

Research Article

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Flurbiprofen-Infused Electrospun Nanofibers-Based Ocular Inserts: A Sustained-Release Platform with Enhanced Tear Pharmacokinetics

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ABSTRACT

Purpose: This study developed and evaluated flurbiprofen-loaded electrospun ocular inserts fabricated from the polycaprolactone (PCL). The primary objective was to design a prolonged release drug delivery system, specifically using FB-PCL-NFIs, as a novel alternative to existing ocular dosage forms for managing diverse ocular disease conditions viz. conjunctivitis, blepharitis, uveitis, scleritis, or dry eye syndrome.

Methods: The FB-PCL-NFIs were fabricated using monoaxial electrospinning technique and primarily characterized for physicochemical properties, contact angle measurements, diffusion, permeation, tensile strength, and stability analyses. The ocular irritancy of the FB-PCL-NFIs was investigated using isotonicity testing and *in ovo* Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay.

Results: The prepared FB-PCL-NFIs benefited from the enhanced ocular retention, sustained FB release, enhanced permeation, and improved patient compliance. The *in vivo* studies confirmed no noticeable signs of irritation suggesting biocompatibility, tolerability, and suitability of the prepared FB-PCL-NFIs for ocular administration. The *in vivo* ocular pharmacokinetic studies performed in adult male New Zealand White (NZW) rabbits confirmed the prolonged ocular residence of FB-PCL-NFIs compared to Ocuflur® IP.

Conclusion: The findings support further evaluation of the inserts for prolonged ocular delivery of FB-PCL-NFIs as promising therapy for ocular irritation associated with various ocular disease.

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1. Introduction

Nanotechnology-enabled medicines have transformed the current epoch of healthcare by creating more effective, targeted, and personalized medical solutions to the variety of illnesses. Several multifunctional nanocarriers such as liposomes, dendrimers, polymeric nanoparticles, nanocochleates, and nanofibers have been developed recently and are being investigated for the treatment of inflammatory illnesses, cancer, neurological diseases, and infectious diseases.¹ Greater drug encapsulation and release can be made possible by fabricating drug-infused electrospun nanofibers (NFs), which makes it crucial to create polymer-based drug delivery systems with desired characteristics viz. high porosity, high surface area to volume ratio, nanometric size, enhanced permeability, lesser systemic toxicity, and improved therapeutic action.^{2,3,4}

Ocular inflammation is a broad group of eye disorders that includes both relatively benign illnesses and the vision-threatening conditions.⁵ The prevalence of ocular inflammation is ~28.7 % of the ocular emergencies. Ocular inflammation could result in total vision loss, if left untreated.⁶ Administration of drugs directly through the ocular route can deliver the therapeutic cargoes directly to the inflamed eye tissues, which further increase the local drug concentrations, decrease the systemic side effects, and improve the patient's adherence to the therapy.^{7,8,9}

The use of drug-infused electrospun polymeric NFs is extremely beneficial over other nanoformulations as former could provide the controlled drug release, enhanced drug retention and penetration across the ocular tissues. Polymeric NFs have recently been investigated for the ocular delivery of variety of drugs viz. itraconazole,¹⁰ dexamethasone acetate,¹¹ melatonin,¹² hyaluronic acid,¹³ gatifloxacin,¹⁴ levofloxacin,¹⁵ ciprofloxacin,¹⁶ ketorolac tromethamine,¹⁷ timolol maleate,¹⁸ and so on. In addition, the NFs have been fabricated using variety of polymers viz. polyvinyl alcohol,¹⁹ polycaprolactone,²⁰ hyaluronic acid,^{21,22} gelatin,²³ and so on.

Polycaprolactone (PCL, approved by FDA for clinical use) is a synthetic, semi-crystalline, biodegradable polyester that is well known for its excellent biocompatibility, mechanical strength, hydrophobicity, and controlled degradation rate. Owing to these inherent traits, PCL is extensively used in drug delivery and biomedical applications.^{24,25} The slow degradation rates make PCL an ideal candidate for achieving prolonged drug release in sustained-release drug delivery systems such as OcuSerts® (ocular implants).²⁶ Moreover, the hydrophobic nature of PCL enables the inclusion of lipophilic drugs and strong electrospinnability of PCL allows for the formation of continuous, uniform, and homogenous electrospun NF mats.²⁷ The electrospun PCL NFs have been investigated for the treatment of various eye-related disorders viz. glaucoma,¹⁸ corneal stroma regeneration,²⁸ post-operative eye inflammation,¹¹ and so on.

Flurbiprofen (FB, 2-(2-fluorobiphenyl-4-yl) propionic acid) is an aryl-propionic acid derivative and non-steroidal anti-inflammatory agent (NSAIA) used to treat eye inflammation. FB works by inhibiting the cyclooxygenase (COX-1 and COX-2) enzymes in arachidonic acid metabolism, thereby blocking the synthesis of prostaglandins (key mediators of inflammation, pain, and miosis) (**Figure 1**). FB is a BCS Class II drug (exhibiting poor aqueous solubility but high permeability) having high log*P* value of 4.2 and mean plasma half-life of 3.5 to 5.5 h. The poor aqueous solubility (9.9 µg.mL⁻¹, regarded as practically water insoluble) of FB limits its therapeutic efficacy and clinical applicability.^{29,30} **Figure S1** (supplementary data) illustrates the chemical structure of FB.

The present study aimed at developing sustained release FB-loaded NFs composed of PCL as a controlled release polymer using monoaxial electrospinning technique. The novelty of this work lies in the fact that the FB-infused PCL-NFs-based ocular inserts have not been investigated yet for the treatment of eye inflammation. The hypothesis of the present investigation is based on the fact that the use of lipophilic PCL would increase the systemic concentration of poorly soluble FB, thereby improving the ocular bioavailability through enhanced ocular retention of the inserts, ultimately resulting in effective relief from the eye inflammation.

2. Materials and methods

2.1 Reagents and chemicals

FB (CAS: 5104-49-4; 244.26 Da; PubChem ID: 329749901; Purity: ~95%) was purchased from Yarrow Chem Products Ltd. (Mumbai, India). PCL (Pellet form, CAS: 24980-41-4; 48-90 kDa) was purchased from Sigma Aldrich Inc. (Bangalore, India). All reagents, chemicals, and solvents of highest possible purity and analytical grades were procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Bidistilled water, prepared on-site, was used throughout the experiments.

2.2 Animals

The *in vivo* pharmacokinetic investigations conferred to the standards prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA), Department of Animal Husbandry and Dairying (DAHD), Ministry of Fisheries, Animal Husbandry and Dairying (MoFAH&D), Government of India. The Institutional Animal Ethics Committee (IAEC; Reg. 651/PO/ReBi/S/02/CPCSEA) at R. C. Patel Institute of Pharmaceutical Education and Research (Shirpur, India) authorized the preclinical study protocol (IAEC/RCPIPER/2024-25/35; Dt. 28 April 2025). All animal experiments complied with the ARRIVE guidelines and carried out in accordance with U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/ EU for animal experiments.

Prior to the *in vivo* experiments, adult male New Zealand White (NZW) rabbits (5-6 lbs., 12–15 weeks old) were accustomed to living circumstances subjected to a 12 h dark-light cycle in rabbit cages (RBS-S-01; stainless steel) kept in semi-climatic chamber ($T_{\text{air}} 65 \pm 5$ °F; RH $\sim 55 \pm 2$ %) for a duration of one week, while being provided with food and water *ad libitum*.

2.3 Fabrication of FB-infused polycaprolactone nanofibers-based ocular inserts (FB-PCL-NFIs)

FB-loaded PCL electrospun nanofibers (FB-PCL-NFs) were fabricated using monoaxial electrospinning technique. The formulation process was optimized based on the polymer concentration, working voltage, and needle tip-to-collector distance. PCL pellets were dissolved in a solvent mixture of dichloromethane, dimethyl formamide and acetone (DCM: DMF: ACT; 4:3:1 v/v) with continuously stirring at 300 rpm for 1 h to prepare a PCL solution (6% w/v). Once PCL was completely dissolved, FB (10% w/v) was added to PCL solution and the mixture was stirred for next 6 h.

The resulting solution was filled in the single channel plastic syringe attached to the 22G needle (0.1 mm ID needle) and electrospun using electrospinning system (E-spin Nano, Physics Instruments Company, Chennai, India) at a flow rate of 1.0 mL.h⁻¹, 20 kV voltage, and 13 cm needle tip-to-collector distance. The electrospun NFs were collected on a rotary collector drum (Diameter: 13 cm; Speed: 1500 rpm) covered with an aluminum sheet. The entire process was carried out at 25 °C. The FB-PCL-NFs were tailored as flat square-shaped inserts (FB-PCL-NFIs; 1 cm² area) and weighed. The NFIs were dried in vacuum oven at 40 °C before study.^{14,17,31} Blank PCL-NFs were prepared by adopting similar procedure without addition of FB (**Figure 1**).

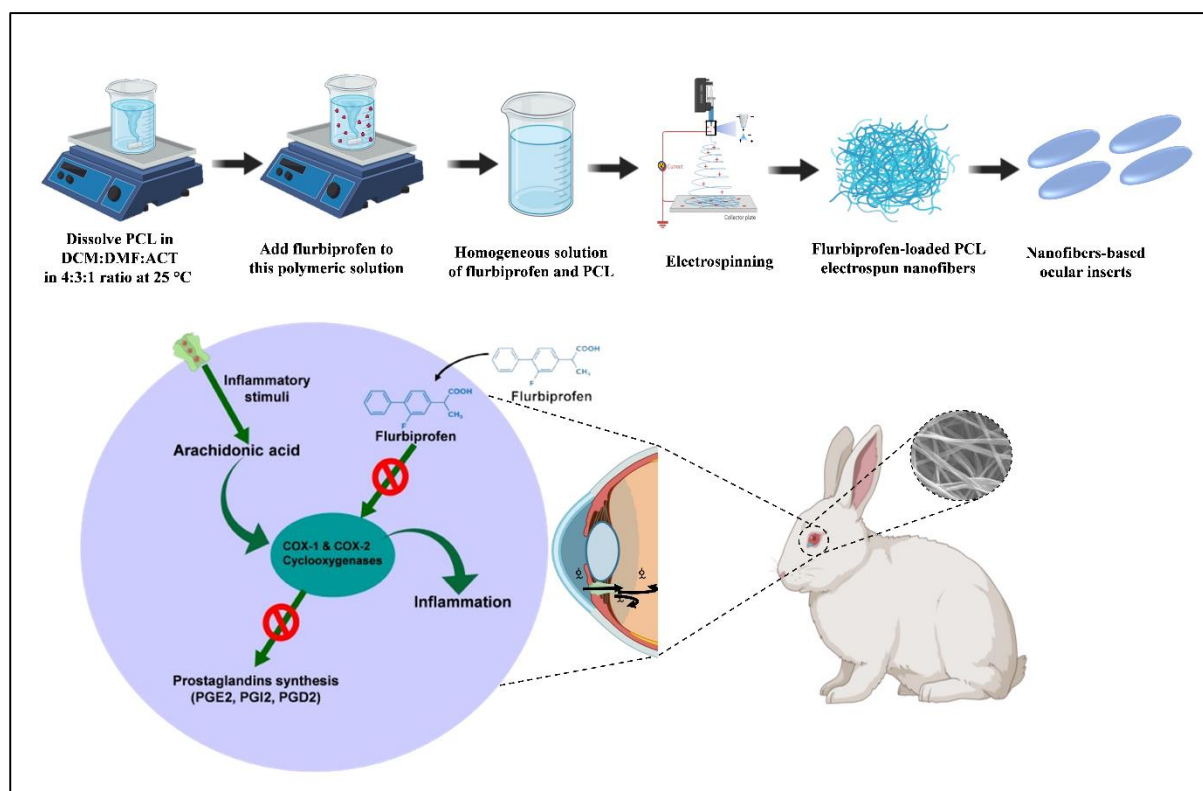


Figure 1: Schematic presentation of preparation of FB-PCL-NFIs using monoaxial electrospinning (upper panel), and mechanism of anti-inflammatory activity of FB-PCL-NFIs by inhibiting the cyclooxygenase enzymes and blocking the synthesis of prostaglandins (lower panel).

2.4 Characterization and evaluation

2.4.1 Physicochemical characterization

To check the suitability of PCL solution for electrospinning, the viscosity of FB-PCL-NFs were investigated using Brookfield's viscometer (DV-E, Brookfield Engineering Laboratories, Middleborough, USA) while the electrical conductivity was determined using microcontroller-based conductivity meter (Type 308; Systronics India Pvt. Ltd., Ahmedabad, India). Each sample was measured in triplicate at 25 ± 0.1 °C.³²

The thickness of the FB-PCL-NFs was assessed using a digital vernier caliper (Digimatic caliper 6"/0-150 mm, Mitutoyo Absolute Digital Caliper, Bengaluru, India).³³ Furthermore, the square shaped sections of FB-PCL-NFs sheet having 1 cm² size were trimmed and weighed using an analytical balance to check weight uniformity. All the determinations were carried out in triplicate.¹⁷

To examine the strength and rigidity of the FB-PCL-NF-based ocular inserts (FB-PCL-NFIs), the tensile strength test was conducted on a texture analyzer (CT3, Brookfield Engineering Laboratories, Middleborough, USA) using TexturePro CT (ver. 1.3) software.¹⁶ The flexibility of the NFs was estimated by the folding endurance test.³⁴

The FB-PCL-NFIs were submerged in double distilled water and the solution pH was recorded using calibrated microcontroller-based automatic pH meter (EQ621; Equiptronics, India).

The swellability of the FB-PCL-NFIs was investigated using gravimetric method at room temperature in a phosphate buffer solution (pH 7.4), which involved measuring the weight change before and after NFIs have absorbed a liquid.³⁵

A pre-weighed sample of FB-PCL-NFIs was dissolved in DCM, sonicated and the FB content was estimated spectrophotometrically at 248 nm.^{36,37}

The wettability and surface hydrophobicity of the FB-PCL-NFIs was estimated using sessile drop method on contact angle goniometer (Ossila Ltd., Sheffield, UK). A sheet of FB-PCL-NFIs was mounted on the glass slide and 3.0 μL droplet of deionized water was cautiously dropped on the surface of the NFIs. Later, the contact angle between the water droplet and surface of NFs was measured.^{38,39}

2.4.2 Morphological characterization

The morphological features of FB-PCL-NFIs were examined by scanning electron microscopy (SEM, JSM-6390, Jeol® Ltd., Tokyo, Japan). Sample was sputter-coated with a thin (400 Å) gold layer and photomicrographs were captured at different magnifications and at an accelerating voltage of 20 kV.⁴⁰

2.4.3 Analytical characterization

The interactions between different constituents involved in the formulation of FB-PCL-NFIs were investigated using an attenuated total reflectance-infrared spectrometer (ATR-IR, ALPHA II Compact, Bruker®, Billerica, USA). Spectra were recorded in solid state with a scanning range of 4000–500 cm^{-1} and a resolution of 2 cm^{-1} .

The thermal properties of FB and PCL within the NFs was investigated using differential scanning calorimeter (DSC, DSC1, Mettler Toledo®, Zurich, Switzerland) at a heating rate of 10 $^{\circ}\text{C min}^{-1}$ from 40–350 $^{\circ}\text{C}$ under 50 mL min^{-1} nitrogen stream.

The crystal characteristics of bulk FB, PCL and the fabricated NFs were studied using X-ray diffractometer (XRD, AXS-D8 advanced, Bruker®, Karlsruhe, Germany) in a scanning range from 5 to 50° on a 2θ scale (step size: 0.02°, scanning speed: 2.0° min^{-1} , voltage: 40 kV, current intensity: 35 mA).

2.4.4 In vitro release

The *in vitro* diffusion characteristics of FB from FB aqueous dispersion and FB-PCL-NFIs was investigated using a multistation diffusion cell (Electrolab, Mumbai, India) installed with Franz's diffusion apparatus (capacity: 15.5 mL; diffusion area: 3.14 cm^2). A 2 cm^2 sheet of FB-PCL-NFs was placed into the donor section filled with 0.5 mL of PBS (pH 7.4) and separated from receiver section that contained 15.5 mL PBS (pH 7.4) by dialysis membrane (12–14 kDa; pore size: 0.45 μm). Samples (300 μL) were collected at specified intervals over 24 hours and replaced with an equivalent volume of fresh medium to maintain the sink conditions. After filtration (Whatman® grade 589/1), the samples were analyzed spectrophotometrically at 248 nm. The cumulative drug release data were fitted to zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas kinetic models to investigate the release kinetics and mechanism of FB from the PCL nanofibers. Model fitting was assessed primarily using the coefficient of determination (R^2), while acknowledging that additional statistical criteria such as residual analysis and uncertainty estimation can provide a more comprehensive evaluation of model performance.^{17,41}

2.4.5 Ex vivo permeation

The *ex vivo* permeation of FB from FB aqueous dispersion and FB-PCL-NFIs was determined using excised goat cornea as a permeation barrier. Fresh goat eyes were obtained from local slaughter house with in approx. 20 min of slaughter and transported to working lab in cold saline solution (pH-7.4). The eyes were not stored prior to the study. The corneas were carefully excised and visually inspected for tissue integrity. Healthy and free from visible damage or abnormalities were used for the *ex-vivo* permeation study. The experimental conditions, sampling and analysis operations were followed in a similar way as *in vitro* diffusion study (section 2.4.4).⁴¹

2.4.6 Evaluation of sterility, irritancy, and toxicity

Sterility test was performed to assure the absence of living microorganisms and suitability of FB-PCL-NFIs for ocular delivery. FB-PCL-NFIs was terminally sterilized using EtO to preserve the delicate NFIs mat at low temperatures to validate the efficacy of terminal sterilization. The NFIs were then positioned in soybean-casein digest broth medium (for aerobes), and sodium thioglycolate broth (for anaerobes) both at 35 ± 0.5 °C in an incubator for 1, 7, 14 and 21 days. The turbidity of the prepared samples including positive and negative controls was visually observed and compared.⁴²

The isotonicity of the prepared FB-PCL-NFIs was observed for enlargement, rupture and shredding of RBCs. The fresh blood sample from the rabbit was split into two portions after dilution (1:200) with Hayem's solution (RBC diluting fluid). First portion was considered as control and left untreated whereas second portion was incubated with FB-PCL-NFIs. Following incubation, the mean diameter of ~500 RBCs was measured using optical digital microscope (DMW2-223, Motic® Instruments Inc., Toronto, Canada) and the mean diameter of the RBCs was determined, and the formulation-treated group was compared with the untreated control for apparent changes in RBC diameter and morphology, including enlargement, shrinkage, rupture, or fragmentation, according to the previously reported method.³⁶

The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay is an *in ovo* alternative to the traditional *in vivo* Draize rabbit eye irritation test. It is used to evaluate the ocular irritancy by assessing alterations in the chorioallantoic membrane of fertile eggs.^{43,44} Fresh and fertile hen eggs (50-60 g; White Leghorn breed) were chosen and incubated at 37 ± 0.5 °C temperature and 60 ± 5 % relative humidity (RH) for 8 days. The eggs were rotated daily for 4 to 5 times to prevent the embryo from sticking to the shell. On 9th day, the eggs were removed from the incubator and the outer eggshell was carefully cut, without hurting the inner layer, to expose the CAM (a highly vascularized membrane). The eggs were divided into four groups. First egg CAM was treated with 0.9% sodium chloride (NaCl) solution (negative control), second with 0.1 M sodium hydroxide (NaOH) solution (positive control), third with FB-PCL-NFIs (test), and fourth with marketed FB eye drop (Ocuflur® IP 0.03% w/v) (standard). After 5 min of treatments, the CAMs were observed for three major irritant effects viz. coagulation, hemorrhage, and hyperemia. Based on the severity of damage and time required for the damage to occur, the cumulative irritation score (IS) was assigned and compared. The mean IS was correlated as: <1: Non-irritant; ≤ 1 to < 5: Slightly irritant; ≥ 5 to < 9: Moderately irritant; ≥ 9 to ≤ 21 : Strongly irritant.^{43,41,45}

2.4.7 *In vivo* studies

The *in vivo* pharmacodynamics study was performed on NZW rabbits to investigate the ocular irritancy potential of the prepared FB-PCL-NFIs. The FB-PCL-NFIs ($1/3$ cm²; square-shaped mat weighing 1.16 mg equivalent to 0.0331 mg of FB) or marketed eye drops (Ocuflur® IP 0.03% w/v) were introduced in the lower cul-de-sac of right eyes of each rabbit, whereas left eyes were treated with PBS and considered as control.⁴⁶ Based on the modified Draize's test, the eyes were examined for inflammatory responses (damage to the cornea, iris, and conjunctivae), and a cumulative numerical score (0 = no alteration, 1 = mild alteration, and 3 = obvious alteration) was assigned. Scores were checked and photographs were captured from day 1 to day 7, post-treatment.⁴⁷

Adult male NZW rabbits were used to evaluate the *in vivo* tear fluid pharmacokinetics profile of FB-PCL-NFIs. Rabbits were divided into two groups each comprising three rabbits, randomized by simple randomization method. Group I (standard) rabbits (right eyes) received 50.0 μ L of the marketed eye drop (Ocuflur® IP 0.03% w/v) whereas Group II received ethylene oxide (EtO) sterilized FB-PCL-NFIs ($1/3$ cm²; square-shaped mat weighing 1.16 mg equivalent to 0.0331 mg of FB) in the lower cul-de-sac of the right eyes of each rabbit. Left eyes were considered

as control. Later, the cul-de-sac of rabbit eyes, both groups, were filled with 50.0 μL of sterile PBS (pH 7.4) at predetermined intervals viz. 2, 4, 8, 12, 24 and 48 h (flush-tear method). After 30 s, tear samples were collected from the inferior conjunctival fornix using Schirmer's tear strips (OpStrip, Medimark, Mumbai, India) and stored at -70°C . To minimize variability associated with the flush-tear and Schirmer-strip sampling method, all tear samples were collected using an identical protocol, including a fixed PBS flushing volume (50 μL), a standardized contact time (30 s), and the same type of Schirmer strip for all animals. Although some variability in tear recovery is unavoidable owing to the nature of ocular tear sampling, the same sampling procedure was consistently followed for all experimental groups to ensure comparability. Drug extraction from the Schirmer strips was carried out using a methanol–chloroform (1:1, v/v) solvent system, and all HPLC determinations were performed in triplicate to ensure analytical reproducibility. Although, the actual sampled volume was difficult to measure precisely due to fluid retention in the Schirmer's strip but, the sampled volume depends on how much of the strip was wetted. The FB was extracted using methanol-chloroform (1:1) mixture in ultracentrifuge tubes, vortexed for 10 min at room temperature, sonicated for 5 min at room temperature, and finally centrifuged using ultracentrifuge (12000 rcf, 15 min, Optima Max-XP, Beckman Coulter Inc., USA) and the free FB in the supernatant was further quantified at 247 using HPLC analysis. The left (PBS-treated) eye of each rabbit served as an internal control for the Draize irritancy assessment only; statistical comparisons of tear pharmacokinetic parameters (C_{max} , T_{max} , AUC_{0-t}) were performed between the treated eyes of Group I (Ocuflur® IP, $n = 3$) and Group II (FB-PCL-NFIs, $n = 3$), and not between contralateral eyes of the same animal. The extraction procedure and chromatographic conditions were maintained consistently for all samples throughout the study to minimize analytical variability. Investigators were masked (unblinded formulator and blinded doser) during ocular dosing, Draize scoring, sample analysis, and data interpretation to minimize the experimental biases.

Chromatographic separations were done using high-performance liquid chromatography (HPLC 1200, Agilent Technologies Inc., Singapore) equipped with G-13148 diode array detector (DAD), Rheodyne™ 7725 sample injector (20 μL), and C_{18} reverse-phase analytical column (Eclipse XDB, 5 μm , 4.6x150 mm). Mobile phase composed of acetonitrile-water (80:20 % v/v) acidified with 0.1 % trifluoro acetic acid (pH 3.5), and flow rate was 0.7 $\text{mL}\cdot\text{min}^{-1}$. FB concentration was detected at 247 nm.^{48,49} Since the administered flurbiprofen dose differed between the FB-PCL-NFIs (0.0331 mg) and the marketed eye drops (0.015 mg), the pharmacokinetic exposure parameters were normalized to the administered dose prior to comparison. Accordingly, dose-normalized C_{max} and AUC_{0-t} values were used to compare the ocular pharmacokinetic performance of the two formulations, thereby enabling an unbiased assessment of formulation-dependent differences in ocular drug delivery. Non-compartmental analysis was utilized to estimate the ocular pharmacokinetic parameters (C_{max} , T_{max} , AUC_{0-t}) from the concentration-time profiles using MATLAB® pharmacokinetic modeling and simulation suit (MathWorks Inc., MA, USA). Statistical data were processed using unpaired student *t*-test and one-way ANOVA on GraphPad Prism (ver.7.04, GraphPad Software Inc., CA, USA). Data were presented as mean $\pm$ SD and statistical significance of data was set at $p < 0.05$.

2.4.8 Stability study and shelf-life estimation

For stability studies, the FB-PCL-NFIs were enclosed in aluminum foils and placed in glass (Borosilicate, Type I) tubes hermetically sealed with a rubber stopper and aluminum crimp. The samples were initially kept for four weeks in a controlled environment ($40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH) in a stability chamber (CHM-10S®, Remi Lab World, Mumbai, India). The sample were investigated for ATR-IR analysis after one-week intervals over a four-week period.⁴⁵ Further, the percent drug content was estimated after two-month intervals over a six-months period. The

projected shelf-life of the fabricated FB-PCL-NFIs was then predicted using Minitab® statistical software, ver. 22.4.0 (Minitab LLC, Pennsylvania, USA), a shelf-life projection software, based on ICH Q1E guidelines.⁵⁰

3. Results and Discussion

3.1 Fabrication of FB-PCL-NFIs

The FB-PCL-NFIs were fabricated using monoaxial electrospinning and modulated to flat square-shaped inserts of uniform size (1 cm²). PCL concentration between 6 to 10 % w/v was tested for electrospinning. High (> 6% w/v) PCL concentrations resulted in increased solution viscosity, which caused clogging of needle tip, thick and irregular NFs whereas 6% w/v concentration produced clear, homogeneous polymer dispersion and resulted in smooth NFs formation. In addition, NFs were benefited from the synergistic properties of the DCM: DMF: ACT solvent combination. DCM facilitated the rapid evaporation for fiber solidification, while DMF enhanced the jet stability, thereby promoted the uniform NFs formation which is ideal for ocular drug delivery applications. Additionally, the high volatility and low boiling point of ACT (a co-solvent) aided in the production of homogeneous, bead-free fibers with smooth texture. ACT also lowered overall solution viscosity and improved charge mobility, resulting in increased jet stability and regulated fiber diameter with minimal structural flaws^{36,17,31}. In a similar way, working voltage of 20 kV, and needle tip-to-collector distance of 13 cm was optimized after a series of experimental trials.

3.2 Characterization and evaluation

3.2.1 Physicochemical characterization

Viscosity estimations suggested that the increased PCL concentrations resulted in linear increase in viscosity due to increased molecular entanglements of polymer chains. A 6% w/v PCL concentration and viscosity of 660 cp was found to be ideal for smooth NFs formation. In contrast, the conductivity was decreased from 9.684 μ S/m to 8.801 μ S/m on increasing PCL concentration from 5 to 6% w/v, which might be due to decreased mobility of ionic species inside the dense polymer matrix. While adequate viscosity is necessary for steady jet production during electrospinning, optimal conductivity aids the elongation of polymer jets under an electric field.³² An increase in the conductivity of polymer solution entails a decrease in fiber diameter.⁵¹

The thickness of the prepared FB-PCL-NFIs was in the range of 0.110 ± 0.01 to 0.120 ± 0.01 mm. Commercially available ocular inserts (Ocusert®) has a thickness of 0.300 mm and it is anticipated that the NFIs with a thickness below 0.15 mm are projected to be well-tolerated by the eyes. The weight of the FB-PCL-NFIs of analogous dimensions was 7.0 ± 0.3 mg. The fabricated ocular NFIs were found to have an ideal thickness and weight for insertion into the cul-de-sac of the eyes, ensuring they are well tolerated after administration.^{17,33}

The FB-PCL-NFIs were found to have the tensile strength of 324 g/cm² (**Figure 2-A**), which is adequate to withstand the repetitive, low-impact mechanical stress induced by continuous blinking motion of the eyes and thereby maintain the NFIs integrity into the eyes. The folding endurance of NFIs was found to be 256 ± 10 times ($n=3$). Mostly, the NFIs are thin, soft, smooth, and more flexible compared to the films.^{16,10,36}

The fabricated NFIs were found to have the surface pH value of 7.5 ± 0.1 , which is well within the physiological pH of eye and ideally compatible with the human tear fluid (pH 6.5 to 7.6). The use of such NFIs may not cause any significant adverse effects viz. redness, burning sensation, or sensitivity on the eye surfaces (Thygesen & Jensen, 1987).^{52,53}

The swelling behavior of NFIs was assessed across a 24 h period in PBS (pH 7.4). The FB-PCL-NFIs showed a time-dependent surge in percent degree of swelling, which was 53.33, 58.82, 65.00, 69.56, and 76.42 % at 1, 3,

6, 12, and 24 h, respectively. A steady and increasing fluid uptake due to capillary permeation mechanism may be attributed to the PCL-NFs porous morphology and high surface area irrespective of PCLs' semi-crystalline structure and intrinsic hydrophobicity. When prolonged surface contact and sustained drug release is required, this slow and regulated hydration behavior of NFIs is highly advantageous.^{12,54}

The FB content in the fabricated FB-PCL-NFIs was determined to be 93.52 ± 0.75 %. This high drug content of NFIs is attributed to their high porosity and large surface area that accommodates high amounts of FB trapped inside the polymer matrix of NFIs. This may lead to formation of smaller dosage form containing adequate amount of FB to be placed easily in the cul-de-sacs, lower the risk of irritation, reduce ocular discomfort, and improve the patients' compliance on ocular administration.^{16,36}

The left and right contact angle of water droplet on the untreated FB-PCL-NFIs surface was found to be 116.57° and 115.55° , respectively. This led to an average contact angle of 116.06° (**Figure 2-B**), indicating hydrophobic nature of the PCL-NFIs surface with low surface energy and limited water penetrability. Also, the hydrophobic surface limits the burst release of FB due to delayed penetration of aqueous tear fluids into the PCL-NFIs matrix.³⁸

3.2.2 Morphological characterization

The scanning electron micrographs (**Figure 2-C**) indicate that the FB-PCL-NFIs are composed of continuous, ultrafine, uniform, and smooth NFs. **Figure 2-C(a)** reveals a broader view, showcasing the ultrafine NFs randomly deposited without beads or lumps. **Figure 2-C(b)** illustrates that the NFs are smooth in texture and continuous with negligible number of beads. **Figure 2-C(c)** indicates a slight entanglement in the NFs. **Figure 2-C(d)** depicts that the fiber mat has a uniform nature, with the diameter of NFs ranging from 200-400 nm, which is appropriate for the fabrication of NFIs.^{14,55}

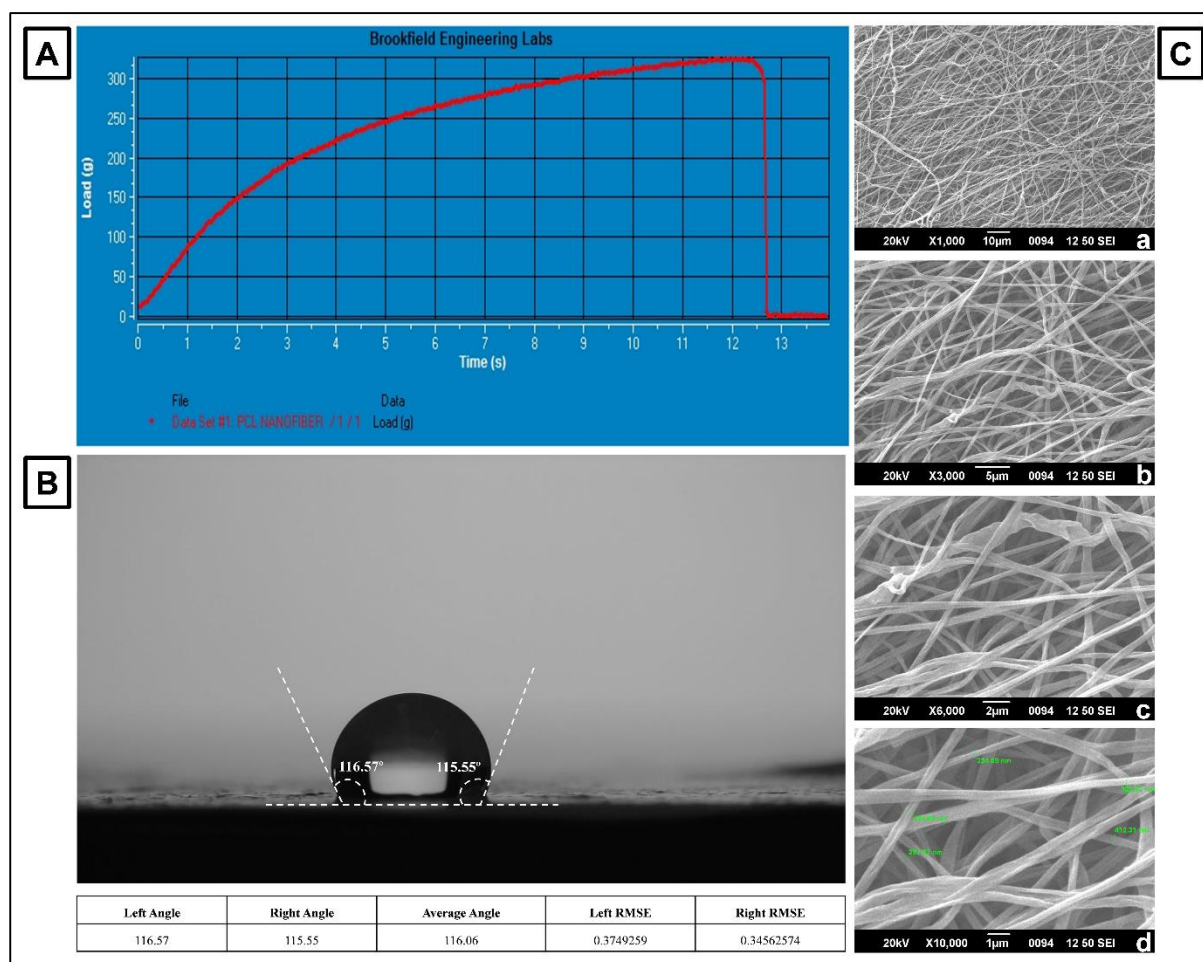


Figure 2: (A) Load-time curve representing the tensile strength FB-PCL-NFIs on texture profile analyzer, (B) Contact angle measurements on the untreated surface of FB-PCL-NFIs, and (C) SEM photomicrographs of FB-PCL-NFIs at different magnifications.

3.2.3 Analytical characterization

Figure 3 shows the ATR-IR spectra of FB, PCL, FB-PCL blend, FB-PCL-NFs, and blank PCL-NFs. The IR spectrum of FB (pink colored) displays its structural identity, primarily associated with the carboxylic acid group, the aromatic rings, and the C-F bond. Strong absorption bands in $1694\text{--}1717\text{ cm}^{-1}$ range associated with the C=O stretching vibrations and a wide band comprising a cluster of short peaks between $2500\text{--}3500\text{ cm}^{-1}$ attributed to the O-H stretching vibrations, both of carboxylic acid group. Peaks between 1450 and 1600 cm^{-1} corresponds to the aromatic C=C stretching vibrations, indicating the presence of biphenyl moiety in the structure of FB. The C-F stretching vibrations are detected in the proximity of $1144\text{--}1212\text{ cm}^{-1}$ linked to the fluorine substitution on the aromatic ring. A band at 2925 cm^{-1} is due to the C-H stretching vibrations. In addition, the bands at 1404 , 1360 , and 1481 cm^{-1} are attributed to the in-plane bending vibrations of O-H bond in carboxylic acid group often coupled with the C-O bending vibrations, C-H bending of methyl group in the side chain, and C-H bending of methylene group (typically from the aromatic ring and alkyl side chain), respectively. The aromatic C-H out-of-plane bending vibrations strongly appear in the fingerprint region (below 1000 cm^{-1}) typically between 607 and 924 cm^{-1} .⁵⁶ The structural soundness of the PCL is validated by the distinctive absorption peaks (primarily due to its ester and methylene functional groups) in its ATR-IR spectrum (purple colored). A Lorentzian-shaped singlet appearing sharply at 1720 cm^{-1} corresponding to C=O stretching vibrations of ester carbonyl group indicates that the PCL is an aliphatic polyester and is crystalline in nature. The symmetric and asymmetric stretching vibrations of C-H

bonds in the methylene groups were identified by peaks at 2942, and 2865 cm^{-1} , respectively. In addition, sharp peaks at 1178, and 1238 cm^{-1} were attributed respectively to the symmetric and asymmetric C-O-C stretching vibration of ester group. Another peak at 1292 cm^{-1} is due to the C-O and C-C stretching vibrations within the PCL chains. The PCL also exhibit methylene rocking vibrations within $\sim 730\text{--}740$ cm^{-1} range. A minor band around 960, that split into two peaks at 932, and 958 cm^{-1} was attributed to the bending vibrations of C-O-C bond in ester group of the PCL.⁵⁷ The characteristic peaks of both FB and PCL were well preserved in the ATR-IR spectrum of FB-PCL physical mixture (1:1, red colored), without any significant band shift. In addition, no new peaks appeared indicating a good physicochemical compatibility and absence of strong chemical interactions between FB and PCL. In ATR-IR spectrum of FB-PCL-NFs (blue colored) showed a strong band at 1722 cm^{-1} correspond to the C=O stretching vibrations of ester carbonyl group of PCL, in addition to the symmetric and asymmetric stretching vibrations of C-H bonds in the methylene groups of PCL were identified by sharp peaks at 2863 and 2941 cm^{-1} , respectively. Furthermore, the bands appearing between 1460–1554 cm^{-1} and ~ 1226 cm^{-1} attributed respectively to the aromatic C=C stretching and C–F stretching vibrations, which are characteristic peaks of FB, were also seen with slightly reduced intensity and minor peak shifting. Since, no new major ATR-IR bands were observed; this finding alone does not exclude all interactions during electrospinning. The study validates the physicochemical compatibility and efficient incorporation of FB inside the matrix of PCL NFs.⁵⁸ Finally, the ATR-IR spectrum of blank PCL-NFs (green colored) showed the presence of all major absorption peaks (due to corresponding stretching and bending vibrations) of specific functional groups in PCL, indicating that the electrospinning did not alter the molecular orientation in PCL chains. In addition, the absence of small peaks around ~ 1670 cm^{-1} relative to the amide bond indicates that no residual solvent (DMF) remained in the PCL-NFs.⁵⁹

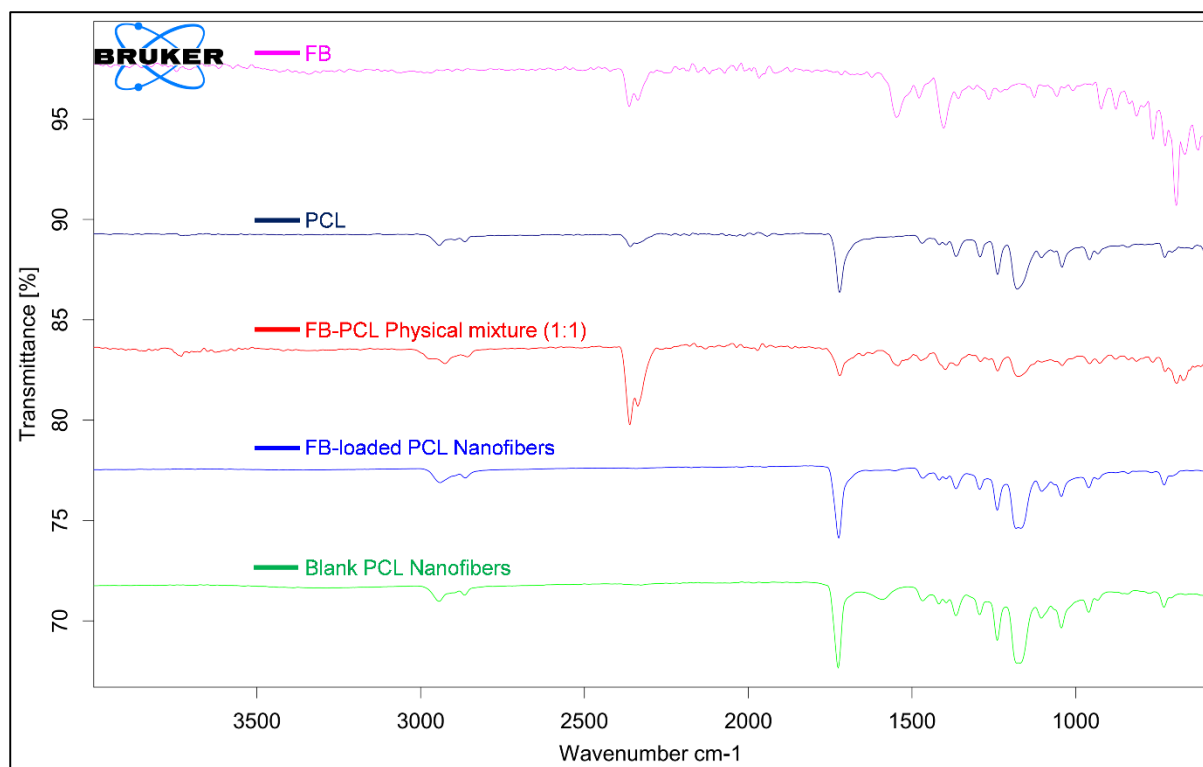


Figure 3: ATR-IR spectra of FB, PCL, FB-PCL blend, FB-PCL-NFs, and blank PCL-NFs.

The DSC thermograms of FB, PCL, FB-PCL-NFs, and blank PCL-NFs are presented in **Figure 4**. The DSC thermogram of bulk FB (black colored) revealed a slightly broad endothermic peak

centered at 98.95 °C indicates the melting of polymorphic form (racemic form II) of FB. The broadness and the relatively low onset temperature could also suggest a glass transition (T_g) followed by recrystallization and melting. The second thermal event was detected at 166.51 °C most likely corresponds to the beginning of thermal degradation of FB and may also be attributed to the melting of residual impurities (Supplier mentions ~95% purity of bulk FB in CoA). The third and last thermal at 237.25 °C may be due to further thermal breakdown or a phase shift of FB occurring at elevated temperatures, usually above 200 °C.^{60,61} The DSC thermogram of PCL (blue colored) shows a sharp endothermic melting peak at 59.56°C, which indicates PCL's semicrystalline nature. The enthalpy of fusion (ΔH_f) was -45.93 J/g, which is in accordance with the literature reports for PCL with moderate crystallinity. The sharpness and position of the peak show the thermal stability of PCL and its suitability for drug delivery applications.⁶²

The DSC thermogram of FB-PCL-NFs (red colored) revealed a slight shifting of melting peak at 60.12 °C and considerably reduced ΔH_f (-14.00 J/g), both observations suggest that the FB was molecularly dispersed within the PCL matrix and the crystallinity of PCL was significantly reduced. In DSC thermogram of blank PCL NFs (green colored), a single thermal event occurring at 58.34 °C indicates the melting transition of the crystalline regions within PCL-NFs. A substantial reduction in the ΔH_f value (-0.42 J/g) indicates the significant reduction in crystallinity of PCL during electrospinning. In addition, the upward trend after the melting peak suggests that the material remains thermally stable beyond its melting point within the observed temperature range (up to ~340 °C), with no signs of degradation.^{63,64}

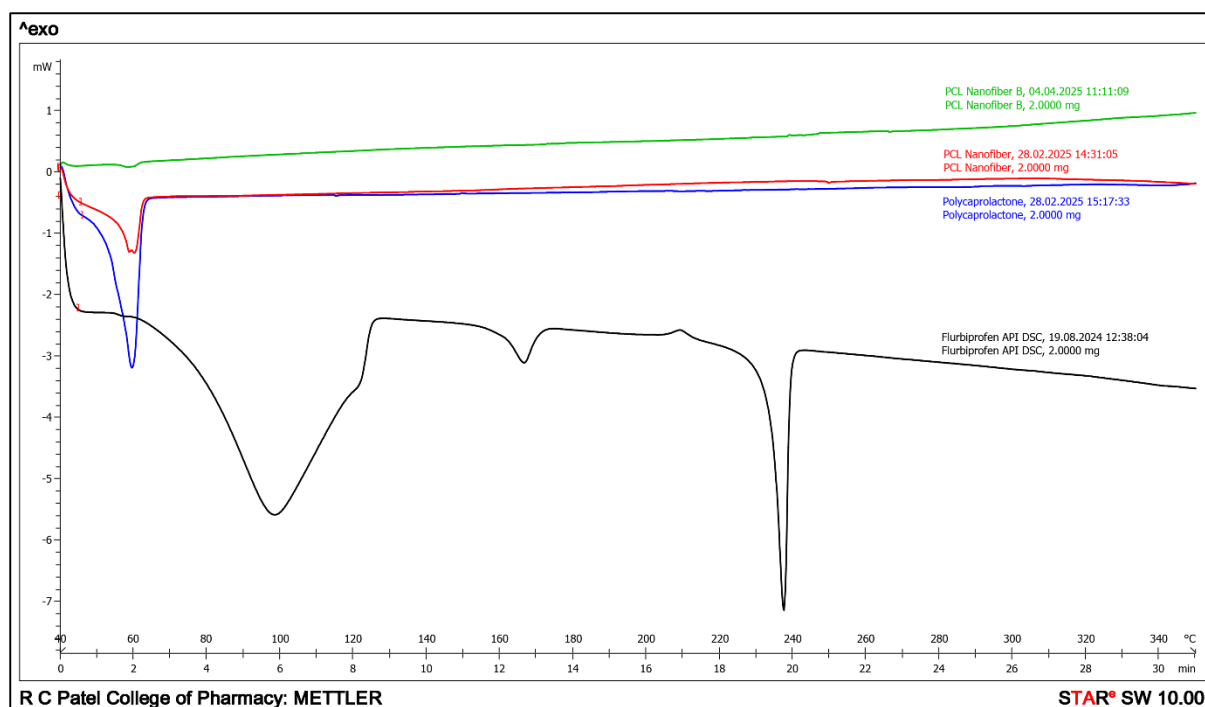


Figure 4: DSC thermograms of FB, PCL, FB-PCL-NFs, and blank PCL-NFs.

Figure 5 illustrates the X-ray diffractograms of FB, PCL, FB-PCL-NFs, and blank PCL-NFs.

The diffractogram for FB (green colored) exhibits highly intense, sharp, and distinct diffraction peaks at various 2θ positions (12.17°, 19.05°, 21.5°, 22.89°, 24.22°, 31.6°, and 36.32°), which indicated that FB exists as a well-ordered crystal structure.⁶⁵ The X-ray diffractogram of PCL (pink colored) showed two prominent and strong diffraction peaks at 21.30° and 23.55° which signify the semi-crystalline nature of PCL.⁶⁶ The sharp characteristic peaks of FB are significantly diminished or absent in the diffractogram of FB-PCL-NFs (red colored). This

indicates that FB, which was initially crystalline, is likely present in an amorphous or molecularly dispersed state within the PCL matrix of the NFs, potentially due to the electrospinning process. The characteristic peaks of PCL are still visible in the diffractograms of FB-PCL-NFs, and blank PCL-NFs (blue colored), suggesting that the PCL matrix retained its semi-crystalline nature.⁶⁷

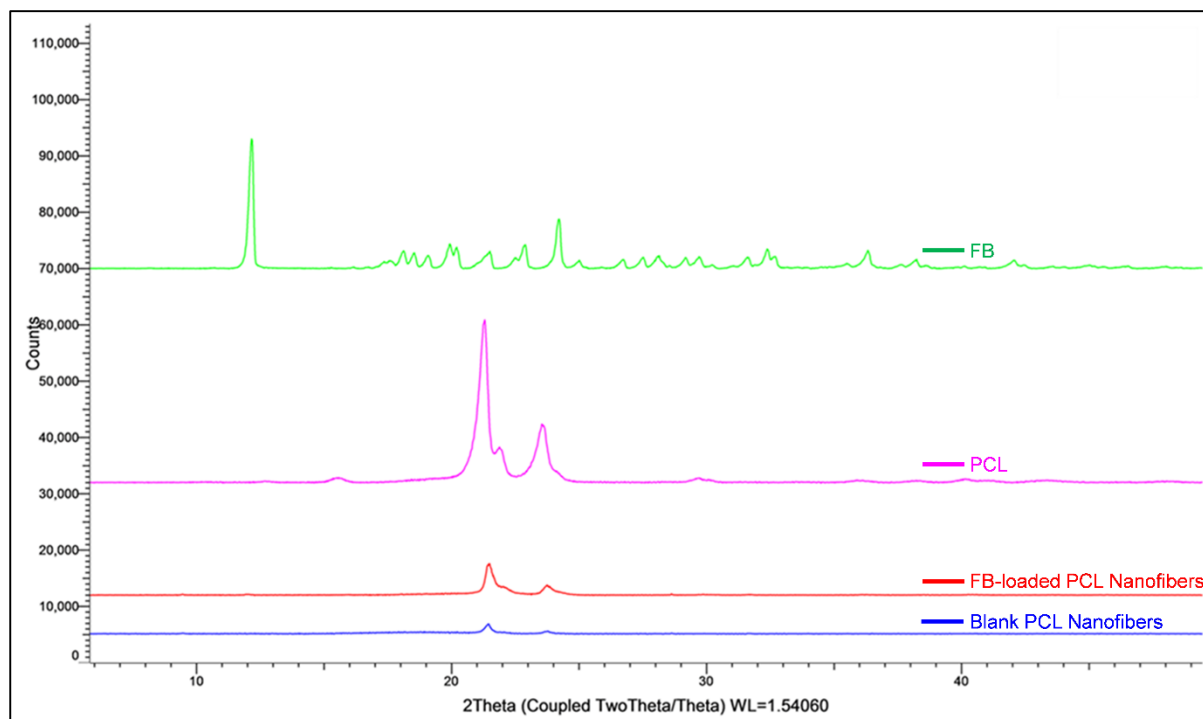


Figure 5: X-ray diffraction spectra of FB, PCL, FB-PCL-NFs, and blank PCL-NFs.

3.2.4 *In vitro* drug release

Figure 6-A(a) illustrates the *in vitro* diffusion profile of FB dispersion and FB-PCL-NFIs in PBS (pH 7.4) through dialysis membrane over 24 h. Around 95% of FB was diffused from the FB dispersion (green colored) within first 3 h while FB was completely diffused within 4 h. Further, FB-PCL-NFIs exhibited biphasic release, where ~30% of FB was released within first hour owing to diffusion of surface-allied or loosely entrapped FB molecules. This initial burst release of FB may also be attributed to the small diameter (high specific surface area) of the fabricated FB-PCL-NFs. In addition, the slightly porous nature of the NFs matrix may also contribute to the rapid FB release. The initial burst release is favorable for achieving rapid therapeutic concentrations at the application site. Subsequently, the release rate decreased gradually, reaching ~68% at 8 h and ~94% at 24 h, which is attributed to the hydrophobic, semi-crystalline, and non-swellable nature of PCL that provides a more sustained drug release due to slow penetration of the release media into the PCL matrix. Also, polymer degradation often alters the mechanism of drug release from purely diffusion-controlled to a combined diffusion-erosion controlled mechanism. However, the slow degradation of the PCL nullifies the likelihoods of such occurrences.⁶⁸

To elucidate the release kinetics of FB from the fabricated nanofibrous inserts, the release data were fitted to zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas kinetic models as shown in **Figure 6-A(b–f)**. Among the investigated models, the Higuchi model exhibited the highest coefficient of determination ($R^2 = 0.9602$), suggesting that diffusion through the polymeric matrix is the predominant mechanism governing FB release from the PCL nanofibers. The comparatively lower R^2 values obtained for the zero-order (0.7459), first-order (0.0751), Hixson–Crowell (0.0794), and Korsmeyer–Peppas (0.4795) models indicate a poorer fit to the experimental data. Although model selection in the present study was primarily based on goodness-of-fit (R^2), it

is acknowledged that complementary statistical approaches, including residual analysis and uncertainty estimation (e.g., RMSE, SSE, Akaike Information Criterion, or confidence intervals), would provide a more rigorous assessment of model suitability. These analyses were beyond the scope of the present investigation and should be incorporated in future studies to strengthen the interpretation of release kinetics.

The release data were further analyzed using various kinetic models and the Higuchi model was observed to be the best-fit for in vitro release data as validated by its higher linearity regression coefficient (0.9602) compared to zero order (0.7459), first order (0.0751), and Hixson Crowell (0.0794) models as shown in **Figure 6-A(b-e)**. Additionally, **Figure 6-A(f)** illustrates the mechanism followed by FB to diffuse from the PCL-NFIs as investigated using Korsmeyer-Peppas model. The kinetic constant (k) and release exponent (n) values were found to be 0.3151 and 0.4608, respectively. The $n < 0.5$ signifies the quasi-Fickian diffusion mechanism, which means diffusion-controlled drug release from a polymer matrix that erodes slowly. As PCL is a hydrophobic polymer, it does not swell much when exposed diffusion medium. The PCL-NFs possess very high surface-area-to-volume ratio. FB molecules located on or near the surface of the NFs may be released very quickly (burst release) that causes a deviation from a purely Fickian mechanism. Following initial burst, the release rate of FB decreases due to slower diffusion of FB from deeper layers of PCL matrix. The FB molecules must travel the longer and more tortuous path through PCL network to reach the surface, leading to sustained FB release.⁶⁹

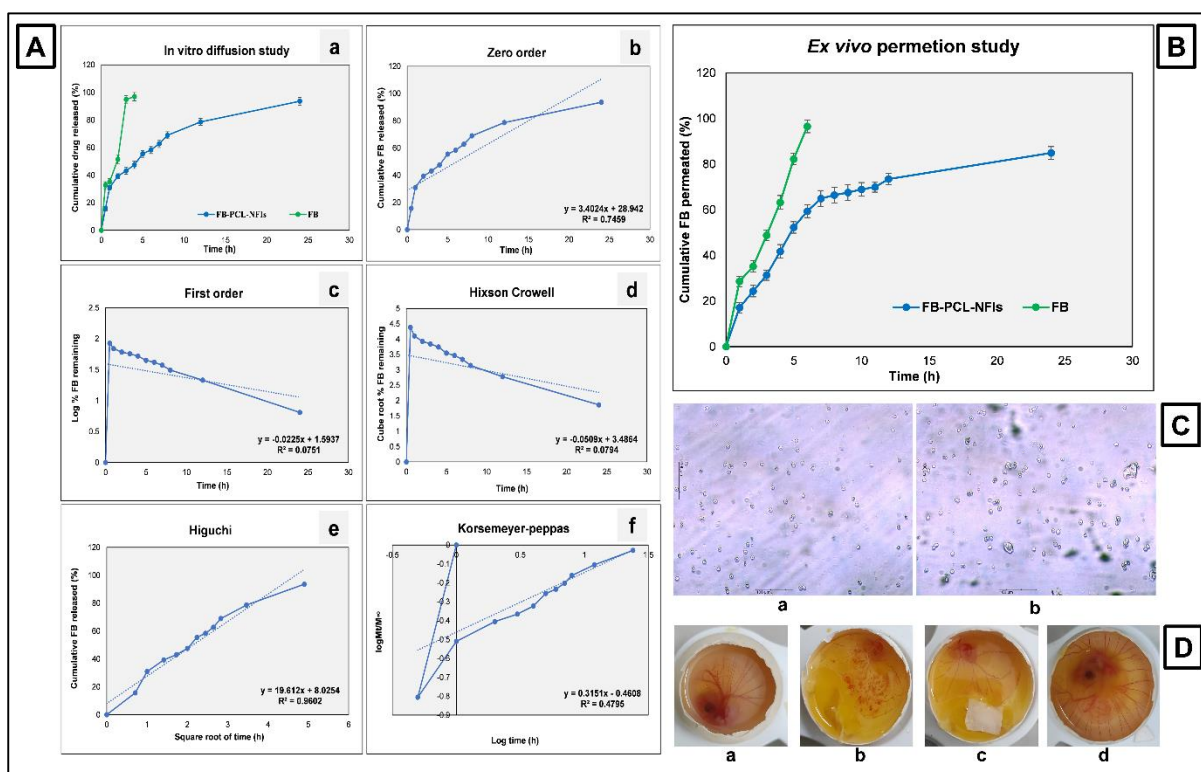


Figure 6: (A) *In vitro* drug release profiles of FB as a function of time from (a) FB aqueous dispersion, and FB-PCL-NFIs through a cellulose dialysis membrane. [Data expressed as Mean $\pm$ SD, $n = 3$]. Kinetics of FB release using (b) Zero order, (c) First order, (d) Hixson Crowell, (e) Higuchi, and (f) Korsmeyer-Peppas kinetic models. (B) *Ex vivo* permeation profile of FB as a function of time from (a) FB aqueous dispersion, and FB-PCL-NFIs through a goat cornea (permeation barrier). [Data expressed as Mean $\pm$ SD, $n = 3$]. (C) Isotonicity test performed on: (a) untreated control RBCs, (b) treatment group comprising RBCs incubated with FB-PCL-NFIs. (D) HET CAM image showing the effect on CAM after exposure to (a) 0.9% NaCl (Negative control), (b) 0.1 M NaOH (Positive Control), (c) FB-PCL-NFIs treated group, and (d) Marketed eye drop (Ocuflur® IP, 0.03% w/v) treated group.

3.2.5 *Ex vivo* permeation

Figure 6-B demonstrates the *ex vivo* permeation profile of FB from FB aqueous dispersion and FB-PCL-NFIs across excised goat cornea over 24 h in PBS (pH 7.4). Around 96% of FB was permeated from FB aqueous

dispersion within 6 h whereas FB-PCL-NFIs released ~89% of FB up to 24 h. Both exhibited high permeability, which may be due to the transcellular transport of hydrophobic FB (a BCS Class IV candidate) through lipid-rich epithelial layer of the cornea, allowing for deeper penetration. NFIs increased the residence time of FB on corneal surface, leading to enhanced retention and prolonged FB permeation over more than 24 h. This ultimately contribute to higher therapeutic concentrations of FB compared to conventional eye drops where drugs usually washed off with tears, due to blinking reflexes, rapid lacrimal fluid turnover rate, and short residence time of drugs in conjunctival sac. Higher permeation of FB from PCL-NFIs over extended period guarantee the efficient management of ocular diseases.⁷⁰ In our opinion, the large surface area of NFs may also contribute to the higher permeation of FB across the corneal tissues.

3.2.6 Evaluation of sterility, irritancy, and toxicity

On sterility testing, the FB-PCL-NFIs, blank PLC-NFs, and negative control exhibited no turbidity indicating absence of microorganisms and validate the sterility of the analyzed samples. In addition, the positive control showed turbidity suggesting the precision of the adopted methodology. EtO-based terminally sterilized NFIs were further employed for the *in vivo* ocular pharmacokinetic studies to investigate the suitability of FB-PCL-NFIs for ocular administration.⁴²

The findings demonstrated that the RBCs incubated with fabricated FB-PCL-NFIs maintained their predominately biconcave, disc-like shape and appeared intact under microscope, suggesting the possible hemocompatibility of the fabricated NFIs. No procedural difference was observed between the untreated control group and treatment group. Moreover, no bulging (hypotonic effect) or shrinkage (hypertonic effect) of cells was observed. These findings are consistent with the previously reported use of RBC morphology and changes in RBC diameter as a primary factor for evaluating formulation for osmotic effect. Additionally, this study represents that the fabricated nanofibers insert does not independently cause irritation or discomfort. Post evaluation, the observed RBCs are shown in **Figure 6-C**.³⁶

Figure 6-D indicates the results of HET-CAM assay, where CAM morphology was examined for three major irritant effects *viz.* coagulation, hemorrhage, and hyperemia. After exposure to negative control (0.9% NaCl), CAM did not show any sign of vascular damage (**Figure 6-D(a)**) and IS was 0, indicating the non-irritant nature of applied sample. In contrast, the exposure to positive control (0.1 M NaOH) resulted in severe irritation responses *viz.* coagulation, hemorrhage, and hyperemia (**Figure 6-D(b)**), with IS of 12. Moreover, exposure of CAM to FB-PCL-NFIs (**Figure 6-D(c)**), and Ocuflur® IP (0.03% w/v) (**Figure 6-D(d)**) did not show any signs of vascular damage including absence of coagulation, hemorrhage, and hyperemia. The IS was just 0.5 and 0.8, respectively, indicating that the fabricated NFIs were non-irritant and appropriate for ocular administration.^{36,41,44}

Table S1 (supplementary data) illustrates the observed IS.

3.2.7 *In vivo* studies

A primary regulatory requirement for any drug delivery systems intended for ocular administration is the absence of any significant ocular adverse effects *viz.* irritation, burning, stinging, and tearing. The FB-PCL-NFIs, in comparison to Ocuflur® IP (0.03% w/v) eye drop, were investigated for estimation of ocular irritancy potential for 7 days in NZW rabbits. In both groups of rabbit eyes treated with FB-PCL-NFIs, and Ocuflur®, no visible symptoms of irritation or injury *viz.* redness, swelling and/or unusual tearing were noticed during the study period. The corneas of the eyes under examination did not exhibit the signs of turbidity and redness. There was no visible swelling in the eyelids. The blood vessels in the iris and conjunctivae were also normal (**Figure 7**). Thus, for both

formulations, no noticeable signs of irritation were found suggesting biocompatibility, tolerability, and suitability of the prepared FB-PCL-NFIs for ocular administration.^{42,46}

The *in vivo* tear pharmacokinetics studies involved insertion of FB-PCL-NFIs into the cul-de-sac of rabbit's eye. The techniques utilized for insertion of NFIs, and tear collection were noninvasive. Although the flush-tear and Schirmer-strip methods are widely used for ocular pharmacokinetic evaluation, some variability in tear recovery is unavoidable due to differences in tear retention and strip wetting capacity. In the present study, this variability was minimized by maintaining a standardized sampling protocol, including a fixed PBS flushing volume, predetermined contact time, and consistent use of Schirmer strips for all experimental groups. Furthermore, the extraction procedure was uniformly applied to all samples, and analytical measurements were performed in triplicate to ensure reproducibility and reliability of the obtained tear concentration data. **Figure S2** illustrates the linearity curve of FB in acetonitrile and water (80:20 % v/v) acidified with 0.1 % trifluoro acetic acid (pH 3.5). Linearity was obtained with good regression coefficient (R^2) values of 0.9987 at 247 nm over a concentration range of 5–25 $\mu\text{g mL}^{-1}$. The developed analytical method demonstrated adequate reproducibility for the quantification of FB in tear samples collected using Schirmer strips. The consistency observed among triplicate determinations supported the reliability of the concentration-time profiles generated during the pharmacokinetic evaluation. Following dose normalization to account for the difference in the administered flurbiprofen dose, the tear fluid concentration-time profiles (**Figure 8**) demonstrated that FB-PCL-NFIs provided a prolonged ocular residence time and sustained release of flurbiprofen compared with Ocuflur® IP. The dose-normalized pharmacokinetic analysis showed that FB-PCL-NFIs achieved sustained ocular exposure over 48 h compared with the commercial eye drops. Although Ocuflur® IP produced an earlier peak concentration ($T_{\text{max}} = 1 \text{ h}$), drug levels declined rapidly and fell below the quantification limit after 8 h. In contrast, FB-PCL-NFIs exhibited a delayed T_{max} (8 h) with gradual drug release that was maintained throughout the 48 h study period.^{17,42}

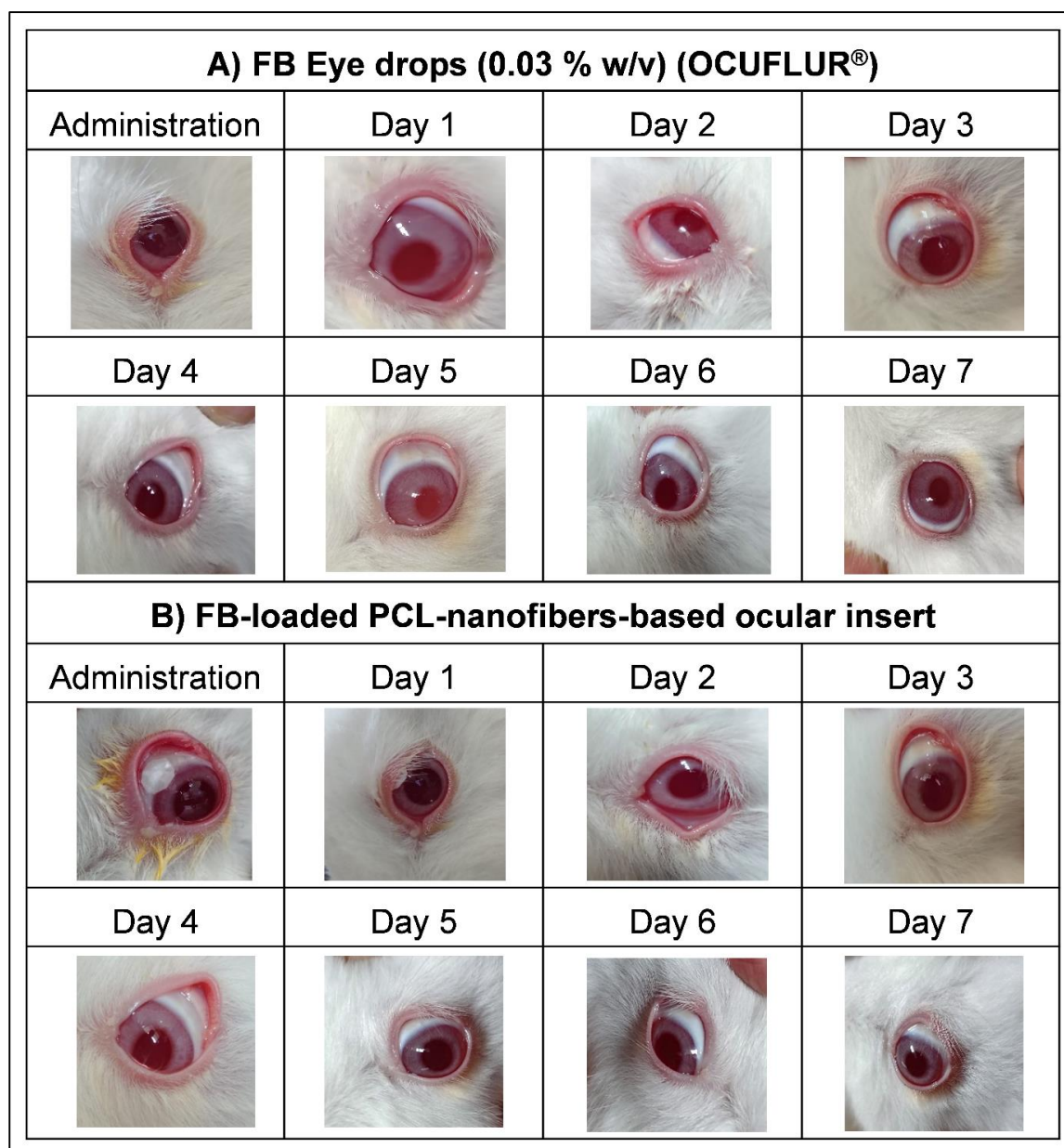


Figure 7: Photographs of NZW rabbit's eyes (cornea and conjunctivae) demonstrating the absence of ocular irritation following treatment with A) Ocuflur® IP (0.03% w/v) and B) FB-PCL-NFIs, investigated over 7 days.

The higher dose-normalized AUC_{0–48} values obtained with FB-PCL-NFIs demonstrate their superior ability to sustain ocular drug exposure. This behavior is attributed to the encapsulation of flurbiprofen within the PCL nanofibrous matrix, which enabled controlled drug release and prolonged precorneal residence compared with the conventional eye drops.

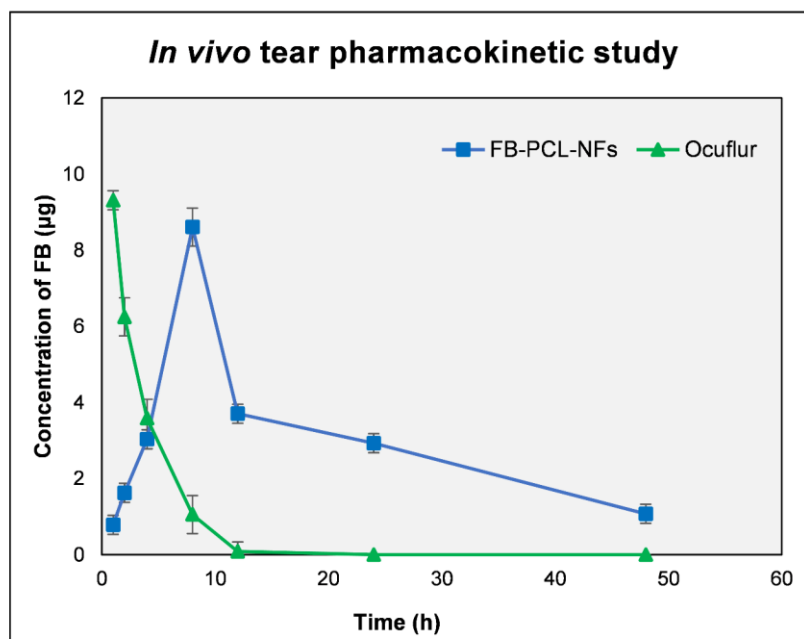


Figure 8: Tear concentrations vs. time profile of FB following ocular administration of FB-PCL-NFIs, and Ocuflur® IP (0.03 % w/v) eye drop in rabbits' eyes during *in vivo* tear pharmacokinetics study.

3.2.8 Stability study and shelf-life estimation

Figure 9 depicts the ATR-IT spectra of FB-PCL-NFIs held for stability analysis. There was no significant change observed in the spectra of studies samples indicating chemical stability during the defined stability conditions (40 ± 2 °C and 75 ± 5 % RH) over a four-week period.⁴⁵ In addition, the percent drug content had shown a very negligible change after six months. The projected shelf-life (t_{90}) of the fabricated FB-PCL-NFIs relative to the lower specification limit was found to be 43.2 months (**Figure 10**).⁵⁰

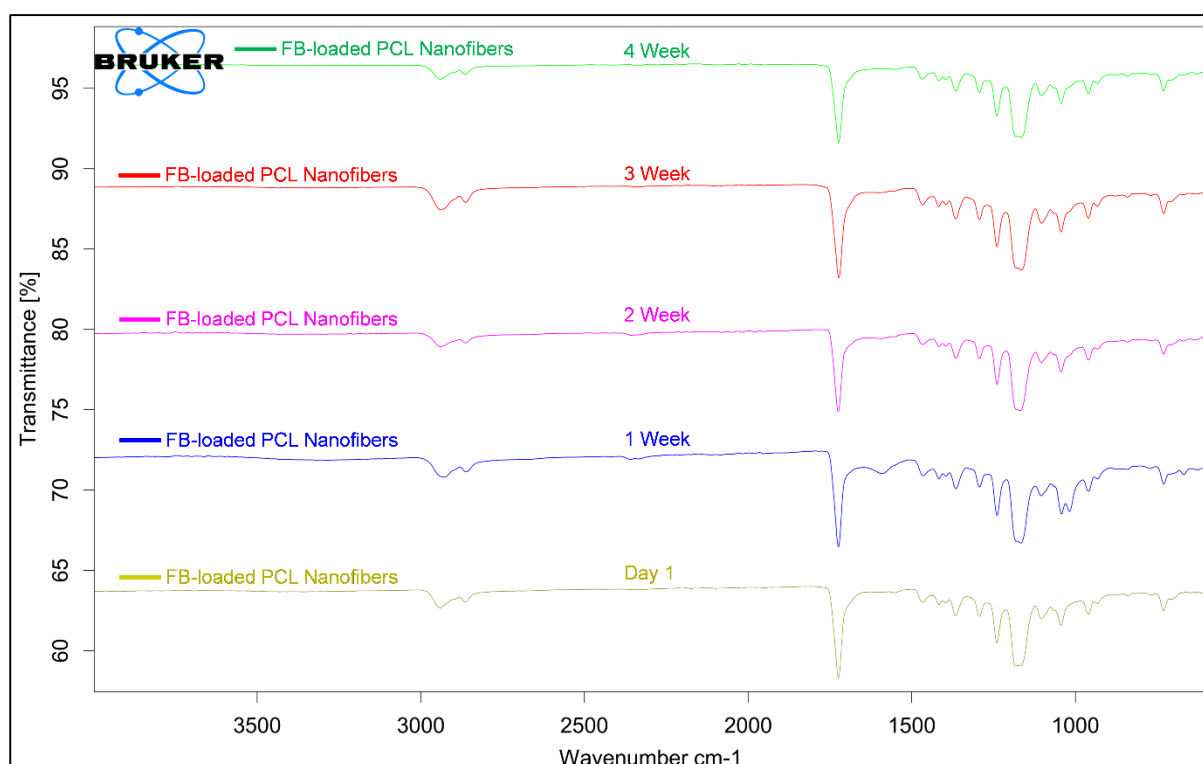


Figure 9: ATR-IR spectra of FLB-PCL-NFIs under defined stability conditions studied over four weeks period.

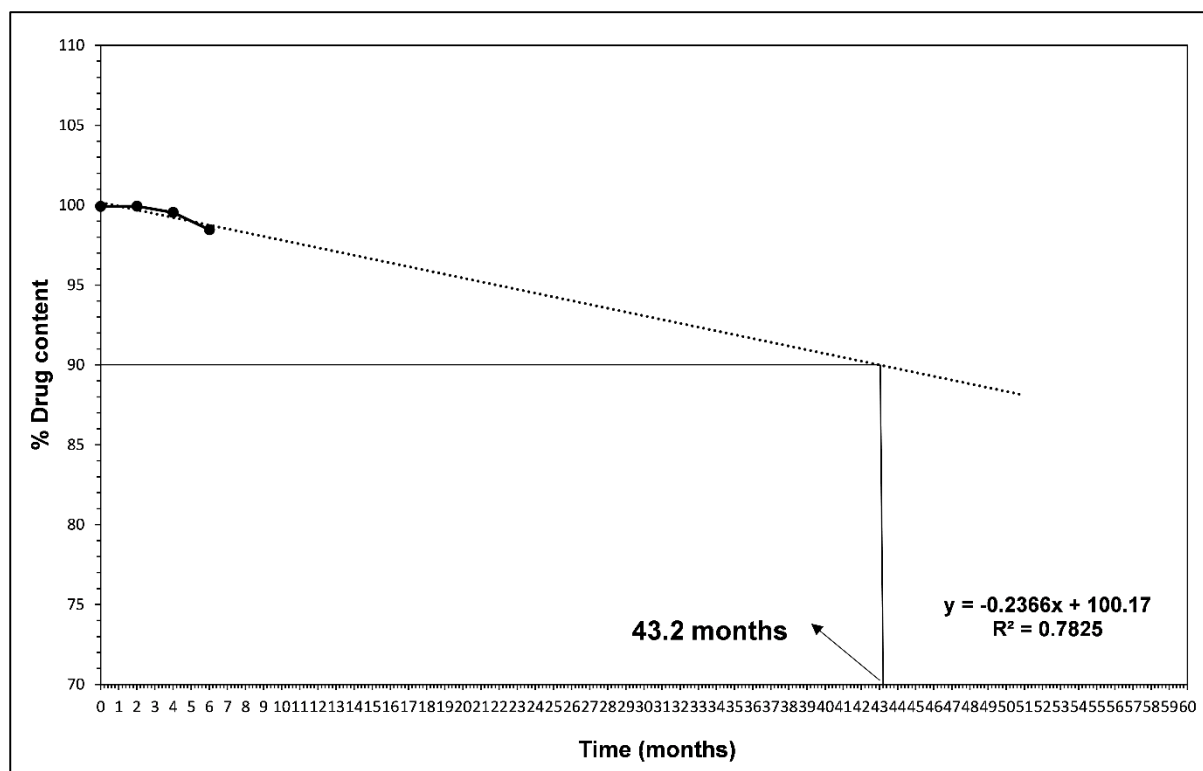


Figure 10: A percent drug content vs. time plot representing the projected shelf-life of FB-PCL-NFIs, following long-term storage.

4. Conclusion and future perspectives

The study successfully investigated the FB-infused PLC-NFs-based ocular inserts for the possible noninvasive treatment of ocular diseases. The prepared FB-PCL-NFIs benefited from the enhanced ocular retention, sustained FB release, enhanced permeation, and improved patient compliance. These merits praise FB-NFIs as potential alternative to the existing conventional therapies, which usually pose various challenges viz. rapid eye flushing, and patients' non-adherence to the repetitive drug delivery. In addition, the NFIs also poses few challenges viz. appropriate placement inside the eye, potential vision interference, and discomfort to the patient. Conventional single-needle electrospinning poses hurdles for large-scale GMP manufacturing and thus, scalable electrospinning technologies viz. multi-needle, needleless, and free-surface electrospinning systems are becoming more compatible with continuous manufacturing under GMP conditions. Further developments directed towards optimizing the design of ocular inserts and improving the insert placement techniques could potentially minimize the patients' uneasiness to the use of inserts. The promising scalability and long-term stability of NFIs may possible open the way to fully realize the clinical efficacy of this budding next-generation drug delivery technology.

Ethics approval and consent to participate:

The Institutional Animal Ethics Committee (IAEC; Reg. 651/PO/ReBi/S/02/CPCSEA) at R. C. Patel Institute of Pharmaceutical Education and Research (Shirpur, India) authorized the preclinical study protocol (IAEC/RCPIPER/2024-25/35; Dt. 28 April 2025).

Consent for publication

All authors reviewed the manuscript and approved for publication.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests.

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Data Availability Statement

Data will be made available on request.

Authors' contributions:

Conceptualization: [VR, CP]

Data curation: [VR, GS, DB, IA, PS, CB]

Formal analysis: [VR, GS, DB, IA, PS, CB]

Investigation: [VR, GS, DB, IA, PS, CB]

Methodology: [VR, GS, DB, IA, PS, CB]

Project administration: [CP]

Resources: [KP, BP, SS, CP]

Software: [VR, GS, DB, IA, PS]

Supervision: [KP, BP, SS, CP]

Validation: [VR, GS, DB, IA, PS, CB]

Writing—original draft: [VR, GS, DB, IA, PS, CB]

Writing—review & editing: [KP, BP, SS, CP]

5. References

1. Kurul F, Turkmen H, Cetin AE, Topkaya SN. Nanomedicine: How nanomaterials are transforming drug delivery, bio-imaging, and diagnosis. *Next Nanotechnol* 2025;7:100129. <https://doi.org/10.1016/j.nxnano.2024.100129>
2. Hawthorne D, Pannala A, Sandeman S, Lloyd A. Sustained and targeted delivery of hydrophilic drug compounds: A review of existing and novel technologies from bench to bedside. *J Drug Deliv Sci Technol* 2022;78:103936. <https://doi.org/10.1016/j.jddst.2022.103936>
3. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date: Bobo et al. *Pharm Res* 2016;33(10):2373-87. <https://doi.org/10.1007/s11095-016-1958-5>
4. Wan X, Zhao Y, Li Z, Li L. Emerging polymeric electrospun fibers: From structural diversity to application in flexible bioelectronics and tissue engineering. *Exploration (Beijing)* 2022;2(1):20210029. <https://doi.org/10.1002/EXP.20210029>
5. Miller JR, Hanumunthadu D. Inflammatory eye disease: An overview of clinical presentation and management. *Clin Med (Lond)* 2022;22(2):100-3. <https://doi.org/10.7861/clinmed.2022-0046>

6. Mukhopadhyay D, Mukherjee S, Francis G, Mukherjee H. An Indian epidemiological overview of ocular emergencies of the year 2022. *Clin Epidemiol Glob Health* 2024;27:101589. <https://doi.org/10.1016/j.cegh.2024.101589>
7. Bairagi RD, Reon RR, Hasan MM, Sarker S, Debnath D, Rahman MT, et al. Ocular drug delivery systems based on nanotechnology: a comprehensive review for the treatment of eye diseases. *Discov Nano* 2025;20(1):75. <https://doi.org/10.1186/s11671-025-04234-6>
8. Pardeshi SR, More MP, Kulkarni AD, Pardeshi CV, Patil PB, Patil AS, et al. Current perspectives in nanomedicine delivery for targeted ocular therapeutics. *Bull Mater Sci* 2023;46(1):35. <https://doi.org/10.1007/s12034-022-02869-0>
9. Onugwu AL, Ugorji OL, Ufondu CA, Ihim SA, Echezona AC, Nwagwu CS, et al. Nanoparticle-based delivery systems as emerging therapy in retinoblastoma: recent advances, challenges and prospects. *Nanoscale Adv* 2023;5(18):4628-48. <https://doi.org/10.1039/d3na00462g>
10. Mehrandish S, Mohammadi G, Mirzaeei S. Preparation and functional evaluation of electrospun polymeric nanofibers as a new system for sustained topical ocular delivery of itraconazole. *Pharm Dev Technol* 2022;27(1):25-39. <https://doi.org/10.1080/10837450.2021.2018609>
11. Da Silva GR, Lima TH, Fernandes-Cunha GM, Oréface RL, Da Silva-Cunha A, Zhao M, et al. Ocular biocompatibility of dexamethasone acetate loaded poly (ϵ -caprolactone) nanofibers. *Eur J Pharm Biopharm* 2019;142:20-30. <https://doi.org/10.1016/j.ejpb.2019.05.010>
12. Romeo A, Kazsoki A, Omer S, Pinke B, Mészáros L, Musumeci T, et al. Formulation and characterization of electrospun nanofibers for melatonin ocular delivery. *Pharmaceutics* 2023;15(4):1296. <https://doi.org/10.3390/pharmaceutics15041296>
13. Lokhande S, Bhandari Y, Ansari M, Khairnar S, Singh K. Development and evaluation of biodegradable nanofiber insert containing hyaluronic acid for treatment of dry eye syndrome. *J Drug Deliv Sci Technol* 2023;87:104847. <https://doi.org/10.1016/j.jddst.2023.104847>
14. Sahu DK, Pradhan D, Halder J, Biswasroy P, Kar B, Ghosh G, et al. Design and optimization of gatifloxacin loaded polyvinyl alcohol nanofiber for the treatment of dry eye infection: In vitro and in vivo evaluation. *J Drug Deliv Sci Technol* 2022;76:103651. <https://doi.org/10.1016/j.jddst.2022.103651>
15. Farkas E, Abboud H, Nagy N, Hofmeister B, Ostorházi E, Tóth B, et al. Formulation and development of nanofiber-based ophthalmic insert for the treatment of bacterial conjunctivitis. *Int J Mol Sci* 2024;25(17):9228. <https://doi.org/10.3390/ijms25179228>
16. Taghe S, Mirzaeei S, Ahmadi A. Preparation and evaluation of nanofibrous and film-structured ciprofloxacin hydrochloride inserts for sustained ocular delivery: pharmacokinetic study in rabbit's eye. *Life (Basel)* 2023;13(4):913. <https://doi.org/10.3390/life13040913>
17. Taghe S, Mirzaeei S, Bagheri M. Preparation of polycaprolactone and polymethacrylate nanofibers for controlled ocular delivery of ketorolac tromethamine: Pharmacokinetic study in Rabbit's Eye. *Eur J Pharm Sci* 2024;192:106631. <https://doi.org/10.1016/j.ejps.2023.106631>
18. Mirzaeei S, Faryadras FB, Mehrandish S, Rezaei L, Daneshgar F, Karami A. Development and evaluation of polycaprolactone-based electrospun nanofibers containing timolol maleate as a sustained-release device for treatment of glaucoma: in vivo evaluation in equine eye. *Res Pharm Sci* 2022;17(5):468. <https://doi.org/10.4103/1735-5362.355196>

19. Zhang Q, Lin Z, Zhang W, Huang T, Jiang J, Ren Y, et al. Fabrication of green poly (vinyl alcohol) nanofibers using natural deep eutectic solvent for fast-dissolving. *Drug Deliv* 2021;11(2):1012-21. <https://doi.org/10.1039/d0ra08755f>
20. Marinceş L, Farkas NI, Barbu-Tudoran L, Barabás R, Toşa MI. Deep eutectic solvent PCL-based nanofibers as drug delivery system. *Mater Chem Phys* 2023;304:127862. <https://doi.org/10.1016/j.matchemphys.2023.127862>
21. Snetkov P, Morozkina S, Olekhovich R, Vu TH, Tyanutova M, Uspenskaya M. Curcumin/usnic acid-loaded electrospun nanofibers based on hyaluronic acid. *Materials (Basel)* 2020;13(16):3476. <https://doi.org/10.3390/ma13163476>
22. Halder J, Dubey D, Rajwar TK, Mishra A, Satpathy B, Sahoo D, et al. Local delivery of methotrexate/glycyrrhizin-loaded hyaluronic acid nanofiber for the management of oral cancer. *Int J Pharm* 2024;660:124311. <https://doi.org/10.1016/j.ijpharm.2024.124311>
23. Laha A, Yadav S, Majumdar S, Sharma CS. In-vitro release study of hydrophobic drug using electrospun cross-linked gelatin nanofibers. *Biochem Eng J* 2016;105:481-8. <https://doi.org/10.1016/j.bej.2015.11.001>
24. Dash TK, Konkimalla VB. Poly-ε-caprolactone based formulations for drug delivery and tissue engineering: A review. *J Control Release* 2012;158(1):15-33. <https://doi.org/10.1016/j.jconrel.2011.09.064>
25. Guarino V, Gentile G, Sorrentino L, Ambrosio L. Polycaprolactone: synthesis, properties, and applications. *Encyclopedia of Polymer Science and Technology* 2002:1-36. <https://doi.org/10.1002/0471440264.pst658>
26. Omer S, Zelkó R. A systematic review of drug-loaded electrospun nanofiber-based ophthalmic inserts. *Pharmaceutics* 2021;13(10):1637. <https://doi.org/10.3390/pharmaceutics13101637>
27. Luraghi A, Peri F, Moroni L. Electrospinning for drug delivery applications: A review. *J Control Release* 2021;334:463-84. <https://doi.org/10.1016/j.jconrel.2021.03.033>
28. Salehi AO, Rafienia M, Mamidi N, Keshel SH, Baradaran-Rafii A. Electrospun aloe vera extract loaded polycaprolactone scaffold for biomedical applications: a promising candidate for corneal stromal regeneration. *J Bionic Eng* 2024;21(4):1949-59. <https://doi.org/10.1007/s42235-024-00520-8>
29. Blazaki S, Tsika C, Tzatzarakis M, Naoumidi E, Tsatsakis A, Tsatsanis C, et al. Pharmacokinetics and efficacy of intraocular flurbiprofen. *Graefes Arch Clin Exp Ophthalmol* 2017;255(12):2375-80. <https://doi.org/10.1007/s00417-017-3812-9>
30. Wu B, Feng J, Zeng T, Guo Q, Zhang Z, Ding C, et al. Flurbiprofen loaded thermosensitive nanohydrogel for ophthalmic anti-inflammatory therapy. *J Drug Deliv Sci Technol* 2022;70:103253. <https://doi.org/10.1016/j.jddst.2022.103253>
31. Garkal A, Bangar P, Mehta T. Thin-film nanofibers for treatment of age-related macular degeneration. *OpenNano* 2022;8:100098. <https://doi.org/10.1016/j.onano.2022.100098>
32. Czarnecka K, Wojasiński M, Ciach T, Sajkiewicz P. Solution blow spinning of polycaprolactone—rheological determination of spinnability and the effect of processing conditions on fiber diameter and alignment. *Materials (Basel)* 2021;14(6):1463. <https://doi.org/10.3390/ma14061463>
33. Bhattarai RS, Das A, Alzhrani RM, Kang D, Bhaduri SB, Boddu SH. Comparison of electrospun and solvent cast polylactic acid (PLA)/poly (vinyl alcohol)(PVA) inserts as potential ocular drug delivery

- vehicles. *Mater Sci Eng C Mater Biol Appl* 2017;77:895-903. <https://doi.org/10.1016/j.msec.2017.03.305>
34. Kamranpour S, Mirzaeei S, Daneshgar F, Najafi F. Fast-dissolving sublingual nanofibers of ondansetron hydrochloride: formulation, physicochemical characterization, and clinical evaluation on post-cataract surgery patients. *Pharm Sci* 2021;28(1):101-11.
 35. Khalil IA, Ali IH, El-Sherbiny IM. Noninvasive biodegradable nanoparticles-in-nanofibers single-dose ocular insert: in vitro, ex vivo and in vivo evaluation. *Nanomedicine (Lond)* 2019;14(1):33-55. <https://doi.org/10.2217/nnm-2018-0297>
 36. Singla J, Bajaj T, Goyal AK, Rath G. Development of nanofibrous ocular insert for retinal delivery of fluocinolone acetonide. *Curr Eye Res* 2019;44(5):541-50. <https://doi.org/10.1080/02713683.2018.1563196>
 37. Ahmad Wsoo M, Izwan Abd Razak S, Shahir S, Ahmed Abdullah Al-Moalemi H, Rafiq Abdul Kadir M, Hasraf Mat Nayan N. Development of prolonged drug delivery system using electrospun cellulose acetate/polycaprolactone nanofibers: Future subcutaneous implantation. *Polym Adv Technol* 2021;32(9):3664-78. <https://doi.org/10.1002/pat.5375>
 38. Khan N, Saneja A. Spectroscopic and structural investigations of quercetin/sulfobutylether- β -cyclodextrin inclusion complex incorporated pH-responsive electrospun nanofibers for colon-specific delivery. *J Mol Struct* 2025;1341:142652. <https://doi.org/10.1016/j.molstruc.2025.142652>
 39. Turanlı Y, Acartürk F. Fabrication and characterization of budesonide loaded colon-specific nanofiber drug delivery systems using anionic and cationic polymethacrylate polymers. *J Drug Deliv Sci Technol* 2021;63:102511. <https://doi.org/10.1016/j.jddst.2021.102511>
 40. Hu J, Prabhakaran MP, Tian L, Ding X, Ramakrishna S. Drug-loaded emulsion electrospun nanofibers: characterization, drug release and in vitro biocompatibility. *RSC Adv* 2015;5(121):100256-67. <https://doi.org/10.1039/c5ra18535a>
 41. Pardeshi SR, More MP, Pardeshi CV, Chaudhari PJ, Gholap AD, Patil A, et al. Novel crosslinked nanoparticles of chitosan oligosaccharide and dextran sulfate for ocular administration of dorzolamide against glaucoma. *J Drug Deliv Sci Technol* 2023;86:104719.
 42. Karami A, Mirzaeei S, Rezaei L, Nokhodchi A. Development and evaluation of polymethacrylate-based ophthalmic nanofiber inserts containing dual drug-loaded dorzolamide and timolol: in vivo study in rabbit's eye. *Biomedicines* 2025;13(1):200. <https://doi.org/10.3390/biomedicines13010200>
 43. Pandit J, Chaudhary N, Emad NA, Ahmad S, Solanki P, Aqil M, et al. Fenofibrate loaded nanofibers based thermo-responsive gel for ocular delivery: Formulation development, characterization and in vitro toxicity study. *J Drug Deliv Sci Technol* 2023;89:104935. <https://doi.org/10.1016/j.jddst.2023.104935>
 44. Patel Z, Gharat S, Momin M. Fluocinolone acetonide loaded chitosan nanofiber scaffolds for treatment of ocular disorders: In vitro characterization, ex-vivo corneal and ex-vivo scleral evaluation. *Curr Eye Res* 2024;49(5):467-76. <https://doi.org/10.1080/02713683.2023.2298917>
 45. Omer S, Nagy N, Szöcs E, Kádár S, Völgyi G, Pinke B, et al. Development of innovative electrospun nepafenac-loaded nanofibers-based ophthalmic inserts. *Int J Pharm* 2023;647:123554. <https://doi.org/10.1016/j.ijpharm.2023.123554>
 46. Aboali FA, Habib DA, Elbedaiwy HM, Farid RM. Curcumin-loaded proniosomal gel as a biofriendly alternative for treatment of ocular inflammation: In-vitro and in-vivo assessment. *Int J Pharm* 2020;589:119835. <https://doi.org/10.1016/j.ijpharm.2020.119835>

47. Gaafar PM, Abdallah OY, Farid RM, Abdelkader H. Preparation, characterization and evaluation of novel elastic nano-sized niosomes (ethoniosomes) for ocular delivery of prednisolone. *J Liposome Res* 2014;24(3):204-15. <https://doi.org/10.3109/08982104.2014.881850>
48. Bertens CJ, Gijs M, Dias AA, van den Biggelaar FJ, Ghosh A, Sethu S, et al. Pharmacokinetics and efficacy of a ketorolac-loaded ocular coil in New Zealand white rabbits. *Drug Deliv* 2021;28(1):400-7. <https://doi.org/10.1080/10717544.2021.1883157>
49. Maddiboyina B, Jhawar V, Desu PK, Gandhi S, Nakkala RK, Singh S. Formulation and evaluation of thermosensitive flurbiprofen in situ nano gel for the ocular delivery. *J Biomater Sci Polym Ed* 2021;32(12):1584-97. <https://doi.org/10.1080/09205063.2021.1927460>
50. Rehman S, Jamil QA, Noreen S, Ashraf MA, Madni A, Mahmood H, et al. Preparation and evaluation of pH-sensitive chitosan/alginate nanohybrid mucoadhesive hydrogel beads: an effective approach to a gastro-retentive. *Drug Deliv* 2024;16(11):1451. <https://doi.org/10.3390/pharmaceutics16111451>
51. Herrero-Herrero M, Gomez-Tejedor JA, Valles-Lluch A. Role of electrospinning parameters on poly (lactic-co-glycolic acid) and poly (caprolactone-co-glycolic acid) membranes. *Polymers (Basel)* 2021;13(5):695. <https://doi.org/10.3390/polym13050695>
52. Abelson MB, Sadun AA, Udell IJ, Weston JH. Alkaline tear pH in ocular rosacea. *Am J Ophthalmol* 1980;90(6):866-869.
53. Thygesen JE, Jensen OL. pH changes of the tear fluid in the conjunctival sac during postoperative inflammation of the human eye. *Acta Ophthalmol* 1987;65(2):134-6. <https://doi.org/10.1111/j.1755-3768.1987.tb06990.x>
54. Renkler NZ, Ergene E, Gokyer S, Tuzlakoglu Ozturk M, Yilgor Huri P, Tuzlakoglu K. Facile modification of polycaprolactone nanofibers with egg white protein. *J Mater Sci Mater Med* 2021;32(4):34. <https://doi.org/10.1007/s10856-021-06505-x>
55. Tawfik EA, Craig DQ, Barker SA. Dual drug-loaded coaxial nanofibers for the treatment of corneal abrasion. *Int J Pharm* 2020;581:119296. <https://doi.org/10.1016/j.ijpharm.2020.119296>
56. Shaheen N, Zaman SU. Development of fast dissolving tablets of flurbiprofen by sublimation method and its in vitro evaluation. *Braz J Pharm Sci* 2019;54(04):e17061. <https://doi.org/10.1590/s2175-97902018000417061>
57. Phillipson K, Hay JN, Jenkins MJ. Thermal analysis FTIR spectroscopy of poly (ϵ -caprolactone). *Thermochim Acta* 2014;595:74-82. <https://doi.org/10.1016/j.tca.2014.08.027>
58. Ali S, Khatri Z, Oh KW, Kim IS, Kim SH. Preparation and characterization of hybrid polycaprolactone/cellulose ultrafine fibers via electrospinning. *Macromol Res* 2014;22(5):562-8. <https://doi.org/10.1007/s13233-014-2078-x>
59. Zahedi P, Fallah-Darrehchi M. Electrospun egg albumin-PVA nanofibers containing tetracycline hydrochloride: Morphological, drug release, antibacterial, thermal and mechanical properties. *Fibers Polym* 2015;16(10):2184-92. <https://doi.org/10.1007/s12221-015-5457-9>
60. Varma MM, Pandi JK. Dissolution, solubility, XRD, and DSC studies on flurbiprofen-nicotinamide solid dispersions. *Drug Dev Ind Pharm* 2005;31(4-5):417-23. <https://doi.org/10.1080/03639040500214613>
61. Joshi R, Garud N. Development, optimization and characterization of flurbiprofen matrix transdermal drug delivery system using Box–Behnken statistical design. *Future J Pharm Sci* 2021;7(1):57. <https://doi.org/10.1186/s43094-021-00199-2>

62. Baptista C, Azagury A, Shin H, Baker CM, Ly E, Lee R, et al. The effect of temperature and pressure on polycaprolactone morphology. *Polymer* 2020;191:122227. <https://doi.org/10.1016/j.polymer.2020.122227>
63. Potrč T, Baumgartner S, Roškar R, Planinšek O, Lavrič Z, Kristl J, et al. Electrospun polycaprolactone nanofibers as a potential oromucosal delivery system for poorly water-soluble drugs. *Eur J Pharm Sci* 2015;75:101-13. <https://doi.org/10.1016/j.ejps.2015.04.004>
64. Soares GO, Lima FA, Goulart GA, Oréfice RL. Physicochemical characterization of the gelatin/polycaprolactone nanofibers loaded with diclofenac potassium for topical use aiming potential anti-inflammatory action. *Int J Polym Mater Polym Biomater* 2022;71(17):1303-18. <https://doi.org/10.1080/00914037.2021.1962875>
65. Akyüz L, Duman F. Encapsulation of flurbiprofen by chitosan using a spray-drying method with in vitro drug releasing and molecular docking. *Turk J Pharm Sci* 2017;14(1):34. <https://doi.org/10.4274/tjps.95867>
66. Norouzi MR, Ghasemi-Mobarakeh L, Itef F, Schoeller J, Fashandi H, Borzi A, et al. Emulsion electrospinning of sodium alginate/poly (ϵ -caprolactone) core/shell nanofibers for biomedical applications. *Nanoscale Adv* 2022;4(13):2929-41. <https://doi.org/10.1039/d2na00201a>
67. Borjigin M, Eskridge C, Niamat R, Strouse B, Bialk P, Kmiec EB. Electrospun fiber membranes enable proliferation of genetically modified cells. *Int J Nanomedicine* 2013;8:855-864. <https://doi.org/10.2147/IJN.S40117>
68. Hashem HM, Motawea A, Kamel AH, Bary EA, Hassan SS. Fabrication and characterization of electrospun nanofibers using biocompatible polymers for the sustained release of venlafaxine. *Sci Rep* 2022;12(1):18037. <https://doi.org/10.1038/s41598-022-22878-7>
69. Xu Y, Li JJ, Yu DG, Williams GR, Yang JH, Wang X. Influence of the drug distribution in electrospun gliadin fibers on drug-release behavior. *Eur J Pharm Sci* 2017;106:422-30. <https://doi.org/10.1016/j.ejps.2017.06.017>
70. Taghe S, Mirzaeei S, Pakdaman N, Kazemi A, Nokhodchi A. Macrolide-loaded nanofibrous inserts with polycaprolactone and cellulose acetate base for sustained ocular delivery: Pharmacokinetic study in Rabbit's eye. *Int J Pharm* 2024;665:124699. <https://doi.org/10.1016/j.ijpharm.2024.124699>