Research highlight

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Topical ocular delivery of a COX-II inhibitor via biodegradable nanoparticles

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Abstract: The present investigation strives to formulate nanoparticles of poly-ε-caprolactone (PCL), containing celecoxib (CXB), a non-steroidal anti-inflammatory agent. The CXB-PCL nanoparticles were formulated by solvent displacement method and optimized based on formulation variables like drug-to-polymer ratio and surfactant concentration. The formulations were characterized for particle dimensions, surface morphology, physicochemical features, percentage drug incorporation efficiency, *in vitro* drug release, *in vitro* trans-corneal permeation, *in vivo* efficacy against arachidonic acid-induced ocular inflammation, and stability study. The prepared nanoparticles were nearly spherical having particle sizes ranging from 89.16±8.2 nm to 191.27±12.1 nm with maximum entrapment efficiency of 97.03±0.20%. The drug release was in sustained fashion (<75% drug released after 8 h) and obeyed zero-order release kinetics. The *trans*-corneal permeation was significantly higher than the aqueous suspension of CXB ($p=0.05$). Further, % hydration level was observed within permissible ranges suggesting ocular tolerability. The anti-inflammatory activity was found better as there was observed an improvement in parameters like lid closure score, PMN counts, and protein content against CXB aqueous suspension. The formulations were stable as evident from accelerated stability testing results. Thus, the CXB-PCL nanoparticles may prove a viable alternative to conventional dosage forms offering enhanced ocular bioavailability and compatibility with ocular milieu.

Keywords: nanoparticles; nanosuspension; ocular inflammation; protein estimation.

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1 Introduction

Drug delivery may bring about a huge difference in ophthalmic therapeutics. Insofar as the drug delivery concerns, the eye is quite fascinating, a distinct multicompartmental organ with different tissues, their grains and fluid flow variables. At present, topical application via eye drops contributes to approximately 90% of all ocular dosage forms. However, this is quite inefficient and, in a few cases, results into grave side effects. There is observed an extent of drug penetration inside the cornea up to approximately 5% of the medicine from eye drop dosage form, and the same travels to the eye interiors, whereas the leftover is dumped or wasted. Moreover, the application of eye drops yields differing degrees of drug availability inside ocular tissues and thereby poses constraints in therapeutic efficacy [1–4]. Hence, novel ocular drug conveyance approaches are extremely essential for enhancing the delivery efficacy and minimizing side effects and for prolonging the healing influence by regulating the extent of drug carriage.

The use of colloidal systems for ocular drug delivery is among such approaches, which offer the feasibility of regulated release of drug and drug targeting, improved endurance of drug, greater loading of actives, with limited toxicity of the materials [5–7].

Amid the various polymers employed for the development of nanocarriers containing medicines, poly-ε-caprolactone (PCL) is experiencing elaborated studies aimed at fabricating formulations being biodegradable. PCL is quasi-crystalline in nature possessing a glass conversion value of -60°C and a thawing behavior in the range of 59–64°C, based on PCL's crystal-like

characteristics. Indeed, this aliphatic polyester is having immense potential for regulated drug applications because of its steadiness, great perviousness for several drugs, well suited, ability to target, outstanding capability of making mixtures with many polymers, extremely poor decay speed [in comparison with several renowned drug vehicles, like poly(D,L-lactide-co-glycolide) (PLGA)], biodegradable, biocompatible, as well as devoid of toxic effects [8, 9]. The PCL has been employed for delivery of a variety of antiphlogistic agents such as dexamethasone, indomethacin, celecoxib, flurbiprofen, aceclofenac, etc., through different formulations [10–15].

Ophthalmic swelling is a usual consequence from cataract surgery, causing pain as well as photophobia among several patients and evidently resulting in grave complications embracing raised intraocular force, posterior capsule murkiness, cystoid macular inflammation, and reduced optical perspicacity. Stimulation of phospholipase A2, subsequent to tissue challenges, converts cell laminar phospholipids to arachidonic acid that makes the base for proceeding activities chiefly through the cyclo-oxygenase (COX) and the lipoxygenase passages. Outputs like arachidonic acid metabolites, accompanied by some biochemical intermediates, react for yielding swelling [16, 17]. COX-2 expression is enhanced with ophthalmic inflammation or injury [18]. The corticosteroids are frequently used topically for the treatment of eye inflammations. Their application is usually linked with a rise in intraocular pressure, cataract progression, as well as threat arising due to infections [19]. Non-steroidal anti-inflammatory drugs (NSAIDs) covering indomethacin, flurbiprofen, diclofenac, aceclofenac, and ketorolac may be used as effective alternatives of steroids for treating ocular inflammation [20–24]. COX-2-specific NSAIDs (such as celecoxib, firocoxib, rofecoxib, lumiracoxib, valdecoxib), which leave COX-1 unaffected and block only COX-2, may be beneficial for the management of diverse ocular inflammations.

Celecoxib {4-[5-(4-methyl phenyl)-3-(trifluoro-methyl)-1-H-pyrazol-1-yl]} being a NSAID possessing selectivity for COX-2 inhibition is employed for the cure of inflammatory arthritis, osteoarthritis, pain, and familial adenomatous polyposis. This is about 300-fold much specific for COX-2 than COX-1 [25].

Therefore, the present investigation strived to formulate and characterize poly-ε-caprolactone nanoparticles containing celecoxib and assess the antiphlogistic effectiveness of optimized preparation versus arachidonic acid-triggered eye inflammation in rabbits.

2 Materials and methods

2.1 Materials

Celecoxib (CXB) was obtained as a gift courtesy of Ranbaxy Research Laboratories (Gurgaon, India). The polymer poly-ε-caprolactone (LCTEL®) of molecular weight ~20 kDa (as per gel permeation chromatography assessment) was procured from DURECT Corporation, Birmingham, AL, USA. Pluronic® F-68 was purchased from BASF Corporation (Parsipanny, NJ, USA). Acetone and mannitol were supplied by SD Fine Chemical Limited (Mumbai, India). Arachidonic acid was procured from Merck Chemical Ltd. (Darmstadt, Germany). The goat eyeballs were bought from an abattoir nearby. The rabbits used were received from the animal house of the institute.

2.2 Methods

2.2.1 Formulation of nanoparticles

The solvent displacement method was used to formulate nanoparticles as discussed by Espuelas et al. [26]. In brief, 100 mg of PCL and CXB (10 mg) were solubilized in acetone (20 ml) by moderate warming and sonication. The solution was then tenderly drizzled into deionized distilled water (40 ml) having 100 mg of Pluronic® F-68 with magnetic agitation of 1000 rpm. The aqueous phase turned milky following the addition of organic phase indicating the formation of colloidal dispersion. The nanoparticulate suspension so resulted was centrifuged at 12,600×g for 1 h (Sorvall™ RC6+, Thermoscientific, Radnor, PA, USA). The supernatant (comprising acetone and water) was drawn off. The pellet was rinsed two times using deionized distilled water, subsequently freeze dried using mannitol (5% w/v) as a cryoprotectant. The preparations were also formulated employing varying drugs: polymer ratio and concentrations of surfactant.

2.3 Characterization of nanoparticles

2.3.1 Particle size and ζ potential study

The nanoparticle dispersion was made in distilled water with prior ultrasonication for 30 s. The average particle size, ζ potential, and polydispersity index (PDI) of the suspension of the nanoparticles containing CXB

were determined via dynamic light-scattering procedure employing Zetasizer (Nano ZS-90, Malvern Instruments, Worcestershire, UK) attached thru the DTS software.

2.3.2 Surface morphology

The transmission electron microscopy (268D, FEI, Eindhoven, The Netherlands) was employed to characterize the morphology of the nanoparticles. The nanoparticle dispersion (5–10 µl) was allowed to spread over copper lattices (Rachna Metal Industries Pvt Ltd, New Delhi, India) covered by colladion in amyl acetate. Following thorough desiccation, the samples were marked with phosphotungstic acid (2% w/v). The image capture and analysis tasks were accomplished using Digital micrograph as well as soft imaging viewer software (Olympus, Singapore).

2.3.3 Encapsulation efficiency of nanoparticles

The amount of celecoxib loaded was determined by suspending 25 mg of the nanoparticle formulation in distilled water (5 ml). The nanosuspension was then centrifuged using an ultracentrifuge at 25,200×g (Sorvall™ RC6+, Thermoscientific, Radnor, PA, USA) for half an hour at 4°C. The supernatant was tested for celecoxib by ultraviolet-visible spectrophotometer (LAMBDA 45, Perkin Elmer, Waltham, MA, USA) at 250 nm. Total drug was assessed after solubilizing nanoparticles in acetonitrile (1 ml of dispersion to 25 ml of acetonitrile). The content of celecoxib encapsulated in the nanoparticles was computed by subtraction of the content that was present in the supernatant from the total concentration utilized to formulate the nanoparticles. The percentage entrapment efficiency (% EE) was calculated using the equation as under:

$$\%\ EE = \frac{\text{Total drug-Free drug}}{\text{Total drug used in formulation}} \times 100 \quad (1)$$

2.3.4 Physicochemical characterization

2.3.4.1 Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

The nanoparticle samples were analyzed using an ATR-FTIR spectrometer (Bruker ALPHA, Billerica, MA, USA). Approximately 2 mg of lyophilized powder was placed on the sample holder. Interferograms readings at a spectral resolution of 4 cm^{-1}; means were calculated for each sample after 32 scans. The spectra were measured using Opus software in the range of 4000–600 cm^{-1}.

2.3.4.2 Powder X-ray diffraction (PXRD)

This profile was determined by utilizing an X-ray diffractometer (X'Pert-PRO, PANalytical, Almelo, The Netherlands) with CuK_ emission produced at 45 kV and 40 mA within a diffraction angle array of 5°–50° 2θ.

2.3.4.3 Differential scanning calorimetry (DSC)

This thermal determination was carried out employing a DSC calorimeter (TA-60, Shimadzu, Tokyo, Japan). Samples were subjected to heating within capped metallic pans in nitrogen flow (50 ml/min) with a rate of scanning, i.e. -10°C/min, ranging from 40°C to 200°C.

2.3.5 *In vitro* drug release

Desired amounts of nanoparticles were suspended in Sorenson's phosphate buffer (pH 7.4) to form nanosuspensions having the CXB (0.1%, w/v). In order to investigate the *in vitro* release of CXB from nanosuspension, the dispersion (1 ml) was kept inside a dialysis sac (Dialysis membrane-110, cutoff: 12–14 kDa, Himedia, Mumbai, India). The dialysis sac was then placed in vials containing 20 ml of Sorenson's phosphate buffer (pH 7.4) at 37°C using a horizontal water bath shaker (Scientech Instruments, New Delhi, India) operating at a speed of 25 oscillations per minute. Aliquots of 5 ml samples were taken at several time pauses and tested for CXB concentration at 250 nm. The volume was supplanted with a similar bulk of new dissolution medium.

2.3.6 *In vitro* transcorneal permeation studies

In vitro transcorneal permeation was done utilizing freshly expunged goat corneas (paired). These corneas were sandwiched amid donor as well as receptor portions of an all-glass modified Franz diffusion cell in the manner as their epithelia met the donor part. A zone amounting to 0.64 cm^2 was available for diffusion from the cornea. Recently formed bicarbonate ringer solution (pH 7.4) was filled into the receptor cautiously to eliminate air bubbles. An aliquot (1 ml) of the formulation or CXB suspension in Sorenson's phosphate buffer (pH7.4) was poured into the donor, which was then, closed using a cover slip, and the receptor liquid temperature was kept at 37°C with continuous agitation by a Teflon-covered magnetic bead. The experiment lasted for 120 min, and samples were taken and tested for CXB concentration by assessing the absorbance at 250 nm by

an ultraviolet-visible spectrophotometer. The findings were represented as % permeation or *in vitro* ophthalmic obtainability. The same was calculated as under:

$$\%\ \text{Permeation} = \frac{\text{amount of drug permeated in receptor}}{\text{Initial amount of drug in donor}} \times 100 \quad (2)$$

When the permeation study was over, every cornea (devoid of sclera) was balanced, immersed in methanol (1 ml), desiccated at 90°C to a constant weight, and weighed again. The weight difference accounts for corneal hydration.

2.3.7 *In vivo* efficacy of CXB-PCL nanoparticles against arachidonic acid-stimulated ophthalmic swelling in rabbits

The arachidonic acid-stimulated rabbit eye inflammation model [27] was considered appropriate for comparing the anti-inflammatory effectiveness of CXB-containing nanoparticles against the aqueous suspension having a similar content of CXB. The study procedure was framed, and permission of the Institutional Animal Ethics Committee (IAEC) was received. The albino rabbits (six) of both sexes having body weights of 1–1.5 kg were grouped randomly in two groups having three rabbits/group. The rabbits were kept in typical ambience providing unrestricted outreach to diet as well as water. The sinister eye of every animal worked as the control and was instilled with 50 µl of isotonic phosphate buffer (pH 7.4) vehicle, whereas the eye dextris received 50 µl of CXB (0.1%, w/v) ocular suspension in isotonic phosphate buffer (pH 7.4) (Group-I) or 50 µl of CXB-PCL (0.1%, w/v) dispersion in isotonic phosphate buffer (pH 7.4) (Group-II). The 50 µl of arachidonic acid (0.5%, v/v formulated in phosphate buffer pH 7.0) was administered to the eyes after 10 min of instillation of the control vehicle or CXB-PCL nanosuspension. The eyes from both groups were afterward assessed for lid closing and polymorphonuclear leukocyte (PMN) relocation. Lid closing was marked as follows: 0, completely open; 1, ⅔ open; 2, ⅓ open; and 3, completely shut. PMN passage was determined by computing the PMN in tears. Normal saline (100 µl) was administered to the lower cul-de-sac of the eye, and following mild mingling, 50 µl of the tears was drawn in a WBC pipette at several time periods subsequent to arachidonic acid application. After appropriate dilution by Turke's fluid, the tally of PMN in the tear fluid was counted using a Neubauer hemocytometer [28]. The tear fluid was subjected to protein content estimation too.

2.3.7.1 Protein estimation

The amount of protein was determined in the tears. The protein content was assessed with the help of the Lowry procedure employing bovine serum albumin as the reference. In order to prepare solution A, sodium potassium tartarate (0.4 g) and copper sulfate (0.2 g) were dissolved in water (q.s. 1 l). Solution B was made by dissolving sodium carbonate (40g) in 1 l of 0.2 N of sodium hydroxide. Similar capacities of solution A and solution B were combined only before application (alkaline copper sulfate solution). Tear volume (20 µl) was blended completely with alkaline copper sulfate solution (5 ml) and kept at 37°C for 15 min. Afterward, to this was added 0.5 ml of Folin phenol reagent (diluted to 1 N) and mixed. Subsequent to 30 min of gestation, the absorbance of the samples was recorded at 660 nm employing an ultraviolet-visible spectrophotometer. This was compared with the same reference content ranging 0–200 µg/ml treated in the same manner [29, 30].

2.3.8 Stability studies

The nanoparticle formulations were placed into rivet-stoppered glass vials covered by metallic foil and put under accelerated stability evaluation by subjecting the CXB-PCL nanoparticles to 40±2°C and 75±5%RH in a stability chamber (HPP108/749, Memmert, Schwabach, Germany) as per Q1A (R2) quality guidelines of ICH. Samples placed on accelerated storage circumstances were drawn out at 0, 1.5, 3, and 6 months, and the CXB amount was determined.

2.3.9 Statistical analysis

The data of the results were tested for statistical impact by one-way analysis of variance (ANOVA) succeeded by Student-Newman-Keuls test or Student's t-test utilizing SigmaStat (version 3.1, Systat Software Inc., San Jose, CA, USA) wherever applicable. A p value <0.05 was assumed substantial.

3 Results and discussions

3.1 Particle size and ζ potential analysis

In the present study, we developed CXB-loaded PCL nanoparticles as appropriate carriers to be used in the treatment of ocular inflammations. The nanoparticles were

successfully formulated by solvent displacement method. The average particle size was of the magnitude ranging from 89.16±8.2 nm to 191.27±12.1 nm (Table 1). Upon an increase in the polymer amount, large-sized nanoparticles were obtained. This is because of enhanced viscosity of the medium at a high polymer amount, which hinders the diffusion of organic phase to aqueous phase resulting in larger particles [31]. There was observed a decrease in size on increasing the Pluronic F-68 content. This was because of the stabilizing effect of poloxamer, which acts by adsorbing at the droplet interface, thus, reducing surface tension and promoting mechanical and steric stabilization. The polydispersity index values were found to be <0.5 suggesting size homogeneity of the prepared nanoparticles. As apparent, average particle sizes are below 200 nm confirming their potential for ocular application. The CXB-PCL nanoparticles possessed ζ potential values ranging from -18.4±1.1 mV to -32.1±1.7 mV (Table 1). The degree of ζ potential offers a signal of the steadiness of the particulate preparations. The surface charge of the developed nanoparticles will allow their dispersion as the range obtained is well above the threshold values for agglomeration such as ±15 mV [32]. Thus, this surface charge is sufficient to confer physical stability to nanosuspension.

3.2 Surface morphology

The nanoparticle formulations were sphere shaped with a smooth surface as depicted by Figure 1. The nanoparticles with such morphological features will possibly be well tolerated by ocular surfaces. It is established that isometric particles possessing obtuse angles and edges lead to lower irritation compared to particles having sharp angles and edges [23, 33].

3.3 Entrapment efficiency of nanoparticles

The CXB-PCL nanoparticles were found to have entrapment efficiency values in the range from 89.94±0.36 to 97.03±0.20% (Table 1). The higher drug entrapment may be due to the lipophilic characteristic of celecoxib making its poor diffusion into the aqueous phase. As the concentration of the polymer was increased, the drug incorporation was also enhanced as larger particles may accommodate more amount of the drug. This behavior was seen with all selected drug:polymer ratios. The lowering of drug encapsulation was found upon an increase in surfactant concentration, which may be owing to the decrease in particle size.

3.4 Physicochemical characterization

The sample such as pristine drug, polymer, mannitol, physical mixture, and CXB-PCL nanoparticles were subjected to physicochemical determinations.

The ATR-FTIR spectral pattern of celecoxib (Figure 2) exhibited a unique S=O symmetric and asymmetric stretching at 1145 and 1344 cm^{-1}, correspondingly. Bands of moderate intensity at 3322 and 3330 cm^{-1} were observed

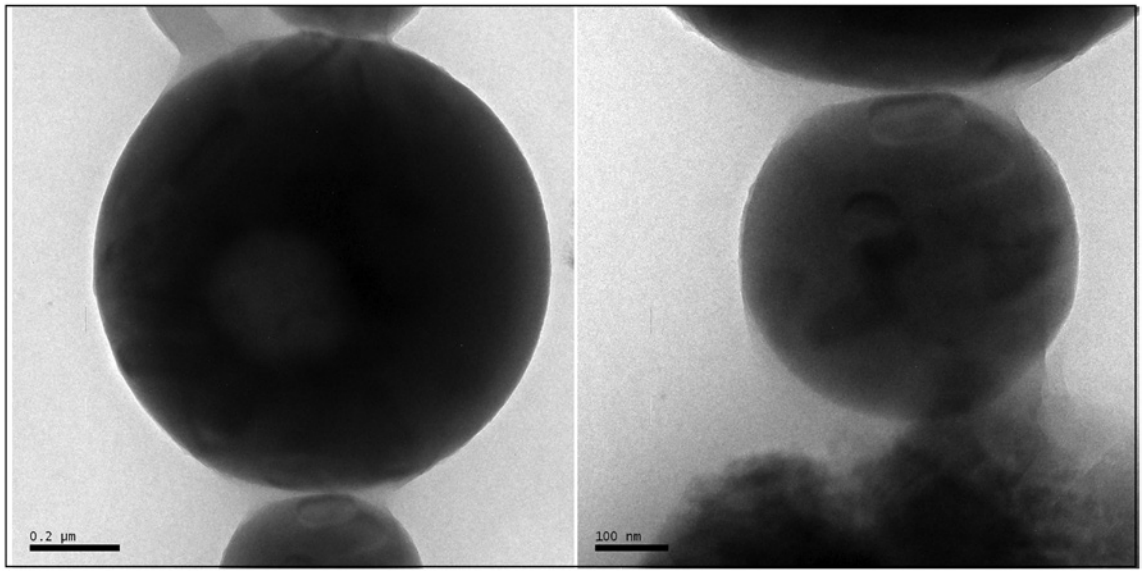

Figure 1: Transmission electron microscope images of CXB-PCL nanoparticles.

Table 1: Particle size, ζ potential, entrapment efficiency, % permeation, and hydration values for CXB-PCL nanoparticles.[a]

Formulation code[b]	Particle size (nm)	ζ potential (mV)	Entrapment efficiency (%)	Permeation (%)[d]	Hydration (%)
A	89.16±8.2	-18.4±1.1	89.94±0.36	6.71±0.47	75.86±0.61
B	146.25±10.4	-25.1±1.5	93.74±0.10	7.48±0.23	78.00±0.35
C	173.12±12.7	-32.1±2.1	96.91±0.05	8.63±0.30[d]	75.15±0.96
D	191.27±12.5	-23.0±1.2	97.03±0.20	8.24±0.64	75.87±0.74
E[c]	153.83±10.3	-27.9±1.7	95.11±0.11	8.54±0.71	77.75±1.03
F[c]	141.29±9.6	-20.1±0.9	93.34±0.23	7.22±0.63	78.09±0.34
G[c]	129.31±10.2	-28.2±1.4	92.51±0.18	8.32±0.68	77.54±0.37
CXB Suspension (0.1% w/v)	–	–	–	4.62±0.66	76.33±0.19

[a]All the data presented are mean±standard deviation (n=3).[b]Formulations made using varying drug:polymer ratios viz. A-1:5, B-1:10, C-1:15, D-1:20. [c]Formulations prepared employing different surfactant concentrations (% w/v), i.e. E-0.6, F-0.9, G-1.2. [d]Statistically significant ($p<0.05$) compared with CXB suspension, as determined by paired t-test.

as a doublet, which might be due to the N-H stretching vibration of the $-SO_2NH_2$ group. The C-N stretching band was recorded at 1558 cm^{-1}. The C-F stretching bands were observed at 786, 900 and 973 cm^{-1}. The presence of bands at 1223 and 1277 may be due to the C-H in the plane-bending aromatic. The band at 1445 cm^{-1} represents a C=C stretching aromatic. PCL displays a characteristic absorption band such as the carbonyl stretching mode at 1716 cm^{-1} (C=O) and asymmetric stretching at 2949 cm^{-1} (CH_2). Further, the appearances of bands at 1236 and 1164 were assigned to asymmetric COC and OCO stretching, respectively. The profile of mannitol was exhibited as OH stretching at 3386 cm^{-1}, OH in plane bending at 1395 cm^{-1}, and CO stretching at 1072 cm^{-1}. In physical mixture and CXB-PCL nanoparticles, the salient peaks for the drug are absent, whereas the peaks for PCL and mannitol are visible. This may be because of the dilution by the polymer as well as mannitol.

The DSC analysis of celecoxib showed the commencement of thermal events at about 54°C, which represents glass transition temperature indicating the amorphous nature of the drug. There is the presence of an exothermic peak at 101–106°C owing to the recrystallization of the amorphous drug to the crystalline state. There was seen as an endotherm at 165.78°C, matching with the melting point of celecoxib (Figure 3). The results are consistent with previous studies [34, 35]. The PCL thermogram was characterized by an endotherm at 62.29°C, which manifested its melting point. The mannitol thermogram was observed having an endotherm at 170.72°C that represented its melting point. The thermal profile of the physical mixture showed endotherms at 62.58°C and 172.94°C, matching the thawing temperatures of PCL and mannitol, separately. Further, the CXB-PCL nanoparticles exhibited a single endotherm at 169.18°C, representing the melting point of mannitol. Thus, the incorporation of the drug did not significantly affect the thermal behavior of the polymer and mannitol heralding that nanoparticles were devoid of any sort of interactions between drug and selected excipients.

The PXRD analysis of CXB revealed its characteristic peaks at 14.84°, 15.88°, 16.12°, 18.46°, 21.54°, and 22.20° 2θ prima facie indicating its crystalline nature (Figure 4). The peaks of PCL were observed at 21.4° and 22° 2θ showing its crystallinity. The mannitol, being a crystalline compound, was found to have several peaks viz. – at 10.46°, 14.64°, 18.80°, 21.12°, 23.42°, 28.34°, 29.54°, 33.60°, 36.08°, 38.72°, and 44.14° 2θ. The physical mixture was observed having a majority of peaks due to mannitol and only a few because of PCL. The CXB-PCL nanoparticle formulation showed peaks representing mannitol and PCL. This corroborates the findings of DSC studies, which obviate the presence of any interaction between drug and selected excipients.

3.5 *In vitro* drug release

There was witnessed a slowed fashion drug release in the case of CXB-PCL nanoparticles. The increase in polymer concentration yielded a fall in drug release. This may be attributed to great viscosity of the particulates, which hindered the diffusion of the dissolution medium inside. The slow degradation feature of PCL further reinforced this behavior as the drug release observed after 8 h was 65.71±1.29% at drug to polymer ratio of 1:20 (Figure 5A). The batches formulated with varying contents of Pluronic F-68 were found to yield higher drug release upon increase in its amount. This can be because of smaller nanoparticles, which failed to hold the drug (Figure 5B).

The drug release data from the optimized formulation (NP Form C) were subjected to treatment using diverse kinetic models [36] like zero-order, first-order, Higuchi,

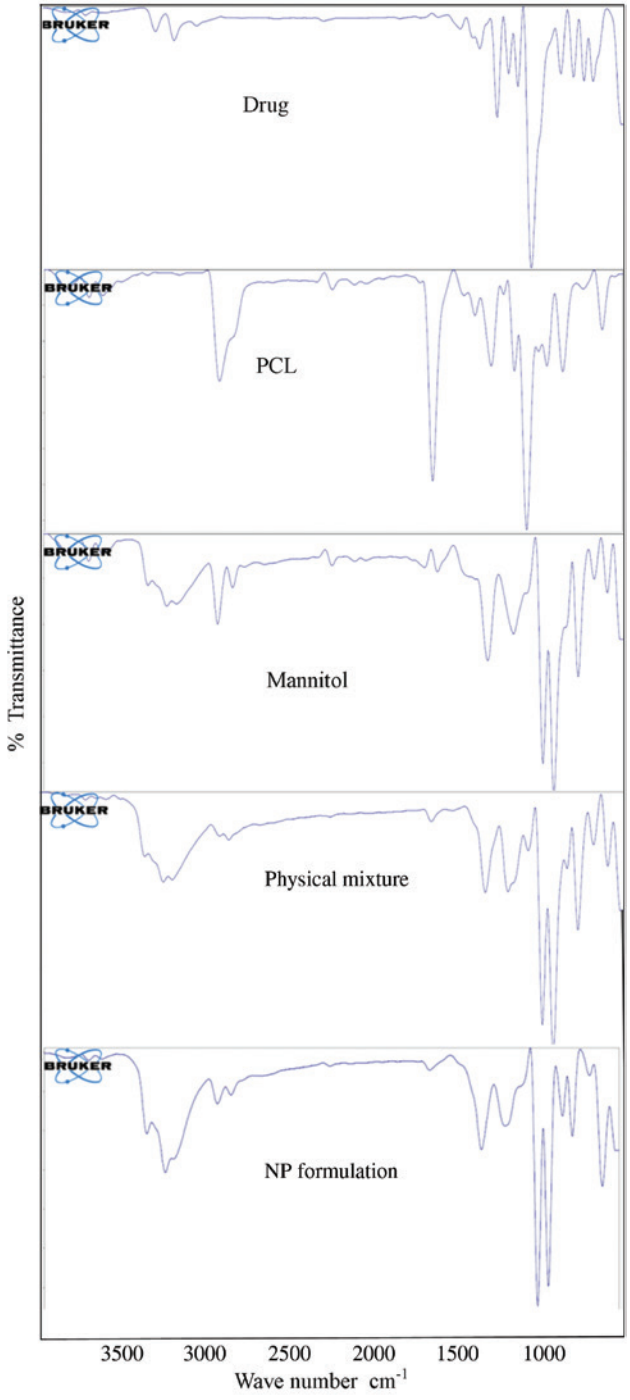

Figure 2: ATR-FTIR spectra of pure celecoxib, PCL, mannitol, physical mixture, and CXB-PCL nanoparticles.

and Korsmeyer-Peppas models to assess the drug-release mechanisms. The release of the drug was found to obey zero-order release kinetics, i.e. a constant fraction of the drug was released irrespective of the concentration. This was concluded on higher tenets of regression coefficients in the zero order (R^2-0.9217) in comparison with Higuchi's square root of time kinetics (R^2-0.7802), first-order kinetics

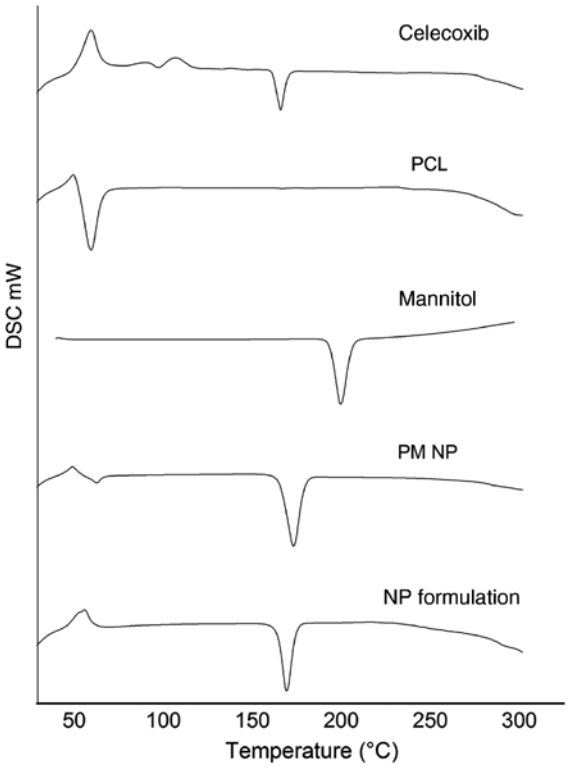

Figure 3: DSC thermograms of pure celecoxib, PCL, mannitol, physical mixture, and CXB-PCL nanoparticles.

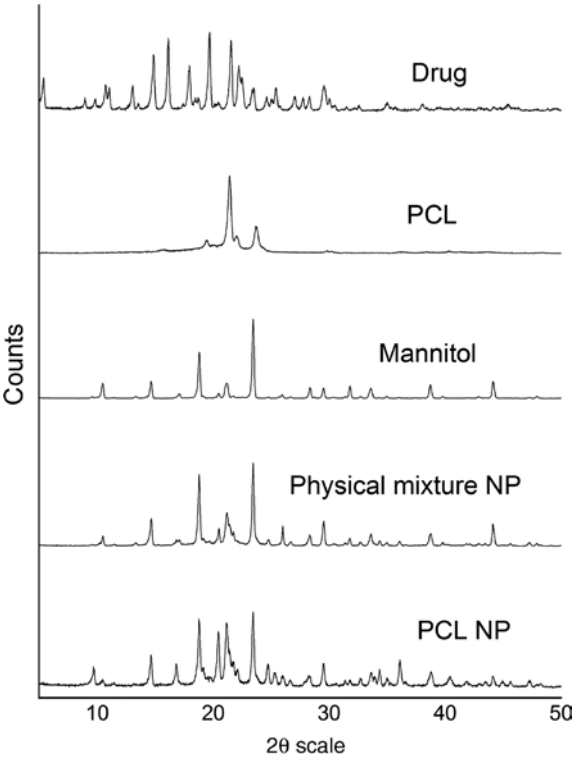

Figure 4: PXRD patterns of pure celecoxib, PCL, mannitol, physical mixture, and CXB-PCL nanoparticles.

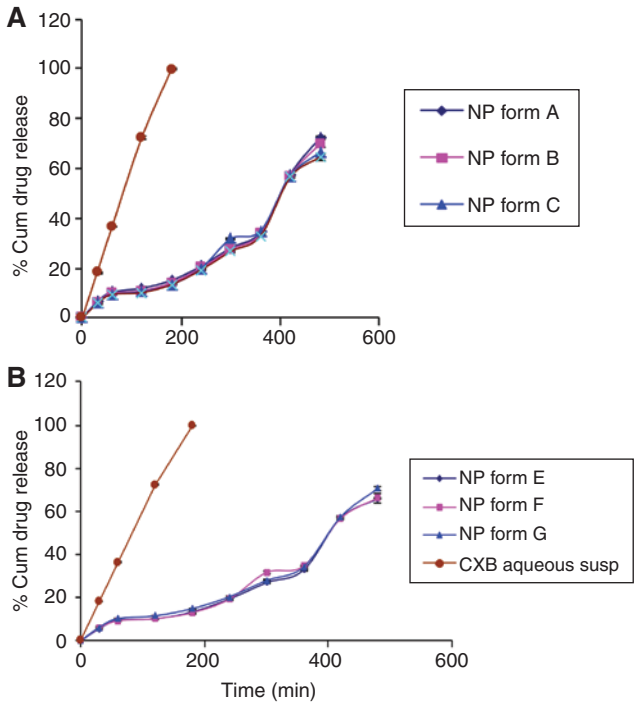

Figure 5: Dissolution profile of CXB-PCL nanoparticles (A) at different drug:polymer ratios (B) at varying Pluronic F-68 concentrations.

(R^2-0.0571), and Korsmeyer-Peppas model (R^2-0.0089). Based on the above data, diffusion may be the underlying mechanism of the drug release.

3.6 *In vitro* transcorneal permeation studies

The transcorneal permeation was approximately double in the case of the CXB-PCL formulation C compared to the CXB suspension. Probably, the smaller size enabled the nanoparticles to traverse the corneal epithelium to some extent, thereby, enhancing the drug transport across the latter. Further, all the formulations were found conducive to the ocular surfaces as the hydration levels were observed within the acceptable range, i.e. 75–80% (Table 1).

3.7 *In vivo* efficacy of CXB-PCL nanoparticles against arachidonic acid-stimulated ophthalmic swelling in rabbits

Arachidonic acid upon topical application triggers the principal symbols of ophthalmic inflammation, such as conjunctival vasodilation, mucous liberation, edema, lid closing, raised intraocular force, PMN relocation, and elevated aqueous humor protein [27]. The effectiveness of the developed CXB-PCL nanoparticles was determined against inflammation by this model and compared with the CXB suspension (0.1% w/v). The lid closure score, PMN count, and protein content are represented in Table 2. It was observed that PMN count as well as protein content was highest in the tear fluid after 4 h of arachidonic acid application. This time point was taken as a yardstick to comment on the efficacy of the developed formulation. The CXB-PCL nanosuspension caused a lowering in the PMN counts to 34.78% against the control, whereas this was 17.39% in the case of the CXB suspension. The lid closure was not prominent upon instillation of the nanosuspension as indicated by small scores compared to the control and the suspension. Further, the nanosuspension caused a significant reduction in tear fluid protein content, i.e. 42.62% (CXB-PCL nanosuspension) versus 35.75% (CXB suspension). Hence, the selected formulation appeared better than the CXB suspension in the improvement of the key parameters of ocular inflammation.

Table 2: Anti-inflammatory effect of celecoxib NP formulations against arachidonic acid-induced ocular inflammation after 4 h of instillation (mean±standard deviation).

Formulation	Lid closure score	PMN count (number/mm³)	Protein content (%)
Control nanosuspension	1.67±0.58	766.67±76.38	0.359±0.02
CXB-PCL nanosuspension	0.33±0.58[a]	500±50[a]	0.206±0.02[a]
Control suspension	1.67±0.58	766.67±76.38	0.358±0.01
CXB suspension	0.67±0.58[b]	633.33±76.38[b]	0.230±0.01[b]

[a]Values are statistically significant for CXB-PCL nanosuspension compared to its vehicle control ($p<0.05$) as determined by one-way analysis of variance followed by Student-Newman-Keuls test.[b]Values are statistically significant for CXB suspension compared to its vehicle control ($p<0.05$) as determined by one-way analysis of variance followed by Student-Newman-Keuls test.

3.8 Stability studies

The inferences from the accelerated stability studies indicate the reduction in the drug entrapment below the permissible extent of 5% of the initial values as per ICH guidelines {Q1 A (R2)}. There was no significant alteration in particle size and ζ potentials of the CXB-PCL nanoparticles subjected to stability studies after 1.5, 3, and 6 months. Hence, storage at room temperature is suggested for these formulations.

4 Conclusion

The PCL nanoparticles containing CXB were formulated via the solvent displacement procedure and optimized based on the formulation variables. The optimized CXB-PCL nanoparticles had appropriate particle size, incorporation efficiency, and sustained drug release. The particulates exhibited improved tolerability and sustained residence at the corneal surface. The formulations effectively reduced inflammation parameters versus the CXB suspension. Therefore, the optimized CXB-PCL nanoparticles may be a promising alternative to the conventional eye drop for the management of ocular inflammation. The exhaustive clinical investigations are required to comment more in this regard.

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