DEVELOPMENT AND OPTMIZATION OF OPHTHALMIC FORMULATION OF TIMOLOL MALEATE

A THESIS

SUBMITTED IN THE PARTIAL FULFILMENT OF

THE REQUIREMENT FOR THE DEGREE OF

MASTER OF PHARMACY

(PHARMACEUTICS)

By

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May, 2015

Annexure I

INDIA'S LARGEST UNIVERSITY

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Discipline: Pharmaceutics

PROJECT/DISSERTATION TOPIC APPROVAL PERFORMA

Name of Student: Ms. Mansa Voleti	Registration No: 11301115
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Session: 2014-2015	Parent Section: Y1307

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PROPOSED TOPICS

1. Development and evaluation of Ophthalmic Formulations
2. Development and Optimization of Ophthalmic Formulation of Timolol
3. Design and Optimization of Timolol Formulation in Ophthalmics

Signature of Supervisor

*Guide should finally encircle one topic out of three proposed topics and put up for approval before Project Approval Committee (PAC)

*Original copy of this format after PAC approval will be retained by the student and must be attached in the Project/Dissertation synopsis and final report.

*One copy to be submitted to Supervisor.

APPROVAL OF PAC CHAIRPERSON:

Signature:

DEDICATED TO MY PARENTS

Statement by the candidate

This is to submit that this written submission in my thesis entitled **"Development and Optimization of Ophthalmic Formulation of Timolol"** represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

Patents related to API, process, product, method and equipment, if any, have been examined to ensure non-infringing approach to the existing patents.

This thesis encompasses the information generated by me based on experimental work carried out in the Institute / Industry. I assure and hold full responsibility for its genuineness.

Voleti Manasa

Forwarded Through

Dr. Amit Bhatia
Assistant Professor
School of pharmaceutical sciences
Lovely Professional University

Certificate by Supervisor

The work described in the thesis entitled **"Development and Optimization of Ophthalmic Formulation of Timolol''** has been carried out by **Ms. Voleti Manasa** under my supervision. I certify that this is her bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Date:

Place:

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Certificate by School

This is certified that the work described in this thesis entitled **''Development and Optimization of Ophthalmic formulation of Timolol''** has been carried out by **Ms. Voleti Manasa** at the school of Pharmaceutical sciences, Lovely Professional University, Punjab.

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Acknowledgement

Thanks God, the merciful and the passionate, for providing me the opportunity to step in the excellent world of science. To be able to step strong and smooth in this way, I would like to express my deepest gratitude to all those people who have helped me through the course of my journey towards producing this thesis.

Indeed I have yet to find words to pay gratitude and sincerely thanks to my honorable supervisor **Dr. Amit Bhatia**, under whose excellent supervision, worthwhile suggestion, keen interest, wise counseling, patience, friendly advice, continuous support and encouragement, that resulted in the successful completion of this work. Apart from the subject of my research I learnt a lot from him, which I am sure, will be useful in different stages of my life.

I am deeply indebted to Dean Madam, **Dr. Monica Gulati** for providing me all the facilities and excellent working environment in the laboratory in order to complete my task.

A special thanks to the faculty of department of pharmaceutical sciences, **Dr. Deepak Kapoor**, for their help and guidance during the research.

I am very grateful to laboratory technicians especially to **Mr. Gopal krishan, Mr. Hans raj, Mr. Vijay Kumar**, who were very helpful during the requirement of the chemicals and spectrophotometric and dissolution studies.

I am obliged to my friends **Mr. Prabhudas Kiran and Saurav Kumar Nayak**. Who always stood by me and gave their helping hands whenever and everywhere and wherever it was required without even asking for it.

I am deeply grateful to **Mr. Ashok Mittal**, Chancellor, Lovely Professional University, for providing me all the required facilities and financial assistance to complete my task at time.

Finally, yet importantly, I would like to express my heartful thanks to my father **Mr. Voleti Srinivas** and my mother **Mrs. Radhakumari Voleti** for their blessings, support, encouragement and wishes for the successful completion of this project.

Voleti Manasa

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List of Abbreviations:

ARMD	: Age Related Macular Degeneration
CMV	: Cyto Megalo Virus
% DR	: % Drug Release
EDTA	: Ethylene Di amine Tetra Acetic acid
FTIR	: Fourier Transform Infra red Spectroscopy
ICH	: International Conference on Harmonization
IPM	: Iso Propyl Myristate
IOP	: Intra Ocular Pressure
HET-CAM	: Hen's Egg Test - Chorioallantoic membrane
KI	: Potassium Iodide
LOQ	: Limit of Quantification
LOD	: Limit of Detection
NaCl	: Sodium Chloride
NaOH	: Sodium Hydroxide
PBS	: Phosphate Buffer Saline
% RH	: % Relative Humidity
% RSD	: Relative Standard Deviation
STF	: Simulated Tear fluid
SLS	: Sodium Lauryl Sulfate
SDS	: Sodium Do decyl Sulfate

Chapter-1

Introduction

The most important sense organ among the five principal senses is the vision. It serves an essential part in our lives and empowers us to capacity ideally. Therefore, it must not be ignored, and full endeavors in protecting of this essential part are obliged to be attempted by science. Frequently occurring disorders such as cataracts, glaucoma and diabetic retinopathy are chronic in character as well as involve constant treatment. The uncomplicated fact that the diseases are chronic makes worse the despair of subjects. Reports of Pascolini and Marriot states that 285 million individuals have a problem with eye sight worldwide. Out of which about 14% are totally blind. This is undoubtedly a lumber, and the greater part of incidents can be endorsed to ageing or to avertable diseases. (Pascolini D et al., 2012)

Eye is an intricate structure intended to congregate a noteworthy quantity of facts about the atmosphere around us. All the Physicians and pharmacists as well as ocularists should be aware of the fundamental anatomy and physiology of the eye. While ocularists hardly ever labor on concrete eyeballs, this knowledge can lend a hand for the ocularists in acquiring a biologically materializing artificial body part, and it will be of larger assistance to the patient. Organization of the Eye includes visual system instigation at the front plane of the eye and succeeding to the primary visual cortex at the back of brain. (Wolff E.1933)

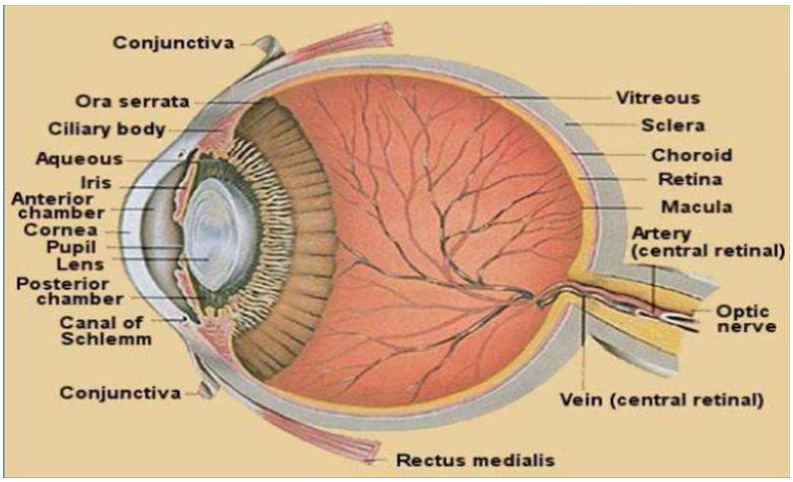


Figure 1.1: Eye structures [source: adapted from www.medicinembbs.blogspot.com/2010/11/eye-structures.html]

1.1. Pathology of Eye:

According to the location of diseases, the ocular disorders are classified as Periocular diseases (which includes pink eye, keratitis, Iritis, corneal ulcer, hyposphagma, lazy eye, and uveitis) Intraocular diseases (including glaucoma, CMV and retinoblastoma) and common diseases (such as cataract, AR macular degeneration and diabetic retinopathy).

1.1.1 Periocular diseases:

- **Conjunctivitis (Pink eye):** Inflammation in conjunctive by bacteria, virus, pollen or pollution.
- **Keratitis:** Corneal inflammation by bacteria or virus.
- **Iritis (acute onset):** pain and inflammation.
- **Corneal Ulcer:** Also known as **"Ulcerative Keratitis"**. Corneal infection by damage of epithelial layer.
- **Subconjunctival haemorrhage (Hyposphagma):** Bleeding underneath the conjunctiva

- **Amblyopia or Lazy Eye:** Debilitated or faint vision without clear surrender in the Eye.

- **Uveitis (eye inflammation):** Uveal inflammation (K.S. Rathore et al.,2009 and R.R.Thakur et al., 2011)

1.1.2 Intraocular diseases:

- **Glaucoma:** The construct up of weight in the front and back councils of the choroid layer that happens when the watery diversion neglects to empty legitimately bringing about incredible loss of sight.

- **Retinoblastoma:** a rare malignant tumor of retina affecting young children.

- **CMV (Cyto Megalo Virus) retinitis:** inflammation of the eye retina that can lead to blindnesss.

1.1.3 Common diseases:

- **Cataract:** Obfuscating of the lens inside the eye which prompts diminish in vision

- **A R Macular Degeneration:** Due to the damage of retina causes loss of vision in centre of the visual field.

- **Diabetic Retinopathy:** Retinopathy including harm to the little veins in retina results from the high glucose blood levels in individuals with inadequately controlled diabetes. (Masood Chowhan et al.,2007, Clive G.Wilson et al., Short BS.2008)

1.2 Routes of ocular delivery:

The principal routes generally used for ocular drug administration into the eye are topical, intraocular and systemic routes. Among these, the topical course is the most all inclusive procedure to manage a drug to the eye. If anterior segment is to be targeted, the drugs ought to be administered by Topical and subconjunctival routes. Intravitreal injections, periocular routes (retrobulbar, per bulbar, subtenon and subconjunctival routes and

implants) are used for the delivery into the posterior segment. (Ahmed I. 2003, Raghava S. et al., 2004)

The most expedient route of administration is topical way, as injections and implants are harmful and coupled with distress and present the problems of retinal detachment and cataracts. (Thilini et al., 2010; Gaudana R et al., 2009). The bioavailability obtained by these topical eye drops to the front council is 5 %.(Robinson JC et al., 1993)

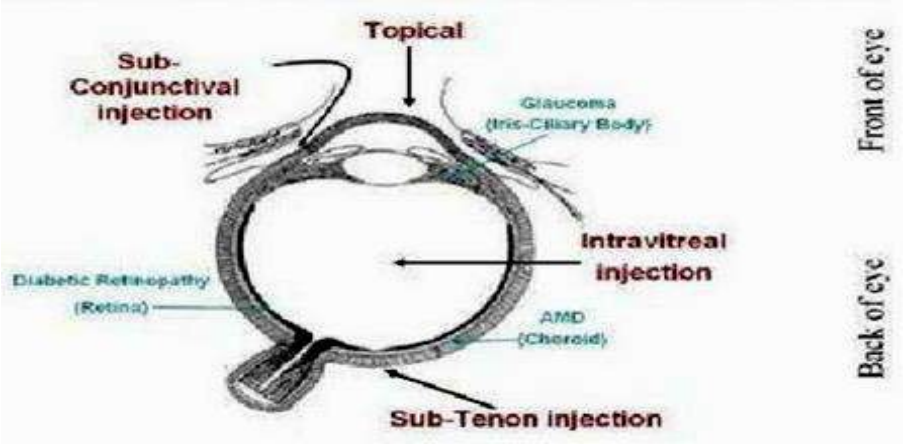


Figure 1.2: Routes of Ocular Delivery

Topically injected formulations are limited by poor bioavailability and penetration, short ocular surface retention time due to nasolacrimaldrainage (Saettone MF et al., 1995). In addition to these, topical delivery systems also involve blurred vision and practical impossibility of delivery to posterior chambers. These disadvantages of topical systems are dependent on blinking, reflex lacrimation of the eye and gravity. (Urtti A.2006; Fraunfelder FW.2006)

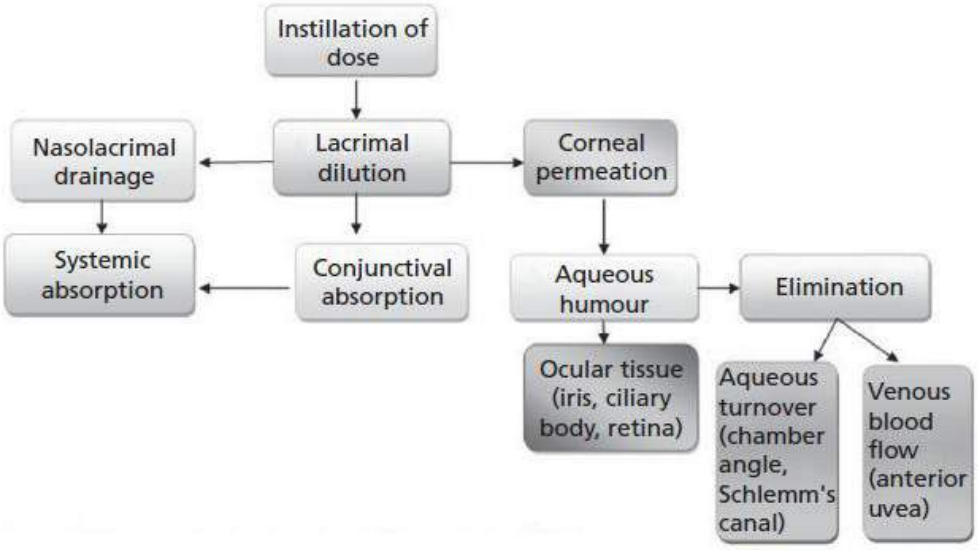


Figure 1.3: Flowchart depicting the topical ophthalmic drug delivery systems

Administration of ocular medications through the anterior segment involves barriers such as cornea and conjunctiva which limit drug absorption. Cornea constitutes a barrier to transcorneal permeation of both hydrophilic and lipophilic compounds. Mediocre lipophilic drugs are permeated through cornea. In addition hydrophilic drugs are more absorbed through sclera-conjunctival route. (Geroski DH and Edelhauser HF.2000)

Higher concentrations of drugs in front council are acquired only 30 minutes after administration. (Uritti.A. et al., 1990; Davis JL et al., 2004) Through the veins of uvea, the elimination of drug occurs through the chamber angle and canal of schlemm (Weiner A.2010).

1.3. Glaucoma:

Ophthalmic diseases are universal and time and again escorted to loss of vision on the off chance that left untreated. One of them is Glaucoma, which involves increase in intraocular pressure which leads to degeneration of the optic nerve which is constituted by retinal ganglion cells and axons resulting in the irreversible loss of vision. (Blomdhal S et al., 1997; Munier A et al., 1998)

Figure 1.4: Progression of glaucoma

The aqueous humor, a fluid present in between the cornea and front surface of crystalline lens.The endothelium of the eye draws nutrients from aqueous humor to cornea. Aqueous fluid circulates out of the eye and replaced by newly produced aqueous humor. To move out of the anterior segment, it flows out of the eye through the trabecular meshwork. This outflow has some resistance that serves to maintain pressure of 15mm of Hg. If there is more resistance, pressure may exceed the eye's tolerance and damage to optic nerve. This condition is known as "Glaucoma".

The pathogenesis includes a mixed bag of starting occasions, bringing on changes in fluid outpouring, bringing about expanded IOP, which prompts optic nerve decay, lastly, dynamic loss of vision.

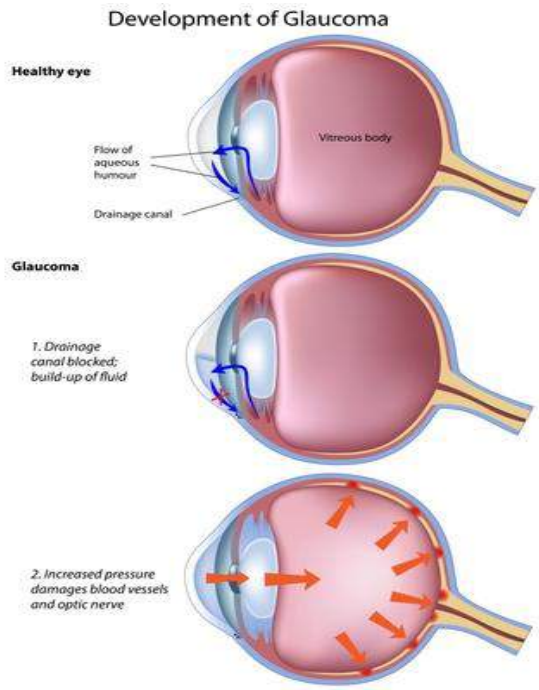


Figure 1.5: Development of glaucoma

1.3.1 Medications used in glaucoma treatment:

Table 1.1: Drug dosage forms used in Glaucoma treatment

Drugs used	Type of dosage form available in market	Mode of action	Side effects
Timolol (β blocker)	Solution, Gel forming solution, tablet	Decrease of aqueous humour production	Bronchospasm,bradycardia,congestive heart failure, depression, confusion, impotence
Levobunolol	Eye drops	Decrease humor synthesis	Bronchospasm,bradycardia,congestive heart failure

Betaxolol	Eye drops, tablets, suspensions	Decrease of aqueous humour production	Bronchospasm,bradycardia,congestive heart failure
Carteolol	Eye drops, solutions	Decrease humor production	Bronchospasm,bradycardia,congestive heart failure,
Brimonidine(adrenergic agonists)	Eye drops, ophthalmic solution.	Decrease of aqueous humour production and decrease resistance to flow	Conjuctival hypermia,allergic reactions,malaise,headache, SNC depression
Pilocarpine(Cholinergic agonist)	Eye drops, tablets,	Increase of aqueous out flow	Eye or brow pain, increased myopia, decreased vision
Latanoprost	Ophthalmic solution	Increase out flow of humor	Conjuctival hypermia, increased iris pigmentation, hypertrichosis
Unoprostone	Eye drops, ophthalmic solutions	Increase out flow of humor	Conjuctival hypermia, increased iris pigmentation, hypertrichosis
Travoprost	Eye drops, ophthalmic solutions	Increase out flow of humor	Conjuctival hypermia, increased iris pigmentation, hypertrichosis
Bimatoprost	Ophthalmic solutions	Increase out flow of humor	Iris pigmentation, hypertrichosis

Dorzolamide (Carbonic anhydrase enzyme)	(Topical)Ophthalmic solution	Decrease of aqueous humour production	Paresthesias, depression, malaise , anorexia, allergic reactions, renal calculi
Brinzolamide	(Topical) Ophthalmic solution	Decrease aqueous humor production	Anorexia, allergic reactions, Renal calculi
Acetazolamide	(Oral) Tablets, Capsules	Decrease aqueous humor	Anorexia, allergic reactions, Renal calculi

1.3.2 Different ocular delivery systems for Glaucoma:

The principal factors to be considered while designing a delivery system are contact time of drug, target region (anterior or posterior), duration for which the system remains contact with the eye, and patient suitability. Lastly, in stipulations of subject suitability the designed delivery approach ought to be simple to handle or apply, secure and remains for long time in contact with eye to maximize the ocular drug absorption and reduce the side effects and damage to ocular cellular system. (Hughes MO, 2004).

Table 1.2: Drug delivery systems for the Glaucoma treatment

Conventional dosage forms and delivery systems	**Novel drug delivery systems**
Oral medications Topical eye drops and gels	Micro and Nano drug delivery systems Contact lens as delivery vehicles

Ocular inserts Surgical implants	Sophisticated surgical implants (MEMS) Injectable systems

1.4. Drug profile: Timolol Maleate

1.4.1 Therapeutic Category:

Timolol maleate is a Beta-adrenoreceptor antagonist. It is non-specific of $beta_1$ and $beta_2$ adrenergic receptors. It does not have noteworthy intrinsic sympathomimetic, direct myocardial depressant or local anaesthetic (membrane stabilising) activity. It is efficacious in sinking intraocular pressure. The patients with open angle glaucoma and aphakic glaucoma are given medications using Timolol. Timolol maleate is designated both for the healing hypertension and reduce cardiovascular mortality. It is combined with other anti-hypertensive agents (thiazide-type diuretics) (Edward R, 1987)

1.4.2. History:

Timolol maleate, belonging to the thiadiazole class of compounds, was first synthesized in the Merck-Frosst Laboratories in Montreal, Canada. The first non-patent literature reference of Timolol maleate appeared in 1972 (Wasson et al., 1972). Timolol maleate, since its introduction in pharmaceutical preparations has gained a wide acceptance as an anti-hypertensive and anti-glaucoma agent.

1.4.3. Description:

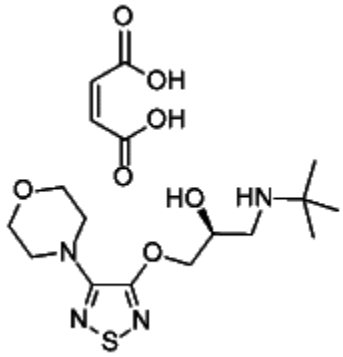


Figure 1.6. Structure of Timolol maleate **(**[source: adapted from http://www.chemicalbook.com/ChemicalProductProperty_EN_CB3711352.htm]

- **Chemical Name:**

The chemical name of Timolol maleate is (S)-1-tert-butylamino-3-(4-morpholino-1, 2, 5-thiadiazol-3-yloxy)-2-proponol, (Z)-butenediote (1:1) salt. The CAS registry number is 26921-17-5.

The other names which have been used for Timolol maleate include the maleate salts of (s)-(-)-3-(3-tert-butyl-amino-2-hydroxypropoxy)-4-morpholino-1, 2, 5-thiazide, (-)-3-morpholino-4-(3-tert-butylamino-2-hydroxypropyl)-1, 2, 5-thiadiazole and (-)-1-(tert-butylamino)-3-[(4-morpholino-1,25-thiadiazol-3-yl)oxy}-2-proponol.

- **Description:**

A white odorless, crystalline powder or colorless crystals

1.4.4. Therapeutic dosage:

- **Adults:**

Ophthalmic solutions: The measurement for glaucoma is 1 drop of 0.25% solution (increased to 1 drop of 0.5%) in one eye and 2 times a day. Hypertensive oral measurements are 15 to 60 mg daily.

- **Children:**

Timolol is not suggested in children. The pediatric measurements are obscure. (Inchem)

1.4.5. Contraindications:

- Asthma patients
- Patients with 2^{nd} and 3^{rd} degree AV block and cardiogenic shock.

1.4.6. Mode of action:

Table 1.3: Mode of action of Timolol

Toxicodynamics	Pharmacodynamics
Negative chronotropic effect Negative inotropic effect Decreases heart rate, supraventricular conduction and cardiac output in therapeutic doses.	Mechanism is not known Decrease secretion of aqueous humor (Zimmerman et al., 1977)

1.4.7. Pharmacokinetics:

A. Absorption:

- **Oral :**

Timolol is 90% absorbed from GIT. (Fourtillan JB et al., 1981). Mass fragmentography is used to investigate kinetic studies of oral Timolol

- **Ocular:**

The onset of ocular Timolol is after 10-20 minutes and lasts for no less than 24 hours. (Zimmerman & Kaufman, 1977b). Timolol is absorbed systemically with 2-5 g/l strengths in serum (Affrime et al., 1980; Alvan et al., 1980).

B. Distribution:

- **Oral :**

Table 1.4: Distribution of Timolol

Property	**Proportion**
Bioavailability	60% (Wilson et al., 1982)
Apparent volume of distribution	1.3 - 1.7 L/kg (Vermeij et al., 1978; Wilson et al., 1982)
Plasma protein binding	10% (approx)

- **Ocular:**

Distribution is all over conjunctiva, cornea, sclera, iris, aqueous humor, liver, kidney and lung.

A. Metabolism:

Metabolism occurs in the liver by hydrolytic cleavage of the morpholino ring with subsequent oxidation. For an oral dose, metabolism is 80 % and elimination is 20% (unchanged in urine) (Tocco et al., 1975). Metabolism is dependent on genetic polymorphism.

B. Elimination:

Table 1.5: Elimination of Timolol

Oral	Ocular
• Kidney • Elimination- 20% and metabolites – 40-60% (Tocco et al, 1975) • Present in breast milk • For a maternal oral dose, milt to plasma ratio is 0.80	**Concentration in breast milk**(5.6 ng/ml) **is 6 times higher than in serum** (0.93 ng/ml) **.** (Lustgarten and Podos, 1983)

1.4.8 Synthesis:

It has been arranged through arrangement of steps starting with D-Mannitol and acetone (Weinstocks et al., 1972). The starting material, I, is reacted with acetone and the product (II) is washed with benzene and crystallized from hexane. This substituted Mannitol (II) is then oxidized in tetrahydrofuran with lead tetra-acetate to the substituted glyceraldehydes (III), hydrogenated with t-butyl amine (IV) and treated with benzaldehyde to form a substituted phenyloxazolidine (V).

1.4.9 Brand names or trade names of Timolol maleate:

Table 1.6: Marketed products of Timolol

Mono component product	Combination product
Betimol® (International)	Combigan® (Indian)
Timoptic® (Indian)	Cosopt®(Indian)
Timoptic-XE® (International)	Timolide®(International)
Timoptic® GFS (International)	

1.4.10. Analytical methods for determination of Timolol maleate:

A. Melting Point:

The melting point of Timolol maleate varies between 201.5^{0}C and 202.5^{0}C as determined by USP class Ia capillary method.

B. Solubilities:

The solubility of Timolol maleate in various vehicles at 25^0 C is given underneath table. These solubilities are expressed regarding current U.S.P definitions. (USP 1985)

Table 1.7: Solubility of Timolol

S.No.	Solvent	Solubility
1	Water	Soluble
2	Methanol	Soluble

3	Ethanol	Soluble
4	Chloroform	Sparingly soluble
5	Propylene glycol	Sparingly soluble
6	Ether	Practically Insoluble
7	Cyclohexane	Practically Insoluble
8	Iso-octane	Practically Insoluble

- **Optical activity:**

Timolol maleate has one chiral carbon, so it is optically active. The active form is levorotatory enantiomer. The optical rotation of 5% (w/v) solution of Timolol maleate in 10N aqueous HCl at 25^0C and 405nm is -12.2^0. The specific optical rotation was -5.7^0 to -6.2^0 determined in 10%w/v of 1M HCl.

C. Physicochemical properties of the Timolol maleate:

Table 1.8: Properties of Timolol

S.No.	Property	Value
1	Melting point	201.5^0C to 202.5^0C
2	Water solubility	2.74 mg/mL
3	Log P	1.83
4	PKa	9.21

5	Physiological charge	1
6	Hydrogen acceptor count	7
7	Hydrogen donor count	2
8	Polar surface area	79.74
9	Refractivity	83.92
10	Polarizability	33.99
11	Rotate bond count	7

Chapter-2

Review of Literature

2.1. Research on Ocular delivery:

A lot of research has been done on Ocular drug delivery; some of them have been discussed here:

Mortazavi S.A. et al. formulated and evaluated ciprofloxacin containing mini tablets for ocular use by direct compression of Ciprofloxacin along with cellulose derivatives such as HPMC, Na.CMC, HEC and EC, Carbopol 974P, solubilizer and lubricant, to achieve continuous and predetermined drug discharge to front eye chamber. (Mortazavi S.A. et al., 2010).

Naval Dinesh et al., formulated Bilayered ocular inserts based on Thiolated sodium alginate (TSA) for improved ocular therapy of Gatifloxacin by Solvent Evaporation technique which contains a mucoadhesive immediate release layer and sustained release layer. It is concluded from the results the films showed improved mucoadhesion and also a strikingly beneficial property of resisting erosion and remaining as the hydrated adhesive layer for the drug release (Naval Dinesh et al., 2014).

Selvaraj et al., fabricated chitosan microspheres of Acyclovir by the method prescribed by Bodmire and Paeratakul. The results declared that the chitosan microspheres of Acyclovir were effective Controlled release preparation to treat eye viral contagions (Selvaraj et al., 2010).

Aparna V et.al, formulated and evaluated ion activated in situ gel ophthalmic drug delivery system of Dorzolamide hydrochloride using sodium alginate as gelling agent in combination with HPC. The results proved that formulation system is a substitute for eye drops in terms of patient conformity and cost (Aparna V et al., 2011).

Basavaraj K Nanjwade et.al, conducted an experimental *in-vitro* and *in-vivo* of discharge model of medicament from Ciprofloxacin ophthalmic formulation by considering three different formulations of aqueous polymeric gel, hydrophobic ointment and conventional solution. The results revealed that one measure of gel created peak drug concentrations in the aqueous humour (Basavaraj K Nanjwade et al., 2011).

J.Padma Preetha et.al formulated and evaluated an ocular delivery approach of Diclofenac sodium, using pH activated Insitu gelation technique by using Sodium alginate. The results proved that the developed formulation system is a substitute for eye drops in terms of patient conformity and cost. (J.Padma Preetha et al., 2010).

2.2. Research on Topical delivery:

Mohammed Mostafa Ibrahim et al., formulated Natural Bioadhesive Biodegradable Nano particle-based Topical Ophthalmic formulation for continued release of Celecoxib by impulsive emulsification solvent diffusion method by optimizing the various formulations which uses different concentrations of Polymers, Emulsifiers and Stabilizers. The result revealed that these formulations provide a immense distribute of impending drug discharge for both the front and back councils of the Eye (Mohammed et al., 2013).

Timothy L Comstock et al., evaluated the safety and efficacy of Loteprednol etabonate ophthalmic ointment 0.5% with medium. They have conducted two randomized, multicentre, double-masked, parallel-group, vehicle-controlled study. From results it can be concluded that LE ointment was effective (Timothy L Comstock et al., 2011).

Subimol S et al., formulated an in situ gel formulation of Diclofenac using Sodium alginate and Hydroxyl propyl methyl cellulose in different concentrations under aseptic

conditions. Ophthalmic formulations in the form of in situ gel system can be applied as solution or suspension that undergoes gelation after administration. The results revealed that this combination serve as a suitable in situ gelling vehicle for ophthalmic use. (Subimol S et al., 2013).

Wu Y et al., synthesised thermo sensitive copolymers, hyaluronic acid-g poly (Nisopropylacrylamide) (HA-g-PNIPAAm), by coupling carboxylic end-capped PNIPAAm to laminated hyaluronic acid via amide bond linkages. HA-g-PNIPAAm microgels attained notably greater CyA concentration levels in corneas (1455.8 ng/g of tissue) than both castor oil formulation and marketed CyA eye drops (Wu Y et al., 2013).

2.3. Research on Timolol maleate:

Roopa Karki et al., developed an approach to formulate small eye drops of Timolol maleate by using altered dropper tip design. This alteration of eye drops delivery system and medication properties can produce smaller drops and can easily decrease the cost of topical therapy in glaucoma and at the same time improve the therapeutic index (Roopa Karki et al., 2011)

Satyabrata bhanja et al., formulated and evaluated mucoadhesive buccal tablets of Timolol maleate by direct compression method and evaluated for various parameters such as weight variation, hardness, surface pH, drug content uniformity, swelling index, bioadhesive strength, and in-vitro drug dissolution. The release studies revealed that all the formulations (F 1-8) showed zero order kinetics followed by Korsemeyer – Peppas, first order and Higuchi's model and mechanism of drug release is non- fickian diffusion. (Satyabrata bhanja et al., 2010)

Mohammed Ali Attia Shafie and Mai Ahmed Hassan Rady evaluated ocular inserts (formulated using various polymers) of Timolol maleate both for in-vitro and in-vivo parameters. It is reported that in-vitro release data of Timolol followed diffusion mechanism and permeability studies revealed that the permeability coefficient depends on

polymer type, the higher the solubility of the polymer, higher the permeability coefficient. (Mohammed Ali et al., 2010)

Himanshu Gupta et al., developed and characterize 99mTc-timolol maleate for evaluating efficacy of in situ ocular drug delivery system.The optimization of the formulation was done by changing chitosan and HPMC ratios.It was found that the radiolabelled drug was stable upto 24 hrs.Ocular iriitation was also tested using hen's egg chorioallantoic membrane test and found to nonirritant. (Himanshu Gupta et al., 2009)

Gabi Shemesh et al., evaluated the safety and efficacy in reduction of intraocular pressure on multiple doses of Cospot.On increasing the dose from twice to thrice a day, it was found that no systemic adverse effects are reported and there is additional decrease in the intraocular pressure. (Gabi Shemesh et al., 2013)

R.K.Janakiraman and C. Ramasamy conducted pre-clinical studies of Timolol maleate matrix tablets which were prepared by using different ratios of polymers. In vitro release studies revealed showed that optimized formulation followed first-order kinetics via non-fickian diffusion. (R.K. Janakiraman et al., 2014)

Kamal Singh rathore et al., formulated and characterized ocular films of Timolol maleate for the treatment of primary open glaucoma.It was concluded ocular films were formulated with the objective of increase in contact time, drug release for long peiod, decrease the number of doses resulting in therapeutic efficacy and patient compliance. (Kamal Singh et al., 2000)

Patel BM et al., developed an analytical approach for concurrent estimation of Timolol and Pilocarpine nitrate in combined pharmaceutical dosage form. The method was validated for various parameters such as linearity, accuracy, precision, recovery according to ICH guidelines and showed that values were accurate and precise. (Patel BM et al., 2014)

Shamim Mushtaq et al., conducted in-vivo studies to test the effects of preserved and unpreserved Timolol maleate on the surface of corneal epithelium of albino

rabbits.Corneal organ culture model showed that topically applied Timolol maleate (0.005), BAC (0.00%) and preservative (0.05%) resulted in deficiency in term of protein expressions. (Shamim Mushtaq et al., 2011)

Akhilesh Dubey and Prabhakara Prabhu formulated stimuli sensitive hydrogels of Timolol maleate and Bromonidine tartrate for the treatment of glaucoma. The formulated hydrogels were evaluated for viscosity, drug release, drug interaction, sterility studies; in-vivo studies. Drug release was 90% up to the end of 8hrs. (Akhilesh Dubey et al., 2014)

N Kanikkannan et al., conducted in-vitro studies to determine the effect the age and species on transdermal iontophoretic transport of Timolol maleate.These studies confirms that there is no interspecies differences in the transdermal permeation. (N Kanikkannan et al., 2011)

Chapter-3

Research Envisaged and Plan of Work

3.1. Rationale of the study:

Glaucoma is an intraocular disease which is the vital reason of blindness in the globe specifically in elder people. It is caused due to the damage of sensitive nerve fibres of optic nerve which carries the images from retina to the brain. The damage of optic nerve and loss of vision is permanent. Only prevention approach for the glaucoma is early detection and receiving treatment. There are no typical early signs and symptoms for the glaucoma. Treatment for glaucoma depends on the character and severity of the disease. It includes medicaments, laser surgery and operative surgery. Reduction of intraocular pressure is the most important possibility in the treatment of glaucoma.

The limitations of the conventional systems are nasolacrimal drainage (ophthalmic drops), blurred vision (ointment). To decrease the elevated intra ocular pressure, the formulation should not show pre-corneal elimination of drugs by solution drainage, lacrimation and non productive absorption. Moreover, the conventional formulations have to meet certain quality checks like Sterilization, pH, Osmolarity and particle size. In the light of these, the present work focuses on development of a novel formulation aimed to achieve continuous association of drugs with cornea, improve the bioavailability and simultaneously act as cosmetic product. The developed formulation is expected to offer improved patient compliance and efficacy due to its combined drug delivery and cosmetic characteristics.

3. Research Envisaged and Plan of Work

3.2. Aim:

Development and Optimization of Ophthalmic Formulation of Timolol

3.3. Objective:

- To develop a formulation of topically applied ophthalmic formulation of Timolol maleate.

- To optimize the formulation and evaluate the formulation for various parameters like Viscosity, Grittiness, spreadability, texture analysis, *in-vitro* permeation study, *in-vitro* ocular irritation test and peroxide content.

3.4. Plan of Work:

- The present research work can be alienated into Pre- formulation and Formulation development and Evaluation.
- Under Pre- formulation development, Physico chemical characterization of the drug followed by UV Estimation of the drug.
- The last Phase of Pre-formulation is to conduct the Compatibility studies between the drug and excipients using FTIR and UV-Spectroscopy.
- Formulation Development includes the preparation of a novel ophthalmic product which is done in two trials.
- In the 1st trial, one formulation out of eight formulations was selected as the fundamental formulation for the further trials. This selection is based on the physical properties and percent drug release.
- By using the selected formulation, six more formulations were developed by incorporating permeation enhancers which may increase the bioavailability further.
- All these formulations are assessed for various parameters like viscosity, grittiness, spreadability, texture analysis, *in-vitro* permeation study, *in-vitro* ocular irritation test and peroxide content.
- The formulation showing the finest values in all the quality determinations will be reported as the final best formulation.

Chapter-4

Experimental Methodology

Table 4.1 List of materials used:

Chemical	Manufacturer
Activated charcoal	Murphy cosmetics Ltd. Amritsar
Bees wax	Loba chemicals, Mumbai, India
Benzalkonium chloride	Loba chemicals, Mumbai, India
Bile salt	Loba chemicals, Mumbai, India
Brij 35	Central drug house (P), New Delhi
Calcium Chloride	Loba chemicals, Mumbai, India
Carnauba wax	Loba chemicals, Mumbai, India
Cetyl alcohol	Central drug house (P), New Delhi
Cholesterol	Central drug house (P), New Delhi
Di sodium hydrogen phosphate	Loba chemicals, Mumbai, India
EDTA	Central drug house (P), New Delhi
Heavy liquid paraffin	Loba chemicals, Mumbai, India
Isopropyl myristate	Loba chemicals, Mumbai, India
Lanolin	Loba chemicals, Mumbai, India
Sodium bicarbonate	Molychem Ltd. Mumbai.
Sodium casein digest media	Loba chemicals, Mumbai, India
Sodium chloride	Loba chemicals, Mumbai, India
Sodium do decyl sulphate (SDS)	Loba chemicals, Mumbai, India
Span 80	Molychem Ltd. Mumbai.
Polaxomer 407	Signet Chemicals Pvt. Ltd.

Potassium di hydrogen phosphate	Central drug house (P), New Delhi
Tocopherol acetate	Loba chemicals, Mumbai, India
Yellow petroleum wax	Loba chemicals, Mumbai, India

Table 4.2 List of equipment used:

Equipment	Source
Autoclave	NavyugUdyog, India
Cone and plate viscometer	Brookfield Rheometer W.B.2000-1213.102
Digital balance	Shimadzu A×200
Hot air oven	NavyugUdyog, India
FTIR	Shimadzu 84005
Incubator	Medisearch systems Private Ltd. India
Laminar Air Flow	Accoindia, India
Magnetic stirrer	Japson Laboratories
Melting point apparatus	VSI Electronic Pvt. Ltd
TA.XT Plus Texture analyzer	Stable Micro Systems Ltd., Godalming, Surrey GU7 1YL, UK.
Trinocular microscope	Kyowa Getnee M.No.10390
UV- Spectrophometer	Shimadzu 1800

4.1 Pre-Formulation

4.1.1. Physico-chemical characterization:

Various Physicochemical characteristics of drug were determined as follows:

a) Appearance:

Timolol maleate was observed for color and appearance.

b) Melting Range determination:

The melting point of the Timolol maleate drug was determined by using capillary method. A capillary tube was taken and heat seal it from one edge. From the open end, the drug was filled to a height of 2.5-3mm from closed edge. The tube was positioned into Melting point apparatus along with the thermometer inside the apparatus. The temperature knob was switched on and with the rise in temperature, the range was noted down. The temperature at which the drug completely melts was noted.

4.1.2. Estimation of Drug by Spectroscopy:

Preparation of standard plot of Timolol maleate in Phosphate buffer saline (pH7.4):

- **Determination of maximum wavelength:**

100mg of Timolol maleate was weighed accurately on a analytical balance and reassigned into a 100ml flask by using a glass funnel. Make up the volume to mark is made up with the phosphate buffer saline (pH 7.4).This is known as the stock solution. 100 µg/ml and 10µg/ml concentrations were prepared using the stock solution and buffer. These solutions were filled in the cuvettes and the spectra was observed for the solutions in the range of 200-400nm.The maximum wavelength (λ_{max}) was found to be 293.60nm.

- **Preparation of Calibration curve:**

  From the stock solution, 5 µg/ml-50 µg/ml concentrations were prepared using buffer solution and the absorbance were observed from 200-400nm range.

  Triplicate readings and their average values were documented. The analytical method validation for linearity, precision, limit of quantification, limit of detection was carried out.

- **Method Validation:**

  After the method development, the method was validated for Accuracy, Precision, Linearity, LOD and LOQ according to ICH Q2 (R1) guidelines.

- **Quantification limit and Detection limit:**

  LOQ and LOD survey were demeanored by taking strengths of 0.5 µg /ml, 0.100 µg/ml, 2 µg/ml, 4.00 µg/ml and 5.00 µg /ml for lower boundary and 25.00 µg /ml, 30.00 µg /ml, 35.00 µg/ml, 40.00 µg/ml and 45.00 µg/ml for higher boundary to observe out the divergence from beers lamberts law.

LOQ = 10* σ/slope
LOD = 3.3* σ/slope

A. Preparation of standard plot of Timolol maleate in Simulated Tear fluid (pH7.4):

- **Determination of maximum wavelength:**

  100mg of Timolol maleate was weighed accurately on a analytical balance and transferred into a 100ml volumetric flask by using a glass funnel. The volume is made up to the mark with the Simulated Tear fluid (pH 7.4).This is known as the stock solution. 100 µg/ml and 10µg/ml concentrations were prepared using the stock solution and tear fluid. These solutions were filled in the cuvettes and the spectra were observed for the solution in the range of 200-400nm.The maximum wavelength (λmax) was found to be 295nm.

- **Preparation of Calibration curve:**

  From the stock solution, 5 µg/ml- 50 µg/ml strengths were prepared using tear fluid solution and the absorbance were observed from 200-400nm range.

Triplicate readings and their average values were recorded. The analytical method validation for linearity, precision, limit of quantification, limit of detection was carried out.

- **Method Validation:**

  After the method development, the method was validated for Accuracy, Precision, Linearity, LOD and LOQ according to ICH Q2 (R1) guidelines.

- **Quantification limit and Detection limit:**

  LOQ and LOD survey were demeanored by taking strengths of 0.5 µg /ml, 0.100 µg/ml, 2 µg/ml, 4.00 µg/ml and 5.00 µg /ml for lower boundary and 25.00 µg /ml, 30.00 µg /ml, 35.00 µg/ml, 40.00 µg/ml and 45.00 µg/ml for higher boundary to observe out the divergence from beers lamberts law.

LOQ = 10* σ/slope
LOD = 3.3* σ/slope

4.1.3. Drug Compatibility study:

a) FTIR :

Compatibility of the drug with the excipients was resolved by IR Spectroscopy using Schimadzu FTIR 8400S model. The mixtures of drug and excipients were made according to the formulation. The spectrum peaks of the drug were compared with other ingredients of formulation with the unique spectral peaks. (John RD.2007; Robert M S and Francis S W, 6^{th} edition).

b) UV-Spectrophotometry:

All the ingredients were mixed in pre-weighed and required ratios and kept at high temperatures and room temperature for 1 week (50^0C and respectively). After 1 week they are taken out and dissolved in suitable solvents and observed under UV-Spectrophotometer.

4.1.4. Results:

1. **Physico chemical characterization:**

a) Appearance:

Timolol maleate is odorless white powder.

b) Melting range determination:

The temperature at which the drug melts was found to be 201.5-202.5 °C.

2. **Estimation of drug using UV Spectrophotometer:**

A. Concentration and Spectrophotometric data for Timolol maleate in Phosphate buffer saline (pH 7.4):

The following data is obtained for the Spectrophotometric estimation of Timolol maleate at 293.60 nm.

Concentration (µg/ml)	Mean Absorbance ± S.D.
0	0.000±0
5	0.113±0.002
10	0.218±0.009
15	0.32±0.001
20	0.421±0.006
25	0.528±0.007
30	0.649±0.031
35	0.761±0.0218
40	0.856±0.0224
45	0.999±0.034

Table 4.3: Spectrophotometric data in PBS buffer

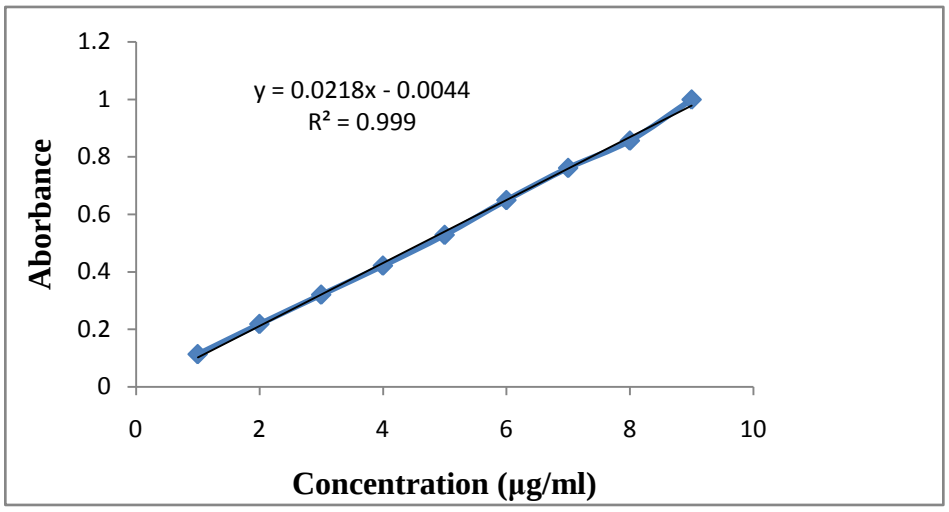


Figure 4.1: Calibration curve of Timolol maleate in PBS (7.4)

- **Analytical method validation:**
  - **Accuracy:**

S. No	TC	Abs	MC	S.D	RSD	Recovery	Mean of recovery	S. D of recovery	Error%
1.	2	0.040	2.036			101.8			
2.	2	0.040	2.036	0.0265	0.013	101.8	101.03	1.3279	- 1.03
3.	2	0.039	1.990			99.5			
4.	20	0.418	19.37			96.85			
5.	20	0.417	19.33	0.2778	0.014	96.65	97.55	1.3892	2.45
6.	20	0.428	19.83			99.15			
7.	40	0.869	40.06			100.1			
8.	40	0.871	40.15	1.112	0.0281	100.3	98.66	2.7442	1.34
9.	40	0.828	38.18			95.45			

Table 4.4: Accuracy of Timolol maleate in PBS

- **Linearity studies:**

Concentration (µg/ml)	Absorbance
5	0.113
10	0.218
15	0.32
20	0.421
25	0.528

Table 4.5: Linearity studies of Timolol maleate in PBS

- **Precision:**

Concentration (µg/ml)	Two days Morn average	Two days Aftn average	Two days Evng average	Average ± S.D.	% R.S.D
0	0.000	0.000	0.000	0.000	0.000
5	0.116	0.112	0.111	0.113±0.002	1.769
10	0.210	0.228	0.216	0.218±0.009	4.12
15	0.319	0.321	0.320	0.320±0.001	0.031
20	0.414	0.425	0.424	0.421±0.006	1.425
25	0.536	0.525	0.523	0.528±0.007	1.325
30	0.625	0.685	0.637	0.649±0.031	4.77
35	0.781	0.766	0.738	0.761±0.0218	2.75
40	0.869	0.871	0.828	0.856±0.024	2.803
45	0.986	1.04	0.972	0.999±0.034	3.40

Morn = Morning Aftn = Afternoon Evng = Evening

Table 4.6: Average inter and intraday readings in PBS

- **LOD and LOQ:**

S.No	Concentration (µg/ml)	I	II	III	Average ± S.D.
1	0	0.000	0.000	0.000	0.000
2	0.5	0.023	0.024	0.025	0.024±0.001
3	1	0.052	0.055	0.056	0.054±0.002
4	2	0.063	0.065	0.068	0.065±0.002
5	4	0.095	0.099	0.100	0.098±0.002
6	5	0.099	0.100	0.106	0.1016±0.003
7	6	0.134	0.135	0.140	0.136±0.003
8	8	0.173	0.172	0.180	0.175±0.004
9	10	0.224	0.223	0.236	0.227±0.007
10	15	0.293	0.294	0.300	0.295±0.003
11	18	0.353	0.353	0.366	0.357±0.007
12	20	0.418	0.417	0.428	0.421±0.006
13	25	0.522	0.525	0.534	0.527±0.006
14	30	0.654	0.654	0.665	0.657±0.006
15	35	0.750	0.754	0.761	0.755±0.005
16	40	0.847	0.841	0.854	0.847±0.006
17	45	0.966	0.966	0.974	0.968±0.004

Table 4.7: LOQ for Timolol in PBS buffer

LOQ = 10*0.006/0.021 = 2.85 µg/ml

LOD = 3.3*0.006/0.021=0.942 µg/ml

- **Concentration and Spectrophotometric data for Timolol maleate in Artificial tear fluid (pH 7.4):**

  The following data is obtained for the Spectrophotometric estimation of Timolol maleate at 295 nm

Concentration (µg/ml)	Mean Absorbance± S.D.
0	0.000±0
5	0.129±0.018
10	0.230±0.004
15	0.373±0.028
20	0.458±0.013
25	0.571±0.019
30	0.717±0.060
35	0.815±0.069
40	0.964±0.063

Table 4.8: Spectrophotometric data of Timolol in Tear fluid

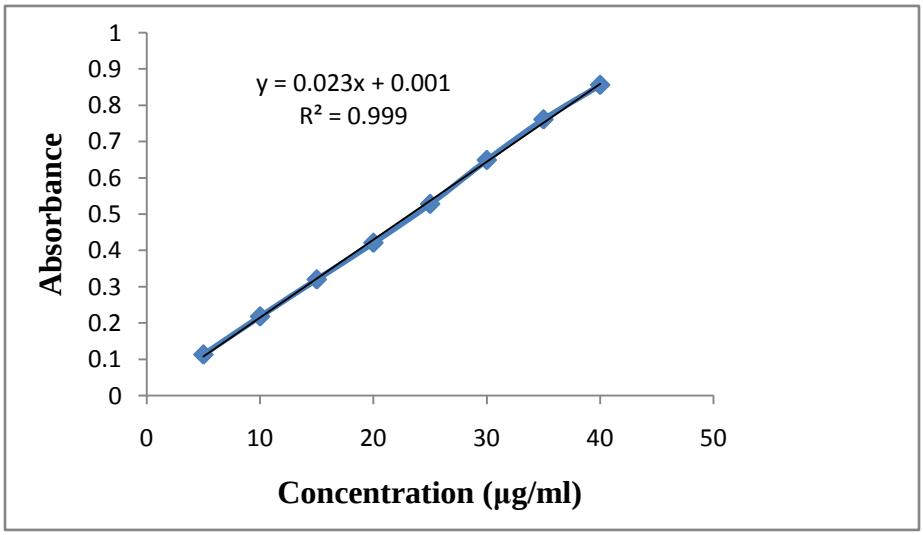


Figure 4.2: Calibration curve of Timolol in Tear fluid

- **Analytical method validation:**
- **Accuracy:**

S. No.	TC	Abs	MC	S.D	RSD	Recovery	Mean of recovery	S. D of recovery	Error%
1.	2	0.049	2.086			104.3			
2.	2	0.045	1.913	0.090	0.045	95.65	99.25	4.503	0.75
3.	2	0.046	1.956			97.8			
4.	20	0.452	19.60			98			
5.	20	0.452	19.60	0.0017	0.0037	98	98.21	0.375	1.79
6.	20	0.455	19.73			98.65			
7.	40	1.015	44.08			110.2			
8.	40	0.984	42.73	0.0595	0.0615	106.8	104.9	6.462	- 4.9
9.	40	0.900	39.08			97.7			

Table 4.9: Accuracy of Timolol in Tear fluid

- **Precision:**

Mean readings of Inter day and intraday investigations of Timolol maleate

Concentration (µg/ml)	Two days Morn average	Two days Aftn average	Two days Evng average	Average ± S.D.	% R.S.D
0	0.000	0.000	0.000	0.000	0.000
5	0.12	0.117	0.151	0.129 ±0.018	13.9
10	0.234	0.233	0.225	0.230 ±0.004	1.73
15	0.387	0.392	0.341	0.373± 0.028	7.5
20	0.462	0.443	0.469	0.458± 0.013	2.83
25	0.575	0.551	0.589	0.571 ±0.019	3.32
30	0.787	0.676	0.689	0.717± 0.060	8.36
35	0.893	0.796	0.758	0.815± 0.069	8.46
40	1.015	0.984	0.893	0.964± 0.063	6.53
45	1.059	1.109	1.101	1.089± 0.026	2.38
50	1.272	1.301	1.318	1.297±0.015	1.156

Morn = Morning Aftn = Afternoon Evng = Evening

Table 4.10: Inter and intraday studies of Timolol in Tear fluid

- **Linearity studies:**

Concentration (µg/ml)	Absorbance
5	0.129
10	0.230
15	0.373
20	0.458
25	0.571

Table 4.11: Linearity estimations of Timolol maleate.

- **LOQ AND LOD:**

S.no	Concentration (µg/ml)	I	II	III	Average ± S.D.
1	0	0.000	0.000	0.000	0.000±0.00
2	2	0.016	0.018	0.016	0.016± 0.001
3	4	0.038	0.038	0.036	0.145± 0.001
4	5	0.111	0.112	0.115	0.112± 0.002
5	6	0.134	0.135	0.113	0.134± 0.001
6	8	0.207	0.204	0.207	0.206± 0.001
7	10	0.229	0.225	0.228	0.227± 0.002
8	15	0.424	0.421	0.420	0.421± 0.002
9	18	0.437	0.439	0.442	0.439± 0.002
10	20	0.452	0.452	0.455	0.453± 0.001
11	25	0.546	0.545	0.552	0.547± 0.003
12	30	0.841	0.838	0.843	0.840± 0.002

13	35	0.928	0.927	0.932	0.929± 0.002
14	40	1.056	1.057	1.057	1.056±0.0005
15	45	1.080	1.084	1.080	1.084± 0.002
16	50	1.210	1.213	1.212	1.211± 0.001

Table 4.12: LOQ studies of Timolol maleate

LOQ = 10*0.003/0.025 = 1.2 µg/ml

LOD = 3.3*0.003/0.025=0.396 µg/ml

3. Drug Compatibility study:

a) FTIR:

The active Pharmaceutical ingredient (Timolol maleate) was estimated using IR spectroscopy using KBR as reference. The main peaks of Timolol maleate observed for corresponding functional groups were reported in the table below.

S.No	Peak observed at (Wave number, cm^{-1})	Represents
1	1072 cm^{-1}	COH stretch (s)
2	1120 cm^{-1}	COC stretch (s)
3	3298 cm^{-1}	NH stretch (m,w)
4	3545 cm^{-1}	OH stretch (s)

Table 4.13: Main peaks of Timolol maleate in FTIR

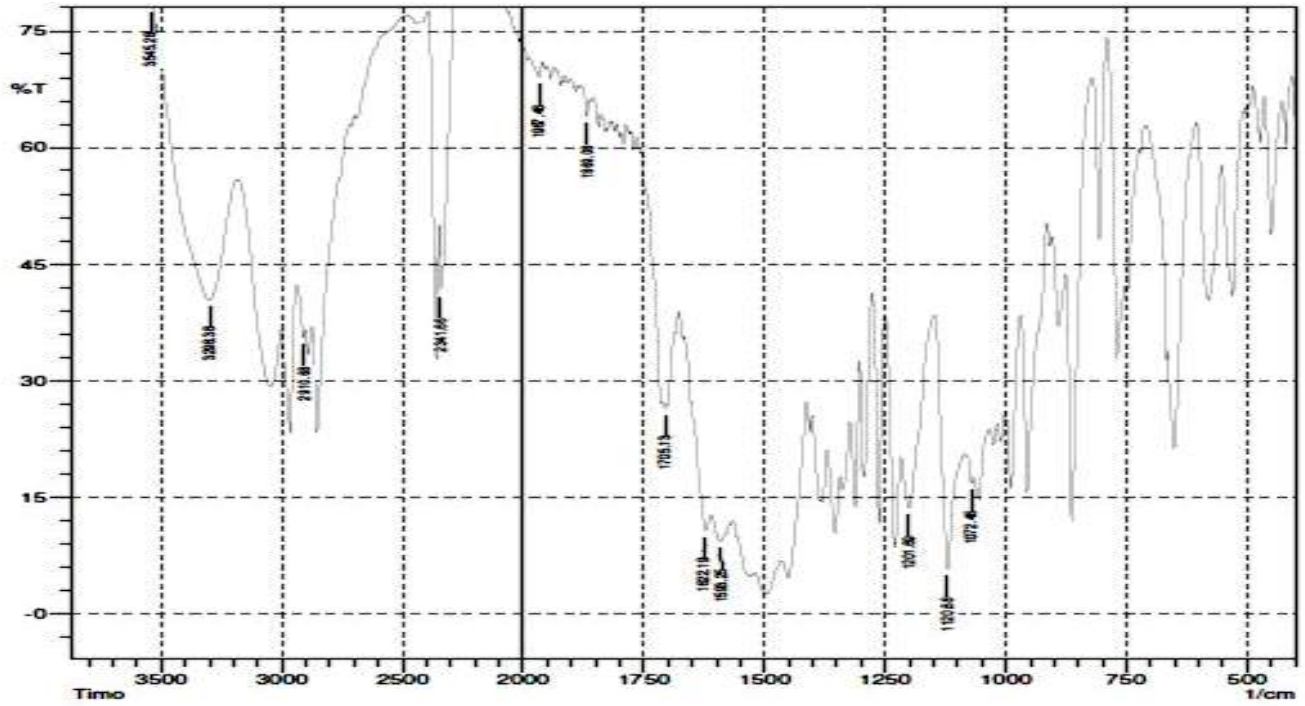


Figure 4.3: FTIR spectra for Timolol maleate

The FTIR peaks and corresponding functional groups for the mixture of drug and excipients were observed as follows:

S.No	Mixture of drug with Excipient	NH stretch (cm^{-1})	OH stretch (cm^{-1})	COC stretch (cm^{-1})	COH stretch (cm^{-1})
1	Heavy Liquid paraffin	3545	3298	1118	1085
2	Lanolin	3537	3300	1122	1080
3	Carnauba wax	3550	3343	1120	1080
4	Bees wax	3547	3327	1122	1089
5	Yellow Petrolatum	3550	3302	1122	1074
6	Cetyl alcohol	3545	3300	1122	1064
7	IPM	3550	3343	1120	1080
8	Cholesterol	3545	3298	1120	1072
9	Tocopherol acetate	3334	3550	1111	1063
10	Benzalkonium chloride	3300	3539	1114	1078
11	Carbon black	3491	3547	1116	1057
12	Polaxomer 407	3300	3539	1114	1078
13	Brij 35	3304	3539	1114	1078

14	SLS	3304	3539	1226	1080
15	Span 80	3306	3549	1226	1072
16	Bile salt	3539	3304	1114	1078
17	EDTA	3531	3549	1120	1072

Table 4.14: Peaks of Drug-Excipient mixtures using FTIR

b) UV-Spectrophotometry:

After a 7 day exposure to the high temperature and room temperature, the UV peaks observed for the mixtures of drug and excipients were given in the Table 4.14.

S.No.	Ingredient	Solvent	Peak observed at
1	Timolol+ Tocopherol	Ethanol	293.00nm
2	Cholesterol+ Timolol	Hot alcohol	297.00nm
3	Lanolin+ Timolol	Ethanol	294.00nm
4	IPM + Timolol	Ethanol	298.00nm
5	IPM+ Timolol	Ethanol	298.00nm
6	IPM+ Timolol	Ethanol	298.00nm
7	IPM+ Timolol	Ethanol	298.00nm
8	Cetyl alcohol+ Timolol	Ethanol	299.00nm
9	Cetyl alcohol+ Timolol	Ethanol	298.00nm
10	Bees wax + Timolol	Chloroform	297.00nm
11	Bees wax +Timolol	Chloroform	297.00nm
12	Liquid paraffin + timolol	Chloroform	297.00nm
13	Liquid paraffin + timolol	Chloroform	297.00nm
14	White petroleum wax+ timolol	Chloroform	294.00nm
15	White petroleum wax+ timolol	Chloroform	294.00nm

Table 4.15: Peaks of Drug-Excipient mixtures using UV-Spectrophotometer

4.1.5. Discussion:

Timolol maleate was odorless and white powder. The melting range of Timolol maleate was found to be between 201.5^0C-202.5^0C. A simple, inexpensive method was developed to estimate the drug by UV-Spectrophotometry.

The estimation of drug using UV analysis was done both in simulated tear fluid (pH7.4) and Phosphate buffer saline (pH7.4) as they both simulate the human tear fluid and human blood respectively. Timolol maleate showed its maximum absorbance (λmax) at 295nm and 293.60nm with regression coefficient of 0.997 and 0.999 respectively in STF and PBS saline.

The method was validated according to ICH (Q2) guidelines. Accuracy of the method developed was assessed by determining the mean recoveries of three different concentrations. Accuracy of estimation of Timolol in PBS acquired the mean recovery of 101.03±1.3279, 97.55±1.3892, 98.66±2.7442 for 2μg/ml, 20 μg/ml and 40 μg/ml respectively. The accuracy of estimation of Timolol in STF obtained the mean recovery of 99.25±4.503, 98.21±0.375, 104.9±6.462 for 2μg/ml, 20 μg/ml and 40 μg/ml respectively. Accuracy determination revealed that the mean recovery values were in the acceptable range of 95-104± S.D.

Linearity studies of Timolol maleate estimation in PBS saline in the range of 2.00 μg / ml to 10.00 μg /ml accomplished linearity with slope (m) 0.0218, intercept (c) is -0.0044 (r^2 = 0.999). Linearity studies of Timolol maleate estimation in STF in the range of 2.00 μg / ml to 10.00 μg /ml achieved linearity with slope (m) 0.023, intercept (c) is 0.001 (r^2 = 0.997).

Precision studies were performed for the Timolol maleate estimation in both Tear fluid and PBS for 2 days and on the same day for same experimental conditions. The obtained % R.S.D values in Tear fluid was less than 10% and 5% in PBS saline. Inter and intraday variation investigations concluded that less R.S.D values confirms that the method is sufficiently precise and variations are least.

LOQ and LOD values for timolol maleate in PBS saline were found to be 2.85μg/ml and 0.942μg/ml respectively. LOQ and LOD for timolol maleate in STF were found to be 1.2 μg/ml and 0.396 μg/ml respectively. These results demonstrated that the analysis was performed in a region above the quantification limit value.

IR-Spectrophotometric analysis was performed in order to check the purity of the drug. IR analysis of the blend of drug and excipients exhibited the same peaks at a difference of 2-10 wave numbers in the same region of the functional groups. The mixture of Activated charcoal and drug presented the broadened peak illustrating that there is some physical adsorption. Besides this, no

other ingredient showed any significant changes confirming that there is no incompatibility between drug and excipients.

UV-Spectrophotometric analysis of the mixtures of drug and excipients was also performed to find the compatibility between the active ingredient and excipients after a 7 day exposure to diverse conditions. The maximum absorbance peak values obtained for the mixtures in different solvents was found to be between 293-298nm. The slight change in the λmax was due to solvent effect and verifying that there are no interactions between the ingredients and drug.

4.2. Formulation Development and Evaluation:

4.2.1. Formulation Development:

4.2.1.1 Screening study of formulations for Novel ophthalmic formulation:

Formulations were prepared using different components. The formulations with good physical properties and percentage drug release were selected and subjected for further trials.

Table 4.16: Formulation Trail1, for the selection of formulation which present the best physical properties and % drug release:

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Liquid paraffin	31	31	31	31	29	29	29	29
Carnauba wax	-	-	-	-	5	5	5	5
Bees wax	23	23	23	23	23	21	21	21
Petroleum wax	23	23	23	23	21	21	21	21
Lanolin	10	10	10	10	10	9	9	9
Cetyl alcohol	8	8	8	8	8	8	8	8
IPM	2.5	3	3	3.5	2.5	3	3	3.5
Cholesterol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Tocopherol acetate	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Benzylkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Charcoal	0.5	-	0.5	-	0.5	-	0.5	-
Drug	1	1	0.5	0.5	1	1	0.5	0.5

Table 4.17: Formulation Trail 2, for the selection of best permeation enhancer

Ingredient	F9	F10	F11	F12	F13	F14
Liquid paraffin	31	31	31	31	31	31
Bees wax	-	-	-	-	-	-
Petroleum wax	23	23	23	23	23	23
lanolin	23	23	23	23	23	23
Cetyl alcohol	10	10	10	10	10	10
IPM	8	8	8	8	8	8
EDTA	0.05	-	-	-	-	-
Polaxomer	-	0.05	-	-	-	-
Bile salt	-	-	0.05	-	-	-
Span	-	-	-	0.05		-
SLS	-	-	-	-	0.05	-
Brij35	-	-	-	-	-	0.05
Cholesterol	0.2	0.2	0.2	0.2	0.2	0.2
Tocopherol acetate	q.s	q.s	q.s	q.s	q.s	q.s
Benzylkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02
Charcoal	0.5	0.5	0.5	0.5	0.5	0.5
Drug	1	1	1	1	1	1

4.2.1.2. Procedure for preparation of Novel ophthalmic formulation:

- Accurate amount of all Ingredients and drug were weighed separately.
- Except Tocopherol, IPM and Benzalkonium chloride all the other ingredients were melted according to their melting point.
- The drug was dissolved in water according to its solubility (1 in 15 of water). When a permeation enhancer i.e., soluble in water is used in the formulation, it was dissolved in water along with the drug.
- The drug solution is dispersed into the molten waxes by stirring.
- Charcoal was added and stirred continuously until it distributes uniformly.
- Then Tocopherol, IPM and Benzalkonium chloride were added and stirred.
- The formulation is transferred to well closed containers of metal or plastic or barrel.
- They can be stored under refrigerator conditions or 40^0C and 75 %RH for stability studies.

4.2.1.3. Evaluation of Formulations:

Following parameters were tested for the developed formulation(s):

1. **Uniformity drug content:**

   Each formulation of 0.5gm was accurately weighed and transferred to beaker containing 50mlof ethanol. Then the solution was filtered using Whitman filter paper and the filtrate was diluted 10 times with ethanol. The absorbance values were determined using UV-Spectrophometer at 298nm. All the determinations were done in triplicate and mean values were considered. The % drug content was expressed in mean ± standard deviation.

2. **Peroxide content:**

   Peroxide content indicates the rancidity in unsaturated fats and oils which are at high risk of autoxidation. It is expressed in Milliequivalents of active oxygen per 1000g sample.

   1g of kajal was weighed into a small beaker and dissolved it in 10ml of petroleum ether with heating. The resultant was filtered with Whitman filter paper for 3 times. Each time the beaker was washed with the hot petroleum ether. To the filtrate, 6ml of 3:2 ratio of acetic acid and chloroform, 0.1ml of KI solution were added and transferred to stoppered

conical flask and allowed to stand for 1 minute with occasional stirring. Then 6ml of distilled water and 2-3 drops of starch indicator were added and titrated with 0.01N sodium thiocynate solution until the color changes from dark blue to light grey color. Then the volume of 0.01N sodium thiocynate consumed was noted. The whole procedure was done in triplicate and the average readings were considered. (BIS 2002)

It was calculated by using the following formula:

$$\text{Peroxide value} = \frac{V*N*1000}{\text{Mass of formulation in grams}}$$

Where,

V = Volume of 0.01N Sodium thiocynate consumed

N = Normality of Sodium thiocynate

3. **Melting range:**

The formulation was melted and it was filled to clean capillary tube by inserting the tube into molten formulation so that the formulation is forced into the tube. The other edge was sealed using burner flame. The tube was positioned in the beaker and stored in refrigerator conditions overnight. Then the tube was attached to thermometer with the help of a thread or rubber band. The tube and thermometer were placed in a 500ml beaker containing 250ml of water which was placed inside a water bath. Note the temperature where the formulation after the formulation completely clears out. Mean of two such determinations was taken as the melting point of formulation. (BIS 2002)

4. **Viscosity:**

The viscosities of the formulations were determined by Cone and plate Viscometer (). Viscosities of the formulations were expressed in centistokes. Measurements were done in triplicate and the average values were reported.

5. Spreadability:

The term spreadability illustrates the ability of the formulation to the extent it spreads on the site of application. It is denoted by 'S". It is expressed in gram.centimeter.seconds^{-1} (gm.cm/sec). Pre-weighed quantity of formulation was taken and placed between the slides (2, movable slide towards upper side and immovable beneath the formulation tied to a load). Then a kilogram weight was positioned on the movable slide to remove any entrapped air bubbles for 5minutes. (Hadi etal., 1989; Vennet Betal., 1994) The weight was removed after 5 min and the time taken for separation of two slides from each other in the direction of load. More the time taken for separation, lesser is the spreadability. (Vennet etal., 1995; Panigrahi etal.,1997)

Spreadability was calculated using the following formula,

$$S = M \times L/T$$

Where,

S = Spreadability of the formulation

M = Mass of the load attached to immovable slide

L = Length of the slide

T = Time taken for separation of two slides

6. Consistency:

It was measured by the distance travelled by a cone shaped material when dropped from a 10cm height into the beaker containing formulation. Consistency is expressed in centimeters (cm) or millimeters (mm). The experiment was performed in triplicate and the results were reported as mean consistency ± standard deviation.

7. Freedom from grittiness:

Around 0.5g of formulation was taken and was spread on the polyethylene sheet. Then the formulation was tested for any abrasive or hard particles by pressing along its length. (BIS 2002).

8. Microscopic evaluation:

A small amount of formulation was taken and was spread evenly on a glass slide. It was observed under a Trinocular microscope under 100X using immersion oil.

9. Dye test:

As the formulation contains both the aqueous phase and oil phase, dye test is performed to distinguish them. A small amount of formulation was taken and was mixed with a water soluble dye, Amaranth. This was evenly spread on a glass slide and observed under a Trinocular microscope under 100X using immersion oil.

10. Drug Permeation studies:

Invitro Permeation studies were performed using a cellophane membrane of molecular weight of 12000D (Glycerin treated). The cellophane membrane was clamped to hollow open end glass tube and was dipped inside the media (100ml) taken in a beaker (receptor). Permeation studies was done in both using PBS saline and STF which was taken in receptor compartment. Exactly 1gm of formulation was weighed and placed on the membrane in donor compartment (hollow glass tube). The solution in receptor compartment is stirred continuously using magnetic stirrer with a Teflon coated magnetic bead. Both the compartments should be in contact with each other and maintained at 37 ± 2^0C.At pre-determined intervals, samples of 5ml were collected and replaced with fresh buffer solutions to maintain the sink conditions. The samples were analyzed and the concentrations of drug in the samples were determined using UV-Spectrophotometer at 295nm for STF and 293.60nm for PBS. The experiment was conducted in triplicate and average values were reported.

11. Invitro eye Irritation test:

Invitro eye irritation severity was determined by HET-CAM bioassay. Fresh, hygienic, fertile chicken eggs were obtained from commercial sources. The eggs were checked for their practicability and development of embryo before use. Three eggs are used for each group in the assay. (Dehelean CA etal.,2011) .These eggs are incubated at 37 ± 2^0C and 58% RH for 8days. Bioassay is conducted on 9^{th} day. Only **F1** and **F10** were selected for the ocular irritation test.

Negative control group:

0.3ml of 0.9% NaCl was applied on the choroiallantoic membrane to give a pattern to measure endpoints and to confirm that the experimental conditions do not inappropriately bring in an irritant response.

Positive control group:

0.3ml of 1% SDS and 0.1N NaOH were applied on choroiallantoic membrane to get the severe response in HET-CAM.

Treatment group:

0.3ml of melted formulation was applied on choroiallantoic membrane on the 9^{th} day. Response is observed in the surrounding environment for 300seconds. The time where hemorrhage, lysis, coagulation was noted.

The Iriitation score was calculated using the following formula,

IS= {(301-H) 5/300} + {(301-L) 7/300} + {(301-C) 9/300}

Where,

H= Time taken to start hemorrhage reaction

L= Time taken to start Lysis reactions

C = Time taken to start Coagulation reactions

The procedure is repeated for 3 times and average irritation scores are reported. According to the Irritation score, severity of the reaction can be confirmed as follows,

If Irritation score is 0, there is no reaction, 1 represents slight reaction, 2 denotes moderate reaction, and 3 illustrate severe reaction.

12. Sterility testing:

Sterility testing is done check the purity of the formulation. It is performed by using Direct Inoculation technique. Soyabean casein digest media was used for the sterility testing which is suitable for the growth of both aerobic bacteria and fungi. Test tubes used for the sterility testing were sterilized in a hot air oven at 170^0C for an hour. Then the media was prepared according to the composition prescribed on the label. 10ml of media was transferred to the sterilized test tubes and the media was sterilized in an autoclave. The formulations were melted and less than 1ml of formulation was transferred into the test tubes containing media in aseptic conditions using LAF. The test tubes were incubated in an incubator at 22.5 ± 2.5^0C. They were observed for growth for 14 days. A

positive control and negative control was used for the comparison of results. If growth is observed, it is reported in CFU/g.

13. FTIR analysis:

The formulations **F1** and **F10** were analyzed using Fourier Transform Infra red Spectroscopy using Schimadzu FTIR 8400S model. The spectrum peaks of the drug were compared with the peaks obtained for the formulations. (John RD.2007; Robert M S and Francis S W, 6^{th} edition)

14. Texture analysis:

Texture analysis was performed by using TA.XT Plus Texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey GU7 1YL, UK). Formulations were put into the female cone of the equipment and it was compressed to make it free from the entrapped air. The excess formulation was removed to provide smooth region. Before performing the test, the formulation should reach room temperature. The test was performed in triplicate and average values were reported. The male cone measures the penetration and detachment force (grams) and work of shear and adhesion put by male cone on formulation. (Cevher E etal. 2008)

15. Stability studies:

The selected formulations F1 and F10 were tested for stability under the conditions of 45^0C and 75% RH and refrigerator conditions for one month.

4.2.1.4. Results:

- **Screening of Formulations:**

For the formulation trial 1, eight formulations (F1 to F8) were developed. F5 to F8 differ from the former four formulations in having Carnauba wax as the additional ingredient and Liquid paraffin, Petroleum wax was decreased by 3% and 2% respectively. The formulations F1 to F4 developed differ in the concentrations of charcoal and drug. The formulations containing charcoal were black in color and the formulations without it were cream or half white in color. All these formulations acquired the shape of the mould in which they are dispensed.

The formulations from F5 to F8 did not satisfy the fundamental properties of kajal formulation. These formulations did not take the shape of the mould in which they are dispensed. Instead of presenting black color, these formulations were changed to light green color in which charcoal was present which are may not be acceptable to the subjects using them. Among all the formulations from F1 to F8, **F1** is selected as best formulation while considering acceptable criteria such as appearance, mould forming ability, and % drug release .

In the formulation trail 2, selected **F1** formulation (trail 1) composition is used for the development of formulations F9 to F14. The concentration of IPM is changed in trial 2 formulations so that the six ocular permeation enhancers such as EDTA, Polaxomer 407, Bile salt, Span 80, SLS, and Brij 35 were selected and incorporated into the formulation. These formulations were developed and evaluated for various parameters like melting range, spreadability, peroxide content, viscosity, freedom from grits, consistency, uniformity content, Drug permeation studies and Invitro eye irritation studies.

- **Evaluation of Formulation:**

Table 4.18. Evaluation parameters of formulations

Formulation	Melting range (^{o}C)	Spreadability (gm.cm/sec)	Peroxide content	Viscosity (Centistokes)	Freedom from grits	Consistency (mm)	Uniformity content (%)
F1	**54±2**	**21.11±1.154**	**2.6±0.288**	**434.63±4.201**	**passed**	**3.33±0.577**	**99.47±0.0458**
F2	54±2	18.66±0.456	2.8±0.288	433.5±3.351	Passed	4.33±0.577	96.84±0.5597
F3	52±2	18.71±1.19	2.6±0.288	327.2±2.251	Passed	3.6±0.577	97.89±0.01
F4	52±2	18.95±0.825	2.3±0.577	323.4±3.593	Passed	4.3±0.577	98.94±0.6359
F5	48±2	22.96±2.7306	4.3±0.288	324.1±3.8105	Passed	4.6±0.577	91.05±0.1058
F6	48±2	24.39±1.2104	2.16±0.288	323.4±3.593	Passed	4±0	87.89±0.3464
F7	50±2	22.15±0.6639	3.3±0.288	327.13±4.539	Passed	4.6±0.577	85.26±0.0458
F8	50±2	20.45±1.1431	3.3±0.288	326.43±3.931	Passed	5±0	89.47±0.1458
F9	54±2	21.91±2.203	2.8±0.288	432.3±1.5307	Passed	3.3±0.577	96.73±0.07
F10	**54±2**	**22.92±3.841**	**2.6±0.288**	**432.8±2.920**	**Passed**	**3**±0	**99.73±0.2631**
F11	52±2	20.45±1.143	2.6±0.577	325.43±3.324	Passed	3±0	97.89±0.0556
F12	54±2	19.50±0.490	2.16±0.288	433.16±2.973	Passed	4±0	98.42±0.0519
F13	54±2	19.9±1.818	2±0	432.8±2.909	Passed	4±0	97.63±0.0818
F14	52±2	19.50±0.4965	2.16±0.288	431.3±0.40	passed	3.6±0.577	99.47±0.0346

- **Drug Permeation studies:**

Table 4.19: Drug permeation studies

Formulation	Mean % Drug release in STF	Mean % Drug release in PBS
F1	**51.05±0.065**	**49.61**±0.105
F2	43.15±0.060	40.05±0.425
F3	39.26±0.015	36.91±0.144
F4	37.17±0.115	36.19±0.454
F5	37.93±0.040	35.11±0.087
F6	35.13±0.1001	34.07±0.42
F7	36.81±0.049	34.63±0.045
F8	32.13±0.1013	30.95±0.269
F9	53.65±0.888	51.77±0.196
F10	**64.38±0.157**	**61.91±1.06**
F11	52.17±0.155	50.95±0.622
F12	56.02±0.196	53.54±0.175
F13	57.01±0.158	56.33±0.2778
F14	59.53±0.1777	57.01±0.108

Microscopic evaluation:

- Microscopic evaluation of the formulation was showed in the figure 4.4 and figure 4.5

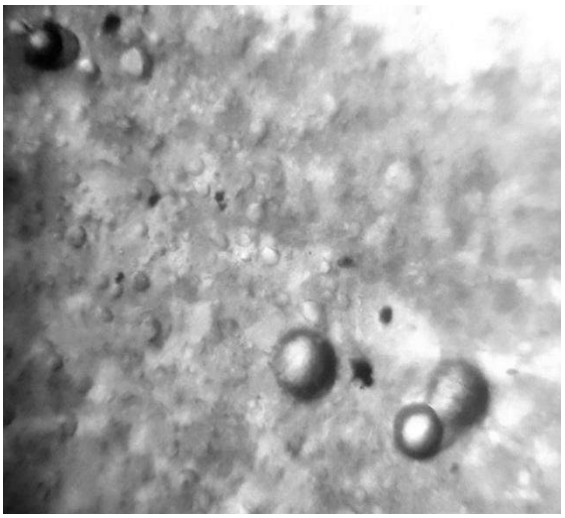


Figure 4.4: Microscopic evaluation of Formulation

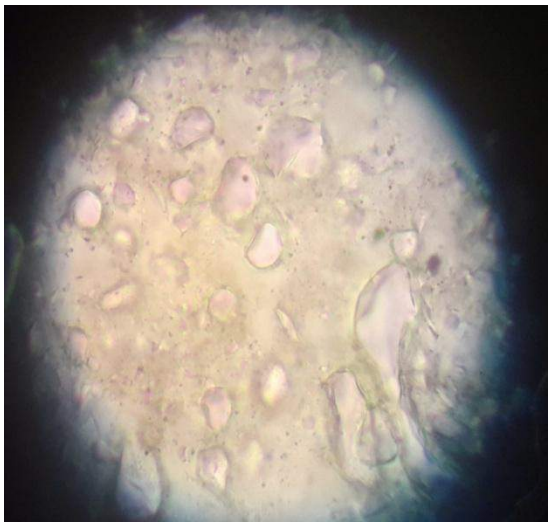


Figure 4.5: Dye test for the formulation

Sterility testing:

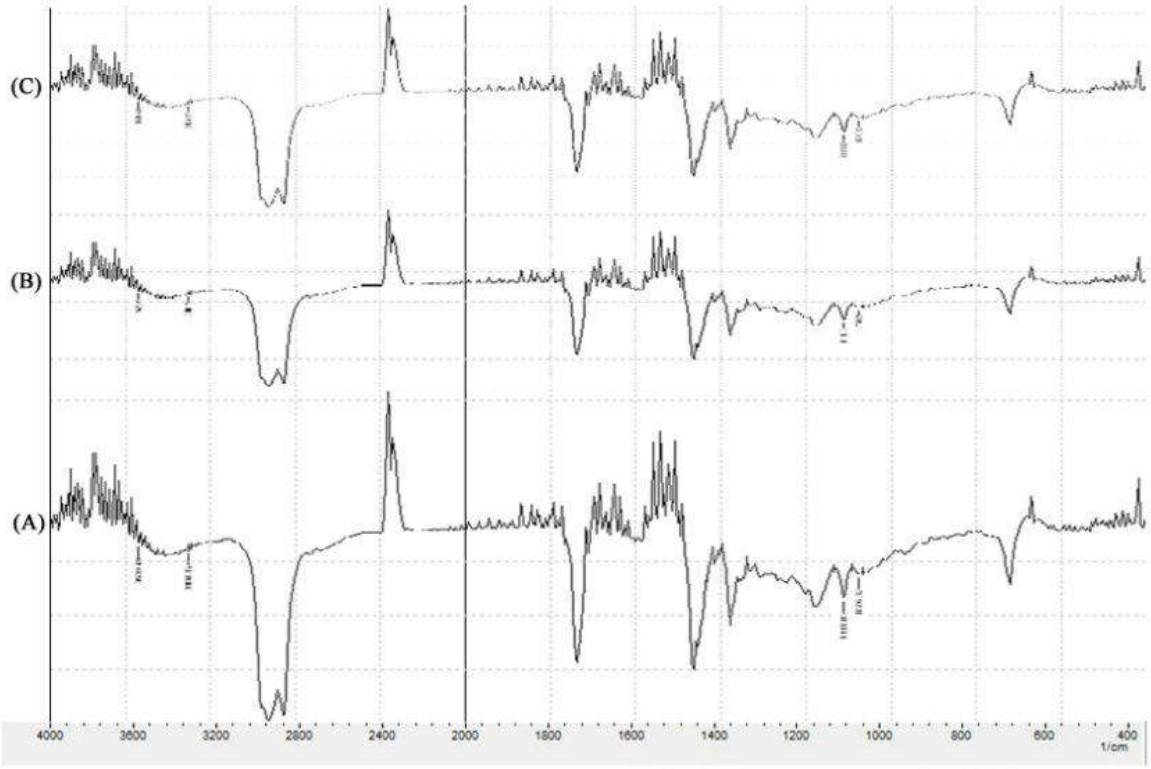
Sterility testing results were depicted in the following figures

Figure 4.6: Sterility testing for F1 to F4 formulations

Figure 4.7: Sterility testing for F5 to F8 formulations

Figure 4.8: Sterility testing for F9 to F14 formulations

- **FTIR studies:**



A: FTIR spectra for the drug; **B:** FTIR spectra for the F1 formulation; **C:** FTIR spectra for the F10 formulation

Figure 4.9: FTIR spectra of formulations

- **Drug permeation studies:**

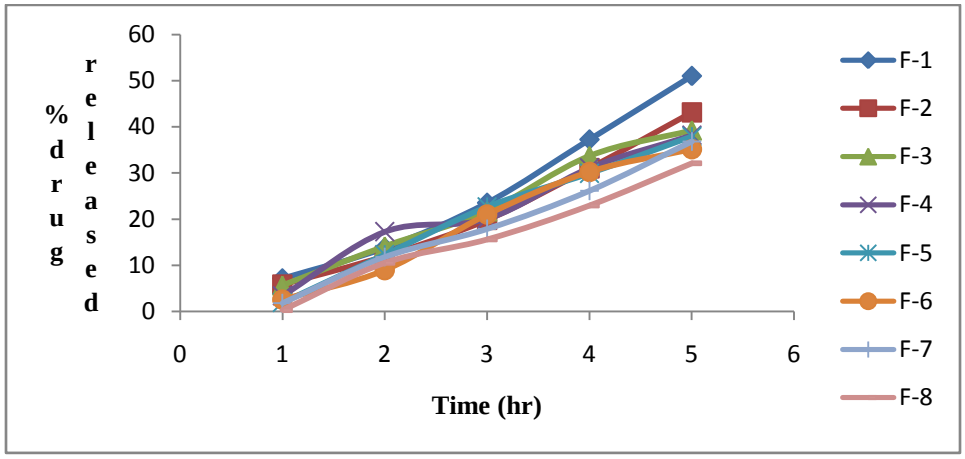


Figure 4.10: Percent Cumulative drug release Vs Time plot for F1-F8 in STF

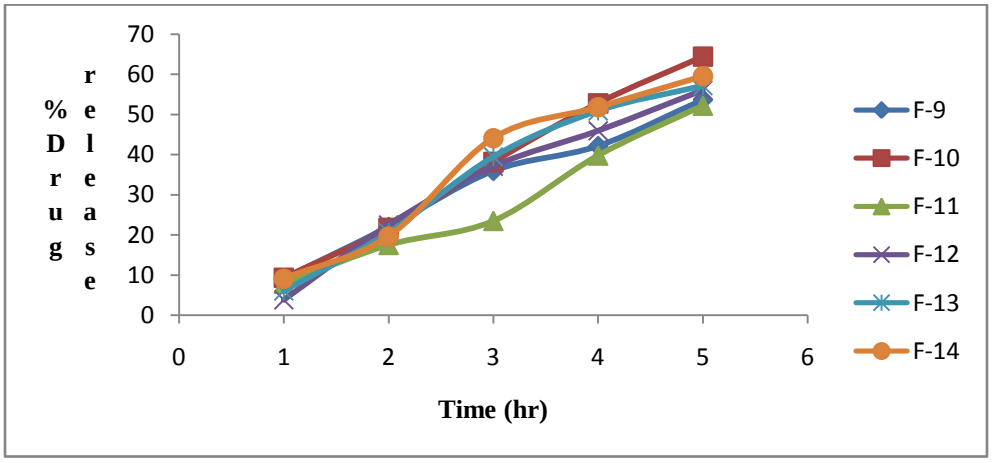


Figure 4.11: Percent Cumulative drug release Vs Time plot for F1-F8 in STF

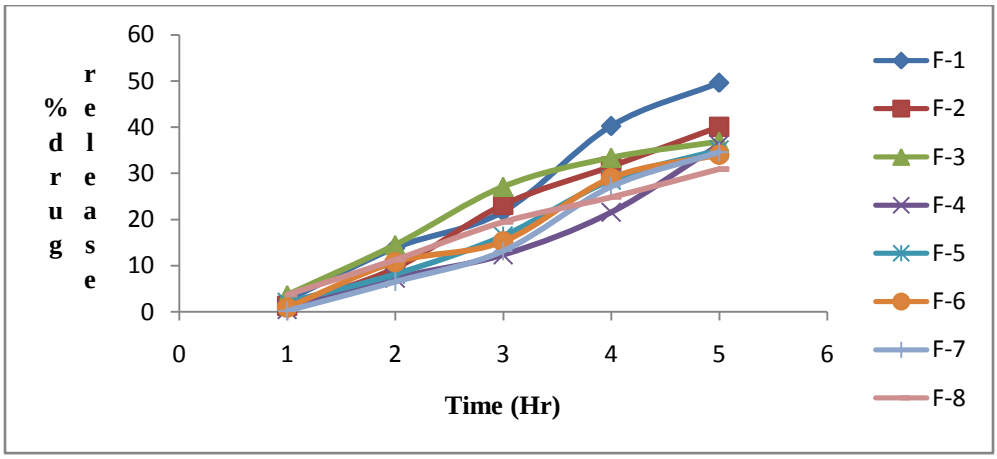


Figure 4.12: Percent Cumulative drug release Vs Time plot for F1-F8 in PBS

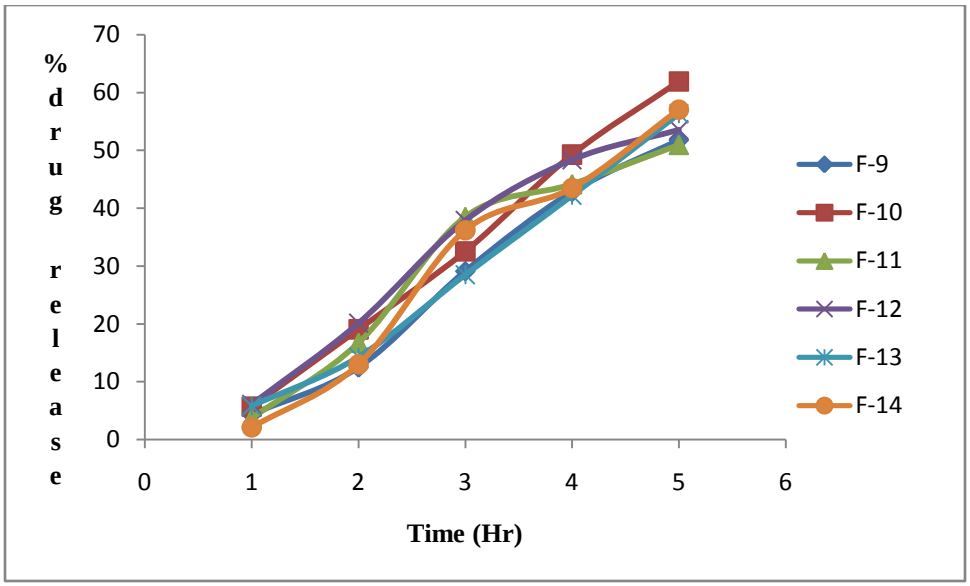


Figure 4.13: Percent Cumulative drug release Vs Time plot for F1-F8 in PBS

Kinetics of Drug release:

Table 4.20: Kinetics of the drug release in both STF and PBS.

Mechanism	F 1 in STF	F 10 in STF	F 1 in PBS	F 10 in PBS
Zero order	0.980	0.997	0.998	0.998
First order	0.952	0.987	0.980	0.980
Higuchi	0.937	0.986	0.982	0.982
Korsmeyer Peppas	0.811	0.885	0.863	0.863

- **Texture analysis:**

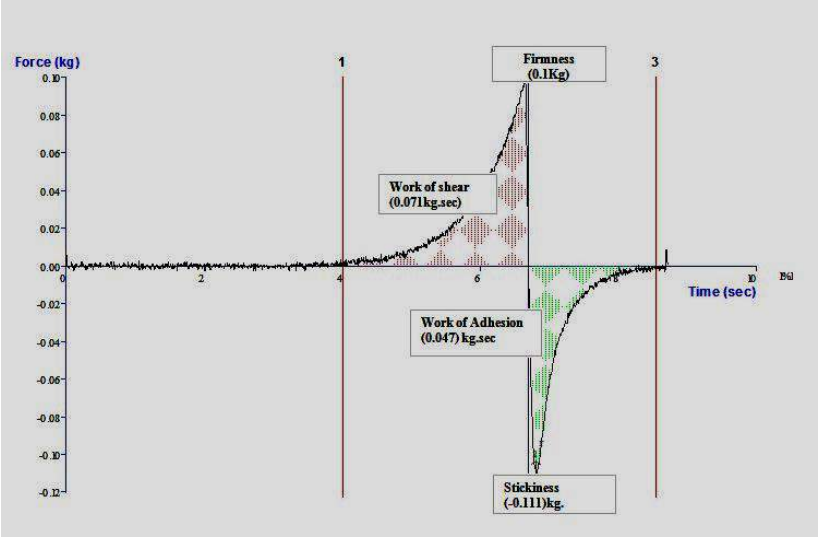


Figure 4.14: Texture analysis for F1

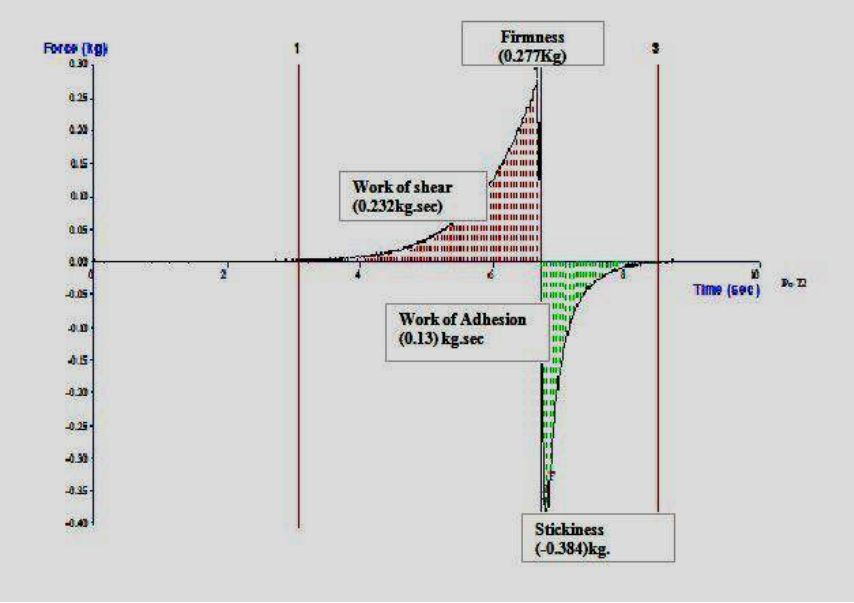


Figure 4.15: Texture analysis for F10

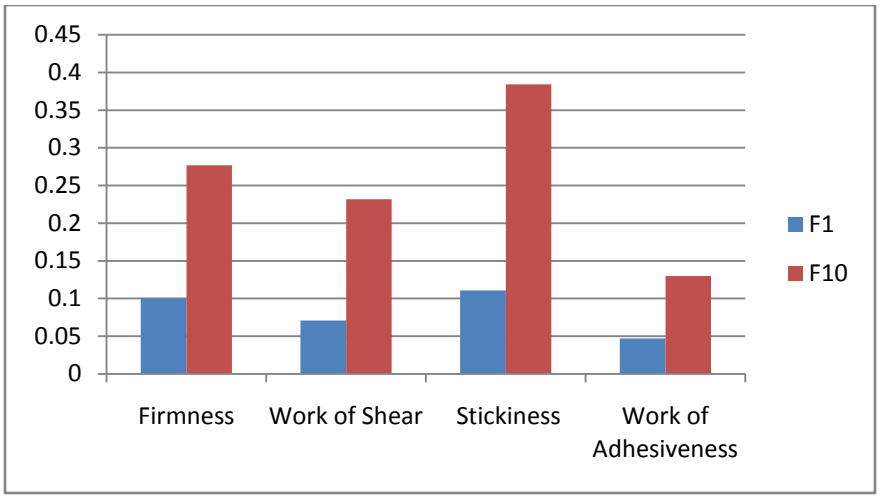


Figure 4.16: Comparative Bar graph for F1 and F10

4.2.1.5. Discussion:

- **Evaluation of Formulation:**

The uniformity drug content analysis corroborates that all the formulations were in the range of 85-100% which is acceptable, confirming that the drug is evenly dispersed in the lipophilic matrix and there is no reaction between the drug and other ingredients.

According to the Bureau of Indian Standards (2002) for kajal specification provided in the IS 15154 (2002): Kajal [PCD 19: Cosmetics], the peroxide content for the kajal preparation should not exceed 10 Milliequivalents per 1000g of formulation. All the formulations F1-F14 reported peroxide content below 5 M.eq/1000g. The results provide the evidence for no rancidity occurred in the formulations due to fats and oils.

All the formulations have shown good melting ranges as per the Bureau of Indian Standards (2002) for kajal specification provided in the IS 15154 (2002): Kajal [PCD 19: Cosmetics]. Only the formulations containing Carnauba wax (F5, F6) have shown some variation from the ideal melting range for cosmetics. The melting range for all the formulations were reported as M.P ± 2^0C in the table 4.18.

The viscosity of all the formulations was expressed in centistokes ± S.D. The obtained % R.S.D values of viscosity of all the formulations were less than 2%. Higher viscosity values of the formulations prove that the formulation applied at the site would not drain out of the eye due to lacrimal drainage such that the Bioavailability is increased and also improves the spreading ability of the formulation.

Among all the formulations, F1, F5, F6, F7, F9, F10, and F11 exhibited more spreadability than that of the formulations F2, F3, F4, F8, F12, F13, and F14. This indicates that the formulations have good spreading ability at the site of application to deliver the standard dose.

Consistency of the formulations is more important factor as it affects the spreading ability and viscosity of the formulation. All the formulations exhibited good consistency property and the results were precise in manner.

The formulations should be free from hard and abrasive foreign particles as they are directly applied on to most sensitive sense organ. So the test for grit particles confirmed that the formulations were free from rigid particles which prevent various irritation reactions.

Microscopic evaluation of the formulation was depicted in the figure 4.4 and figure 4.5. As the drug is dissolved in water and dispersed in lipophilic matrix, the globules represent that the drug molecules and surrounding matrix represents the lipophilic base. Dye test evaluation proves that the formulation is w/o emulsion type. It is confirmed from the color of scattered globules which appear red to pink in color due to the addition of water soluble dye Amaranth.

Drug permeation studies revels that **F1** formulation showed the highest release in trial 1 i.e. 51.05% in STF and 49.61% in PBS. So it is selected as basic formulation for the trail 2. Among the formulations in trial 2, **F10** formulation exhibited highest %DR of 64.38% in STF and 61.91% in PBS buffer. In trail 2, the % drug release order in both PBS and STF for permeation enhancers is as follows:

Polaxomer 407 > Brij35 > SLS > EDTA > Span 80 > Bile salt.

Kinetic studies of the drug release reveal that the drug release follows Zero order release and Higuchi mechanism.

The invitro ocular irritation bioassay shows that the formulations **F1** and **F10** has less than 1 as mean irritation score which indicates zero irritation severity and no reaction. This confirms that there will be no hypersensitive reaction on the biomembrane.

Kinetics of the drug release mechanism reveals that the Formulations **F1** and **F10**

Sterility testing of the formulations provided evidence that there was no microbial growth after incubation for 14 days. Both the Positive controls ascertain that the media is suitable for the growth of both aerobic bacteria and fungi. Negative control of the test did not promote any growth in the media. They were visibly depicted in the figure 4.6, figure 4.7 and figure 4.8.

FTIR analysis of the formulations did not show any significant variations in the spectrum peaks from the original spectral peaks of the drug. This confirms that there is no interaction between the drug and other ingredients when developed into formulation. Also the drug did not undergo any significant changes itself in the formulation.

Texture analysis of F1 and F10 formulations shows that F10 formulation required high force to penetrate and withdraw the male cone. These results confirmed that F10 formulation was 3 times stickier than the F1.

Stability testing concludes that F1 formulation found to melt at high temperatures where as F10 retained its original nature.

Chapter-5

Summary and Conclusion

Glaucoma is an intraocular disease which is the vital reason of blindness worldwide specifically in elder people. It is caused due to the damage of sensitive nerve fibres of optic nerve which carries the images from retina to the brain. The damage of optic nerve and loss of vision is permanent.

The major problem associated with ophthalmic formulations is precorneal elimination of the drugs by drainage and lacrimal washing leading to their incomplete absorption and bioavailability. Besides these problems, the conventional dosage forms also should satisfy certain parameters such as sterilization, pH, osmolarity and particle size. Hence, there is need of novel formulation and strategies which can overcome the problems. In the current world the attempts are going on to develop and explore the potential of some topical formulations.

The present research focuses on development of a novel ophthalmic formulation which provides continuous contact of drugs with cornea, improved bioavailability and patient compliance due to its combined drug delivery and cosmetic characteristics.

In pre-formulation studies, different suitable components were selected. Formulations were developed using a simple and non tedious process and they were evaluated for the parameters such as viscosity, grittiness, spreadability, texture analysis, *in-vitro* permeation study and peroxide content. From the first trial of eight formulations, a formulation (F1) was selected based on percent drug release, physical characteristics and it was used for the further trials. In the next trial (2), six permeation enhancers (Polaxomer 407, EDTA, Bile, Span 80, SLS, Brij35) were used in the selected formulation in trial 1. These formulations were compared in terms of percent drug release and uniformity content. Finally the formulation containing Polaxomer 407 (F10) was selected in trial2. Finally, one formulation from trial 1 and trial 2 each were selected

for texture analysis and *in-vitro* eye irritation test. In texture analysis, all the parameters for both formulations were found to be appropriate and F10 was found to be stickier than F1. In *in-vitro* eye irritation test, both the formulations were found to be same.

In stability testing, F1 formulation found to melt at high temperatures where as F10 retained its original nature. Based on all the observations, F10 was found to be more appropriate and can be used for the further studies including invivo evaluation.

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Chapter-7

Supplementary Data

1. Preparation of Simulated Tear fluid (7.4 pH):

Weigh 6.7g of NaCl, 0.2g of $NaHCo_3$, 0.08g of $CaCl_2$.$2H_2O$ using a analytical balance and dissolve all pre-weighed chemicals the in 100ml of distilled water 1000ml volumetric flask. Then make up the volume upto 1000ml using distilled water. Adjust the pH to 7.4 with HCl or NaOH.

2. Preparation of Phosphate Buffer Saline (7.4 pH):

Weigh 2.38g of $NaHPO_3$, 0.19g of KH_2PO_4 , 8g of NaCl using a analytical balance and dissolve all pre-weighed chemicals in 100ml of distilled water in a 1000ml volumetric flask. Then make up the volume upto 1000ml using distilled water. Adjust the pH to 7.4 with HCl or NaOH.

3. Preparation of 0.01N Sodium thiosulphate solution:

Dissolve 2.5g of Sodium thiosulphate in 100ml of distilled water in a 1000ml volumetric flask. Then make up the volume upto 1000ml using distilled water.

4. Preparation of Starch Indicator solution:

Weigh 1g of starch and dissolve it in 100ml of distilled water. Transfer this solution to 100ml of boiling water. Te supernant liquid can be used as starch indicator.

5. Preparation of Potassium Iodide solution:

Dissolve 14.4g of Potassium iodide in 10g of water to give potassium iodide solution.

6. Preparation of 0.9% Sodium Chloride solution:

Weigh 0.9g of NaCl and dissolve it in 100ml of distilled water to give 0.9% Sodium chloride solution.

7. Preparation of 1% SDS solution:

Dissolve 1g of SDS in 100ml of Distilled water to give 1% SDS solution.

8. Preparation of 0.1N Sodium Hydroxide solution:

Dissolve 4g of Sodium hydroxide in 100ml of distilled water in a 1000ml volumetric flask. Then make up the volume upto 1000ml using distilled water.

Appendices

- Manasa, V., Amit Bhatia, Deepak N Kapoor, Novel Drug Delivery systems for the treatment of glaucoma. Presented at 29th Annual conference of IPGA held at Lovely Professional University, Phagwara.
- Manasa, V., Amit Bhatia, Gagandeep, Deepak N Kapoor, Development and Evaluation of Fast dissolving formulation of Promethazine. Presented at 66th Indian Pharmaceutical Congress on 23-25th Jan, 2015 held at HITEX, Hyderabad.
- Manasa, V., Amit Bhatia, Deepak N Kapoor, Development and Evaluation of Fast dissolving formulation of Promethazine Presented at Lovely Professional University on 27-28th Apr, 2015 held at LPU, Punjab and was awarded 3rd prize for the presentation.