Characterisation of 2-HP-β-cyclodextrin-PLGA nanoparticle complexes for potential use as ocular drug delivery vehicles

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ABSTRACT
Aim: 2-HP-β-cyclodextrin-PLGA nanoparticle complexes were prepared to enhance the aqueous humour delivery of Triamcinolone acetonide.

Materials & methods: Drug-loaded 2-HP-β-CD/PLGA nanoparticle complexes prepared by adapting a quasi-emulsion solvent evaporation technique. In vitro drug release, in vitro transcorneal permeation study, histopathological study and in vivo transcorneal penetration of PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes were evaluated.

Results: Particle size distributions of 2-HP-β-CD/PLGA nanoparticle complexes were 149.4 ± 3.7 nm and presented stable system. Corneal penetration studies revealed steady sustained drug release (First-order); 2-HP-β-CD/PLGA nanoparticle complexes increased ocular bioavailability by increasing dispersion in the tear film and improving drug release.

Conclusion: 2-HP-β-CD/PLGA nanoparticle complex formulation is a promising alternative to conventional eye drops.

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Introduction

Normal ocular structure comprises of special physiological structures, such as the corneal barrier, blood-aqueous barrier, and blood-retinal barrier, that may obstruct the delivery of ocular drugs, and thus, hinder them from achieving satisfactory bioavailability [1,2]. The application of drug delivery systems such as nano eye drops, contact lenses, conjunctival implants, and non-implantable ocular surface drug delivery devices increases the amount of penetration into the aqueous humour by topical eye drops [3–5]. Polymeric carrier materials are widely used because of their suitable biomechanical, physical, and chemical properties, strong plasticity and low irritation potential, all of which make them biocompatible and safe for application [6,7]. Polylactic-co-glycolic acid (PLGA) polymer can overcome the limitations caused by these physiological barriers. It can improve the permeability, mucosal adhesion, sustained release, and controlled release properties of different drug molecules that, in turn, improves the bioavailability of the drug. Silva-Abreu et al. [8] investigated the in vitro scleral permeability of pioglitazone-loaded PLGA nanoparticles to demonstrate that the nanoparticles had better permeability and could be more effective against inflammation. Kalam et al. [9] examined the pharmacokinetics of aqueous extracts of tacrolimus PLGA nanoparticles after aqueous eye drops. The results showed that the nanoparticles had longer $t_{1/2}$ in aqueous humour and the bioavailability was significantly higher than that of aqueous solutions as control group. The PLGA nanoparticles loaded with sparfloxacin can be retained in the anterior cornea for a long time and are not easily eliminated by systemic circulation [10]. Since PLGA is a copolymer of lactic acid (LA) and glycolic acid (GA) monomers, its rate of degradation in the body, drug encapsulation, and in vitro release can be adjusted by changing the ratio of the two monomers and the molecular weight that each contributes to the polymer. Nanoparticles loaded with bevacizumab were prepared using PLGA (molecular weight of 7000–17,000 and a 50/50 copolymerisation ratio). The average retention time and bioavailability of bevacizumab-loaded PLGA nanoparticles in the vitreous increased by 3.28 and 1.39 times, respectively, compared to that in the control group [11]. Numerous studies have shown that the cells have a significant size dependence on the uptake efficiency and targeting of the vector. The PLGA nanoparticles with particle sizes between 1 and 1000 nm can better dissolve, couple, or entrap drugs in the eye for sustained long-term release. Hamed et al. [12] injected a brinzolamide-loaded PLGA nanoparticle with an average particle size distribution of 100 nm under the conjunctiva of normal rabbits and compared their results with the same-site injection of a brinzolamide suspension. The results of intraocular pressure monitoring showed that nanoparticles can reduce intraocular pressure for 10 days; the reduction and duration of intraocular pressure were better for nanoparticles than for the brinzolamide suspension. The effect of improving drug release and penetration can be achieved by modifying a specific polymer on the surface of the PLGA carrier. Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic inner cavity that can interact with small molecules to form a total inclusion complex or form a partial inclusion complex with a macromolecular drug through a hydrophobic side chain [13]. Estefanía Vega et al. [14] added hydroxypropyl-β-cyclodextrin to PLGA and flurbiprofen to PLGA-PEG di-block copolymers. In vitro excised corneal permeability results indicated that the presence of CDs reduces the burst effect and allows a more sustained release of the drug. The strong hydrophilicity of CDs not only provides the drug with the ability to penetrate the tear film but also increases drug concentration on the surface of the eye, thereby enhancing the ability of the drug to enter the eye. Zikhona et al. [15] compared the effect of CD-modified nano-drug delivery system for the eyes on corneal permeability in vitro; the results showed that in the presence of CDs, drug penetration was > 50% within 15 min. The control group had a penetration of less than 30% in 30 min.

The work presented here focussed on the preparation of a hydrophilic polymer and PLGA nanoparticles loaded with triamcinolone acetonide (TA-NPs) for ocular drug delivery. Triamcinolone acetonide was selected as a model drug because of its highly lipophilic nature. Polylactic-co-glycolic acid, with its excellent encapsulation efficiency in combination with 2-hydroxypropyl-beta-cyclodextrin (2-HP-β-CD), was used as a hydrophilic polymer so that it could increase the hydrophilicity of the nanoparticles and aid in making suitable formulations for ocular application.

Materials and methods

Materials

Triamcinolone acetonide (TA) was purchased from Jin Hui Pharmaceutical Group Co., Ltd (Tianjin, China). Poly (lactic-co-glycolic acid) (PLGA, 50:50, MW 10,000 Da) copolymer was obtained from Dai Gang Bioengineering Co., Ltd (Jinan, China). Polyvinyl alcohol (PVA) was supplied by Cola International Trade Co., Ltd (Shanghai, China), while 2-HP-β-CD was gifted by Qian Hui Biotechnology Co., Ltd (Zibo, China). All other reagents and solvents were analytical grade reagents.

Preparation of PLGA NPs and 2-HP-β-CD/PLGA nanoparticle complexes

PLGA NPs were prepared by the emulsion solvent evaporation method based on the previous description with slight modification [16,17]. Briefly, 10 mg of TA and 100 mg of PLGA (LA/GA = 50:50) in 5 ml of acetone/ethanol (4:1) was slowly injected into 40 ml of 2% (w/v) PVA aqueous solution in ice bath using a probe sonicator set at 150 W of energy output (JY88-IIIN, Scientz Biotechnology Co, Ltd, People’s Republic of China) for 600 s with pulses of 3 s on and 3 s off to form an oil-in-water (O/W) emulsion. Then this emulsion was diluted with DI water, followed by the evaporation of organic solvents under magnetic stirring for overnight to obtain a PLGA nanoparticle nanosuspension.

To prepare 2-HP-β-CD/PLGA-nanoparticle complexes, 2-HP-β-CD (1.5% w/v) was dissolved in the PVA aqueous phase. All other steps were the same as in the original method, and
the 2-HP-β-CD/PLGA-nanoparticle complexes were obtained. 2-HP-β-CD/PLGA-nanoparticle complexes is a composite system comprising 2-HP-β-CD/PLGA nanoparticles and a small amount of 2-HP-β-CD inclusion compound. PLGA NPs and 2-HP-β-CD/PLGA-nanoparticle complexes were dried in a lyophilizer using mannitol (1% w/v) as cryoprotectant to prepare for subsequent experiments.

Characterisation of the two types of nanoparticle complexes

Particle size and zeta-potential

The particle size (diameter, nm), polydispersity index (PDI), and zeta-potential of PLGA NPs and the 2-HP-β-CD/PLGA nanoparticle complexes were determined using a laser diffraction instrument (Delsa Nano C® particle size and ζ-potential analyser, Beckman Coulter Inc., CA, USA). All measurements were performed in triplicates, and the results are reported as means ± SD of the three replicates (n = 3) for each formulation.

Morphological observations

The surface morphology of PLGA NPs and 2-HP-β-CD/PLGA nanoparticle complexes were determined using transmission electron microscopy (TEM) (JEM-1400, JEOL, JAPAN). Few microliters of samples of the NPs suspension were dropped on a copper grid and dried at room temperature. After complete drying, the samples were stained using 2% w/v phosphotungstic acid solution and observed using TEM.

Loading capacity and entrapment efficiency

Since triamcinolone acetonide is a fat-soluble drug, the experiment first uses low-speed centrifugation (1000 rpm, 10 min) to separate the nanoparticles from the undissolved drug, and the undissolved drug sinks to the bottom layer; the upper layer of liquid is removed to an ultrafiltration tube (10 min) to separate the nanoparticles from the undissolved drug, and the undissolved drug sinks to the bottom layer; the upper layer of liquid is removed to an ultrafiltration tube and centrifuged (9000 rpm, 30 min, 4 °C) to separate a small amount of dissolved drug from the nanoparticles. The loading capacity (LC) and entrapment efficiency (EE) were calculated by the following equation.

\[
\%\text{LC} = \frac{W_1 - W_2}{W_N} \times 100
\]

\[
\%\text{EE} = \frac{W_1 - W_2}{W_0} \times 100
\]

where \(W_0\) = total amount of TA; \(W_1\) = amount of TA in the upper liquid at low speed centrifugation; \(W_2\) = amount of TA in ultrafiltration filtrate; \(W_N\) = weight of NPs.

Evaluation of the interactions of nanoparticle complexes

Fourier transform infra-red spectroscopy (FTIR)

Many studies have employed FTIR spectra to study various miscible polymer blend systems [18,19]. Furthermore, the existence of a certain group can be inferred, as well as the combination method of the nanoparticle system can be deduced. FTIR spectra were obtained using FTIR-spectrometer (Perkin–Elmer, spectrum 100, Waltham, USA). Dried samples were mixed with KBr powder and compressed into discs. The each samples pellet was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 400–4000 cm\(^{-1}\).

Differential scanning calorimetry (DSC)

Differential scanning calorimetry can be used to evaluate the changes in the crystal forms of the drug in the preparations and the interaction between them [20]. The change in enthalpy was the main parameter that was used to judge whether the excipients had altered. DSC thermograms were obtained using an automatic thermal analyser DSC system (DSC400, Perkin–Elmer, Norwalk, CT, USA). The dried samples were loaded and sealed into an aluminium pan and scanned between 30 and 300 °C at heating rate of 10 °C/min, under constant purging nitrogen atmosphere.

X-ray diffraction (XRD)

XRD was used to investigate the physical form (crystalline or amorphous) of drug dispersion within the PLGA matrix of the nanoparticles. XRD analysis was carried out using a D8-Advance X-ray diffractometer (Bruker, Germany) equipped with a position-sensitive detector allowing all angles between 8°Cand 80°Cto be read simultaneously at a scan rate of 1°/min. The system was operated at 40 kV and 40 mA, using the Cu Kα as the radiation source (\(\lambda = 1.54\) Å).

In vitro drug-release studies

In vitro release studies were evaluated in phosphate buffer saline (PBS, pH 7.4), simulated tear fluids (STF, pH 7.4) [21], and simulated aqueous fluids (SAF, pH 7.4) [22] using a dialysis method with a molecular weight cut-off of 8000–14,000. Two millilitres of the formulation was placed into a dialysis tube (8 mm diameter, 14,000. Two millilitres of the formulation was placed into a dialysis tube and immersed in 25 ml of release media maintained at 34.5 °C in a shaking water bath (100 rpm). At predetermined intervals, 2.0 ml of the release medium was withdrawn and the same volume of fresh release medium was added to maintain sink conditions. The amount of TA released was quantified by HPLC. The release study was carried out in triplicate. The equation (Equation (3)) used to calculate the cumulative percentage of drug release was as follows:

\[
Q = (C_n * 25 + \sum_{i=1}^{n-1} C_i)/C_0 * 100
\]

where \(C_n\) is the drug concentration (μg mL\(^{-1}\)) at time \(t\), \(C_i\) is the drug concentration at the \(i\)-th sampling point, \(C_0\) is the total amount of drug in the solution, and \(V\) is the sampling volume.

In vitro transcorneal permeation study

The transcorneal experiment was performed with a modified Franz vertical diffusion cell [23]. New Zealand white rabbit was humanely killed by an intravenous injection of excess urethane, and the whole eyes were enucleated. The method of dissection of the cornea was the same as those reported.
previously [24]. The receptor compartment was filled with 5 ml of SAF (pH 7.4), while 0.5 of sample (equivalent to 0.1 mg of TA) were applied to the epithelial side. The opening of the donor compartment was sealed with a cover slip and the receptor compartment was maintained at 34 °C with constant stirring, using a magnetic stir bead. At predetermined intervals, 2.0 ml of permeation medium was withdrawn and the same volume of fresh permeation medium was added. The amount of TA permeated was quantified by HPLC. All the experiments were conducted in three repeats. The cumulative permeation amount, $Q_n$ (Equation (4)), the apparent permeability coefficient, $P_{app}$ (Equation (5)), and the steady state flow rate, $J_{ss}$ (Equation (6)), were calculated for each sample.

$$Q_n = V_0 \left( C_n + \frac{V}{V_0} \sum_{i=1}^{n-1} C_i \right) = V_0 C_n + V \sum_{i=1}^{n-1} C_i = 5C_n + 2 \sum_{i=1}^{n-1} C_i$$

where $C_n$ is the drug concentration at time $t$, $C_i$ is the drug concentration at the time point before the last sampling time point $t$, $V_0$ is the total volume of the medium in the receiving pool (5 ml), and $V$ is the sampling volume (2 ml).

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot C_0 \cdot A \cdot 3600} \text{ cm s}^{-1}$$

where $C_0$ is the initial drug concentration in the supply pool, and $\Delta Q/\Delta t$ is the steady-state slope of the linear portion of the cumulative permeated amount of drug in the receiving chamber ($Q$) plotted versus time ($t$).

$$J_{ss} = C_0 P_{app}$$

**Histopathological study**

The in vivo evaluation of the tested formulations for possible ocular irritation potential was conducted following the low-volume eye test procedure, which was actually a modification of the Draize Test [25–27]. The tested eyes were observed at 1, 2, 4, 12, 24, 48, and 72 h to compare changes in the cornea, iris, and the conjunctival secretion while controlling for the bulbar conjunctival oedema. Long-term irritation tests were the same as those of single-dose eye irritation but lasted for 7 days. After irritation tests, the rabbits were sacrificed by air injection. The eyeball was fixed in 4% formaldehyde and the receptor compartment was maintained at 34 °C, while 0.5 mmol of SAF (pH 7.4) was used as the perfusate at a perfusion rate of 0.15 ml/min. After perfusion for 1 h to alleviate the local minimally invasive state, 180 µl (equivalent to 36 µg of TA) of the preparation was dropped into the conjunctival sac of the eye. After administration, samples were collected continuously every 30 min for 6 h. Each sample was processed without treatment, and thus, could be directly tested using HPLC.

**Results**

**Characterisation of the two types of nanoparticle complexes**

Particle size distributions of the PLGA nanoparticles and 2-HPC-β-CD/PLGA nanoparticle complexes are shown in Figure 1(A,B), respectively. The average particle size of the PLGA nanoparticles and 2-HPC-β-CD/PLGA nanoparticle complexes were 163.2 ± 2.8 and 149.4 ± 3.7 nm, respectively. The PDI values were all within 0.2, i.e., the particle size distribution range was narrow, and the system was relatively uniform. As shown in Figure 1, the PLGA nanoparticles and 2-HPC-β-CD/PLGA nanoparticle complexes (Figure 1(A,B), respectively) showed negative values of zeta-potential (−16.14 ± 1.23 and −14.05 ± 0.39 mV, respectively).

The TEM image of PLGA nanoparticles and 2-HPC-β-CD/PLGA nanoparticle complexes are shown in Figure 2(A,B), respectively. The particle size observed under TEM was larger than that recorded using the laser particle size analyser, which may be due to sample drying that causes nanoparticle aggregation during pre-treatment of sample for TEM. Under magnification of 20,000× and 50,000×, PLGA nanoparticles (Figure 2(A-2,A-3)) and 2-HPC-β-CD/PLGA nanoparticle complexes (Figure 2(B-2,B-3)) appeared to be spherical and uniformly dispersed. Their particle size was uniform, which further indicated that the prepared nanoparticle system was similar as well.

The loading capacity and entrapment efficiency of PLGA NPs and 2-HPC-β-cycloexetrin-PLGA nanoparticle complexes was 0.19 ± 0.014% and 18.4 ± 0.7%, 0.52 ± 0.035% and 76.0 ± 4.8% respectively. The addition of cycloextrin effectively increases the entrapment efficiency, which should benefit from the increased solubility of the drug and the stability of the system due to the presence of cycloextrin.

**Evaluation of interactions in the nanoparticle complexes**

The nature of interactions between the drugs and PLGA or 2-HPC-β-CD was established with FTIR spectroscopy since any kind of physicochemical interaction that may take place, such as...
as the formation of hydrogen bonds between the drugs and PLGA or 2-HP-β-CD, will automatically lead to frequency shifts or splitting of the absorption peaks. The FTIR spectra of pure TA, the physical mixtures of each component, PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes were interpreted. The results are shown in Figure 3. From the infra-red spectrum, it can be seen that due to the vibrations of the carbon-carbon double bond (C=C) and ketonic carbonyl (C=O), the TA showed four strong absorption peaks between 1708 cm⁻¹ and 1600 cm⁻¹. The vibration of the aliphatic C-H bond was observed at 2960 cm⁻¹ and 2871 cm⁻¹. The physical mixture also had the same characteristic peaks in these wavelength ranges. In the infra-red spectrum of PLGA nanoparticles (Figure 3(A)) and 2-HP-β-CD/PLGA nanoparticle complexes (Figure 3(B)), all the characteristic peaks of TA disappeared, while the ketonic carbonyl absorption peak of 1708 cm⁻¹ exhibited a blue-shift to 1740 cm⁻¹. The characteristic peak of TA at 2960 cm⁻¹ exhibited a red-shift to the amorphous state of 2931 cm⁻¹. It is speculated that the interaction between TA and the
nanoparticles may be due to the self-assembly of the carrier via hydrogen bonding and the like. The modification of PLGA nanoparticles by 2-HP-β-CD did not affect the presence of TA in the nanoparticles.

Differential scanning calorimetry (DSC) of the PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes were carried out in order to examine the possible changes in the specific heat of PLGA. The result is shown in Figure 4. It can be seen from the DSC spectrum that TA exhibited a melting endothermic peak at about 290°C, where the physical mixture also had a characteristic peak, but its absorption peak signal was weak, which may be due to the low content of TA in the sample. In PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes, the characteristic absorption peaks of TA disappeared. This amorphization was in accordance with the XRD patterns (Figure 5), which indicated that TA was not in a crystalline state [29] within the nanoparticles, as well as with the FTIR spectra (Figure 3), which indicated the conversion of drug to amorphous and distribution at nano/uniform level in the NPs.

For further identification of the physical state of the drugs incorporated in PLGA nanoparticles, XRD analysis was used and the patterns of pure drugs, as well as TA-loaded PLGA nanoparticles and TA-loaded 2-HP-β-CD/PLGA nanoparticle complexes were obtained. The results from this analysis are shown in Figure 5. It can be seen from the XRD pattern that the characteristic map of TA showed a plurality of sharp high-intensity peaks at 8–30°, indicating that TA existed in the form of crystals. The physical mixture also exhibited a weak characteristic peak at the corresponding position, which may be due to the small amount of raw material in the physical mixture. Furthermore, the amorphous substance containing PVA, PLGA, or the like was more in amount, resulting in poor crystallinity. However, it was still apparent that the drug was present in its crystalline form in the physical mixture, and no change in its crystallinity occurred. In PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes, the crystal diffraction peak of TA disappeared, which may be due to TA forming a molecular dispersion or an amorphous nanodispersion within the PLGA matrix of the nanoparticles [30,31].
In vitro drug-release studies

In vitro drug release studies can preliminarily predict the release behaviour of nanoparticles in vivo. Since the bioavailability of the drug is usually low in the form of local eye drops, frequent administration is required, and it is difficult to achieve a therapeutic effect. Therefore, it is important to prepare a nanoparticle capable of releasing and maintaining sufficient drug concentrations for a certain period of time. It can be seen from Figure 6 that the cumulative drug release from 2-HP-β-CD/PLGA nanoparticle complexes in PBS, artificial tears, and artificial aqueous humour is more than 90%. The release amount and release rate of 2-HP-β-CD/PLGA nanoparticle complexes were significantly higher than that of the PLGA nanoparticles and TA aqueous suspension.

In vitro transcorneal permeation study

Cyclodextrins have been shown to reduce drug irritation after being topically administered to the eye and to enhance chemical stability of drugs in aqueous eye drop formulations. However, increasing the amount of dissolved drug in the aqueous tear fluid by addition of CDs, while keeping the tear fluid saturated with drug, does not lower the drug activity [32–34]. By modifying the PLGA nanoparticles with 2-HP-β-CD, the overall hydrophilicity of the nanoparticles increased; therefore, nanoparticles were able to better disperse in the tear film.

The results of in vitro drug transcorneal permeation test are shown in Figure 7. The cumulative permeation amount of
2-HP-β-CD/PLGA nanoparticle complexes (27.591 ± 10.05 μg) was significantly higher than that of the PLGA nanoparticles (3.973 ± 2.10 μg) and the TA aqueous suspension (3.567 μg) after 12 h.

The cumulative permeability amount \( (Q_c) \) was fitted with time \( (t) \) to calculate the apparent permeability coefficient \( (P_{app}) \). The \( P_{app} \) of the TA aqueous suspension was \( 9.83 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \). As shown in Table 1, the \( P_{app} \) of TA aqueous suspension was low, which is attributable to the hydrophobic nature of the drug itself that limits its diffusion through the stromal layer. The \( P_{app} \) of 2-HP-β-CD/PLGA nanoparticle complexes were 4.93 times of PLGA nanoparticles and 7.19 times of TA aqueous suspension, respectively. Furthermore, there was a significant difference \( (p < .05) \). It can be further illustrated that the modification of 2-HP-β-CD can produce a significant penetration-enhancing effect on the PLGA nanoparticles.

The steady-state flow rate \( (J_{ss}) \) of TA aqueous suspension was \( 0.17 \times 10^{-3} \text{ μg} \cdot \text{s}^{-1} \cdot \text{cm}^2 \). The results of the steady-state flow of nanoparticles are shown in Table 1. As compared to the PLGA nanoparticles and TA aqueous suspension, the steady-state flow of 2-HP-β-CD/PLGA nanoparticle complexes was significantly improved. Maintaining a drug concentration at saturation in the tear water sample layer helped maintain the driving force for drug diffusion into the aqueous humour and increased the amount of drug that passed through the cornea [35].

From the cumulative drug permeation amount-time curve (Figure 7), it was deduced that TA in the nanoparticles penetrated the excised cornea at a continuously increasing rate over a period of 8 h. In order to further elucidate the release characteristics of PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes, the model was used to fit the cumulative drug permeation amount and time in 8 h, and the release characteristics of different nanoparticles and TA aqueous suspension were investigated. The first-order release model equation, the Higuchi model equation, the Ritger-Peppas model equation, and the zero-order model equation were fitted; the correlation was determined using the model correlation coefficient. The results are given in Table 2.

### Histopathological study

The Draize method was used to evaluate the eye irritation potential of PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes, with saline solution and TA aqueous suspension as control materials. For all formulations, the corneal and iris scores were zero (Table 3). Although conjunctival hyperaemia was observed in the group administered the 2-HP-β-CD/PLGA NP complexes, there was no significant difference between the groups administered normal saline solution and TA aqueous suspension. Conjunctival hyperaemia led to conjunctival sensitivity to exogenous compounds. The total scores of all formulations were valued between 0 and 3 in a single-dose or long-term eye irritation test. These results showed that PLGA nanoparticles did not stimulate any action in the rabbit eye tissues and were less irritating than the aqueous suspension.

Results from histological analysis of the corneal sections of different formulations after long-term irritation are shown in Figure 8. As can be seen from Figure 8, satisfactory epithelium and stroma structure with a little oedema were maintained after the administration of normal saline solution (Figure 8(A-1–A-3), respectively). After long-term irritation tests, the corneal epithelial cells of eyes were treated with PLGA nanoparticles that exhibited some slight oedema (Figure 8(B-1–B-3), respectively). However, there was no significant difference between the two groups \( (p > .05) \).
**In vivo transcorneal penetration**

In this study, microdialysis was used for pharmacokinetic experiments. As compared to traditional keratotomy, microdialysis is a local minimally invasive sampling technique that can help reduce the sample size of animals and also the differences caused by having many animals as subjects in the experiment. The dialysis window of the microdialysis probe has a certain molecular weight cut-off value; if the collected sample does not contain biological macromolecules, such as proteins and enzymes, it can be directly injected and analysed without further processing [36]. Therefore, sample loss that occurs during sample preparation can be avoided, and the measured results are also closer to the true drug concentration in the body. In the previous study, the positive and negative recovery rates of microdialysis probes during in vitro and in vivo reverse recovery of the anterior chamber were examined. The experimental results showed that these rates of recovery were similar, and there was no significant difference. It was indicated that the in vivo reverse recovery rate can be used to infer the true concentration of the tested drug. Both in vitro and in vivo recovery rates decreased with increasing perfusion rate, regardless of the concentration of perfusate. In the pharmacokinetic experiments, the concentration of the drug in the animal changes with time, and the probe recovery rate is only applicable if it is not affected by the concentration. Taking into account the sensitivity and practical application of the detection instrument, this study used a perfusion rate of 0.5 \( \mu \text{L} \cdot \text{min}^{-1} \) for pharmacokinetic experiments.

It can be seen from the drug concentration-time curve in the aqueous humour ([Figure 9](#)) that the drug permeation of 2-HP-\( \beta \)-CD/PLGA nanoparticle complexes after eyedrop administration is significantly better than that of PLGA nanoparticles and aqueous drug solution. The pharmacokinetic properties of the drug in aqueous humour after administration of eye drops were investigated. The data are shown in [Table 4](#). The peak concentration \( (C_{\text{max}}) \) of 2-HP-\( \beta \)-CD/PLGA nanoparticle complexes group was significantly higher than that of the PLGA nanoparticles group and the TA aqueous solution group, which were 23.2 times and 36.8 times higher, respectively. It was observed that the PLGA nanoparticles that were modified by 2-HP-\( \beta \)-CD were beneficial for the absorption of drugs and improved their bioavailability.

The area under the curve (AUC) for the 2-HP-\( \beta \)-CD/PLGA nanoparticle complexes in aqueous humour increased by 20.5 times and 44.9 times as compared to the AUC before...
modification and that of the aqueous solution, respectively; the mean residence time (MRT) increased by 1.16 times and 1.15 times, respectively, as well. These data indicate that the drug can maintain an effective concentration in the aqueous humour for a longer period of time.

**Discussion**

PLGA has been widely reported for ocular delivery. However, due to the special environment of the ocular surface and special structure of the cornea, the overall performance of the TA-loaded PLGA nanoparticles has yet to be improved. In our current work, we have developed and evaluated a new colloidal system, 2-HP-β-CD-modified PLGA nanoparticle complexes, for TA delivery that gives better hydrophilicity to the nanoparticles and improves their corneal penetration.

The particle size and zeta-potential of nanoparticles play an important role in the uptake of nanoparticles by cells [37] by affecting their transport and penetration. It has been reported in literature that nanoparticles with a particle size of less than 200 nm can carry drugs around the cornea and deliver them to the posterior segment of the eye [38]. The average particle size of PLGA nanoparticles and 2-HP-β-CD/PLGA nanoparticle complexes prepared in this study were all less than 200 nm, and the PDI values were all within 0.2, which was consistent with the particle size requirements for delivery to the posterior segment of the eye. The size and zeta-potential of the 2-HP-β-CD/PLGA nanoparticle complexes were both smaller than that of the PLGA nanoparticles, probably because the 2-HP-β-CD in the aqueous phase provided a lipophilic cavity for the system. It was seen in the TEM image that the outer layer of the PLGA nanoparticle was light in colour; the outer layer of 2-HP-β-CD/PLGA nanoparticle complexes was not present, probably due to the modification of 2-HP-β-CD, increasing the hydrophilicity of the outer layer of 2-HP-β-CD/PLGA nanoparticle complexes.

Moving on, DSC, XRD, and FTIR studies were performed to determine if the drug incorporated into the nanoparticle system was crystalline, amorphous, or in a combined form. The PLGA nanoparticles and the 2-HP-β-CD/PLGA nanoparticle complexes did not exhibit peaks indicating the presence of the drug, suggesting that the drug was encapsulated in the polymer matrix or might have taken an amorphous form within the polymer matrix. Combining the results of the three spectra, it can be further inferred that TA may exist in the nanoparticles in an amorphous state. It can be seen from the comparative results of the map that modification of the 2-HP-β-CD does not affect the form that the drug takes when it is present within the nanoparticles.

**In vitro** release profile showed an initial rapid release of 2-HP-β-CD/PLGA nanoparticle complexes. This initial rapid release of the drug may be due to the trancer drug adsorbed on the surface of the 2-HP-β-CD/PLGA nanoparticle complexes [39,40], and also due to some of the drug being released from the 2-HP-β-CD. In this initial release, the effective drug concentration can be achieved in a short time, which is helpful in improving the bioavailability of the drug. In the medium term, TA continues to be released from the polymer due to hydration and swelling of the polymer [41–43]. The 24h cumulative release of 2-HP-β-CD/PLGA nanoparticle complexes was higher than 90%, and the release amount and release rate were significantly higher than PLGA nanoparticles and TA suspension. The reason may be that the addition of cyclodextrin to the polymer system can enhance drug release by acting as a channelling or wicking agent or by promoting erosion of the matrix [44,45]. It is also possible that cyclodextrin promotes hydration of polymer systems [46]. It was seen in the TEM image that the surface of the 2-HP-β-CD/PLGA nanoparticles is rougher than the surface of the PLGA nanoparticles, and there are more black spots, which may be grooves or voids on the surface of the nanoparticle. These all promote the erosion of the matrix and the release of the drug.

The results of in vivo transcorneal penetration experiments show that 2-HP-β-CD-modified PLGA nanoparticles can significantly increase the cumulative permeation of nanoparticles so that it becomes much higher than that of the PLGA nanoparticles and TA aqueous suspension. Pharmacokinetic experiments indicated that 2-HP-β-CD-modified PLGA significantly increased the area under the drug concentration-time curve of the nanoparticles in the aqueous humour, the average residence time in the body, and the peak drug

![Graph](image)

**Figure 9.** Concentration–time curves of TA in the aqueous humor (mean ± SD, n = 6). PLGA; Polylactic-co-glycolic acid; 2-HP-β-CD, 2-HP-β-cyclodextrin.

| Formulation          | \(C_{\text{max}}\) mg L\(^{-1}\) | AUC\(_{0\rightarrow t}\) h\(^{\text{h}}\)\(/\text{mg} L\(^{-1}\) | MRT h | \(T_{\text{max}}\) h | VRT h\(^{2}\) |
|---------------------|------------------------|------------------|------|----------------|--------|
| 2-HP-β-CD-NPs       | 13.25 ± 2.87           | 24.531 ± 1.64    | 2.299 ± 0.18 | 1.5 ± 0 | 1.55 ± 0.44 |
| NPs                 | 0.57 ± 0.28            | 1.199 ± 0.35     | 2.286 ± 0.23 | 1.5 ± 0 | 1.363 ± 0.18 |
| Aqueous solution    | 0.36 ± 0.17            | 0.546 ± 0.17     | 1.982 ± 0.15 | 1.5 ± 0 | 0.658 ± 0.12 |

\(C_{\text{max}}\): peak concentration; AUC\(_{0\rightarrow t}\): area under the curve; MRT: mean residence time; \(T_{\text{max}}\): the peak time; VRT: variance of residence time.

Table 4. Pharmacokinetic parameters of TA in the aqueous humour after topical instillation (n = 3).
concentration in the aqueous humour. 2-HP-β-CD/PLGA nanoparticles complexes significantly increased the bioavailability of TA in the eye. This result is consistent with the in vitro transcorneal permeation study. This indicates that a hydrophilic modification is added to facilitate corneal permeation of the nanoparticles. This may be due to the fact that hydrophobic PLGA is easily lost by tears flushing into the nasolacrimal duct, while hydrophilic 2-HP-β-CD can penetrate the tear film by carrying a lipophilic molecule that is poorly soluble in water. Therefore, the drug can be passively transported into the anterior chamber of the eye to improve the bioavailability of the drug.

In summary, our findings suggest that 2-HP-β-CD/PLGA nanoparticle complexes may achieve desired therapeutic effects by improving dispersion of TA in the tear film and enhancing corneal permeation, supporting improved bioavailability and effective delivery of drugs to eye.

Conclusion

The results of this study indicate that 2-HP-β-CD can significantly improve the pharmacokinetic behaviour of TA-loaded PLGA nanoparticles in the eye. This effect may be attributable to the action of 2-HP-β-CD and tear film characteristics. We can conclude that the combination of 2-HP-β-CD and PLGA nanoparticles can increase the penetration of the drugs, and thus, their concentration in the aqueous humour. Therefore, the TA-loaded 2-HP-β-CD/PLGA nanoparticle complex formulation is a promising alternative to conventional eye drops that needs to be clinically evaluated.

Disclosure statement

No potential conflict of interest was reported by the authors.

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