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ORIGINAL ARTICLE

Preparation and evaluation of solid lipid nanoparticles of baicalin for ocular drug delivery system in vitro and in vivo

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Abstract

Purpose: To prepare and evaluate the solid lipid nanoparticles of baicalin (BA-SLN) for ocular drug delivery system. *Methods*: The BA-SLN was prepared by emulsification/ultrasonication method. The appearance of BA-SLN was examined by the negative stain method. The mean diameter and zeta potential of BA-SLN were determined using a Zetasizer. The entrapment efficiency of BA-SLN was determined by Sephadex-G50 column. And the solid-state characterization of BA-SLN was analyzed by DSC and X-ray. The release of drug from BA-SLN was evaluated using dialysis bag diffusion technique. The effects of SLN on corneal permeability of baicalin were investigated in vitro, using isolated rabbit corneas. The in vivo ocular irritation of BA-SLN was tested by pathological section observation using rabbits. The pharmacokinetics was evaluated by microdialysis in the rabbit aqueous humors. *Results*: The results showed that the BA-SLN had an average diameter of 91.42 ± 1.02 nm with a zeta potential of –33.5 ± –1.28 mV and the entrapment efficiency of 62.45 ± 1.67%. In vitro release studies indicated that the BA-SLN retained the drug entity better than the baicalin ophthalmic solutions (BA-SOL). In the pharmacokinetics studies, the AUC value of BA-SLN was 4.0-fold versus the BA-SOL ($P < 0.01$), and the Cmax value of BA-SLN versus the BA-SOL was 5.3-fold ($P < 0.01$). *Conclusion*: SLN can be used as a carrier to enhance ocular bioavailability of baicalin.

Key words: Baicalin, solid lipid nanoparticles, ocular drug delivery system, pharmacokinetics, microdialysis

Introduction

In ocular drug delivery, the physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, resulting in a short duration of the therapeutic effect. When a drug solution is dropped into the eye, the effective tear drainage and blinking action of the eye result in a 10-fold reduction in the drug concentration within 4–20 minutes[1]. The limited permeability of the cornea also contributes to the poor absorption of ocular drugs. Due to tear drainage, most of the administered dose passes through the naso-lacrimal duct into the GI tract, which may cause side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary.

Ocular therapy would be significantly improved if the pre-corneal residence time of drugs were increased. Controlled and sustained delivery systems, such as liposomes, emulsions, and biodegradable nanoparticles have been proved to improve the corneal penetration of drugs[2] and prolong the retention of drugs on the ocular surface as well[3]. Solid lipid nanoparticles (SLN) have been proposed as a new type of drug carrier system[4]. The advantage of SLN lies in that the lipid matrix is made from physiological lipids and polymers from natural or synthetic sources, which could avoid the danger of acute and chronic toxicity. Because of its nonirritant and non-toxic property[4], SLNs were regarded as one of the most suitable carriers in ocular remedies. Sustained and controlled drug release properties of SLN can be beneficial for ophthalmic preparations. In addition, a good

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biocompatibility of SLN may help to resolve the problem of the poor bioavailability of conventional ophthalmic solutions. The above factors indicated that SLN is one of the ideal ophthalmic drug delivery systems.

The aim of this study was to develop the baicalin SLN for ophthalmic drug delivery system. The baicalin, β-D-gluopyranosiduronic acid, 5,6-dihydroxy-4-oxo-2-phenyl-4H-1-benzopyran-7-yl, is a flavonoid purified from the medicinal plant *Scutellaria baicalensis* Georgi, which has been used in traditional Chinese medicine for thousands of years. It has many significant biological activities to eyes, such as anti-inflammatory, antibacterial and anti-cataract effects[5,6]. Baicalin is practically insoluble in water and soluble in alcohol, whose molecular weight and melting point is 236.27 and 222–224°C, respectively. After administration into the rabbit eyes, the concentrations of baicalin in aqueous humor of rabbits were determined using high-performance liquid chromatography (HPLC). The pharmacokinetics of free and nanoparticle-encapsulated baicalin were studied.

Materials and methods

Materials

Baicalin was purchased from Zhong Xin Pharmaceuticals (> 98%, Tianjin, China). Soya phospholipids SL-100 was acquired from Lipoid (Rheinland-Pfalz, Germany). Poloxamer 188 was obtained from BASF (Deutschland, Germany). Triglyceride was purchased from Tianjin North Tianyi chemical agent factory. Sephadex-G50 was purchased from Sigma (St. Louis, MO, USA). Lidocaine hydrochloride injection was acquired from Shanghai Hefeng Pharmaceutical Co. Ltd. (Shanghai, China). Ofloxacin ophthalmic solution was obtained from Hubei Qianjiang Pharmaceutical Co. Ltd. (Qianjiang, China). The linear microdialysis probes (LM-10, 10 mm membrane) were acquired from Bioanalytical Systems Inc. (West Lafayette, IN, USA). A microinjection pump was purchased from CMA (Solna, Svorige, Sweden). All other chemicals and reagents were of analytical grade.

Animals

New Zealand White rabbits, with weight of 2.5–3.0 kg, were provided by the Chinese Academy of Medical Sciences of Radiation Research Institute. The animals, housed in standard cages in a light-controlled room at 19 ± 1°C and 50 ± 5%RH, were given a standard pellet diet and water ad libitum. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 92–93, revised in 1985) and were approved by the Department of Laboratory Animal Research at Tianjin University of Traditional Chinese Medicine. The procedures involving animals were reviewed and approved by the Animal Ethical Committee at Tianjin University of Traditional Chinese Medicine.

Stability test of baicalin in different pH solutions

Baicalin was added into the phosphate buffer at pH 6.5 and 7.4, respectively. And the samples were withdrawn at 0, 0.5, 1.0, 1.5, 2.0, 4.0, 8.0, and 10.0 hours. Then 20 μL of sample solution was injected for HPLC determination (Cometro 6000, Hunters Point, NY, USA).

The solubility of baicalin test

The excess baicalin was added into the distilled water and the phosphate buffer at pH 6.5, which were maintained at 35 ± 1°C for 24 hours using a magnetic stirring (SCZL-4B, Henan, China) at a rotating speed of 200 rpm/min, to make sure the excess baicalin always remain. After 24 hours, the samples were centrifuged for 10 minutes at 880 × g. The collected supernatant was filtered with 0.22 μm microspore filter, and the filtrate was diluted with the distilled water and the phosphate buffer at pH 6.5, respectively. Then 20 μL of sample solution was injected for HPLC determination (Cometro 6000).

Preparation of baicalin solid lipid nanoparticles

BA-SLN was prepared by emulsification/ultrasonication method. An orthogonal experimental design was used to optimize the formulation. The aqueous phase was prepared by adding poloxamer 188 0.67 g to 100 mL ultrapure water maintained at 70 ± 1°C under magnetic stirring (SCZL-4B, Henan, China) at a rotating speed of 400 rpm/min until completely dissolved. The oil phase was prepared by adding baicalin 0.007 g, triglyceride 0.5 g and soya phospholipids SL-100 0.5 g to 5 mL anhydrous alcohol maintained at 70 ± 1°C with continuous stirring (SCZL-4B) at a rotating speed of 400 rpm/min until the organic solvent was completely evaporated. The coarse hot oil-in-water emulsion was prepared by slowly adding hot aqueous phase to the oil phase maintained at 70 ± 1°C under constant stirring (SCZL-4B) at a rotating speed of 400 rpm/min for 0.5 hours. Then the coarse emulsion was then treated by probe-type ultrasonic (JY92-4B, Zhejiang, China) for 10 minutes and filtered using 0.22 μm microporous membrane. The BA-SLN suspensions were thus obtained.

Characterization of BA-SLN

The appearance of BA-SLN was examined by the negative stain method[7]. A drop of the sample was applied to a film-coated copper grid. Phosphotungstic acid solutions were then dropped onto the grid. The stained sample was examined using a transmission electron microscope (JEOL, Tokyo, Japan).

The mean diameter and zeta potential of BA-SLN were determined using a Zetasizer (Nano-ZS, Malvern Instruments, Malvern, UK). Each batch was analyzed in triplicate.

The BA-SLN was separated from free drug using Sephadex-G50 column (1.5 × 25 cm) to assay the entrapment efficiency of BA-SLN[7]. Suspension of 1 mL BA-SLN was eluted by distilled water at the rate of 0.5 mL/min in

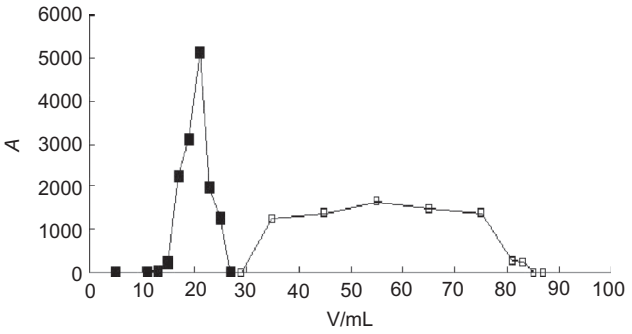

Figure 1. Separation curve of baicalin from BA-SLN. ■: BA-SLN; □: free baicalin.

a Sephadex-G50 column. The opalescence part of the eluate was collected. Both concentrations of BA-SLN in eluate collected and in the suspension were assayed using HPLC (Cometro 6000), after dilution with anhydrous ethanol. The separation curve was shown in Figure 1. Entrapment efficiency (*EE*%) can be calculated by the following formula:

$$EE\% = \frac{C}{C_0} \times 100 \quad (1)$$

where C is the amount of drug encapsulated and C_0 is the total amount of drug in the BA-SLN.

Recovery of incorporated and non-incorporated baicain accounted for more than 96% of the used dose.

Differential scanning calorimetry analysis

Differential scanning calorimetric (DSC) analysis was performed using a Perkin-Jade DSC apparatus. The samples were sealed in aluminum pans under nitrogen air atmosphere at a flow rate of 50 mL/min and evaluated in 30–270°C temperature ranges at 10°C per minute. Poloxamer 188, soya phospholipids SL-100, triglyceride, baicalin, physical mixture of excipients : baicalin (100:1) and freeze-dried BA-SLN formulation were evaluated.

X-ray diffractometry analysis

In X-ray studies, an automatic X-ray diffractometer (Rigaku D/max 2500v/pc, Tokyo, Japan) equipped with a PW R18 X-ray generator was used. Nickel-filtered Cu kα1 radiation having a wavelength of 1.54056 Å, operating at 40 kW and 200 mA in the range (2θ) of 3–60°, was used. Poloxamer 188, soya phospholipids SL-100, triglyceride, baicalin, physical mixture of excipients : baicalin (100:1), and freeze-dried BA-SLN formulation were evaluated.

In vitro release studies

The release of drug from BA-SLN was evaluated using dialysis bag diffusion technique (cut-off 5 kDa, Billerica, MA, Milipore). The dissolution medium was the freshly prepared phosphate buffer at pH 6.5. Two-milliliter volume of BA-SOL and BA-SLN were accurately pipetted into the dialysis bag, and to this a little amount of dissolution media was added, which was then sealed at both ends. The dialysis bag was placed in 200 mL dissolution medium maintained at 35 ± 1°C using a Drug Dissolution Tester (ZRS-8G, Tianjin, China) with the paddle rotation at 100 rpm. Samples, each 1 mL in volume, were withdrawn at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 10 hours and replaced by an equal volume of receptor medium. The release of baicalin was analyzed using HPLC.

Corneal permeation studies of BA-SLN

After the rabbits were killed by injecting air intravenously through the marginal ear vein, freshly excised rabbit corneas were immediately mounted on Franz-type diffusion cells, which were maintained at a constant temperature 35 ± 1°C, under stirring conditions using a transdermal diffusion machine at a rotating speed of 200 rpm (TK-20A, Shanghai, China). The corneal area available for diffusion was 0.50 cm^2. Preheated (35°C), 0.5 mL of BA-SOL and BA-SLN suspension was added to the epithelial and the phosphate buffer at pH 6.5 was added to the endothelial (4.5 mL) compartment. To ensure oxygenation and agitation, an $O_2 : CO_2$ (95:5) mixture was bubbled through each compartment at a rate of 3–4 bubbles/s. Samples of medium from the endothelial side were withdrawn every 40 minutes from the sampling port and were replaced with a preheated equal quantity of fresh phosphate buffer at pH 6.5 to maintain a constant volume. Each experiment was continued for 4 hours and repeated for three times.

The apparent corneal permeability coefficients (P_{app}) were calculated according to the equation below[8].

$$P_{app} = \frac{\Delta Q}{\Delta t \times C_0 \times A \times 60 (\mathrm{cm\ s^{-1}})} \quad (2)$$

where $\Delta Q/\Delta t$, the steady-state slope of the linear portion of the plot of the amount of drug in the receiving chamber (Q) versus time (t); A, the area of exposed corneal surface (0.5 mm^2); and C_0, the initial concentration of drug in the donor cell and 60 represents the conversion of minutes to seconds.

Determination of corneal hydration levels

To determine the wet corneal weight, W_a, each corneal sample was carefully removed from the scleral ring and weighed. And the excess water on the cornea sample was removed by filter paper. It was then desiccated at 60°C for 16 hours to determine the corresponding dry corneal weight, W_b[9].

The percentage corneal hydration level (HL%) was determined both for untreated corneas (removed no later than 30 minutes after the death of the rabbit) and for corneas recovered from permeation tests performed in BA-SOL and BA-SLN. The percentage corneal HL% was defined as follows:

$$HL\% = 1 - \left(\frac{W_b}{W_a}\right) \times 100 \quad (3)$$

Ocular irritation studies of SLN

Ocular irritation studies were performed according to the Draize technique[10] using six New Zealand white rabbits, each weighing 2.5–3.0 kg. BA-SLN were instilled into the left eyes and SLN without baicalin were instilled into the right eyes, 0.1 mL every 4 hours, four times a day for a period of 7 days. The condition of the ocular tissue was monitored after 4, 12, 24, 48, and 72 hours after the end of the last instillation. Evaluation was done using the Draize technique. After the in vivo studies, the pathological sections of the eyes were also made.

Pharmacokinetics studies

Rabbits (n = 3) had been treated with ofloxacin 0.3% ophthalmic solution for 4 days before surgery. Then the animals were anesthetized with lidocaine hydrochloride injection. A custom-designed LM-10 microdialysis probe was implanted into the anterior chamber of each eye as described[11]. Probe inlet and outlet lines were tunneled beneath the conjunctiva, under the upper eyelid, and exited between the ears. The leads were protected with a latex glove pocket affixed to the top of the head. The probe was introduced as previously described method[12]. The anchor was sutured to the sclera with 7-0 Vicryl, and conjunctiva was sutured over the anchor. Exterior wound surfaces were treated with ofloxacin 0.3% ophthalmic solution. Animals were used for experimentation after 2 days of recovery. Slit-lamp (YZ2, Changzhou, China) was taken after recovery to estimate fibrin formation and the condition of the eye before the use of rabbits in experiments.

Conscious rabbits (n = 3) were placed in rabbit restrainers, which permitted free movement of the head. Following a 1-hour equilibration period with perfusion of saline solution through the probe, different concentrations of standard baicalin saline solutions (6.04×10^{-2}, 0.1208, 0.1812, 0.2416, 0.3624, 0.4832 μg/mL) were perfused through the probe at a rate of 3 μL/min, and dialysate were collected for 10 minutes after 30 minutes of perfusion. A 20-μL aliquot of each fraction was analyzed using HPLC. In vivo recovery was defined as (Higuchi, 1960): $R = (C_{in} - C_{out})/(C_m - C_{out})$ (C_{in}, the concentration of standard solutions; C_{out}, the concentration of dialysate; and C_m, the concentration in aqueous humor). A linear equation was plotted by ($C_{in} - C_{out}$) versus C_{out}, and the slope of the line gave the recovery (R).

After the disturbance of standard solutions was minimized by perfusion of saline solution through the probe, 100-μL of BA-SOL containing 0.1% (w/v) baicalin and BA-SLN suspensions containing 0.1% (w/v) baicalin were administrated into the rabbit eyes. The samples were collected every 10 minutes at the first hour and 20 minutes at the remaining hours until baicalin could not be detected. At the end of the experiment, euthanasia was performed under deep anesthesia with an intravenous injection of sodium pentobarbital through the marginal ear vein.

Chromatographic analysis

Baicalin was assayed using reversed-phase HPLC (Cometro 6000). A C18 column (Diamonsil, 200 × 4.6 mm, 5 μm) was used with the mobile phase of methanol-water containing 0.05% (w/v) folic acid (55:45) at the detection wavelength of 280 nm. The flow rate was 1.0 mL/min. The calibration curve was linear in the range of 5.84×10^{-2}~0. 4672 μg/mL (r^2 = 0.9999). The sensitivity was 12 ng.

Statistical analysis

The data obtained were expressed as mean ± SD. The experimental data were analyzed using statistical analysis t-test. Differences were considered to be significant at $P < 0.05$.

Results and discussion

Stability of baicalin in different pH solutions

Because baicalin is a weak acidic compound with several reaction points, which is not stable in strongly acidic or basic conditions, baicalin would be hydrolyzed to form baicalin aglycone, and would be decomposed to polyhydroxy flavone aglycones by the baicalin enzymes contained in the root *S. baicalensis*[13,14]. Table 1 showed that the stability of baicalin was significantly influenced by the pH value of solutions. However, the pH value of the tear is around 7 and the baicalin was unstable. GBR was a kind of physiological solution, which can preserve the cornea for 6 hours[15], but baicalin was not stable because of the basic property of GBR solution. So when the pH value of solutions is over 7.0, which should not be used as the receiving solution and the dissolution medium for baicalin in vitro.

The solubility of baicalin

The solubilities of baicalin in distilled water and in phosphate buffer at pH 6.5 were 0.1410 ± 0.024 mg/mL and 4.898 ± 0.212 mg/mL, respectively. So baicalin would be dissolved in the phosphate buffer at pH 6.5.

According to the results of the stability test and solubility test of baicalin, the phosphate buffer at pH 6.5 was

Table 1. Stability of baicalin in different pH phosphate buffer solutions.

	Concentration% (w/v)								
Medium	0 h	0.5 h	1.0 h	1.5 h	2.0 h	4.0 h	6.0 h	8.0 h	10 h
pH 6.5 PBS	100	102.7	99.7	99.2	98.6	98.7	99.3	98.8	95.4
pH 7.4 PBS	100	98.4	95.3	92.7	89.6	81.2	74.7	69.4	62.5

selected as the receiving solution and the dissolution medium for baicalin.

The physicochemical properties of BA-SLN

Photograph from transmission electron microscope clearly showed that the shape of BA-SLN was spherical (Figure 2). And Table 2 shows that the average size, average Zeta potential, and entrapment efficiency of BA-SLN were 91.42 ± 1.02 nm, −33.5 ± −1.28 mV, 62.45 ± 1.67%, respectively.

DSC and X-ray diffraction analysis

The drug entrapment is further characterized using DSC and X-ray diffraction (XRD). It reveals the state of the encapsulated drug whether it is dispersed in a microcrystalline form, with or without polymorph change or transition change in amorphous form. The thermograms of bulk poloxamer 188, triglyceride, soya phospholipids SL-100, physical mixture, and freeze-dried BA-SLN were shown in Figure 3. The thermogram of physical mixture showed that the melting process for poloxamer 188 and triglyceride took place with maximum bi-peak at 40–70°C, and the peak of soya phospholipids SL-100 was at 130–180°C. But there was no peak at the temperature range of the thermogram of freeze-dried BA-SLN. The baicalin peak was lost in the BA-SLN, pointing out the solubilization of baicalin in the lipid[16].

Similar results were observed using XRD studies (Figure 4). XRD diffractogram of poloxamer 188, soya phospholipids SL-100, and triglyceride were compared with that of physical mixture and that of freeze-dried BA-SLN. Drug peak in BA-SLN was diminished or suppressed, which can be attributed to the dilution factor because of high concentration of polymer without any qualitative fraction[17].

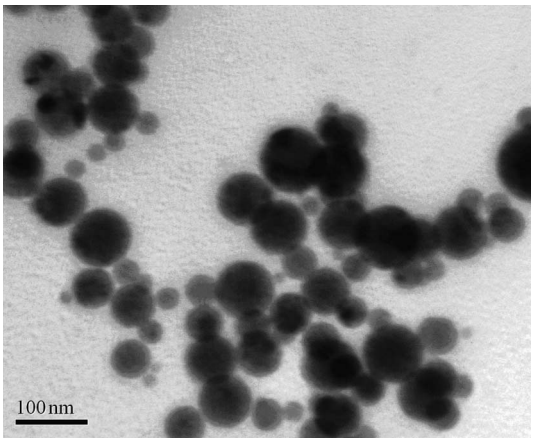

Figure 2. Transmission electron micrograph of BA-SLN.

Table 2. The results of physicochemical properties of BA-SLN ($n = 3$).

Drug	Size(nm)	ζ-potential (mv)	*EE*(%)
BA-SLN	91.42 ± 1.02	−33.5 ± 1.28	62.45 ± 1.67

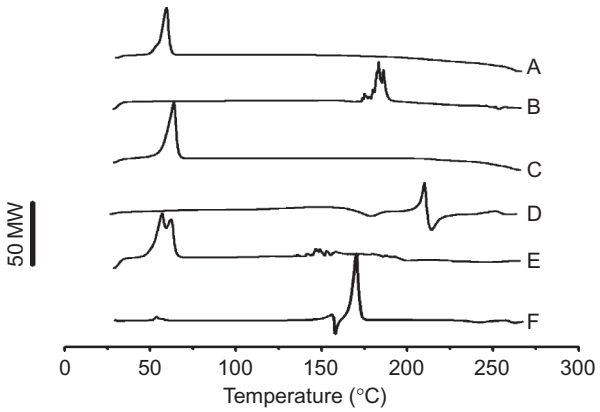

Figure 3. DSC thermograms. A: poloxamer 188; B: soya phospholipids SL-100; C: triglyceride; D: baicalin; E: physical mixture of excipients: baicalin (100:1); F: freeze-dried SLN formulation.

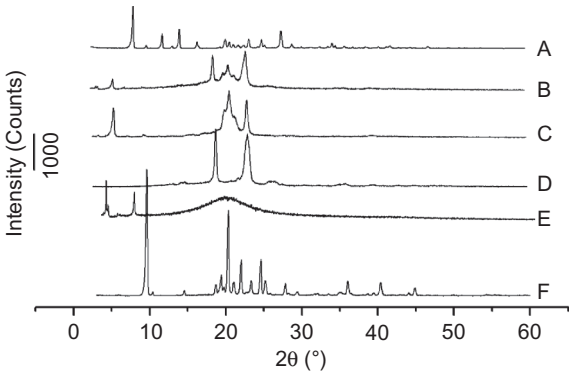

Figure 4. XRD diffractograms. A: poloxamer 188; B: soya phospholipids SL-100; C: triglyceride; D: baicalin; E: physical mixture of excipients : baicalin (100:1); F: freeze-dried SLN formulation.

Effect of BA-SLN on the corneal permeability of baicalin

Table 3 listed the apparent permeation coefficients (P_{app}) and the corneal hydration levels (HL) determined from the permeant in BA-SOL and BA-SLN. It indicated that the cumulative penetration amount of BA-SLN was much higher than that of BA-SOL. The results of Table 3 indicated that BA-SLN increased the P_{app} and J_{ss} with 1.4-fold versus the BA-SOL ($P < 0.01$).

The results showed that the trans-corneal amount of BA, which was encapsulated by SLN, was obviously increased and the effect of drug was also improved.

This SLN formulation of BA, which showed high in vitro ocular transport, would offer two advantages in

Table 3. The corneal permeability coefficients of baicalin ($n = 3$).

Drug	Papp × 106/cm/s	$J_{ss} \times 10^4$/μg/s/cm^2	*HL*(%)
BA-SOL	3.56 ± 0.44	3.85 ± 0.48	79.01 ± 0.86
BA-SLN	4.93* ± 0.26	5.47* ± 0.29	80.08 ± 1.46

*, $P < 0.01$ versus BA-SOL.

Table 4. Release kinetics of BA-SLN.

Drug	First-order r^2	Higuchi r^2	Korsmeyer–Peppas r^2	Korsmeyer–Peppas n
BA-SLN	0.9532	0.9653	0.9312	0.2452

terms of ocular drug delivery. First, encapsulation of the BA into the lipophilic particle would facilitate transport through the corneal route. Lipid formulations with phospholipid are also known to enhance the absorption of diclofenac sodium[18]. Second, the particulate nature of the formulation would ensure adherence to the surrounding membranes preventing tear wash out and providing sustained release of BA, as SLN are highly adhesive[18,19].

The percentage of corneal HL is a parameter frequently used to evaluate damage to this tissue. Previous report showed that the normal cornea has a HL of 76–80%[20]. An 83–92% HL denotes damages to the epithelium or endothelium[8]. As shown in Table 4, the corneal hydration values after the studies were not higher than 83%. This indicated that the BA-SLN did not cause any damage to the epithelium or endothelium during the studies.

In vitro release

Figure 5 exhibited the cumulative amount of baicalin released versus time profiles for different drug-containing solutions. For ophthalmic solutions, almost all the baicalin was released within 3 hours. There was a 17.6% of baicalin released from BA-SLN after 1 hour, approximately 62% after 3 hours, and the remaining amount of drug was found to be released in a sustained manner, over a period of 10 hours. The system displayed some extended release characteristics. These results indicated that BA-SLN has a better ability to retain drugs than the ophthalmic solution.

Data obtained from in vitro release studies of BA-SLN were fitted to various kinetic equations such as first order, Higuchi model, and Korsmeyer–Peppas model. The release of drug from nanoparticles was diffusion controlled as indicated (Table 4) by the higher r^2 (0.9653) values in Higuchi model. So it indicated that the release behavior was followed by Higuchi model. Meanwhile, the n values, which means the release parameter, obtained from the Korsmeyer–Peppas model were less than 0.45, according to the theory of the Korsmeyer–Peppas model, the mechanism of drug release from the nanoparticles was Fickian when $n < 0.45$[21,22].

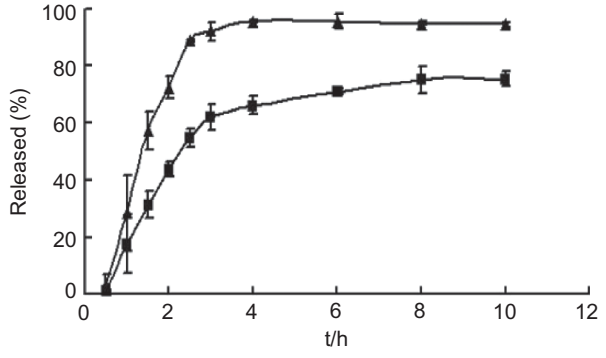

Figure 5. Cumulative amount of baicalin released as a function of time from BA-SLN and BA-SOL. ▲: BA-SOL; ■: BA-SLN.

Ocular irritation studies

The results of the ocular irritancy studies (Table 5) indicated that the BA-SLN was nonirritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible.

Pharmacokinetics studies

As it was shown in Figure 6, the linear regression between perfusate (C_{in}) and dialysate (C_{out}) was $C_d - C_p = -0.3993 \times C_p - 0.022$ ($R^2 = 0.9903$), so the recovery (R) in vivo was $39.92 \pm 5.81\%$.

The area under the aqueous humor conentration curve (AUC) versus time was estimated by the linear trapezoidal method with extrapolation to infinite time. Concentration at peak (C_{max}), time to peak (T_{max}), and terminal rate constant (K_e) were calculated with noncompartmental techniques[23]. Individual aqueous humor parameters for each eye were calculated. All parameters were reported as mean ± SD.

Aqueous humor pharmacokinetics parameters were presented in Table 6. As shown in Figure 7, the AUC value of BA-SLN was much higher than that of the BA-SOL, which was 4.0-fold versus the BA-SOL ($P < 0.01$), and the C_{max} value of BA-SLN versus the BA-SOL was 5.3-fold ($P < 0.01$). The T_{max} value of BA-SLN was longer than that of the BA-SOL, and K_e value of BA-SLN was lower than that of the BA-SOL.

Table 5. Ocular irritating test ($n = 6$).

Preparation	Average score
Blank of SLN	0
BA-SLN	0

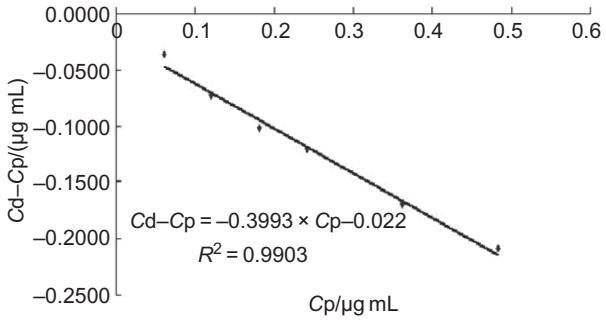

Figure 6. In vivo recovery of microdialysis probe in aqueous humor.

Table 6. Pharmacokinetics parameters of baicalin in aqueous humor after topical administration rabbit ($n = 3$).

Drug	AUC (μg/mLh)	C_{max} (μg/mL)	T_{max}/h	K_e/h	$t_{1/2}$/h
BA-SOL	0.0314 ± 0.0136	0.0751 ± 0.0248	0.167 ± 0	3.8196 ± 0.8419	0.1869 ± 0.0373
BA-SLN	0.1229 ± 0.0312*	0.3947 ± 0.1137*	0.222 ± 0.096	3.6401 ± 1.5856	0.2271 ± 0.1275

*, $P < 0.01$ versus BA-SOL.

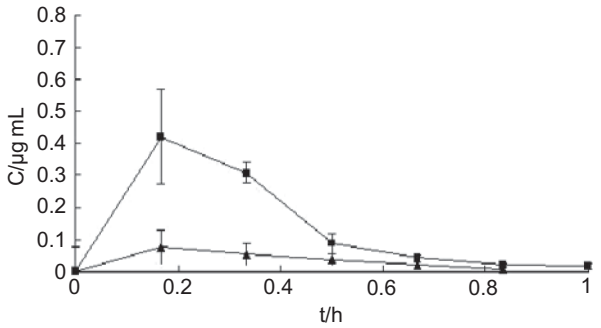

Figure 7. Aqueous humor baicalin concentration–time profiles following a 100-μL topical dose in conscious rabbits. ■: BA-SLN; ▲: BA-SOL.

Conclusion

In this study, baicalin SLN were successfully prepared by the emulsification/ultrasonication method. In vitro studies indicated that SLN can prolong the drug release and enhance the apparent permeation coefficients of drug. The pharmacokinetics studies in rabbits showed that the SLN can significantly enhance the bioavailability of baicalin. The developed formulation is a viable alternative to conventional ophthalmic solution to enhance bioavailability.

Declaration of interest

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