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SUSTAINED DELIVERY OF MOXIFLOXACIN FOR OCULAR APPLICATIONS.

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SUSTAINED DELIVERY OF MOXIFLOXACIN FOR OCULAR APPLICATIONS.

A Dissertation

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by

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ABSTRACT

Moxifloxacin is available as a marketed solution (Vigamox(R) which has to be administered 3 or more times a day leading to poor patient compliance and low ocular bioavailability. The overall goal of this research was to use formulation strategies and develop patient compliant dosage forms of moxifloxacin which are sustained-release so as to improve its retention and thereby ocular bioavailability. The first strategy that was explored was to develop sustained release inserts of moxifloxacin hydrochloride using hot-melt extrusion. The inserts showed sustained release and antibacterial activity up to 24h and could potentially be a once-a-day application and improve patient compliance

Another strategy that was utilized was to develop a sustained release nanoemulsion of moxifloxacin with a mucoadhesive agent (HPMC or PVP) as a non-invasive, cost-effective alternative delivery system that is known to enhance the retention and permeation of drugs. The nanoemulsions were formulated with a lower amount of surfactant as compared to the nanoemulsion which is published in the literature (Shah et al., 2019), and were found to stable at room temperature for up to 45 days. The nanoemulsion was also filtered through various 0.22-micron filters and they did not show any significant change in physicochemical properties after filtration.

DEDICATION

This dissertation is dedicated to my parents Bharati and Amit Thakkar.

They made me who I am today.

LIST OF ABBREVIATIONS

MOX: Moxifloxacin

HME: Hot-melt extrusion

API: Active Pharmaceutical Ingredient

BK: Bacterial keratitis

NE: Nanoemulsion

DoE: Design of Experiments

CCD: Central composite design

RSM: Response surface methodology

PG: Propylene glycol

FS: Eudragit™ FS-100

PDI: Polydispersity index

FDA: Food and Drug Administration

HPMC: Hydroxy propyl methyl cellulose

PVP: Polyvinyl pyrrolidone

GRAS: Generally regarded as safe

PRESS: predicted residual sum of squares

ANOVA: Analysis of variance

IR: Immediate release

PBS: Phosphate buffered saline

ZP: Zeta potential

RT: Room temperature

MIC: Minimum Inhibitory concentration

HP-B-CD- Hydroxy propyl beta cyclodextrin

PEO: Polyethylene oxide

NLC: Nanostructure lipid carriers

2FI: 2 factorial interaction

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Microbial keratitis is an acute infection of the cornea; failure to promptly eliminate it can lead to serious ocular morbidity, corneal scarring and subsequently loss of vision. In the United States, approximately 71,000 cases of microbial keratitis are reported annually, with an increasing incidence in the recent years.[1,2] Microbial keratitis can be caused by bacteria, fungi, amoeba or viruses.[3] However, bacteria are the leading cause of ocular infections such as conjunctivitis, keratitis, endophthalmitis and blepharitis. Amongst these, bacterial keratitis (BK) is a sight-threatening infection of the cornea and accounts for approximately 90% of all microbial keratitis case.[4,5] It requires urgent antimicrobial treatment because if left untreated in can develop in endophthalmitis and can eventually cause corneal blindness. BK and endophthalmitis are both potentially destructive infections of the eye if not diagnosed early. Endophthalmitis is ocular infection in the posterior segment of the eye that is most due to keratitis or intraocular surgery like cataract. [3]

The impact of BK can be adjudged from its prevalence rate that accounts for nearly two million cases annually.[6,7] Furthermore, it remains one of the most common global causes of irreversible blindness.[8] While the eye has several defense mechanisms to counter infections, there are several predisposing factors like physical/chemical trauma, contact lens wear, ocular surface disease and systemic immunosuppression that could damage the cornea and trigger BK.[9-11] Amongst these, the use of contact lens presents a major risk factor for BK worldwide.[12] Anatomical location of BK is of paramount importance because if it occurs in the central or paracentral cornea it can be sight-

threatening, even if the causative agent is eradicated.[13] The causative organisms vary primarily based on geographical locations and climatic conditions. For example, the gram-positive organisms *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common causative agents in northern United States, whilst the gram-negative *Pseudomonas aeruginosa* infections are most common in southern United States.[14,15]

According to the American Academy of Ophthalmology, the majority of the BK cases can be treated with empirical antibiotic therapy.[16] In the clinic, monotherapy with fluoroquinolone-based ophthalmic solutions have been the predominant choice for ocular infections because of their broad-spectrum activity, excellent tissue penetrance, and patient tolerability.[17] There are diverse generation of fluoroquinolones such as 0.3 % ciprofloxacin (second generation), 0.3% ofloxacin and 1.5% levofloxacin which are FDA approved for the treatment of keratitis. Although, 0.5% moxifloxacin (fourth generation) is not FDA-approved for keratitis, it is frequently used as an off-label treatment because of its broad spectrum of activity, enhanced antibacterial potency and high penetration across ocular tissues. Moreover, moxifloxacin (MOX) has shown to be safe and effective for ophthalmic use with very low risk of recognized quinolone-related toxicity.[18]

Chemistry

MOX is an antibiotic with an empirical formula of $C_{21}H_{24}FN_3O_4$ and a molecular weight of 401g/mol

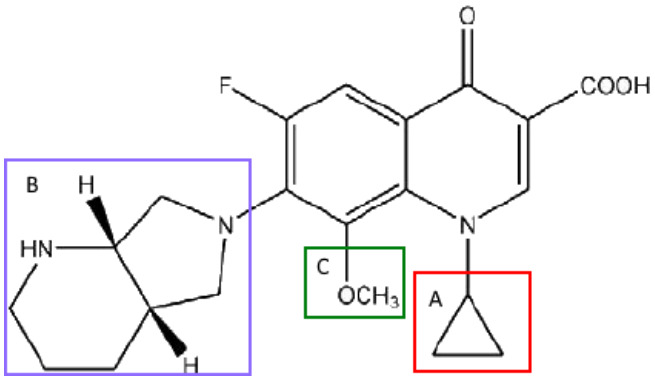


Figure 1: Chemical structure of MOX showing, (A) cyclopropyl substitution, (B) bulky diazabicyclononyl ring, and (C) methoxy group.

Structural modifications made to the parent 4-quinolone structure led to the development of newer generation of fluoroquinolones with enhanced antimicrobial activity, safety and tolerability.[19] Substitution at the N-1 nitrogen atoms of the parent moiety is crucial for both the potency as well as the spectrum of activity of the molecule. In case of MOX the N-1 atom is substituted with a cyclopropyl ring (A) providing it with enhanced activity against anaerobic and gram-positive isolates. Furthermore, at the C-7 position there is addition of a bulky diazabicyclononyl ring (B) that imparts it increased gram-positive activity as well as increased affinity for DNA gyrase while impeding efflux from the bacterial cell. Moreover, addition of the methoxy group (C) at the C-8 position leads to increased affinity against anaerobes and also decreases the selection of resistant bacterial populations.[20] These advances in the molecular structure provide MOX with enhanced potency against gram-positive organisms than earlier-generation fluoroquinolones, while maintaining similar activity against gram-negative bacteria.

From Fig 1 it is evident that MOX possesses two ionizable moieties. For the acidic moiety (C-7 secondary amino group) has a higher value of 9.29. Therefore, MOX is present as zwitterion with an isoelectric point at pH 8.[21] MOX is electrically neutral at the isoelectric point. Thereby it can

easily penetrate the biological membranes. However, the loss of charge of MOX leads to decreased aqueous solubility which could in turn lead to precipitation of drug. Therefore, MOX is available as its hydrochloride salt in its marketed formulation (Vigamox®) to improve its aqueous solubility.[21] Vigamox® has a near neutral pH of 6.8 adjusted using hydrochloric acid (HCL)/sodium hydroxide. Vigamox® is a sterile ophthalmic solution containing 0.5% Moxifloxacin HCL, boric acid, sodium chloride and purified water.[21] Solubility information of the base and MOX-HCL was not available in literature.

Mechanism of Action

DNA gyrase (Topoisomerase II) and Topoisomerase IV are essential bacterial enzymes that are involved in translation, replication and repair of the bacterial DNA. The role of these enzymes is as followed:

1) DNA gyrase: It is responsible for separation of the daughter chromosomes which is crucial for initiating the bacterial DNA replication process.
2) Topoisomerase IV: Functions to disconnect the interlinking between daughter chromosomes resulting into replicates of two separate daughter cells.

Since both of these enzymes are crucial for bacterial survival, inhibition of either of them could lead to bacterial death. MOX binds strongly to both of these enzymes thereby being bactericidal and showing dual activity. This dual activity of MOX reduces the likelihood of developing resistant organisms because two simultaneous mutations are required to establish resistance.[20,22] Furthermore, the bacterial efflux mechanism is hindered owing to the bicyclic side chain at the C-7 position, enhancing the potency of MOX.[22]

Spectrum of Activity

MOX has a broad spectrum of antibacterial activity as it has enhanced activity against gram

positive organisms while maintaining activity against gram negative organisms. Kowalski et al. determined the MIC90s of ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin. against 177 bacterial keratitis isolates. They found that the MIC90 for moxifloxacin was significantly lower against most gram positive bacteria especially for *Staphylococcus aureus* (3.0 µg/mL in moxifloxacin and gatifloxacin versus 64.0 µg/mL in levofloxacin, ciprofloxacin, and ofloxacin) as compared to the older generation of fluoroquinolones. In another study by Sueke et al the fluoroquinolones were tested against 722 bacterial keratitis isolates in the United Kingdom and they found that moxifloxacin showed the lowest MICs for both gram negative and gram positive bacteria.[23] The MIC90 of moxifloxacin against *S. pneumonia* which is a common cause causative agent in bacterial keratitis was found to be 0.25 µg/ml.[24] These factors make MOX a first choice in the management of ocular bacterial infections like conjunctivitis, keratitis and endophthalmitis.

Challenges in current MOX therapy

Moxifloxacin (Vigamox®) solution has been used widely in treating bacterial infections of the eye like conjunctivitis, keratitis and endophthalmitis. However, one of the challenges associated with the marketed solution is the low bioavailability (~5%) through the topical route due to the pre-corneal losses, nasolacrimal drainage and tear turn over. Vigamox® does not contain any mucoadhesive/ viscosity enhancer as an excipient. The excipients are simply NaCl to maintain tonicity and boric acid which is a preservative. This is results in the need for repeated applications (3 times a day) to achieve therapeutic concentrations and efficacy, which often results in low patient adherence and an increase in cost of therapy.[25]

1.2 Objective

The overall objective of this research project was to overcome the challenges associated with the current MOX therapy options for ocular delivery, by developing and investigating alternate sustained release dosage forms in order to improve and enhance ocular retention and, thus, ocular bioavailability of MOX. This could improve treatment and therapeutic outcomes in ocular bacterial infections like keratitis, conjunctivitis.

1.3 Specific aims

1. Develop and optimize sustained release inserts of MOX, for ocular drug delivery, using hot- melt extrusion (HME), and, compare their efficacy with the marketed MOX formulation (Vigamox®), *in vitro* and *ex vivo*.
    i. Optimize MOX-HME inserts for sustained release using the Design of Experiments approach specifically using Central Composite Design,
    ii. Evaluate physicochemical and release characteristics from the MOX-HME inserts,
    iii. Determine stability of the optimized inserts at room temperature for [insert time duration],
    iv. Evaluate ex vivo permeation of MOX from the optimized MOX-HME inserts across excised rabbit corneas.
2. Develop and optimize MOX nanoemulsion (MOX-NE) formulations containing mucoadhesive agents, to improve the retention and permeation of MOX as well to evaluate their *in vitro* and *ex vivo* performance and compare it with Vigamox® as an alternative dosage form in ocular bacterial infections.
    i. Formulate a MOX-NE with low surfactant load
    ii. Optimize MOX-NE with various mucoadhesive polymers

iii. Evaluate the physicochemical characteristics, pH and viscosity of MOX-NE.

iv. Evaluate stability of the optimized MOX-NE up to a minimum of four weeks at room temperature.

v. Evaluate permeation of the MOX-NE with mucoadhesive agent across excised rabbit corneas and compare it to MOX-NE and Vigamox®,

CHAPTER 2: DEVELOPMENT AND OPTIMIZATION OF HOT-MELT EXTRUDED MOXIFLOXACIN HYDROCHLORIDE INSERTS FOR OCULAR APPLICATIONS USING THE DESIGN OF EXPERIMENTS

2.1 Introduction

Topical eye drops have been the mainstay in the management of the infections of the anterior ocular segment. However, they face certain challenges such as high pre-corneal losses, nasolacrimal drainage and low ocular residence time, all of which leads to poor ocular bioavailability following topical application.[25] This results in the need for frequent dosing, which potentially leads to patient non-compliance resulting in ineffective and a costlier therapy. While gels and ointments stay longer on the ocular surface, they get diluted by the tear fluid quickly and leak out, thus, reducing bioavailability. Moreover, ointments with oil base are also associated with blurred vision due to their greasiness.[26,27]

Ocular inserts are solid or semi-solid drug containing polymeric devices that are usually placed in the cul de sac, conjunctiva, or in the upper fornix. Recently ocular inserts have been gaining in popularity because of their ability to increase retention at the ocular surface. This leads to prolonged delivery and, thereby, may reduce the frequency of administration and possibly decrease systemic exposure and toxicity.[28] Furthermore, addition of preservatives can be avoided thereby reducing the side effects associated with them.[29] Inserts with various desired characteristics can be formulated through the selection of appropriate polymers to impart properties including biocompatibility, biodegradability and mucoadhesivity.[30] A majority of the ocular inserts in the

laboratory scale are fabricated using the solvent cast method. However, this method could present safety issues from the trace amounts of residual solvent.[31] Furthermore, it also suffers from scalability issues, batch-to-batch variation, air entrapment and time-consuming solvent removal process.[32] Hot-melt extrusion (HME) particularly overcomes these challenges as it a solvent-free, easily scalable and economical process.[32] HME is a successful and versatile technology with varying applications in the pharmaceutical industry, including improving solubility of poorly soluble compounds, formulation of abuse deterrent products, as well as in the production of transdermal, transmucosal and topical drug delivery systems.[33] Currently there are two Food and Drug Administration (FDA) approved ophthalmic inserts that are prepared by HME (Lacrisert®, Ozurdex®).[34] The goal of the current research was to develop and optimize sustained release ocular inserts of MOX-HCL using HME (MOX-HCL-HME).

Quality by Design (QbD) and design of experiment (DoE) approaches have become commonplace in the development of pharmaceutical formulations. The conventional optimization process is based on changing one factor at a time which can be time consuming. [35] On the contrary, multiple factors can be varied simultaneously in the DoE. Moreover, DoE also allows for the employment of response surface methodology (RSM) to optimize the drug delivery systems by investigating the relationships between the chosen factors and their responses using minimum number of runs. [36] Thus, a central composite design (CCD) was used to develop and optimize MOX-HCL-HME inserts that would prolong the pre-ocular residence time and the duration of ocular absorption phase. The design was employed to understand the effect of the controlled release polymer Eudragit™ FS-100 (FS) and the plasticizer propylene glycol (PG) (independent variables) on drug release at 2 h and 6 h (dependent variables) and the physicochemical properties of the hot-melt extruded inserts. The optimized formulation was then evaluated for stability, crystallinity, surface

morphology and *ex vivo* permeation through isolated rabbit cornea.

2.2 Materials and Methods

Materials:

MOX-HCL was purchased from Combi-Blocks (San Diego, CA) and Polyox® WSR N10 (PEO N10) was purchased from Colorcon (Irvine, CA). Eudragit™ FS-100 (FS) was a kind gift from Evonik (Darmstadt, Germany) and propylene glycol (PG) was purchased from Sigma-Aldrich (St. Louis, MO). D-α-Tocopherol polyethylene glycol succinate (TPGS) was purchased from Spectrum (Gardena, CA, USA). High-performance liquid chromatography (HPLC)-grade solvents and other analytical grade chemicals were obtained from Fisher Scientific (Hampton, NH, USA). Fresh whole eyes of male albino New Zealand rabbits were procured from Pel-Freez Biologicals (Rogers, AR, USA). Vigamox® was obtained from the health centre pharmacy at the University of Mississippi (MS, USA)

Methods:

HPLC chromatographic conditions

MOX concentration in all *in vitro* samples was analyzed by HPLC using an Alliance Waters e2695 separations module and Waters 2489 UV/Vis dual absorbance detector using a published method.[37] A detection wavelength (λ_{max}) of 254 nm was used. The run time for each sample was 10 min. The standard curve of MOX was from 5-100 ng/ml. The results were analyzed through Empower software. The mobile phase consisted of 18 mM of potassium dihydrogen phosphate with 0.1 % w/v of trimethylamine (TEA) pH 2.8 (adjusted with phosphoric acid) and methanol in the ratio of 60:40 v/v.[37] Phenomenex Luna® C_8 column (250 x 4.6 mm, 5 μ) was used for the analysis.

Preparation of MOX inserts using HME

The polymers, plasticizer and MOX were mixed in a mortar and pestle until they were visually

uniform. The batch size was 10 g. This physical mixture was then fed into the 6-mm counter-rotating mini extruder (Haake Minilab, Thermo Electron, Germany) using standard screw configuration. All the formulations were extruded at a temperature of 90°C and screw speed of 50 rpm. Upon cooling at room temperature the extruded formulations were pre-marked for 1 mm cuts, using a Thermo Fischer™ Scientific 0–150 mm digital caliper, and then cut using a scalpel blade. such that the weight of the inserts was approximately 5 mg with a drug load of 5-10% w/w. These inserts were further used for all other studies.

Preliminary Trials

The goal of these preliminary trials was to identify the most critical factors that affect the responses. The effect of the immediate release polymer: PEO and control release polymer: FS (varied between 0 to 45% w/w) was investigated. Various concentrations (0 to 10% w/w) of plasticizers (PG and TPGS) were added to study their impact on the inserts. Drug load was also varied between 5% w/w to 10% w/w to understand its effect on the drug release. Since the number of factors that potentially affect the responses is too large for in-depth exploration, these preliminary trials allowed us to select only critical factors from a list of many potential ones.

Optimization of MOX-HME inserts using CCD

A 2-factor 3-level CCD design was applied to optimize the MOX-HME inserts. The concentration of PG (X_1), the concentration of FS (X_2) and % drug release at 2 h (Y_1), at 6 h (Y_2) were the independent and dependent variables, respectively, for the optimization of MOX-HME inserts. The levels of FS and PG used for the design was chosen based on the preliminary trials and showed in Table 1. This design involves preparation of 13 formulations including 4 factorial points (F-2, F-3, F-7, F-8) augmented by 4 axial points (F-1, F-5, F-6, F-9) and 5 center points (F-4, F-10, F-11, F-12, F-13) that allow for estimation of curvature as shown in Table 2 and Figure 2. The levels

of factorial points are ±1 and those on axial design are ±α. For two-factor design, the value of $\alpha = \sqrt{2}$ ensures that the design is rotatable and all points are equidistant from the center.

The formulations were prepared according to the compositions given by the design (Table 2) with drug load (MOX) kept constant at 5% w/w and PEO was used to make up 100%. The Design Expert® version 11 (Stat-Ease Inc., Minnesota, USA) was used to generate and analyze the experimental design.

Table 1: Levels of independent and dependent variables used in central composite design for optimization of moxifloxacin hydrochloride ocular inserts

Independent factors	**Levels**		
	-1	0	+1
X_1 - concentration of PG (%)	4	7	10
X_2 - concentration of FS (%)	25	37.5	50
Dependent factors	Constraints		
Y_1 - % release at 2h	Minimize		
Y_2 - % release at 6h	Minimize		

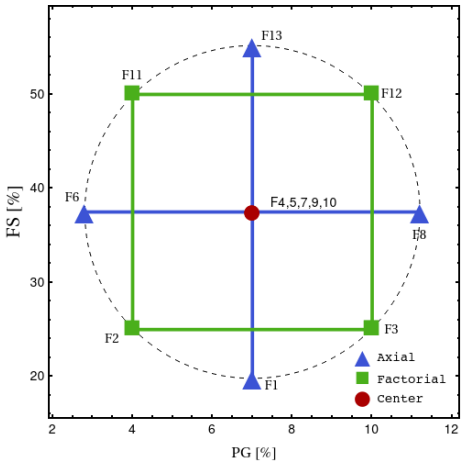


Figure 2: Schematic layout of central composite design used for the optimization of moxifloxacin hydrochloride ocular inserts

Table 2: Composition of moxifloxacin hydrochloride ocular inserts as per central composite design

Formulation code	X_1 FS %	X_2 PG %
F-1	37.5	2.8
F-2	25.0	4.0
F-3	50.0	4.0
F-4	37.5	7.0
F-5	37.5	7.0
F-6	19.8	7.0
F-7	37.5	7.0
F-8	55.2	7.0
F-9	37.5	7.0
F-10	37.5	7.0
F-11	25.0	10.0
F-12	50.0	10.0

F-13	37.5	11.2

The adjusted and predicted R^2, and predicted residual sum of squares (PRESS) values from the sequential model comparison were used to select the best model for fitting among linear, two-factor interaction (2FI), quadratic and cubic models. Analysis of variance (ANOVA) and F-statistics was performed on the best-fitted model to study the significance of the various model terms. Contour plots and response surfaces were generated to understand the effect of each factor on the responses.

Numerical optimization was employed using Design Expert® to simultaneously achieve two goals of minimizing the drug release at 2h and 6h. For the goal of minimizing a quantity, the desirability is a linear ramp function between the low value and high value and is defined by

$$d_i = \frac{High_i - Y_i(X_1, X_2)}{High_i - Low_i}$$

In the above equation, i=1 provides the desirability for minimizing Y_1, while i=2 is the desirability for minimizing Y_2. The program allows in assigning a weight to each goal in order to adjust the shape of its particular desirability function. In this study, both goals were deemed equally important and hence were assigned equal weights. The overall desirability function was calculated by the weighted geometric mean of both individual goals.

$$D = \sqrt{d_1 d_2}$$

The optimum values of X_1 and X_2 were obtained by maximizing this desirability function, D, that varies from zero to one (least to most desirable, respectively).[38] The formulation was prepared using the suggested optimum values and was then compared to the model prediction in order to validate that the model can predict actual outcomes at the optimal settings determined from the analysis.

Control formulations

Immediate release (IR) MOX-HME (MOX-HME-IR) insert**:** MOX-HME-IR inserts were prepared as per the composition of the optimized MOX-HME inserts but without the FS polymer, and with PEO making-up the bulk of the insert. These immediate release inserts were used as a control formulation for *ex vivo* permeation and *in vitro* release studies.

Commercial ophthalmic MOX solution**:** Vigamox® (Alcon Pharmaceuticals, USA), a 0.5%w/v commercial ophthalmic solution of MOX, was used as a control formulation for the ex vivo permeation studies.

Differential scanning calorimetry (DSC)

The DSC profiles of pure MOX, polymers (PEO N10 and FS), physical mixtures and the extruded formulations were collected to understand changes in crystallinity, evaluate thermal stability and to assess the drug-excipient compatibility. DSC studies were performed using a DSC 25 (TA instruments, New Castle, DE) with approximately 5-10 mg of the samples sealed in aluminum pans. The samples were exposed to a temperature range of 25-260°C with a ramp of 10°C/min and with constant nitrogen purging at 50 mL/min.

Fourier transform infrared spectroscopy (FTIR)

The interaction between pure MOX, pure PEO N10 and FS, as well as their physical mixture and the hot-melt extruded formulations were analyzed using FTIR spectroscopy. The infrared spectra (IR) were obtained using a Cary 660 series (Agilent Technologies, Santa Clara, CA) and MIRacle ATR (Attenuated Total reflectance).

Thickness, weight, and surface pH

The extruded formulations were pre-marked for 1 mm cuts, using a Thermo Fischer™ Scientific 0–150 mm digital caliper, and then cut using a scalpel blade such that the weight of the inserts was

approximately 5 mg and the dimensions were 2 mm x 1 mm with thickness of approximately 1.0 mm. What balance was used to weigh them? To measure the pH, the inserts were soaked in 3 mL of phosphate buffered saline (PBS) pH 7.4 ± 0.1, in a centrifuge tube. The glass electrode of the InLab® Micro pH probe (Mettler Toledo, Columbus, OH) was brought in contact with the surface of the films to measure the pH. The buffers with pH 4,7,10 were used to calibrate the pH meter.

Uniformity of drug content

Three sections from each formulation, were randomly selected, cut and then weighed such that they weighed approximately 5 mg and had dimensions (2 mm x 1 mm) with thickness of approximately 1.0 mm. Thereafter, they were dissolved in a 1:1 ratio of methanol and dimethyl sulfoxide, followed by sonication for 20 min to dissolve the drug. This solution was then diluted appropriately to obtain a concentration of 50 ng/ml that is within the standard curve and it was analyzed using the HPLC method outlined above.

***In vitro* release**

The inserts, weighing approximately 5 mg, were placed at the bottom of 20 mL scintillation vials. A stainless steel mesh (#10) was placed on top of them with a magnetic stirrer above the mesh. This whole system was maintained at a constant temperature of 34 ± 0.2 °C with continuous magnetic stirring. Ten mL of the release media, phosphate-buffered saline (PBS) pH 7.4 with 5% hydroxyl propyl-β-cyclodextrin (HPβCD) to maintain sink conditions, was added to each vial and aliquots of 0.5 mL were withdrawn at predetermined time points and replaced with an equal amount of the release media. The release media was selected based on the solubility of the drug (visual estimation) in various media studied. MOX-HME-IR inserts composed primarily of PEO without FS were used as a control against the optimized MOX-HME inserts. The concentration of the MOX in the aliquot was analyzed using the HPLC method discussed above. Release kinetics

for the optimized MOX-HME inserts were evaluated. Zero-order, first-order, Korsmeyer-Peppas and Higuchi models were applied and the R^2 values were compared to determine the best fit and the possible mechanism of release.

Stability study

Stability of the optimized MOX-HME insert formulation was studied at room temperature (25 ± 2º C). Briefly, approximately 50 mg of the optimized MOX-HME inserts were weighed, placed in 5 mL glass vials and stored at room temperature (n=3). The formulation was evaluated for change in drug content, weight, physical state as formulations prepared with hot melt extrusion often face the challenge of drug recrystallization upon storage which could alter the and release characteristics and therefore bioavailability of the formulation. Therefore, the release characteristics of the formulation was evaluated at various time at 0.5hr, 1hr, 1.5hr, 2hr, 2.5hr, 3hr, 6hr, 12hr and 24hr before and after storage.

Scanning electron microscopy (SEM)

The surface morphology of the pure MOX, physical mixtures and optimized MOX-HME inserts was studied with a JSM-7200FLV scanning electron microscope (JOEL, Peabody, MA, USA) with an accelerating voltage of 5 kV. All the samples were placed on the SEM stubs and fixed using double-adhesive tape. The samples were sputter-coated with Platinum under an argon atmosphere using a fully automated Denton Desk V TSC Sputter Coater (Denton Vacuum, Moorestown, NJ, USA) prior to imaging.

Evaluation of antibacterial activity

The anti- *methicillin-resistant Staphylococcus aureus* activity of MOX from the release samples were evaluated against *methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 1708, obtained from the American Type Culture Collection (ATCC, Manassas, VA). Susceptibility testing was performed using a modified version of the Clinical and Laboratory Standards Institute

(CLSI) methods [CLSI, 2012].[39] MRSA inoculate was prepared by correcting the OD630 of microbe suspensions in incubation broth cation-adjusted Mueller-Hinton at pH 7.0 containing 5% Alamar Blue™. Total 20 uL volume of samples were taken from each time points and 180 µL of inoculate was added to the 96 well microplate. Crude MOX in release media was used as a positive control and the release media which is (PBS pH-7.4 + 5%w/v HPβCD) was used as a negative control. Plates were read, at 544ex/590em using the Bio-Tek plate reader prior to and after incubation at 35°C for 24 h. Minimum inhibitory concentration (MIC) was defined as the lowest test concentration that affords no visual growth.

***Ex vivo* transcorneal permeation**

Freshly excised rabbit whole eyes stored in Hank's balanced salt solution on ice was shipped from Pel-Freez® Biologicals. Upon receipt, corneas were isolated and washed with PBS, pH 7.4. They were then mounted on Valia-Chien cells (PermeGear® Inc., Cranford, NJ) with a spherical joint. The jacketed cells were maintained at a constant temperature of 34 ± 0.2 °C. The receiver chamber was filled with 5% w/v HPβCD in PBS pH 7.4. The optimized MOX-HME insert and the MOX-HME-IR insert formulation were cut such that they approximately weighed 5 mg, to be equivalent in Mox quantity to 50 µL of Vigamox™, and were then placed in the donor chamber. The inserts were wetted with 50 µL of PBS pH 7.4 and were evaluated for transcorneal permeation. At pre-determined time points aliquots of 700 µL were drawn and replaced with the receiver solution. The aliquots were analyzed for MOX content using the HPLC procedure described above. The transcorneal permeability (P_{app}) of MOX was calculated using the following equation:

$$\text{Permeability} = \frac{\text{cumulative amount of MOX transported/corneal surface area}(0.636\ \text{cm}^2)}{\text{donor concentration}}$$

2.3 Results and Discussion

In recent years there has been an active interest in ocular inserts and efforts to introduce them to the pharmaceutical market continue as they offer several advantages[31,40,41]: accurate dosing compared to eye drops which suffer from high pre-corneal losses upon instillation; ability to target internal ocular tissues through the conjunctival-scleral routes; ability to deliver drugs to the posterior segment of the eye and also offer direct contact to the ocular tissues for extended periods of time. The MOX inserts that have been reported so far are either prepared by the solvent cast method or are non-biodegradable i.e. they need to be removed after use.[42,43] In an earlier study, MOX was also loaded into a nanoemulsion (NE; MOX-NE) at 0.5% w/v by Shah et al., 2019.[44] However, the MOX-NE formulation showed complete drug release within 3 h. Furthermore, the reported MOX-NE formulation contained benzalkonium chloride (0.005% w/w) as a preservative, the addition of which can be avoided in inserts. Also, the MOX-NE formulation contained high amounts of surfactant Tween® 80 (12%w/v) and Soluphor® P (24%w/v) which could lead to irritation and toxicity.[45] In another report by Gade et al., 2019, MOX was loaded into nanostructured lipid carrier (NLC; MOX-NLC) *in situ* gel at 0.2% w/v. But, there lack of comparison with the commercial solution in terms of permeation makes it difficult to gauge an improvement, if any. Also, scalability and commercialization are more challenging with NLC formulations. Currently, Vigamox® a 0.5%w/v ophthalmic solution of MOX, is administered 3 times a day. The overarching objective of this study was to develop a biodegradable sustained release ocular insert of MOX using HME which is a simple, versatile, solvent-free, continuous manufacturing technology with ease of scalability. Furthermore, the optimized insert could provide sustained release and increased retention at the ocular surface, thereby reducing the dosing frequency to once-a-day application and serving as an alternative delivery system in the

management of BK.

Formulation development

MOX-HCL inserts were developed using PEO and FS as immediate and controlled release biodegradable polymers, respectively, and PG and TPGS as plasticizers. PEO is a non-ionic hydrophilic polymer with low melting temperature of 68 °C, which makes it a suitable choice for HME process. In addition, PEO exhibits thermoplastic behavior and has been proven safe for ocular delivery.[31,41] Eudragit™ polymers have been widely used in various ocular drug delivery systems and are considered safe.[46] FS is a methyl acrylate-methyl methacrylate-methacrylic acid terpolymer which shows pH dependent solubility. FS has a free carboxylic acid moiety that makes it soluble only above pH 7.[47] Therefore, it has been used in various colon targeting delivery systems. However, this research would be the first to investigate FS as a sustained release polymer for ocular drug delivery.

Preliminary Trials

Preliminary trials were conducted to select the most critical factors affecting drug release. To select a suitable plasticizer, TPGS and PG were added to the insert at concentrations between 0 and 10% w/w. It was observed that TPGS did not have a significant impact on the release, as opposed to PG, which had a substantial, although non-linear, effect on the drug release. Consequently, TPGS was eliminated from the design and PG was selected as the plasticizer.

Preliminary trials also helped us evaluate the effect of the polymers PEO and FS (varied between 0-45% w/w) on the release profile. It was found that FS behaved as a controlled release polymer and an increase in its concentration led to a decrease in drug release. Therefore, during further optimization using CCD, the range of FS concentrations was raised to 25-50 % w/w to achieve our goal of sustained MOX-HCL release up to 24 h. Finally, varying the MOX-HCL load at 5 or 10

% w/w did not significantly affect the release or any other characteristics. Therefore, the drug load was kept constant at 5% w/w for the development of MOX-HCL-HME inserts.

Characterization and optimization of MOX-HME inserts using CCD

MOX-HCL-HME inserts were developed as per the composition given in Table 2. The inserts were cut such that they weighed approximately 5-5.5 mg with dimensions of about 2 mm x 1 mm. The thickness was found to be in the range of 1.00-1.18 mm (Table 3). Surface pH was in the range of 7.1 - 7.3 which was suitable for ocular administration. The drug content in the inserts varied from 93.1 ± 4.02 % to 102.4 ± 2.08 % (Table 3).

Table 3:Weight, thickness, surface pH and drug content of hot-melt extruded moxifloxacin hydrochloride ocular inserts (mean ± SD, n = 3)

Formulation	Weight (mg)	Thickness (mm)	Surface pH	MOX-HCl Content (%)
F-1	5.0 ± 1.1	1.0 ± 0.05	7.2 ± 0.1	97.5 ± 2.3
F-2	5.1 ± 1.1	1.0 ± 0.06	7.2 ± 0.1	102.5 ± 3.4
F-3	5.0 ± 0.9	1.0 ± 0.03	7.1 ± 0.1	93.1 ± 4.0
F-4	5.2 ± 0.4	1.0 ± 0.04	7.2 ± 0.1	102.4 ± 2.0
F-5	5.0 ± 1.1	1.1 ± 0.07	7.2 ± 0.1	100.7 ± 4.0
F-6	5.1 ± 1.0	1.0 ± 0.03	7.1 ± 0.1	96.6 ± 2.7
F-7	5.1 ± 1.2	1.1 ± 0.05	7.3 ± 0.1	98.6 ± 1.4
F-8	5.2 ± 1.3	1.0 ± 0.02	7.3 ± 0.1	94.1 ± 0.7
F-9	5.2 ± 1.2	1.1 ± 0.04	7.2 ± 0.1	102.3 ± 1.7
F-10	5.1 ± 1.2	1.1 ± 0.06	7.1 ± 0.2	98.3 ± 3.3

F-11	5.3 ± 1.8	1.0 ± 0.03	7.2 ± 0.2	96.3 ± 2.2
F-12	5.3 ± 1.1	1.1 ± 0.05	7.3 ± 0.1	99.2 ± 1.7
F-13	5.5 ± 1.6	1.1 ± 0.08	7.2 ± 0.1	100.4 ± 3.7

The DoE approach was used to determine the relationship between the independent factors (FS and PG concentrations) and drug release at 2 h and 6 h as dependent factors over the entire design space. The independent and dependent variables that were explored in this study are listed in Table 1. All independent variables (PG and FS concentrations) and responses (drug release at 2 h and 6 h) with their coded and actual levels are described in Table 1. The CCD was selected with a total of 13 experiments comprising four factorial points ($2^{\#\text{ of factors}}$), four axial points (2 × no of factors) and the center point replicated five times.

The cumulative MOX release profiles from all the formulations as a function of time is plotted in Figure 3a. To elucidate the effect of FS concentration, the drug-release profiles at FS concentrations of 19.8% w/w (F6), 37.5% w/w (F4) and 55.2% w/w (F8), with PG concentration at 7% w/w, is plotted in Figure 3b. Based on the data we can infer that an increase in FS concentration led to a more sustained release profile.

Similarly, Figure 3c illustrates the effect of different PG concentrations (2.8% w/w (F1), 7% w/w (F4) and 11.2% w/w (F13)) on the drug release at FS=37.5% w/w. It was observed that PG had a mild effect on release such that as the PG concentration was increased from 2.8% (F1) to 7% (F4), drug release decreases; however, at larger PG concentration of 11.2% (F13) we observed a faster release. The drop in release from PG at 2.8% to 7% is significant with a "*slight*" increase in release at PG=11.2% This points towards a quadratic or a higher-order relationship between the drug release and the PG concentration.

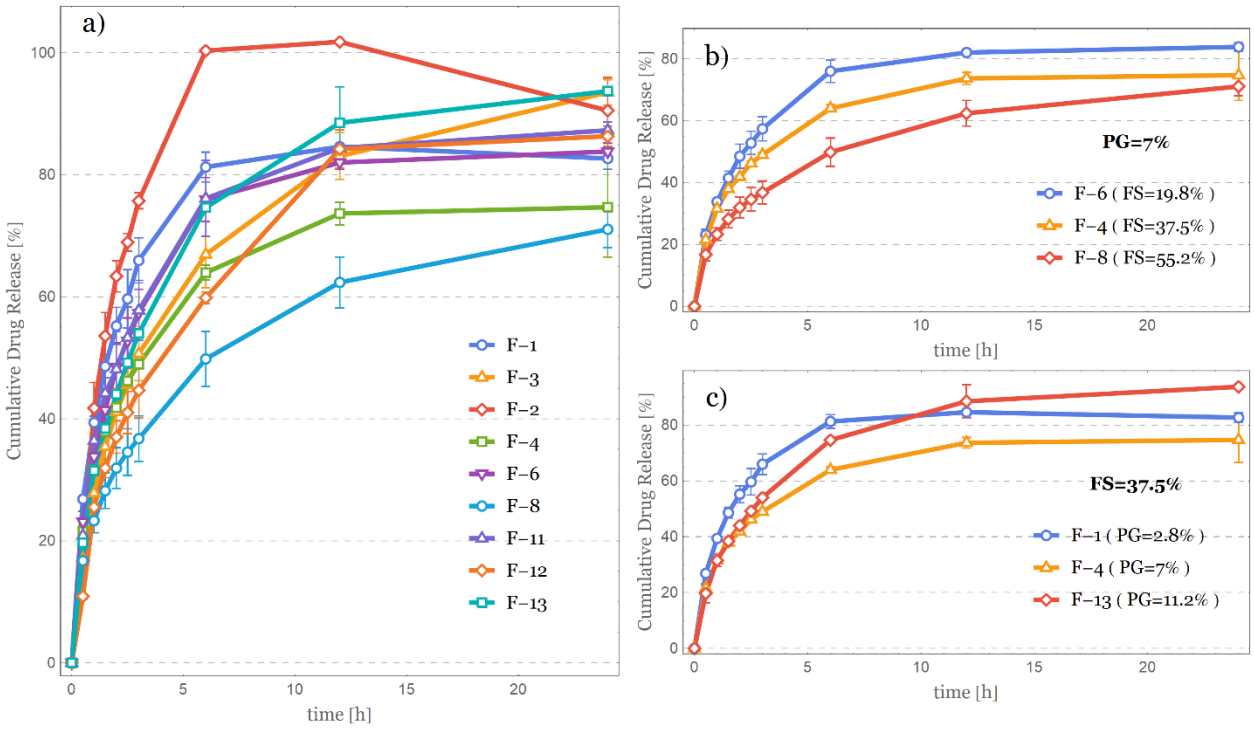


Figure 3: a) In vitro release profiles of moxifloxacin (MOX) from hot-melt extruded moxifloxacin hydrochloride ocular inserts in the design space. b) MOX release profile from formulations with PG=7% w/w and FS concentrations of 19.8%, 37.5% and 55.2% w/w. c) MOX release profile from formulations with FS=37.5% w/w and PG concentrations of 2.8%, 7% or 11.2% w/w.

The first step towards an optimal statistical analysis is selecting the model that best describes and fits the data. Therefore, a sequential model comparison was carried out to analyze the results for the response variables (Table 4). The drug release was fitted to linear, 2FI, cubic, and quadratic models, to select the best model that determines the relationship between input factors and response. Higher-order models such as cubic models are aliased (i.e. number of terms in the model is larger than the number of unique points in the design) and were not suitable for prediction in the current study. The best-fit was chosen for each response based on a significant p-value from the sequential model sum of squares analysis. Compared to other models, the quadratic model

provided the best-fit for Y_1 and Y_2 as evident from the smallest standard deviation, large R^2 values, and small predicted residual sum of squares (PRESS).

Table 4:Fit summary of dependent variables as per central composite design

Source	Std. Dev.	R^2	Adjusted R^2	PRESS
Fit summary for Y_1				
Linear	5.09	0.71	0.65	528.76
2FI	4.80	0.77	0.69	624.02
Quadratic	2.66	0.94	0.90	353.12
Fit summary for Y_2				
Linear	8.60	0.61	0.54	1461.76
2FI	8.52	0.66	0.54	1993.51
Quadratic	4.76	0.91	0.85	1127.09

Next, the analysis of variance (ANOVA) test was applied to study the significance of various terms in the quadratic model and determine the effect of each factor on Y_1 and Y_2. To this end, the model was separated into individual terms and tested independently. A summary of the ANOVA for the responses is provided in Table 5 including the sum of the squared differences, degrees of freedom (number of parameters used to compute the source's sum of squares), mean square (sum of squares divided by the degrees of freedom), p-value and F value (test for comparing the source's mean square to the residual mean square). The statistically significant *p values* (0.0003 and 0.0011) for both the responses Y_1 and Y_2, respectively, indicates that the selected model provides a good fit for the data. The small residual in the model indicates insignificant unexplained variation in the

response. The robustness of the model is also evident from the large F values implying that the model is significant with only a 0.03% and 0.11% chance that an F-value this large could occur due to noise for Y_1 and Y_2, respectively.[48]

Table 5:ANOVA and F-value of dependent variables as per central composite design

Source	Sum of Squares	df	Mean Square	F-value	p-value
		ANOVA for Y_1			
Model	846.62	5	169.32	23.87	0.0003
A-PG	185.43	1	185.43	26.14	0.0014
B-FS	451.47	1	451.47	63.64	< 0.0001
AB	52.06	1	52.06	7.34	0.0302
A^2	155.06	1	155.06	21.86	0.0023
B^2	0.0005	1	0.0005	0.0001	0.9933
Residual	49.66	7	7.09		
		ANOVA for Y_2			
Model	1771.22	5	354.24	15.65	0.0011
A-PG	221.60	1	221.60	9.79	0.0166
B-FS	968.61	1	968.61	42.78	0.0003
AB	85.56	1	85.56	3.78	0.0930
A^2	495.17	1	495.17	21.87	0.0023
B^2	5.63	1	5.63	0.248	0.6334
Residual	158.50	7	22.64		

The quadratic-model equation for the responses Y_1 and Y_2 as a function of the factors X_1 and X_2 are given by

$$Y_1 = +41.85 - 4.81\ X_1 - 7.51\ X_2 + 3.61\ X_1\ X_2 + 4.72\ X_1^2 + 0.0088\ X_2^2 \quad \text{Eq. 1}$$

$$Y_2 = +63.96 - 5.26\ X_1 - 11.00\ X_2 + 4.63\ X_1\ X_2 + 8.44\ X_1^2 + 0.8994\ X_2^2 \quad \text{Eq. 2}$$

The intercepts (+41.85 for Y_1 and +63.96 for Y_2) are the averaged response of 13 runs. In the regression equation above, a term's positive coefficient demonstrates an increase in the factor leads to an increase in the response, while the opposite is true for negative coefficient.[49] The non-linear terms ($X_1\ X_2$, X_1^2, X_2^2) are responsible for the curvature in the response while X_1X_2 signifies any interaction between PG and FS. The three-dimensional response surface, as well as two-dimensional contour plots encode the main effects of the FS and PG on the drug release (Figure 3).

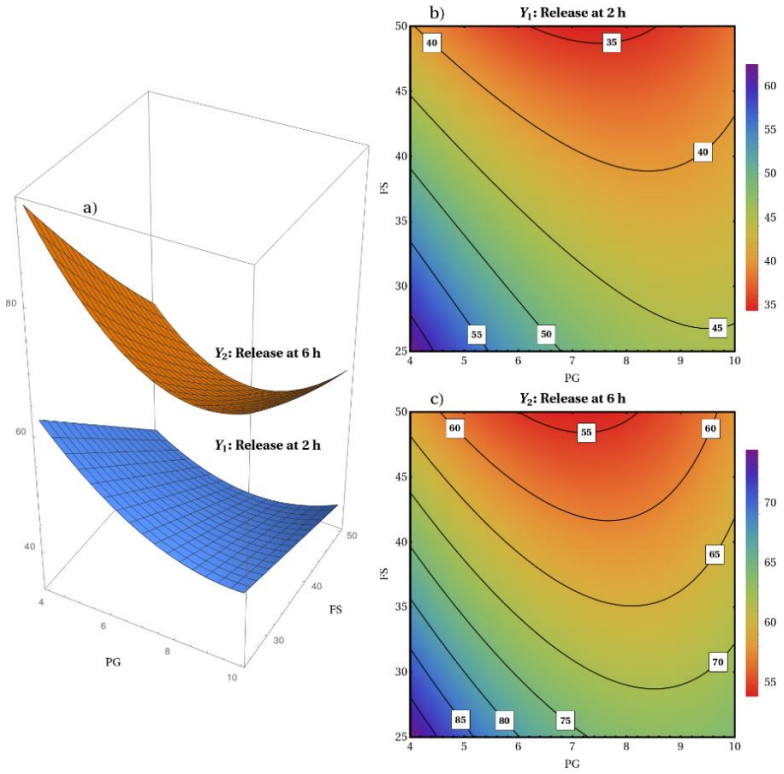


Figure 4:Left (a): The response surface plot for MOX release at 2h (Y1, shown in blue) and 6h (Y2, shown in red) as a function of PG and FS concentrations. Right (b): Contour plots of Y1 (top) and Y2 (bottom) variables as function of PG and FS concentrations

Both Y_1 and Y_2 showed a linear behavior as a function of FS concentration (X_2) as evident from the coefficient of X_2^2 and a p-value >0.05 indicating an insignificant contribution of the X_2^2 term. The negative coefficient of X_2 signifies that as we increase the FS concentration, the release at both 2 h and 6h decreases. This behavior can also be observed in Figure 4a.

An interaction between the PG and FS concentration was also inferred from the positive coefficient of X_1 X_2 (3.61 and 4.63 for Y_1 and Y_2 respectively) This implies that one factor moderates the effect of another factor, i.e., the effect of a factor on the response variable is different at different levels of another factor. This can also be seen from Figure 4a. While at all PG concentrations, Y_1 and Y_2 are linearly decreasing functions of X_2, the slope of the release depends

on PG. In Figure 5a, at low PG concentration (4% w/w), Y_1 and Y_2 values decrease very steeply with increasing FS concentration with a slope of -0.62 and -1.07, respectively. However, as FS is increased to 10% w/w, Y_1 and Y_2 have a relatively weaker dependence on FS as evident from the smaller slope of -0.22 and -0.56 respectively.

The release had a quadratic dependence on the PG concentration (X_1). This implies that Y_1 and Y_2 are not strictly decreasing as a function of X_1. For small and moderate PG concentrations (X_1<7% w/w) in the study, Y_1 and Y_2 decrease as a function of PG. However, as PG concentration was increased above 7%, the curve reaches a minimum and then starts increasing again at very high PG concentrations, as can be seen in Figure 5b. This implies that release as a function of PG has a minimum close to PG=7% w/w. This also explains the behavior observed in Figure 3b when FS concentration was fixed to 37.5% while PG was varied from 2.8% (F1), 4% (F4) and 11.2% (F6). This "U-shaped" effect of the plasticizer has also been observed by Zhu et al.[50] Finally, a larger coefficient for X_2 (FS) indicated that it had a more potent impact on the release compared to PG; however, this likely due to a larger range of allowed FS concentration.

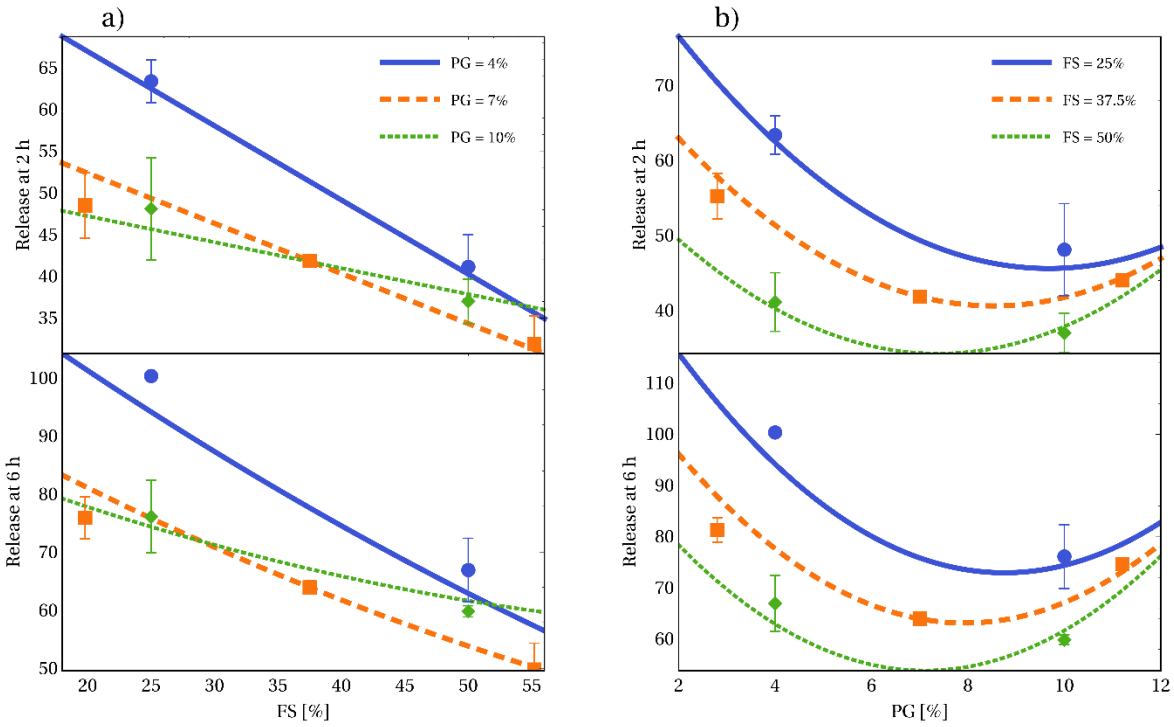


Figure 5:a) The linear effect of FS on the release at select values of PG. The slope is different at different PG concentration indicating interaction between PG and FS. b) The quadratic effect of PG on the release at select values of FS. Points indicate the actual observations while lines are the fits provided in Eq. 1 and 2.

Design Expert® was used to find the X_1 and X_2 that simultaneously minimize Y_1 and Y_2. Using numerical optimization, the maximum desirability value of 0.93 was obtained at the optimum value of PG=7.23% w/w and FS=50% w/w. The optimum values of PG and FS can also be gauged from Figures 3 and 4. Since the drug release linearly decreases with FS, maximum FS from the design was chosen. At FS=50% w/w, minimum release was achieved at PG=7.23% w/w (as shown by the green-dotted line in Fig 5b. For this optimized formulation, the drug release at 2h and 6h were predicted to be Y_1=34.3% and Y_2=56.9%. In order to ascertain that the model can predict actual outcomes, n=3 films were formulated using the predicted optimal concentrations. The

experimental values of Y_1=35.18% and Y_2=60.76% for the optimized formulation agrees well with the predictions.

Comparative *in vitro* release studies of the optimized MOX-HCL-HME inserts

This study was conducted to understand the release rate and the drug release pattern of MOX from the optimized MOX-HCL-HME inserts into the release media. The *in vitro* release of MOX from the MOX-HCL-HME-IR insert and optimized MOX-HME insert is depicted in Figure 6. The MOX-HME-IR insert did not have FS and was primarily made up of PEO and had the same drug load and PG content as that of the optimized insert, MOX-HME. From Figure 6, it is evident that almost 100% of MOX is released from the MOX-HME-IR within 30 mins, thereby acting as an immediate release platform in this case. On the contrary, the optimized MOX-HME insert released only 14.5 ± 3.2 % MOX within the first 30 mins and thereafter a sustained release of MOX was observed with 80.0 ± 3.1% MOX being released at the end of 24 h. This is an improvement over the previously reported MOX-NE (0.5% w/v) formulation, that showed more than 90% MOX release in 3h.[44] Gade et al., 2019 also reported 82.3 ± 7.8% release in 24h from MOX-NLC (0.2%w/v) formulation. However, the MOX-NLC formulation release study utilized a dialysis membrane which acted as a diffusion barrier, while, in the current study, the MOX-HME inserts were placed directly in the release medium.[51]

Furthermore, to understand the mechanism of release from the inserts, the data was fitted to the zero-order model (R^2 = 0.8941), first order model (R^2 = 0.9734), Higuchi (R^2 = 0.9970) and Korsmeyer-Peppas model (R^2 = 0.9923). The highest value of R^2 was observed for the Higuchi's model (R^2 = 0.9970) indicating that the release was directly proportional to square root of time. The Higuchi model was closely followed by the Korsmeyer-Peppas model (R^2 = 0.9923) indicating that the release was proportional to t^n where n was 0.57 indicating that the release is governed by

both erosion and diffusion mechanisms. This is in agreement with the earlier studies that used FS in melt extrusion.[52]

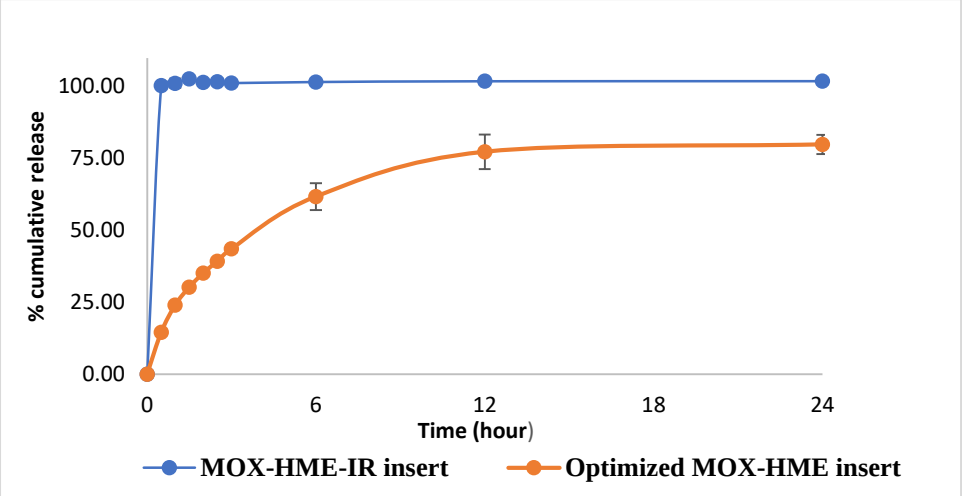


Figure 6:In vitro release profile of the optimized MOX-HME insert in comparison with the MOX-HME-IR insert (mean ± SD, n=3)

Antibacterial activity of the MOX-HME inserts

The goal of this study was to understand the correlation between the release profile and the antibacterial activity. We observed that there was no bacterial growth in any of the release study samples from 0.5 h to 24 h, indicating that MIC against MRSA had been achieved across all time points (Figure 6A). It is to be noted that the release study samples were diluted 10-fold prior to testing of the antibacterial activity. Thus, MIC levels would be achieved at time points much earlier than 30 minutes. Furthermore, with the disc diffusion method, we observe that the zone of inhibition increases as additional amount of drug is released into the medium as a function of time (Figure 6B). Thus, the antibacterial activity correlates very well with the release data, and the latter can thus be used to predict therapeutic efficacy.

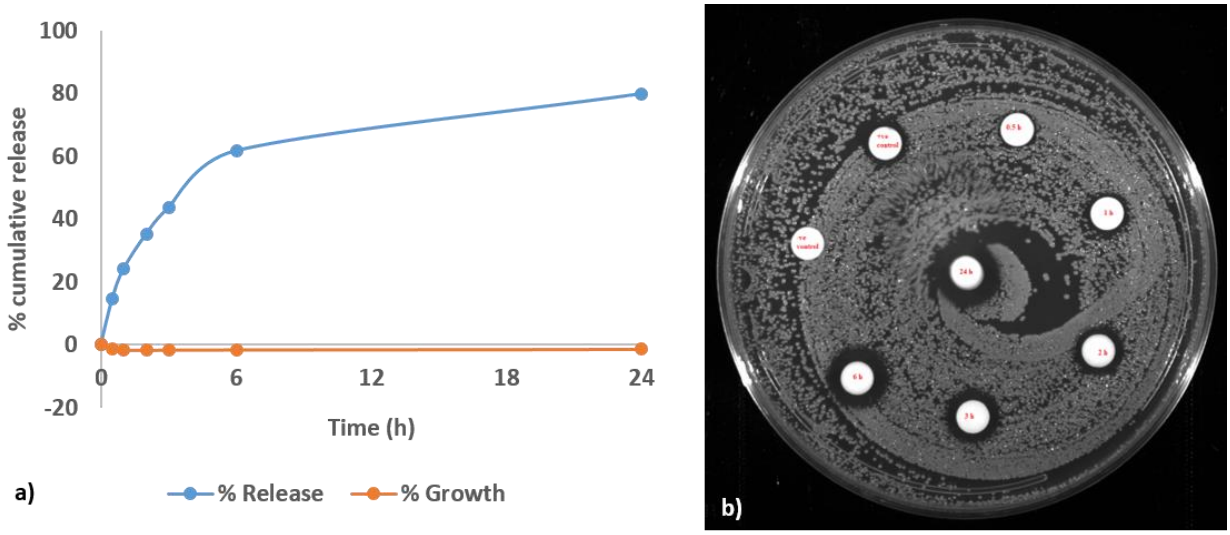


Figure 7: Correlation of moxifloxacin hydrochloride (MOX-HCL) release profile from the optimized MOX-HME insert with antibacterial activity on methicillin-resistant Staphylococcus aureus a) % growth and release profile against time, b) Correlation of release profile of the optimized MOX-HME insert with the antibacterial activity using disc diffusion method.

***Ex vivo* permeation**

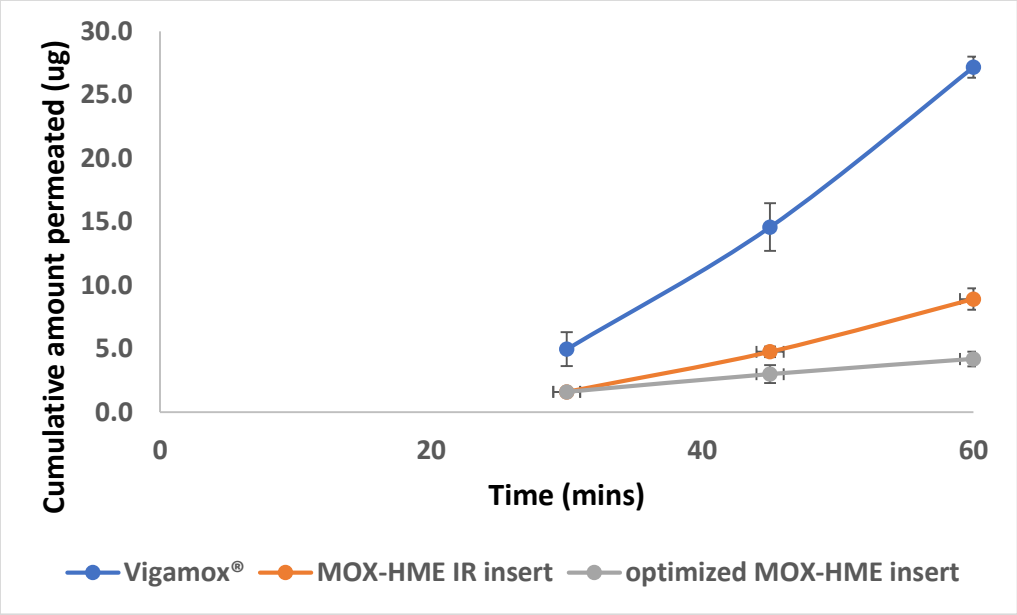


Figure 8:Comparison of cumulative amount of drug permeated across Vigamox, MOX-ME-IR insert and optimized MOX-HME insert across isolated rabbit cornea.

Amount (ug) of MOX-HCL permeated across rabbit cornea from the commercial MOX solution (Vigamox®), MOX-HME-IR and the optimized MOX-HCL-HME insert was studied and is shown in Figure 8. The amount permeated from Vigamox® was the highest at 30 min 5 ± 1.33 ug and the amount of MOX-HCL permeated from the optimized insert was 1.59 ± 1.65 ug. However, this amount was still enough to meet the MIC necessary to show therapeutic efficacy (MIC_{90} = 0.2 µg/mL against *S. pneumoniae* and an MIC of 0.125 µg/mL against *S. aureus*)

Flux of MOX-HCL across rabbit cornea from the Vigamox®, MOX-HME-IR and the optimized MOX-HCL-HME insert was studied and is shown in Figure 9. The flux from Vigamox® (1.2 ± 0.07 µg/min/cm^2) was found to be higher than that of the optimized MOX-HME insert formulation (0.21 ± 0.05 µg/min/cm^2). Similar observations were reported by Polat et al., 2020, wherein the commercial solution obtained higher permeability rate as compared to corresponding besifloxacin ocular inserts.[28] These observations can be explained as MOX is in its solubilized form in

Vigamox®, while the inserts are solid dosage forms where more drug is in the solid state resulting in low flux.

Therefore, for a more appropriate comparison for the optimized MOX-HME inserts, MOX-HME-IR (PEO based insert of MOX), as a control formulation as shown in Figure 9. We observed that the MOX-HME-IR insert had approximately 3-fold higher flux (0.70 ± 0.06 μg/min/cm^2) of as compared to the optimized MOX-HME insert (0.21 ± 0.05 μg/min/cm^2). This is because the MOX-HME-IR insert is made primarily of PEO and lacks the controlled release FS polymer. Since PEO is an immediate release polymer the drug is able to permeate out of the matrix at a faster rate as compared to the optimized sustained release insert. Importantly, the transcorneal permeation studies demonstrate that at 30 min the concentration of MOX in the receiver chamber, which represents the aqueous humor, from the optimized MOX-HME inserts, is 0.5 ± 0.2 μg/mL which is more than the minimum concentration necessary for therapeutic efficacy ($MIC_{90} = 0.2$ μg/mL against *S. pneumoniae* and an MIC of 0.125 μg/mL against *S. aureus*).[24,44,51]

Two previous studies have examined ocular inserts of MOX. The study by Pawar et al., 2012, used goat cornea for transcorneal studies, making it difficult to directly compare with the current study, which uses rabbit cornea that exhibits traits similar to human eyes.[43] In another study, Sebastian-Morello et al., 2018, reported 26.0 ± 3.2 μg MOX accumulation in the receiver chamber, across rabbit cornea, at the end of a 3h experiment from MOX loaded soluble inserts of 1 cm^2.[42] This is comparable to the 21.6 ± 4.2 μg of MOX that was observed in the receptor compartment with the optimized MOX-HME inserts at the end of 3h in the current study. However, both the inserts reported by Pawar et al., 2012 and Sebastian-Morello et al., 2018 were prepared by the solvent cast method and suffer from many limitations such as solvent residue, scalability issues like batch to batch variation, air entrapment. Moreover, the inserts formulated by Pawar et al., 2012 were

non-biodegradable i.e., they need to be removed after use. In contrast, MOX-HME inserts circumvent the usual drawbacks associated with the solvent cast method in addition to being biodegradable.

Besides ocular inserts, other formulations of MOX such as MOX-NE and MOX-NLC have also been reported in the literature.[44,51] However, the permeation study of MOX-NLC was performed on goat cornea and therefore, cannot be used as a comparator against the current study. In case of the MOX-NE formulation, permeation study was performed using Franz diffusion cells through rabbit cornea. In these studies, a transcorneal flux of 0.53 μg/min/cm^2 for MOX-HCL was observed, which was higher than the optimized MOX-HCL-HME inserts (0.21 ± 0.05 μg/min/cm^2). The higher flux from the MOX-NE formulation could be due to the solubilized form of MOX (as was the case for Vigamox®). Interestingly, we observed that the MOX-HME-IR had a slightly higher transcorneal flux (0.7 μg/min/cm^2) compared to the MOX-NE formulation. It can thus be inferred that the MOX-NE formulation and MOX-HME-IR have higher flux due to their faster release rates, whereas the optimized MOX-HCL-HME insert displayed a sustained release thereby leading to lower transcorneal flux. However, and importantly, the MOX-HCL-HME successfully achieved MIC_{90} concentrations in the receiver chamber within 30 min. Moreover, the MOX-HCL-HME inserts presents added advantages in that it avoids the high surfactant load (36%w/v) and preservatives added to the MOX-NE reported by Shah et al.,2019.

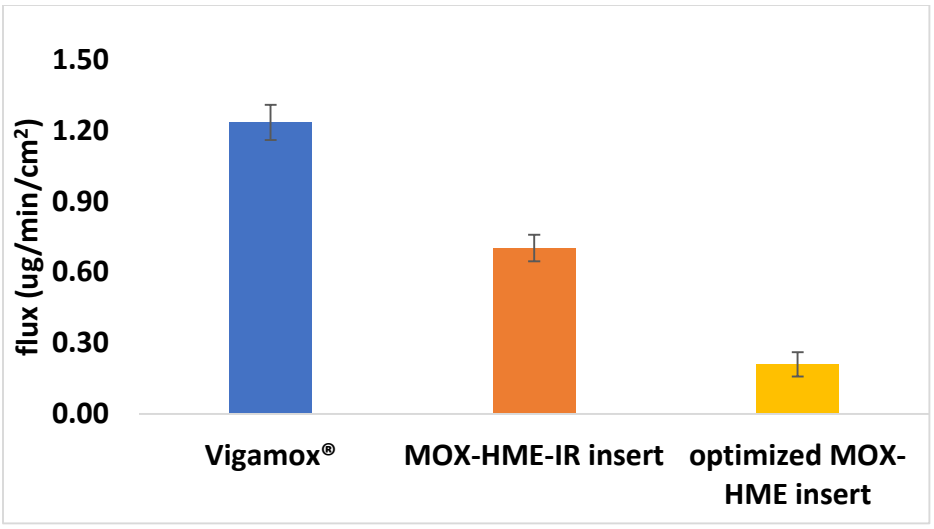


Figure 9:Plot of flux (µg/min/cm2) for moxifloxacin hydrochloride permeation across cornea from Vigamox®, MOX-HME-IR insert and the optimized MOX-HME insert (mean ± SD, n=4).

Stability

The optimized MOX-HCL-HME inserts were analyzed to evaluate their physical and chemical stability upon storage at room temperature. The inserts did not show any significant change in drug content during the four-month study period (Figure 8). The inserts were also evaluated for drug release at initial time point and following 4-month storage and no change in drug release rate was observed (Figure 8).

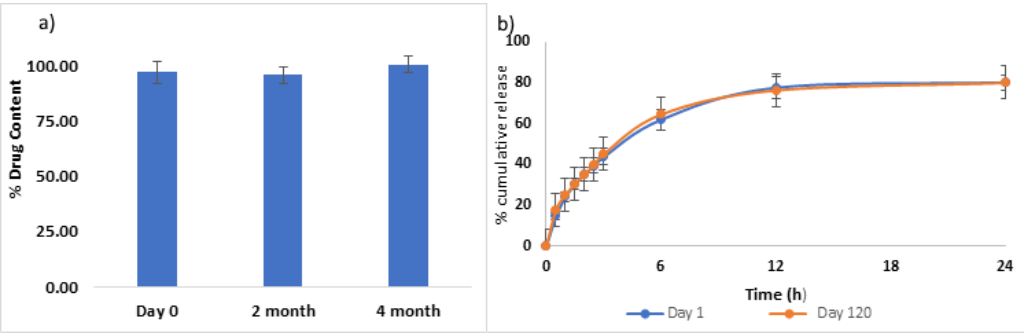


Figure 10:(a) Drug content and (b) in vitro release profile of the optimized MOX-HME insert upon storage at room temperature over four months (mean ± SD, n=3)

DSC

The MOX-HCL-HME inserts were studied for thermal characteristics and compatibility using DSC. As seen from Figure 9, the DSC thermogram of pure PEO showed an endothermic melting peak at 68°C whereas pure FS presents a glass transition temperature (T_g) around 50°C. The characteristic low T_g along with its thermoplastic properties makes FS a suitable carrier for the HME process.[52] Pure MOX-HCL was crystalline in nature and showed a melting point at 247°C. The physical mixture of the optimized formulation was evaluated prior to extrusion and the absence of the MOX-HCL peak indicated that the drug was miscible in the PEO-FS polymer matrix. Moreover, the absence of the drug peak in the MOX-HCL-HME thermogram suggested that the drug is converted into its amorphous form or had been molecularly dispersed within the polymer matrix. The HME process converts the drug into an amorphous form due to the molecular mixing between the drug and polymer.[41] However, as the amorphous form is unstable, some drugs have a tendency to revert to its stable crystalline state which could alter the formulation properties with time.

Therefore, the MOX-HCL-HME inserts were evaluated for change in crystallinity upon storage at room temperature for 4 months (last time-point tested) as change in solid state can affect the release rates of the formulation ultimately affecting bioavailability. No MOX-HCL peak was observed in thermogram of the inserts, indicating the absence of any recrystallization event on storage. Thereby, we can infer that the optimized MOX-HCL-HME inserts were stable at room temperature upon storage.

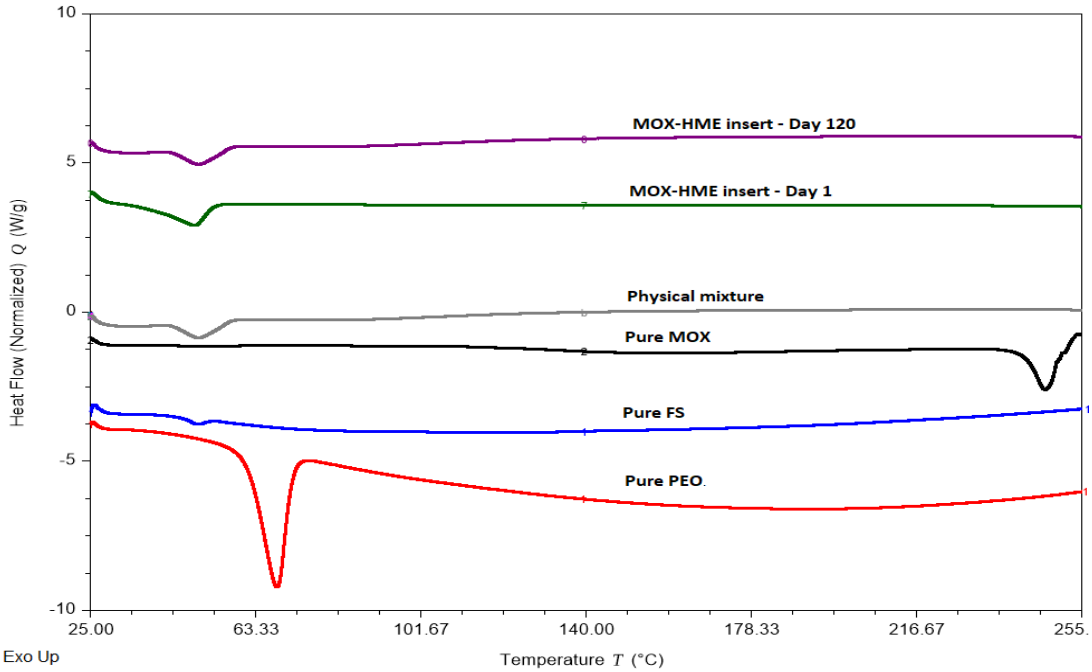


Figure 11:Overlay of DSC thermograms to evaluate drug-polymer compatibility

FTIR

FTIR spectrum of pure MOX-HCL, pure FS, pure PEO, physical mixture (PM) of the optimized formulation and the optimized MOX-HCL-HME inserts were investigated for interactions between the drug and the polymer. FTIR spectra of pure MOX-HCL exhibited characteristic bands corresponding to C=O stretching at 1760 cm^{-1} and primary amine group peaks at 3524 and 3470 cm^{-1}.[51] The FTIR spectra of pure PEO showed aliphatic -CH stretching at 2880 cm^{-1}. The characteristic C=O group of the free carboxylic acid group of FS was seen around 1726 cm^{-1} (Figure 10). In the physical mixture and the optimized formulation while most of the peaks of PEO and FS were present intact. However, there was an absence of the amine group (–NH) peak of MOX-HCL indicating potential weak hydrogen bonding interaction with carbonyl function group of FS. This could be explained since the NH group is a potential H-donor shifts its position reflect possible interruption between the drug molecules and formation of potential drug-polymer interactions which could help stabilize the formulation.[53]

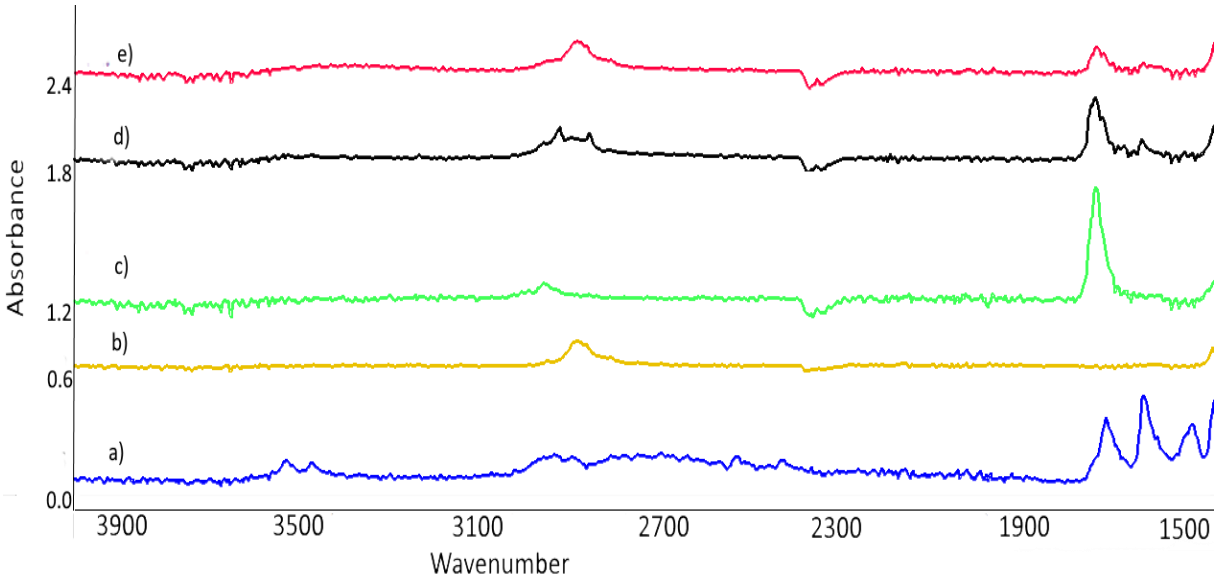
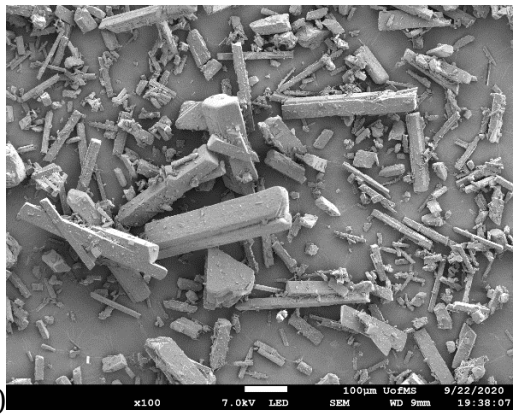
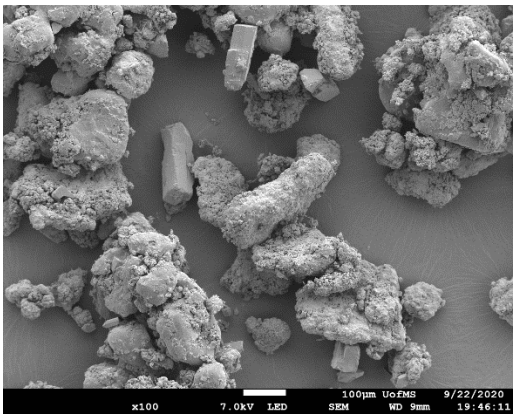


Figure 12: FTIR spectra for a) pure MOX-HCL, b) pure PEO, c) pure FS, d) physical mixture of the optimized formulation, and d) optimized MOX-HME insert

SEM

The SEM images of pure MOX-HCL, physical mixture of the optimized formulation and optimized MOX-HCL-HME insert are shown in Figure 11. Pure MOX-HCL exhibited crystalline structure (Figure. 11a). The crystalline nature of MOX-HCL is still preserved in the physical mixture of the drug and polymer (Figure. 11b). The optimized MOX-HCL-HME insert showed a smooth surface and the absence of MOX-HCL crystals on the surface indicating molecular dispersion of drug within the polymer (Figure. 11c). Thus supporting the DSC finding of amorphous drug within the formulation.

a) 

b) 

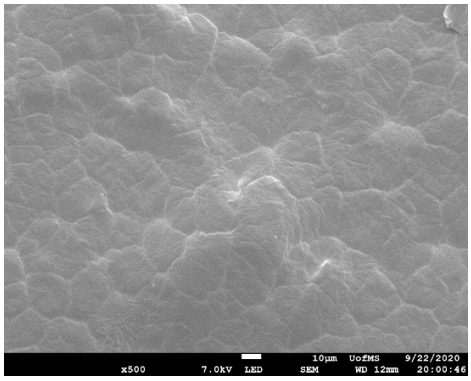
c) 

Figure 13:SEM images for a) pure MOX, b) physical mixture of the optimized formulation, and c) optimized MOX-HME insert

2.4 Conclusions

Ocular inserts have attracted considerable attention over the years as a platform to enhance the ocular bioavailability of drugs. In this study, MOX-HCL loaded inserts were prepared using HME and their *in vitro* and *ex vivo* performance was investigated. CCD was used to optimize the inserts for sustained release over 24 h. The optimized formulation also demonstrated stability at room temperature for 4 months with no change in the release profile as well as in its solid state. Moreover, the optimized MOX-HCL-HME inserts showed sustained transcorneal delivery *ex-vivo* as compared to commercial ophthalmic solution and the immediate release inserts. To conclude,

the optimized MOX-HCL-HME have been proposed as alternative delivery systems for MOX-HCL in the treatment of BK as they can potentially reduce the dosing frequency to once a day thereby improving the patient compliance. However, *in vivo* ocular biodistribution and efficacy studies in BK induced animal models are additional studies that are needed for the development of an ocular dosage form for the treatment of BK.

Acknowledgment

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Funding

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Declaration of interest

The authors report no conflicts of interest.

CHAPTER 3: DEVELOPMENT AND CHARACTERIZATION OF MOXIFLOXACIN NANOEMULSION WITH A MUCOADHESIVE AGENT TO ENHANCE BIOAVAILIBILITY FOR OCULAR INFECTIONS

3.1 Introduction

Moxifloxacin hydrochloride (MOX), is an 8-methoxy, fourth generation fluoroquinolone anti-infective, with broad spectrum activity and is well tolerated compared with minimal side effects. It is often the preferred drug of choice as compared to other fluoroquinolones due to its higher intraocular bioavailability.[54] Additionally, it has affinity for two essential bacterial enzymes (topoisomerase II and topoisomerase IV) resulting in enhanced potency and reducing the possibility of resistance as compared to older fluoroquinolones. Owing to these attributes it is widely used off label for the treatment of keratitis and as a prophylaxis agent in cataract and refractive surgeries. It is currently marketed as Vigamox®, a 0.5% moxifloxacin solution which is isotonic and has a near neutral pH of 6.8 and has been FDA approved for bacterial conjunctivitis. [20] However, the limitations in drug absorption with the eye drops result in frequent administration of at least 3 times or more per day. [55]

Nanoemulsions (NEs) are colloidal dispersions, which are widely used in ophthalmic drug delivery as they are non-invasive, cost-effective enhance bioavailability via sustained drug release.[56] NEs, especially the oil in water (o/w) nanoemulsions have been frequently used in ocular formulations. They possess several advantages including high solubilizing capacity as well enhanced penetration of various drugs. Additionally, the low surface tension of NEs results in good spreading on cornea

consequently achieving proper mixing with pre-corneal film, thus improving the contact time between the drug and cornea and resulting in resulting in sustained release. Moreover, the sterilization of NEs is simple and inexpensive.[57] These properties of NEs, make it an ideal vehicle for the delivery of MOX.

Although NEs are commercially available for ocular formulations (Restasis®, Cyclokat®), the low viscosity of NEs is a potential challenge. To address this, various approaches including the addition of cationic agents to the NEs, *in situ* gelling agents and mucoadhesive agents have been explored to increase the NEs residence time on the ocular surface. In the current study, addition of a mucoadhesive agent to the MOX-NE to further improve the pre-corneal residence time of the drug at corneal surface and thus improve its ocular bioavailability was adopted. Some polymers that have been routinely used in ophthalmic formulations to improve the residence time by including mucoadhesives and viscosity enhancing polymers such as hypromellose (HPMC), sodium carboxy methyl cellulose, Carbopol®, xantham gum, and polyvinyl pyrrolidone (PVP). The objective of this project was to investigate various mucoadhesive polymers and consequently develop a stable MOX loaded NE with a mucoadhesive agent so as to further prolong the residence time and enhance the bioavailability of MOX. Thus providing an alternative delivery system of MOX for ocular bacterial infections.

3.2 Materials and Methods

Materials:

MOX was purchased from Fischer Scientific (Hanover park, IL). Oleic acid (OA), Tween® 80, Poloxamer 407 and glycerin were purchased from Spectrum Pharmaceuticals (Handerson, NV). All the other chemicals were obtained from Fischer Scientific (Hampton, NH, USA). Hydroxy propyl methyl cellulose (HPMC K4M) was a kind gift from Colorcon. Polyvinylpyrrolidone (PVP

Plasdone™ K29/32) was purchased from Ashland (Willmington, DE, USA). Solvents used for analysis were of High-Performance Liquid Chromatography (HPLC) grade.

Methods

HPLC chromatographic conditions

MOX concentration in all samples was analyzed using HPLC, Alliance Waters e2695 separations module and Waters 2489 UV/Vis dual absorbance detector using a published method. A detection wavelength (λ_{max}) of 254 nm was used. The run time for each sample was 10 min. The standard curve of MOX was from 5-100 ng/ml. The samples were analyzed through Empower software. The mobile phase consisted of 18 mM of potassium dihydrogen phosphate with 0.1 % w/v of trimethylamine (TEA) pH 2.8 (adjusted with phosphoric acid) and methanol in the ratio of 60:40 v/v.[37] Phenomenex Luna® C_8 column (250 x 4.6 mm, 5 µ) was used for the analysis.

Screening of oils

The solubility of MOX in various oils was determined by adding 10 mg of MOX in 200 mg of the oils (castor oil, soybean oil, sesame oil, OA, Miglyol® 829, Labrafac® Lipophile WL 1349, and Capryol 90®, Isopropyl myristate, Transcutol®) in 3-mL glass vials. They were then mixed using a vortex mixer for approximately 5 minuts. Further, the MOX-oil mixtures were heated at 80±2ºC, under continuous magnetic stirring for 20 mins. The mixtures were then allowed to cool at room temperature for 24 hrs and were visually examined for MOX precipitation. The oil that did not show any precipitation were chosen for further studies.

Preparation of MOX-NE formulations

Oil in water (O/W) type MOX-NE formulations were prepared using the hot homogenization followed by ultra-sonication. The oil phase was prepared by weighing 50 mg (0.5% for 10 ml batch) of MOX and solubilizing it within the selected oil by heating at 80±2ºC to obtain a clear

liquid. The aqueous phase was prepared by adding Tween® 80, Poloxamer 407 and glycerin in double distilled water. The aqueous phase was also heated at 80±2ºC. The hot aqueous phase then added to the heated oil phase, under continuous mixing to form a pre-mix. This pre-mix was then homogenized using a T25 digital Ultra-Turrax (IKA, Germany) at 11,000 rpm for 5 min at 65±2ºC to form a primary emulsion. This primary emulsion was then allowed to cool at 25°C for 10 mins before subjecting it to ultra-sonication at 40% amplitude for 10 mins (pulse on: 10 s, pulse off: 15 s) using Sonics Vibra-Cell™ Sonicator (Newtown, CT, USA) to form MOX-NE.

Characterization of MOX-NE

Determination of droplet size, polydispersity index (PDI), and zeta potential (ZP)

The MOX-NE formulations were evaluated for their droplet size, PDI and ZP using the Zetasizer Nano ZS Zen3600 (Malvern Instruments, MA, USA) at 25ºC in disposable, folded, clear capillary cells (cite travatoprost NE). The samples were diluted 1:100 times with bi-distilled water and measured at 25ºC in triplicate.

Measurement of the pH

The pH of the formulations was measured using InLab® Micro pH probe meter (Mettler Toledo, Columbus, OH).

Drug content

For drug content analysis, 100 ul amount of MOX-NE was taken from 3 different regions (bottom, middle, and top) of the formulation and added to 900 ul of methanol (extracting solvent) in an centrifuge tube. The samples were then centrifuged at 13,000 rpm for 20 min using the AccuSpin 17R centrifuge (Fisher Scientific, Hanover, IL, USA). Furthermore, the supernatant was diluted with mobile phase such that the final concentration is 50 ug/ml and analyzed for MOX content using the HPLC method described above.

Physicochemical stability

Stability study of the formulations was assessed by storing the MOX-NE formulations at refrigerated (4±2ºC), room temperature (25±2ºC). Stability of the formulations was assessed by evaluating them for droplet size, PDI, ZP viscosity and change in MOX content at the aforementioned conditions.

Viscosity

Viscosity of the MOX-NE and formulations with 0.4% HPMC K4M and PVP-K29/32 was measured at different angular velocities at 25°C using the Brookfield viscometer (LVDV-ii+ P, Middleborough, MA, USA). 500 µl of the NE samples was taken using a pipette and placed in the plate of the viscometer and the measurements were carried in triplicate (Top-middle-bottom) from the same formulation. The rheology data analysis was accomplished using Rheocalc software (Version3.3 Build 49-1, USA)

Antimicrobial Efficacy of MOX Formulations

The antimicrobial activity of MOX formulations were evaluated against methicillin-resistant *Staphylococcus aureus* ATCC 1708 (MRS), *Escherichia coli* ATCC 2452, *Pseudomonas aeruginosa* ATCC BAA-2018, *Klebsiella pneumonia*e ATCC 2146 and vancomycin resistant *Enterococcus faecium* (VRE) ATCC 700221. All microbial strains are obtained from the American Type Culture Collection (ATCC, Manassas, VA). Susceptibility testing was performed using a modified version of the CLSI methods [CLSI, 2012]. MOX formulations were serially diluted using assay medium (cation-adjusted Mueller-Hinton @ pH 7.0) and diluted samples were transferred to 96 well assay plates. Inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth to afford recommended inocula as per CLSI protocol. 5% Alamar Blue™ was added in VRE and MRS plates. Crude MOX was included as a positive control in each

assay. The optical density was measured using the Bio-Tek plate reader prior to and after incubation at 35°C for 24h.

Minimum Inhibitory Concentrations (MICs), defined as the lowest test concentration that allows no visual growth, were calculated for all formulations. All experiments were performed in triplicates.[39]

Sterilization method

Sterilization of the MOX-NE formulations by filtration was explored. 500 µl of the samples were passed through various 0.22-µm filter membranes using a 13 mm stainless steel Swinny Filter Holder (Millipore Sigma, MA, USA). The different filter membranes tested were Nylon, Durapore™, Fluoropore™ and Millipore Express PLUS (PES) was purchased from (Millipore Sigma, MA, USA). The filtrate was collected in a 2 ml glass vial, and the volume collected was noted using a pipette. The effect of filtration on the physical and chemical characteristics of the MOX-NE formulations was also evaluated.

3.3 Results and Discussions

Screening of oils

Ocular NEs contain usually contain 5-20 wt % of oil as the dispersed phase. The selection of oil/lipid phase is critical as the API is dissolved in an oil phase prior to dispersion in aqueous phase. It is crucial to determine the solubility of the drug in oil phase which constitutes the dispersed phase in o/w water emulsion. The selected oil needs to well tolerated and compatible with other excipients included in the nanoemulsion. Most common oil phase of ocular NEs includes castor and soybean oils and other vegetable oils, medium chain triglycerides, long-chain unsaturated fatty acids.[58] The solubility of MOX in various oils was determined using visual examination as described above and the results are shown in Table 6. MOX showed no

precipitation in oleic acid upon cooling and therefore, was chosen as an oil. Oleic acid is a long-chain unsaturated free fatty acid that frequently used in topical administration due to its biocompatibility and well tolerated safety profile. Additionally, oleic acid, has often been used in ophthalmic formulations as it acts as a powerful penetration enhancer as it increases the fluidity of intercellular lipid barriers.[59]

Table 6: Oil screening study for moxifloxacin

Oil	**Solubility**	**Oil**	**Solubility**
Soybean oil	(-)	Miglyol® 829	(-)
Castor oil	(-)	Labrafac® Lipophile WL 1349	(-)
Oleic acid	(+)	Transcutol	(-)
Sesame oil	(-)	Isopropyl myristate	(-)

(+): MOX is soluble in the oil and does not precipitate on cooling; (-): MOX is either soluble in the oil, but precipitates on cooling or is insoluble in the oil.

Effect of various oil and surfactant concentrations

The NE formulations consisting of oil, surfactants, co-surfactants and drug should be monophasic liquid at ambient temperature and should be stable physically stable so as to prevent any creaming, phase separation and aggregation. Surfactants are necessary for the emulsification of o/w phases to form the NEs as they reduce the interfacial tension between the two phases. Surfactants with an HLB value more than 10 are usually recommended for o/w NEs. Tween 80 which has an HLB of 15 and is a non-ionic surfactant that is widely used in ocular formulations was selected for as it safe and non-toxic.[56] Thus we evaluated the effect of various concentrations of oil (oleic acid) and surfactant (Tween 80®) concentrations on the physical stability of the nanoemulsions.

Table 7: Effect of oleic acid and Tween 80 concentrations on physical stability of emulsions

Oleic Acid (%w/v)	Tween 80 (%w/v)	MOX (%w/v)	Glycerin (%w/v)	Water	Stability after 21 days
5.83	2.38	0.5	2.25	Q.S 10 ml	(+)

3.00	4.67	0.5	2.25	Q.S 10 ml	(-)
5.00	0.75	0.5	2.25	Q.S 10 ml	(-)
1.00	0.75	0.5	2.25	Q.S 10 ml	(-)
3.00	2.38	0.5	2.25	Q.S 10 ml	(-)
5.00	4.00	0.5	2.25	Q.S 10 ml	(+)
3.00	2.38	0.5	2.25	Q.S 10 ml	(-)
1.00	4.00	0.5	2.25	Q.S 10 ml	(-)

Oleic acid was chosen based on the oil screening studies discussed earlier. The oil concentration was varied between 1-5% w/v as these concentrations are within the acceptable range for ocular delivery. Tween® 80 is widely used surfactant in ocular formulations and is considered safe up to 4 % w/w in ophthalmic emulsions according to inactive ingredients list for ophthalmic products approved by the FDA. Hence our chosen concentrations (0.75-4.00%) are within the allowable limits. From these studies we found that only those formulations that had 5% w/v of oleic acid were stable up to 21days and did not show any phase separation or creaming. All the other formulations at day 21 either cracked or showed phase separation indicating the amount of oleic acid seems to be playing a critical role in stabilization of the NE. The two stable formulations had 5% w/v oleic acid with Tween ®80 concentrations of 2.38 and 4.00 %w/v. Amongst these two formulations, the lower concentration of Tween ®80 (2% w/v) was selected for further studies.

Stability of MOX-NE

The physical stability of a formulation is an essential parameter to be considered during formulation development. On long-term storage, the NE formulation needs to be stable. Post one-month storage at 4°C, there was no significant change observed in either size, PDI or assay of the MOX-NE formulation. Thus, we can infer the MOX-NE formulation was stable at 4°C up to at least a month. The formulation needs to be evaluated for stability at room temperature.

Table 8: Stability of MOX-NE at refrigerated conditions (4°C).

Mox (%w/v)	**Oil (%w/v)**	**Tween ®80 (%w/v)**	**Glycerin (%w/v)**	**Water**
0.5	5	2	2.25	Q.S 10ml
Physical Characteristics				
	Day 1	**Day 30**		
Size (nm)	144.3 ± 5.0	158.4 ± 1.3		
PDI	0.20 ± 0.00	0.11 ± 0.02		
Zeta potential (mV)	-40.1 ± 1.1	-44.6 ± 3.1		
Assay (%)	100.8 ± 3.6	101.8 ± 1.6		

Addition of mucoadhesive agent to MOX-NE

Since the above MOX-NE formulation was found to be stable, it was further developed with the addition of mucoadhesive agents. Carbopol 940 was also explored as mucaodhesive polymer but initial trials revealed stability issues and therefore was not selected for further studies. PVP and HPMC were stable and compatible, therefore, were further explored. HPMC has been widely used by researchers in ophthalmic formulations over the past few years due to its biocompatibility and solubility in water. According to the inactive ingredients database that is approved by the FDA for ophthalmic products HPMC(K4M) can be used up to a concentration of 0.5% w/v in ophthalmics. In the current study HPMC K4M which is a non-ionic viscoelastic polymer was selected because it is inert, non-toxic, exhibits mucoadhesive property and high swelling capacity which can further aid in providing sustained drug delivery. [60]

PVP is a polymer that is extensively used in the pharmaceutical field as a binder, lubricant, wetting agent, complexation agent (e.g with iodine) and stabilizer. PVP is widely used because of its

amphiphilic properties due to its protein like lactam bond which contributes for hydrophilicity and methylene component which contributes for lipophilicity. Due to this amphiphilic nature it has good solubility in water and organic solvents and is compatible with hydrophilic and hydrophobic compounds.[61] Owing to these properties PVP was chosen.

In this study HPMC K4M and PVP both were evaluated at a concentration of 0.4% w/v. The physical and chemical characteristics of the MOX-NE formulations upon addition of these agents is given in Table 9. It was found that upon addition of the PVP and HPMC K4M to the MOX-NE there was no significant difference observed in the size, PDI and ZP of the MOX-NE formulation. The assay for all the formulations were found to be in the range of 97.1 ± 5.2% to 101.9 ± 1.7%. The pH of all three formulations was found to be similar around 5.5 with no significant difference between them. It was observed that the MOX-NE and MOX-NE with 0.4% w/v PVP had similar viscosity of 3.8 ± 0.17 cPs and 4.4 ± 0.21 cPs respectively. Therefore, we can infer that PVP did not show any significant effect on the viscosity of the formulation (it was an 18% increase in viscosity so why say this?]. However, on the contrary, the viscosity of the formulation with HPMC K4M was found to significantly higher than the (13.4 ± 2.6 cPs) other formulations. Typical a viscosity of ocular formulations of less than 25 cPs is considered suitable in terms of patient compliance, ease of application and increasing the retention time.[56] All the free formulations are well within the desired viscosity range while HPMC K4M shows the highest viscosity however, more comparison in terms of stability, permeation profiles of these formulations needs to be explored before selecting the mucoadhesive?.

Table 9: Characterization of the MOX-NE formulations upon addition of mucoadhesive agents.

Formulation	**Size (nm)**	**PDI**	**Zeta Potential (mV)**	**% Assay**	**pH**	**Viscosity (cP)**

MOX-NE	122.2 ± 1.9	0.08 ± 0.02	-40.2 ± 0.9	97.1 ± 5.2	5.85 ± 0.11	3.8 ± 0.17
MOX-NE with PVP	130.8 ± 2.2	0.12 ± 0.02	-36.6 ± 0.9	101.9 ± 1.7	5.53 ± 0.07	4.4 ± 0.21
MOX-NE with HPMC-K4M	131.1 ± 2.4	0.17 ± 0.01	-34.9 ± 1.4	101.5 ± 2.9	5.47 ± 0.02	13.4 ± 2.6

Stability at 25°C

NEs often show instabilities including coalescence, creaming, phase inversion over time upon storage. Therefore, this study was done to evaluate the physicochemical stability of the three optimized NEs at room temperature for 45 days (last time point evaluated). MOX-NE, MOX-NE with PVP and MOX-NE with HPMC K4M showed a slight increase in particle size around 10 nm (Table 10). PDI for all three formulations was below 0.2 for 45 days (Table 10). There were no significant changes observed in the zeta potential and assay, therefore, it can be concluded that all the three formulations were stable at 25°C for 45 days.

Table 10: Stability of the optimized MOX-NE, MOX-NE with 0.4% PVP and HPMC AT 25°C

Formulation	**Time**	**Size (nm)**	**PDI**	**Zeta Potential (mV)**	**%Assay**
MOX-NE	Day 1	122.2 ± 1.9	0.08 ± 0.02	-40.2 ± 0.9	97.7 ± 1.4
	Day 45	128.0 ± 0.6	0.14 ± 0.03	-39.8 ± 0.7	95.4 ± 1.8
MOX-NE with PVP K30	Day 1	130.8 ± 2.2	0.12± 0.02	-36.4 ± 0.9	104.9 ± 2.1
	Day 30	138.5 ± 2.1	0.16± 0.06	-37.2 ± 0.6	102.8 ± 1.9
	Day 45	138.6 ± 2.1	0.14± 0.04	-37.6 ± 0.8	98.1 ± 1.5
MOX-NE	Day 1	131.1 ± 2.4	0.16± 0.01	-34.9 ± 1.4	101.5 ± 2.9
	Day 30	140.9 ± 1.3	0.18± 0.03	-35.1 ± 0.9	97.6 ± 2.9

with HPMC-K4M	Day 45	139.3 ± 0.4	0.14± 0.01	-34.2 ± 0.4	96.4 ± 1.5

Effect of filtration

Ophthalmic formulations are required to be sterile. The major methods that are employed for sterilization are autoclaving and aseptic filtration. Sterilization by filtration also known as terminal sterilization, is widely adopted for NEs as it is an efficient way to sterilize without the need for heat and can be used for thermolabile drugs. Besides, autoclaving may also change the physiochemical properties of NEs as they are performed at high temperatures or could also lead to hydrolysis of the lipids which can destabilize the NEs. NEs are considered good candidates for sterilization by filtration due to their small size, which is less than the maximum nominal pore size of membrane filters used in this sterilization technique (0.22 μm or 220 nm). Thus, a globule size larger than 220 nm might clog the filter pores and could lead to loss of active ingredient. The optimized formulations MOX-NE, MOX-NE with PVP and MOX-NE with HPMC-K_4M were filtered through several filters. It was found that all three formulations could be easily filtered through Durapore™, Nylon and PES membranes but faced resistance through Fluoropore™. Durapore™ is a hydrophilic polyvinylidene fluoride membrane filter, PES is also a hydrophilic membrane filter and Fluoropore™ is a hydrophobic polytetrafluoroethylene membrane filter. We can infer that all the formulation passed through the hydrophilic filters but faced resistance from the hydrophobic filter materials: this could be due to the hydrophilic nature of the polymers (PVP and HPMC) used. Filtration could affect the particle size and distribution along with the drug content during the sterilization process. Therefore, the physiochemical characterization of all the formulation was investigated. There was no significant difference observed in the particle size

before and after filtration in MOX-NE, MOX-NE with PVP and MOX-NE with HPMC. The particle size was below 150 nm for all the formulations pre-filtration as well as post-filtration.

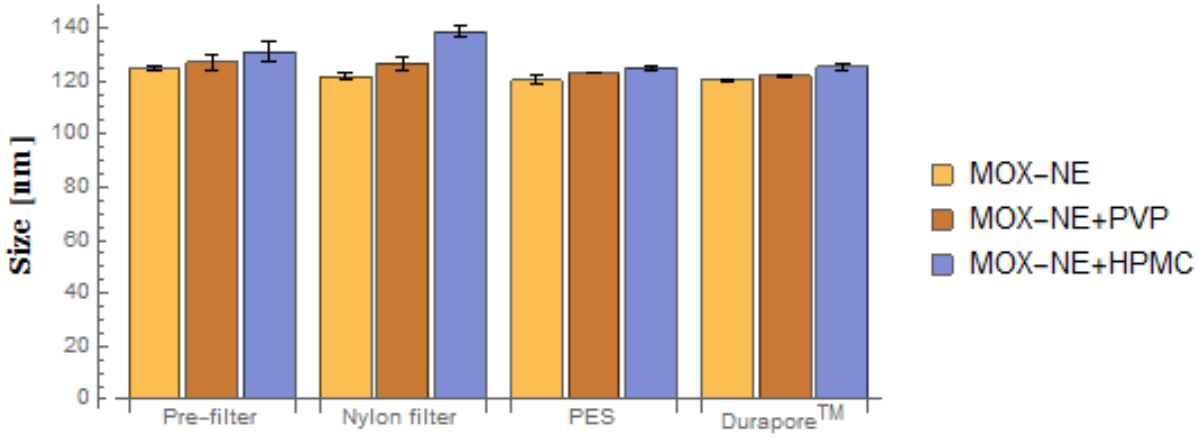

Figure 14: Effect of filtration on the size of MOX-NE, MOX-NE with PVP, MOX-NE with HPMC.

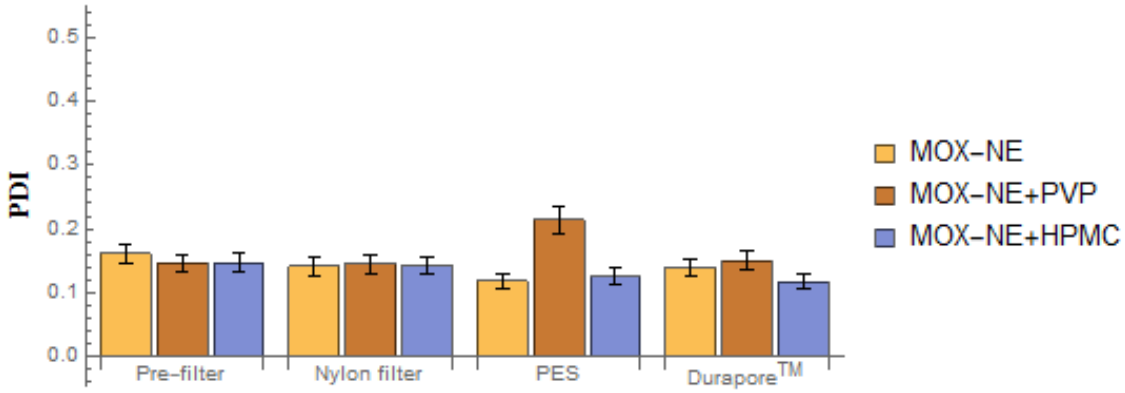

Figure 15: Effect of filtration on the PDI of the formulations; MOX-NE,MOX-NE with PVP, MOX-NE with HPMC

All three formulations exhibited a narrow size distribution (PDI < 0.3) before as well as after filtration. There was no significant difference observed in the PDI of the formulations post-filtration (Figure 13).

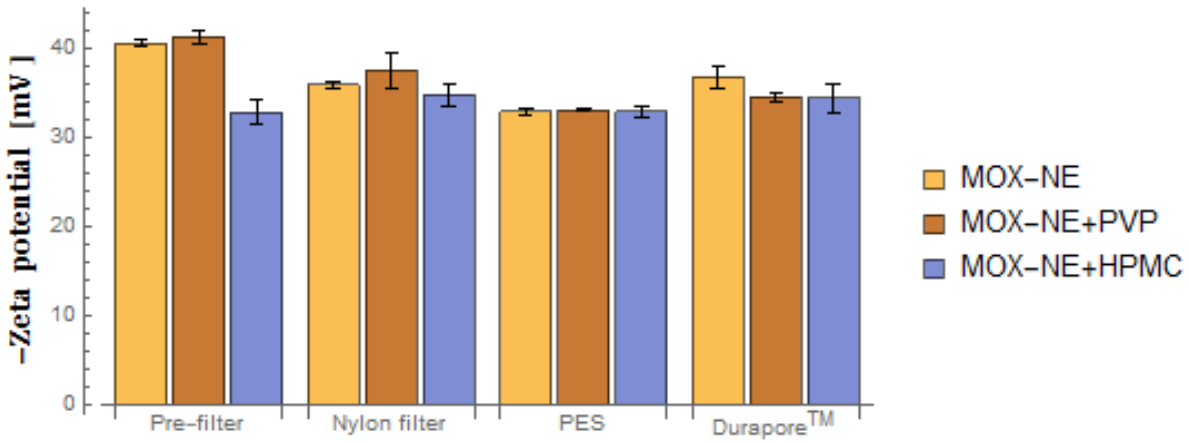

Figure 16: Effect of ZP on the formulations before and after filtration.

ZP is an important indicator the stability of the formulation. However, there no significant difference observed in the zeta potential of MOX-NE, MOX-NE with PVP and MOX-NE with HPMC upon filtration through difference filters. The ZP ranged between -35-40 mV.

There was no significant change in assay observed before and after filtration. The was found to be 97.39 ± 2.1% before filtration and was found to be 95.69 ± 3.5% after filtration.

Antimicrobial efficacy

Staphylococcus are the most common causative agents of corneal infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with increasing persistent and complicated eye infections.[62] *Pseudomonas aeruginosa* is another species which is known to cause severe cases of BK and corresponding corneal blindness.[63] Both of these species represent a threat to visual acuity. The goal of this study was to evaluate the antimicrobial efficacy of the optimized MOX-NE MOX-NE-PVP and HPMC-K4M and compare it with Vigamox® and the crude MOX solution against MRSA and *Pseudomonas aeruginosa.* We observed that the MIC values (6.25 ug/ml) obtained for all the three formulations that were investigated was the same as Vigamox®. This implies that the MOX-NE, MOX-NE-PVP and HPMC-K4M are as effective as the commercial formulation in terms of their antimicrobial activity.

3.4 Conclusion

NEs are widely used in ocular drug delivery because they are non-invasive, cost effective, exhibit high penetration capacity, have improved drop drainage compared to gels or ointments. In the current study a stable MOX-NE with low surfactant concentration was formulated as compared to Shah et al.,2019 which used 12% w/v of Tween®80 and 24%w/v of Soluphor®P to prepare MOX-NE. This MOX-NE was further optimized by adding mucoadhesive agents - PVP and HPMC K4M to prolong the ocular retention time and thus improve bioavailability. MOX-NE with HPMC K4M showed a higher viscosity (13 cPs) compared to MOX-NE and MOX-NE with PVP. All the three formulations were found to be stable upon storage at room temperature up to 45 days and the MOX-NE was found to be stable at 4°C for one month. Evaluation of the ocular permeation profiles are needed in order to evaluate the different formulations designed to provide an alternative delivery system of MOX for ocular infections.

CHAPTER 4: CONCLUSION

Moxifloxacin is available as a marketed solution (Vigamox(R) which has to be administered 3 or more times a day leading to poor patient compliance and low ocular bioavailability. The overall goal of this research was to use formulation strategies and develop patient compliant dosage forms of moxifloxacin which are sustained release so as to improve its retention and thereby ocular bioavailability. The first strategy that was explored was to developed sustained release inserts of moxifloxacin using hot melt extrusion. The inserts showed sustained release and antibacterial activity up to 24h and could potentially be a once-a-day application.

Another strategy that was utilized was to develop a sustained release nanoemulsion of moxifloxacin with a mucoadhesive agent (HPMC or PVP) as it non-invasive, cost-effective and known to enhance the retention and permeation of drugs. The nanoemulsions was formulated with lower amount of surfactant within the range by the inactive ingredients for ophthalmic products that have been approved by the FDA and was found to stable at room temperature upto 45 days. The nanoemulsion also did not show any significant change in physicochemical properties after filtration.

Long term stability of the nanoemulsion and a comparative permeation profiles of the nanoemulsions with and without a mucoadhesive, need to be evaluated to provide more insight into the feasibility of this alternative sustained delivery dosage form for moxifloxacin.

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VITA

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PUBLICATIONS

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