FORMULATION OF NANOEMULSIONS FOR THE ENHANCEMENT OF BIOAVAILABILITY OF SOME DRUGS

THESIS SUBMITTED TO THE KAKATIYA UNIVERSITY

FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

PHARMACEUTICAL SCIENCES

By

AJMEERA DIVYA

M.Pharm

Under the Supervision of

Prof. M. SARANGAPANI

M.Pharm, Ph.D., PGDBM

UNIVERSITY COLLEGE OF PHARMACEUTICAL SCIENCES

KAKATIYA UNIVERSITY

WARANGAL, TELANGANA, INDIA.

2020

FORMULATION OF NANOEMULSIONS FOR THE ENHANCEMENT OF BIOAVAILABILITY OF SOME DRUGS

SYNOPSIS OF THE

THESIS SUBMITTED TO THE KAKATIYA UNIVERSITY
FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

PHARMACEUTICAL SCIENCES

By

AJMEERA DIVYA
M.Pharm

Under the Supervision of

Prof. M. SARANGAPANI
M.Pharm, Ph.D., PGDBM

UNIVERSITY COLLEGE OF PHARMACEUTICAL SCIENCES
KAKATIYA UNIVERSITY
WARANGAL, TELANGANA, INDIA.

2020

UNIVERSITY COLLEGE OF PHARMACEUTICAL SCIENCES
KAKATIYA UNIVERSITY
WARANGAL, (T.S.) INDIA

Prof. M. Sarangapani
M.Pharm, Ph.D., PGDBM

Ph. No:9246896242
Email: panimanda@gmail.com

CERTIFICATE

I certify that **Mrs. Ajmeera Divya** has prepared her thesis entitled "**Formulation of Nanoemulsions for the Enhancement of Bioavailability of Some Drugs**" for the award of PhD degree of the Kakatiya University under my guidance. She has carried out the work at the Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University and Jeeva Life Sciences, Hyderabad.

I further declare that the contents of this thesis is free from plagiarism.

(Prof. M. SARANGAPANI)
RESEARCH SUPERVISOR

Jeeva Life Sciences
(An ISO 9001:2015 Certified Company)

CERTIFICATE

I certify that Mrs. Ajmeera Divya, Research Scholar (PhD) from University College of Pharmaceutical Sciences, Kakatiya University, Warangal has learned and practiced animal handling and biological sample collection and carried out In Vivo studies in Rabbits for her thesis entitled "**Formulation of Nanoemulsions for the Enhancement of Bioavailability of Some Drugs**" at Jeeva Life Sciences, Hyderabad for a period of three months.

During this period, she has performed the following tasks with perfection.

1. Animal Handling.
2. Anesthetizing the animal through I.V, I.M & S.C routes.
3. Blood sample collection.
4. Aqueous Humour collection.

(Dr. UTTAM KUMAR)
CHAIRMAN

Jeeva Life Sciences ★ Hyderabad ★

3-224/98, Plot No- 98, Shanti Nagar,Uppal,RR Dist-500039
Phone no: 09291485088

KAKATIYA UNIVERSITY

Warangal – 506009, Telangana India

CERTIFICATE OF PLAGIARISM CHECK

1.	Name of the Research Scholar (Full Name)	**AJMEERA DIVYA**
2.	Course of the Study	**Ph.D.**
3.	Title of the Thesis (Full)	**FORMULATION OF NANOEMULSIONS FOR THE ENHANCEMENT OF BIOAVAILABILITY OF SOME DRUGS**
4.	Name of the Supervisor (Full Name)	**Prof. M. SARANGAPANI**
5.	Department	**PHARMACY / FACULTY OF PHARMACEUTICAL SCIENCES**
6.	Percentage (%) Acceptable or Maximum Limit (as per UGC 2018 regulations)	**10 %**
7.	Similarity Percentage (%) of the content	
8.	Software Used	**URKUND**
9.	Date of Verification (D/M/Y)	

Report on Plagiarism check, Items with % of similarity is attached

Co-ordinator, Anti-Plagiarism Cell **Supervisor**

Chairperson
Board of Studies **Head** **Dean**

DECLARATION

I declare that the thesis entitled "**Formulation of Nanoemulsions for the Enhancement of Bioavailability of Some Drugs**" has been prepared by me under the guidance of Prof. M. Sarangapani, Professor of Pharmacy/Faculty of Pharmaceutical Sciences, Kakatiya University**.** No part of this thesis has formed the basis for the award of any degree or fellowship previously.

I further declare that the contents of this thesis is free from plagiarism.

(AJMEERA DIVYA)

ABSTRACT

Background: Medical or non-surgical treatment of most common eye problems involves oral as well as the topical eye-drop instillation of solutions or suspensions and topical application of eye ointments or gels among which the eye drops are generally the preferred means as they are easy to prepare and sterilize for the formulator and convenient to use for the patients. However, the drawbacks those are associated with these conventional eye drops such as the poor drug bioavailability and short residence time in tears result in several daily administrations to accomplish the preferred therapeutic effects, dropping the patient compliance and sometimes serious systemic side effects. Furthermore, physiology, anatomy, and biochemistry of eyes act as major barriers for drug delivery to the ocular environment. Thus it is in need of developing new dosage forms for effective treatment of various eye problems.

Objective: To develop novel cationic nanoemulsions (NEs) for ophthalmic delivery of three different categories of drugs namely Timolol maleate (anti-glaucoma, reduces the aqueous humor secretion in glaucoma condition), Ciprofloxacin (anti-bacterial, fights with different micro organisms responsible for conjuctivitis) and Indomethacin (anti-inflammatory, relieves postoperative pain) with improved ocular bioavailability.

***Methodology*:** Based on the solubility profile of drugs, oil phase, surfactant and co-surfactant were selected to construct pseudo ternary phase diagrams and NEs regions were recognized. The sonication method was applied for preparing NEs and evaluated for various physicochemical properties. Optimization was done using 3^2 factorial designs and the optimized NEs were studied for the surface morphology, antimicrobial activity, *ex vivo* permeation across the bovine cornea and ocular irritancy in rabbits. Further, drug pharmacokinetics of optimized NEs and marketed products were assessed in rabbit aqueous humor and plasma.

***Results*:** The optimized NEs showed acceptable physicochemical characteristics for ocular use, sustained drug release profiles and improved *ex vivo* transcorneal permeation compared to drug solutions and were considered safe for ocular delivery with the individual Iirr scores of less than 4. Further, they have improved the ocular bioavailability of drugs with significantly higher $AUC_{(0–24h)}$ in rabbit aqueous humour and lower $AUC_{(0-24h)}$ in rabbit plasma compared to the conventional marketed products.

***Conclusion*:** From the obtained results, we can strongly suggest that the optimized NEs as effective alternate dosage forms of selected drugs for ophthalmic use.

PREFACE

The major eye disorders are being treated either by surgical or non surgical medication. When it comes to non surgical medication, topical ocular products are preferred and beneficiary, however, the current commercial topical ophthalmic products found to face challenges to reach the ocular environment due to different physiological, anatomical and dosage form related aspects. In the present study, three major eye problems including Glaucoma, Eye infections and Postoperative treatment were studied. Based on the available literature, it is evident that the usage of the conventional topical eye drops as well as systemic dosage forms of beta blockers, antibiotics and NSAIDs will leads to severe systemic and ocular side effects. Hence a single drug for each eye problem which include Timolol maleate, a beta blocker with well established record of first line usage in anti glaucoma therapy, Ciprofloxacin Hydrochloride, a broad spectrum antibiotic proved to be effective against various bacterial eye infections and Indomethacin, an NSAID with proven ocular usage in pre and post operative treatment regimen were chosen.

The purpose of this entire study was to develop topical ophthalmic eye drops which can be better tolerated by the patients with reduced ocular as well as systemic side effects and better therapeutic effects for the medical management of glaucoma, bacterial eye infections and post operative care. An attempt was made to develop nanoemulsions of selected drug moieties with different categories and solubility profiles for topical ocular application in the form of eye drops where we found comparatively less work done on selected drugs in selected area of interest and believed that an additional research is a high priority. Nano emulsions were chosen to develop as they can load both hydrophilic as well as hydrophobic drug moieties with improved pre corneal residence time, corneal permeation and absorption. This thesis includes formulation development and pharmacokinetic studies in rabbits while no pharmacodynamic or clinical studies were carried out. We hope that this work will serve as a point of reference to future investigations in developing nano drug delivery systems for ocular therapy.

Sarangapani Manda (Investigator-1)

Divya Ajmeera (Investigator-2)

PRESENTATIONS AND PUBLICATIONS

Poster Presentations

1. Formulation of nanoemulsions for the enhancement of bioavailability of some drugs. 2nd International Pre-Conference Workshop on "Pharmacovigilance: Adverse Drug Monitoring and Orphan Drugs" with training on VIGIFLOW Software. Anurag Group of Institutions, **Hyderabad**, India. 15th &16th September, 2017.
2. Development of nanoemulsions to improve the ocular bioavailability of Timolol maleate. "Telangana State Science Congress (**TSSC-2018**)". National Institute of Technology, **Warangal,** India. 22nd -24th December, 2018.
3. Development of nanoemulsions to improve the ocular bioavailability and patient compliance in Glaucoma treatment using Timolol maleate. "8th World Glaucoma Congress (**WGC-2019**)". Melbourne Convention and Exhibition Centre, **Melbourne**, **Australia**. 27th -30th March, 2019.
4. Design and optimization of a new cationic nanoemulsion as an effective topical ophthalmic drug delivery system for ciprofloxacin: *in vitro*, *ex vivo* and *in vivo* characterization. International Conference on "Advances in Microbial Biotechnology and Biotherapeutics (**AMTB-2020**)". Department of Microbiology, University College of Science, Osmania University, **Hyderabad**, India. 26th -28th March 2020. (To be presented, postponed due to Covid-19).

Oral Presentations

1. Formulation of nanoemulsions for improving the ocular bioavailability of Ciprofloxacin. 3rd international conference on "Innovations in Pharmaceutical Sciences (**ICIPS-2018**)". Guru Nanak College of Pharmacy, **Hyderabad**, India. 3rd &4th August, 2018.
2. Formulation of nanoemulsions for improving the ocular bioavailability of Indomethacin. 9th National level Conference on "Emerging innovations in pharmaceutical sciences (**DRAVYAKA-2018**)". Geethanjali College of Pharmacy, **Hyderabad**, India. 2nd &3rd November, 2018.
3. Formulation and evaluation of Ciprofloxacin nanocarrier for ocular delivery. 10th National level Conference on "Rare Diseases- Orphan Drugs and their

Prevalence in Public (**DRAVYAKA-2019**)". Geethanjali College of Pharmacy, **Hyderabad**, India. 13th &14th November, 2019. (Awarded with 2nd **best oral** presentation).

4. Development of nanoemulsions to improve the ocular bioavailability and patient compliance in postoperative treatment using indomethacin eye drops. 2nd International Conference on "Integrative Biology &Applied Genetics (**ICIBAG-2020**)". Department of Genetics &Biotechnology, Osmania University, **Hyderabad**, India. 20th &21st March, 2020. (To be presented, postponed due to Covid-19).

Workshops

1. Two Day National Workshop on "Applications of Advanced Analytical Techniques in Pharmaceutical Sciences and Nanotechnology". Center for Pharmaceutical Sciences & Center for Nano Science &Technology, Institute of Science & Technology, JNTU, **Hyderabad**, India. 16th &17th September, 2016.
2. USFDA-CDSCO-DIA workshop on "Global Anti-Infective Drug Development: Challenges and Opportunities". **Mumbai**, India. 8th March, 2019.

Journal Publications

1. Divya Ajmeera*, Sarangapani Manda, Krishnaveni Janapareddi, Susmitha Kolluri, Shanthipriya Ajmera. Design and optimization of ciprofloxacin hydrochloride nanoemulsion for effective ocular delivery: *In vitro, ex vivo* and *in vivo* characterization. International Journal of Advanced Science and Technology. 2020;29(3):7036-7054.
2. Divya Ajmeera*, Sarangapani Manda, Krishnaveni Janapareddi, Susmitha Kolluri. Development of nanoemulsion to improve the ocular bioavailability and patient compliance in postoperative treatment using indomethacin. International journal of applied pharmaceutics. 2020;12(3):1-9.
3. Divya Ajmeera*, Sarangapani Manda, Shanthi Priya Ajmera 3. Most common eye infections: diagnosis, signs, symptoms and treatment options with a note on the potential of nanoemulsions for ocular delivery of antimicrobial agents.

(Manuscript submitted as a review article to Research Journal of Pharmacy and Technology).

E-book

1. Divya Ajmeera*, Sarangapani Manda, Krishnaveni J, Susmitha Kolluri. Development of nanoemulsions to improve the ocular bioavailability and patient compliance in Glaucoma treatment using Timolol maleate. Medical Treatment and Non-Incisional Surgery. 8th World Glaucoma Congress 2019 - Abstract Book. P-WT 128. Pg: 260- 261. [Full length paper available at https://www.worldglaucomacongress.org/wp-content/uploads/ninja-forms/8/timolol-final-manuscript.-PDF.pdf]

ISBN book Chapters

1. Divya Ajmeera*, Sarangapani Manda, Susmitha Kolluri, Shanthipriya Ajmera. Glaucoma- a Silent Blinding Disease: Route cause, Diagnosis, Treatment and the Potential of Nanoemulsions as Topical Ophthalmic Delivery Systems. Education for Future. 2020/22 (In press).
2. Divya Ajmeera*, Sarangapani Manda. Postoperative care and treatment in eye surgery. Education for Future. 2020/54 (In press).

ACKNOWLEDGMENTS

I will forever be indebted to all those who have supported me throughout the study and gave me the possibility to complete this work. I, therefore, take this opportunity to express my gratitude to all of them

I would like to express my earnest gratitude and appreciation to my research supervisor **Prof. M. Sarangapani**, Dean, Faculty of Pharmaceutical Sciences, University College of Pharmaceutical Sciences, Kakatiya University for his valuable instruction, guidance, positive appreciation and his constructive criticism over research findings helped me in the successful completion of the research work. I am grateful for his fatherly love and generous care throughout this work.

I profusely thank **UGC, New Delhi** for providing financial support through the "National Fellowship for Higher Education for ST Students" throughout this project without which this work would not have been possible.

I would like to convey my sincere thanks to **Prof. G. Achaiah**, Principal, University College of Pharmaceutical Sciences, Kakatiya University and all of the former Principals of the University College of Pharmaceutical Sciences, Kakatiya University, **Prof. Y. Narsimha Reddy, Dr. G. Sammaiah, Prof. Ravi Kumar, Prof. V. Kishan** for providing requisite infrastructure and institutional facilities throughout my research tenure.

I extend my sincere thanks to **Dr. N. Prasad**, Chairperson, Board of Studies, University College of Pharmaceutical Sciences, Kakatiya University for his constant support and encouragement.

I am extremely thankful to **Dr. J. Krishnaveni**, Assistant Professor, University College of Pharmaceutical Sciences, Kakatiya University for her valuable constructive suggestions and involvement during the planning and development of this research work.

I am also grateful to **Prof. Raghu Rama Rao** former Dean& BOS, Faculty of Pharmaceutical Sciences, University College of Pharmaceutical Sciences, Kakatiya University for his advice and assistance in keeping my progress on schedule.

I am profoundly grateful to **Prof. S. Girisham**, Department of Microbiology, Kakatiya University for motivating me and supporting my research ambitions.

I am greatly thankful to **Prof. K. Gopal Kishan Rao**, Department of Central Instrumentation Center, Kakatiya University for providing analytical assistance.

I extend my gratitude to **Prof. C. Veeresham, Dr. Shayeda, Dr. V. Swaroopa Rani and Mr. B. Nagaraju** and all contractual teachers of UCPSc, KU, Warangal for their co-operation and support.

I greatly appreciate and acknowledge **Dr. Suresh Bandari**, former Principal, St. Peter's Institute of Pharmaceutical Sciences, Warangal for providing me with samples of excipients for the research work and also for allowing me to utilize the laboratory facilities.

I greatly appreciate and acknowledge the support by **Dr. Raghunandan**, Principal, Balaji Institute of Pharmaceutical Sciences, Narsampet for carrying out IR analysis.

I greatly appreciate and acknowledge **Dr. M. Laxman,** Professor, Dept of Veterinary Pathology and Officer-in-Charge, RUSKA LABS, College of Veterinary Science, PVNR TVU, Rajendranagar, Hyderabad for carrying out Transmission Electron Microscopy.

It gives me great pleasure in acknowledging the assistance given by **Dr. B.D.P. Kala Kumar**, Professor &Head, Dept of Vety Pharmacology &Toxicology, College of Veterinary Science, PVNR TVU, Rajendranagar, Hyderabad for his professional guidance and teaching in conducting animal studies without which the Principal objective of the work would not have been fulfilled.

Particularly, I would like to give special appreciation to **Dr. Uttam Kumar**, Chairman of Jeeva Life Sciences, Hyderabad for his timely supply and maintenance of animals and also for providing laboratory facilities to conduct *in vivo* studies.

I gratefully acknowledge the support and generous care of my seniors **Dr. K. Swathi,** Associate professor, Sri Padmavati Mahila Visvavidyalayam, Tirupathi, **Dr. G. Sandhya Rani,** Principal, Pathfinder College of pharmacy throughout the research tenure.

My warm regard goes to **Mrs. K. Susmitha**, my co scholar and my best friend for being with me in all my hardships, motivating me and pushing me forward to achieve my dreams.

My special thanks to my colleagues and friends particularly, **Dr. Saritha**, **Mr. Ravi, Ms. Aparanjitha, Mrs. Sravanti, Mrs. Kalpana Devi, Mrs. Divya, and Mr. Ajay Kumar** for their timely suggestions and support throughout the journey and also for making my workplace joyful with their smiles and cooperation.

I cannot ignore to appreciate the silent contributions made by all of the non-teaching staff of UCPSc, KU, Warangal that directly or indirectly helped me to complete this research work.

I give my deepest gratitude and love with a deep sense of reverence, to my parents, **Uday Singh** & **Radha,** without them none of this would have been possible. They always believed that I could do it and gave me the liberty to choose whatever I desired. I cannot payback for their unconditional love and support. I thank my sisters& brothers-in-law **Prema Latha**& **Venkat**, **Swathi**& **Ramu** and **Shanthi Priya**& **Mohan Lal** for their selfless love and care. Special thanks to my brother-in-law, **Mohan Lal** for his dedicated efforts and contributions in pursuit of my PhD Degree. I thank my Uncle, **Bala Ram Naik,** the person who inspired me to reach where I am today. I extend my thanks to all of my family members for showing constant love and affection despite my ignorance through all these years.

My heart full regard goes to my husband, **Naveen Kumar** for standing beside me with continued and unfailing love, support and understanding which contributed a lot for the completion of my thesis. I earnestly value his contribution throughout my journey and am grateful for his belief in me.

(**AJMEERA DIVYA**)

LIST OF ABBREVIATIONS

kg	:	Kilogram
g	:	Gram
mg	:	Milligram
µg	:	Microogram
ng	:	Nanogram
l	:	Liter
ml	:	Milliliter
µl	:	Micrliter
mol	:	Mole
nm	:	Nanometer
rpm	:	Rotations per meter
h	:	Hour
min	:	Minute
sec	:	Second
%	:	Percentage
ºC	:	Degree Celsius
Conc.	:	Concentration
RT	:	Retention time
Cm	:	Centimeter
mm	:	Millimeter
mN	:	Millinewtons
mOsm	:	Milliosmole
HPLC	:	High performance liquid chromatography
UFLC	:	Ultrafast liquid chromatography
max	:	Maximum wavelength
AUC	:	Area under the curve
Cmax	:	Maximum concentration
Tmax	:	Time to reach maximum concentration
HLB	:	Hydrophilic-lipophilic balance
TEM	:	Transmission electron microscopy
mPas	:	Millipascal
mV	:	Millivolts
SD	:	Standard deviation
n	:	Number
R^2	:	Regression coefficient
w/v	:	Weight by volume
w/w	:	Weight by weight
v/v	:	Volume by volume
fig.	:	Figure
p	:	Probability

CONTENTS

LIST OF TABLES

LIST OF FIGURES

He performs wonders that cannot be fathomed, miracles that cannot be counted.

With all glory to God,

I dedicate this dissertation to my beloved family and friends.

1.1 NANOEMULSIONS

1.1.1 Introduction

Richard P. Feynman, a Nobelist honoree in 1959 first projected the concept of nano scale structures by saying “There is plenty of room at the bottom” (Feynman RP, 1960). This announced the Nanotechnology to the world. Nanotechnology, by name, scales up to one billionth of a meter. Usually, they are well-thought-out to be in the range of 10 nm to 1000nm (Santram L, et al. 2018; Patil PA, et al. 2016). As time progressed and due to advancements in science and technology, dosage forms have evolved from simple mixtures and tablets to highly developed systems called novel drug delivery systems and Nanoemulsions stands one of the promising novel drug delivery systems in the world of nano materials (Sanjeeb SK, et al. 2008).

Nanoemulsion also denoted as miniemulsion, ultrafine emulsion and the submicron emulsion is a heterogeneous system of liquid-in-liquid dispersions containing oil and water stabilized by surfactant and co-surfactant molecules with a droplet of the sub-micron size range of 5-200nm (Thakur N, et al. 2012; RiadQidra KE, 2015). It is kinetically and thermodynamically stable, optically clear and transparent (Ankur G, et al. 2016; Vivek PC and Dhaval S, 2017). Nanoemulsions can be categorized into three types based on the formulation components i.e. Oil in water (O/W); where oil phases are dispersed in continuous aqueous phases, Water in oil (W/O); where water phases are dispersed in the continuous oil phase and multiple emulsion (either O/W/O or W/O/W), multiphase system where an O/W or W/O emulsion is dispersed in oil or water respectively (Thakur N, et al. 2012). Nanoemulsions can be of neutral, anionic and cationic based on the charge over nano droplet surface. Nanoemulsions possess higher solubilization capacity compared to simple micellar solutions and are advantageous over other dispersions like emulsions and suspensions due to their thermodynamic stability leading to longer shelf life (Gupta PK, et al. 2009). Because of their small size, nanoemulsions have high surface area per unit volume, optical transparency, tunable rheology, even size distribution and robust stability without any apparent flocculation or coalescence during long-term storage. Their long-term stability, ease of preparation and high solubilization of drug molecules make them a promising drug delivery means (Azeem A, et al. 2009).

1.1.2 Preparation Methods

Mixing oil and aqueous phases forms a coarse emulsion in the presence of emulsifier that may change into nanoemulsion spontaneously or by applying high shear generally used to reduce the droplet size to the nanoscale. Nanoemulsion preparation requires the contribution of a large amount of either surfactants or energy and occasionally combination of both.

1.1.2.1 High energy methods

The high-energy methods include high-pressure homogenization, ultrasonication and micro fluidization that involve mechanical devices to generate strongly disruptive forces that can breakdown oil and water phases to nanosized droplets. Nanoemulsion production by High -pressure homogenization and micro fluidization can be used at a laboratory and industrial scale as well, whereas the ultrasonication is the method mainly used at the laboratory scale.

High-pressure homogenization

High-pressure homogenization is a highly efficient method used for producing nanoemulsions with extremely small droplet size as low as 1nm and desired polydispersity index utilizing several forces such as hydraulic shear, strong turbulence and cavitation. In this method, two liquids, aqueous phase and oil phase along with surfactant/ cosurfactants are allowed to pass through a small orifice at high pressure (500-5000psi) (Lovelyn C and Anthony AA, 2011; Setya S, et al. 2014). Firstly, the emulsion is formed in a large fraction of the dispersed phase and maybe diluted afterward. During emulsification by using the high phase volume ratios, the problem of coalescence may arise, however, this can be reduced by adding an excess amount of surfactant. Surfactant mixtures that show more reduction in surface tension over the sole components can be used (Setya S, et al. 2014). If possible, the surfactant is added in the disperse phase and then added to the continuous phase; this often leads to smaller droplets. Increasing the intensity in steps is also useful, particularly with nanoemulsions having a highly viscous disperse phase. The major drawback of this method is the generation of much heat that may lead to deterioration of emulsion components specifically those are thermolabile and can produce only oil-in-water type liquid nanoemulsion having less than 20 percent of oil component while

nanoemulsions of high viscosity with a mean droplet diameter less than 200nm cannot be prepared (Gurpreet and Signh SK, 2017).

Microfluidization

Microfluidization is a two-step process and depending on formulation leads to emulsions with droplet sizes smaller than 600 nm. Smaller droplet sizes may be obtained by using a dual-channel microfluidization method (RiadQidra KE, 2015). The aqueous phase and oily phase are combined to yield a coarse emulsion. The coarse emulsion is circulated repeatedly through a device called microfluidizer which utilizes a high-pressure positive displacement pump (500 - 20,000 psi), bypassing the rapidly flowing stream of the coarse emulsion through stainless steel microchannels to create strong dimensional flow until a stable nanoemulsion of desired particle size is obtained (Lovelyn C and Anthony AA, 2011; Setya S, et al. 2014). Then a uniform nanoemulsion is produced by removing the large droplets from the bulk emulsion under nitrogen.

Ultrasonication

This method is a feasible alternate to high-pressure homogenization and can produce kinetically stable nanoemulsions (Setya S, et al. 2014). A premixed emulsion is prepared and brought it in contact with a Probe sonicator agitating at an ultrasonic frequency of 20-40 kHz (de Oca-Avalos JM, et al. 20017). Ultra-sonic waves are responsible for the generation of mechanical vibration and cavitation that provides the necessary energy input for the droplet to break into nano droplets (Thakur N, et al. 2012).

1.1.2.2 Low energy methods

Stable nanoemulsion can be prepared by a low-energy emulsification method but they are only applicable to certain combinations of oils and emulsifiers. Low energy approaches have been recently developed based on the phase behavior and intrinsic properties of the emulsion constituents to promote the formation of ultra-small droplets including phase inversion composition (PIC), phase inversion temperature (PIT), and spontaneous emulsification. The main drawback of low-energy methods is that they often require high levels of synthetic surfactant.

Phase inversion composition (PIC)

In this method, an emulsion is formed when water is added to an oil-surfactant mixture. The greater affinity of surfactant towards water helps in the emulsification process, where a chemical energy is released by the conversion from positive to negative (obtaining water-in-oil nanoemulsions) or from negative to positive (obtaining oil-in-water nanoemulsions) due to curvature changes in surfactant molecules and is responsible the formation nano droplets (Setya S, et al. 2014; Manjit J, et al. 2015).

Phase inversion temperature (PIT)

This method is based on the temperature depended solubility of non-ionic surfactants. In this method, the emulsion components such as oil, surfactants and water are mixed at room temperature. At low temperatures, the headgroups of the non-ionic surfactants are highly hydrated (hydrophilic), which favors the formation of oil-in-water nanoemulsions and the surfactant monolayer exhibits positive curvature (Setya S, et al. 2014; Manjit J, et al. 2015). As temperature increases gradually, the surfactant headgroups become progressively dehydrated (lipophilic) and at higher temperatures, the surfactant gets completely solubilized in the oily phase and the initial o/w emulsion undergoes phase inversion favoring the formation of water-in-oil nanoemulsions (de Oca-Avalos JM, et al. 20017). Hence, the surfactant monolayer exhibits negative curvature. As this method involves heating mechanisms, it possibly will remain difficult to incorporate thermolabile drugs. However, the usage of a mixture of surfactants with appropriate features can be able to reduce the PIT of dispersion, minimizing the degradation of emulsion components (Gurpreet K and Singh SK, 2017).

Spontaneous emulsification

This method earned a great consideration from inventers of diverse fields including pharmaceutical sciences it generates nanoemulsions at room temperature without the use of any organic solvent and heat (Setya S, et al. 2014). Kinetically stable nanoemulsions with small droplet size as low as 50 nm can be produced by mixing the dispersed phase with a surfactant which is having a high affinity to the continuous phase and then adding this homogeneous mixture to the continuous phase

with mild stirring at a constant temperature (de Oca-Avalos JM, et al. 20017). The phase conversions during the emulsification process are responsible for the spontaneous nano emulsification and involve lamellar liquid crystalline phases. Nanoemulsions obtained from the spontaneous nano emulsification process are not thermodynamically stable, although they might have high kinetic energy and long term colloidal stability (Thakur N, et al. 2012; Manjit J, et al. 2015).

1.1.2.3 Miscellaneous

Solvent displacement method in which both, low and high energy methods can be improvised by dissolving the oil phase in an organic solvent and a later step involving evaporation. This kind of approach is particularly effective for protein stabilized emulsions.

Solvent displacement method

This method produces nanoemulsions at room temperature with simple stirring. Henceforth, researchers in pharmaceutical sciences are using this technique for producing nanoemulsions (RiadQidra KE, 2015). Water-miscible organic solvents like acetone are used to prepare the organic phase and this is transferred to an aqueous phase holding surfactant to produce nanoemulsion by diffusion of organic solvent which is removed from the nanoemulsion by proper way, such as vacuum evaporation (Setya S, et al. 2014; RiadQidra KE, 2015). The main drawback of this method is the need of the high solvent to oil ratio to prepare small-sized droplets that require supplementary energy for removal of the solvent (Setya S, et al. 2014).

Bubble bursting at liquid/air interface and evaporative ripening are the emerging techniques of nanoemulsion production. However, the physics involved in the formation of nanoemulsion is more based on the observation or experience rather than theory or pure logic and rational scale-up procedure need to be explored (Lovelyn C and Anthony AA, 2011).

1.1.3 Applications of nanoemulsions

Nanoemulsions as suitable vehicles are finding applications in diverse areas such as food, cosmetics, pharmaceuticals, and material synthesis and gene therapy. Coming to the drug delivery aspects, nanoemulsions have already proved as ideal drug delivery systems at the research level also few products available in the market.

1.1.3.1 Cosmetics

Nanoemulsions gained rising interest as good vehicles for cosmetics mostly used as moisturizers and creams due to its lipophilic interior with small-sized droplets that transports the lipophilic active in a controlled and effective manner supporting the skin penetration and upsurging their concentration in the skin (Ankur G, et al. 2016). Nanoemulsion is beneficial as its components hold their bioactive properties like strengthening the skin barrier function by reducing the trans-epidermal water loss and creating a smooth skin feel (Patel PR and Joshi JR, 2012). Moreover, its ability to overcome the stability problems associated with microemulsions like creaming, sedimentation, flocculation or coalescence made nanoemulsion suitable for the cosmetic world (Shah P, et al. 2010).

1.1.3.2 Anti-Microbial Agent

Antimicrobial nanoemulsions are O/W systems of droplet size ranging 200-600nmwhich has a broad-spectrum activity against bacteria, fungi, enveloped viruses (e.g. HIV) and spores. When nanoemulsion comes in contact with lipid-containing organisms, there occurs an electrostatic attraction between the cationic charge of emulsion and anionic charge on the pathogen (Haritha, et al. 2012; Patil PA, et al. 2016). When enough nanoparticles fused with the pathogen release part of the energy trapped within the emulsion. Both active ingredients along with this released energy destabilize the Pathogen lipid membrane ensuing the cell lysis and death. Moreover, the topical anti-microbial activity of nanoemulsion is selective towards microbes without damaging healthy cells thus possess unique safety levels (Thakur N, et al. 2012; Shah P, et al. 2010).

1.1.3.3 Cancer therapy

Vast research was made in this area of cancer therapy using nanoemulsion strategy. Nanoemulsions achieve targeting effect with prolonged drug delivery and innovations were made periodically resulted in different advanced therapies for the treatment using various anti-cancer drugs as well as diagnostic (magnetic nanoemulsions) purposes (Shah P, et al. 2010).

1.1.3.4 Bio-terrorism attack

Nanoemulsions have proven to prevent diseases that are prone through bioterrorism attacks by harmful pathogens like anthrax, gangrene, clostridium, Ebola and to treat the contaminated wounds (Patil PA, et al. 2016; Vivek PC and Dhaval S, 2017). Nanoemulsions are more advantageous over conventional preparations due to the liberty to formulate into a wide variety of dosage forms like cream, foam, liquid, spray, etc. Hence nanoemulsions are evolved as ideal systems in this area (Shah P, et al. 2010; Lovelyn C and Anthony AA, 2011).

1.1.3.5 Mucosal Vaccines

The nanoemulsion can be used as an effective vaccine delivery system to transport either recombinant proteins are incapacitated organisms such as attenuated viruses to a mucosal surface to yield an immune response (Patil PA, et al. 2016). The nanoemulsion causes applied proteins to be adjuvant and help uptake by the antigen-presenting cells resulting in the production of specific IgG and IgA antibody due to which, systemic and mucosal immune response develops.The first applications, an influenza vaccine and HIV vaccine are under clinical trials which can soon become an innovative non-invasive immunization model (Shah P, et al. 2010; Thakur N, et al. 2012).

1.1.3.6 Cell culture technology

To improve the cell growth in cell culture technics, the culture medium can be supplemented with enriched molecules or blood serum. It is very difficult to provide the media with a large number of such oil-soluble substances made available to the culture cells (Thakur N, et al. 2012; Patil PA, et al. 2016). However, increased uptake of oil-soluble components in cell culture can be possible with nanoemulsions

stabilized by phospholipids that improve the uptake of oil-soluble substances through fine oil droplets of nanoemulsions leading to improved culture cell growth (Shah P, et al. 2010; Patil PA, et al. 2016).

1.1.3.7 Oral Drug Delivery System

Nanoemulsions are widely used to improve the oral bioavailability of poorly soluble drugs, offer several benefits over conventional oral formulations. As it can dissolve a large amount of hydrophobics and it is further stabilized by a surfactant molecule, the dose can be reduced (Thakur N, et al. 2012; Patil PA, et al. 2016; hence dose-dependent toxicity is omitted. Moreover, Pharmaceutical drugs of peptides and proteins can be protected from enzyme degradation in GIT by incorporating into the oil matrix (Raj Kumar M, et al. 2014).

1.1.3.8 Transdermal Drug Delivery System

Transdermal drug delivery is an interesting area of research in drug delivery. Many pharmaceutical drugs show low skin permeation, which fallouts in poor efficacy (Thakur N, et al. 2012). A solvent-free topical vehicle is more efficacious in terms of percutaneous absorption with possibly devoid of adverse effects (Raj Kumar M, et al. 2014). Nanoemulsion enhances the therapeutic efficacy and also the bioavailability of the drugs due to increased permeation through the skin without any adverse effects as it is non-irritant (Gupta PK, et al. 2010; Patel PR and Joshi JR, 2012).

1.1.3.9 Intranasal drug delivery

Because of the impervious nature of the endothelium, targeting drugs to the brain has become problematic, especially in the case of hydrophilic drugs and those with high molecular weight (Patil PA, et al. 2016). As the olfactory region of the nasal mucosa offers a direct connection between the nose and brain, intranasal drug delivery appears to be a fortunate way to overcome the difficulties for the direct access of drugs to the target site. Intranasal drug delivery, a non-invasive and well-tolerated system considered as a reliable route for the administration of drugs due to low enzymatic activity, high availability of immunoreactive sites and its moderately permeable epithelium (Haritha, et al. 2012; Patil PA, et al. 2016). The intranasal drug delivery of nanoemulsions can leads to the targeting of drugs to the brain significant

with results in the treatment of central nervous system disorders (Lovelyn C and Anthony AA, 2011).

1.1.3.10 Pulmonary Drug Delivery

Till today, the nanoemulsion system has not yet been fully exploited for pulmonary drug delivery and very limited literature available in this area. The cationic submicron emulsions were studied by Bivas-Benita, et al. 2004 and reported them as promising carriers for lung delivery of DNA vaccines due to their ability to transfect pulmonary epithelial cells, which perhaps induce cross-priming of antigen-presenting cells and directly activate dendritic cells that lead to antigen-specific T-cells stimulation (Lovelyn C and Anthony AA, 2011).

1.1.3.11 Parenteral Drug Delivery

Nanoemulsions are capable of dissolving the maximum quantity of hydrophobic drugs and able to protect drugs from hydrolysis and enzymatic degradation. This made nanoemulsions the ideal carriers for parenteral delivery (Gupta PK, et al. 2010). As the emulsions release the drugs in a sustained and controlled manner for a longer duration, the dose of the drug and frequency of administration are significantly reduced during the treatment period (Patel PR and Joshi JR, 2012).

1.1.3.12 Ocular and otic drug delivery

The main downside related to the systemic administration of ocular and otic drugs is that only an insignificant portion of the administered drug reaches the target. Ocular and otic drug delivery can be reformed into nano-sized systems that can overcome drawbacks of conventional dosage forms (Vivek PC and Dhaval S, 2017). Nanoemulsions that are stable upon dilution remain effective drug delivery systems for ophthalmic use due to their several advantages including sustained drug release and high ability to improve the drug permeation all the way through the ocular barriers leading to increased therapeutic levels of drug into the deeper layers of the ocular structure and the aqueous humor (Haritha, et al. 2012; Raj Kumar M, et al. 2014). At present, there is a lack of research results dealing with the targeting of ocular or otic drugs to certain cellular targets in the organs. But then again, it is possible to develop nanocarriers for aiming drug to appropriate cells in the ocular and

otic fields with the evidence of the pathophysiological and/or passive targeting approaches used in cancer chemotherapy (Patil PA, et al. 2016; Patel PR and Joshi JR, 2012).

1.1.3.13 Phytopharmaceuticals

Significant consideration has been focused in recent years on the formulation of novel drug delivery systems for herbal drugs. Plant bioactive loose their stability in highly acidic pH and are subjected to pre systemic metabolism. That may lead to drug levels below the therapeutic concentration in the blood. However, encapsulation of plant bioactive would minimize their pre systemic degradation and eliminate serious side effects associated with accumulation in the non-targeted areas. Nanoemulsions are one of the ideal strategies for delivering phytopharmaceuticals to the targeted tissues (Patil PA, et al. 2016).

1.2 MOST COMMON EYE DISEASES

Macular Degeneration, Cataracts, Glaucoma, Diabetic Retinopathy, Dry Eyes Syndrome, Conjunctivitis (Pinkeye), Retinal Detachment, Uveitis, Night Blindness (Nyctalopia), Color Blindness, Nearsightedness (Myopia), Farsightedness (Hypermetropia), Astigmatism, Presbyopia, Proptosis, Macular Edema are the most common eye problems known (Usman M, 2019).

1.2.1 Macular Degeneration

Macular Degeneration, frequently referred as age-related macular degeneration (AMD), is an incurable eye disease with its two basic types, i.e. 'dry AMD' and 'wet AMD' involving in the damage to the macula, the central portion of the retina that helps us read, recognize colors and faces, and get a detailed image of an object (Valencia H, 2016).

Signs and Symptoms of Macular Degeneration

In the earlier stages of macular degeneration, there are no definite signs and symptoms exist rather gradual or sudden change in the quality of vision occurs. However, the later stages include the symptoms like vision blurriness, formation of blind spots, difficulty seeing in dim light, objects appear smaller than their actual size, etc., (Andrew AD, 2019).

Treatment Options

There is no single cure that able to treat macular degeneration fully. However, there are some medications like vascular endothelial growth factor inhibitors that help prevent the development of abnormal blood vessels in the eye and are generally administered by injecting into the eye cavity. Example: Lucentis (ranibizumab) and Avastin (bevacizumab). Laser therapy is another option to destroy the abnormal blood vessels (Usman M, 2019; AMDF, 2019).

1.2.2 Cataracts

Cataracts are another form of the most widely existing eye problem associated with the development of cloudy areas in the lens interfering with the passage of light through the retina and hence the difficulty in the vision (NEI, 2019; Mayo Clinic, 2018).

Signs and Symptoms of Cataracts

The symptoms are lacking as the cataract formation is a slow process. However, some of the symptoms include blurred vision, problem seeing at night, problem seeing through light, seeing 'halos' around lights, faded view of colors, etc (Brazier Y, 2017 ; Bailey G, 2019).

Treatment Options

At early stages, antiglare sunglasses and magnifying lenses can help. However, removing and replacing the cloudy lens with an artificial one by doing the surgery is the only effective treatment (Whitney S, 2019; Usman M, 2019).

1.2.3 Glaucoma

Glaucoma is a group of optic neuropathies that are developed by elevated intraocular pressure in the eye that results in progressive degeneration of retinal ganglion cells. The increased pressure results in a cupping of the optic nerve and may cause vision loss (Usman M, 2018).

Glaucoma is of two basic types and were explained below.

1.2.3.1 Open-angle Glaucoma

Open-angle Glaucoma which is frequently referred to as 'wide-angle glaucoma' is the most common form of Glaucoma and usually painless. In open-angle glaucoma, the trabecular meshwork looks normal with the iridocorneal angle open as wide as it should be, however, over time, the eye's drainage canals become clogged, causing the flow of fluid flow restricted and an increase in intraocular pressure and following damage to the optic nerve (Kimberly H, 2016).

Signs and Symptoms of Open-Angle Glaucoma

No specific symptom initially rather, the rise in intraocular pressure will be the only indication and in the later stages the time vision is affected with symptoms like tunnel vision, peripheral vision loss and gradually the damage gets permanent (Kozarsky A, 2019).

1.2.3.3 Angle-closure Glaucoma

Angle-closure glaucoma, which is frequently referred to as acute or chronic angle-closure or narrow-angle glaucoma the iridocorneal angle gets too narrow and the normal flow of aqueous humour between the iris and lens is blocked thus build an extreme pressure in the eye which is a medical emergency and needs immediate treatment. If left untreated, that can result in permanent blindness in a day or two (Kimberly H, 2016).

Signs and Symptoms of Angle-Closure Glaucoma

Angle-closure glaucoma often occurs suddenly and is associated with symptoms like severe pain in eyes together with nausea and vomiting most of the time, unexpected vision interruption in dim light, halos in light, blurred vision, redness of the eyes, etc (Kozarsky A, 2019).

Treatment Options

The vision loss by glaucoma is irreversible, hence the treatment is helpful only before the complete vision loss and it includes medical management using from eye drops to pills, traditional and laser surgery, or a combination both in certain conditions (Andrew AD, 2016).

1.2.4 Diabetic Retinopathy

Diabetic retinopathy is an eye condition due to diabetes complication, in which eyes get affected by the damage to the blood vessels in the back of the eye i.e., the light-sensitive tissues of the retina that can develop in both type 1 or type 2 diabetes, especially for those having diabetes with fluctuating blood sugar levels for a long time (Michael D, 2019).

Signs and Symptoms of Diabetic Retinopathy

The early stages might not have any detectable symptoms but, eventually, in later stages, the symptoms like dark spots or floaters, difficulty color identification, blurred vision and finally loss of vision might appear (NEI, 2019; Usman M, 2019).

Treatment Options

There is no cure for the retinopathy in advanced stages whereas photocoagulation, a kind of laser treatment and vitrectomy, a surgical procedure that removes the vitreous gel helps in preventing vision loss if treated before the retina get damaged severely (Adam F, 2017).

1.2.5 Dry Eyes Syndrome

Dry eye syndrome is a condition in which tears fail to provide adequate lubrication due to various reasons like defects in tear production or poor quality of tears etc (Chitra B, 2016).

Signs and Symptoms of Dry Eyes Syndrome

One can experience a burning sensation in eyes, redness of eyes, sensitivity to light, eye fatigue and issues in wearing contact lenses, etc. in dry eyes syndrome condition (Richard A, 2019)

Treatment Options

Different treatment options are available depending upon the cause of the eye problem and include over-the-counter topical medications like artificial tears, gels and ointments to treat mild dry eyes, prescription dry eye medications like Lifitegrast and Cyclosporine; Corticosteroid eye drops to treat eye inflammation and Pilocarpine, an

alternative cholinergic, devices that stimulate the nerves and glands of tear production for temporary relief from dry eyes, surgical options involving the insertion of silicone-based punctal plugs to plug the tear ducts to detect tear drainage from the eye and the changes in lifestyle like avoiding exposures to warm conditions and light, screens, etc can help to some extent (Usman M, 2019; Chitra B, 2016).

1.2.6 Conjunctivitis

Conjunctivitis, usually known as pinkeye is one of the most commonly existing eye problems that involve the inflammation of the conjunctival tissue and is highly contagious but rarely serious when identified and treated early (Michael SO, 2020).

Signs and Symptoms of Conjunctivitis

The signs and symptoms of conjunctivitis include red, blurry excessive tearing itching and burning eyes with thick yellowish discharge covering whole eyelashes and swelling in the conjunctiva (Mary L, 2017; Michael SO, 2020).

Treatment Options

The treatment options vary depending upon the cause of conjunctivitis.

In allergic conjunctivitis with mild intensity, artificial tears are sufficient, in case of severe allergic conjunctivitis, antihistamines or non-steroidal anti-inflammatory drugs may be and in case of persistent allergic conjunctivitis, topical steroid eye drops may be prescribed (NHS inform, 2020). Antibiotic eye drops or ointments for example, chloramphenicol or fusidic acid are prescribed for bacterial conjunctivitis that requires a treatment course of 3 to 4 days for recovery (Gregersen PR, 2019). Viral conjunctivitis, similar to the common cold, cannot be treated with antibiotic drops or ointments until the completion of the virus course for 2-3 weeks whereas the chemical conjunctivitis is treated with saline water to thoroughly flush the eyes and in some cases using topical steroids (Usman M, 2019).

1.2.7 Retinal Detachment

Retinal detachment is the condition where a small area of the retina being torn and eventually the retina is separated from its underlying tissues (Usman M, 2019).

Retinal detachment can be of Rhegmatogenous, a tiny break in the retina causes the fluid inflow underneath the retina, that eventually separates the retina from its pigment epithelium involved in the nourishment of the retina, Tractional, the retina detaches from its pigment epithelium due to the scar tissue contraction on the retinal surface, and or Exudative, due to eye injury, trauma or other retinal diseases in which the retina suffers no tears or breaks despite the fluid leaking underneath it (NEI, 2019).

Signs and Symptoms of Retinal Detachment

The retinal detachment is not associated with pain, but it always shows certain signs like floaters in the affected eye, appearance of light flashes in one or both eyes, blurred vision, progressively recedes the side vision and the feel of a shadow through the field of vision (NEI, 2019).

Treatment Options

To repair a detached retina, most of the time various surgeries are done based on the condition.

Photocoagulation is the procedure in which the laser is used to burn around the tear site to make scar that affixes the retina to the back of the eye (Erickson AG, 2017).

Retinopexy, where a gas bubble is used to move retina back into place and a laser or freezing probe is used to seal the holes once the retina is back in place (Erickson AG, 2017).

Cryopexy, a kind of laser surgery is used to treat small holes and breaks where tiny burns are made around the damaged area to patch up the retina back to original and then freeze the patch up area to reattach the retina (Usman M, 2019).

Scleral buckling is the procedure in which a band is placed around the outside of the eye to push the wall of the eye into the retina getting it back into place followed by Retinopexy and Cryopexy (Erickson AG, 2017).

Vitrectomy, which involves the vitreous humor removal using a specialized instrument to make a tiny incision in the sclera, and gas is injected into the eye to push the retina back to its normal position (Usman M, 2019).

1.2.8 Uveitis

Uveitis is a group of eye diseases causing inflammation in the uvea that may result in eye tissue damage leading to the loss of eyesight (Cafasso J, 2017).

Signs and Symptoms of Uveitis

The main symptoms include blurred vision, redness and pain in the eye, and sensitivity to light. One affected with immune system disorders like AIDS, rheumatoid arthritis, ulcerative colitis, etc. is at a high risk to get Uveitis (Mayo Clinic, 2018).

Treatment Options

The prime objective of the uveitis treatment is to reduce the inflammation and depends on the basic cause and the affected area of the eye.

Medication includes anti-inflammatory drugs in eye drops, such as corticosteroids, a corticosteroid pill or injection if eye drops are unable to reduce the inflammation, antibiotics or antiviral medications to control the infection, with or without corticosteroids if the cause is an infection and finally immunosuppressive and cytotoxic drugs are used to treat uveitis if it does not respond to the corticosteroids (NEI, 2019; Usman M, 2019).

Vitrectomy, a surgical procedure is the only choice for removing the excess vitreous from the eye and the posterior uveitis, a very difficult to treat condition is treated by implanting a device that in the eye for slow release of corticosteroids for about two to three years (Usman M, 2019).

1.2.9 Nyctalopia

Nyctalopia or night blindness is a kind of vision impairment at night or in other dim light environments. Night blindness is not considered as a disease rather a

symptom of some other eye problem like nearsightedness (The Editors of Encyclopaedia Britannica, 2018).

Signs and Symptoms of Night Blindness

The only symptom is increased difficulty seeing things in the dark. However, based on the cause, the symptoms like eye pain, blurry vision, sensitivity to light, headaches, nausea and vomiting may also occur (Aaron K, 2018).

Treatment Options

Nyctalopia due to cataracts, nearsightedness or deficiency of vitamin A is treated with eyeglasses or contact lenses (Alan K, 2018).

1.2.10 Color Blindness

Color blindness or color deficiency a genetic condition occurs when there is a problem in the pigments of eye cones and the one with color deficiency is unable to differentiate colors includes red-green colorblindness and blue-yellow color blindness. Achromatopsia, a rare case and the worst form of color blindness, where, the cones all color pigments and hence can't see any color at all (Usman M, 2018).

Signs and Symptoms of Color Blindness

The Signs and Symptoms of color blindness include difficulty to differentiate two colors or different shades of a single colo (Whitney S, 2019).

Treatment Options

Still, there is a lack of reliable cure to color blindness; however, glasses and contact lenses are available to assist color deficiencies (Gretchyn B, 2019).

1.2.11 Myopia

Myopia or nearsightedness is an eye condition where images are focused in front of the retina rather than focusing on the retina that occurs due to irregular refraction (Smith A, 2020).

Signs and Symptoms of Nearsightedness

Major symptoms include blurred vision while looking at distant objects, headaches due to eyestrain and difficulty seeing objects while driving, mostly at night (Usman M, 2018).

Treatment Options

Corrective glasses, contact lenses or refractive surgery depending upon the intensity of myopia (Whitney S, 2020).

1.2.12 Hypermetropia

Hypermetropia or farsightedness is an eye condition where problem focusing a clear vision forms when looking at distanced objects while getting a blurred vision of near objects (Mary L, 2016; Usman M, 2018).

Signs and Symptoms of Farsightedness

The primary symptom of farsightedness is the difficulty with headaches in focusing near objects objects (Mary L, 2016; Usman M, 2018).

Treatment Options

Corrective glasses, contact lenses and surgery including PRK- Photorefractive Keratectomy, LASIK- Laser in Situ Keratomileusis, LASEK -Laser epithelial keratomileusis and Refractive lens exchange are the regular ways of treating hypermetropia (Mary L, 2016; Usman M, 2018).

1.2.13 Astigmatism

Astigmatism or keratoconus is an eye disorder in which the abnormally curved cornea makes the vision to get out of focus (Whitney S, 2019).

Signs and Symptoms of Astigmatism

Some of the symptoms of astigmatism include eye irritation, blurred vision, difficulty in seeing things at night, headaches due to eyestrain (Whitney S, 2019; Usman M, 2018).

Treatment Options

Corrective eyeglasses, contact lenses, laser surgery as well as other refractive surgery procedures including LASIK and photorefractive keratectomy and radial keratotomy are used to treat astigmatism (Kivi R and Elizabeth B, 2017; Usman M, 2018).

1.2.14 Presbyopia

Presbyopia, an eye disease characterized by the lack of ability to focus on nearby objects due to gradual loss of vision generally associated with aging (Brian Wachler SB, 2018).

Signs and Symptoms of Presbyopia

Presbyopia progresses slowly, after 40 years to 65 years of age and the symptoms are blurred vision and losing the ability to read from normal distance and eyestrain accompanied by headaches (Gretchyn B, 2019).

Treatment Options

Corrective eyeglasses, contact lenses, refractive surgery are the treatments that aim at assisting the eyes to focus on nearby objects (Brian Wachler SB, 2018).

1.2.15 Proptosis

Proptosis or exophthalmos, a phenomenon of eyeball protrusion caused either naturally due to anatomical defects or by various health conditions like thyroid and Graves' disease (Troy B, 2020; Christopher JB, 2019).

Signs and Symptoms of Proptosis

The symptoms include eye irritation and pain, blurred vision, sensitivity to light, lacrimation, and Diplopia, a condition where double vision is formed due to eye muscle weakness (Usman M, 2018; Christopher JB, 2019).

Treatment Options

Most of the time, the lubrication of cornea is suggested followed by eye surgery to improve the coverage of the exposed eye surface and to remove tumors if

any (Usman M, 2018; Christopher JB, 2019). Systemic corticosteroids for example, prednisone 1mg/kg oral dose for 1 week are prescribed for orbital congestion due to inflammatory orbital pseudotumor or thyroid eye disease (Christopher JB, 2019). FDA has approved Tepezza as the first drug for treating thyroid eye disease in January 2020 (Troy B, 2020).

1.2.16 Macular Edema

Macular edema is the swelling and thickening of the macula due to the unwanted fluid buildup inside the macula that result in distorted vision. Any eye disease that damages the retinal blood vessels may cause macular edema; most common is the diabetic retinopathy.

Signs and Symptoms of Macular Edema

The symptoms of macular edema vary from a little blurred vision in or around the central field of vision to considerable vision loss. If the disease attacks a single eye, then the person can notice the blurriness only when the condition gets into an advanced stage.

Treatment Options

The treatment options vary with the basic cause and condition of the disease Non-steroidal anti-inflammatory (NSAID) eye-drops serves best for treating cystoid macular edema, a condition after cataract surgery for example, Prednisone (NaturalEyeCare, 2020). Steroids in the form of pills, eye-drops or injections are prescribed when inflammation causes macular edema (Usman M, 2018). However, FDA has approved three sustained-release implants of corticosteroids namely Ozurdex for dexamethasone; Retisert and Iluvien for fluocinolone acetonide (NEI, 2019; NaturalEyeCare, 2020). The leaking blood vessels can be sealed and vision can be stabilized by laser surgery. Anti- vascular endothelial growth factor (VEGF) drugs can control the growth of abnormal blood vessels in the retina and help prevent unwanted fluid buildup for example, Avastin, Eylea, Lucentis and Ozurdex through intravitreal injection (IVI) of Bevacizumab, aflibercept, Ranibizumab, and dexamethacone respectively Vitrectomy is used to remove the vitreous humour when the vitreous pulling is the reason for the macular edema (NEI, 2019; ASRS, 2016).

1.3. HUMAN EYE ANATOMY

1.3.1 Introduction

The human eye is the most amazing sensory as well as the principal organ of vision. The adult eyeball is more or less spherical with a diameter of 24mm anteroposteriorly, an average axial length of 23.5mm (Kristina I and David LG, 2009; Carolyn S, 2012). The horizontal section of human eye was illustrated in fig.1.

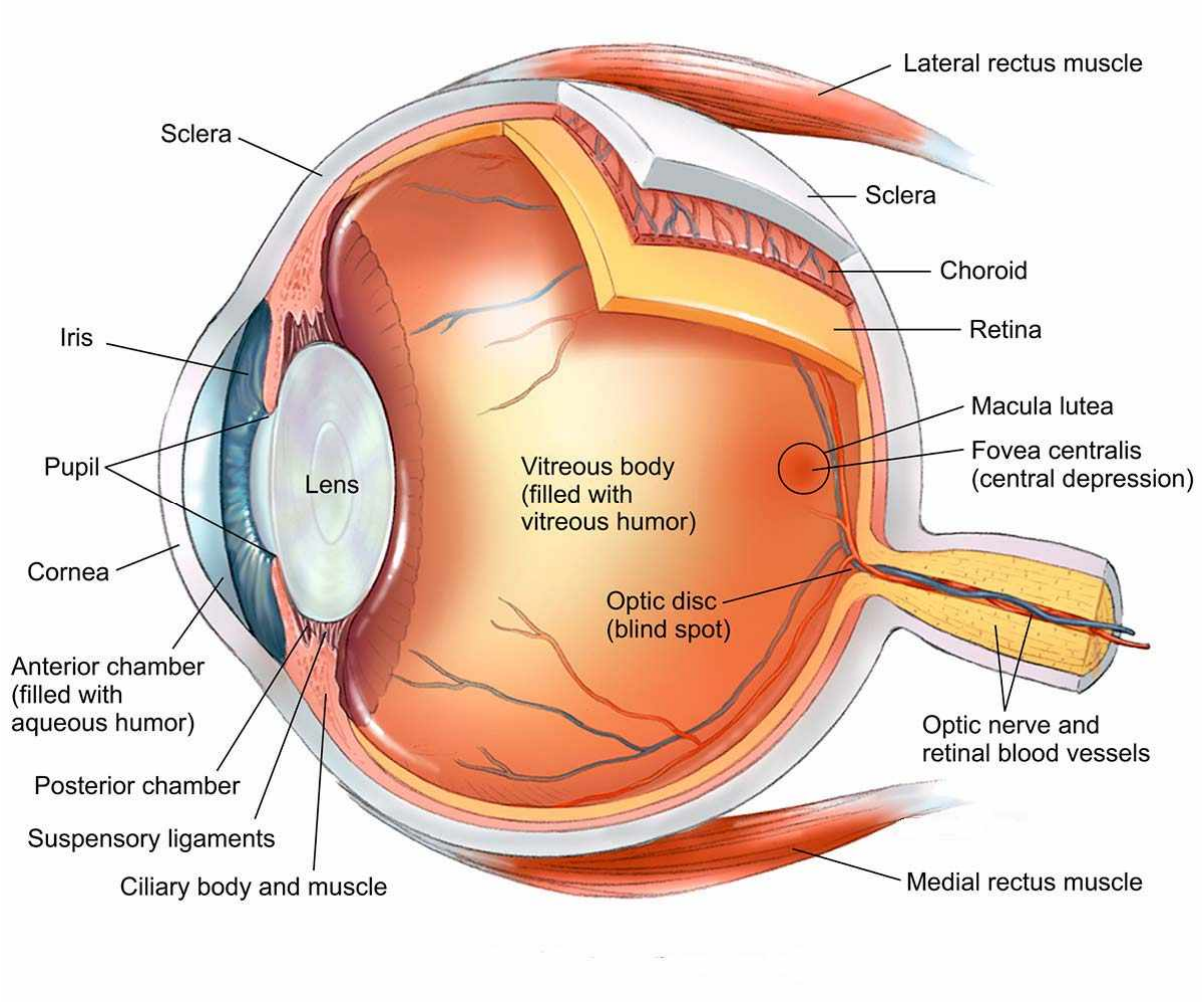


Fig.1: Human eye anatomy- horizontal section [www.Carlson-Art.com]

1.3.2 Structure and Function

From a physiological point of view, the eye can be divided into two segments, an anterior segment consisting of the cornea, the conjunctiva, the ciliary body, the iris, aqueous humour, lens, and lachrymal system, and a posterior segment composed of the retina, the vitreous humour, the choroid and the sclera.

1.3.2.1 Conjunctiva

The conjunctiva is a highly vascularized clear thin fibrous membrane that covers the sclera and inner surface of the eyelids forming an inferior and superior conjunctival sac (Wu Y, et al. 2019).

The three portions, the bulbar conjunctiva, which covers the sclera, the palpebral conjunctiva, which covers the backside of the eyelid, and the fornix, where both conjunctivae meet collectively form the conjunctiva (Carolyn S, 2012).

The conjunctiva acts as a protective barrier on the ocular surface due to the presence of the tight junctions on the apical surface of its cells and it protects the eyes by secreting mucus that contributes to the lubrication and antimicrobial peptides that prevent the entry of microorganisms. In most places, it is loosely attached and thereby permits free movement of the eyeball (Krishna MB and Lorenzetti OJ, 1993).

1.3.2.2 The lachrymal apparatus

The lachrymal apparatus is the physiologic system containing the orbital structures for tear production and drainage. It consists of the lachrymal gland, which secretes the tears that form a precorneal tear film that covers and lubricates the conjunctival and corneal surfaces (Achouri D, et al. 2012).

The tear film is composed of a thin outer lipid layer, a thicker middle layer and a thin inner mucous layer. Tears are drained by the lacrimal canaliculi which lead into the upper part of the nasolacrimal duct. The act of blinking exerts a suction-pressure-pump action in removing the tears from the eyes into the nasal cavity. The precorneal tear film maintains the integrity of the cornea to keep the surface of the eyeball wet for comfort and normal functioning (Krishna MB and Lorenzetti OJ, 1993). The lachrymal apparatus anatomy was illustrated in fig.2. It washes away irritating material, e.g. dust and grit as it contains protective substances.

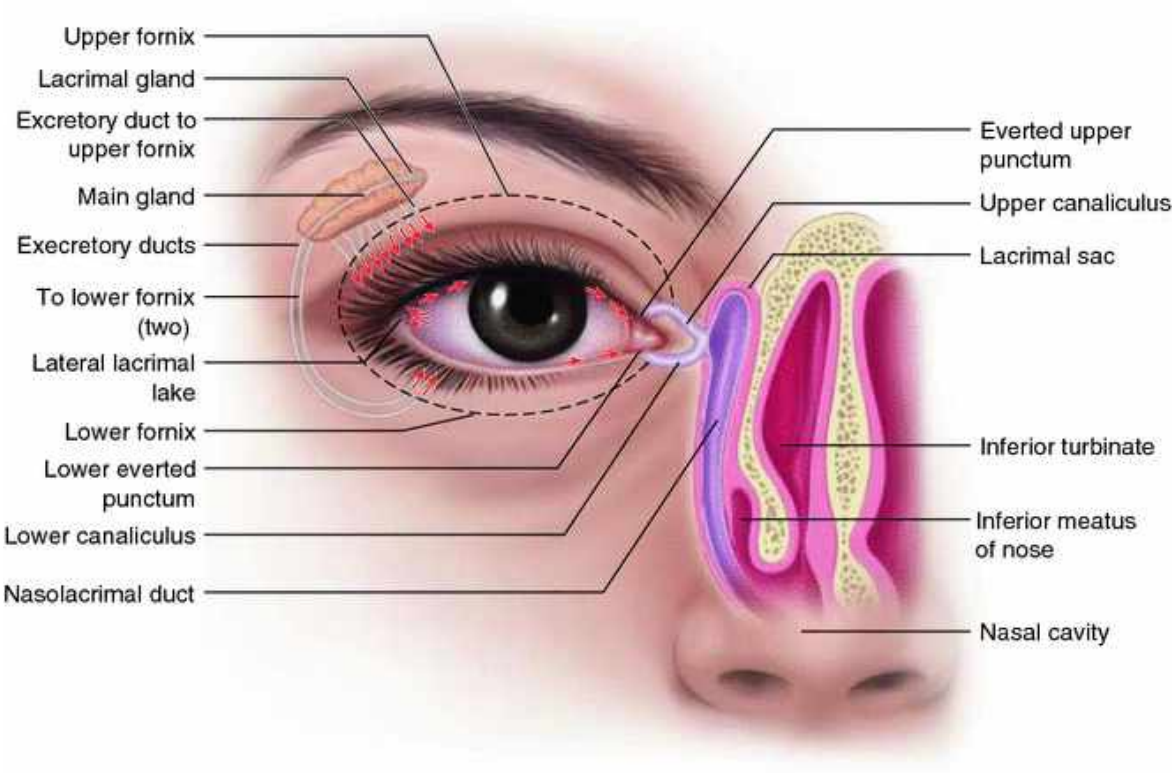


Fig.2: Lacrimal apparatus anatomy

[Ansari M.W., Nadeem A. (2016) The Lacrimal Apparatus. In: Atlas of Ocular Anatomy. Springer, Cham]

Anatomically, the eyeball consists of three layers: the fibrous, vascular, and nerve layers, and additionally, lens and the ocular media. Following is a description of these layers and their functions.

1.3.2.3 Fibrous layer-outer coat

The outermost layer of the eyeball is a fibrous layer which is also known as the protective layer. This protective layer consists of two important components one is the sclera, the white of the eyeball and the other one is transparent, avascular cornea.

Sclera

The sclera is the whitish portion of the eye, avascular, opaque and elastic, made of highly resistant fibrous tissue that occupies the five-sixths of the eyeball. These fibers are arranged in a random order, creating irregular bundles causing the sclera to appear white (Ameeduzzafar, et al. 2016). The scleral dimensions include 0.6mm at equator and 0.3mm at the insertion point of the rectus muscle and the overall thickness approximates to 1mm (Krishna MB and Lorenzetti OJ, 1993). The sclera maintains the intraocular pressure and serves as the attachment portion for the

extraocular muscles (Wu Y, et al. 2019). The blood supply to the sclera is both from anterior ciliary arteries as well as from the long and short posterior ciliary arteries.

Cornea

The cornea is a very transparent and avascular fibrous layer that occupies one-sixth of the anterior part of the eyeball. It is convex with a diameter of 12mm horizontally and 10-11mm vertically. The corneal thickness is 0.5mm at the middle and 1mm at the edge (Ameeduzzafar, et al. 2016; Carolyn S, 2012). The corneal fibres are similar to scleral fibres but are arranged in a more orderly parallel pattern.

Physically, the cornea is made up of five major layers. The corneal epithelium forms the outermost layer and the supplementary layers comprise Bowman's membrane, stroma, Descemet's membrane and the endothelium layer (Wu Y, et al. 2019).

a. Corneal epithelium

The corneal epithelium is made of stratified squamous kind of epithelial cells of five to six layers in a systematic arrangement and contains the mainstream of nerve endings, making it sensible to touch and pain. It consists of surface microvilli to hold the precorneal tear film and flatter the corneal surface. It is hydrophobic not allowing fluid to permit through, which aids uphold the transparency (Ameeduzzafar, et al. 2016). These five to six layers of epithelial cells are capable of reinforcing for every seven days allowing the epithelium to restore itself after trauma. The epithelium is braced to a basement membrane, which is impervious to water-soluble composites. It is lipid-soluble, which is a significant factor in the manufacturing of eye drops, confirming the possibility of absorption (Hugo A, et al. 2013).

b. Bowman's layer

The Bowman's layer is a homogeneous sheet of modified stroma present immediately to the basement membrane consisting of clear, collagen fibrils, randomly oriented and surrounded by mucoprotein as a ground substance. It aids the cornea to retain its form and makes a smooth surface. It prevents the entry of the fluid and micro-organisms into the stroma. Once destroyed, this layer cannot be regenerated (Achouri D, et al. 2012).

c. Stroma

The stroma is owed to the hydrophilic nature consists of lamellae of collagen, cells and pounded substance comprises approximately 90% of entire corneal thickness with one-third of the corneal cells in the form of keratocytes (Wu Y, et al. 2019). Corneal transparency is maintained with its compact layer of collagen fibrils running analogous to the outward. Numerous pores in the inner structure allow the corneal nerves to enter from the stroma into the epithelium. Swelling and stretching of these nerves result in severe pain (Krishna MB and Lorenzetti OJ, 1993).

d. Descemet's membrane

Descemet's membrane is the subsequent layer of the stroma and denotes the basement membrane of the endothelium. It consists of collagen and glycoproteins secreted by the endothelium. This is a hard, robust layer impervious to chemicals and trauma, but it can be restored if injured (Carolyn S, 2012).

e. Endothelium

The endothelium is a single layer of plane, polygonal cells with tight junctions and the most posterior layer of the cornea. Being a permeable membrane, it brings in oxygen through aqueous while fluid and waste products transportation. The number of endothelial cells begins to decrease with age. Endothelial cells cannot regenerate but involve the concealment of lost cells (Carolyn S, 2012).

1.3.2.4 Vascular layer – middle coat

Inside the outer coat, there is a highly vascular layer also known as the uveal tract mainly composed of cribriform tissue which brings nutrition and is situated between the sclera and retina. The vascular layer consists of one posterior part called the choroid. As moving from the forward, a part of the middle layer becomes thickened and this thickened triangular part is called the ciliary body. In the middle of the ciliary body, there is another vascular diaphragm with a central aperture (pupil) is called iris.

Choroid

The choroid a huge, pigmented and vascular tissue that forms the middle coat of the eye's posterior part (Kristina I and David LG, 2009). It lies in between retina and sclera, primarily composed of a dense capillary plexus, as well as small arteries and veins. The two types of choroidal blood vessels that are found in the choroidal vasculature are the choriocapillaris, and the large-caliber arteries and veins. The ophthalmic artery is the major blood supply for both the choroid and the retina. The choroid supplies oxygen and nourishment to the most of the back of the eye specifically the outer layers of the retina (rods, cones, and retinal pigment epithelium). The four layers of the choroid include the lamina fusca, the stroma, the choriocapillaris, and Bruch's membrane (Carolyn S, 2012).

Ciliary body

The ciliary body is a thin vascular part of the uveal tissue also known as the intermediate uvea. It continues with the choroid and retina posteriorly and is attached to the iris and the scleral spur interiorly (Barar J, et al. 2008). The ciliary body has a muscle called ciliaris, vascular stroma and epithelium. The total dimension of the ciliary body is about 6-6.5 mm anteroposteriorly (Carolyn S, 2012).

It is divided into two parts:

a. The pars plana

The pars plana is the most posterior part of the ciliary body that continues with the choroid and retina with 4.5-mm thickness (Carolyn S, 2012).

b. The pars plicata

The pars plicata forms the anterior 2 mm and the loops of muscle covered by epithelium which actively secrete aqueous humour called ciliary process (Carolyn S, 2012).

There are numerous capillaries in the ciliary body. Systemic drugs pass through the ciliary body vasculature to enter into the anterior and posterior chambers of the eye, then diffusing into the iris where they can enter into the aqueous humour (Carolyn S, 2012).

Iris

Iris is the colored thin circular disc perforated centrally by the pupil and forms a diaphragm in front of the crystalline lens. It controls the amount of light transmitted into the eye by altering the size of the pupil (Barar J, et al. 2008). It prevents the excess light from entering into the eye and helps form clear images on the retina. The two iris muscles, the sphincter and the dilator regulate pupil size through the cholinergic and adrenergic innervations respectively. The sphincter muscle contraction constricts the pupil (Miosis), while dilator musculature contraction dilates the pupil (Mydriasis) (Kristina I and David LG, 2009). .

The iris consists of three layers:

a. Anterior layer

The anterior layer mainly comprises melanocytes and collagen fibres.

b. Middle stroma

It forms the major part of the iris and comprises melanocytes, collagen, fibroblasts, blood vessels and nerves. The color of the iris is based on the number and percentage of melanin granules in the superficial stroma.

c. Posterior layer

The pigment epithelium and dilator muscle form the posterior layer (Kristina I and David LG, 2009; Carolyn S, 2012).

1.3.2.5 Innermost layer- Retina or sensory layer

The retina is a complex assembly of multiple layers that consists of vascular, glial and neural cells and nerve fibres. Though there are 10 distinct layers in the retina, this can be broadly divided into two major segments, an outer layer (pigment epithelium) and an inner layer (neuro-epithelium) where the functions of light reception and transmission are located.

The outer pigment layer

The outer part of the retina has pigmented cells that are useful in the light absorption and prevent the reflection so the image should not get distracted. It has a

very intimate strong relation with the vascular layer. This outermost part of the retina does not have rods and cones. Pigment layer continues all over the choroid, ciliary body and behind the iris (Kristina I and David LG. 2009; Krishna MB and Lorenzetti OJ, 1993).

The innermost layer or neural layer

The innermost layer of the retina is a neural layer, having rods and cones (light receptors). These are a very special type of transducers that convert electromagnetic energy into the chemical energy of action potential through multiple cells that go into the central nervous system. The anterior part of the neural layer is not sensitive to light as the rods and cones do not extend forward (Kristina I and David LG. 2009; Krishna MB and Lorenzetti OJ, 1993).

1.3.2.6 Lens

The lens is a biconvex transparent disc that helps light to focus properly on the retina and flexible held in position with the help of suspensory ligaments /zonules attached to the ciliary body. It is located behind the iris separating the aqueous and the vitreous humour and is the second major refracting structure in the eye. It is composed of fibres from epithelial cells. It is characterized by its plasticity and its ability to change the curvature radius and refractive index, allowing active control of light penetration (Krishna MB and Lorenzetti OJ, 1993).

Transparency is maintained by being avascular, containing no nerves or connective tissue. The capsule, epithelium, and lens substance (cortex and nucleus) are the components of the lens. the entire lens is covered by an elastic membrane, the capsule that molds the shape of the lens and beneath the anterior lens capsule, cube-shaped epithelial cells are located (Carolyn S, 2012).

Lens substance forms the mass of the lens and comprises densely packed cells and fibres migrating to the center of the lens, making the lens more rigid and compact. A thick layer of younger cells having high water content is the cortex, and the nucleus which is located at the center of the lens is made up of older cells. The elastic nature of the lens is due to the high degree of protein content (Krishna MB and Lorenzetti OJ, 1993; Carolyn S, 2012).

1.3.2.7 Intraocular fluid system of the eye

The vitreous humour and the aqueous humour collectively form the intraocular fluid system of the eye. One purpose of these two intraocular fluids is to keep the eyeball appropriately distended so that the optical function of the eyeball can be maintained.

Vitreous humour

The vitreous body or humour is a transparent, colorless and gelatinous mass mainly consists of a very fine network of fibrils made of elongated proteoglycan fibres and occupies two- third i.e., nearly 80 percent of the eyeball (Carolyn S, 2012). The composition of vitreous humour includes mucopolysaccharide and hyaluronic acid. This is not freely circulated, only water and dissolved substances may get diffused into it. The vitreous functions to maintain the transparency of the optical media and to provide constant internal pressure for support of the internal structures of the eye (Krishna MB and Lorenzetti OJ, 1993; Carolyn S, 2012).

Aqueous humour

The aqueous humour is a clear watery fluid with 99% water content that is continuously being produced, freely circulated and continuously being drained. It is secreted by the ciliary processes of the ciliary body at the posterior chamber at a rate of 2µl/min, is directed into the anterior chamber through the pupil bathing the cornea and anterior segment and thus fills the anterior chamber between the cornea and iris, and also fills the posterior chamber that is situated between the iris and the zonular fibres and lens (Krishna MB and Lorenzetti OJ, 1993). The trabecular meshwork is a special network of porous tissue that shows normal physiological resistance to the aqueous humour drainage that contributes to the intraocular pressure. The aqueous humour has to pass through the trabecular meshwork at an iridocorneal angle, where the cornea and iris meet. It eventually drains into the canal of Schlemm then continues through collection channels, aqueous veins and episcleral veins (Carolyn S, 2012). The major role of the aqueous humour is to provide nutrients to the avascular parts of the eye particularly to the lens and the cornea; eliminate the waste from avascular tissues and regulate the intraocular pressure thereby helps the eyeball maintain its shape (Wu Y, et al. 2019).

1.4 OCULAR DRUG ABSORPTION

The solutions, suspensions and ointments are the most frequently employed ophthalmic dosage forms. Nevertheless, they are quickly drained out from the cul-de-sac due to increased tear flow and lacrimal drainage into the nasal cavity upon instillation on to the eye (Peng CC, et al. 2011).

1.4.1 Possible drug absorption pathways

The fate of an ophthalmic dosage form after instillation on to the eye can be better explained by taking into consideration of the topical instillation of an ophthalmic drug solution. The topical eye drops are usually administered onto the tear film, by instillation into the cul-de-sac. An instilled eye drop is primarily diluted by tear-film and drug loss occurs quickly due to low cul-de-sac capacity, tear fluid turnover and blinking, and most of the instilled dose will reach the systemic circulation through the nasolacrimal duct (Farkouh A, et al. 2016).

The possible drug absorption pathways for the topical oclar absorption (fig. 3) include corneal, sclera- conjunctival and nasolacrimal where the later three collectively known as noncorneal absorption pathways.

Corneal absorption

The cornea serves as the primary route of ocular absorption where the medicaments diffuse from the tear fluid to the anterior segment of the eye. The corneal absorption may occur either by the paracellular pathway through which the hydrophilic drugs prefer to penetrate the corneal epithelium or by the transcellular pathway through which the lipophilic drugs primarily penetrate across the corneal epithelium (Wadhwa S, et al. 2009). In trans-corneal penetration the drugs often bind to the corneal tissues where the cornea acts as a drug pool, releasing the drug into the aqueous humor at a slow rate. The aqueous humour then, distribute the drugs to the intraocular tissues that include iris, ciliary body, lens, vitreous body and retina subsequently eliminated either by the aqueous humor turnover or by the venous blood flow that opens into the anterior uvea.

Frequently, the corneal to noncorneal absorption ratio is higher for lipophilic drugs for example, 5:1 for pilocarpine, 70:1 for hydrocortisone and 12:1 for timolol

while the cornea serves as a potent barrier to drug absorption in case of hydrophilic drugs thus cannot diffuse across the epithelium passively (Shirasaki Y, 2008). Literature suggests that the passage of hydrophilic drugs across the epithelium can be achieved by active transport. However, there occurs a huge difference between the corneal transporter-mediated uptake and elimination among different species, hence difficult to relate corneal absorption in animal models to humans (Abelson MB, 2009).

Noncorneal absorption

It is the scleral uptake and accumulation in the ciliary body through which the hydrophilic drugs often reach the ocular tissues whereas lipophilic drugs can better permeate the cornea. It is further recommended for a hydrophilic drug, the ocular penetration takes place rapidly via the trans-sclera-conjunctival absorption rather than trans-corneal absorption (Patel A, et al. 2013). Both trans-sclera-conjunctival absorption and trans-nasal absorption lead to the loss of a major part of the instilled dose into the systemic circulation result in severe side effects. Example: cardiovascular effects of the beta-blockers while treating open-angle glaucoma (Ahmed I and Patton TF, 1985).

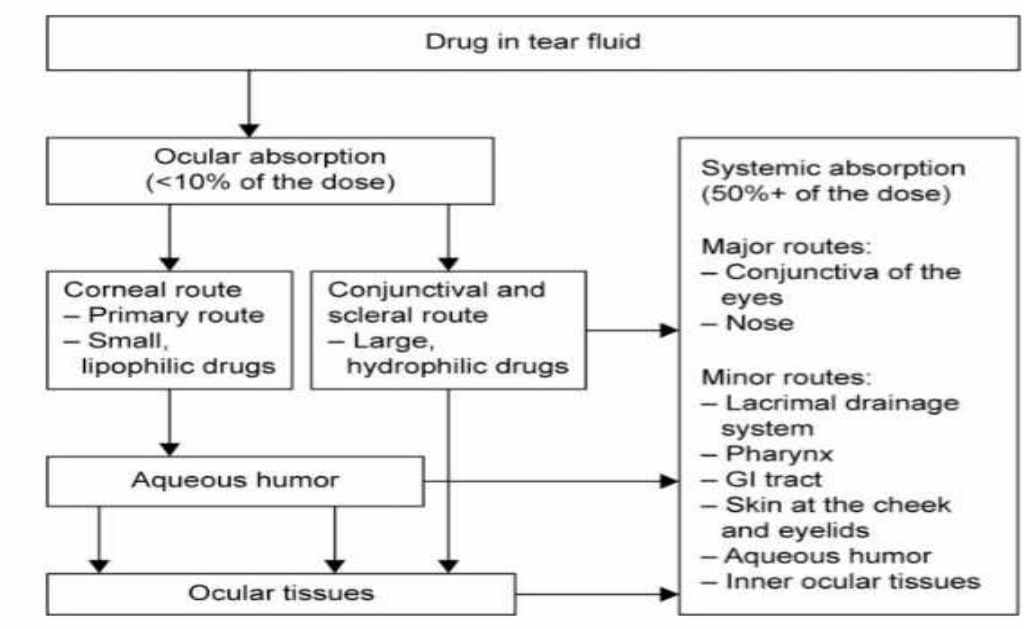


Fig. - 3: Possible absorption pathways of a drug administered into the eye [Farkouh A, Frigo P, Czejka M. Systemic side effects of eye drops: a pharmacokinetic perspective. Clinical Ophthalmology. 2016;2016(10):2433—2441]

1.4.2 Approaches to increase the Corneal Absorption

The corneal absorption can be improved either by increasing precorneal residence time or by enhancing corneal permeation.

1.4.2.1 Formulation considerations

Formulations with high viscosity (not more than 70 cPs which may cause lid caking and discomfort by interfering with vision) will have an elevated residence time on the corneal surface, facilitating the drug availability to absorption for a longer time (Chun DK, et al**.** 2008).

Changes in the drug lipophilicity and solubility can enhance the ability to enter the ocular tissue. The increased lipophilicity of moxifloxacin enhanced the corneal permeation and the increased aqueous solubility lead to the increased concentration gradient between the corneal epithelium and the tear film subsequently improved absorption (Robertson SM, 2005).

Prodrugs

Formation of prodrug with high corneal permeability is one of the successful approaches which can be converted to its respective active drug either chemically or enzymatically after passing through the cornea. For example, dipivefrin, a prodrug that is hydrolyzed to epinephrine after passing through the corneal epithelium (Abelson MB, 2009).

The cationic surfactants

Preservative Benzalkonium and other cationic surfactants found to have a high affinity for membrane proteins possibly altering corneal ionic resistance and are suggested for increased corneal permeability (Peng CC, 2011).

Mucoadhesive polymers

Mucoadhesive polymers that can enhance corneal penetration have also been used to increase corneal absorption. For example, chitosan hydrochloride was found to be a successful mucoadhesive polymer that significantly enhances corneal permeability probably due to elevated residence time on the corneal surface (Baranowski P, et al. 2014).

Other factors that influence ocular absorption include solution osmolality and pH. Hypertonic solutions with an osmolarity above 400 mOsm can cause discomfort and induced lacrimation thus increase the loss of drug whereas the hypotonic solutions having an Osmolarity as low as 100 mOsm are found comfortable and can create a solvent drag for water-soluble drugs thus improve bioavailability. The pH of an ocular solution is supposed to be within the comfort range i.e., 6 to 8 while lowering to below 3 or exceeding to above 10.5 results in tissue damage (Abelson MB, 2009).

1.4.2.2 Alternative drug delivery systems

Several new topical delivery systems are being researched as alternatives to the standard eye drop therapy.

Ocular inserts

The biodegradable inserts usually placed under the eyelid mostly to treat the eye conditions like dry eye syndrome; however, the release kinetics differ from patient-to-patient based on the enzyme composition of individuals' tear film, tear fluid production and drainage rates and an unmanageable rupture as the insert degrades resulting in discomfort. When compared to the biodegradable inserts, the non-biodegradable inserts found to be more controllable and can deliver the drugs for longer periods. However, they suffered a lack of acceptance. Example: Ocusert Pilo, a non-surgical insert loaded with pilocarpine in glaucoma treatment was suffered from poor compliance due to ejection and also found expensive (Del Amo EM, et al. 2008).

Colloidal drug delivery systems

Liposomes and nano delivery systems are the two methods of ophthalmic drug delivery which can facilitate drug transfer across the corneal membrane with comparatively higher drug absorption than the ophthalmic solutions. Example: idoxuridine antiviral drug encapsulated into liposomes for treatment of herpes simplex keratitis. Drugs can be incorporated into (nanoemulsions) or adsorbed by the particles (polymeric nanoparticles), where the smaller particles provide lower irritation to the eyes hence, they are better bearable by the patients and lead to higher drug absorption due to their slow elimination rate (Rinda Devi B, et al. 2018).

1.4.2.3 Miscellaneous

Different new methods are available for overcoming the drawbacks of the conventional eyedrops and are frequently referred to as ocular devices.

Metered-dose delivery systems

Visine Pure Tears, they are the multidose delivery devices that can deliver a standard eye drops by controlling drop size and avoiding the need for preservatives (Abelson MB, 2009).

Small-volume Nebulizers

It is advancement in the metered delivery system which delivers the formulation to the eye by spraying. Example: vitamin B12 bioavailability was compared between a small-volume nebulizer and standard topical eye drops in a clinical study where the former being showed significantly high bioavailability (Baranowski P, et al. 2014).

Iontophoresis

Ocular devices based on iontophoresis use an electrical current is used for driving the ionized drugs into cells. Example: iontophoresis enhanced the ocular absorption of antibiotics like gentamicin and ketokonazole; carboplatin, a small cationic compound and was also found safe and well-tolerated in the management of active corneal graft rejection (Baranowski P, et al. 2014; Abelson MB, 2009).

1.5 CHALLENGES TO OCULAR DRUG DELIVERY

The topical route is the safest and convenient method of administrating eye medication usually in the form of eye drops which are the majority of existing marketed topical eye products. However, their bioavailability is limited by several intrinsic barriers due to the complex structure, physiology and defensive mechanisms of the eye towards foreign materials and drugs as well. Typically, 1–10% of the instilled dose enters the corneal absorption and approximately 1-2% reaches the aqueous humor (Margareth RCM, et al. 2011; Bucolo C, et al. 2012).

1.5.1 The major barriers to topical ocular drug delivery

Fig. 4 illustrates the physiological and anatomical barriers to ocular drug delivery.

Tear film

The topical eye drops are usually administered onto the tear film, by instillation into the cul-de-sac. Generally, the volume of an eye drop measures approximately 30µl and as the capacity of the cul-de-sac is the only 15µl, the excess will get rolled off within a fraction of 15 – 30 seconds leading to the drug wastage (Gaudana R, et al. 2010). From the remaining of the instilled drug, some fraction can be absorbed into corneal and noncorneal pathways such as conjunctiva and sclera while the major fraction is diluted with tear fluid and enters into the nasolacrimal drainage subsequently reaching the systemic circulation resulting in adverse side effects. Thus only 2-5% of the administered drug is available for the corneal absorption to reach the aqueous humour and ocular tissues resulting in poor bioavailability (Hugo A, et al. 2013; Ranjan KS, et al. 2014). The factors such as pH and tonicity of the formulation can also accelerate the tear clearance and remove the drug solution from the cul-de-sac in a few minutes. The tear proteins can bind with the drug molecule and result in poor bioavailability (Achouri D, et al. 2012).

Corneal barrier

The variation in the polarity among the corneal limits the permeation of both lipophilic and hydrophilic drugs through the cornea. Though the cornea is made of five layers, three of them namely the epithelium, the stroma, and the endothelium are the main barriers to absorption. The drug which is absorbed by the cornea can diffuse across these three layers to reach the anterior segment of the eye and get circulated to the ocular tissues by the aqueous humour where a fraction can bind to some ocular tissues like the lens, ciliary bodies, etc. and some fraction get cleared by aqueous humour drainage (Hugo A, et al. 2013).

a. The corneal epithelium

The corneal epithelium is lipophilic with 5–6 layers of closely packed cells connected by tight junctions. These layers form an effective barrier to most therapeutic drugs particularly hydrophilic compounds allowing the transcellular permeation for the lipophilic drugs having a log p value of 2–3. However, as the

corneal epithelium is negatively charged, the hydrophilic cationic compounds can be permeated through it compared to anionic forms (Ameeduzzafar, et al. 2016).

b. The stroma

The stroma mainly consists of hydrated collagen and proteoglycan scatter among keratocytes thus forms a barrier to diffusion of highly lipophilic drugs (Hugo A, et al. 2013).

c. The corneal endothelium

The corneal endothelium, a monolayer of hexagonal-shaped cells is lipophilic and acts as the rate-limiting barrier to diffusion of most hydrophilic drugs, however, its permeability depends on molecular weight. The leaky tight junctions' in the endothelium help the passage of macromolecules between the aqueous humor and stroma (Li G, et al. 2012).

Thus to travel across the cornea, it is advantageous for those molecules having both hydrophilic and lipophilic properties within the same structure (Achouri D, et al. 2012).

Conjunctiva

The amount of drug that is absorbed by the conjunctiva is much more than that of the cornea because of the larger intercellular spaces of the conjunctival epithelium and hence more permeable compared to the cornea. However, both the conjunctiva and the cornea act as the rate-limiting factors for the water-soluble drugs (Achouri D, et al. 2012). Also, conjunctiva has a rich supply of capillaries and lymphatics and hence, the administrated drugs in the conjunctiva may be cleared through blood and lymph. The fraction of the drug that is absorbed through the conjunctiva enters systemic circulation by pinocytosis and/ or through paracellular transport across the vascular endothelial layer and efficient elimination of absorbed drugs from the conjunctival space takes place by the conjunctival lymphatic system (Ameeduzzafar, et al. 2016).

Sclera

The scleral permeability strongly depends on the molecular dimensions and hydrophobicity of the substances where the smaller molecules and hydrophilic drugs diffused easily while the larger molecules and the lipophilic drugs are restricted (Achouri D, et al. 2012).

Blood–ocular barrier

The blood–ocular barrier is a natural physical barrier located between the ophthalmic vasculature and the major parts of the eye and prevents the passage of many substances as well as drugs across it as a part of protecting the eye from toxic materials maintaining its physiology.

a. Blood-aqueous barrier

The blood-aqueous barrier is formed by the two distinct cell layers, one is the endothelium of the iris/ciliary blood vessels and the other is the non-pigmented ciliary epithelium, both having complexes of tight junctions whose function is to control the transport of solutes between the anterior and the posterior chambers of the eye (Achouri D, et al. 2012). The solutes that are dissolved in the aqueous humor can simply infiltrate into the anterior surface of the iris as the iris endothelial vasculature has tight junctions of the leaky type that show less restriction to the entry of solutes from the aqueous humor into the systemic circulation. Thus, the drugs are absorbed by the iris pigments, later washed out into the iris blood vessel (Ameeduzzafar, et al. 2016).

The larger and more hydrophilic drugs are eliminated by the aqueous humor turnover whereas the small and lipophilic drugs enter the uveal blood circulation where their rapid elimination than larger and more hydrophilic drugs take place (Barar J, et al. 2008).

Generally, the aqueous humor is continuously drained off at a turnover of 2.0 – 3.0 µl/min and hence, the drugs do not pass from the anterior segment to the posterior segment much efficiently and thus, most of the ophthalmic therapies fall short in providing an efficient pharmacological effect in the retina and vitreous which are present in the posterior segment (Barar J, et al. 2008).

The ionic concentration gradients facilitate the passive permeability which is controlled by the expansion of the tight junctions and the transcellular transport occurs through vesicles at the iris capillaries/ pigmented epithelium (Ameeduzzafar, et al. 2016).

b. Blood-retinal barrier

The blood-retinal barrier is located in the posterior part of the eye where the inner layer consists of retinal capillary endothelial cells and the outer layer consists of the retinal pigmented endothelial cells. It has specialized transport processes in conjunction with tough barrier restrictiveness for the selective transport of nutrients/compounds between the choroid and retina. The Na^+/ K^+ -ATPase in the outer layer regulates the balance of intracellular Na^+ and K^+ (Ameeduzzafar, et al. 2016; Barar J, et al. 2008). The inner layer cells possess intercellular tight junctions and display poor permeability to proteins and small hydrophilic compounds selectively protect the retina from the molecules in the blood. Hence, systemic or intravitreal drug administration is needed for achieving significant drug concentrations and pharmacological effects within the vitreous and the retina. However, higher doses of drugs involved in the systemic application (Achouri D, et al. 2012).

1.5.2 Defensive mechanisms of eye

Despite the ease of access, the eyes are naturally provided with very efficient defensive mechanisms that include blinking, tear turnover, nasolacrimal drainage and induced lacrimation which cause the quick elimination of substances from the eye surface to protect the eyes from foreign materials (Rinda Devi B, et al. 2018)

1.5.3 Ocular transporters

The efflux transporters of ocular tissues include P-glycoprotein, breast cancer resistance protein and multidrug resistance protein that can throw the drug molecules out of the cytoplasm and cell membrane lowering the bioavailability and may develop drug-resistant in some cases, for example, P-glycoprotein found to efflux lipophilic compounds in normal as well as in cancerous cells (Ameeduzzafar, et al. 2016). Amino acids and peptides act as influx transporters for ocular drug delivery facilitating the translocation of xenobiotics as well as essential nutrients across the

biological membranes. Endocytosis, a receptor mediated transport is reported in delivering nanoparticles due to the presence of specialized receptors like *caveolin-1* on the ocular barriers (Barar J, et al. 2008). However, the improvements in bioavailability depend upon the type of influx transporter, size and charge over the particle surface in the formulation (Ameeduzzafar, et al. 2016).

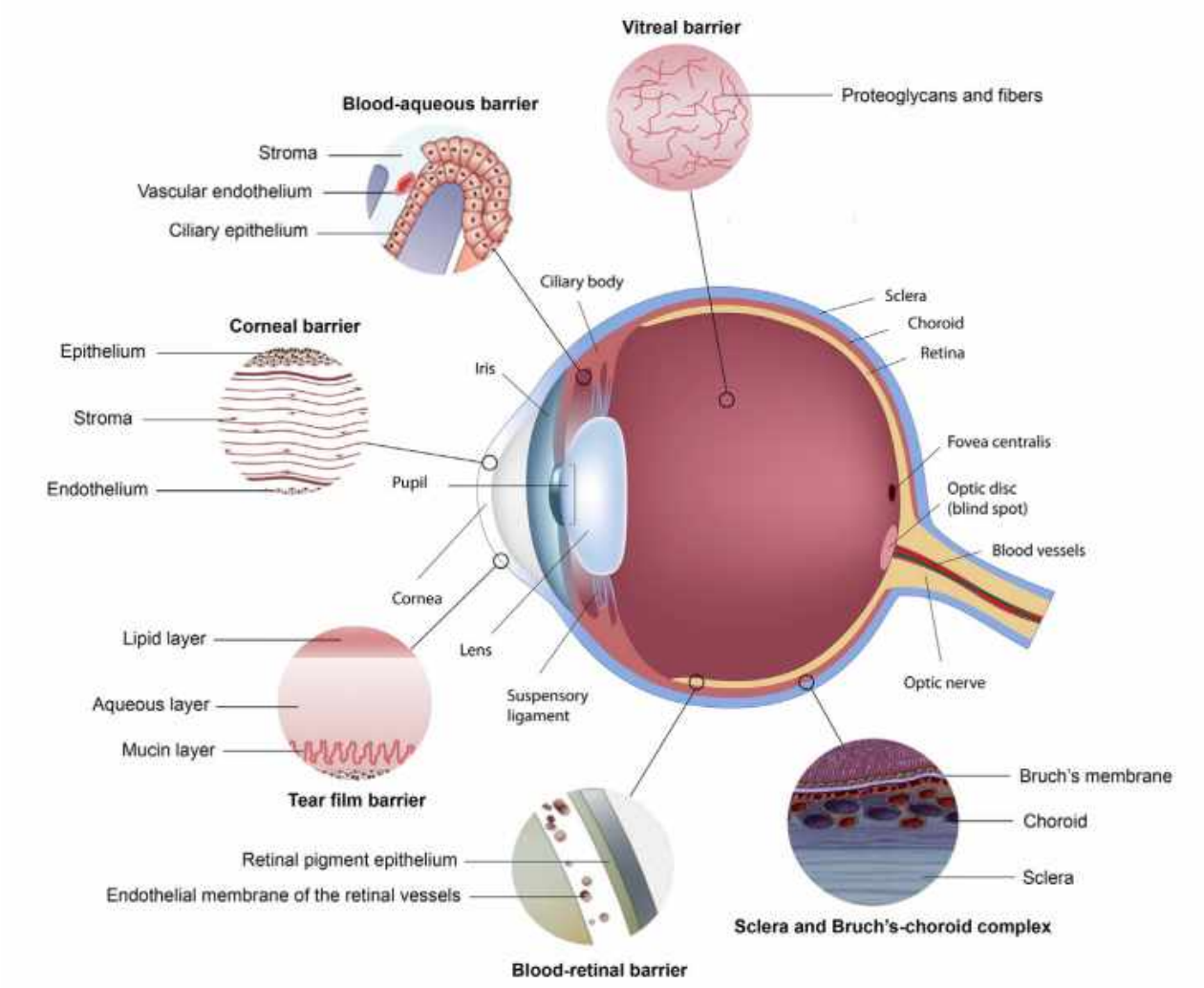


Fig - 4: Physiological and anatomical barriers to ocular drug delivery
[D. Huang, et al., Overcoming ocular drug delivery barriers through the use of physical forces, Adv. Drug Deliv. Rev. 2017:1-17]

1.6 AN INSIGHT INTO MAJOR EYE PROBLEMS

1.6.1 GLAUCOMA

1.6.1.1 Introduction

Glaucoma is a group of optic neuropathies that are developed by elevated intraocular pressure in the eye that results in progressive degeneration of retinal

ganglion cells. The increased pressure results in cupping of the optic nerve and may cause permanent vision loss.

Glaucoma frequently referred to as a '*Silent Blinding Disease'* because it shows no specific symptom initially rather, the rise in intraocular pressure will be the only indication that appears in the later stages the time vision is affected with symptoms like tunnel vision, peripheral vision loss and gradually the damage gets permanent if left untreated.

Glaucoma represents the second most important cause of permanent sight loss in the world (Jama. 2015; Ciotu IM, et al. 2015). In the year 1996, it was estimated that the number of Glaucoma patients by the year 2000 is of about 68.8 million, of whom 6.7 million are blind (Quigley HA, 1996) and in the year 2006, it was estimated that the number of Glaucoma patients by the year 2010 is of 60.5 million, increasing to 79.6 million by the year 2020, where 4.5 million people with open-angle glaucoma and 3.9 million people with angle-closure glaucoma are blind, growing to 5.9 and 5.3 million people in 2020, correspondingly (Quigley HA and Broman, 2006).

1.6.1.2 Route cause

Glaucoma is the result of elevated intraocular pressure, which is mainly determined by the balance between the production and the drainage of the aqueous humor through the trabecular meshwork. The persistent changes in the aqueous humor composition may induce apoptosis in the cells of the trabecular meshwork and in the head of the optic nerve leading to elevated intraocular pressure that further leads to optic nerve damage (Sacca CS and Izzotti A, 2008). The intraocular pressure in the normal eye is around 10-21 mm Hg whereas, in the glaucomatous eye, it can exceed up to 70 mm Hg.

1.6.1.3 Diagnosis

The changes in the optic disc, for the increase in the cup to disc ratio that indicates the atrophy of the nerve fibres and also the asymmetry between the disc cupping of both eyes, is also essential because the extent of cupping varies between both eyes (Fingeret M, et al. 2005). The visual field loss can be detected only after

50% damage of the nerve fibres using computer-assisted field testing that detects the long term changes and declines visual fields (Weinreb RN, et al. 1989).

Optic disk photography

The stereoscopic photography, that gives a three-dimensional full- color image of optic nerve head and the stereoscopic observation of the optic nerve using an ophthalmoscope or slit-lamp biomicroscopy are the most common methods to detect glaucomatous neuropathy. However, in recent years, remarkable advances took place in the computer-based techniques to check the optic nerve head (Sehi M, et al. 2007).

Confocal scanning laser ophthalmoscope

It is an imaging tool in which several stereometric parameters are generated by the device software and provides a quantitative three-dimensional composite image of the optical nerve head and also the posterior segment of the eye (Parikh RS, et al. 2008).

Scanning laser polarimetry

It is an instrument having a confocal scanning laser ophthalmoscope with a polarized laser beam. It is a non-invasive method in which a polarized laser beam is passed through the retinal nerve fibre layer that creates a measurable phase shift which can be correlated to the thickness of the retinal nerve fibre layer (Gooch N, et al. 2012).

1.6.1.4 Treatment for glaucoma

The principal objective of glaucoma treatment is to lower the intraocular pressure that includes medical management, usually through topical therapies that include topical eye drops, eye ointments, creams and gels and systemic therapies that include oral and invasive methods like intracorneal and intravitreous injections. The surgical procedures include either laser or incisional surgery.

1.6.1.4.1 Surgical procedures

Traditional and laser surgery, or a combination both in certain conditions can treat glaucoma aiming to reduce the pressure in the eye. Canaloplasty is the other new

surgical procedure that restores the eyes' natural outflow system and helpful for the treatment of open-angle glaucoma.

Surgical procedures are expensive and require pre/postoperative care. Incisional procedures may result in tissue damage and lead to infections. Examples: Laser therapy, Laser-assisted sclerectomy, Trabeculectomy, Canaloplasty, implant surgery.

1.6.1.4.2 Non inscisional Medical management of glaucoma

Prostaglandins

Prostaglandins in the form of eye drops are used to reduce the pressure build-up in the eyes by relaxing the eye muscle that results in the better outflow of intraocular fluids. The Possible side effects include burning, stinging and change in eye color**.** Examples: Latanoprost, Travoprost, Bimatoprost and Tafluprost.

Beta-blockers

The beta-blockers decrease the aqueous humor production in the eye and were usually prescribed in combination with prostaglandins. The beta-blockers are responsible for the lessening of the cAMP levels in the ciliary body as the beta receptors are expressive all over the eyes that lead to the decreased rate of aqueous humor production (Grieshaber MC and Flammer J, 2010).

The beta-blockers have the potential to reduce the heart rate and hence, cause adverse effects in individuals with certain cardiovascular disorders. Example: Timolol, betaxolol, carteolol, metipranolol, and levobutaxolol.

Alpha-adrenergic agonists

Alpha-adrenergic agonists can be prescribed alone or in combination with various anti-glaucoma drugs in the form of eye drops which decrease the aqueous humour production rate. The vasoconstriction in the ciliary body due to the activation of alpha-2 receptors results in a decreased aqueous humor production (Deepak S and Ahmad Aref A, 2014).

The side effects include redness and itching in eyes, the elevation of upper eyelids and pupil dilatation. Example: Brimonidine.

Carbonic anhydrase inhibitors

The carbonic anhydrase inhibitors are used either in the form of pills orally or as eye drops topically and are usually used along with other anti-glaucoma eye drops as a combination therapy but not alone. The carbonic anhydrase inhibitors inhibit the carbonic anhydrase II, IV and XII isozymes which are present in the ciliary processes of the eye and thus reduce the secretion of bicarbonate and aqueous humour, and subsequently lower the intraocular pressure (Mincione F, et al. 2008).

Common side effects of eye drops include bitter taste due to nasolacrimal drainage, redness and burning in the eyes lid whereas the systemic side effects include depression, kidney stones loss of appetite, fatigue, weight loss of libido and tingling fingers and toes. Example: Dorzolamide, Brinzolamide, acetazolamide, methazolamide and ethoxzolamide.

Para sympathomimetics or Miotics

The para sympathomimetics act by the pupil constriction that helps open the narrowed or blocked angle where drainage occurs thus increase the aqueous humour outflow from the eyes and thereby reduction in the intraocular pressure. The common side-effects include pupil constriction, brow ache, burning eyes and trouble night vision. Examples: Pilocarpine, carbachol, echothiopate and demecarium.

Hyperosmotic agents

Hyperosmotic agents are the potent ocular hypotensive drugs which are prescribed only in acute intraocular hypertension conditions as the emergency treatment or as preoperative medication and the kidney and heart function must be monitored before administration of these agents to the patients because of their serious side effects (Deepak S and Ahmad Aref A, 2014).

The possible side effects include fluid and electrolyte imbalance, peripheral edema, metabolic acidosis, gastrointestinal symptoms, hypotension, or tachycardia. Example: Mannitol, glycerol and isosorbide

Miscillaneous

The anti-VEGF agents are generally given as an intravitreal injection in glaucoma surgeries to manage neovascular glaucoma (Slabaugh M and Salim S, 2017). The intravitreal injection of anti-VEGF agents may be associated with systemic adverse events and devastating ocular complications like soreness, floaters, or foggy vision. Example: Avastin and Lucentis

1.6.1.5 Pros and cons of current glaucoma treatment options

Glaucoma being the second most important cause of permanent sight loss and the numbers of glaucoma cases are increasing year by year, it is necessary to diagnose and treat effectively. Mostly the treatments for Glaucoma involve medical management where different drugs are administered either by topical (solutions, suspensions, ointments, creams and gels) oral (tablets and pills) or parenteral (IV and intraocular injections) routes and surgical procedures where incisions and laser are widely used. Among the medical management options, the topical route is widely accepted due to ease of application, avoiding first-pass effect, ease of preparation, etc while the oral and parenteral suffering from drawbacks like serious systemic and ocular side effects, poor patient compliance and the surgical procedures are expensive and requires pre/postoperative care.

1.6.2 EYE INFECTIONS

1.6.2.1 Introduction

Fortunately, the eye is protected by several means, for example, it is placed inside a tough bony cavity to save from external injuries, covered by eyelids and eyelashes to save from external particles and, other intrinsic defensive mechanisms, for example, blinking, tear film composition and rapid turnover and efflux transporters. Despite all these protective means, the eye is a highly sensitive organ, there are chances where a little dust particle can create severe irritation, pain and discomfort or may results in infections upon attack by microbes and may get injured by other foreign bodies. Moreover, as the eyes are supplied with end arteries, continued by the optic nerve and have direct contact with the meningeal layers of the brain, most of the common eye problems may end up with sight-threatening

conditions requiring emergency medical support and in some cases complete blindness if the diagnosis and treatment not done properly.

1.6.2.2 Route cause

The eye infections are the regular problem that may affect people of any age and can affect one or both eyes. The causative organisms for the eye infection may include bacteria, fungi, viruses, or other microbiological means of various allergies.

1.6.2.3 Diagnosis of eye infection

It is typically difficult to find out the cause of ocular infections i.e., the type of causative organism and infective or non-infective mere by the clinical features because most of the time, they may not distinctive. However, there are various methods currently available for the diagnosis of ocular infections.

In vivo confocal microscopy

Confocal microscopy is an eye-catching non-invasive procedure that allows repeated observations aiding the diagnosis, management, and follow up of cases with microbial keratitis. The confocal microscopy is particularly helpful in cases like deep corneal infiltration or microbial keratitis developed after intracorneal implants or refractive surgery (Paladini I, et al. 2013; Jalbert I, et al. 2003; Cruzat A, et al. 2010).

Conventional microbiological methods

Clinical samples from the site of infection are collected and subjected to an initial smear examination that provides rapid diagnosis of eye infections and helps initiate specific treatment early in the disease (Sharma S, 2012).

Culture methods for bacteria, fungi & Acanthamoeba

The culture methods involve the inoculation of the collected clinical samples on appropriate culture media. Prior knowledge of expected organisms helps to select the type of media and the incubation conditions. Fungi associated with eye infections are usually fast-growing saprophytic fungi that can grow on blood agar media, and bacteria can grow on chocolate agar media Dahlgren MA, et al. 2007; Sharma S, 2012).

Culture of ocular samples for viruses

The samples may be processed using various techniques in the virology laboratory and the selection of technique depends on the sample type and the specific virus expected. The samples must be collected in an appropriate transport medium like sucrose phosphate broth or Hank's balanced salt solution for viral diagnosis while sending it to the laboratory with an exception for the smears. However, the molecular methods are considered ideal for viral diagnosis as the virus isolation is lengthy and requires expensive special technical set up of the virology laboratory (Sharma S, 2012; Sagar A, 2018)).

1.6.2.4 Types of eye infections

Eye infections can be categorized as viral, bacterial, or fungal infections based on the causative organism and each of them require different treatments.

1.6.2.4.1 Conjunctivitis

Conjunctivitis mostly referred to as pink eye or red eye involves irritation and swelling in the conjunctival mucosa due to infection in the conjunctival blood vessels and the eyes become pink or red (Lambert L, 2017).

Conjunctivitis is extremely contagious that can be infected due to bacteria (bacterial conjunctivitis) or virus (viral conjunctivitis) and can also be resulted from various allergies or chemical exposure (Amir A, et al. 2013).

Symptoms

Eye color changes to pink or red, Lacrimal discharge from eyes, itchiness or irritation in the eyes and an elevated tear production especially from a single eye (Mary L, 2017).

Treatment

The treatment depends on the type of conjunctivitis i.e., based on the causative organism.

Bacterial conjunctivitis is treated with antibiotics either in eye drops or ointments, and or oral medication is used to kill bacteria in the eyes (Bremond-Gignac D, et al. 2011).

Viral conjunctivitis has no treatment and the symptoms can get fade out after 7 to 10 days as in cold. However, Antihistamines like diphenhydramine and loratadine as oral and eye drops and anti-inflammatory eye drops can help relieve allergic symptoms (Azari AA, et al. 2013).

1.6.2.4.2 Keratitis

Keratitis is a corneal infection in which swelling, ulceration and or infiltration of inflamed corneal cells occur due to bacterial, viral, fungal, or parasitic attack or due to injury or trauma. The other possibilities to develop keratitis are wearing contact lenses, other illness that weakens immune system and also due to treatments for an existing eye problem like corticosteroid eyedrops (Pachigolla G, et al. 2007).

Symptoms

Redness, swelling, pain and discomfort in the eye mostly while blinking, blurred vision, sensitivity to light and elevated tear production (Claire S, 2018).

Treatment

To treat bacterial keratitis, antibacterial agents are prescribed either in eye drops or as oral medication based on the severity of the infection and to treat the fungal keratitis, antifungal agents either in the form of the eye drops or as oral medication, however, this may take weeks to months. Oral antiviral medications or eye drops can help stop the viral keratitis in a few days up to a week however, symptoms may reoccur even with treatment (Tim J, 2018).

1.6.2.4.3 Endophthalmitis

It is a severe eye inflammation resulting from a bacterial or fungal infection where Candida fungal infections are the most common type (Vasumathy V, 2014). The other causes include certain eye surgeries and intravitreal injections which are rare but are associated with sight-threatening complications (Yam JC and Kwok AK, 2004).

Symptoms

Inflammation around the eye and eyelids, mild to severe pain in the eye, blurred vision, formation and discharge of eye pus and sensitivity to lights (McBean B, 2017).

Treatment

First, the antibiotics are injected directly into the eye to stop the infection using a special needle and then, to relieve inflammation, a corticosteroid may be injected (McBean B, 2017; Vasumathy V, 2014).

1.6.2.4.4 Blepharitis

Blepharitis is a bacterial infection in which the oil glands inside the eyelid get clogged and result in the eyelid inflammation especially at the base of the eyelashes (Jackson W, 2008; Markus M, 2018).

The other possibilities to develop blepharitis include dandruff, allergy due to eye makeup certain and under medication affecting the immune system (Markus M, 2018).

Symptoms

The common symptoms include eyelid redness, itchiness and swelling in the eye, oiliness in the eyelid, sensitivity to light and elevated tear production (Tim J, 2018).

Untreated blepharitis may result in complications like trichiasis, abnormal eyelash growth, scarring in the eyelids and constant irritation may lead to the corneal injury that subsequently leads to small corneal ulcers (Jackson W, 2008).

Treatment

Antibiotics either in eye drops or as oral medication and ointments are applied to the eyelids. Corticosteroids are also used as eye drops or ointments to relieve the inflammation. Further, the lubricating eye drops are prescribed to moisten the eyes and prevent irritation (Mary L, 2018).

1.6.2.4.5 Cellulitis

Cellulitis is an infection in the eye tissues and can be termed according to the affected area preseptal cellulitis (eyelids), periorbital cellulitis (anterior to the orbital septum) and post septal cellulitis (tissues behind the septum). Mostly infected by the bacteria, such as Staphylococcus or from others like sinus infections (Rutar T, et al. 2005).

Symptoms

Usually, the symptoms of cellulitis include redness and swelling in the eyelids without any pain or discomfort. However, the orbital cellulitis shows painful uncomfortable symptoms can lead to blindness when left untreated (Corey W, 2019).

Treatment

Inflammation can be relieved by applying a lukewarm, moist, clean towel on the eye for about 20 minutes, oral antibiotics, like amoxicillin, and for children under 4 are prescribed with intravenous (IV) antibiotics (Corey W, 2019). In rare cases where the infection becomes very severe surgery is the option to relieve pressure from the eye (Tim J, 2018).

1.6.2.4.6 Sty

A sty is a bacterial infection and found in either form of hordeolum and chalazion. Hordeolum is a lump that develops from an oil gland near the eyelids edges while chalazion is a condition where the lump develops from an oil gland that present inside the eyelid and this mostly caused due to the bacterium staphylococcus (Practicia SB, 2017).

Symptoms

Symptoms include eyelid swelling, irritation and pain in the eyelid along with increased tearing (Andrew AD, 2019).

Treatment

Pain relievers, for example, acetaminophen (Tylenol) help relieve pain and swelling antibiotic topical eye drops and ointments to help kill the infectious

overgrowth. Surgery is the option where the lump is filled with pus and does not rupture (Tim J, 2018; Andrew AD, 2019).

1.6.2.5 Practical approach to the treatment of eye infections

Mostly the treatments for eye infections involve medical management where different drugs are administered either by topical (solutions, suspensions, ointments, creams and gels) oral (tablets and pills) or parenteral (IV and intraocular injections) routes and surgical procedures where incisions and laser are widely used.

Topical dosage form

Sodium sulfacetamide, chloramphenicol, gentamicin, tobramycin, azithromycin, neomycin, trimethoprim and polymyxin B combination, ciprofloxacin, ofloxacin, gatifloxacin, and erythromycin (Bowman RJ, et al. 2000).

Intravitreal injection

Anti-vascular endothelial growth factor (anti-VEGF), Corticosteroids, Voriconazole, and Trifluorothymidine (Moshfeghi AA, 2008).

Intracameral injection

1 mg cefuroxime in 0.1 ml normal saline is given at the end of cataract surgery, to prevent endophthalmitis (Garcia-Saenz MC, et al. 2010).

Intrastromal route

Amphotericin B prescribed in cases of partial response to topical treatment (Lekhanon K, et al. 2015).

Subconjunctival route

Miconazole, Fluconazole - prescribed in patients with low adherence to topical treatment (NadaWM, et al. 2017).

Oral route

Ketoconazole, Posaconazole, Fluconazole are associated with topical therapy in deep keratitis or those affecting intraocular tissues (Muller GG, et al. 2013)

Cationic Antiseptics

Chlorhexidine - Postoperative endophthalmitis, used for preoperative washing preceding intraocular surgery and CHX was established as an antimicrobial agent in 1954 (Bowes MH, et al. 1984).

1.6.2.6 Pros and cons of current eye infection treatment

The current practical approach to the treatment of eye infections is having some major drawbacks. The invasive methods suffer from lack of patient compliance, the oral medication has huge chances of systemic side effects and hence, the topical route stands as an acceptable non-invasive route of drug administration to treat eye diseases. However, simple aqueous-based solutions also facing their drawbacks like dilution by tears and aqueous humour, drainage into the nasolacrimal duct and that subsequently leads to repeated instillations of drug per day, drug loss into the systemic circulation which is dangerous sometimes making patient compliance poor and thus reduces the clinical efficacy of the drugs. Their degradation could lead to insufficient therapeutic action with side effects. Therefore, safe and effective antimicrobials for the treatment and prevention of ocular infections must be adapted to the type of microbes suspected. Hence, it is necessary to replace the usual topical antimicrobials with novel discoveries for the topical route that aid in the more effective treatments to avoid the drawbacks of conventional dosage forms (Bremond-Gignac D, 2011).

1.6.3 EYE SURGERY, POSTOPERATIVE CARE AND TREATMENT

1.6.3.1 Introduction

Eye surgery is an efficient approach to treat various eye disorders or conditions that are not manageable through medication. The patients need to follow a bunch of guidelines provided by the surgeons following eye surgery up to some

weeks to months depending on condition, treatment type, and occupation to prevent infection and other complications in the healing area.

1.6.3.2 Types of eye surgery

Some of the common surgical procedures that can correct eye problems like cataracts glaucoma and others are as follows.

Refractive surgery

Several surgical procedures use a laser to reshape the cornea and correct myopia, hyperopia, astigmatism, and presbyopia (Wachler BSB, 2018).

Example: LASIK, stands for 'laser-assisted in situ keratomileusis' is the refractive surgery which reshapes the cornea by using a laser to remove cells from beneath the corneal surface and the other PRK, stands for 'photorefractive keratectomy' which is similar to the LASIK but that uses a laser to remove cells on the surface of the cornea and contrary to LASIK, PRK does not engage creating a "flap" in the cornea. Both the procedures can correct nearsightedness, farsightedness, or astigmatism (Wiley C, 2014; Kim TI, et al. 2019).

Cataract surgery

Cataract, a condition where the clouding of eye lens occurs naturally with age making the vision blurry and dull. The surgical procedure involves the removal of the cloudy lens and replaces it with an artificial intraocular lens (NEI, 2019).

In cataract surgery, the lasers are to create corneal incisions for getting access to the lens so that the anterior capsule of the lens can be removed, and then the cataract can be fragmented (Thompson V, 2015).

Glaucoma surgery

Traditional and laser surgery or a combination of both in certain conditions can treat glaucoma aiming to reduce the pressure in the eye. Example: Trabeculectomy, Glaucoma implant surgery, and Minimally invasive glaucoma surgery (MIGS) are the traditional surgery options whereas Laser

Trabeculoplasty and Selective Laser Trabeculoplasty (SLT) are the two laser surgery options (Yvonne O, 2018).

Canaloplasty is the other new surgical procedure that restores the eye's natural outflow system and helpful for the treatment of open-angle glaucoma (Lewis RA, 2019).

Diabetic Retinopathy Surgery

Diabetic Retinopathy, where the high blood sugar levels can damage blood vessels in the eyes that cause fluid and blood leakage into the retina (NEI, 2019).

The surgical procedure uses a laser to reduce swelling in the eye and helps new blood vessels to grow and also helps prevent future blood vessel leakage. However, it may need more future laser surgeries (Ahdoot M, 2018).

Vitrectomy is another choice of surgical procedure in diabetic retinopathy that removes leaked blood and scar tissue from the eye and also helps prevent reoccurrence of blood vessel leakage (Simon B and Susanne B, 2015).

Macular Degeneration Surgery

The macula tends to break down leading to the leaky blood vessels as age grows. Photocoagulation and photodynamic therapy (PDT) are the two in laser surgery in which the unwanted leaky blood vessels are burnt away to close by using a laser. This procedure prevents bleeding and also stops the overgrowth of blood vessels (Elder G, 2017).

Miscellaneous

Corneal transplant surgery, Conjuctivoplasty, Ciliarotomy, coremorphosis, Penetrating keratoplasty, etc.

1.6.3.3 Post-operative care

The patients need to follow a bunch of guidelines provided by the surgeons following eye surgery up to some weeks to months depending on condition, treatment type, and occupation to prevent infection and other complications in the healing area.

General instructions

The general instructions include protecting the eye from external injury, chemicals, dirt, or dust by wearing protective sunglasses; maintaining eye hygiene; avoiding physical exertion like lifting weights and hard coughing must be treated using cough suppressant; avoiding washing hair for at least 2 weeks, eye make-ups for 4 weeks and tasks which require sharp focus; wearing safety goggles at all times and UV protective sunglasses on sunny days for not less than12 months (Timothy MG, 2014; Ronald LF, et al. 2017; Kyari F, et al. 2016).

Instructions for systemic medications

Medications like aspirin or ibuprofen and vitamin E are avoided for five days after surgery whereas the diabetics are advised to take their insulin once they can continue eating and rest. All oral medications can be continued the same day of surgery at their normal schedule and dosage (Tiihonen PS, et al. 2016; Ronald LF, et al. 2017).

Instructions for eye medications

Usually, taking Diamox or Neptazane is stopped after glaucoma surgery. However, antibiotic drops, for example, Ocuflox, Zymar, Zymaxid, and Vigamox can be used for 5 to 7 days after surgery and steroid drops; for example, Pred Forte or Durezol can be used after surgery for 1□3 months (Ronald LF, et al. 2017).

1.6.3.4 Practical approach to Post-operative treatment

Postoperative pain and irritation symptoms in the eyes are most common during the first hours after surgery which is associated with a surgical trauma induced prostaglandin production (Tiihonen PS, et al 2013). Prostaglandins promote vasodilatation, leukocyte migration, miosis, and disruption of the blood-ocular barrier and may result in various unwanted physiological and psychological outcomes; hence, it is necessary to provide postoperative treatment (Ahuja M, et al. 2008). However, there are no specific rigid guidelines in the postoperative treatment because it is purely patient-specific, and mostly based on the cause and condition of the pain (Clifford LS, 2019).

Postoperative treatment regimens include topical antibiotics, corticosteroids, and NSAIDs. However, the possible complications of postoperative medications must be measured. Example: Corticosteroids can elevate the intraocular pressure possible with myopic and glaucoma patients. Rarely but significant corneal reactions like epithelial defects, ulceration, and melting have been reported with topical NSAIDs (Haripriya A, et al. 2016; Vijayalaxmi P and Lucy N, 2016; Clifford LS, 2019).

Oral medication may result in severe systemic side-effects due to poor ocular bioavailability and higher concentrations in the systemic circulation. Hence, the topical route is preferred to administer ophthalmic dosage forms due to benefits like the ease of application, targetability, reduced side effects and also cost-effectiveness (Patel A, et al. 2013).

Topical antibiotics

Topical antibiotics reduce the risk of postoperative endophthalmitis. Example: Staphylococcus species is the most common organism concerned with acute postoperative infectious endophthalmitis and topical gatifloxacin and moxifloxacin are the most prescribed antibiotics due to their broad-spectrum activity. Others include subconjunctival and intracameral antibiotics such as cefazolin or cefuroxime that may results in leakage from surgical incisions leading to the potential risk for intraocular toxicity (Bradley SL and Linda TM, 2013).

Topical corticosteroids

In general, topical corticosteroids for example, dexamethasone, difluprednate, fluorometholone, prednisolone, etc., are prescribed to control postoperative inflammation; however, they found increasing intraocular pressure, delaying in corneal epithelial and stromal wound healing, and more susceptible to microbial infections. Hence, the use of non-steroidal anti-inflammatory drugs becomes a choice of treatment (Peter K, 1996; Michel Weber et al, 2013; Clifford LS, 2019).

Topical nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are safe and effective in the treatment of postoperative pain, and they can be prescribed to all postoperative surgical treatments unless contraindicated (Peter K, 1996). The topical NSAIDs are

now becoming the standard for postoperative treatment mainly in cataract surgery by decreasing the risk of postoperative cystoid macular edema (Thiel B, et al. 2017). Previous studies suggest that the use of NSAIDs alone or in combination with corticosteroids could be more effective than using corticosteroids alone in the prevention and treatment of cystoid macular edema (Peter K, 1996; Eric D, 2012). Analgesics such as acetaminophen and non-steroidal anti-inflammatory drugs such as dexamethasone are given to overcome the pain and to control of corneal abrasion for 1–2 hourly use in the first few days of corneal surgery (Munish A, et al. 2013). According to the latest clinical study report, bromfenac 0.09% and nepafenac 0.1% were found well tolerated in the treatment of pain and inflammation after cataract surgery with positive outcomes (Bradley SL and Linda TM, 2013). However, NSAIDs and COX-2 inhibitors should be carefully used in colorectal surgery as they can increase the risk of anastomotic leak (Clifford LS, 2019).

1.6.3.5 Pros and Cons of conventional topical analgesics in postoperative treatment

Postoperative pain and irritation symptoms are relatively common during the first hours after surgery. Topical NSAIDs are presently considered as the standard choice of postoperative management along with antibiotics and corticosteroids. Topical NSAIDs are prescribed routinely by eye surgeons due to their safety and effective treatment in the management of postoperative pain and inflammation. Topical eye drops are available mostly in the form of solutions, ointments, and suspensions. The solutions are convenient to use and do not interfere with vision (Sheetu W, et al. 2009; Peng CC, et al. 2011). However, frequent administration is required for aqueous solutions due to poor ocular bioavailability and drug loss through the nasolacrimal drainage which may lead to extremely unwanted pharmacokinetics that may result in ocular and systemic toxicity (Peng CC, et al. 2011). Ointments suffer from poor patient acceptance because of blurred vision and matted eyelids, and suspensions lead to particle irritation, poor bioavailability, and changes in polymorphism and particle size upon storage (Patel A, et al. 2013; Shulin D, 1998) and due to poor ocular bioavailability, higher concentrations of the conventional form of analgesic used during surgery may result in ocular and systemic side-effects and thus suggesting for the necessity of new alternatives.

2.1 INTRODUCTION

In ancient times itself, people are aware of the importance and the sensitivity of eyes and is being given in the proverb from “Neeti Satakam” which explains the four key requisites of life by Chanakya quoting “Sarvasya gatrasya sirah pradhanam ***Sarvendriyanam Nayanam Pradhanam*** Shannam rasanam lavanam pradhanam Bhavet paneeyan udakam prdhanam” that explains the importance of head among the body, eyes among all the Senses, Salt among all the tastes and water among all the liquids (Panchajanya K and Devi, 2015).

The eye is protected by several means, for example, it is placed inside a tough bony cavity to save from external injuries, covered by eyelids and eyelashes to save from external particles and, other intrinsic defensive mechanisms, for example, blinking, tear film composition and rapid turnover and efflux transporters (Wikibooks, 2020). Despite all these protective means, the eye is a highly sensitive organ, there are chances where a little dust particle can create severe irritation, pain and discomfort or may results in infections upon attack by microbes and may get injured by other foreign bodies (Huang D, et al. 2017). Moreover, as the eyes are supplied with end arteries, continued by the optic nerve and have direct contact with the meningeal layers of the brain, most of the common eye problems may end up with sight-threatening conditions requiring emergency medical support and in some cases complete blindness if the diagnosis and treatment not done properly (Lauralee S, 2018).

2.2 DRAWBACKS OF CURRENT TREATMENT OPTIONS AND NEED FOR ALTERNATIVE

Mostly the treatments for eye disorders involve medical management where different drugs are administered either by topical (solutions, suspensions, ointments, creams and gels) oral (tablets and pills) or parenteral (IV and intraocular injections) routes and surgical procedures where incisions and laser are widely used (Peng CC, et al. 2011; Usman M, 2018).

Among the medical management options, the topical route is widely accepted due to ease of application, avoiding first-pass effect, ease of preparation, etc., (Le Bourlais C, et al. 1998; Maria SM, et al. 2018) while the oral and parenteral suffering

from drawbacks like serious systemic and ocular side effects, poor patient compliance and the surgical procedures are expensive and requires pre/postoperative care (Patel A, et al. 2013; Mansi K, 2017; Sarah B, 2019).

Though the topical dosage forms are preferable, the complex physiology of eye results into short residence time in tears, poor bioavailability, need for several daily administrations to get desired pharmacological action, reduction in the patient comfort levels leading to the reduced therapeutic and clinical efficacy of most of the eye products (Le Bourlais C, et al. 1998; Patel A, et al. 2013; Reimondez TS, et al. 2015; Riad K, et al. 2015; Wu Y, et al. 2019).

Therefore, it is needed to develop an alternative dosage form that resembles an eye drops, overcome the physiological barriers, involves an economical production and improves the patient compliance both in terms of comfort levels as well as reduced dosing frequency without compromising the therapeutic efficacy in fact with an expectation of improved therapeutic efficacy either by improving ocular absorption and bioavailability or by reducing the systemic absorption and hence reduced systemic side effects and or combination of both. The advantages, as well as the drawbacks that are associated with conventional therapies, are summarized in table 1.

Table – 1

Pros and cons of current conventional ocular treatment options

Conventional therapy Formulation	Advantages	Drawbacks
Eye solutions	Convenient to use and do not interfere with vision	Dilution by tears and aqueous humour, drainage into the nasolacrimal duct leads to multiple instillation of the drug during a day making patient compliance poor thus reducing the clinical efficacy of the drugs.
Ointments & gels	longer contact time and greater shelf life	Blurred vision and lack of patient compliance
Oral	Ease of administration and stability	Uncertain/minimal local effect. Due to poor ocular bioavailability, higher concentrations of drugs used may result in systemic side-effects
Invasive methods	Targeting	Lack of patient compliance, expensive procedures

3.1 REVIEW OF LITERATURE ON EMULSION AND EMULSION BAED SYSTEMS

Different researchers have been developed ocular nanoemulsions and novel nanoemulsion based systems to improve the bioavailability of various drugs for ocular use through topical administration as novel eye drops and a few of them are described in brief hereunder.

3.1.1 Emulsions

Yamaguchi M, et al. (2005) Prepared difluprednate ocular nanoemulsion using 0.05% w/v difluprednate 5.0% w/v castor oil and 4.0% w/v polysorbate 80. The lipid emulsion formulation had a mean droplet diameter of 104.4 nm. The formulation was stable for 6 months at 40 °C with no phase separation and no change in particle size. Studies revealed that the aqueous humor concentrations of difluprednate found 5.7-fold higher in the case of the lipid emulsion formulation when compared to that of 0.05%, w/v ophthalmic suspension at 1 h after instillation.

Ammar HO, et al. (2009) formulated dorzolamide hydrochloride ocular nanoemulsions using a single-dose crossover design where, seventeen drug-loaded (2.22% w/w) nanoemulsions were prepared using isopropyl myristate, Tween 80, Cremophor EL and Propylene glycol as emulsion components and benzalkonium chloride (0.01% w/w) as a preservative. The nanoemulsions showed sustained drug release compared to the drug solution and marketed product with mean droplet diameter of 8.4–12.7 nm, viscosity of 4.19□±□0.29 to 9.24□±□0.11, a refractive index of 1.356□±□0.002 to 1.358□±□0.001, the surface tension of 44.1□±□0.28 to 51.9□±□0.40, pH of 4.18□±□0.42 to 6.66□±□0.53 and Osmolarity of 500□±□36 to 1,320□±□25. They concluded that the physicochemical properties of dorzolamide hydrochloride ocular nanoemulsions found suitable for ophthalmic use with better thermodynamic stability and showed higher therapeutic efficacy, faster onset of action and prolonged effect when tested in normal tensive albino rabbits.

Lallemand F et al. (2012) developed cyclosporine A cationic ocular nanoemulsion using cyclosporine A (0.05 to 0.2 % w/w) as active ingredient, medium-chain triglyceride (1 to 2 %w/w) as Internal phase, Cetalkonium chloride (0.005 %w/w) as Cationic agent Tylopaxol (0.2 %w/w) and Poloxamer 188 (0.01

%w/w) as Surfactants Glycerol(1.5 to 2.5 %w/w) as Osmotic agent NaOH as pH adjuster and External phase(up to 100 %w/w). The formulations showed a mean droplet diameter of 150 to 300 nm, a viscosity of 1.1 m^2/s, pH 5.5–7, the zeta potential of +20 to +40 mV, surface tension 41 mN/m and 180 to 300 mOsm/kg. The researchers reported that the 0.2% cyclosporine A cationic emulsion showed 2 times greater drug delivery to the ocular tissues when compared to an anionic emulsion which contained 0.01% BAK and 0.2% deoxycholic acid as a mild detergent. Further, single and multiple dosing with cyclosporine cationic emulsions (0.05%w/w and 0.1 %w/w) was compared to Restasis, an anionic emulsion of 0.05%w/w cyclosporine (Allergan). Single-dose PK data confirmed the improved corneal absorption of 0.05% cationic emulsion (Cmax of 1372 ng/g and AUC of 26477ng/g.h) when compared to Restasis (Cmax of 748 ng/g and AUC of 14210 ng/g.h).

Morsi NM, et al. (2014) formulated acetazolamide ocular nanoemulsions using isopropyl myristate, Oleic acid, peanut oil, tween 80 cremophor EL and transcutol P as nanoemulsion components where 1 %w/w ACZ was sonicated with different surfactant/cosurfactant/oil blends, DMSO was used as cosolvent and benzalkonium chloride (0.02%w/w) as a preservative. The formulations showed a mean droplet diameter of 23.81 to 90.26 nm, a viscosity of 157 to 2736 Cp, a refractive index of 1.347 to 1.366, the surface tension of 32.5 - 38.7 mN/m, pH of 4.9 to 5.5 to 6.66□±□0.53 and osmolality of 860 to 1750 mOsm/kg. However, the formulation with a composition of 3 peanut oil, 25.65%w/w Tween 80, 25.65%w/w Cremophor EL, 5.7%w/w transcutol P and 39%w/w water found to be with acceptable physicochemical properties, highest drug release rate, better thermodynamic stability, 6 times higher drug permeability coefficient compared with reported permeability coefficient of acetazolamide, found non-irritant to rabbits' eyes and showed highest therapeutic activity with 1.8 and 2 times higher AUC compared to marketed eye drops and oral products respectively.

Chi-Hsien L, et al. (2015) developed lutein (a macular pigment that helps to maintain the eye health) loaded ocular nanoemulsions using corn oil (1.8 g), transcutol (0.2g), Span 20 (3g), Pluronic F68 (2g) and cyclodextrin (2g) and water (72g) and found improved stability, entrapment efficacy and enhanced scleral lutein accumulation. They further studied the combined effect of lutein loaded ocular

nanoemulsions with cyclodextrins on their partition coefficients in the porcine sclera by analyzing the penetration depth of lutein. A combination of nanoemulsion and 2% hydroxyl ethyl β-cyclodextrin found with 119±6 µg/ g/h i.e., 9.2-fold higher lutein accumulation in the porcine sclera when compared to the lutein suspension alone.

Li X, et al. (2016) developed dexamethasone acetate and polymyxin B sulfate ocular nanoemulsion with a concept of combinatorial formulation using Eutanol: G-Lipoid S 100 (70:30), 0.01%w/w cetylpyridinium chloride and 2.6%w/w glycerol as nanoemulsion components where 0.05%w/w of dexamethasone and 0.1%w/w of polymyxin B were loaded to yield a combination product. The optimized combination product had a pH of 5.31, a viscosity of 2.45 mPas, and an osmolality of 374 mOsm/kg. The formulation had high stability and shelf life for 180 days of storage at 4 °C and room temperature and showed no potential cytotoxicity in the *in vitro* cytotoxicity test.

Panatier LF, et al. (2016) developed ocular nanoemulsion by incorporating 5-methoxy-6, 7-methylenedioxycoumarin extracted from Pterocaulon balansae using medium-chain triglycerides and egg-lecithin as nanoemulsion components. The nanoemulsions found to have a mean droplet diameter of 200 to 300 nm regardless of the amount of extract incorporated (1.0–5.0 mg/ml). However, the concentration of coumarin and time of incubation resulted in variation in the effect on trophozoite viability. The formulation composed at 1.25 mg/ml of coumarins showed a 95% reduction of trophozoite viability after a 24 h incubation period which found similar to that of chlorhexidine indicating the potential of the formulations for the treatment of ocular keratitis caused by Acanthamoeba.

Soltani S, et al. (2016) formulated ketotifen fumarate ocular nanoemulsion where the nanoemulsions showed increased permeability, entrapment efficiency, positive zeta potential and improved patient compliance with reduced dosing frequency. The formulations showed a mean droplet diameter of 182 to 314.30 nm, encapsulation efficiency up to 95% and 65.51% to 88.82% of drug release at 24 hr. The mechanism of drug release for the best formulation was found to be a fickian diffusion mechanism. Formulations containing high polymer concentration (1:15) offered a faster drug release and a higher drug penetration whereas those formulations

with low polymer concentration (1:7.5) offered the sustained release of the drug and slower drug permeation through the cornea.

Moghimipour E, et al. (2017) Prepared celecoxib ocular nanoemulsion for using transcutol P, oleic acid, tween 80, span 20 and propylene glycol as nanoemulsion components. Eight nanoemulsion formulations were prepared using a factorial design where 3 variables at 2 levels are applied. The formulations showed a mean droplet diameter of 6.96 to 26.65 nm, a viscosity of 118-245 Cp, a pH of 6.5 to 6.9, a percentage drug release of 82.6% at 24 h and percentage drug permeation through rabbit cornea of 6.1 to 15.73%. The researchers reported that with a change in nanoemulsion components their properties like partitioning, flux, and permeability coefficient were significantly altered and the optimized formulation had a permeability coefficient of 0.13 cm/h which is 7.23 times higher and flux of 0.65 mg cm2 /h which is 21.68 times higher than that of control 1% drug suspension.

Mahboobian MM, et al. (2017) developed brinzolamide ocular nanoemulsions using Brij 35 and Tyloxapol, Triacetin, Cremophore RH40, Labrasol, Transcutol P and Capryol90 as nanoemulsion components where 0.4%w/w incorporated into oil and surfactant to the co-surfactant mixture at 5% and 20%w/w respectively by applying cross-over design study. The formulations showed a mean droplet diameter of 7.53 to 48.67 nm, viscosity of 2.74-23.96 cP, a refractive index of 1.367-1.384, pH of 5.89 to 6.56 to 6.66□±□0.53 and osmolality of 645.3 to 1551 mOsm/kg. The brinzolamide ocular nanoemulsions showed sustained release of drug with enhanced ocular bioavailability where the optimized formulation with a composition of triacetin, labrasol and transcutol-P had higher $AUC_{(0\text{-}6h)}$ value (129.60±11.53) compared to that of the marketed product, Azopt, 1% aqueous suspension (97.00±7.92) indicating improved therapeutic efficacy.

Kalpesh CA, et al. (2017) developed Chloramphenicol ocular emulsion by applying constrained simplex-centroid design using mixtures of Neem Oil, olive oil and Peppermint oil and PEG 400 as nanoemulsion components where 1% chloramphenicol, 5-19% oil mixtures, 25-39% water, 55-69% PEG-400 were used. The nanoemulsions showed globule size in the range of 15±0.1 to 55±1 μM. The optimized formulation had a composition of water: PEG-400: optimized oil mixture in the ratio of 34.02:58.67:7.31 with pH 7.4 and osmolarity of 3.09 mOsm/L. However,

the tonicity was adjusted using 0.799 g/100 ml NaCl. chloramphenicol emulsion showed relatively higher drug permeability across both Cellophane and goat ocular membrane compared to Chloramphenicol eye caps with 0 score for ocular irritation.

Shah J, et al. (2019) prepared moxifloxacin ocular nanoemulsions using mixtures of tween 80, soluphor® P, ethyl oleate and water and were chosen by testing their solubility in oil, surfactants, and cosurfactants. Seven formulations were developed by applying simplex lattice design and assessed the influence of formulation parameters. The formulations showed a mean droplet diameter of 32.41 to 81.04nm, a viscosity of 3.28 to 6.50 and a pH of 7.04 to 6.19. The optimized formulation exhibited 100% drug release within 3 h and had a flux value of 32.01 µg/cm2 /h across the corneal membrane which was comparable with that of control (31.53 µg/cm2 /h). However, the aqueous humour concentrations of the drug from nanoemulsion found greater with Cmax; 555.73 ± 133.34 ng/ml and delayed Tmax(2h) indicating the reduction in dosing frequency and better therapeutic efficacy than control. Also, the $AUC_{(0\text{-}8h)}$ (1859.76 ± 424.51 ng·h/ml) was found 2 times higher than control with $p < 0.0005$ confirmed the significantly improved aqueous humour bioavailability along with good tolerance revealed from the ocular irritation study. The optimized moxifloxacin nanoemulsion found stable for 3 months without significant changes in physicochemical properties when stored under refrigerator temperature.

3.1.2 Emulsion based system

Ammar HO, et al. (2010) developed dorzolamide thermosensitive hydrochloride ocular nanoemulsion using triacetin, Miranol C2M, Poloxamers and water as nanoemulsion components; 0.884% NaCl used as tonicity adjuster and 0.01% benzalkonium chloride as preservative. The in situ gel was prepared by dispersing the optimized nanoemulsion in the aqueous solutions of Poloxamer 407 which is having the tendency of thermoreversible gelation and also surface-active properties thus increasing the viscosity of the formulation and hence prolonged drug residence time and therapeutic effect.the formulation with a composition of 4.55% Miranol C2M, 7.80% triacetin, 13.65% Poloxamer 407, 3.41% Poloxamer 188 and 70.59% water was found optimum due to its better biological performance, faster

onset of action, and prolonged effect and also a superior pharmacodynamic activity compared to drug solution as well as the marketed product in albino rabbits.

Pathak MK, et al. (2013) developed pH triggered fluconazole ocular nano emulsified in-situ using capmul MCM, Tween 80 and transcutol P as nanoemulsion components. The nanoemulsion was further transformed into nano emulsified sols by dispersing in the solution of carbopol 934 which can convert into in-situ gels at corneal pH 7.4. The optimized formulation was showed greater drug permeation across the corneal membrane with greater flux value (419.30 µg/cm2) compared to control formulation (112.92 µg/cm2) and found non-irritant along with an extended residence time more than 6 h resulted into reduced dosing frequency hence, improved patient compliance.

Wang SJ, et al. (2013) developed cyclosporine A ocular nanoemulsion using 0.5 to 9.79%w/w of polyethoxylated castor oil as a solubilizer, 90 to 99.29%w/w of phosphate buffer as an aqueous medium, 0.1 to 3%w/w ethanol as co-solvent, 0.1 to 3%w/w glycerine and 0.1 to 5%w/w sodium hyaluronate as thickening agents where 0.01 to 1%w/w of cyclosporine was incorporated. The optimized formulation had a composition of 0.05%w/w cyclosporine, 4%w/w polyethoxylated castor oil, 1 %w/w ethanol, 2.2%w/w glycerine, 0.1%w/w sodium hyaluronate, quantity sufficient of phosphate buffer and showed mean droplet diameter of 17.4nm, pH of 7.2 and osmolarity of 300 mOsm/l. The optimized nanoemulsions were mixed with in situ hydrogel matrix which was prepared by allowing gellan gum to swell overnight in water.incorporated in situ hydrogel system. The formulation with higher gellan gum concentration showed a slower drug release rate comparatively and found stable up to 4 weeks without any significant changes in the particle size.

Saadia TA, et al. (2014) developed terbinafine hydrochloride ion-sensitive ocular nanoemulsion in situ gels using 5% isopropyl myristate, 5% Miglyol® 812, a mixture of Cremophor® EL and PEG 400 at 1:2 ratio and 40% water as nanoemulsion components and gellan gum as gelling agent where 0.5%w/w drug was loaded into the emulsion. The drug pharmacokinetics in rabbit aqueous humour were evaluated by applying a non-blind, two-treatment, randomized, parallel design where, the formulation showed controlled drug release and significantly ($P < 0.01$) improved the

ocular drug bioavailability with higher Cmax, delayed Tmax, prolonged mean residence time when compared to oily drug solution.

Kim HS, et.al. (2016) evaluated the clinical efficacy and safety of 0.05% cyclosporine A ocular nanoemulsion (Clacier™) and compared with that of commercially available 0.05% cyclosporine A conventional emulsion (Restasis®). The patients under the treatment of severe to moderate Dry Eye Syndrome were randomly divided into two groups and administered topically one group with Clacier and the other with Restasis twice daily for 12 weeks and at the end of the 12th week, the corneal staining scores were recorded. The corneal staining scores were improved in patients received Clacier and also Restasis and are comparable to each other. The alleviated clinical signs and symptoms of Dry Eye Syndrome in the patients resembled that of the commercially available Restasis. However, a faster improvement in the tear film stability was reported in the case of Clacier. Further, the prepared nanoemulsion did not increase the risk of adverse events.

Patel N, et al. (2016) developed loteprednol etabonate thermosensitive cationic ocular nano emulsified in-situ ophthalmic gel by applying 3^2 factorial design and using Capryol 90 as oil phase), tween 80 as a surfactant and transcutol P as cosurfactant where 0.5%w/w drug was loaded and were preserved using 0.01%w/w of benzalkonium chloride. The prepared nanoemulsions were optimized using nanoemulsion and it was optimized using 3^2 factorial design. The optimized nanoemulsion was further transformed into nano emulsified sols by dispersing in the solution of 407 and Poloxamer 188 which can converts into in-situ gels at corneal temperature. The formulations showed mean droplet diameter of 15.32 ± 1.3 to 53.45 ± 0.8 nm, viscosity 2400–1400 cPs, refractive index of 1.366 ± 0.04 to 1.430 ± 0.02, pH of 5.44 to 6.49, Osmolarity of 223.18 to 260.23 mOsmol/L and drug content of 99.24 to 96.90%. The optimized nanoemulsion had a composition of 0.69% w/w oil, 0.99%w/w Smix in 1:3 ratio, 13% Poloxamer 407 and 4% w/w Poloxamer 188 were showed mean globule size 41.50 ± 0.4 nm PDI of 0.64, zeta potential of 31.22 ± 2.5 mV, 1.352 ± 0.04, % transmittance of 99.05 ± 0.20, PH of 5.70 ± 0.29 osmolarity 280.16 mOsmol/L and drug content of 99.05 ± 0.10%. When incorporated into the polymeric solution, the optimized nanoemulsion had mean globule size of 70.85 ± 0.25 nm, PDI of 0.45, the zeta potential of 31.65 ± 1.9 mV, a refractive index of 1.387

± 0.07, % transmittance of 98.20 ± 0.45, PH of 7.40 ± 0.29, the osmolarity of 262.16 mOsmol/L and drug content of 98.82 ± 0.30 %. The drug release kinetics revealed that the optimized nanoemulsion incorporated in-situ gels showed higher Cmax and $AUC_{(0–10\ h)}$ delayed Tmax extended mean residence time and 2.54 fold improved drug bioavailability in the rabbit aqueous humour compared to marketed formulation.

4.1 THE RATIONALE FOR THE SELECTION OF DOSAGE FORM

4.1.1 Topical ophthalmic delivery systems

Eye drops as drug solutions are convenient to use, and they do not interfere with the vision of patients while the longer contact time and superior drug stability are the advantages that the ointments and the eye gels have in common (Gooch N, et al. 2012).

However, the bioavailability of topical ophthalmic dosage forms is affected by several factors like tear turnover, limited capacity of the cul-de-sac, dilution by tears, conjunctival surface and nasolacrimal drainage that allow the drug to enter the systemic circulation result in side-effects coupled with drug loss. The drug accumulation in the ocular tissues due to binding to the proteins that limit the amount of free drug available to act and passage across the blood-ocular barrier.

Multiple instillations required in case of solutions and vision interruptions in case of ointments and gels, invasive methods in case of intraocular injections and incisional surgeries reduce patient compliance and hence the clinical efficacy of the drugs (Januleviciene I, et al. 2012).

4.1.2 Potential of nanoemulsions as topical ophthalmic delivery systems

Nanoemulsions are currently considered as potential drug delivery systems for topical ophthalmic use due to a variety of advantages in a vast area of interest.

Pharmaceutical considerations

They stand best in case of formulation aspects, that includes, a variety of drug molecules can be chosen due to its capacity to solubilize both hydrophilic as well as lipophilic molecules, the effortless and the economical preparation methods, the excellent long-term stability and can be sterilized easily by filtration thus reduces the cost of production (Gurpreet K, et al. 2018).

Therapeutic efficacy

They stand best in case of pharmacological aspects that include the low toxicity and irritancy; smaller droplet size, hence, the large effective surface area and

improved permeability collectively promote corneal absorption thus a significant improvement in ocular bioavailability (Ammar HO, et al. 2009).

Clinical efficacy

Finally, they stand best in case of consumer benefit effects that include, due to low viscosity they can be administered as easy as normal eye drops without any visual interference, sustained release of a drug can decrease the frequency of application without compromising the therapeutic efficacy thus improves the patient acceptability and comfort levels (Lallemand F, et al. 2012).

4.1.3 Nanoemulsions in overcoming the limitations of conventional dosage forms

Nanoemulsions overcome the limitations of conventional dosage forms by enhancing the drug solubility, acting as bionic tear film, enhancing the precorneal residence time on the ocular surface and enhancing the corneal penetration.

Enhancing the drug solubility

Because of the poor solubility, the lipophilic drugs cannot be delivered as aqueous solutions Also the water-soluble drugs when administered as aqueous solutions, they suffer from the dosage form based drawbacks like frequent instillations, low bioavailability and unwanted systemic effects due to noncorneal absorption pathways and nasolacrimal drainage. As the nanoemulsions can incorporate both lipophilic and hydrophilic drugs, both can be formulated into emulsions or micro/nanoemulsions which can increase the drug loading. Besides upon instillation, the drug encapsulated droplets act as drug depots to continuously release the drug to the tear film as the drug gets absorbed into the corneal epithelium (Li G, et al. 2013; Peng CC, et al. 2011).

Acting as bionic tear film

The tear film is composed of three layers i.e., the outer lipid layer that lubricates the eyeball and protects the aqueous layer by preventing from evaporation; the middle aqueous layer that consists about 90% of the tear film maintaining the ocular surface even and supply oxygen to the corneal epithelial cells; and the inner mucous layer that changes the lipophilicity of the corneal epithelium from

hydrophobic to hydrophilic thus maintains the uniform distribution of the aqueous layer throughout the ocular surface.

As nanoemulsions have both the aqueous and oil phases, the properties similar to those of tear film, the phase of the emulsion can improve the aqueous layer of the tear film and can moisten the cornea. The oil phase can combine with the lipid layer of the tear film and aids in the reduction of fluid evaporation. The emulsifiers used in the preparation of nanoemulsions found to improve the wettability of the tear film and have excellent ocular biocompatibility. This property of nanoemulsion is highly applicable in the treatment of dry eye syndrome. Furthermore, the increased viscosity of nanoemulsions when compared to the ophthalmic solutions can increase in precorneal residence time in the tears (Li G, et al. 2013).

Enhancing the precorneal residence time on the ocular surface

As the oil components in the nanoemulsions interact with the lipid layer of the tear film they can enable the emulsion to stay for a long time in the conjunctival sac. Also when cationic surfactants are used to form positively charged droplets in the nanoemulsion, electrostatic interactions take place with the anionic corneal surface and reduces the contact angle which results in the increased spreading coefficient and hence improved wettability thus improve the precorneal residence time of the formulation (Li G, et al. 2013; Patel A, et al. 2013).

It is also reported that nanoemulsions can prolong the preocular residence time when mucoadhesive polymers are used to form insitu gels or bioadhesive liposomes.

Enhancing the corneal permeation

The non-ionic surfactants can open the tight junctions of the corneal epithelium which renders the topically applied drugs impermeable to it. It is also reported that the incorporation of the P-glycoprotein inhibitors can enhance drug penetration by inhibiting the anti-adherent effects of the P-glycoprotein inhibitors present on the ocular epithelial cells. Further, the incorporation of penetration enhances aid in the corneal permeability (Li G, et al. 2013; Hegde RR, et al. 2013).

4.2 RATIONALE FOR DRUG SELECTION

The major eye disorders are being treated either by surgical or non surgical medication. When it comes to non surgical medication, topical ocular products are preferred and beneficiary, however, the current commercial topical ophthalmic products found to face challenges to reach the ocular environment due to different physiological, anatomical and dosage form related aspects. In the present study, three major eye problems including Glaucoma, Eye infections and Postoperative treatment were studied. Based on the available literature, it is evident that the usage of the conventional topical eye drops as well as systemic dosage forms of beta blockers, antibiotics and NSAIDs will leads to severe systemic and ocular side effects. Hence a single drug for each eye problem which include Timolol maleate, a beta blocker with well established record of first line usage in anti glaucoma therapy, Ciprofloxacin Hydrochloride, a broad spectrum antibiotic proved to be effective against various bacterial eye infections and Indomethacin, an NSAID with proven ocular usage in pre and post operative treatment regimen were chosen.

Due to the solubility of timolol maleate and ciprofloxacin hydrochloride in water, the conventional dosage forms mostly formulated into simple aqueous solutions and hence suffer from poor bioavailability and side effects. A vast research is underway using these drugs as a monotherapy or in combinations. The available conventional topical dosage forms are of mostly solutions, suspensions, ointments, gels and a few gel forming solutions with their own advantages and drawbacks as well and many of times with severe proven side effects. So it is necessary to develop alternative dosage forms that will improve ocular absorption, reduce the systemic absorption, and thus prevent systemic/ocular side effects and improve ocular therapy. While in the case of indomethacin, as it is a lipophilic moiety, it becomes difficult to formulate into aqueous-based dosage forms, hence, mostly available as topical polymeric solutions/suspensions, oral dosage forms like pills/capsules/suspensions and suffer from poor ocular bioavailability and side effects. To our knowledge, though there is a vast research done on different alternative topical dosage forms for indomethacin like oily suspensions and nano particulate colloidal systems, very few commercial products are available as topical eye drops mostly as polymeric solution/suspension being marketed in a very few countries mostly of Europe.

Hence to overcome the drawbacks of conventional dosage forms, these three drugs were selected for the study to check the possibilities of enhancing their bioavailability by mostly focusing on the prolonged preocular residence time by increasing viscosity of the formulation, increasing lipophilicity/ solubility of drugs.

Drug Profile

Drug-I, Timolol Maleate

Timolol Maleate is the maleate salt form of timolol, a non-selective ß-blocking agent known for its antihypertensive property available for both systemic and ophthalmic use for treating arrhythmia and glaucoma respectively as monotherapy or in combinations (Costagliola C, et al, 2009). The systemic use of Timolol maleate reduces hypertension by decreasing the heart rate and cardiac output by binding to the beta-1 adrenergic receptors found in the heart and vascular smooth muscle; vasodilatation by binding to the beta-2-adrenergic receptors found in the bronchial and vascular smooth muscle help prevent the recurrence of myocardial infarction and also found useful preventing migraines (Costagliola C, et al, 2009; Toris C, 2010).

Timolol has been said to be a gold standard drug and emerged as an important warhead in glaucoma treatment due to its ability to reduce the intraocular pressure mainly by reducing the blood flow to the ciliary processes and cAMP synthesis with less ocular side effects when compared to other antiglaucoma drugs (Kamal Singh R, et al. 2010).

Marketed eye products

The marketed eye products of Timolol as monotherapy include Timolol Osl, Timoptic, Istalolol, Betimol, etc., and in combination with other antiglaucoma drugs include dorzolamide + timolol (Cosopt), brinzolamide + timolol (Azarga) which are carbonic anhydrase inhibitors and brimonidine + timolol (Combigan) which is an alpha, -2 selective adrenergic agonist.

Ophthalmic Dosage

Initially, one drop of 0.25% timolol maleate ophthalmic solution is recommended twice a day and when the adequate drug response is not seen with this

dosage, one drop of 0.5% solution twice a day will be recommended (Brown & Burk UK Ltd., 2020; Rising Pharmaceuticals, Inc., 2019).

Possible side effects

Timolol maleate is available as an aqueous solution in the form of eye drops and shows some minor side effects like eye irritation, blurred vision, headache, depression, nausea, etc., after using Timolol maleate ophthalmic solution which can be disappeared in some time and the major side effects like irregular heartbeat, breathlessness, weight gain, dizziness, swelling on legs, etc which are life-threatening if not treated immediately (Pacific Pharma, Inc., 2019)

Drug-II, Ciprofloxacin Hydrochloride

Ciprofloxacin is a fluoroquinolone broad spectrum antibiotic with high potent bactericidal activity against a wide range of bacteria including both gram-positive and gram-negative; and used for the treatment of various bacterial infections (Rita G, 2019). Ciprofloxacin ophthalmic use is intended to treat bacterial eye infections including conjunctivitis and corneal ulcers. Ophthalmic ciprofloxacin comes as a topical solution and an ointment (to treat conjunctivitis) (ASHP, 2018).

Marketed products for eye infection

Ciprofloxacin mostly in its hydrochloride salt form is available as Eye drops, eye ointment and oral tablet either as monotherapy or in combination for the treatment of ocular infections and are sold under various brand names for example, Eye drops-Ciloxan, Civox, Ciplox (Ciprofloxacin+ Carbamazepine), Beuflox (ciprofloxacin + dexamethasone), etc., eye ointment-Aprocin and oral tablet-Bactin, Cipro R , Dumaflox, etc.

Ophthalmic Dosage

Ciprofloxacin ophthalmic solution is recommended to apply once every 15 minutes to once every 4 hours and the treatment extends for 7 to 14 days or longer based on the disease condition while Ciprofloxacin ophthalmic ointment is recommended to apply 3 times a day for 2 days followed by twice a day for 5 days (ASHP, 2018) while oral tablets are usually prescribed with a dosage of 500-750 mg 12 hourly for 7-14 days (IBN SINA, 2014).

Possible side effects

The common side effects of ophthalmic use of ciprofloxacin include eye pain or discomfort, dry eyes or watery eyes, blurred vision, red or puffy eyelids, mild itching or irritation. The major signs of an allergic reaction may include severe discomfort and burning in the eyes, eye swelling in the face, lips, tongue, or throat, after using, redness, crusting or drainage and breathing difficulty (Drugs.com, 2019&2020; IBN SINA, 2014).

Drug-III, Indomethacin

Indomethacin is a nonsteroidal anti-inflammatory drug that works by inhibiting the cyclooxygenase which catalyzes the prostaglandin production thus fights the inflammation and commonly prescribed for reducing pain, fever, stiffness, and swelling (Brayfield A, 2014).

The topically applied indomethacin is mainly used to manage and prevent ocular pain and inflammation, macular edema followed by cataract surgery and to maintain mydriasis throughout cataract surgery (Halim Mohamed MA and Mahmoud AA, 2011; Cho H, et al. 2009). The other common uses include reducing discomfort after refractive surgery and also used along with antibiotics to treat allergic conjunctivitis (Bucolo C, et al. 2011).

Marketed products for ophthalmic use

Eye drops either in polymeric suspension or polymeric solution are available for ocular use of Indomethacin. Indom is the topical ophthalmic polymeric suspension by Alfa-Intes and Indocollyre ™ is the topical ophthalmic polymeric solution of indomethacin by Bosch& Lomb. However, many brands available for systemic medication like oral suspension (Indocin) and tablets or capsules for example, Indoflam ™, Indocid ™, Indocap ™, etc.

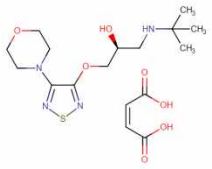
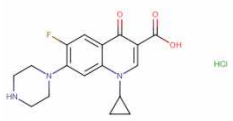
Possible side effects

The possible side effects of eye products include conjunctive redness itching and burning, increased intraocular pressure, edema in cornea and eyelids corneal epithelium alterations, and streaked keratitis (Alfa Intes Industry Terapeutic S.R.L., 2007)

Usually indomethacin products are sold with boxed warning as the drug carries significant risk of serious or even life threatening adverse effects. Indomethacin can increase the risk of fatal heart attack or stroke, even in the absence of other risk factors and can cause an increased risk of serious gastrointestinal adverse events which can be fatal. It is dangerous if indomethacin is used by one who is allergic to it, or has ever had an asthma attack or severe allergic reaction after taking an NSAID for example aspirin (Iroko Pharmaceuticals, 2018).

Table – 2

Basic profile of selected drugs

Character	Timolol Maleate (Oral, topical)	Ciprofloxacin (Oral, intravenous and topical)	Indomethacin (Oral, rectal, intravenous and topical)
Chemical structure And IUPAC name	 (2S)-1-(tert-butylamino)-3-{[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy}propan-2-ol; (2Z)-but-2-enedioic acid	 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride	 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid
Physico-chemical	Formula:$C_{17}H_{28}N_4O_7S$ MWt: 432.49 g/mol M.P: 202- 203°C Log P: 1.8	Formula: $C_{17}H_{18}FN_3O_3$ MWt: 331.346 g/mol M.P: 255- 257° C Log P: 0.28	Formula: $C_{19}H_{16}ClNO_4$ MWt: 357.787 g/mol M.P:155- 162°C Log P: 4.27
Pharmaco-kinetic	Bioavailabilty: 60% Metabolism: Hepatic (80%) Elimination half life: 2.5-5 hours Excretion: Renal	Bioavailabilty: 70% Metabolism: Hepatic Elimination half life: 3.5 hours Excretion: Renal	Bioavailabilty: 100% oral Metabolism: Hepatic Elimination half life: 2.6-11.2 hours Excretion: Renal (60%), fecal (33%)
Water solubility	Slightly Soluble, 2.74 mg/ml	Sparingly Soluble, 30 mg/ml (at 20 °C)	Practically insoluble, 0.937 mg/L (at 25 °C)
Mechanism of action	Blocks both β-1 and β-2 adrenergic receptors, reduces IOP by reducing aqueous humor production	Bactericidal, inhibits the enzyme bacterial DNA gyrase and prevents replication of bacterial DNA	Inhibits two isoforms of COX, COX-1 and COX-2, with greater selectivity for COX-1

4.3 RATIONALE FOR THE SELECTION OF FORMULATION COMPONENTS

4.3.1 Oils

The oils used in this study were the medium-chain triglycerides and their derivatives which can improve solubility, permeability and bioavailability of actives.

4.3.1.1 Capryol 90

Description: Capryol 90, chemically known as Propylene glycol Monocaprylate is a Colorless oily liquid with little or no odor.
Melting point: 3 °C
Solubility: Water-insoluble.
HLB value: 6
Uses: Capryol 90 is used as a nonionic surfactant/co-surfactant/ emulsifier/ solubilizer and found applicable for emulsions/ microemulsions based oral or topical dosage forms including eye drops and nasal drops.

4.3.1.2 Captex 355

Description: Captex 355, chemically known as Glyceryl Tricaprylate/Tricaprate is colorless to the slightly yellowish oily liquid with no odor.
Melting point: 55 °C
Solubility: Insoluble in water.
HLB value: 10
Uses: Captex 355 is a mixture of triglycerides of saturated fatty acids, mainly of caprylic acid and of capric acid used in the preparation formulations such as oral emulsions, microemulsion, self-emulsifying systems.

4.3.1.3 Captex 200

Description: Captex chemically known as propylene glycol dicaprylate/dicaprate is a clear, odorless, and colorless to light yellow oily liquid.
Melting point: NA
Solubility: Insoluble in water.
HLB value: 13

Uses: Captex 200 used as lubricant, emollient, non-irritant moisturizing agent and solubilizer mostly in skincare creams, lotions and other cosmetic products. It is usually associated with low viscosity, very good stability, and low irritation.

4.3.1.4 Captex 8000

Description: Captex 8000 chemically known as glyceryl tricaprylate is odorless Clear liquid.
Boiling Point: >260°C
Solubility: Insoluble in water
HLB value: 13
Uses: Preferred lipophilic vehicle /bioavailability enhancer, carrier, solubilizer, emollient, lubricant, viscosity modifier in various delivery systems.

4.3.1.5 Labrafil M 1944

Description: Labrafil M 1944, chemically known as Oleoyl polyoxyl-6 glycerides is a yellow color liquid with faint no odor obtained from partial hydrolysis and esterification of kernel oil
Melting point: 44 °C
Solubility: Dispersible in water.
HLB value: 10
Uses: Labrafil M 1944 is used as a solubilizer for actives in lipid-based emulsions/ microemulsions that found applicable for oral or topical drug delivery.

4.3.1.6 Labrafil M 2125

Description: Labrafil M 2125, chemically known as Oleoyl Corn oil-PEG-6 esters is a yellow colored liquid with weak, characteristic odor obtained from partial hydrolysis and esterification of kernel oil.
Melting point: 44 °C
Solubility: Dispersible in water
HLB value: 9
Uses: Labrafil M 2125 consists of mono, di and tri unsaturated polyglycolyzed glycerides used as a nonionic surfactant/ solubilizer/ bioavailability enhancer for lipid-based formulations.

4.3.1.7 Capmul PG 8 NF

Description: Chemically it is an octanoic acid ester with 1,2-propanediol, clear liquid with fatty odor.
Melting point: 44 °C
Solubility: Partially soluble in water.
HLB value: 5-6
Uses: As a solvent/ viscosity modifier/ penetration enhancer/ bioavailability enhancer/ carrier in pharmaceutical formulations mostly to produce stable emulsions.

4.3.1.8 Capmul MCM C8

Description: Capmul MCM C8, chemically known as glyceryl monocaprylate is a Liquid or soft solid medium-chain fatty acid i.e., mono and diglyceride of caprylic acid with a slightly fatty odor.
Melting point: 16.7 °C
Solubility: Partially soluble in water.
HLB value: 5-6
Uses: Carrier/ solubilizer/ emulsifier/ co-emulsifier/ bioavailability enhancer/ penetration enhancer particularly in dermatological water-oil systems.

4.3.1.9 Caprol® Microexpress

Description: It is a patent-pending blend of different emulsifiers and emollients such as Caprylic/Capric Glycerides, PEG-6 Caprylic/Capric Glycerides, Polyglyceryl-6 Dioleate and Polyglyceryl-3 Oleate that form a clear nonsticky microemulsion when mixed with water.
Appearance: Liquid
Solubility: Water soluble.
Uses: Caprol® Microexpress acts as an emulsifier, solubilizer and carrier of actives, fragrances, and other water or oil soluble products mostly used in creams, lotions, sun care, perfume and cosmetic products.

4.3.1.10 Capmul GMO-50

Description: Chemically it is glyceryl monooleate, clear to opaque; light yellow to yellow liquid / semi-solid with a bland, fatty odor.

Melting point: 57–65 °C

pH: 4.5-5.5

Solubility: Insoluble at room temperature but dispersible in hot water.

HLB value: 3-4

Uses: Commonly used as a food additive as a thickening agent and can be used alone as a primary solubilizer, or in conjunction as an emulsifier with other emulsifiers.

4.3.1.11 Acconon-E

Description: Acconon-E is chemically Polypropoxylated stearyl alcohol, colorless oily liquid Mild soapy odor low irritation.

Melting point: Not determined

Solubility: Insoluble in water.

HLB value: 6-8

Uses: Used as an emulsifier in microemulsion/ emollient/ dispersing agent/ moisturizer and solubilizer in different formulations like cleansing agents, specifically in baby and sensitive skin cleansing lotions and creams, low irritation shampoos.

4.3.2 Surfactants and co-surfactants

The nonionic surfactants and co-surfactants were chosen for the study mainly based on the criteria of less toxicity or no toxicity and those can produce stable nanoemulsions.

4.3.2.1 Tween 80

Description: Tween 80 chemically Polyoxyethylene 20 sorbitan monooleate a viscous, yellow liquid with a characteristic odor.

Boiling point: >100 °C

Solubility: Water soluble.

HLB value: 15.0

Uses: Tween 80 is a non-ionic surfactant, widely used as solubilizing agent /emulsifier in pharmaceuticals, food products and cosmetics industry mostly in facial cleansers and hair care products.

4.3.2.2 Tween 20

Description: chemically Polyoxyethylene 20 sorbitan monolaurate clear, yellow to yellow-green viscous liquid faint characteristic odor.
Melting point: 98.9 °C
Solubility: Water soluble.
HLB value: 3-4.
Uses: Polysorbate 20 is used as a wetting agent/ dispersing agent/ w/o type emulsifier in foods, cosmetics and pharmaceuticals.

4.3.2.3 Tween 40

Description: Tween 40 chemically polyoxyethylene sorbitan monopalmitate odorless amber colored oily liquid.
Melting point: 0.1 °C
Solubility: Soluble in water.
HLB value: l5.6
Uses: Tween 40 is an effective emulsifier also used as a solubilizer, stabilizer and fiber lubricant.

4.3.2.4 Span 80

Description: Chemically span 80 is sorbitan monooleate, odorless, yellow and viscous liquid.
Boiling point: 463.43°C
Solubility: Soluble in organic solvents but dispersible in water.
HLB value: 4.3
Uses: Used as w/o type emulsifier/ solubilizer/ stabilizer/ softener in cosmetic and pharmaceutical formulations. Also found applicable for oil field chemicals, plastics, textiles, paints, etc.

4.3.2.5 Span 20

Description: Sorbitan monolaurate, Span 20 is a non-toxic, odorless, and amber to brown viscous oily liquid.
Boiling point: 401.18°C
Solubility: Insoluble in water.

HLB value: 8.6

Uses: Span 20 is used as a wetting agent and lubricant in pharmaceuticals, cosmetics, textiles, etc and it acts as a water/oil type emulsifier when used in combination polysorbates particularly with its ethoxylated derivative Tween 20.

4.3.3 Viscosity modifiers/permeation enhancers

Polymers that can enhance the viscosity of the formulation and permeability of the actives with lubricating properties were chosen to prolong the residence time and to reduce the corneal irritation of the formulation.

4.3.3.1 PEG 400

Description: PEG 400 is chemically a low-molecular-weight polyethylene glycol frequently referred to as Carbowax is a thick, hygroscopic, and colorless liquid substance with a characteristic odor.

Melting point: 64-66 °C

Solubility: Soluble Water.

HLB value: 16

Uses: Due to its low toxicity, it is found as one of the ingredients in various drug formulations mainly for enhancing the solubility and bioavailability of poorly water-soluble drugs. For ophthalmic use, it can provide temporary relief from burning sensation and protect from irritation due to dryness of the eye.

4.3.3.2 PEG 200

Description: PEG-200, polyethylene glycol monostearate is a clear, viscous, colorless, odorless hygroscopic liquid polymer.

Melting point: 64-66 °C

Solubility: Soluble in water.

HLB value: 11.6

Uses: PEG 200 has broad applications such as surfactant/ solvent/ humectant/ lubricant/ viscosity modifier/ defoaming agent in a wide variety of products mostly in cosmetics and pharmaceuticals.

4.3.3.3 Propylene glycol

Description: Propylene glycol chemically 1,2-Dihydroxypropane, colorless, odorless and viscous liquid

Melting point: -59 °C

Solubility: Miscible with water.

HLB value: 3.4

Uses: Propylene glycol is used as a solvent, carrier, humectant, lubricant, permeation enhancer, viscosity modifier in various pharmaceutical preparations including oral, injectable and topical dosage forms and also used in foods such as caffeine contained drinks, ice creams and sweeteners.

4.3.4 Tonicity imparting agents

Based on the literature, glycerol was chosen as a tonicity imparting agent for topical nanoemulsions preparation as it enhances the tear film.

4.3.4.1 Glycerol

Description: Glycerol chemically 1, 2, 3-propanetriol is a simple polyol compound frequently referred to as Glycerin, colorless odorless hygroscopic liquid.

Melting point: 17.8°C

Solubility: Miscible in water.

HLB value: 3.8

Uses: Glycerol is used as preservative agent/ humectants/ lubricating agent pharmaceutical formulations such as in eye preparations. It founds its application in different FDA approved wound and burn treatments due to its antimicrobial and antiviral properties.

4.3.5 Preservative cum charge imparters

Quarternium ammonium chloride compounds were chosen for the study as they can act as preservatives and also can create a positive charge to the cationic nanoemulsions which aid in bioadhesive property towards the negatively charged ocular surface.

4.3.5.1 Benzalkonium chloride

Description: Chemically it is diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride, white crystalline powder with mild odor

Melting point: 34-37°C.

Solubility: Soluble in water, give soapy solution in acetone, ethanol and chloroform, slightly soluble in ether.

HLB value: 12

Uses: Frequently used as a preservative in eye drops mostly within the typical concentration of not more than 0.02% and also used as a cationic surfactant in nanoemulsions.

4.3.5.2 Cetalkonium chloride

Description: chemically it is Benzyldimethyl-n- hexadecyl ammonium chloride, white crystalline powder with mild odor.

Melting point: 50°C

Solubility: Soluble in water, give soapy solution in acetone, ethanol and chloroform, slightly soluble in ether.

HLB value: 12

Uses: Cetalkonium chloride is used in pharmaceutical products either as an active ingredient or as excipients.

Table – 3

Category and source of different materials

Category	Material	Source
Active pharmaceutical ingredient	Timolol Maleate/ Ciprofloxacin/ Indomethacin	Alphamed Formulations Pvt Ltd, Aliabad, India/ Lead Pharma, Kukatpally, Hyderabad, Telangana India/ Sreeji Pharma International, Vadodara, India.
Oils/surfactants/co-surfactants/viscosity promoters/tonicity modulators/ penetration enhancers	Capryol 90, Capmul MCM C8, Capmul MCM L8, Labrafil M 1994, Labrafil M 2125 and Acconol E / Captex 100, Captex 200, Captex 355, Captex 8000 /Tween 20, Tween 40, Tween 80, Span 20,80/ PEG 200,400/ propylene glycol and glycerin.	Gettefosse, Saint Priest Cedex, France & Abitec Corporation, Mumbai, India &Himedia Laboratories, Mumbai, India.
Analytical solvents/ preservatives	HPLC grade methanol and acetonitrile/ benzalkonium chloride and cetalkonium chloride	S D Fine Chemicals, India.
Bacterial isolates	Klebsiella pneumonia, Staphylococcus aureus and Proteus mirabilis	Department of Microbiology, Palamuru University, India
Universal solvent	Double-distilled Water	Department of Microbiology, KU, Telangana, India
Animal model	Adult albino rabbits/ Male and female/ 2-3kg	Jeeva Life Sciences, Hyderabad, India.
Marketed products	TIMOLET-0.25% ophthalmic solution/ CIPLOX-0.3% ophthalmic solution/ INDOCOLLYRE-0.1%ophthalmic solution	Pulse Pharmacy India Pvt. Ltd/ Cipla/ Bosch & Lomb.

5.1 AIM

To enhance the effectiveness of the ocular drug therapy, our study was aimed to design, prepare, optimize and evaluate the nanoemulsions of different drugs for topical administration.

5.2 OBJECTIVES

i. To design, prepare and optimize nanoemulsions of different selected drugs
ii. To evaluate the prepared formulations for the physical characteristics
iii. To perform the *in vitro* diffusion studies of prepared formulations, fitting the obtained data for response surface analysis and perform *ex vivo* permeation studies of the optimized formulations
iv. To carry out *in vivo* studies of the optimized formulations by evaluating the drug pharmacokinetics in aqueous humour as well as plasma and comparison with the marketed products.

5.3 PLAN OF WARK

i. Literature review- 6 months
ii. Screening of excipients for formulation of nanoemulsions- 6 months
iii. Formulation development -12 months
iv. *In vitro* characterization-12 months
v. *Ex vivo* transcorneal permeation-3 months
vi. *In vivo* studies-3 months
vii. Data interpretation, publications and thesis writing-18 months

Table – 4

List of selected methods and instruments to excecute the Plan of work

ANALYTICAL METHOD FOR ESTIMATION OF PURE DRUG	UFLC (Ultra fast liquid chromatography)
SOLUBILITY STUDIES	Shake Flask/ Gyratory Shaker/ vertexed -in Solvents (Oils and surfactants); Sample Analysis- UFLC.
DRUG EXCEPIENT COMPATIBILITY	FTIR (Fourier transformed infrared spectroscopy) analysis
OPTIMIZATION OF FORMULATION COMPOSITIONS	Pseudo ternary phase diagram -Water titration method; Design expert
PREPARARTION METHOD	Ultrasonic emulsification - Probe Sonicator
PHYSICOCHEMICAL CHARACTERIZATION	pH of Products- pH Meter Rheological Properties- Brookfield Viscometer Characterization of droplet size, zetapotential and PDI-Zetasizer Surface tension- Dunoy ring method-KRUSS tentiometer Osmolarity-Mathematical equation
ACCELERATED STABILITY STUDIES	According to latest ICH guidelines
IN VITRO STUDIES DRUG RELEASE	*In vitro* Drug Release- Franz Diffusion Cell Method/dialysis membrane; Sample Analysis-UFLC
OPTIMIZATION	3^2 factorial design; response surface analysis- Design expert software
SURFACE MORPHOLOGY	Transmission electron microscopy
ANTIMICROBIAL ACTIVITY	Optimized ciprofloxacin NE-standard cup plate method
EX VIVO STUDIES	*Ex vivo* Permeation Studies - Franz Diffusion Cell/ bovine cornea; Sample Analysis- UFLC
IN VIVO STUDIES	Eye irritation studies and pharmacokinetic studies- aqueous humor and blood collection-rabbits; Sample Analysis – UFLC
STATISTICAL ANALYSIS	One sample t-test

6.1 PREFORMULATION STUDIES

6.1.1 Melting point

Usually, by comparing the physical properties of a drug substance with that of a certified pure sample mostly from the standard monographs help verify its purity. Among them, the melting point stands one of the simplest ways to check the purity of a drug substance as every pure drug substance will have a specific melting point and a decrease in melting point represents the presence of impurities (Suico, et al. 2020).

A pinch of the drug was filled in an end closed capillary tube and positioned in a melting point apparatus. The temperature where the drug started melting was observed and noted as the melting point. The noted value was compared with the standard value to determine drug purity.

6.1.2 Quantitative analysis of drug by ultrafast liquid chromatography (UFLC)

The prominence UFLC series offers ten times faster analysis without compromising the precision quality and reliability offered by the ultra-performance liquid chromatography (UPLC) and can be easily performed on an existing instrument as it supports its use at a pressure below 30mpa. The small particle size and length of UFLC column results in shorter retention times and less solvent consumption leading to highly selective and rapid analysis with an excellent resolution where the same components can be eluted within 3.5 min by using UFLC with superior reproducibility compared to the conventional column (Gangadasu BR, et al. 2015).

6.1.3 Stock solution and working sample preparation

100 mg of drug was allowed to dissolve in 50 ml methanol of HPLC grade and the final volume was adjusted up to 100 ml to prepare a concentration of 1000µg/ml stock solution. For the calibration curve, serial dilutions of stock solution were made to prepare the final working concentrations 10-5000ng/ml using the mobile phase as diluent.

6.1.4 UFLC Conditions

The quantitative analysis of drugs was performed using the reported methods. UFLC (Model- Shimadzu UFLC L220) containing LC-20AD isocratic pump, SPD-

20A UV/Vis detector, and rheodyne injector was used. The sample analysis was carried out on a C8 column (100 mm x 4.6 mm, the particle size of 5µm). The flow rate was set at 1.0 ml/min and about 20 µl of sample solutions were injected into the rheodyne injector in triplicate using Hamilton microsyringe and measured at respective λmax. The chromatographic data analysis was done by using the LC solution software. The mobile phase compositions used in the study were given in table 5. A standard plot of concentration of the drug (ng/ml) vs. peak area (mV/sec) was plotted. The linearity of the calibration curve was established by the correlation coefficient value obtained from the graph.

Table – 5

The mobile phase composition and λmax used for drug analysis

Drug	Mobile phase (%v/v)	λmax (nm)
Timolol maleate	7.4 pH phosphate buffer: methanol (40:60)	295
Ciprofloxacin	0.25M phosphoric acid: acetonitrile (30:70)	279
Indomethacin	0.5 % Ortho-phosphoric acid: methanol: acetonitrile (40:20:40)	270

6.1.5 Selection of oil and surfactants

The drug solubility in various formulation components i.e., oils, surfactants and co-surfactants will affect the drug loading which is a key factor in the development of nanoemulsion systems and also determines the performance of developed formulation (Ahmad U, et al. 2019). Medium-chain fatty acid derivatives which can solubilize a good amount of a variety of drug substances (Azeem A, 2009) were chosen as oils to produce nanoemulsions. Further, the nonionic surfactants which are less toxic and proved to be less/nonirritant compared to ionic surfactants were chosen because large amount of surfactants may irritate eyes (Ali MS, et al. 2014).

The solubility of drugs in quite a few solvents was evaluated by adding a surplus amount of drug in 2 ml of each solvent in individual stopper vials of 5 ml capacity and mixed properly using cyclomixer for about 10 min. To attain an

equilibrium, the vials were set aside for 72h at 25 °C in an isothermal shaker. The samples were centrifuged for half an hour at 3000 rpm. The supernatant was collected and filtered using a 0.22 mm syringe filter. The concentration of drug in the filtrate was analyzed by the UFLC (Azeem A, et al. 2009).

6.1.6 Drug - excipients compatibility

Any potential interaction between drug and excipients can affect the physical or chemical properties of drug, bioavailability and stability of dosage form. Hence, it is important to check and confirm that the selected formulation components are in good compatibility with drug and do not compromise its stability and safety (WatermanKC, 2004; Bapi G, et al. 2018).

The principle involved in the IR spectroscopy is measuring the energy difference between the excited and ground states of a molecule. Fourier transformed infrared spectroscopy (FTIR) analysis used for identifying the functional groups with their means of attachment thus helps assess the drug excipients interaction in terms of polymerization, cross-linking as well as drug loading in the formulation (Gurpreet K, et al. 2018).

FTIR was carried out to evaluate the interaction of excipients with the drug. For pure powdered drug KBR pellet method was used and for physical mixture, polished sodium chloride salt plates were used to check the interaction between the components of the formulation (Pavia DL, 2008).

6.2 FORMULATION DEVELOPMENT

6.2.1 Construction of pseudo-ternary phase diagrams

The co-surfactants can improve the fluidity of the interface, aid in drug solubility and reduce the required surfactant volume (Abdul Sisak MA, et al. 2017). However, the suitability of components and their appropriate ratio will affect the hydrophilic-lipophilic balance (HLB), another vital factor in developing nanoemulsions (Kanke PK, et al. 2019). The pseudo ternary phase diagrams help to identify the appropriate ratio of surfactant mixtures that can form a large existence area of the stable nanoemulsions.

The pseudo ternary phase diagrams of selected oil, surfactant and co surfactant were developed using the water titration method (Januleviciene I, et al. 2012). Different surfactant -co-surfactant mixtures (Smix) in ratios 1:1, 1:2, 1:3, 2:1 and 3:1 were prepared. Then, altered weight ratios of oil and Smix mixtures from 1:9 to 9:1 were prepared (Goosh N, et al. 2012). Water was added in increments of 50µl until turbidity occurs. Clear, transparent and stable mixtures were marked as points in the phase diagram using CHEMIX software (Thielen TL, et al. 2000).

6.2.2 Optimization of nanoemulsion formulation compositions

Formulation design and development involves a combination of various components and optimization of process and formulation parameters. Factorial designs can be used to select and assess certain factors involved in the successful formation of nanoemulsions and also study their effect on the performance of formulation by analyzing various parameters thus determining the optimized conditions (Kuncahyo I, et al. 2019).

3^2 factorial design where, 2-factors at 3-levels were used to select the compositions for formulating nanoemulsions with Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) and a total of 9 compositions were suggested by the software. The oil and Smix contents were chosen as the independent variables and the response variables chosen were the droplet size (nm) and percentage drug release (%) (Nirav P, et al. 2016).

6.2.3 Preparation of nanoemulsions

NEs were prepared by the ultrasonic emulsification method by using probe sonicator. Ultrasonic emulsification is known to be a reliable method for the production of long-term stable nanoemulsions that can reduce the droplet size of primary emulsion to size less than 0.2 µ (Gurpreet K and Singh SK, 2018). This involves either of two phenomena (1) waves generated by acoustic field travel through the liquid and produce micro turbulences and an interfacial movement occurs. Because of this, the boundary phase becomes unstable and the dispersed phase eventually breaks into droplets in the continuous phase. (2) The application of ultrasonic waves of low-frequency and high power generates cavities that lead to the formation of microbubbles or voids in the medium. They grow over numerous wave

cycles until they collapse aggressively. This bubble implosion causes locally extreme conditions such as very high shear, liquid jets, and extreme heating- cooling rates. These extreme forces are responsible for the primary droplets of dispersed phase to break down into nano-sized droplets and mix them homogeneously into the continuous phase (Seid MJ, et al. 2006; Kentish S, et al. 2008).

Nanoemulsion with o/w systems is recommended for the lipophilic drugs and those with w/o systems for hydrophilic drugs. The oil phase contained selected oil and Smix, the aqueous phase contained 1% propylene glycol and 3% glycerol dissolved in water. The pre-weighed quantity of the drug and the cationic surfactant were added to the internal phase by vortexing until clear solutions were obtained. The emulsions were prepared by the gradual addition of the drug dissolved internal phase to the external phase with continuous stirring at 250 rpm on a magnetic stirrer to produce a primary emulsion, the most common method for preparing highly concentrated emulsions (Pons R, et al. 1994; Kumar H, et al. 2018). The primary emulsion was then sonicated to get a nanoemulsion following a cycle of 5s on and 3s off for about 10 min at 50 amplitude (Priya, et al. 2015).

6.3 FORMULATION EVALUATION

6.3.1 *In vitro* characterization

i. Size analysis

The small size of emulsion globules is responsible for its stability and also contributes to the improved ocular absorption. Particularly droplets with nano size elevate the interfacial area thereby facilitate the drug diffusion to the targeted tissues at greater rates which results in the enhanced clinical efficacy of the drug substance (Frederic L, et al. 2011). Hence, it is of utmost importance to verify whether the prepared emulsions are in the required nano size range or not.

Mean globule size, surface charge (Zeta potential) and size distribution of the nanoemulsions (PDI) were determined by photon correlation spectroscopy using a Zetasizer S-90 1000 HS (Malvern Instruments, UK) at 25°C and 90° angle. The samples were prepared by diluting at a ratio of 1: 500 with water before the measurement (Patel A, et al. 2013).

ii. pH measurements

The pH is important in case of ophthalmic dosage forms as eyes are sensitive to pH changes and the pH of the prepared nanoemulsions was measured by digital pH meter (DPH 504, Global electronics, India) (Porela-Tiihonen S, et al. 2013).

iii. Evaluation of rheological properties

In general, viscosity contributes to emulsion stability and efficient drug release. But when it comes to ophthalmic use, viscosity also plays a major role in formulation flow and retention time on the corneal membrane. The quantity of emulsion components including oil, surfactant, co surfactant and water will affect the viscosity. Different researchers reported different effects on viscosities for example; the decrease in water content lowered the viscosity (Musa SH, et al. 2017), whereas the increase in oil increased the viscosity (Ali MS, et al. 2014). Further, the addition of surfactants will decrease the interfacial tension between water and oil that may lower the viscosity vice versa. But it is purely dependent on the emulsion component quantities so it becomes necessary to check the viscosity of each prepared nanoemulsion.

Literature suggests that the O/W nanoemulsions are less greasy and posses lower viscosities and exhibit faster drug release when compared to the W/O nanoemulsions (Chime SA, et al. 2014).

The viscosity of the NEs was measured in triplicate using a digital cone and plate viscometer (Model DV-II+Pro, Brookfield, USA) with spindle number 40.

iv. Refractive index

The refractive index of the nanoemulsion is measured and compared with that of water to confirm its transparency. Nanoemulsion with a refractive index similar to that of water indicates the emulsion is transparent and does not interfere with the vision upon application (Harika K, et al. 2015).

The refractive index of nanoemulsions was determined using an Abbes refractometer (Navi Mumbai, India) at 25±0.5° C (Haripriya A, et al. 2016). Briefly, the prism box was opened and one drop of the NEs was placed between the two

prisms. Then the prism box was closed and the refractor was placed below the prism box. The scale was focused on viewing through the left eyepiece and the shadow was observed viewing through the right eyepiece. Then the scale knob was rotated until the scale touched the intersection of cross wire and the reading was noted.

v. Surface tension measurements

The surface tension is an essential parameter that ensures uniform spreading of emulsion on the corneal surface and the low interfacial tension will improve the effective surface area for drug exchange. Also the formation and other nanoemulsion properties, for example, the viscosity are interconnected by the interfacial tension.

Surface tension measurements were carried out by the Du Nouy ring method using an electronic tensiometer (model- K100, KRÜSS, Germany) (Thiel B, et al. 2017). In this method, as a ring moves from one phase to another, tension is developed by the withdrawn liquid lamella and the force that is acting on the ring is measured from which the interfacial tension can be calculated using the following mathematical formula.

$$\sigma = F/L \cos\theta$$

vi. Osmolarity determination

Osmolarity is an important parameter by which one can predict the irritability and the physiological acceptance of the formulation by ocular tissues (Morsi NM, et al. 2017). Osmolarity of the prepared nanoemulsions was calculated by using the following equation (Ashara K, et al. 2017).

$$\text{Milli osmoles per liter} = \frac{\text{Mass(g)}}{\text{Molecular Wt}\left(\frac{\text{g}}{\text{ml}}\right)} \times \text{particle number} \times 1000$$

vii. Thermodynamic stability studies

Stability testing helps to check the influence of environmental factors, including temperature, humidity and light with time on the efficiency and integrity of the final product. The accelerated stability study predicts the stability profile of a drug product that helps in selecting the best one from a series of prepared formulations (Sanjay B, et al. 2012).

Prepared nanoemulsions were subjected to six heat and cool cycles at 45 ºC and 4 ºC respectively followed by centrifugation at 3500 rpm for 30 min and then, exposed to three freeze and thaw cycles at −21 ºC 25 ºC respectively. The storage period for the formulations at each cycle was 48h (Mahboobian MM, et al. 2017; Jaiswal P, et al. 2014). The stability of nanoemulsions was confirmed by checking the changes in droplet size and drug content before and after subjecting to thermodynamic stability studies (Eskandar M, et al. 2017).

viii. Drug content estimation

The accuracy of the method of preparation and excipient interference can be predicted by estimating drug content. A small fixed volume of nanoemulsion was taken and diluted with methanol (Mowafaq GM and Abulfadhel NJ, 2017). Then the samples were analyzed by using UFLC and the concentrations were calculated from the calibration curve (Khalid N, et al. 2017).

ix. *In vitro* drug release

In vitro release testing is an important analytical tool in investigating the fundamental information regarding the dosage form performance, the release mechanism and kinetics, which assumes greater significance for complex dosage forms like nanosystems enabling a rational and scientific approach to the dosage form development (Susan D, 2014).

The drug release from the NEs was studied by utilizing vertical Franz diffusion cell of 20mm internal diameter and 15ml receiver capacity. The donor and receiver compartments were separated by the dialysis membrane of 12,000 Da (Himedia Laboratories Pvt. Ltd, Mumbai, India). The dissolution medium, simulated tear fluid (STF) of pH 7.4 was prepared with a percentage composition of NaCl, NaHCO3 and CaCl2. 2H2O at 0.67, 0.2 and 0.008 respectively (Usha GK, et al. 2016). 1ml of the test formulation was filled in the donor compartment whereas 15ml of the STF was filled in the receiver compartment. 1 ml of STF with a dose equivalent amount of drug was served as control. The setup was kept under continuous stirring with a Teflon-coated magnetic bar up on a magnetic stirrer at 100 rpm maintaining a constant temperature of 37±0.5 ºC. 1 ml sample was collected from the receiver

compartment and replaced with the same amount of fresh STF at every predetermined time points (0.5, 1, 2, 4, 6 and 12h) for a period of 4h. The concentration of drug in samples was analyzed using UFLC.

x. Optimization using Response surface analysis:

The response surface analysis was carried out using Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) to optimize the prepared nanoemulsions**.** The experimental data were fitted into different statistical models and the computer generated results were analyzed to identify the significant model and further, the response surface analysis by contour and 3D surface plots were used to study the effects of independent variables on the response variables i.e., mean globule size (nm) and percentage drug release (%).

xi. Transmission electron microscopy (TEM)

TEM is an advanced microscopic method that enables imaging of the disperse phase with higher resolution and therefore reveals the surface morphology thus helps to verify the nanoemulsion formation. However, because of high equipment costs, we have planned to study the morphology of the optimized nanoemulsions only.

TEM was carried out by following the standard protocol practicing at RUSKA Labs, PVNRT V University, Hyderabad. A sample drop was placed on a piece of Para-film with the copper grid coated with carbon, kept aside for 5-10 min, washed with distilled water and the excess was drained with help of filter paper, strained using 2% Uranyl acetate, air-dried and observed under the transmission electron microscope (Hitachi, H-7500) at various magnifications (Laxman M, 2014).

xii. Antimicrobial activity

Generally, *in vitro* antimicrobial activity is performed for the antimicrobial agents where their activity against microbial pathogens is evaluated that further ensure the drug release from the nanoemulsion.

To know the changes in the efficiency of ciprofloxacin, the standard spread plate method was used to study the antibacterial effect of optimized ciprofloxacin formulation against 3 different bacterial stains. 100µl of the test sample was added to

the wells on the sterile agar plates previously inoculated with the organisms, allowed for diffusion of solutions for 2h, and incubated at 37 ºC for 24h. The growth inhibition zones were measured after the incubation period. The nanoemulsion without antibiotic was used as negative control and a standard antibiotic disc was used as the positive control (Singh D, et al. 1982).

6.3.2 *Ex vivo* studies

The rate of drug transfer from disperse phase to continuous phase and thereby from continuous phase to the receiver phase through the biological membrane corresponds to the drug release patterns from the nanoemulsion and this can be predicted by determining the mass transfer constants of the drugs at the equilibrium state through the biological membrane which probably depends on the concentration of drug in the aqueous phase in case of hydrophilic drug and vice versa in case of a lipophilic drug.

i. Preparation of corneas

Bovine eyeballs were procured from a slaughterhouse and transported immediately to the laboratory in normal saline maintained at 4 ºC (Kim HS, et al. 2017). The eyeballs were carefully dissected and the corneas were excised in such a way that some part of the sclera was attached and washed with cold saline. Then, placed in cold freshly prepared STF of pH 7.4 (Ammar HO, et al. 2010).

ii. Transcorneal Permeation studies

Permeation studies were carried out by using Franz-diffusion cells of 20mm internal diameter and 15 ml receiver capacity. The STF was used as a diffusion medium. 200 µl of the samples were withdrawn at different time intervals and replaced with an equal volume of STF (pH 7.4) at each time interval (0.5, 1, 3, 2 and 4h) and samples were analyzed using UFLC (Morsi NM, et al. 2014). The amount of drug permeated per unit area through the excised cornea (µg/cm2) versus time (h) graphs were plotted and the equations used for calculating flux (J, µg/cm^2 hr) and trans corneal permeability coefficient (Papp, cm/s) were as follows (Tayel SA, et al. 2013).

$$J=dQ/dtA$$

Where, J, M and A indicate flux, the cumulative amount of drug permeated and the membrane surface area respectively.

$$Papp = J/Cd$$

Where Cd indicates the initial drug concentration in the donor phase.

iii. Corneal hydration

The viability of excised cornea must be studied to ensure the quality of *ex vivo* studies because there are chances of corneal damage due to the environmental factors and also the formulation components.

Each corneal membrane was weighed immediately after the ex-vivo transcorneal permeation experiment, dried overnight by desiccating, and re-weighed (Morsi NM, et al. 2014). The difference in weights was used to calculate the percentage corneal hydration level (HL %) and the equation used was as follows (Yu S, et al. 2015).

$$HL\% = 1 - \frac{Wd}{Ww} \times 100$$

Where Ww and Wd are the corneal weights in wet and dry conditions respectively.

6.3.3 In vivio studies

i. Animal Models of Ocular Pharmacokinetics

Despite the numerous physiological differences with human eyes, rabbits remain the animal of choice of ocular pharmacokinetic studies because of their relatively large eyes; same time rodents are too small to test ocular delivery systems, unlike systemic medications where mice or rats are preferred. Rabbit eyes differ from human eyes in terms of slow blink rate that leads to higher corneal surface drug concentrations and lower drainage; less sensitive to variations in the emulsion viscosity, presence of a nictitating membrane which may absorb the drug and act as a reservoir and they lack eye pigmentation that may involve the pharmacokinetics of drug substances (Krishna MB and Lorenzetti JO, 1993).

The study protocol (IAEC/49/UCPSc/KU/2018) was approved by the institutional animal ethical committee, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India. Fig. 5 illustrates the study schedule.

The study was performed at Jeeva Life Sciences, Hyderabad.

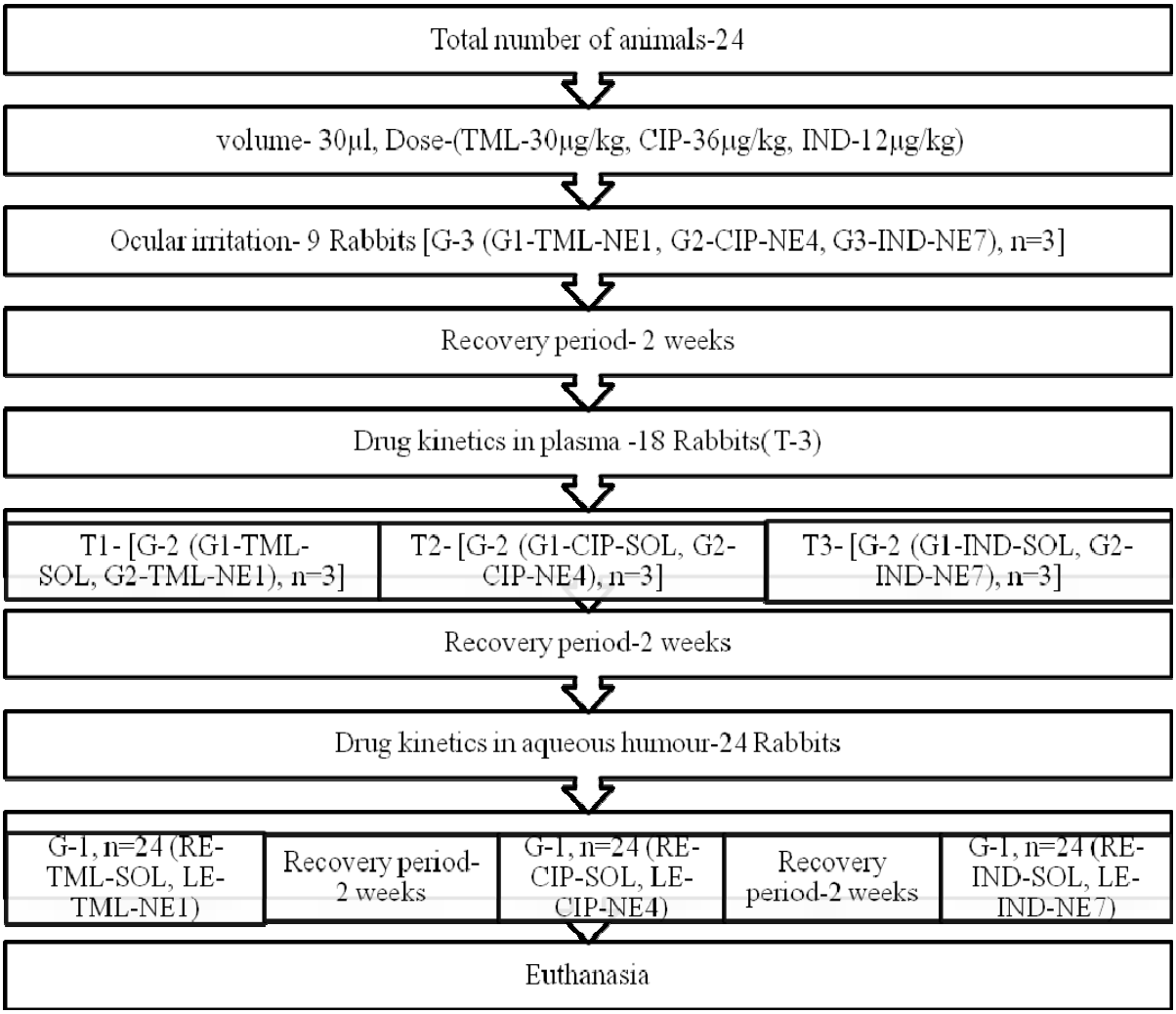


Fig. - 5: Flow chart illustrating the study schedule

ii. Ocular irritation studies

Ocular irritancy of optimized nanoemulsions was studied in healthy adult New Zealand albino rabbits free from visible ocular abnormalities, weighing about 2 – 3 kg of either sex (Lallemand F, et al. 2012). 30 µl of test formulation previously sterilized using a 0.2 µm syringe filter was instilled into the right eye of each rabbit (n=3) and observed for ocular irritation reactions like redness, conjunctival chemosis and discharge for 24h keeping the untreated eye as a control. The scoring was given from 0 to 4 for the absence to highest observed abnormality and an on the whole ocular irritation index (Iirr) was calculated by summing up the total scores for each category. The Iirr more than 4 was considered the presence of clinically significant irritation (Seung YK, et al. 2016).

iii. *In vivo* pharmacokinetics

a. Drug kinetics in plasma

18 Rabbits were divided into 3 treatment groups of six animals each. Each treatment group was further divided into 2 groups of three animals each. In every treatment group, Gruop1 received control (Marketed formulation) and Group 2 received Test (optimized nanoemulsion) samples of 30 µl each. 200µl of blood samples were collected into heparinized tubes at 0.5, 0.75, 1, 2, 4, 8, 12 and 24h time points through the marginal vein. After collection, samples were immediately stored at temperature (-20 °C) until the UFLC analysis (Marongiu ML, et al. 2007; Parasuraman S, et al. 2010).

b. Drug kinetics in aqueous humour

To study and compare the pharmacokinetics of optimized nanoemulsions with that of marketed formulation, 24 animals were used corresponding to eight sampling points of 0.5, 0.75, 1, 2, 4, 8, 12 and 24h. Each animal was placed in individual restraining boxes, 30 µl of test sample and control (marketed product) were topically applied on to the right eye and left eye respectively (Rajendra SK, et al. 2011). 100µl of aqueous humor was collected from three animals at each time point by inserting 22 G needle of an insulin syringe into the anterior segment of the eye through the cornea without causing any injury to iris and lens (Mark Fisher L, et al. 1989). Before collecting the samples, a combination of 35 mg/kg ketamine hydrochloride and 5 mg/kg xylazine was used to anesthetize the rabbits by injecting intramuscularly. After collection, samples were immediately stored at temperature (-20 °C) until the UFLC analysis (Kumar H, et al. 2018).

iv. Analysis of biological (aqueous humour & blood) samples

a. Calibration sample preparation

Different calibration standards ranging from 10–500 ng/ml were prepared by adding 10 µl of known working solution of drug to 90 µl of drug-free rabbit aqueous humor/blood. All samples were vortexed to ensure complete mixing and 20 µl was used for HPLC analysis (Hassib ST, et al. 2016).

b. Test sample preparation

The aqueous humor/ blood samples collected were mixed with 200 µl of acetonitrile, vortexed and centrifuged for 30 minutes at 3000rpm. 20 µl of the organic phase was used for HPLC analysis (Ismail K, et al. 2016; Nirav P, et al. 2016; Janis V, et al. 2015).

7.1 TIMOLOL MALEATE NANOEMULSIONS

7.1.1 Melting Point

The melting point of timolol maleate (TML) was found to be 202ºC meeting the standard melting point range of 202-203 ºC indicating the purity of the drug (Nagarani B, et al. 2016).

7.1.2 Calibration curve:

The calibration curve was plotted between peak area and concentration (ng/ml) showed in fig. 6. The concentration range of 50 to 1000 ng/ml showed linearity with a regression coefficient (R^2) value of 0.996. The retention time was found to be 3.1 ± 0.12 min as shown in fig.7.

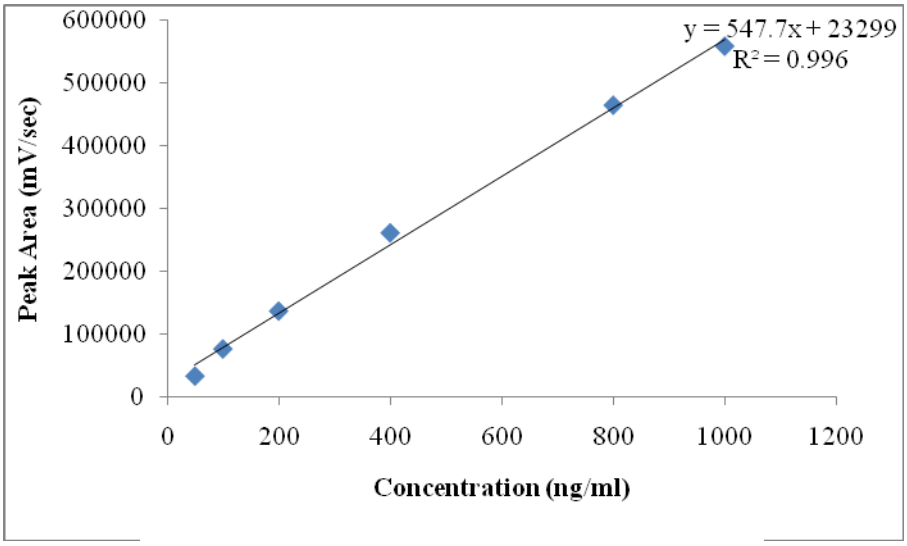


Fig. – 6: Calibration curve of timolol maleate

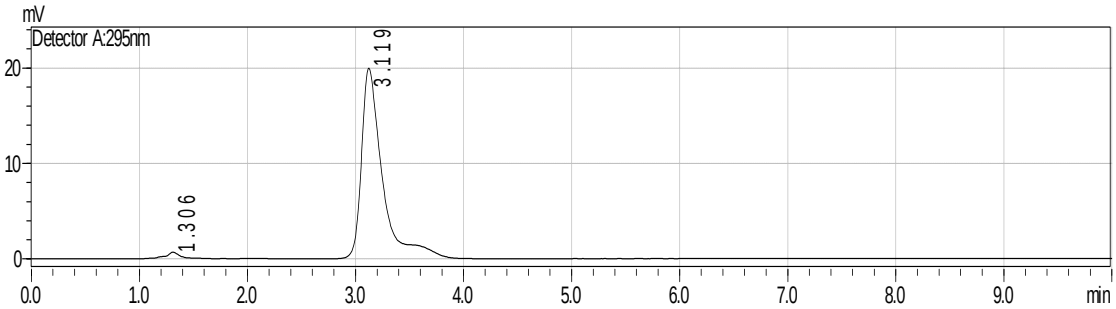


Fig. – 7: UFLC Chromatogram of timolol maleate (RT- 3.1 min)

7.1.3 Solubility study:

To develop a nanoemulsion of TML for ocular delivery, it should possess good solubility in the components of the system, to avoid any drug precipitation upon

dilution. The solubility of TML in various oils, surfactants and co-surfactants were depicted in fig. 8. Among oils, capryol 90 showed better solubility. Therefore, capryol 90 was selected as the oil phase. Among surfactants and co-surfactants TML had the highest solubility in tween 80 and span 80 hence they were selected as surfactant and co-surfactant respectively for the phase study. Among co solvents/ viscosifying agents/permeation enhancers propylene glycol was selected for the study as it had better solubility comparatively.

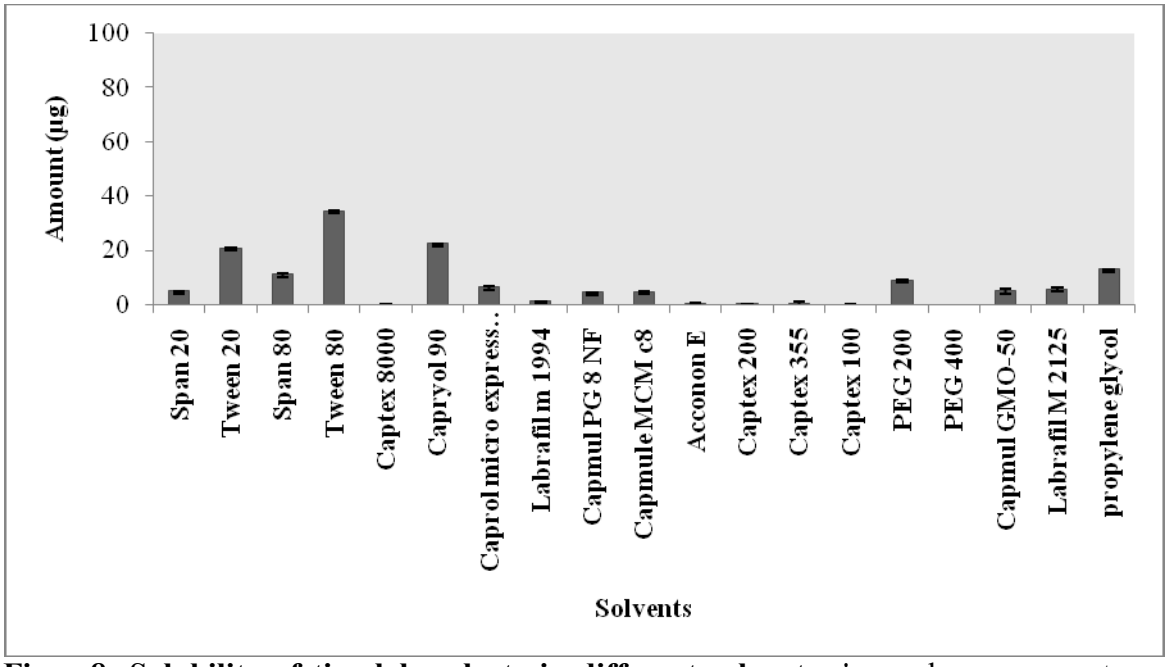


Fig. - 8: Solubility of timolol maleate in different solvents, *error bars represent standard deviations of three replicates

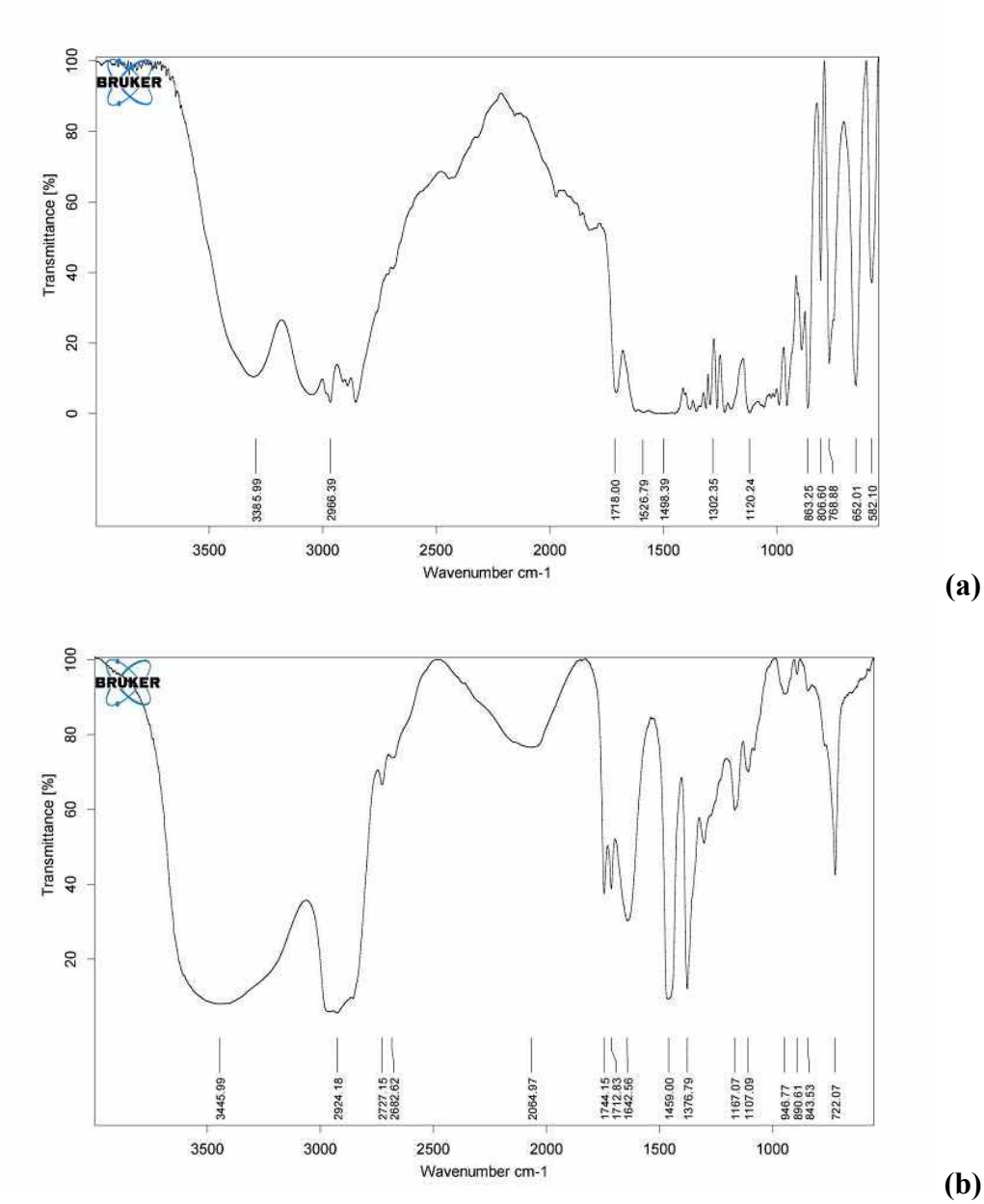
7.1.4 Drug – Excipients Compatibility

The major functional groups of TML that are identified from the FTIR spectral analysis were depicted in table 6 and the characteristic peaks were illustrated in fig.9. In brief, O-H stretching of carboxylic acid were found at 3385.99 cm^{-1} and 3445.99 cm^{-1}, methyl C-H stretching were found at 2966.39 cm^{-1} and 2924.18 cm^{-1}, C=O stretching of amide were found at 1718.00 cm^{-1} and 1712.15 cm^{-1}, C=N stretching were found at 1698.39 cm^{-1} and 1642.56 cm^{-1} in pure drug and physical mixture respectively (Nagarani B, et al. 2016; Swati J, et al. 2016). Thus all the characteristic peaks of drug were found in both spectra confirming the chemical integrity of the drug in the presence of selected excipients and hence, there exists a good compatibility between drug and excipients.

Table – 6

Timolol maleate functional group Identification from FTIR data

Functional group	Wave number (cm^{-1})	
	Pure Drug	Physical mixture
O-H	3385.99	3445.99
C-H stretch	2966.39	2924.18
C=O	1718.00	1712.15
C=N	1698.39	1642.56



(a)

(b)

Fig. – 9: Timolol maleate FT-IR spectra (a) pure drug and (b) physical mixture

7.1.5 Phase behavior

Pseudo ternary phase diagrams were constructed separately for each surfactant to the co surfactant ratio and were given in fig. 10. A large nanoemulsion area was observed for the ratio 1:3 (w/w) and the same was used to optimize the final formulation compositions.

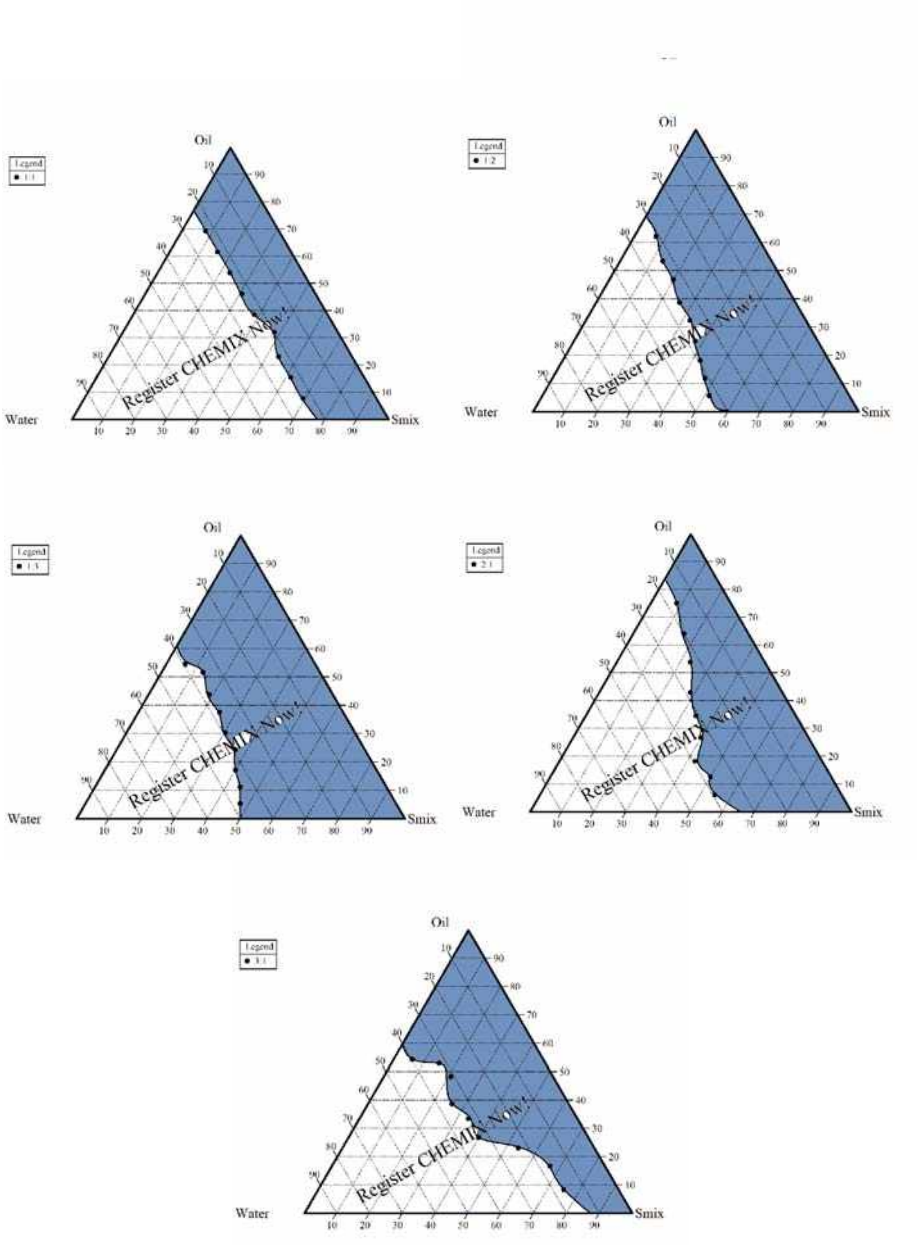


Fig. - 10: Pseudo ternary phase diagrams of capryol 90 and Smix (tween 80: span 80) Ratios; (a) 1:1, (b) 1:2, (c) 1:3, (d) 2:1 and (e) 3:1

7.1.6 Optimization of formulation composition and Preparation of Timolol Maleate nanoemulsions (TML-NEs):

Based on the phase behavior of the components, three different levels of oil and Smix were incorporated into the design expert software and the computer has generated a total of nine combinations which were shown in table 7. The final compositions used to prepare TML- NEs were shown in table 8.

Table – 7

3^2 factorial design for the selection of formulation composition for timolol maleate nanoemulsions

Formulation code	**Coded value**		**Actual value**	
	X1	**X2**	**X1**	**X2**
TML-NE1	0	1	22.5	65
TML-NE2	-1	1	20	65
TML-NE3	-1	0	20	60
TML-NE4	1	1	25	65
TML-NE5	1	-1	25	55
TML-NE6	0	-1	22.5	55
TML-NE7	0	0	22.5	60
TML-NE8	1	0	25	60
TML-NE9	-1	-1	20	55
Independent variables	**Levels**			
	Low (-1)	**Medium (0)**	**High (1)**	
X1- amount of oil (%w/v) X2-amount of Smix(%w/v)	20 55	22.5 60	25 65	
Dependent variables	Y1- Mean droplet size (nm) Y2- Percentage drug release (%)			

Table – 8

Formulation composition of timolol maleate nanoemulsions

Formulation code	Oil	Smix	Water	1% Propylene glycol	3% Glycerol	Drug	Benzalkonium chloride
TML-NE1	22.5	65	5	2.5	4.748	0.25	0.02
TML-NE2	20	65	10	2.5	2.248	0.25	0.02
TMLNE3	20	60	15	2.5	2.248	0.25	0.02
TMLNE4	25	65	5	2.5	2.248	0.25	0.02
TMLNE5	25	55	10	2.5	7.248	0.25	0.02
TMLNE6	22.5	55	15	2.5	4.748	0.25	0.02
TMLNE7	22.5	60	5	2.5	9.748	0.25	0.02
TMLNE8	25	60	10	2.5	2.248	0.25	0.02
TMLNE9	20	55	15	2.5	7.248	0.25	0.02

*all the values are represented as %w/v

7.1.7 Physicochemical Evaluation:

The nanoemulsions found suitable for ocular delivery with satisfactory physicochemical characteristics and the complete profile was depicted in table 9. The mean globule size for all of the TML-NEs was found less than 200nm confirming the successful formation of nanoemulsions (Panchal SS, et al. 2017). This small size could be related to the penetration of the co-surfactant molecules into the surfactant film. This would decrease the fluidity and surface viscosity of the interfacial film, lower the radius of curvature of the droplets and thus form transparent systems (Lucia P, et al. 2020). Though the human eye can tolerate a pH of 3.5 to 8.5, usually a pH of 5.5-7.4 is recommended for the ophthalmic products and the ideal pH for maximum comfort to the eye is of 7.2□±□0.2 (Horn G, 2015). The pH values of the TML-NEs were found in the range of 5.8± 0.3 to 6.7 ± 0.5 falling under the recommended value. Refractive index measurements tell about the possibility of discomfort to the patient after the administration of eye drops. It is recommended that eye drops should have refractive index values near to that of tear fluid (1.340 to 1.360) or not higher than 1.476 (Ammar HO, et al. 2009). TML-NEs had refractive index values ranging from 1.341 to 1.363 which are within the recommended value. Viscosity ranged from 43.1±0.1 to 102.3±0.5 mPas. In general, 15-150 mPas of viscosity is recommended as

this low viscosity will ensure the transparency and hence no visual impairment upon eye drop instillation into the eyes without compromising the prolonged retention time (Baranowski P, et al. 2014). The positive charge helps to improve the retention time and also helps in improving corneal absorption due to electrostatic interactions with the corneal membrane (Lallemand F, et al. 2012). The surface tension was found in the range of 41.5 ± 1.8 to 52.1 ± 3.4 mN/m ensuring the good spread ability of the TML-NEs over the corneal membrane (Morsi NM, et al. 2014). The glycerine acted as tonicity adjusting agent and the osmolarity values of TML-NEs were found within the range of 303-395 mOsm/L falling under the recommended values of 231-446 mOsm/L (Gan L, et al. 2013; Morsi NM, et al. 2014).

Table – 9

Physicochemical characteristic profile of timolol maleate nanoemulsions

Formulation code	Droplet size (nm)	Poly dispersity index	Zeta potential (mV)	Viscosity (mPas)	pH	Refractive index	Surface tension (mN/m)	Osmolarity (mOsm/L)
TML-NE1	129.5±1.8	0.102±0.031	21.1±5.25	43.1±0.1	6.5±0.2	1.347 ± 0.007	52.1 ± 3.4	353
TML-NE2	148.9±1.1	0.017±0.001	25.8±5.86	46.4±0.1	6.5±0.4	1.356 ± 0.002	47.4 ± 2.5	303
TML-NE3	157.7±0.1	0.201±0.110	14.5±4.83	55.5±0.2	6.6±0.9	1.361± 0.002	48.2 ± 3.7	324
TML-NE4	138.9±1.3	0.209±0.005	18.7±3.51	48.5±0.1	6.7±0.5	1.345 ± 0.005	46.3 ± 1.9	362
TML-NE5	223.6±1.6	0.091±0.010	22.6±4.09	102.3±0.5	6.6±0.4	1.346 ± 0.001	41.5 ± 1.8	374
TML-NE6	186.2±1.5	0.213±0.002	17.2±4.6	94.4±0.2	5.8±0.6	1.395 ± 0.001	43.1 ±4.0	352
TML-NE7	151.8±1.9	0.406±0.021	13.6±5.45	52.6±0.0	5.8±0.3	1.356 ± 0.001	46.7 ± 2.5	329
TML-NE8	180.8±1.9	0.302±0.004	12.4±4.82	76.0±0.1	5.9±0.2	1.359 ± 0.002	50.4 ± 2.6	395
TML-NE9	167.5±2.0	0.334±0.140	19.4±3.84	65.3±0.0	5.9±0.8	1.357± 0.005	44.6 ±3.5	344
Required	≤200	≤1.00	±20-40	15-150	5.5-7.4	1.340-1.360 (NMT1.476)	40-50	231-446

*Values are expressed as mean ±SD (n=3)

7.1.8 Stability:

TML-NEs remained clear with no phase separation or drug precipitation, indicating their excellent physical stability. All the formulations were found to be consistent concerning their mean globule size, drug content, phase separation and transparency during the stability study. The physical data comparing their mean globule size and drug content before and after stability studies were given in table 10.

Table – 10

Physical evaluation of timolol maleate nanoemulsions before and after stability studies

Formulation code	Particle size(nm)		Drug content (%)	
	Freshly prepared	After stability studies	Freshly prepared	After stability studies
TML-NE1	129.5±1.8	131.65± 1.2	96.41 ± 0.89	95.65± 0.21
TML-NE2	148.9±1.1	149.41 ± 1.3	94.68 ± 0.23	94.41 ± 0.24
TML-NE3	223.6±1.6	224.73 ± 0.9	91.81 ± 0.12	92.73 ± 0.13
TML-NE4	138.9±1.3	139.42 ± 2.8	94.56 ± 0.73	93.42 ± 0.82
TML-NE5	186.2±1.5	188.18 ± 1.4	98.29 ± 0.99	98.18 ± 0.14
TML-NE6	180.8±1.9	183.54 ± 2.4	95.76 ± 0.65	95.54 ± 0.45
TML-NE7	151.8±1.9	156.48 ± 0.9	97.12 ± 1.09	96.48 ± 0.22
TML-NE8	157.7±0.1	153.12 ± 2.5	93.13 ± 0.11	93.12 ± 0.51
TML-NE9	167.5±2.0	165.92 ± 0.6	96.19 ± 0.86	95.92 ± 0.46

*Values are expressed as mean ±SD (n=3)

7.1.9 *In vitro* release studies:

The release results of nanoemulsions revealed that the prepared formulations were found to exhibit sustained release of 59.27±1.59 to 96.41±2.31 at 24h where the control solution showed 99.03 ±3.11 % drug release within 2.5 h. The *in vitro* drug release profiles of the TML-NEs and the control solution were graphically illustrated in fig. 11. The variation in drug release between TML-NEs can be emphasized from the formulation composition variables and the droplet size of the formulations (Jaiswal P, et al. 2014).

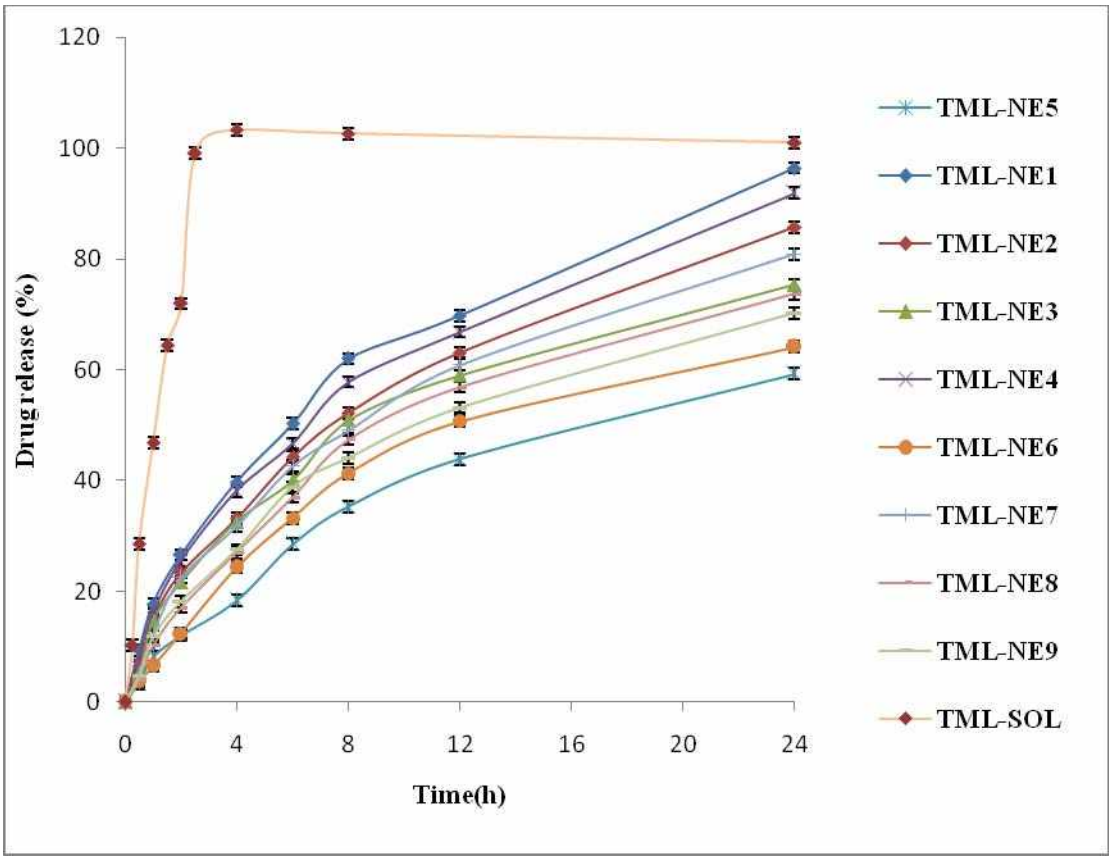


**Fig. - 11: *In vitro* drug release profiles of timolol maleate nanoemulsions and control drug solution, ***error bars represent standard deviations of three replicates

7.1.10 Optimization by response surface analysis:

The experimental data were fitted into the different models where the quadratic model found significant and the results were showed in table 11. The model fit summary, statistics and ANOVA analysis were depicted in table 12, 13 and 14 respectively. For the quadratic model, the predicted R^2 of mean globule size and percentage drug release were found to be 0.9617 and 0.6919 respectively which are in reasonable agreement with the adjusted R^2 of 0.9903 and 0.8440 respectively; i.e. the difference is less than 0.2. Adeq precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 39.7690 for mean globule size and 10.2422 for percentage drug release indicated an adequate signal. The model found significant with the F-value of 163.74 for mean globule size and 22.64 for percentage drug release. P-values less than 0.0500 indicate that the model terms are significant and those values greater than 0.1000 indicate that the model terms are not significant. In the case of mean globule size A, B, AB, A^2, B^2 were found significant model terms and for pecentage drug release, B was found as a significant model term (Yafinaz L, et al. 2013).

The final equation in terms of coded factors for mean globule size (Y_1) and percentage drug release (Y_2):

$$Y_1 = +149.84 + 7.10\ X_1 - 28.88\ X_2 - 13.20\ X_1X_2 + 13.73\ X_1^2 + 8.98\ X_2^2$$

$$Y_2 = +77.48 - 1.07\ X_1 + 13.39\ X_2$$

Table - 11

Fit summary of the experimental data of timolol maleate nanoemulsions for responses Y_1 and Y_2

Source	Sequential p-value		Adjusted R^2		Predicted R^2		
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	
Linear	0.0071	0.0016	0.7443	0.8440	0.4558	0.6919	
2FI	0.0552	0.0706	0.8629	0.9087	0.4981	0.8267	
Quadratic	0.0088	0.2350	0.9903	0.9420	0.9617	0.7491	**Suggested**
Cubic	0.5990	0.3694	0.9895	0.9763	0.7612	0.4595	

Table – 12

Fit statistics of the experimental data of timolol maleate nanoemulsions by quadratic model for responses Y_1 and Y_2

Std. Dev.	Y_1	2.83	**R^2**	Y_1	0.9963
	Y_2	4.89		Y_2	0.8830
Mean	Y_1	164.99	**Adjusted R^2**	Y_1	0.9903
	Y_2	77.48		Y_2	0.8440
C.V. %	Y_1	2.16	**Predicted R^2**	Y_1	0.9617
	Y_2	6.31		Y_2	0.6919
Adeq Precision				Y_1	39.7690
				Y_2	10.2422

Table – 13

ANOVA for the experimental data of timolol maleate nanoemulsions by quadratic model for response Y_1

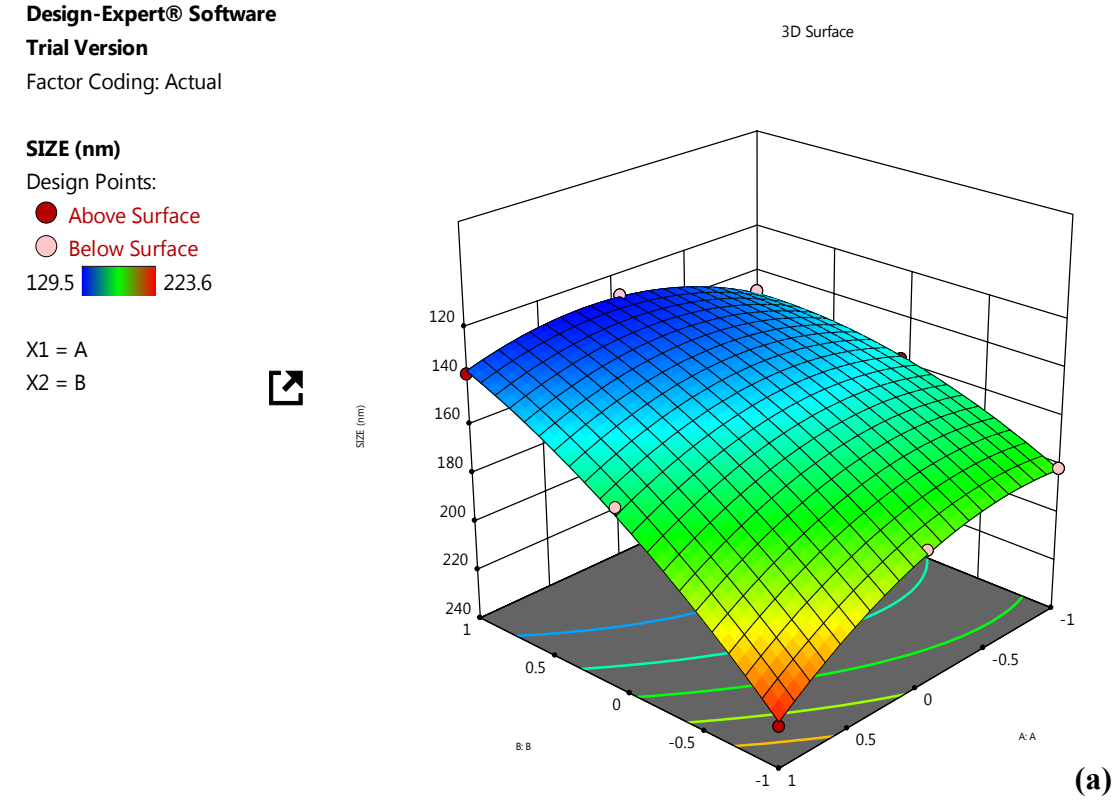
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6543.51	5	1308.70	163.74	0.0007	**Significant**
A-A	302.46	1	302.46	37.84	0.0086	
B-B	5005.48	1	5005.48	626.27	0.0001	
AB	696.96	1	696.96	87.20	0.0026	
A^2	377.21	1	377.21	47.19	0.0063	
B^2	161.40	1	161.40	20.19	0.0206	

Table – 14

ANOVA for the experimental data of timolol maleate nanoemulsions by quadratic model for response Y_2

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1082.31	2	541.16	22.64	0.0016	**Significant**
A-A	6.83	1	6.83	0.2856	0.6123	
B-B	1075.48	1	1075.48	45.00	0.0005	
Residual	143.41	6	23.90			
Cor Total	1225.72	8				

From the response surface analysis, it was evident that the oil and Smix compositions showed a significant effect on mean globule size and percentage drug release. The effects were represented as 3D surface plots and contour plots in fig.12&13 respectively. It is prominently noticed that an increase in oil content provided a decrease in globule size to some extent and a further increase in oil content lead to a slight increase in globule size and an opposite effect was seen for percentage drug release (Ketan H, et al. 2010). However, by increasing the Smix content the globule size decreased and the percentage drug release increased. The drug release was found to be affected by their droplet size (Navneet S, et al. 2016). The decrease in droplet size increased the total number of oil globules and a subsequent increase in their surface area which led to an increase in drug release (Ammar HO, et al. 2009). Thus the formulation TML-NE1 with the medium oil and high Smix was found to have lowest mean globule size and significantly superior drug release; hence it was chosen as the optimized formulation and was subjected to further study.



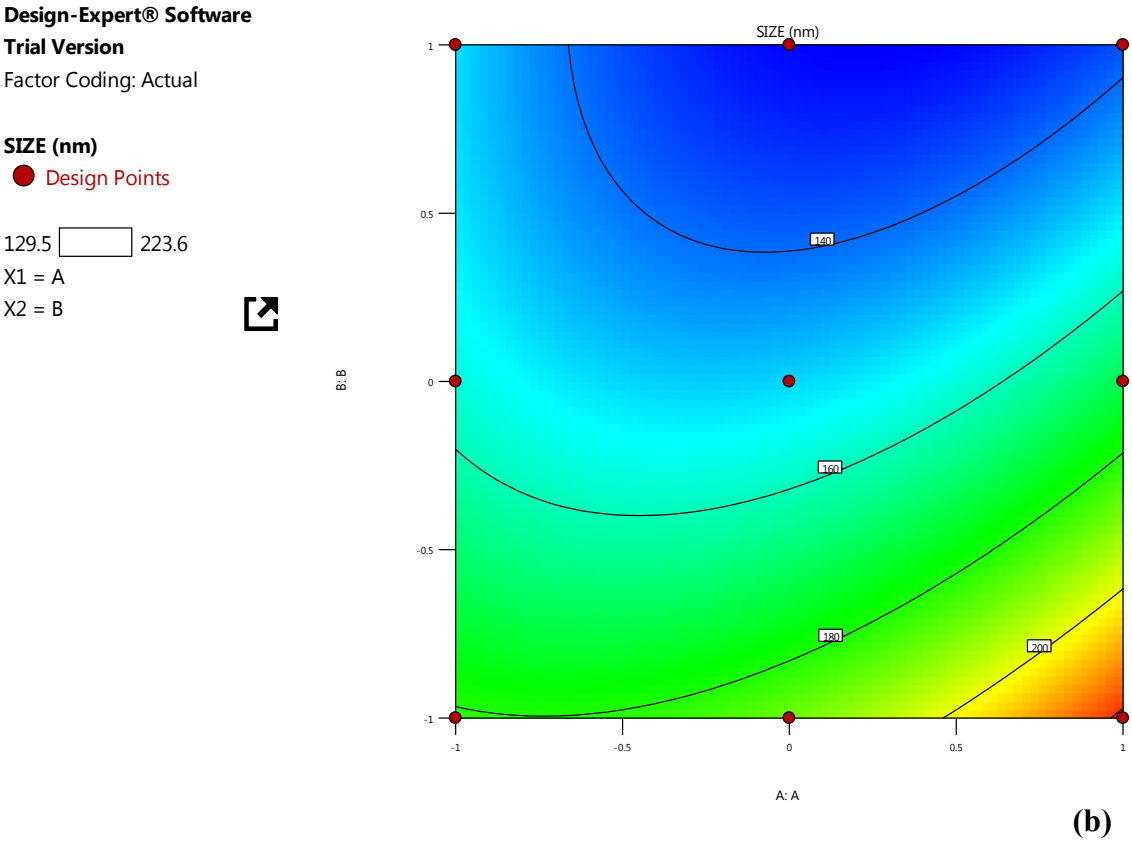
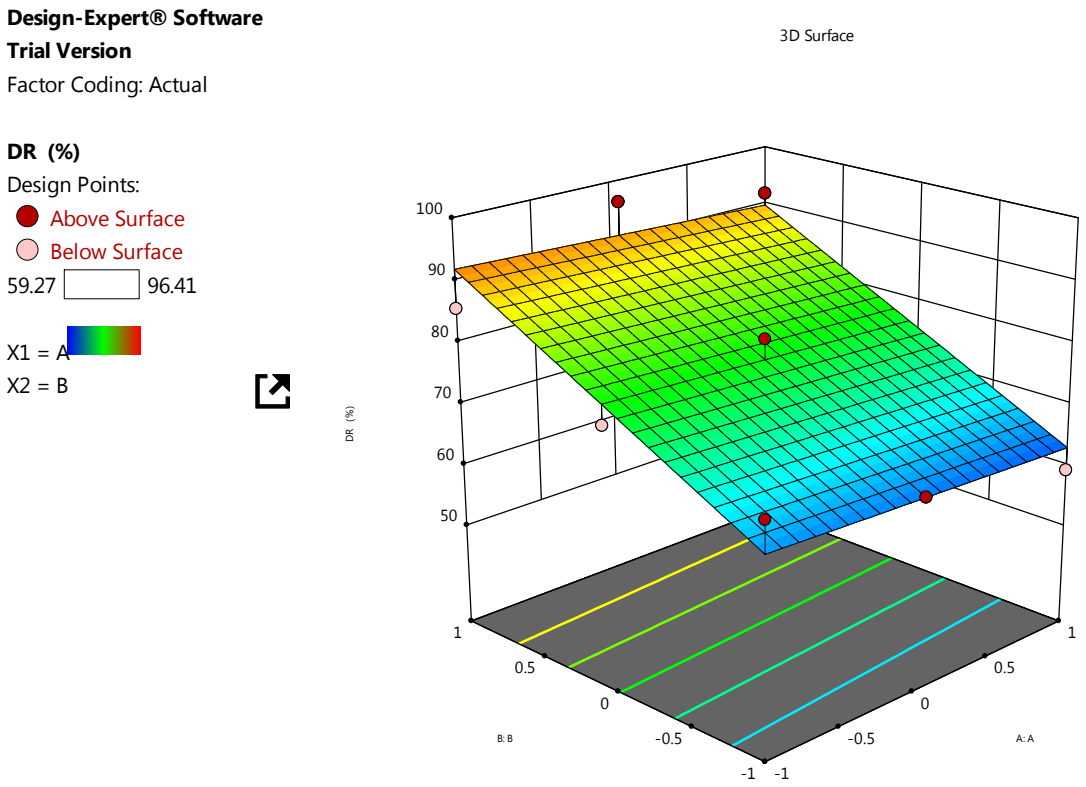
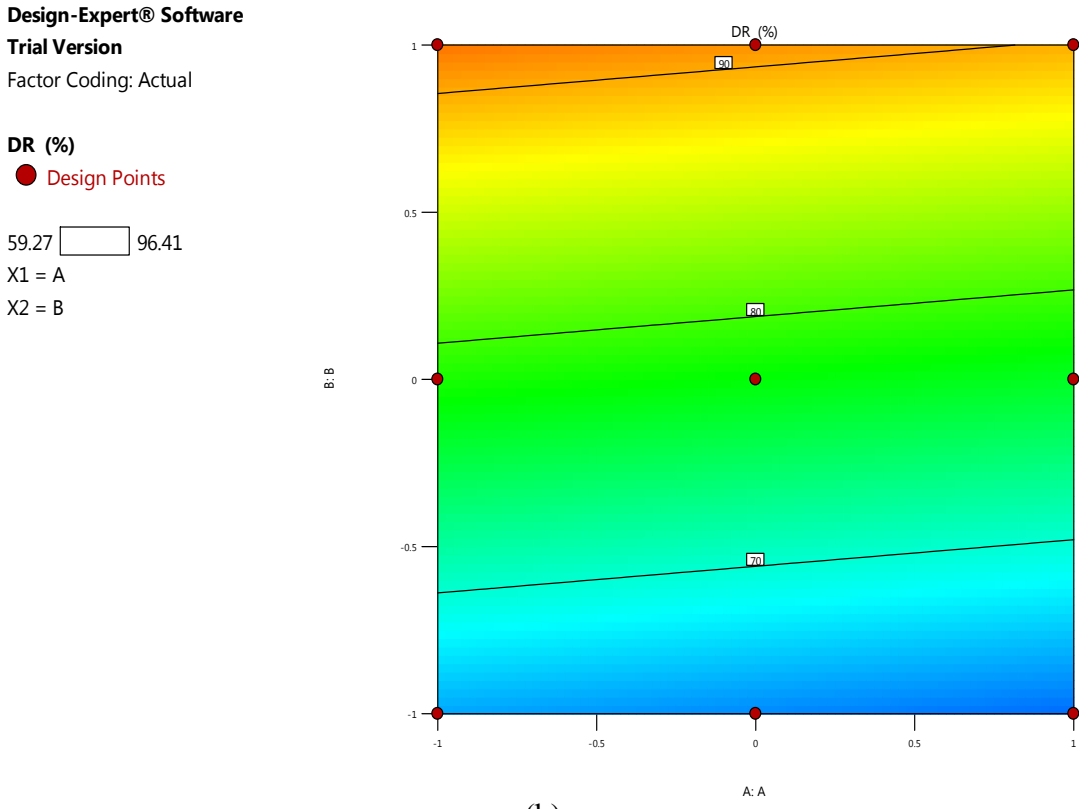


Fig. – 12: Response surface plots showing the effect of oil and Smix on mean globule size (nm) of timolol maleate nanoemulsions (a) 3D plot and (b) contour plot.



(a)



(b)

Fig. – 13: Response surface plots showing the effect of oil and Smix on percentage drug release (%) of timolol maleate nanoemulsions (a) 3D plot and (b) contour plot.

7.1.11. Surface morphology

The droplets in the optimized nanoemulsion appeared spherical and the droplet size was in agreement with the results obtained from droplet size analysis using the Zeta sizer and the TEM image was given in fig. 14.

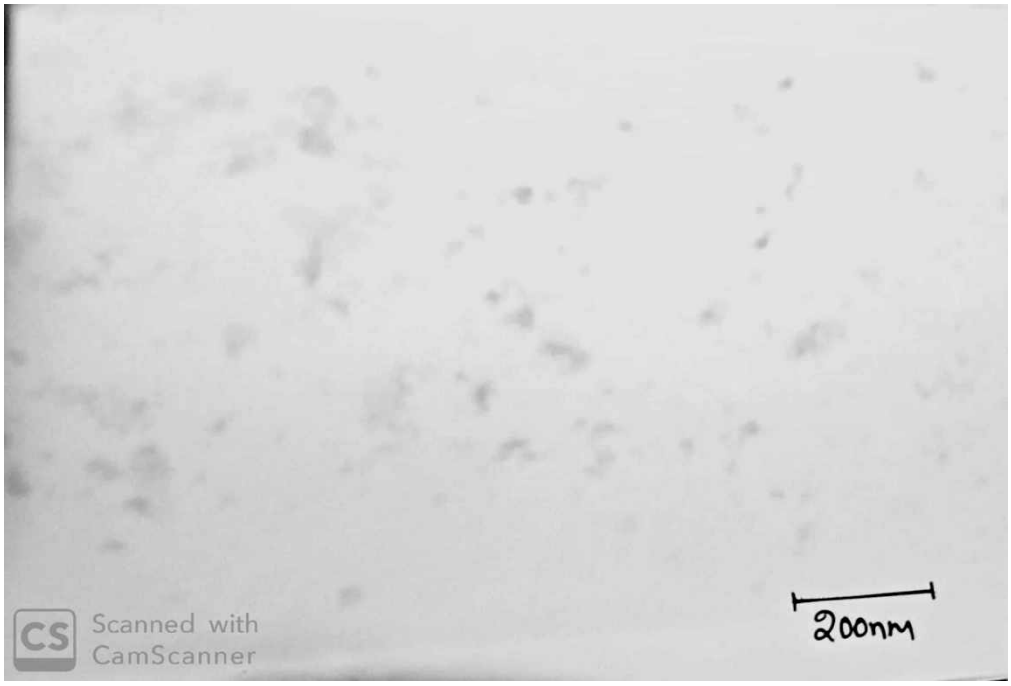


Fig. – 14: TEM image of optimized timolol maleate nanoemulsion

7.1.12 *Ex vivo* corneal permeation studies using bovine corneas:

The drug permeated through the corneal membrane at 4 h was found to be 775 ± 15 µg/ cm^2 and 262.5±12 µg/cm^2 from the optimized formulation and control solution respectively. The results for the drug solution were found quite opposite to the *in vitro* drug diffusion rate. This can be explained by the structural complexity of the corneal membrane that opposes the aqueous solution because of the outer lipophilic layer (Kumar H, et al., 2018). In the case of nanoemulsions, the nano sized droplets can be easily infiltrated by endocytosis and the permeation enhancing ability of the formulation ingredients will cause temporary changes in the tight junctions of the corneal membrane that enhances the permeation by transcellular pathway (Elsayed I, et al. 2017). The drug permeation profiles for TML-NE1 and the control solution were illustrated in fig. 15 and the permeability parameters were depicted in table 15. TML-NE1had greater flux value of 246.815 µg/cm^2/h and permeation coefficient of 2.74×10^{-5} cm/sec when compared to that of control 83.518 µg/cm^2/h and0.92×10^{-5} cm/sec while both TML-NE1 and the control solution showed corneal hydration

values of less than 70% indicating that there was no corneal damage occurred during *ex vivo* experiments (Morsi NM, et al. 2014).

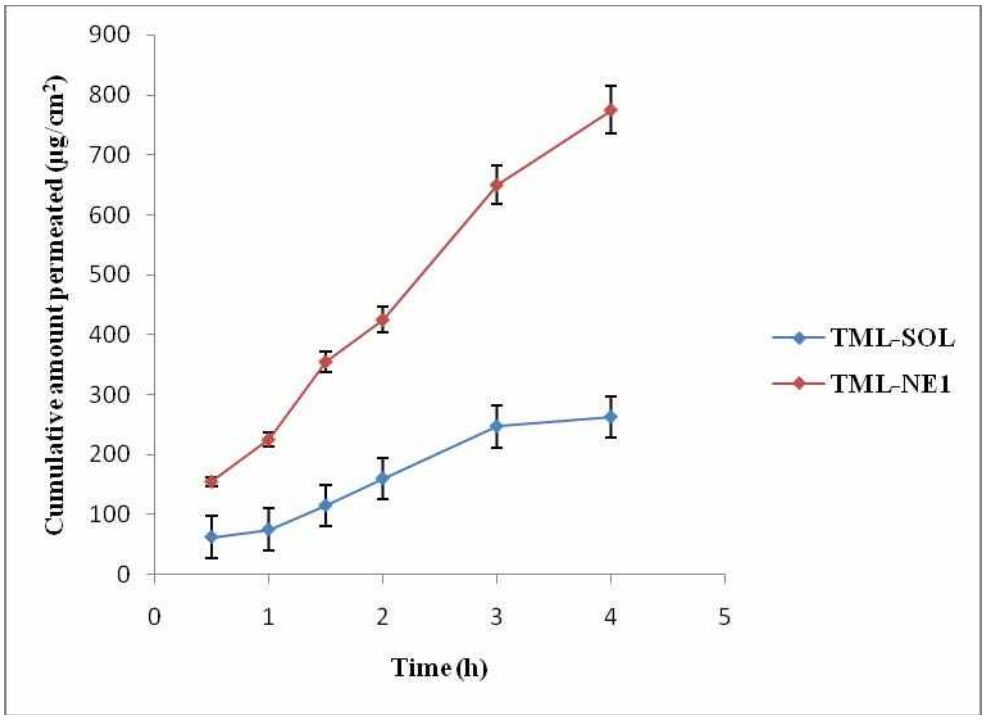


**Fig. – 15: *Ex vivo* transcorneal drug permeation profiles of optimized timolol maleate nanoemulsion and control drug solution, ***error bars represent standard deviations of three replicates

Table – 15

***Ex vivo* drug permeation parameters of optimized timolol maleate nanoemulsion and control drug solution**

Parameter	Control	Test formulation
Flux (µg/cm^2/h)	83.518	246.815
Papp(cm/sec)	0.92×10^{-5}	2.74×10^{-5}
Corneal hydration (%)	61.8±1.05	58.6±1.2

*Corneal hydration values expressed in mean±SD, n=3

7.1.13 *In vivo* studies

7.1.13.1 Ocular irritation test:

TML-NE1 found nonirritant with a minimal score of 2 in the Draize eye test. The rabbits showed slight redness of the conjunctiva which disappeared completely within 15 min but no lachrymation or chemosis were observed throughout the study

indicating the optimized formulation is nonirritant and could be tolerated by the rabbit eye (Patil N, et al 2013). The observations recorded were depicted in table 16.

Table – 16

Draize eye test results of optimized timolol maleate nanoemulsion in rabbits

Time point	Parameter									score
	Conjunctival discharge			Conjunctival chemosis			Conjunctival redness			
	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3	
5min	-	-	-	-	-	-	-	-	-	0
10min	-	+	-	-	-	-	-	-	-	1
15min	-	-	-	-	-	-	+	+	-	1
30min	-	-	-	-	-	-	-	-	+	1
1h	-	-	-	-	-	-	-	-	-	0
2h	-	-	-	-	-	-	-	-	-	0
3h	-	-	-	-	-	-	-	-	-	0
6h	-	-	-	-	-	-	-	-	-	0
9h	-	-	-	-	-	-	-	-	-	0
12h	-	-	-	-	-	-	-	-	-	0
24h	-	-	-	-	-	-	-	-	-	0
Total score- 3										
Inference- non-irritant										

7.1.13.2 Analytical method for estimation of TML in rabbit aqueous humour using UFLC

The calibration curve of TML in rabbit aqueous humour was plotted between peak area and concentration (ng/ml) showed in fig. 16. The concentration range of 2 to 100 ng/ml showed linearity with a regression coefficient (R^2) value of 0.993. The retention time was found to be 3.1 ± 0.12 min as shown in fig. 17.

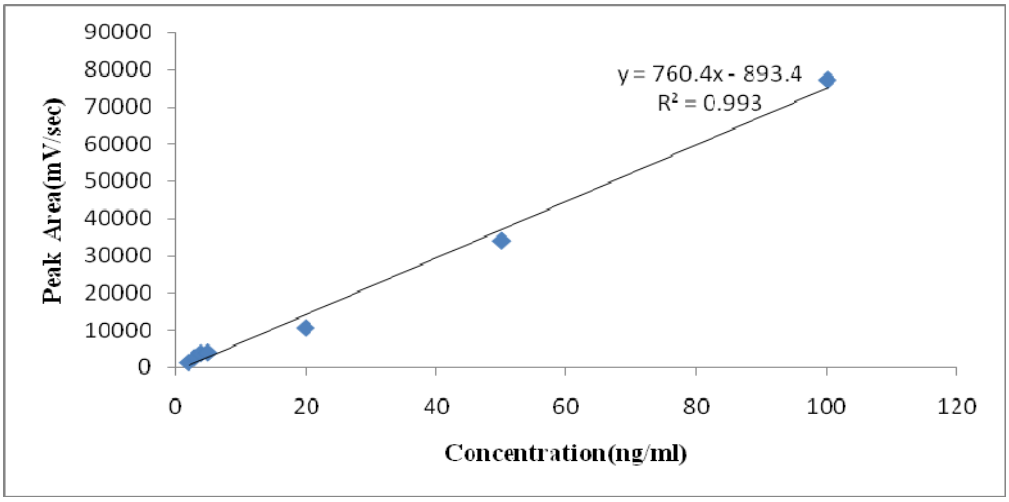


Fig. – 16: Calibration curve of timolol maleate in rabbit aqueous humour

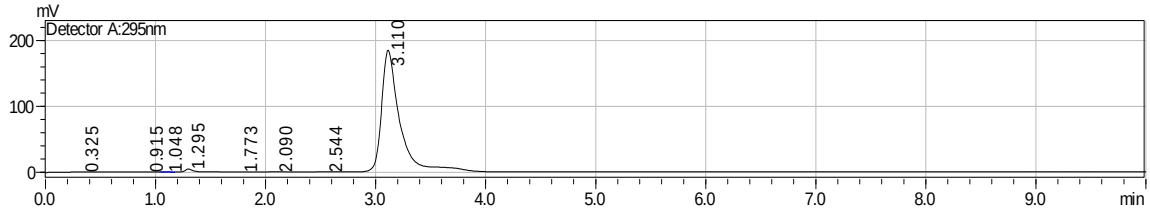


Fig. – 17: UFLC Chromatogram of timolol maleate (RT- 3.1 min) in rabbit aqueous humour

7.1.13.3 Analytical method for estimation of TML in rabbit plasma using UFLC

The calibration curve of TML in rabbit aqueous humour was plotted between peak area and concentration (ng/ml) showed in fig. 18. The concentration range of 50 to 400 ng/ml showed linearity with a regression coefficient (R^2) value of 0.994. The retention time was found to be 3.1 ± 0.12 min as shown in fig. 19.

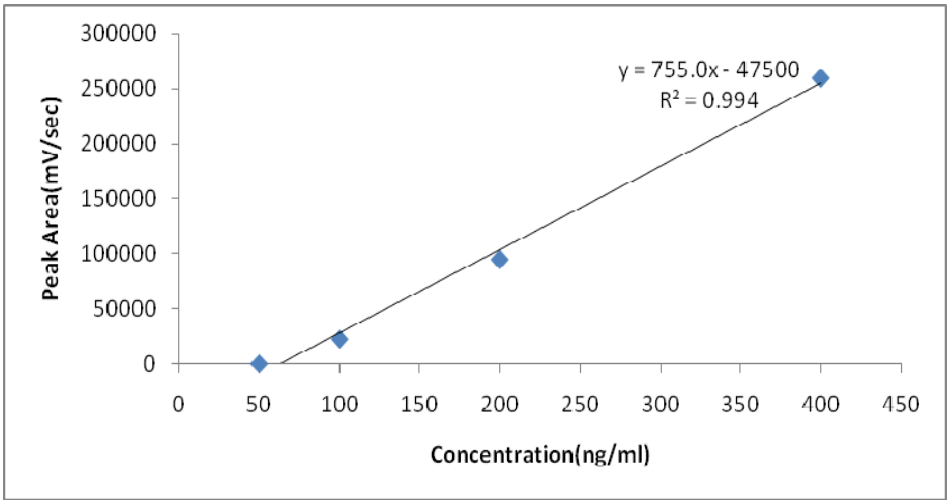


Fig. - 18- Calibration curve of timolol maleate in rabbit plasma

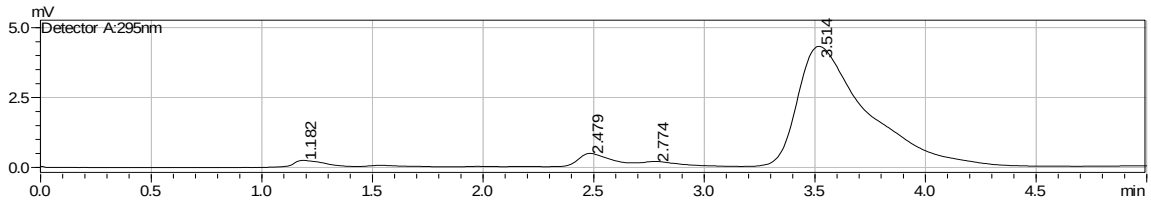


Fig. – 19: UFLC Chromatogram of timolol maleate (RT- 3.5 min) in rabbit plasma

7.1.13.4 Pharmacokinetic evaluation:

The plasma levels and aqueous humor levels of TML confirmed the lowest systemic absorption and the improved corneal absorption of TML-NE1 when compared to the marketed TML ophthalmic solution. It was found that the optimized TML-NE1 had 2.28 fold rise in ocular bioavailability and 3.39 fold reduction in systemic bioavailability when compared to the marketed product. The reason for improved corneal absorption can be explained by the small globule size, improved retention time due to viscosity and the positive charge on the droplets imparted by benzalkonium chloride involved in the electrostatic interaction with the negatively charged corneal membrane and that lead to improved residence time on cornea (Philippe D, et al. 2013). Different graphs were plotted comparing the pharmacokinetic profiles of TML-NE1 and the marketed product in aqueous humor and plasma and were shown in fig. 20-24.

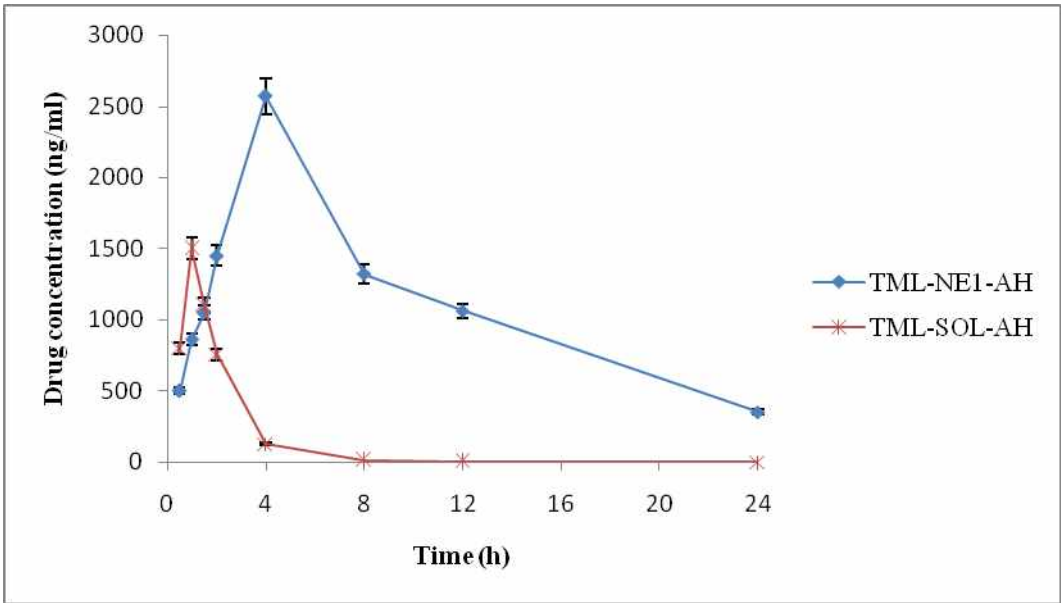


Fig. - 20: Pharmacokinetic profile of drug from optimized timolol maleate nanoemulsion and conventional marketed product in rabbit aqueous humour, *error bars represent standard deviations of three replicates

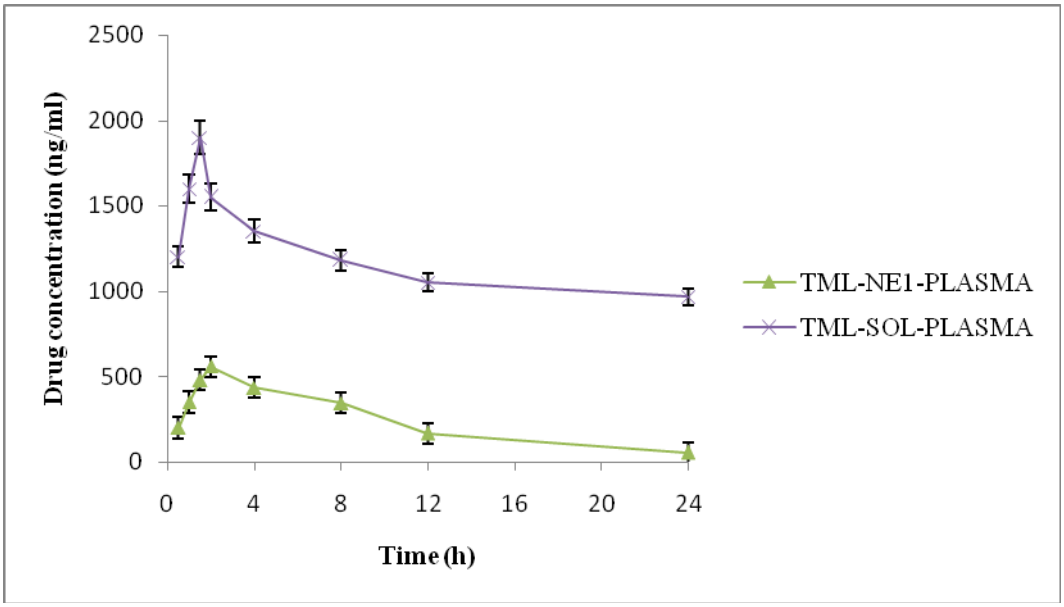


Fig. - 21: Pharmacokinetic profile of drug from optimized timolol maleate nanoemulsion and conventional marketed product in rabbit plasma, *error bars represent standard deviations of three replicates

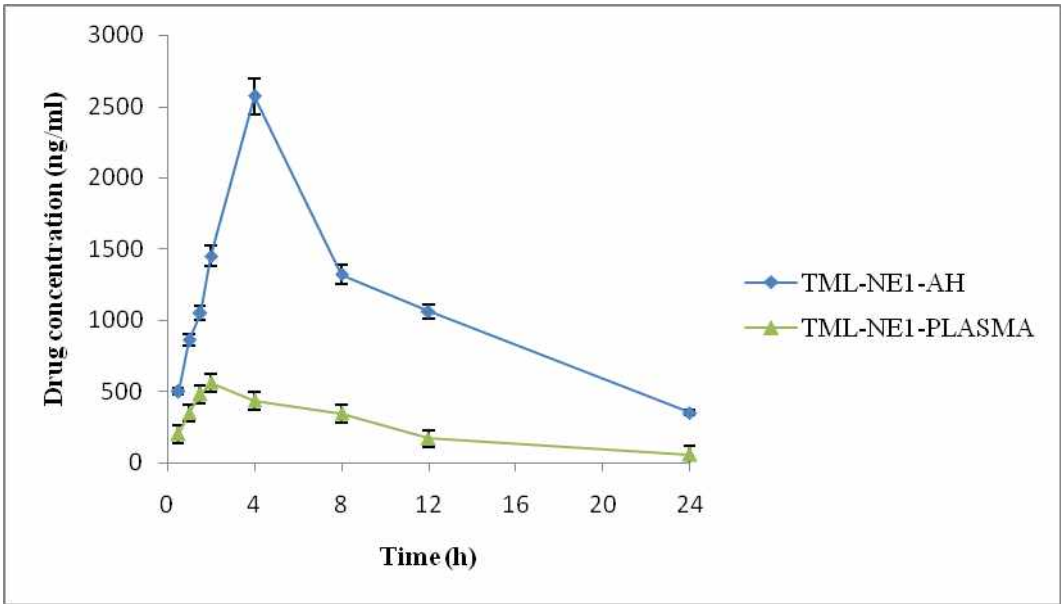


Fig. - 22: Pharmacokinetic profiles of drug from optimized timolol nanoemulsion in rabbit aqueous humour and rabbit plasma, *error bars represent standard deviations of three replicates

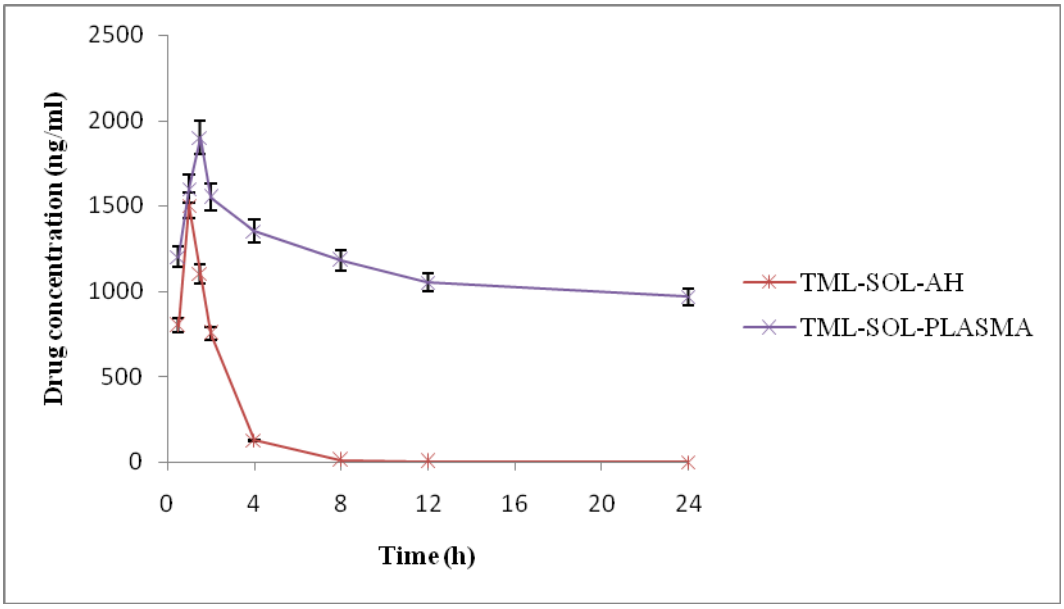


Fig. - 23: Pharmacokinetic profiles of TML from conventional marketed product in rabbit aqueous humour and in rabbit plasma, *error bars represent standard deviations of three replicates

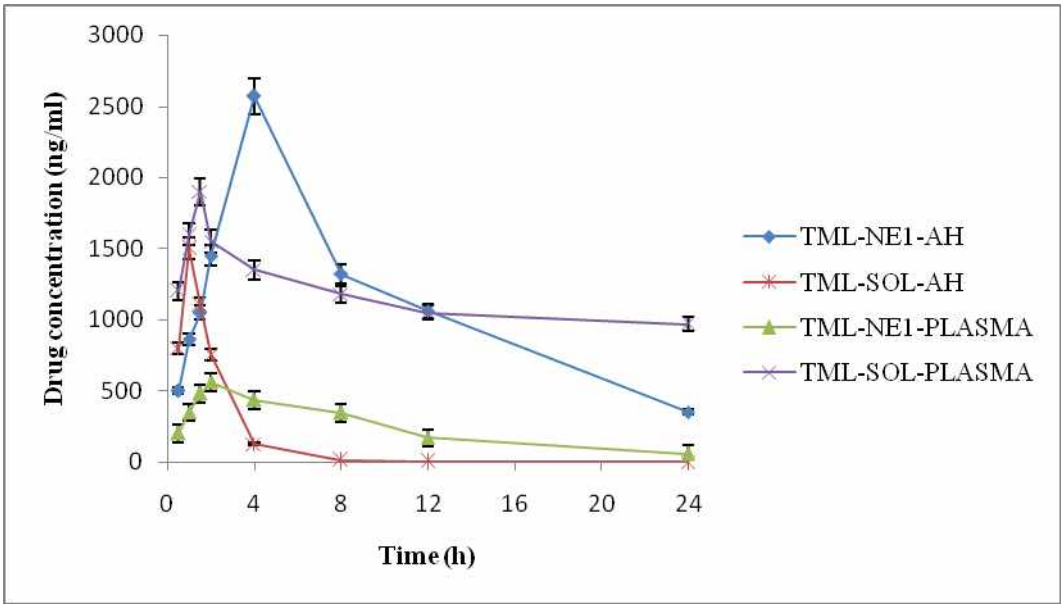


Fig. - 24: Comparison between aqueous humour and plasma pharmacokinetic profiles of drug from optimized timolol maleate nanoemulsion and conventional marketed product in rabbits, *error bars represent standard deviations of three replicates

The pharmacokinetic parameters like maximum drug concentration (Cmax) and the time required to achieve maximum concentration (Tmax) were obtained from the concentration versus time plot and area under the curve (AUC) was calculated by trapezoidal model (Nagarval RC, et al. 2012) and were summarized in table 17.

Table – 17

Pharmacokinetic parameters of optimized timolol maleate nanoemulsion and conventional marketed product in rabbits

Parameter	Aqueous humor		Plasma	
	Control	Test formulation	Control	Test formulation
$AUC_{(0\text{-}24h)}$ (ng/ml/h)	7790.98	17818.14	20398.28	4488.34
Tmax (h)	1	4	1.5	2
Cmax (ng/ml)	1500.32	2569.11	1900.08	560.72

The obtained results were found to be statistically significant with P values 0.0353 in aqueous humour and <0.0001 in plasma when analyzed by a one-sample t-test using GraphPad Prism software version, 8.0.2 and were depicted in table 18.

Table – 18

Statistical ananlysis of pharmacokinetic data of optimized timolol maleate nanoemulsion and conventional marketed product

One sample t-test	TML-NE1	
	Aqueous humour	Plasma
Actual mean	536.9	1350
Number of values	8	8
t, df	t=2.603, df=7	t=12.15, df=7
P value	0.0353	<0.0001
Significant (alpha=<0.05)	Yes	Yes
95% confidence interval	49.20 to 1025	1087 to 1613
R^2	0.4919	0.9547

7.2. CIPROFLOXACIN NANOEMULSIONS

7.2.1 Melting Point

The melting point of ciprofloxacin hydrochloride was found to be in the range of 328°C meeting the reported standard melting point of >300°C indicating the purity of the drug (Sagar KS, et al. 2016).

7.2.2 Calibration Curve

The calibration curve plotted for peak area against a concentration (ng/ml) was shown in fig. 25. The linearity was observed for the concentration range of 20 to 400 ng/ml with a regression coefficient (R^2) value of 0.992. Fig. 26 shows the UFLC chromatograph of CIP and the retention time was found to be 1.36 ± 0.01 min. The λ_{max} was found to be 279 nm.

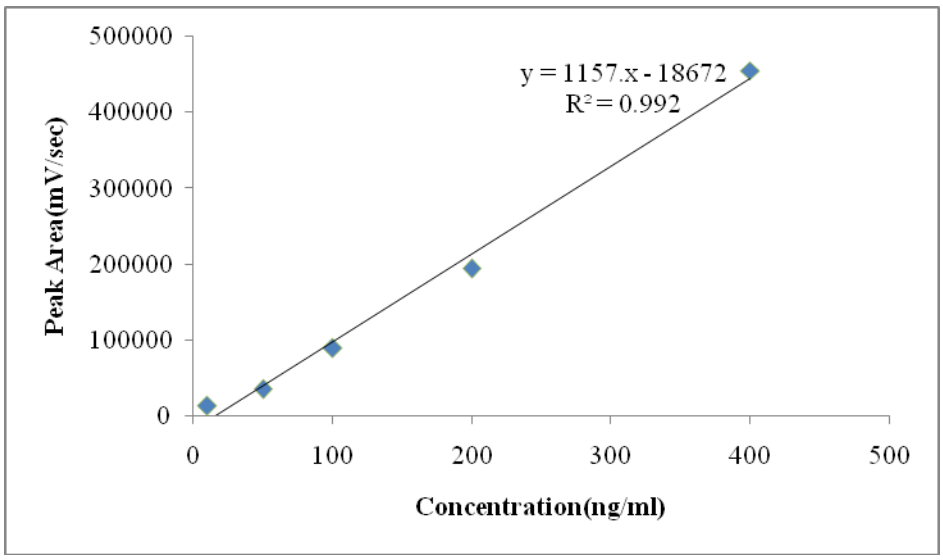


Fig. – 25: Calibration curve of ciprofloxacin

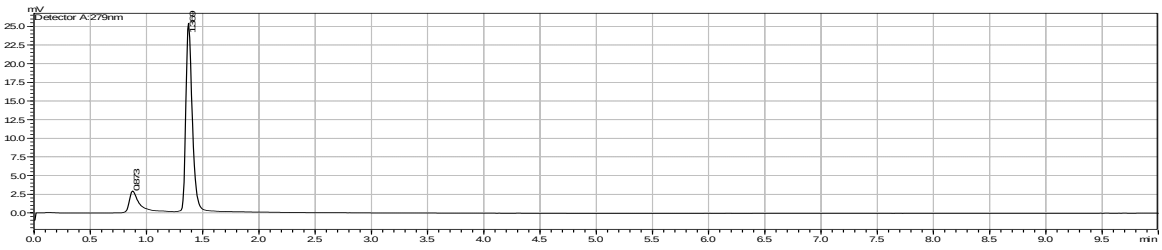


Fig. – 26: UFLC chromatogram of ciprofloxacin (RT- 1.367 min)

7.2.3 Solubility Study

The solubility of CIP in various oils, surfactants and co-surfactants were depicted in fig. 27. Among oils, caprol micro express showed very good solubility. Therefore, it was selected as the oil phase. Among surfactants and co-surfactants CIP had the highest solubility in polysorbate 20 and span 20 hence they were selected as surfactant and co-surfactant respectively for the phase study.

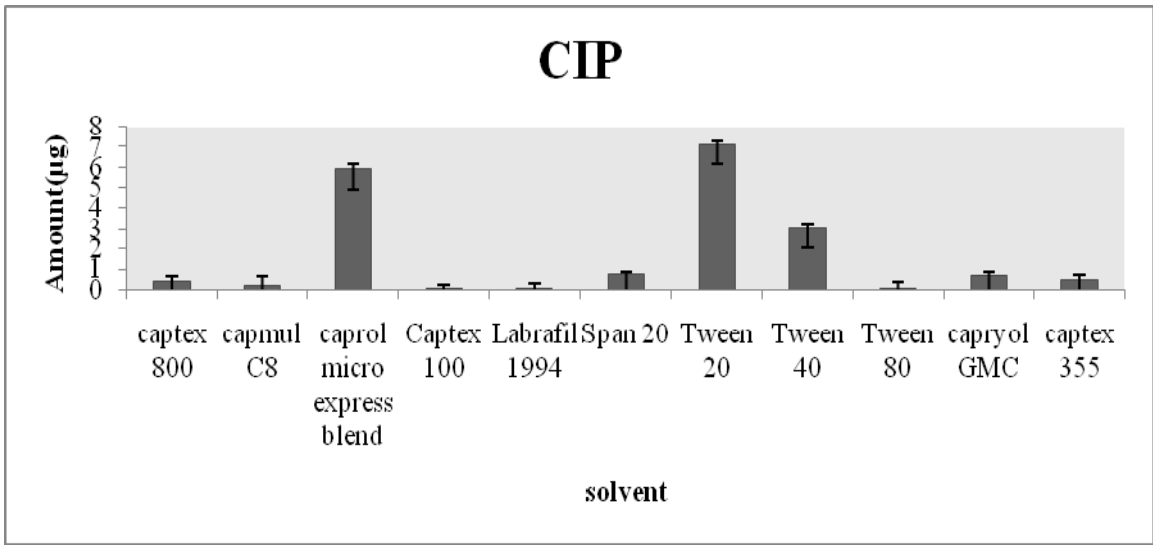


Fig. – 27: Ciprofloxacin solubility in various solvents, *error bars represent standard deviations of three replicates

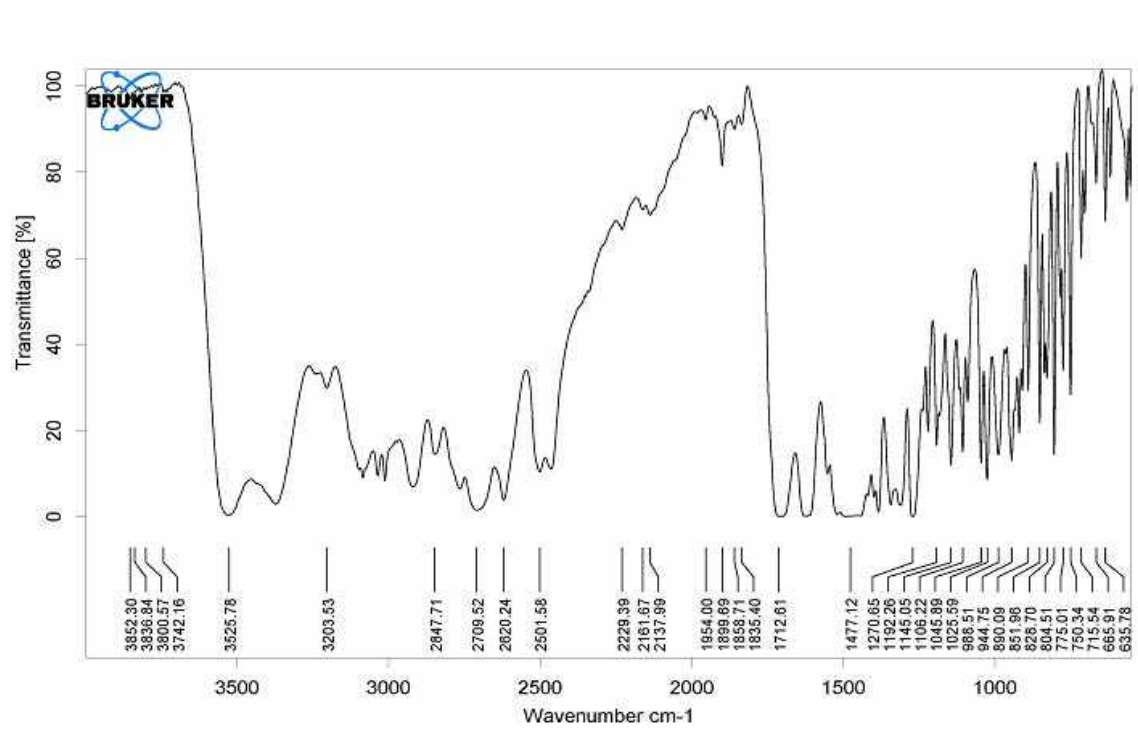
7.2.4 Drug – Excipient Compatibility Studies

The major functional groups of CIP that are identified from the FTIR spectral analysis were depicted in table 19 and the characteristic peaks were illustrated in fig.28. In brief, OH stretching of carboxylic acid were found at 3525.78 cm^{-1} and 3440.61 cm^{-1}, methyl C-H stretching were found at 2847.71 cm^{-1} and 2923.71 cm^{-1}, C=O stretching were found at 1712.61 cm^{-1} and 1741.80 cm^{-1}, C-F stretching were found at 1025.59 cm^{-1} and 1000.23 cm^{-1} in pure drug and physical mixture respectively (Makwana SB, et al. 2016; Sagar KS, et al. 2016). Thus all the characteristic peaks of drug were found in both spectra confirming the chemical integrity of the drug in the presence of selected excipients and hence, there exists a good compatibility between drug and excipients.

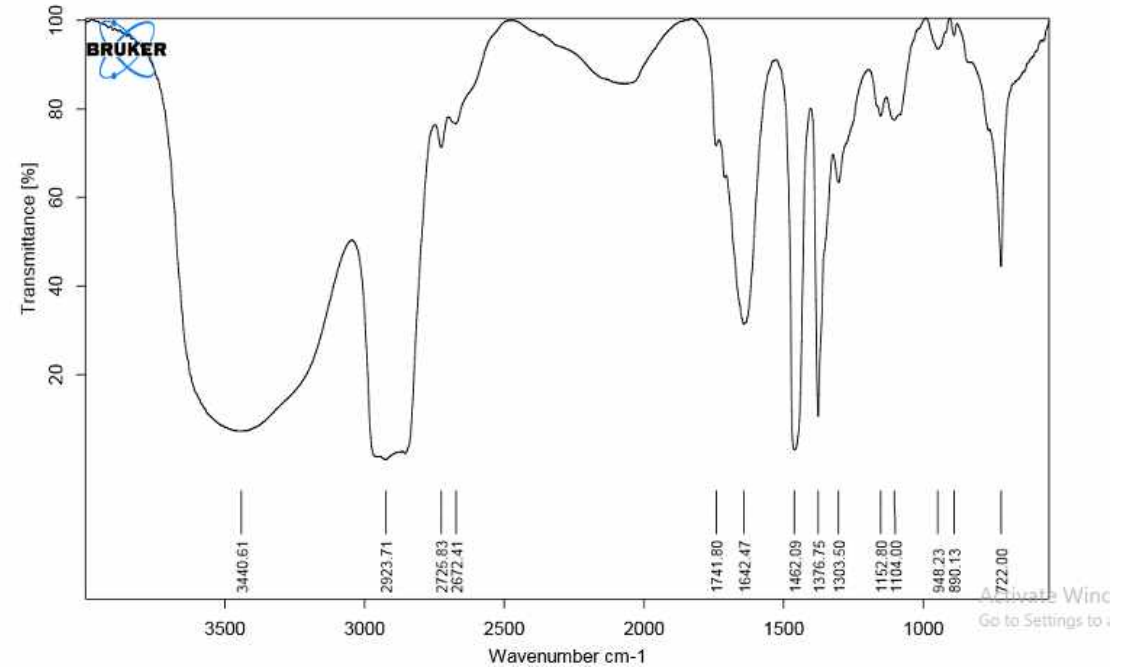
Table – 19

Ciprofloxacin functional group identification from FTIR spectral data

Functional group	Wave number(cm^{-1})	
	Pure Drug	Physical mixture
O-H	3525. 78	3440.61
C-H	2847.71	2923.71
C=O	1712.61	1741.80
C-F	1025.59	1000.23



(a)



(b)

Fig. – 28: Ciprofloxacin FT-IR spectra (a) pure drug and (b) physical mixture

7.2.5 Phase Behavior and Optimization of Formulation Compositions

Pseudoternary phase diagrams were constructed separately for each surfactant to the co- surfactant ratio and were given in fig. 29. A large w/o area was observed for the co-surfactant concentration in the ratio 1:3 (w/w) and the same was used to optimize the final formulation compositions. 3^2 factorial design was applied to finalise the compostitions and the factors under consideration were shown in table 20 while the final nanoemulsion compositions were depicted in table 21.

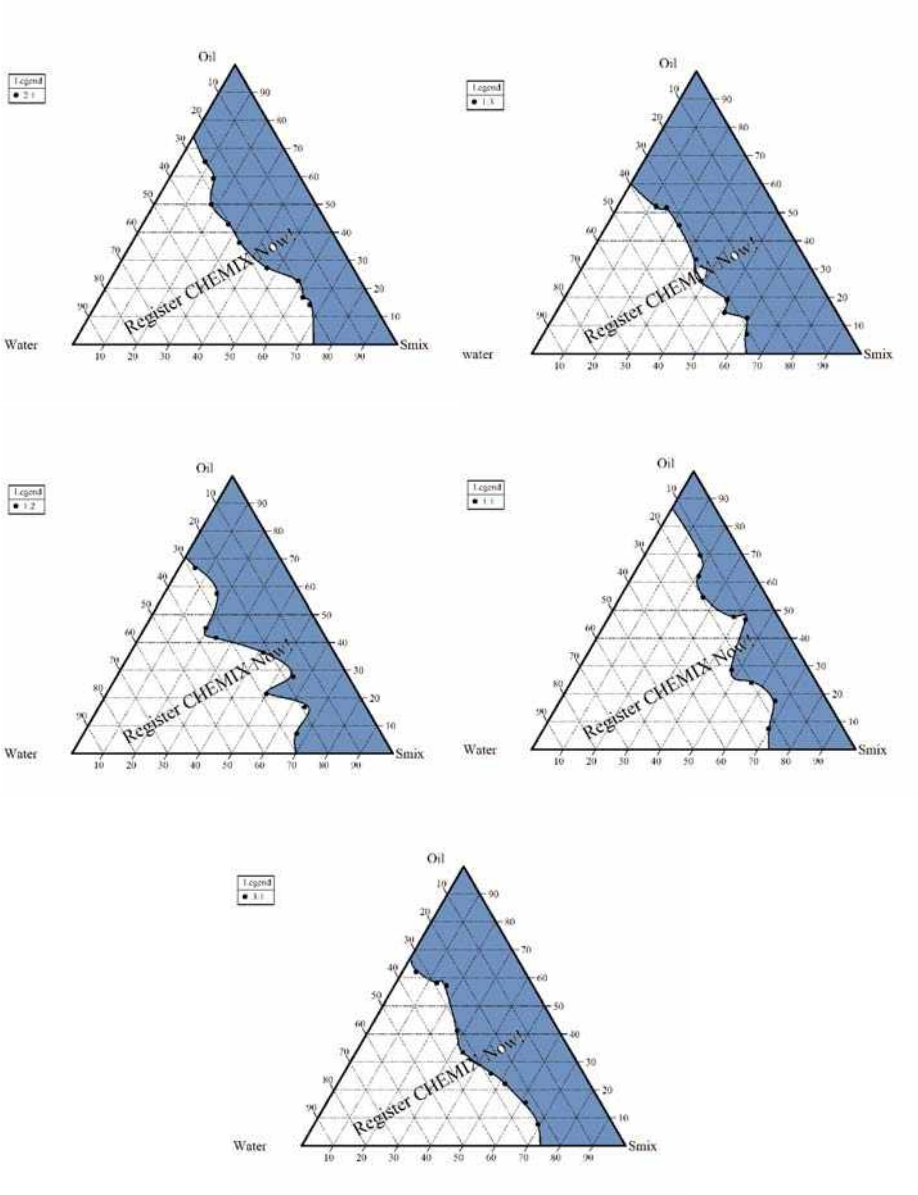


Fig. – 29: Pseudo ternary phase diagrams of caprol micro express blend and Smix (tween 20: span 20) Ratios; (a) 1:1, (b) 1:2, (c) 1:3, (d) 2:1 and (e) 3:1

Table – 20

3^2 factorial design for the selection of formulation composition for ciprofloxacin nanoemulsions

Formulation code	Coded value		Actual value	
	X1	X2	X1	X2
CIP-NE1	-1	-1	25	40
CIP-NE2	0	-1	27.5	40
CIP-NE3	1	-1	30	40
CIP-NE4	-1	0	25	45
CIP-NE5	0	0	27.5	45
CIP-NE6	1	0	30	45
CIP-NE7	-1	1	25	50
CIP-NE8	0	1	27.5	50
CIP-NE9	1	1	30	50
Independent variables	Levels			
	Low (-1)	Medium (0)	High (1)	
X1- amount of oil (%w/w) X2-amount of Smix(%w/w)	25 40	27.5 45	30 50	
Dependent variables	Y1- mean droplet size(nm) Y2- percentage permeation (%)			

Table – 21

Formulation composition of ciprofloxacin nanoemulsions

Formulation code	Oil	Smix	Water	1% Propylene glycol	3% Glycerol	Drug	Benzalkonium chloride
CIP-NE1	25	40	10	12.5	12.5	0.3	0.02
CIP-NE2	27.5	40	12.5	10	10	0.3	0.02
CIP-NE3	30	40	15	7.5	7.5	0.3	0.02
CIP-NE4	25	45	10	10	10	0.3	0.02
CIP-NE5	27.5	45	12.5	7.5	7.5	0.3	0.02
CIP-NE6	30	45	15	5	5	0.3	0.02
CIP-NE7	25	50	10	7.5	7.5	0.3	0.02
CIP-NE8	27.5	50	12.5	5.25	5.25	0.3	0.02
CIP-NE9	30	50	15	2.5	2.5	0.3	0.02

*all the values are represented as %w/v

7.2.6 Physicochemical Characterization.

The results obtained from the physicochemical characterization of CIP-NEs were summarized in table 22. The droplet size of nanoemulsions found < 200 nm ranged from 121.4±1.6 to 186.2±1.9 nm confirming the formation of nanoemulsions (Panchal SS, et al. 2017). The polydispersity index values found <1.00 ranged from 0.031±0.021 to 0.512±0.001 confirming the homogeneity of due to narrow size distribution of droplets (Siafaka PI, et al. 2015). The zeta potential values ranged from +16.32±4.12 to +24.15±5.25 mV and the positive sign indicates the ability to interact with negatively charged corneal epithelial cells and can improve the contact time with corneal membrane (Hao J, et al. 2011). It is evident from the previous research reports that the ophthalmic formulations with a viscosity between 15-150 cP have improved corneal retention time and the viscosity of prepared formulations ranged from 40.5±0.0 to 64.2±0.2 cP. (Honar S and Zahi F, 2013). The pH of prepared formulations found near to the biological pH with values ranged from 5.5 ± 0.4 to 6.7 ± 0.5. The pH of tear solution is 7.4 and the acceptable range for ophthalmic formulations is 5 to 8 as the human eye can tolerate pH changes within this range (Horn G, 2015). The refractive index of formulations ranged from 1.340 ± 0.001 to 1.386± 0.005 and researchers reported that formulations with refractive index NMT 1.476 found non-irritant (Ammar HO, et al. 2009). The surface tension of formulations ranged from 44.1 ± 1.9 to 52.6 ± 2.6 mN/m and the acceptable range is 40-50 mN/m. This low surface tension is advantageous for ophthalmic delivery as it improves the spreading of formulation onto the corneal membrane (Radomska Soukharev AN, et al. 2005). The acceptable range of osmotic pressure for ophthalmic formulations is 231-446 mOsm/L and that of prepared formulations ranged from 355 to 398 mOsm/L (Gan L, et al. 2013; Morsi NM, et al. 2014).

Table – 22

Physical data of different evaluation parameters of ciprofloxacin nanoemulsions

Formulation code	Droplet size (nm)	Poly dispersity index	Zeta potential (mV)	Viscosity (mPas)	pH	Refractive index	Surface tension (mN/m)	Osmolarity (mOsm/L)
CIP-NE1	145.7±1.5	0.512±0.001	+24.15±5.25	45.4±0.1	5.9 ± 0.9	1.346 ± 0.007	45.3 ± 3.4	398
CIP-NE2	167.8±1.5	0.031±0.021	+23.83±5.86	61.6±0.1	6.3 ± 0.2	1.347 ± 0.002	49.9 ± 2.5	356
CIP-NE3	186.2±1.9	0.209±0.110	+18.35±4.83	50.9±0.2	5.8 ± 0.6	1.368± 0.002	46.8 ± 3.7	384
CIP-NE4	121.4±1.6	0.318±0.005	+19.70±3.51	58.1±0.1	6.7 ± 0.5	1.348 ± 0.005	44.1 ± 1.9	397
CIP-NE5	153.3±1.6	0.070±0.000	+21.00±4.09	52.6±0.5	5.8 ± 0.7	1.346 ± 0.001	51.5 ± 1.8	365
CIP-NE6	176.5±2.0	0.253±0.012	+23.20±4.06	64.2±0.2	6.2 ± 0.6	1.369 ± 0.001	47.1 ±4.0	382
CIP-NE7	130.1±1.1	0.237±0.011	+16.61±5.45	40.5±0.0	6.5 ± 0.3	1.340 ± 0.001	49.9 ± 2.5	355
CIP-NE8	160.1±0.8	0.318±0.003	+16.32±4.12	46.3±0.1	5.5 ± 0.4	1.368 ± 0.002	52.6 ± 2.6	361
CIP-NE9	180.8±1.1	0.272±0.040	+18.74±3.84	52.0±0.0	6.6 ± 0.8	1.386± 0.005	47.5 ±3.5	385
Required	≤200	≤1.00	±20-40	15-150	5.5-7.4	1.340-1.360 (NMT1.476)	40-50	231-446

*Data represents mean ±SD, n=3

7.2.7 Stability

CIP-NEs remained clear with no phase separation or drug precipitation, indicating their excellent physical stability. All the formulations were found to be consistent with their mean globule size, drug content, phase separation and transparency during the stability study. The physical data comparing their mean globule size and drug content before and after stability studies were given in table 23.

Table – 23

Physical evaluation of ciprofloxacin nanoemulsions before and after stability studies

Formulation code	Particle size (nm)		Drug content (%)	
	Freshly prepared	After stability studies	Freshly prepared	After stability studies
CIP-NE1	145.7±1.5	148.2± 0.5	97.12 ± 0.02	96.17 ± 0.14
CIP-NE2	167.8±1.5	169.4 ± 0.3	94.55 ± 1.12	94.07 ± 1.05
CIP-NE3	186.2±1.9	187.7 ± 0.7	98.34 ± 1.52	98.52 ± 0.20
CIP-NE4	121.4±1.6	122.2 ± 0.5	96.32 ± 1.57	96.18 ± 1.12
CIP-NE5	153.3±1.6	158.8 ± 0.4	95.97 ± 2.02	95.73 ± 1.03
CIP-NE6	176.5±2.0	175.4 ± 0.5	93.35 ± 1.65	93.21 ± 1.36
CIP-NE7	130.1±1.1	132.8 ± 0.1	94.36 ± 1.79	93.75 ± 2.42
CIP-NE8	160.1±0.8	161.2 ± 0.7	96.22 ± 1.91	95.89 ± 1.24
CIP-NE9	180.8±1.1	182.5 ± 0.3	95.16 ± 1.14	94.99 ± 1.13

*Data represents mean ±SD, n=3

7.2.8 *In vitro* Release

The drug release profiles of prepared formulations were compared to that of aqueous solution and the results were shown in fig. 30. The drug was diffused faster from solution compared to the prepared formulations and their sustained drug release confirmed the characteristic lipophilic nature of nanoemulsions (Shen Y, et al. 2015). The higher viscosity of the nanoemulsions can also be the reason for the slow drug release according to Fick's law of diffusion (Floury J, et al. 2000).

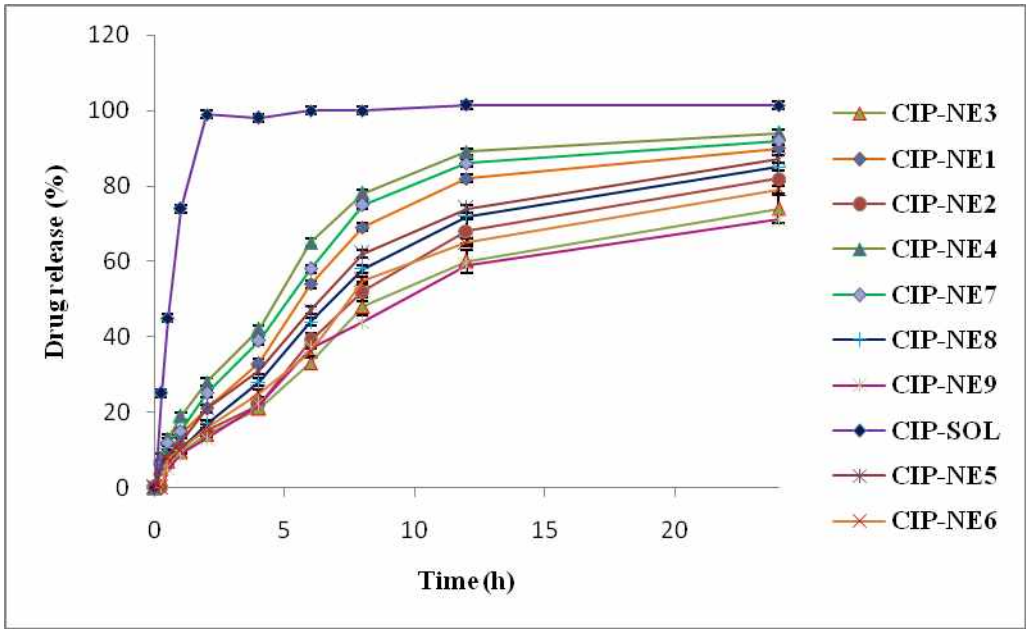


Fig. – 30: ***In vitro*** **drug release profiles of ciprofloxacin nanoemulsions and control drug solution,** *error bars represent standard deviations of three replicates

7.2.9 Optimization of Nanoemulsions

7.2.9.1 Model Fitting Analysis

All the responses observed for 9 formulations were fitted in to different experimental models using Design Expert®. The best-fitted model for Y_1 (Mean globule size, nm) and Y_2 (Drug release, %) was found to be a quadratic model. The fit summary and statistics were summarized in the table 24 and 25 respectively. The predicted R^2 values were found in reasonable agreement with the adjusted R^2 values. ANOVA of quadratic model for responses Y_1 and Y_2 were shown in table 26. The model found significant with F-values 68.63 and 42.77 for responses Y_1 and Y_2 respectively. The final equations in terms of coded factors are as follows.

$$Y_1=152.44+24.33\ X_1-4.67\ X_2+2.25\ X_1\ X_2-3.67\ X_1^2+11.33\ X_2^2$$

$$Y_2= +88.67\ -8.67\ X_1+1.33\ X_2\ +0.2500\ X_1\ X_2-2.00\ X_1^2-5.00\ X_2^2$$

Table – 24

Fit summary of the experimental data of ciprofloxacin nanoemulsions for responses Y_1 and Y_2

Source	Sequential p-value		Adjusted R^2		Predicted R^2		
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	
Linear	0.0006	0.0017	0.8877	0.8405	0.8219	0.7465	
2FI	0.5973	0.8930	0.8732	0.8094	0.7631	0.4396	
Quadratic	0.0362	0.0188	0.9769	0.9775	0.8952	0.8995	Suggested
Cubic	0.1411	0.2379	0.9986	0.9962	0.9685	0.9130	

Table – 25

Fit statistics of the experimental data of ciprofloxacin nanoemulsions for responses Y_1 and Y_2

Std. Dev.	Y_1	3.41	**R^2**	Y_1	0.9913
	Y_2	1.21		Y_2	0.9916
Mean	Y_1	157.56	**Adjusted R^2**	Y_1	0.9769
	Y_2	84.00		Y_2	0.9775
C.V. %	Y_1	2.16	**Predicted R^2**	Y_1	0.8952
	Y_2	1.44		Y_2	0.8995
Adeq Precision				Y_1	22.4252
				Y_2	24.1412

Table – 26

ANOVA for the experimental data of ciprofloxacin nanoemulsions by quadratic model for responses Y_1 and Y_2

Source	Sum of Squares		df		Mean Square		F-value		p-value		
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	
Model	3987.36	519.58	5	5	797.47	103.92	68.63	70.58	0.0027	0.0026	**Significant**
A-A	3552.67	450.67	1	1	3552.67	450.67	305.73	306.11	0.0004	0.0004	
B-B	130.67	10.67	1	1	130.67	10.67	11.24	7.25	0.0440	0.0743	
AB	20.25	0.2500	1	1	20.25	0.2500	1.74	0.1698	0.2785	0.7080	
A^2	26.89	8.00	1	1	26.89	8.00	2.31	5.43	0.2256	0.1020	
B^2	256.89	50.00	1	1	256.89	50.00	22.11	33.96	0.0182	0.0101	

7.2.9.2 Response Surface Analysis

A response surface analysis was used to study the interaction effects of two factors at one time. A total of 9 runs with triplicate center points were generated and the responses so observed are shown in fig. 31&32. The size analysis showed that as the oil content increased, the droplet size increased thus had a linear effect on droplet size. This observation suggested that an increase in the interfacial surface by increasing the oil content (Azeem A, et al. 2009). The droplet size reduced as the Smix content increased from low to medium and further increase in Smix resulted in a slight increase in the droplet size. This might be due to the formation of mixed micelles at high surfactant levels (Seyfoddin A, et al. 2013). At a low level of oil content, the % drug release was more and as the oil content increased the drug release decreased thus had an invert effect on % drug release. The % drug release increased as the smix content increased from low to medium and further increase in Smix resulted in a slight decrease in the % drug release. It was observed that the high % drug release from the optimized formulations was due to their nano size in addition to the presence of formulation ingredients. Thus the formulation with low oil and medium Smix content, CIP-NE4 found to have the smallest average droplet size and highest % drug release and can be expected with a significant influence on bioavailability.

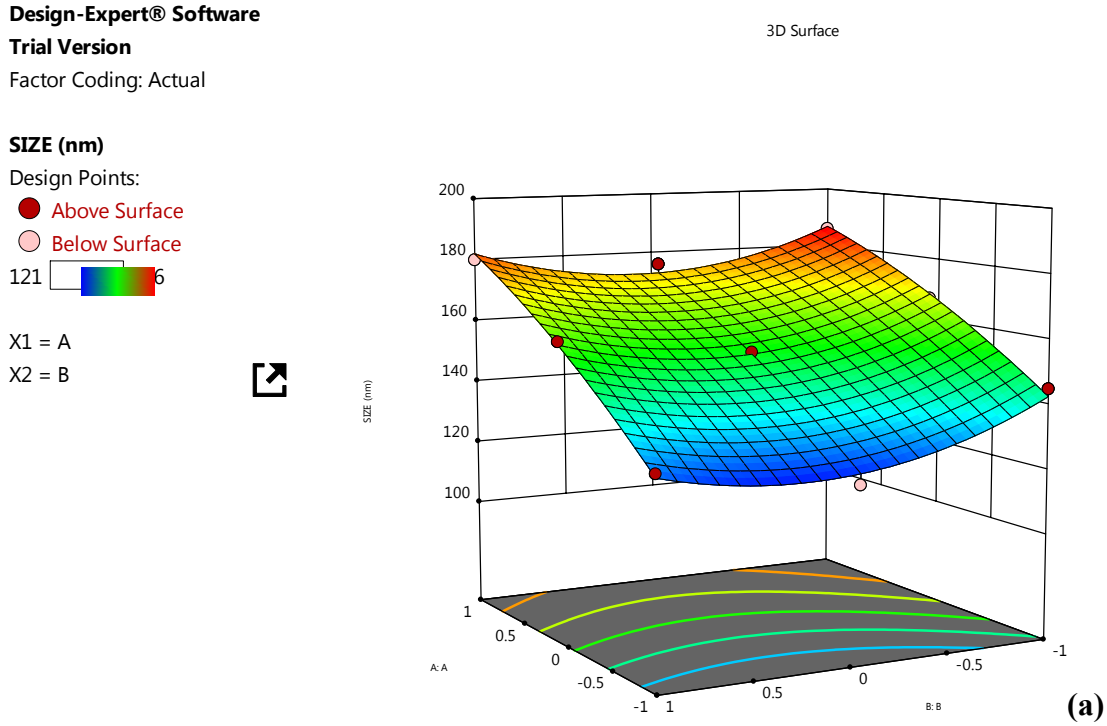


Fig. – 31: Response surface plots showing the effect of oil and Smix on mean globule size (nm) of ciprofloxacin nanoemulsions (a) 3D plot and (b) contour plot.

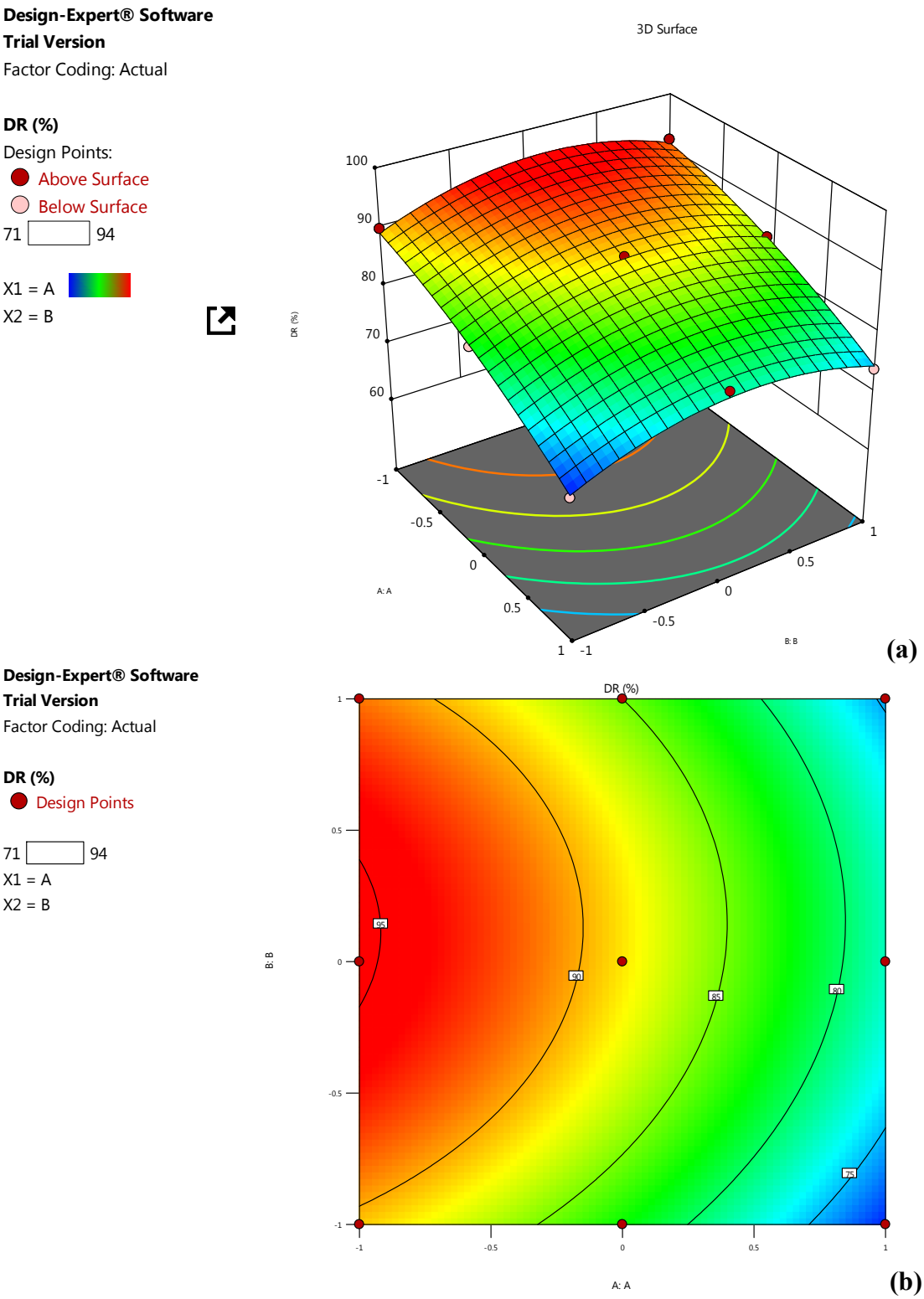


Fig. – 32: Response surface plots showing the effect of oil and Smix on percentage drug release (%) of ciprofloxacin nanoemulsions (a) 3D plot and (b) contour plot.

7.2.10 Transmission Electron Microscopy (TEM)

The droplets in the nanoemulsions appeared spherical and the droplet size was in agreement with the results obtained from droplet size analysis using the Zetasizer and the TEM image of optimized formulation was given in fig. 33.

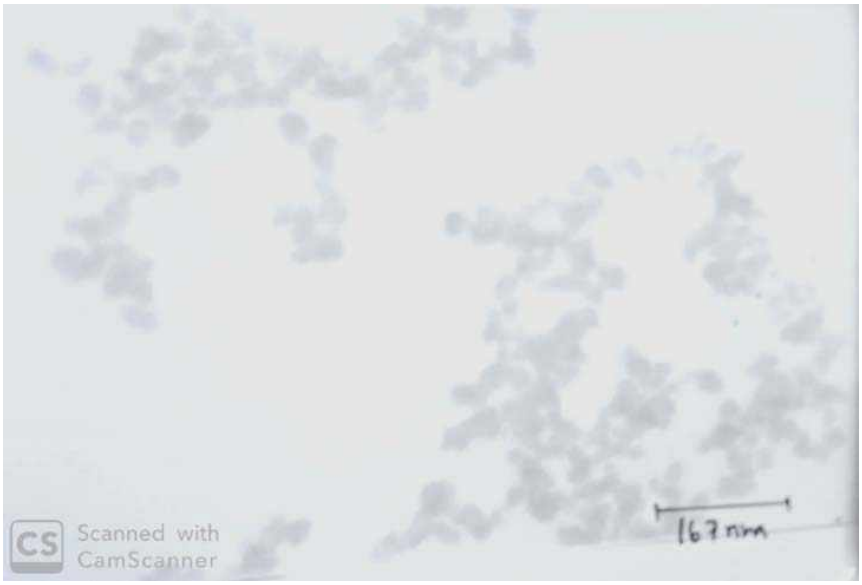


Fig. – 33: TEM image of optimized ciprofloxacin nanoemulsion

7.2.11 Antibacterial Activity

Both positive control and the test samples inhibited the growth whereas the negative control showed no zone of inhibition. The average zone of inhibition values and the image were shown in table 27. In the image, K indicates Klebsiella pneumonia, Sta indicates Staphylococcus aureus and P indicates Proteus mirabilis. The results confirmed the antibacterial activity of CIP released from the nanoemulsion against Gram +ve and Gram -ve organisms and indicated no influence of formulation ingredients on the activity of CIP. The zone of inhibitions were showen in fig. 34.

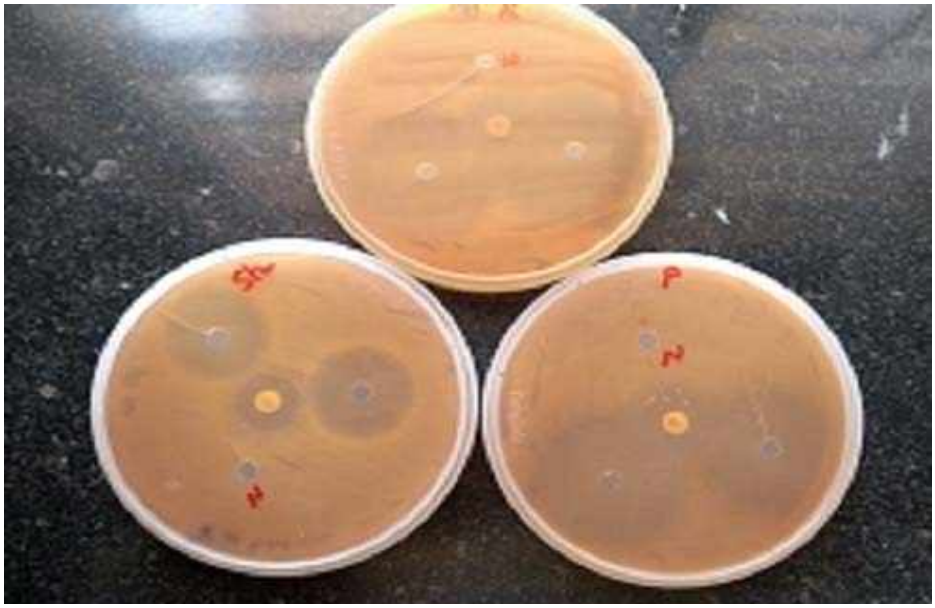


Fig. – 34: Antibacterial activity of optimized ciprofloxacin nanoemulsion against different micro organisms

Table – 27

Antibacterial activity of optimized ciprofloxacin nanoemulsion against different micro organisms

Organisms used	Zone of inhibition (mm)
Klebsiella *pneumoniae* (Gram –ve)	35.25±1.06
Staphylococcus *aureus* (Gram+ve)	24.75±0.35
Proteus *mirabilis* (Gram –ve)	34.00±1.41

*Data represents mean ±SD, n=2

7.2.12 *Ex vivo* Permeation

The *Ex vivo* drug permeation profiles and Drug permeation parameters were shown in fig. 35 and table 28 respectively. The drug permeation from CIP-NE4 was found to be significantly high with higher flux and higher permeability coefficient compared to the drug solution. The results for the drug solution were found quite opposite to the *in vitro* drug diffusion rate. This can be explained by the structural complexity of the corneal membrane that opposes the aqueous solution because of the outer lipophilic layer (Silva AC, et al. 2012). In the case of CIP-NE4, two factors played a major role in permeation enhancement; one is the droplet size and the other is the permeation enhancing the ability of the formulation ingredients used. The nano droplets can be easily infiltrated by endocytosis and the formulation ingredients will

cause temporary changes in the tight junctions of the corneal membrane that enhances the permeation by the transcellular pathway (Shell JW, 1985). Furthermore, the sustained effect could be due to the viscous nature (Tommaso DC, et al. 2011).

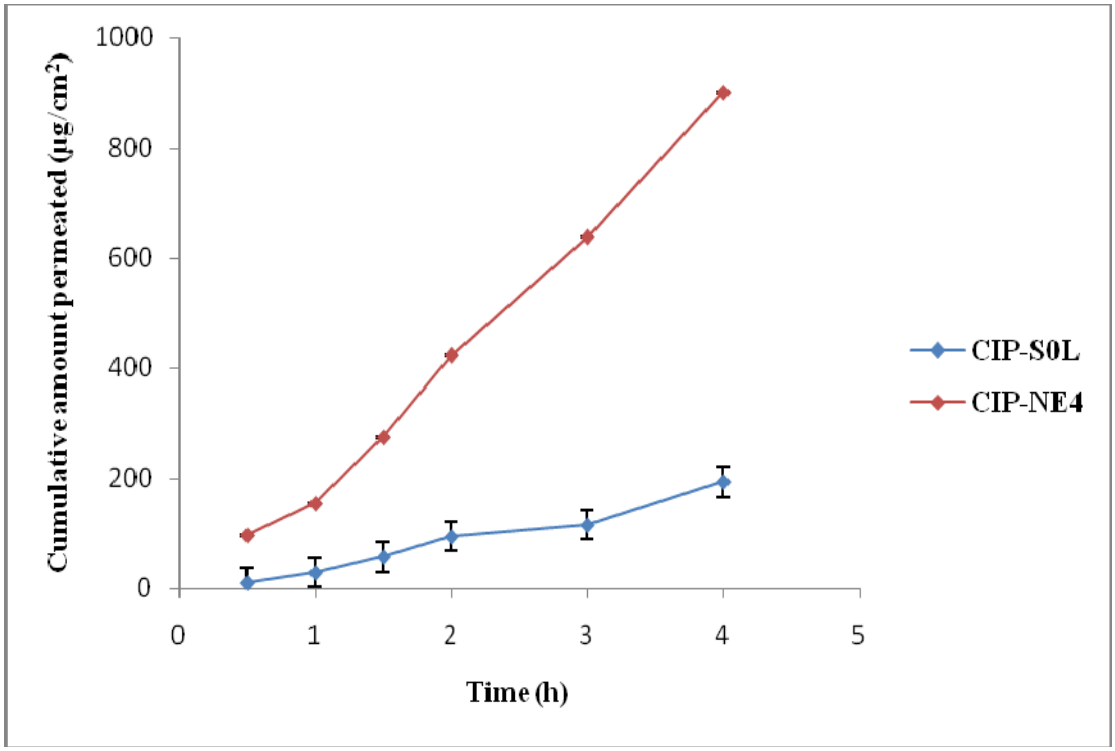


Fig. – 35: ***Ex Vivo*** **transcorneal drug permeation profiles of optimized ciprofloxacin nanoemulsion and control drug solution,** **error bars represent standard deviations of three replicates

Table - 28

Drug permeation parameters of optimized ciprofloxacin nanoemulsion and control drug solution

Parameter	Control	Test formulation
Flux (µg/cm^2/h)	61.783	287.261
Papp(cm/sec)	0.57×10^{-5}	2.65×10^{-5}
Corneal hydration (%)	72.2±0.13	66.5±0.24

*Corneal hydration values expressed in mean±SD, n=3

7.2.13 *In Vivo* Studies

7.2.13.1 Ocular Irritantion test

The scores given for each observation were summarized in the table 29. The individual scores were found 0 to 1 and the Iirr was found less than 4 indicated absence of clinically significant irritation and hence the optimized nanoemulsion was considered safe for ocular delivery.

Table – 29

Draize eye test results of optimized ciprofloxacin nanoemulsion in rabbits

Time point (h)	Parameter									score
	Conjunctival discharge			Conjunctival chemosis			Conjunctival redness			
	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3	
5min	-	-	-	-	-	-	-	-	-	0
10min	-	-	-	-	-	-	+	-	-	1
15min	-	-	-	-	-	-	+	-	+	1
30min	-	-	-	-	-	-	-	-	-	0
1h	-	-	-	-	-	-	-	-	-	0
2h	-	-	-	-	-	-	-	-	-	0
3h	-	-	-	-	-	-	-	-	-	0
6h	-	-	-	-	-	-	-	-	-	0
9h	-	-	-	-	-	-	-	-	-	0
12h	-	-	-	-	-	-	-	-	-	0
24h	-	-	-	-	-	-	-	-	-	0
Total score-2										
Inference- non-irritant										

7.2.13.2 Analytical method for estimation of CIP in rabbit aqueous humour using UFLC

The calibration curve of CIP in rabbit aqueous humour was plotted between peak area and concentration (ng/ml) showed in fig. 36. The concentration range of 10 to 200 ng/ml showed linearity with a regression coefficient (R^2) value of 0.994. The retention time was found to be 1.35 ± 0.12 min as shown in fig. 37.

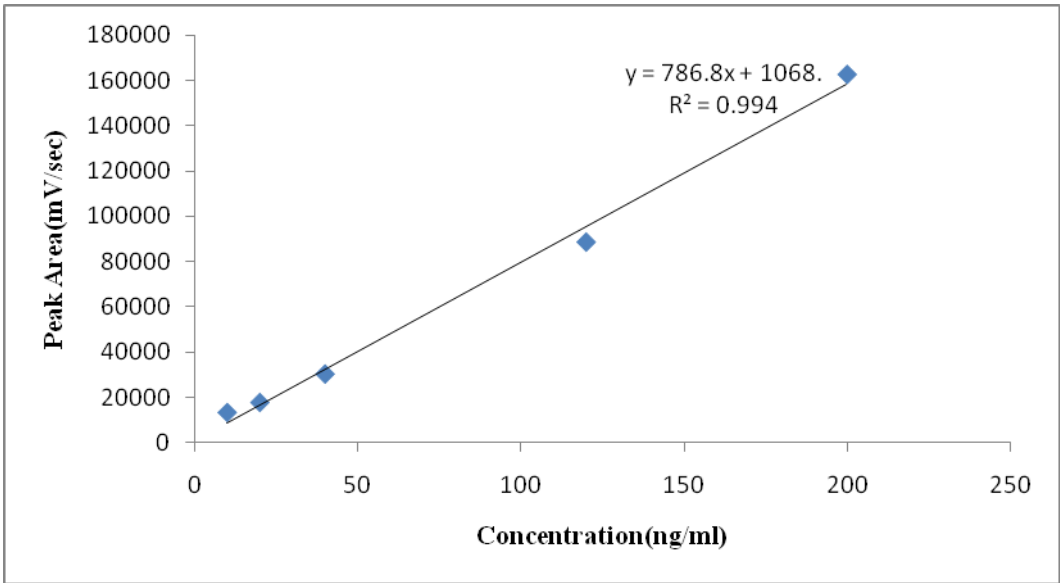


Fig. - 36: Calibration curve of ciprofloxacin in rabbit aqueous humour

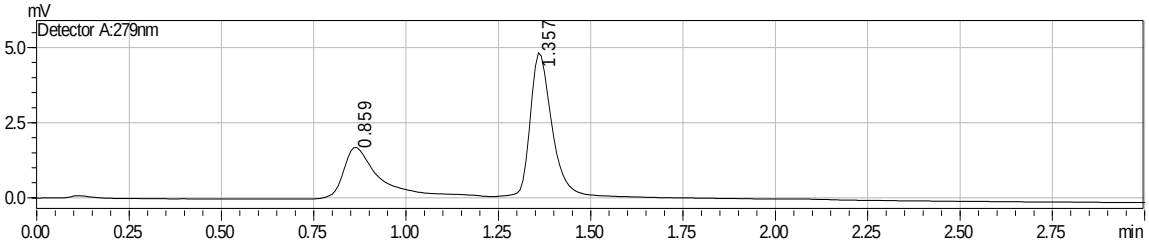


Fig. – 37: UFLC chromatogram of ciprofloxacin (RT- 1.35) in rabbit aqueous humour

7.2.13.3 Analytical method for estimation of CIP in rabbit plasma using UFLC

The calibration curve of CIP in rabbit aqueous humour was plotted between peak area and concentration (ng/ml) showed in fig. 38. The concentration range of 10 to 200 ng/ml showed linearity with a regression coefficient (R^2) value of 0.998. The retention time was found to be 1.36 ± 0.12 min as shown in fig. 39.

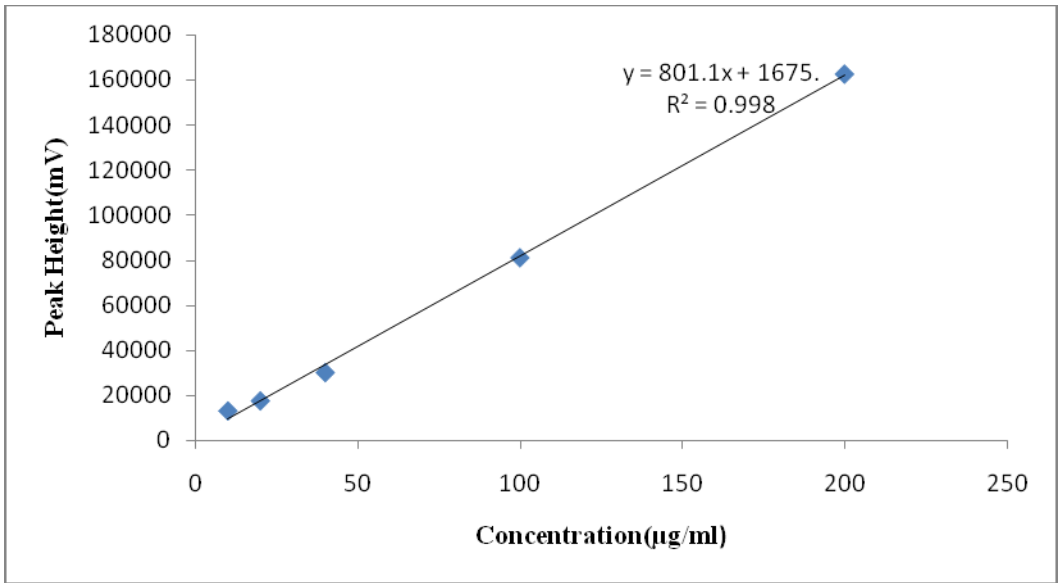


Fig. – 38: Calibration curve of ciprofloxacin in rabbit plasma

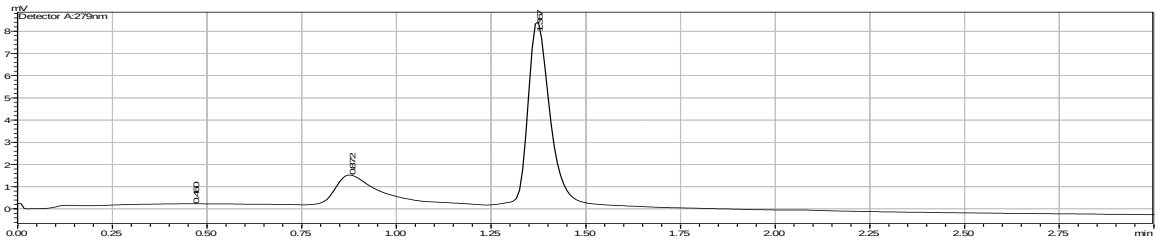


Fig. – 39: UFLC chromatogram of ciprofloxacin (RT- 1.36) in rabbit plasma

7.2.13.4 Pharmacokinetic evaluation

The plasma levels and aqueous humor levels of CIP confirmed the lowest systemic absorption and the improved corneal absorption of CIP-NE4 when compared to the marketed TML ophthalmic solution. It was found that the optimized CIP-NE4 had 2.9 fold rise in ocular bioavailability and 4.37 fold reduction in systemic bioavailability when compared to the marketed product. The reason for improved corneal absorption can be explained by the small globule size, improved retention time due to viscosity and the positive charge on the droplets imparted by benzalkonium chloride involved in the electrostatic interaction with the negatively charged corneal membrane and that lead to improved residence time on cornea (Philippe D, et al. 2013). Different graphs were plotted comparing the pharmacokinetic profiles of CIP-NE4 and the marketed product in aqueous humor and plasma and were shown in fig. 40-44.

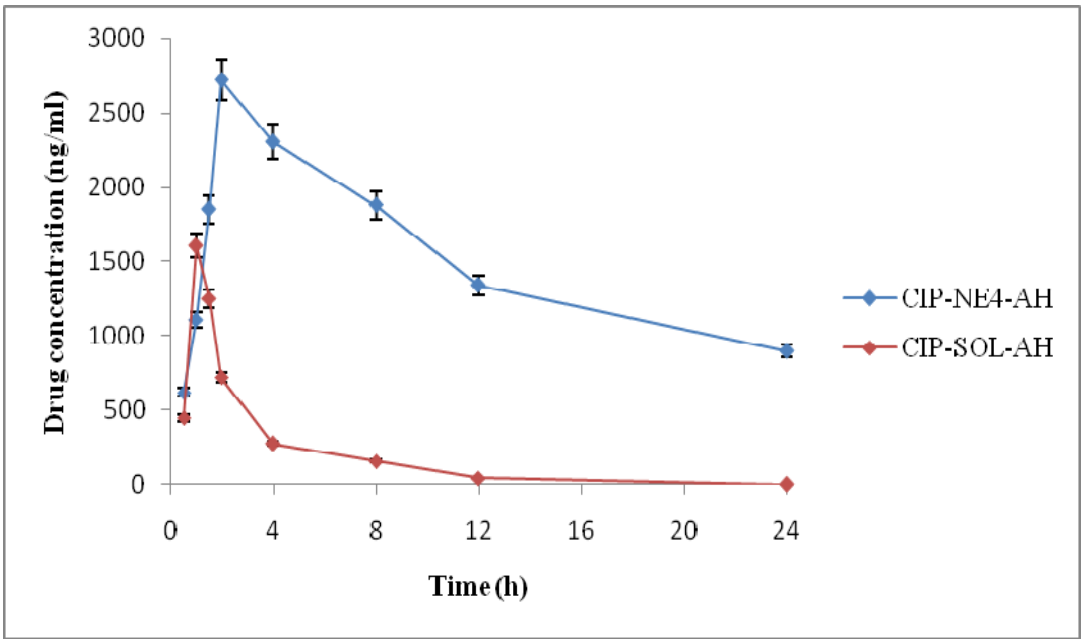


Fig. - 40: Pharmacokinetic profiles of drug from optimized ciprofloxacin nanoemulsion and conventional marketed product in rabbit aqueous humour, *error bars represent standard deviations of three replicates

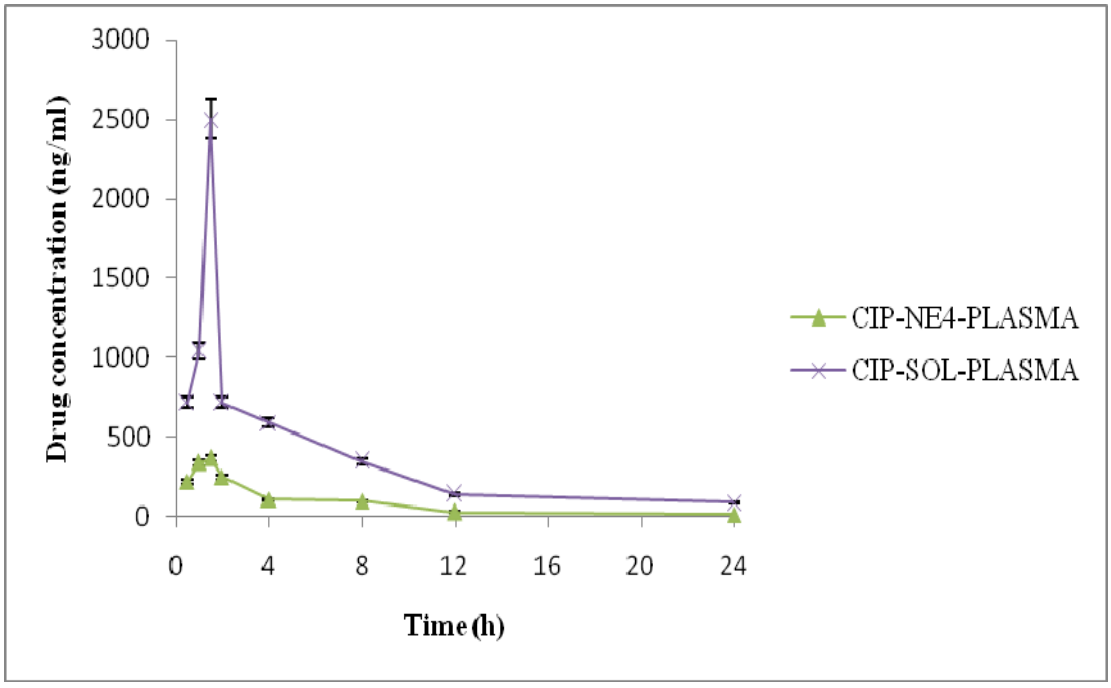


Fig. - 41: Pharmacokinetic profiles of drug from optimized ciprofloxacin nanoemulsion and conventional marketed product in rabbit plasma, *error bars represent standard deviations of three replicates

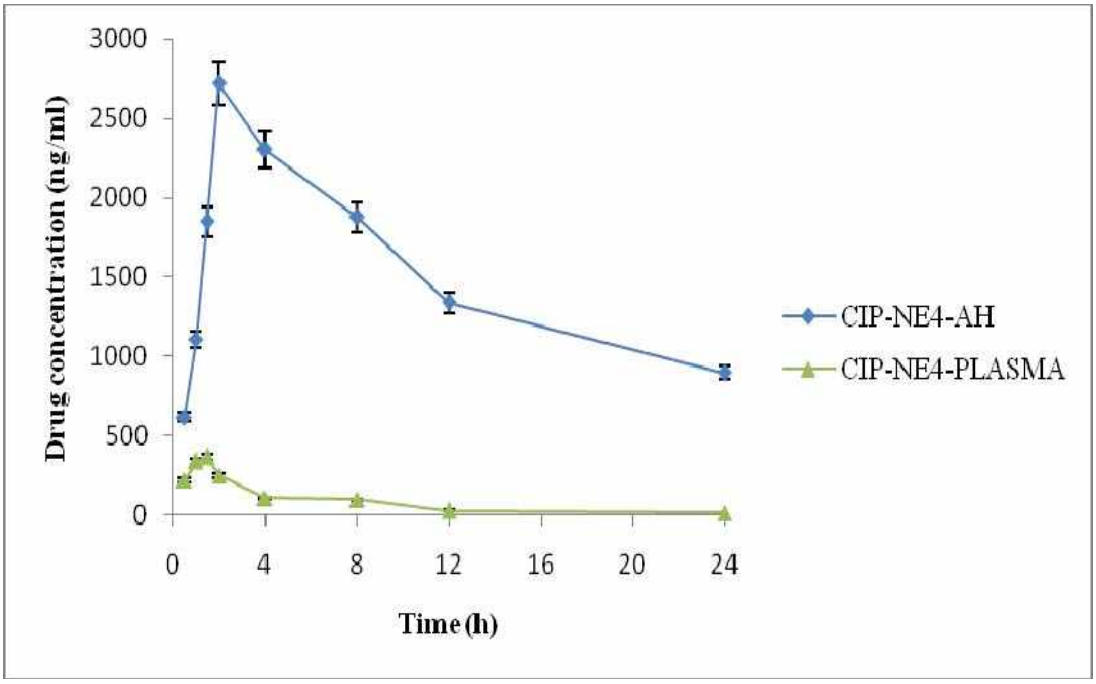


Fig. - 42: Pharmacokinetic profile of drug from optimized ciprofloxacin nanoemulsion in rabbit aqueous humour and rabbit plasma, *error bars represent standard deviations of three replicates

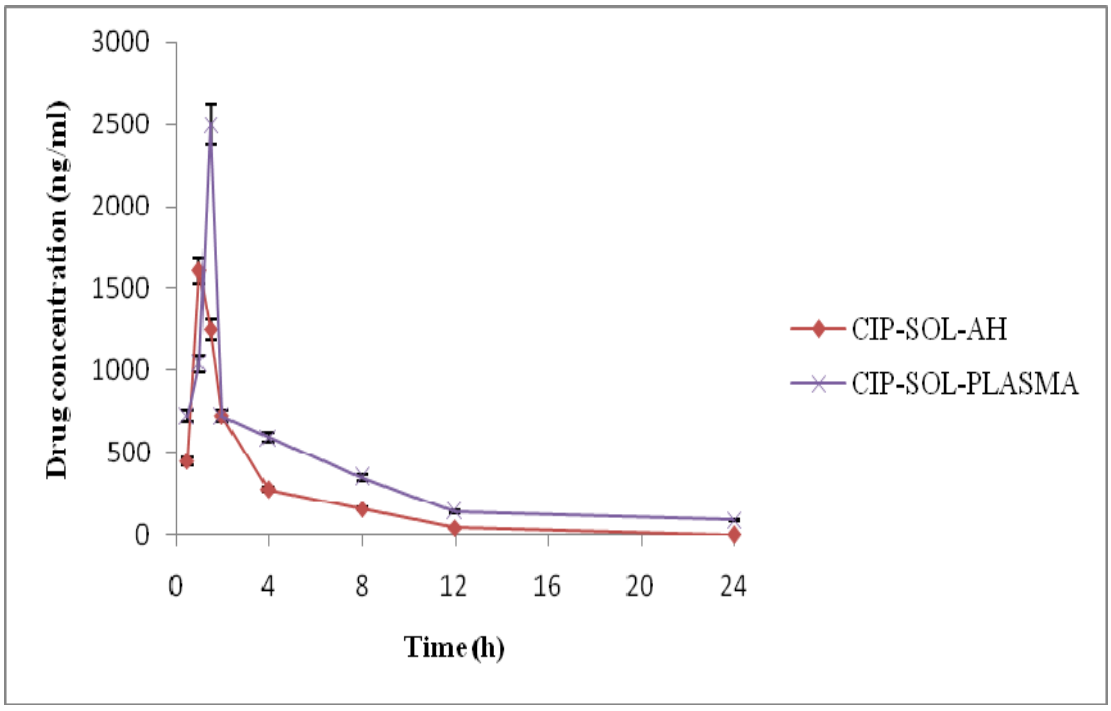


Fig. - 43: Pharmacokinetic profiles of CIP from conventional marketed product in rabbit aqueous humour and rabbit plasma, *error bars represent standard deviations of three replicates

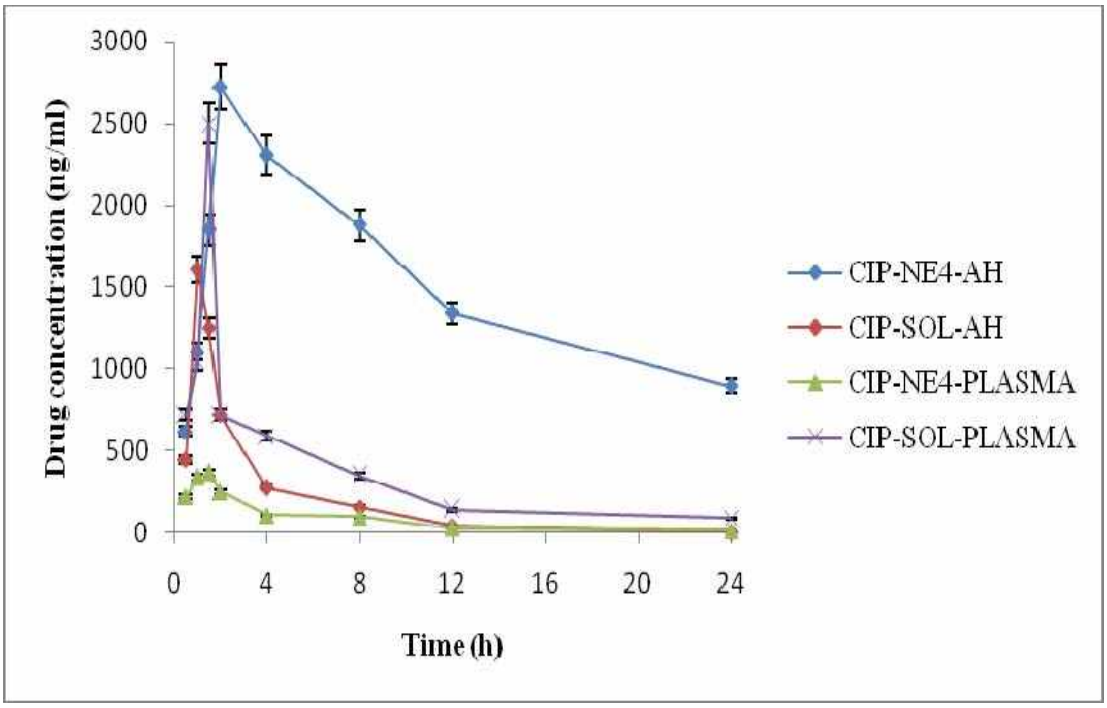


**Fig. - 44: Comparison between pharmacokinetic profiles of CIP from optimized nanoemulsion and conventional marketed product in rabbit aqueous humour and rabbit plasma, ***error bars represent standard deviations of three replicates

The pharmacokinetic parameters like maximum drug concentration (Cmax) and the time required to achieve maximum concentration (Tmax) were obtained from the concentration versus time plot and area under the curve (AUC) was calculated by trapezoidal model (Nagarval RC, et al. 2012) and were summarized in table 30. Cmax of CIP in aqueous and plasma was achieved within 1h and 2h; 2h and 4h after instillation of control (marketed ophthalmic solution) and test formulation (CIP-NE4) respectively.

Table – 30

Pharmacokinetic parameters of optimized ciprofloxacin nanoemulsion and conventional marketed product in rabbits

Parameter	Aqueous humor		Plasma	
	Control	Test formulation	Control	Test formulation
$AUC_{(0\text{-}24h)}$ (ng/ml/h)	8557.81	24826.19	11580.22	2646.46
Tmax (h)	1	2	1.5	1.5
Cmax (µg/ml)	1607.23	2720.34	2500.11	370.72

The obtained results were found to be statistically significant when analyzed by a one-sample t-test using GraphPad Prism software version, 8.0.2 and were depicted in table 31.

Table – 31

Statistical analysis of pharmacokinetic data of optimized ciprofloxacin nanoemulsion and conventional marketed product

One sample t-test	CIP—NE4	
	Aqueous humour	**Plasma**
Actual mean	563.0	768.8
Number of values	8	8
t, df	t=2.701, df=7	t=2.828, df=7
P value	0.0306	0.0255
Significant (alpha=<0.05)	Yes	Yes
95% confidence interval	70.19 to 1056	125.9 to 1412
R^2	0.5104	0.5332

7.3. INDOMETHACIN NANOEMULSIONS

7.3.1 Melting Point

The melting point of indomethacin (IND) was found to be in the range of 153°C meeting the standard melting point range of 152–154°C and 160–162°C for α and γ polymorphic forms respectively (Eman A, et al. 2012; Karima FA, et al. 2015). Thus indicates the purity of the drug.

7.3.2 Calibration curve

The calibration curve was plotted between peak area and concentration (ng/ml) as shown in fig 45. The linearity was seen at a concentration range of 2.5 to 100 ng/ml with a regression coefficient (R^2) value of 0.998. The retention time was found to be 5.4 ± 0.34 min as shown in fig 46.

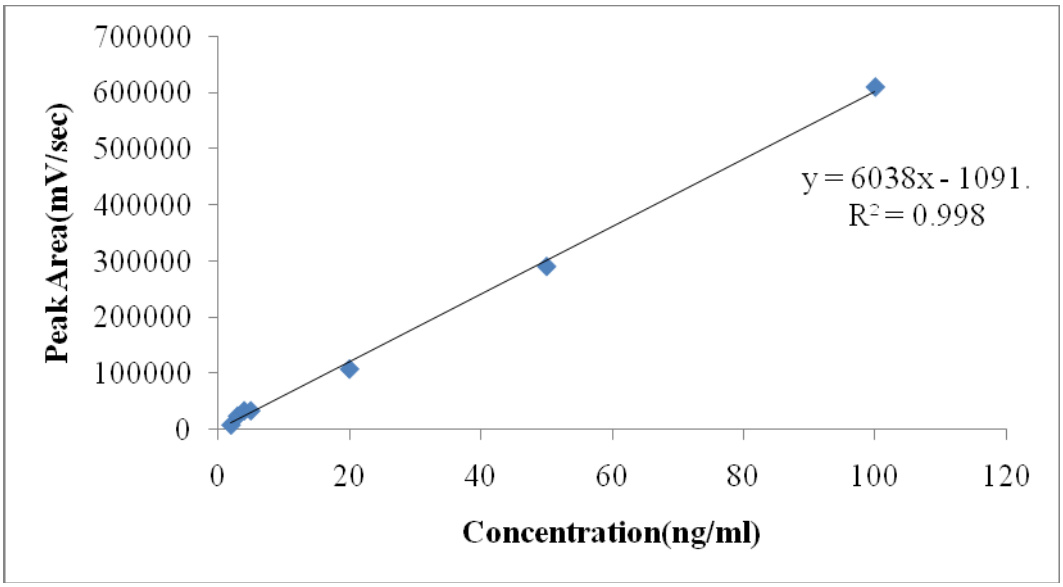


Fig. - 45: Calibration curve of indomethacin

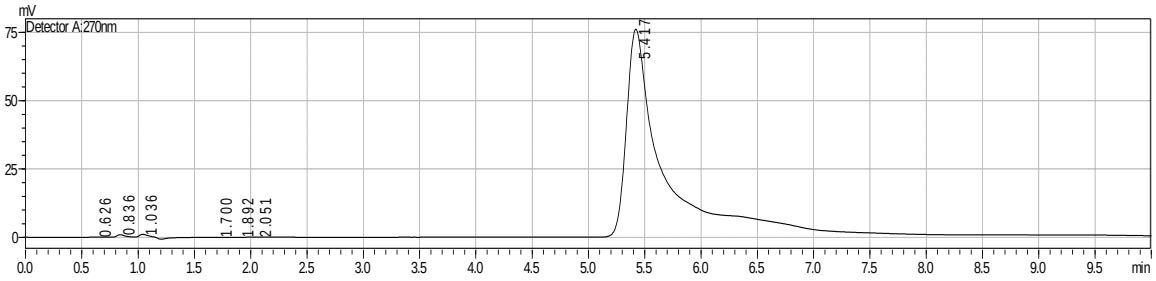


Fig. - 46: UFLC chromatogram of indomethacin (RT- 5.4)

7.3.3 Solubility study

The solubility of IND in various oils, surfactants and co-surfactants were depicted in fig 47. Among oils, captex 8000 showed very good solubility. Therefore, it was selected as the oil phase. Among surfactants and co-surfactants, IND had the highest solubility in span 20 and tween 20; hence they were selected as surfactant and co-surfactant respectively for the phase study.

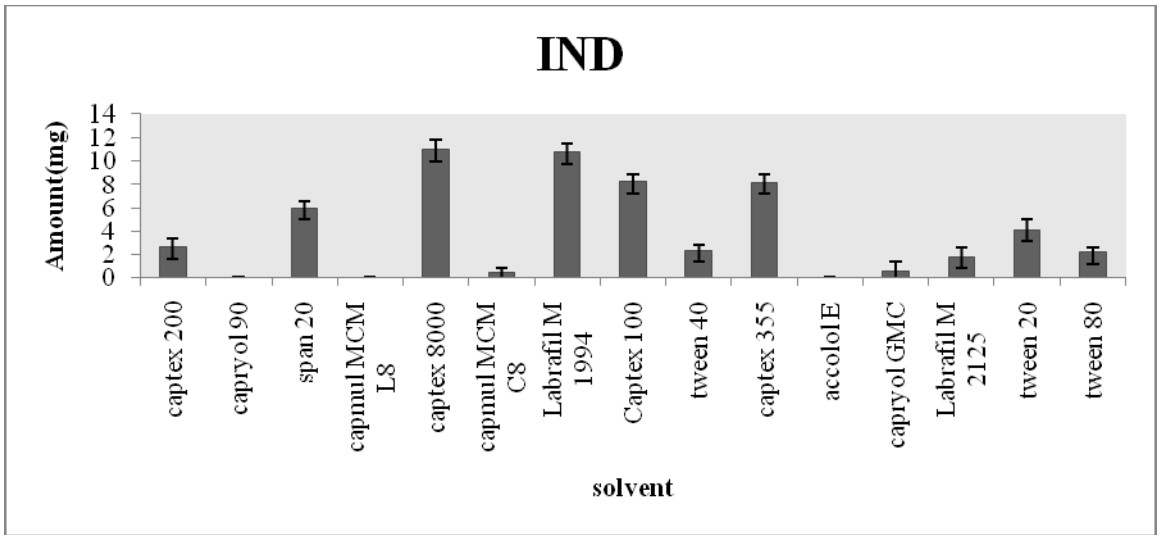


Fig. - 47: Indomethacin solubility in various solvents, *error bars represent standard deviations of three replicates

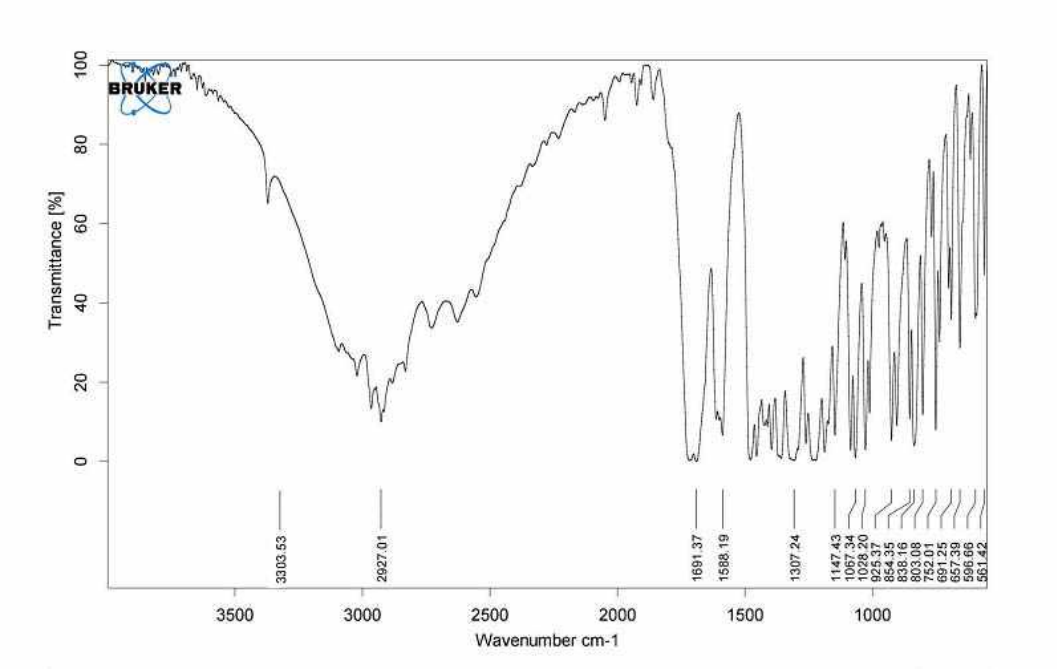
7.3.4 Drug – Excipient Compatibility Studies

The major functional groups of IND that are identified from the FTIR spectral analysis were depicted in table. 32 and the characteristic peaks were illustrated in fig.48. In brief, O-H stretching of carboxylic acid were found at 3340.58 cm^{-1} and 3450.58 cm^{-1}, methyl C-H stretchings were found at 2927.01 cm^{-1} and 2897 cm^{-1}, C=O stretching were found at 1691.37 cm^{-1} and 1641.93 cm^{-1}, C-O-C stretching were found at 1147.43 cm^{-1} and 1153.64 cm^{-1}, C-Cl stretching were found at 752.01 cm^{-1} and 722.26 cm^{-1} in pure drug and physical mixture respectively (Dupeyron D, et al. 2013;Garbacz P, et al. 2018). Thus all the characteristic peaks of drug were found in both spectra confirming the chemical integrity of the drug in the presence of selected excipients and hence, there exists a good compatibility between drug and excipients.

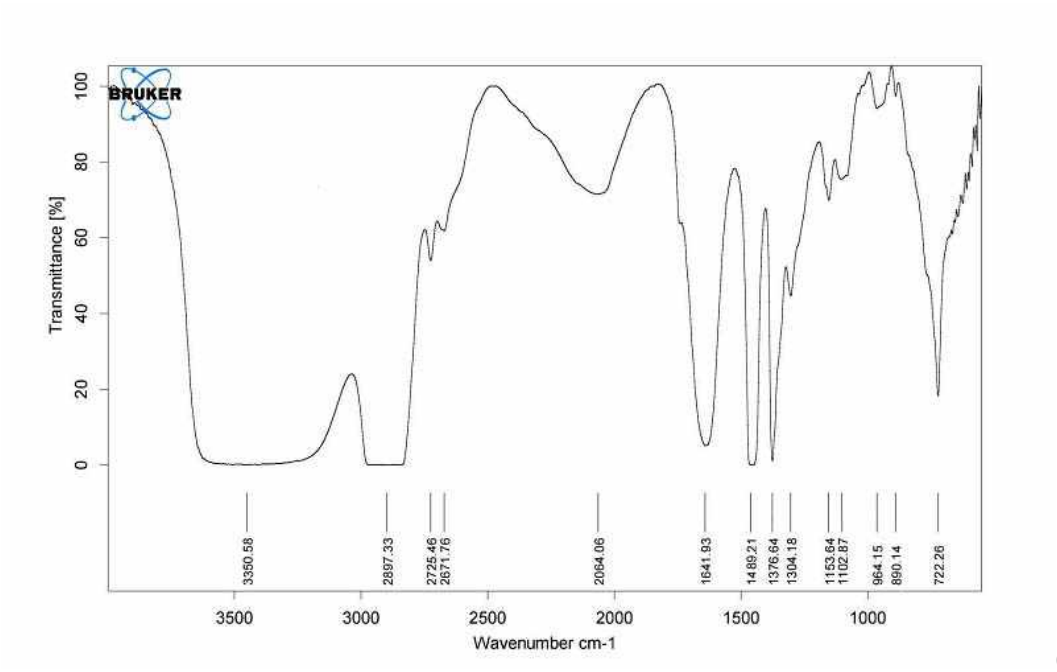
Table – 32

Identified indomethacin functional groups from FTIR spectral data

Functional group	Wave number (cm^{-1})	
	Pure Drug	Physical mixture
O-H	3340.58	3450.58
C-H	2927.01	2897.33
C=O	1691.37	1641.93
C-O-C	1147.43	1153.64
C-Cl	752.01	722.26



(a)



(b)

Fig. – 48: Indomethacin FTIR spectra (a) pure drug and (b) physical mixture

7.3.5 Phase behavior and optimization of nanoemulsion

Pseudo ternary phase diagrams were constructed separately for each Smix ratio and were given in fig. 49. A large NE area was observed for the Smix ratio 1:3 and the same was used to optimize the final formulation compositions.

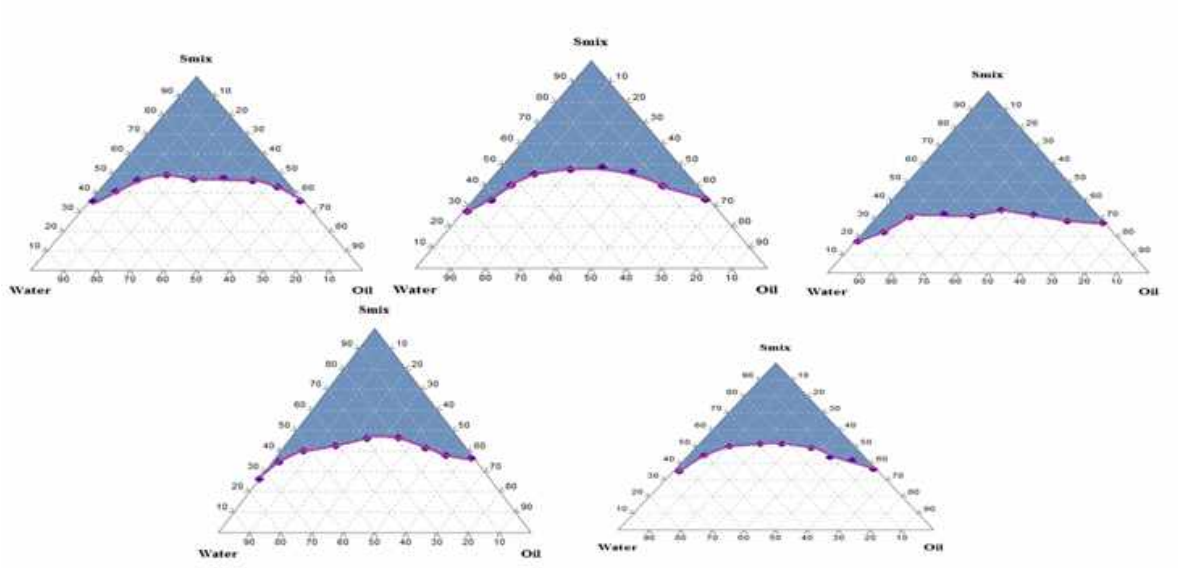


Fig. - 49: Ternary phase diagrams of captex 8000 and Smix (tween 20 and span 20) at ratios 1:1, 1:2, 1:3, 2:1 and 3:1 from right to left

7.3.6 Optimization of formulation composition and Preparation of IND-NEs

Based on the phase behavior of the components, three different levels of oil and Smix were incorporated into the design expert software and the computer has generated a total of nine combinations which were shown in table 33. The final compositions used to prepare IND-NEs were shown in table 34.

Table – 33

3^2 factorial design for the selection of formulation composition of indomethacin nanoemulsions

Formulation code	Coded value		Actual value	
	X1	X2	X1	X2
IND-NE1	1	1	15	60
IND-NE2	-1	0	12.5	55
IND-NE3	-1	1	12.5	60
IND-NE4	1	0	15	55
IND-NE5	0	-1	10	50
IND-NE6	-1	-1	12.5	50
IND-NE7	0	1	10	60
IND-NE8	0	0	10	55
IND-NE9	1	- 1	15	50

Independent variables	Levels		
	Low (-1)	Medium (0)	High (1)
X1- amount of oil (%w/w)	10	12.5	15
X2-amount of Smix (%w/w)	50	55	60
Dependent variables	Y1- Mean droplet size (nm) Y2- Cumulative drug release (%)		

Table – 34

Formulation composition of indomethacin nanoemulsions

Formulation code	oil	Surfactant mix	1% Propylene glycol	3% Glycerine	Aqueous phase	Drug	Cetalkonium chloride
IND-NE1	15	60	2.5	1	22.5	0.1	0.01
IND-NE2	10	55	2.5	1	32.5	0.1	0.01
IND-NE3	10	60	2.5	1	27.5	0.1	0.01
IND-NE4	15	55	2.5	1	27.5	0.1	0.01
IND-NE5	12.5	50	2.5	1	35	0.1	0.01
IND-NE6	10	50	2.5	1	37.5	0.1	0.01
IND-NE7	12.5	60	2.5	1	25	0.1	0.01
IND-NE8	12.5	55	2.5	1	30	0.1	0.01
IND-NE9	15	50	2.5	1	32.5	0.1	0.01

*Values are represented as %w/v

7.3.7 Physicochemical evaluation

The IND-NEs found suitable for ocular delivery with satisfactory physicochemical characteristics and the complete profile was depicted in table 35. The mean globule size for all of the IND-NEs was found less than 200nm ranging from 129.8±1.1 to 191.4±1.6 nm confirming the successful formation of NE. This small size could be related to the penetration of the co-surfactant molecules into the surfactant film. This would decrease the fluidity and surface viscosity of the interfacial film, lower the radius of curvature of the droplets and thus form transparent systems (Lucia P, et al. 2020). The zeta potential of IND-NEs ranged from +13.20±4.6 to +23.45±4.82. The positive charge helps to improve the retention time and also helps in improving corneal absorption due to electrostatic interactions with the corneal membrane (Lallemand F, et al. 2012). The viscosity ranged from 15.3±0.1 to 32.7±0.0 mPas. In general, 15-150 mPas of viscosity is recommended as this low viscosity will ensure the transparency and hence no visual impairment upon eye drop instillation into the eyes without compromising the prolonged retention time (Baranowski P, et al. 2014). Refractive index measurements tell about the possibility of discomfort to the patient after the administration of eye drops. It is recommended that eye drops should have refractive index values near to that of tear fluid i.e., 1.340 to 1.360 or not higher than 1.476 and for the prepared IND-NEs, the refractive index values ranged from 1.346±0.007 to 1.386±0.005 which are within the recommended value. The ideal pH for maximum comfort to the eye is of 7.2□±□0.2. However, the human eye can tolerate a pH of 3.5 to 8.5. The pH values of the IND-NEs were found in the range of 5.5± 0.4 to 6.9 ± 0.9 and hence provide better comfort to the eyes (Ammar HO, et al. 2009). The surface tension of the IND-NEs was found to be in the range of 32.0 ± 2.6 to 52.3 ± 3.4 mN/m ensuring the good spreadability of the IND-NEs over the corneal membrane. The osmolarity values of IND-NEs were found within the range of 303-395 mOsm/l falling under the recommended values (Morsi NM, et al. 2014).

Table – 35

Physical data of different evaluation parameters of indomethacin nanoemulsions

Formulation code	MGS (nm)	PDI	ZP (mV)	Viscosity (mPas)	pH	RI	Surface tension (mN/m)	Osmolority (mOsm/l)
IND-NE1	152.8±1.9	0.070±0.000	+14.1±5.25	15.3±0.1	6.9 ± 0.9	1.346 ± 0.007	52.3 ± 3.4	382
IND-NE2	183.8±1.1	0.209±0.110	+17.8±5.86	16.8±0.1	6.7 ± 0.2	1.347 ± 0.002	49.5 ± 2.5	337
IND-NE3	180.1±0.8	0.253±0.012	+15.3±4.83	20.7±0.2	6.8 ± 0.6	1.368± 0.002	46.4 ± 3.7	324
IND-NE4	157.7±1.5	0.031±0.021	+14.7±3.51	18.4±0.1	6.9 ± 0.5	1.348 ± 0.005	44.1 ± 1.9	352
IND-NE5	137.3±1.6	0.272±0.040	+22±4.09	22.3±0.5	6.8 ± 0.7	1.346 ± 0.001	41.5 ± 1.8	368
IND-NE6	191.4±1.6	0.512±0.001	+13.2±4.6	24.4±0.2	5.9 ± 0.6	1.369 ± 0.001	43.1 ±4.0	372
IND-NE7	116.4±1.5	0.318±0.003	+22.6±5.45	20.1±0.0	6.8 ± 0.3	1.340 ± 0.001	39 ± 2.5	379
IND-NE8	128.9±1.1	0.237±0.011	+23±4.82	26.0±0.1	5.5 ± 0.4	1.368 ± 0.002	32 ± 2.6	381
IND-NE9	176.5±2.0	0.318±0.005	+18.4±3.84	32.7±0.0	6.9 ± 0.8	1.386± 0.005	37.9 ±3.5	355

*Values are expressed as mean ±SD (n=3), MGS- mean globule size, PDI- poly dispersity index, ZP- zeta potential, RI- refractive index.

7.3.8 Stability

IND-NEs remained clear with no phase separation or drug precipitation, indicating their excellent physical stability. All the formulations were found to be consistent concerning their mean globule size, drug content, phase separation and transparency during the stability study. The physical data comparing their mean globule size and drug content before and after stability studies were given in table 36.

Table – 36

Physical evaluation data of indomethacin nanoemulsions before and after stability studies

Formulation code	Mean globule size (nm)		Drug content (%)	
	Freshly prepared	After stability studies	Freshly prepared	After stability studies
NF1	152.8±1.9	150.12± 0.44	99.52 ± 0.12	99.47 ± 0.54
NF2	183.8±1.1	185.42 ± 0.13	96.51 ± 1.06	95.07 ± 1.65
NF3	180.1±0.8	182.71 ± 0.42	97.35 ± 1.83	96.52 ± 2.20
NF4	157.7±1.5	159.14 ± 0.56	95.72 ± 1.27	94.18 ± 1.32
NF5	137.3±1.6	138.18 ± 0.54	99.87 ± 2.52	98.73 ± 2.03
NF6	191.4±1.6	194.62 ± 0.34	98.34 ± 1.05	98.25 ± 1.36
NF7	116.4±1.5	118.24 ± 0.55	96.96 ± 1.99	96.15 ± 2.42
NF8	128.9±1.1	129.13 ± 0.81	98.92 ± 1.92	97.87 ± 1.25
NF9	176.5±2.0	179.02 ± 0.65	98.46 ± 1.24	98.39 ± 1.86

*Values are expressed as mean±SD (n=3)

7.3.9 *In vitro* release studies

To facilitate comparison between release behaviors of different NE formulae, the *In vitro* drug release profiles of the IND-NEs were graphically illustrated in fig. 50. The release results of NE revealed that the prepared formulations were found to exhibit sustained release with 67.91±2.01 to 95.90±1.93 % drug release at 24h in comparison to control which showed 99.81 ±5.21 % drug release within 2 h. The variation in drug release between IND-NEs can be emphasized from the formulation composition variables and the droplet size of the formulations (Jaiswal P, et al. 2014).

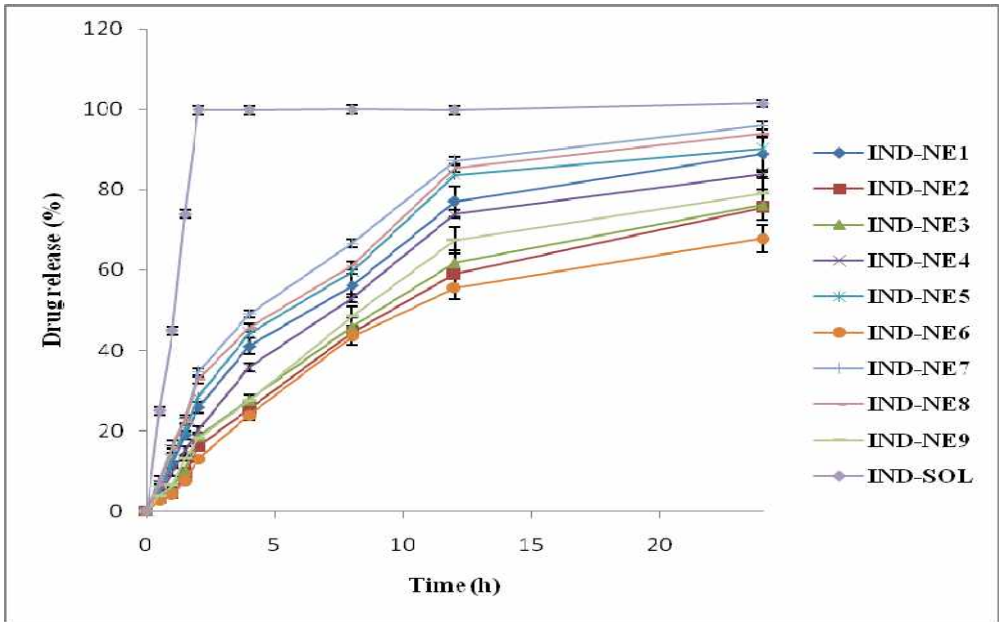


Fig. - 50: *In vitro* drug release profiles of indomethacin nanoemulsions and control drug solution, *error bars represent standard deviations of three replicates

7.3.10 Optimization by response surface analysis

The experimental data were fitted into the quadratic model and the predicted R^2 of mean globule size and % drug release were found to be 0.9505 and 0.8177 respectively and were in reasonable agreement with the adjusted R^2 of 0.9852 and 0.9599 respectively; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 27.662 for mean globule size and 17.793 for % drug release indicated an adequate signal. The model found significant with the F-value of 107.49 for mean globule size and 39.33 for % drug release. P-values less than 0.0500 indicate that the model terms are significant. The model terms A, B, AB, A^2, B^2 were found significant for both mean globule size and % drug release. The fit summary, statistics and ANOVA for quadratic model were depicted in table 37, 38 & 39 respectively.

The final equation in terms of coded factors for mean globule size (Y_1) and % drug release (Y_2):

$$Y_1 = 125.811 - 11.2833\,X_1 - 9.28333\,X_2 - 3.15\,X_1X_2 + 46.0833\,X_1^2 + 2.58333\,X_2^2$$

$$Y_2 = 94.1444 + 5.37167\,X1 + 3.81167\,X2 + 0.36\,X1X2 - 14.4817\,X1^2 - 1.56167\,X2^2$$

Table – 37

Fit summary of the experimental data of indomethacin nanoemulsions for responses Y_1 and Y_2

Source	Sequential p-value		Adjusted R^2		Predicted R^2		
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	
Linear	0.4597	0.2451	-0.0290	0.1656	-0.6620	-0.3275	
2FI	0.8382	0.9414	-0.2235	-0.0001	-2.1811	-1.6189	
Quadratic	0.0006	0.0037	0.9852	0.9599	0.9505	0.8177	**Suggested**
Cubic	0.8301	0.1134	0.9694	0.9985	0.3029	0.9648	

Table – 38

Fit statistics of of the experimental data of indomethacin nanoemulsions by quadratic model for responses Y_1 and Y_2

Std. Dev.	Y_1	3.22	**R^2**	Y_1	0.9944
	Y_2	1.87		Y_2	0.9850
Mean	Y_1	158.26	**Adjusted R^2**	Y_1	0.9852
	Y_2	83.45		Y_2	0.9599
C.V. %	Y_1	2.04	**Predicted R^2**	Y_1	0.9505
	Y_2	2.24		Y_2	0.8177
Adeq Precision				Y_1	27.6617
				Y_2	17.7933

Table – 39

ANOVA for the experimental data of indomethacin nanoemulsions by quadratic model for responses Y_1 and Y_2

Source	Sum of Squares		Df		Mean Square		F-value		p-value		
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	
Model	5581.35	685.13	5	5	137.03	103.92	39.33	70.58	0.0014	0.0062	**Significant**
A-A	763.88	173.13	1	1	173.13	450.67	49.70	306.11	0.0033	0.0059	
B-B	517.08	87.17	1	1	87.17	10.67	25.02	7.25	0.0059	0.0154	
AB	39.69	0.5184	1	1	0.5184	0.2500	0.1488	0.1698	0.1456	0.7254	
A^2	4247.35	419.44	1	1	419.44	8.00	120.40	5.43	0.0003	0.0016	
B^2	13.35	4.88	1	1	4.88	50.00	1.40	33.96	0.3393	0.3219	

From the response surface analysis, it was evident that the oil and Smix compositions showed a significant effect on mean globule size and % drug release. The effects were represented as 3D surface plots and contour plots in fig. 51& 52 respectively. It is prominently noticed that an increase in oil content provided a decrease in globule size to some extent and a further increase in oil content lead to a slight increase in globule size and an opposite effect was seen for percentage drug release. However, by increasing the Smix content the globule size decreased and the percentage drug release increased. Zainol S, et al. (2012) reported an increase in the mean globule size when the oil composition of levodopa lipid emulsions was increased. Koteswari P, et al. (2011) developed felodipine nanoemulsions and reported that the mean globule size reduced from 231.8 to 162.7 nm by increasing the surfactant concentration from 1 to 1.5%. In the present study, the drug release was found to be affected by their droplet size. The decrease in droplet size increased the total number of oil globules and subsequent increase in their surface area which led to an increase in drug release (Jaiswal P, et al. 2014). Thus the formulation IND-NE7 with the medium oil and high Smix was found to have the lowest mean globule size and significantly high drug release, hence it was chosen as the optimized formulation and was subjected to further study. Similar results were reported by Yadav P, et al. (2020) where low oil and high surfactant levels of emulsion composition for ezetimibe showed the lowest globule size of 24.4 ± 2.07 nm and high % drug release.

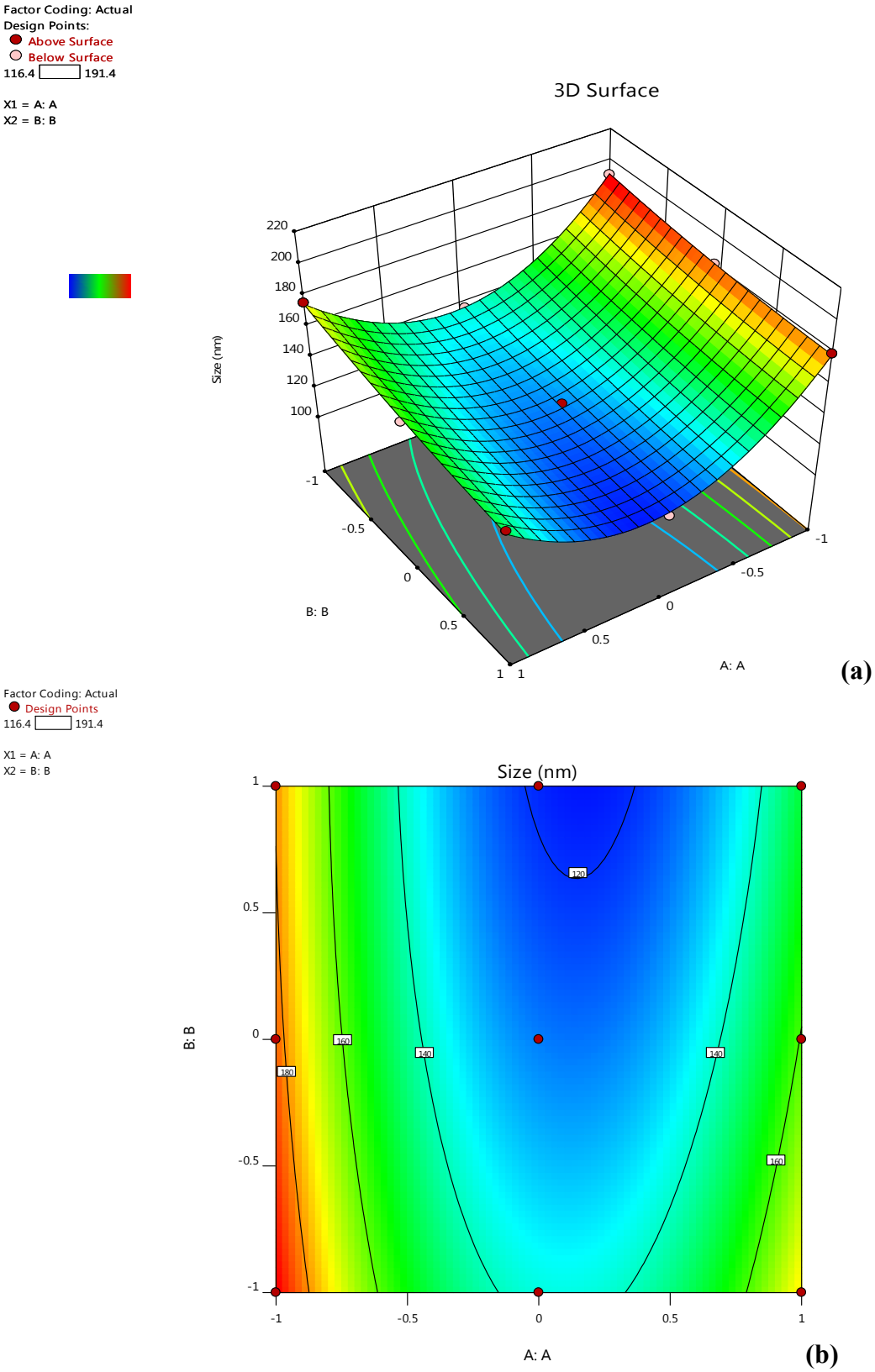


Fig. - 51: Response surface plots showing the effect of oil and Smix on mean globule size (nm) of indomethacin nanoemulsions (a) 3D plot (b) Contour plot

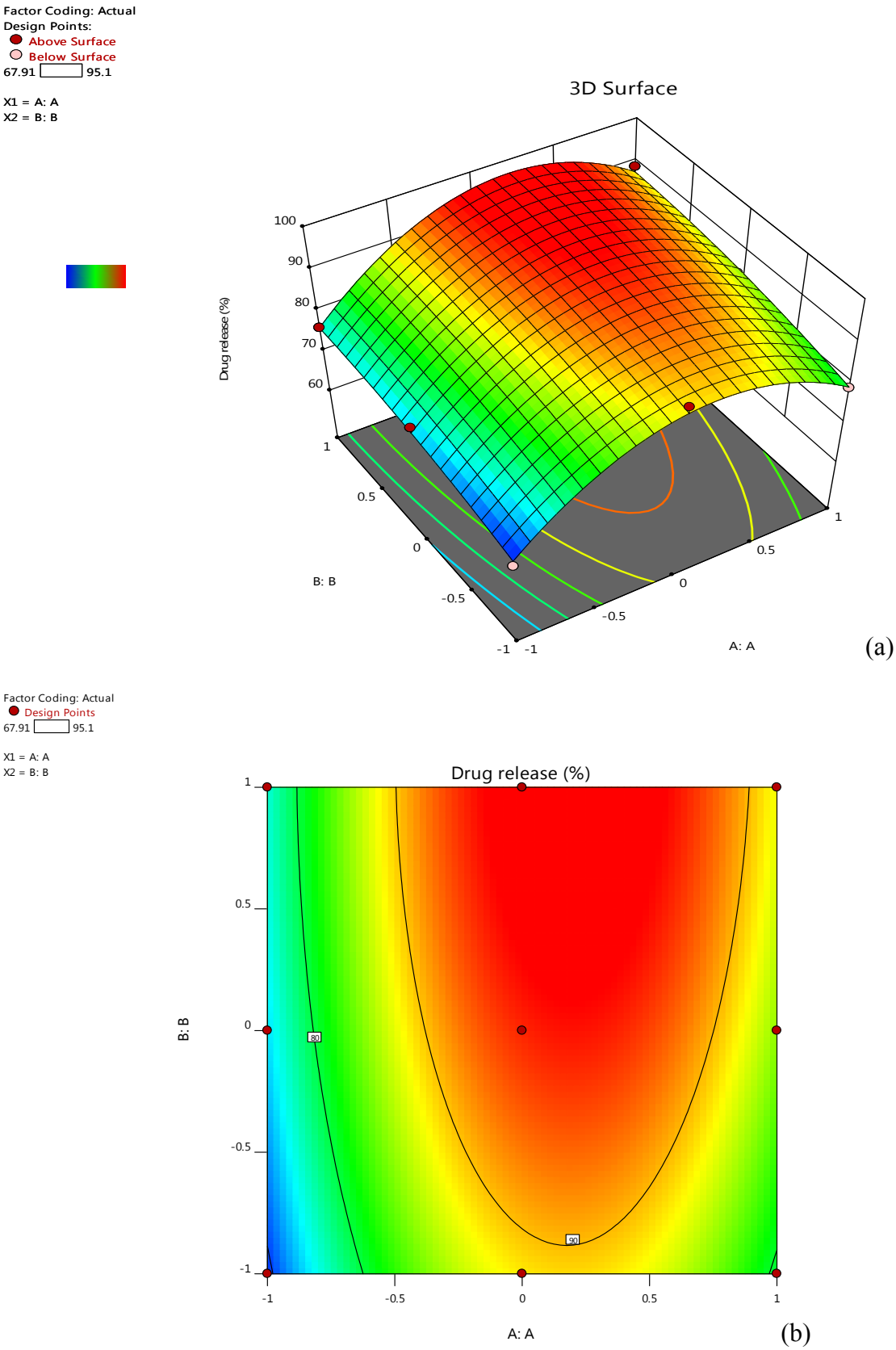


Fig. – 52: Response surface plots showing the effect of oil and Smix on percentage drug release (%) of indomethacin nanoemulsions (a) 3D plot (b) Contour plot

7.3.11 Surface morphology:

The droplets in the IND-NE7 appeared dark, and spherical with size more or less similar and less than 100nm. The image can be seen in fig. 53.

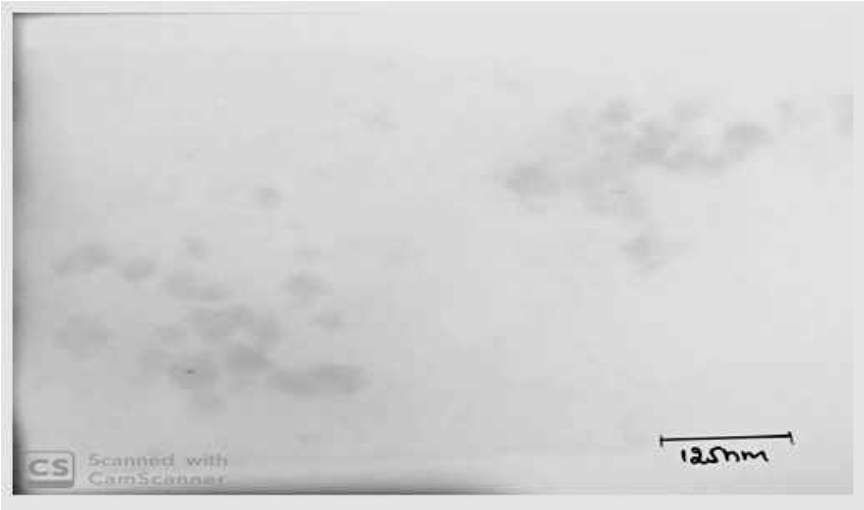


Fig. - 53: TEM image of optimized indomethacin nanoemulsion

7.3.12 *Ex vivo* corneal permeation studies using bovine corneas

The drug permeated through the corneal membrane at 4 h from the IND-NE7 and drug solution was found to be 524 ± 1.5 µg/ cm^2 and 175±2.6 µg/cm^2 respectively. The results for the drug solution were found quite opposite to the *in vitro* drug diffusion rate. This can be explained by the structural complexity of the corneal membrane that opposes the aqueous solution because of the outer lipophilic layer (Silva AC, et al. 2012). In the case of IND-NE7, the nano-sized globules can be easily infiltrated by endocytosis and the permeation enhancing the ability of the formulation ingredients will cause temporary changes in the tight junctions of the corneal membrane that enhances the permeation by transcellular pathway. Similar results were reported by Sayed, et al. (2017) where the high *in vitro* drug diffusion rate from the drug solution was reversed in case of corneal permeation and the nano vesicles showed more corneal permeation compared to the drug solution due to the above said reasons. The graphical representation of the comparative *ex vivo* drug permeation profiles of test and control were shown in fig. 54 and the drug permeation parameters were depicted in table 40.

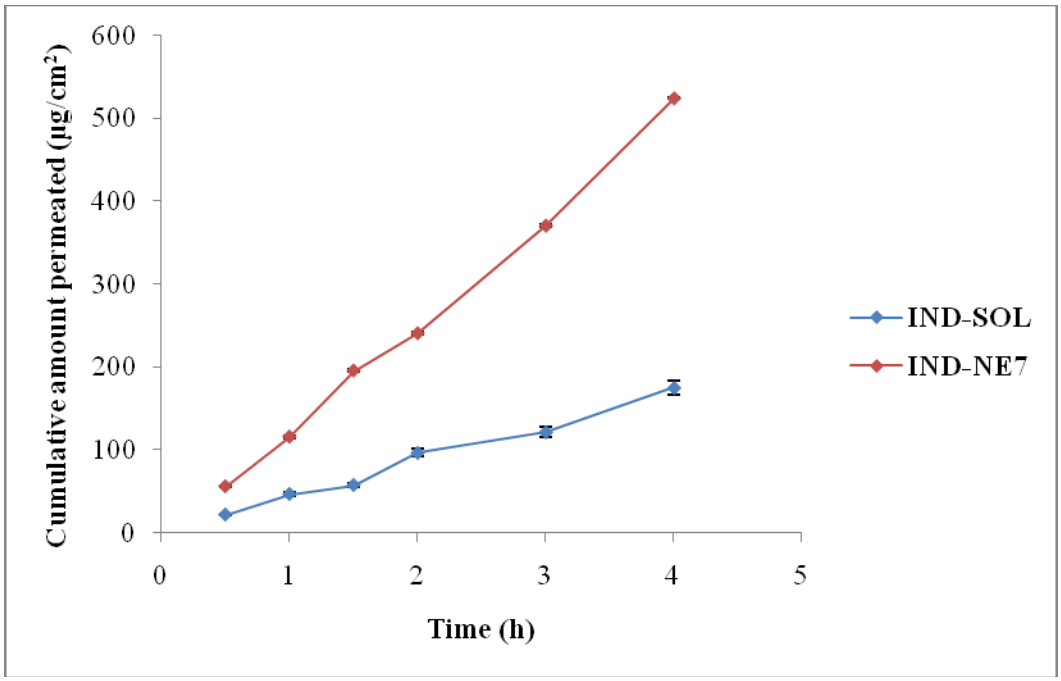


Fig. - 54: *Ex vivo* transcorneal drug permeation profiles of optimized indomethaci nanoemulsion and control drug solution, *error bars represent standard deviations of three replicates

Table – 40

***Ex vivo* drug permeation parameters of optimized indomethaci nanoemulsion and control drug solution**

Parameter	Control	Test formulation
Flux (µg/cm^2/h)	55.732	166.878
Papp(cm/s)	1.54×10^{-5}	4.63×10^{-5}
Corneal hydration (%)	72.2±0.13	66.5±0.24

*Values are expressed as mean ±SD (n=3)

7.3.13 *In vivo* studies

7.3.13.1 Ocular irritation test

IND-NE7 found nonirritant with a minimal score of 1 in the Draize eye test. The rabbits showed slight redness of the conjunctiva which disappeared completely within 15 min but no lachrymation or chemosis was observed throughout the study indicating the optimized formulation is nonirritant and could be tolerated by the rabbit eye. The observations recorded were depicted in table 41.

Table – 41

Draize eye test results of optimized indomethacin nanoemulsion in rabbits

Time point (h)	Parameter									score
	Conjunctival discharge			Conjunctival chemosis			Conjunctival redness			
	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3	
5min	-	-	-	-	-	-	-	-	-	0
10min	-	-	-	-	-	-	+	-	+	1
15min	-	-	-	-	-	-	-	-	-	0
30min	-	-	-	-	-	-	-	-	-	0
1h	-	-	-	-	-	-	-	-	-	0
2h	-	-	-	-	-	-	-	-	-	0
3h	-	-	-	-	-	-	-	-	-	0
6h	-	-	-	-	-	-	-	-	-	0
9h	-	-	-	-	-	-	-	-	-	0
12h	-	-	-	-	-	-	-	-	-	0
24h	-	-	-	-	-	-	-	-	-	0
Total score- 1										
Inference- non-irritant										

7.3.13.2 Analytical method for estimation of IND in rabbit aqueous humour using UFLC

The calibration curve of IND in rabbit aqueous humour was plotted between peak area and concentration (ng/ml) showed in fig. 55. The concentration range of 2 to 400 ng/ml showed linearity with a regression coefficient (R^2) value of 0.992. The retention time was found to be 3.1 ± 0.12 min as shown in fig. 56.

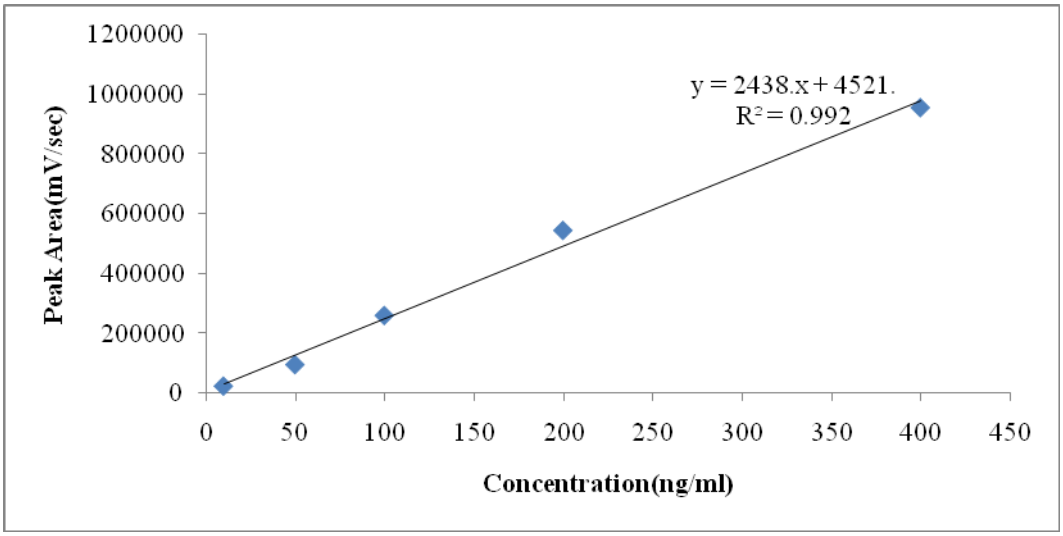


Fig. – 55: Calibration curve of indomethacin in rabbit aqueous humour

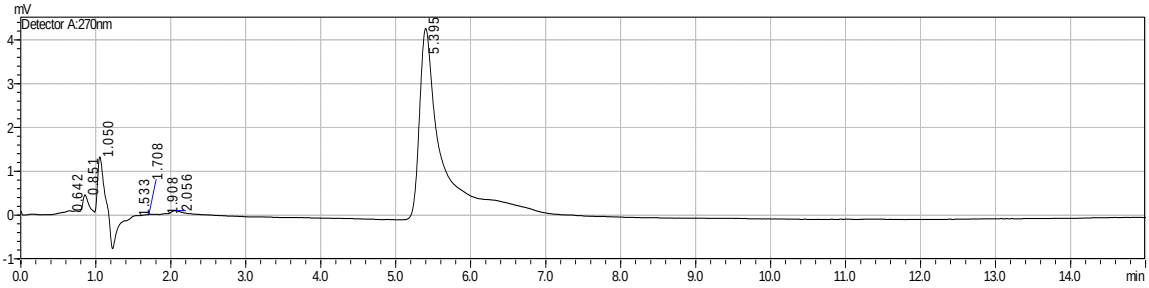


Fig. – 56: UFLC Chromatogram of indomethacin (RT- 5.4) in rabbit aqueous humour

7.3.13.3 Analytical method for estimation of IND in rabbit plasma using UFLC

The calibration curve of IND in rabbit plasma was plotted between peak area and concentration (ng/ml) showed in fig. 57. The concentration range of 2 to 500 ng/ml showed linearity with a regression coefficient (R^2) value of 0.998. The retention time was found to be 3.1 ± 0.12 min as shown in fig. 58.

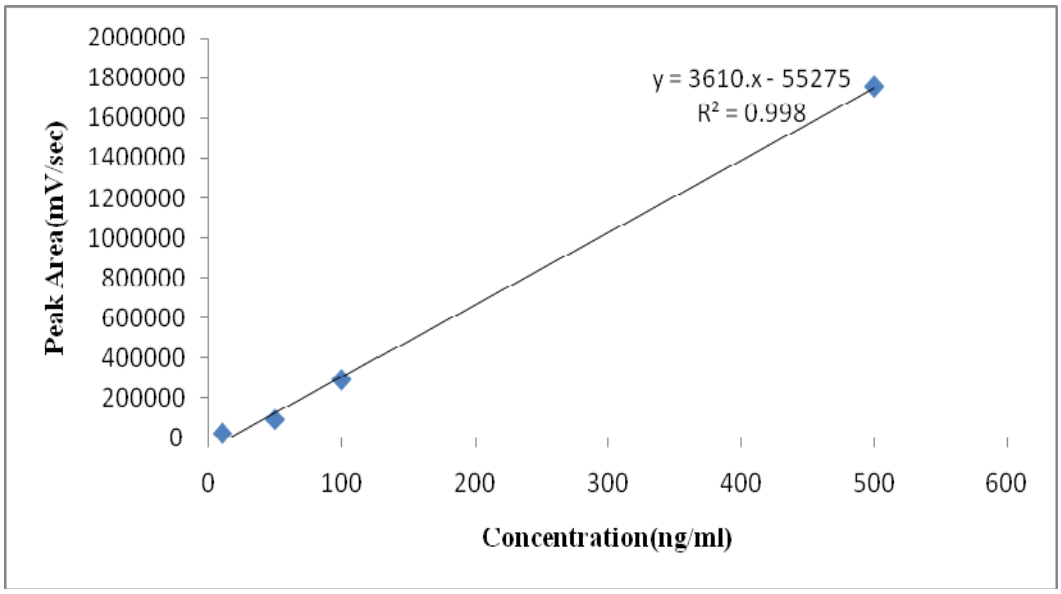


Fig. – 57: Calibration curve of indomethacin in rabbit plasma

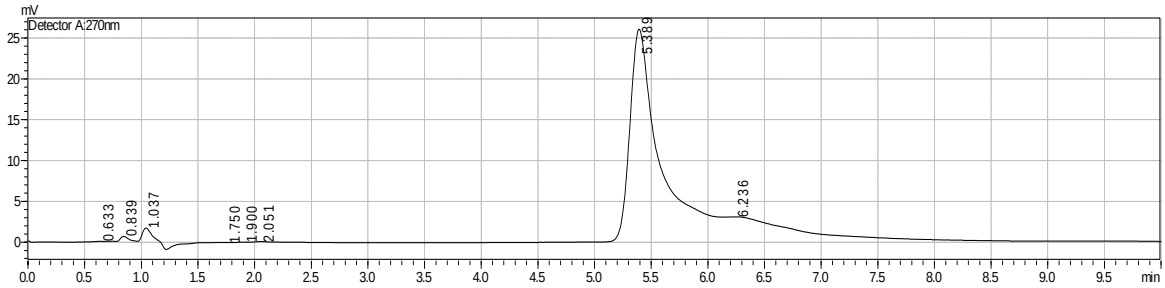


Fig – 58: UFLC chromatogram of indomethacin (RT- 5.389) in rabbit plasma

7.3.13.4 Pharmacokinetic evaluation

The graphical representation of the comparative *In vivo* drug concentration profiles of test and control was shown in fig 59- 63 and the pharmacokinetic parameters were depicted in table 42. The AUC $_{(0–24h)}$ for IND-NE7 and the marketed formulation were found to be 1514.99 ng/ml/h and 974.14 ng/ml/h in aqueous humour; 2266.83 ng/ml/h and 778.15 ng/ml/h in plasma respectively. The plasma levels and aqueous humor levels of IND confirmed that 1.55 fold increased bioavailability in the aqueous humour and 2.91 fold decreased bioavailability in systemic circulation of IND-NE7 when compared to the marketed IND ophthalmic solution. The reason for improved corneal absorption can be explained by the small globule size, improved retention time due to viscosity and the positive charge on the droplets imparted by cetalkonium chloride involved in the electrostatic interaction with the negatively charged corneal membrane and that lead to improved residence

time on cornea (Daull P, et al. 2014). Ban J, et al. (2017) reported similar results for dexamethasone lipid nanoparticles which showed a 2.7 fold increased bioavailability in aqueous humour compared to the drug solution due to the nano-size and positive charge on the nanoparticles.

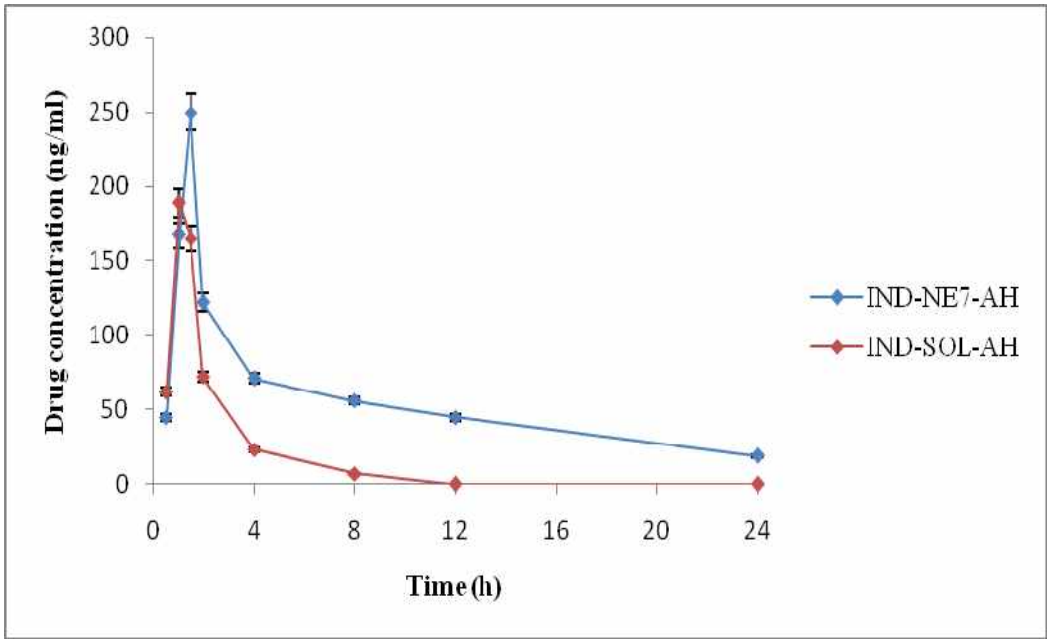


Fig. - 59: Pharmacokinetic profiles of optimized indomethacin nanoemulsion and conventional marketed product in rabbit aqueous humour, *error bars represent standard deviations of three replicates

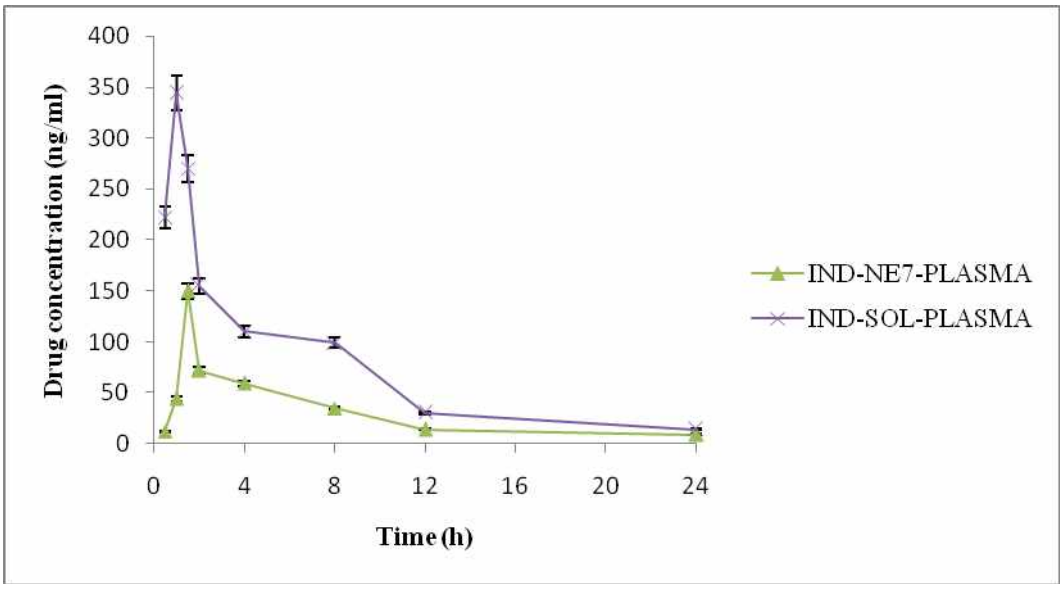


Fig. - 60: Pharmacokinetic profiles of optimized indomethacin nanoemulsion and conventional marketed product in rabbit plasma, *error bars represent standard deviations of three replicates

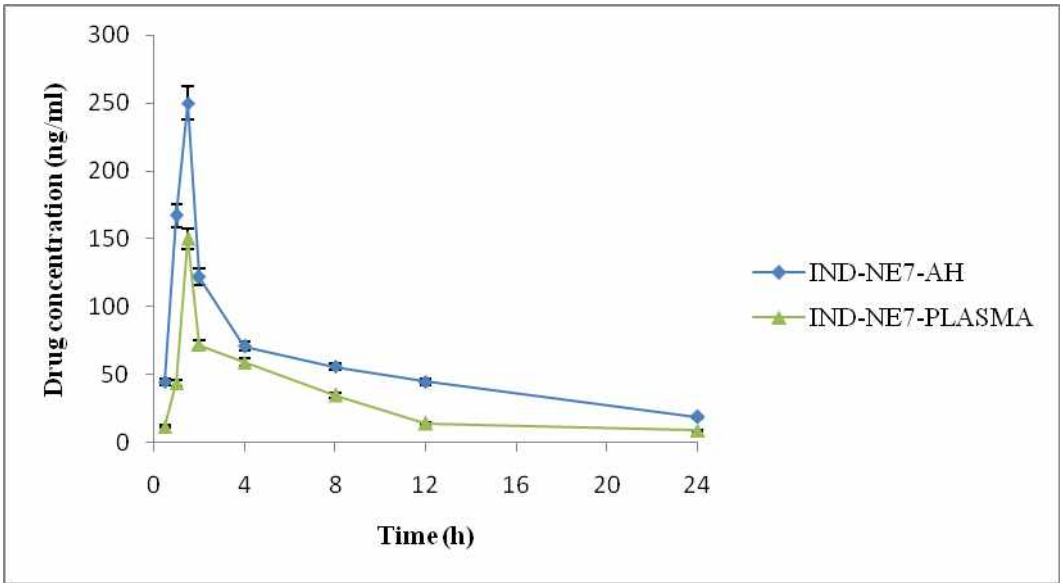


**Fig. - 61: Comparative Pharmacokinetic profiles of drug from optimized indomethacin nanoemulsion in rabbit aqueous humour and rabbit plasma, ***error bars represent standard deviations of three replicates

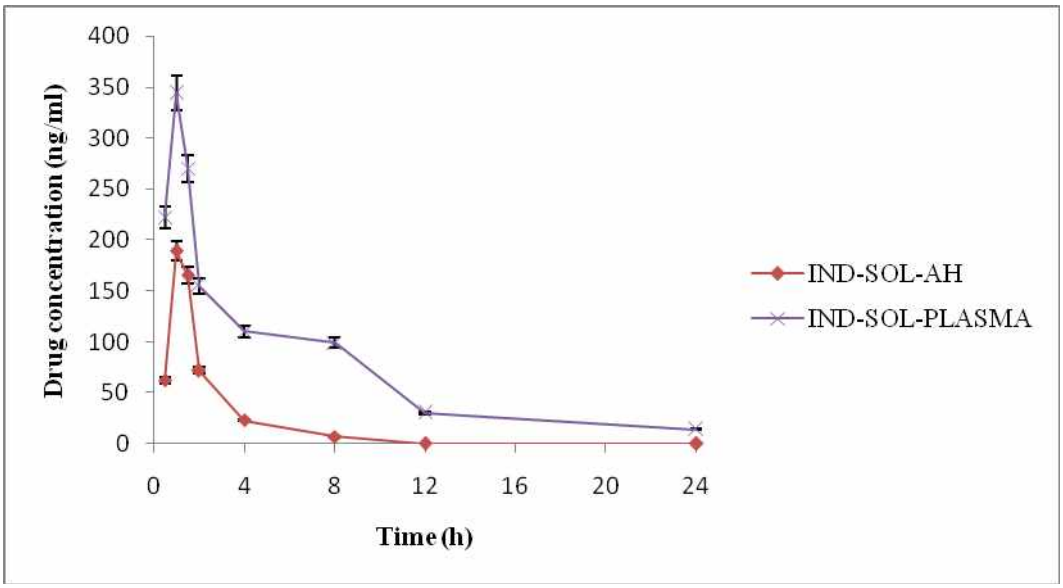


**Fig. - 62: Comparative Pharmacokinetic profiles of IND from conventional marketed product in rabbit aqueous humour and rabbit plasma, ***error bars represent standard deviations of three replicates

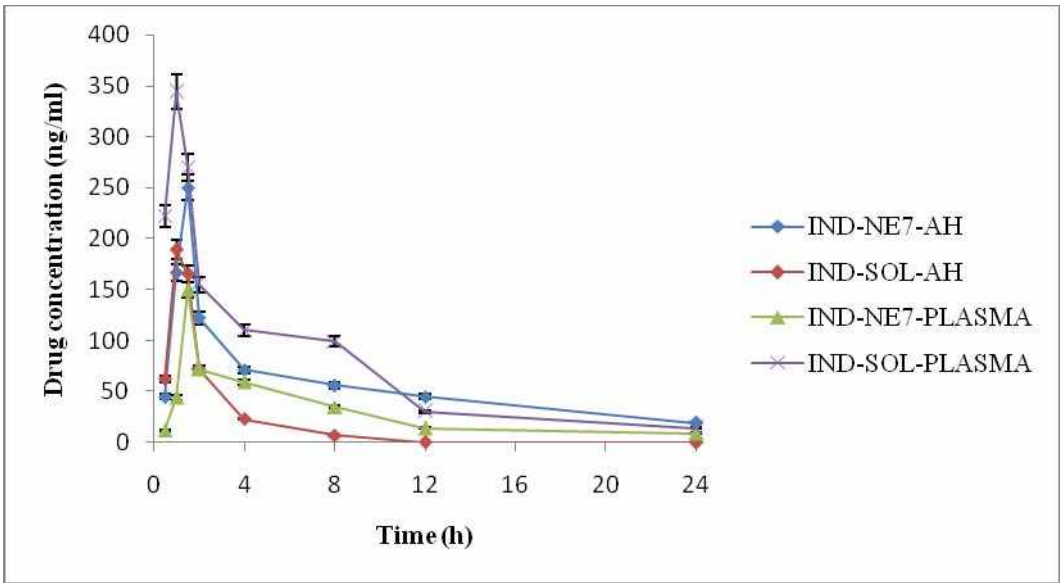


Fig. - 63: Comparative pharmacokinetic profiles of drug from optimized indomethacin nanoemulsion and conventional marketed product in rabbit aqueous humour and rabbit plasma, *error bars represent standard deviations of three replicates

Table – 42

Pharmacokinetic parameters of optimized indomethacin nanoemulsion and conventional marketed product in rabbits

Parameter	Aqueous humor		Plasma	
	Control	Test formulation	Control	Test formulation
$AUC_{(0\text{-}24h)}$ (ng/ml/h)	974.14	1514.99	2266.83	778.15
Tmax (h)	1	1.5	1	1.5
Cmax (ng/ml)	189.15	250.45	270.22	150.73

*Values are expressed as mean ±SD (n=3)

The obtained results were found to be statistically significant when analyzed by a one-sample t-test using GraphPad Prism software version, 8.0.2 and were depicted in table 43.

Table – 43

Statistical analysis of pharmacokinetic data of optimized indomethacin nanoemulsion and conventional marketed product

One sample t-test	INDO-NE7	
	Aqueous humour	**Plasma**
Actual mean	57.56	155.5
Number of values	8	8
t, df	t=2.362, df=8	t=3.793, df=7
P value	0.0458	0.0068
Significant (alpha=<0.05)	Yes	Yes
95% confidence interval	1.373 to 113.7	58.57 to 252.4
R^2	0.4109	0.6727

8.1 SUMMARY

- The melting point of Timolol maleate was evaluated using melting point apparatus to determine drug purity and the melting point was found to be 201ºC meeting the standard melting point range of 202-203 ºC indicating the purity of the drug.
- Quantitative analysis of Timolol maleate was done using ultrafast liquid chromatography where the concentration range of 50 to 1000 ng/ml showed linearity with a regression coefficient (R^2) value of 0.996.
- Medium-chain fatty acid derivatives that can solubilize a good amount of a variety of drug substances were chosen as oils and nonionic surfactants which are less toxic and proved to be less/nonirritant compared to ionic surfactants were chosen to produce nanoemulsions. Further, screening of the oil and surfactants was done by performing the drug solubility studies in various formulation components i.e., oils, surfactants and co-surfactants using a gyratory shaker.
- Based on the solubility profile, capryol 90 was selected as the oil phase, tween 80 and span 80 were selected as surfactant and co-surfactant respectively and propylene glycol was selected as co-solvent/ viscosifying agent/permeation enhancer and glycerol as tonicity adjusting agent.
- FTIR spectroscopy was carried out to check and confirm whether the selected formulation components are in good compatibility with drug and do not compromise its stability and safety and the obtained spectra confirmed that the drug maintained its chemical integrity in the presence of selected excipients and hence, there exists good compatibility between drug and excipients.
- The pseudo ternary phase diagrams of selected oil, surfactant and co-surfactant were developed using the water titration method to identify the appropriate ratio of surfactant mixtures that can form a large existence area of the stable nanoemulsions and a large nanoemulsion area was observed for the surfactant to co-surfactant (Smix) ratio of 1:3 (w/w).
- 3^2 factorial designs where, 2-factors at 3-levels were used to select the compositions for formulating nanoemulsions with Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) using the oil and Smix contents as the independent variables and the response variables chosen were the droplet size

(nm) and percentage drug release (%) where a total of 9 compositions were suggested by the software and the formulation codes were given as TML-NE1-9.

- Timolol maleate nanoemulsions (TML-NEs) were prepared by the ultrasonic emulsification method where the primary emulsion was produced by the gradual addition of the drug dissolved internal phase to the external phase with continuous stirring, the most common method for preparing highly concentrated emulsions and the primary emulsion was then sonicated by using probe sonicator to get a nanoemulsion.
- Mean globule size, surface charge (Zeta potential) and size distribution (polydispersity index, PDI) of the prepared TML-NEs were determined by photon correlation spectroscopy using a Zetasizer and the mean globule size for all of the TML-NEs was found less than 200nm ranging from 129.5±1.8 to 223.6±1.6 confirming the successful formation of nanoemulsion. The zeta potential values found in the range of 12.4 ± 4.82 to 25.8±5.86 indicated the ability to interact with negatively charged corneal epithelial cells and hence able to improve the contact time with the corneal membrane. The polydispersity index values found <1.00 ranging from 0.07±0.001 to 0.406±0.021 indicating narrow size distribution of droplets and thus confirmed the homogeneity of formulations.
- The pH of the prepared nanoemulsions was measured by digital pH meter and the pH of prepared formulations found near to the biological pH with values ranging from 5.8±0.3 to 6.7±0.5.
- The viscosity of the NEs was measured in triplicate using a digital cone and plate viscometer and the viscosity of formulations found within the acceptable range of 43.1±0.1 to 102.3±0.5 cP.
- The refractive index of nanoemulsions was determined using an Abbes refractometer and the refractive index of formulations ranged from 1.345 ± 0.005 to 1.395± 0.001 confirming the safety and comfort for applying onto the eye surface.
- Surface tension measurements were carried out by the Du Nouy ring method using an electronic tensiometer where the TML-NEs found to have low surface tension values ranging from 41.5 ± 21.8 to 52.1 ±3.4 mN/m indicating

the ability to improve the spreading of formulation onto the corneal membrane.

- The osmotic pressure of prepared formulations was calculated by using the standard mathematical equation and it was found that the values ranged from 303 to 395 mOsm/l which are within the values recommended for ophthalmic products thus further confirming the safety and comfort for applying onto the eye surface.
- The stability of nanoemulsions was confirmed by checking the changes in droplet size and drug content before and after subjecting to thermodynamic stability studies where the by Prepared nanoemulsions were subjected to six heat and cool cycles followed by centrifugation and then, exposed to three freeze and thaw cycles. The results showed that there were no considerable changes in particle size and drug content of TML-NEs before and after stability test and thus found to be stable enough for further evaluation.
- The drug release from the NEs was studied by utilizing vertical Franz diffusion cell in triplicate and from the *in vitro* diffusion studies, it is evident that the prepared formulations were found to exhibit sustained drug release of 59.27±1.59 to 96.41±2.31% at 24h time point when compared to that of control which released 99.03 ±3.11 % of drug within 2.5 h.
- The response surface analysis was carried out using Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) to optimize the prepared nanoemulsions**.** The experimental data were fitted into the quadratic model. The model found significant with the F-value of 163.74 for mean globule size and 22.64 for % drug release. The P-values found less than 0.0500 when analyzed using ANOVA indicating that the model terms are significant.
- Further, from the response surface analysis by contour and 3D surface plots, it is evident that the oil and Smix compositions showed a significant effect on mean globule size and % drug release. The variation in drug release can be emphasized from the formulation composition variables and the droplet size of the formulations. The drug release was found to be affected by their droplet size i.e. as the size decreased the release was increased and thus the formulation TML-NE1 with the medium oil and high Smix was found to have lowest mean globule size and significantly superior drug release; hence it was chosen as the optimized formulation and was subjected to further study.

- The optimized formulation, TML-NE1 was subjected to Transmission Electron Microscopy to study the surface morphology and the droplets in the nanoemulsion appeared spherical in shape where the droplet size value was in agreement with the results obtained from the Zetasizer.
- Permeation studies across the bovine cornea were carried out by using Franz-diffusion cells. The drug permeated through the bovine corneal membrane at 4 h from the optimized formulation and solution was found to be 524 ± 1.5 µg/ cm^2 and 175±2.6 µg/cm^2 respectively. The results for the drug solution were found quite opposite to the *in vitro* drug diffusion rate. This can be explained by the structural complexity of the corneal membrane that opposes the aqueous solution because of the outer lipophilic layer.
- The flux for TML-NE1 and control were found to be 246.815µg/cm^2/h and 83.518µg/cm^2/h respectively. The transcorneal permeability coefficient for TML-NE1 and control was found to be 2.74×10^{-5} cm/sec and 0.92×10^{-5} cm/sec respectively. The improved drug permeation in case of nanoemulsion can be explained by the nano-sized droplets that can be easily infiltrated by endocytosis and the permeation enhancing ability of the formulation ingredients will cause temporary changes in the tight junctions of the corneal membrane that enhances the permeation by transcellular pathway.
- The corneal hydration studies were carried out to ensure the quality of the *ex vivo* studies by calculating the percentage corneal hydration level (HL %) and was found to be 58.6±1.2 for TML-NE1 and 61.8±1.05 confirming the viability of the excised cornea.
- The *in vivo* study protocol (IAEC/49/UCPSc/KU/2018) was approved by the institutional animal ethical committee, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India and the study was performed at Jeeva Life Sciences, Hyderabad.
- Ocular irritancy of optimized nanoemulsion was studied in healthy adult New Zealand albino rabbits free from visible ocular abnormalities by following the modified Draize eye test. The ocular irritation index (Iirr) value for TML-NE1 was found less than 4 i.e., 3 indicating that the formulation is nonirritant to rabbit eyes.

- The *in vivo* pharmacokinetics in rabbit aqueous humour, as well as rabbit plasma, were carried out to study the ocular and systemic pharmacokinetics of drug from TML-NE1 and to compare and with that of the marketed formulation.
- Quantitative analysis of Timolol maleate in rabbit aqueous humour was done by using calibration curves plotted in rabbit aqueous humour following ultrafast liquid chromatography where the linearity with a regression coefficient (R^2) value of 0.993 was obtained at a concentration range of 2 to 100 ng/ml and the quantitative analysis of Timolol maleate in rabbit plasma was done by using calibration curves plotted in rabbit plasma following where the linearity with a regression coefficient (R^2) value of 0.994 was obtained at a concentration range of 50 to 400ng/ml.
- The aqueous humor levels and the plasma levels of timolol maleate from TML-NE1 and marketed product were 17818.14 ng/ml/h and 4488.34 ng/ml/h; 7790.98 ng/ml/h and 20398.28 ng/ml/h respectively which indicated the lowest systemic absorption and the improved corneal absorption of TML-NE1 when compared to the marketed formulation. The reason for improved corneal absorption can be explained by the positive charge on the droplets imparted by Benzalkonium chloride that involved in the electrostatic interaction with the negatively charged corneal membrane and that lead to improved residence time on the cornea.
- The obtained *in vivo* pharmacokinetic data was found to be statistically significant when analyzed by a one-sample t-test using GraphPad Prism software version, 8.0.2 with P values 0.0353 in rabbit aqueous humour and <0.0001 in rabbit plasma respectively.
- The melting point of ciprofloxacin hydrochloride was evaluated using melting point apparatus to determine drug purity and the melting point was found to be 255ºC meeting the standard melting point range of 255-257°C indicating the purity of the drug.
- Quantitative analysis of Timolol maleate was done by using calibration curves in rabbit aqueous humour and rabbit plasma following ultrafast liquid chromatography where the concentration range of 20 to 400 ng/ml showed linearity with a regression coefficient (R^2) value of 0.992.

- Medium-chain fatty acid derivatives that can solubilize a good amount of a variety of drug substances were chosen as oils and nonionic surfactants which are less toxic and proved to be less/nonirritant compared to ionic surfactants were chosen to produce nanoemulsions. Further, screening of the oil and surfactants was done by performing the drug solubility studies in various formulation components i.e., oils, surfactants and co-surfactants using a gyratory shaker.
- Based on the solubility profile, caprol micro express was selected as the oil phase, tween 20 and span 20 were selected as surfactant and co-surfactant respectively and propylene glycol was selected as co-solvent/ viscosifying agent/permeation enhancer and glycerol as tonicity adjusting agent.
- FTIR spectroscopy was carried out to check and confirm whether the selected formulation components are in good compatibility with drug and do not compromise its stability and safety and the obtained spectra confirmed that the drug maintained its chemical integrity in the presence of selected excipients and hence, there exists good compatibility between drug and excipients.
- The pseudo ternary phase diagrams of selected oil, surfactant and co-surfactant were developed using the water titration method to identify the appropriate ratio of surfactant mixtures that can form a large existence area of the stable nanoemulsions and a large nanoemulsion area was observed for the surfactant to co-surfactant (Smix) ratio of 1:3 (w/w).
- 3^2 factorial designs where, 2-factors at 3-levels were used to select the compositions for formulating nanoemulsions with Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) using the oil and Smix contents as the independent variables and the response variables chosen were the droplet size (nm) and percentage drug release (%) where a total of 9 compositions were suggested by the software and the formulation codes were given as CIP-NE1-9.
- Ciprofloxacin hydrochloride nanoemulsions (CIP-NEs) were prepared by the ultrasonic emulsification method where the primary emulsion was produced by the gradual addition of the drug dissolved internal phase to the external phase with continuous stirring, the most common method for preparing highly concentrated emulsions and the primary emulsion was then sonicated by using probe sonicator to get a nanoemulsion.

- Mean globule size, surface charge (Zeta potential) and size distribution (polydispersity index, PDI) of the prepared CIP-NEs were determined by photon correlation spectroscopy using a Zetasizer and the mean globule size for all of the CIP-NEs was found less than 200nm ranging from 121.4±1.6 to 186.2±1.9 confirming the successful formation of nanoemulsion. The zeta potential values found in the range of 16.32±4.12 to 24.15±5.25 indicated the ability to interact with negatively charged corneal epithelial cells and hence able to improve the contact time with the corneal membrane. The polydispersity index values found <1.00 ranging from 0.031±0.021 to 0.512±0.001 indicating narrow size distribution of droplets and thus confirmed the homogeneity of formulations.
- The pH of the prepared nanoemulsions was measured by digital pH meter and the pH of prepared formulations found near to the biological pH with values ranging from 5.5 ± 0.4 to 6.7 ± 0.5.
- The viscosity of the NEs was measured in triplicate using a digital cone and plate viscometer and the viscosity of formulations found within the acceptable range of 40.5±0.0 to 64.2±0.2 cP.
- The refractive index of nanoemulsions was determined using an Abbes refractometer and the refractive index of formulations ranged from 1.340 ± 0.001 to 1.386± 0.005 confirming the safety and comfort for applying onto the eye surface.
- Surface tension measurements were carried out by the Du Nouy ring method using an electronic tensiometer where the TML-NEs found to have low surface tension values ranging from 44.1 ± 1.9 to 52.6 ± 2.6 mN/m indicating the ability to improve the spreading of formulation onto the corneal membrane.
- The osmotic pressure of prepared formulations was calculated by using the standard mathematical equation and it was found that the values ranged from 355 to 398 mOsm/l which are within the values recommended for ophthalmic products thus further confirming the safety and comfort for applying onto the eye surface.
- The stability of nanoemulsions was confirmed by checking the changes in droplet size and drug content before and after subjecting to thermodynamic stability studies where the by Prepared nanoemulsions were subjected to six

heat and cool cycles followed by centrifugation and then, exposed to three freeze and thaw cycles. The results showed that there were no considerable changes in particle size and drug content of CIP-NEs before and after stability test and thus found to be stable enough for further evaluation.

- The drug release from the NEs was studied by utilizing vertical Franz diffusion cell in triplicate and from the *in vitro* diffusion studies, it is evident that the prepared formulations were found to exhibit sustained drug release of 71.56±0.79 to 94.64±2.31 % at 24h time point when compared to that of control which released 98.99 ±1.67 % of drug within 2 h.
- The response surface analysis was carried out using Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) to optimize the prepared nanoemulsions**.** The experimental data were fitted into the quadratic model. The model found significant with the F-value of 68.63for mean globule size and 22.64 for % drug release. The P-values found less than 0.0500 when analyzed using ANOVA indicating that the model terms are significant.
- Further, from the response surface analysis by contour and 3D surface plots, it is evident that the droplet size reduced as the Smix content increased from low to medium and further increase in Smix resulted in a slight increase in the droplet size. At a low level of oil content, the % drug release was more and as the oil content increased the drug release decreased thus had an invert effect on % drug release. The % drug release increased as the Smix content increased from low to medium and further increase in Smix resulted in a slight decrease in the % drug release. Thus the formulation with low oil and medium Smix content, CIP-NE4 found to have lowest mean globule size and significantly superior drug release; hence it was chosen as the optimized formulation and was subjected to further study.
- The optimized formulation, CIP-NE4 was subjected to Transmission Electron Microscopy to study the surface morphology and the droplets in the nanoemulsion appeared spherical in shape where the droplet size value was in agreement with the results obtained from the Zeta sizer.
- Permeation studies across the bovine cornea were carried out by using Franz-diffusion cells. The drug permeated through the bovine corneal membrane at 4 h from the optimized formulation and solution was found to be 902 ± 1.5 µg/ cm^2 and 194 ±11 µg/cm^2 respectively. The results for the drug solution were

found quite opposite to the *in vitro* drug diffusion rate. This can be explained by the structural complexity of the corneal membrane that opposes the aqueous solution because of the outer lipophilic layer.

- The flux for CIP-NE4 and control were found to be 287.261 μg/cm^2/h and 61.783 μg/cm^2/h respectively. The trans corneal permeability coefficient for TML-NE1 and control was found to be 2.65×10^{-5}cm/sec and 0.57×10^{-5} cm/sec respectively. The improved drug permeation in case of nanoemulsion can be explained by the nano-sized droplets that can be easily infiltrated by endocytosis and the permeation enhancing ability of the formulation ingredients will cause temporary changes in the tight junctions of the corneal membrane that enhances the permeation by transcellular pathway.
- The corneal hydration studies were carried out to ensure the quality of the *ex vivo* studies by calculating the percentage corneal hydration level (HL %) and was found to be 66.5±0.24 for CIP-NE4 and 72.2±0.13confirming the viability of the excised cornea.
- To know the changes in the efficiency of ciprofloxacin, the standard spread plate method was used to study the antibacterial effect of optimized ciprofloxacin formulation against selected microorganisms. The average zone of inhibition values found to be 35.25±1.06, 24.75±0.35 and 34.00±1.41 against *Klebsiella pneumoniae* (Gram –ve), *Staphylococcus aureus* (Gram+ve) and *Proteus mirabilis* (Gram –ve) respectively and indicated no influence of formulation ingredients on the activity of CIP.
- The *in vivo* study protocol (IAEC/49/UCPSc/KU/2018) was approved by the institutional animal ethical committee, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India and the study was performed at Jeeva Life Sciences, Hyderabad.
- Ocular irritancy of optimized nanoemulsion was studied in healthy adult New Zealand albino rabbits free from visible ocular abnormalities by following the modified Draize eye test. The ocular irritation index (Iirr) value for CIP-NE4 was found less than 4 i.e., 2 indicating that the formulation is nonirritant to rabbit eyes.

- The *in vivo* pharmacokinetics in rabbit aqueous humour, as well as rabbit plasma, were carried out to study the ocular and systemic pharmacokinetics of drug from CIP-NE4 and to compare and with that of the marketed formulation.
- Quantitative analysis of ciprofloxacin in rabbit aqueous humour was done by using calibration curves plotted in rabbit aqueous humour following ultrafast liquid chromatography where the linearity with a regression coefficient (R^2) value of 0.994 was obtained at a concentration range of 10 to 200 ng/ml and the quantitative analysis of ciprofloxacin in rabbit plasma was done by using calibration curves plotted in rabbit plasma following where the linearity with a regression coefficient (R^2) value of 0.998 was obtained at a concentration range of 10 to 200ng/ml.
- The aqueous humor levels and the plasma levels of timolol maleate from CIP-NE4 and marketed product were 24826.19 ng/ml/h and 2646.46 ng/ml/h; 8557.81 ng/ml/h and 11580.22 ng/ml/h respectively which indicated the lowest systemic absorption and the improved corneal absorption of CIP-NE4 when compared to the marketed formulation. The reason for improved corneal absorption can be explained by the positive charge on the droplets imparted by benzalkonium chloride that involved in the electrostatic interaction with the negatively charged corneal membrane and that lead to improved residence time on the cornea.
- The obtained *in vivo* pharmacokinetic data was found to be statistically significant when analyzed by a one-sample t-test using GraphPad Prism software version, 8.0.2 with P values 0.0306 in rabbit aqueous humour and 0.0255 in rabbit plasma respectively.
- The melting point of indomethacin was evaluated using melting point apparatus to determine drug purity and the melting point was found to be 153ºC meeting the standard melting point range of 152–154 ºC indicating the purity of the drug.
- Quantitative analysis of indomethacin was done using ultrafast liquid chromatography where the concentration range of 2 to 100 ng/ml showed linearity with a regression coefficient (R^2) value of 0.998.

- Medium-chain fatty acid derivatives that can solubilize a good amount of a variety of drug substances were chosen as oils and nonionic surfactants which are less toxic and proved to be less/nonirritant compared to ionic surfactants were chosen to produce nanoemulsions. Further, screening of the oil and surfactants was done by performing the drug solubility studies in various formulation components i.e., oils, surfactants and co-surfactants using a gyratory shaker.
- Based on the solubility profile, captex 8000 was selected as the oil phase, tween 20 and span 20 were selected as surfactant and co-surfactant respectively. Propylene glycol was selected as co-solvent/ viscosifying agent/permeation enhancer and glycerol as tonicity adjusting agent and cetalkonium chloride was chosen as a cationic surfactant.
- FTIR spectroscopy was carried out to check and confirm whether the selected formulation components are in good compatibility with drug and do not compromise its stability and safety and the obtained spectra confirmed that the drug maintained its chemical integrity in the presence of selected excipients and hence, there exists good compatibility between drug and excipients.
- The pseudo ternary phase diagrams of selected oil, surfactant and co-surfactant were developed using the water titration method to identify the appropriate ratio of surfactant mixtures that can form a large existence area of the stable nanoemulsions and a large nanoemulsion area was observed for the surfactant to co-surfactant (Smix) ratio of 1:3 (w/w).
- 3^2 factorial designs where, 2-factors at 3-levels were used to select the compositions for formulating nanoemulsions with Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) using the oil and Smix contents as the independent variables and the response variables chosen were the droplet size (nm) and percentage drug release (%) where a total of 9 compositions were suggested by the software and the formulation codes were given as IND-NE1-9.
- Indomethacin nanoemulsions (IND-NEs) were prepared by the ultrasonic emulsification method where the primary emulsion was produced by the gradual addition of the drug dissolved internal phase to the external phase with continuous stirring, the most common method for preparing highly

concentrated emulsions and the primary emulsion was then sonicated by using probe sonicator to get a nanoemulsion.

- Mean globule size, surface charge (Zeta potential) and size distribution (polydispersity index, PDI) of the prepared IND-NEs were determined by photon correlation spectroscopy using a Zetasizer and the mean globule size for all of the IND-NEs was found less than 200nm ranging from 128.9±1.1 to 191.4±1.6 confirming the successful formation of nanoemulsion. The zeta potential values found in the range of 13.2 ± 4.6 to 22.6±5.45 indicated the ability to interact with negatively charged corneal epithelial cells and hence able to improve the contact time with the corneal membrane. The polydispersity index values found <1.00 ranging from 0.031±0.021 to 0.512±0.001indicating narrow size distribution of droplets and thus confirmed the homogeneity of formulations.
- The pH of the prepared nanoemulsions was measured by digital pH meter and the pH of prepared formulations found near to the biological pH with values ranging from 5.5±0.4 to 6.9±0.9.
- The viscosity of the NEs was measured in triplicate using a digital cone and plate viscometer and the viscosity of formulations found within the acceptable range of 15.3±0.1 to 32.7±0.0 mN/m.
- The refractive index of nanoemulsions was determined using an Abbes refractometer and the refractive index of formulations ranged from 1.340 ± 0.001 to 1.386± 0.005 confirming the safety and comfort for applying onto the eye surface.
- Surface tension measurements were carried out by the Du Nouy ring method using an electronic tensiometer where the IND-NEs found to have low surface tension values ranging from 32 ± 2.6 to 52.3 ±3.4 mPas. indicating the ability to improve the spreading of formulation onto the corneal membrane.
- The osmotic pressure of prepared formulations was calculated by using the standard mathematical equation and it was found that the values ranged from 324 to 382 mOsm/l which are within the values recommended for ophthalmic products thus further confirming the safety and comfort for applying onto the eye surface.

- The stability of nanoemulsions was confirmed by checking the changes in droplet size and drug content before and after subjecting to thermodynamic stability studies where the by Prepared nanoemulsions were subjected to six heat and cool cycles followed by centrifugation and then, exposed to three freeze and thaw cycles. The results showed that there were no considerable changes in particle size and drug content of IND-NEs before and after stability test and thus found to be stable enough for further evaluation.
- The drug release from the IND-NEs was studied by utilizing vertical Franz diffusion cell in triplicate and from the *in vitro* diffusion studies, it is evident that the prepared formulations were found to exhibit sustained drug release of 67.91±2.01 to 95.90±1.93 % at 24h time point when compared to that of control which released 99.81±5.21 % of drug within 2 h.
- The response surface analysis was carried out using Design- Expert (Version 12, Stat-Ease Inc., Minneapolis, MN) to optimize the prepared nanoemulsions**.** The experimental data were fitted into the quadratic model. The model found significant with the F-value of 107.49 for mean globule size and 39.33 for % drug release. The P-values found less than 0.0500 when analyzed using ANOVA indicating that the model terms are significant.
- Further, from the response surface analysis by contour and 3D surface plots, it is evident that the oil and Smix compositions showed a significant effect on mean globule size and % drug release where an increase in oil content provided a decrease in globule size to some extent and a further increase in oil content lead to a slight increase in globule size and an opposite effect was seen for percentage drug release. However, by increasing the Smix content, the globule size decreased and the percentage drug release increased. Thus the formulation IND-NE7 with the medium oil and high Smix was found to have the lowest mean globule size and significantly high drug release; hence it was chosen as the optimized formulation and was subjected to further study.
- The optimized formulation, IND-NE7 was subjected to Transmission Electron Microscopy to study the surface morphology and the droplets in the nanoemulsion appeared spherical in shape where the droplet size value was in agreement with the results obtained from the Zetasizer.

- Permeation studies across the bovine cornea were carried out by using Franz-diffusion cells. The drug permeated through the bovine corneal membrane at 4 h from the optimized formulation and solution was found to be 524 ± 1.5 µg/ cm^2 and 175±2.6 µg/cm^2 respectively. The results for the drug solution were found quite opposite to the *in vitro* drug diffusion rate. This can be explained by the structural complexity of the corneal membrane that opposes the aqueous solution because of the outer lipophilic layer.
- The flux for IND-NE7 and control were found to be 166.878 µg/cm^2/h and 83 and 55.732 µg/cm^2/h respectively. The trans corneal permeability coefficient for IND-NE7 and control was found to be 4.63×10^{-5} cm/sec and 1.54×10^{-5} cm/sec respectively. The improved drug permeation in case of nanoemulsion can be explained by the nano-sized droplets that can be easily infiltrated by endocytosis and the permeation enhancing ability of the formulation ingredients will cause temporary changes in the tight junctions of the corneal membrane that enhances the permeation by transcellular pathway.
- The corneal hydration studies were carried out to ensure the quality of the *ex vivo* studies by calculating the percentage corneal hydration level (HL %) and was found to be 66.5±0.24% for IND-NE7 and 72.2±0.13% confirming the viability of the excised cornea.
- The *in vivo* study protocol (IAEC/49/UCPSc/KU/2018) was approved by the institutional animal ethical committee, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India and the study was performed at Jeeva Life Sciences, Hyderabad.
- Ocular irritancy of optimized nanoemulsion was studied in healthy adult New Zealand albino rabbits free from visible ocular abnormalities by following the modified Draize eye test. The ocular irritation index (Iirr) value for IND-NE7 was found less than 4 i.e., 1 indicating that the formulation is nonirritant to rabbit eyes.
- The *in vivo* pharmacokinetics in rabbit aqueous humour, as well as rabbit plasma, were carried out to study the ocular and systemic pharmacokinetics of drug from IND-NE7 and to compare and with that of the marketed formulation.

- Quantitative analysis of indomethacin in rabbit aqueous humour was done by using calibration curves plotted in rabbit aqueous humour following ultrafast liquid chromatography where the linearity with a regression coefficient (R^2) value of 0.992 was obtained at a concentration range of 10 to 400 ng/ml and the quantitative analysis of indomethacin in rabbit plasma was done by using calibration curves plotted in rabbit plasma following where the linearity with a regression coefficient (R^2) value of 0.998 was obtained at a concentration range of 10 to 500ng/ml.
- The aqueous humor levels and the plasma levels of indomethacin from IND-NE7 and marketed product were 1514.99 ng/ml/h and 778.15 ng/ml/h; 974.14 ng/ml/h and 2266.83ng/ml/h respectively which indicated the lowest systemic absorption and the improved corneal absorption of IND-NE7 when compared to the marketed formulation. The reason for improved corneal absorption can be explained by the positive charge on the droplets imparted by cetalkonium chloride that involved in the electrostatic interaction with the negatively charged corneal membrane and that lead to improved residence time on the cornea.
- The obtained *in vivo* pharmacokinetic data was found to be statistically significant when analyzed by a one-sample t-test using GraphPad Prism software version, 8.0.2 with P values 0.0458 in rabbit aqueous humour and 0.0068 in rabbit plasma respectively.

8.2 CONCLUSION

Topical ocular cationic nanoemulsions for timolol maleate, ciprofloxacin hydrochloride and indomethacin were designed and prepared using design expert software with various oil and surfactant mixtures and studied for the physicochemical properties such as particle size, zeta potential, pH, viscosity, surface tension and osmolarity. All the prepared nanoemulsions were found to have appropriate physicochemical properties with values recommended for ocular use. Further, the prepared nanoemulsions were tested for stability by evaluating the change in the particle size and drug content values before and after the stability testing. The changes in droplet size and drug content of nanoemulsions before and after stability test were found to be negligible and indicated good stability. The *in vitro* drug release behavior of all the nanoemulsions was studied using the diffusion method and compared with

that of drug solution as control formulation. From the results of *in vitro* diffusion studies, it is evident that the prepared nanoemulsions exhibited sustained release in comparison to control. The obtained experimental data were fitted into design expert software to study the effect of formulation ingredients on the parameters like particle size and percentage drug release and the prepared nanoemulsions were optimized through response surface analysis. The formulations coded as TML-NE1, CIP-NE4 and IND-NE7 were chosen as optimized nanoemulsions as they were found to show lower particle size and better percentage drug release comparatively and were further studied for the surface morphology using Transmission Electron Microscopy, antimicrobial activity (CIP-NE4), *ex vivo* permeation across the bovine cornea, ocular irritancy and *in vivo* pharmacokinetics in rabbit aqueous humour as well as in rabbit plasma and compared with the marketed products. The droplets in the nanoemulsions appeared spherical and the droplet size values were in agreement with the results obtained from the Zetasizer. The results from the antibacterial activity revealed that there is no influence of formulation ingredients on the activity of ciprofloxacin against both Gram (+ve) and Gram (-ve) organisms. *Ex vivo* studies showed enhanced drug permeation through bovine cornea when compared to the control formulations. The optimized NEs were considered safe for ocular delivery with the individual Iirr scores of less than 4. The improved corneal absorption together with lesser systemic absorption resulted in enhanced ocular drug bioavailability with higher $AUC_{(0\text{-}24h)}$ values in rabbit aqueous humour and lower $AUC_{(0\text{-}24h)}$ values in rabbit plasma when compared to marketed products. The pharmacokinetic data were found statistically significant when analyzed using a one-sample t-test with P values less than 0.05. The suitability of prepared nanoemulsions for the topical administration in the form of eye drops due to low viscosity and transparency; improved spreading ability over the corneal membrane due to lower surface tension; improved corneal absorption and hence improved ocular bioavailability due to the nano-sized droplets that can be easily infiltrated by endocytosis and the permeation enhancing ability of the formulation ingredients by causing temporary changes in the tight junctions of the corneal membrane; prolonged corneal retention due to the positive charge on the droplets that involved in the electrostatic interaction with the negatively charged corneal membrane imparted by cationic surfactants; lesser systemic absorption and hence reduced chances of systemic side effects due to comparatively greater viscosity than solution that lead to reduced nasolacrimal drainage; the sustained drug release and hence

improved therapeutic efficacy with reduced dosing frequency that would results into improved patient compliance. Thus, the TML-NE1 offers intensive treatment of glaucoma with a decrease in the number of instillations per day and a decrease or disappearance of systemic side effects of TML with an improvement in patient compliance. The CIP-NE4 offers improved therapeutic efficacy in controlling ocular infections with sustained drug release for 24 h that would provide longer antibacterial effect with less dosing frequency along with the ease of application and leading to improved patient compliance. The IND-NE7 offers an effective postoperative treatment with increased ocular bioavailability and improved patient compliance with a decrease in the number of instillations per day and a decrease or disappearance of systemic side effects of IND that are associated with conventional dosage forms.

REFERENCES

1) Aaron K, Marie AG. Do I have night blindness?. December 14, 2018. https://www.medicalnewstoday.com/articles/324004
2) Abdul Sisak MA, Rusli D, Suria R. Study on the effect of oil phase and co-surfactant on microemulsion systems. Malaysian J Anal Sci. 2017;21(6):1409-16.
3) Abelson MB. Secret world of pharmacokinetics. Review of ophthalmology. 2009. https://www.reviewofophthalmology.com/article/the-secret-world-of-pharmacokinetics
4) Achouri D, Alhanout K, Piccerelle P, Andrieu V. Recent advances in ocular drug delivery. Drug Dev Ind Pharm. 2012:1-19.
5) Adam F, Daniel M. What to know about diabetic retinopathy. August 25, 2017. https://www.medicalnewstoday.com/articles/183417
6) Adnan A, Rizwan M, Farhan JA, Zeenat I, Roop KK, Aqil M, et al. Nanoemulsion components screening and selection: a technical note. AAPS Pharm Sci Tech 2009;10:69-76.
7) Affo W, Mensah-Brown H, Awuku JF, Markwo A. Quantitative Analysis of Ciprofloxacin Sodium Chloride Pharmaceutical Infusions Using Ultraviolet-visible Spectroscopy. ARPN Journal of Science and Technology. 2013;3(3):215-227.
8) Agarwal P, Rupenthal ID. *In vitro* and *ex vivo* corneal penetration and absorption models. Drug Delivery Transl Res. 2016; 6:634–47.
9) Ahdoot M. Diabetic Retinopathy. Progressive Ophthalmology website. August 09, 2019. https://www.thenewyorkeyedoctor.com/diabetic-retinopathy.html
10) Ahmad N, Alam MA, Ahmad FJ, Sarafroz M, Ansari K, Sharma S, Amir M. Ultrasonication techniques used for the preparation of novel Eugenol in the treatment of wounds healings and anti-inflammatory. J Drug Delivery Sci Technol. 2018; 46:461-73.
11) Ahmed I, Patton TF. Importance of the noncorneal absorption route in topical ophthalmic drug delivery. Invest Ophthalmol Vis Sci. 1985;26(4):584-587.
12) Ahuja M, Dhake AS, Sharma SK, Majumdar DK. Topical ocular delivery of NSAIDs. AAPS J. 2008;10:229–41.

13) Alan K. What is Night Blindness?. WebMD Medical Reference. February 3, 2018. https://www.webmd.com/eye-health/night-blindness
14) Ali MS, Alam MS, Alam N, Siddiqui MR. Preparation, characterization and stability study of dutasteride loaded nanoemulsion for treatment of benign prostatic hypertrophy. Iran J Pharm Res. 2014;13:1125–40.
15) Ali MS, Alam MS, Alam N, Siddiqui MR. Preparation, characterization and stability study of dutasteride loaded nanoemulsion for treatment of benign prostatic hypertrophy. Iran J Pharm Res. 2014;13(4):1125-40.
16) American Macular Degeneration Foundation (AMDF).What is Macular Degeneration?. 2019. https://www.macular.org/what-macular-degeneration
17) Ammar HO, Salama HA, Ghorab M, Mahmoud AA. Nanoemulsion as a potential ophthalmic delivery system for dorzolamide hydrochloride. AAPS Pharm Sci Tech. 2009; 10(3):808-19.
18) Amo del EM, Urtti A. Current and future ophthalmic drug delivery systems: A shift to the posterior segment. Drug Discovery Today. 2008;13:135-143.
19) Andrew AD, Melissa CS. Sty. October 10, 2019. https://www.emedicinehealth.com/sty/article_em.htm
20) Andrew AD, William C. Glaucoma. June 21, 2016. https://www.medicinenet.com/glaucoma/article.htm#what_are_glaucoma_risk factors
21) Andrew AD, William CS. Macular Degeneration. July 25, 2019. https://www.medicinenet.com/macular_degeneration/article.htm
22) Ankur G, Burak EH, Alan HT, Patrick DS. Nanoemulsions: formation, properties and applications. Soft Matter. 2016;12:1-16.
23) Appel LE, Ray RJ, Lyon DK, West JB, McCray SB, Crew MD, Friesen DT, Herbig SM, Lo JB, inventors; Pfizer Inc, assignee. Multiparticulate crystalline drug compositions having controlled release profiles. United States patent, US 7,887,844. 2011 Feb 15.
24) Araujo J, Gonzalez E, Egea MA, Garcia ML, Souto EB. Nanomedicines for ocular NSAIDs: safety on drug delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2009;5(4):394-401.
25) Ashaben P, Kishore C, Vibhuti A, Ashim KM. Ocular drug delivery systems: an overview. World J Pharmacol. 2013;2(2): 47–64.

26) Ashwini AC, Milind JU, Minal NB, Neha SR. Effect of trans - esterification of oil on nanoemulsion gel for topical drug delivery. IJPSR. 2018;9(2):589-98.
27) Asiya M, Mohammed A, Neha M, Zrien N, Gaurav KJ, Farhan JA. Transungual delivery of ketoconazole nanoemulgel for the effective management of onychomycosis. AAPS Pharm Sci Tech. 2015;17(6):1477-1490.
28) At a glance: Diabetic Retinopathy. National Eye Institute (NEI). May 29, 2019. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/diabetic-retinopathy
29) Azari AA, Barney NP. Conjunctivitis: a systematic review of diagnosis and treatment. JAMA. 2013;310(16):1721-29.
30) Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, et al. Nanoemulsion components screening and selection: a technical note. APPS Pharm Sci Tech. 2009; 10(1):69-76.
31) Bactin - Ciprofloxacin-Antibacterial Film Coated Tablet. IBN SINA. https://drugbd.blogspot.com/2014/10/bactin-ciprofloxacin-antibacterial-film.html
32) Bailey G, Thompson V. Cataracts: 3 common types, causes, symptoms and treatments. June 23, 2019. https://www.allaboutvision.com/conditions/cataracts.htm
33) Balata GF, Essa EA, Shamardl HA, Zaidan SH, Abourehab MA. Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol. Drug Des Dev Ther. 2016; 10:117-128.
34) Ban J, Zhang Y, Huang X, Deng GH, Hou DZ, Chen YZ, et al. Corneal permeation properties of a charged lipid nanoparticles carrier containing dexamethasone. Int J Nanomed. 2017;12:1329–39.
35) Baranowski P, Karolewicz B, Gajda M, Pluta J. Ophthalmic drug dosage forms: characterization and research methods. Sci World J. 2014;2014:1-14.
36) Barar J, Aghanejad A, Marziyeh F, Yadollah O. Advanced drug delivery and targeting technologies for the ocular diseases. BioImpacts. 2016;6(1):49-67.
37) Beg S, Jena SS, Patra CN, Rizwan M, Swain S, Sruti J, Rao MB, Singh B. Development of solid self-nano emulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. Colloids and surfaces B: Biointerfaces. 2013;101:414-23.

38) Bekerman I, Paul G, Michael V. Variations in Eyeball Diameters of the Healthy Adults. J Ophthalmol. 2014;2014:1-5.
39) Bhuwanesh SP, Kumar B, Jain SK, Shafaat, K. (2012). Development and characterization of a nanoemulsion gel formulation for transdermal delivery of carvedilol. Int J Drug Del Res. 2012;4(1):151-61.
40) Bogaard JD. Evaluation of Therapeutic Interventions for the Treatment of Eye Disorders (Doctoral dissertation, University of Illinois at Chicago), 2017.
41) Bowes MH, Michael SO, Wilhelmus KR. Experimental Evaluation of Chlorhexidine Gluconate for Ocular Antisepsis. Antimicrob. Agents Chemother. 1984:793-96.
42) Bowman RJ, Sillah A, Van Dehn C, Goode VM, Muqit MM, Johnson GJ, Milligan P, Rowley J, Faal H, Bailey RL. Operational comparison of single-dose azithromycin and topical tetracycline for trachoma. Invest Ophthalmol Vis Sci. 2000; 41(13):4074–79
43) Boyka T, Ivanka P, Alexander Z, Plamen P. Performance liquid chromatographic assay of indomethacin and its related substances in tablet dosage forms. Int J Pharm Pharm Sci. 2012;4:549-52.
44) Bradley SL and Linda TM. Postoperative care in cataract surgery. Curr Opin Ophthalmol. 2013, 24:66–73.
45) Brayfield, A, ed. (14 January 2014). "Indometacin". Martindale: The Complete Drug Reference. London, UK: Pharmaceutical Press. Retrieved 22 June 2014.
46) Brazier Y. What you need to know about cataracts. December 19, 2017. https://www.medicalnewstoday.com/articles/157510
47) Brian Wachler SB. WebMD Medical Reference. December 13, 2018. https://www.webmd.com/eye-health/eye-health-presbyopia-eyes
48) Bucolo C, Melilli B, Piazza C, Zurria M, Drago F. Ocular Pharmacokinetics Profile of Different Indomethacin Topical Formulations. J. Ocul. Pharmacol. 2011; 10(10): 1-6.
49) Cafasso J, Marie AG. Uveitis. December 20, 2017. https://www.healthline.com/health/uveitis#diagnosis
50) Carolyn S. Anatomy and Physiology of the Eye. Distance learning course. 2010-12. http://www.bsmcpss.com/resources/study-guides/OPH%2003%2014-118.pdf

51) Cataract Surgery. National Eye Institute (NEI). May 29, 2019.
52) Cataracts. National Eye Institute (NEI). August 3, 2019. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts
53) Chi-Hsien L, Kuan-Yu L, Wei-Chi W, Yu-Jui C, Wei-Shiou L, Ching-Yun H. In vitro scleral lutein distribution by cyclodextrin containing nanoemulsions. Chem Pharm Bull. 2015;63(2):59-67.
54) Chikte AA, Umekar MJ, Borkhade MN, Raut NS. Effect of transesterification of oil on nanoemulsion gel for topical drug delivery. Int J Pharm Sci Res. 2018;9:589-98.
55) Chime SA, Kenechukwu FC, Attama AA Nanoemulsions — Advances in Formulation, Characterization and Applications in Drug Delivery. Application of Nanotechnology in Drug Delivery. Ali Demir Sezer, IntechOpen. July 25, 2014.
56) Chitra B, Kim S. Dry Eye Syndrome. January 6, 2016. https://www.healthline.com/health/dry-eye-syndrome
57) Cho H, Wolf KJ, Wolf EJ. Management of ocular inflammation and pain following cataract surgery: focus on bromfenac ophthalmic solution. Clin Ophthalmol. 2009;3:199-210.
58) Christopher JB. Proptosis (Exophthalmos). Merck and the Merck Manuals. July 20, 2019. https://www.merckmanuals.com/professional/eye-disorders/symptoms-of-ophthalmologic-disorders/proptosis
59) Chun DK, Shapiro A, Abelson MB. Ocular pharmacokinetics. In: Abelson MB, ed. Principles and Practices of Ophthalmology. Canada: Elsevier, 2008.
60) Ciotu IM, Stoian I, Gaman L, Popescu MV, Atanasiu V. Biochemical changes and treatment in glaucoma. J Med Life. 2015;8(1):28-31.
61) Ciprofloxacin (Ophthalmic). Drugs.com. Last updated on Mar 12, 2020. https://www.drugs.com/ppa/ciprofloxacin-ophthalmic.html
62) Ciprofloxacin Eye Drops. Drugs.com. Sep 12, 2019. https://www.drugs.com/cdi/ciprofloxacin-eye-drops.html
63) Ciprofloxacin Ophthalmic. American Society of Health-System Pharmacists, Inc. Feb 15, 2018. https://medlineplus.gov/druginfo/meds/a605005.html

64) Claire S, Marie AG. What is keratitis?. December 18, 2017. https://www.medicalnewstoday.com/articles/320347#types

65) Clifford LS, Bruce IG, Rajesh RK. Innovations in Topical Ocular Corticosteroid Therapy for the Management of Postoperative Ocular Inflammation and Pain. Am J Manag Care. 2019; 25:S215-S226.

66) Colin EW, Diego P, Stefano F, Aires L, Klara L, Yadollah O. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function – a review. Clin Exp Ophthalmol. 2010;38: 2–11

67) Conjunctivitis. NHS inform. February 13, 2020. https://www.nhsinform.scot/illnesses-and-conditions/eyes/conjunctivitis

68) Corey W, Marie AG. What to Know About Orbital Cellulitis. December 16, 2019. https://www.healthline.com/health/orbital-cellulitis

69) Costagliola C, Roberto DO, Mario RR, Michele R, Lucia Z, Francesco P. Pharmacotherapy of intraocular pressure: part I. Parasympathomimetic, sympathomimetic and sympatholytics. Expert Opin. Pharmacother. 2009;10(16):2663-77.

70) Costagliola C, Roberto DO, Mario RR, Michele R, Lucia Z, Parmeggiani F. Pharmacotherapy of intraocular pressure: part I. Parasympathomimetic, sympathomimetic and sympatholytics. Expert Opin Pharmacother. 2009; 10(16):2663-2677.

71) Cristhian Yarce. The HLB system: a timesaving guide to emulsifier selection. Wilmington (DE): ICI Americas Inc; 1992.

72) Daull P, Lallemand F, Garrigue JS. Benefits of cetalkonium chloride cationic oil-in-water nanoemulsions for topical ophthalmic drug delivery. J Pharm Pharmacol. 2014;66:531–41.

73) Deepak S, Ahmad Aref A. Glaucoma management: relative value and place in therapy of available drug treatments. Ther Adv Chronic Dis. 2014;5(1):30-43.

74) Di Tommaso C, Behar-Cohen F, Gurny R, Moller M. Colloidal systems for the delivery of cyclosporin A to the anterior segment of the eye. In Annales Pharmaceutiques Francaises. Elsevier Masson. 2011;69(2):116-123.

75) Diabetic Retinopathy. National Eye Institute (NEI). August 3, 2019. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/diabetic-retinopathy

76) Dupeyron D, Kawakami M, Ferreira AM, Cáceres-Vélez PR, Rieumont J, Azevedo RB, Carvalho JC. Design of indomethacin-loaded nanoparticles: effect of polymer matrix and surfactant. Int J Nanomedicine. 2013;8: 3467-77.

77) Edelhauser HF, Rowe-Rendleman CL, Robinson MR, Dawson DG, Chader GJ, Grossniklaus HE, Rittenhouse KD, Wilson CG, Weber DA, Kuppermann BD, Csaky KG. Ophthalmic drug delivery systems for the treatment of retinal diseases: basic research to clinical applications. Investigative ophthalmology & visual science. 2010;51(11):5403-20.

78) Edward SP, Davson H. Human eye. Encyclopædia Britannica, inc. June 22, 2018. https://www.britannica.com/science/human-eye

79) Elder G. Macular Degeneration Treatments. Education/vision/eye-diseases. September 6, 2017. https://www.docshop.com/education/vision/eye-diseases/macular-degeneration/treatment

80) Elsayed I, Sayed S. Tailored nanostructured platforms for boosting transcorneal permeation: box–behnken statistical optimization, comprehensive *in vitro*, *ex vivo* and *in vivo* characterization. Int J Nanomed. 2007; 12: 7947–62.

81) Eric D. Current use of Non-steroidal Anti-inflammatory drugs in the treatment of ocular inflammation related to cataract surgery. European Ophthalmic Review. 2012: 173-177.

82) Erickson AG. Sampson SR. Retinal Detachment. March 17, 2017 https://www.healthline.com/health/retinal-detachment

83) Farkouh A, Peter F, Martin C. Systemic side effects of eye drops: a pharmacokinetic perspective. Clin Ophthalmol. 2016:10 2433–41.

84) Feynman RP. There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics. Published in Engineering and Science magazine. 1960; 23(5):1-13.

85) Fingeret M, Medeiros FA, Susanna R, Weinreb RN. Five rules to evaluate the optic disc and retinal nerve fiber layer for glaucoma. Optometry. 2005; 76(11):661-68.

86) Florence AT, Attwood D. Physicochemical properties of drugs in solution: physicochemical principles of pharmacy. 4th ed. Pharmaceutical Press. 2006: 69-73.

87) Floury J, Desrumaux A, Lardieres J. Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. Innovative Food Sci Emerging Technol. 2000;1(2):127-34.

88) Francis CA, Zheng Y, Xu Y, Yao L, Back A, Hong Y, Chen C, inventors; Cooper Vision International Holding Co LP, assignee. Dimensionally stable silicone hydrogel contact lenses. United States patent US 8,445,614. May 21, 2013.

89) Gan L, Wang J, Jiang M, Bartlett H, Ouyang D, Eperjesi F, Liu J, Gan Y. Recent advances in topical ophthalmic drug delivery with lipid-based nanoemulsions. Drug discovery today. 2013; 18(5-6):290-97.

90) Garbacz P, Wesolowski M. DSC, FTIR and Raman Spectroscopy Coupled with Multivariate Analysis in a Study of Co-Crystals of Pharmaceutical Interest. Molecules. 2018;23(9):2136.

91) Garcia-Saenz MC, rias-Puente A, Rodriguez-Caravaca G, Banuelos JB. Effectiveness of intracameral cefuroxime in preventing endophthalmitis after cataract surgery Ten-year comparative study. J Cataract Refract Surg. 2010;36(2):203–07.

92) Garg P, Sharma S, Rao GN. CIP-resistant Pseudomonas keratitis. Ophthalmology. 1999;106(7):1319-23.

93) Georgeta C, Vicențiu V, Ioana O, Ana MM, Dan FA, Cornelia I, et al. Development and evaluation of new micro emulsion-based hydrogel formulations for topical delivery of fluconazole. AAPS Pharm Sci Tech. 2015;16:889-904.

94) Glaucoma: A review. JAMA. 2014;311(18):1901–1911.

95) Gooch N, Molokhia SA, Condie R, Burr RM, Archer B, Ambati BK, Wirostko B. Ocular drug delivery for glaucoma management. Pharmaceutics. 2012;4(1):197-211.

96) Goskonda VR, Khan MA, Hutak CM, Reddy IK. Permeability characteristics of novel mydriatic agents using an *in vitro* cell culture model that utilizes SIRC rabbit corneal cells. Journal of pharmaceutical sciences. 1999; 88(2):180-4.

97) Gregersen PR, Henderson R. Conjunctivitis: how to treat eye inflammation: The causes, symptoms and treatment of inflammation of the conjunctiva. October 31, 2019.

https://www.netdoctor.co.uk/conditions/eyes/a5325/conjunctivitis-inflammation-of-the-eye/

98) Gretchyn B, Gary H. Color blindness: Types of Color Blindness. April 20, 2019. https://www.allaboutvision.com/conditions/colordeficiency.htm

99) Gretchyn B, Thompson V. Presbyopia: What causes it and how to treat it. April 2019. https://www.allaboutvision.com/conditions/presbyopia.htm

100) Grieshaber MC, Flammer J. Is the medication used to achieve the target intraocular pressure in glaucoma therapy of relevance?- an exemplary analysis on the basis of two beta-blockers. Prog Retin Eye Res. 2010;29(1):79-93.

101) Gupta SK, Niranjan DG, Agrawal S.S., Srivastava S, Saxena R. Recent advances in pharmacotherapy of glaucoma. Indian J Pharmacol. 2008; 40(5):197–208.

102) Gurpreet K, Singh SK. Review of Nanoemulsion formulation and characterization techniques. Indian J Pharm Sci. 2018;80(5):781-789.

103) Halim Mohamed MA, Mahmoud AA. Formulation of indomethacin eye drops via complexation with cyclodextrins. Curr Eye Res. 2011;36(3):208-16.

104) Hao J, Fang X, Zhou Y, Wang J, Guo F, Li F, Peng X. Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box-Behnken design. International journal of nanomedicine. 2011;6:683.

105) Harika K, Debnath S, Babu MN. Formulation and evaluation of nanoemulsion of amphotericin B. Int J Novel Trends Pharm Sci. 2015;5:114-22.

106) Haripriya A, Baam ZR, Ravindran RD, Postoperative cataract care: the Aravind perspective. Community EyeHealth. 2016;29(94):25-26.

107) Harwansh RK, Patra KC, Pareta SK, Singh J, Rahman MA. Nanoemulsions as vehicles for transdermal delivery of glycyrrhizin. Braz J Pharm Sci 2011;47:769-78.

108) Hassib ST, Elkady EF, Sayed RM. Simultaneous determination of timolol maleate in combination with some other anti-glaucoma drugs in rabbit aqueous humor by high performance liquid chromatography-tandem mass spectroscopy. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1022:109□17.

109) Hegde RR, Verma A, Ghosh A. Microemulsion: New Insights into the ocular drug delivery. ISRN Pharm. 2013;2013:1-11.

110) Hippalgaonkar K, Majumdar S, Kansara V. Injectable lipid emulsions-advancements, opportunities and challenges. AAPS PharmSciTech. 2010;11(4):1526-1540.

111) Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2). Trop J Pharm Res. 2013;12(2):265-73.

112) Horn G, inventor; GNT LLC, assignee. Ophthalmic lipophilic drug delivery vehicle formulations. United States patent, US 8,999,938. Apr 7, 2015.

113) Huang D, ShanChen Y, Rupentha DI. Overcoming ocular drug delivery barriers through the use of physical forces, Adv. Drug Deliv. Rev. 2017; 139:1-157.

114) Human Physiology/Print Version. WIKIBOOKS. *The Free Textbook Project* April 6, 2020). https://en.wikibooks.org/wiki/Human_Physiology/Print_Version

115) Hussein NF, Randa Tag A, Abdelmagid DH, Basant AH. Sustained ocular delivery of Dorzolamide- HCl via proniosomal gel formulation: in-vitro characterization, statistical optimization, and *in vivo* pharmacodynamic evaluation in rabbits. Drug Delivery. 2018;25(1): 1340–49.

116) Indocin- indomethacin suspension. Iroko Pharmaceuticals, LLC. May 28, 2018. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=06de3c9d-63bf-47c1-f78c-4fbdd482928a

117) Indom-Indometacina 0.5% eye drops, suspension. Alfa Intes Industry Terapeutic S.R.L. June, 2007. https://edoc.com/indom

118) Jackson W. Blepharitis: current strategies for diagnosis and management. Can J Ophthalmol. 2008; 43(2):170-79.

119) Jaiswal M, Dudhe R, Sharma PK. nanoemulsion: an advanced mode of drug delivery system. 3Biotech. 2015;5(2):123–27.

120) Jaiswal P, Aggarwal G, Harikumar SL, Singh K. Development of self-microemulsifying drug delivery system and solid-self-microemulsifying drug delivery system of telmisartan. Int J Pharm Investig. 2014;4(4):195-206.

121) Januleviciene I, Lina S, Barsauskaite R. Ophthalmic Drug Delivery in Glaucoma—A Review. Pharmaceutics. 2012;4:243-51.

122) Januleviciene I, Siaudvytyte L, Barsauskaite R. Ophthalmic drug delivery in glaucoma-a review. Pharmaceutics. 2012;4(1):243-51.

123) Jigar S, Anroop BN, Shery J, Rakesh KP, Hiral S, Tamer MS, Mohamed AM. Nanoemulsion based vehicle for effective ocular delivery of moxifloxacin using experimental design and pharmacokinetic study in rabbits. Pharmaceutics. 2019;11(230):1-16.

124) Jing X, Deng L, Gao B, Xiao L, Zhang Y, Ke X, Lian J, Zhao Q, Ma L, Yao J, Chen J. A novel polyethylene glycol mediated lipid as drug delivery carrier for paclitaxel. Nanomedicine: Nanotechnology, Biology and Medicine. 2014;10(2):371-80.

125) Kalam MA, Alshamsan A, Aljuffali IA, Mishra AK, Sultana Y. Delivery of gatifloxacin using microemulsion as vehicle: formulation, evaluation, transcorneal permeation and aqueous humor drug determination. Drug delivery. 2016;23(3):886-97.

126) Kalpesh CA, Ketan VS. The Study of Chloramphenicol for Ophthalmic Formulation. Int J Sci Res Rev. 2018;7(1): 173-181.

127) Kamal Singh R, Rajesh Kumar N, Sidhraj Singh S. Timolol maleate a gold standard drug in glaucoma used as ocular films and inserts: an overview. Int J Pharm Sci Rev Res. 2010;3(1)23-29.

128) Kanke PK, Pathan IB, Jadhav A, UsmanMRM. Selection of oil and surfactants- Formulation and evaluation of febuxostat nanoemulsion for transdermal drug delivery. J Pharm BioSci 2019;7(1):1-7.

129) Kanoujia J, Kushwaha PS, Saraf SA. Evaluation of gatifloxacin pluronic micelles and development of its formulation for ocular delivery. Drug delivery and transl res. 2014;4(4):334-43.

130) Kassem MA, Rahman AA, Ghorab MM, Ahmed MB, Khalil RM. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. Int J pharm. 2007;340(1-2):126-33.

131) Kentish S, Wooster T, Asho Kumar M., Simons L. The use of Ultrasonics for the nanoemulsion preparation. Innovative Food Sci. Emerging Technol. 2010;9(2):170-75.

132) Khalid N, Gaofeng S, Brenden HJ, Kobayashid I, Mitsutoshi N, Colin Barrowa J. Formulation and characterization of O/W nanoemulsions encapsulating high concentration of astaxanthin. Food Res Int 2017;102:364–71.

133) Khaw PT, Shah P, Elkington AR. Glaucoma: Diagnosis. BMJ. 2004; 328(7431): 97-99.

134) Khurana S, Bedi PM, Jain NK. Preparation and evaluation of solid lipid nanoparticles based nanogel for dermal delivery of meloxicam. Chem phys lipids. 2013;175:65-72.

135) Kim SJ, Sachoenberger SD, Jennifer T E, Justis EP, Steven Y, Sophie B J. Topical nonsteroidal anti-inflammatory drugs and cataract surgery. Ophthalmology. 2015;122(11):2159-68.

136) Kim TI, Alio Del Barrio JL, Wilkins M, Cochener B, Ang M. Refractive surgery. Lancet. 2019;393(10185):2085-98.

137) Kimberly H, George K. Glaucoma. January 29, 2016. https://www.healthline.com/health/glaucoma

138) Kivi R, Elizabeth B, Deborah W. Astigmatism. April 12, 2017. https://www.healthline.com/health/astigmatism

139) Koteswari P, Rama Krishna S, Prabhakar Reddy V, Lakshmi MN. Formulation and preparation of felodipine nanoemulsions. Asian J Pharm Clin Res. 2011;4(1):116-17.

140) Kozarsky A. Glaucoma. WebMD Medical Reference. November 01, 2019 https://www.webmd.com/eye-health/glaucoma-eyes#1

141) Krishna MB, Lorenzetti OJ. Development of Ophthalmic Formulations. Pharmaceutical dosage forms: parenteral medications. Vol. 2. 2nd ed. Revised and expanded. Marcel Dekker, INC. New York. 1993.

142) Kristina I, David LG. Anatomy of Eyes. Encyclopedia of Biometrics. January, 2009.

143) Kumar H, Kumar V. Ultrasonication assisted the formation and stability of water-in-oil nanoemulsions: optimization and ternary diagram analysis. Ultrason Sonochem. 2018;49:79-88.

144) Kumar S, Ali J, Baboota S. Design expert® supported optimization and predictive analysis of selegiline nanoemulsion via the olfactory region with enhanced behavioral performance in Parkinsons disease. Nanotechnology. 2016; 27(43):43-51.

145) Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf, B. 2010; 75(1):1-8.

146) Kuncahyo I, Choiri S, Fudholi A, Martien R, Rohman A. Assessment of Fractional Factorial Design for the Selection and Screening of Appropriate Components of a Self-nanoemulsifying Drug Delivery System Formulation. Adv Pharm Bull. 2019;9(4):609–618.
147) Kyari F, Abdul MM. The basics of good postoperative care after glaucoma surgery. Community Eye Health. 2016;29(94):29-31.
148) Lallemand F, Daull P, Simon B, Ronald B, Garrigue JS. Successfully improving ocular drug delivery using the cationic nanoemulsion, novasorb. J Drug Delivery 2012;1-16.
149) Lambert L. Diagnosing a red eye: an allergy or an infection?. S Afr Pharm J 2017;84(1):24-30.
150) Lauralee S. Human Physiology. Cells to systems. January 16, 2018. https://issuu.com/jgbo/docs/sherwood-ilovepdf-compressed__2_-1-
151) Laxman M. Electron microscopy as a tool in research and disease diagnosis. Lead paper compendium published and presented in IAVP annual conference XXXI, held at Anand, Gujarat, India. Nov 13-15, 2014;1:39-160.
152) Lekhanont K, Manachai N, Nutthida N, Pitak S. Treatment With Intrastromal and Intracameral Voriconazole in 2 Eyes With Lasiodiplodia theobromae Keratitis. Case Rep Med. 2015;94(6):1-5.
153) Lewis RA. Canaloplasty: A Surgical Alternative for Glaucoma. Glaucoma Research Foundation website. October 29, 2019. https://www.glaucoma.org/treatment/canaloplasty-a-new-surgical-alternative.php
154) Li G, Jing W, Ming J, Hanah B, Defang O, Frank E, Jianping L, Yong G. Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. Drug Discovery. 2013; 18(5/6):290-97.
155) Li GP, Jiang M, Ouyang D, Frank E, Wang J, Liu J, Yong G. Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. Drug Disc. Today. 2012;18(5):1010-1016.
156) Li X, Muller RH, Keck CM, Bou-Chacra NA. Mucoadhesive dexamethasone acetate-polymyxin B sulfate cationic ocular nanoemulsion – novel combinatorial formulation concept. Pharmazie. 2016;71:327–33.
157) Lin HR, Sung KC. Carbopol/pluronic phase change solutions for ophthalmic drug delivery. J Controlled Release. 2000;69(3):379-88.

158) Lina Formica M, Adrian Calles J, Daniel Palma S. Polysaccharide-based nanoemulsions for ocular drug delivery. Current pharm des. 2015; 21(33):4851-68.
159) Lou J, Hu W, Tian R, Zhang H, Jia Y, Zhang J, et al. Optimization and evaluation of a thermoresponsive ophthalmic in situ gel containing curcumin-loaded albumin nanoparticles. Int J Nanomed 2014;9:2517-25.
160) Lovelyn C, Anthony AA.Current State of Nanoemulsions in Drug Delivery. J Biomater Nanobiotechnol. 2011;2:626-39.
161) Lucia P, Diego RP, Giulia B, Marco C, Giovanni FP. An overview of micro- and nanoemulsions as vehicles for essential oils: formulation, preparation and stability. Nanomaterials. 2020;10:2-24.
162) Macular Edema. National Eye Institute (NEI). July 8, 2019. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/macular-edema
163) Macular Edema. NaturalEyeCare,Inc. 2020. https://www.naturaleyecare.com/eye-conditions/macular-edema/
164) Mahboobiana MM, Ali S, Ilva DR, Reza A, Seyed MF. Formulation Development and Evaluation of the Therapeutic Efficacy ofBrinzolamide Containing Nanoemulsions. Iranian Journal of Pharmaceutical Research. 2017;16(3):847-57.
165) Mainardes RM, Urban MC, Cinto PO, Khalil NM, Chaud MV, Evangelista RC, Daflon Gremiao MP. Colloidal carriers for ophthalmic drug delivery. Curr drug targets. 2005;6(3):363-71.
166) Majumdar S, Hingorani T, Srirangam R. Evaluation of active and passive transport processes in corneas extracted from preserved rabbit eyes. J pharm sci. 2010; 99(4):1921-30.
167) Makwanan SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Results Pharma Sci. 2016;6:1-6.
168) Mansi K. Cataract Surgery – pre and post care. July 6, 2017. https://www.thehealthsite.com/diseases-conditions/cataract-surgery-pre-and-post-care-f0717-503791/

169) Maria SM, Maria ACP, Vicent R, Cristina BF, Alicia LC. *Ex vivo* rabbit cornea diffusion studies with a soluble insert of moxifloxacin. Drug Delivery Transl Res. 2018;8:132–39.

170) Markus MG, Ann Marie Griff, January 5, 2018. https://www.medicalnewstoday.com/articles/185155

171) Marongiu ML, Gulinati A, Floris B. A procedure for rabbit blood serial collection. Ital J Anim Sci. 2007; 6 (1):773-79.

172) Mary L, Colin TL. Sight Hypermetropia. The Information Standard. November2, 2016. https://patient.info/eye-care/long-sight-hypermetropia#

173) Mary L, Helen H. Conjuctivitis. Patient website. February 24, 2017. https://patient.info/doctor/conjunctivitis

174) Mary L, Helen H. Infective Conjunctivitis. The Information Standard. February 24, 2017. https://patient.info/eye-care/eye-problems/infective-conjunctivitis

175) Mary L, John C. Blepharitis and how to treat it. May 2, 2018. The Information Standard. https://patient.info/eye-care/swollen-eyelid/blepharitis

176) Mayo Clinic. Cataracts. June 23, 2018. https://www.mayoclinic.org/diseases-conditions/cataracts/symptoms-causes/syc-20353790

177) McBean B, Marie AG. What Is Endophthalmitis?. September 22, 2017. https://www.healthline.com/health/endophthalmitis#causes

178) Michael D. What Is Diabetic Retinopathy?. WebMD Medical Reference. June 22, 2019. https://www.webmd.com/diabetes/diabetic-retinopathy

179) Michael SO. A Patient's Guide to Conjunctivitis (Pink Eye): Here's what you need to know about this common, irritating eye infection. January 16, 2020. https://health.usnews.com/conditions/eye-disease/conjunctivitis

180) Mincione F, Scozzafava A, Supuran CT. The development of topically acting carbonic anhydrase inhibitors as antiglaucoma agents. Curr Pharm Des. 2008;14(7):649-54.

181) Morsi NM, Mohamed MI, Refai H, Elsorogy HM. Nanoemulsion as a novel ophthalmic delivery system for acetazolamide. Int J Pharm Pharm Sci. 2014; 6(11): 227-36.

182) Moshfeghi AA. Rate of endophthalmitis after anti-vegf intravitreal injection. Retina Today. 2008:75–76.

183) Mowafaq GM, Abulfadhel NJ. Formulation and characterization of nimodipine nanoemulsion as ampoule for oral route. Int J Pharm Sci Res. 2017;8:591-602.

184) Muller GG, Newton KJ, Silvestre de Castro R. Antifungals in eye infections: drugs and routes of administration. Rev Bras Oftalmol. 2013; 72 (2): 132-41.

185) Munish A, Avinash SD, Surendra SK, Dipak MK. Topical Ocular Delivery of NSAIDs. AAPS J. 2008;10(2):229-41.

186) Musa SH, Basri M, Fard Masoumi HR, Shamsudin N, Salim N. Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: *in vitro* diffusion and *in vivo* hydrating action. Int J Nanomed. 2017;12:2427-41.

187) Muza MR, Taha UW, Neeraj M, Hasham SS, Roqia A, Faheem SA. Development and characterization of drug-loaded selfsolidnano- emulsified drug delivery system for treatment of diabetes. Mater Sci Res India. 2018;15:1-11.

188) Nada WM, Aswad MA, El-Haig WM. Combined intrastromal injection of amphotericin B and topical fluconazole in the treatment of resistant cases of keratomycosis: a retrospective study. Clin Ophthalmol. 2017;11:871-74.

189) Nagarani B., A.Sravanthi, M.Santosh Kumar, R.Srikanth. Design & characterization of timolol maleate Osmotic drug delivery system. Int J Pharm Anal Rese. 2016;5(3):504-17.

190) Nagarwal RC, Kumar R, Pandit JK. Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: *in vitro* characterization and *in vivo* study in rabbit eye. Eur J Pharm Sci. 2012;47(4):678-85.

191) Nanjawade BK, Manvi FV, Manjappa AS. In situ-forming hydrogels for sustained ophthalmic drug delivery. J Control Release. 2007;122(2):119-34.

192) Nauman K, Gaofeng S, Brenden JH, Isao K, Mitsutoshi N, Colin JB. Formulation and characterization of o/w nanoemulsions encapsulating a high concentration of astaxanthin. Food Research International. 2017;102:364-71.

193) Navneet Sharma, Parshotam Madan, Senshang Lin. Effect of process and formulation variables on the preparation of parenteral paclitaxel-loaded biodegradable polymeric nanoparticles: A co-surfactant study. Asian J Pharm Sci. 201611:404-16.

194) Neeraj M, Gurpreet K. Investigations on polymeric nanoparticles for ocular delivery. Adv Polym Technol. 2019:1-14.

195) Nirav P, Nakrani H, Mihir R, Navin S. Development of loteprednol etabonate-loaded cationic nano emulsified in-situ ophthalmic gel for sustained delivery and enhanced ocular bioavailability. Drug Delivery. 2016; 23(9):3712-23.

196) Pachigolla G, Blomquist P, Cavanagh HD. Microbial keratitis pathogens and antibiotic susceptibilities: A 5-year review of cases at an urban county hospital in north Texas. Eye Contact Lens. 2007;33(1):45–49.

197) Panchal SS, Patidar RK, Jha AB, Allam AA, Ajarem J, Butani SB. Anti-inflammatory and antioxidative stress effects of oryzanol in glaucomatous rabbits. J Ophthalmol. 2017;2017: 1-13.

198) Parasuram RR, Guruprasad S. Nanoemulsion based emulgel formulation of lipophilic drug for topical delivery. Int J Pharm Tech Res. 2016;9:210-23.

199) Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother.2010; 1(2): 87-93.

200) Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010;1:87-93.

201) Parikh RS, Parikh SR, Navin S, Arun E, Thomas R, Practical approach to medical management of glaucoma, Indian J Ophthalmol. 2008;56(3):223-30.

202) Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: an overview. World J Pharmacol 2013;2:47-64.

203) Patel HC, Ghanshyam P, Seth AK, Patel JD, Patel SR. Formulation and evaluation of o/w nanoemulsion of Ketoconazole. Pharma science monitor. Int J pharm sci. 2013;4(4/):1-15.

204) Patel N, Nakrani H, Raval M, Sheth N. Development of loteprednol etabonate-loaded cationic nano emulsified in-situ ophthalmic gel for sustained delivery and enhanced ocular bioavailability. Drug delivery. 2016; 23(9):3712-23.

205) Patel P, Shastri D, Shelat P, Shukla A. Ophthalmic drug delivery system: challenges and approaches. Systematic Reviews in Pharmacy. 2010;1(2):113.

206) Patel RA, Joshi JR. An overview on nanoemulsion: a novel approach. Int J pharm Sci Res. 2012;3(12):4640-50.

207) Patel S. Development and Evaluation of a Nanomicellar Eye Drop Formulation of Dexamethasone for Posterior Uveitis (Doctoral dissertation, University of Toledo). 2014.

208) Patil PA, Bhutkar BR. Biomedical Application of Nanoemulsion- A Features Review. Int J Res Methodol. 2010;1(3):37-58.

209) Patricia SB, Chunha JP. Sty (Definition, Causes, Pictures, and Treatment). December 04, 2017. https://www.medicinenet.com/sty_stye/article.htm

210) Pavia DL, Lampman GM, Kriz GS, Vyvyan JA. Introduction to Spectroscopy. 4th ed. Cengage Learning, USA. 2008.

211) Peng CC, Bengani LC, Jung HJ, Leclerc J, Gupta C, Chauhan A. Emulsions and microemulsions for ocular drug delivery. J Drug Del Sci Tech. 2011;21(1):111-121.

212) Peter K. The emerging roles of topical non-steroidal anti-inflammatory agents in ophthalmology. British J Ophthalmol. 1996: 80: 480-85.

213) Peter WJM, Natalia NP, Sukhmanpreet C, Lgiz AS, Charlene L, Irina IS, *et al.* Crown ethers: novel permeability enhancers for ocular drug delivery. Mol Pharm. 2017;14;3528-38.

214) Pons R, Carrera I, Erra P, Kunieda K, Solans C. Novel preparation methods for highly concentrated water-in-oil emulsions. Colloids and Surfaces.1994; 91(3):259-266.

215) Pranita S, Amrita B. Nanoemulsions-a review. Int J Res Pharm Chem. 2016;6:312-22.

216) Pratap SB, Brajesh K, Jain SK, Shafaat K. Development and characterization of a nanoemulsion gel formulation for transdermal delivery of carvedilol. Int J Drug Dev Res 2012;4:151-61.

217) Priya S, Marina K, Suchetha Kumari N. Nanoemulsion components screening of quetiapine fumarate: effect of surfactant and co-surfactant. Asian J Pharm Clin Res. 2015;8(6):136-40.

218) Quanying B, Bryan N, Yan W, Stephanie C, Diane JB. *In vitro* and *ex vivo* correlation of drug release from ophthalmic ointments. J Controlled Release. 2018; 276:93–101.

219) Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006;90(3):262-67.

220) Quigley HA. Number of people with glaucoma worldwide. Br J Ophthalmol. 1996; 80:389-93.

221) Radomska-Soukharev AN, Wojciechowska JO. Microemulsions as potential ocular drug delivery systems: phase diagrams and physical properties depending on ingredients. Acta Pol Pharm. 2005;62(6):465-71.

222) Rahul T, Vikas P, Saket A, Vandana S, Dharmendra J. Therapeutic challenges in ocular delivery of lipid based emulsion. Egy J Bas App Sci. 2018; 5:121–29.

223) Reddy PD, Swarnalatha D.Recent advances in Novel Drug Delivery Systems. Indian J Phys Ther Res. 2010;2(3):2025-27.

224) Rajendra SK, Gajanan J, Miller O, Uday BK. Ocular pharmacokinetics of dorzolamide and brinzolamide after single and multiple topical dosing: implications for effects on ocular blood flow. Drug Metab Dispos. 2011;39 (9):1529-37.

225) Rajesh G, Ramarao A. Gastroretentive Floating-Bioadhesive Drug Delivery System for Rebamipide: Design, *in vitro* and *in vivo* evaluation. Int J Pharm Sci Nanotechnol. 2019:4534-43.

226) Rathore KS, Nema RK, Sisodia SS. Timolol maleate a gold standard drug in glaucoma used as ocular films and inserts: an overview. Int J Pharm Sci Rev Res. 2010;3(1):23-29.

227) Reddy BG, Reddy NG, Dhanalakshmi K. Comparison of UPLC with UFLC: Liquid Chromatography Int. J. Pharm. Sci. Rev. Res. 2015;31(1):97-101.

228) Richard A. Dry eye syndrome: Symptoms and causes. June, 2019. https://www.allaboutvision.com/conditions/dryeye-syndrome.htm

229) Rinda Devi B, Pallabitha C, Zahraa HF, Al-Saedi, Pradeep KK, Sai HSB. Ocular Drug Delivery Barriers-Role of Nanocarriers in the Treatment of Anterior Segment Ocular Diseases. Pharmaceutics. 2018;10(28):1-31.

230) Ripal G, Hari Krishna A, Ashwin P, Ashim M. Ocular Drug Delivery. The AAPS Journal. 2010; 12(3):348-59.

231) Ripal Gaudana,1 Hari Krishna Ananthula,1 Ashwin Parenky,1 and Ashim K. Mitra1,2. Ocular Drug Delivery. AAPS J. 2010;12(3):348-60.

232) Rita G. Ciprofloxacin (Ciproxin): an antibiotic for bacterial infections. May 08, 2019. https://www.netdoctor.co.uk/medicines/infection/a28009/ciprofloxacin-uses-actions/

233) Robertson SM, Curtis MA, Schlech BA, et al. Ocular pharmacokinetics of moxifloxacin after topical treatment of animals and humans. Surv Ophthalmol. 2005;50(1):32-45.

234) Ronald LF, David GG, Smith OU, Davinder SG, Michelle RB, Matthew EE. Eye Surgery Postoperative Instructions. Glaucoma Associates of Texas website. April 05, 2017. http://glaucomaassociates.com/patient-information/eye-surgery-postoperative-instructions/

235) Rutar T, Zwick OM, Cockerham KP, Horton JC. Bilateral blindness from orbital cellulitis caused by community-acquired methicillin-resistant Staphylococcus aureus. Am J Ophthalmol. 2005;140(4):740-42

236) Sacca SC, Izzotti A. Oxidative stress and glaucoma: injury in the anterior segment of the eye. Prog Brain Res. 2008;173:385-407.

237) Sagar KS, Pravin C, Patil H, Patil P. Formulation and evaluation of gastroretentive ciprofloxacin hcl effervescent tablet. Int J Res Pharm Nano Sci. 2016;5(1):1-8.

238) Sahil A, Brian Ta. Ocular side-effects of corticosteroids. Moran Eye Center. July, 2018. http://morancore.utah.edu/medical-student-education-outline/ocular-side-effects-of-corticosteroids/

239) Saieede S, Parvin ZM, Mohammad BJ, Mitra J. Comparison of Different Nanosuspensions as Potential Ophthalmic Delivery Systems for Ketotifen Fumarate. Adv Pharm Bull. 2016;6(3): 345-52.

240) Sanjay B, Dinesh S, Neha S. Stability Testing of Pharmaceutical Products. J App Pharm Sci. 2012;2(03):129-38.

241) Santram L, Hemant TN, Mahesh Pralhad P, Sunil SP, Gautam PV. Nanoemulsion: A brief review on development and application in Parenteral Drug Delivery. Adv Pharm J. 2018;3(2):43-54.

242) Sarah B. Laser eye surgery costs. August 21, 2019. https://www.finder.com.au/laser-eye-surgery-costs

243) Sayed S, Ibrahim E. Tailored nanostructured platforms for boosting transcorneal permeation: box–behnken statistical optimization, comprehensive *in vitro*, *ex vivo* and *in vivo* characterization. Int J Nanomed 2017;12:7947–62.

244) Sehi M, Guaqueta DC, Feuer WJ, Greenfield DS. Advanced imaging in glaucoma study group, scanning laser polarimetry with variable and enhanced

corneal compensation in normal and glaucomatous eyes. Am J Ophthalmol. 2007;143(2):272-79.

245) Setya S, Negi P, Razdan BK, Talegaonkar S. Design, development and *in vitro* investigation of water in oil nanoemulsion for transdermal delivery. World J Pharm PharmSci. 2014;3:1495-512.

246) Seyfoddin A, Al-Kassas R. Development of solid lipid nanoparticles and nanostructured lipid carriers for improving ocular delivery of acyclovir. Drug Dev Ind Pharm. 2013;39(4):508-19.

247) Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A, Khar RK, Ali M. Formulation development and optimization using nanoemulsion technique: a technical note. AAPS Pharm Sci Tech. 2007; 8(2): E12-7.

248) Shah J, Nair AB, Jacob S, Patel RK, Shah H, Shehata TM, et al. Nanoemulsion based vehicle for effective ocular delivery of moxifloxacin using experimental design and pharmacokinetic study in rabbits. Pharmaceutics. 2019;11:230-46.

249) Sharma S, Diagnosis of infectious diseases of the eye, Eye (Lond). 2012 Feb; 26(2):177-84

250) Sheetu W, Rishi P, Shivani RP, Vyas SP. Nanocarriers in ocular drug delivery: an update review. Curr Pharm Des. 2009; 15: 2724-2750.

251) Shell JW. Ophthalmic drug delivery systems. Drug Dev Res. 1985; 6(3):245-61.

252) Shen Y, Ling X, Jiang W, Du S, Lu Y, Tu J. Formulation and evaluation of Cyclosporin A emulgel for ocular delivery. Drug delivery. 2015;22(7):911-17.

253) Shirasaki Y. Molecular design for enhancement of ocular penetration. J Pharm Sci. 2008;97:2462-96.

254) Shital PS, Rajesh PK, Abhishek JB, Ahmed AA, Jamaan A, Shital BB. Anti-inflammatory and antioxidative stress effects of oryzanol in glaucomatous rabbits. J Ophthalmol 2017;2017:1-9.

255) Shulin D. Recent developments in ophthalmic drug delivery. Pharm Sci Technol Today. 1998;1:325-38.

256) Siafaka PI, Titopoulou A, Koukaras EN, Kostoglou M, Koutris E, Karavas E, Bikiaris DN. Chitosan derivatives as effective nanoemulsions for ocular release of timolol drug. Int J Pharm. 2015;495(1):249-64.

257) Silva AC, Amaral MH, González-Mira E, Santos D, Ferreira D. Solid lipid nanoparticles (SLN)- based hydrogels as potential carriers for oral transmucosal delivery of Risperidone: Preparation and characterization studies. Colloids Surf, B. 2012;93:241-48.

258) Simon B, Susanne B. Surgery for Proliferative Diabetic Retinopathy. Clinical Gate. Chapter 111. March 03, 2015:1-26.

259) Slabaugh M, Salim S. Use of Anti-VEGF agents in glaucoma surgery. J Ophthalmol. 2017;2017:16452-69.

260) Smith A, Marie AG. What to know about myopia. March 26, 2020 https://www.medicalnewstoday.com/articles/myopia

261) Sneha P, Marina K, Suchetha Kumari N. Nanoemulsion components screening of quetiapine fumarate: effect of surfactant and co-surfactant. Asian J Pharm Clin Res. 2015;8:136-40.

262) Soneta, B. Development and Characterization of Fluocinolone Acetonide Loaded Nanosized Lipid Carriers for Ocular Delivery via Subconjunctival Route: Synergistic Effect of Vitamin a Palmitate. Dissertation, University of Mississippi. 2018.

263) Sonia HT, Ehab EF, Rawda SM. Simultaneous determination of timolol maleate in combination with some other anti-glaucoma drugs in rabbit aqueous humor by high-performance liquid chromatography-tandem mass spectroscopy. J Chromatogr B. 2016;1022:109–17.

264) Sophie JB, Audina B, Antonio C, Netan C, Thomas C, Pravin UD et al. Macular Edema. Retinal Health Series. The Foundation of the American Society of Retina Specialists (ASRS). 2016. https://www.asrs.org/patients/retinal-diseases/20/macular-edema

265) Suico J. How Can We Check the Purity of a Substance?. sciencing.com. April 4, 2020. https://sciencing.com/can-check-purity-substance-5950683.html

266) Sunil T, Roshan I, Badri PN. Formulation and *in vitro-in vivo* correlation of timolol maleate ocular insert. Indonesian Journal of clinical pharmacy. 2015; 4(4): 281-88.

267) Susan DS. A review of in vitro drug release test methods for nano-sized dosage forms. Advances in Pharmaceutics. 2014;2014:1-12.

268) Susanna PT, Kai K, Merja K, Sinikka P, Hannu K. A prospective study on postoperative pain after cataract surgery. Clin Ophthalmol 2013;7:1429–35.

269) Susanna PT, Kai K, Merja K, Sinikka P, Hannu K. A prospective study on postoperative pain after cataract surgery. Clin Ophthalmol. 2013;7:1429-35.
270) Swati J, Nirupama S, Bhanudas SK. Optimization of Thermoreversible In Situ Nasal Gel of Timolol Maleate. Scientifica. 2016:2016;1-11.
271) Syafinaz Z, Mahiran B, Emilia AM. Formulation optimization of a palm-based nanoemulsion system containing levodopa. Int J Mol Sci 2012;13:13049-64.
272) Syed, Kok KP. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. Acta Poloniae Pharmaceutica: Drug Rese. 2014;71(2):301-09.
273) Talegaonkar S, Tariq M, Alabood RM. Formulation of nanoemulsion for the transdermal delivery of ondansetron. Bull Pharm Res. 2011;1(3):18-30.
274) Tangri P, Khurana S. Basics of ocular drug delivery systems. Int J Res Pharm Biomed Sci. 2011;2(4):1541-52.
275) The Editors of Encyclopaedia Britannica. Night blindness. Encyclopædia Britannica. Encyclopædia Britannica, inc. January 16, 2018. https://www.britannica.com/science/night-blindness
276) Thiel B, Sarau A, Ng D. Efficacy of Topical Analgesics in Pain Control for Corneal Abrasions: A Systematic Review. Cureus. 2017;9(3):e1121.
277) Thompson V. Cataract surgery: Everything you need to know. J Cataract Refractive Surg. February 2015. https://www.allaboutvision.com/conditions/cataract-surgery.htm
278) Tian L. Ternary polyelectrolyte/protein complex for tumor pH targeting and intracellular delivery (Doctoral dissertation, The University of Utah).2014.
279) Tiihonen PS, Kaarniranta K, Kokki H. Postoperative pain after cataract surgery. J Cataract Refract Surg. 2013;39(5):789-98.
280) Tiihonen PT, Kaarniranta K, Merja K, Sinikka P, Hannu K. A prospective study on postoperative pain after cataract surgery. Clin Ophthalmol 2013; 7:1429–35.
281) Tim J, Marie AG. 8 Common Eye Infections and How to Treat Them. October 23, 2018. https://www.healthline.com/health/infected-eye#pink-eye
282) Timolol 0.5% w/v Eye Drops Solution. Brown & Burk UK Ltd. 19 Feb 2020. https://www.medicines.org.uk/emc/product/10888/smpc#
283) TIMOLOL MALEATE solution/ drops. Rising Pharmaceuticals, Inc. September 13, 2019.

https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=2ed21930-1e05-46de-8acd-cb6f4798a7fa

284) Timolol maleate- timolol maleate solution/ drops. Pacific Pharma, Inc. December 22, 2019. https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=e16c33fd-aa86-49ad-a767-c79ade4a62c7

285) Timothy MG. Postoperative Care after Cataract Surgery. Restoration Eye Care website. March 2014. https://restorationeyecare.com/wp-content/uploads/2014/03/Postoperative-Care-after-Cataract-Surgery.pdf

286) Toris C. Pharmacotherapies for Glaucoma. Curr Mol Med. 2010;10(9):824-40.

287) Troy B, Kim JM. An Overview of Proptosis. February 05, 2020. https://www.verywellhealth.com/bulging-eyes-and-proptosis-causes-and-evaulation-3422004

288) Umesh DL, Rohan KB, Rasika VZ, Amol AT. Development and validation of stability indicating reverse-phase high-performance liquid chromatography method for timolol. Int J Pharm Tech Res. 2014;6(5):1429-35.

289) Usama A and Akhtar J, Patel P. Preformulation Studies: An Integral Part of Formulation Design, Pharmaceutical Formulation Design - Recent Practices. Intech Open. June 9, 2019. https://www.intechopen.com/books/pharmaceutical-formulation-design-recent-practices/preformulation-studies-an-integral-part-of-formulation-design)

290) Usha GK, Sree HN, Balakeshwa R, Prakash RB, Subramanian R. Enhanced trans-corneal permeability of valacyclovir by polymethacrylic acid copolymers based ocular microspheres: *in vivo* evaluation of estimated pharmacokinetic/pharmacodynamic indices and simulation of aqueous humor drug concentration-time profile. J Pharm Innov. 2016;11:82–91.

291) Usman M. Most Common Eye Problems – Signs, Symptoms and Treatment Options. Irisvision.com. Feb 12, 2019. https://irisvision.com/most-common-eye-problems-signs-symptoms-and-treatment/

292) Uveitis. Mayo Clinic. June 05, 2018. https://www.mayoclinic.org/diseases-conditions/uveitis/symptoms-causes/syc-20378734

293) Uveitis. National Eye Institute (NEI). July 11, 2019. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/uveitis

294) Valencia H, Alana B. Macular Degeneration. August 4, 2016. https://www.healthline.com/health/macular-degeneration#symptoms

295) Vasumathy V. Endophthalmitis : An Update Delhi J Ophthalmol 2014; 25 (1): 13-19

296) Vasumathy V. Endophthalmitis : An Update. Delhi J Ophthalmol 2014;25 (1):13-19.

297) Vijayalakshmi P, Lucy N. Postoperative care for pediatric cataract patients. Community Eye Health Journal. 2016; 29(94): 32-34.

298) Wachler BSB, Mary AD. A Guide to Refractive and Laser Eye Surgery. WebMD Medical Reference. December 13, 2018. https://www.webmd.com/eye-health/overview-refractive-laser-eye-surgery#2

299) Waterman KC, Adami RC. Accelerated aging: prediction of chemical stability of pharmaceuticals. Int J Pharm. 2005;293(1-2):101-125 .

300) Weber M, Laurent K, Friedrich EK, Zagorski Z, Catherine MA. Efficacy and safety of indomethacin 0.1% eye drops compared with ketorolac 0.5% eye drops in the management of ocular inflammation after cataract surgery. Acta Ophthalmol. 2013:91:e15-e21.

301) Weinreb RN, Aung T, Felipe AM. The Pathophysiology and Treatment of

302) Weinreb RN, Dreher AW, Bille JF. Quantitative assessment of the optic nerve head with the laser tomographic scanner. Int J Ophthalmol. 1989; 13(1-2):25-29.

303) Whitney S. What Are Cataracts?.WebMD Medical Reference. August 18, 2019. https://www.webmd.com/eye-health/cataracts/what-are-cataracts#1

304) Whitney S. What Is Color Blindness?. WebMD Medical Reference. September 06, 2019. https://www.webmd.com/eye-health/color-blindness#1

305) Whitney S. What Is Myopia (Nearsightedness)?.WebMD Medical Reference. February 07, 2020. https://www.webmd.com/eye-health/nearsightedness-myopia#2

306) Whitney S. Astigmatism. WebMD Medical Reference. November 11, 2019. https://www.webmd.com/eye-health/astigmatism-eyes#1

307) Wiley C. 6 Eye Surgery Options to Correct your Sight. Angie's List website. Jan. 3, 2014. https://www.angieslist.com/articles/6-eye-surgery-options-correct-your-sight.htm

308) Yadav P, Vaibhav R, Verma A. Application of Box–Behnken design and desirability function in the development and optimization of self-nano emulsifying drug delivery system for enhanced dissolution of ezetimibe. Future J Pharm Sci. 2020;6:2-20.

309) Yafinaz, Basri M, Basri H.B, Ahmad FS, Roghayeh AK. Formulation optimization of a palm-based nanoemulsion system containing levodopa. Int J Mol Sci. 2013;13:13049-64.

310) Yam JC, Kwok AK. Update of the management of postoperative endophthalmitis. Hong Kong Med J. 2004;10(5):337-43.

311) Yamaguchi M, Yasueda S, Isowaki A, et al. Formulation of an ophthalmic lipid emulsion containing an anti-inflammatory steroidal drug, difluprednate. Int J Pharmaceutics. 2005;301:121-28.

312) Yvonne O. Glaucoma Surgery Series: The Risks and Benefits of Glaucoma Surgery. Bright Focus Foundation website. April 23, 2018. https://www.brightfocus.org/glaucoma/article/glaucoma-surgery-series-risks-and-benefits-glaucoma-surgery

313) Zainol S, Basri M, Basri HB. Formulation optimization of a palm-based nanoemulsion system containing levodopa. Int J Mol Sci 2012;13:13049–64.