Topical ophthalmic lipid nanoparticle formulations (SLN, NLC) of indomethacin for delivery to the posterior segment ocular tissues

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ABSTRACT

Purpose: The objective of the present study was to formulate indomethacin (IN)-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) and to investigate their potential use in topical ocular delivery.

Methods: IN SLNs (0.1% w/v) and NLCs (0.8% w/v) were prepared, characterized and evaluated. Their in vitro release and flux profiles across the cornea and sclera-choroid-RPE (trans-SCR) tissues and in vivo ocular tissue distribution were assessed. Furthermore, chitosan chloride (CS) (mol. wt. < 200 kDa), a cationic and water-soluble penetration enhancer, was used to modify the surface of the SLNs, and its effect was investigated through in vitro transmembrane penetration and in vivo distribution tissue studies.

Results: For the IN-SLNs, IN-CS-SLNs and IN-NLCs, the particle size was 226 ± 5, 265 ± 8, and 227 ± 11 nm, respectively; the zeta potential was −22 ± 0.8, 27 ± 1.2, and −12.2 ± 2.3 mV, respectively; the polydispersity index (PDI) was 0.17, 0.30, and 0.23, respectively; and the entrapment efficiency (EE) was 81 ± 0.9, 91.5 ± 3.2 and 99.8 ± 0.2%, respectively. The surface modification of the SLNs with CS increased the ocular tissue distribution were assessed. Furthermore, chitosan chloride (CS) (mol. wt. < 200 kDa), a cationic and water-soluble penetration enhancer, was used to modify the surface of the SLNs, and its effect was investigated through in vitro transmembrane penetration and in vivo distribution tissue studies.

Conclusion: The results suggest that lipid-based particulate systems can serve as viable vehicles for ocular delivery. The NLC formulations demonstrated increased drug loading capability, entrapment and delivery to anterior and posterior segment ocular tissues.

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1. Introduction

Indomethacin (IN), 2-[(4-chlorophenyl) carboxyl]-5-methoxy-2-methyl-1H-indol-3-yl] acetic acid, a topical non-steroidal anti-inflammatory drug (NSAID) is used in the treatment of the ocular inflammatory disorders such as conjunctivitis, uveitis, cystoid macular edema, and anterior segment inflammation, including post-operative pain following cataract surgery [1–3]. The compound elicits its anti-inflammatory action through the inhibition of COX-2 enzymes, which are essential for prostaglandin biosynthesis, and thus possesses analgesic and anti-pyretic properties [4]. The potential effects of prostaglandins include elevation of intraocular pressure, vasodilatation, disruption of blood ocular barriers, and leukocyte migration; hence, potent inhibition of COX-2 enzymes may provide therapeutic effects [5]. NSAIDs are employed in the treatment of diabetic retinopathy and age-related macular degeneration [6]. Formulating IN as a topical ophthalmic solution is challenging due to its poor solubility and stability [7]. Indosol, which is an aqueous solution of IN complexed in TRIS-sodium salt (tromethamine), has been widely used in ophthalmic research to treat inflammation of the anterior segment and the uvea [8]. Currently, topical ophthalmic formulations of IN are not marketed in the United States. Indocilyre® (hydro-poly(ethylene glycol) (PEG) ophthalmic) 0.1% w/v eyedrops, which are commercially available in Europe, are associated with poor ocular bioavailability [9]. Conventional dosage forms and other formulation approaches have been employed to improve the intraocular penetration of IN into the posterior ocular tissues. The formulation strategies that
have been tested include the application of solutions, suspensions, polymeric nanoparticles, surfactant-based systems, implants, nanomicelles, emulsions and gelling systems [10–12].

The ocular bioavailability of drugs from topical solutions is very poor, and it has been reported that less than 5–10% of the administered drug reaches the inner ocular tissues. Approaches for improving the pre-corneal residence time and transcorneal permeability characteristics could enhance intracocular bioavailability [13,14]. In recent years, colloidal nanoparticulate systems have gained popularity as a promising ocular drug delivery platform [15]. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are colloidal nanoparticulate systems designed and developed to deliver lipophilic drugs. These particulates are composed of biocompatible and biodegradable materials and are in the nanometer size range. All excipients used in these formulations are generally regarded as safe, and process scale-up is feasible. Several studies have demonstrated superior ocular bioavailability of therapeutic agents from these colloidal nanoparticulate systems, possibly because of improved retention and phagocytosis by epithelial cells. Furthermore, adsorption of polymers, such as chitosan, on the surface of SLNs may further improve the retention of the nanoparticles on the epithelial surface and increase cellular uptake of the nanoparticles [16,17]. Chitosan possesses favorable biological characteristics, such as biodegradability, biocompatibility and mucoadhesive properties [18,19]. The ability of chitosan and its derivatives in ophthalmic solutions to modulate characteristics of the epithelial barrier through the transient opening of the tight junctions, which results in enhanced transmembrane absorption, has been widely reported in the literature [20–22]. In this study, chitosan was used to modify the surface characteristics of SLNs (chitosan adsorbed onto the SLN surface), and the ocular penetration of IN from the chitosan-coated SLNs was evaluated.

Varying the lipid component in the colloidal framework could also improve drug release characteristics and chemical stability. Alpha Rx developed Ocusolin™, a gentamicin loaded-SLN, is still in preclinical trials.

NLCs, however, appear to be a viable alternative to SLNs in terms of drug loading efficiency and are prepared by incorporating liquid lipids within the solid lipid structure. Depending on the ratio and concentration of the solid and liquid lipids, NLCs with different structural matrices can be obtained [23,24].

The objective of the current study was to develop and characterize various formulations, such as SLNs, CS-SLNs and NLCs, and to evaluate the ocular delivery and disposition of IN from these topically administered formulations.

2. Materials and methods

Compritol® 888 ATO (glyceryl behenate) was a gift from Gattefosse (Paramus, NJ, USA). Miglyol 812® and 829® were kindly supplied by Sasol, Hamburg, Germany GmbH. PROTASANT® ultrasound Chitosan (Chitosan chloride < 200 kDa) was received from NovaMatrix (Philadelphia, PA, USA). Amicon® Ultra centrifugal filter devices with regenerated cellulose membranes (molecular weight cut-off of 100 kDa), poloxamer 188, hydroxypropyl-beta-cyclodextrin (average molecular weight: 1380; degree of substitution: 0.6), Tween® 80, indomethacin, high-performance liquid chromatography (HPLC)-grade solvents, and other chemicals (analytical grade) were obtained from Fisher Scientific (Hampton, NH, USA). Whole eyes of male albino New Zealand rabbits were obtained from Pel-Freez Biologicals (Rogers, AR, USA). Male albino New Zealand rabbits were procured from Harlan Labs (Indianapolis, IN, USA).

2.1. Saturation solubility studies

Saturation solubility, as a function of pH, was studied using the standard shake-flask method. An excess amount of IN was added to screw-capped glass vials containing 200 mM phosphate buffer at different pH values, namely 5.5, 6.0, 6.5, 7.0, and 7.4, and phosphate buffer with various solubilizers, such as HPβCD, RMβCD, poloxamer 188, and Tween® 80. To achieve uniform mixing, samples were stirred at 100 rpm for 24 h at 25°C in a reciprocating water bath (Fisher Scientific). After 24 h, the samples were centrifuged (AccuSpin 17R), and the supernatant was analyzed for drug content.

2.1.1. Chromatography system for in vitro sample analysis

Samples were analyzed for IN content using an high performance liquid chromatography (HPLC)-UV method. The system comprised a Waters 717 plus Autosampler, a Waters 2487 Dual λ Absorbance detector, a Waters 600 controller pump, and an Agilent 3395 Integrator. A Phenomenex Luna® C18 4.6 mm × 250 mm column was used for the analysis. The mobile phase used was methanol, water, and orthophosphoric acid (70:29.05:0.05). The detection wavelength λmax for IN was 270 nm. The flow rate was set to 1 mL/min during the analysis.

2.2. Formulations

2.2.1. IN-TSOL, IN-SOL and IN-CS-SOL formulations

IN-TSOL was prepared by dissolving 0.1% w/v IN in 1% w/v Tween® 80 solution, which was used to investigate the release characteristics compared to the test formulations and to evaluate barrier resistance. Additionally, IN-SOL was prepared by dissolving IN (0.1% w/v final concentration) in an aqueous solution containing Tween® 80 (1% w/v) and propylene glycol (29.3% w/v). Sodium hydroxide (1 N) was added in small increments to adjust the pH. IN-CS-SOL was prepared by adding chitosan chloride (CS; 0.1% w/v final concentration) to IN-SOL. The pH of the final formulations was maintained at 6.8 because Indocollyre®, a formulation marketed in Europe, is at this pH.

2.2.2. IN-HPβCD and IN-CS-HPβCD solution formulations

IN-HPβCD formulation was prepared by dissolving IN (0.1% w/v) in 2.5% w/v HPβCD solution prepared in isotonic phosphate-buffered saline (IPBS; pH 6.8). IN-CS-HPβCD was prepared by the addition of 0.1% w/v CS to the IN-HPβCD formulation. The final pH of the formulations was adjusted to 6.8.

2.2.3. Indomethacin solid lipid nanoparticles (IN-SLN) and IN-SLN-HPβCD formulations

The solubility of IN in a wide variety of lipids was visually evaluated to select suitable lipid excipients for formulating the SLNs/NLCs. IN-loaded SLNs were prepared based on a hot homogenization method. Solid lipid, namely Compritol® 888 ATO, was melted, and IN (5% w/w with respect to the lipid) was dissolved therein to obtain a clear lipid phase. Simultaneously, an aqueous phase prepared using the surfactants poloxamer 188 (0.25% w/v) and Tween® 80 (0.75% w/v) and glycerin (2.25% w/v) in bidistilled water, was heated. The hot aqueous phase was added to the melted lipid phase while stirring, and the premix was then subjected to emulsification at 16,000 rpm for 6 min using a T 25 digital Ultra-Turrax. The pre-emulsion obtained was homogenized under high pressure, using previously optimized process parameters (15–20 K psi; 6 cycles; 6 min), using a thermostated Emulsiflex C5 (Avestin) resulting in the formation of a hot emulsion dispersion [9]. The sample cylinder was preheated and maintained at 80 ± 2°C during the entire process using an electrical cord harness that was wrapped and securely fastened to the sample cylinder.
and connected to an external thermostat. The hot emulsion obtained was slowly cooled to room temperature to form the IN-SLNs. The final concentrations of Compritol® 888 ATO and IN in the formulation were kept constant at 2% w/v and 0.1% w/v, respectively.

Additionally, a variation of the IN-SLN formulation was prepared wherein 2.5% w/v HPβCD (final concentration) was added to the aqueous phase described above prior to the preparation of the SLNs. The pH of the resulting formulations was adjusted to 6.8 using NaOH (1 N).

2.2.4. Indomethacin nanostructured lipid carriers (IN-NLCs)

The NLCs contained both solid (Compritol 888® ATO) and liquid lipids (Miglyol® 812 or 829), unlike the SLNs, which contained only solid lipids. The total amount of lipid employed in the NLCs was 4 and 8% w/v, of which Compritol 888® ATO constituted 60% and Miglyol® 812 or 829 made up the remaining 40% of the lipids. The concentration of surfactants (Tween 80® and poloxamer 188) and propylene glycol in the NLC formulations was maintained identical to that in the SLNs. Drug loading in all NLC formulations was kept constant at 0.8% w/v. The prepared formulations were then characterized and evaluated with respect to in vitro release and transcorneal permeation and in vivo ocular tissue levels.

2.2.5. Chitosan-coated IN solid lipid nanoparticles (IN-CS-SLNs)

CS (mol. wt. < 200 kDa) was used for surface modification of the SLNs. CS (0.1% w/v) was incorporated into the aqueous phase prior to preparation of the SLNs, as described above (in vitro studies). CS at a concentration of 0.25% w/v was used in the SLNs for the in vivo experiments. Surface modification of the CS-coated formulations was confirmed through zeta potential measurements.

2.3. Particle size, polydispersity index (PDI) and zeta potential measurements

The hydrodynamic radius and the PDI of the particle size, as well as the zeta potential, were measured using an iontophoresis system (Zetasizer Nano ZS Zen3600, Malvern Instruments, Inc.) at 25 °C and with 173° backscatter detection in disposable folded capillary clear cells. The measurements were obtained using a helium-neon laser of 633 nm, and the particle size analysis data were evaluated based on the volume distribution.

Zeta potentials were measured at 25 °C in folded capillary cells using the same instrument. To measure the particle size distribution and zeta potential, the SLN samples were diluted (1:500) with water. Distilled and 0.2-μM filtered waters were used for these measurements and were performed in triplicate.

2.4. Assay and entrapment efficiency (EE)

The lipid in the IN-SLN dispersion, the IN-CS-SLN and the IN-NLCs were determined by photon correlation spectroscopy using a Zetasizer Nano ZS Zen3600 (Malvern Instruments, Inc.) at 25 °C and with 173° backscatter detection in disposable folded capillary clear cells. The measurements were obtained using a helium-neon laser of 633 nm, and the particle size analysis data were evaluated based on the volume distribution.

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Zeta potentials were measured at 25 °C in folded capillary cells using the same instrument. To measure the particle size distribution and zeta potential, the SLN samples were diluted (1:500) with water. Distilled and 0.2-μM filtered waters were used for these measurements and were performed in triplicate.
contents in the IN-SLN, IN-CS-SLN and IN-NLC formulations were 0.1% w/v, 0.1% w/v and 0.8% w/v, respectively. Three milliliters of the optimized IN-SLNs, IN-CS-SLNs or IN-NLCS was added to the donor chamber after adjusting the pH to 6.8. The donor IN concentration was maintained at 0.1% w/v in the SLN formulation and 0.8% w/v in the NLC formulation. The receiver chamber medium consisted of 3.2 mL RMjCD (2.5% w/v) in IPBS solution for all the transport studies. The difference in the volume of liquid between the two half-cells (3 mL on one side and 3.2 mL on the other) creates a pressure difference across the corneal membrane. Due to this difference in hydrostatic pressure, the cornea bulges out, mimicking the natural curvature of the cornea [25–28]. The circumference of the corneal/scleral limbus is clamped together with air tight clamps such that the scleral membrane was exposed between the donor and receptor chamber and fastened securely with respect to IN peaks in any of the tissues. The limit of detection (LOD) for various ocular tissues was determined and corresponded to 10 ng for AH, 10 ng for VH, 5 ng for the cornea, 5 ng for the sclera, 10 ng for the RPE, and 10 ng for the IC.

2.9. Trans-SCR permeability studies of IN formulations

The scleral tissue with the retinal pigment epithelium and choroid layers excised from whole eyes, obtained from Pel-Freez Biologicals, were used to determine the in vitro trans-SCR (sclera-choroidal RPE) permeability of IN from the formulations. After excision, the scleral membranes were washed with IPBS (pH 7.4) and mounted on Vialia-Chien cells (PermeGear, Inc®). The scleral tissues were mounted as an inverted cup onto diffusion cells between the donor and receptor chamber and fastened securely with air tight clamps such that the scleral membrane was exposed to the donor compartment (episcleral side) and the RPE-choroidal tissues were in contact with the receptor compartment (vitreous body side). Five milliliters of 2.5% w/v RMjCD solution prepared in IPBS (pH 7.4) was used as the media in the receiver chamber during the course of the study for 2.5 h. Five hundred microliters of IN-SOL, IN-HpjCD, IN-SLNs (pH 6.8 and 7.4), and IN-SLNs + HpjCD were added to the donor chambers, and the concentration was maintained at 0.1% w/v. Aliquots (600 µL) were withdrawn from the receiver chamber at predetermined time points up to 3 h and replaced with an equal volume of 2.5% w/v RMjCD in IPBS. The samples were stored at −80 °C until further analysis of IN using the chromatography system described in Section 2.1. Additionally, the transcorneal permeabilities of IN from the IN-SOL formulation (control) and IN-HpjCD in the presence of CS as a penetration enhancer were also determined.

2.10. In vivo bioavailability studies

In vivo bioavailability of IN was determined in Male New Zealand White albino Rabbits, weighing between 2 and 2.5 kg, procured from Harlan Labs. All the animal studies conformed to the tenets of the Association for Research in Vision and Ophthalmology statement on the use of animals in ophthalmic vision and research and the University of Mississippi Institutional Animal Care and Use Committee approved protocols. Rabbits were anesthetized with a combination of ketamine (35 mg/kg) and xylazine (3.5 mg/kg) injected intramuscularly and were maintained under anesthesia throughout the experiment. The IN formulations, namely the IN-SOL, IN-HpjCD, and IN-SLNs, were evaluated in vivo. All the above IN topical formulations (100 µL) were given as two doses (50 µL), 30 min apart, (T−30 min and 0 min) to reduce precorneal loss. Additionally, the IN-CS-SOL, IN-SLNs, IN-CS-SLNs (n = 6) and IN-NLCS were administered to conscious rabbits to delineate the effects of anesthesia on the ocular bioavailability of IN. CS was used at a concentration of 0.25% w/v (in vivo). Two hours post-topical application, the rabbits were euthanized with an overdose of pentobarbital injected through a marginal ear vein. The eyes were washed thoroughly with ice-cold IPBS and were immediately enucleated. All the intraocular tissues were separated and stored at −80 °C, and further analysis was carried out using the HPLC-UV system (Section 2.1). All experiments were performed in triplicate.

2.10.1. Biosample preparation for the determination of IN content in ocular tissue homogenates

The in vitro analytical HPLC-UV method described above was employed for sample analysis following method validation. A protein precipitation technique was employed to determine the amount of IN in the ocular tissue homogenates. Briefly, tissues including the cornea, sclera, iris-ciliary (IC), and retina-choroid (RC) were cut into small pieces, and a mixture of ice-cold acetoni-trile and 0.1% v/v formic acid was added (1 mL) to precipitate proteins from each individual tissue. The supernatant was then collected via centrifugation for 1 h at 13,000 rpm prior to the analysis. The aqueous humor (AH) (200 µL) and vitreous humor (VH) (500 µL) were precipitated by adding an ice-cold mixture of acetoni-trile and formic acid, 200 µL of each for the AH and 500 µL of each for the VH corresponding to a ratio of 1:1. Quantification of IN was performed using standard calibration curves constructed from various ocular tissues, such as the cornea (20–500 ng), the sclera (20–500 ng), the AH (10–200 ng), the VH (10–200 ng), the IC (10–200 ng), and the RPE (10–200 ng). All the standard curves had a coefficient of determination \( r^2 \geq 0.96 \). The recovery of IN was evaluated by spiking the drug in pure AH and VH and comparing the expected IN concentration with the standard concentration. Recovery values were determined for AH (93.1) and VH (91.5). Interference was not observed from co-eluted protein residues with respect to IN peaks in any of the tissues. The limit of detection (LOD) for various ocular tissues was determined and corresponded to 10 ng for AH, 10 ng for VH, 5 ng for the cornea, 5 ng for the sclera, 10 ng for the RPE, and 10 ng for the IC.

2.11. Data analysis

The steady-state flux (SSF) for transcorneal and trans-SCR experiments was calculated by dividing the rate of transport by the surface area. The slope of the cumulative amount of IN transported versus time plot was used to obtain the rate of IN transport across the excised rabbit cornea. The flux was calculated using the Eq. (2):

\[
\text{Flux (J) = (dM/dt)/A}
\]  

where \( M \) is the cumulative amount of drug transported, and \( A \) is the surface area of the corneal membrane (0.636 cm²) exposed to the permeant (drug).

The transcorneal permeability was determined by normalizing the SSF to the donor concentration, \( C_d \), according to Eq. (3).

\[
\text{Permeability (Papp) = Flux/Cd}
\]  

2.12. Statistical analysis

One-way-ANOVA coupled with a post hoc test was employed to analyze the differences between groups (in vivo). The difference in data obtained was considered statistically significant at the level of \( p < 0.05 \).
3. Results

3.1. Morphometrical and physico-chemical characteristics

The particle size, zeta potential, PDI and EE of IN-SLNs, the IN-CS-SLNs and the IN-NLCs were observed to be 226 ± 5, 265 ± 8, and 227 ± 11 nm; –22 ± 0.8, 27 ± 1.2, and –12.2 ± 2.3 mV; 0.17, 0.30, and 0.23; and 81 ± 0.9, 91.5 ± 3.2 and 99.8 ± 0.2%, respectively. The quantitative compositions of the various IN formulations are presented in Table 1. The particle size of the IN-NLCs increased as the total lipid content increased, and IN-NLC-F1 appears to be a promising formulation, exhibiting a lower hydrodynamic radius and better physico-chemical characteristics compared to the other formulations (Table 2).

3.2. Solubility of IN in the presence of cyclodextrins and surfactants

Cyclodextrins and surfactants are frequently employed as solubilizers in topical ophthalmic formulations. The solubilities of IN in 0.25 and 0.5% w/v poloxamer 188 at pH 7.4 were measured as 251.8 ± 78 and 657 ± 127 µg. The solubility of IN in 0.5% w/v Tween® 80 at pH 7.4 was determined to be 1055 ± 106 µg. The solubility profiles of IN in 5% w/v HPβCD and RMβCD were found to be similar. However, the solubility of IN is highly pH-dependent and tends to be predominantly solubilized at high pH (Fig. 1).

3.3. Stability, moist-heat sterilization and FTIR studies

IN-CS-SLNs and IN-NLCs exhibited good stability when compared to the IN-SLN formulation. The particle size of the IN-SLN formulation after storage for 90 days at 40 °C was increased by 65%, whereas the IN-CS-SLNs and IN-NLCs displayed a 15–20% increase in particle size (Fig. 2). Additionally, the EE of the SLN formulations decreased by 12% compared to the IN-CS-SLNs (6%) and IN-NLCs (5%) (Fig. 3A). The zeta potential and PDI of the IN formulations were not changed significantly under the storage conditions tested here (Figs. 3B and 3C). Fig. 4 shows the effect of sterilization on the physicochemical characteristics of the IN lipid-based formulations. No significant differences were observed post-sterilization. The particle size of the IN-CS-SLNs and IN-NLC formulations slightly increased compared to the IN-SLNs following autoclaving. Additionally, the FTIR spectra revealed a slight drug-excipient interaction in the lipid formulations (Fig. 5).

3.4. In vitro release studies

Based on the solubility profile of IN in different solubilizing agents, RMβCD in IPBS at pH 7.4 was chosen as the receptor media for the in vitro release and transcorneal permeability experiments. The in vitro releases of IN from the IN-TSOL, the IN-SLNs, the F-1IN-NLCs, and the F-2 IN-NLCs were observed to be 81.6 ± 2.1 µg, 18.7 ± 0.9 µg, 31.8 ± 3.9 µg, and 23.3 ± 3.1 µg within the time period tested (6 h). The in vitro release of IN from these formulations is depicted in Fig. 6.

3.5. Transcorneal permeability studies

The transmembrane permeabilities of IN from the IN-SLNs, the F-1IN-NLCs, and the F-2 IN-NLCs were observed to be 1.93 ± 0.17 × 10⁻⁵, 1.34 ± 0.13 × 10⁻⁵, and 1.2 ± 0.1 × 10⁻⁵ cm/s, respectively. The transcorneal flux of IN was increased by twofold when CS was used as a permeation enhancer in the SLN formulation. Moreover, the effect of including CS as a penetration enhancer in the solution formulations, namely in IN-SOL and IN-HPβCD, was also investigated. CS significantly enhanced the transcorneal flux of IN by ~3.5 and ~2-fold for the IN-SOL and IN-HPβCD formulations, respectively (Fig. 7).

3.6. Trans-sclera-choroid-RPE (SCR) permeability studies

Trans-SCR permeability experiments were carried out to assess the scleral penetration capability of IN from the formulations compared to the corneal absorption route. The trans-SCR permeability of IN-HPβCD was markedly higher compared to IN-SOL. The trans-SCR permeabilities of the SLN increased in the order of IN-SLNs (pH 6.8) < IN-SLNs (pH 7.4) < IN-SLNs + HPβCD (pH 6.8). The SLNs in combination with HPβCD demonstrated a higher trans-SCR permeability than the SLN formulation alone (Fig. 8).

3.7. In vivo bioavailability studies

Based on the transcorneal and trans-SCR data obtained, the IN formulations were investigated for their ocular bioavailability and disposition of IN 2 h post-topical administration in anesthetized and conscious rabbits. The IN-SOL formulation could not deliver the drug to the posterior ocular tissues. However, the IN-SOL formulation was able to achieve drug levels in the anterior segments of the eye, including 463.5 ± 15 ng/g in the cornea and 224 ± 8.6 ng/g in the scleral tissues. CS as a penetration enhancer further improved the ocular bioavailability of IN-SOL. Significant drug levels were attained from the IN-HPβCD formulation in most of the ocular tissues tested, namely in the cornea (3267.3 ± 1867.6 ng/g), the sclera (575.7 ± 433.5 ng/g), the AH (877.4 ± 492.5 ng/g), the RC (94.4 ± 79.9 ng/g) and the IC (1203.9 ± 547 ng/g). Significantly higher levels of IN were observed in the posterior segments of the eye for the use of the SLN formulation compared to the IN-SOL and IN-HPβCD formulations. The formation of SLNs in combination with 0.25% w/v CS achieved higher levels of IN in conscious rabbits (n = 6). The NLC formulations, however, were the most effective in terms of drug loading

Table 1

<table>
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<tr>
<th>Formulation composition</th>
<th>IN formulations</th>
<th>IN-HPRCD</th>
<th>IN-SLNs</th>
<th>IN-HPβCD</th>
<th>IN-SLNs</th>
<th>IN-SLNs</th>
<th>IN-CS-SLNs</th>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HPβCD (mg)</td>
<td>–</td>
<td>–</td>
<td>250</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Water (mL)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
and ocular IN levels. The ocular tissue IN concentrations obtained from the above formulations are shown in Figs. 9 and 10.

4. Discussion

The objective of the current work was to develop IN-loaded lipid-based nanoparticles and to investigate the in vitro corneal permeation and in vivo ocular disposition of IN from these formulations. Most NSAIDs are inherently weak acidic drugs with poor corneal penetration due to their ionization at lacrimal pH. Lowering the pH of these formulations increases corneal penetration but also increases potential irritation. Additionally, it has been reported that due to the anionic nature of NSAIDs, they are incompatible with preservatives such as benzalkonium chloride and could form insoluble complexes [29–31]. IN (pKa of 4.5) exhibits pH-dependent solubility, which increases as a function of higher pH (acidic to neutral/alkaline: 1.5 µg/mL at pH 1.2 and 105.2 µg/mL at pH 7.4). IN displayed a solubility of 0.64 ± 0.02 mg/mL in phosphate buffer at pH 7.0, which is consistent with previously reported data [32,33]. Additionally, the solubility of IN was increased by ~5 and ~6-fold with 5% w/v HPβCD and RMβCD in phosphate buffer at pH 7.0.

SLNs and NLCs are colloidal nanoparticulate dispersions that can be administered topically in the form of eyedrops. A major advantage of the nanoparticulate systems is their uptake by epithelial cells, which allows for greater penetration into the surface layers [34–36]. Moreover, the small size, biocompatibility and mucoadhesive properties of SLNs improve their interactions and prolong the pre-ocular residence time of drugs, thus enhancing drug bioavailability [37,38]. The literature suggests that surface modification of SLNs by coating with hydrophilic agents such as poly (ethylene) glycol derivatives (PEGs) or chitosan can further improve ocular penetration, mainly due to enhancing interactions

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Table 2
Composition of lipid mixtures used in the NLC formulations. The particle size characteristics, PDI, zeta potential, entrapment efficiency and assay values for each formulation are presented below.

<table>
<thead>
<tr>
<th>IN-NLC formulations</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
<th>F-5</th>
<th>F-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN (0.8% w/v)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Compritol (60%)</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>Miglyol 812 (40%)</td>
<td>160</td>
<td>-</td>
<td>320</td>
<td>640</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Miglyol 829 (40%)</td>
<td>-</td>
<td>160</td>
<td>-</td>
<td>-</td>
<td>320</td>
<td>640</td>
</tr>
<tr>
<td>Total lipid (%; mg)</td>
<td>4%; 400</td>
<td>4%; 400</td>
<td>8%; 800</td>
<td>16%; 1600</td>
<td>8%; 800</td>
<td>16%; 1600</td>
</tr>
<tr>
<td>Particle size (nm)</td>
<td>227</td>
<td>279.1</td>
<td>304.1</td>
<td>519.2</td>
<td>385.1</td>
<td>629.1</td>
</tr>
<tr>
<td>Polydispersity index (PDI)</td>
<td>0.235</td>
<td>0.259</td>
<td>0.433</td>
<td>0.58</td>
<td>0.456</td>
<td>0.381</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>−12.2</td>
<td>−5.57</td>
<td>−0.92</td>
<td>0.027</td>
<td>−2.58</td>
<td>−0.304</td>
</tr>
<tr>
<td>Entrapment efficiency (%) EE</td>
<td>99.8</td>
<td>99.74</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99.9</td>
</tr>
<tr>
<td>Assay (%)</td>
<td>96.8</td>
<td>97.8</td>
<td>97.4</td>
<td>91.3</td>
<td>96.2</td>
<td>92.5</td>
</tr>
</tbody>
</table>

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Fig. 1. pH-dependent saturation solubility of IN in phosphate buffer, 5% w/v HPβCD in phosphate buffer, and 5% w/v RMβCD in phosphate buffer (µg/mL). The results are depicted as the mean ± SD (n = 3).

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Fig. 2. Particle size characteristics of various IN formulations following storage at 4 °C, 25 °C/60% RH, and 40 °C/75% RH. The data represent the mean ± S.D (n = 3).
with the ocular mucosa and increasing cellular uptake and internalization [39,40]. Additionally, previous reports have demonstrated the ability of chitosan nanoparticles to produce a sharp, reversible decrease in the transepithelial electrical resistance (TEER) and to improve the permeability of model macromolecules [41]. The mechanism of mucoadhesion is possibly through the electrostatic interaction between the positively charged amino groups of chitosan and the negatively charged sialic acid residues of ocular mucosa [42].

The corneal route is a major absorption pathway for topically administered medications [43]. Compared to other formulations, IN-SLN demonstrated higher transcorneal permeability, which may be ascribed to endocytosis or transcytosis uptake mechanisms [44,45]. The in vitro transcorneal permeability of IN from the NLC formulations was comparatively lower than that of the SLNs, probably because of higher entrapment in the oily phase and thus lower partitioning into the membrane. Reports have suggested that chitosans with a moderate degree of deacetylation (65–80%) and a relatively high molecular weight (170–200 kDa) is required for the exertion of optimal transepithelial penetration and low toxicity [46–48]. The in vitro transcorneal flux of IN from the IN-SOL, IN-HP\(\beta\)CD, and IN-SLN formulations increased by 3.5-fold, 2-fold, and 2-fold, respectively, in the presence of CS, which is consistent with previous reports.

Numerous reports have demonstrated that sclera is more permeable to hydrophilic than to lipophilic molecules and approximately 10 times more permeable than the cornea [49,50]. Based on trans-scleral transport studies, the higher permeability across several static layers of ocular tissues (sclera, Bruch's membrane-choroid, RPE, and neural retina) demonstrates the diffusional ability of drugs to the back of the eye through the conjunctival-scleral pathway [51,52]. To investigate drug delivery to the posterior ocular segments, scleral diffusion of IN was assessed in trans-SCR experiments. The higher observed trans-scleral permeability of IN from the SLNs compared to the IN-SOL and IN-HP\(\beta\)CD formulations demonstrated that lipid carriers could enhance accumulation in
scleral tissue, prolonging the ocular residence time \textit{in vivo}. The increased permeability of IN from SLNs with HPβCD in external aqueous phase could be due to the complexation effect of cyclodextrin with the free drug. The trans-scleral permeability of SLNs at pH 7.4 (2.99 ± 0.1 \times 10^{-5} \text{cm/s}) was considerably higher than that of the SLNs at pH 6.8 (2.13 ± 0.3 \times 10^{-5} \text{cm/s}), which is likely due to an increase in the ionized fraction of IN at pH 7.4.

Castelli et al. [53] fabricated SLNs and NLCs of IN and characterized the formulations with respect to drug distribution and entrapment efficiencies in the lipid matrices. However, the loaded drug in the SLNs and NLCs of IN was maintained at 2 and 1.5\% w/w with respect to the total lipid, whereas in the present study, drug loading of IN in the SLNs and NLCs was achieved at 5 and 20\% w/w, respectively. The IN lipid formulations demonstrated higher drug loading and entrapment efficiencies at lower lipid contents compared to Castelli’s formulations. Bucolo et al. [54] investigated the ocular pharmacokinetics of IN following a multiple dosing treatment regime (30 \mu L/eye; four times in 8 h) of 0.5\% IN + hydroxypropyl methylcellulose, IN-HPMC and Indocollyre\textsuperscript{e} eye-drops in conscious rabbits. The drug levels in the AH, RC and VH obtained 2 h post-topical administration of IN-HPMC and Indocollyre\textsuperscript{e} solution were 360 ± 40 and 100 ng/mL, 65 and 20 ng/g, and 7 ± 2 and 5 ± 2 ng/mL, respectively. Campos et al. [42] studied the ocular distribution of chitosan-fluorescein nanoparticles (0.25\% w/v, dose: 100 \mu L, 250 \mu g) and their interaction with the corneal and conjunctival epithelia in conscious rabbits. The drug levels in
the cornea and conjunctiva 2 h post-topical application were 760 ± 60 and 1000 ± 150 ng/g, respectively. In another study, Klang et al. [55] formulated positively charged submicron emulsions (0.1% w/v) and compared the formulation with Indocollyre®/C210. The drug concentrations obtained in the cornea and conjunctiva 1 h after topical instillation of 50 μL of the test formulation were nearly 40 and 30% lower, respectively, compared to the marketed formulation. The drug concentrations obtained in the AH and sclera-retina were found to be 75 ± 38 ng/mL and 450 ± 200 ng/g (submicron emulsion) vs 110 ± 50 ng/mL and 450 ± 200 ng/g (Indocollyre®), respectively. Yamaguchi et al. [56] investigated ocular tissue IN concentrations upon use of chitosan-coated emul-

Fig. 5. FTIR spectral images of the IN + Compritol physical mixture, Compritol, IN, IN-SLN, IN-CS-SLN and IN-NLC F-1 formulations.

Fig. 6. In vitro release of IN from various formulations across Spectra/Por® membranes at 34 °C. Receiver solution consisted of IPBS containing 2.5% w/v RMjCD (pH 7.4). The results are depicted as the mean ± S.D (n = 3).
Fig. 7. Transcorneal flux across isolated rabbit cornea from various IN topical formulations at 34 °C. The receiver solution consisted of IPBS containing 2.5% w/v RMβCD (pH 7.4). The results are depicted as the mean ± S.D (n = 4).

Fig. 8. Trans-SCR permeability of IN from various topical ocular formulations at 34 °C. The receiver solution consisted of IPBS containing 2.5% w/v RMβCD (pH 7.4). The results are depicted as the mean ± S.D (n = 3).

Fig. 9. IN ocular tissue concentrations (ng/g of tissue) obtained from the IN-SOL, IN-HPβCD, and IN-SLN formulations 2 h post-topical administration in the anesthetized rabbit model. The data represent the mean ± S.D. (n = 3). AH: Aqueous humor; VH: Vitreous humor; IC: Iris-Ciliary; RC: Retina-Choroid. (N.D., not detected). Different symbols, such as μ and β, indicate significant differences (p < 0.05) of the IN-HPβCD and IN-SLN formulations compared to IN-SOL. ¥ represents a significant difference in the ocular tissue concentrations of IN for use of the IN-SLNs compared to all other formulations.
sion (0.1% w/v) formulations 1 h post-topical instillation (50 μL) in both eyes of anesthetized male Japanese albino rabbits. The drug levels attained were predominantly in the cornea (3596 ± 425 ng/g), aqueous humor (434 ± 90 ng/mL) and conjunctiva (868 ± 188 ng/g). In another study, an IN (0.1% w/v) ophthalmic solution was prepared using Poloxamer 407 (10% w/w) and compared with an Indoclyre® formulation in terms of AH concentration 2 h after a multiple post-topical administration regime of 150 μL (6 × 25 μL at 90 s intervals). The IN concentration in the AH was enhanced by ~2-fold compared to the use of Indoclyre® [7].

In comparison with all the ocular tissue levels obtained with the above-discussed formulations, the IN-CS-SLNs delivered significantly higher levels of IN to the anterior and posterior segment ocular tissues, which could be attributed to mucoadhesive and epithelial barrier-modulating properties of chitosan. Compared to IN-SOL without CS, the incorporation of CS in the IN-SOL formulation improved penetration of IN into the AH and IC bodies, but retinal tissue IN levels remained below the detection limit. The addition of viscosity modifiers, such as hydroxypropyl methylcellulose, in the IN-CS-SOL formulation may further prolong the pre-corneal residence and thus increase ocular drug levels. IN concentrations were reduced by ~4–5-fold in conscious animals compared to the anesthetized model, delineating the effects of anesthesia on ocular pharmacokinetics. In comparison, at higher doses (0.8% w/v; 8-fold dose) the IN-NLCs delivered ~4–5-fold higher concentrations than the IN-CS-SLNs, which could be due to higher drug loading, EE and pre-ocular retention of IN for the use of the NLC formulation. NLCs are superior to SLNs in terms of higher IN levels in the ocular tissues. The IN-CS-SLNs demonstrated superior trans-membrane IN permeation characteristics compared to the IN SLNs, confirming the penetration-enhancing properties of chitosan. It is worth noting that the IN-CS-SLNs, containing a tenth of the loaded drug of the IN-NLCs, induced IN concentrations in the inner ocular tissues (the AH, IC and RPE-choroid) that were 3–4-fold lower than those obtained for IN-NLCs. The corneal and scleral IN concentrations achieved using the IN-NLCs were significantly higher, indicating the effect of the increased drug loading in the formulation. Thus, both the IN-CS-SLNs and IN-NLCs are viable platforms for the delivery of IN to the posterior segment ocular tissues.

5. Conclusion

Targeting NSAIDs to the posterior segment of the eye via a topical route is a challenging task due to formulation constraints and the anatomical, physiological and efflux barriers present in ocular tissues. IN-loaded NLC formulations displayed higher drug-loading capabilities and entrapment efficiencies, which resulted in higher IN levels in the ocular tissues. The IN-CS-SLNs demonstrated superior trans-membrane IN permeation characteristics compared to the IN SLNs, confirming the penetration-enhancing properties of chitosan. It is worth noting that the IN-CS-SLNs, containing a tenth of the loaded drug of the IN-NLCs, induced IN concentrations in the inner ocular tissues (the AH, IC and RPE-choroid) that were 3–4-fold lower than those obtained for IN-NLCs. The corneal and scleral IN concentrations achieved using the IN-NLCs were significantly higher, indicating the effect of the increased drug loading in the formulation. Thus, both the IN-CS-SLNs and IN-NLCs are viable platforms for the delivery of IN to the posterior segment ocular tissues.

Conflict of interest

The authors have declared no conflict of interests regarding this article.
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