Characterization and evaluation in vivo of baicalin-nanocrystals prepared by an ultrasonic-homogenization-fluid bed drying method

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[ABSTRACT]
AIM: To improve the absorption and bioavailability of baicalin using a nanocrystal (or nanosuspension) drug delivery system.

METHODS: A tandem, ultrasonic-homogenization-fluid bed drying technology was applied to prepare baicalin-nanocrystal dried powders, and the physicochemical properties of baicalin-nanocrystals were characterized by scanning electron microscopy, photon correlation spectroscopy, powder X-ray diffraction, physical stability, and solubility experiments. Furthermore, in situ intestine single-pass perfusion experiments and pharmacokinetics in rats were performed to make a comparison between the microcrystals of baicalin and pure baicalin in their absorption properties and bioavailability in vivo.

RESULTS: The mean particle size of baicalin-nanocrystals was 236 nm, with a polydispersity index of 0.173, and a zeta potential value of −34.8 mV, which provided a guarantee for the stability of the reconstituted nanosuspension. X-Ray diffraction results indicated that the crystallinity of baicalin was decreased through the ultrasonic-homogenization process. Physical stability experiments showed that the prepared baicalin-nanocrystals were sufficiently stable. It was shown that the solubility of baicalin in the form of nanocrystals, at 495 μg mL⁻¹, was much higher than the baicalin-microcrystals and the physical mixture (135 and 86.4 μg mL⁻¹, respectively). In situ intestine perfusion experiments demonstrated a clear advantage in the dissolution and absorption characteristics for baicalin-nanocrystals compared to the other formulations. In addition, after oral administration to rats, the particle size decrease from the micron to nanometer range exhibited much higher in vivo bioavailability (with the AUC(0-∞) value of 206.96 ± 21.23 and 127.95 ± 14.41 mg L⁻¹h⁻¹, respectively).

CONCLUSION: The nanocrystal drug delivery system using an ultrasonic-homogenization-fluid bed drying process is able to improve the absorption and in vivo bioavailability of baicalin, compared with pure baicalin coarse powder and micronized baicalin.

[KEY WORDS] Baicalin; Nanocrystals; Ultrasonic-homogenization-fluid bed drying; Characterization; Absorption; Bioavailability


Introduction

Baicalin, (BG, 5, 6-dihydroxyflavone-7-O-D-glucuronic acid, Fig. 1), a glucuronide isolated from the root of Scutellaria baicalensis Georgi (Lamiaceae), is widely used in traditional Chinese medicine for treating viral hepatitis [1], upper respiratory and gastrointestinal tract infections [2], and cardiovascular disorders [3]. Various pharmacological effects of BG were demonstrated in recent studies, such as anticancer [4], anti-inflammatory [5], antioxidative [6], antileukemic [7], antiproliferative [8], etc. However, the low lipid and water solubility of BG lead to low oral bioavailability, which causes low exposure of baicalin in the blood, and limits its therapeutic effects and clinical application [9]. Although a baicalin–phospholipid complex [10], a dispersible tablet of baicalin [11], and a baicalin-polyvinylpyrrolidone coprecipitate [12] have been prepared with an improvement in dissolution and bioavailability,
In order to improve the physical and chemical stability of nanosuspension systems, water has to be removed. Methods used for solidification include two main techniques: freeze drying and spray drying [24]. Freeze drying is used more frequently than spray drying. Although the lyophilization process is time-consuming, and the residual moisture content of the obtained dried powder is relatively high, spray drying provides a relatively high yield and a low residual moisture content [25]. Similar results could be produced based on both fluid bed drying and spray drying. In addition, a fluidized bed can achieve step granulation, which improves the handling properties of the powder, such as an enhancement of the flowability or reduction of dust formation, a higher dissolution rate by reducing lump formation and flotation of the powder, and a higher bulk density, resulting in lower transport costs and a better compactability for the tableting process [26]. Consequently, the prepared nanosuspension was solidified by fluid-bed drying, and the physicochemical properties of the granulated powder of BG-NS were characterized by photon correlation spectroscopy (PCS), powder X-ray diffraction, and scanning electron microscopy (SEM). To verify the advantages of BG-NS, the intestinal absorption properties and bioavailability were also evaluated through the in situ intestinal single-pass perfusion model and pharmacokinetics in rats, comparing with a BG-microsuspension (BG-MS) of the bulk drug in the presence of the corresponding surfactant concentrations under the same conditions.

Materials and Methods

Materials. Standard BG was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Pure BG (95%) was obtained from Shanxi Yongjian Pharmaceutical Co., Ltd (Xi’an, China). SDS and poloxamer-188 were purchased from the BASF Corp. (Ludwigshafen, Germany). Ethyl carbamate was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phenolsulfonaphthalein (PSP) was purchased from Solarbio (Beijing, China). Methanol for HPLC was HPLC-grade was purchased from Promptar Co. Ltd. (Elk Grove, CA, USA); All other chemicals were of analytical grade and were used as received.

Animals. Wistar rats (adult male, 200–220 g) used in the experiments received care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals. The studies were approved by the Animal Ethics Committee of China Military Institute of Chinese Materia Medica.

Preparation of BG-MS and BG-NS. In order to investigate the requirements of the combination of stabilizers, different compositions of stabilizers were added to both the BG-MS and BG-NS preparations as shown in Table 1. Two formulations were chosen, and those of BG-MS had the same corresponding compositions and concentration as those of BG-NS under the same conditions.
BG-MS was prepared by the ultrasonic method as follows. BG bulk drug was weighed into a sample vial and stabilizers were added as shown in Table 1, and the mixture stirred with an appropriate amount of distilled water. The BG suspension was then micronized and homogenized by the ultrasonic probe (Bandelin Sonopuls HD2200, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) for 5 min. BG-NS was produced through the ultrasonic-homogenization process in the same way as with BG-MS in the previous treatment. The produced BG-MS was homogenized at high pressure using a piston-gap high pressure homogenizer Emulsiflex-C3 (Avestin Inc., Ottawa, Canada). Initially, five cycles at 500 bar were conducted as a pre-milling step, and then twenty cycles at 1 000 bar were run to obtain the nanosuspension.

Solidification by fluid-bed drying. The BG-NS and BG-MS suspensions were solidified and dried using a fluid-bed dryer (MINI-Glatt, Glatt, Germany) for further physico-chemical characterization and processing into solid dosage forms. Briefly, PVP-K30 (5%, M/V) was added to the obtained aqueous suspensions, and then bottom sprayed on the fluidizing pill cores bed at a rate of 1 mL·min⁻¹ using a peristaltic pump. The inlet drying air temperature was 50 °C with the fluidizing pressure of 0.29 bar, and a materialized pressure of 1.57 bar. Spraying was continued until all of the suspensions were used. Finally, the moist granules were dried by fluidizing them with an inlet air temperature of 50 °C for 30 min.

Morphology. The granulated powder morphology was observed using a scanning electron microscope (S-4800, Hitachi Technologies Corporation, Tokyo, Japan). Samples were coated with gold and palladium using a vacuum evaporator and were examined at an accelerating voltage of 15 kV.

Particle size and zeta potential measurements. The freshly liquid suspensions and reconstituted suspensions of (BG-NS and BG-MS) were analyzed for their average particle size and polydispersity index (PI) by photon correlation spectroscopy (PCS) using a Zeta-sizer (3000 SH, Malvern Instruments Ltd., Malvern, UK) at a fixed angle (173°) at 25 °C. A dilution of the samples in deionized water was performed until a concentration of 1 mg mL⁻¹ was obtained in order to enable measurements. Each sample was measured in triplicate.

The zeta (ζ) potential of the different samples was performed using the same Malvern instrument. Zeta potential measurements were also made at a similar concentration (1 mg mL⁻¹) in deionized water at 25 °C, with a dielectric constant of 79, refractive index of 1.33, viscosity of 0.89 cP, cell voltage 150 V, and current of 5 mA. Data from the analysis of ζ potential are presented as a mean and SD of triplicate runs.

X-Ray analysis. Powder X-ray diffraction (PXRD) patterns were recorded on a Powder X-ray diffractometer (D/Max-2500 PC, Rigaku, Japan) with Ni-filtered Cu-Kα radiation. The samples were analyzed over the range from 3° to 40° of 2θ at a rate of 4° per minute. PXRD patterns were determined for pure BG, poloxamer-188, SDS, the physical mixture (BG-PM), and the BG-MS, and BG-NS granulated powders. Each batch was analyzed in triplicate.

Physical stability. BG-NS samples were stored at room temperature. Particle sizes, zeta potential, and PI were determined after storage for 5, 15, 30, 60 and 90 days to evaluate their physical stability using the same method as for the determination of particle size and zeta potential.

Solubility. Solubility determinations were performed on pure BG, BG-PM, and the dried powders of BG-NS and BG-MS by adding the excess into water (6 mL) in sealed glass containers at 25 °C. The liquids were agitated for 24 h, then centrifuged to remove residual solid (15 min, 4 000 r·min⁻¹). The supernatant was filtered through a 0.22 μm filtration membrane, and the concentration of BG in the supernatant was determined by HPLC.

In situ intestinal perfusion in rats. Three different test perfusates of BG were prepared as follows:

- Treatment 3 (T3): BG-PM in Krebs-Ringer buffer.

Preparation of perfusates: The BG preparations were diluted to the desired volume with Krebs-Ringer buffer [27], in addition, vitamin C (Vit C) was employed as the antioxidant to improve the stability of BG in the intestine perfusates. Phenolsulfonphthalein (PSP) was added as a non-absorbable marker [28] in the intestinal perfusate (pH 7.4), which consisted of 2 mg mL⁻¹ Vit C, 20 μg mL⁻¹ PSP, and 30 μg mL⁻¹ BG.

The in situ rat intestine absorption experiment was carried out using the recirculating perfusion technique described previously [27-28]. Rats were fasted for 12 h before the experiment and were fixed after being anesthetized by intraperitoneal administration of ethyl carbamate (20%, 6 mL·kg⁻¹). A longitudinal midline incision (2–3 cm) was made after the abdominal area was shaved and cleaned, and then incisions were made at both ends of the small intestine (duodenum to ileum). Meanwhile, the bile duct was ligated with suture thread. The tubes were connected to the chosen intestinal segment and an infusion pump (BT600FJ, Baoding Chuangrui Precision Pump Co., Ltd., Baoding, China). Tubing between the intestine and the pump was water-jacketed and maintained at 37 °C. Initially, the intestinal perfusion (100 mL) was perfused at a constant rate of 5 mL·min⁻¹ through the chosen segment. When the perfusate emerged at the distal end of the segment, the flow rate was decreased to 2.5
mL·min⁻¹ and the timer was reset. At intervals of 15 min, the effluent perfusate samples were collected and the PSP blank perfusate without BG was simultaneously added quantitatively into the perfusate. Each perfusion experiment lasted for 2 h. Concentrations of BG and PSP were analyzed by HPLC and UV spectrophotometry, respectively.

**Dose-toxicity studies.** Wistar rats (adult male, 200–220 g) were orally administered with pure BG or BG-NS (85, 170, 340 and 680 mg·kg⁻¹), suspended in distilled water before use. Two rats were used for each dose and housed separately. Animals were administered with a pre-determined dose using 20 mL·kg⁻¹ as the volume to be gavaged in a 200 g rat. Control rats (two for each dose) were administered with the appropriate vehicle without the drug. Following the administration, the animals were observed immediately, and twice daily thereafter, for a period of 14 days. Clinical signs of toxicity such as decreased physical activity, ruffled fur, hunched posture, and loss or gain in the body weight were recorded.

**Pharmacokinetic experiments in rats.** Wistar rats (adult male, 200–220 g), fasted for 12 h before experiments, were orally administered different preparations of BG (340 mg·kg⁻¹) which was suspended in distilled water before use (n = 5). The dose of BG for oral administration was according to the dose-toxicity studies and a previous study [29]. Blood samples were collected in heparinized centrifuge tubes through the orbital sinus before dosing, and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 16 and 24 h following the oral dose of BG under light ethere anesthesia. After being centrifuged at 3000 rpm for 10 min, the plasma samples were separated and frozen at −20 °C until analysis. Before analysis, methanol (400 μL) was added to each rat plasma sample (200 μL). The mixtures were vortexed (MS3, IKA®, Staufen, Germany) for 2 min and centrifuged (H 2050R, Xiang Yi Centrifuge Instrument Co., Ltd., Hunan, China) at 8 000 r·min⁻¹ for 5 min. Thereafter, a sample (20 μL) of the obtained supernatant was directly injected into the HPLC system for analysis, as described in the analytical methods.

**Analytical methods.** The concentrations of BG in water, perfusate, and plasma were measured using the same HPLC system (Agilent 1100, Agilent, Santa Clara, USA). The HPLC system consisted of a quaternary pump, degasser, diode array detector and HP Chemstation Data system. Analyses were performed on a Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm, Agilent) protected by an Alltima C18 guard column (20 mm × 4 mm, 5 μm). The mobile phase consisted of solvent A (methanol) and solvent B (0.5% phosphoric acid, V/V) in the ratio 59 : 41. The flow rate was 1.0 mL·min⁻¹ and sample injection volume was 20 μL. The DAD detector was set at the wave-length of 276 nm. The column temperature was maintained at 35 °C.

**Statistical analysis.** Pharmacokinetic analysis was performed using statistics software (DAS® 2.0, Boying Corporation, Beijing, China). Results are presented as mean ± SEM; Data were subjected to a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc test, and differences were considered statistically significant at P < 0.05.

**Results and Discussion**

**Morphology of the granulated powder of BG-NS and BG-MS.** For an early screening, SEM imaging was performed to characterize the morphology of the BG-NS and BG-MS granulated powders. As shown in Fig. 2, the images of the BG-NS preparation were close to granulated in shape, typically in the range of 200–300 nm, depending on the formulation 2 (a complex surfactants composition of poloxamer-188 and SDS), and on the conditions used for fluid-bed drying. Under the same conditions, the BG-MS powder showed irregular shapes with a wide size range of about 5–20 μm. Whereas, the granulated powders of BG-MS and BG-NS from the formulation 1 (with the absence of SDS) exhibited an increase in particle size along with obvious adhesion, indicating that poloxamer-188 was not sufficient to stabilize the nanoparticles during the fluid-bed drying process. Drying of nanosuspensions can create stress on the particles that can cause aggregation. For instance, fluid-bed drying may lead to crystallization of polymer-188, a polymeric surfactant, thereby compromising its ability to prevent aggregation. Drying can also create additional thermal stresses (due to water removal) that may destabilize the particles [30]. Images of the fluid-bed dried powders resulting from the different formulations indicated that a combination of surfactants was required to stabilize the BG-NS and BG-MS preparations. Poloxamer-188 in combination with SDS (a charged surfactant) stabilized the BG-NS against aggregation or Ostwald ripening. Consequently, formulation 2, with complex surfactants, was selected for the preparation of BG-NS and BG-MS.

**Particle size and zeta potential analysis.** The freshly prepared liquid suspensions were diluted in deionized water, and the particle size was measured by photon correlation spectroscopy (PCS). The mean diameter of the BG-NS particles was 236 nm, with a polydispersity index (PI) of 0.173 (Fig. 3). This corresponded to the information obtained from the SEM images in Fig. 2. On the other hand, the mean diameter of BG-MS was 16.2 μm (Table 2), which was about 70-fold less than that of the BG-NS particles. In addition, a higher PI value of 0.432 was obtained for the BG-MS preparation. A PI value of 0.1–0.3 indicates a narrow size distribution, whereas a PI value greater than 0.3 indicates a very broad size distribution [31-32]. According to the data presented above, the particle size reduction and the narrow size distribution could be attributed to the high pressure homogenizing process with cavitation forces, as well as collision and shear forces determining the fragmentation of the drug microparticles down to the nanometer level [33].
Fig. 2  SEM images of the dried powders of BG-MS depending on F1 (A), BG-NS depending on F1 (B), BG-MS depending on F2 (C), and BG-NS depending on F2 (D)

Fig. 3  Size distribution of BG-NS by intensity

The zeta potential had an important effect on the storage stability of the colloid dispersion system, and it was reflected in the electrostatic barriers which could prevent the nanoparticles from aggregation and agglomeration. Particle aggregation was likely to occur if the particles possessed too low a zeta potential to provide sufficient electric repulsion or steric repulsion between each other. Generally, a zeta potential of at least $-30 \text{ mV}$ for electrostatically stabilized systems or $-20 \text{ mV}$ for sterically stabilized systems was desired to obtain a physically stable nanosuspension \[^{[34]}\]. Therefore, the zeta potential could predict the storage stability of nanosuspension. In this study, the zeta potential of the fresh BG-NS preparation was ($-34.8 \pm 2.5 \text{ mV}$) (Table 2). This value provided a guarantee for the stability of reconstituted nanosuspension.

Table 2  The particle size, PI value and zeta potential of BG-NS and BG-MS in fresh liquid suspensions and reconstituted liquid dispersion (mean ± SEM, n = 3)

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Fresh liquid suspension</th>
<th>Reconstituted liquid dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size/µm</td>
<td>PI</td>
</tr>
<tr>
<td>BG-NS</td>
<td>0.236 ± 0.005 (^a)</td>
<td>0.173 ± 0.062 (^a)</td>
</tr>
<tr>
<td>BG-MS</td>
<td>16.2 ± 2.1</td>
<td>0.432 ± 0.121</td>
</tr>
</tbody>
</table>

\(^a\) P <0.05 vs the BG-MS group; \(^b\) P <0.05 vs the corresponding values of fresh liquid suspension groups

Redispersibility. To confirm the ability of the granulated powders to be redispersed in water, the particle size and zeta potential analysis of the reconstituted liquid dispersion were also performed. The granulated powders were found to be wetted easily and redispersed in water. As seen in Table 2, the average particle size of BG-NS in the reconstituted liquid dispersion was 248 nm (PI 0.181), which was similar to the fresh liquid nanosuspension (236 nm with a PI value of 0.173). Furthermore, the results also showed no significant changes in zeta potential before and after fluid-bed drying for BG-NS. This can be attributed to its narrow particle size distribution and a sufficient zeta potential, which contribute to preventing the aggregation or Ostwald ripening. For BG-MS, a slightly higher in particle size and PI value was observed in the reconstituted liquid dispersion than that of fresh liquid suspension. The reason
may be due to the broad size distribution in the original suspensions which promoted Ostwald ripening and subsequently irreversible aggregation [32,34].

**Powder X-ray diffraction (PXRD) analysis.** The solid state of the BG-NS granulated powder was characterized by powder X-ray diffraction. Fig. 4 shows the X-ray diffraction patterns of the granulated powders of BG-NS and BG-MS, BG-PM, pure BG, poloxamer-188, and SDS. The crystalline diffraction peaks were found in the similar patterns of the samples obtained by different processes, suggesting that BG in nanosuspension had the same crystalline structure as that of BG-MS and pure BG. The crystalline state of BG was unaltered following the ultrasonic-homogenization. In addition, a distinct decrease in the peak intensity of BG-NS was observed compared to that of BG-MS and pure BG, indicating that the crystallinity of BG was decreased through the ultrasonic-homogenization process [19].

**Fig. 4** PXRD diffractograms of BG-NS (A), BG-MS (B), BG-PM (C), pristine BG (D), poloxamer-188 (E), and SDS (F)

**Physical stability.** The measurements of the zeta potential and particle size allowed predictions to be made regarding the storage stability of the nanosuspensions. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. Figures 5 and 6 showed that particle sizes, zeta potential, and PI were very stable with a slight change \( P > 0.05 \) after storage for 5, 15, 30, 60, and 90 days. It was established that the BG-NS prepared by the ultrasonic-homogenization-fluid bed drying method was sufficiently stable.

**Fig. 5** Particle size (nm) and PI as a function of storage time for BG-NS at room temperature (mean ± SEM, \( n = 3 \))

**Fig. 6** Particle size (nm) and zeta potential (mV) as a function of store time for BG-NS at room temperature (mean ± SEM, \( n = 3 \))

**Solubility.** The saturation solubility of bulk BG was 53.2 μg·mL\(^{-1}\) (as shown in Fig. 7). BG-MS and BG-PM exhibited an improvement in solubility over pure BG coarse powder, which may due to increased wettability of the drug and the inhibition of drug particle aggregation by the surfactants [35]. On the other hand, the size reduction may contribute to a higher saturation solubility of BG-MS compared with BG-PM. However, the BG in dried powder of BG-NS was significantly more soluble (495 μg·mL\(^{-1}\)) than the BG-MS and BG-PM preparations (135 and 86.4 μg·mL\(^{-1}\), respectively). The enhancement of BG-NS solubility by the ultrasonic-homogenization process over BG-MS was attributed to the nano-sized dispersed state of the drug. According to the Ostwald–Freundlich equation (1), the saturation solubility will be increased sharply when the particle size is reduced to nanometer level [36].

\[
\log(\frac{C_S}{C_\infty}) = \frac{(2νσ)}{2.303RTρr}
\]

where \( C_s \) is the solubility, \( C_\infty \) is the solubility of the solid consisting of large particles, \( σ \) is the interfacial tension, \( ν \) is the molar volume of the particle material, \( R \) is the gas constant, \( T \) is absolute temperature, \( ρ \) is the density of the solid, and \( r \) is the radius of particle material.

**In situ intestinal absorption experiment.** In situ perfusion in rats is one of the most important methods in drug absorption research. In the present study, the absorption activities of the four different preparations (BG-NS, BG-MS, BG-PM, and pure BG) in the small intestine were investigated. In order to distinguish the effects of various preparations, different formulations were added to the perfusate immediately before the intestinal perfusion experiment.
began. Fig. 8 shows the typical chromatograms of blank perfusate, perfusate with BG standard, and sample perfusate. Under the chromatographic conditions of the experiment, there was no endogenous interference at the retention position of BG. The regression equation was $Y = 28.972X - 2.347, \, r = 0.9997 \, (n = 6)$. The method was validated and found to be linear over the concentration range of 0.892–30.12 μg·mL$^{-1}$.

Fig. 8 Typical chromatograms of BG. (A) blank perfusate, (B) perfusate with BG standard, (C) sample perfusate (1) BG

As shown in Fig. 9, the average perfusate concentration of BG versus time profiles after small intestinal perfusion of different preparations were obtained, and it was observed that each absorption profile consisted of two phases (an increase and a decrease of BG concentration in the effluent perfusate).

In the BG increasing phase, in fact a dissolution process, BG-NS showed a sharp increase of BG concentration, and took the lead in coming to the $c_{\text{max}}$, which was distinctly superior compared to the BG-MS, BG-PM, and pure BG samples. The enhanced dissolution velocities of BG from BG-NS can be interpreted by the Noyes–Whitney equation (2):

$$\frac{dc}{dt} = D \times A (C_S - C_X) / h$$

where $D$ is the diffusion coefficient; $A$ is the surface area; $C_s$ is the saturation solubility; $C_X$ the concentration of the solid in the bulk dissolution medium, and $h$ is the diffusional distance. According to the Prandtl equation, the diffusional distance $h$ is reduced for small particles. Thus, the simultaneous increase in the $C_s$ and decrease in the $h$ lead to an increased concentration gradient $(C_s - C_X)/h$, enhancing the dissolution velocity, together with the surface area increase $^{[37]}$. Whereas the dissolution velocity of BG from BG-MS was much lower than that of BG-NS, it was higher than that of BG-PM. This may be mainly attributed to the particle size level and crystallinity of the dried powder. It could be inferred from the Ostwald–Freundlich and Noyes–Whitney equations that particles at the nanometer level would increase the dissolution velocity significantly more than those in the micron range. Additionally, as discussed in the PXRD analysis, the crystallinity of BG in the BG-NS powders decreased compared to that in the BG-MS, BG-PM, and pure BG samples, which should lead to an increase in the drug dissolution rate $^{[19]}$.

In fact, the BG decreasing phase is the real absorption process (as shown in Fig. 9). From the absorption phase (40–120 min), a series of absorption parameters (including the absorption rate constant ($K_a$), the absorption half-life ($t_{1/2}$), and the hourly absorption percentage ($P$) of the drug) were obtained on the basis of the principle of Fick. As shown in Table 3, the absorption of pure BG in the small intestine was poor, and this result was in good agreement with a previous study $^{[28]}$. Nevertheless, preparations of BG-MS and BG-PM exhibited an increase in absorption in the small intestine perfusion, and this proved to be greater for BG-MS than for BG-PM (with the average $K_a$ value of 0.132 5 and 0.051 3 h$^{-1}$, respectively). This may be attributed to the improvement in solubility and dissolution of BG with the corresponding surfactants discussed above. Whereas the size reduction to micron level probably led to the higher absorption of BG for BG-MS compared with BG-PM (with the average $K_a$ value of 0.132 5 and 0.051 3 h$^{-1}$, respectively). This may be attributed to the improvement in solubility and dissolution of BG with the corresponding surfactants discussed above. Whereas the size reduction to micron level probably led to the higher absorption of BG for BG-NS compared with BG-PM. As for BG-NS, the average value of $K_a$ was 0.2409 h$^{-1}$, which was an approximate two-fold increase compared with BG-MS. Such an advantage of BG-NS in absorption activities can be ascribed to its better properties of solubility and dissolution, following a decrease in particle size from the micron to nanometer range $^{[35-36]}$. On the other hand, nanoparticles show excellent adhesion to biological surfaces such as the epithelial gut wall, and are able to deliver active pharmaceutical ingredients (API) across a number of biological barriers (i.e., different types of mucus and epithelia, and cell membranes), an advantage for drug absorption in the gastrointestinal tract $^{[19-20]}$. 

Fig. 9 Average perfusate concentration versus time profiles of BG after small intestinal perfusion of different BG preparations (30 μg·mL$^{-1}$; BG-NS, BG-MS, BG-PM, and pure BG)

In the increasing phase, a dissolution process, BG-NS showed a sharp increase of BG concentration, and took the lead in coming to the $c_{\text{max}}$, which was distinctly superior compared to the BG-MS, BG-PM, and pure BG samples. The enhanced dissolution velocities of BG from BG-NS can be interpreted by the Noyes–Whitney equation (2):
Table 3 The absorption rate constants ($K_a$, absorption half-life ($t_{1/2}$), and hourly absorption percentage ($P$) of different preparations in intestine perfusion in rats (mean $\pm$ SEM, $n = 5$)

<table>
<thead>
<tr>
<th>Preparation</th>
<th>$K_a$ ($h^{-1}$)</th>
<th>$t_{1/2}$ ($h$)</th>
<th>$P$ ($%$/$h$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG-NS</td>
<td>0.240 ± 0.048 g·h$^{-1}$</td>
<td>2.88 ± 0.45 h$^{-1}$</td>
<td>13.64 ± 1.52 h$^{-1}$</td>
</tr>
<tr>
<td>BG-MS</td>
<td>0.132 ± 0.023 g·h$^{-1}$</td>
<td>5.23 ± 0.89 h$^{-1}$</td>
<td>8.38 ± 0.78 h$^{-1}$</td>
</tr>
<tr>
<td>BG-PM</td>
<td>0.051 ± 0.010 g·h$^{-1}$</td>
<td>13.51 ± 2.32 h$^{-1}$</td>
<td>5.13 ± 0.65 h$^{-1}$</td>
</tr>
<tr>
<td>Pure BG</td>
<td>0.0067 ± 0.0023 g·h$^{-1}$</td>
<td>102.64 ± 15.12 h$^{-1}$</td>
<td>0.73 ± 0.35 h$^{-1}$</td>
</tr>
</tbody>
</table>

$^a P < 0.05$ vs the pure BG group; $^b P < 0.05$ vs the BG-PM group; $^c P < 0.05$ vs the BG-NS group.

Dose-toxicity studies. BG-NS is an investigational preparation, and thus, there are no reports regarding the maximum tolerated dose (MTD) of BG-NS that can be administered to rats. Consequently, dose-toxicity studies were performed to determine the MTD of BG as an ordinary suspension and nanosuspension formulation. The results showed that animals treated with bulk BG and the nanosuspension at 85, 170 and 340 mg·kg$^{-1}$ showed no visible signs of toxicity.

After administration of pure BG at a dose of 680 mg·kg$^{-1}$, one animal was moribund for an initial five min followed by a rapid recovery. In addition, for BG-NS at a dose of 680 mg·kg$^{-1}$, the animals were moribund for a period of ten min, followed by a slow recovery over 1 h. However, no deaths were observed. There was no significant difference in the body weight at any of the doses of the BG ordinary suspension and the nanosuspension formulation for the duration of the study (14 days) compared with the control group. Therefore, the MTD of BG was determined as 340 mg·kg$^{-1}$.

Bioavailability analysis in rats. To confirm the superiority of BG-NS in improving the oral bioavailability, a pharmacokinetics test was carried out in rats, and the pharmacokinetic parameters of BG-NS, BG-MS, and pure BG were compared after oral administration. Fig. 10 shows the typical chromatograms of blank plasma, plasma with BG standard, and plasma sample. It was indicated that under the chromatographic conditions of this experiment, there was no endogenous interference at the retention position of BG. The regression equation was $Y = 28.972X - 2.347$, $r = 0.9997$ ($n = 6$). The method was validated and found to be linear over the concentration range of 0.892–30.12 µg mL$^{-1}$.

![Fig. 10 Chromatograms of BG bioavailability. (A) blank plasma, (B) plasma with BG standard, and (C) plasma sample](image)

The mean plasma BG concentration was plotted as a function of time and is shown in Fig. 11. As for BG-NS, BG-MS, and pure BG, the double peaks of maximum concentrations were obvious, which may be attributed to the enterohepatic circulation, as reported by Xing et al. [38]. The pharmacokinetic parameters after oral administration of BG-NS, BG-MS, and pure BG are summarized in Table 4. After oral administration, due to the improved characteristics of absorption followed by decreasing particle size and corresponding surfactants, BG-NS and BG-MS exhibited higher plasma concentration and AUC$_{0-\infty}$ compared to pure BG. The peak plasma concentration ($c_{\text{max}}$) of BG-NS (16.51 and 14.71 mg·L$^{-1}$, respectively) was approximately two-fold that of BG-MS (7.71 and 9.48 mg·L$^{-1}$, respectively). AUC$_{0-\infty}$ for BG-NS (206.96 mg·L$^{-1}$·h$^{-1}$) was significantly greater ($P < 0.01$) than that of BG-MS (127.95 mg·L$^{-1}$·h$^{-1}$). Since the analysis is in the absorption phase, the decrease in particle size from the micron to the nanometer range in BG-NS could afford better properties of solubility and dissolution [36-37]. Thus improved absorption, consequent on those advantages for BG-NS, led to higher bioavailability in vivo. On the other hand, for the nanoparticles, excellent adhesion to biological surfaces would prolong drug residence time on the surface of the intestinal bacteria, and then contribute to the transformation from baicalin to baicalein for easier absorption [39-40], resulting in an earlier and higher second peak for BG-NS compared to BG-MS, as shown in Fig. 11. Furthermore, following the higher initial absorption phase, BG-NS may also enhance the in vivo bioavailability through enterohepatic circulation [38].

In conclusion, compared with pure BG coarse powder, the two preparations of baicalin-microsuspension, (BG-MS) and baicalin-nanosuspension (BG-NS) produced by ultrasonic and ultrasonic-homogenization process, showed significant improvement in an absorption evaluation in situ in rats at a dose 340 mg·kg$^{-1}$ (mean $\pm$ SEM, $n = 5$).
The drying process is able to improve the absorption and bioavailability of baicalin-phospholipid complex by using HPLC [J]. Biomed Chromatogr, 1999, 13 (7): 493-495.


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