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Development of inclusion complex of brinzolamide with hydroxypropyl-β-cyclodextrin

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ABSTRACT

Glaucoma is an accumulative optic neuropathy resulted from increasing intraocular pressure. Brinzolamide (BRZ) is a kind of carbonic anhydrase inhibitors for glaucoma treatment. In this study, brinzolamide-hydroxypropyl-β-cyclodextrin (BRZ-HP-β-CD) inclusion complex was prepared by solvent evaporation method to improve the solubility of BRZ and enhance the therapeutic effect of BRZ. The formation of the inclusion complex was confirmed by Fourier transform infrared spectroscopy, differential scanning calorimeter and nuclear magnetic resonance spectroscopy. The solubility of BRZ increased about 10-fold after the formation of the BRZ-HP-β-CD inclusion complex. The *in vitro* corneal accumulative permeability of the inclusion complex increased 2.91-fold compared to the commercial available formulation (AZOPT®). In addition, BRZ-HP-β-CD inclusion complex (0.5% BRZ) had an equivalent efficiency of lowering intraocular pressure with AZOPT® (1% BRZ) *in vivo*. These results identified the BRZ-HP-β-CD inclusion complex might have a promising future as a novel formulation of BRZ for glaucoma treatment.

1. Introduction

Glaucoma is an accumulative optic neuropathy. Increased intraocular pressure (IOP) is a primary risk factor for progressive damage (Heijl, Leske, Bengtsson, Hyman, & Hussein, 2002). There are two major forms of glaucoma in patients: open-angle glaucoma and angle-closure glaucoma. For patients with open-angle glaucoma (defined as having optic nerve damage), lowering IOP is effective and always recommended (Quigley, 2011). In clinical practice, eye drops are the first line treatments for most glaucoma patients including prostaglandin analog, β-adrenergic antagonists and carbonic anhydrase inhibitors (CAIs). CAIs, such as acetazolamide, methazolamide, dorzolamide and brinzolamide, could decrease the production of the fluid (aqueous humor) and therefore lower the intraocular pressure.

Brinzolamide (BRZ), a CAI, is a well-known therapeutic agent for glaucoma and intraocular high-pressure, particularly for the primary open-angle glaucoma (POAG) (Siesky et al., 2008). The aqueous solubility of BRZ is very low at pH 7.4 (25 °C). Therefore, the commercial available formulation of BRZ (AZOPT®) is a sterile aqueous suspension of 1% BRZ with a physiologic pH of approximately 7.5 (Iester, 2008). The granular sensation may cause uncomfortable after instillation and arouse undesirable tear wash to wipe the drug off rapidly. Therefore the solution formulation is necessary to be developed to improve the compliance of patients.

Cyclodextrins (CDs) are a structurally related group of natural products formed during bacterial digestion of starch. They are cyclic oligosaccharides that consist of (α-1,4)-linked α-D-glucopyranose units with a lipophilic central cavity and a hydrophilic outer surface (Loftssona & Jarvinen, 1999). The lipophilic central cavity could trap lipophilic drug and thereby enhance the water solubility, stability and bioavailability of the drug. Hydroxypropyl-β-cyclodextrin (HP-β-CD), a type of CD with high water solubility, low renal toxicity and hemolytic activity, is commonly used for ocular drug delivery and has been approved by FDA. Many *in vitro* and *in vivo* studies also proved that the inclusion complex of poor water soluble drug by HP-β-CD could increase drug solubility, stability, corneal permeability and ophthalmic bioavailability (Bozkir, Denli, & Basaran, 2012).

The main purpose of this research was to develop a novel formulation of BRZ for ocular application to improve its solubility and therapeutic effect. Brinzolamide-hydroxypropyl-β-cyclodextrin

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(BRZ-HP-β-CD) inclusion complex was prepared by solvent evaporation method and confirmed by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimeter (DSC) and nuclear magnetic resonance spectroscopy (^{1}H NMR). The *in vitro* corneal permeability and *in vivo* lowering IOP efficiency of the inclusion complex were evaluated and compared with AZOPT®.

2. Experimental

2.1. Materials

BRZ (purity > 99%) was purchased from Dalian Meilun Biology Technology Co., Ltd (Dalian, China). HP-β-CD (purity > 99%, DS = 7) was purchased from Xi'an Deli Chemicals Corporation (Xi'an, China). AZOPT® was obtained from Alcon® (UK). All other reagents were of analytical grade unless otherwise stated. A Millipore Milli-Q Water Purification System was used to generate the water for the study.

2.2. Solubility studies

2.2.1. pH-solubility

An excess amount of BRZ was added to a series of pure aqueous solutions with different pH from 5 to 9 (Jansook et al., 2010). The suspension was shaken at 25 °C for 72 h in an air bath. Excess solid drug was always present during the process. After equilibration, samples were filtered through 0.22 μm membrane. The subsequent filtrate (dissolved drug) was diluted with mobile phase and determined by reversed-phase high performance liquid chromatography (HPLC, Waters, Milford, MA, USA) equipped with a C18 column (4.6 mm diameter, 250 mm length, 5 μm particle size, Global Chromatography Co., Ltd, China). The mobile phase was acetonitrile and water at a volume ratio of 40:60 and a flow rate of 1.0 mL/min. 20 μl sample was injected and the BRZ was detected at 254 nm.

2.2.2. Phase solubility

An excess amount of BRZ was added to a series of HP-β-CD aqueous solutions ranging from 0 to 400 mg/mL. All the samples were sonicated for 30 min in an ultrasonic bath (Scientz SB25-12D, Ningbo Scientz Biotechnology CO., LTD, Ningbo, China) and shaken at 25 °C for 72 h to reach the equilibrium. Then the samples were filtered through 0.22 μm membrane. BRZ concentration in the subsequent filtrate was determined by HPLC. The apparent stability constant (K_S) and the complexation efficiency (CE) were determined from the slope of the linear phase-solubility diagrams according to the following equation (Jansook et al., 2010):

$$K_S = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

$$\text{CE} = \frac{\text{slope}}{1 - \text{slope}} = \frac{[D/(\text{CD complex})]}{[\text{CD}]} = K_S \cdot S_0 \quad (2)$$

where S_0 is the intrinsic solubility of the drug in the absence of HP-β-CD, D is the total drug solubility and CD is the HP-β-CD concentration in moles per liter.

2.3. Preparation of BRZ-HP-β-CD inclusion complex

The BRZ-HP-β-CD inclusion complex was prepared by dissolving BRZ and HP-β-CD in ethanol in a round bottomed flask. After 1 h ultrasound treatment, the ethanol was removed in vacuum. Pure water was added to the flask to dissolve the complex. After filtered through 0.22 μm membrane, sterile inclusion complex eye drops were obtained (Li et al., 2012). The inclusion complex was diluted with acetonitrile to further determine the concentration of BRZ by HPLC as described in Section 2.2.1.

2.4. Characterization of BRZ-HP-β-CD inclusion complex

2.4.1. Ultraviolet–visible spectroscopy (UV/vis)

The UV–visible absorption spectra of BRZ, HP-β-CD and the inclusion complex were recorded using a Perkin-Elmer Lambda 35 UV/vis spectrometer (USA) in the range of 200–600 nm.

2.4.2. Differential scanning calorimetry (DSC)

DSC analysis was performed using a differential scanning calorimeter (TA, DSC TA-Q200, USA). Around 5–6 mg samples (BRZ, HP-β-CD, physical mixture and the inclusion complex) were placed in flat-bottomed aluminum pans respectively and heated at a constant rate of 10 °C/min in a nitrogen atmosphere with a scanning range of 35–300 °C.

2.4.3. Infrared spectroscopy (FTIR)

FTIR spectra of HP-β-CD, the inclusion complex, physical mixture and BRZ were monitored as KBr disk using a Bruker Vector 22 FT-IR Spectrometer (Bruker, Switzerland).

2.4.4. ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded at 25 °C on an ASCEND™ 400 MHz NMR spectrometer (Bruker, Switzerland). Before tests, samples (BRZ, HP-β-CD, physical mixture and the inclusion complex) were dissolved in 0.6 mL DMSO-d_6 respectively, and equilibrated for at least 48 h before measurement.

2.5. In vitro permeability and release

2.5.1. Corneal permeability

The transcorneal permeation experiments were performed using a modified diffusion chamber (Palma et al., 2009). The cell, made of acrylic plastic, consisted of a donor and a receiver compartment (with a volume of 1.0 and 2.0 mL respectively). No significant adsorption of the tested formulations to the diffusion chamber was observed over the 2 h period of the permeability experiments. The receptor solution consisted of glutathione bicarbonate ringer buffer (pH 6.85). Before use, the receptor solution was aerated with a mixture of 95% O_2 and 5% CO_2 to maintain the oxygenation of cornea. Fivealbino rabbits were humanely sacrificed. The corneas, with a 2 mm ring of sclera, were immediately excised and assembled in a perfusion apparatus after rinsing with cold saline. A 2 mL aliquot of the receptor solution was added to the endothelial side, while 0.5 mL of BRZ-HP-β-CD inclusion complex or AZOPT® formulation was added to the epithelial side. The temperature of the diffusion chamber was maintained at 34.0 ± 0.5 °C by a thermostatic water bath. Sample aliquots from the receptor chamber were withdrawn at 15, 30, 60, 90, 120, 180 and 240 min and immediately replaced by previously aerated fresh receptor medium. Samples were filtered through a 0.22 μm membrane and kept at 4 °C before analyzing by HPLC. Three corneas were used in each group. The apparent permeability coefficients (P_{app}) of the test formulations were calculated using the following equation (Goskonda, Hill, Khan, & Reddy, 2000):

$$P_{app} = \left(\frac{1}{AC_0}\right)\left(\frac{dM}{dt}\right) \quad (3)$$

where dM/dt is the flux across the cornea (μg/cm^2 h), A is the available corneal surface area for diffusion (cm^2), and C_0 is the initial drug concentration (μg/mL) in the donor compartment at the zero point. Flux per unit surface area ($1/A \times dM/dt$) was calculated from the slope of the linear portion of the cumulative amount permeated per unit surface area *versus* time plot. The experiments were performed in triplicates.

2.5.2. Determination of corneal hydration levels (HL)

Corneal hydration levels were investigated by measuring total water content by gravimetric method (Vega et al., 2008). At the end of the experiment, each cornea (free from sclera) was weighed (W_1) and dried for 6 h at 100 °C to determine dry corneal weight (W_2). The corneal hydration level (HL%), defined as $[1-(W_2/W_1)]\times 100$, was determined both on untreated and treated corneas.

2.5.3. In vitro release studies

The release rate of BRZ from inclusion complex was measured by the following procedures (Zhao et al., 2011). Four formulations including 0.2%, 0.5% BRZ-complex, free drug and AZOPT® were respectively transferred into a dialysis bag with a molecular weight cut off (MWCO) of 1000 Da, followed by immersion of the dialysis bag into a container filled with 45 mL artificial tears. 1 mL solutions from the outer phase of the dialysis bag were withdrawn at specific time intervals. The concentration of BRZ was analyzed by HPLC method as describe in Section 2.2.1. The data were expressed as means of triplicate determinations.

2.6. In vivo study

2.6.1. Animals

White New Zealand normotensive rabbits (1.9–2.5 kg) were used. The rabbits were provided with food and water *ad libitum* in a temperature controlled room (21 ± 5 °C). They were exposed to 12 h light and 12 h dark cycles. All experimental procedures and animal care conformed to the ARVO (Association for Research in Vision and Ophthalmology) regulation on the use of animals in research, and were approved by the Institutional Animal Care and Use Committee of Sichuan University. After a week of adaptation, animals were admitted to the experiment.

2.6.2. IOP measurements

Intraocular pressure was measured in mmHg using a Tono-pen XL® tonometer (Reichert, NY, USA), calibrated daily or when disordered according to the manufacturer's instructions. Before IOP measurement, a topical anesthetic agent containing 0.4% oxybuprocaine hydrochloride (Benoxil ophthalmic solution, 50 μL, Santen, Osaka, Japan) was applied to the cornea. IOP was measured twice a day until the animals were accustomed to the experimental procedure. Then the normal baseline of each animal was obtained before treatment. The measurements were always carried out at the same time of the day. They were made three times and a mean was taken. Eighteen rabbits were divided into three groups with half female and half male. In group A and B, 50 μL BRZ-HP-β-CD inclusion complex solution was applied with a concentration of 0.2% or 0.5% (w/v), respectively. In group C, 50 μL AZOPT® (BRZ, 1% w/v) suspension was administered. All the treatments were given topically. For all the rabbits, the right eye received the BRZ formulations, while the left eye (contralateral side) was used as control without treatment to minimize the diurnal, seasonal and individual variations. IOP was measured at 30, 60, 120, 150, 180, 240 and 300 min (Palma et al., 2009). The results were expressed as the mean change in IOP from the IOP baseline in six animals (mean mmHg ± standard error of the mean (S.E.)). Intraocular pressure values of both eyes were compared at the same time point by paired Student's *t*-test.

2.7. Statistical analysis

The obtained data were expressed as mean ± standard. Statistical significance was assessed with paired Student's *t*-test using SPSS 13.0. A probability $P<0.05$ was considered significant throughout the study.

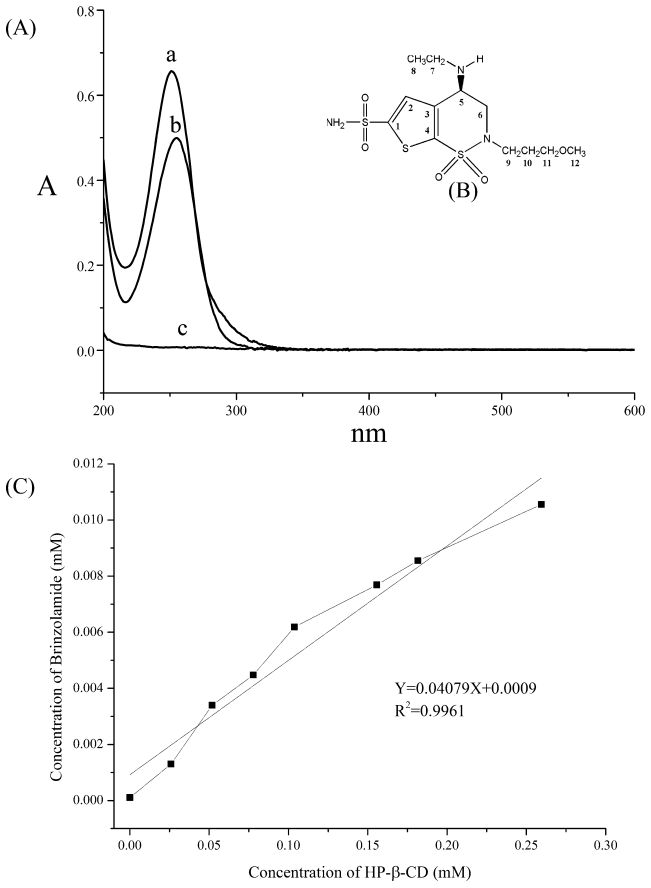

Fig. 1. The UV/vis spectra of BRZ (a), BRZ-HP-β-CD inclusion complex (b) and HP-β-CD (c) in acetonitrile (A), the chemical structure of BRZ (B) and the phase-solubility diagram of BRZ-HP-β-CD host-guest system at 25 °C (C).

3. Results and discussion

3.1. UV/vis spectroscopy

The influence of the inclusion complexation on the absorption of BRZ was observed by UV/vis spectroscopy. The absorption spectra of BRZ, HP-β-CD and BRZ-HP-β-CD inclusion complex were shown in Fig. 1. There was no absorption for HP-β-CD within 240–600 nm. The spectrum of BRZ-HP-β-CD exhibited a characteristics absorption peak at 252 nm, which was very similar to the peak of BRZ. However, a slightly blue shift was observed in the curve of BRZ-HP-β-CD inclusion complex. The reason might be the more hydrophilic environment surrounding BRZ (Hu, Zhang, Song, Gu, & Hu, 2012). These results indicated that the HPLC method developed for BRZ measurement with a detection wavelength of 252 nm could also be applied to determine the BRZ concentration in the inclusion complex.

3.2. Solubility studies

3.2.1. pH-solubility

In this study, the solubility of BRZ was investigated from pH 5.0 to 9.0, which was considered to be the nonirritating pH range for eyes. BRZ has very low water solubility at physiologic pH and higher solubility in both acid and basic solution as shown in Table 1. BRZ is an amphoteric compound as the chemical structure shown in Fig. 1. The pKa values of BRZ are 5.9 (amine) and 8.4 (primary sulfonamide). It could act as an acid or a base (ampholyte) depending upon the pH values. BRZ at the physiologic pH, despite the

Table 1
The solubility of BRZ at different pH (mean ± SD, $n = 6$, 25 °C).

pH value	Mean ± SD (mg/mL)
5.0	1.096 ± 0.018
5.5	1.156 ± 0.030
6.2	0.959 ± 0.055
7.4	0.749 ± 0.038
9.0	2.756 ± 0.072

lowest water-solubility, was preferable to corneal penetration due to its high lipophilicity (octanol/water distribution coefficient was 6.6) (DeSantis, 2000). Thus, it is necessary to increase BRZ water-solubility at pH 7.4 through novel formulation techniques for the enhanced therapeutic efficacy.

3.2.2. Phase solubility

Solubility study showed that 2.5% (w/v) HP-β-CD can solubilize 0.5% (w/v) BRZ in water. The phase solubility of BRZ-HP-β-CD inclusion complex was shown in Fig. 1. The K_S was found to be 32.6 M^{-1} and the CE was 0.043.

3.3. Characterization of BRZ-HP-β-CD inclusion complex

3.3.1. Differential scanning calorimetry (DSC)

The physical characteristics of the guest molecules may be different after trapped in cyclodextrin cavities (Zhao et al., 2011). Therefore, the DSC thermograms of HP-β-CD, inclusion complex, physical mixture (PM) and BRZ were measured (shown in Fig. 2A). The thermogram of HP-β-CD showed an endothermic effect ranging from 100 to about 120 °C, and the peak emerged near 110 °C corresponding to the release of water in HP-β-CD. The unique melting peak of BRZ emerged an onset of 121 °C and a maximum of 131 °C. Besides, the negative peaks near 220 °C could attribute to oxidative degradation process of BRZ in pure drug and physical mixture. Compared to the inclusion complex, the drug endothermic peak, negative peak and the HP-β-CD peaks both presented in the physical mixture, indicating the interactions between BRZ and HP-β-CD, and the physical mixture system was still the simple mixture of those components. Concerning the complex inclusion, the peaks of BRZ and HP-β-CD were combined into a broad peak from 100 to 160 °C, indicating some interactions between these components. The DSC results suggested that BRZ was successfully included into the cavity of HP-β-CD in the inclusion complex.

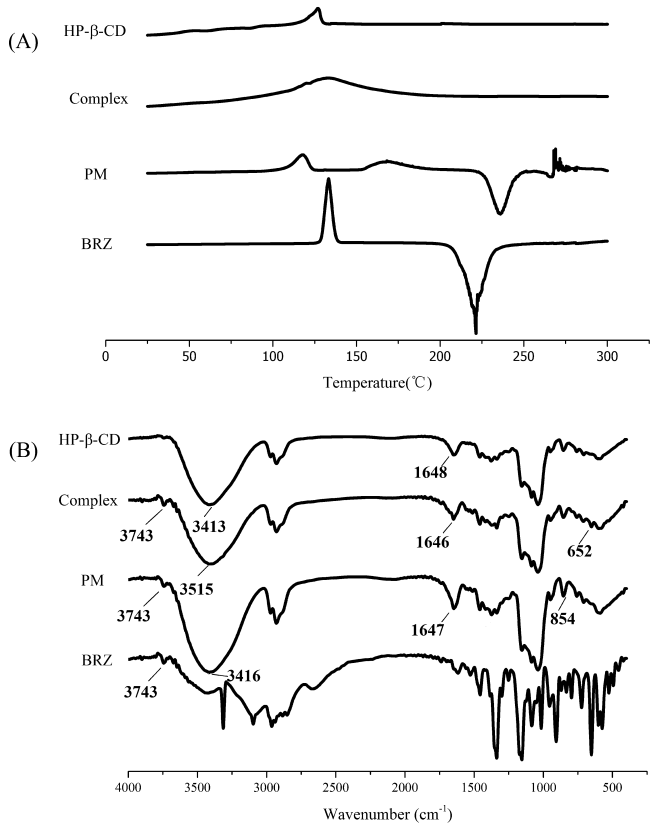

Fig. 2. Characterization of HP-β-CD, inclusion complex, physical mixture (PM) and BRZ (A, DSC thermograms; B, FTIR spectra).

3.3.2. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of the HP-β-CD, complex inclusion, physical mixture and BRZ were showed in Fig. 2B. In the spectrum of HP-β-CD, the most characteristic peaks were O—H stretch (3413 cm^{-1}), C—H stretch (2929 cm^{-1}) and the C—O stretch (1643 cm^{-1}). The broad peak at 3400 cm^{-1} could attribute to the influence of hydrogen bond. The most distinct peaks of BRZ laid in the O_2S—NH_2 stretch (1136 cm^{-1}), the C—NH—C stretch (3313 cm^{-1}) and the O=S=O (sulphone) stretch (1155 cm^{-1}). Moreover, the characteristic absorption bands at 2962 and 2670 cm^{-1} represented the C—C stretch of the —CH_3. The absorption peaks at 3096 and 652 cm^{-1} attributed to the existence of heterocycle including N and S elements. The spectrum of physical mixture was equivalent to the simple combination of BRZ and HP-β-CD, in which the characteristic absorption peaks of BRZ were easily observed including 3743 and 652 cm^{-1}, indicating the natural structure of BRZ still existed without any interaction with HP-β-CD. For the BRZ-HP-β-CD inclusion complex, the spectrum was very similar to that of HP-β-CD and the characteristic peaks of BRZ were almost entirely disappeared. The FT-IR spectrum of the BRZ-HP-β-CD inclusion complex still showed other difference to the physical mixture except the disappearance of the characteristic peaks of BRZ. The physical mixture bands located at 3416, 1647, 854 and 652 cm^{-1} had been shifted and diminished in inclusion complex bands. The FTIR results were corresponding to the DSC findings, indicating BRZ was included in the cavity of HP-β-CD.

3.3.3. ^1H NMR spectroscopy

The ^1H NMR spectra of HP-β-CD, inclusion complex, physical mixture and BRZ were shown in Fig. 3. Numerous peaks were found in the spectrum of BRZ: 1.022 (3H, t, —C**H**$_3$) for the protons on site 8 of BRZ, 1.814 (2H, m, —C**H**$_2$—CH_2—), 2.504 (DMSO-d_6), 2.593 (2H, q, —NH—C**H**$_2$—), 3.173 (1H, m, =N—C**H**$_2$—), 3.227 (2H, m, —OC**H**$_3$), 3.360 (2H, t, —C**H**$_2$—O—), 3.440 (1H, m, =N—C**H**$_2$—), 3.787 (2H, m, —**CH**$_2$—) for the proton on site 6, 4.122 (1H, t, NH—C**H**—) for the proton on site 5, 7.665 (1H, s, —C**H**—) for the proton on site 5, 8.039 (2H, m, SO_2N**H**$_2$). In the spectrum of HP-β-CD, the signals for H-1, H-2, H-3, H-4, H-5 and H-6 appeared at 5.034, 3.223, 4.871, 3.585, 3.755 and 4.585, respectively. The H-3 and H-5 protons of HP-β-CD were considered to be the most common binding sites in inclusion complexes. The signals relating to the BRZ almost disappeared in the spectrum of inclusion complex. There were two possible reasons. First, the amount of BRZ was quite small in the inclusion complex sample. Second, the guest compounds may be affected by the signals of HP-β-CD when they accessed to the cavity. The characteristic peaks of BRZ were easy to be found in the spectrum of the physical mixture. At the same time, the chemical shifts relating to HP-β-CD with the absence of BRZ were compared to the inclusion complex and the physical mixture: $\triangle\delta 1 = \delta$ (complex) $- \delta$ (HP-β-CD), $\triangle\delta 2 = \delta$ (PM) $- \delta$ (HP-β-CD). As shown in Table 2, a significant variation of the H-3 proton of HP-β-CD shifted 0.030 ppm in the spectrum of inclusion complex. However, this distinct change was not shown in the physical mixture, and no other obvious shift was observed. The ^1H NMR investigations suggested that BRZ was

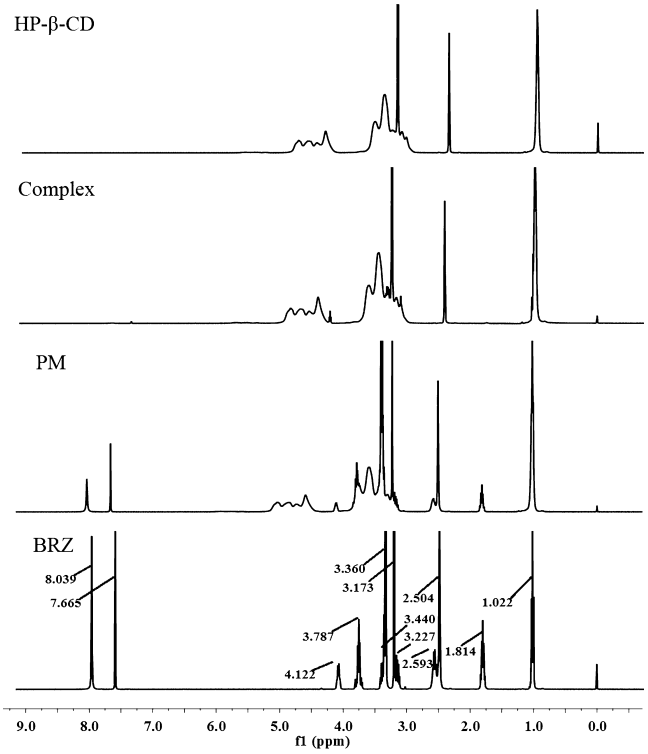

Fig. 3. ^{1}H NMR spectra of HP-β-CD, inclusion complex (complex), physical mixture (PM) and BRZ.

Table 2
Chemical shift change values relating to the signals of HP-β-CD.

Proton	Inclusion complex	Physical mixture	HP-β-CD	△δ1	△δ2
H-1	5.035	5.036	5.034	0.001	0.002
H-2	3.227	3.226	3.223	0.004	0.003
H-3	4.869	4.871	4.871	−0.002	0.000
H-4	3.585	3.585	3.585	0.000	0.000
H-5	3.785	3.752	3.755	0.030	−0.003
H-6	4.593	4.586	4.585	0.008	0.001

included in the HP-β-CD cavity and the H-5 proton was found to serve as the binding sites between BRZ and HP-β-CD in the inclusion complex.

3.4. In vitro permeability and release

3.4.1. In vitro permeability and corneal hydration levels (HL)

The corneal permeation characteristics and HL of BRZ-HP-β-CD inclusion complex and BRZ commercial formulation (AZOPT®) were shown in Table 3. No dramatic difference in HL was found ($p > 0.05$), but there was a significant increase of BRZ cumulative permeation from BRZ-HP-β-CD inclusion complex compared with AZOPT®. The permeation coefficient of BRZ-HP-β-CD with 2.5% HP-β-CD was much larger than the one with 4% HP-β-CD ($p < 0.05$), as shown in Fig. 4A. The high concentration of HP-β-CD might prevent the drug releasing from the inclusion complex, or the BRZ might be re-trapped after releasing when there was a large amount of excess free HP-β-CD around.

Table 3
Corneal hydration levels (HL%) and permeation coefficient (P_{app}) of different BRZ formulations (mean ± SD, $n = 3$).

Formulation	HL%	P_{app} ($\times 10^5$ cm/s)
AZOPT®	81.12 ± 3.03	2.71 ± 0.08
2.5% HP-β-CD	79.59 ± 3.05	7.89 ± 0.21
4.0% HP-β-CD	78.04 ± 3.03	3.21 ± 0.17

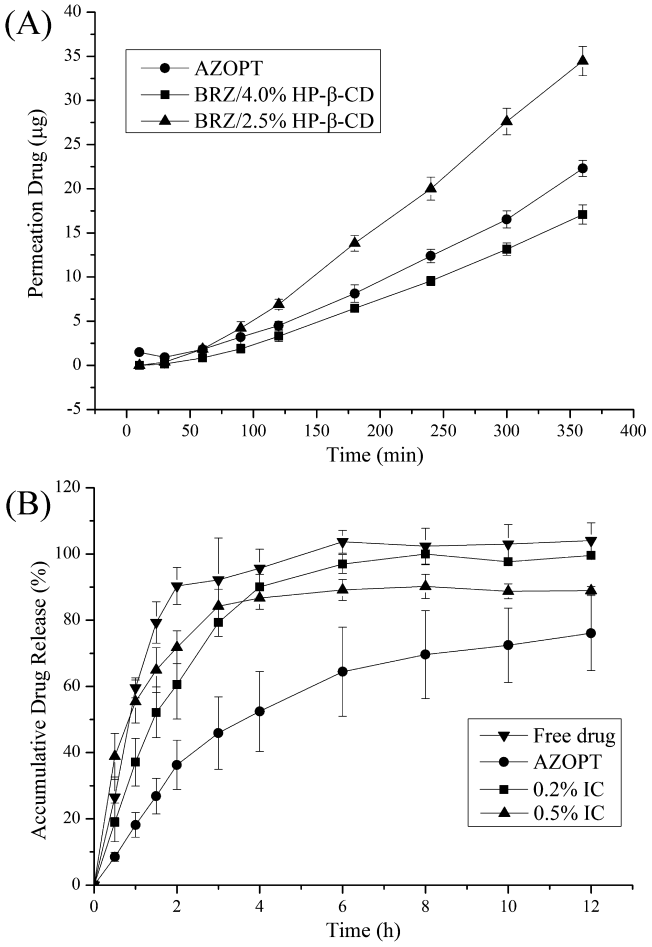

Fig. 4. Drug permeated through rabbit cornea *versus* time (min) (A): ●, AZOPT 1% (w/v); ▲, BRZ/2.5% (w/v) HP-β-CD; ■, BRZ/4.0% (w/v) HP-β-CD ($n = 3$). Accumulative drug release of BRZ from different formulations (B): ▼, Free drug; ●, AZOPT 1% (w/v); ▲, inclusion complex 0.5% (w/v); ■, inclusion complex 0.2% (w/v) ($n = 3$).

3.4.2. In vitro release studies

The *in vitro* release experiments were carried out to evaluate the successful inclusion of BRZ into cyclodextrin and the sustained release characteristic of the complex. As shown in Fig. 4B, the dialysis bag would delay the release of free drug at the first 2 h. The BRZ release from the inclusion complex was slower than free drug but faster than AZOPT®, which proved that BRZ had indeedly accessed into the complex.

3.5. IOP measurements

The IOP of different BRZ formulations were shown in Fig. 5. Both 0.2% and 0.5% (w/v) BRZ-HP-β-CD inclusion complex formulations had higher capacities of lowering IOP compared to AZOPT® at the first 0.5 h. Then, AZOPT® sharply decreased the IOP at 1 h, which was better than inclusion formulations. The lowering effect of AZOPT® maintained at 2–3 mmHg for 4 h, while the 0.2% and 0.5% (w/v) inclusion complex were 4 and 5 h respectively. For the 0.2% (w/v) BRZ-HP-β-CD inclusion complex, IOP elevated fast after 4 h. It might be due to the lower viscosity and the less BRZ amount compared to the 0.5% formulation. Even though the dosage of BRZ was just half in BRZ-HP-β-CD inclusion complex (0.5%, w/v) compared with the commercial AZOPT® (1%, w/v), their lowering IOP efficacy

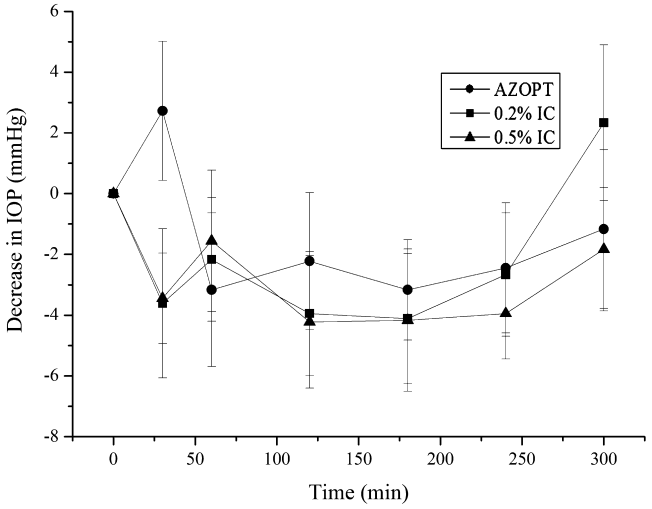

Fig. 5. *In vivo* intraocular pressure profile in the treated eyes for the different formulations of BRZ: ●, AZOPT® 1% (w/v), ▲, inclusion complex 0.5% (w/v),■, inclusion complex 0.2% (w/v) (mean ± SE, $n = 6$).

were similar. The reason might be the higher corneal permeability of inclusion complex. The slow release of AZOPT® lead to a lower concentration of BRZ in the eyes, which would also decrease its IOP lowering efficacy.

4. Conclusion

In this study, BRZ-HP-β-CD inclusion complex was successfully prepared by solvent evaporation method. The formation of the inclusion complex was proved by DSC, FTIR and ^{1}H NMR. ^{1}H NMR also indicated the binding site of the inclusion complex mainly located in the H-5 of HP-β-CD. The stability constant was found to be 32 M^{-1} for 1:1 BRZ-HP-β-CD inclusion complex. The solubility and dissolution behavior of BRZ were significantly improved by the inclusion technique. Moreover, both *in vitro* and *in vivo* investigations showed that BRZ-HP-β-CD inclusion complex had higher cornea permeability and better reducing IOP capacity than the commercially available formulation. All the results suggested that BRZ-HP-β-CD inclusion complex might be a promising novel formulation of BRZ for glaucoma treatment.

Acknowledgements

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