Formulation, development, and evaluation of innovative electrospun nanofiber-based ophthalmic inserts

Ph.D. thesis

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1. Introduction

Delivering drugs to the eye presents significant challenges due to various biological barriers hindering medication from reaching its target site effectively. Factors such as the blinking reflex and the constant renewal of the tear film on the cornea limit the duration drugs remain in the precorneal region. Topical administration via the cornea is the main technique for treating the inner layers of the eye. It is more complicated when targeting the posterior eye segment because of the long diffusional distance, additional barriers, and dilution of the drug in the vitreous humor. Conditions affecting the posterior portion of the eye pose substantial unmet therapeutic needs. Diseases that go untreated can result in blindness or visual impairment. Drugs can either diffuse via the trans-scleral channel, which reaches the choroids, and then diffuse over the blood-retinal barrier, or they can diffuse through the vitreous humor and reach the posterior part of the eye.

The topical route is the most popular and preferred method for administering the medication to the eyes; nevertheless, treating various eye diseases through conventional formulations can be difficult due to the limited bioavailability. Diseases of the posterior eye segment are treated using intravitreal, periocular, and/or transscleral injections, particularly when using recently developed medications; however, effective, safe, and comfortable drug delivery techniques are required. Because of these obstacles to drug permeability through the mentioned barriers, there is a clear demand for smart drug delivery systems that are capable of delivering the drug to the targeted site of action while being safe, effective, and comfortable.

Many alternatives have been tried in the last few decades. Ocular inserts attracted much interest since they decrease overall dosage and dosing frequency, provide slow and controlled drug release, and increase the drug residence on the eye's surface. Furthermore, compared to liquid preparations, they are stable and do not require preservatives, which lowers the possibility of adverse effects and increases the drug's shelf life. Electrospinning is one of the novel methods employed to prepare ocular inserts. The fabricated nanocarriers have the ability to interact with the ocular mucosa, thereby increasing the retention time of the associated drug onto the eye, consequently increasing the permeability across the corneal and conjunctival epithelium.

2. Objectives

General objective (ultimate goal)

The main objective of my work was to develop and formulate electrospun nanofiber-based ophthalmic inserts (OIs) using different model drugs and different polymeric matrices in an attempt to find a drug delivery system that is as efficient as invasive ways in targeting different ophthalmic diseases while maintaining the relative safety and self-convenience of the conventional eye drops with the overall goal of increasing patients' comfortness, acceptability, and reducing diseases burden.

Specific objectives

The details necessary for the complete accomplishment of this work are as follows:

I. Comprehensive literature searching about the nanofiber-based OIs to justify the research and to conduct a theoretical study to collect the necessary information regarding the active ingredients (nepafenac (NEPA), cysteamine hydrochloride (CysH)), polymers (polyvinyl alcohol (PVA) and poloxamer 407 (PO-407)), and other excipients (tetraetoxysilane (TEOS), hydroxypropyl-β-cyclodextrin (HP-β-CD), and polysorbate 80 (PS-80)).

II. To conduct preliminary experiments to set the precursor solutions' suitable composition and define the optimal electrospinning parameters.

III. Evaluation of the effect of different PVA grades, the addition of PO-407, and PS-80 on the morphology and physicochemical properties of the fibers using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), respectively.

IV. To formulate nepafenac-loaded and cysteamine-loaded fibrous webs and to evaluate their morphological and physicochemical characteristics.

V. To study the *in vitro* drug release and *in vitro* and *ex vivo* permeability.

VI. To study the cytocompatibility with hen eggs' chorioallantoic membrane (HET-CAM) of fertilized chick embryos.

VII. To study the effect of applying elevated humidity and temperature levels on the morphological and physicochemical characteristics.

VIII. To study the effect of applying elevated humidity and temperature levels on the morphological and physicochemical characteristics.

3. Methods

3.1. Literature searching

A comprehensive literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines to search for relevant studies and report construction. A systematic search was performed in PubMed, Ovid Medline, Web of Science, ScienceDirect, Scopus, Reaxys, Google Scholar, and Google Patents/Espacenet. Search results from all databases were exported to the Mendeley reference manager, and the articles underwent two successive screening processes. The relevant articles were double-checked by the reviewers based on the inclusion criteria, and the required information was extracted and tabulated into the following variables: polymer base, loaded drug/concentration, dimensions of inserts used in the study, diameter of nanofibers, *in vivo* animal model, and effects/properties of presented system.

3.2. Preparation of electrospinning solutions

3.2.1. Preparation of polyvinyl alcohol (PVA)/poloxamer 407 (PO-407) solutions

Three different solutions of different grades of PVA were prepared at concentrations of 15%, 17.5%, and 20% w/w for low molecular weight PVA, 13%, 14%, and 15% for intermediate molecular weight PVA, and 5%, 7.5%, and 10% for high molecular weight PVA. The required amount of polymers and distilled water were used to make each PVA solution. PVA solutions (P) were prepared by stirring while heated. Parallelly, PO-407 solutions were made by dissolving the necessary amount in cold water. The solutions were mixed to prepare PVA/PO-407 solutions (PP) with 85:15, 80:20, and 75:25 m:m mass ratios. PS-80 (0.5% and 1% w/w)) was also added to the PVA/PO-407 (80:20 m:m) to prepare other solutions (PPP).

3.2.2. Preparation of tetraetoxysilane (TEOS)/ polyvinyl alcohol (PVA) solutions

To prepare the TEOS/PVA precursor solutions, a solution of TEOS:EtOH:H2O:HCl 1:3:8:0.04 molar was prepared in a tightly sealed glass container at 60 °C. After 3 h of hydrolysis, solutions of 10,12 and14 % (w/w) PVA water: ethanol 8:2 (m:m) were added in 4 different ratios (mass ratio of the TEOS: PVA solutions were 1:4, 2:3, 3:2, and 4:1) and stirred for 1 h at 60 °C.

3.2.3. Preparation of nepafenac (NEPA)/ Hydroxypropyl-β-cyclodextrin (HP-β-CD) solution in water

To determine the solubility of NEPA in water in the presence of HP-β-CD, aqueous solutions of 50mM HP-β-CD containing 1 mg of NEPA powder were heated in sealed vials at 60°C for one hour. The concentrations of the resulting solutions were measured to determine the dissolved amount. The solutions' absorbance was measured at λ_{max} 238 nm using a Jasco 530 UV-VIS spectrophotometer coupled with an inline probe. The measurements were performed in triplicate, and the previously created calibration curve was used to calculate the average quantities.

3.2.4. Preparation of nepafenac-loaded (NEPA-loaded) solutions

Nine different NEPA 0.1%w/w precursor solutions were prepared by varying the concentrations of HP-β-CD (50 mM, 100 mM, and 150 mM) and PVA: PO-407 (100:00, 85: 15, and 80: 20). The PVA polymer was dissolved in distilled water and stirred while heated to make clear PVA solutions. PO-407 solutions were prepared by dissolving the required amounts in cold water. PVA/PO-407 solutions were prepared by mixing together PO-407 and PVA solutions and then stirred at room temperature until complete homogenization. NEPA/ HP-β-CD was added to the PVA or PVA/PO-407 solutions and subjected to stirring at 60 °C for 60 min, followed by stirring at room temperature for 2 hours until complete solubility of NEPA.

3.2.5. Preparation of cysteamine-loaded (Cys-loaded) solutions

To prepare Cys-loaded solutions, 5.5 mg and 11 mg of CysH were added to 1g of plain PVA/PO-407 and TEOS/PVA viscous solutions to get the final solution concentration of 0.55% (w/w) and 1.1% (w/w). The drug-loaded solutions were then stirred at room temperature for 2 hours until complete homogenization.

3.3. Electrospinning of the solutions

The fibers were prepared using laboratory-scale electrospinning equipment (SpinSplit Ltd., Budapest, Hungary). The electrospinning procedure was adjusted to a flow rate of 0.08–0.1 μL/sec, an applied voltage of 10–20 kV, and effective distances of 10, 12.5, and 15 cm between the grounded collector and the needle. The samples were collected on aluminum foil and stored in a desiccator until further analysis. The

process was conducted at ambient conditions of 22 ± 1 °C room temperature and 40 ± 5 % relative humidity.

3.4. Morphological characterization

The morphological properties of the prepared samples were examined using a JEOL JSM-6380LA scanning electron microscope (SEM). The fibrous samples' diameter was studied using ImageJ software (US National Institutes of Health, 138 Bethesda, MD, USA). Excel 2010 was used to determine the average fiber diameters ± the standard deviation for each sample measurement, skewness, and kurtosis. OriginPro 2018 software (v9.5.1, OriginLab Corporation, Northampton, MA, USA) was used to create the histograms and fit the data to a Gaussian (normal) distribution.

3.5. Solid state characterization

3.5.1. Fourier Transform Infrared Spectroscopy (FTIR)

The physicochemical properties, compatibility, and supramolecular interactions between polymers and other excipients were studied using Fourier transform infrared spectroscopy (FTIR) (Jasco FT/IR-4200 spectrophotometer (Jasco Inc., Easton, MD, USA)).

3.5.2. X-ray diffraction (XRD)

Diffraction patterns were measured on a PANalytical X'Pert3 Powder diffractometer (Malvern Panalytical B.V., The Netherlands). Data were collected by PANalytical Data Collector Application, version 5.5.0.505 (Malvern Panalytical B.V., The Netherlands).

3.6. *In vitro* release study and release kinetics

The *in vitro* releases of NEPA and Cys from the fibrous samples were studied according to a modified method analogy to the basket method reported by Pharmacopoeia Hungarica (Ph.Hg. VIII). The method was developed to fit small-volume physiological compartments such as the buccal cavity and ophthalmic sac. A Jasco 530 UV-VIS spectrophotometer coupled with an inline probe was used to detect the absorbance of the released drugs at a predetermined time interval at λ_{max} 238 nm and λ_{max} 412 nm for NEPA and Cys, respectively.

3.7. Permeability studies of nepafenac-loaded (NEPA-loaded) fibers

3.7.1. *In vitro* corneal parallel artificial membrane permeability assay (PAMPA)

The electrospun samples were dissolved in phosphate-buffered saline (PBS) to create solutions with 1 mg/ml nepafenac concentration, followed by a 20-fold dilution to simulate the tear effect on the applied formulations. Each matching well of the acceptor plate (Multiscreen Acceptor Plate, MSSACCEPTOR; Millipore) was filled with 10% w/v of HP-β-CD solution, whereas in the case of the reference (Nevanac®), the acceptor wells were filled with PBS. Samples were measured in six replicates. The donor plate was filled with 150 µL sample solutions. The system was incubated for 4 hours at 35°C (Heidolph Titramax 1000, Heidolph Instruments, Swabach, Germany). A parallel experiment was conducted using the same method while omitting the sink effect of 10% w/v of HP-β-CD. After that, initial donor and acceptor samples were collected and analyzed by HPLC-DAD. Effective permeability (P_e) and membrane retention (MR) were then calculated.

3.7.2. *Ex vivo* corneal permeability studies on porcine eyes

To study the *ex vivo* permeability of the NEPA samples, porcine eyes obtained freshly from the slaughterhouse were used. Before the experiment, the electrospun samples were dissolved in a physiological saline solution (PSS). The porcine eyes were first placed into Poly(tetrafluoroethene) (PTFE) inserts, where the cornea was uncovered and surrounded by a PTFE ring. The orifice above the corneal surface was first washed with PSS, and then the devices were pre-incubated at 35 °C in a water bath for 5 minutes (min). After that, PSS was removed from the corneal surface, and 100 µL of the undiluted formulations were pipetted on each cornea using 3 eyes for each formulation, then the devices were incubated for 1 min. After that, the samples on the eye surface were diluted by adding PSS to the device's orifice to reach a 20-fold diluted concentration (~0.05 mg/mL) of the original dose, and the eyes were incubated with these diluted formulations for another 14, 29, or 59 min. At these endpoints, diluted formulations were removed from the precorneal area, the devices were disassembled, then aqueous humor was drained using a 26G needle, and finally the cornea was excised and NEPA was extracted with 2 mL of acetonitrile:water 50:50 (v/v) using an orbital shaker (Heidolph Titramax 100, Heidolph Instruments, Swabach, Germany) for 60 min at 450 rpm. Samples of precorneal fluid, aqueous humor, and corneal extract

were analyzed by HPLC-DAD, and the NEPA concentration was calculated using a calibration curve (75). The corneal retention (CR) of NEPA, the apparent permeability ($P_{app}C$) of NEPA into the cornea, and the apparent permeability ($P_{app}Aq$) of NEPA into the aqueous humor were calculated.

3.7.3. *Ex vivo* cornea Raman mapping

Parallel with the corneal permeability, the distribution of NEPA in the excised cornea was investigated using Raman mapping. Raman spectroscopic analysis was performed with a Thermo Fisher DXR Dispersive Raman Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a Charge-coupled Device (CCD) camera and a diode laser with a wavelength of 780 nm. The OMNIC for Dispersive Raman 8.2 software (Thermo Fisher Scientific) was used for chemical evaluation.

3.8. Cytocompatibility Study

The potential for ocular irritation caused by the developed NEPA-loaded and Cys-loaded nanofibers was investigated using the HET-CAM test. The test is based on observing whether hyperemia, hemorrhage, and coagulation would occur upon exposing the chorioallantoic membrane (CAM) of 9-day-old chicken embryos to the developed formulations. Images were taken using a Nikon SMZ25 stereomicroscope (Unicam Ltd., Hungary). Image processing was conducted using Nikon's proprietary software, QCapture Pro.

3.9. Accelerated stability study

A stability chamber (Sanyo type 022, Leicestershire, UK) was used to store the samples for four weeks under stressful conditions (40±2 °C, 75±5% relative humidity). The morphological and physicochemical characteristics of the NEPA-loaded and Cys-loaded electrospun samples were evaluated by SEM and FTIR, respectively.

3.10. Statistical analysis

The statistical analysis and figures were prepared using OriginPro 2018 software (v9.5.1., OriginLab Corporation, Northampton, MA, USA). Skewness and kurtosis were calculated using Microsoft Excel 2010 functions to assess the normality of fiber diameter distribution.

4. Results

4.1. Results of literature analysis and systematic review

A literature search was conducted to assess the feasibility of upscaling the electrospinning process for industrial application and to conduct a systematic review aimed at gathering theoretical information that could be used to manipulate the applicability of nanotechnology in ophthalmic formulations. It has been concluded that the scalability of electrospinning for pharmaceutical applications is possible and that more advancements in the pharmaceutical sector will be made in a matter of time. A comprehensive review of literature and patents yielded 795 articles and 197 patents, from which 13 articles and 15 patents were selected after the screening and extraction processes. The analysis of the articles revealed that most studies focused on increasing residence time and bioavailability. Moreover, findings indicated the ability to modulate drug release durations ranging from minutes to a month based on the polymers used to fabricate nanofibers. These formulations demonstrated compatibility with ocular administration, exhibiting negligible or minimal signs of eye irritation and toxicity when studied *in vivo* involving New Zealand albino rabbits or in vitro models.

4.2. Solution of nepafenac (NEPA)/hydroxypropyl-beta-cyclodextrin (HP-β-CD) in water

The solution of NEPA in water was successfully obtained by adding HP-β-CD, which is consistent with the published studies. Accordingly, each 1mg/mL (3.9 mM) of NEPA can be dissolved by adding approximately 0.069g (50 mM) of HP-β-CD to produce a clear yellow solution.

4.3. Morphological evaluation

4.3.1. Polyvinyl alcohol (PVA)/poloxamer 407 (PO-407)/polysorbate 80 (PS-80) sample

The addition of PS-80, at a concentration of 0.5–1% (w/w), induced slight liquefication of the extremely viscous precursor solutions of PVA/PO-407. With most solution compositions, the overall effect was random fiber deposition. However, several beads were observed, and the fibers became thinner when a high concentration of PS-80 (1% w/w) was added. Low molecular weight PVA (Mw~67 kDa) presented a more noticeable effect. Homogenous normal distribution and compound distribution were among the distribution curves produced by the various solution compositions.

4.3.2. Tetraetoxysilane (TEOS)/ polyvinyl alcohol (PVA)/ samples

The electrospun samples' morphology and the precursor solutions' ability to form fibers demonstrated significant variability. Beady fibrous structures were seen with increasing TEOS concentration while increasing PVA concentration favored the formation of a fibrous structure at the same TEOS: PVA ratio. Gels, rather than very viscous solutions, were formed with some samples. As a result, there was no fiber formation. The average fiber diameters varied significantly. The fiber diameter increases as the polymer concentration increases at the same TEOS: PVA ratio.

4.3.3. Nepafenac-loaded (NEPA-loaded) fibers

The SEM images for the different NEPA-loaded samples revealed randomly oriented, bead-free fiber depositions without gel droplets on the fiber surfaces. The incorporation of HP-β-CD improved fiber morphology. A uniform, clear fiber surface was obtained with formulations containing the highest HP-β-CD amount. Low concentrations of HP-β-CD, 50 mM (F2 and F3), resulted in a lower average fiber diameter compared to formulations with double and triple concentrations (F5 and F6) and (F8 and F9), respectively. The histograms revealed various diameter distributions, even though there was no significant difference between the average fiber diameters at p-value < 0.05 ($p=0.16921$).

4.3.4. Cysteamine-loaded (Cys-loaded) fibers

SEM images of the Cys-loaded electrospun samples of PVA/PO-407-based and TEOS/PVA-based polymer systems loaded with CysH revealed randomly oriented fibers without notable gel droplets or beads on their surfaces. The addition of CysH to PVA/PO-407 resulted in a concentration-dependent decrease in the average fiber diameter; formulations loaded with 1.1% w/w (F4, F8, and F12) displayed lower fiber diameter than formulations with 0.55% w/w (F2, F6, and F10). Compared to neat fibers, adding the active substance (CysH) decreased beads and enhanced the ability to form fibers in TEOS/PVA-based samples.

4.4. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted to study the structural changes in the components of NEPA-loaded and Cys-loaded electrospun nanofibers. The spectra presented the formation of amorphous solid dispersion in all cases (CysH/PVA/PO-407,

CysH/TEOS/PVA, and PVA/PO-407/NEPA/HP-β-CD samples). The spectra of all individual components, including NEPA, CysH, EDTA, PS-80, PVA, PO-407, TEOS, and HP-β-CD, and electrospun fibrous samples' spectra have been detected.

4.5.X-ray diffraction (XRD) for Nepafenac-loaded (NEPA-loaded) fibers

The NEPA crystallinity in its purest form was confirmed by the existence of sharp peaks. In the physical mixture, the peak intensities decreased once the NEPA was mixed with PO-407, PVA, or HP-β-CD. The disappearance of these peaks from the nanofibers (F3, F6, and F9) confirmed the formation of an amorphous solid dispersion from crystalline NEPA through electrospinning of NEPA, HP-β-CD, PVA, and PO-407.

4.6. Determination of the drug content

All formulations (F1–F9) of NEPA-loaded nanofibers had varying drug contents, ranging from 100.01 (% w/w) ± 0.03 to 101.05 (% w/w) ± 0.02. The drug content of all formulations containing CysH ranged from 99.74 ± 0.04 (%) (w/w) to 100.5 ± 0.05 (%) (w/w). Both experiments' results demonstrate that the drug content is consistent throughout the polymeric fiber structure. These findings confirm the uniform precipitation of the drug/polymer mixture following solvent evaporation.

4.7. *In vitro* drug release and release kinetics

4.7.1. Nepafenac-loaded (NEPA-loaded) fibers

NEPA was fully released in less than 60 minutes (16.5–40 minutes) from the NEPA-loaded nanofibers (0.1% w/w). Some variations were observed in the dissolution profiles with varying HP-β-CD and PO-407 amounts. Higher HP-β-CD levels resulted in a relatively longer drug release, while higher PO-407 levels released the NEPA faster than those without PO-407. The nine formulations' overall releases did not differ significantly. The release kinetics of NEPA from the nanofibrous matrix were studied using Weibull distribution. All formulations (F1-F9) were successfully fitted to the Weibull model, and R^2 values were greater than 0.9, indicating linear regression. For most formulations, β values were < 0.75, indicating Fickian diffusion, while two formulations were $0.75 < \beta < 1$, which means a combined release mechanism.

4.7.2. Cysteamine-loaded fibers

The in vitro releases of CysH from PVA/PO-407 or TEOS/PVA showed complete and fast release in less than 10 minutes regardless of the matrix type, whether it is soluble (PVA/PO-407) or insoluble (TEOS/PVA). All formulations from the different matrices were successfully fitted to the Weibull model (R^2 >0.9), indicating linear regression. In the case of the TEOS/PVA system, the values were $0.75 < \beta < 1$, indicating a Case II transport. Most values for the PVA/PO-407 matrix were < 0.75, indicating Fickian diffusion.

4.8.Permeability studies of Nepafenac-loaded fibers

4.8.1. *In vitro* corneal parallel artificial membrane permeability (PAMPA) assay

The PAMPA permeability assay results showed no significant difference between the three formulations regardless of the HP-β-CD content per formulation (50 mM, 100 mM, and 150 mM for F3, F6, and F9, respectively) when 10% of the donor's HP-β-CD content was added to the acceptor wells to create a more desirable environment for the poorly soluble NEPA molecules. Nonetheless, their permeability values were substantially lower than the Nevanac® value ($p < 0.05$). Owing to this, corneal-PAMPA could not distinguish between the three formulations.

When the experiments were conducted using PBS acceptor media, omitting the reverse sink effect of 10% w/v HP-β-CD, different tendencies within the samples containing various amounts of HP-β-CD were obtained. NEPA flow to the acceptor compartment increased as a result of increasing formulations' HP-β-CD amounts. For the formulations F3, F6, and F9, the average permeabilities ± SD (10-6/cm) were 11.9 ± 2.21, 13.8 ± 1.59, and 21.6 ± 2.968, respectively. Due to its lowest HP-β-CD content and comparable average permeability values across the two experiments, F3 was selected for further *ex vivo* investigation.

4.8.2. *Ex vivo* corneal permeability studies on porcine eyes

NEPA concentrations for the F3 formulation have been measured at 15, 30, and 60 minutes in the precorneal area, cornea, and aqueous humor. The results showed that F3 has a significantly higher concentration than Nevanac® in the precorneal area**,** and the p-values for the difference in the concentrations of NEPA in the precorneal fluid between F3 and commercial Nevanac® at 15, 30, and 60 minutes were found to be

0.0269, 0.0013, and <0.0001, respectively. Regarding the cornea, although there is a slight increase in the Nevanac® compared to the F3 formulation, it was only a numerical difference without statistical significance. The NEPA concentrations in the Nevanac® and F3 formulation in the aqueous humor were not significantly different at 15 and 30 minutes. At 60 minutes, Nevanac® had a significantly higher concentration than the F3 formulation, with a p-value of 0.0242.

4.8.3. *Ex vivo* cornea Raman mapping

Our conclusion that F3 might be bioequivalent to Nevanac® in vivo is supported by the NEPA distribution results of both F3 and Nevanac® within the porcine corneal tissues. In both cases, NEPA distribution at 15 minutes was limited to the outer layers of the cornea, but at 30 and 60 minutes, it was distributed to the innermost layer (stroma) with a more homogenous feature.

4.9. Cytocompatibility Study

The applied samples, including NEPA-loaded, Cys-loaded nanofibers of different bases, the empty polymeric nanofibers, and the negative control (Phosphate buffer), resulted in no noticeable redness, coagulation or bleeding, which indicates the tolerability and cytocompatibility of the formulation components when compared to a positive control (0.1 N NaOH). The latter induced a strong hemorrhage on the surface of a chick CAM at embryonic day 9.

4.10. Accelerated stability study

The time-dependent changes in the morphology and physicochemical characteristics of the NEPA-loaded and Cys-loaded nanofibers exposed to stressful conditions were studied by SEM and FTIR, respectively. The results from both experiments suggested stable electrospun nanofibers loaded with NEPA and CysH for both freshly prepared samples and samples kept under stressful conditions. All formulations maintained their fibrous morphology over the period of study (4 weeks).

5. **Conclusions**

Despite the emergent development in pharmaceutical technologies, ocular drug delivery remains a challenging process due to the complex structure of the eye. Nanofiber-based ophthalmic inserts are considered smart candidates for delivering various drugs and biopharmaceuticals to different layers of the eye. They can be considered a novel strategy to provide satisfactory ocular delivery with improved drug ocular bioavailability with lower adverse effects for better disease control and improvement of patients' adherence and quality of life.

Nanofiber-based OIs loaded with NEPA and CysH have been successfully formulated. SEM results showed smooth fibers and FTIR spectra, which reflected good compatibility between the formulation components. The formation of amorphous solid dispersion was detected by FTIR and confirmed by XRD. The release and pharmacokinetics studies showed immediate release formulation with the release pattern following Fickian diffusion in the case of NEPA-loaded and PVA/PO-407-based Cys-loaded formulations, while it was case transport II in the case of TEOS/PVA-based Cys-loaded formulations.

The components of the formulations showed acceptable cytocompatibility when tested on HET-CAM of fertilized chicken embryos. The short-term stability studies carried out under stressful conditions suggest stable formulations. In the case of NEPA, the formulations showed considerable *in vitro*, and *ex vivo* permeability and the results suggest bioequivalence to the commercial Nevanac® suspension that has been confirmed by the Raman mapping within corneal tissues.

Based on the presented results of different polymeric combinations and different model drugs, it can be concluded that the electrospun nanofiber webs could be considered promising candidates as ophthalmic inserts, which can be used as an alternative to conventional systems for NEPA and CysH ocular delivery or any drugs with similar physicochemical properties with reasonable corneal permeation and better stability.

6. Novelty and new findings

- A comprehensive literature review provided information to develop a strategy to bridge the gap considering nanofiber-based OIs [**I, II**].
- An ophthalmic system was developed using an electrospinning method combining the advantages of nanocarriers and ophthalmic inserts in targeting different eye diseases [**III, IV**].
- The added value of formulated NEPA-loaded nanofibrous webs is to avoid the disadvantageous properties of conventional eyedrops by improving formulation stability and the ocular solubility of a BCS class IV [**III**].
- Stable immediate-release CysH-loaded nanofibrous webs were developed to avoid conventional eyedrops' disadvantageous properties and improve the stability issues of the loaded drugs [**IV**].
- Various polymeric matrices (soluble PVA/PO-407 and insoluble TEOS/PVA) were developed, and the optimum electrospinning parameters were determined; thus, a novel ophthalmic drug delivery platform was developed [**IV**].

7. Bibliography

7.1. Publications related to the thesis

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7.2. Other related publications

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