding to the melting point of Pluronic F68. No endotherm corresponding to the melting of pure crystalline baicalein was observed in the physical mixture or SFD product. These results suggest that upon heating in DSC, baicalein progressively dissolves in Pluronic F68 and dissolves completely below the melting temperature of crystalline baicalein. Therefore, no melting peak of baicalein could be observed in the solid dispersion with Pluronic F68. Similar behaviors of the solid dispersions using Pluronic F68 as a carrier were reported before, such as for the solid dispersion of nifedipine with Pluronic F68 and Gelucire 50/13 (27) and the solid dispersion of rofecoxib with Pluronic F68 (28). DSC appears to be unsuitable for determining the degree of crystallinity of the prepared solid dispersion system in the present study.

In order to further ascertain if baicalein undergoes a polymorphic change during the preparation of solid dispersion and to test for the possible intermolecular interactions

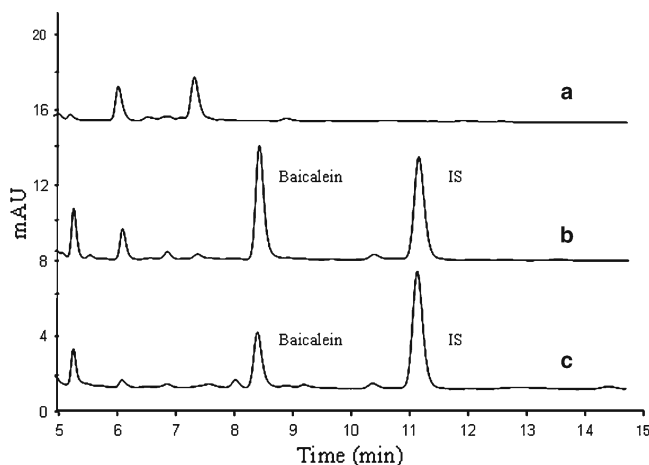


**Fig. 6.** DSC thermograms of pure baicalein, Pluronic F68, PM of baicalein with Pluronic F68, and SFD baicalein powders



**Fig. 7.** FTIR spectra of **a** pure baicalein, **b** Pluronic F68, **c** PM of baicalein with Pluronic F68, and **d** SFD baicalein powders

between baicalein and the constituents of the dispersion matrix, FTIR was used and the results are presented in Fig. 7. In the FTIR spectra of crystalline baicalein (Fig. 7a), absorption bands of O–H stretching vibrations were at 3,412  $\text{cm}^{-1}$ , indicating the intra-molecular hydrogen bond of baicalein. Absorption peaks of 1,391 ( $\delta_{\text{OH}}$ ), 1,299 ( $\nu_{\text{C-OH}}$ ), and 1,163 ( $\nu_{\text{C-OH}}$ ) are the correlation peaks of O–H bands. Absorption bands of C=O stretching vibrations were at 1,656  $\text{cm}^{-1}$ ; C=C (benzene ring) stretching vibrations were at 1,619, 1,587, and 1,472  $\text{cm}^{-1}$ , and C–H stretching vibrations were at 3,092  $\text{cm}^{-1}$  (29). These bands were also observed for the physical mixture of baicalein with Pluronic F68 (Fig. 7c). The carrier Pluronic F68 exhibits relatively broad peaks (Fig. 7b) due to its large molecular size and its partially amorphous nature (27). The absence of any other new peaks in the solid dispersion indicates that baicalein did not undergo any polymorphic change during preparation, confirming the PXRD results. Comparing the spectra of SFD powder to the physical mixture, no characteristic peaks were significantly shifted except the absorption band of Pluronic F68 at 2,890  $\text{cm}^{-1}$  was shifted to 2,877  $\text{cm}^{-1}$  (Fig. 7d), indicating the lack of significant interactions between baicalein and Pluronic F68 at the molecular level in the SFD-processed solid dispersion (27,30).



**Fig. 8.** Representative HPLC chromatograms of **a** blank rat plasma sample, **b** blank rat plasma sample spiked with standard baicalein and IS, and **c** rat plasma sample collected after a single intragastric dose of baicalein

### Stability Studies

A stability study was conducted for the SFD baicalein–Pluronic F68 1:4 at 40°C for 6 months to examine any changes in baicalein content, dissolution profile, and crystallinity by PXRD. As shown in Table I, the content of baicalein remained unchanged and no significant decline of dissolution rate was observed in the dissolution profiles between the initial and 6-month samples ( $p > 0.05$ ). SFD baicalein–Pluronic F68 powder exhibited no significant changes in peak densities between the initial and 6-month samples in PXRD (Fig. 5), suggesting that the SFD baicalein sample was physically and chemically stable during the 6-month storage in a low humidity environment.

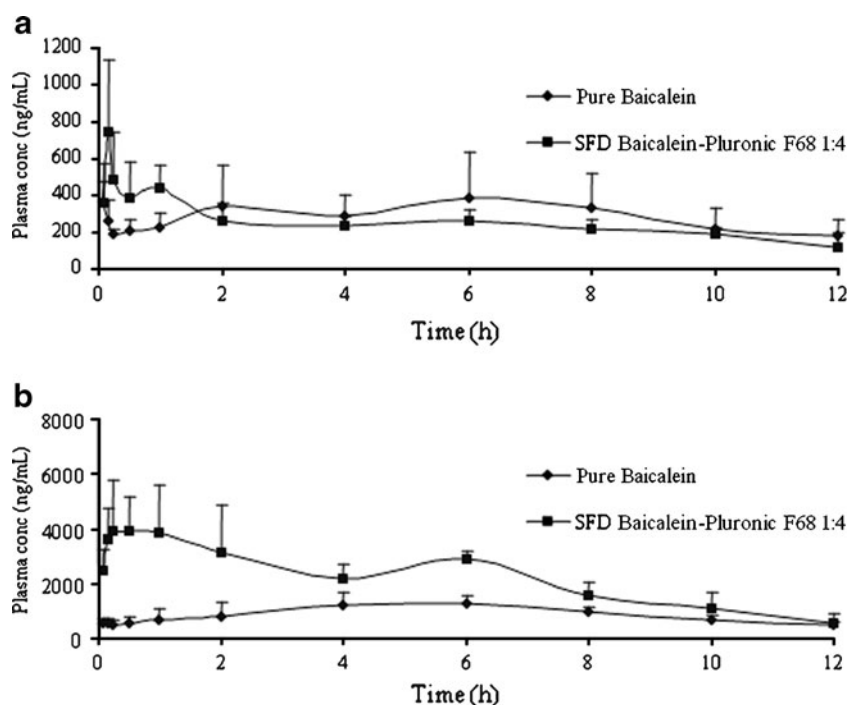
### In Vivo Bioavailability Study

The representative chromatograms of baicalein with plasma internal standards are shown in Fig. 8. No interference of internal components was found in the quantification of baicalein in rat plasma. As shown in Fig. 9, after oral administration of pure baicalein, the mean plasma concentrations at each time point of the free form (Fig. 9a) were much lower than total baicalein after enzyme hydrolysis (Fig. 9b), indicating that it is the baicalein glucuronides or sulfates, not the free form, that accounted for the majority of the total baicalein in the circulation. It is expected that the conjugated metabolites of baicalein, not the free form, are responsible for the *in vivo* effects of baicalein. Therefore, the

**Table I.** Accelerated Stability Study at 40°C in SFD Baicalein Formulation (Mean  $\pm$  SD,  $n=3$ )

Time (months)	Baicalein content (%)	% of baicalein dissolved within 10 min	% of baicalein dissolved within 2 h
0	21.63 $\pm$ 0.97	79.61 $\pm$ 3.58	86.16 $\pm$ 5.27
1	19.47 $\pm$ 0.96	75.14 $\pm$ 1.36	78.80 $\pm$ 0.56
2	21.31 $\pm$ 0.15	72.53 $\pm$ 2.29	78.96 $\pm$ 1.29
3	22.38 $\pm$ 0.38	75.72 $\pm$ 4.32	87.60 $\pm$ 3.57
6	18.73 $\pm$ 0.16	73.37 $\pm$ 2.34	81.36 $\pm$ 1.15





**Fig. 9.** Plasma concentration–time profile of baicalein after a single oral dose of baicalein to rats at 75 mg/kg. **a** Baicalein in free form and **b** baicalein after hydrolysis (mean  $\pm$  SD,  $n=5$ )

subsequent bioavailability study used parameters calculated by the concentrations of total baicalein after enzyme hydrolysis for comparison with the SFD-processed formulation. The pharmacokinetic parameters of baicalein were computed as described in “Data Analysis” and tabulated in Table II. The  $C_{max}$  and  $AUC_{0-12}$  of baicalein in SFD processed solid dispersion increased by 3.6- and 2.3-fold, respectively, compared to the pure baicalein. Additionally, baicalein in the solid dispersion was absorbed more rapidly with the  $T_{max}$  reduced from 6 to  $\sim 2$  h ( $p < 0.05$ ). The accelerated and increased oral absorption of baicalein from the solid dispersion may be attributed to the more rapid and complete release of baicalein from SFD powder than from the pure baicalein as shown in Fig. 3, indicating that dissolution is a rate-limiting step in the absorption of baicalein from the solid state.

It was reported that baicalin, one of the mono-glucuronidation metabolites of baicalein, is one of the major conjugated metabolites after oral administration of baicalein (18). The activity of baicalin reported in *in vitro* studies could in part explain the *in vivo* effects of baicalein after oral dosing. Due to the fact that no other authentic standard of baicalein glucuronides or sulfates was available, baicalein was

determined in plasma samples before and after treatment with  $\beta$ -glucuronidase/sulfatase to calculate the concentration of baicalein as free and total forms (free and conjugated baicalein). The plasma profiles of the baicalein as free and total forms (Fig. 9) indicated that the conjugation metabolism of baicalein occurred very rapidly and extensively *in vivo*, which was due to the extensive first-pass conjugation metabolism in the gut and liver (15).

## CONCLUSIONS

The SFD technology was demonstrated to produce stable, amorphous, high surface area, and free-flowing baicalein powders with Pluronic F68 at a 1:4 (drug/carrier) weight ratio, which could not be obtained by the conventional solvent evaporation method. Pluronic F68 underwent recrystallization during spraying into the liquid nitrogen and could be used as a carrier to inhibit the majority of baicalein from recrystallizing. The SFD produced powders exhibited a significantly enhanced dissolution rate compared to the powders formed by the conventional solvent evaporation method and significantly improved the oral bioavailability of

**Table II.** Pharmacokinetic Parameters of Baicalein After Enzyme Hydrolysis in Rats (Mean  $\pm$  SD,  $n=5$ )

Parameter	Unit	Bulk baicalein	Solid dispersion
$T_{max}$	h	6.00 $\pm$ 1.41	1.63 $\pm$ 2.25*
$C_{max}$	ng/mL	1,401.20 $\pm$ 303.21	4,984.00 $\pm$ 1,286.37*
$AUC_{0-12}$	h ng/mL	10,641.76 $\pm$ 2,375.59	24,814.87 $\pm$ 6,190.48*
MRT	h	5.91 $\pm$ 0.69	4.49 $\pm$ 0.59*
Rel. BA <sup>a</sup>	%	100	233.18

\* $p < 0.05$  in two-tailed Student's *t* test compared with pure baicalein

<sup>a</sup> Relative bioavailability compared with pure baicalein

baicalein in rats. In summary, the SFD technology is an effective particle engineering process for preparing solid dispersions with a low glass transition/melting point carrier to improve the dissolution rate and oral bioavailability of BCS II APIs.

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