



Application of TPGS as an efflux inhibitor and a plasticizer in baicalein solid dispersion

Meng Tong^a, Xiaoyan Wu^b, Shuya Zhang^a, Di Hua^a, Shukun Li^a, Xiangyu Yu^a, Jing Wang^{a,*}, Zhenhai Zhang^{a,*}

^a Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, 100 Shizi Road, Nanjing, Jiangsu 210028, China

^b Pharmacy, Nanjing Drum Tower Hospital, The affiliated hospital of Nanjing University Medical School, Nanjing 210008, China

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ABSTRACT

The oral bioavailability and efficacy of baicalein is dramatically limited by its low solubility and effect of efflux. In our study, we chose PVP-VA64 as a carrier and TPGS as a plasticizer and efflux inhibitor to prepare a solid dispersion of baicalein using hot-melt extrusion technology to improve its solubility and bioavailability. The hot-melt process and formulation were optimized, and a BAC-PVP VA64-TPGS solid dispersion (BPT-SD) was prepared. BAC exists in an amorphous or molecular state in BPT-SD. BPT-SD comprised irregular lumps and small particles without BAC or carrier characteristics. The dissolution efficiency of BPT-SD improved under sink conditions. FTIR showed a strong hydrogen bond between BAC and PVP-VA64 in BPT-SD. BPT-SD maintained good physical stability for 6 months. The apparent permeability coefficient of BAC in the Caco-2 cell model confirmed that BPT-SD had higher gastrointestinal membrane permeability. A rat pharmacokinetic study showed that BPT-SD had higher C_{max} and AUC_{0-24h} , shorter T_{max} , and 2.88-fold higher bioavailability than BAC. A behavioral experiment in chronic unpredictable mild stress (CUMS) mice confirmed the antidepressant efficacy of BAC. BPT-SD reversed depression-like behavior in CUMS mice and improved BAC bioavailability. BAC preparation into a solid dispersion significantly enhanced dissolution performance and bioavailability.

1. Introduction

Baicalein (BAC, structure shown in Fig. 1) is a natural active phenolic flavonoid compound used in traditional Chinese medicine. It is extracted from the roots of *Scutellaria baicalensis* Georgi (Gao et al., 1999). BAC has antineuritic (Zhang et al., 2017), anticancer (Yu et al., 2018), hepatoprotective (Huang et al., 2019b), antiviral (Zandi et al., 2012), antibacterial (Qian et al., 2015), and antidepressant (Liu and Liu, 2017; Zhang et al., 2018a) properties and other clinically important pharmacological effects. In recent years, BAC has shown great potential for the treatment of depression. According to reports, BAC exerts a neuroprotective effect by regulating the cAMP/PKA, GSK3 β , NF- κ B, and NLRP3 signaling pathways and prevents depression-like behavior induced by chronic unpredictable mild stress (CUMS) (Liu and Liu, 2017; Zhang et al., 2018a; Zhang et al., 2018b). However, BAC has very low

solubility, poor stability, and low oral bioavailability (Huang et al., 2014), which are key factors restricting the pharmaceutical application of BAC.

Solid dispersion technology is an effective method for improving the dissolution rate of poorly soluble drugs. Hot-melt extrusion (HME) technology is a new type of solid dispersion technology that can promote the transformation of the physical state of a raw material drug in a carrier material at the molecular level to form an amorphous system. (Wu et al., 2014) The dissolution rate and apparent solubility are markedly increased, thereby improving oral absorption and bioavailability. For high-melting-point drugs, adding plasticizers is the most effective method to increase the solubility of the raw material. Plasticizers can reduce the viscosity of the extrudate, increase flexibility, lower the operating temperature and T_g of the polymer, and avoid thermal degradation of the drug and carrier (Huang et al., 2019a).

Abbreviations: BAC, baicalein; CUMS, chronic unpredictable mild stress; DSC, differential scanning calorimetry; DMEM, dulbecco's modified eagle medium; DMSO, dimethyl sulfoxide; FTIR, fourier transform infrared spectroscopy; FST, forced swimming test; HBSS, hanks' balanced salt solution; HME, hot-melt extrusion; OFT, open field test; XRD, X-ray diffraction; SDS, sodium dodecyl sulfate; SEM, scanning electron microscopy; SPT, sucrose preference test; TST, tail suspension test; TEER, trans-epithelium electrical resistant; T_g, glass transition temperature; TPGS, D- α -tocopherol polyethylene glycol succinate.

* Corresponding authors.

E-mail addresses: wangjing3968@126.com (J. Wang), david23932@163.com (Z. Zhang).

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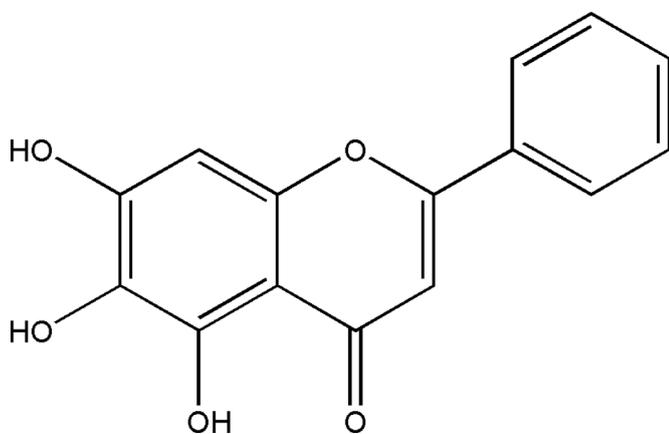


Fig. 1. Structural formula of BAC.

Commonly used plasticizers include surfactants, glycerin, and PEG. Zhang et al. (2014) prepared a solid dispersion of BAC using HME technology. After oral administration in rats, the relative bioavailability of the two solid dispersion preparations was approximately 2.4 and 2.9 times higher than that of the pure drug, respectively. However, the formulation did not improve the effect of efflux, which may have affected its bioavailability. TPGS is synthesized by the esterification of vitamin E succinate and polyethylene glycol (PEG) 1000. TPGS is a natural water-soluble derivative of vitamin E and has an amphiphilic structure containing a hydrophilic polar head and a lipophilic alkyl tail. In addition, TPGS can inhibit the efflux of P-glycoprotein and improve the bioavailability of P-glycoprotein blocking drugs (Collnot et al., 2010, 2007). TPGS can be an excellent solubilizer for hydrophobic drugs (Childs et al., 2013), emulsifiers (Ke et al., 2005), efflux inhibitors (Zhang et al., 2014b), plasticizers (Repka and McGinity, 2000), and solid dispersion carriers (Beig et al., 2017).

Based on the above discussion, a novel solid dispersion system was prepared to enhance oral bioavailability of BAC using a hot melt extrusion technique with PVP VA64 and TPGS. TPGS was added as an efflux inhibitor and a plasticizer. *In vitro* and *in vivo* experiments were carried out to evaluate this solid dispersion system with crystalline BAC as control.

2. Materials and methods

2.1. Materials

BAC (purity > 95%) was purchased from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). PVP VA64 and TPGS were kindly supplied by Chineway Pharmaceutical Tech. Co. Ltd. (Shanghai, China). Fluoxetine hydrochloride (FLU) (positive control drug) was obtained from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, China). All other chemicals were of analytical grade.

2.2. Animals

Male Sprague–Dawley (SD) rats (220 ± 20 g) used in the pharmacokinetic study were purchased from the SLAC Lab Animal Center of Shanghai (Shanghai, China) and allowed to acclimate to the environment for 7 days prior to the experiments.

Male BALB/c mice (20 ± 2 g), which were used for CUMS, were purchased from the Yangzhou University Comparative Medical Center (Yang Zhou, China) and allowed to acclimate to the environment for 2 weeks prior to CUMS induction.

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Jiangsu Provincial Academy of Chinese Medicine. License number: SCXK (Su) 2018–0018

2.3. Preparation of BPT-SD

Based on our preliminary experiments, we selected Kollidon® VA64 to prepare BAC solid dispersion. HME was conducted using a Process 11 twin-screw hot-melt extruder (Thermo Electron Corporation, MA, USA). Importantly, the operations were conducted in the dark because BAC is photolabile when exposed to light. We designed an orthogonal experiment to optimize the hot-melt preparation process parameters and formulas.

2.4. *In vitro* dissolution study

2.4.1. Equilibrium solubility test

We added excess BAC to the pH 1.2 hydrochloric acid solution and pH 6.8 phosphate buffer solution containing different proportions of SDS, equilibrated in a shaker at 37 °C for 48 h. The equilibrium solution was transferred to a centrifuge tube and centrifuged at 13,000 × g for 10 min. The supernatant was removed and measured according to the chromatographic conditions. All samples were tested in triplicate.

2.4.2. Dissolution test

The dissolution of pure BAC powder, BAC-PVP VA64-TPGS physical mixture (BPT-PM), and BAC-PVP VA64-TPGS solid dispersion (BPT-SD) were examined using a ZRS-8GD dissolution apparatus (Tianfa Technology, China) at a paddle rotation speed of 50 × g.

Samples equivalent to 15 mg of BAC were accurately weighed and added to 900 mL dissolution solution at 37 °C. Two milliliters of the sample were withdrawn at different time points (5, 10, 15, 30, 45, 60, 75, 90, and 120 min) and filtered through 0.45 μm polyester filters. Another 2 mL of fresh dissolution fluid was added to maintain a constant dissolution volume. The filtrate was subjected to HPLC quantification using an Agilent HPLC system (LC 1100, Agilent, La Jolla, CA, USA). Each test was performed in triplicate.

Chromatographic separation was conducted on the Agilent HPLC system (Agilent Corporation) equipped with an ultraviolet absorbance detector set at 276 nm. The separation was performed at 30 °C on an X-Bridge C₁₈ column (5, 250 × 4.6 mm, Waters Corporation, USA). The mobile phase consisted of methanol: 0.1% phosphoric acid (65/35, v/v). The mobile phase was freshly prepared prior to each series of analyses. The retention time was 6.8 min, and the total run of each sample was 10 min.

2.5. Physical characterization of BPT-SD

2.5.1. DSC

DSC was performed to characterize the state of the drug in the solid dispersion using a 200F3 instrument (NETZSCH, Germany). Approximately 8 mg of each sample was placed in an aluminum pan. The measurements were conducted at a heating rate of 10 °C/min from 30 to 300 °C (Zhang et al., 2014a).

2.5.2. XRD

X-ray diffraction (XRD) was conducted using a Bruker-D8 X-ray diffractometer (Bruker Corporation, Germany). The samples were exposed to Cu Kα radiation (40 kV, 40 mA) over a 2θ range from 5° to 90°, with a step size of 0.04° and a step time of 0.5 s.

2.5.3. SEM

SEM measurements were conducted with a ZEISS-SUPRA40 electron microscope (ZEISS Co., Germany) at 15 kV and 15 μA. The samples were coated with gold prior to the measurements.

2.5.4. FTIR

Fourier transform infrared spectroscopy (FTIR) was performed using a Bruker IFS-55 interferometer (Bruker, Germany). The samples were ground and mixed thoroughly with potassium bromide (KBr). Then, they

were compressed into tablets for scanning at a resolution of 4 cm^{-1} and a range of 400 to 4000 cm^{-1} (Wang et al., 2017).

2.6. Solubility test

We added excess BAC, BPT-PM, and BPT-SD to the hydrochloric acid buffer solution containing 0.5% SDS at pH 1.2, equilibrated in a shaker at $37\text{ }^\circ\text{C}$ for 48 h. The equilibrium solution was transferred to a centrifuge tube and centrifuged at $13,000 \times g$ for 10 min. The supernatant was removed and measured according to the aforementioned chromatographic conditions (Zhang et al., 2014a). All samples were tested in triplicate.

2.7. Stability study

When exposed to environments of high humidity or high temperature, the physicochemical properties of BPT-SD may deteriorate. We evaluated the long-term stability of BPT-SD. The BPT-SD powder packaged in an airtight container was stored at $40 \pm 2\text{ }^\circ\text{C}$ and $75 \pm 5\%$ relative humidity. After 6 months, the dissolution and XRD were examined and compared with the original state (Nepal et al., 2010). All samples were tested in triplicate.

2.8. Caco-2 cell transport experiment

2.8.1. Caco-2 cell culture

The human colon carcinoma cell line Caco-2 was used to study BAC transport. Briefly, Caco-2 cells were stored in an incubator at $37\text{ }^\circ\text{C}$, with 95% relative humidity, and 5% CO_2 . Caco-2 cells were seeded at a density of approximately 1×10^4 cells/ cm^2 in 24-well Transwell® plates to reach confluence and differentiation. At 21 days post-seeding, the cells developed well with TEER values greater than $600\ \Omega \cdot \text{cm}^2$ (Xiaoyan et al., 2017).

2.8.2. Cytotoxicity of BPT-SD

In the CCK-8 assay, the dye of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] is reduced by cellular dehydrogenase to form a water-soluble orange-colored product (formazan) (Cai et al., 2019). The CCK-8 assay was performed as described previously by Li et al. (2020) with some modifications. Caco-2 cells were incubated in high-glucose and L-glutamine DMEM supplemented with 1% (v/v) penicillin-streptomycin and 10% (v/v) fetal bovine serum. The cells were seeded at a density of 2.5×10^4 cells/well in 96-well plates and incubated at $37\text{ }^\circ\text{C}$ and 5% CO_2 for 72 h. The culture medium was removed, and BAC was dissolved in DMSO. This solution was then diluted to different concentrations (1.01–40.4 $\mu\text{g}/\text{mL}$) by adding DMEM to the wells where the DMSO dosage was less than 0.5%. After 24 h of incubation, the cells were washed three times with 200 μL PBS/well (Shen et al., 2018). Then, 200 μL CCK-8 containing medium (5 mg/mL CCK-8 in DMEM) was added to dissolve the formazan crystals, and absorbance was measured at 570 nm using a microplate reader (Synergy HT, BioTek, USA). Relative cell viability (%) was calculated by comparing the absorbance of the treated cells with that of control cells (Slütter et al., 2009).

2.8.3. Transport experiment

The transport assay was performed in the apical-basolateral direction. The test solutions of BAC, BAC+PVP VA64, and BAC+PVP VA64+TPGS1000 at doses of 1, 5, and 20 $\mu\text{g}/\text{mL}$ of BAC were prepared by adding the appropriate amount of each formulation to DMSO and diluted with the Hanks' balanced salt solution (HBSS, pH 7.4) to corresponding concentration. Caco-2 cell monolayers were washed twice with prewarmed HBSS before permeability studies. The experiments were initiated by adding 0.5 mL of the test solution to the apical chamber (AP) and 1.5 mL of HBSS to the basolateral chamber. The plates

were agitated on an orbital shaker at $50 \times g$ throughout the incubation period. Samples (150 μL) were then taken from the basolateral chamber and immediately replaced with the same volume of HBSS at $37\text{ }^\circ\text{C}$ and predetermined time intervals of 30, 60, 90, and 120 min. The samples were stored at $-20\text{ }^\circ\text{C}$ until analysis.

The concentration of BAC in the transport buffer samples was determined using HPLC analysis. The apparent permeability coefficient (Papp) across the Caco-2 cell monolayer was calculated for permeability studies using the following equation:

$$P_{\text{app}} = \left(\frac{dQ}{dt} \right) \left(\frac{1}{AC_0} \right)$$

where dQ/dt ($\mu\text{g}/\text{min}$) is the transport rate, A is the surface area of the monolayer, and C_0 is the initial concentration of BAC ($\mu\text{g}/\text{mL}$).

2.9. Pharmacokinetic study

2.9.1. Animal experiment

For the pharmacokinetic study, SD rats (180–220 g) were randomly divided into three groups ($n = 8$). Briefly, BAC, BPT-PM, and BPT-SD (dispersed in 0.5% aqueous sodium carboxymethyl cellulose) were orally administered at a dose of 75 mg/kg (Liu et al., 2006). Blood samples were collected in heparinized tubes from the orbital vein at predetermined times (0, 5, 15, 30, 60, 120, 180, 240, 300, 360, 480, 600, 720, and 1440 min) after oral administration. Blood samples were immediately centrifuged at $13,000 \times g$ for 10 min to obtain plasma.

2.9.2. Plasma sample preparation

Plasma (150 μL) and internal standard solution (Daidzin, 50 μL) were spiked with 400 μL of methanol, followed by vigorous vortexing for 5 min to precipitate the plasma proteins. The samples were centrifuged at $16,000 \times g$ for 10 min at $4\text{ }^\circ\text{C}$. After centrifugation, 20 mL of the supernatant was injected into the HPLC system to determine BAC concentration. (Lai et al., 2010)

After oral administration of baicalein, a prototype drug baicalein, in rats, was almost completely metabolized after 2 h, and the detectable drug concentration was extremely low. Baicalin is the main form of BAC after being absorbed and metabolized in the body; therefore, baicalin was chosen as the test index (Pan et al., 2013).

2.9.3. HPLC analysis of plasma samples

For the stationary phase, an Agilent C18 column ($250 \times 4.6\text{ mm}$, $5\ \mu\text{m}$) was maintained at $25\text{ }^\circ\text{C}$. The mobile phase comprised a mixture of 0.1% H_3PO_4 and acetonitrile, which was run in a gradient elution program. The flow rate was 1.0 mL/min.

2.10. Effect of BPT-SD in CUMS mouse model

2.10.1. CUMS and experimental design

A total of 84 BALB/c mice were randomly assigned to seven groups of 12 mice each, using a randomization method. The groups were Control, CUMS, CUMS-FLU (10 mg/kg), CUMS-BAC (20 and 40 mg/kg), and CUMS-SD (20 and 40 mg/kg) (Zhang et al., 2018b). The CUMS procedure was conducted as previously described (Willner et al., 1987) with slight modifications. The specific details of the CUMS procedure are as follows: (1) cage shaking (1 time/s, 10 min); (2) cage tilt (45° , 8 h); (3) food and water deprivation (24 h); (4) soiled cage (200 mL water in 100 g sawdust bedding); (5) overnight illumination (8 h); (6) restrained in a bottle; (7) tail pinched (1 cm from the beginning of the tail); (8) light/dark perversion; (9) stroboscopic illumination (120 flashes/min, 1 h); and (10) cold water swimming ($4\text{ }^\circ\text{C}$, 5 min). Each animal was individually exposed to one stressor per day. The control animals ($n = 12$) were housed in a separate room and had no contact with the stressed animals.

After the fourth week of the CUMS paradigm, when depressive-like

Table 1
Equilibrium solubility of BAC in different dissolution media.

Dissolution medium	Equilibrium solubility($\mu\text{g/ml}$)
pH 1.2 buffer	6.49 \pm 1.60
pH 1.2 + 0.1%SDS	13.70 \pm 0.71
pH 1.2 + 0.3%SDS	36.83 \pm 0.33
pH 1.2 + 0.5%SDS	59.58 \pm 1.81
pH 6.8 buffer	6.40 \pm 0.84
pH 6.8 + 0.1%SDS	9.64 \pm 2.20
pH 6.8 + 0.3%SDS	29.57 \pm 1.61
pH 6.8 + 0.5%SDS	59.72 \pm 0.39

behaviors occurred, drugs were administered once per day during the last 15 days. Behavioral tests were performed 1 h after the final drug administration.

2.10.2. Behavioral tests

Sucrose preference test (SPT) was performed using the traditional method, with minor modifications. Briefly, before initiating the CUMS procedure, the mice were individually housed. Two bottles of 1 m/v% sucrose solution were placed in each cage for 24 h to allow the mice to adapt to the solution. Then, one bottle of sucrose solution was replaced with water (24 h). The mice were deprived of food and water for 8 h before the test and only provided two bottles with 1% sucrose solution and water for 2 h.

Sucrose preference (%) = [sucrose consumption (g)/water consumption (g) + sucrose consumption (g)] \times 100%

The goal of the open field test (OFT) was to observe the autonomous behavior, exploratory behavior, and tension of experimental animals in a new environment and evaluate the level of anxiety in the experimental animals. The open field apparatus was 39 \times 39 \times 40 cm and divided equally into nine sections (13 \times 13 cm). The mice were individually placed in the center of the cage in a quiet environment. After 2 min of adaptation, the mice were allowed to explore freely during a 4-min testing period, and the total distance was recorded (LiuLiu, 2017).

The forced swimming test (FST) was performed to evaluate depressive-like behavior according to the time of immobility. The mice were placed in a cylinder (25 cm high, 10 cm diameter) filled with 19 cm

of water at 25 \pm 1 $^{\circ}\text{C}$. On the first day, the rats or mice were forced to swim in deep water at 25 $^{\circ}\text{C}$ for 15 min and then returned to the cage. After being forced to swim for 6 min the next day, the immobility time was recorded during the last 4 min of the test. There was no chronological effect in the swimming experiment; that is, the results of the experiment at different times of the day were consistent (Yu et al., 2018).

The tail suspension test (TST) reflected the desperate behavior of mice and was used to detect variability in animal behavior. The mouse was fixed on a tail suspension instrument approximately 35 cm from the ground and held head downward. The duration of the test was 6 min, and the immobility time, defined as the time when the mouse was completely immobile, was recorded during the last 4 min of the test (Li et al., 2015; Zhang et al., 2018a).

2.11. Statistical analysis

Data are presented as mean \pm standard error of the mean for all data. The data processing software DAS 3.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China) was used to calculate the pharmacokinetic parameters. Statistical software (SPSS 22.0, SPSS Inc., Chicago, IL, USA) was used for analyses. Statistical analyses were performed using one-way ANOVA. If the distribution was normal, Student's *t*-test was also used. If the distribution was not normal, the Wilcoxon rank-sum test was used. *p* < 0.05 indicated statistically significant differences.

3. Results and discussion

3.1. Preparation of BPT-SD

In this study, we prepared a solid dispersion of BAC using HME technology, and the formulation and process were optimized using an orthogonal design. DSC, XRD, SEM and FTIR analysis and pharmaceutical research including solubility, dissolution, permeability, bioavailability and pharmacodynamics were studied to characterize the particles obtained by HME process (Kim et al., 2008). In the pre-experiment, we

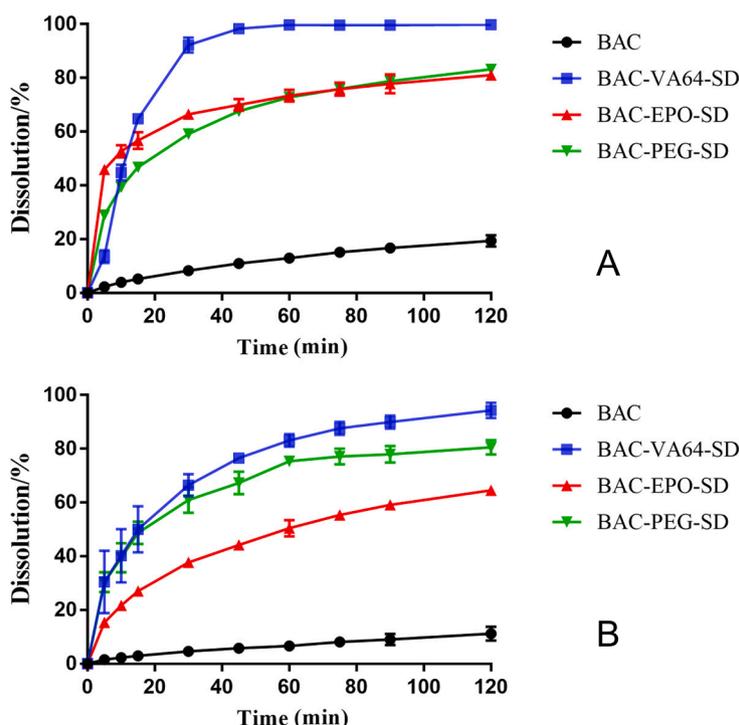


Fig. 2. Dissolution curves of pure BAC, BAC-PVP VA64 PMs, and BAC-PVP VA64 SDs at different pH values (A pH 1.2; B pH 6.8) under sink conditions (*n* = 3).

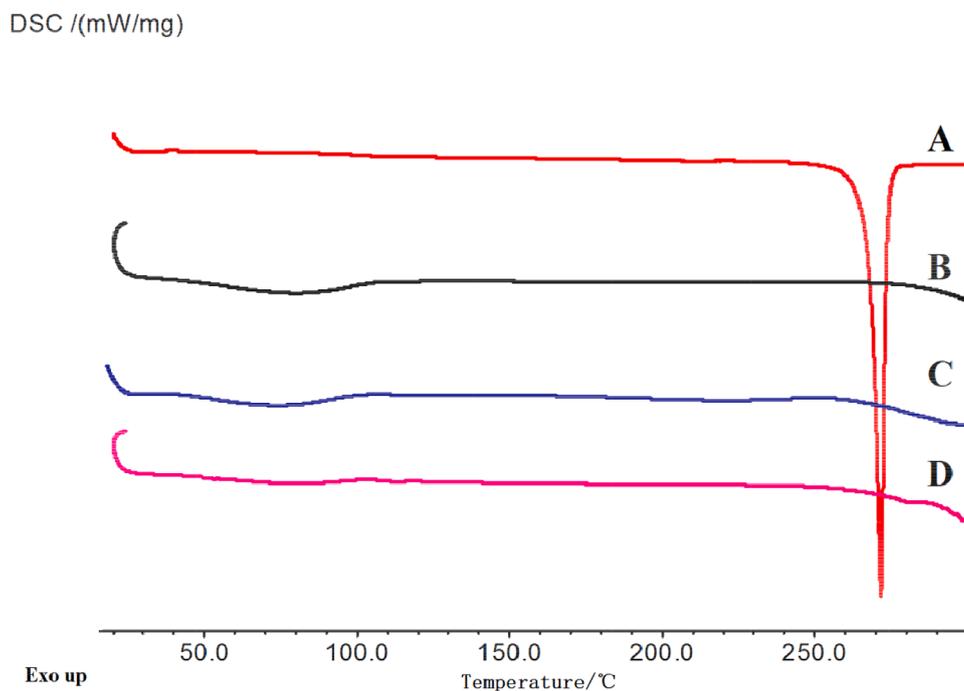


Fig. 3. DSC thermograms of BAC (A), PVP VA64 (B), BPT-PM (C), and BPT-SD (D).

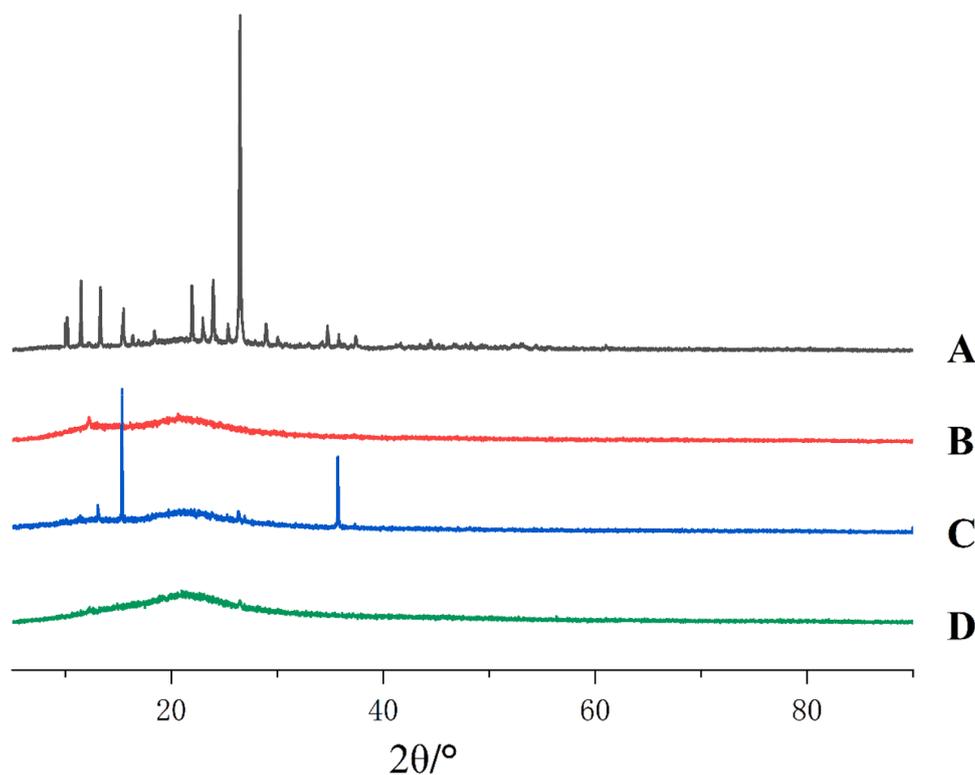


Fig. 4. XRD patterns of BAC (A), PVP VA64 (B), BPT-PM (C), and BPT-SD (D).

selected different carriers according to the literature (Zhang et al., 2004a; Li et al., 2015). Finally, PVP VA64 was selected as the preferred carrier because of its good solubility and stability. Because of the high melting point of BAC (approximately 265 °C), we considered adding a plasticizer to improve the compatibility of BAC and the carrier PVP VA64 (Huang et al., 2019a). The Hansen solubility parameters reflect the degree of mixing between the drug and its corresponding carrier. The solubility parameter value of BAC was $17.2 \text{ Mpa}^{1/2}$, and the

difference $\Delta\delta$ between PVP-VA64 was less than $7 \text{ Mpa}^{1/2}$, indicating that it may be compatible with BAC (Fule et al., 2016).

Using the cumulative dissolution rate at 15 min as an indicator and comparing the solid dispersions under different processes, the final optimal process was as follows: speed was $50 \times \text{g}$, temperature in zones 2–8 was 140–170 °C, and the ratio of BAC to carrier was 1:6, which contained 10% TPGS. This is a novel solid dispersion system prepared using the HME technique with a hydrophilic polymer and plasticizer,

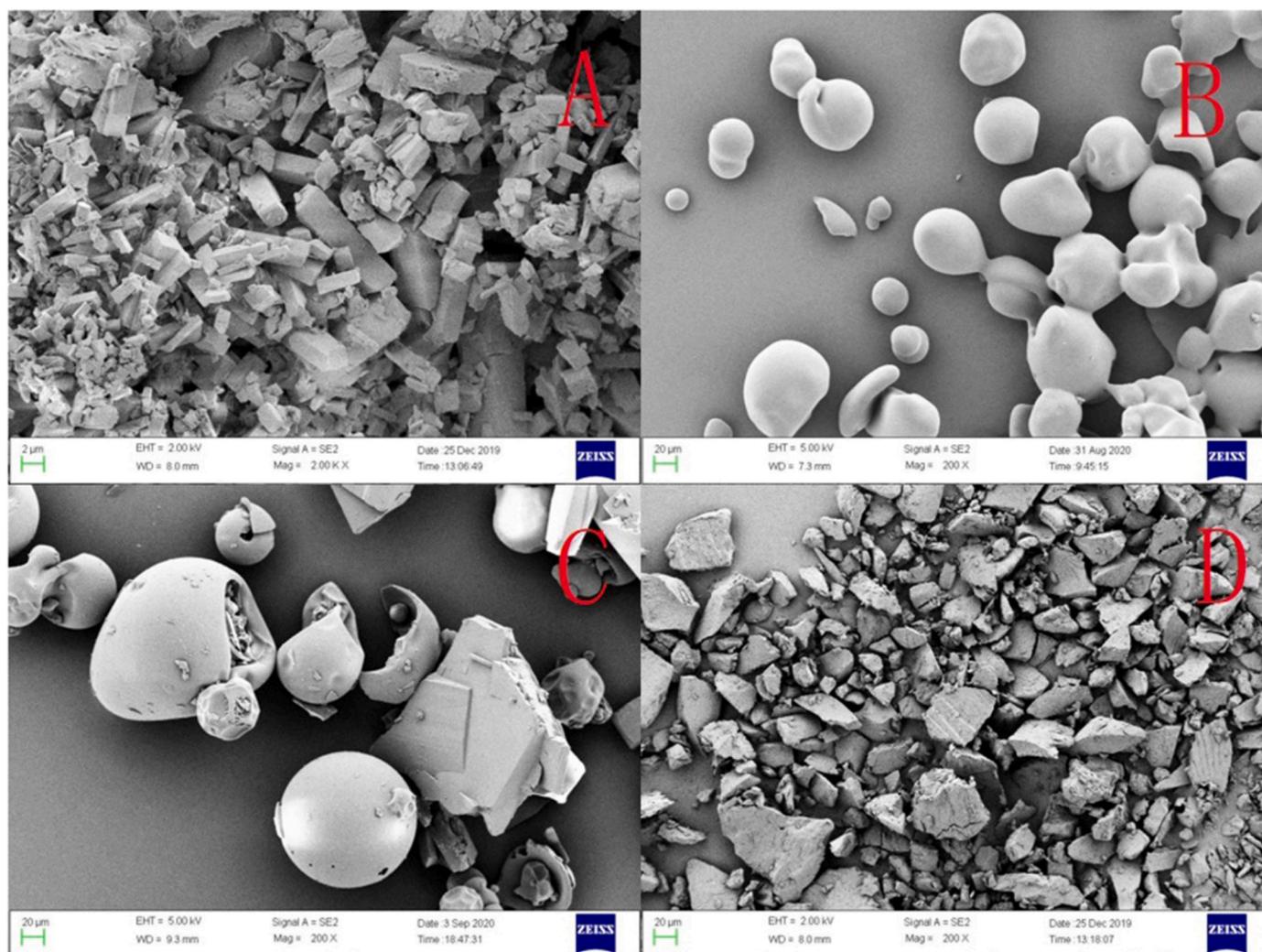


Fig. 5. SEM images of BAC (A), PVP VA64 (B), BPT-PM (C), and BPT-SD (D).

without an organic solvent. (Adler et al., 2016)

3.2. In vitro dissolution study

3.2.1. Equilibrium solubility test

We carried out an equilibrium solubility test during the prescription screening phase. The results are shown in Table 1. BAC is a chemical component with low solubility. We found that the equilibrium solubility of BAC in pH 1.2 and pH 6.8 dissolution media was only 6.49 ± 1.60 and 6.40 ± 0.84 $\mu\text{g/ml}$ respectively which was difficult to reach the sink condition. Therefore, SDS was selected to improve the solubility of BAC. After screening, 0.5% SDS was added into the dissolution medium which not only met the sink condition, but also reached the limit of detection. Referring to Zhang et al.'s research (Zhang et al., 2014a), we finally chose 0.5% SDS.

3.2.2. Dissolution test

As shown in Fig. 2, in the hydrochloride buffer containing 0.5% SDS (Fig. 2,A), the bulk drug and physical mixture showed less than 10% dissolution in 15 min, and 11.2% and 12.53% dissolution, respectively, in 120 min. The dissolution of BPT-SD in 15 min was 75.89%; after 30 min, it exceeded 80% and reached 96.26% in 120 min. In the phosphate buffer containing 0.5% SDS (Fig. 2,B), the dissolution of BAC and BPT-PM in 5 min was less than 5%, and the dissolution in 120 min was less than 20%. The BPT-SD dissolved in 5 min. The dissolution rate exceeded 90% within 120 min. It can be seen that under this process, the

dissolution efficiency of BAC was greatly improved.

3.3. DSC

DSC is mainly used to detect whether the crystal form of BAC changes during the preparation of solid dispersions (Zhang et al., 2014a). As shown in Fig. 3, BAC (Fig. 3,A) has a sharp endothermic peak at 271.6 °C, which is the characteristic peak of the melting point of BAC. PVP VA64 (Fig. 3,B) showed no significant endothermic peaks. BPT-PM (Fig. 3,C) also showed no obvious endothermic peak, which may be due to the drug being dissolved in the carrier at a high temperature. There was no significant endothermic peak in BPT-SD (Fig. 3,D), and the drug was present in the carrier in an amorphous or molecular state (Wang et al., 2019)

3.4. XRD

XRD was used to determine the crystallinity of different samples. As shown in Fig. 4, BAC (Fig. 4,A) has multiple crystal diffraction peaks at 10.14° , 11.34° , 13.22° , 15.32° , 26.30° , and 34.02° (Wen et al., 2018). PVP VA64 (Fig. 4,B) did not show obvious crystal diffraction peaks, indicating that PVP VA64 was in an amorphous or molecular state. BPT-PM (Fig. 4,C) showed the diffraction peak of BA, but it was weakened by the mixing of some polymers, which indicated that the physical mixture was simple. However, the XRD patterns of the solid dispersions (Fig. 4,D) did not show the crystal diffraction peak of BAC, indicating

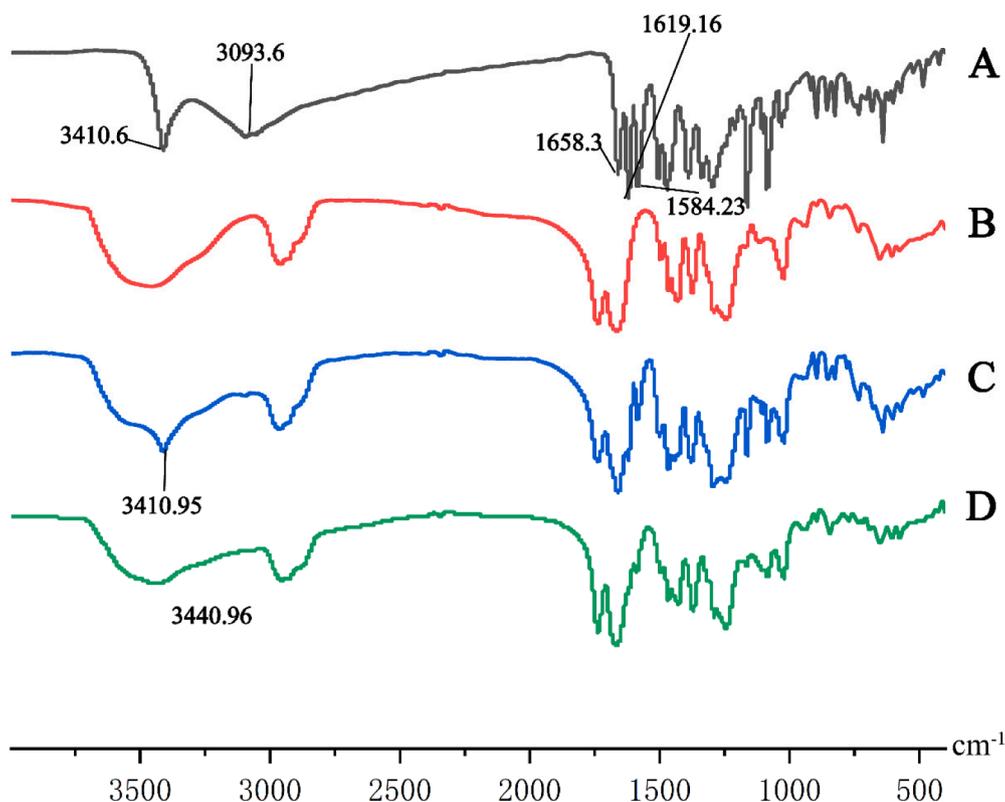


Fig. 6. FTIR results of BAC (A), PVP VA64 (B), BPT-PM (C), and BPT-SD (D).

Table 2

Solubility of BAC and solid dispersions ($n = 3$).

Sample	Solubility/ $\mu\text{g mL}^{-1}$
BAC	40.28 ± 2.08
BPT-PM	43.31 ± 1.28
BPT-SD	$316.07 \pm 20.50^{**}$

Compared with BAC, $**p < 0.01$.

that the crystalline nature of BAC disappeared. Based on the DSC results, it is speculated that BAC was dispersed in the solid dispersion in an amorphous or molecular state. (He et al., 2011)

3.5. SEM

SEM was used to observe the surface and crystal structures of BAC, BPT-PM, and BPT-SD. The results are presented in Fig. 5. BAC (Fig. 5,A) mostly had columnar crystals accompanied by large lumps with obvious crystal characteristics. PVP VA64 (Fig. 5,B) had a smooth spherical or ellipsoidal shape. The characteristics of BAC and PVP VA64 were clearly visible in BPT-PM (Fig. 5,C). BPT-SD (Fig. 5,D) had no raw material characteristics, consisting primarily of irregularly shaped blocks with granular, loose, and porous surfaces (Fan et al., 2020).

3.6. IR

FTIR was used to study the possible interactions between BAC and

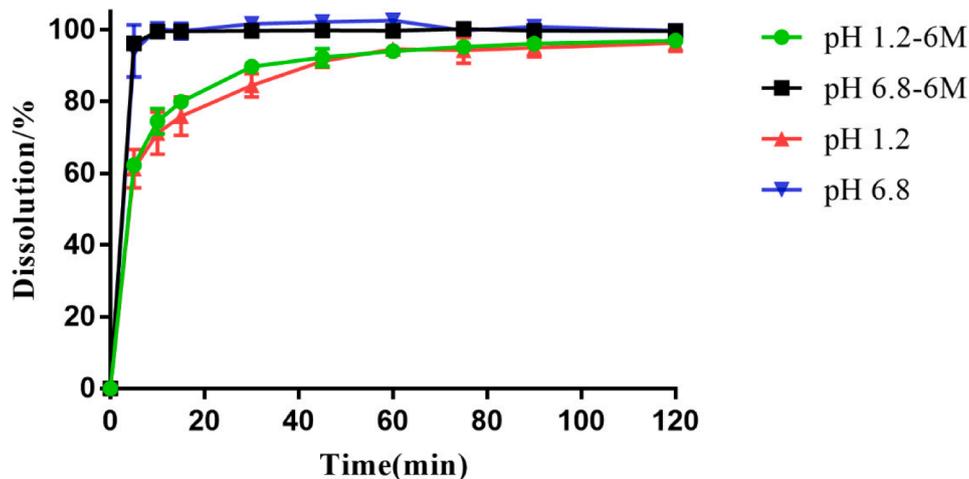


Fig. 7. Dissolution curves of BAC at different pH values under the accelerated condition.

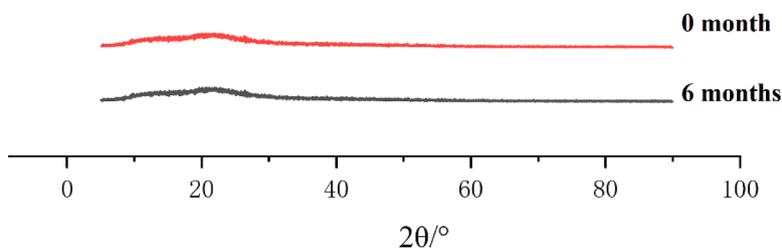


Fig. 8. XRD of BAC under accelerated conditions.

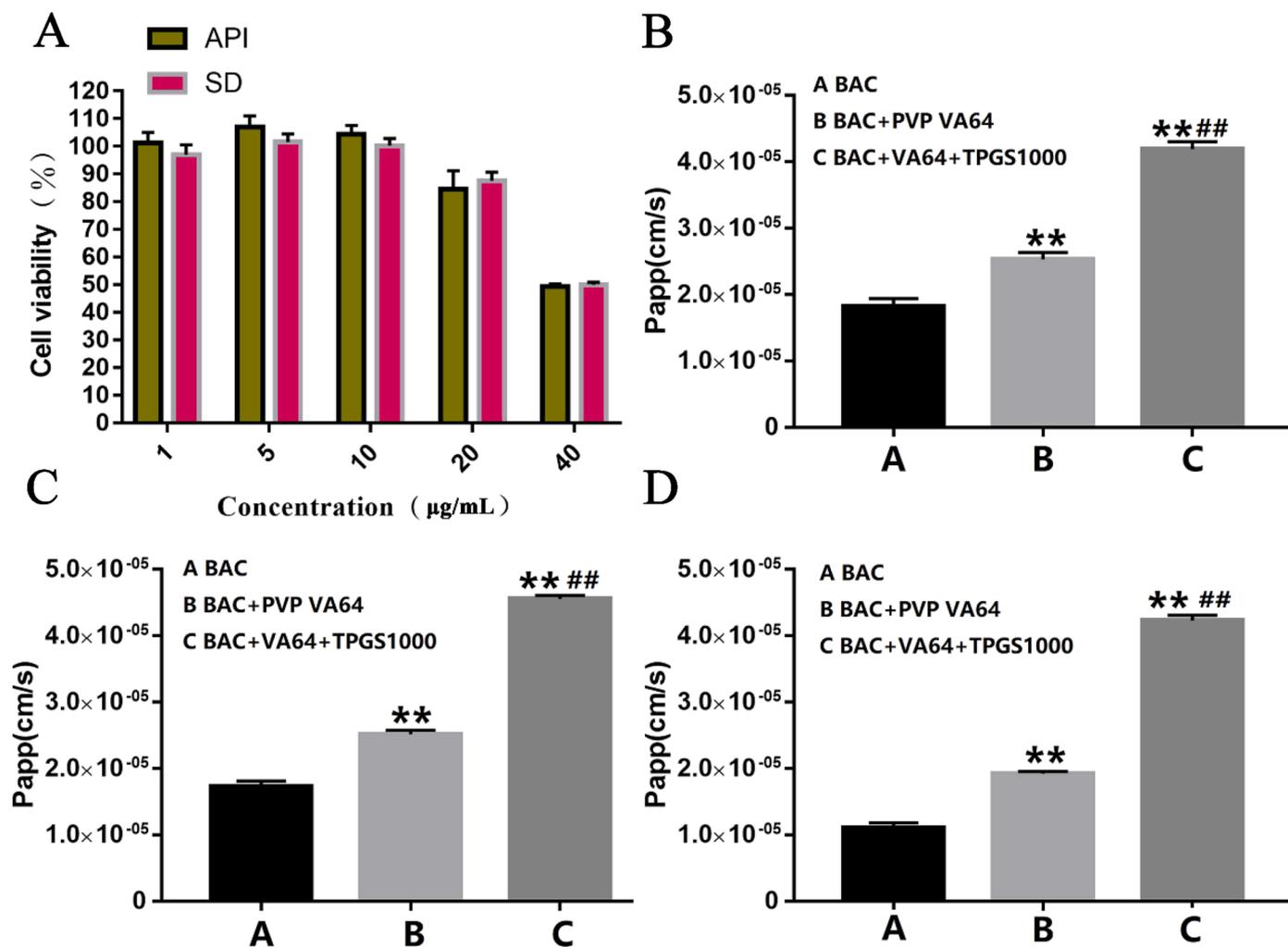


Fig. 9. Results of cell cytotoxicity (A) and the effect of BPT-SD (B. 5 µg/mL; C. 10 µg/mL; D. 20 µg/mL) on the apparent permeability coefficient of BAC across Caco-2 monolayer ($n = 3$). * $p < 0.05$, ** $p < 0.01$, compared to BAC, ## $p < 0.01$, compared to BAC-PVP VA64.

the polymer. The results are shown in Fig. 6. In the FTIR spectrum of BAC crystals, the band at 3410.6 cm^{-1} corresponded to the OC-H stretching vibrations of the hydroxyl group, indicating the intramolecular hydrogen bonds of BAC. The absorption peak at 3093.6 cm^{-1} for the binding peaks was derived from the intermolecular hydroxyl group, and the peaks at 1658.3 , 1619.16 , and 1584.23 cm^{-1} (Zhang et al., 2014) were caused by the absorption of the benzene ring.

In BPT-PM, the characteristic peaks at 3410.95 cm^{-1} of BAC were clearly observed, indicating that simple physical mixing resulted in no interactions between BAC and the polymer. However, in BPT-SD the broadened peak at 3410.95 cm^{-1} of BAC was weakened and shifted to the left at 3440.96 cm^{-1} , which indicated intermolecular hydrogen bonds between the drug and carrier after HME (Huang et al., 2014; Wen

et al., 2018)

3.7. Solubility test

The solubilities of BAC, physical mixture, and solid dispersion are shown in Table 2. The equilibrium solubility of BAC was $40.28 \mu\text{g}\cdot\text{mL}^{-1}$ and BPT-PM was $43.31 \mu\text{g}\cdot\text{mL}^{-1}$. However, the solid dispersion with PVP-VA64 as a carrier greatly improved its solubility, which was 7.85 times higher ($p < 0.01$) because of the presence of a hydrophilic carrier. After the preparation of BPT-SD, its molecular order was disrupted; it had a larger surface energy, and the contact area between BAC and the medium increased, resulting in greater solubility.

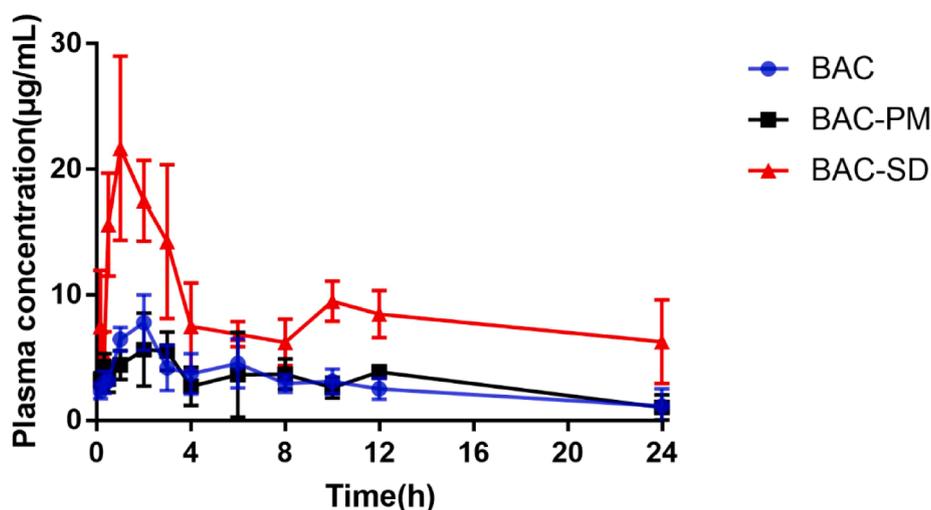


Fig. 10. Plasma concentration-time profiles of pure BAC and BPT-SD at a 1:6 ratio and BPT-PM at a 1:6 ratio in rats following oral administration ($n = 6$).

Table 3

Pharmacokinetic parameters of drugs in mice after oral administration of BAC, BPT-PM, and BPT-SD ($n = 6$).

Parameter	BAC	BPT-PM	BPT-SD
C_{max} ($\mu\text{g/mL}$)	8.13 ± 1.15	6.97 ± 1.90	$23.09 \pm 5.79^{**}$
T_{max} (h)	1.50 ± 0.58	1.87 ± 0.96	1.10 ± 0.55
$AUC_{0-24\text{ h}}$ ($\mu\text{g/mL}\cdot\text{h}$)	71.97 ± 26.92	98.67 ± 33.57	$207.4 \pm 27.36^{**}$

Compared with BAC, * $p < 0.05$, ** $p < 0.01$.

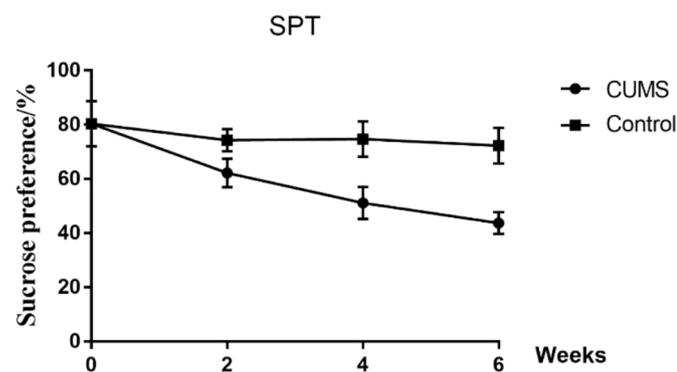


Fig. 11. Sucrose preference of the Control group and CUMS group over 6 weeks ($n = 8$).

3.8. Preliminary stability study

3.8.1. In vitro dissolution study

The stability results are presented in Fig. 7. After 6 months, the dissolution of BPT-SD was consistent with the original curve, indicating that BPT-SD could maintain stability for at least 6 months. It is possible that in the solid content, BAC interacts with PVP VA64 and TPGS could play a plasticizing effect, further increasing its stability and theoretically reducing crystals and amorphous or molecular solids (Huang et al., 2019b).

3.8.2. XRD

Physical stability is an important issue in thermodynamically unstable systems. The free energy of amorphous or molecular crystals is higher than that of stable crystals. Therefore, XRD was used to study the physical stability of amorphous or molecular drugs in SD rats. As shown in Fig. 8, after 6 months of accelerated stability experiments, the XRD of

BPT-SD did not show a new diffraction absorption peak, indicating that BAC and PVP VA64 still existed in an amorphous or molecular state and exhibited good physical stability.

3.9. Caco-2 cell transmembrane transport results

3.9.1. Cytotoxicity assessment

The CCK-8 method was used to determine the cytotoxicity of BAC and BPT-SD in Caco-2 cells at different concentrations. As shown in Fig. 9,A, after 24 h of measurement, the survival rate of Caco-2 cells in the test group treated with 1,5,10, and 20 $\mu\text{g/mL}$ was greater than 80%, indicating that BAC and BPT-SD were not toxic to Caco-2 cells in this concentration range. Based on the results of the CCK-8 experiments, BAC concentrations of 5,10, and 20 $\mu\text{g/mL}$ were selected to conduct drug transport experiments across the membrane.

3.9.2. Transmembrane transport results

According to previous reports, BAC can upregulate the expression of P-glycoprotein in Caco-2 cells and affect its transport activity. TPGS inhibits the efflux of P-glycoprotein and improves the bioavailability of P-glycoprotein blocking drugs (Collnot et al., 2010); therefore, we considered adding TPGS to improve the transmembrane transport capacity of BAC.

The results of the BAC and BPT-SD transfers are shown in Fig. 9. The transport results (Fig. 9,B) showed that the Papp values of 20 $\mu\text{g/mL}$ BAC, BAC-PVP VA64, and BAC-PVP VA64-TPGS were 1.126×10^{-5} , 1.966×10^{-5} , and 4.231×10^{-5} , respectively. The Papp values of BAC-PVP VA64 and BAC-PVP VA64-TPGS were significantly increased ($p < 0.01$) by 1.75- and 3.76-fold, respectively, compared with that of BAC. In addition, the Papp values of 10 $\mu\text{g/mL}$ of BAC-PVP VA64 and BAC-PVP VA64-TPGS were increased by 1.46- and 2.64-fold ($p < 0.01$) (Fig. 9,C), and the Papp values of 5 $\mu\text{g/mL}$ B and C were increased by 1.4- and 2.31-fold ($p < 0.01$) (Fig. 9,D). This showed that the preparation process of the solid dispersion could effectively improve the cell membrane permeability of BAC. Compared with B, the Papp value of C was significantly increased ($p < 0.01$), indicating that TPGS inhibited the transport of P-glycoprotein and increased the concentration of the drug. Moreover, the resistance value of the Caco-2 cell membrane did not change significantly before and after the experiment, indicating that the integrity of the cell membrane did not change during the experiment.

3.10. Pharmacokinetic study

Based on the drug-time curve (Fig. 10) and the pharmacokinetic

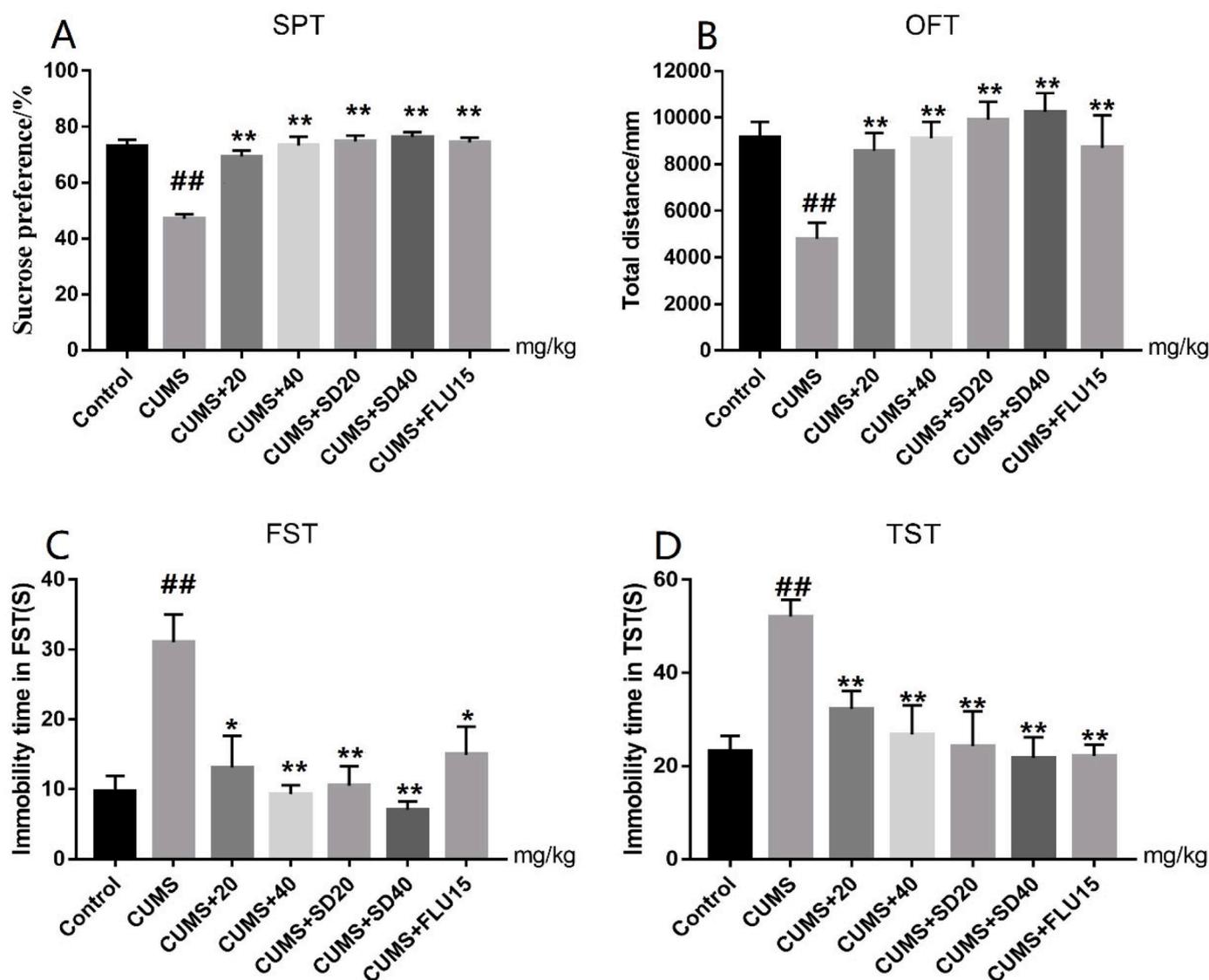


Fig. 12. Effects of BAC on sucrose preference index in SPT (A), total distance in OFT (B), and immobility time in FST (C) and TST (D) in CUMS mouse model. Results are shown as the mean \pm SD ($n = 12$). ## $p < 0.01$ versus the Control group; * $p < 0.05$, ** $p < 0.01$ versus the CUMS group.

parameter table (Table 3), after intragastric administration, the T_{max} of the BAC group was 1.5 ± 0.58 h, C_{max} was 8.13 ± 1.15 $\mu\text{g/mL}$, and AUC_{0-24} h was 71.97 ± 26.92 $\mu\text{g}\cdot\text{h/L}$. The T_{max} of the BPT-SD group was 1.1 ± 0.55 h, C_{max} was 23.09 ± 5.79 $\mu\text{g/mL}$, and AUC_{0-24} h was 207.4 ± 27.36 $\mu\text{g}\cdot\text{h/L}$. Compared with the BAC group, the BPT-PM group did not show significant increase in C_{max} and AUC_{0-24} h. The C_{max} and AUC of the BPT-SD group significantly increased ($p < 0.01$) by 2.84- and 2.88-fold, respectively. Therefore, the preparation of BAC into a solid dispersion could significantly improve the bioavailability of BAC.

The results of the pharmacokinetic test of BAC showed that baicalin had a double peak phenomenon with baicalin as the detection index (Lai et al., 2010). After oral administration, BAC is directly absorbed in the intestine and catalyzed by glucuronyl transferase (UGT) to generate baicalin, which enters the blood circulation, thus forming the first peak. Baicalin in the blood passes through the hepato-intestinal circulation and is metabolized into BAC in the intestine through the flora and subsequently absorbed (Kotani et al., 2006). The absorbed baicalin is again glucuronated in the body and excreted by the small intestine, thus forming the second peak.

3.11. Effects of BAC on depressive-like behaviors

BAC (20 mg/kg) could maintain neuronal survival, promote neuronal maturation, and rescue neurons from apoptosis via inhibiting activation of GSK3 β /NF- κ B/NLRP3 signal pathway that is known to be associated with inflammation, thus exerting neuroprotective effects and preventing CUMS-induced depressive-like behaviors. (Zhang et al., 2018a) Lack of anhedonia is a typical symptom of depression. As shown in Fig. 11, after 4 weeks of CUMS, the sugar water preference of CUMS mice decreased significantly ($p < 0.01$), indicating that the model was successful.

3.11.1. SPT

As shown in Fig. 12,A, compared with the blank group, the sucrose consumption in the CUMS group significantly reduced ($p < 0.01$). However, BAC (20 and 40 mg/kg), BPT-SD (20 and 40 mg/kg), and the positive drug FLU reversed the change in sucrose preference. The reversal effect of BPT-SD was slightly higher than that of BAC but not substantial.

3.11.2. OFT

Compared with the blank group, the total exercise distance of the

CUMS group was significantly reduced, which indicated that the exercise capacity of the CUMS group was significantly reduced. As shown in Fig. 12,B, compared with the CUMS group, BAC (20 and 40 mg/kg), BPT-SD (20 and 40 mg/kg), and FLU (15 mg/kg) significantly improved exercise capacity. Among them, the exercise capacity between the BPT-SD-treated group and the blank group did not change significantly ($p > 0.05$), indicating that it would not cause excitability of the central nervous system.

3.11.3. FST

As shown in Fig. 12,C, compared with that in the blank group, the immobility time in the CUMS group was significantly increased. BAC (20 and 40 mg/kg, $p < 0.05$, $p < 0.01$), BPT-SD (20 and 40 mg/kg, $p < 0.01$), and FLU (15 mg/kg, $p < 0.05$) significantly reduced the immobility time.

3.11.4. TST

As shown in Fig. 12,D, compared with that in the blank group, the immobility time in the CUMS group significantly increased. BAC (20 and 40 mg/kg, $p < 0.01$), BPT-SD (20 and 40 mg/kg, $p < 0.01$), and FLU (15 mg/kg, $p < 0.01$) significantly reduced immobility time.

In summary, these results indicated that BAC could significantly improve depression-like behaviors caused by CUMS; however, there was no significant difference between the effects of BPT-SD and BAC.

3.12. Limitations of the study

Preparation of BPT-SD is often difficult because of the non-uniform mixing with the plasticizer TPGS, which is usually waxy and semi-solid at room temperature attributable to its low melting point. The process can be performed on ice with a mortar and pestle to ensure even mixing. For evaluating pharmacodynamics, the behavioral gap between BAC and its solid dispersion is not very evident. This needs to be further investigated to elucidate depression-related indicators in the brain (such as the amount of dopamine and serotonin) by experiments such as western blotting. Further, this can help to explore the differences of the effects of BAC and BPT-SD on the expression of related proteins in the brain.

4. Conclusions

In our study, PVP VA64 was selected as the carrier, and TPGS was used as a plasticizer to prepare BPT-SD, which can improve the dissolution rate of BAC, thereby increasing its oral bioavailability. The HME process is a new, environmentally friendly, and efficient preparation method, that can render BAC highly dispersed in the carrier and maintain good physical stability. This study can improve the dissolution of BAC, inhibit the P-glycoprotein efflux of BAC, and provide a reference for TPGS as a plasticizer and efflux inhibitor to prepare solid dispersions by HME.

CRedit authorship contribution statement

Meng Tong: Writing – original draft, Writing – review & editing, Investigation. **Xiaoyan Wu:** Writing – review & editing, Formal analysis. **Shuya Zhang:** Validation, Software, Methodology. **Di Hua:** Data curation, Writing – original draft, Resources. **Shukun Li:** Validation, Formal analysis, Investigation. **Xiangyu Yu:** Investigation, Formal analysis. **Jing Wang:** Conceptualization, Funding acquisition, Writing – review & editing. **Zhenhai Zhang:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors report no conflicts of interest in this work.

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