FORMULATION AND EVALUATION OF SUSTAINED RELEASE OCULAR DRUG DELIVERY SYSTEM FOR AN ANTI-GLAUCOMA DRUG

A THESIS SUBMITTED

TO

SAURASHTRA UNIVERSITY, RAJKOT

FOR

THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACY

FACULTY OF PHARMACY (MEDICINE)

Research Guide:

Dr. Jayvadan K. Patel
[M. Pharm., Ph. D., LLB, FICS]

Submitted By:

Mr. Hitesh B. Gevariya
[M. Pharm]

JANUARY - 2013

CERTIFICATE

This is to certify that the thesis entitled **"Formulation and evaluation of sustained release ocular drug delivery system for an anti-glaucoma drug"** represents bonafide and genuine research work of **Mr. Hitesh B. Gevariya** carried out under my guidance and supervision. The work presented in this dissertation was carried out at Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India and is upto my satisfaction.

Research Guide

Dr. Jayvadan K. Patel
[M. Pharm, Ph. D., LLB, FICS]
Professor of Pharmaceutics and Principal,
Nootan Pharmacy College,
S. P. Vidyadham, Kamana Crossing,
Visnagar-384315, Gujarat, India.

Forwarded through

Prof. (Dr.) B. N. Suhagia,
[M. Pharm, Ph. D., LLB., FICS]
Professor and Dean,
Faculty of Pharmacy,
Dharmsinh Desai University,
Nadiad-387001, Gujarat, India.

DECLARATION

I hereby declare that the thesis entitled "**Formulation and evaluation of sustained release ocular drug delivery system for an anti-glaucoma drug**" is a bonafide and genuine research work carried out by me, under the guidance of **Dr. Jayvadan Patel,** Professor of Pharmaceutics and Principal, Nootan Pharmacy College, Visnagar, Gujarat, India. The results presented in this dissertation are original and has not been submitted in part or full for the award of any degree or diploma to any university.

Date:
Place: Rajkot

Mr. Hitesh B. Gevariya
Reg. No: 4200
Department of Pharmaceutical Sciences, Saurashtra University, Rajkot -360005.

Forwarded By

Guide

Dr. Jayvadan K. Patel
[M.Pharm.,PhD.,LLB.,FICS]
Professor of Pharmaceutics and Principal,
Nootan Pharmacy College,
S. P. Vidyadham, Kamana Crossing,
Visnagar-384315, Gujarat, India.

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DECLARATION BY THE CANDIDATE

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Date:
Place: Rajkot

Mr. Hitesh B. Gevariya
Reg. No: 4200
Department of
Pharmaceutical Sciences,
Saurashtra University,
Rajkot- 360005.

Acknowledgement

By the grace and mercy of ***The God Krishna*** almighty, the most Beneficent and Merciful, this thesis entitled "Formulation and evaluation of sustained release ocular drug delivery system for an anti-glaucoma drug" has taken a final shape.

No research is ever the outcome of single individual's talent or efforts. I have seen and experienced the countless blessing showered on me by my parents, all family members, teachers, friends and all my well-wishers knowing the God's hand is there, always guiding me and leading me to greater heights. It provides me pleasure to convey my gratitude to all those who have directly or indirectly contributed to make this work a success. I must make special mention of some of the personalities and acknowledge my sincere indebtedness to them.

Teacher is a guide, philosopher and friend, which I could experience in my respected dear and esteemed guide. I consider myself the luckiest to work under the guidance of ***Dr. Jayvadan K .Patel,*** M. Pharm., Ph.D., LLB, FICS, Professor of Pharmaceutics and Principal, Nootan Pharmacy College, Visnagar. I take this opportunity to express my deep gratitude, sincere and heartful thanks to my reverend guide. His discipline, principles, simplicity, caring attitude and provision of fearless work environment will be cherished in all walks of my life. I am highly indebted to him for his valuable advices, support, shared intelligent thought and everlasting encouragement throughout my course. To work under the guidance of such an eminent person has been a great and inexplicable experience, which will go a long way down my memory lane in my life. My hearty thanks go to him.

It is a proud privilege to express my sincere thanks to ***Prof. Dr. B. N. Suhagia***, Dean, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, with a deep sense of gratitude for his encouragement, co-operation, kind suggestion and providing the best facilities during this work. He always motivated me, shared his valuable ideas with me and kept the environment charged. It would be right to honor him as co-

guide for this investigation. I thank him for providing unceasing encouragement, precious and erudite suggestions and directions, constant and untiring guidance along with the freedom of work that he gave me.

Words are an inadequate medium to express my deep sense of gratitude to ***Dr. N.R.Sheth,*** Professor and Head, Department of Pharmaceutical Science, Saurashtra University, Rajkot for his keen interest, propelling inspiration, informative and critical discussions, valuable suggestions and directions and selfless support as and when needed.

I can't forget smiling encouragement and enthusiasm of ***Dr. D. O. Shah***, Director, and ***Dr. Atinder Shukla***, Asso. Prof., Shah-Schulman Centre for Surfacescience and Nanotechnology, Dharmsinh Desai University, Nadiad and I owe my warmest and humble thanks to them for helping me to carry out DSC, XRD, FTIR, gonioscopy and zeta potential.

I consider it as a great priviledge to express my heartful gratitude and sincere thanks to ***Mr. Virendra kagathara, Ms. Roshani Patel, Dr. Ranju Pal,*** Asst. Prof., Dept. of Pharmacology, and friends ***Dr. Rajesh and Dr. Padurang,*** M.S.(ophthal), for helping me in performing *in vivo* studies using rabbits.

It gives me immense pleasure to express my sincere gratitude to ***Dr. Rajnikant Mardia*** and ***Dr. S. P. Chauhan*** for their help in FTIR and HPLC. I sincerely thank Ciron Labs. Ltd., Mumbai, for providing gratis sample of betaxolol HCL for research work.

Memories play an important part in keeping special people close at heart. I can't forget the sweet memories of the time that I have spent with my colleagues, friends and faculty members of Faculty of Pharmacy, DDU, Nadiad. I owe a lot to them for valuable suggestions, constant moral support, kind co-operation and timely help in the fulfillment of this research work.

I wish to thank *all the staff members of my college* for their involvement and friendly attitude during my work. I would like to mention names of ***Dr. S. P. Adeshara,*** Ex-Dean, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad and Ex-Drug Commissioner, FDCA, Gujarat state***, Dr. Tejal soni, Dr. Mehul Patel, Mr. Tejas Patel, Mr. Ketan ranch*** and ***Mr. Furquan Maulvi,*** *who* deserve special words of praise for

all the help they render throughout the course of my work. I thank storekeeper, Lab. Assistants, Peons and ***Mr. Pragnesh Patel***, Librarian for his constant help in procuring required materials and books for the research work.

I acknowledge with gratitude the help and support I received from my wife, ***Saguna*** who suffered a lot to keep me free from social responsibility and her constant emotional support is the actual driving force that enabled me to reach this stage today. I can never forget to appreciate my little baby - ***Parv*** for keeping me tension free and always laughing by his naughty and giggling acts.

The completion of this thesis is not only fulfillment of my dreams but also the dreams of my parents who have taken lots of pain for me in completion of my higher studies.

I dedicate this work to my God, Guruji, Adorable Parents, Wife, Son, Sisters – ***Harsha*** and ***Kiran***, Nephew – ***Himanshu*** and ***Dvij*** for giving me encouragement and moral support all the time.

Last but not least, I express my gratitude and apologize to everybody whose contributions, I could not mention here.

With thanks to one and all…

Hitesh B. Gevariya

(M.Pharm, Pharmaceutics)

DEDICATED TO

MY GOD, GURU, PARENTS,

BELOVED WIFE AND 'PARV'

ABBREVIATIONS

PEO	Poly Ethylene Oxide
SA	Sodium Alginate
PVP	Poly Vinyl Pyrrolidone
PMMA	Polymethyl Methacrylate
EC	Ethyl Cellulose
PEG 400	Poly Ethylene Glycol 400
DBP	Dibutyl Phthalate
DCM	Dichloromethane
BX	Betaxolol
%	Percentage
FTIR	Fourier Transform Infrared
UV	Ultra Violet
HPLC	High Performance Liquid Chromatography
mg	Milligram
g	Gram
nm	Nanometer
mm	Millimeter
cm	Centimeter
µL	Micro liter
mL	Milliliter
µg, mcg	Microgram
mM	Millimolar
wt.	Weight
w/w	Weight by weight
w/v	Weight by volume
h, hr	Hour, Hours
Min	Minutes
Sec	Seconds
^{o}C	Degree centigrade
rpm	Revolutions per minute
t	Time

CDR	Cumulative drug release
Abs	Absorbance
Conc	Concentration
STF	Simulated Tear Fluid
PBS	Phosphate Buffer Saline
IP	Indian Pharmacopoeia
ATGM	Alternate Thioglycollate Medium
SCDM	Soyabean Casein Digest Medium
RSD	Relative Standard Deviation
SD	Standard Deviation
PMA	Percent Moisture Absorbance
PML	Percent Moisture Loss
PA	Physical Appearance
RDC	Remaining Drug Content
SRT	Sterility Testing
RH	Relative Humidity
WVT	Water Vapour Transmission
FE	Folding Endurance
EB	Elongation at Break
RT	Room Temperature

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AIM OF PRESENT WORK

Aim of present work

Eye being a most delicate organ, ocular drug delivery is a challenge for the formulator. A drop of an aqueous solution, irrespective of instilled volume is eliminated completely from the eye within 5 to 6 minutes of its application and only a small amount (1-3%) actually penetrates the cornea and reaches the intraocular tissue. Recent trend in ocular research is to formulate a dosage form which not only prolongs the residence of system in eye but also helps to reduce the elimination of the drug. In the same area many drug delivery systems including insitu gel, microemulsions, nanoparticles, liposome, niosomes, ocular inserts etc. are being investigated worldwide by many researchers.

Ophthalmic inserts offer many advantages over conventional dosages forms, like increased ocular residence, possibility of releasing drug at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf life. Design, construction and technology of ocular insert in a controlled and sustained ocular delivery device are gaining rapid improvement to overcome these constraints.

Niosomes in topical ocular delivery are preferred over other vesicular systems because of the following reasons: (1) chemical stability; (2) low toxicity because of their non-ionic nature; (3) handling surfactants with no special precautions or conditions; (4) the ability to improve the performance of the drug via better availability and controlled delivery at a particular site; (5) being biodegradable, biocompatible and non-immunogenic.

There are only a few ocular inserts available on the market, made of EVA as a rate controlling membrane. Likewise, poly (methylmethacrylate) (PMMA) is also an excellent film-forming polymer but the films of PMMA alone are brittle. It offers more resistance to the diffusion of drug molecules, and is less explored as a polymer for ocular delivery of drugs. The current literatures indicate that no inserts are made of hydrophobic monolithic systems using betaxolol. Therefore, varying the ratio of these polymers in the composition of the films provides better control of drug release characteristics. Betaxolol is selective beta-1-adrenegic receptor blocker and used in the treatment of ocular hypertension and chronic open angle glaucoma. Ocular administration of Betaxolol has the disadvantage of low bioavailability due to extensive and

highly variable defense mechanisms of the eye. In addition, Betaxolol eye drops requires bid dosing. Owing to these disadvantages, an Ocular patch of Betaxolol was designed and developed.

The advantage of vesicular systems does not only reside in providing prolonged and controlled action at the corneal surface but also involves providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action. The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. In vesicular dosage forms, the drug is encapsulated in lipid vesicles, which can cross cell membrane. Vesicles, therefore, can be viewed as drug carriers which can change the rate and extent of absorption as well as the disposition of the drug.

Hence this investigation has been designed to study the drug release kinetics of betaxolol from a hydrophobic matrix system of PMMA cast with incorporating different proportions of PEO. Various niosomal formulations of betaxolol are formulated and evaluated in this study. New ocular insert devices - Nioserts are designed and developed.

It was our prime objective to develop ocular drug delivery system that:

1. Release the drug for prolonged period of time preferably 24 hr.
2. Provide an increased ocular residence time resulting in prolonged drug delivery in eye.
3. Reduce precorneal drug loss and thereby obtaining greater therapeutic efficacy.
4. Show better *in-vivo* performance than conventional dosage forms.
5. Avoid the systemic side effects.

CHAPTER 1

INTRODUCTION

Chapter 1 - INTRODUCTION

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1. INTRODUCTION

In the recent years considerable attention has been focused on the development of new drug delivery systems. The therapeutic efficacy and safety of drugs administered by conventional methods can be improved by more precise spatial and temporal placement with in the body through a controlled drug delivery. Basically, there are three modes of drug delivery i.e. Targeted Delivery, Controlled Delivery and Modulated Delivery.

1) Targeted delivery refers to the systemic administration of drug carrier with the goal of delivering the drug to specific cell types, tissues or organ.

2) Controlled release refers to the use of delivery device with the objective of releasing the drug into the patient's body at a predetermined rate, or at a specific time or with specific release profiles.

3) Modulated release of a drug delivery device refers to the release of drug at a variable rate, controlled by environmental conditions, biofeedback, sensor input or an external control device[(1)].

1.1 Ocular drug delivery system

For many decades, treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables as drug carriers. Even today, these conventional drug deliveries are the primary pharmaceutical products commonly seen in the prescription and over the counter drug market place. These types of conventional dosage systems are known to provide a prompt release of drug. Therefore, to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day. This results in a significant fluctuation in drug levels.

Recently, several new techniques for drug delivery are made which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity or targeting the delivery of the drug to a tissue. These advancements have led to the development of several novel drug delivery systems that could revolutionize the method of medication and provide a number of therapeutic benefits[(2)].

Ophthalmic products are preparations designed for application to the eye either for the treatment of disease, for the relief of symptoms, for diagnostic purpose or an adjuvant to surgical procedure. The conventional ocular preparations are eye drops and ophthalmic ointments. As soon as the eye drop solution is instilled into cul-de-sac, it is rapidly drained away from the precorneal cavity by constant tear flow and lachrymal-nasal drainage. Only about 1-2% of instilled dose is absorbed into the target tissues and relatively concentrated solution is required for installation to achieve an adequate level of therapeutic effect. The frequent periodic instillation of eye drops becomes necessary to maintain a continuous sustained level of medication.

A basic concept in ophthalmic research and development is that the therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. The viscosity enhancing agents such as methylcellulose are added to eye drop preparations or ophthalmic drug is formulated in water-insoluble ointment formulation to sustain the duration of intimate drug-eye contact. But these dosage forms give only marginally more sustained drug-eye contact than eye drop solutions and do not yield a constant drug bioavailability as originally hoped. The ocusert system has succeeded in significantly reducing dosing and also in remarkably improving the therapeutic efficacy of ophthalmic drugs[(2)].

The ocusert systems are the only medicated inserts marketed in western countries. The prolonged constant-rate release pattern achieved by inserts such as ocusert and ocufit type can be considered as most desirable condition for long term therapy; both because of efficiency as well as the reduction of ocular and systemic side effects.

Drugs that could be considered for delivery by log-acting inserts, in addition to anti glaucoma drugs, are antibiotics and anti-bacterials which require several applications daily in standard vehicles for treatment of Acanthamoeba Keratitis, trachoma etc. Other drugs selected for topical administration with inserts are antiviral, antifungal, anti-filarial, anti-allergenic, anti-inflammatory (Steroidal and NSAID), fibrinolytes, immuno suppressant and growth factors[(3)].

1.2 Anatomy and physiology of the eye

Eye is the most marvelous of the sense organs as it makes us aware of various objects all around us, near and far away. Eye is nearly spherical in shape except that its front portion i.e., transparent cornea bulges a bit forward. The eye is protected by the eyelashes, eyelids, tears and blinking. The eyelashes catch foreign materials as the blink reflex prevents injury by closing the lids, blinking occurs frequently during waking hours to keep the corneal surface free of mucous and moistened by the tears secreted by the lacrimal glands. Tears wash away irritating agents and are bactericidal, preventing infections. The protective operations of the eye lids and lacrimal system are such that, there is a rapid removal of material instilled into the eye unless the material is suitably small in volume, chemically and physiologically compatible with surface tissues. The eye is one of the most delicate and yet most valuable of the sense organs and is a challenging subject for topical administration of drugs to the eye[(4)].

1.2.1 Accessory structures of the eye

The accessory structures of the eye include the eyelids, eyelashes, eyebrows, the lacrimal (tearing) apparatus and extrinsic eye muscles. The lacrimal apparatus is a group of structures that produces and drains lacrimal fluids or tears[(5)].

1.2.2 Anatomy of the eyeball

The eyeball measures about 2.5 cm in diameter, only a small portion (about 1/6th part) of the globular eye is exposed in front, the rest is hidden in bony socket of the orbit on a cushion of fat and connective tissue. The wall of the human eyeball consists essentially of three layers: Fibrous tunic, Vascular tunic and Retina[(5, 6)].

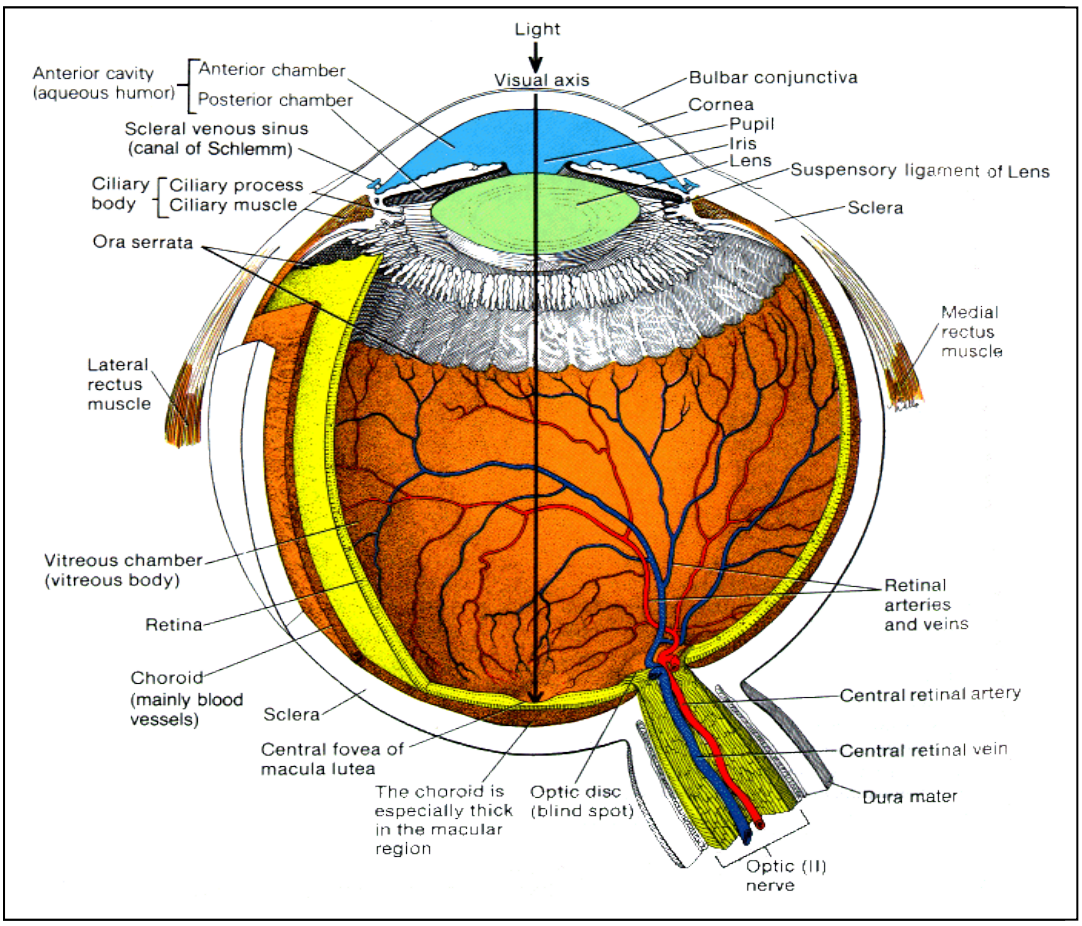

Figure 1.1: Anatomy of the eye

1. Fibrous tunic

Fibrous tunic, the outermost coat of the eyeball, consists of the anterior cornea and posterior sclera.

The **cornea** is a transparent coat that covers the colored iris. Cornea mainly consists of the following structures from the front to back, (I) Epithelium, (II) Bowman's membrane, (III) Stroma, (IV) Descemet's Membrane, (V) Endothelium. The cornea is 0.5 to 1mm in thickness and normally it possesses no blood vessels except at the corneosclerar junction. The **sclera**, the "white" of the eye, is a layer of dense connective tissue made up densely of collagen fibers and fibroblasts. The sclera covers the entire eyeball except the cornea. At the junction of the sclera and cornea is an opening known as the scleral venous sinus (canal of Schelmm).

2. Vascular tunic

This middle layer is mainly vascular, consisting of the choroid, ciliary body and iris.

Choroid lines the posterior five-sixths of the inner surface of the sclera. It is very rich in blood vessels.

Ciliary body is the anterior continuation of the choroids consisting of ciliary muscle and secretary epithelial cells. The major function of the ciliary body is the production of aqueous humor. Systemic drugs enter the anterior and posterior chambers largely by passing through the ciliary body vasculature and then diffusing in to the iris where they can enter the aqueous humor. The ciliary body is one of the major ocular sources of drug-metabolizing enzymes, responsible for drug detoxification and removal from the eye.

Iris is the visible colored part of the eye and extends interiorly from the ciliary body lying behind the cornea and in front of the lens. The pigment granules of the iris epithelium absorb light as well as lipophilic drugs. This type of binding is characteristically reversible, allowing release of drug overtime. As a result, the iris can serve as a reservoir for some drugs, concentrating and then releasing them for longer than otherwise expected[5, 7].

3. Retina

The innermost layer is the retina, consisting of the essential nervous system responsible for vision. Retina lines the posterior three quarters of the eyeball and is the beginning of the visual pathway[5]. The retina is situated between the clear vitreous humor in its inner surface and the choroids on its outer surface. Retina consists of two distinct chambers, anterior and posterior[8].

1.2.3 Lens

Behind the pupil and iris, within the cavity of the eyeball, is the lens. Protein called crystallins, arranged like the layers of an onion, make up the lens. The lens is held in place by the zonules, which run from the ciliary body and fuse into the outer layer of the lens capsule. The lens tends to develop cataract or opacities with age, interfering with vision[8].

1.2.4 Interior of the eyeball

The lens divides the interior of the eyeball into two cavities; Anterior cavity and Vitrous chamber.

The **anterior cavity** consists of two chambers

The anterior chamber that lies between the cornea and the iris.

The posterior chamber that lies behind the iris and in front of the lens. Aqueous humor is formed by ciliary bodies and occupies the posterior and anterior chambers, having a volume of about 0.2mL. The fluid is constantly generated by pigmented and non- pigmented epithelium of ciliary body[5, 7].

The **Vitreous chamber** is filled with a viscous fluid, vitreous humor, which is a viscoelastic connective tissue composed of small amounts of glycosaminoglycans, including of hyaluronic acid and proteins such as collagen[8].

1.2.5 Conjunctiva

The conjunctiva membrane covers the outer surface of the white portion of the eye and the inner surface of the eyelids. In most places it is loosely attached and thereby permits free movement of the eyeball, this makes possible sub-conjunctival injection. The conjunctiva forms an inferior and a superior sac except for the cornea, the conjunctiva is the most exposed portion of the eye[8].

1.3 Common eye disorders

A number of disorders can affect the structure of the eye, with outcomes ranging from moderate discomfort to significant loss of vision. The health care provider should be familiar with the signs and symptoms of common eye disorders and understand the decision making process behind treatment[9].

1.3.1 Inflammatory conditions

1. Hordeolum and Chalazion
2. Blepharitis
3. Dacryocystitis
4. Conjunctivitis
5. Trachoma
6. Anterior Uveitis

1.3.2 Corneal ulcer

1.3.3 Glaucoma

Glaucoma is a group of diseases of the eye characterized by damage to the ganglion cells and the optic nerve. If left untreated, these effects may lead to

various degrees of loss of vision and blindness. Increased intraocular pressure (IOP) remains the most important risk factor for the development of glaucoma. Glaucoma is typically classified as either open angle or angle closure (closed angle), based upon causes of increased intraocular pressure[10].

1.3.3.1 Glaucoma classified according to etiology

A) Primary glaucoma

1. Open angle glaucoma

a) Primary open angle glaucoma (chronic open angle glaucoma, chronic simple glaucoma)

b) Normal pressure glaucoma (low pressure glaucoma)

2. Angle-closure glaucoma

a) Acute

b) Sub acute

c) Chronic

d) Plateau iris

B) Congenital glaucoma

1. Primary congenital glaucoma
2. Glaucoma associated with other development ocular abnormalities
   a) Anterior chamber cleavage syndromes
      Axenfeld's syndrome
      Sieger's syndrome
      Peter's anomaly
   b) Aniridia
3. Glaucoma associated with extraocular development abnormalities
   a) Sturge-Weber syndrome
   b) Marfen's syndrome
   c) Neurofibromatosis
   d) Lowe's syndrome
   e) Congenital rubella

C) Secondary glaucoma.

1. Pigmentary glaucoma
2. Exfoliation syndrome
3. Due to lens changes (phacogenic)
    a) Dislocation
    b) Intumescence
    c) Phacolytic
4. Due to uveal tract changes
    a) Uveitis
    b) Posterior synechiae (seclusio pupillae)
    c) Tumor
5. Iridocorneoendothelial (ICE) syndrome
6. Trauma
    a) Hyphema
    b) Angle contusion / recession
    c) Peripheral anterior synechiae
7. Postoperative
    a) Ciliary block glaucoma (malignant glaucoma)
    b) Peripheral anterior synechiae
    c) Epithelial downgrowth
    d) After corneal graft surgery
    e) After retinal detachment surgery
8. Neovascular glaucoma
    a) Diabetes mellitus
    b) Central retinal vein occlusion
    c) Intraocular tumor
9. Raised episclera venous pressure
    a) Carotid-cavernous fistula
    b) Sturge-Weber syndrome
10. Steroid-induced

D) Absolute glaucoma: The end result of any uncontrolled glaucoma is a hard, sightless, and often painful eye.

1.3.3.2 Etiology (Study of causes of disease)

Optic nerve damage caused by the different types of glaucoma is a result of a variety of initiating factors. Genetic predisposition, physical changes, systemic diseases, or medications may increase a person's risk of developing damage that may be broadly classified as intraocular pressure dependent (most commonly) or intraocular pressure independent. Increased intraocular pressure remains the major etiologic risk factor for the development of glaucoma. Myopia may be an additional risk factor, especially in younger patients. Glaucoma can occur as a secondary manifestation of systemic disorders or trauma.

1.3.3.3 Pathogenesis

There are five stages in the pathogenesis of glaucoma: (1) a variety of initial events, causing (2) Changes in aqueous outflow, resulting in (3) Increased IOP, which leads to (4) Optic nerve atrophy, and finally, (5) Progressive loss of vision. This description highlights the importance of aqueous humor production and elimination in the progression of glaucoma and subsequent complications.

1.3.3.4 Open-angle glaucoma

In open-angle glaucoma, a physical blockage occurs within the trabecular meshwork that retards elimination of aqueous humor. The obstruction is presumed to be between the trabecular sheet and the episcleral veins, into which the aqueous humor ultimately flows. The impairment of aqueous drainage elevates the intraocular pressure to between 25 and 35 mm Hg (normal intraocular pressure is 10 to 20 mm Hg), indicating that the obstruction is usually partial. This increase in intraocular pressure is sufficient to cause progressive cupping of the optic disk and eventually visual field defects.

1.3.3.5 Angle-closure glaucoma

In angle-closure glaucoma, increased intraocular pressure is caused by papillary blockage of aqueous humor outflow and is more severe. The basic requirements leading to an acute attack of angle closure are a papillary block, a narrowed anterior chamber angle and a convex iris. When a patient has a narrow anterior chamber or a pupil that dilates to a degree where the iris

comes in greater contact with the lens, there is interference with the flow of aqueous humor from the posterior to the anterior chamber. Because aqueous humor is continually secreted, pressure from within the posterior chamber forces the iris to bulge forward. This may progress to complete blockage.

The pathologic complications of angle closure and open angle glaucoma include the formation of cataracts, adhesion of the iris to the cornea, atrophy of the optic nerve and retina, complete blockage of aqueous outflow, and ultimately, blindness.

1.3.3.6 Congenital glaucoma

Congenital glaucoma is a rare disorder in which intraocular pressure is increased as a result of developmental abnormalities of the ocular structures in the newborn or infant. It may occur in association with other congenital abnormalities and anomalies such as homocystinuria and Marfan's syndrome.

1.3.3.7 Normal-tension glaucoma

The etiology and pathogenesis of normal tension glaucoma remain to be completely understood. Normal tension glaucoma is thought to be related, at least in part, to decreased blood flow to the optic nerve. This may eventually cause neuronal damage. In addition, these eyes appear to be more susceptible to pressure related damage within the normal or high normal range, and therefore a pressure lower than normal is often necessary to prevent further visual loss.

1.3.3.8 Drug-induced glaucoma

Several therapeutic classes of drugs, such as those with anti-cholinergic, adrenergic, or corticosteroid effects, have been implicated in inducing or worsening glaucoma. Medications affect open angle and closed angle glaucoma differently. Drugs that dilate the pupil, for instance, may precipitate an acute attack of angle closure glaucoma but usually do not produce harmful effects in those with open angle glaucoma. Dilation of the pupil in angle closure glaucoma may cause the peripheral iris to bulge forward, blocking the trabecular meshwork. The aqueous humor is prevented from reaching the outflow channels, which results in increased IOP. Because excessive resistance to outflow in open angle glaucoma is caused primarily by changes

within the trabecular outflow channels, dilation of the pupil usually will not increase the intraocular pressure.

1.4 Absorption of drugs in eye[11]

It is often assumed that drugs administered into the eye are rapidly and totally absorbed. However, contrary to this belief, the moment drug is placed in lower cul-de-sac of eye, several factors immediately begins to affect the bioavailability of drug. Absorption of drugs takes place either through corneal or non corneal routes. The non-corneal route involves absorption across the sclera and conjunctiva into the intraocular tissues. This route is however, not productive as it restrains the entry of drug into aqueous humor. Maximum absorption thus takes place through cornea, which leads the drug into aqueous humor. The goal of ophthalmic drug delivery system has traditionally been to maximize ocular drug absorption rather than to minimize the systemic absorption.

1.5 Drug elimination from lacrimal fluid[11]

Ophthalmic liquid dosage form like solutions, suspensions or liposome is either drained from conjunctival sac into nasolacrimal duct or is cleared from precorneal area resulting in poor bioavailability of drugs. Drugs are mainly eliminated from the precorneal lacrimal fluid by solution drainage, lacrimation and non productive absorption to the conjunctiva of the eye. These factors and the corneal barrier limit the penetration of the topically administered drug into the eye. Only a few percentage of applied dose is delivered into intraocular tissue, while the major part (50-100%) of the dose is absorbed in systemic route. Precorneal constraints include,

a) Spillage of drug by overflow.
b) Dilution of drug by tear turnover.
c) Nasolacrimal drainage / systemic drug absorption.
d) Conjunctival absorption.
e) Enzymatic metabolism.

1.5.1 Transcorneal penetration

Transcorneal penetration of drug is mainly affected by corneal barriers, physiochemical properties of drugs and active ion transport systems present at cornea.

1.5.1.1 Corneal barriers

Corneal epithelium is the main barrier for drug absorption into eye. Corneal epithelium acts as a protective barrier against foreign molecules and also as a barrier to ion transport. The corneal epithelium consists of a basal layer of columnar cells, squamous cells, and polygonal shaped superficial cells.

In a healthy corneal epithelium, intracellular tight junctions completely surrounds the most superficial cells, nevertheless the intracellular spaces are wider between wing cells and basal cells. These allow the paracellular diffusion of large molecules through these layers of cell only. Tight junctions serve as a selective barrier for small molecules and they completely prevent the diffusion of macro molecules via the paracellular route. Corneal stroma is a highly hydrophilic tissue; it acts as a rate limiting barrier for ocular absorption of most lipophilic drugs. The corneal endothelium is responsible for maintaining normal corneal hydration.

1.5.1.2 Physiochemical properties of drug

Transcellular or paracellular pathway is the main route for drugs to penetrate across corneal epithelium. Hydrophilic drugs penetrate primarily through the paracellular pathway, which involves passive or altered diffusion through intracellular spaces while lipophilic drugs prefer the transcellular route. For topically applied drugs passive diffusion along their concentration gradients, either transcellular or paracellular permeation, is the main permeation mechanism. Lipophilicity, solubility, molecular size and shape, charge and degree of ionization also affect the route and rate of penetration in cornea.

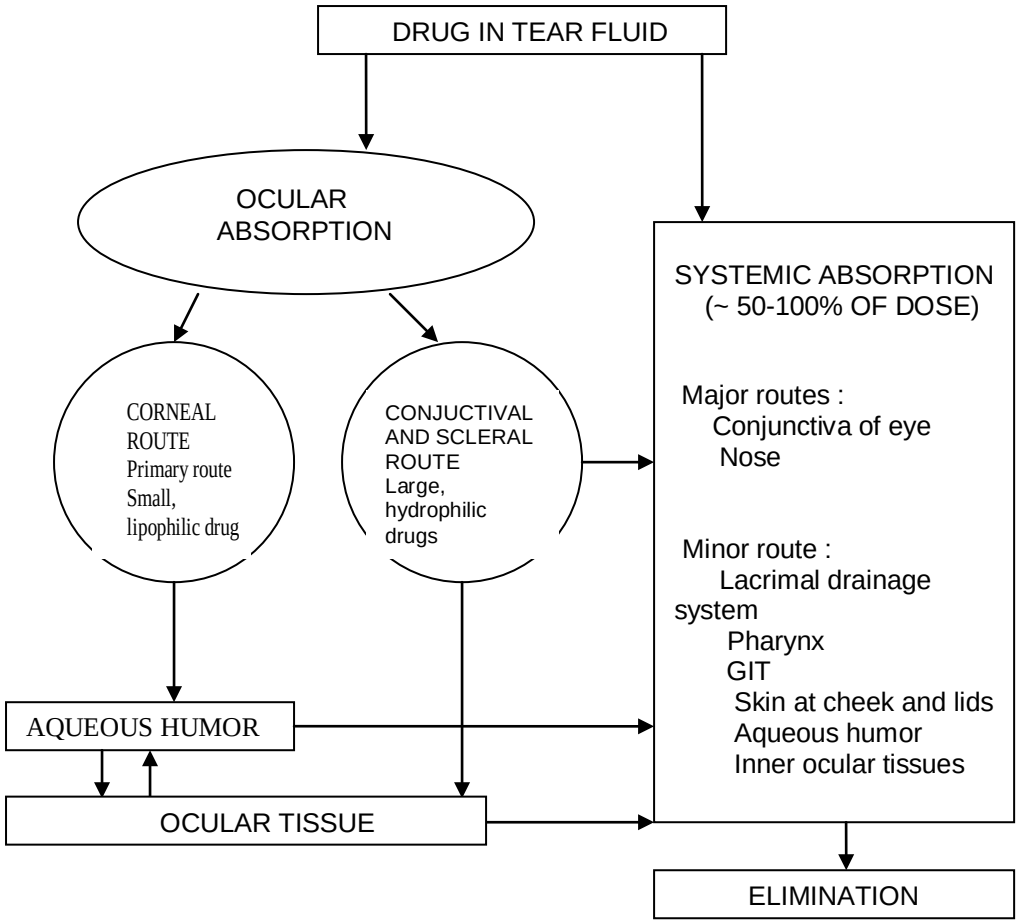

Figure 1.2: Factors and corneal barrier limitations for penetration of topically administered drug

1.5.2 Non–corneal absorption

Apart from corneal route topically applied ocular drugs may be absorbed through non-corneal route. This route involves drug penetration across the bulbar conjunctiva and underlying sclera in to the uveal tract and vitreous humor. This route is important for hydrophilic and large molecules, such as insulin and p-aminoclonidine, which have poor corneal permeability.

1.6 Drawback of traditional ophthalmic formulations [11]

1. They have poor bioavailability because of
   a) Rapid precorneal elimination
   b) Conjunctival absorption
   c) Solution drainage by gravity
   d) Induced lacrimation
   e) Normal tear turnover

2. Frequent instillation of concentrated medication is required to achieve a therapeutic effect.
3. Systemic absorption of the drug and additives drained through nasolacrimal duct may result in undesirable side effects.
4. The amount of drug delivered during external application may vary. The drop size of ocular medication is not uniform and those delivered is generally not correct.
5. Presence of viscous vehicles can cause blurred vision.

1.7 Novel ophthalmic delivery systems

To overcome the drawbacks of conventional ophthalmic dosage form, many progresses have been done to improve the pre-corneal drug absorption and minimize pre-corneal drug loss.

1.7.1 Mucoadhesives

Mucoadhesives are retained in the eye by virtue of non-covalent bonds established with the corneal conjunctival mucin for extending pre-ocular residence time[12, 13].

1.7.2 Phase transition system

These are liquid dosage forms which shift to the gel or solid phase when instilled in the *cul-de-sac*. After converting into gel it remains in contact with the cornea of the eye for prolonged period of time due to which drug elimination also slow down[14, 15].

1.7.3 Niosomes

Niosomes are the vesicles, containing non-ionic surfactants, that can entrap both hydrophilic and lipophillic drugs either in aqueous layer or in vesicular membrane made of lipid materials[16]. It helps in preventing the metabolism of the drug by enzymes present at the tear/corneal surface[17].

1.7.4 Liposomes

Liposomes are microscopic vesicles composed of membrane like lipid layers surrounding aqueous compartments. The lipid layers are comprised mainly of phospholipids[18]. They have the ability to entrap hydrophilic compound in the aqueous compartment and to incorporate hydrophobic molecule in the lipid bilayers[19].

1.7.5 Nanoparticles

Nanoparticles are solid particles of polymeric nature ranging in size from 10-1000 nm. The drugs are bound to small particles, which are then dispersed into aqueous vehicle[20]. Due to very small in size these are not washed away with tears quickly[21].

1.7.6 Contact lenses

Contact lenses are substitutes for spectacles and are enjoying a certain degree of popularities. Use of soft contact lenses soaked in drug solution have been suggested for slow but prolonged drug delivery but particularly to corneal tissue[22].

1.7.7 Pharmacosomes

They are the vesicles formed by the amphiphilic drugs. Any drug possessing a free carboxyl group can be esterified to the hydroxyl group of a lipid molecule thus generating an amphiphillic prodrug. These are converted to pharmacosomes on dilution with tear[23].

1.7.8 Ophthalmic inserts

Inserts are defined as a thin disks or small cylinders made with appropriate polymeric material and fitting into the lower or upper conjunctival sac. Their long persistence in preocular area can result in greater drug availability with respect to liquid and semisolid formulation[24].

1.8 Requisites of controlled ocular delivery systems[11]

1. To overcome the side effects of pulsed dosing (frequent dosing and high concentration) produced by conventional systems.
2. To provide sustained and controlled drug delivery.
3. To increase the ocular bioavailability of drug by increasing corneal contact time. This can be achieved by effective coating or adherence to corneal surface, so that the released drug effectively reaches the anterior chamber.
4. To provide targeting within the ocular globe so as to prevent the loss to other ocular tissues.
5. To circumvent the protective barriers like drainage, lacrimation and diversion of exogenous chemicals into the systemic circulation by the conjunctiva.

6. To provide comfort and compliance to the patient and yet improve the therapeutic performance of the drug over conventional systems.
7. To provide the better housing of the delivery system in the eye so as the loss to other tissues besides cornea is prevented.

Ocular drugs and delivery systems are currently undergoing a process of design optimization due to inherent physiological and anatomical constraint of the eye leading to limited absorption of topically applied drugs.

Two major approaches are being undertaken to improve topical delivery of drugs which are:

1. Approaches to prolong the contact time of drug with corneal surface.
2. Approaches to enhance corneal permeability either by mild or transient structural alteration of corneal epithelium or by modification of chemical structure of the drug molecules.

1.9 Ophthalmic inserts

Ophthalmic inserts are defined as elliptical flexible wafer, multilayered system consisting of drug as core surrounded by rate controlling membrane and designed to be placed in *cul-de-sac* between sclera and eyelid[11].

1.9.1 Advantages of ophthalmic insets[3, 11]

1. Increasing the contact time and thus improve bioavailability.
2. Providing prolonged drug release and thus a better efficacy.
3. Reduction of systemic side effects and thus reduces adverse effects.
4. Reduction of the number of administration and thus better patient compliance.
5. Administration of an accurate dose in the eye and thus a better therapy.
6. Increased shelf life with respect to aqueous solutions.
7. Exclusion of preservative, thus reducing the risk of sensitivity reactions.
8. A possibility of incorporating various novel chemical technological approaches such as pro-drugs, micro particulates, salts acting as buffers.

1.9.2 Disadvantages of ocular inserts[3]

1. Initial discomfort, their movement around the eye.
2. Occasional inadvertent loss during sleep or while rubbing the eye.
3. Interference with vision and a difficult placement.

1.9.3 Desired criteria for ocular insert are[3, 12]

1. Comfort
2. Lack of explosion
3. Ease of handling and insertion
4. Non-interference with vision and oxygen permeability
5. Reproducibility of release kinetics
6. Sterility
7. Stability
8. Ease of manufacture

1.9.4 Classification of ophthalmic inserts[11]

Ophthalmic inserts, based upon their solubility behaviour, are classified as given below;

A. Insoluble ophthalmic inserts
B. Soluble ophthalmic inserts
C. Bio erodible ophthalmic inserts

A. Insoluble ophthalmic inserts

They have been sub-classified into:

a) Diffusion Inserts, E.g. 'Ocuserts'
b) Osmotic inserts
c) Contact lenses presoaked in drug solution

a) Diffusion inserts or ocuserts

Ocusert system is a novel ocular drug delivery system based on porous membrane. A central reservoir of drug is surrounded by the polymeric membrane, which allows a constant movement of the drug in to the tissue by diffusion. A controlled rate of delivery is provided by interaction between the membrane molecule and the drugs. The first major important factor in the rate of drug release is the driving force, which is maintained by saturated concentration of the drug in the reservoir.

As long as gradient exist, there is zero-order drug delivery through the membrane. Second one is the rate of release of the drug outside the membrane. The eye (*cul-de-sac*) provides an excellent environment for this type of drug delivery system and prevents a build up of stagnant drug around the module. Thus the gradient and force are preserved and drug continues to

move out of reservoir. Pilocarpine ocusert has been marketed in U.S. by Alza Corporation for the treatment of glaucoma. It is 13.4mm long, 5.7 mm broad, 0.3 mm thick and weighs 19mg to be placed in *cul-de-sac* between sclera and eyelid. The ocusert system provides nearly steady zero-order delivery rate of pilocarpine from the unit for 7 days in the aqueous tear environment of the *cul-de-sac.*

When the insert placed in the eye, water from the tear fluid begins to penetrate the matrix, then swelling and consequently polymer chain relaxation and drug diffusion take place. The dissolution of the matrix, which follows the swelling process, depends on polymers structure, linear amorphous polymer dissolve much faster than cross-linked or partially crystalline polymers.

The release rate of diffusional devices present three distinct regions as shown under

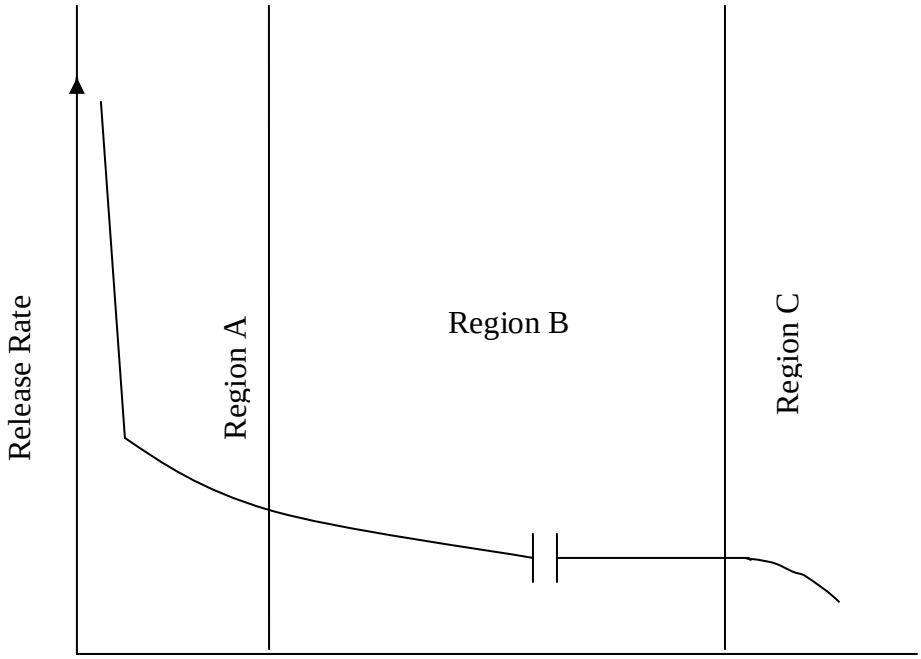

Figure 1.3: Release rate from diffusion inserts

Region A: An initial usually high drug release rate corresponding to the establishment of equilibrium between the reservoir and the eye surface.

Region B: Rate decreases to a plateau corresponding to a steady drug release rate.

Region C: A final decrease of the release rate corresponding to the exhaustion of the drug.

E.g. Pilocarpine Ocusert

The principle for its operation can be described by the Fick's diffusion equation.

$$J = -DA\, dc/dx \qquad [1.1]$$

Where,

J – Solute flux

D – Diffusion coefficient for the drug within the polymer membrane

A – Area of membrane

dc/dx – drug concentration gradient within the membrane along the direction of drug flow.

The use of a hydrophobic membrane that does not interact with the environment so as to change the shape (area) or diffusional characteristics as well as reservoir with excess drug (saturated solution) to provide thermodynamic force for the drug to diffuse continuously through the rate-controlling membrane should provide a steady zero-order release rate. Two kinds of this drug delivery system are available as ocusert Pilo 20 (5mg of pilocarpine with release rate of 20 mcg/hr) and as ocusert pilo 40 (11mg of pilocarpine with release rate of 40mcg/hr).

b) Osmotic insert

The osmotic inserts are generally composed of a centre part surrounded by a peripheral part and are of two types.

Type 1: The central part is composed of a single reservoir of a drug with or without an additional osmotic solute dispersed throughout polymeric matrix, so that the drug is surrounded by the polymer as discrete small deposits. The second peripheral part of this inserts compresses a covering film made up of insoluble semi permeable polymer. The osmotic pressure against the polymer matrix causes its rupture in the form of apertures. Drug is then released through these apertures from the deposits near the surface of the devices.

Type 2: The central part is composed of two distinct compartments. The drug and the osmotic solutes are placed in two separate compartments, the

drug reservoir being surrounded by an elastic impermeable membrane and the osmotic solute reservoir by a semi permeable membrane. The second peripheral part is similar to that of type 1. The tears diffuse into the osmotic compartment inducing an osmotic pressure that stretches the elastic membrane and contracts the compartment including drug, so that the active component is forced to the single drug release aperture.

c) Contact lenses

Contact lenses are shaped structures comprising covalently cross linked hydrophilic or hydrophobic polymer that forms a three dimensional network or matrix capable of retaining water, aqueous drug solution or solid components. Contact lenses are classified into 5 groups.

a) Rigid
b) Semi-rigid
c) Elastomeric
d) Soft hydrophilic
e) Bio-polymeric

The soft hydrophilic contact lenses are very popular because they are easy to fit and are tolerated better. The drug incorporation in to contact lens depends on whether its structure is hydrophilic or hydrophobic. When contact lens (including 35-80% water) is soaked in solution, it absorbs the drug. Drug release depends markedly on the amount of drug, the soaking time of the contact lens and the drug concentration in the soaking solution.

Advantages

1. Concerning the high cost of contact lenses it should be mentioned that disposable contact lenses have been commercially available and it should be possible to incorporate a drug into such devices at an acceptable cost.
2. The contact lenses are the only class of ophthalmic inserts that have the ability to correct any refractive error that the patient may have and there by provide improved visual acuity, while the medication is being administered.

Disadvantages

1. Cleaning and rinsing procedures can induce deterioration of the devices.

2. The cost of medicated contact lens is high.

B. Soluble ophthalmic inserts

They can be broadly divided in to two types, the first one being based on natural polymers and the other one synthetic or semi synthetic polymers.

I. Natural polymers

Natural polymer used to produce soluble ophthalmic inserts is collagen. The therapeutic agent is preferably absorbed by soaking the insert in a solution containing the drug, drying and rehydrating it before use on the eye. The amount of drug loaded will depend on the amount of binding agent present, concentration of the drug solution in to which the composite is soaked as well as duration of soaking. As the collagen dissolves, the drug is gradually released from the interstices between the collagen molecules.

II. Synthetic or semi-synthetic polymer

The soluble ophthalmic inserts, containing synthetic and semi-synthetic polymers, offer the additional advantages of being of a generally simple design, of being based on products well adapted for ophthalmic use and easily processed by conventional methods. The main advantage is decreased release rate, but still controlled by diffusion.

C. Bioerodible ophthalmic inserts

These are composed of matrix of homogenous dispersion of a drug coated with hydrophobic material, which is substantially impermeable to the drug. The main components used for the production of this type of inserts are the so-called Bioerodible polymers i.e., materials that undergo hydrolysis of chemical bonds and hence dissolution.

Bioerosion is defined here as the property of a material to innocuously disintegrate or breakdown from a unit structure or entity, over a prolonged period of time, in response to the environment in the eye. When bioerosion process takes place geometrically of inserts is modified and it is difficult to adequately control the release. The bioerodible polymers used in erodible inserts are carboxymethylcellulose, hydrophobic polyacrylic acids, hydroxypropylmethylcellulose, and silicone based pro-polymers. There are three devices, which are marketed to date namely Lacrisert, Soluble Ocular Drug Inserts (SODI) and Minidisc.

1.10 Niosomes

Vesicles prepared from self-assembly of hydrated non-ionic surfactants molecules are called niosomes. Niosomes and liposomes are equiactive in drug delivery potential and both increase drug efficacy as compared with that of free drug. Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy.

These types of vesicles were first reported in the cosmetic industries. Non-ionic surfactants used in formation of niosomes are polyglyceryl alkyl ether, glucosyl dialkyl ether, crown ether, polyoxyethylenealkyl ether, ester-linked surfactants, and steroid-linked surfactants and spans, and tweens series. Niosomes preparation is affected by processes variables, nature of surfactants, and presence of membrane additives and nature of drug to be encapsulated[25].

1.10.1 Structure of niosomes

Niosomes are microscopic lamellar structures, which are formed on the admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media[16]. Structurally, niosomes are similar to liposomes, in that they are also made up of a bilayer. However, the bilayer in the case of niosomes is made up of non-ionic surface active agents rather than phospholipids as seen in the case of liposomes. Most surface active agents when immersed in water yield micellar structures; however some surfactants can yield bilayer vesicles which are niosomes.

Niosomes may be unilamellar or multilamellar depending on the method used to prepare them. The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle, while the hydrophobic chains face each other within the bilayer. Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself. The figure below will give a better idea of what a niosome looks like and where the drug is located within the vesicle.

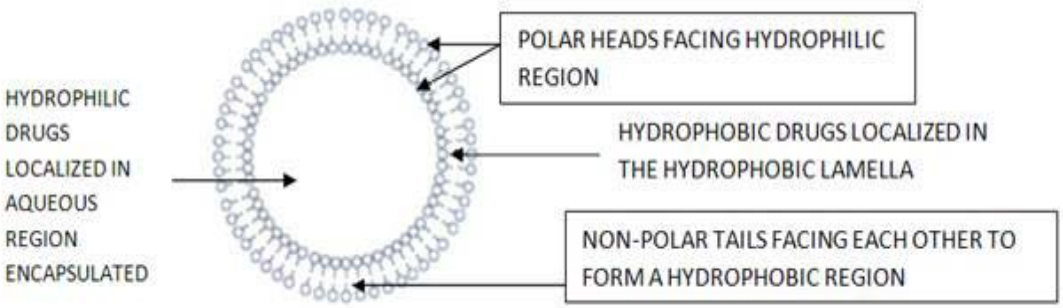

Figure 1.4: Structure of niosomes

A typical niosome vesicle would consist of a vesicle forming ampiphile i.e. a non-ionic surfactant such as Span-60, which is usually stabilized by the addition of cholesterol and a small amount of anionic surfactant such as diacetyl phosphate, which also helps in stabilizing the vesicle[(26)].

1.10.2 Advantages of niosomes

Use of niosomes in cosmetics was first done by L'Oreal as they offered the following advantages[(16)]

- The vesicle suspension being water based offers greater patient compliance over oil based systems
- Since the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as amphiphilic drug moieties, they can be used for a variety of drugs.
- The characteristics such as size, lamellarity etc. of the vesicle can be varied depending on the requirement.
- The vesicles can act as a depot to release the drug slowly and offer a controlled release.

Other advantages of niosomes are

- They are osmotically active and stable.
- They increase the stability of the entrapped drug.
- Handling and storage of surfactants do not require any special conditions.
- Can increase the oral bioavailability of drugs.
- Can enhance the skin penetration of drugs.
- They can be used for oral, parenteral as well topical use.

- The surfactants are biodegradable, biocompatible, and non-immunogenic. They improve the therapeutic performance of the drug by protecting it from the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.
- The niosomal dispersions in an aqueous phase can be emulsified in a non-aqueous phase to control the release rate of the drug and administer normal vesicles in external non-aqueous phase.

1.10.3 Salient features of niosomes

- Niosomes can entrap solutes in a manner analogous to liposomes.
- Niosomes are osmotically active and stable.
- Niosomes possess an infra structure consisting of hydrophobic and hydrophilic mostly together and so also accommodate the drug molecules with a wide range of solubility.
- Niosomes exhibits flexibility in their structural characteristics (composition, fluidity and size) and can be designed according to the desired situation.
- Niosomes can improve the performance of the drug molecules by delayed clearance from the circulation.
- Improve bioavailability to the particular site, just by protecting the drug from biological environment.
- Offer controlled delivery of drug at a particular site.
- No special conditions are required for handling and storage of Niosomes.

1.10.4 Types of niosomal systems[16]

1. **Small unilamellar vesicles** (SUV, size 0.025-0.05 μm) are commonly produced by sonication, and French Press procedures. Ultrasonic electrocapillary emulsification or solvent dilution techniques can be used to prepare SUVs.
2. **Multilamellar vesicles** (MLV, size >0.05 μm) exhibit increased-trapped volume and equilibrium solute distribution, and require hand-shaking method. They show variations in lipid compositions.
3. **Large unilamellar vesicles** (LUV, size >0.10 μm), the injections of lipids solubilised in an organic solvent into an aqueous buffer, can result in

spontaneous formation of LUV. But the better method of preparation of LUV is Reverse phase evaporation, or by Detergent solubilisation method.

1.10.5 Methods/ techniques of preparation of niosomes

By using following general steps niosomes can be prepared:

• Hydration of mixture of the surfactants/lipids at elevated temperature,

• Sizing of niosomes,

• Removal of the unentrapped material from the vesicles by different methods.

1.10.5.1 Hydration stage

Niosomes widely differ in their properties depending on the method used for production and composition of bilayer. The method of preparation of niosome is based on liposome technology. The basic process of preparation is the same i.e. hydration by aqueous phase of the lipid phase which may be either a pure surfactant or a mixture of surfactant with cholesterol. The bioactive material, which is to be entrapped, is dissolved in the aqueous phase/organic phase. The methods used for preparation of niosomes are listed as follows.

1.10.5.2 Ether injection method

This method was reported in 1976 by Deamer and Bangham, in which a lipid solution in di-ethyl ether is slowly introduced into warm water typically the lipid mixture is injected into an aqueous solution of the material to be encapsulated (using syringe type infusion pump) at 55-65 ^{o}C and under reduced pressure. Vaporization of ether leads to the formation of single layered vesicles (SLVs) depending upon the conditions used, the diameter of vesicles varies. Baillie *et al.,*[27] used this method for entrapment of 5, 6 carboxy florescein whereas, Hunter *et al.,*[25] used it for the entrapment of sodium stibogluconate (pentosam). The particle size of the niosomes formed depends on the conditions used, and can range anywhere between 50-1000µm[28].

1.10.5.3 Thin film hydration technique (Hand shaking method)

In this method a mixture of the vesicle forming agents such as the surfactant and cholesterol are dissolved in a volatile organic solvent such as diethyl ether or chloroform in a round bottom flask. The organic solvent is removed at room temperature using a rotary evaporator, which leaves a thin film of solid mixture deposited on the walls of the flask. This dried surfactant film can then be rehydrated at 50-60 ^{o}C using the aqueous phase, with gentle agitation to

yield multilamellar niosomes. The multilamellar vesicles thus formed can further be processed to yield unilamellar niosomes or smaller niosomes using sonication, microfluidization or membrane extrusion techniques[28].

Baillie *et al* reported Hand shaking method for the entrapment of 5, 6 carboxy fluorescein[27]. Chandraprakash *et al* entrapped methotrexate in niosomes prepared by Hand shaking method using lipophilic surfactants like span 40, span 60 and span 80, cholesterol and di-cetyl phosphate in ratio of 47.5: 47.5: 5. The tissue distribution of methotrexate was improved after entrapping with niosomes[29]. Rogerson *et al* prepared doxorubicin entrapped niosomes using pure surfactant or a mixture of surfactants and cholesterol[30]. Azmin *et al* modified this method for preparation of methotrexate entrapped niosomes[26].

1.10.5.4 Reverse phase evaporation

The novel key in this method is the removal of solvent from an emulsion by evaporation. Water in oil emulsion is formed by bath sonication of a mixture of two phases, and then the emulsion is dried to a semi-solid gel in a rotary evaporator under reduced pressure. The next step is to bring about the collapse of certain portion of water droplets by vigorous mechanical shaking with a vortex mixture. In these circumstances, the lipid monolayer, which encloses the collapse vesicles, is contributed to adjacent intact vesicles to form the outer leaflet of the bilayer of large unilamellar niosomes. The vesicles formed are unilamellar and have a diameter of 0.5 µm. Recently a great deal of interest is being shown in formulation of proniosomes. Proniosomes are dry formulations of surfactant coated carrier, which on rehydration and mild agitation give niosomes. Proniosomes have the advantage of circumventing the problems of physical stability such as aggregation, fusion and leaking, chemical stability such as hydrolysis, providing the convenience of transportation, distribution, storage and dosing. Proniosomes are usually prepared by dissolving spray coated surfactant in a organic solvent on to inert carriers such as sorbitol and maltodextrin[31, 32].

Briefly, method involves the creation of a solution of cholesterol and surfactant (1:1 ratio) in a mixture of ether and chloroform. An aqueous phase containing the drug to be loaded is added to this, and the resulting two phases are sonicated at 4-5 °C. A clear gel is formed which is further sonicated after the

addition of phosphate buffered saline (PBS). After this the temperature is raised to 40 °C and pressure is reduced to remove the organic phase. This results in a viscous niosome suspension which can be diluted with PBS and heated on a water bath at 60 °C for 10 min to yield niosomes[33, 34].

1.10.5.5 Trans membrane pH gradient (inside acidic) / Drug uptake process (remote loading)

In this method, a solution of surfactant and cholesterol is made in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask, similar to the hand shaking method. This film is then hydrated using citric acid solution (300mM, pH 4.0) by vortex mixing. The resulting multilamellar vesicles are then treated to three freeze thaw cycles and sonicated. To the niosomal suspension, aqueous solution containing drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 using 1M disodium phosphate (this causes the drug which is outside the vesicle to become non-ionic and can then cross the niosomal membrane, and once inside it is again ionized thus not allowing it to exit the vesicle). The mixture is later heated at 60 °C for 10 min to give niosomes[35].

1.10.5.6 The "Bubble" method

It is a technique which has only recently been developed and which allows the preparation of niosomes without the use of organic solvents. The bubbling unit consists of a round bottom flask with three necks, and this is positioned in a water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck, while the third neck is used to supply nitrogen. Cholesterol and surfactant are dispersed together in a buffer (pH 7.4) at 70 °C. This dispersion is mixed for a period of 15 sec with high shear homogenizer and immediately afterwards, it is bubbled at 70 °C using the nitrogen gas to yield niosomes[36].

1.10.5.7 Formation of niosomes from proniosomes

To create proniosomes, a water soluble carrier such as sorbitol is first coated with the surfactant. The coating is done by preparing a solution of the surfactant with cholesterol in a volatile organic solvent, which is sprayed onto the powder of sorbitol kept in a rotary evaporator. The evaporation of the organic solvent yields a thin coat on the sorbitol particles. The resulting

coating is a dry formulation in which a water soluble particle is coated with a thin film of dry surfactant. This preparation is termed Proniosome[37].

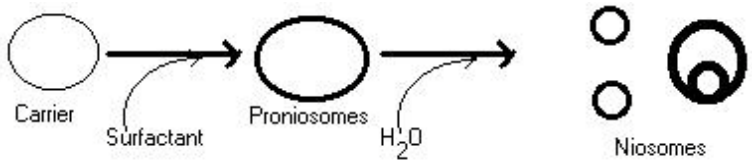

Figure 1.5: Formation of niosomes from proniosomes

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

1.10.5.8 Microfludisation

This is a recent technique to prepare small MLVS. A Microfludizer is used to pump the fluid at a very high pressure (10,000 psi) through a 5 µm screen. Hereafter; it is forced along defined micro channels, which direct two streams of fluid to collide together at right angles, thereby affecting a very efficient transfer of energy. The lipids can be introduced into the fluidizer. The fluid collected can be recycled through the pump until vesicles of spherical dimensions are obtained. This results in greater uniformity, small size and better reproducible niosomes.

1.10.6 Sizing of niosomes[38]

The size ranges of niosomes have a major effect on their fate *in-vivo* and *in-vitro*. Hence, size reduction stage of niosome is essential after hydration stage. The more commonly used methods for niosome size reduction found in literature are given below

1. Probe sonication [26, 27]: produced $C_{16}G_3$ niosomes in the 100–140 nm size range.
2. Extrusion through 100 nm Nucleopore filters[39] yielded sodium stilbogluconate $C_{16}G_3$ niosomes in the 140 nm size range.
3. In some instances the combination of sonication and filtration (220 nm Millipore® filter) has been used like DOX loaded Span 60 niosomes in the 200 nm size range[40].

4. The achievement of sub-50 nm sizes is possible by the use of a microfluidizer.
5. High-pressure homogenization also yields vesicles below 100 nm in diameter.

1.10.7 Removal of unentrapped materials

Lipophilic drugs are fully associated with niosomes due to their high affinity to the lipid bilayer. But other drugs exhibit less attachment tendency to lipid bilayer hence has entrapment efficiency less than 100%. Small fraction of unentrapped drugs can cause unacceptable side effects (anti-cancer drugs). The methods that have been used for the removal of unentrapped material identified in literature are listed below.

1. Exhaustive dialysis[27, 41]
2. Separation by gel filtration (eg. Sephadex G50)[40, 42]
3. Centrifugation (7000 *g* for 30 min)[30, 43]
4. Ultracentrifugation (150000 *g* for 1.5 h)[44]

1.10.8 Components of niosomes

Niosomes mainly contains following types of components:

1.10.8.1 Non-ionic surfactants

The non-ionic surfactants orient themselves in bilayer lattices where the polar or hydrophobic heads align facing aqueous bulk (media) while the hydrophobic head or hydrocarbon segments align in such a way that the interaction with the aqueous media would be minimized. To attain thermodynamic stability, every bilayer folds over itself as continuous membrane i.e. forms vesicles so that hydrocarbon /water interface remains no more exposed[38]. Mainly following types of non-ionic surfactants are used for the formation of niosomes:-

a) Alkyl ethers: L'Oreal described some surfactants for the preparation of niosomes containing drugs/chemicals as

1) Surfactant-I (Mol.Wt.473) is C16 monoalkyl glycerol ether with average of three glycerol units.
2) Surfactant-II (Mol.Wt.972) is diglycerol ether with average of the seven glycerol units.

3) Surfactant III (Mol.Wt.393) is ester linked surfactant. Other than alkyl glycerol, alkyl glycosides and alkyl ethers bearing polyhydroxyl head groups are also used in formulation of niosomes[27, 45].

b) Alkyl esters: Sorbitan esters are most preferred surfactant used for the preparation of niosomes amongst this category of surfactants. Vesicles prepared by the polyoxyethylene sorbitan monolaurate are relatively soluble than other surfactant vesicles. For example polyoxyethylene (polysorbate 60) has been utilized for encapsulation of diclofenac sodium[33].

c) Alkyl amides: Alkyl amide (e.g. galactosides and glucosides) have been utilized to produce niosomal vesicles[46].

d) Fatty acid and amino acid compounds: Long chain fatty acids and amino acid moieties have also been used in some niosomes preparation.

1.10.8.2 Cholesterol

Sterols are important components of the cell membrane and their presence in membrane affect the bilayer fluidity and permeability. Cholesterol is a sterol derivative, which is mainly used for the formulation of niosomes. Although it may not show any role in the formation of bilayer, its importance in formation of niosomes and manipulation of layer characteristics cannot be discarded. In general, incorporation of cholesterol affect properties of niosomes like membrane permeability, rigidity, encapsulation efficiency, ease of rehydration of freeze dried niosomes and their toxicity. It prevents the vesicle aggregation by the inclusion of molecules that stabilize the system against the formation of aggregates by repulsive steric or electrostatic forces that leads to the transition from the gel to the liquid phase in niosome systems. As a result of this, the niosome become less leaky in nature[46].

1.10.8.3 Charged molecule

Some charged molecules are added to niosomes to increase stability of niosomes by electrostatic repulsion which prevents coalescence. The negatively charged molecules used are diacetyl phosphate (DCP) and phosphotidic acid. Similarly, stearylamine (STR) and stearyl pyridinium chloride are the well known positively charged molecules used in niosomal preparations. These charged molecules are used mainly to prevent aggregation of niosomes. Only 2.5-5 mol percentage concentration of charged

molecules is tolerable because high concentration can inhibit the niosome formation.

1.10.9 Formulation aspects of niosomes

Niosomes are formed by self-assembly of non-ionic surfactants in aqueous media as spherical, unilamellar, multilamellar system and polyhedral structures in addition to inverse structures which appear only in non-aqueous solvent[40].

1.10.9.1 Nature of surfactants

Van Abbe[47] explained that the non-inonic surfactants are preferred because the irritation power of surfactants decreases in the following order: cationic> anionic> ampholytic> non-ionic. The ether type surfactants with single alkyl chain as hydrophobic tail, is more toxic than corresponding dialkylether chain. The ester type surfactants are chemically less stable than ether type surfactants and the former is less toxic than the latter because ester-linked surfactant is degraded by esterase to triglycerides and fatty acid *in* vivo[25]. The surfactants with alkyl chain length from C12-C18 are suitable for the preparation of niosomes. Span series surfactants having hydrophilic lipophilic balance (HLB) number of between 4-8 can form vesicles[42]. Guinedi *et al*.[17] prepared niosomes from Span 60 and Span 40 to encapsulate acetazolamide (ACZ). Highest drug entrapment efficiency was obtained with Span 60 in a molar ratio of 7: 6 with cholesterol. They found that both the surfactants were nonirritant with ocular tissues however; only reversible irritation of substantia propia was observed in the rabbit eye.

1.10.9.2 Charge inducer

Charge inducer is used to impart charge on the vesicles to increase its stability by preventing fusion of vesicles and providing higher value of zeta potential. The commonly used positively charge inducers are stearylamine, cetyl pyridinium chloride and negatively charge inducers are lipoamino acid and dicetyl phosphate. Aggarwal and his coworkers[48] formulated niosomes by reverse phase evaporation method to encapsulate ACZ using Span 60, cholesterol, positively (stearyl amine), and negatively (dicetyl phosphate) charge inducers. Drug entrapment efficiency varied with the charge and the percent entrapment efficiency was found to be 43.75%, 51.23% and 36.26%

for neutral, positively charged and negatively charged niosomes, respectively. The positively charged niosomes, although showed good corneal permeability and IOP lowering capacity, were however seemed to be inappropriate in terms of the corneal cell toxicity.

1.10.9.3 Bioadhesive polymer

Bioadhesive polymers are the other membrane additives that are used to provide some additional properties to the niosomes. Carbopol 934P-coated niosomal formulation of ACZ, prepared from Span 60, cholesterol, stearylamine or dicetyl phosphate exhibited more tendency for the reduction of intraocular pressure compared to that of a marketed formulation (Dorzox)[48]. Aggarwal and Kaur[49] prepared chitosan and carbopol-coated niosomes to entrap antiglaucoma agent timolol maleate by reverse phase evaporation method. Polymer coating extended the drug release up to 10 hr (releasing only 40-43% drug). However, in comparison, chitosan coated niosomes showed a better sustained effect.

1.10.9.4 Steric barrier

Some researchers[50] examined the aggregation behavior of monomethoxypoly (ethylene glycol) cholesteryl carbonates in mixture with diglycerol hexadecyl ether and cholesterol. They obtained non-aggregated, stable, unilamellar vesicles at low polymer levels with optimal shape and size homogeneity at cholesteryl conjugate/lipids ratios of 5-10 mol%. Higher levels up to 30 mol% led to the complete solubilization of the vesicles into disk-like structures of decreasing size with increasing polyethylene glycol content. This study revealed the bivalent role of the derivatives; while behaving as solubilizing surfactants, they provided an additional efficient steric barrier, preventing the vesicles from aggregation and fusion over a period of at least 2 weeks.

1.10.9.5 Isotonic stabilizer

Development of a topically effective formulation of ACZ is difficult because of its unfavorable partition coefficient, solubility, permeability coefficient, and poor stability at the pH of its maximum solubility. Based on these factors and the ability of niosomes to come into complete contact with corneal and conjunctival surfaces, niosomal drug delivery system has been investigated to

enhance the corneal absorption of ACZ. Boric acid solution (2%) is isotonic with tears and could be used as a vehicle for the ACZ niosomal formulations because the pH of maximum stability for ACZ is 4.0. A recent study revealed that boric acid solution can maintain the pH between 4.0 and 5.0. In addition, the pharmacodynamic studies showed more than 30% fall in IOP which was sustained up to 5 hr[51].

1.10.10 Factors affecting formation of niosomes

1.10.10.1 Structure of surfactants

The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of Surfactants can predict geometry of vesicle to be formed. Critical packing parameters can be defined using following equation,

$$\text{CPP (Critical Packing Parameters)} = v/l_c \times a_0 \qquad [1.2]$$

Where v = hydrophobic group volume

l_c = the critical hydrophobic group length

a_0= the area of hydrophilic head group

From the critical packing parameter value type of miceller structure formed can be ascertained as given below,

If CPP < ½ then formation of spherical micelles

If ½ < CPP < 1 formation of bilayer micelles

If CPP > 1 formation of inverted micelles

1.10.10.2 Membrane composition

The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives. In case of polyhedral niosomes formed from $C_{16}G_2$, the shape of these polyhedral niosome remains unaffected by adding low amount of solulan C24 (cholesteryl poly-24-oxyethylene ether), which prevents aggregation due to development of steric hindrance**.** In contrast spherical Niosomes are formed by $C_{16}G_2$: cholesterol:solulan (49:49:2). The mean size of niosomes is influenced by membrane composition such as polyhedral niosomes formed by C16G2:

solulan C24 in ratio (91:9) having bigger size (8.0 ± 0.03µm) than spherical/tubular niosomes formed by $C_{16}G_2$: cholesterol:solulan C24 in ratio (49:49:2) (6.6±0.2µm)[44]. Addition of cholesterol molecule to niosomal system provides rigidity to the membrane and reduces the leakage of drug from noisome[43].

1.10.10.3 Nature of encapsulated drug

The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size[39]. The aggregation of vesicles is prevented due to the charge development on bilayer.

1.10.10.4 Temperature of hydration

Hydration temperature influences the shape and size of the niosome. For ideal condition it should be above the gel to liquid phase transition temperature of system. Temperature change of niosomal system affects assembly of surfactants into vesicles and also induces vesicle shape transformation[38, 44]. Arunothayanun *et al*. reported that a polyhedral vesicle formed by $C_{16}G_2$: solulan C24 (91:9) at 25 °C which on heating transformed into spherical vesicle at 48 °C, but on cooling from 55 °C, the vesicle produced a cluster of smaller spherical niosomes at 49 °C before changing to the polyhedral structures at 35 °C**.** In contrast vesicle formed by $C_{16}G_2$: cholesterol: solulanC24 (49:49:2) shows no shape transformation on heating or cooling[38].

Along with the above mentioned factors, volume of hydration medium and time of hydration of niosomes are also critical factors. Improper selection of these factors may result in formation of fragile niosomes or creation of drug leakage problems.

1.10.11 Characterization of niosome

1.10.11.1 Size

Shape of niosomes assumed to be spherical, their mean diameter can be determined by using laser light scattering method[31]. Also, diameter can be determined by using electron microscopy, molecular sieve chromatography,

ultracentrifugation, photon correlation microscopy and optical microscopy[26, 52].

1.10.11.2 Bilayer formation

Assembly of non-ionic surfactants to form bilayer vesicle is characterized by X-cross formation under light polarization microscopy[53].

1.10.11.3 Number of lamellae

It is determined by using NMR spectroscopy, small angle X-ray scattering and electron microscopy[52].

1.10.11.4 Membrane rigidity

Membrane rigidity can be measured by means of mobility of fluorescence probe as function of temperature[53].

1.10.11.5 Entrapment efficiency (EE)

The entrapment efficiency (EE) is expressed as EE = amount entrapped/total amount added * 100. It is determined after separation of unentrapped drug, on complete vesicle disruption by using about 1mL of 2.5% sodium lauryl sulfate, briefly homogenized and centrifuged and supernatant assayed for drug after suitable dilution[54]. Entrapment efficiency is affected by following factors:

Surfactants

The chain length and hydrophilic head of non-ionic surfactants affect entrapment efficiency, such as stearyl chain C18 non-ionic surfactant vesicles show higher entrapment efficiency than lauryl chain C12 non-ionic surfactant vesicles. The tween series surfactants bearing a long alkyl chain and a large hydrophilic moiety in the combination with cholesterol at 1:1 ratio have highest entrapment efficiency for water soluble drugs[53]. HLB value of surfactants affects entrapment efficiency, such as HLB value of 14 to 17 is not suitable for niosomes but HLB value of 8.6 has highest entrapment efficiency and entrapment efficiency decreases with decrease in HLB value from 8.6 to 1.7[55]. The entrapment efficiency is affected by phase transition temperature of surfactants, i.e. span 60 exhibits highest entrapment efficiency in series having highest transition temperature (Tc)[56].

Cholesterol contents

The incorporation of cholesterol into bilayer composition of niosome induces membrane-stabilizing activity and decreases the leakiness of membrane[30].

Hence, incorporation of cholesterol into bilayer increases entrapment efficiency. The permeability of vesicle bilayer to 5, 6-carboxy flourescein (CF) is reduced by 10 times due to incorporation of cholesterol[41].

1.10.12 Specialized systems

1.10.12.1 Aspasomes (Ascorbyl palmitate vesicles)

Rambhau *et al*., reported that ascorbyl palmitate in combination with cholesterol and negatively charged lipid diacetyl phosphate forms vesicles called aspasome. The film hydration method was used for preparation of aspasomes, followed by sonication. The aqueous solution of azidothymidine was entrapped in aqueous region of bilayers. The cholesterol content in aspasomes exhibits very less effect for vesicle size and percent entrapment that affect release rate of azidothymidine. Aspasome with 45% of cholesterol shows maximum retardation in release rate than other composition. Aspasomes have inherent antioxidant properties that have potential applications toward disorder caused by reactive oxygen species. Transdermal permeation of aspasomal drug is much higher than aqueous dispersion and aqueous solution of drug[57].

1.10.12.2 Niosomes in carbopol gel

Niosomes prepared from nimesulide, span and cholesterol and incorporated in carbopol-934 gel (1%w/w) base contain propylene glycol (10%w/w) and glycerin (30%w/w). *In vitro* diffusion studies of such niosomal gel, plain drug gel and marketed gel were carried out in diffusion cell using human cadaver skin. The mean flux value and diffusion co-efficient were found to be 5 to 7 times lower for niosomal gel as compared to plain drug gels. Skin retention of drug was maximum (58.19%) in niosomal gel formulation after 24 hr of diffusion studies. This formulation also evaluated for inhibition of edema using carrageenan-induced rat paw edema method. It was found that the percent of inhibition of edema in niosomal gel i.e.66.68±5.19% is high as compared to plain gel[55].

1.10.12.3 Polyhedral niosomes

Polyhedral niosomes can be obtained from mixture of $C_{16}EO_5$ and solulan-C24 in low concentration of cholesterol. A.T.Florence *et al*., worked on extrusion of polyhedral niosomes by capillary and studied some properties of

extruded polyhedral niosomes[58]. When polyhedral niosomes extruded under certain condition into aqueous media fuse to produce long continuous stable tubules by controlling factor such pressure need to extrude niosomes and composition of vesicles. The applied shear stress on vesicle affects its release pattern such as increasing sheer stress by narrowing size of micropipette aperture increases higher release pattern of entrapped materials[59].

1.10.12.4 Vesicles in water and oil system (V/W/O)

Yoshioka *et al*., reported that the emulsification of an aqueous niosomes into an oil phase form vesicle in water in oil emulsion (V/W/O)[42]. On addition of niosomes suspension formed from mixture of sorbitol mono stearate, cholesterol and solulan C24 to oil phase at 60 ^{o}C. There is formation of vesicle in water in oil emulsion but cooling to room temperature forms vesicle in water in oil gel (V/W/O gel). The (V/W/O gel) can entrap protein and also protect it from enzymatic degradation after oral administration and controlled release. The release of entrap material is lowest in case of V/W/O gel as compared to W/O gel and niosomal suspension[60]. Florence *et al*., studied on immunogenic properties of V/W/O gel and (W/O) gel, reported that both exhibit immunoadjuvant tendency.

1.10.12.5 Niosomes in hydroxypropyl methylcellulose

Reddy *et al*., studied on anti-inflammatory effect of noisome after incorporating into hydroxypropyl methyl cellulose semi-solid base containing 10% glycerin. The bio availability and reduction of carageenan induced higher rat paw edema in case of noisome formulated in hydroxylpropyl methyl cellulose as compared to plain formulation of flurbiprofen[61].

1.10.13 Applications of niosomes

The application of niosomal technology is widely varied and can be used to treat a number of diseases. There are very less niosomal formulations found in market. But some experimentally evaluated application of niosomal formulation identified in literatures, either proven or under research, are listed below.

1.10.13.1 Drug targetting

One of the most useful aspects of niosomes is their ability to target drugs. Niosomes can be used to target drugs to the reticulo-endothelial system. The

reticulo-endothelial system (RES) preferentially takes up niosome vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins. These opsonins mark the niosome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of drugs can also be used for treating parasitic infections of the liver[16].

Niosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulins bind readily to the lipid surface of the niosome) to target them to specific organs. Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells[62].

1.10.13.2 Anti-neoplastic treatment

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism; prolong circulation and half life of the drug, thus decreasing the side effects of the drugs.

Daunorubicin HCl

Niosomal daunorubicin hydrochloride exhibited an enhanced anti-tumor efficacy when compared to free drug. The niosomal formulation was able to destroy the Dalton's ascitic lymphoma cells in the peritoneum within the third day of treatment, while free drug took around six days and the process was incomplete. The hematological studies also prove that the niosomal formulation was superior to free drug treatment. An enhanced mean survival time was achieved by the niosomal formulation that finally substantiates the overall efficacy of the niosomal formulation[54].

Doxorubicin

Rogerson *et al*., studied distribution of niosomal doxorubicin prepared from C16 monoalkyl glycerol ether with or without cholesterol. Niosomal formulation exhibited an increased level of doxorubicin in tumor cells, serum and lungs, but not in liver and spleen. Doxorubicinloaded cholesterol-free niosomes decreased the rate of proliferation of tumor and increased life span of tumorbearing mice. The cardio toxicity effect of doxorubicin was reduced by

niosomal formulation. Niosomal formulation changes the general metabolic pathway of doxorubicin[30].

Methotrexate

Azmin *et al*., quoted in their research article that niosomal formulation of methotrexate exhibits higher AUC as compared to methotrexate solution, administered either intravenously or orally. Tumoricidal activity of niosomally-formulated methotreaxate is higher as compared to plain drug solution[26].

Bleomycin

Niosomal formulation of bleomycin containing 47.5% cholesterol exhibits higher level drug in the liver, spleen and tumor as compared to plain drug solution in tumor bearing mice. There is no significant difference in drug concentration with niosomal formulation in lung as compared to plain drug solution. Also, there is less accumulation of drug in gut and kidney in case of niosomal formulation[63].

Vincristine

Niosomal formulation of vincristine exhibits higher tumoricidal efficacy as compared to plain drug formulation[64]. Also, niosomal formulation of carboplatin exhibits higher tumoricidal efficacy in S-180 lung carcinoma-bearing mice as compared to plain drug solution and also less bone marrow toxic effect[65].

Leishmaniasis

Leishmaniasis is a disease in which a parasite of the genus *Leishmania* invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of niosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment[25].

1.10.13.3 Delivery of peptide drugs

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated. In an *in vitro* study conducted by Yoshida *et al*, oral

delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide[56].

1.10.13.4 Use in studying immune response

Due to their immunological selectivity, low toxicity and greater stability; niosomes are being used to study the nature of the immune response provoked by antigens[66].

1.10.13.5 Niosomes as carriers for haemoglobin

Niosomes can be used as carriers for haemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anemic patients[67].

1.10.13.6 Transdermal drug delivery systems utilizing niosomes

One of the most useful aspects of niosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing niosomal technology is widely used in cosmetics; in fact, it was one of the first uses of the niosomes. Topical use of niosome entrapped antibiotics to treat acne is done. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug[26].

Recently, transdermal vaccines utilizing niosomal technology is also being researched. A study conducted by P. N. Gupta *et al* has shown that niosomes (along with liposomes and transfersomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in niosomes allows only a weak immune response, and thus more research needs to be done in this field.

1.10.13.7 Ophthalmic drug delivery

It is difficult to achieve excellent bioavailability of drug from ocular dosage form like ophthalmic solution, suspension and ointment due to the tear production, impermeability of corneal epithelium, non-productive absorption and transient residence time. But to achieve good bioavailability of drug various vesicular systems are proposed to be use, in experimental level, like niosomes, liposomes. Bioadhesive-coated niosomal formulation of acetazolamide prepared from span 60, cholesterol stearylamine or dicetyl phosphate exhibits more tendencies for reduction of intraocular pressure as compared to marketed formulation (Dorzolamide). The chitosan-coated

niosomal formulation timolol maleate (0.25%) exhibits more effect for reduction intraocular pressure as compared to a marketed formulation with less chance of cardiovascular side effects[48].

1.10.13.8 Other applications

Diagnostic imaging with niosomes

Niosomal system can be used as diagnostic agents. Conjugated niosomal formulation of gadobenate dimeglcemine with [N-palmitoyl-glucosamine (NPG)], PEG 4400, and both PEG and NPG exhibit significantly improved tumor targeting of an encapsulated paramagnetic agent assessed with MR imaging[68].

Niosome formulation as a brain targeted delivery system for the vasoactive intestinal peptide (VIP)

Radiolabelled (I^{125}) VIP-loaded glucose-bearing niosomes were injected intravenously to mice. Encapsulated VIP within glucose-bearing niosomes exhibits higher VIP brain uptake as compared to control[69].

Niosomes can also be utilized for sustained drug release and localized drug action to greatly increase the safety and efficacy of many drugs. Toxic drugs which need higher doses can possibly be delivered safely using niosomal encapsulation.

It is obvious that niosome appears to be a well preferred drug delivery system over liposome as niosome being stable and economic. Also niosomes have great drug delivery potential for targeted delivery of anti-cancer, antiinfective agents. Drug delivery potential of niosome can enhance by using novel concepts like proniosomes, discomes and aspasome. Niosomes also serve better aid in diagnostic imaging and as a vaccine adjuvant.

Marketed products

Lancome has come out with a variety of anti-ageing products with niosomes. L'Oreal is also conducting research on anti-ageing cosmetic products.

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CHAPTER 2

LITERATURE REVIEW

Chapter 2 - LITERATURE REVIEW

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2. LITERATURE REVIEW

2.1 Ocular inserts

Sasaki Hitoshi *et al*., (1993)[1] developed disc type ophthalmic inserts of beta-blockers with various polymers and drug release from the inserts were investigated. Release of tilisolol from ten different types of polymer inserts showed a variety patterns. In the inserts prepared with poly(2-hydroxypropyl methacrylate)HPM and poly(2-hydroxyethyl methacrylate), the release data were fitted to a simple power equation and it was found that the release characteristics of tilisolol from these systems followed behavior conforming to a non-Fickian mechanism. Medium pH and medium temperature influenced the release of tilisolol from the HPM insert. Various beta-blockers also showed controlled release from their HPM inserts. Macromolecular dye and insulin showed a slow release with an initial burst effect. Release data for an HPM insert under various conditions were also fitted to a simple power equation. The *in vivo* release pattern of tilisolol from an HPM insert in rabbit conjunctival sac reflected the *in vitro* release pattern.

John H. Draize *et al*., (1944)[2] modified the interpretation of Friedinwald procedure for the objective measurement of injuries to rabbit eyes and extended the same principle to the evaluation of other physiological effects. In the "Modified Draize Technique", they have transformed quantitative observations of physiological effects to reasonably quantitative objective measurements and also they have applied the principal of assigning numerical values to physiological phenimena in order to obtain data easily subject to arithmatical interpretation. It has been considered as the official method in the Federal Hazardous Substance Act, USA.

Kauffmann *et al*., (1971)[3] studied the various medical properties of soft contact lens indicating their ability to take up and release medications. Both humans and animals were used for the study.

Loucas S.P. and Hadded H.M., (1972)[4] concluded that solid state dosage forms of pilocarpine in the cul-de-sac gave more uniform release.

Grass *et al*., (1984)[5] carried out *in vitro* dissolution and mitosis studies in rabbits to evaluate the sustained action properties of pilocarpine nitrate in polymeric films and gels. Significant prolongation of drug release was

demonstrated when the polymer systems were compared to simple aqueous or viscous solutions.

M. F. Saettone *et al*., (1984)[6] formulated polymeric ophthalmic inserts containing pilocarpine with four different types of polyvinyl alcohol, PVA, and two types of hydroxypropylcellulose. Pilocarpine was present as the nitrate, or as the salt with polyacrylic acid, PAA. In-vivo miosis vs time experiments on albino rabbits, showed that all inserts increased significantly the bioavailability of pilocarpine, with respect to a standard solution of pilocarpine nitrate. Two PVA inserts, containing the PAA-salt of pilocarpine, were particularly effective. The preparations were also submitted to in-vitro release tests and to differential scanning calorimetry, to ascertain the release mechanism, and to verify, via the thermal behaviour, possible interactions between drug and polymers. The chemical and physicochemical factors, most likely to influence the ophthalmic bioavailability of pilocarpine from the present preparations, are briefly reviewed.

K.P. Rao *et al*., (1988)[7] used pepsin-treated telopeptide-poor foetal calf skin collagen as a carrier for a controlled release of pilocarpine nitrate. Three types of collagen-pilocarpinenitrate drug delivery systems were developed. *In vitro* release of pilocarpinenitrate from these systems was studied. The release studies indicated that after an initial boost release, pilocarpine was released at a constant rate following zero-order kinetics. The release of the drug can be manipulated based on the type of modification made on the collagen carrier. The release rate of pilocarpinenitrate could be regulated from 5 to 15 days depending on the modification made on the collagen carrier. Collagen film, because of its biological inertness, structural stability and good biocompatibility, proved to be the most promising carrier for ophthalmic drug delivery systems.

Attia *et al*., (1988)[8] evaluated *in vivo* (in rabbits) performance of dexamethasone ophthalmic film and concluded that ophthalmic film delivery system may target the drug to the eye tissue in which the drug is otherwise poorly available.

Dumortier *et al*., (1994)[9] compared the lachrymal and plasmatic kinetic of morphine from a thermosensitive gel, an insert and a simple solution and evaluated the lacrimation in rabbits. It was observed that the inserts prolonged

the lachrymal and plasmatic kinetics of morphine. In lachrymal fluid, the maximum concentration was delayed from 2.9 to 51.4 min.

Marco fabrizio saettone *et al*., (1995)[10] discussed the advantages, disadvantages and requirement for the success of ocular inserts. They examined few inserts which are available in the market or are being developed by pharmaceutical companies for drug delivery. They discussed S.O.D.I., Ocusert, Collagen Shields, Ocufit, Minidisc and NODS with special attention to biological / clinical performances and potential for future applications and development.

Gautler and Gurny, (1995)[11] reviewed the technologies involved in the development of various types of ophthalmic inserts. The design, conception, release mechanism, *in vivo-in vitro* assays, limitations and therapeutic rationale for the use of ophthalmic inserts were discussed.

N. Udupa *et al*., (1996)[12] incorporated norfloxacin and its betacyclodextrin complex into polymeric matrices and showed sustained drug release. The *in vitro* and *in vivo* release of complex was found to be better than the plain drug.

Manvi *et al*., (1997)[13] developed timolol maleate circular ophthalmic insert by solvent casting technique using cellulose acetate as polymer with PEG 600 and diethyl phthalate as plasticizer in two different concentration. Plasticizer system influences their effect on drug release. The correlation was obtained in both *in-vivo* and *in-vitro* method.

R. Gurny *et al*., (1998)[14] developed a soluble ocusert having gentamicin sulphate and dexamethasone phosphate and demonstrated the concomitant release of both drugs during the first 10 hr treatment, followed by an adequate concentration of gentamicin sulphate above the MIC of 4 µg/mL during 50 hr by using CAP solid dispersion.

Patrizia chetoni *et al*., (1998)[15] developed rod-shaped mucoadhesive ophthalmic inserts fitting the upper or lower conjuctival fornix using silicone elastomer and polyacrylic acid(PAA) or polymethacrylic acid (PMA) interpenetrating polymer network grafted on surface. They showed 90% release of oxytetracyclin HCL after 12 days from this inserts.

R. Vijaya muthu manikander *et al*., (1998)[16] reported that diclofenac sodium ocular inserts using 4% HPMC released the drug over an extended period of 11 hr.

Samual H. Yalkowsky *et al*., (1999)[17] formulated a gelfoam based ocular device containing 1.7 mg of Phenylephrine and 0.6 mg of Tropicamide. The *in vivo* results showed that the mydriatic response produced by the proposed device is larger and longer lasting than that produced by eye drops with an equivalent amount of Phenylephrine and Tropicamide.

Saisivam *et al*., (1999)[18] proved zero order release of ciprofloxacin hydrochloride from ocuserts made up of HPMC(2%) as drug reservoir and EC (6%) as a rate controlling membrane.

Karatas *et al*., (2000)[19] prepared ophthalmic inserts containing Indomethacin using water soluble polymers such as hydroxypropl cellulose (HPC), methlycellulose (MC), hydroxypropyl methlycellulose (HPMC) and polyvinyl alcohol (PVA), according to the film casting (FC) and compression molding (CM) methods and studied the effects of different polymers and methods on *in vitro* drug release. The hydrophilicity of inserts was tested by measuring their water vapor absorption. *In vitro* insert hydration was also determined by measuring their water absorption. Drug release was reduced by the use of high viscosity and less soluble polymers. Inserts prepared by the compression molding technique exhibited higher release rates than those prepared by film casting method.

S. Jayaprakash *et al*., (2000)[20] investigated different polymers (HPMC, PVP, MC, EC) for ocuserts and proved zero order release of ketorolac tromethamine from ocuserts consisted of 3% HPMC and 4% EC as a rate controlling membrane.

G. Di Colo *et al*., (2001) prepared and evaluated ocular inserts of ofloxacin using poly(ethylene oxide) alone and with neutralized eudragit L100 by powder compression technique. These inserts were able to form insitu mucoadhesive gels which eroded slowly to release the drug. The inserts based on the PEO – EUD Na17 compound yielded a profile typical of a zero order controlled delivery system[21].

They also studied correlation between PEO molecular weight and insert properties potentially related to its therapeutic efficacy and showed that low

molecular weight PEO were suitable for ocuserts while high molecular weight PEO were not suitable[22].

They also evaluated PEO based inserts, containing Chitosan microparticles, which produced microstructural changes and hence accelerated both insert erosion and ofloxacin release. Chitosan increased the diffusive contribution to the release mechanism with respect to the inserts based on plain PEO. Thus they suggested ability of chitosan-hydrochloride to enhance corneal permeability[23].

Ging-Ho Hsiue *et al*., (2001)[24] investigated pilocarpine trapped in a matrix diffusion-controlled drug delivery system using hydrophilic inserts of Poly(2-hydroxyethyl methacrylate) (pHEMA) to ensure an increased bioavailability of pilocarpine and prolong the length of time in which the medication remains in the eyes of the test subjects. The physical and chemical properties of pilocarpine were investigated to elucidate the mechanism of drug–polymer interaction and the effect on drug release behavior of controlled release polymeric devices. *In vitro* release studies indicated that pilocarpine continued to be released from the inserts for a 24 h period. The results of intraocular pressure tests performed on albino rabbits were consistent with the observed *in vitro* behavior. The pressure decrease was significant for a period longer than 48 h. It confirms that the inserts, as sustainable releasing devices, are promising carriers for ophthalmic drug delivery systems

Margit Hornof *et al*., (2003)[25] developed a mucoadhesive ocular insert for the controlled delivery of ophthalmic drugs. The inserts tested were based either on unmodified or thiolated poly(acrylic acid).Water uptake and swelling behavior of the inserts as well as the drug release rates of the model drugs fluorescein and two diclofenac salts with different solubility properties were evaluated *in vitro*. Fluorescein was used as fluorescent tracer to study the drug release from the insert in humans. The mean fluorescein concentration in the cornea/tearfilm compartment as a function of time was determined after application of aqueous eye drops and inserts composed of unmodified and of thiolated poly(acrylic acid). The acceptability of the inserts by the volunteers was also evaluated. Inserts based on thiolated poly(acrylic acid) were not soluble and had good cohesive properties. A controlled release was achieved for the incorporated model drugs. The *in vivo* study showed that inserts based

on thiolated poly(acrylic acid) provide a fluorescein concentration on the eye surface for more than 8 hr, whereas the fluorescein concentration rapidly decreased after application of aqueous eye drops or inserts based on unmodified poly(acrylic acid). Moreover, these inserts were well accepted by the volunteers. The present study indicates that ocular inserts based on thiolated poly(acrylic acid) are promising new solid devices for ocular drug delivery.

Hitoshi Sasaki *et al*., (2003)[26] prepared a unique one-side-coated insert that releases from only uncoated side. It was prepared by attaching a polypropylene tape on the one side of the polymer disc of poly(2-hydroxypropylmethacrylate) containing Tilisolol as a model drug. Ocular application of the one-side-coated insert produced the constant concentrations of tilisolol in the tear fluid over 180 min. A release of Tilisolol from the one-side-coated insert was twice slower than from the uncoated insert.

Naseem A. Charoo *et al*., (2003)[27] prepared ocuserts using sodium alginate and ciprofloxacin hydrochloride as a drug reservoir, Eudragit and PVA films as a rate controlling membrane and demonstrated zero order release of the drug over an extended period of 12 hr.

P. M. Dandagi *et al*., (2003)[28] prepared ketorolac tromethamine ocular films using PVP and Sod. CMC. *In vitro* drug release studies were carried out by using bichambered donor receptor compartment model designed with open end cylinder using transparent cellulose type semi permeable membrane. Formulations were also subjected to *in vivo* drug release study after sterilization by U.V. radiation followed by sterility test as per I.P. method.

F. V. Manvi *et al*., (2004)[29] prepared diclofenac sodium circular ocular inserts using gelatin(18% w/v and 20% w/v) as a polymer and glycerin as a plasticizer in two different concentration (70 % w/v and 50 % w/v) on dry weight of gelatin. *in vitro* and *in vivo* release of the inserts were determined. It was confirmed by strong positive correlation between the two results indicating that inserts could control drug release and might improve ocular bioavailability and reduce toxicity of diclofenac sodium.

Samanta *et al*., (2004)[30] developed ciprofloxacin hydrochloride ocuserts and evaluated their potential for prolonged ocular delivery. Inserts were fabricated

with sodium alginate films loaded with drug and then treated with calcium chloride. *In vitro* release of the drug from the inserts followed Higuchi's and first order kinetic models. The *in vivo* studies were carried out in rabbit eyes by measuring tear fluid concentration against time.

P. M. Dandagi *et al*., (2004)[31] formulated ocular films with cromolyn sodium using PVA and sodium alginate. Films showed extended release of drug for 12 hr following zero order kinetics and non-fickian mechanism. They also demonstrated strong *in vitro-in vivo* correlation.

Venkateshwara Rao *et al*., (2004)[32] prepared norfloxacin ocular inserts using PVP, EVA, HPMC. They have developed new technique of *in vitro* evaluation in a fabricated flow through cell. All the films prepared were found to be uniform in thickness, and the partition coefficient of norfloxacin and its betacyclodextrin complex was 0.048 and 0.853, respectively. I.R. spectra revealed complexation of norfloxacin with b-cyclodextrin. *In vitro* results revealed that 2 patch/insert formulations, V1 and V2, followed perfect zero order kinetics release (n = 1), and 3 formulations, V3, V4 and V5, released the drug by super case II kinetics (n > 1). The study confirmed the improved solubility of norfloxacin when complexed with b-cyclodextrin and that it can be delivered through films made of HPMC matrix cast with EC alone or with a combination of PVP K30.

D. Dhachinamorthi *et al*., (2005)[33] prepared ofloxacin ocuserts using different polymers such as HPMC, MC and EC at different concentrations. The Physicochemical parameters and *in vitro* release of the drug from the formulations were studied using commercial semipermeable membrane. A zero-order release formulation with 3% HPMC, 4% EC was subjected to *in vivo* after sterilization. The expected zero order release for one day was observed in formulation mentioned as above.

V. Sankar *et al*., (2005)[34] used MC and Sodium CMC as polymers for ocuserts and showed sustained release of diclofenac sodium. Ocuserts were evaluated for uniformity of drug content, *in vitro* drug release and stability studies. It was concluded that ocuserts prepared with 4% SCMC and 1% MC showed sustained release of drug and it was found to be stable at 30 and 40 ^{o}C for 2 month.

Yasmin sultana *et al*., (2005)[35] worked on pefloxacin mesylate ocular inserts using Eudragit RS 100, Eudragit RL 100 and PVP K-30 and demonstrated drug release for 5 days with good long term stability of formulations.

S. Jayaprakash *et al*., (2005)[36] prepared timolol maleate ocuserts using different polymers such as HPMC, EC, and Eudragit RL 100 at various concentrations. The zero order release was observed in the formulation containing 1.25% HPMC and 1.25% EC as a drug reservoir and 2% HPMC as rate controlling membrane which was subjected for *in vivo* studies. The expected zero order release for one day was observed in the formulation mentioned as above.

Hiratani H. *et al*., (2005)[37] evaluated the ocular *in vivo* release of timolol maleate from molecularly imprinted soft contact lenses on male Nippon albino rabbit. They concluded that imprinted soft contact lenses are promising drug devices able to provide greater and more sustained drug concentration in tear fluid with lower dosage than conventional eye drops.

J. K. Pandit *et al*., (2006)[38] prepared soluble ocular inserts of ciprofloxacin using high and low molecular weight PVA and performed microbiological evaluation. Inserts showed matrix diffusion kinetic release showing an anomalous release mechanism (erosion controlled release).

Aysegul Karatas and Tamer Baykara, (2006)[39] prepared inserts of water-soluble ketorolac tromethamin (KT) and water-insoluble indomethacin (IND) using hydrogels such as Poly (butyl methacrylate) (pBMA), Poly (2-hydroxyethyl methacrylate) (pHEMA), and poly (2-hydroxypropyl methacrylate) (pHPMA), and a plasticizer such as Polyethylene glycol 300 (PEG) by film casting method. Swelling properties of these inserts was determined and they were irradiated with an absorbed dose of 1.2 Mrad by means of a Co- 60 source. The effects of these parameters on the drug release were examined. The mechanism of drug release was identified by means of the semi-empirical equation developed by Korsmeyer and Peppas. Swelling of the hydrogels and release of drugs from the hydrogels increased with size of side chain, hydroxyl groups on the side chain of the acrylate and using PEG 300 in the formulation, which increase hydrophilicity. Water–soluble KT showed higher release than water–insoluble IND. It was also

observed no effect of irradiation dose on the release of drugs from the inserts. Release of KT mainly fit to the Fickian diffusion mechanism, whereas drug release of IND mainly showed the non-Fickian release mechanism according to their n exponent values.

A. S. Mundada and B. K. Shrikhande, (2006)[40] prepared reservoir using natural hydrophilic polymer viz. gelatin while rate-controlling membrane was prepared using hydrophobic ethyl cellulose. Since targeted prolong release was observed in formulation CF2 and CF5, these formulations were further subjected to *in vivo* drug release study using rabbits as an animal model. *In vitro* drug release kinetic data was treated according to Zero, First, and Higuchi kinetics to access the mechanism of drug release. Correlation between *in vitro* and *in vivo* drug release was found to be strong revealing the efficacy of the formulation.

Leo H. Koole *et al*., (2007)[41] prepared the ophthacoil, a flexible and tubular device for delivery of drugs to the tear film of the eye. Poly(2-hydroxyethyl methacrylate)- and poly(2-hydroxyethyl methacrylate-*co*-1-vinyl-2-pyrrolidone)-microspheres were prepared by suspension polymerization. The resultant particles were swollen in a highly concentrated solution of either the dye fluorescein sodium or the antibiotic chloramphenicol. The loaded particles were placed in the central cavity of the ocular device. *In vitro* release profiles showed a six-fold increase of the capacity for the dye fluorescein sodium, but not for the antibiotic chloramphenicol. Flexibility measurements revealed that by introducing microspheres in the central cavity of the device, flexibility did not decrease. Finally, a preliminary *in vivo* evaluation of the device (n = 5) was done for a 2 hr-period to assess the tolerance of the device in the human eye. Ophthalmologic examinations and photographs of the eye indicated no signs of irritation. Volunteers reported that the presence of the device in the eye could be noticed, but no irritation was reported.

Tanwar Y.S. *et al*., (2007)[42] prepared polyvinyl alcohol (PVA) ofloxacin films by mercury substrate method. The weight and thickness of the inserts were in the range of 57.3-126.0 mg and 55.6-99.3 microns. Tensile strength and percent elongation at break varied with the nature of rate-controlling membrane and film thickness. Moisture vapour transmission through films followed zero-order kinetics and decreased with increase in film thickness.

The drug content varied from 99.53-99.86%. The method of exposure to UV radiation was used for sterilization of ocular inserts and no microbial growth was observed in any formulation during sterility testing by direct inoculation method. Ocular insert F3 with rate-controlling membrane of Eudragit RS100, when inserted into the eye of rabbit showed controlled release up to 24 hr. There was a good correlation between *in vitro* and *in vivo* release data. The developed formulation was effective against selected microorganism during *in vitro* antimicrobial efficacy studies.

S.Ramkanth *et al*., (2009)[43] prepared diclofenac sodium ocuserts by using different polymers such as hydroxy propyl methyl cellulose (HPMC), hydroxy propyl cellulose (HPC), methyl cellulose (MC) and ethyl cellulose (EC) at various concentrations and combinations using dibutyl phthalate (DBP) as plasticizer. The *invitro* drug release was studied using commercial semi permeable membrane. A zero order release formulation F3 were sterilized by ethylene oxide and subjected to *in vivo* studies. IR spectral observation show there is no interaction of drug with polymer which indicates the intactness of drug in formulation. Ocular toxicity test and accelerated stability studies were also carried out for the formulation F3.

2.2 Niosomes

Baillie a. J. *et al*., (1985)[44] prepared vesicles on hydration of a mixture of a single or double alkyl-chain, non-ionic surfactant with cholesterol. These vesicles, or 'niosomes', are capable of entrapping and retaining water soluble solutes such as carboxyfluorescein, are osmotically active and can be formulated to release entrapped solute slowly. The physical characteristics of the vesicles were found to be dependent on the method of production and three such methods, based on liposome technology, are described. The vesicles have been characterized by photon correlation spectroscopy, freeze fracture electron micrography, measurement of solute entrapment efficiency, and solute release rates. Vesicular forms of the single chain surfactant which could be formed under certain conditions in the absence of cholesterol are also described.

Rogerson *et al*., (1987)[45] studied the effect of encapsulation of adriamycin into niosomes, and its resultant chemical purity by means of HPLC and high-

speed scanning spectrophotometry (the simultaneous use of which allowed investigation of potential non-fluorescent drug degradation products), and the process shown not to adversely affect the drug. Efficiency of entrapment of aqueous solutions of the drug was apparently dependant on neither vesicle composition nor method of production, and evidence of a degree of surfactant-adriamycin association was provided by the high entrapment values. Light-induced drug degradation was reduced by niosome encapsulation, and efflux of entrapped adriamycin was decreased by inclusion of cholesterol into the vesicles, in a manner similar to that reported for liposome preparations. Thus only chemically pure adriamycin was entrapped in, and released from, niosomes.

Florence *et al*., (1988)[46] prepared multilamellar niosomes from a c_{16} triglyceryl ether with and without cholesterol and containing doxorubicin (adriamycin) were administered to s180 tumour-bearing mice by bolus injection. Although in-vitro drug release from cholesterol-containing niosomes is delayed, in-vivo there was little difference between the two preparations when plasma levels were compared. As previously observed, half-lives of the drug were prolonged compared with free solution profiles. Liver uptake was not significantly affected by niosome encapsulation of doxorubicin. Tumour levels of drug were higher following administration of cholesterol-containing niosomes and this was reflected in the more effective reduction in tumour growth.

Alexander *et al*., (1994)[47] formulated multilamellar vesicles (niosomes) of a series of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan trioleate (Span 85) using a mechanical shaking technique without sonication. 5(6)-Carboxyfluorescein (CF) was used as a model solute to investigate entrapment efficiency and release. For Span 80, cholesterol and dicetyl phosphate (DCP) in the molar ratio 47.5:47.5:5.0, entrapment efficiency increased linearly with increasing concentration of lipid. Entrapment efficiency per mmol lipid, however, was constant at about 34%, independent of the lipid concentration. Entrapment efficiency increased with increasing cholesterol content when vesicles were prepared by changing the molar ratio of non-ionic surfactant to cholesterol. Most efficient entrapment of CF occurred with Span 60 (HLB 4.7). Mean size of the un-sonicated niosomes showed a regular

increase with increasing HLB from Span 85 (HLB 1.8) to Span (HLB 8.6). The release rate of CF from vesicles depended on the surfactant used in the preparation of the vesicles.

Yoshioka *et al*., (1995)[48] developed the niosomes-in-water-in-oil (v/w/o) system. The properties of the surfactant used to form the vesicles, the surfactant or surfactant mixture used to stabilize the emulsion and the nature of the oil phase can be changed to provide systems of different capacities for drug or antigen and different release characteristics. The same nonionic surfactant is used as the principle amphipile to form the niosomes and to stabilize the w/o emulsion, thus promoting stability by decreasing transfer of surfactant between the stabilizing monolayers and the vesicle bilayers. The *in vitro* release of carboxyfluoroscein and 5-fluorouracil encapsulated within the niosomes of the v/w/o system has been investigated, the nature of the oil phase and surfactant-oil interactions being important in determining the rate of solute release. Initial studies of the system *in vivo,* as an adjuvant for tetanus toxoid, using cottonseed oil as the external oil phase, showed enhanced immunological activity over the free antigen or vesicles.

Uchegbu and S. P. Vyas, (1998)[49] gave summary of the achievements in the field of niosome research to date. They suggested that the self assembly of surfactants into niosomes is governed not only by the nature of the surfactant but by the presence of membrane additives, the nature of the drug encapsulated and the actual method of preparation. Methods of niosome preparation and the number of different morphologies that have been identified are detailed. The influence of formulation factors on niosome stability is also examined as are methods to optimise drug loading. *In vivo* these systems have been evaluated as immunological adjuvants, anti-cancer:anti-infective drug targeting agents and carriers of anti-inflammatory drugs. Niosomes have also been used in diagnostic imaging. Efforts to achieve transdermal and ophthalmic drug delivery with some formulations are also discussed.

Alhaique *et al*., (1998)[50] prepared niosomes from polysorbate 20 and cholesterol by means of two different methods: by direct sonication of an aqueous dispersion of the various components (bulk) or by solubilization of the components, evaporation of the organic solvent to form a film inside the

vessel used for the preparation and then by sonication (film). The influence of the preparation technique on the properties of the obtained structures was studied. Vesicles with bigger dimensions and higher entrapment efficiency were obtained when sonication was carried out after the film formation. Vesicle formation in the presence of ionic surfactants was investigated in order to evaluate the effect of charged components on vesicle dimensions, entrapment efficiency and stability. Dimethyldioctadecylammonium bromide (DDOA) and cetylpyridinium chloride (CPy) were used to introduce a positive charge in the vesicle structure, while dicetylphosphate (DCP) was used for a negative charge. Better resistance to osmotic stress and higher entrapment efficiency values were obtained with vesicles containing DCP and CPy.

Uchegbu *et al*., (2000)[51] prepared polymeric vesicles and niosomes bearing glucose or transferrin ligands for drug targeting. A glucose-palmitoyl glycol chitosan (PGC) conjugate was synthesised and glucose-PGC polymeric vesicles prepared by sonication of glucose-PGC/ cholesterol. N-palmitoylglucosamine (NPG) was synthesised and NPG niosomes also prepared by sonication of NPG/ sorbitan monostearate/ cholesterol/ cholesteryl poly-24- oxyethylene ether. TEM imaging confirmed the presence of glucose units on the surface of PGC polymeric vesicles and NPG niosomes. Transferrin was coupled to PGC vesicles at a level of 0.60 ± 0.18 g of transferring per g polymer. The proportion of FITC-dextran positive A431 cells was 42% (FITC-dextran solution), 74% (plain vesicles) and 90% (transferrin vesicles).

Blazek-Welsh and David G., (2001)[52] developed proniosomes, a dry formulation using a sorbitol carrier coated with nonionic surfactant, which can be used to produce niosomes within minutes by the addition of hot water followed by agitation. The sorbitol carrier in the original proniosomes was soluble in the solvent used to deposit surfactant, so preparation was tedious and the dissolved sorbitol interfered with the encapsulation of one model drug. A novel method is reported here for rapid preparation of proniosomes with a wide range of surfactant loading. A slurry method has been developed to produce proniosomes using maltodextrin as the carrier. The time required to produce proniosomes by this simple method is independent of the ratio of surfactant solution to carrier material and appears to be scalable.

David G. Rhodes *et al*., (2001)[53] developed a novel method for producing proniosomes with a maltodextrin carrier, which provides for rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free-flowing powder which could be rehydrated by addition of warm water. Successful rehydration of surfactant to produce niosomes from dried film requires that the film be as thin as possible to avoid the clumping and precipitation that occurs when pure, granular surfactant is hydrated directly. The appearance of a coarse, broken surface on the proniosomes correlates with inefficient rehydration and occurrence of aggregation and precipitate in the final niosome suspension.

Joke A. Bouwstra *et al*., (2001)[54] prepared vesicles composed of the single chain non-ionic surfactant octaoxyethylenelaurate-ester (PEG-8-L), the sucrose laurate-ester L-595 and cholesterol sulfate (CS) to monitor local dynamic properties of lipid molecules in vesicle bilayers and to study the elasticity of vesicle bilayers. Studies with the spin label probes 5-, 12- and 16-doxyl stearic acid (DSA) indicated that both the order parameter and the rotational correlation times increased when the doxyl group was positioned closer to the headgroup region. These findings indicate that the fluidity of membranes decreased near the headgroup region. Comparing 16-DSA incorporated in vesicle formulations with either 30 or 70 mol% showed no difference in alkyl chain mobility as was reflected by the order parameter. The rotational correlation times, however, showed a slowdown from 0.38 to 0.71 and 1.13 ns when the PEG-8-L molar content was decreased from 100 to 70 and 30 mol% for PEG-8-L:L-595:CS vesicles, respectively. Extrusion measurements indicated an increase in elasticity of vesicle bilayers as the molar content of PEG-8-L was increased from 10 to 90 mol%.

Rambhau *et al*., (2004)[55] developed azidothymidine (AZT) Aspasomes (Ascorbyl palmitate (ASP) vesicles) containing 18–72 mol% cholesterol and a negatively charged lipid (dicetyl phosphate) by film hydration method followed by sonication. Differential scanning calorimetric data of aspasome dispersion and anhydrous mixtures of ascorbyl palmitate, cholesterol and dicetyl phosphate confirm the formation of bilayered vesicles with ascorbyl palmitate. Cholesterol content in aspasome did not exhibit any relation with vesicle size, zeta potential or percent entrapment. A substantial change in release rate of

azidothymidine from aspasome was noticed on varying the proportion of cholesterol. A preparation with 45 mol% of cholesterol showed maximum retardation in release rate, than other compositions. The antioxidant potency of Aspasomes was assessed by measuring the protection offered by this preparation against quinolinic acid induced lipoperoxidation of whole human blood *in vitro*, where in the lipoperoxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS) levels. Aspasome rendered much better antioxidant activity than ascorbic acid. Transdermal permeation of aspasomal AZT, ASP-AZT aqueous dispersion and AZT-solution across excised rat skin was investigated *in vitro* using Franz diffusion cell. Permeation of aspasomal AZT was much higher than the other two preparations. However, ASP-AZT aqueous dispersion has also enhanced permeation of AZT significantly over the AZT-solution, indicating skin permeation enhancing property of ascorbyl palmitate.

Behrooz Nasseri, (2005)[56] studied mechanical characteristics of non-ionic bilayer membranes composed of sorbitan monostearate, cholesterol and poly-24-oxyethylene cholesteryl by measuring the modulus of surface elasticity (μ), a measure of membrane strength, as a function of cholesterol content and temperature. The modulus of surface elasticity increased slowly with increasing cholesterol concentration, with a sharp increase around 40 mol% cholesterol (on average an increment of 0.43×106 Nm−2 per molar percentage), and displayed a maximum of 6.5×106 Nm−2 around 47.5 mol% cholesterol. Further cholesterol resulted in a decrease inμ. Generally the interaction of cholesterol with the sorbitan monostearate should increase the rigidity of the membrane. However, the latter effect may be due to the formation of cholesterol clusters at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayers compromising their uniformity, strength and permeability. This behaviour was evident when measurements were carried out above and below 25 ^{o}C.

Aggarwal and I. P. Kaur, (2005)[57] prepared chitosan (REVTMbio1) or Carbopol (REVTMbio2 and 3) coated niosomal timolol maleate (0.25%) formulations by reverse phase evaporation (REV) and compared to timolol solution (TMS; 0.25%) in terms of *in vitro* release and IOP lowering

pharmacodynamic effect. The *in vitro* release phase of timolol (91% release in 2 h) was extended significantly by its incorporation into niosomes and further by the polymer coating (40–43% release upto 10 h). The developed formulations were evaluated for their pharmacodynamics in albino rabbits, by measuring intraocular pressure (IOP) using a non-contact pneumatonometer, and were compared to a marketed in situ gel forming solution of timolol (Timolet GFS, 0.5%; Sun Pharma). REVTMbio1 formulation showed a more sustained effect of upto 8 h (vis a vis 6 h for carbopol-coated niosomes). TMS in comparison showed effect for only 2 h though the peak effect was slightly more (14%). Lowering of IOP in the contralateral eye (20–40% as compared to 100% in case of TMS), considerably reduces with REV and REVbio formulations indicating lesser systemic side effects. Moreover, the results of REVTMbio1formulation containing 0.25% of timolol maleate compared well with the 0.5% marketed gel formulation, indicating our formulation to be significantly better considering that similar effect is obtained at half the concentration.

Ibrahim Alsarra *et al*., (2005)[58] investigated permeation of a potent nonsteroidal anti-inflammatory, ketorolac, across excised rabbit skin from various proniosome gel formulations using Franz diffusion cells. Each of the prepared proniosomes significantly improved drug permeation and reduced the lag time ($P<0.05$). Proniosomes prepared with Span 60 provided a higher ketorolac flux across the skin than did those prepared with Tween 20 (7- and 4-fold the control, respectively). A change in the cholesterol content did not affect the efficiency of the proniosomes, and the reduction in the lecithin content did not significantly decrease the flux ($P>0.05$). Each of the prepared niosomes achieved about 99% drug encapsulation. Vesicle size was markedly dependent on the composition of the proniosomal formulations.

Samar Mansour *et al*., (2005)[59] formulated niosomes from Span 40 or Span 60 and cholesterol in the molar ratios of 7:4, 7:6 and 7:7 using reverse-phase evaporation and thin film hydration methods. The results showed that the type of surfactant, cholesterol content and the method of preparation altered the entrapment efficiency and drug release rate from niosomes. Higher entrapment efficiency was obtained with multilamellar niosomes prepared from Span 60 and cholesterol in a 7:6 molar ratio. Niosomal formulations have

shown a fairly high retention of acetazolamide inside the vesicles (approximately 75%) at a refrigerated temperature up to a period of 3 months. Each of the tested acetazolamide niosomes prepared by either method produced a significant decrease in IOP compared to the solution of free drug and plain niosomes. Multilamellar acetazolamide niosomes formulated with Span 60 and cholesterol in a 7:4 molar ratio were found to be the most effective and showed prolonged decrease in IOP. Histological examination of corneal tissues after instillation of niosomal formulation for 40 days showed slight irritation in the substantia propria of the eye which is reversible and no major changes in tissues were observed.

S.P. Vyas (2005)[60] encapsulated DNA encoding hepatitis B surface antigen (HBsAg) in niosomes composed of span 85 and cholesterol as constitutive lipids using reverse phase evaporation method. Prepared niosomes were characterized for their size, shape and entrapment efficiency. The immune stimulating activity was studied by measuring serum anti-HBsAg titer and cyokines level (IL-2 and IFN-γ) following topical application of niosomes in Balb/c mice and results were compared with naked DNA and liposomes encapsulated DNA applied topically as well as naked DNA and pure recombinant HBsAg administered intramuscularly. The proposed system is simple, stable and cost effective compared to liposomes.

Fadda *et al*., (2007)[61] formulated minoxidil loaded liposome and niosome formulations to improve skin drug delivery. Multilamellar liposomes were prepared using soy phosphatidylcholine at different purity degrees (Phospholiponw 90, 90% purity, soy lecithin (SL), 75% purity) and cholesterol (Chol), whereas niosomes were made with two different commercial mixtures of alkylpolyglucoside (APG) surfactants (Oramixw NS10, Oramixw CG110), Chol and dicetylphosphate. Minoxidil skin penetration and permeation experiments were performed *in vitro* using vertical diffusion Franz cells and human skin treated with either drug vesicular systems or propylene glycol–water–ethanol solution (control). Penetration of minoxidil in epidermal and dermal layers was greater with liposomes than with niosomal formulations and the control solution. These differences might be attributed to the smaller size and the greater potential targeting to skin and skin appendages of liposomal carriers, which enhanced globally the skin drug delivery.

Ajay Solanki *et al*., (2007)[62] used 3-factor, 3-level Box-Behnken design to optimize niosomes. Contour plots were constructed to show the effects of X1, X2 and X3 on the PDE. A model was validated for accurate prediction of the PDE by performing checkpoint analysis. The computer optimization process and contour plots predicted the levels of independent variables X1, X2, and X3 (0, -0.158 and –0.158 respectively), for maximized response of PDE with constraints on vesicle size. The Box-Behnken design demonstrated the role of the derived equation and contour plots in predicting the values of dependent variables for the preparation and optimization of piroxicam proniosomes.

Indu P. Kaur *et al*., (2007)[63] prepared carbopol coated acetazolamide niosomes by reverse phase evaporation method. The pharmacodynamic studies showed 33% fall in IOP with the developed formulation, and the effect was sustained for 6 h after instillation. The effect compared well with a four times higher concentration of dorzolamide (Dorzox®), a topical CAI available in the market. In the present study, the aqueous humor disposition of the drug from the developed bioadhesive coated niosomal formulation (ACZREVbio) is compared with the aqueous suspension of the drug (containing 1% (w/v) Tween 80 as a dispersing agent) at similar concentrations. The concentration of acetazolamide absorbed in the aqueous humor at various times from the control suspension and from ACZREVbio was determined by microdialysis in male albino rabbits. The peak concentration of drug absorbed in the aqueous humor from the ACZREVbio formulation (14.94 µg/mL) was almost two times of that obtained with the equivalent amount of acetazolamide control suspension (6.93 µg).An important observation was the fact that a high drug concentration of 12.02 µg reached immediately, i.e., 20 min after instillation of ACZREVbio indicating a high penetration being achieved, while a meagre concentration of only 3.53 µg is obtained at 60 min after instillation of the control suspension.

Abbas Pardakhty *et al*., (2007)[64] studied niosomes of polyoxyethylene alkyl ethers (Brij^{TM}) for encapsulation of insulin prepared by film hydration method. Without cholesterol, brij 35 and brij 58 did not form niosomes, apparently because of relatively large polar head groups in comparison with their alkyl chains. The size of vesicles depended on the cholesterol content, charge incorporation or hydrophilicity of surfactants. Entrapment of insulin in bilayer

structure of niosomes protected it against proteolytic activity of chymotrypsin, trypsin and pepsin *in vitro*. The maximum protection activity was seen in brij 92/cholesterol (7:3 molar ratios) in which only 26.3±3.98% of entrapped insulin was released during 24 h in simulated intestinal fluid (SIF). The kinetic of drug release for most formulations could be best described by Baker and Lonsdale equation indicating diffusion based delivery mechanism.

Attia *et al*., (2007)[65] prepared acyclovir niosomes by thin film hydration method in a trial to improve its poor and variable oral bioavailability. The lipid mixture consisted of cholesterol, span 60, and dicetyl phosphate in the molar ratio of 65:60:5, respectively. The percentage entrapment was ~11% of acyclovir used in the hydration process. The vesicles have an average size of 0.95 μm, a most probable size of 0.8 μm, and a size range of 0.4 to 2.2 μm. Most of the niosomes have unilamellar spherical shape. *In vitro* drug release profile was found to follow Higuchi's equation for free and niosomal drug. The average relative bioavailability of the drug from the niosomal dispersion in relation to the free solution was 2.55 indicating more than 2-fold increase in drug bioavailability. The niosomal dispersion showed significant increase in the mean residence time (MRT) of acyclovir reflecting sustained release characteristics.

Massimo Fresta *et al*., (2007)[66] prepared a novel niosomes of ammonium glycyrrhizinate, a natural compound, using a new non ionic surfactant, *α*,*ω*-hexadecyl-bis-(1-aza-18-crown-6)(Bolasurfactant)-Span80-cholesterol (2:3:1 molar ratio). The tolerability of Bola-surfactant both as free molecules or assembled ion niosome vesicles was evaluated *in vitro* on cultured of human keratinocyte cells (NCTC2544). Human tolerability was evaluated on volunteers. The ability of Bola-niosomes to promote intracellular delivery was evaluated by confocal laser scanning microscopy (CLSM) studies. Human stratum corneum and epidermis (SCE) membranes were used *in vitro* to investigate the percutaneous permeation. The anti-inflammatory activity of ammonium glycyrrhizinate was evaluated *in vivo* on human volunteers with a chemically induced erythema. Experimental data show that Bola-niosomes are characterized by a mean size of 400 nm and are able to provide an encapsulation efficiency of 40% with respect to the drug amount used during preparation. Bolaniosomes were also able to significantly improve (p <0.001)

the percutaneous permeation of ammonium glycyrrhizinate. Bola-niosomes showed a suitable tolerability both *in vitro* and *in vivo*. Ammonium glycyrrhizinateloaded Bola-niosomes determined a significant (p <0.001) and noticeable improvement of the *in vivo* anti-inflammatory activity of the drug.

Ibrahim *et al*., (2008)[67] formulated and evaluated proniosomal transdermal carrier systems for flurbiprofen using span 20 (Sp 20), span 40 (Sp 40), span 60 (Sp 60) and span 80 (Sp 80) without and with cholesterol at percentages ranging from 0% to 50%. The effect of surfactant type and cholesterol content on drug release was investigated. Drug release was tested by diffusion through cellophane membrane and rabbit skin. Drug release from the prepared systems was compared to that from flurbiprofen suspensions in distilled water and HPMC (hydroxypropylmethylcellulose) gels. In case of Sp 20 and Sp 80, the added amount of cholesterol affected the preparation type to be either proniosomal alcoholic solutions or liquid crystalline gel systems. On the other hand, both Sp 40 and Sp 60 produced gel systems in presence or absence of cholesterol. Due to the skin permeation barrier, rabbit skin showed lower drug diffusion rates compared to cellophane membrane.

Jim Jiao, (2008)[68] summarized the surface and thermodynamic properties of polyoxyethylated nonionic surfactants (Polysorbates, Tyloxapol, Poloxamers, Cremophor EL, Brij, and alpha-Tocopherol TPGS), evaluated the recent advancement of these surface active agents in ophthalmic topical drug delivery, and analyzed advantages and potential pitfalls of using them as ocular formulation ingredients to address solubility, compatibility, and bioavailability issues. The effects of these surfactants on biopharmaceutics of the ocular drugs are assessed and information on their safety to the eye tissues over chronic exposure is provided.

Ghada Abdelbary and Nashwa El-gendy, (2008)[69] prepared niosomal formulations using various surfactants (Tween 60, Tween 80 or Brij 35), in the presence of cholesterol and a negative charge inducer dicetyl phosphate (DCP) in different molar ratios and by employing a thin film hydration technique. Results showed a substantial change in the release rate and an alteration in the %EE of gentamicin sulphate from niosomal formulations upon varying type of surfactant, cholesterol content and presence or absence of DCP. In-vitro drug release results confirmed that niosomal formulations have

exhibited a high retention of gentamicin sulphate inside the vesicles such that their *in vitro* release was slower compared to the drug solution. A preparation with 1:1:0.1 molar ratio of Tween 60, cholesterol and DCP gave the most advantageous entrapment (92.02%±1.43) and release results (Q8h=66.29%±1.33) as compared to other compositions. Ocular irritancy test performed on albino rabbits, showed no sign of irritation for all tested niosomal formulations.

Junyaprasert V B *et al.*, (2008)[70] studied an influence of different types of membrane additives including negative charge (dicetylphosphate, DCP), positive charge (stearylamine, STR) and non-ionic molecule (cholesteryl poly-24-oxyethylene ether, SC24) on the physicochemical properties of drug-free and drug-loaded span 60, unilamellar niosomes. The results show that incorporation of salicylic acid to the niosomes did not affect zeta potential values; however, addition of the membrane additives changed the zeta potential depending on the type of the additives. The particle sizes of all developed niosomes were between 217 to 360 nm. The entrapment efficiency (%E.E.) of all salicylic acid niosomes at pH 3 was higher than that of niosomes at pH 5, indicating that salicylic acid in unionized form was preferably incorporated in niosomes. Furthermore, the positively charged niosomes showed the highest %E.E. of salicylic acid owing to electrostatic attraction between STR and salicylic acid. In addition, all niosomes showed no leakage of the salicylic acid after 3 months of storage indicating the good stability.

Adnan Azeem *et al.,* (2009)[71] offered some recent advances on niosomes as sustained and targeted drug delivery. The review considers the current status and explores the potential of niosomes in drug delivery with special attention to their role in drug targeting. Their methods of preparation, formulation aspects, advantages, limitations, and applications are also discussed.

Pratap S. Jadon *et al*., (2009)[72] studied niosomes to improve poor and variable oral bioavailability of griseofulvin using span 20, span 40, and span 60. The lipid mixture consisted of surfactant, cholesterol, and dicetyl phosphate in the molar ratio of 125:25:1.5, 100:50:1.5, and 75:75:1.5, respectively. The niosomal formulations were prepared by thin film method

and ether injection method. Result indicated that the niosomes prepared by thin film method with span 60 provided higher entrapment efficiency. The niosomal formulation exhibited significantly retarded *in vitro* release as compared with free drug. The *in vivo* study revealed that the niosomal dispersion significantly improved the oral bioavailability of griseofulvin in albino rats after a single oral dose. The maximum concentration (Cmax) achieved in case of niosomal formulation was approximately double (2.98 µg/mL) as compared to free drug (1.54 µg/mL). Plasma drug profile also suggested that the developed niosomal system also has the potential of maintaining therapeutic level of griseofulvin for a longer period of time as compared to free griseofulvin. The niosomal formulation showed significant increase in area under the curve0-24 (AUC; 41.56 µg/mL h) as compared to free griseofulvin (22.36 µg/mL h) reflecting sustained release characteristics.

D. Cosco *et al*., (2009)[73] proposed innovative of 5-fluorouracil (5-FU) niosomes made up of α,ω-hexadecylbis-(1-aza-18-crown-6) (bola), Span 80® and cholesterol (2:5:2 molar ratio) as suitable delivery systems for the treatment of breast cancer. The bola-niosomes, after sonication procedure, showed mean sizes of ~200 nm and a loading capacity of ~40% with respect to the amount of 5-FU added during the preparation. Similar findings were achieved with PEG-coated bola-niosomes (bola, Span 80®, cholesterol, DSPE-mPEG2000, 2:5:2:0.1 molar ratio respectively). *In vivo* experiments on MCF-7 xenograft tumor SCID mice models showed a more effective antitumoral activity of the PEGylated niosomal 5-FU at a concentration ten times lower (8 mg/kg) than that of the free solution of the drug (80 mg/kg) after a treatment of 30 days.

Kandasamy Ruckmani and Veintramuthu Sankar, (2010)[74] studied effects of process-related variables like hydration and sonication time, rotation speed of evaporation flask, and the effects of charge-inducing agent and centrifugation on zidovudine entrapment and release from niosomes. Formulation of zidovudine niosomes was optimized by altering the proportions of Tween, Span and cholesterol. Non-sonicated niosomes were in the size range of 2-3.5 µm and sonicated niosomes formulated with Tween 80 and dicetylphosphate (DCP) had a mean diameter of 801 nm. Zidovudine niosomes formulated with Tween 80 entrapped high amounts of drug and the

addition of DCP enhanced drug release for a longer time (88.72% over 12 h). The mechanism of release from Tween 80 formulation was the Fickian type and obeyed first-order release kinetics. Niosomes can be formulated by proper adjustment of process parameters to enhance zidovudine entrapment and sustainability of release.

Ebtessam A Essa *et al*., (2010)[75] investigated various formulations and processing factors on vesicular Z-average particle size. The selected variables were membrane additives, [including cholesterol (CHO), dicetylphosphate (DCP) and stearylamine (SA)], sonication time as well as drug loading factor (using mannitol and estradiol). Sorbitan monopalmitate (span 40) niosomes were prepared by the conventional thin film hydration method. The results indicated that CHO increased the vesicular size, with 2:1 and 1:1 (span 40:CHO) ratios showing the same size. Sonication reduced the vesicle size by 23, 35 and 42% after 10, 20 and 30 min, respectively. After 30 min, the effect of sonication was minor. The addition of charge inducing agents changed the zeta potential depending on the type of the additives. Surface charge increased the size by 24 and 11% when using DCP and SA, respectively. Drug incorporation increased the vesicle size to an extent based on its aqueous solubility. There were about 35 and 6.2% increase in vesicular size for estradiol and mannitol, respectively, supporting the partitioning of lipophilic drug within the fatty acyl side chains of the bilayer membrane.

Ahmed A. Aboelwafa *et al*., (2010)[76] investigated the effects of formulation variables on development of carvedilol (CAR) proniosomal gel. Different nonionic surfactants; polyoxyethylene alkyl ethers, namely Brij 78, Brij 92, and Brij 72; and sorbitan fatty acid esters (Span 60) were evaluated for their applicability in preparation of CAR proniosomal gels. A 2^3 full factorial design was employed to evaluate individual and combined effects of formulation variables, namely cholesterol content, weight of proniosomes, and amount of CAR added on performance of proniosomes. Proniosomes prepared with Brij 72 and Span 60 showed better niosome forming ability and higher EE% than those prepared with Brij 78 and Brij 92. Higher EE % was obtained by increasing both weight of proniosomes and amount of CAR added, and decreasing cholesterol content. Release rate through cellulose membrane was inversely affected by weight of proniosomes. In Span 60 proniosomes, on

increasing percent of cholesterol, a decrease in release rate was observed. While in Brij 72 proniosomes, an enhancement in release rate was observed on increasing amount of CAR added. Permeation experiments showed that skin permeation was mainly affected by weight of proniosomes and Span 60 proniosomal gels showed higher permeation enhancing effect than Brij 72.

Mohamed Nasr, (2010)[77] prepared celecoxib proniosomes and evaluated the influence of proniosomal formulation on the oral bioavailability of the drug in human volunteers. Proniosomes were prepared by sequential spraying method, which consisted of cholesterol, span 60, and dicetyl phosphate in a molar ratio of 1:1:0.1, respectively. The average entrapment was about 95%. The prepared proniosomes showed marked enhancement in the dissolution of celecoxib as compared to pure drug powder. The bioavailability of 200 mg single dose of both celecoxib proniosomal formulation and a conventional marketed celecoxib capsule was studied in human volunteers. The obtained results show that the proniosomal formulation significantly improved the extent of celecoxib absorption than conventional capsule. The mean relative bioavailability of the proniosomal formulation to the conventional capsule was 172.06±0.14%. The mean T_{max} for celecoxib was prolonged when given as proniosomal capsule. There was no significant difference between the values of K_{el} and $t_{1/2}$ for both celecoxib preparations.

Indu Pal Kaur *et al*., (2010)[78] established the pharmacokinetic and pharmacodynamic superiority of the developed ocular formulation of Timolol (TM). Aqueous humor concentration of TM in male albino rabbits, after instillation of one drop of TM solution (TMS) or TMREVbio was measured using the microdialysis method. Peak concentration of drug in aqueous humor from TMREVbio (12.46 µg/mL achieved at 60 min) was almost 1.7 times that of the control drug solution (TMS, 0.25%; 7.2 µg/mL). An important observation was that the high drug concentrations achieved upon TMREVbio administration were maintained for up to 2 hr. AUC for TMREVbio formulation was 2.34 times that of the TMS. Results confirm a sustained and controlled effect of the developed formulation.

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CHAPTER 3

DRUG AND EXCIPIENTS PROFILE

Chapter 3 - DRUG AND EXCIPIENTS PROFILE

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3. DRUG AND EXCIPIENTS PROFILE

3.1 Drug profile

3.1.1 Betaxolol Hydrochloride[1-6]

3.1.1.1 Chemical name[7]: 2-propanol, 1-4-2-(cyclopropylmethoxy) ethylphenoxy-3-(1-methylethyl) amino-, hydrochloride

3.1.1.2 Molecular formula: $C_{18}H_{29}NO_3$. HCl

3.1.1.3 Molecular weight: 343.9

3.1.1.4 Chemical structure

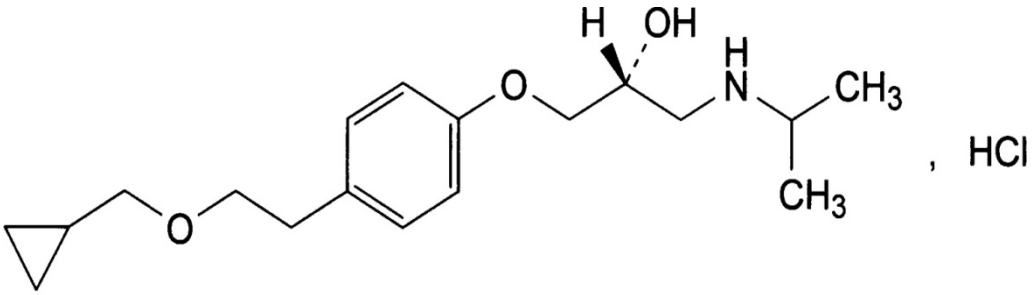


Figure 3.1: Chemical structure of Betaxolol HCl

3.1.1.5 Properties

State: Solid white powder

Melting point: 113-117 ^{o}C

Solubility: 451 mg/L in water. It is freely soluble in chloroform, methylene chloride, ethanol and methanol.

$CaCO_2$ permeability: 4.81 [ADME Research, USCD]

pKa: 9.4

3.1.1.6 Pharmacodynamics

Betaxolol is a competitive, β1-selective (cardioselective) adrenergic antagonist. Betaxolol is used to treat hypertension, arrhythmias, coronary heart disease, glaucoma, and is also used to reduce non-fatal cardiac events in patients with heart failure. Activation of β1-receptors (located mainly in the heart) by epinephrine increases the heart rate and the blood pressure, and the heart consumes more oxygen. Drugs such as betaxolol that block these receptors therefore have the reverse effect: they lower the heart rate and blood pressure and hence are used in conditions when the heart itself is deprived of oxygen. They are routinely prescribed in patients with ischemic

heart disease. In addition to its effect on the heart, betaxolol reduces the pressure within the eye (intraocular pressure). Betaxolol blocks beta-receptors that are found on the ciliary body. This action reduces the amount of aqueous humour that is secreted into the eyeball by the ciliary body. Betaxolol also blocks beta-receptors found on the blood vessels that supply the ciliary body. This causes the blood vessels to constrict, and reduces the amount of watery fluid that filters out of the blood vessels to form aqueous humour. The reduction in intraocular pressure reduces the risk of damage to the optic nerve and loss of vision in patients with elevated intraocular pressure due to glaucoma.

3.1.1.7 Absorption

Absorption of an oral dose is complete. There is a small and consistent first-pass effect resulting in an absolute bioavailability of 89% ± 5% that is unaffected by the concomitant ingestion of food or alcohol.

3.1.1.8 Metabolism

Primarily hepatic. Approximately 15% of the dose administered is excreted as unchanged drug, the remainder being metabolites whose contribution to the clinical effect is negligible. Half life of drug is 12-14 hr and protein binding is 50%.

Dosing: The usual dose is 1 to 2 drops into each affected eye twice daily.

3.1.1.9 Toxicity

Oral LD_{50}s are 350 to 400 mg betaxolol/kg in mice and 860 to 980 mg/kg in rats. Predicted symptoms of overdose include bradycardia, congestive heart failure, hypotension, bronchospasm, and hypoglycemia.

3.1.1.10 Side effects

Ophthalmic betaxolol can cause side effects which are usually mild and transient. The most common side effect is transient ocular (temporary eye) discomfort. Rarely, betaxolol eye drops can result in side effects that are seen with oral beta-adrenergic blockers. For example, persons can experience fatigue, insomnia, nausea, dizziness, lightheadedness, depression, slow heart rate, low blood pressure, cold extremities, and shortness of breath or wheezing.

3.2 Polymer profile

3.2.1 Polyethylene oxide[8-10]

3.2.1.1 Nonproprietary names: USPNF - Polyethylene oxide.

3.2.1.2 Synonym: Polyox*;* polyoxirane; polyoxyethylene.

3.2.1.3 Chemistry: The USPNF 23 describes Polyethylene oxide as a nonionic homopolymer of ethylene oxide, represented by the formula $(CH_2CH_2O)_n$, where n represents the average number of oxyethylene groups. It may contain up to 3% of silicon dioxide.

3.2.4 Category: Mucoadhesive; tablet binder; thickening agent.

3.2.5 Application in pharmaceutical formulation or technology

Polyethylene oxide can be used as a tablet binder at concentrations of 5–85%. The higher molecular weight grades provide delayed drug release via the hydrophilic matrix approach.

The relationship between swelling capacity and molecular weight is a good guide when selecting products for use in immediate- or sustained-release matrix formulations.

Polyethylene oxide has been shown to be an excellent mucoadhesive polymer. Low levels of Polyethylene oxide are effective thickeners, although alcohol is usually added to water-based formulations to provide improved viscosity stability. Polyethylene oxide films demonstrate good lubricity when wet. This property has been utilized in the development of coatings for medical devices. Polyethylene oxide can be radiation crosslinked in solution to produce a hydrogel that can be used in wound care applications.

3.2.1.6 Description: White to off-white, free-flowing powder. Slight ammoniacal odor.

3.2.1.7 Typical properties:

Angle of repose: 34°

Density (true): 1.3 g/cm^3

Melting point: 65–70 °C

Moisture content: <1%

Solubility: Polyethylene Oxide is soluble in water and a number of common organic solvents such as acetonitrile, chloroform, and methylene chloride. It is insoluble in aliphatic hydrocarbons, ethylene glycol, and most alcohols.

3.2.1.8 Stability and storage conditions: Store in tightly sealed containers in a cool, dry place. Avoid exposure to high temperatures since this can result in reduction in viscosity.

3.2.1.9 Incompatibilities: Polyethylene Oxide is incompatible with strong oxidizing agents.

3.2.1.10 Safety: Animal studies suggest that Polyethylene Oxide has a low level of toxicity regardless of the route of administration. It is poorly absorbed from the gastrointestinal tract but appears to be completely and rapidly eliminated. The resins are neither skin irritants nor sensitizers, and they do not cause eye irritation.

3.2.1.11 Regulatory status: It is included in the FDA Inactive Ingredients Guide (sustained-release tablets). It is also included in the Canadian List of Acceptable Non-medicinal Ingredients.

3.2.2 Polyvinyl pyrollidone[11]

3.2.2.1 Nonproprietary names

- BP: Povidone
- JP: Povidone
- PhEur: Povidonum
- USP: Povidone

3.2.2.2 Synonyms

E1201; Kollidon; Plasdone; poly[1-(2-oxo-1-pyrrolidinyl)ethylene]; polyvidone; polyvinylpyrrolidone; PVP; 1-vinyl-2-pyrrolidinone polymer.

3.2.2.3 Chemical name

1-Ethenyl-2-pyrrolidinone homopolymer

3.2.2.4 Empirical formula and molecular weight

$(C_6H_9NO)_n$ 2500–3 000 000

The USP 28 describes povidone as a synthetic polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the differing degree of polymerization of which results in polymers of various molecular weights. It is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a *K*-value, in the range 10–120. The *K*-value is calculated using Fikentscher's equation[12]

$$\log z = c\left[\frac{75k^2}{1+1.5kc}\right]+k \qquad [3.1]$$

where *z* is the relative viscosity of the solution of concentration *c* (in % w/v), and *k* is the *K*-value × 10^{-3}.

Alternatively, the *K*-value may be determined from the following equation:

$$K\text{-value} = \sqrt{\frac{300c\log z\,(c+1.5c\log z)^2+1.5}{0.15c+0.003c^2}} \qquad [3.2]$$

where *z* is the relative viscosity of the solution of concentration *c* (in % w/v).

3.2.2.5 Structural formula

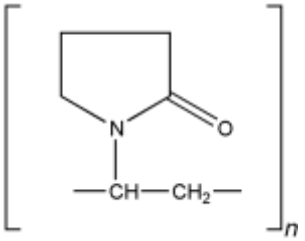


Figure 3.2: Structure of PVP

3.2.2.6 Functional category

Disintegrant; dissolution aid; suspending agent; tablet binder.

3.2.2.7 Applications in pharmaceutical formulation or technology

Although povidone is used in a variety of pharmaceutical formulations, it is primarily used in solid-dosage forms. In tableting, povidone solutions are used as binders in wet-granulation processes[13]. Povidone is also added to powder blends in the dry form and granulated *in situ* by the addition of water, alcohol, or hydroalcoholic solutions. Povidone is used as a solubilizer in oral and parenteral formulations and has been shown to enhance dissolution of poorly soluble drugs from solid-dosage forms[14]. Povidone solutions may also be used as coating agents.

Povidone is additionally used as a suspending, stabilizing, or viscosity-increasing agent in a number of topical and oral suspensions and solutions. The solubility of a number of poorly soluble active drugs may be increased by mixing with povidone. Special grades of pyrogen-free povidone are available and have been used in parenteral formulations.

3.2.2.8 Description

Povidone occurs as a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder. Povidones with *K*-values equal to or lower than 30 are manufactured by spray-drying and occur as spheres. Povidone K-90 and higher *K*-value povidones are manufactured by drum drying and occur as plates.

3.2.2.9 Typical Properties

Acidity/alkalinity: pH = 3.0–7.0 (5% w/v aqueous solution).

Density (bulk): 0.29–0.39 g/cm^3 for Plasdone.

Density (tapped): 0.39–0.54 g/cm^3 for Plasdone.

Density (true): 1.180 g/cm^3

Flowability:

- 20 g/s for povidone K-15;
- 16 g/s for povidone K-29/32.

Melting point: softens at 150°C.

Moisture content

povidone is very hygroscopic, significant amounts of moisture being absorbed at low relative humidities.

Particle size distribution

- Kollidon 25/30: 90% >50 µm, 50% >100 µm, 5% >200 µm;
- Kollidon 90: 90% >200 µm, 95% >250 µm.

Solubility

Freely soluble in acids, chloroform, ethanol (95%), ketones, methanol, and water; practically insoluble in ether, hydrocarbons, and mineral oil. In water, the concentration of a solution is limited only by the viscosity of the resulting solution, which is a function of the *K*-value.

Viscosity (dynamic)

The viscosity of aqueous povidone solutions depends on both the concentration and the molecular weight of the polymer employed. Dynamic viscosity of 10% w/v aqueous povidone (Kollidon) solutions at 20°C for K-28/32 is 5.5-8.5 mPa s.

3.2.2.10 Stability and storage conditions

Povidone darkens to some extent on heating at 150 °C, with a reduction in aqueous solubility. It is stable to a short cycle of heat exposure around 110–130 °C; steam sterilization of an aqueous solution does not alter its properties. Aqueous solutions are susceptible to mold growth and consequently require the addition of suitable preservatives.

Povidone may be stored under ordinary conditions without undergoing decomposition or degradation. However, since the powder is hygroscopic, it should be stored in an airtight container in a cool, dry place.

3.2.2.11 Incompatibilities

Povidone is compatible in solution with a wide range of inorganic salts, natural and synthetic resins, and other chemicals. It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin, and other compounds. The efficacy of some preservatives, e.g. thimerosal, may be adversely affected by the formation of complexes with povidone.

3.2.2.12 Safety

Povidone has been used in pharmaceutical formulations for many years, being first used in the 1940s as a plasma expander, although it has now been superseded for this purpose by dextran[15].

Povidone is widely used as an excipient, particularly in oral tablets and solutions. When consumed orally, povidone may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes[15]. Povidone additionally has no irritant effect on the skin and causes no sensitization.

Evidence also exists that povidone may accumulate in the organs of the body following intramuscular injection[16].

A temporary acceptable daily intake for povidone has been set by the WHO at up to 25 mg/kg body-weight. LD_{50} (mouse, IP): 12 g/kg

3.2.2.13 Regulatory status

Accepted for use in Europe as a food additive. Included in the FDA Inactive Ingredients Guide (IM and IV injections; ophthalmic preparations; oral capsules, drops, granules, suspensions, and tablets; sublingual tablets;

topical and vaginal preparations). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

3.2.4 Poly(methyl methacrylate)[8]

3.2.4.1 Empirical formula : $(C_5H_8O_2)_n$

3.2.4.2 Synonyms: methyl methacrylate polymer; PMMA.

3.2.4.3 Properties

- Hard, rigid, transparent (very clear to see through)
- softening point is 125 ^{o} C
- Absorbs very little visible light but there is a 4% reflection at each polymer-air interface for normal incident light.
- PMMA is a polar material and has a rather high dielectric constant
- **Solubility**

  PMMA prepared by free radical polymerization is amorphous and is therefore soluble in solvents with similar solubility parameters such as benzene, toluene, chloroform, methylene chloride, esters, ethyl acetate, and amyl acetate.

3.2.4.4 Stability

PMMA has good resistance to alkalis (sodium hydroxide, etc.), aqueous inorganic salts (the Pacific Ocean) and dilute acids. PMMA has a better resistance to hydrolysis than PMA probably by virtue of the shielding of the methyl group.

3.2.4.5 Use

Poly(methyl methacrylate) has been used as a material for intraocular lenses, for denture bases, and as a cement for dental prostheses.

3.3 Other excipients and materials

3.3.1 Cholesterol[8]

3.3.1.1 Synonyms - Cholesterin; cholesterolum.

3.3.1.2 Chemical name - Cholest-5-en-3β-ol

3.3.1.3 Empirical formula - $C_{27}H_{46}O$

3.3.1.4 Molecular weight- 386.67

3.3.1.5 Structural formula

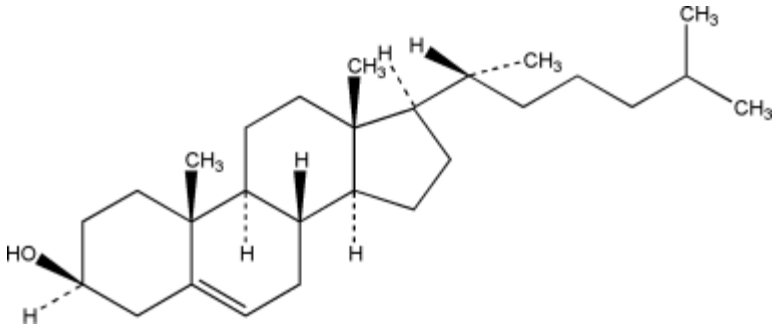


Figure 3.3: Structure of cholesterol

3.3.1.6 Functional category - Emollient; emulsifying agent.

3.3.1.7 Applications in pharmaceutical formulation or technology

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity.

Cholesterol also has a physiological role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones.

3.3.1.8 Description

Cholesterol occurs as white or faintly yellow, almost odorless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires a yellow to tan color.

3.3.1.9 Typical Properties

Boiling point: 360 °C

Density: 1.052 g/cm^3 for anhydrous form.

Dielectric constant D^{20}: 5.41

Melting point: 147–150 °C

Solubility[17]: Freely soluble in acetone, benzene, chloroform, ether and vegetable oils. It is practically insoluble in water.

Specific rotation $[\alpha]^{20}_{D}$: −39.5° (2% w/v solution in chloroform); −31.5° (2% w/v solution in ether).

3.3.1.10 Stability and storage conditions

Cholesterol is stable and should be stored in a well-closed container, protected from light.

3.3.1.11 Incompatibilities

Cholesterol is precipitated by digitonin.

3.3.1.12 Method of manufacture

The commercial material is normally obtained from the spinal cord of cattle by extraction with petroleum ethers, but it may also be obtained from wool fat. Purification is normally accomplished by repeated bromination. Cholesterol may also be produced by entirely synthetic means.

Cholesterol produced from animal organs will always contain cholestanol and other saturated sterols.

3.3.1.13 Safety

Cholesterol is generally regarded as an essentially nontoxic and nonirritant material at the levels employed as an excipient[18]. It has, however, exhibited experimental teratogenic and reproductive effects, and mutation data have been reported[19].

Cholesterol is often derived from animal sources and this must be done in accordance with the regulations for human consumption. The risk of bovine spongiform encephalopathy (BSE) contamination has caused some concern over the use of animal-derived cholesterol in pharmaceutical products. However, synthetic methods of cholesterol manufacture have been developed.

3.3.1.14 Regulatory status

It is included in the FDA Inactive Ingredients Guide (injections, ophthalmic, topical, and vaginal preparations).

It is included in nonparenteral medicines licensed in the UK and in the Canadian List of Acceptable Non-medicinal Ingredients.

3.3.2 Span 60[8]

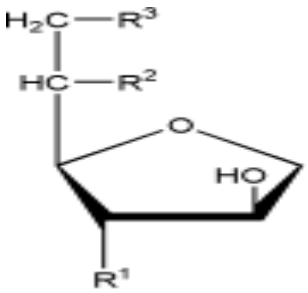
3.3.2.1 Synonym: Sorbitan monostearate

3.3.2.2 Chemical Name: Sorbitan mono-octadecanoate

3.3.2.3 Chemical Formula: $C_{24}H_{46}O_6$

3.3.2.4 Molecular Weight: 431

3.3.2.5 Structural formula



$R^1 = R^2 = OH$, $R^3 = R$ (see below) for sorbitan monoesters

$R^1 = OH$, $R^2 = R^3 = R$ for sorbitan diesters

$R^1 = R^2 = R^3 = R$ for sorbitan triesters

where R = $(C_{17}H_{35})COO$ for isostearate

$(C_{11}H_{23})COO$ for laurate

$(C_{17}H_{33})COO$ for oleate

$(C_{15}H_{31})COO$ for palmitate

$(C_{17}H_{35})COO$ for stearate

The sesquiesters are equimolar mixtures of monoesters and diesters.

Figure 3.4: Structure of span

3.3.2.6 Functional category

Emulsifying agent; nonionic surfactant; solubilizing agent; wetting and dispersing/suspending agent.

3.3.2.7 Applications in pharmaceutical formulation or technology

Sorbitan monoesters are a series of mixtures of partial esters of sorbitol and its mono- and dianhydrides with fatty acids. Sorbitan diesters are a series of mixtures of partial esters of sorbitol and its monoanhydride with fatty acids.

Sorbitan esters are widely used in cosmetics, food products, and pharmaceutical formulations as lipophilic nonionic surfactants. They are mainly used in pharmaceutical formulations as emulsifying agents in the preparation of creams, emulsions, and ointments for topical application. When

used alone, sorbitan esters produce stable water-in-oil emulsions and microemulsions but are frequently used in combination with varying proportions of a polysorbate to produce water-in-oil or oil-in-water emulsions or creams of varying consistencies.

Sorbitan monolaurate, sorbitan monopalmitate and sorbitan trioleate have also been used at concentrations of 0.01–0.05% w/v in the preparation of an emulsion for intramuscular administration.

3.3.2.8 Description: Cream Solid

3.3.2.9 Typical properties

Acid value: 5-10

Flash point:>149 °C

HLB value: 4.7

Hydroxyl value: 235-260

Iodine number: ≤1

Melting point: 53-57

Moisture content: ≤1

Saponification value: 147-157

Solubility: sorbitan esters are generally soluble or dispersible in oils; they are also soluble in most organic solvents. In water, although insoluble, they are generally dispersible.

Surface tension of 1% aqueous solution: 46 mN/m

3.3.2.10 Stability and storage conditions

Gradual soap formation occurs with strong acids or bases; sorbitan esters are stable in weak acids or bases. Sorbitan esters should be stored in a well-closed container in a cool, dry place.

3.3.2.11 Safety

Sorbitan esters are widely used in cosmetics, food products, and oral and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. However, there have been occasional reports of hypersensitive skin reactions following the topical application of products containing sorbitan esters[20]. When heated to decomposition, the sorbitan esters emit acrid smoke and irritating fumes.

The WHO[21] has set an estimated acceptable daily intake of sorbitan monopalmitate, monostearate, and tristearate, and of sorbitan monolaurate and monooleate at up to 25 mg/kg body-weight calculated as total sorbitan esters.

LD_{50} (rat, oral)[19]: 31 g/kg. Very mildly toxic by ingestion.

3.3.2.12 Handling precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended.

3.3.2.13 Regulatory status

Certain sorbitan esters are accepted as food additives in the UK. Sorbitan esters are included in the FDA Inactive Ingredients Guide (inhalations; IM injections; ophthalmic, oral, topical, and vaginal preparations). Sorbitan esters are used in nonparenteral medicines licensed in the UK. Sorbitan esters are included in the Canadian List of Acceptable Non-medicinal Ingredients.

3.3.3 Tween 60[8]

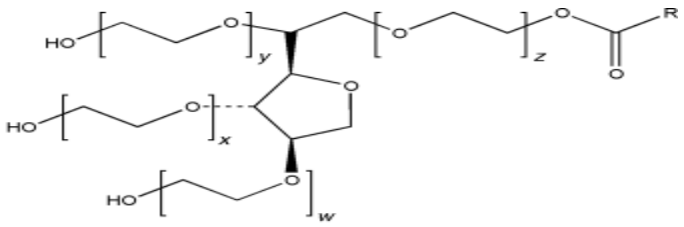
3.3.3.1 Synonym: Polysorbate 60

3.3.3.2 Chemical name : Polyoxyethylene 20 sorbitan monostearate

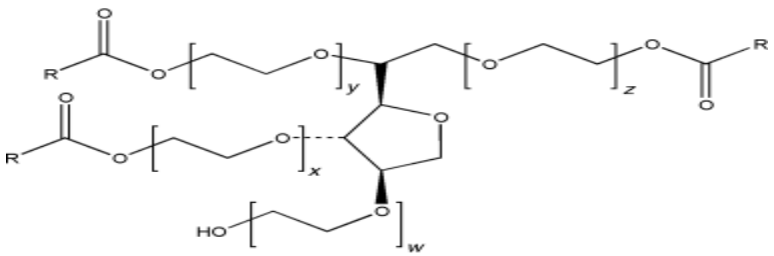
3.3.3.3 Empirical formula: $C_{64}H_{126}O_{26}$

3.3.3.4 Molecular weight.: 1312

3.3.3.5 Structural formula



Polyoxyethylene sorbitan monoester



Polyoxyethylene sorbitan triester

Figure 3.5: Structure of polysorbates

$w + x + y + z = 20$ (Polysorbates 20, 40, 60, 65, 80, and 85)

$w + x + y + z = 5$ (Polysorbates 81)

$w + x + y + z = 4$ (Polysorbates 21 and 61)

R = fatty acid

3.3.3.6 Functional category

Emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.

3.3.3.7 Applications in pharmaceutical formulation or technology

Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides. The resulting product is therefore a mixture of molecules of varying sizes rather than a single uniform compound.

Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for *p*-glycoprotein.[22] Polysorbates are also widely used in cosmetics and food products.

3.3.3.8 Description

Cream Solid. Polysorbates have a characteristic odor and a warm, somewhat bitter taste. Their colors and physical forms at 25°C is yellow oily liquid, although it should be noted that the absolute color intensity of the products may vary from batch to batch and from manufacturer to manufacturer.

3.3.3.9 Typical properties

Acid value: 2%

Acidity/alkalinity: pH = 6.0–8.0 for a 5% w/v aqueous solution.

Flash point: 149 °C

HLB value: 14.9

Hydroxyl value: 81-96

Specific gravity at 25 ^{o}C : 1.1

Viscosity (dynamic) : 600 mPa s

Iodine number: ≤1

Melting point: 53-57 ^{o}C

Moisture content: 3

Saponification value: 45-55

Solubility: Tween is generally insoluble in oils; they are also soluble in methanol and water.

Surface tension of 0.1% w/v aqueous solution: 42.5 mN/m

3.3.3.10 Stability and storage conditions

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

3.3.3.11 Incompatibilities

Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates[(23)].

3.3.3.12 Safety

Polysorbates are widely used in cosmetics, food products, and oral, parenteral, and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. There have, however, been occasional reports of hypersensitivity to polysorbates following their topical

and intramuscular use[24]. The WHO has set an estimated acceptable daily intake for polysorbates 20, 40, 60, 65, and 80, calculated as total polysorbate esters, at up to 25 mg/kg body-weight[21].

Polysorbate 60: LD_{50} (rat, IV): 1.22 g/kg[19]. Moderately toxic by IV route.

3.3.3.13 Regulatory status

Polysorbates 60, 65, and 80 are GRAS listed. Polysorbates 20, 40, 60, 65, and 80 are accepted as food additives in Europe. Polysorbates 20, 40, 60, and 80 are included in the FDA Inactive Ingredients Guide (IM, IV, oral, rectal, topical, and vaginal preparations). Polysorbates are included in parenteral and nonparenteral medicines licensed in the UK. Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, 85, and 120 are included in the Canadian List of Acceptable Non-medicinal Ingredients.

3.3.4 Polyethylene Glycol 400[8, 25]

3.3.4.1 Synonym: Polyoxyethylene glycol, Lutrol E, Macrogol 400, Carbowax

3.3.4.2 Description*:* Polyethylene glycol is an addition polymer of ethylene oxide and water. Liquid grades occur as a clear, colorless as slightly yellow-colored viscous liquids.

3.3.4.3 Molecular weight: 380-420

3.3.4.4 Typical properties

Flash point	: 238 ^{o}C
Density	: 1.11-1.14 g/cm^3 at 25 °C
Glass Transition Temperature	: 120 °C
Refractive index	: 1.467
Viscosity	: 131 at 25 ^{o}C

3.3.4.5 Incompatibilities: Polyethylene glycol due to the two terminal hydroxyl groups can exhibit some oxidizing activity.

3.3.4.6 Health and safety: Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials[26].

However the most serious effects associated with polyethylene glycols are hyperosmolarity metabolic acidosis following the topical use of polyethylene glycols in burn patients.

3.3.4.7 Stability and storage conditions: Polyethylene glycols are chemically stable in air and in solution. Polyethylene glycols should be stored in well closed container in a cool, dry place.

3.3.4.8 Applications in pharmaceutical formulation or technology

Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations including parenteral, topical, ophthalmic, oral, and rectal preparations. It has been used experimentally in biodegradable polymeric matrices used in controlled-release systems[27].

Polyethylene glycols liquids are useful as plasticizer in transdermal films to avoid ruptures to provide strength to the formulation.

3.3.5 Dibutyl Phthalate[8]

3.3.5.1 Synonyms: benzenedicarboxylic acid; butyl phthalate; Celluflex DBP; DBP; dibutyl-o-phthalate; di-n-butyl phthalate; Elaol; Ergoplast FDB; Genoplast B; Hatcol DBP; Hexaplast M/B; Kodaflex DBP; Monocizer DBP; Palatinol C; phthalic acid dibutyl ester; Polycizer DBP; PX 104; RC Plasticizer DBP; Staflex DBP; Unimoll DB; Vestimol C; Witcizer 300.

3.3.5.2 Description*:* Dibutyl phthalate occurs as an odorless, oily, colorless, or very slightly yellow-colored, viscous liquid.

3.3.5.3 Molecular weight: 278.34

3.3.5.4 Typical properties:

Boiling point : 340 °C

Density : 1.0465 g/cm^3 at 20 °C

Flash point : 171 °C

Melting point : -35 °C

Partition coefficient: Octanol : water log k_{ow} = 4.50

Refractive index: nD_{20} = 1.491–1.495

Solubility: very soluble in acetone, benzene, ethanol (95%), and ether; soluble 1 in 2500 of water at 20 °C.

Viscosity (dynamic): 20 mPa s at 20 °C.

3.3.5.5 Incompatibilities

Dibutyl phthalate reacts violently with chlorine. It also reacts with oxidizing agents, acids, bases, and nitrates.

3.3.5.6 Health and safety

Dibutyl phthalate is generally regarded as a relatively nontoxic material, although it has occasionally been reported to cause hypersensitivity reactions. It is widely used in topical cosmetic and some oral pharmaceutical formulations[19].

3.3.5.7 Stability and storage conditions

Dibutyl phthalate should be stored in a well-closed container in a cool, dry, location. Containers may be hazardous when empty since they can contain product residues such as vapors and liquids.

3.3.5.8 Applications in pharmaceutical formulation or technology

Dibutyl phthalate is used in pharmaceutical formulations as a plasticizer in film-coatings. It is also used extensively as a solvent particularly in cosmetic formulations such as antiperspirants, hair shampoos and hair sprays.

3.4 Preparation of Buffer Solutions

3.4.1 Saline pH 7.4, Phosphate-buffered[7]

Dissolve 2.38 g of disodiumhydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 mL. Adjust the pH if necessary.

3.4.2 Simulated Tear Fluid (pH 7.4)[28]

Dissolve KCL (1.7893 gm), NaCl (6.3118 gm), $NaHCO_3$ (2.1842 gm), $CaCl_2$ (44.4 mg) and $MgCl_2$ (47.6 mg) in 1 liter of distilled water and adjust the pH 7.4 by adding required amount of 0.1 N HCl.

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CHAPTER 4

EXPERIMENTAL SETUP

4. EXPERIMENTAL SETUP

Table 4.1: Materials used in present investigation

Sr. No.	Material	Sources
1	Betaxolol HCl	Ciron Drugs Ltd., Mumbai
2	PVP	Loba Chemie, Mumbai
3	Sodium alginate	Loba Chemie, Mumbai
4	Poly ethylene oxide	Alfa Aesar, USA
5	EC	SD Fine Chemicals, Mumbai
7	PMMA	Himedia , Mumbai
8	Potassium dihydrogen orthophosphate	Qualigens Fine Chemical, Mumbai
9	Sodium hydroxide	SD Fine Chemicals, Mumbai
10	PEG 400	Qualigens Fine Chemical, Mumbai
11	Dibutyl phthalate	Qualigens Fine Chemical, Mumbai
12	Chloroform	SD Fine Chemicals, Mumbai
13	Dichloromethane	SD Fine Chemicals, Mumbai
14	Acetone	SD Fine Chemicals, Mumbai
15	Span 20,40,60,80	SD Fine Chemicals, Mumbai
16	Tween 20,60,80	SD Fine Chemicals, Mumbai
17	Cholesterol	Loba Chemie, Mumbai
19	ATGM, SBCDM	Himedia, Mumbai

Table 4.2: Softwares used in present investigation

Software	Make/Developer
Design-Expert® version 8.0.7 trial	Stat-Ease, Inc., Minneapolis MN, USA.
SpectraTreats 3.11.01Rel1	Elico Limited, India.
Microsoft Office 2007	Microsoft Corporation, USA.
EndNote X5	Thomson Reuters, USA

Table 4.3: Instruments used in present investigation

Sr. No.	Material	Sources
1	Electronic balance	Shimadzu, Japan.
2	Digital pH meter	Elico, India.
3	USP XXIII dissolution apparatus	Campbell, Mumbai.
4	Hot air oven	Technico, Chennai.
5	UV-Visible spectrophotometer 1700	Shimadzu, Japan.
6	FT/IR-4100 type A	Jasco, Japan.
7	Orbital shaking incubator	Remi, Mumbai
8	Sonica ultrasonic cleaner	Spincotech , India
9	HPLC (Model- LC 20 AT)	Shimadzu, Japan.
10	BOD incubator	Technico, Chennai.
11	Magnatic stirrer – KMS 400	Jasco, Japan.
12	Rotary flash evaporator	EIE instruments Pvt. Ltd, Ahmedabad, India
13	Freeze dryer-Alpha 1-4 LD plus	Martin Christ, Germany
14	DSC 1/700	Mettler Toledo, Germany
15	XRD – D8 Advance	Bruker, Germany
16	TEM (H 7500)	Hitachi Ltd., Japan
17	SEM	Jeol Ltd., Japan
18	Zetasizer	Malvern Instruments Ltd., UK
19	Laminar flow hood	Esco, Singapore
20	Filter paper	Whatman Inc., Clifton, NJ, USA
21	Tonometer	Schiotz, Germany
22	Viscometer (DV-II+Pro)	Brookfield, Middleboro, USA
23	Theta optical tensiometer	Biolin scientific, Sweden

CHAPTER 5

LONG ACTING BETAXOLOL OCULAR INSERTS BASED ON POLYMER COMPOSITE

Chapter 5 - Long acting betaxolol ocular inserts based on polymer composite

TABLE OF CONTENTS

5. LONG ACTING BETAXOLOL OCULAR INSERTS BASED ON POLYMER COMPOSITE

5.1 Aim of present investigation

Ocular drug delivery is a challenge for the formulator. A drop of an aqueous solution, irrespective of instilled volume is eliminated completely from the eye within 5 to 6 min of its application and only a small amount (1-3%) actually penetrates the cornea and reaches the intraocular tissue[1].

Recent trend in ocular research is to formulate a dosage form which not only prolongs the residence of system in eye but also helps to reduce the elimination of the drug. In the same area many drug delivery systems including insitu gel[2], microemulsions[3], nanoparticles[4], liposome[5], niosomes[6], ocular inserts[7] etc. are being investigated worldwide by many researchers. The developmental technology of new ocular dosage forms has witnessed tremendous improvement during last few decades and its use is expected to increase in near future.

Ophthalmic inserts offer many advantages over conventional dosages forms, like increased ocular residence, possibility of releasing drug at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf life. Design, construction and technology of ocular insert in a controlled and sustained ocular delivery device are gaining rapid improvement to overcome these constraints[7, 8].

Betaxolol is selective beta-1-adrenegic receptor blocker and used in the treatment of ocular hypertension and chronic open angle glaucoma[9]. There are only a few ocular inserts available on the market, made of EVA as a rate controlling membrane[10, 11]. Likewise, poly (methylmethacrylate) (PMMA) is also an excellent film-forming polymer but the films of PMMA alone are brittle[12]. It offers more resistance to the diffusion of drug molecules, and is less explored as a polymer for ocular delivery of drugs. The current literatures indicate that no inserts are made of hydrophobic monolithic systems using betaxolol.

Release of drug through polymer films is dependent on properties of polymers and plasticizers. In the present investigation polymers have been blended on varying ratios to combine the advantages of the individual polymers.

Advantages of polymer blends include easy fabrication of devices, controlling drug release, manipulation of drug loading and other devices properties such as hydration, degradation rate and mechanical strength.

There are two major ocular therapeutic systems existing. Those are matrix and reservoir type ocular therapeutic systems. The present work aimed to develop a matrix-dispersion-type ocular drug delivery system of Betaxolol HCl using blend of polymers. Matrix type of ocular systems have been investigated and found to be effective in drug delivery. There are reports describing the use of polymers like HPMC[13], HPC, EC and Carbopol[14], PVP[15], Eudragit and PVA[16] for ocular delivery systems. These ocular delivery systems are neither extremely hydrophobic nor extremely hydrophilic. Therefore, varying the ratio of these polymers in the composition of the films provides better control of drug release characteristics. Ocular administration of Betaxolol has the disadvantage of low bioavailability due to extensive and highly variable defense mechanisms of the eye. In addition, Betaxolol has a half-life of 12 to 14 hr and requires bid dosing. Owing to these disadvantages, an ocular insert of Betaxolol was designed and developed.

Hence this investigation has been designed to study the drug release kinetics of betaxolol from a hydrophobic matrix system of PMMA cast with incorporating different proportions of PEO. With the addition of hydrophilic polymer to PMMA, the films become resilient and do not break easily and it was ascertained that the diffusion might improve. Matrix type Inserts of betaxolol HCl was formulated using polymer blend of Polyethylene oxide and PMMA with a plasticizer, dibutyl phthalate. Formulation was designed for 24 hr therapy.

It was our prime objective to develop ocular inserts that

1. Release the drug for prolonged period of time preferably 24 hr.
2. Provide an increased ocular residence time resulting in prolonged drug delivery in eye.
3. Deliver a drug at a controlled rate over a period of time.
4. Show better *in-vivo* performance than conventional dosage forms.

5.2 Estimation of betaxolol hydrochloride

5.2.1 Method of estimation

UV visible spectroscopic method for analysis of Betaxolol HCl was adopted in present work. An accurately weighed quantity of Betaxolol HCl (100mg) was dissolved in 100 mL of simulated tear fluid (STF, pH 7.4) to generate a stock solution having concentration of 1mg/mL. Stock solution (10 mL) was further diluted to 100 mL to produce standard solution having concentration of 100μg/mL. The standard solution was serially diluted with STF pH 7.4 to get working standard solutions having concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μg/mL. The absorbance of the solutions was measured at 274.0 nm using double beam UV visible spectrophotometer against STF pH 7.4 as a blank. The plot of absorbance v/s concentration (μg/mL) was plotted (Figure 5.1) and data was subjected to linear regression analysis in Microsoft Excel®[17].

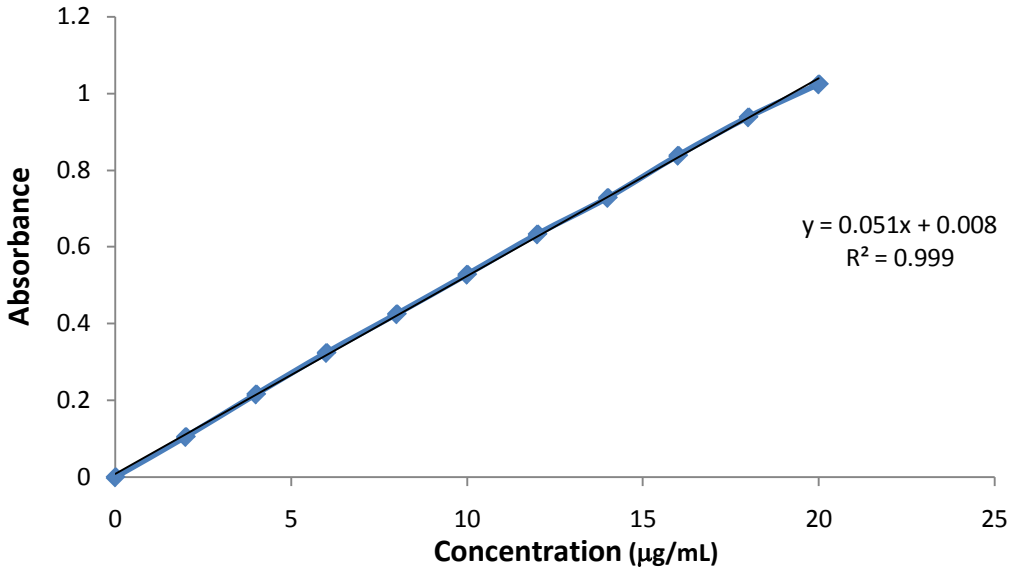

Figure 5.1: Standard curve of betaxolol HCl in STF pH 7.4

5.2.2 Validation of method of estimation[17]

5.2.2.1 Linearity and range

The prepared aliquots (2-20 µg/mL) were scanned for absorbance at λ_{max} value 274 nm. The absorbance range was found to be 0.105-1.024. These solutions obeyed Beer-Lambert's law in above concentration range with correlation coefficient value 0.999.

5.2.2.2 Accuracy and precision

Accuracy and precision were investigated by analyzing three concentrations of Betaxolol HCl (i.e. 3, 6 and 9 mg) in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). The data evaluated was summarized in Table 5.1.

Table 5.1: Results of recovery and precision of betaxolol HCl

Sr. No.	Amount of drug (mg)	Amount found (mg)	% Recovery	%RSD (n=3) intra-day	%RSD (n=3) inter-day
1	3	2.99	99.83	0.144	0.138
2	6	5.98	99.72	0.096	0.166
3	9	9.03	100.42	0.190	0.071

Intra-day and Inter-day relative standard deviation (%RSD) values and also the low RSD values obtained indicated good intermediate precision of method.

5.3 Experimental

5.3.1 Determination of solubility and partition coefficient of betaxolol

5.3.1.1 Solubility

The solubility of Betaxolol was determined in different solvents. An excess quantity of the drug was added in 5 mL of each solvent in screw capped glass test tubes and shaken for 12 hr at room temperature. The solution was filtered, diluted and the solubility was determined by UV visible spectrophotometer[18, 19].

5.3.1.2 Partition coefficient[19]

The partition coefficient of Betaxolol HCl was determined in n-octanol: phosphate buffer pH 7.4 system. An accurately weighed (500 mg) amount of Betaxolol HCl was added into 10 mL each of n-octanol and aqueous phase in a screw capped tube. The mixture was shaken for 24 hr until equilibrium was reached. Phases were separated; the aqueous phase was filtered, diluted and the amount of Betaxolol HCl solubilised in aqueous phase was determined by measuring the absorbance at 274 nm spectrophotometrically.

The partition coefficient of Betaxolol HCl was calculated from the ratio between the concentration of Betaxolol HCl in organic and aqueous phase using following equation:

$$Partition\ Coefficient = \frac{\text{Concentration in Organic Phase}}{\text{Concentration in Aquous Phase}} \quad [5.1]$$

5.3.2 Preliminary screening

Preliminary study was carried out for screening of various polymers and their concentrations.

5.3.2.1 Selection of polymers

Polymers were selected from among PVP, PEO, HPMC, EC and PMMA based on the type of films formed at different concentrations, the strength of the films formed and appearance of the films. Bearing in mind all these factors, it was concluded that PVP and PEO with PMMA form very good films (Table 5.2). In order to idealize a film for ocular inserts, even combinations of hydrophilic-hydrophobic polymers at different concentrations were tried. Finally, it was deduced that PMMA and PEO can be a better combination to give a sustained release for a prolonged period and also fulfill the other requirements at various concentration.

Table 5.2: Formulation compositions for preliminary screening of polymers for films of Betaxolol

Ingredients	A1	A2	A3	A4	A5	A6	A7
Betaxolol HCl	10	10	10	10	10	10	10
PEO	167	---	---	167	---	---	150
PVP	---	240	---	---	240	---	150
HPMC	---	---	167	---	---	167	---
Ethyl Cellulose	333	360	333	---	---	---	---
PMMA	---	---	---	333	360	333	---
PEG 400	---	---	---	---	---	---	100
Dibutyl Phthalate	150	180	150	150	180	150	---
Solvent	S1	S1	S2	S2	S1	S2	Water

Note: All values are in mg.

Solvent: S1: Dichloromethane: Acetone (1:1) and S2: Chloroform: DCM (6:4)

This investigation was aimed to use composite polymer in matrix type ocular formulation. For screening the polymers and polymer blend a formulation study was carried out. Above Table 5.2 shows some of the important formulations screened for actual study. Formulation A1, A2 and A3 were prepared using EC and hydrophilic polymers combination. It was found that good film was not obtained in case of A3 whereas A1 and A2 gave the film but film obtained was not good in appearance and contents were not distributed uniformly. Formulation A4 to A6 were prepared using PMMA and hydrophilic polymers combination. All the films were good in appearance and uniformity. Considering above result it was decided to prepare the ocular films by using the PEO in combination with PMMA. Formulation A7 prepared with PEO and PVP was better as compared to films of HPMC with PEO and PVP. It was found that alone PMMA was not able to form good film whereas EC alone was able to form good uniform film but it was brittle.

5.3.2.2 Water absorption test for polymers[20]

Water uptake measurement was performed by using an apparatus consisting of G15 Buchner sintered glass filter of 3.8 cm diameter with one end

connected to a graduated 1 mL pipette via silicone rubber tube. The lower part of the filter and pipette were filled with distilled water at room temperature just before the experiment. The evaporation of water was minimized with aluminum foil wrapped around the filter.

Polymer discs of PEO were prepared by directly compressing 200 mg of the dried polymer in a hydraulic press. The prepared discs were placed at the center of the filter and the uptake of water for 24 hr was determined by obtaining the change in volume of water in the graduated pipette. The mean of 5 determinations were taken to represent the water uptake for the polymer.

5.3.3 Design of experiment

A 2-factor 3-level full factorial design was used for the formulation and optimization of inserts. This design is suitable for exploring quadratic response surface and constructing second order polynomial models. The non linear quadratic model generated by the design in the form:[21]

$$Y = X_0 + X_1A + X_2B + X_3A^2 + X_4B^2 + X_5AB + E \quad [5.2]$$

Where, Y is the measured response associated with each factor level combination: X_0 is an intercept: X_1 - X_5 are the regression coefficients: A and B are the factors studied and E is the associated error term. Independent factors used in the design are listed in Tables 5.3 and 5.4 show applied 3^2 full factorial design.

Table 5.3: Independent factors for formulations of betaxolol ocular inserts

Independent variable	Factor A Drug to polymer ratio (Drug : Total polymer)			Factor B Polymer to polymer ratio (PMMA : PEO)		
	Low	Medium	High	Low	Medium	High
Coded levels	-1	0	1	-1	0	1
Actual levels	1:2	1:4	1:6	9:1	8:2	7:3

Table 5.4: A 3^2 Full factorial design layout of betaxolol ocular inserts

Run	Factor A Drug to polymer ratio		Factor B Polymer to polymer ratio (PMMA:PEO)	
	Coded	Actual	Coded	Actual
1	-1	1:2	-1	9:1
2	-1	1:2	0	8:2
3	-1	1:2	1	7:3
4	0	1:4	-1	9:1
5	0	1:4	0	8:2
6	0	1:4	1	7:3
7	1	1:6	-1	9:1
8	1	1:6	0	8:2
9	1	1:6	1	7:3

5.3.4 Formulation of ocular inserts

The monolithic films of Betaxolol HCl with PMMA and PEO were prepared by solvent evaporation technique[22]. Chloroform was used as solvent. The composition of ocular films of Betaxolol HCl is shown in Table 5.5.

Table 5.5: Formulation compositions for ocular inserts of betaxolol

Inserts	Drug (mg)	Total polymer (% w/v)	PMMA:PEO (Ratio)
F1	0.5	1	9:1
F2	0.5	1	8:2
F3	0.5	1	7:3
F4	0.5	2	9:1
F5	0.5	2	8:2
F6	0.5	2	7:3
F7	0.5	3	9:1
F8	0.5	3	8:2
F9	0.5	3	7:3

All formulations contain dibutyl phthalate as plasticizer 30 % w/w of polymer weight

The weighed quantities of polymers were dissolved in 13 mL solvent and plasticizers (30% w/w of polymers) were incorporated. To this solution Betaxolol HCl (65 mg) was added and mixed thoroughly with the help of magnetic stirrer for 10 min at 25 rpm. Polymeric solution was sonicated for 30 sec to remove the air. Polymeric solution was then poured into a petridish (6.8 cm diameter) placed on a flat even surface. The rate of evaporation was controlled by inverting the funnel over the petridish. After drying at room temperature for 24 hr, circular ocular inserts of diameter 6 mm were cut using fabricated mould, sterilized under UV for 10 min and 60 min and packed in aluminum foil and stored in desiccators until further use.

5.4 Evaluation and statistical optimization

5.4.1 Physicochemical evaluation/characterization of ocular inserts

5.4.1.1 Physical appearance

All the ocular films were visually inspected for color, clarity and smoothness.

5.4.1.2 Surface pH[23]

Surface pH of the inserts was determined by allowing them to swell in a closed petri dish at room temperature for 30 min in 0.1 mL of distilled water. The swollen devices were removed and placed on pH paper to determine the surface pH. After 1 min the color developed was compared with the standard color scale.

5.4.1.3 Thickness[23]

Thickness of the film is an important factor while considering its drug release from ocular delivery systems. If thickness varies from one film to another, the drug release from the film also varies. So it is must to keep the thickness of the film uniform to get reproducible results. In the present study, the thickness of the formulated films was measured using digital micro meter of sensitivity of 0.01mm (mitutoyo, Japan). Average of 3 readings was taken and standard deviation values were calculated.

5.4.1.4 Weight uniformity[23]

As weight variation between the formulated films can lead to difference in drug content and *in vitro* behavior, evaluation was carried out by weighing 10 films by an electronic balance (least count – 0.1 mg). The average weight and standard deviation were then calculated and reported.

5.4.1.5 Tensile strength and percentage elongation at break[24]

The tensile strength of ocuserts refers to tension or force required to tear off the insert apart into two pieces. This was determined with an instrument assembled in the laboratory.

Instrument: A small strip of ocular film measuring 5 cm×1 cm was cut with a sharp blade. One end of the film was fixed by placing in the film holder. Another end of the film was fixed with the help of forceps having triangular ends to keep the strip straight while stretching and a hook was inserted. A thread was tied to the hook, passed over the pulley and a small pan attached to the other end to hold weights. A small pointer was attached to the thread that travels over the graph paper affixed on the base plate.

Procedure: To determine elongation and tensile strength, the film was pulled by means of a pulley system. Weights were gradually added (5g/min) to the pan to increase the pulling force till the film was broken. Elongation was determined simultaneously by noting the distance traveled by the pointer on the graph paper before the film was broken. The weight necessary to break the film was noted as break force. Percentage elongation at break and tensile strength was calculated using the following formula.

$$\%\, elongation\ at\ break = \mathrm{IB} - \mathrm{Io} \times 100 \qquad [5.3]$$

Where Io is the original length of the film and

IB is the length of the film at break when stress was applied.

$$\mathrm{Tensile\,strength} = \frac{\mathrm{Break\ \ force}}{\mathrm{ab}\,(1+\Delta \mathrm{L}/\mathrm{L})} \qquad [5.4]$$

Where a, b and L are width, thickness and length of the strip respectively and ΔL is the elongation at break.

Break force = weight required to break the film (Kg).

5.4.1.6 Folding endurance[25]

The flexibility of polymeric films can be measured quantitatively in terms of folding endurance. Folding endurance was determined by repeatedly folding a small strip of ocular film (2×2 cm) at the same place till it breaks. The number of times film could be folded at the same place, without breaking gives the value of folding endurance.

5.4.1.7 Moisture uptake[22, 25]

The percentage moisture uptake test was carried out to check the physical stability or integrity of the film. Ocular films were weighed individually and placed in a desiccator containing 100 mL of saturated solution of sodium chloride (~ 75 % humidity). After three days, films were taken out and reweighed; the percentage moisture uptake was calculated by using following formula.

$$\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial Weight}}{\text{Initial weight}} \times 100 \qquad [5.5]$$

5.4.1.8 Percentage moisture content[22, 25]

The percentage moisture loss test was carried out to check the integrity of the film at dry condition. Ocular films were weighed individually and placed in a desiccator containing anhydrous calcium chloride. After three days, films were taken out and reweighed; the percentage moisture loss was calculated by using following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final Weight}}{\text{Initial weight}} \times 100 \qquad [5.6]$$

5.4.1.9 Water vapor transmission studies[26]

The glass vials of 5 mL capacity were washed thoroughly and dried to constant weight in an oven. One gram of fused calcium chloride was taken in vials and the polymer films were fixed over the brim with the help of an adhesive. These pre-weighed vials were stored in humidity chamber at RH 80% with temperature of 25 ^{o}C for a period of 24 hr. The weight gain was determined every hour up to a period of 24 hr to note the weight gain. Water vapor transmission (WVT) was calculated by taking the difference in weight of film before and after the study for a total period of 24 hr.

5.4.1.10 Determination of drug content[23]

The films were dissolved in 5 mL chloroform. The drug was extracted from chloroform using phosphate buffer. The volume was adjusted to 100 mL with phosphate buffer pH 7.4 and the solutions were filtered through filter. Chloroform was evaporated at 60 ^{o}C. The drug content in each formulation was determined spectrophotometrically at 274 nm. Similarly, a blank solution was prepared using dummy film. Average drug content of three films was determined.

5.4.1.11 Surface morphology

Surface characteristics of polymer blend were studies by scanning electron microscopy. Films were mounted on an aluminum stub using double-sided adhesive carbon tape and coated with gold palladium using JEOL JFC 1600 auto fine coater for 90 sec. Samples were examined using scanning electron microscope JSM-6380 LV (Jeol Ltd., Tokyo, Japan) at 20 kv accelerating voltage.

5.4.1.12 Hydrophilicity

Contact angle was measured at R.T. by optical tensiometry using contact-angle meter (Theta optical tensiometer, Biolin scientific AB, Sweden) equipped with T200 60 fps digital camera. Drops of STF (pH 7.4) were prepared with a precision syringe (1 mL, Hamilton 1001TPLT) and were dropped onto the surface of the polymer. The static contact angle was measured at contact time t=10 s.

5.4.1.13 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) scans of pure drug and drug loaded ocular insert were performed using DSC 1/700 (Mettler Toledo, Germany). The analysis was performed with a heating range of -25 ^{o}C to 250 °C and at a rate of 10 °C/min in nitrogen atmosphere. The sample weight was approximately 6 mg.

5.4.1.14 X-Ray diffraction

X-Ray diffraction (XRD) patterns of pure betaxolol and ocular film were recorded using a powder X-Ray diffractometer (XRD-D8 Advance, Bruker, Germany) over the interval 10-80° $2\theta^{-1}$. The experimental condition was: generator tension (voltage) of 38 kV, generator current of 34 mA, scan step time of 30.6 sec^{-1} and scan step size of 0.049° $2\theta^{-1}$.

5.4.2 *In vitro* drug release study[27]

Since there was no specific official method prescribed for *in vitro* studies of ocular inserts, we fabricated an open flow through assembly, simulating the condition of the ocular cavity, by using the classical standard cylindrical tube which has the diameter of 15 mm. Dialysis membrane (Dialysis membrane 110, mw cut-off 12000-14000, Hi Media, India), immersed in water for one hour to remove the preservatives followed by rinsing in phosphate buffered saline (PBS) solution, acted as corneal epithelium, was tied to one end of

open cylinder which acted as donor compartment. An ocular insert was placed inside this compartment with 0.7 mL of simulated tear fluid (STF pH 7.4). Then, the glass tube was suspended in the dissolution flask of a USP dissolution apparatus such that entire surface of the membrane was in contact with the receptor compartment containing 250 mL of STF (pH 7.4). The content of the receptor compartment was stirred continuously at 25 rpm. Samples of 1 mL were withdrawn from the receptor compartment at periodic intervals and replaced by equal volume of fresh solution. The samples were analyzed spectrophotometrically at 274 nm against reference standard using STF as blank.

5.4.3 Data treatment and analysis

The following parameters were determined from the *in vitro* data obtained for the diffusion of Betaxolol hydrochloride through the membrane.

5.4.3.1 Diffusion rate

Diffusion rate is the milligrams of Betaxolol HCl diffused through membrane per unit time. It can be determined by dividing the milligrams of Betaxolol HCl diffused by time in hour[28].

$$\mathrm{D_r} = \frac{Q}{T} \qquad [5.7]$$

5.4.3.2 The steady-state flux[29]

Absorption is a passive diffusion process and can be described by Fick's second law equation

$$\mathrm{J_S} = \frac{dQ}{dt} \times \frac{1}{A} \qquad [5.8]$$

Where J_S is the steady-state flux in micrograms/square centimeter per hour, *dQ* is the change in quantity of material passing through the membrane into the receptor compartment expressed in micrograms, *A* is the active diffusion area in square centimeters, and *dt* is the change in time in hr.

The steady-state flux values of the Betaxolol HCl through the membrane were calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area versus time plot.

5.4.3.3 Permeability coefficient[29]

To determine the permeability coefficient, the following equation was used:

$$\mathrm{K_P} = \frac{J_S}{C_d} \qquad [5.9]$$

Where K_P is the permeability coefficient, J_S is the flux calculated at the steady time, and C_d is the donor concentration.

5.4.3.4 Kinetics of permeation

For finding out the mechanism of drug release from ocular system, the diffusion data obtained from the above experiments was treated with the different release kinetic equations[30].

Zero order release equation:

$$Q = K_0 t \quad [5.10]$$

Higuchi's square root of time equation:[25]

$$Q = K_H t^{1/2} \quad [5.11]$$

Where, Q is amount of drug release at time t, K_0 is zero order release rate constant, K_H is Higuchi's square root of time release rate constant.

5.4.3.5 Mechanism of release

Korsmeyer and Peppas equation is used to determine the mechanism of drug release[31, 32].

$$F = (M_t/M) = K_m t^n \quad [5.12]$$

Where, M_t is drug release at time t, M is total Amount of drug in dosage form, F is fraction of drug release at time t, K_m is constant which depends on geometry of dosage form and n is diffusion exponent indicating the mechanism of drug release, where for cylinder value of n is 0.45 indicate fickian diffusion, between 0.45 and 0.89 indicate anomalous transport and 0.89 indicate case-II transport.

5.4.4 Statistical analysis

The selected responses obtained from the various systems were tested for significant differences. Statistical analysis of data was carried out using analysis of variance (ANOVA). The individual response was evaluated using F-test and F value and P value were generated. The statistical analysis was conducted using Design-Expert® version 8.0.2 trial (Stat-Ease, Inc., Minneapolis MN).

The following responses were selected for statistical analysis:

- Diffusion rate
- Flux

5.4.5 Drug-excipients compatibility studies[33]

The FTIR spectra of the pure drug and physical mixture (betaxolol, PEO and PMMA) were taken as KBr pellets in the range of 4000–650 cm^{-1} (FT/IR-4100 type A spectrophotometer, Jasco, Japan). The infrared analysis of optimized insert was carried out in the same range by ATR-IR spectroscopy (Perkin Elmer Model 1600 FT-IR spectrophotometer with ATR mode Perkin Elmer, USA).

5.4.6 Sterility testing [34]

Sterility is one of the most vital requirements for an ophthalmic preparation. The tests for sterility are intended for detecting the presence of viable forms of microorganisms in ophthalmic preparations. The principle governing these tests is that if the microorganisms are placed in a medium which provides nutritive material and water, kept at a favourable temperature, the organisms will grow and their presence can be indicated by turbidity in the originally clear medium. In the present study, two media namely, alternate thioglycolate medium (ATGM) and soyabean-casein digest medium (SBCD) were used to investigate the presence/absence of aerobic, anaerobic bacteria and fungi, in the formulated sterilized ocular inserts.

5.4.6.1 Preparation of culture medium

a) ATGM was used to detect the growth of aerobic and anaerobic bacteria. 7.25 g of readymade ATGM was dissolved in 250 mL of purified water and the pH was adjusted to 7.1±0.2 with 1M NaOH. This was sterilized in an autoclave at 115 ^{o}C for 30 min. The medium was freshly prepared and allowed to cool just prior to use.

b) SBCD medium was used to detect the growth of aerobic bacteria and fungi. 7.25 g of readymade SBCD was dissolved in 250 mL of purified water and the pH was adjusted to 7.1±0.2 with 1M NaOH. This was sterilized in an autoclave at 115 ^{o}C for 30 min. The medium was freshly prepared and allowed to cool just prior to use.

5.4.6.2 Test procedure

Sterilized inserts were directly inoculated in above medium aseptically as described in IP 2007 and labeled as ‘Test’. Simultaneously, positive and negative controls were also prepared and all the three tubes were incubated at specified temperatures.

5.4.7 *In vivo* studies

Approval for the use of animals in the study was obtained from the Institutional Animal Ethics Committee (1338/c/CPCSEA). New Zealand rabbits of either sex weighing 2.5 to 3.1 kg were used for *in vivo* studies. The rabbits were housed singly in restraining cages during the experiment and allowed food and water *ad libitum*. Free lag and eye movement was allowed. No ocular abnormalities were found on external and slit-lamp examination prior to beginning of the study.

5.4.7.1 *In vivo* release study[24]

On the day of experiment the sterilized ocuserts were placed into the lower *cul-de-sac* of rabbits. The inserts were inserted into one eye of seven rabbits at same time and another eye served as control. After 1, 2, 4, 6, 10, 22 and 24 hr, the inserts were carefully removed and analyzed for remaining drug content by UV analysis. The amount of drug remaining was subtracted from initial drug content of inserts to find the amount of drug released in the rabbit eye. Observations for any fall out of insert were also recorded throughout the experiment.

5.4.7.2 Corneal residence time evaluation

Precorneal residence time of ocular drugs has been assessed by certain invasive techniques[35] and non-invasive techniques[36, 37]. These approaches however, require isolation of ocular tissues or the use of radioisotopes. In the present study, an effort was therefore made to develop a non-invasive method to assess the precorneal residence of the drug from the formulated delivery system based on HPLC technique.

i) Tear sampling and analysis

Tear samples equivalent to 1 µL were collected from the left eye after application of test delivery system at 0, 0.10, 1, 2, 4, 6, 10, 22, 24 hr post dosing. Glass capillary tubes having 320 µm internal diameter and 1 µL premark were placed near the canthus of the eye without applying pressure. Tear fluid was drained into the tubes due to capillary action. Samples equivalent to 1 µL were mixed with 50 µL of mobile phase and injected into HPLC chamber. Data were collected and interpreted and results were tabulated.

ii) HPLC Condition[38]

Mobile phase: methanol:acetonitrile:0.1% diethylamine (pH adjusted to 3 with orthophosphoric acid) (30:60:10, %v/v)

Flow Rate: 1.0 mL/min

Column: Phenomenex C18 Column. (Luna C-250, 34.6 mm, 5 mm)

Detector: SPD-M20A Prominence diode array detector

Retention Time: 4.08 min

Injection Volume: 20 µl by Rheodyne 7725*i* injector

Standard solution: 2 µg/mL of Betaxolol in HPLC grade water.

5.4.7.3 Ocular safety study[39, 40]

The ocular safety of administered delivery system is based on the Draize Irritancy Test (Table 5.6). The observations based on scoring approach (Table 5.7) established the safety of the developed ocular inserts in rabbit eye.

Table 5.6: Draize irritancy test for ocular safety

Ocular tissue	Scoring scale	Calculations	Total
Cornea: Opacity (O)	0,1,2,3,4	O×A×5	80
Area involved (A)	0,1,2,3,4		
Iris: Values for congestion and hemorrhage (I)	0, 1, 2	I×5	10
Conjunctiva : Redness (R)	0, 1, 2, 3		
Chemosis (C)	0, 1, 2 , 3, 4	(R+C+D)×2	20
Discharge (D)	0, 1, 2, 3		
	Total Maximum		110

Note: Score of 0 is normal, 3 and 4 is severe in case of O, R, C and D.
Score of 0 is none, 1,2,3,4 is the extent of cornea covered for A.
Score of 0 is normal and 2 are severe in case of I.

Table 5.7: Safety evaluation chart

Score	Rating
0.0– 0.5	Non irritating
0.5 – 2.5	Practically non irritating
2.5 – 15	Minimally irritating
15.0 – 25.0	Mildly irritating
25.0 – 50.0	Moderately irritating
50.0 – 80.0	Severely irritating
80.0 – 110.0	Extremely irritating

5.4.7.4 *In vivo* anti-glaucoma activity evaluation[41, 42]

i) Animal care and handling

The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2 °C relative humidity 44–56% and light and dark cycles of 12:12 hr, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1338/c/CPCSEA).

ii) Steroid induced model[43]

Eyes of the 12 rabbits were treated 3 times daily by 0.5% dexamethasone eye drops for 2 weeks. Dexamethasone sodium phosphate (Taj Pharmaceuticals Ltd., Mumbai) was dissolved in phosphate buffer saline to a final concentration of 0.5 % at pH 7.4. The intraocular pressure was measured at noon twice a week by Schiotz tonometer.

iii) Antiglaucoma activity evaluation

For evaluation, the rabbits with induced glaucoma were randomly divided in three groups (n=4). First group was treated with marketed preparation of betaxolol HCl eye drop equivalent to 0.50 mg. In second group placebo film and in third group medicated film (F8) of betaxolol HCL was inserted in to lower *cul de sac* of rabbits. The intraocular pressure (IOP) changes were recorded up to 26 hr at specified time intervals. The ocular hypotensive activity is expressed as the average difference in IOP according to the equation $\Delta IOP = IOP_{'0'time} - IOP_{'t'time}$[42, 44].

5.4.8 Ageing study[27]

The optimized inserts (F8) were stored in amber colored glass bottles at 3 different temperatures 4 ^{o}C, Room temperature and 40±0.5 ^{o}C for a period of 6 months. The samples were withdrawn after 60, 120 and 180 days and analyzed for physical appearance, drug content, folding endurance and sterility.

5.5 Results and discussion

In the present study ocular films of betaxolol HCl were prepared using combination of polymers (PMMA and PEO). A 3^2 full factorial design was employed and so nine formulations were obtained. Films were evaluated for their use as ocular delivery system with a view to obtain sustained release.

5.5.1 Preliminary screening

Solubility of Betaxolol HCl was determined in different solvents and the observations are shown in Table 5.8. The maximum solubility was found in water and ethanol and least in ethyl acetate.

Partition coefficient of Betaxolol HCl in n-octanol and phosphate buffer pH 7.4 was found to be 1.07. PEO was found to take up 1.6 folds of its own weight of water over a period of 24 hr.

Table 5.8: Solubility profile of betaxolol HCl in different solvents

SR. No.	Solvents	Solubility
1.	Ethyl acetate	+
2.	Chloroform	++++
3.	Methylene chloride	+++
4.	Methanol	++++
5.	Ethanol	++++
6.	Distilled water	++++
7.	0.1N NAOH	++
8.	0.1N HCl	+++

+	Practically insoluble	++	Slightly soluble
+++	Soluble	++++	Freely soluble

5.5.2 Physicochemical evaluation

In the present investigation solvent evaporation technique is adopted and it was found to be giving thin uniform films. All prepared ocular films have good appearance with smooth surface. Films prepared were semitransparent. Surface texture was smooth and uniform.

Weight and thickness measurements of films were carried out and low standard deviation values in film weight and thickness measurements ensure the uniformity of weight and thickness in each film. A good weight uniformity of all formulation indicates an even distribution of drug and the polymers in the matrix film prepared by solvent evaporation technique. It was also accounted that weight and thickness of films were increasing with increasing polymer concentration. However there was no significant effect of PMMA:PEO ratio on weight and thickness of the film. Weight variation and thickness data of ocular films is shown in Table 5.9. Formulations were not thick enough to produce any irritation while placing and being in *cul-de-sac* as inferred from ocular irritancy test.

Table 5.9: Physical properties of prepared ocular films of betaxolol

RUN	Weight of films(mg)*	Thickness (mm)#	Tensile strength# Kg/cm^2	%Elongation at break# (EB)	Folding endurance#
F1	4.09± 0.069	0.118± 0.016	0.75 ± 0.03	3.26± 0.21	69±2
F2	4.2± 0.046	0.13±0.0063	0.68 ± 0.01	8.13± 0.23	90.3±2.08
F3	4.11± 0.052	0.148±0.007	0.57 ± 0.01	12.63± 0.4	99.3±2.51
F4	6.43± 0.094	0.19 ± 0.015	0.84 ± 0.02	2.95± 0.32	65.3±3.51
F5	6.49± 0.082	0.20± 0.0072	0.72 ± 0.01	7.18± 0.43	81±2.61
F6	6.44± 0.044	0.21± 0.0054	0.65 ± 0.01	12.17± 0.21	94.6±2.08
F7	8.89± 0.057	0.24± 0.0032	1.34 ± 0.05	2.28± 0.32	61.3±2.08
F8	9.09± 0.04	0.248± 0.004	0.92 ± 0.02	7.89± 0.46	73.3±2.52
F9	8.91± 0.052	0.25± 0.0154	0.80 ± 0.02	11.93± 0.54	89.6±3.78

All readings are in the form of Mean±SD, # Average of 3 runs, *Average of 10 determinations

The **surface pH** of the prepared inserts were found in between 6.5 to 7.5, indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range[45].

Tensile strength for all inserts is shown in Table 5.9. It shows that formulation F7 shows highest tensile strength among all batches i.e. 1.34 ± 0.05 kg/cm^2. It was also found that inserts containing more amount of PMMA shows higher tensile strength whereas increasing amount of PEO in insert lower the tensile strength. Increasing values of **EB** with increase in PEO amount suggests that PEO increases elasticity of the films.

Folding endurance values revealed that maximum folding endurance was found at low concentration of polymer. Formulation F3 shows highest folding endurance. Decrease in folding endurance at higher concentration of polymer, may be due to increase in thickness of films. At Low amount of polymer addition of PEO in PMMA films, increase folding endurance significantly but at high amount of polymer, the effect was less.

Ocular inserts were also evaluated for % moisture content, % moisture uptake and water vapour transmission (WVT) rate. Results were tabulated in Table 5.10**.**

Moisture content values of inserts were found in range of 2.50 ± 0.07 to 6.05 ± 0.17 %. It also shows that moisture content of inserts increase with increasing amount of PEO. This is due to hydrophilic properties of PEO. PEO content was more in formulation F9 and so more moisture content was found. It was also noticed that moisture uptake properties of formulation also depend on PEO content. Moisture uptake value was more for formulation F9 as it contains more amount of PEO. Same observation was also found in water vapour transmission rate of inserts. Water vapour transmission rate was found in range of 0.81×10^{-3} to 4.20×10^{-3} gm/cm^{2}h.

Films containing more amount of PMMA show less WVT rate. These could be due to the hydrophilicity of the films was changed towards higher hydrophobic property when the higher amount of PMMA was present as compared to PEO.

Table 5.10: Evaluation of prepared ocular films of betaxolol

Inserts	% Moisture content (%MC ± SD)[a]	% Moisture uptake (%MU ± SD)[a]	Water vapour transmission rate (gm/cm^2h)[a]× 10^{-3}
F1	2.50 ± 0.07	04.17 ± 0.14	0.85±0.01
F2	2.78 ± 0.05	05.00 ± 0.09	1.21±0.03
F3	4.17 ± 0.12	09.37 ± 0.16	1.84±0.02
F4	2.94 ± 0.04	05.15 ± 0.12	0.96±0.05
F5	4.17 ± 0.10	08.33 ± 0.08	1.36±0.03
F6	5.47 ± 0.15	10.94 ± 0.12	3.06±0.02
F7	3.85 ± 0.09	07.69 ± 0.07	1.14±0.03
F8	4.81 ± 0.08	09.62 ± 0.09	2.21±0.02
F9	6.05 ± 0.17	11.08 ± 0.11	4.20±0.05

[a] Average ± SD of three determination has been reported

The water uptake or absorption behavior of the polymeric film plays an important role at the beginning stage of drug release from dosage form[(46)]. Thus, the film with higher moisture uptake supposed to give higher drug release rate initially.

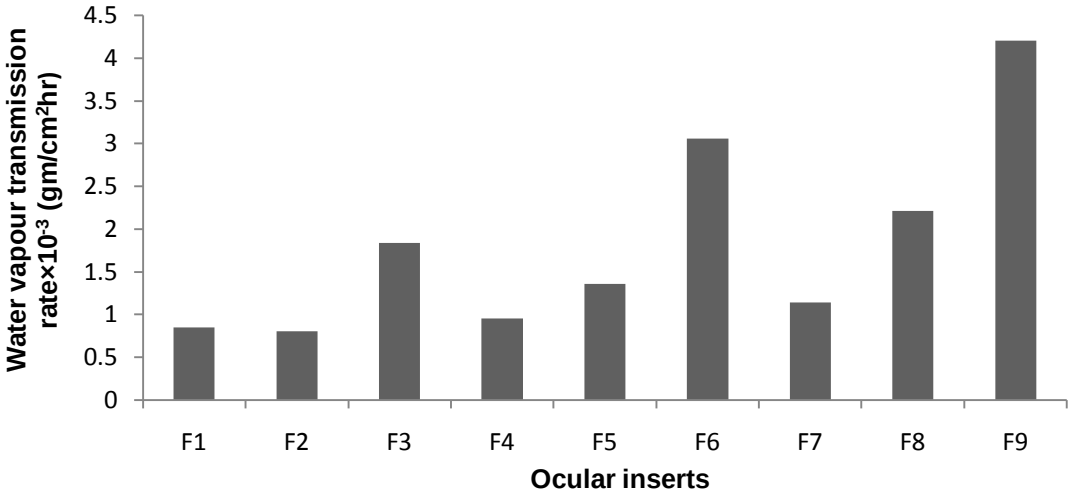

Figure 5.2: Comparative WVT rate of ocular formulation of betaxolol

Good uniformity in the **drug content** among the batches was observed for all the formulations. Drug content was found in the range of 99.07% to 102.13%.

Table 5.11: Drug content of prepared ocular films of betaxolol HCl

Inserts	Drug content* (mg ± SD)	Drug content* (% ± SD)
F1	0.511±0.0076	102.13±1.51
F2	0.501±0.010	100.2±2.03
F3	0.502±0.006	100.5±1.21
F4	0.506±0.0031	101.27±0.61
F5	0.497±0.0045	99.46±0.90
F6	0.495±0.0021	99.07±0.42
F7	0.499±0.0042	99.87±0.83
F8	0.501±0.0023	100.13±0.46
F9	0.503±0.0042	100.53±0.83

***** Average of five readings±SD

The drug content analysis of the prepared formulations have shown that the process employed to prepare films in this study was capable of giving films with uniform drug content and minimum batch variability.

5.5.3 Surface morphology (SEM)

SEM study (Figure 5.3) revealed that surface of the ocular films are smooth indicating the complete miscibility of PEO with PMMA. This finding is similar to that of J.baldrian [(47)] who suggested that when the concentration of PEO is less than or equal to 20wt. %, the polymers are completely miscible and the blend is amorphous. This result is also supported by DSC and XRD studies.

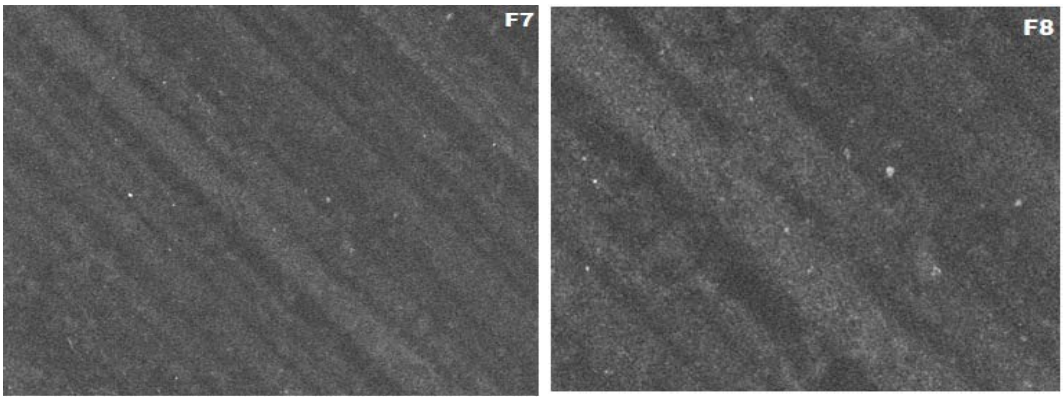

Figure 5.3: Scanning electron microscopy (SEM) images of inserts F7 and F8. Experimental condition: magnification= ×1000, Acc. V 20 kV, signal SEI, ___ 10 µm

5.5.4 Hydrophilicity

Static STF Contact angles (θs) of films were below 50 indicating increasing hydrophilicity of the surface as the concentration of PEO increased in the matrix. As θ_s is only related to the outermost 10Å of each sample, the surface composition of blend is greatly correlated with θ_s [48]. This result also reveals that PEO was uniformly distributed throughout the bulk of PMMA and concentration of PEO at surface increases with bulk concentration, making the insert hydrophilic enough to be wetted by tear fluid and adhere to corneal surface for longer duration.

5.5.5 Drug-excipients compatibility study

Interpretation of IR spectrum [49] of Betaxolol (Figure 5.4A) shows characteristic peaks at 3237 cm^{-1} (hydroxyl group O-H stretching), 2928 cm^{-1} (methyl C-H stretching), 2858 cm^{-1} (methylene C-H stretching), 1612 cm^{-1} (aromatic ring C=C-C stretching), 1557 cm^{-1} and 1513 cm^{-1} (secondary amine N-H bending), 1474 cm^{-1} (methylene C-H bending), 1376 cm^{-1} (methyl C-H sym. bending), 1246 cm^{-1} (alkyl aryl ether c-o-c stretching), 1179 cm^{-1} (aromatic in plane C-H bending), 1087 cm^{-1} (aliphatic ether c-o-c stretching) and 1050 cm^{-1} (amine C-N stretching).

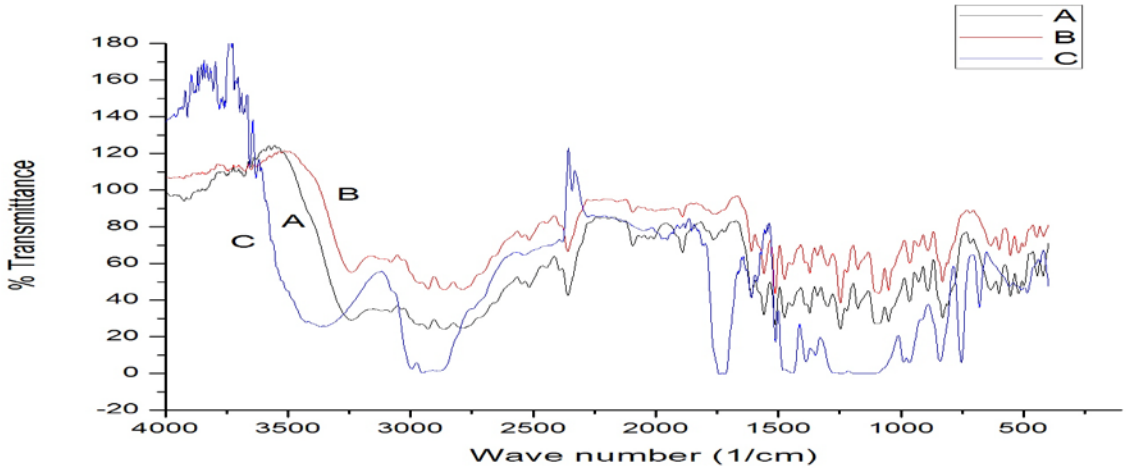

Figure 5.4: IR spectra of (A) Betaxolol HCL (B) Mixture of PMMA/ PEO/BX (C) Ocular insert F8

FTIR spectra of mixture (Figure 5.4B) and insert F8 (Figure 5.4C), in comparison to IR spectra of pure drug, show no substantial shifting of the position of the functional groups, indicating no major interaction between drug and polymers. However, broadening and reduced intensity of peaks in IR spectra of insert indicates encapsulation of betaxolol in polymeric matrix. Although shifting and broadening of drug peak at 3237 cm^{-1} in the IR spectra

of insert F8 indicate that hydrogen bonding has occurred between the lone electron pairs of the oxygen atom of polymers and the hydrogen atom of the hydroxyl group of the drug.

5.5.6 Differential scanning calorimetry

From the overlay of the DSC thermograms, it has been observed that Betaxolol is crystalline in nature (Figure 5.5).

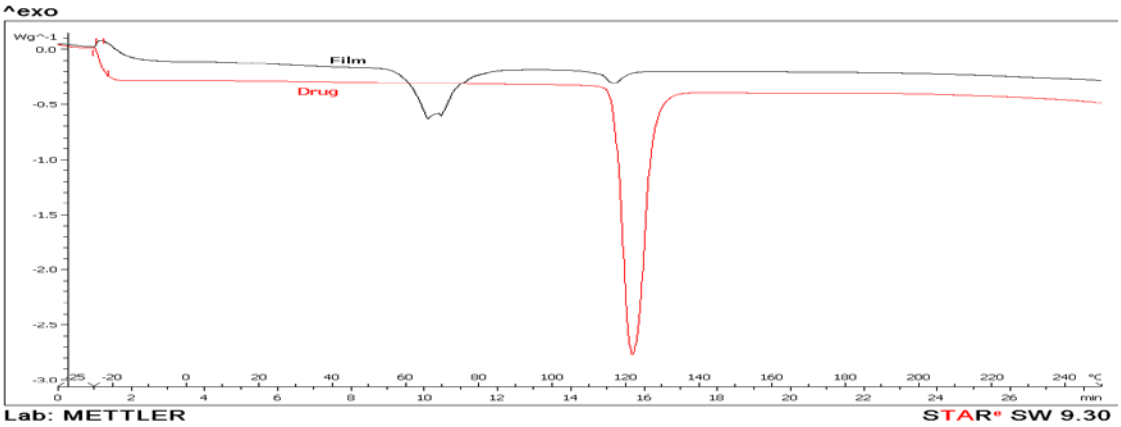

Figure 5.5: DSC spectra of pure drug and ocular film

Thermogram exhibited a sharp melting endotherm at an onset temperature of 112.82 ^{o}C, a peak temperature of 119.76 ^{o}C and a heat of fusion of 6.97 J/g. While the thermogram of film shows crystallization of betaxolol from glass at 67.18 ^{o}C followed by fusion at 116.89 ^{o}C. The thermal behavior of film suggested that the drug is present in the film as semicrystalline form as the fusion peak in the film is very weak compared to the pure drug.

5.5.7 X-ray diffraction (XRD) study

XRD spectrum of Betaxolol (Figure 5.6) revealed that the drug is crystalline in nature.

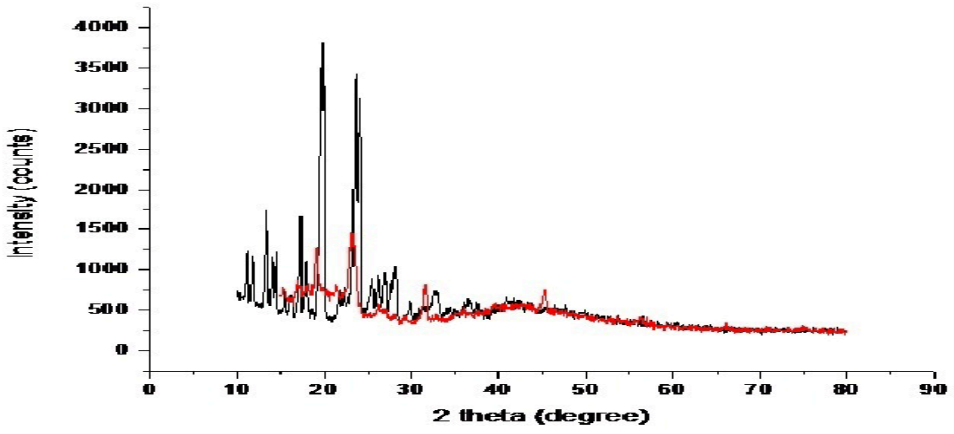

Figure 5.6: XRD patterns of Betaxolol (black line) and Insert F8 (red line)

XRD pattern of film showed that characteristic peaks of betaxolol were reduced in number and intensity indicating that the drug crystallinity was decreased in the inserts.

5.5.8 *In vitro* drug diffusion studies

In this study, Matrix-type inserts of Betaxolol HCl was prepared with polymer blend (PMMA and PEO). All formulations prepared were subjected to *in vitro* diffusion study to ensure the effect of polymer concentration and polymer blend. The data obtained for *in- vitro* study were tabulated and represented graphically.

Table 5.12 shows percentage cumulative drug release profile (average of 3 runs) for all formulations F1 to F9. The Maximum drug release was found at lower polymer concentration and as the polymer amount was increased from 1% to 3% w/v, the release was found to be decreased. Ocusert F7 shows minimum drug release i.e. 79.26% in 24 hr.

Order of cumulative drug permeated was found as follows:

Eye drops>F3> F6 > F2 > F5 > F1 > F9 > F4 > F8 > F7

From *in vitro* release results, insert F8 was selected as optimized formulation and subjected to further studies.

Table 5.12: Percent cumulative amount of betaxolol permeated through membrane

Time (hr)	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
	% CP	% CP	% CP	% CP	% CP	% CP	% CP	% CP	% CP
1	5.43	7.21	9.43	3.14	4.78	7.31	2.16	5.46	6.74
2	9.45	13.41	16.2	8.25	7.98	14.65	5.92	9.27	12.45
4	17.72	22.84	26.36	15.76	16.84	23.76	9.25	17.45	21.35
6	28.43	33.24	41.28	24.33	30.65	38.54	15.24	23.34	32.18
8	39.44	44.34	52.34	35.98	41.54	48.87	22.67	32.65	41.82
10	50.15	58.45	65.87	48.73	56.87	62.87	31.24	41.79	51.84
12	62.12	67.42	76.89	59.16	65.8	71.25	37.14	50.08	62.14
22	98.78	99.87	100.21	99.65	100.25	99.74	67.24	87.88	98.87
24	98.87	99.79	100.22	99.45	100.73	99.65	79.26	100.45	99.15

Diffusion profiles of all formulation were subjected to data treatment. Diffusion data were treated with zero order, Higuchi and Peppas equation. Diffusion parameters like Diffusion rate, Flux and Permeability coefficient were also determined.

Drug permeation profile of all formulations is shown in Figure 5.7. It shows that formulation F8 shows almost 100 percent drug release. Lowest drug release was found in case of F7 as it contained more amount of hydrophobic polymer compare to all others.

5.5.8.1 Effect of polymer concentration

In vitro permeation profile shows that concentration of polymer in the film plays a very important role on drug permeation. ANOVA study shows that factor A (drug to polymer ratio) has significant effect on maximum drug permeated i.e. C_{max}. Figure 5.7 shows that as concentration of polymer increases there was decrease in drug permeation.

The Maximum average diffusion rate found was 0.0306 mg/h in case of formulation F3 whereas F7 shows minimum drug release with diffusion rate of 0.017 mg/h. Flux values attained for all formulation are shown in Table 5.13.

Table 5.13: Permeation parameters of betaxolol through membrane

Inserts	Diffusion rate D_r	Flux J_S	Permeability coefficient K_P
	mg/h	mg/cm^{2}h	cm/h
F1	0.0247	0.013958	2.79×10^{-2}
F2	0.0273	0.015459	3.09×10^{-2}
F3	0.0306	0.017299	3.46×10^{-2}
F4	0.0236	0.013364	2.67×10^{-2}
F5	0.0252	0.014275	2.86×10^{-2}
F6	0.0286	0.016195	3.24×10^{-2}
F7	0.0170	0.009609	1.92×10^{-2}
F8	0.0221	0.012514	2.50×10^{-2}
F9	0.0256	0.014499	2.90×10^{-2}

Result of flux also favors that drug release was inversely proportional to polymer concentration. Among all inserts, maximum flux was found in case F3 i.e. 0.01730 mg/cm^{2}h.

Diffusion of molecules through polymer matrix was rate limiting step for permeation of molecule. The process of drug release in most controlled release devices is governed by diffusion, and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. It has been reported that diffusion rate of molecule is inversely proportional to diffusion path[(50)].

Thickness of inserts increases with increasing the amount of polymer. This lead to an increase in diffusion path of molecules and so less release was observed in case of formulation containing high concentration of polymer.

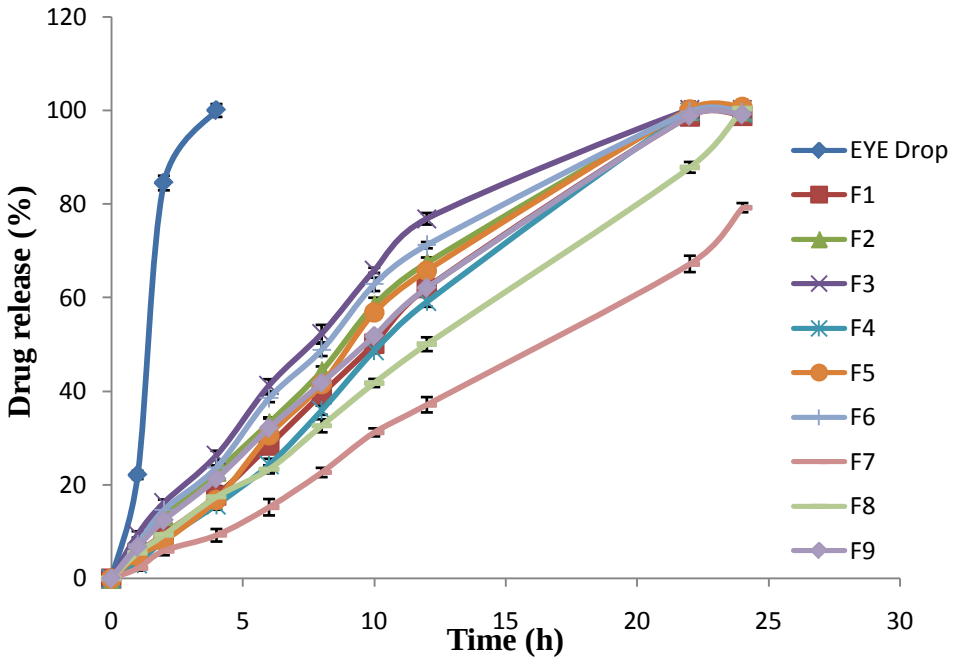

Figure 5.7: *In vitro* drug release profiles of betaxolol inserts and eye drops

5.5.8.2 Effect of polymer composition

The effect of polymer blend on drug permeation profile was also studied and results are shown in Table 5.13 and Figure 5.7. *In vitro* permeation study shows that change in polymer blend will alter the drug permeation profile.

ANOVA study shows that factor B (HPMC to EC ratio) has significant effect on drug permeation profile.

Average diffusion rate of formulation F1 to F3 was found 0.0247, 0.0273 and 0.0306 mg/h respectively. This shows that diffusion rate was increased with increase in PEO concentration. Similar observations were also found in case of batches F4 to F6 and F7 to F9. Result of flux also indicates that drug release was directly proportional to PEO concentration.

Drug permeation data represented in Table 5.13 shows that increasing concentration of PMMA will retard the drug release and so drug permeation. Release rates were increased when the concentration of PEO increased in the formulations. This is because as the proportion of this polymer in the matrix increased, there was an increase in the amount of water uptake and hydration of the polymeric matrix and thus more drugs was released[51].

The drug release was found to increase on increasing the concentration of hydrophilic polymer in the polymer matrix. The polyether chains of PEO can form strong hydrogen bonds with water, therefore, when inserts are brought into contact with an aqueous medium, the polymer tends to hydrate, forming a superficial gel which eventually erodes as the polymer dissolves[52]. This is due to the fact that dissolution of aqueous soluble fraction of the polymer matrix leads to the formation of gelaneous pores. The formation of such pores leads to decrease in the mean diffusion path length of drug molecules to release into the diffusion medium and hence, to cause higher release rate.

The kinetic treatment of diffusion data of different batches is shown in Table 5.14. It can be concluded that Korsemeyer and Peppas model fit the best for all the inserts as correlation coefficient value for all the inserts were more than 0.9867. This is followed by Higuchi and zero order equation. From the n value it can be seen that all the formulations follow non fickian diffusion of drug release[30]. This can be supported by the good fit of Higuchi equation. The drug was released by diffusion from the polymer matrix. Results also indicated that inserts show zero order drug release at high amount of polymer. Drug release from such matrices may be controlled by polymer swelling or erosion, or drug diffusion in the hydrated gel, or by these processes altogether[52].

Table 5.14: Kinetics modeling of permeation profile of ocular inserts containing betaxolol

Inserts	Zero order R^2	Higuchi R^2	Korsmeyer-Peppas R^2	N	Release mechanism
F1	0.9869	0.9789	0.995	0.9573	Anomalous transport
F2	0.9714	0.9869	0.9944	0.8522	Anomalous transport
F3	0.933	0.9836	0.9867	0.7785	Anomalous transport
F4	0.9904	0.9721	0.9937	1.095	Super case-II transport
F5	0.9752	0.9826	0.9871	1.024	Super case-II transport
F6	0.9517	0.9891	0.9881	0.8423	Anomalous transport
F7	0.9951	0.9445	0.9935	1.107	Super case-II transport
F8	0.9986	0.9552	0.9963	0.9215	Anomalous transport
F9	0.9859	0.9847	0.9979	0.8661	Anomalous transport

5.5.9 Statistical analysis

5.5.9.1 ANOVA for diffusion rate (DR)

ANOVA for response surface quadratic model was generated by software. Below is the ANOVA table for diffusion rate.

Table 5.15: Result of analysis of variance for measured response diffusion rate

Source	Sum of squares	df	Mean square	F Value	p-value Prob > F
Model	1.218E-04	5	2.435E-05	27.15	0.0106
A-Drug: Polymer	5.340E-05	1	5.340E-05	59.54	0.0045
B-PMMA:PEO	6.337E-05	1	6.337E-05	70.66	0.0035
AB	1.823E-06	1	1.823E-06	2.03	0.2493
A^2	3.125E-06	1	3.125E-06	3.48	0.1588
B^2	4.500E-08	1	4.500E-08	0.050	0.8372
Residual	2.691E-06	3	8.969E-07		
C or Total	1.245E-04	8			

The Model F-value of 27.15 implies the model is significant. There is only a 1.06% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case A and B are significant model terms. The polynomial equation derived from the coefficients of estimate in term of coded factor is

DR = $0.026-2.983E-03A+3.250E-03B+6.750E-04AB-1.250E-03A^2+1.500E-04B^2$ [5.13]

$R^2 = 0.9784$

Prob > F less than 0.05 indicate that drug : Polymer ratio and PMMA : PEO ratio has significant effect on diffusion rate of Betaxolol HCl. Following Figure (Figure 5.8) shows the effect of significant interaction terms on diffusion rate.

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. Response surface 3D plot shows that as amount of polymer increased, DR was reduced due to increased diffusion path at high amount of polymer whereas increase in PEO amount in insert resulted in increase in DR because PMMA retards water penetration and so diffusion of molecules through the film.

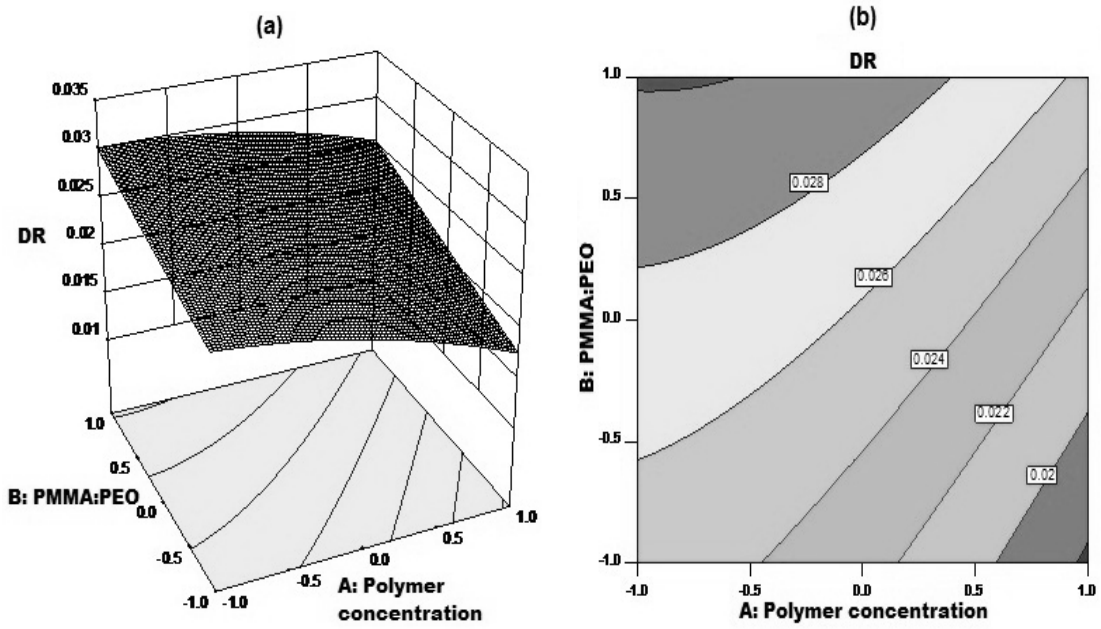

Figure 5.8: (a) Response surface plot (3D) and (b) contour plot showing the effect of drug to polymer ratio and PMMA to PEO ratio on the diffusion rate (DR)

Same observations were also indicated by counter plot. Contour plot shows that at high level of Polymer amount, PMMA:PEO has more significant effect compared to low level of polymer amount. It also shows that increasing amount of PEO has significant effect on DR at high amount of polymer.

It is well known that the addition of hydrophilic component to an insoluble film former leads to enhanced release rate constant. This may be due to dissolution of the aqueous soluble fraction of the film, which leads to creation of pores and decrease of mean diffusion path length of the drug molecule to be released[(53)].

5.5.9.2 ANOVA for flux (J)

ANOVA for response surface quadratic model was generated by software. Below is the ANOVA table for Flux.

Table 5.16: Result of analysis of variance for measured response flux

Source	Sum of squares	df	Mean square	F Value	p-value Prob > F
Model	3.903E-005	5	7.806E-00	26.92	0.0107
A-Drug:Polymer	1.698E-005	1	1.698E-005	58.58	0.0046
B-PMMA:PEO	2.039E-005	1	2.039E-005	70.35	0.0036
AB	5.999E-007	1	5.999E-007	2.07	0.2459
A^2	1.042E-006	1	1.042E-006	3.59	0.1543
B^2	1.018E-008	1	1.018E-008	0.035	0.8633
Residual	8.697E-007	3	2.899E-007		
C or Total	3.990E-005	8			

The Model F-value of 26.92 implies the model is significant. There is only a 1.07% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case A and B are significant model terms. The polynomial equation derived from the coefficients of estimate in term of coded factor is:

Flux=$0.015-1.682E-03A+1.844E-03B+3.872E-04AB-7.217E-04A^2+7.133E-05B^2$ [5.14]

$R^2 = 0.9782$

From the value of Prob>F, it has found that amount of polymer and polymer ratio had significant effect on steady state flux. It was also noticed that PMMA:PEO had more significant effect than drug:polymer ratio. Figure 5.9 shows the effect of significant interaction terms on flux (J).

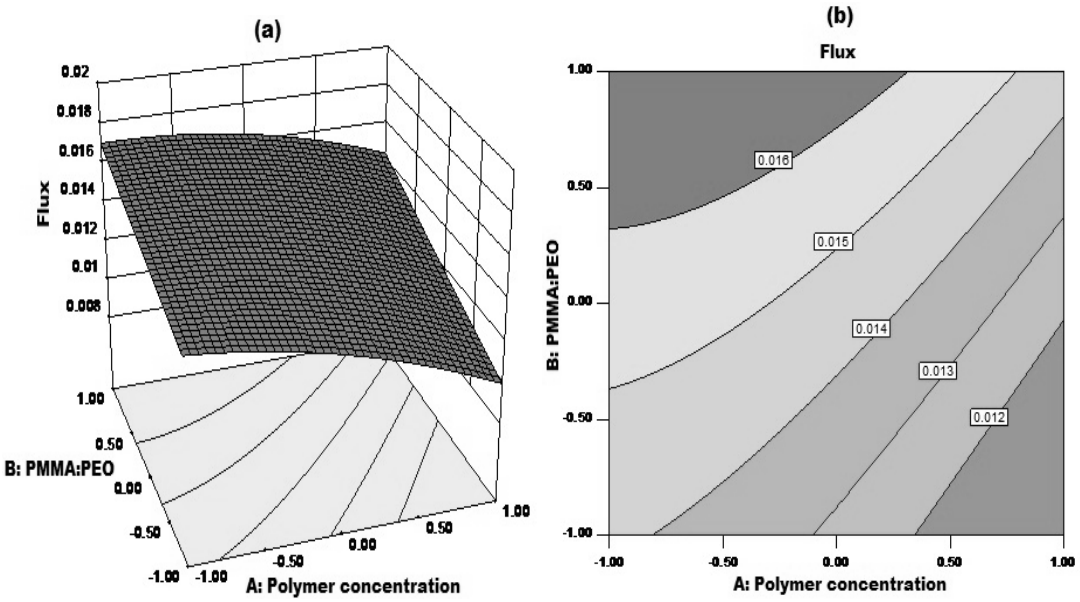

Figure 5.9: (a) Response surface plot (3D) and (b) contour plot showing the effect of drug to polymer ratio and PMMA to PEO ratio on the flux

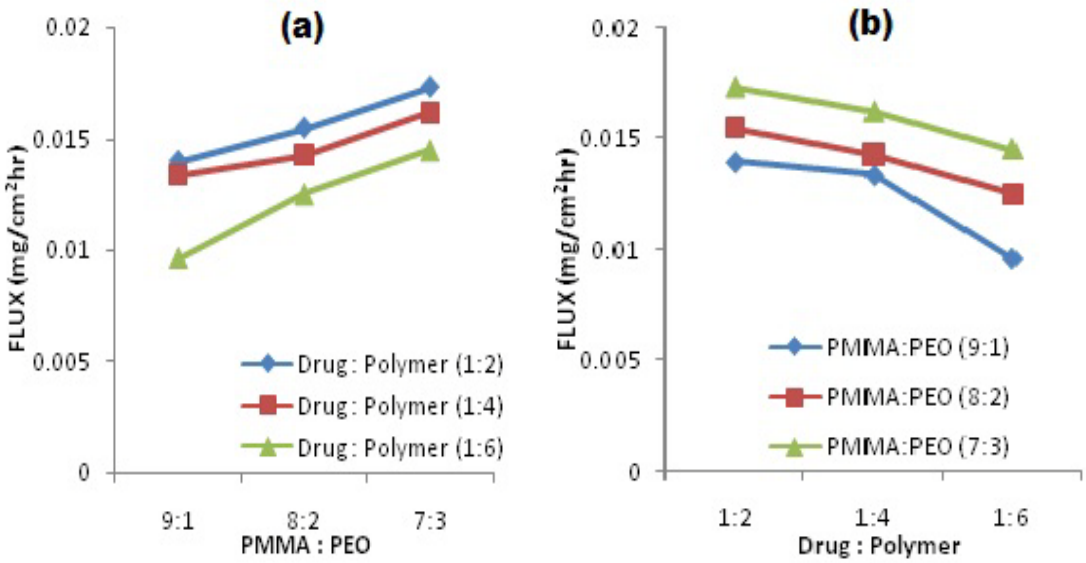

Figure 5.10: (a) Effect of drug to polymer ratio and (b) Effect of PMMA to PEO ratio on the measured response flux

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. Response surface 3D plot shows the interaction effect of independent variable on flux. It shows that increasing polymer concentration retards drug permeation so flux value was decreased. Increasing concentration of PEO enhances the drug diffusion and so flux value was increased. Contour plot shows that retarding effect of PMMA was less significant at low level of drug:polymer ratio compare to high level. It shows that increasing amount of PEO has significant effect on flux at high amount of polymer. Amount of polymer had significant effect on flux when both polymers (PMMA:PEO) used in 9:1 combination.

5.5.9.3 ANOVA for Q12h (%)

ANOVA for response surface quadratic model and reduced model was generated by software. Below is the ANOVA table for percent amount released at 12 hr (Q12h).

Table 5.17: Result of analysis of variance for measured response Q12h

Source	Sum of squares	df	Mean square	F Value	p-value Prob > F
Full Model	1092.02	5	218.40	28.15	0.0100
A-Drug:Polymer	542.83	1	542.83	69.98	0.0036
B-PMMA:PEO	448.24	1	448.24	57.78	0.0047
AB	26.16	1	26.16	3.37	0.1636
A^2	74.54	1	74.54	9.61	0.0533
B^2	0.25	1	0.25	0.032	0.8703
Residual	23.27	3	7.76		
C or Total	1115.30	8			
Reduced Model	991.07	2	495.54	23.93	0.0014
A-Drug:Polymer	542.83	1	542.83	26.22	0.0022
B- PMMA:PEO	448.24	1	448.24	21.65	0.0035
Residual	124.22	6	20.70		
C or Total	1115.30	8			

The Model F-value of 28.15 implies the model is significant. There is only a 1.00% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case A and B are significant model terms. The polynomial equation derived from the coefficients of estimate in term of coded factor is

$$\textbf{Q12h} = 65.17 - 9.51\,A + 8.64B + 2.56\,AB - 6.10A^2 + 0.35B^2 \quad [5.14]$$

$R^2 = 0.9791$

For reduced model, polynomial equation is

$$\textbf{Q12h} = 61.33 - 9.51A + 8.64B \quad [5.15]$$

$R^2 = 0.8886$

Response surface 3D plot (Figure 5.11a) shows the interaction effect of independent variable on Q12h. It shows that increasing polymer concentration retards drug permeation so Q12h value was decreased. Increasing concentration of PEO enhances the drug diffusion and so Q12h value was increased. The relationship between the dependent and independent variables was further elucidated using contour plots (Figure 5.11b). It shows that retarding effect of PMMA was less significant at low level of drug:polymer ratio compare to high level. It shows that increasing amount of PEO has significant effect on Q12h at high amount of polymer.

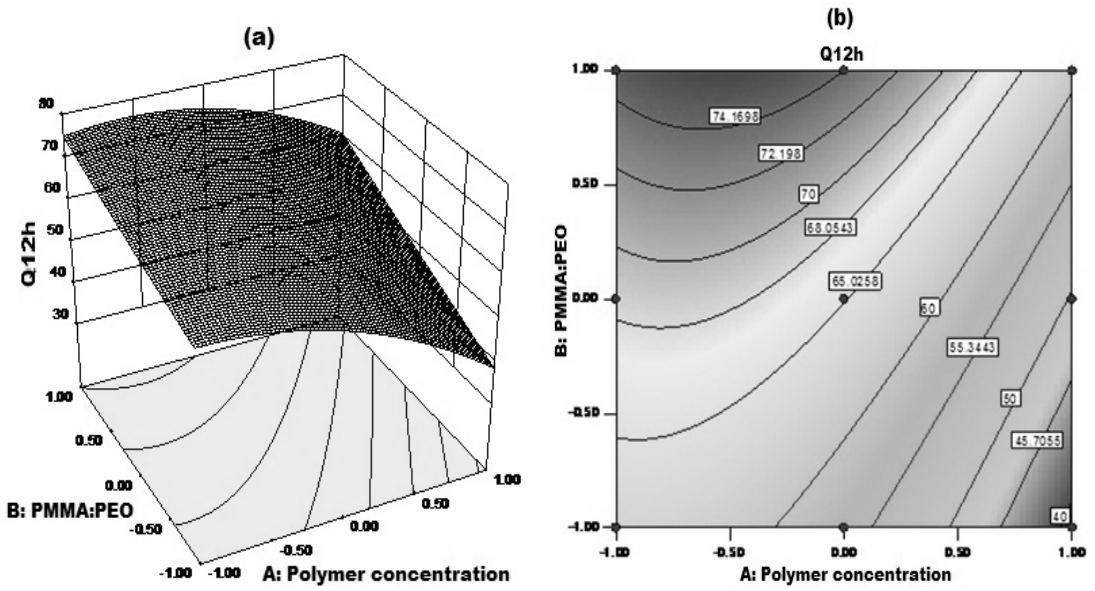

Figure 5.11: (a) Response surface plot (3D) and (b) contour plot showing the effect of drug to polymer ratio and PMMA to PEO ratio on the Q12h

5.5.9.4 Checkpoint analysis

To validate the evolved mathematical models, two checkpoints were selected. From the contour plot, two sets of A and B were selected and two batches were prepared using the same procedure keeping the other process variables constant with the amounts of A and B at the selected checkpoint. The experiment was repeated three times and experimentally obtained mean flux, DR and Q12h values were compared with predetermined (predicted) values (Table 5.18). Good correlation was found between observed and predicted values. Hence, it might be concluded that the evolved model might be used for theoretical prediction of responses within the factor space.

Table 5.18: Observed and predicted results of check point batches of inserts

Batch	A	B	Diffusion rate		Flux		Q12h (%)	
			Observed	Predicted	Observed	Predicted	Observed	Predicted
CF1	0.5	-1	0.02019	0.02036	0.01138	0.01152	46.54	47.94
CF2	-0.5	1	0.02965	0.02983	0.01653	0.01688	77.81	76.14

5.5.10 Sterility testing

The sterility testing of ocusert F8 was performed for aerobic, anaerobic bacteria and fungi by using alternate thioglycollate medium and soyabean casein digest medium as per the IP'07 procedure.

Test for aerobic bacteria: Here, *Bacillus subtilis* was used as a test organism. As shown in Table 5.19 and Figure 5.12A, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' tube. The results suggest that the ocusert F8 tested for aerobic bacteria were passed the test for sterility.

Test for anaerobic bacteria: Here, *Bacterioides vulgatus* was used as test organism. As shown in Table 5.19 and Figure 5.12B, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' tube. The results suggest that the ocusert F8 tested for anaerobic bacteria were passed the test for sterility.

Table 5.19: Sterility test observations in ATGM

Sr. no.	Samples	Days					
		1	2	3	5	7	14
1	Negative Control	-	-	-	-	-	-
2	Positive control	√	√	√	√	√	√
3	Surface Sterilized						
	10 min	-	-	√	√	√	√
	60 min	-	-	-	-	-	-

(-) Absence of microbial growth, (√) Presence of microbial growth.

Test for fungi: Here, *Candida albicans* was used as test organisms. As shown in Table 5.20 and Figure 5.12C, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' test tube. The results suggest that the ocusert F8 tested for fungi were passed the test for sterility.

The overall results of the sterility test showed that the surface sterilized ocusert F8 passed the sterility test and hence they were sterile preparations.

Table 5.20: Sterility test observations in SBCD medium

Sr. no.	Samples	Days					
		1	2	3	5	7	14
1	Negative Control	-	-	-	-	-	-
2	Positive control	√	√	√	√	√	√
3	Surface Sterilized						
	10 min	-	-	√	√	√	√
	60 min	-	-	-	-	-	-

(-) Absence of microbial growth, (√) Presence of microbial growth

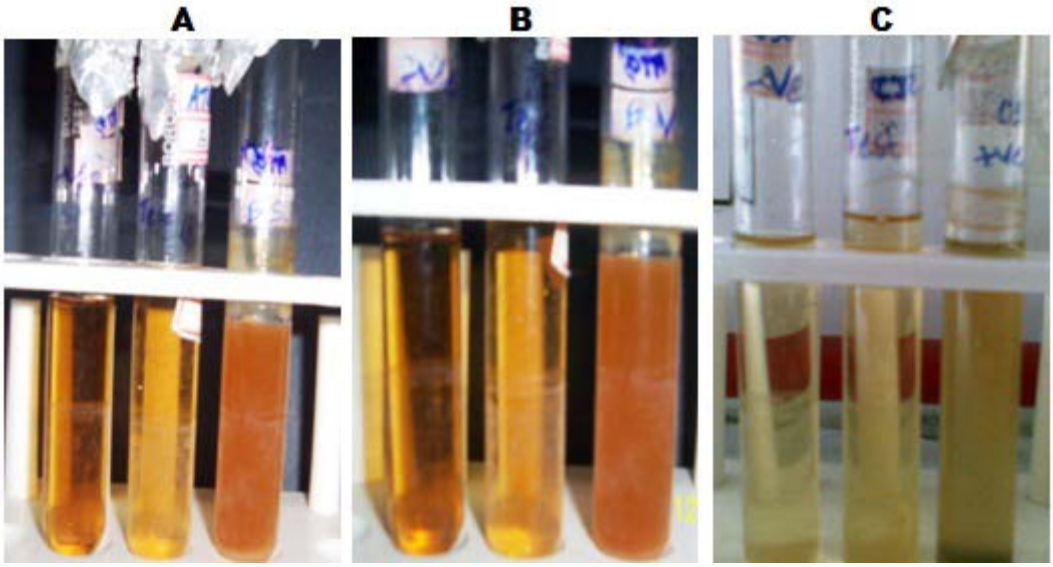

Figure 5.12: Sterility testing for ocusert F8

A–Aerobic bacteria(ATGM), B-Anaerobic bacteria (ATGM), C- Fungi (SBCDM)

5.5.11 *In vivo* studies

5.5.11.1 *In vivo* release study

The results of *in vivo* release study of the ocusert F8 is shown in Table 5.21. The ocusert showed 99.87% of drug release after 24 hr which was comparable to *in vitro* drug release (Table 5.21). Thus there was good *in vitro* – *in vivo* correlation for the ocusert F8 (Figure 5.13) indicating the effectiveness of the formulation to be used *in vivo*.

Table 5.21: Comparison of *in vivo-in vitro* drug release data

Time(h)	*In vivo* % DR	*In vitro* % DR
1	2.88	5.46
2	7.03	9.27
4	13.67	17.45
6	21.39	23.34
10	40.31	41.79
12	48.78	50.08
22	89.29	87.88
24	99.87	100.45

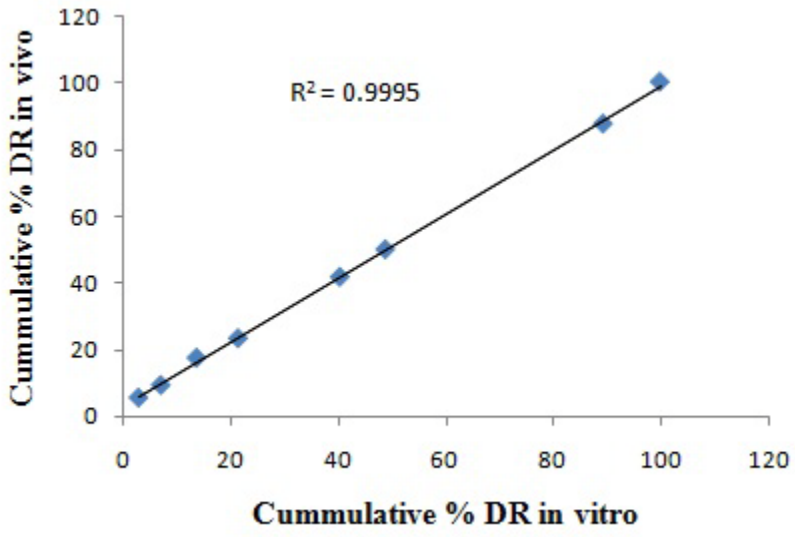

Figure 5.13: *In vitro – in vivo* correlation for formulation F8

Difference factor of 0.0004 and similarity factor of 80.29 for the *in vitro-in vivo* release data of formulation F8 indicates no significant difference between *in vitro* release and *in vivo* performance of the inserts.

5.5.11.2 Corneal residence evaluation

The precorneal residence of betaxolol after application of ocuserts and eye drops in rabbit eyes is shown in Table 5.22 and Figure 5.14.

Table 5.22: Tear fluid concentration of betaxolol

Time (h)	Tear fluid concentration (µg/mL)	
	Insert F8	Eye drop
0.1	0.75	50.14
1	3.16	12.35
2	4.38	6.85
4	5.81	2.14
6	5.18	1.24
8	4.89	0.08
10	5.12	N.D.
12	4.74	N.D.
22	3.11	N.D.
24	2.12	N.D.

N.D.- Not detectable

There was a significant improvement in precorneal residence of betaxolol after application of the formulated insert as compared to eye drops. In case of ocuserts, the levels of drug concentration in tear fluid were maintained for 24 hr while for eye drops concentration was very less after 6 hr. The increase in corneal residence may be attributed to the controlled release of drug from the ocuserts as proved by *in vivo-in vitro* release studies.

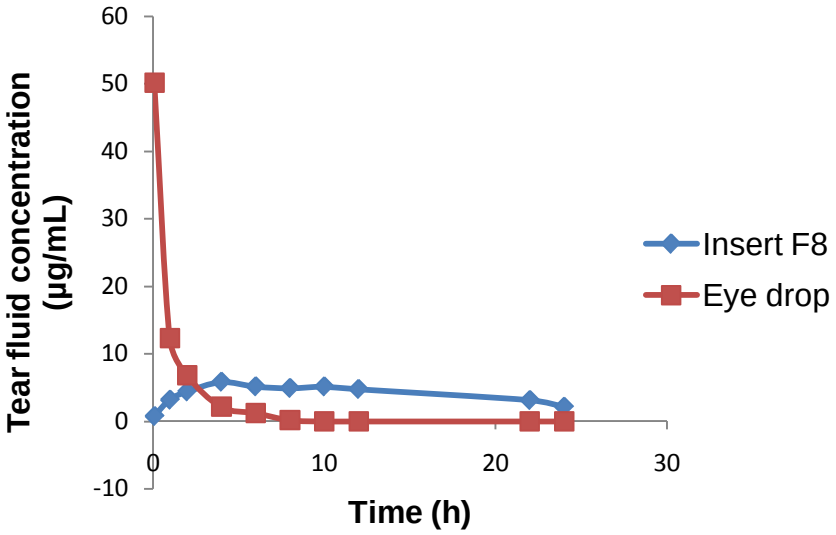

Figure 5.14: Tear fluid concentration of betaxolol

5.5.11.3 Ocular safety studies

The ocular safety study observations were recorded as per Annexure I. The ocular safety score of the formulation F8 was found to be 3 at the end of 24 hr and therefore, considered as minimally irritating[40]. This mild irritation might be due to the organic solvent used in the preparation of the ocular inserts. Thus, it can be concluded that they were safe for ocular administration.

5.5.11.4 *In vivo* antiglaucoma efficacy evaluation

In vivo antiglaucoma activity was carried out on rabbits using steroid induced glaucoma model. The data obtained was represented graphically as shown in Figure 5.15. *In vivo* IOP lowering study results revealed that in comparison to eye drops, formulation F8 showed better control of IOP up to 24 hr ($P < 0.001$). A single instillation of one drop of 0.5% betaxolol eye drops controlled IOP well to the base level up to the period of 8 hr and then after IOP was increased, while same dose of betaxolol from insert F8 controlled IOP up to

24 hr. Peak effect was observed at 4 hr and 6 hr in case of eye drops and F8 respectively. The interesting finding of the study was that IOP was also lowered effectively in control eyes of eye drop treated group, which was not the case for inserts treated group. This can be an indirect measure of systemic absorption of the drug following eye drops treatment, indicating that ocusert provides better control over systemic side effects.

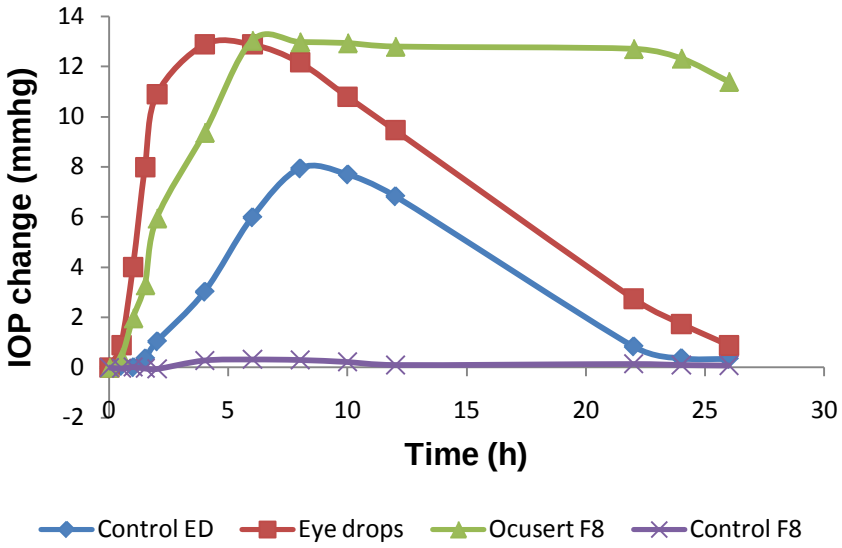

Figure 5.15: IOP lowering activity of ocusert F8 and eye drops

5.5.12 Ageing study

Ageing study of the ocusert F8 was performed at R.T., 4 ^{o}C and 40 ^{o}C for the period of 6 months. The results (Table 5.23) showed that there was no change in physical appearance of ocuserts. The drug content showed no marked change after six months.

Table 5.23: Ageing study results of ocular insert F8

Time	4 ^{o}C		R.T.		40 ^{o}C	
(days)	RDC	FE	RDC	FE	RDC	FE
0	0.504±0.016	93±2	0.505±0.016	92±3	0.503±0.016	91±2
60	0.0503±0.036	94±3.5	0.496±0.029	89±2.5	0.501±0.063	87±2.5
120	0.495±0.028	90±1.5	0.502±0.042	91±2	0.498±0.058	84±2.6
180	0.501±0.032	91±3	0.501±0.038	93±2.5	0.497±0.045	84±2.5

RDC-Remaining drug content, FE-Folding endurance

Folding endurance values were also comparable to initial values indicating good physical integrity after six months of storage even at 40 ^{o}C. All the inserts were found sterile. These results concluded that ocusert F8 was chemically, physically and microbiologically stable at RT for six months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life.

5.6 Conclusion

Attempt has been made to use blend of polymers in design of sustained ocular delivery system of Betaxolol HCl. Study was mainly focused on investigating influence of Hydrophilic (PEO) and hydrophobic (PMMA) polymers and their concentration on ocular delivery using factorial design statistically. Inserts of all batches had desired ocular physicochemical properties. Both polymers amount and their ratio had significant influence on dependent variables studied. The ratio of hydrophilic and hydrophobic polymeric film formers affected the mechanical properties, percentage moisture uptake, WVT rate, rate of drug release and consequently the permeation of the Betaxolol HCl. Due to addition of hydrophilic polymer, the surface of inserts was hydrophilic enough to be easily wetted by tear film. The blend of PEO in PMMA matrix was found to be homogenous and blend was amorphous in nature. No phase separation was observed in polymer composite as revealed from SEM, DSC and XRD studies. It was found that drug permeation was decreased with increasing polymer concentration. It was also concluded that presence of PEO in films favors the drug release and so permeation whereas PMMA retards drug release. Analysis of variance study shows that both the studied factors had significant effect on drug permeation profile. The results indicate that the polymeric film composed of PMMA and PEO at the ratio of 8:2 and dibutyl phthalate as a plasticizer was suitable for developing an ocular drug delivery system for Betaxolol HCl.

Thus the present work showed that incorporation of hydrophilic polymer into hydrophobic matrix system can be successfully done in order to model ocular inserts providing promising controlled release delivery system. The control of IOP, systemic absorption and hence possible side effects using inserts was found to be better than conventional eye drops. Thus, on the basis of *In vivo* antiglaucoma activity, ocular safety test and stability studies, it can be concluded that this betaxolol ocular insert can be a promising once-a-day controlled release formulation after due considerations of human *in vivo* studies.

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CHAPTER 6

PREPARATION AND CHARACTERIZATION OF BETAXOLOL NIOSOMES

Chapter 6 - Preparation and characterization of betaxolol niosomes

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6. PREPARATION AND CHARACTERIZATION OF BETAXOLOL NIOSOMES

6.1 Aim of present investigation

Technological innovations in drug delivery systems in recent years promise radical change in the field of pharmacotherapy. Many predict that, by the next ten years, drugs will be more specific in pharmacodynamic action, more site selective and will be administered less often and in lower quantities[1, 2]. There are many potentially effective drug delivery systems and vehicles. They differ in their drug capacities, structure, *in vivo* and storage stability, modes of administration and possible applications. Each of them has favorable attributes and limitations. Major technical challenges include drug loading, size, cost and stability. Major biological challenges include immunity, drug denaturation, targeting and toxicity.

During the last 25 years intensive investigations have been undertaken on the use of delivery systems to carry pharmaceuticals to target sites. The purpose of using these delivery systems is to convey active materials to target site. In this way the tissue is protected against the side effects of the drug and at the same time the stability of the drug can be maintained. In this regard, many micromolecular carriers such as liposomes, niosomes, pharmacosomes etc. have been designed and studied extensively to modulate biodistribution characteristic of the drug.

Ocular drug delivery is a challenge for the formulator. A drop of an aqueous solution, irrespective of instilled volume is eliminated completely from the eye within 5 to 6 minutes of its application and only a small amount (1-3%) actually penetrates the cornea and reaches the intraocular tissue[3]. Recent trend in ocular research is to formulate a dosage form which not only prolongs the residence of system in eye but also helps to reduce the elimination of the drug. In the same area many drug delivery systems including insitu gel[4], microemulsions[5], nanoparticles[6], liposome[7], niosomes[8], ocular inserts[9] etc. are being investigated worldwide by many researchers. The developmental technology of new ocular dosage forms has witnessed tremendous improvement during last few decades and its use is expected to increase in near future.

Since excessive loss of drug (betaxolol) through nasolacrimal drainage can cause respiratory and cardiovascular side effects[10], it is important to minimize the systemic absorption and enhance ocular bioavailability of drug. This problem can be addressed by use of suitable carrier systems. Niosomal vesicular system is one of the potential approaches, which can be suitably used[8].

These structures are analogous to phospholipid vesicles (liposomes) and are able to encapsulate hydrophilic and lipophilic solutes and serve as drug carriers. The low cost, higher stability, entrapping of more substances, ease of handling and storage and availability of prepared materials in pure form have led to the exploitation of these compounds as alternative to liposomes[11-14]. Niosomes, therefore, are promising drug carrier and have the potential to reduce the side effects of drugs and increased therapeutic effectiveness in various diseases. As of today more than 50 drugs are tried in niosome formulations by intravenous, per oral, transdermal, inhalation, ocular and nasal routes of administration.

Niosomes in topical ocular delivery are preferred over other vesicular systems because of the following reasons: (1) chemical stability; (2) low toxicity because of their non-ionic nature; (3) handling surfactants with no special precautions or conditions; (4) the ability to improve the performance of the drug via better availability and controlled delivery at a particular site; (5) being biodegradable, biocompatible and non-immunogenic[15].

Until now, a great deal of attention has been paid on incorporation of a great number of drugs in niosomes such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine[16].

The advantage of vesicular systems does not only reside in providing prolonged and controlled action at the corneal surface but also involves providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action. The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. In vesicular dosage forms, the drug is encapsulated in lipid vesicles, which can

cross cell membrane. Vesicles, therefore, can be viewed as drug carriers which can change the rate and extent of absorption as well as the disposition of the drug[17].

Aim of the present study was to develop a suitable niosomal preparation of betaxolol with an optimal ocular pharmacodynamics extended over a prolonged period, and a limited systemic absorption and side effects. Hence this investigation has been designed to study the drug release kinetics of betaxolol from niosomal formulations.

It was our objective to develop niosomes that:

1. Release the drug for prolonged period of time.
2. Provide an increased ocular residence time resulting in prolonged drug delivery.
3. Delivers a drug at a controlled rate over a period of time.
4. shows better in-vivo performances than conventional dosage forms.

In context to above intention, following criteria were aimed to achieve:

1. Niosomes should be discrete and spherical.
3. Drug entrapment should be more than 50 %.
3. More than 90 % of drug should be released within 24 hr.

Based on the aforementioned reasons, the purpose of the current study was to prepare betaxolol encapsulated niosomes possessing a high drug loading capacity in order to be used as ophthalmic carriers for topical ocular glaucoma treatment.

6.2 Experimental

6.2.1 Preliminary screening

Preliminary study was carried out for screening of various nonionic surfactants.

6.2.1.1 Selection of surfactants

Surfactants were selected from among Span 20, 40, 60, 80 and Tween 60, 80 based on the size of niosomes, shape of niosomes and appearance of the niosomal suspension.

Table 6.1: Formulation compositions for preliminary screening of surfactants for niosomes of betaxolol

Trial	Cholesterol	Span				Tween		PEO (mg)
		20	40	60	80	60	80	
1	1	2						-
2	1	2						10
3	1		2					-
4	1		2					10
5	1			2				-
6	1			2				10
7	1				2			-
8	1				2			10
9	1					2		-
10	1					2		10
11	1						2	-
12	1						2	10

Table 6.2: Physical evaluation of test batches of niosomes

Trial	Appearance	Shape of niosomes	Size of niosomes
1	Transparent	Not formed properly	NA
2	Transparent gel	Not formed properly	NA
3	White creamy Sus	Spherical	Medium
4	Translucent gel	Spherical	Medium to Large
5	White creamy Sus	Spherical	Medium
6	Translucent gel	Spherical	Medium to Large
7	White creamy gel	Spherical	Large
8	Translucent gel	Spherical	Large
9	White creamy Sus	Spherical	Medium
10	Translucent gel	Spherical	Medium to Large
11	Transparent	Not formed properly	NA
12	Transparent gel	Not formed properly	NA

For screening the surfactants, a formulation study was carried out. Above Table 6.2 shows some of the important formulations screened for actual study. Formulations were prepared using surfactant and cholesterol with or without PEO. It was found that niosomes were not obtained in case of span 20 and tween 80 whereas span 60 and tween 60 gave the niosomes that were good in appearance, shape and size. Considering above result it was decided to prepared the ocular niosomes by using the span 60 and tween 60.

6.2.1.2 Drug-excipients compatibility study

Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug and excipients. The drug, 1:1 physical mixture of cholesterol/drug/span/tween (each 10 mg) was mixed with 400 mg of potassium bromide. About 100 mg of this mixture was compressed to form a pellet using a hydraulic press at 10 tones pressure. Pellets were scanned in the range of 4000-400 cm^{-1} in FTIR spectrophotometer (FT/IR-4100 type A spectrophotometer, Jasco, Japan). The IR spectrum of physical mixtures was compared with that of pure drug to detect any appearance or disappearance of peaks.

6.2.2 Design of experiment

A 2-factor 3-level full factorial design (3^2) was used for the formulation and optimization of niosomes by studying the influence of the two independent variables on the responses (Y1 and Y2) percentage drug entrapment (PDE) and vesicle size (Mean volume diameter - MVD). This design is suitable for exploring quadratic response surface and constructing second order polynomial models. Values of selected variables at different levels, after measuring the responses either simple linear ($Y= X_0 + X_1A + X_2B$) or interactive ($Y= X_0 + X_1A + X_2B + X_5AB$) or quadratic ($Y = X_0 + X_1A + X_2B + X_3A^2 + X_4B^2 + X_5AB + E$) models can be evolved by carrying out multiple regression analysis of the data and F statistics to identify statistically significant terms. The reduced equation, an equation containing only statistically significant terms is then used for drawing contour plots to see the influence of selected variables when changing from low to high level. The non linear quadratic model generated by the design in the form[18]

$$Y = X_0 + X_1A + X_2B + X_3A^2 + X_4B^2 + X_5AB + E \quad [6.1]$$

Where, Y is the measured response associated with each factor level combination: X_0 is an intercept: X_1 - X_5 are the regression coefficient: A, B are the factor studied and E is the associated error term. The independent factors used in the design are listed in Table 6.3 and Table 6.4 shows applied 3^2 full factorial design.

Table 6.3: Variables and their levels in full factorial design

Independent variables	Levels		
	Low	Medium	High
A=molar ratio of Span 40/60:cholesterol	2:1	1:1	1:2
B=amount of PEO	0	5	10
Transformed values	-1	0	1
Dependent variables			
Y1 = Percentage drug entrapment (PDE)			
Y2 = Vesicle size (MVD)			

Table 6.4: A 3^2 Full factorial design layout of betaxolol niosomes

Run	Factor A molar ratio of Surfactant:cholesterol		Factor B Amount of PEO (mg)	
	Coded	Actual	Coded	Actual
1	-1	2:1	-1	0
2	-1	2:1	0	5
3	-1	2:1	1	10
4	0	1:1	-1	0
5	0	1:1	0	5
6	0	1:1	1	10
7	1	1:2	-1	0
8	1	1:2	0	5
9	1	1:2	1	10

6.2.3 Preparation of niosomes by thin film hydration method

Niosomes were prepared by thin film hydration method as reported earlier.[19] Span 60/tween 60 and cholesterol in different molar ratios of 1:2, 1:1, and 2:1 were dissolved in 10 mL of chloroform in a round bottom flask to get 1.5 mmol of lipid concentration. The flask was then attached to a rotary flash evaporator (EIE-R, Ahmedabad, India) and the temperature of water bath was maintained at 60 °C. The flask was rotated at 150 rpm for 2 h. The combination of heat and vacuum evaporated chloroform and resulted in the formation of a thin film. The film was kept overnight in vacuum desiccator for the removal of traces of chloroform. Accurately weighed, 100 mg of betaxolol hydrochloride was dissolved in 10 mL of phosphate-buffered saline solution (PBS, pH 7.4) and sterilized through 0.22 µm membrane filter. The drug solution was added to the flask containing the film and was rotated at 150 rpm for another 1 h to peel off the surfactant/cholesterol film. The hydration of film led to the formation of vesicles.

The niosomal suspension was left to mature overnight at 4 °C. For sterility, all the above mentioned steps were done under aseptic conditions. All glasswares were sterilized by autoclaving (121 °C for 15 min), phosphate buffered saline was passed through a 0.22 µm membrane filter, and the entire procedure was carried out in a laminar flow hood (Esco, Singapore).

Formulations were sonicated three times in a bath-sonicator (Spincotech, India) for 2 min with 5-min interval between successive times.

The formulation was lyophilized at a surfactant:cryoprotectant (sucrose) ratio of 1:2.5 after standard pre-freezing at −20 °C.

6.3 Evaluation and statistical analysis

6.3.1 Appearance and morphology

6.3.1.1 Visual observation

Sedimentation, flocculation and turbidity were visually observed and reported as degrees of sedimentation using the criteria used by junyapraset[20]. The experiment was performed in triplicate.

6.3.1.2 Optical microscopy

The size and shape of vesicles in nonsonicated formulations were observed by optical microscopy[19] using a calibrated eyepiece micrometer, and photographs were taken at ×400 magnification with a digital camera (Olympus, 8.1 megapixel, Tokyo, Japan). A small droplet of the vesicle suspension was placed on a glass microscope slide, diluted with a few drops of distilled water and covered with a glass cover slip. The samples were examined for vesicle formation, crystal formation and vesicular size and shape.

6.3.1.3 Characterization of sonicated vesicles by transmission electron microscopy (TEM)

Morphology analysis of niosomes was carried out using transmission electron microscopy. A drop of the niosome colloidal suspension was placed onto a carbon-coated copper grid and left for 1 min thus allowing niosomes to adhere to the grid. The excess of the niosome suspension was then drawn off by a piece of filter paper. A drop of negative stain solution, 1% (w/v) phosphotungstic acid solution, was placed on the carbon grid thus staining the niosomes. After 3 min, the excess staining agent was removed by adsorbing the drop with the tip of a filter paper and the sample was then air-dried. The grid was allowed to air dry thoroughly and then examined using a transmission electron microscope[11].

6.3.2 Determination of un-entrapped and entrapped drug

Betaxolol niosomal formulations were centrifuged at 15,700 rpm for 90 min at 4 °C using centrifuge (Remi Cooling Centrifuge, Mumbai, India). Concentration of the free drug in the supernatant was determined by measuring absorbance at 274 nm with a UV spectrophotometer (Shimadzu, UV 1800 PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated[21]. This process was repeated thrice to ensure that free drug was completely removed.

$$\%drug\ entrapment = \frac{total\ drug - drug\ in\ supernatant}{total\ drug} \times 100 \qquad [6.2]$$

Percent drug entrapment was confirmed by lysing the niosomes. The vesicles were separated from the supernatant, washed with PBS solution (2×5 mL), and centrifuged for another hour. The drug entrapment efficiency of isolated

vesicles was determined by a slight modification of the technique reported earlier[19]. The amount of entrapped drug was estimated by lysis of the vesicles. Few drops of chloroform were added to the centrifuge tube to disrupt the isolated vesicles. Certain volume of PBS solution was added to extract the entrapped drug. Simultaneously, the tube was maintained at a temperature of 60 °C to evaporate the organic solvent. The PBS solution containing entrapped drug was filtered and analyzed for drug content with a spectrophotometer. The drug entrapment efficiency was calculated by the following equation:

$$\text{Entrapment efficiency } (\%) = \frac{\text{amount of drug entrapped(mg)}}{\text{amount of drug added(mg)}} \times 100 \qquad [6.3]$$

6.3.3 pH measurement

The pH of niosomes was measured by a pH meter (Accumet® basic model AB15, Fisher Scientific, Pennsylvania, USA). The pH measurement was performed at 25 °C.

6.3.4 Viscosity measurement

The viscosity of all formulations was determined by Brookfield viscometer (Model DV-II+Pro, Middleboro, USA) at 25 °C[22]. The spindle CPE 41 was rotated at different angular velocity of 2 rpm. Each determination was carried out in triplicate.

6.3.5 Zeta potential measurement

Zeta potential of suitably diluted niosome dispersion was determined using zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method using Zetasizer (Malvern instruments). The temperature was set at 25 °C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements were obtained directly from the measurement.

6.3.6 Particle size measurement

The z-average diameter of sonicated vesicles was determined by dynamic light scattering[23] using a Zetasizer. For the measurement, 100 µl of the formulation was diluted with an appropriate volume of PBS, pH 7.4 and the vesicle diameter and polydispersity index were determined. The measurement was taken repeated 3 times for each sample.

6.3.7 *In vitro* drug release study [24]

We fabricated an open flow through assembly, simulating the condition of the ocular cavity, by using the classical standard cylindrical tube which has the diameter of 15 mm. Dialysis membrane (Dialysis membrane 110, mw cut-off 12000-14000, Hi Media, India), immersed in water for 1 h to remove the preservatives followed by rinsing in phosphate buffered saline (PBS) solution, acted as corneal epithelium, was tied to one end of open cylinder which acted as donor compartment. Niosomes containing entrapped betaxolol obtained after centrifugation of 2 mL of the formulation were resuspended in 1 mL of PBS (pH 7.4), and used for the release study. The niosome preparation or free betaxolol solution was placed inside this compartment. Then, the glass tube was suspended in the dissolution flask of a USP dissolution apparatus such that entire surface of the membrane was in contact with the receptor compartment containing 250 mL of STF (pH 7.4). The content of the receptor compartment was stirred continuously at 25 rpm. Samples of 1 mL were withdrawn from the receptor compartment at periodic intervals and replaced by equal volume of fresh solution. The samples were analyzed spectrophotometrically at 274 nm against reference standard using STF as blank.

Results were the mean values of three runs. The mechanism of drug release from niosomal formulations was determined using the following mathematical models: zero-order kinetics, first-order kinetics, Higuchi kinetics, and the Korsmeyer-Peppas model[25-27].

6.3.8 Statistical analysis

The selected responses obtained from the various systems were tested for significant differences. Statistical analysis of data was carried out using analysis of variance (ANOVA). The individual response was evaluated using F-test and F value and P value were generated. The statistical analysis was conducted using Design-Expert® version 8.0.2 trial (Stat-Ease, Inc., Minneapolis MN).

The following responses were selected for statistical analysis

- Entrapment efficiency (PDE)
- Vesicle Size (MVD)

6.3.9 Physical state of drug

6.3.9.1 Differential scanning calorimetry (DSC) analysis

DSC thermograms of pure drug and drug loaded niosomes were recorded using DSC 1/700 (Mettler Toledo, Germany) in an inert atmosphere of nitrogen (150 mL/min). The analysis was performed with a heating range of -25 ^{o}C to 250 °C and at a rate of 10°C per minute. The sample weight was approximately 6 mg.

6.3.9.2 X-Ray diffraction analysis

X-Ray diffraction (XRD) patterns of pure betaxolol and ocular film were recorded using a powder X-Ray diffractometer (XRD-D8 Advance, Bruker, Germany) over the interval 0-40° $2\theta^{-1}$. The experimental conditions were: generator tension (voltage) of 38 kV, generator current of 34 mA, scan step time of 30.6 sec^{-1} and scan step size of 0.049° $2\theta^{-1}$.

6.3.10 Sterility testing [28]

Sterility is one of the most vital requirements for an ophthalmic preparation. The tests for sterility are intended for detecting the presence of viable forms of microorganisms in ophthalmic preparations. The principle governing these tests is that if the microorganisms are placed in a medium which provides nutritive material and water, kept at a favourable temperature, the organisms will grow and their presence can be indicated by turbidity in the originally clear medium. In the present study, two media namely, alternate thioglycolate medium (ATGM) and soyabean-casein digest medium (SBCD) were used to investigate the presence/absence of aerobic, anaerobic bacteria and fungi, in the formulated sterilized ocular inserts.

6.3.10.1 Preparation of culture medium

a) ATGM was used to detect the growth of aerobic and anaerobic bacteria. 7.25 gms of readymade ATGM was dissolved in 250 mL of purified water and the pH was adjusted to 7.1±0.2 with 1M NaOH. This was sterilized in an autoclave at 115 ^{o}C for 30 min. The medium was freshly prepared and allowed to cool just prior to use.
b) SBCD medium was used to detect the growth of aerobic bacteria and fungi. 7.25 gms of readymade SBCD was dissolved in 250 mL of purified water and the pH was adjusted to 7.1±0.2 with 1M NaOH. This was sterilized in

an autoclave at 115 ^{o}C for 30 min. The medium was freshly prepared and allowed to cool just prior to use.

6.3.10.2 Test procedure

Direct inoculation method was used. Here specified amount of niosome suspension was mixed with both medium and incubated. Simultaneously, positive and negative controls were also prepared and all the three tubes were incubated at specified temperatures.

6.3.11 *In vivo* studies

New Zealand rabbits of either sex weighing 2.5 to 3.1 kg were used for *in vivo* studies. The rabbits were housed singly in restraining cages during the experiment and allowed food and water *ad libitum*. Free lag and eye movement was allowed. The animals were housed at controlled temperature (25±2 °C), and humidity (60±5%), with a 12/12-h light-dark cycle. Institutional Animal Ethics Committee (IAEC) (Registration no. 1338/c/CPCSEA) approved the pharmacodynamic study. The animal experiment was conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations, as per the spirit of ethics committee.

6.3.11.1 Ocular safety study [29, 30]

The ocular safety of administered delivery system can be tested based on the Draize Irritancy Test (as discussed in section 5.4.7.3). The observations based on scoring approach were used to establish the safety of the developed ocular inserts in rabbit eye. Three healthy albino rabbits (2.7–2.9 kg) were selected to evaluate the ocular safety of the optimized niosomal formulations. For feeding, conventional laboratory diets were used with an unrestricted supply of drinking water. A single dose of 100-µL niosomal formulation was instilled into the conjunctival sac of left eye of each animal (initially to one animal) and the untreated eye served as a control. Each of the animals was observed visually with a slit lamp for the severity of ocular reactions such as corneal ulceration, iritis, conjunctival redness, and conjunctival edema at various intervals of 1, 24, 48, and 72 hr. The animal experiment was conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations, as per the spirit of ethics committee. This study was approved by IAEC (Registration no. 1338/c/CPCSEA).

6.3.11.2 *In vivo* anti-glaucoma activity evaluation [31, 32]

i) Steroid induced model [33]

Eyes of the 12 rabbits were treated 3 times daily by 0.5% dexamethasone eye drops for 2 weeks. Dexamethasone sodium phosphate (Taj Pharmaceuticals Ltd., Mumbai) was dissolved in phosphate buffer to a final concentration of 0.5 % at pH 7.4. The intraocular pressure was measured at noon twice a week by Schiotz tonometer.

ii) Antiglaucoma activity measurement on rabbit

The optimized vesicular formulation was tested for its intraocular pressure lowering activity on albino rabbits (2.5–3.1 kg) and the results were compared to that of a marketed betaxolol solution (0.5%). The dispersions of niosomes, NS5 and NT6 were adjusted to a concentration of 0.5% betaxolol. The animals were divided into three groups (n=4). Group I received formulation NS5, Group II administered with formulation NT6 and Group III was treated with the marketed eye drops formulation. The IOP was measured at different intervals with a standardized tonometer (ShiØtz, Germany). A single 50-µl dose of 0.5% betaxolol preparation was instilled onto the corneal surface of rabbit's left eye; [32] then after 30 min, and subsequently every 1 h interval, the IOP was measured for a period up to 24 hr. The right eye was left as a control in all the experimental animals. The ocular pressure lowering activity was expressed similarly to that reported by Winum and his associates [34] as the average difference in IOP between the treated and control eye.

6.3.12 Ageing Study [20]

The prepared formulations were tested for stability by storing them at 4±1 °C, RT and at 40±2 °C. Formulations were assessed for vesicular fusion, pH variation, sterility testing, vesicles size and shape and number of vesicles per cubic millimeter before and after storage for 180 days. Residual drug content was also assessed every 60th day. Size of the vesicular systems was determined by dynamic light scattering method. The shape of the vesicle was observed under light microscope. The number of vesicles per cubic millimeter was calculated by method described elsewhere [35] using a hemocytometer by applying the following formula.

$$\text{Number of niosomes per mm}^3 = \frac{\text{Total number of niosomes} \times \text{dilution} \times 400}{\text{Total number of small squares counted}} \qquad [6.4]$$

6.4 Results and discussion

In the present study niosomes of Betaxolol HCl were prepared using surfactants (Span 60 and Tween 60). A 3^2 full factorial design was employed and so nine formulations were obtained for each surfactant. Niosomes were evaluated for their use as ocular delivery systems with a view to obtain sustained release.

6.4.1 Drug-excipients compatibility study

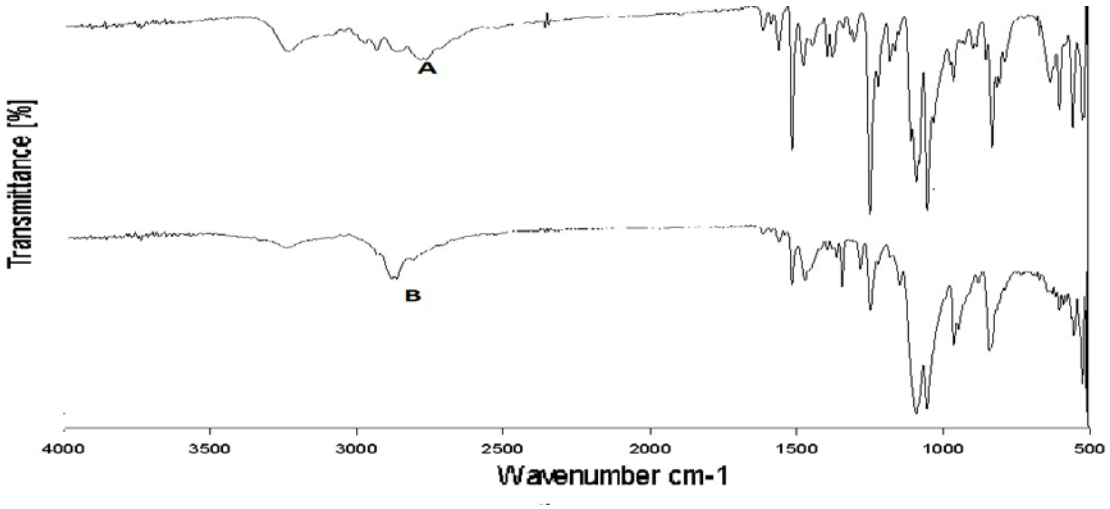

Figure 6.1: IR Spectra of (A) Betaxolol HCL (B) Mixture of BX/span60/tween60/cholesterol

Interpretation of IR spectrum of Betaxolol (Figure 6.1A) shows characteristic peaks at 3237 cm^{-1} (hydroxyl group O-H stretching), 2928 cm^{-1} (methyl C-H stretching), 2858 cm^{-1} (methylene C-H stretching), 1612 cm^{-1} (aromatic ring C=C-C stretching), 1557 cm^{-1} and 1513 cm^{-1} (secondary amine N-H bending), 1474 cm^{-1} (methylene C-H bending), 1376 cm^{-1} (methyl C-H sym. bending), 1246 cm^{-1} (alkyl aryl ether c-o-c stretching), 1179 cm^{-1} (aromatic in plane C-H bending), 1087 cm^{-1} (aliphatic ether c-o-c stretching) and 1050 (amine C-N stretching). FTIR spectrum of mixture (Figure 6.1B), in comparison to IR spectrum of pure drug, shows no substantial shifting of the position of the functional groups, indicating no major interaction between drug and polymers.

6.4.2 Appearance and morphology

All niosomes appeared as translucent white dispersions without sedimentation, indicating that the niosomes were physically stable due to small and uniform vesicle sizes obtained after the sonication process. The formulations with an addition of the membrane additive-PEO were more turbid and whitish.

The photomicrographs (×40) of betaxolol hydrochloride niosomes before sonication are shown in Figures 6.2a, 6.2b and 6.2c. Niosomes were spherical large unilamellar vesicles. It is also clear that the vesicles containing more amount of PEO are larger in size than those having fewer amounts or no PEO indicating that inclusion of PEO may have increased the internal space of niosomes, which is coherent to the entrapment efficiency results.

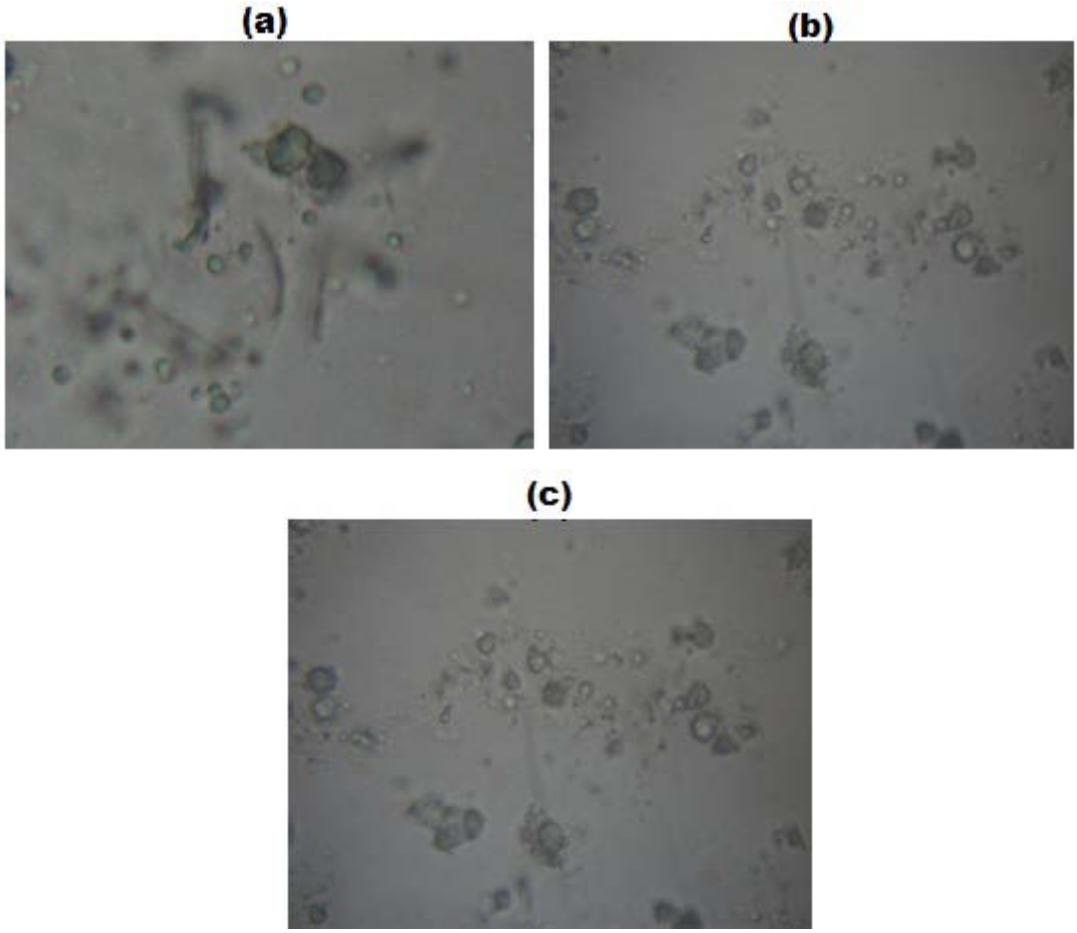

Figure 6.2: Photomicrographs of Betaxolol niosomes before sonication (a) without PEO-NS4 (b) with 5 mg PEO-NS5 (c) with 10 mg PEO-NS6

Similar results were observed in other formulations (data not shown). Under the optical microscope, aggregation/fusion of the vesicles could be occasionally observed before size reduction as depicted in Figure 6.2. After sonication process, nonaggregated niosomes were obtained. Unfortunately, information concerning microstructure of niosomes could not be visualized by the low magnification power of optical microscope (lower of detection ~200 nm); therefore TEM was employed to elucidate niosome morphology.

Negative stain transmission electron micrographs of betaxolol niosomes (NS4 and NS5) are shown in Figure 6.3a and 6.3b respectively. It is demonstrated that the vesicles are well identified and present in a nearly perfect sphere-like shape having a large internal aqueous space.

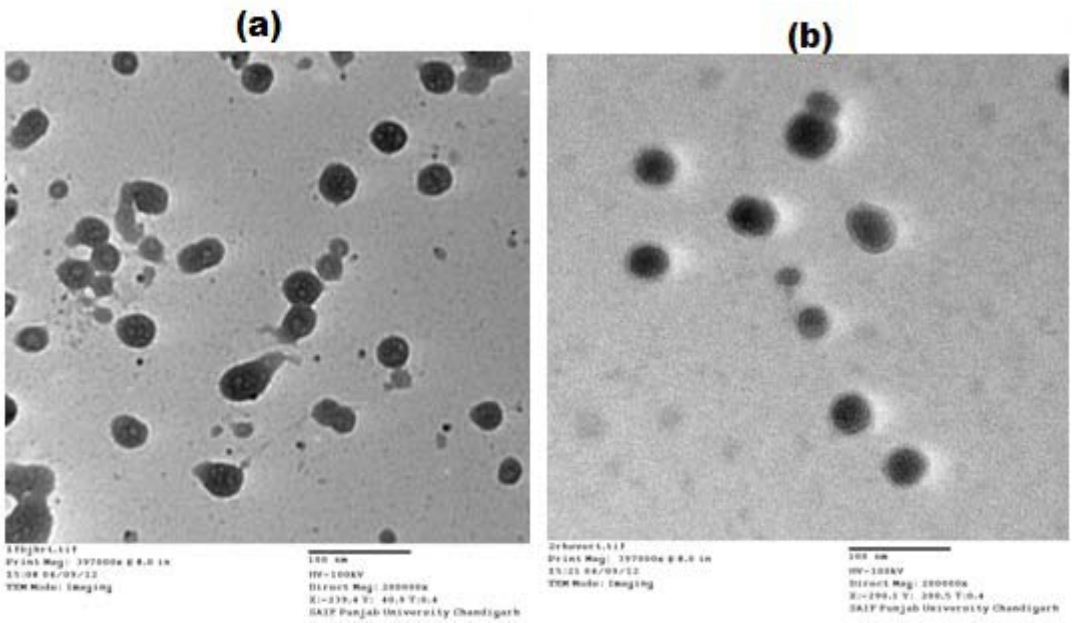

Figure 6.3: TEM images of (a) Niosome NS4 (b) Niosome NS5

Moreover, by comparison of both the structures, it could be concluded that NS4 niosomes has less internal aqueous volume than NS5 and the structure of NS4 is like polygonal/irregular while that of NS5 is like perfect sphere. Aggregation was seen in NS4 niosomes while NS5 niosomes remained as separate entity. This may be attributed to the addition of small amount of PEO which acted as membrane stabilizer with probable mechanism of steric hindrance.

6.4.3 Optimization (PDE and MVD)

The vesicles were characterized for drug entrapment efficiency, size, zeta potential and polydispersity index.

It was observed that increase in surfactant/cholesterol ratio from 2:1 to 1:1 increased the drug entrapment efficiency from 36.21% (NT1) to 43.84% (NT4) in case of tween niosomes; however, above the ratio of 1:1, the drug entrapment efficiency of the vesicles decreased to 33.52% for NT7 (Table 6.5). Similar results were observed in case of span niosomes also. Such differences in drug entrapment efficiencies as well as drug content were found to be statistically significant ($p<0.05$). Guinedi and his co-workers[36] reported that an increase in span 60/ cholesterol ratio from 7:4 to 7:6 increased acetazolamide entrapment efficiency from 21.48% to 32.21%; however, the same was decreased to 21.36% with further increase in Span 60/cholesterol ratio to 7:7. However, cholesterol content beyond a certain extent starts disrupting the regular bilayer structure leading to lower drug entrapment efficiency[37].

Table 6.5: Characterization of span60 niosomes of betaxolol

Batch Code	A	B	PDE±SD*	Vesicle size		Zeta potential	PDI
				Before SC(µm)	After SC(nm)*		
NS1	2:1	0	30.12±0.36	<2	43.21±1.21	-33.1±0.3	0.411
NS2	2:1	5	36.64±1.02	3.2	102.7±2.62	-24.7±0.7	0.254
NS3	2:1	10	34.82±0.52	3.4	117.27±1.38	-17.8±0.3	0.268
NS4	1:1	0	38.42±1.62	<2	60.84±1.48	-34.9±1.2	0.428
NS5	1:1	5	51.64±1.14	3.6	124.21±3.21	-21.2±0.8	0.385
NS6	1:1	10	48.63±0.73	3.7	134.82±0.98	-18.8±0.6	0.208
NS7	1:2	0	29.24±1.53	<2	49.32±0.84	-31.6±1.1	0.276
NS8	1:2	5	37.54±0.68	<2	108.65±1.25	-20.4±0.4	0.218
NS9	1:2	10	36.64±2.15	3.12	118.87±1.15	-16.5±0.8	0.352

* Average ± SD of three determinations has been reported

Table 6.6: Characterization of tween60 niosomes of betaxolol

Batch Code	A	B	PDE±SD*	Vesicle size		Zeta potential	PDI
				Before SC(µm)	After SC(nm)*		
NT1	2:1	0	36.21±0.48	<2	68.65±0.84	-35.3±1.3	0.398
NT2	2:1	5	48.42±1.24	3.3	92.38±2.38	-24.2±1.1	0.224
NT3	2:1	10	47.94±0.84	3.2	115.87±2.78	-13.6±0.6	0.262
NT4	1:1	0	43.84±2.12	<2	77.37±1.83	-39.2±0.8	0.408
NT5	1:1	5	57.28±1.82	3.5	108.57±2.34	-26.4±1.2	0.415
NT6	1:1	10	58.48±0.75	3.7	121.96±2.38	-15.7±0.5	0.213
NT7	1:2	0	33.52±2.15	<2	66.82±1.24	-29.6±1.4	0.292
NT8	1:2	5	42.63±0.38	<2	95.52±2.35	-19.2±0.8	0.253
NT9	1:2	10	41.97±1.12	3.2	118.21±3.15	-14.1±1.1	0.418

* Average ± SD of three determinations has been reported

Maximum and minimum PDE observed in niosomes were 29.24% and 51.64% in span niosomes and 58.58% and 33.52% in tween niosomes respectively. This shows wide variation, which indicates that dependent variable is strongly affected by the independent variables selected for the study which was further confirmed by statistical optimisation using ANOVA.

6.4.4 Statistical analysis

6.4.4.1 ANOVA for percentile drug entrapment of span 60 niosomes

ANOVA for response surface quadratic model was generated by software. Below is the ANOVA table for PDE of niosomes of Span 60.

The Model F-value of 22.01 implies the model is significant. There is only a 1.43% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case B, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The polynomial equation derived from the coefficients of estimate in term of coded factor is

$$\textbf{PDE} = 49.98 - 0.31A + 3.72B - 0.67AB - 12.07A^2 - 5.63B^2 \quad [6.5]$$

The value of correlation coefficient $\mathbf{R^2 = 0.9735}$ indicating the good fit of the model.

Table 6.7: Result of ANOVA for measured response PDE of span niosomes

Source	Sum of squares	df	Mean square	F value	p-value Prob > F
Full Model	439.93	5	87.99	22.01	0.0143
A-Span 60: cholesterol Molar Ratio	0.57	1	0.57	0.14	0.7307
B-Amount of PEO	83.03	1	83.03	20.77	0.0198
AB	1.81	1	1.81	0.45	0.5493
A^2	291.13	1	291.13	72.84	0.0034
B^2	63.39	1	63.39	15.86	0.0283
Residual	11.99	3	4.00		
Cor Total	451.92	8			
Reduced Model	437.55	3	145.85	50.75	0.0004
B-Amount of PEO	83.03	1	83.03	28.89	0.0030
A^2	291.13	1	291.13	101.30	0.0002
B^2	63.39	1	63.39	22.06	0.0054
Residual	14.37	5	2.87		
Cor Total	451.92	8			

Equation 6.5 represents the effects of individual and combined variables on PDE of betaxolol niosomes. Small values of coefficients of A and AB (having p >0.05) in equation 6.5 are regarded as the least contributing factors in the entrapment of betaxolol in the niosomes. Positive value of B factor indicates that entrapment of drug increases with increase in the PEO amount. Negative signs of coefficients of A terms indicate low to medium level of cholesterol favors the increased PDE of BX niosomes. When the coefficient values of two independent key variables (A and B) compared, the value of variable B (3.72) was found to be maximum and hence amount of PEO was considered to be a major contributing variables for PDE of BX niosomes.

Prob > F less than 0.05 indicate that surfactant:cholesterol ratio and amount of PEO has significant effect on entrapment efficiency of Betaxolol HCl.

Following graph (Figure 6.4) shows the effect of significant interaction terms on PDE.

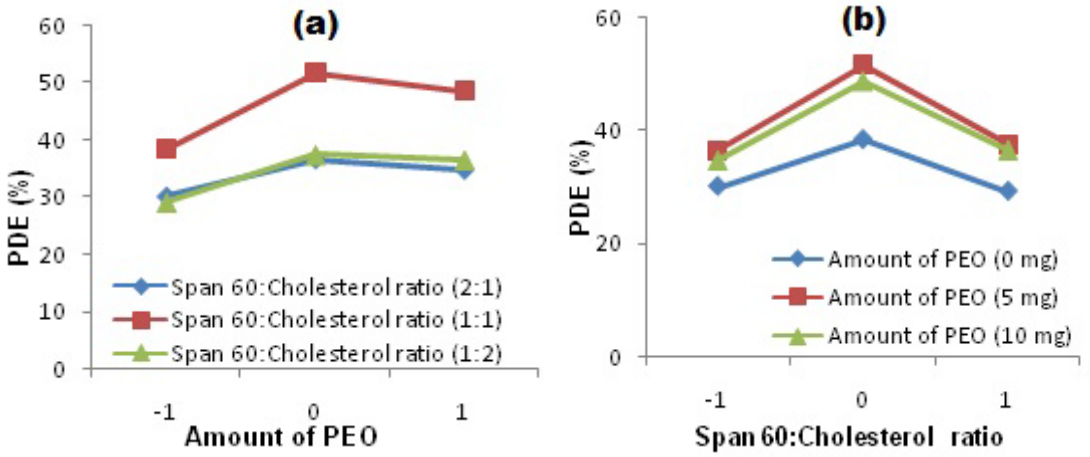

Figure 6.4: Effect of (a) amount of PEO and (b) span60:cholesterol ratio on PDE of betaxolol niosomes

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots (Figure 6.5). Response surface 3D plot shows that as amount of polymer increased, PDE was increased. This may probably be due to increased internal aqueous volume with high amt of polymer.

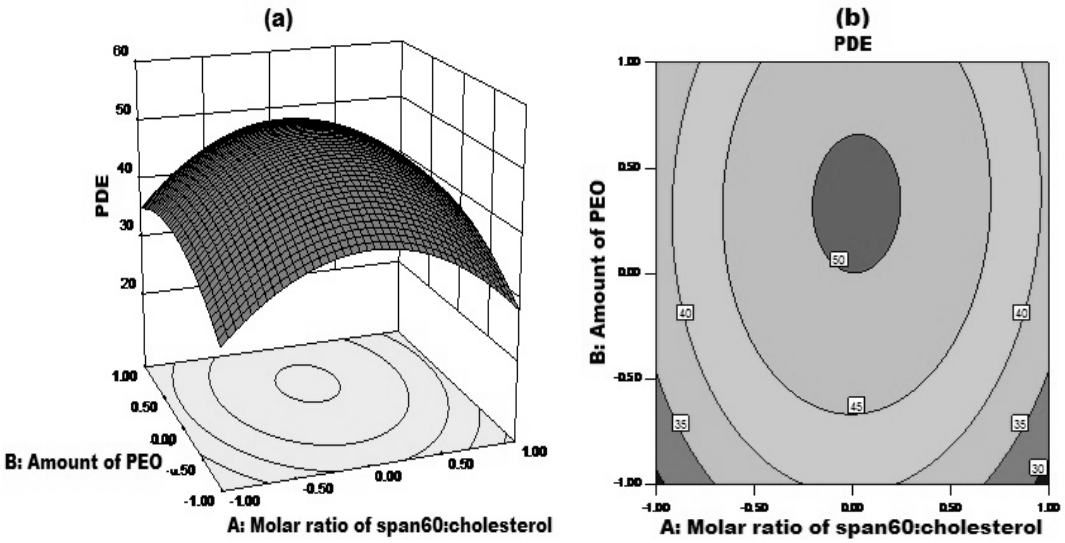

Figure 6.5: (a) Response surface plot (3D) and (b) Contour plot showing the effect of molar ratio of span60: cholesterol and amount of PEO on the percentage drug entrapment (PDE)

Same observations were also indicated by counter plot. Contour plot shows that at medium span60: cholesterol ratio, amount of PEO has more significant effect compared to low level and high level of ratio. It also shows that increasing ratio level has significant effect on PDE at high amount of PEO. It is well known that increase in cholesterol content in vesicles increases the entrapment of drug by increasing the rigidity of the membrane and hence reducing the leakage of the drug from it.

6.4.4.2 ANOVA for percentile drug entrapment (PDE) of tween 60 niosomes

ANOVA for response surface quadratic model was generated by software. Below is the ANOVA table for PDE.

Table 6.8: Result of ANOVA for response PDE of tween niosomes

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Full Model	567.21	5	113.44	44.06	0.0052
A-Tween 60: cholesterol Molar Ratio	34.80	1	34.80	13.52	0.0348
B-Amount of PEO	202.07	1	202.07	78.48	0.0030
AB	2.69	1	2.69	1.04	0.3820
A^2	260.76	1	260.76	101.27	0.0021
B^2	66.89	1	66.89	25.98	0.0146
Residual	7.72	3	2.57		
C or Total	574.94	8			
Reduced Model	564.52	4	141.13	54.21	0.0010
A-Tween 60: cholesterol Molar Ratio	34.80	1	34.80	13.37	0.0217
B-Amount of PEO	202.07	1	202.07	77.61	0.0009
A^2	260.76	1	260.76	100.15	0.0006
B^2	66.89	1	66.89	25.69	0.0071
Residual	10.41	4	2.60		
C or Total	574.94	8			

The Model F-value of 44.06 implies the model is significant. There is only a 0.52% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant.

In this case A, B, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The polynomial equation derived from the coefficients of estimate in term of coded factor is

$$\textbf{PDE} = 57.06 - 2.41A + 5.80B - 0.82AB - 11.42A^2 - 5.78B^2 \quad [6.6]$$

$$\textbf{PDE} = 57.06 - 2.41A + 5.80B - 11.42A^2 - 5.78B^2 \quad [6.7]$$

The values of correlation coefficient R^2 = 0.9866 for full model and 0.9836 for reduced model indicate the good fit of the model. Equation 6.6 represents the effects of individual and combined variables on PDE of betaxolol niosomes. Small values of coefficients of AB (having p >0.05) in equation 6.6 are regarded as the least contributing factors in the entrapment of betaxolol in the niosomes. Positive value of B factor indicates that entrapment of drug increases with increase in the PEO amount. Negative signs of coefficient of A term indicate low to medium level of cholesterol favors the increased PDE of BX niosomes. When the coefficient values of two independent key variables (A and B) compared, the value of variable B (5.80) was found to be maximum and hence amount of PEO was considered to be a major contributing variables for PDE of BX niosomes.

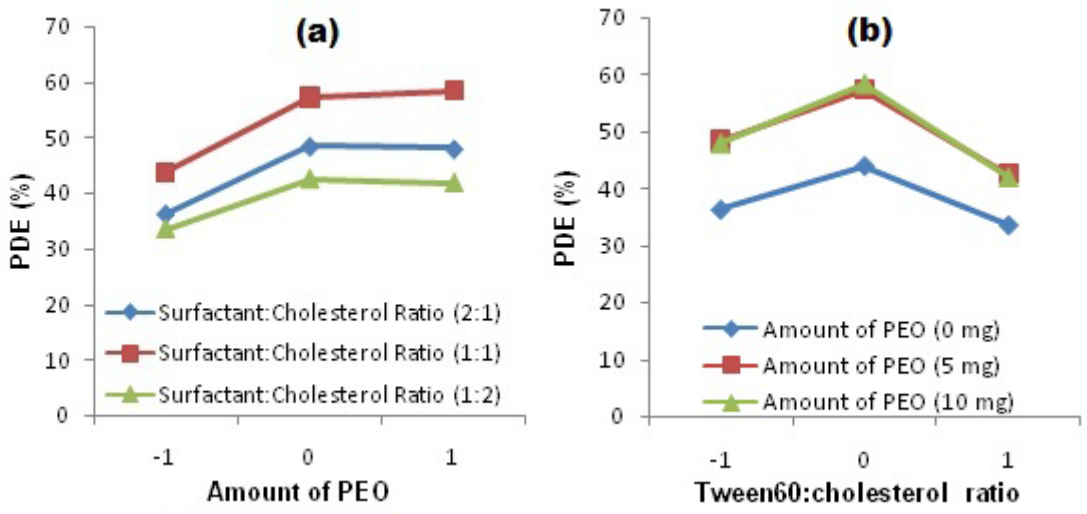

Figure 6.6: Effect of (a) amount of PEO and (b) tween60:cholesterol ratio on PDE of betaxolol niosomes

Prob > F less than 0.05 indicate that surfactant:cholesterol ratio and amount of PEO has significant effect on entrapment efficiency of Betaxolol HCl. The graph (Figure 6.6) shows the effect of significant interaction terms on PDE.

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots (Figure 6.7). Response surface 3D plot shows that as amount of polymer increased, PDE was increased. This may probably be due to increased internal aqueous volume with high amt of polymer. Same observations were also indicated by counter plot. Contour plot shows that at medium span60: cholesterol ratio, amount of PEO has more significant effect compared to low level and high level of ratio. It also shows that increasing ratio level has significant effect on PDE at high amount of PEO. It is well known that increase in cholesterol content in vesicles increases the entrapment of drug by increasing the rigidity of the membrane and hence reducing the leakage of the drug from it.

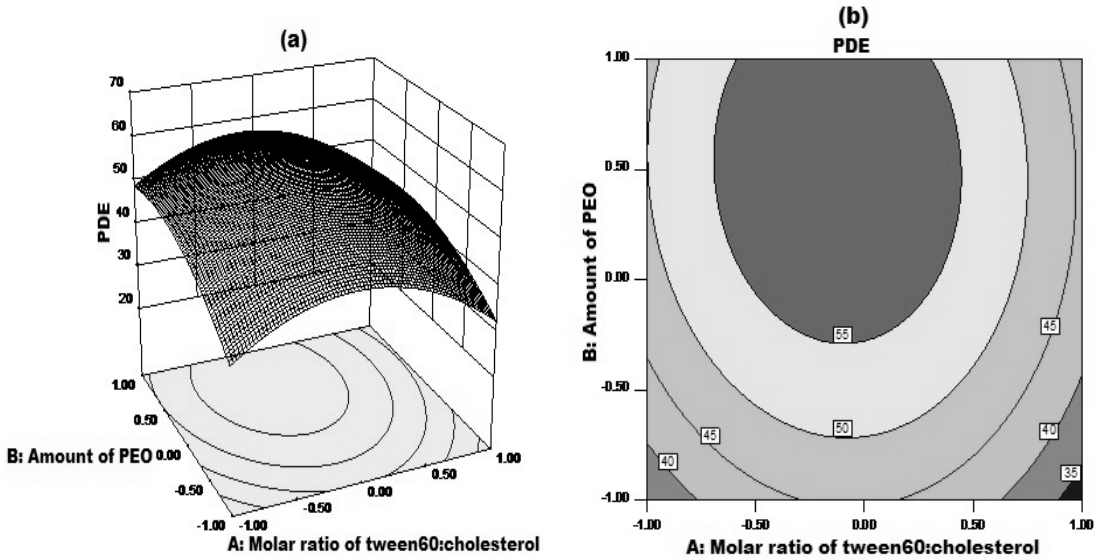

Figure 6.7: (a) Response surface plot (3D) and (b) Contour plot showing the effect of molar ratio of tween60: cholesterol and amount of PEO on the percentage drug entrapment (PDE)

6.4.4.3 ANOVA for mean volume diameter (MVD) of span 60 niosomes

ANOVA for response surface quadratic model was generated by software. Below is the ANOVA table for MVD.

Table 6.9: Result of ANOVA for response MVD of span niosomes

Source	Sum of squares	df	Mean square	F Value	p-value Prob > F
Model	9676.61	5	1935.32	865.64	< 0.0001
A-Span60:chol ratio	31.10	1	31.10	13.91	0.0336
B- Amount of PEO	7890.90	1	7890.90	3529.49	< 0.0001
AB	5.09	1	5.09	2.27	0.2286
A^2	552.45	1	552.45	247.10	0.0006
B^2	1197.07	1	1197.07	535.43	0.0002
Residual	6.71	3	2.24		
Cor Total	9683.31	8			

The Model F-value of 865.64 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case A, B, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The polynomial equation derived from the coefficients of estimate in term of coded factor is:

$$\textbf{MVD} = 122.93 + 2.28\,A + 36.26B - 1.13AB - 16.62\,A^2 - 24.46B^2 \quad [6.8]$$

The value of correlation coefficient R^2 = 0. 0.9993 indicating the good fit of the model.

Equation 6.8 represents the effects of individual and combined variables on PDE of betaxolol niosomes. Small values of coefficients of AB (having p >0.05) in equation 6.8 are regarded as the least contributing factor affectind MVD. Positive value of A and B factor indicates that vesicle size increases with increase in the the cholesterol as well as PEO amount. When the coefficient values of two independent key variables (A and B) compared, the value of variable B (36.26) was found to be maximum and hence amount of

PEO was considered to be a major contributing variables for vesicle size of BX niosomes (Figure 6.8).

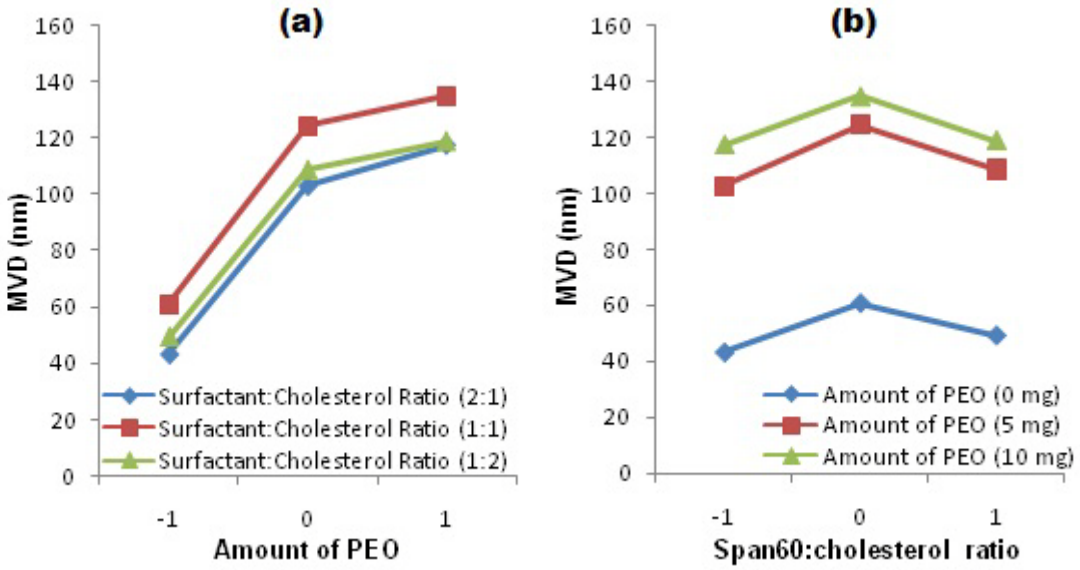

Figure 6.8: Effect of (a) amount of PEO and (b) span60:cholesterol ratio on MVD of betaxolol niosomes

Prob > F less than 0.05 indicate that surfactant:cholesterol ratio and amount of PEO has significant effect on entrapment efficiency of Betaxolol HCl. The graph (Figure 6.8) shows the effect of significant interaction terms on PDE.

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots (Figure 6.9. Response surface 3D plot shows that as amount of polymer increased, MVD was increased. This may probably be due to increased internal aqueous volume with entrapment of high amt of polymer and drug. Same observations were also indicated by counter plot. Contour plot shows that at medium span60: cholesterol ratio, amount of PEO has more significant effect compared to low level and high level of ratio. It also shows that increasing ratio level has significant effect on PDE at high amount of PEO. It is well known that increase in cholesterol content in vesicles increases the entrapment of drug by increasing the rigidity of the membrane and hence reducing the leakage of the drug from it.

Kapadia et al.[38] reported that increase in Span 60/cholesterol ratio from 7:4 to 7:6 increased the size of acyclovir-loaded vesicles from 3.69 to 3.76 µm; however at ratio of 7:7, the same was found to decrease to 3.73 µm.

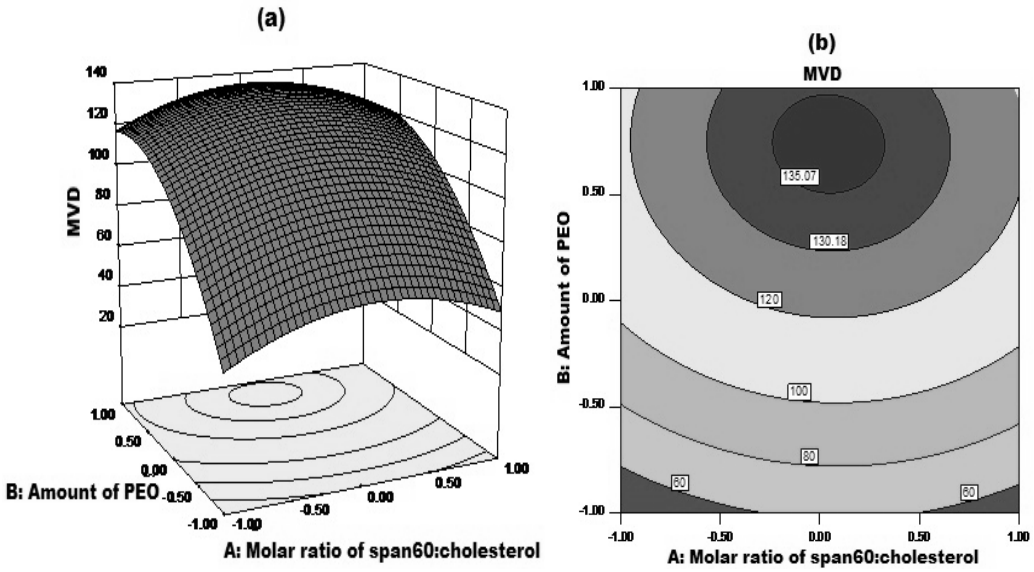

Figure 6.9: (a) Response surface plot (3D) and (b) Contour plot showing the effect of span60:cholesterol ratio and amount of PEO on the MVD

In this study, we noticed that the size of nanovesicles was gradually increased with the increase in cholesterol content. It has long been realized that incorporation of more cholesterol into the vesicles would yield larger particles because of the reduction in fluidity of the bilayer. This would enhance the rigidity of bilayer membrane above the phase transition temperature, resulting in an increased elastic modulus, which inhibited curving of the bilayer[39, 40]. The larger size may also contribute to the higher drug entrapment efficiency of the vesicles[36, 41].

6.4.4.4 ANOVA for mean volume diameter (MVD) of tween 60 niosomes

ANOVA for response surface quadratic model was generated by software. Below is the ANOVA table for MVD.

The Model F-value of 64.06 implies the model is significant. There is only a 0.30% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case B and A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Table 6.10: Result of ANOVA for response MVD of tween niosomes

Source	Sum of squares	df	Mean square	F Value	p-value Prob > F
Model	3645.59	5	729.12	64.06	0.0030
A-Tween60:chol ratio	2.22	1	2.22	0.20	0.6886
B- Amount of PEO	3417.71	1	3417.71	300.27	0.0004
AB	435	1	4.35	0.38	0.5803
A^2	189.15	1	189.15	16.62	0.0266
B^2	32.16	1	32.16	2.83	0.1914
Residual	34.15	3	11.38		
Cor Total	3679.73	8			

The polynomial equation derived from the coefficients of estimate in term of coded factor is:

$$\textbf{MVD} = 105.31 + 0.61\,A + 23.87B + 1.04AB - 9.73\,A^2 - 4.01B^2 \quad [6.9]$$

The value of correlation coefficient $R^2 = 0.9907$ indicating the good fit of the model.

Equation 6.9 represents the effects of individual and combined variables on MVD of betaxolol niosomes. Small values of coefficients of A, AB and B2 (having p >0.05) in equation 6.9 are regarded as the least contributing factor affecting MVD. Positive value of A and B factor indicates that vesicle size increases with increase in the the cholesterol as well as PEO amount. When the coefficient values of two independent key variables (A and B) compared, the value of variable B (23.87) was found to be maximum and hence amount of PEO was considered to be a major contributing variables for vesicle size of BX niosomes (Figure 6.10).

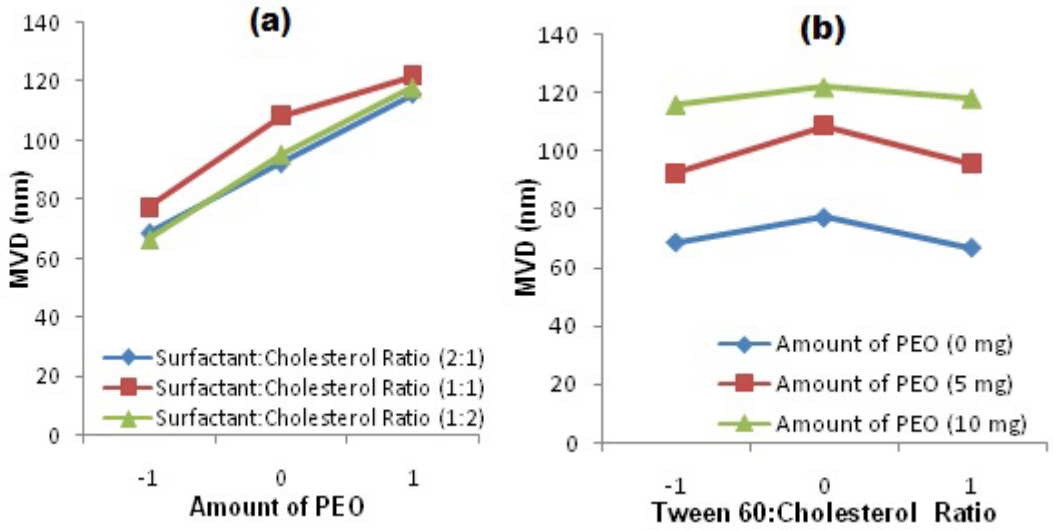

Figure 6.10: Effect of (a) amount of PEO and (b) tween60:cholesterol ratio on MVD of betaxolol niosomes

Prob > F less than 0.05 indicate that surfactant:cholesterol ratio and amount of PEO has significant effect on entrapment efficiency of Betaxolol HCl. The graph (Figure 6.10) shows the effect of significant interaction terms on PDE.

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots (Figure 6.11). Response surface 3D plot shows that as amount of polymer increased, MVD was increased. This may probably be due to increased internal aqueous volume with entrapment of high amt of polymer and drug.

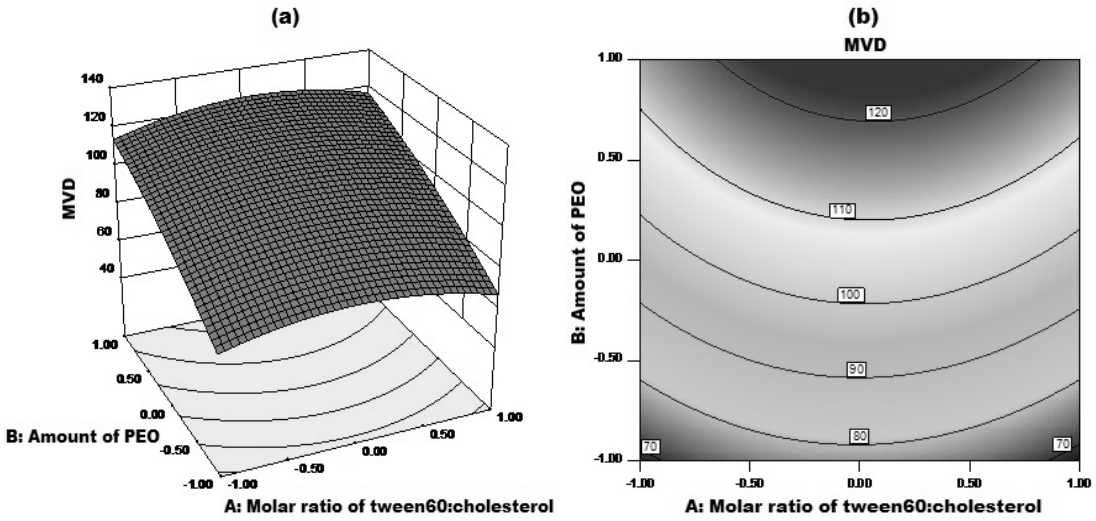

Figure 6.11: (a) Response surface plot (3D) and (b) Contour plot showing the effect of tween60: cholesterol ratio and amount of PEO on the MVD

Same observations were also indicated by counter plot. Contour plot shows that at medium span60: cholesterol ratio, amount of PEO has more significant effect compared to low level and high level of ratio. It also shows that increasing ratio level has significant effect on PDE at high amount of PEO. It is well known that increase in cholesterol content in vesicles increases the entrapment of drug by increasing the rigidity of the membrane and hence reducing the leakage of the drug from it.

6.4.4.5 Checkpoint analysis

To validate the evolved mathematical models, two checkpoints were selected. From the contour plot, two sets of A and B were selected and two batches were prepared using the same procedure keeping the other process variables constant with the amounts of A and B at the selected checkpoint. The experiment was repeated three times and experimentally obtained mean PDE and MVD values were compared with predetermined (predicted) values (Table 6.11). Good correlation was found between observed and predicted values. When compared with the predicted PDE and MVD using students t-test the differences were found to be insignificant (P>0.05). Hence, it might be concluded that the evolved model is valid for theoretical prediction of responses within the factor space.

Table 6.11: Observed and predicted results of checkpoint batches of betaxolol niosomes

Batch	A	B	PDE		MVD	
			Observed	Predicted	Observed	Predicted
CS1	0	0.5	49.29	50.44	113.63	116.24
CS2	1	-0.5	34.92	34.65	80.31	82.73
CT1	0.5	0	51.49	53	101.20	103.18
CT2	-0.5	0.5	57.73	56.87	115.83	112.51

CS- span niosomes, CT-tween niosomes

Considering above results, formulations NS5 and NT6 were selected as optimized formulations and subjected for further studies.

Polydispersity index is a parameter that gives an estimate of the width of distribution of the vesicles. The higher the polydispersity index, the wider is the size distribution. This parametric value was in the range of 0.208–0.428, and was found to follow bimodal intensity size distribution irrespective of the ratio of Span 60 and cholesterol. Cholesterol generally abolishes the gel to sol transition [(42)] resulting in vesicles that are less leaky[(43)]. Increase in cholesterol content results in higher microviscosity which is indicative of more rigidity of vesicular lamella[(16)].

Zeta potential values for span niosomes were within -16.5±0.8 and -34.9±1.2 and for tween niosomes between -13.6±0.6 and -39.2±0.8. This high negative zera-potential is important in preventing aggregation. It has been reported that a physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta-potential of 30mV.[(13)] The decrease in zeta potential after inclusion of PEO was due to the absorption of polyoxyethylene chain of PEO on the surface of the niosomes, leading to the shift of shear plane and subsequently reduction of zeta potential values.

The **viscosity** of ophthalmic solutions is often increased to prolong their retention in precorneal area because the rate of solution drainage decreases with increasing viscosity. Part of the viscous solution is also incorporated in the precorneal tear film and in the marginal tear strip[(44)]. It is also true that the products with a high viscosity are not well tolerated in the eye, causing lacrimation and blinking until the original viscosity of tear is regained. The drug diffusion from a viscous formulation into eye becomes difficult. Finally, the ocular administration is not so easy. A recent report indicated that the niosomal suspension having an optimal viscosity of 1.20 cps could prolong the ocular residence time, compared to solutions; and will not create lacrimation and blinking or blurred vision[(45)].

We examined that the dispersion of niosomes in a merely viscous PEO (0.05%) solution offer viscosity in the range of 0.42±0.02 and 2.19±0.08 cps (Table 6.12), respectively, with their increasing cholesterol and PEO content and therefore, the ocular niosomal formulation obtained at the intermediate ratio could be most suitable for ocular administration.

Table 6.12: pH and viscosity of betaxolol niosomes

Batch Code	pH n=3	Viscosity (cps) n=3	Batch Code	pH n=3	Viscosity (cps) n=3
NS1	7.22±0.06	0.42±0.02	NT1	7.32±0.03	0.47±0.03
NS2	7.26±0.12	1.19±0.05	NT2	7.36±0.15	1.11±0.08
NS3	7.32±0.05	1.94±0.04	NT3	7.32±0.09	1.72±0.05
NS4	7.18±0.09	0.49±0.04	NT4	7.28±0.11	0.48±0.04
NS5	7.42±0.11	1.28±0.09	NT5	7.42±0.08	1.23±0.06
NS6	7.38±0.14	2.12±0.05	NT6	7.38±0.09	2.02±0.07
NS7	7.29±0.02	0.47±0.06	NT7	7.39±0.11	0.52±0.06
NS8	7.46±0.16	1.29±0.11	NT8	7.41±0.12	1.36±0.03
NS9	7.37±0.04	2.19±0.08	NT9	7.37±0.08	2.16±0.05

The **pH values** of the prepared formulations varied between 7.18 to 7.46, indicating that the niosomal suspension did not have an irritation potential as the pH values are within the accepted ocular range[46].

6.4.5 *In vitro* drug release

In this study, niosomes of Betaxolol HCl was prepared using two different surfactants in varying ratio of surfactant to cholesterol with or without PEO. All formulation prepared were subjected to *in vitro* release study to ensure the effect of both the factors. The data obtained for *in-vitro* study were tabulated and represented graphically.

Tables 6.13 and 6.14 and Figures 6.12 and 6.13 show percentage cumulative drug release profiles for all formulations of span60 i.e. NS1 to NS9 and tween60 i.e. NT1 to NT9 respectively. All the formulations showed sustain release of betaxolol as compared to betaxolol eye drops. Batch NS6 showed lowest release (37.14%) and NS1 showed faster release (76.89%) within 12 hr. In case of tween niosomes NT7 showed faster release (79.98%) and NT6 showed 50.62% release within 12 hr.

Table 6.13: *In vitro* drug release profiles of betaxolol niosomes of span60

Time (h)	Eye drops	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9
1	22.14	9.43	7.21	5.43	6.74	5.46	2.16	4.78	3.14	3.52
2	84.53	16.2	13.41	9.45	12.45	9.27	5.92	7.98	8.25	7.26
4	100.03	26.36	22.84	17.72	21.35	17.45	9.25	16.84	15.76	14.27
6		41.28	33.24	28.43	32.18	23.34	15.24	30.65	24.33	21.62
8		52.34	44.34	39.44	41.82	32.65	22.67	41.54	35.98	30.34
10		65.87	58.45	50.15	51.84	41.79	31.24	56.87	48.73	39.26
12		76.89	67.42	62.12	62.14	50.08	37.14	65.8	59.16	47.18

Table 6.14: *In vitro* drug release profiles of betaxolol niosomes of tween60

Time (h)	Eye drop	NT1	NT2	NT3	NT4	NT5	NT6	NT7	NT8	NT9
1	22.14	11.32	9.86	7.25	9.82	7.82	6.83	11.84	10.14	7.12
2	84.53	19.54	15.25	12.24	17.26	14.35	9.16	20.14	17.28	13.18
4	100.03	29.62	26.22	21.82	26.89	20.14	14.25	31.26	28.42	21.16
6		42.18	37.88	30.46	37.12	27.32	20.64	43.14	38.26	31.84
8		52.84	46.42	39.28	48.32	34.24	25.92	54.48	49.25	42.36
10		66.72	56.28	47.28	56.46	42.73	33.25	65.24	58.82	50.22
12		75.97	64.64	55.28	65.11	50.62	42.83	79.98	67.18	61.62

Order of cumulative drug released was found in following manner

NS1> NS2 > NS7 > NS4 > NS3 > NS8 > NS5 > NS9 > NS6

NT7> NT1 > NT8 > NT4 > NT2 > NT9 > NT3 > NT5 > NT6

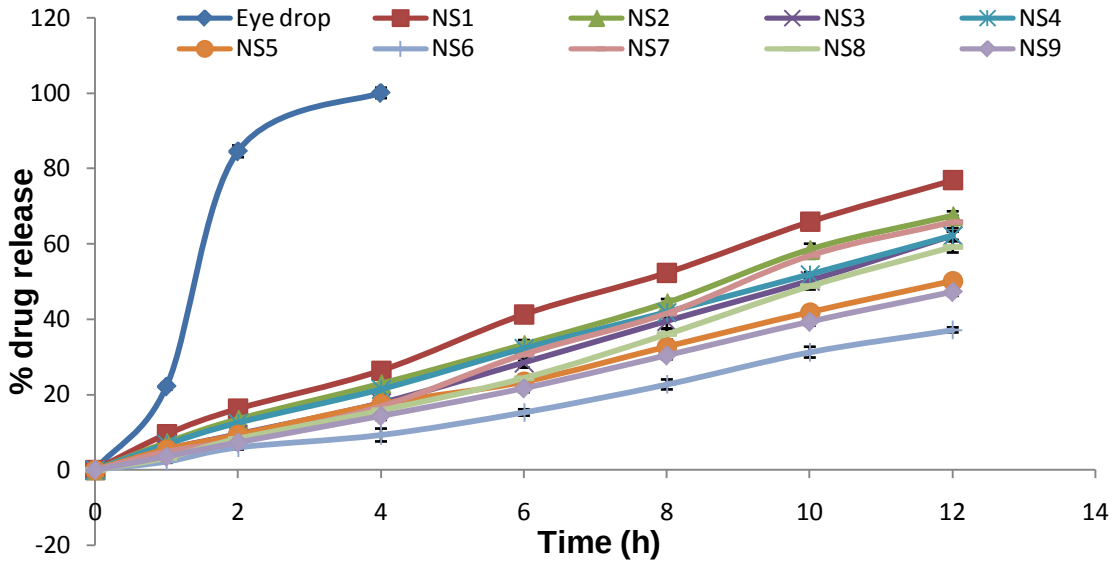

Figure 6.12: Drug release profiles of eye drop and span niosomes

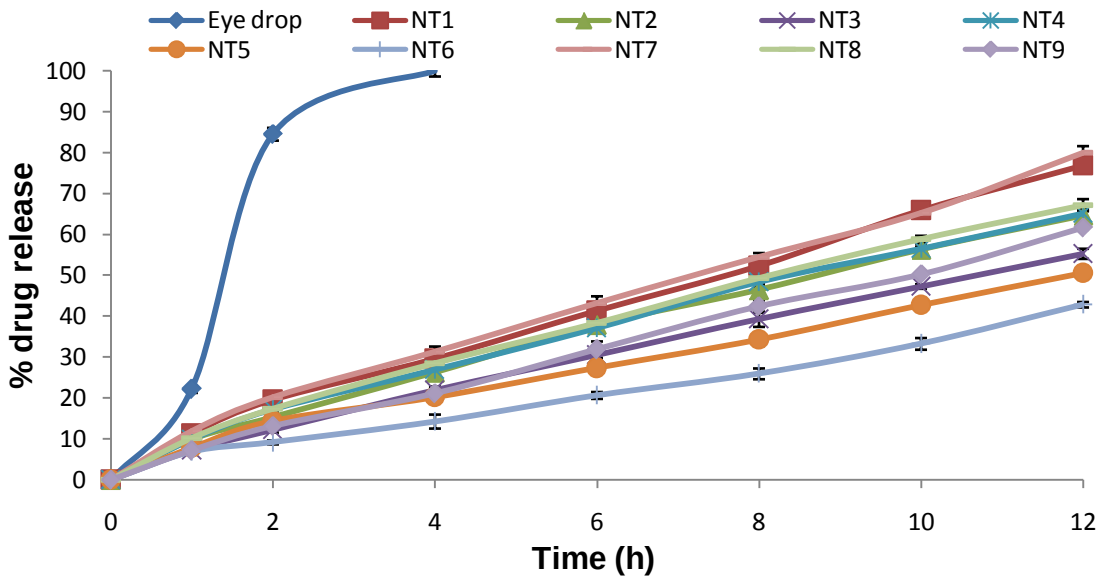

Figure 6.13: Drug release profiles of eye drop and tween niosomes

It was observed that, as the proportion of cholesterol increases, the release of betaxolol from niosomes decreases. This may be due to the fact that cholesterol increases the rigidity of the bilayer of niosomes. It also abolished the gel to liquid phase transition and promoted the formation of a less ordered liquid-crystalline state as vesicles[47]. This is a desirable feature as the leakage of content from the niosomes can be prevented[48].

In vitro drug release data also revealed that the increase in amount of PEO decreased the release of the drug from niosomes.

The time-point approach (Q_{12h}) was adopted to compare the drug release potential. The Q_{12h} values (i.e., the % cummulative amount of drug released at the end of 12 h) were 76.89%, 62.14% and 65.8% for NS1, NS4 and NS7 respectively, for the formulations with their increasing surfactant:cholesterol ratio (Table 6.13). A statistically significant difference was observed in their Q_{12h} values and, hence, in their drug-release potential ($p<0.05$). As compared to eye drop release, the vesicles continued to liberate its content comparatively at a slower rate up to 12 hr. The formulation with 1:1 ratio of span 60 and cholesterol (NS6) exhibited slower and extended drug-release profile. The trend was followed by vesicular formulation of span 60 and cholesterol in the molar ratios of 2:1 and 1:2, respectively. Similar results were found in case of tween 60 niosomes. This is in good agreement with the fact that cholesterol causes a decrease in density of head groups at the interfaces of bilayer, and an increase in the package of phospholipid tails in the middle of bilayer, thereby reducing their permeability to encapsulated compound[(49)]. Moreover, it is reported that cholesterol in phospholipid vesicular preparations could reduce the leakage of encapsulated material by decreasing the membrane fluidity[(50)]. However, cholesterol beyond 50% starts disrupting the vesicular membrane which serves as the reason for faster drug release from the vesicles.

In case of formulations with span60, it was revealed that the release kinetics of drug appeared to follow zero order release kinetics (r2>0.9881) because high correlation coefficient was observed in the zero-order plot rather than Higuchi's and first-order models (Table 6.15). This indicated that the drug release was independent on concentration of drug entrapped.

The kinetic modeling of drug-release profiles of tween60 vesicles have been represented in Table 6.16. It was revealed that the release kinetics of drug appeared to follow the mixed release kinetics of zero order (r2>0.9875) as well as Higuchi's release kinetics (r2>0.9802). However, zero-order release kinetics predominated at the medium and higher ratio of surfactant to cholesterol (Table 6.16). On the other hand, the best fit with higher correlation was found with the Higuchi's equation at low and high ratios, i.e., the drug release were proportional to square root of time, indicating that the drug release from vesicles was diffusion controlled.

Table 6.15: Kinetics modeling of release profile of span 60 niosomes containing betaxolol

Release Kinetic	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9
Zero order	0.9987	0.9974	0.9965	0.9997	0.9971	0.9881	0.9934	0.9928	0.9982
First order	0.9287	0.9263	0.9375	0.9186	0.9328	0.9101	0.9328	0.9035	0.9139
Peppas	0.9958	0.9968	0.9946	0.9986	0.9956	0.9888	0.9908	0.9964	0.9993
Higuchi	0.975	0.9672	0.9565	0.9758	0.9635	0.9401	0.9538	0.9483	0.964
n	0.849	0.895	0.990	0.889	0.891	1.109	1.101	1.155	1.042

Table 6.16: Kinetics modeling of release profile of tween 60 niosomes containing betaxolol

Release Kinetic	NT1	NT2	NT3	NT4	NT5	NT6	NT7	NT8	NT9
Zero order	0.9987	0.9971	0.9987	0.9964	0.9963	0.9875	0.9981	0.9969	0.9984
First order	0.9305	0.9194	0.9126	0.9104	0.9226	0.9757	0.9272	0.9106	0.9213
higuchi	0.9754	0.988	0.9863	0.9886	0.9734	0.9373	0.9767	0.9895	0.9743
peppa	0.9953	0.9975	0.9995	0.9984	0.9914	0.9717	0.9967	0.9995	0.9969
n	0.757	0.771	0.822	0.755	0.720	0.732	0.749	0.759	0.856

6.4.6 Differential scanning calorimetry

From the overlay of the DSC thermograms, it has been observed that Betaxolol is crystalline in nature (Figure 6.14).

Thermogram exhibited a sharp melting endotherm at an onset temperature of 112.82 ^{o}C, a peak temperature of 119.76 ^{o}C and a heat of fusion of 6.97 J/g. While the thermogram of niosomes shows crystallization of betaxolol from glass at 67.18 ^{o}C followed by fusion at 116.89 ^{o}C.

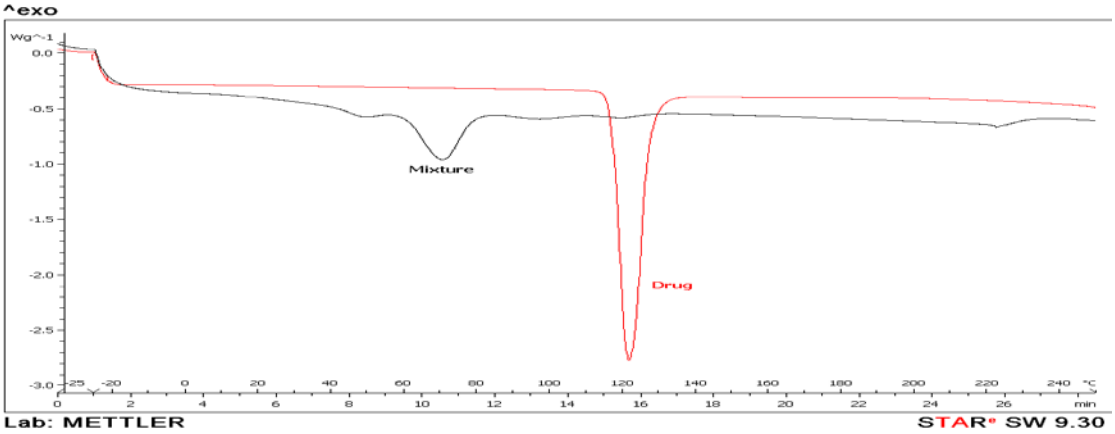

Figure 6.14: DSC spectra of pure drug and NS5(mixture)

The thermal behavior of niosomes suggested that the drug is encapsulated in the niosomes as semicrystalline form as the fusion peak in the niosomes is very weak compared to the pure drug.

6.4.7 X-Ray Diffraction (XRD)

XRD spectrum of Betaxolol (Figure 6.15) revealed that the drug is crystalline in nature. XRD pattern of niosomes showed that characteristic peaks of betaxolol were reduced in number and intensity indicating that the drug crystallinity was decreased and drug was completely entrapped within the niosomes.

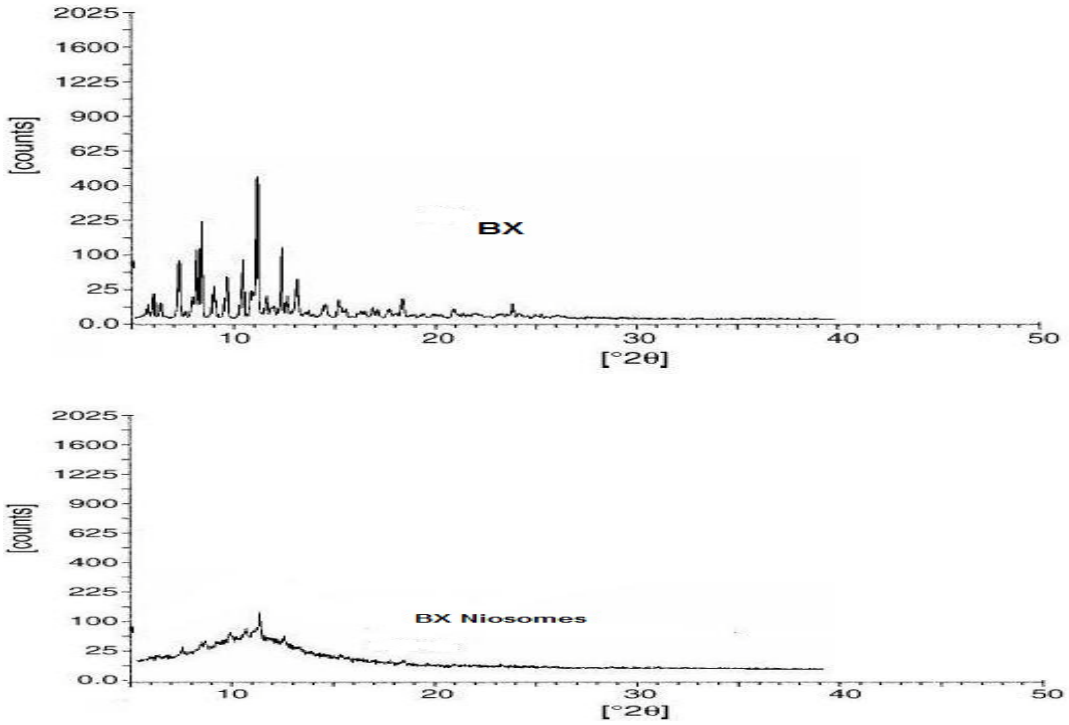

Figure 6.15: XRD patterns of Betaxolol (BX) and NS5 (BX Niosomes)

6.4.8 Sterility testing

The sterility testing of niosomes was performed for aerobic, anaerobic bacteria and fungi by using alternate thioglycollate medium and soyabean casein digest medium as per the IP'07 procedure.

6.4.8.1 Test for aerobic bacteria

Here, *Bacillus subtilis* was used as a test organism. As shown in Table 6.17, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' tube. The results suggest that the niosomes tested for aerobic bacteria were passed the test for sterility.

6.4.8.2 Test for anaerobic bacteria

Here, *Bacterioides vulgatus* was used as test organism. As shown in Table 6.17, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' tube. The results suggest that the niosomes tested for anaerobic bacteria were passed the test for sterility.

Table 6.17: Sterility test observations in ATGM

Sr. no.	Samples	Days					
		1	2	3	5	7	14
1	Negative Control	-	-	-	-	-	-
2	Positive control	√	√	√	√	√	√
3	Test	-	-	-	-	-	-

(-) Absence of microbial growth, (√) Presence of microbial growth

6.4.8.3 Test for fungi

Here, *Candida albicans* was used as test organisms. As shown in Table 6.18, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' test tube. The results suggest that the niosomes tested for fungi were passed the test for sterility.

Table 6.18: Sterility test observations in SBCD medium

Sr. no.	Samples	Days					
		1	2	3	5	7	14
1	Negative Control	-	-	-	-	-	-
2	Positive control	√	√	√	√	√	√
3	Test	-	-	-	-	-	-

(-) Absence of microbial growth, (√) Presence of microbial growth

The overall results of the sterility test showed that the niosomes passed the sterility test and hence they were sterile preparations.

6.4.9 *In vivo* studies

6.4.9.1 Corneal residence evaluation

The precorneal residence data of betaxolol after application of equivalent doses containing niosomes and eye drops in rabbit eyes is shown in Table 6.19 and Figure 6.16. There was a significant improvement in precorneal resident of betaxolol after application of the formulated niosomes as compared to eye drops. In case of niosomes, the levels of drug concentration in tear fluid were maintained for 12 hr while for eye drops concentration was very less after 6 hr. The increase in corneal residence may be attributed to the sustained release of drug from the niosomes as proved by *in vitro* studies.

Table 6.19: Tear fluid concentration of betaxolol

Time (h)	Tear fluid concentration (µg/mL)		
	NT6	NS5	Eye drop
0.1	0.1	0.12	20.48
1	1.02	1.08	10.42
2	3.24	2.73	6.85
4	4.12	3.84	2.14
6	3.82	3.8	1.24
8	3.12	2.62	0.18
10	2.13	1.62	0.02
12	1.1	0.42	0
22	0.2	0.14	0
24	0.05	0.04	0

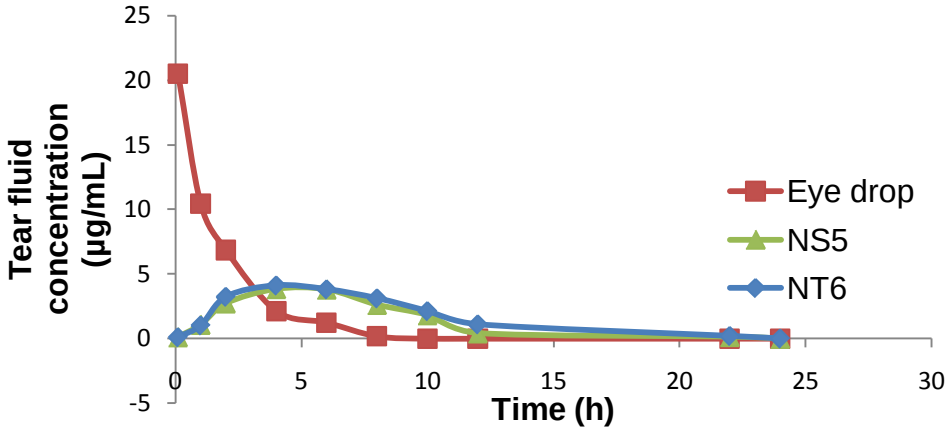

Figure 6.16: Tear fluid concentration of betaxolol

6.4.9.2 Ocular safety studies

The ocular safety score of the formulation NS5 and NB6 was found to be 1 and 0 at the end of 24 hr respectively and therefore, considered as non irritating[30]. Thus, it can be concluded that they were safe for ocular administration.

6.4.9.3 *In vivo* antiglaucoma efficacy evaluation

In vivo antiglaucoma activity was carried out on rabbit eyes using steroid induced glaucoma model. The data obtained was represented graphically as shown in Figure 6.17. The graph (Figure 6.17) shows that both selected niosomal formulations NS5 and NT6 showed better control over IOP as compared to eye drops. Both formulations showed peak effect (t_{max}) at 5 hr as compared to eye drops (3 hr). On administration of niosomes, IOP was under control for the period of 12 hr while eye drops was effective in controlling IOP for the period of 9 hr.

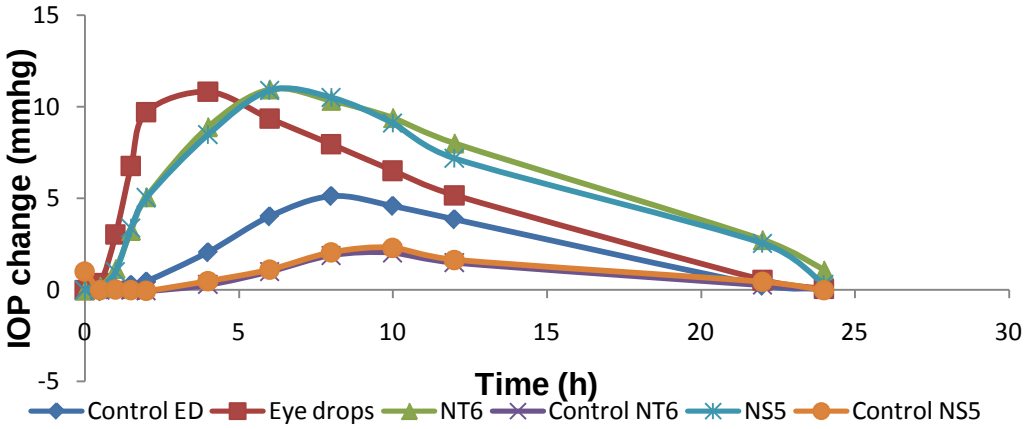

Figure 6.17: IOP lowering activity of betaxolol niosomes and eye drop

The interesting finding of the study was that IOP was also lowered effectively in control eyes of eye drop treated group, which was not the case for niosome treated group. This can be an indirect measure of systemic absorption of the drug following eye drops treatment, indicating that niosomes provide better control over systemic side effects.

6.4.10 Ageing study

Ageing study of the betaxolol niosomes was performed at R.T., 4 ^{o}C and 40 ^{o}C for the period of 6 months. The results showed that there was no change in physical appearance of formulations.

The drug content of the formulations were determined and it was observed that the percentage of drug leaching (percent of un-entrapped drug) was increased by 5.43%, 22.13% and 31.5% respectively (Table 6.20), for the preparations stored at refrigerated temperature, room temperature and 40 ^{o}C for NS5. Similar results were observed for NT6 niosomes.

The formulation stored at cold temperature was subjected to vesicular size analysis. The z-average diameter of the vesicles was found to increase from 124.21 to 211.8 nm and 121 nm to 222.48 for NS5 and NT6 respectively, the PDI increased from 0.387 to 0.598 and 0.213 to 0.568 indicating vesicle aggregation at RT and 40 ^{o}C. However, at refrigerated temperature no evidence of vesicular fusion was seen because an increase in vesicle size and PDI did not vary widely. Thus, we could suggest that the nanovesicular formulation should be stored at refrigerated temperature to maintain its better physical stability. Both formulations complied test for sterility at the end of 180 days. Sedimentation data revealed that both the optimized formulations had shown complete sedimentation at the end of 120 days at RT and 40 ^{o}C.

In addition, the pH shifted from 7.41 to 6.64 and from 7.38 to 6.51 for the formulations NS5 and NT6 respectively stored at 40^{o}C. No appreciable change in pH was noted for the formulation stored at cold temperature and room temperature; however, the formulation stored at 40^{o}C exhibited a considerable shift in pH of the nanovesicular suspension.

Table 6.20: Ageing study results of niosomes

		NS5 (days)				NT6 (days)			
		0	**60**	**120**	**180**	**0**	**60**	**120**	**180**
RDC	**4°C**	100	98.92	99.18	94.57	100	98.48	97.86	95.14
	RT	100	97.42	89.74	77.87	100	97.32	88.42	75.50
	40° C	100	93.18	79.26	68.5	100	93.65	78.32	68.26
pH	**4°C**	7.42	7.42	7.40	7.38	7.38	7.41	7.37	7.35
	RT	7.41	7.38	7.15	7.01	7.38	7.19	7.06	6.84
	40° C	7.41	7.13	7.06	6.64	7.38	7.02	6.95	6.51
MVD	**4°C**	124.21	128.21	125.65	132.41	121.96	120.28	124.61	124.41
	RT	124.21	128.32	159.84	198.48	121.96	138.21	198.86	214.28
	40° C	124.21	156.63	198.48	211.26	121.96	188.81	204.64	222.48
Sedimentation	**4°C**	-	-	+	++	-	-	+	++
	RT	-	+	++	+++	-	+	++	+++
	40° C	-	++	+++	+++	-	++	+++	+++

- no sedimentation, +(1-25%) partial sedimentation, ++ (26-75%) nearly complete sedimentation, +++ complete sedimentation

These results concluded that niosomes was chemically, physically and microbiologically stable at refrigeration temperature for six months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life.

6.5 Conclusion

Betaxolol containing niosomes were prepared using two different surfactants span and tween and evaluated for *in vitro* and *in vivo* tests. Morphological studies revealed that all the formulations were spherical in shape and existed as separate particles. Drug entrapment was higher enough to incorporate required dose of drug in minimum possible concentrated niosomal suspension. The release of drug from niosomes was controlled by diffusion for a prolonged period of time. Both the optimized formulations showed better control of IOP over eye drops, indicating that niosomes can be a choice of drug delivery for the treatment of glaucoma as a sustained ocular drug delivery system.

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CHAPTER 7

FORMULATION AND EVALUATION OF BETAXOLOL NIOSERTS

Chapter 7 - Formulation and evaluation of betaxolol nioserts

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7. FORMULATION AND EVALUATION OF BETAXOLOL NIOSERTS

7.1 Aim of present investigation

There are many potentially effective drug delivery systems and vehicles. They differ in their drug capacities, structure, *in vivo* and storage stability, modes of administration and possible applications. Each of them has favorable attributes and limitations[1].

Recent trend in ocular research is to formulate a dosage form which not only prolongs the residence of system in eye but also helps to reduce the elimination of the drug. In the same area many drug delivery systems including insitu gel[2], microemulsions[3], nanoparticles[4], liposome[5], niosomes[6], ocular inserts[7] etc. are being investigated worldwide by many researchers. The developmental technology of new ocular dosage forms has witnessed tremendous improvement during last few decades and its use is expected to increase in near future.

Ophthalmic inserts offer many advantages over conventional dosages forms, like increased ocular residence, possibility of releasing drug at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf life. Design, construction and technology of ocular insert in a controlled and sustained ocular delivery device are gaining rapid improvement to overcome these constraints[7,8].Niosomal vesicular system is one of the potential approaches, which can be suitably used[9].

In our last attempt (chapter 6), we developed niosomal suspension of betaxolol. It showed sustained release of betaxolol for 24 hr (*in vitro*) but in *vivo* antiglaucoma activity was only upto 12-14 hr due to precorneal washout of niosomes from *cul-de-sac*. Stability data of this formulations suggested that on a longer storage of niosomes, physical instability (agglomeration) and drug leakage had occurred. This may cause dose inaccuracy and therapeutic inefficiency.

Owing to these disadvantages, ocular inserts containing Betaxolol Niosomes – NIOSERT was designed and developed, in order to prolong the residence of drug/niosome in tear fluid as well as to enhance physical and chemical stability of niosomes.

Hence this investigation has been designed to study the drug release kinetics of betaxolol from a niosert developed by incorporating niosomes in matrix system of PEO and PVP. In the present investigation two different polymers have been blended to combine the advantages of the individual polymer. Advantages of polymer blends include easy fabrication of devices, controlling drug release, manipulation of drug loading and other devices properties such as hydration, degradation rate and mechanical strength. Formulation was designed for 24 hr therapy.

It was our aim to develop nioserts that:

1. Release the drug for prolonged period of time preferably 24 hr, *in vitro* as well as *in vivo*.
2. Provide an increased ocular residence time resulting in prolonged drug delivery in eye.
3. Deliver a drug at a controlled rate over a period of time.
4. Show better *in-vivo* performance than conventional dosage forms.
5. Improve stability of niosomes.

7.2 Experimental

7.2.1 Preliminary screening

Preliminary study was carried out for screening of various polymers.

7.2.1.1 Selection of polymers

Polymers were selected from among Sodium alginate, PVP, PEO and HPMC based on the type of films formed at different concentrations, the strength of the films formed and appearance of the films. Bearing in mind all these factors, it was concluded that PVP and PEO form very good films.

Table 7.1: Formulation compositions for preliminary screening for films incorporating betaxolol niosomes

Ingredients	F1	F2	F3	F4	F5	F6	F7
NS5 (mL)	10	10	10	10	10	10	10
PEO*	240	120	---	---	180	120	---
PVP*	---	120	100	240	---	---	200
HPMC*	---	---	140	---	---	120	---
SA*	---	---	---	---	100	---	100

*All values are in mg. PEG400 (30% w/w) added as plasticizer

This investigation was aimed to use composite polymer in matrix type ocular formulation. For screening the polymers and polymer blend a formulation study was carried out. Above Table 7.1 shows some of the important formulations screened for actual study.

Formulation F2, F5 and F6 were prepared using PEO with PVP, SA and HPMC respectively. It was found that film was not good in case of F5 and F6; whereas F2 gave good uniform, smooth surfaced film as compared to rest other formulations. F1, F3, F4 and F7 films were more sticky and soft, so being difficult to handle. Considering above results, it was decided to prepare the ocular film by using the PEO in combination with PVP in equal proportions.

7.2.1.2 Water absorption test for polymers[10]

Water uptake measurement was performed by using an apparatus consisting of G15 Buchner sintered glass filter of 3.8 cm diameter with one end

connected to a graduated 1 mL pipette via silicone rubber tube. The lower part of the filter and pipette were filled with distilled water at room temperature just before the experiment. The evaporation of water was minimized with aluminum foil wrapped around the filter.

Polymer discs of PEO and PVP were prepared by directly compressing 200 mg of the dried polymer in a hydraulic press. The prepared discs were placed at the center of the filter and the uptake of water for 24 hr was determined by obtaining the change in volume of water in the graduated pipette. The mean of 5 determinations were taken to represent the water uptake for the polymer.

7.2.1.3 Drug-excipients compatibility studies

Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug and the polymer. The drug and 1:1 physical mixtures of drug and polymer (each 10 mg) were prepared and mixed with 400 mg of potassium bromide. About 100 mg of this mixture was compressed to form a pellet using a hydraulic press at 10 tones pressure. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer. The IR spectrum of physical mixture was compared with those of pure drug and polymers to detect any appearance or disappearance of peaks.

7.2.2 Formulation of nioserts

The matrix films of Betaxolol HCl using equal proportions of PEO and PVP (1:1) were prepared by solvent evaporation technique[(11)]. In the present study PBS (pH 7.4) was used as solvent.

The weighed quantities of polymers were dissolved in 10 mL of niosomal suspension and plasticizer (30% w/w of polymer amount) was incorporated. Polymeric suspension was sonicated for 2 min under vacuum to remove the air. Polymeric suspension was then poured into a petridish (6.8 cm diameter) placed on a flat even surface. The rate of evaporation was controlled by inverting the funnel over the petridish. After drying at 40 ^{o}C for 24 hr keeping in desiccator, circular ocular inserts of diameter 6 mm were cut using fabricated mould and packed in aluminum foils and stored in desiccators until further use. The composition of ocular films of Betaxolol HCl is shown in Table 7.2.

Table 7.2: Formulation compositions for ocular inserts of betaxolol

Formulation	Betaxolol (0.5mg)	Total polymer (% w/v)	Loading dose (%)
BNS1	Drug	1	0
BNS2	NS5	1	0
BNS3	NS5	1	20
BNS4	Drug	2	0
BNS5	NS5	2	0
BNS6	NS5	2	20
BNS7	Drug	3	0
BNS8	NS5	3	0
BNS9	NS5	3	20
All formulations contain PEG400 as plasticizer 30 % w/w of polymer weight			

Formulations BNS1, BNS4 and BNS7 were containing pure drug while rest of formulations incorporated niosome NS5 formulation containing equivalent (5 mg) of betaxolol.

7.2.3 Evaluation

7.2.3.1 Physicochemical evaluation of nioserts

i) Physical appearance

All the ocular films were visually inspected for color, clarity and smoothness.

ii) pH [12]

pH of the inserts was determined by allowing them to dissolve in a test tube containing 5 mL of distilled water at room temperature for 30 min. pH was measured using calibrated pH meter.

iii) Thickness [12]

Thickness of the film is an important factor while considering its drug release from ocular delivery systems. If thickness varies from one film to another, the drug release from the film also varies. So it is must to keep the thickness of the film uniform to get reproducible results. In the present study, the thickness of the formulated films was measured using digital micro meter of sensitivity of

0.01mm (mitutoyo, Japan). Average of 10 films was taken and standard deviation values were calculated.

iv) Weight uniformity (12)

As weight variation between the formulated films can lead to difference in drug content and *in vitro* behavior, evaluation was carried out by weighing 10 films by an electronic balance (least count – 0.1 mg). The average weight and standard deviation were then calculated and reported.

v) Tensile strength and percentage elongation at break (13)

Percentage elongation at break and tensile strength was calculated using the following formulae (Refer section 5.4.1.5).

$$\%\ elongation\ at\ break = \mathrm{IB} - \mathrm{Io} \times 100 \qquad [7.1]$$

Where Io is the original length of the film and

IB is the length of the film at break when stress was applied.

$$\text{Tensile strength} = \frac{\text{Break force}}{\mathrm{ab}(1 + \Delta \mathrm{L}/\mathrm{L})} \qquad [7.2]$$

Where a, b and L are width, thickness and length of the strip respectively and ΔL is the elongation at break.

Break force = weight required to break the film (Kg).

vi) Folding endurance (14)

The flexibility of polymeric films can be measured quantitatively in terms of folding endurance. Folding endurance was determined by repeatedly folding a small strip of ocular film (2×2 cm) at the same place till it breaks. The number of times film could be folded at the same place, without breaking gives the value of folding endurance.

vii) Moisture uptake (11, 14)

The percentage moisture uptake test was carried out to check the physical stability or integrity of the film. Ocular films were weighed individually and placed in a desiccator containing 100 mL of saturated solution of sodium chloride (~ 75 % humidity). After three days, films were taken out and reweighed; the percentage moisture uptake was calculated by using following formula.

$$\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial Weight}}{\text{Initial weight}} \times 100 \qquad [7.3]$$

viii) Percentage of moisture content (11, 13)

The percentage moisture loss test was carried out to check the integrity of the film at dry condition. Ocular films were weighed individually and placed in a desiccator containing anhydrous calcium chloride. After three days, films were taken out and reweighed; the percentage moisture loss was calculated by using following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \qquad [7.4]$$

ix) Water vapor transmission studies (15)

The glass vials of 5 mL capacity were washed thoroughly and dried to constant weight in an oven. Fused calcium chloride (1 g) was taken in vials and the polymer films were fixed over the brim with the help of an adhesive. These pre-weighed vials were stored in humidity chamber at RH 80% with temperature of 25 ^{o}C for a period of 24 hr. The weight gain was determined every hour up to a period of 24 hr to note the weight gain. Water vapour transmission (WVT) was calculated by taking the difference in weight of film before and after the study for a total period of 24 hr.

x) Determination of drug content (12)

Polymeric films were dissolved in 5 mL distilled water in separate volumetric flasks and stirred using magnetic stirrer. Nioserts were dissolved in 1mL chloroform and drug was extracted with PBS (pH 7.4) which was kept at 60 ^{o}C to remove chloroform. The volume was adjusted to 100 mL with phosphate buffer pH 7.4 and the solutions were filtered through filter. The drug content in each formulation was determined spectrophotometrically at 274 nm. Similarly, a blank solution was prepared using dummy film. Average drug content of three films was determined.

xi) Scanning Electron Microscopy

Films were mounted on an aluminum stub using double-sided adhesive carbon tape and coated with gold palladium using JEOL JFC 1600 auto fine coater for 90 seconds. Samples were examined using scanning electron microscope JSM-6380 LV (Jeol Ltd., Tokyo, Japan) at 20 kv accelerating voltage.

7.2.3.2 *In vitro* drug release study [16]

The *in vitro* drug release from different ophthalmic inserts were studied by using the classical standard cylindrical tube which has the diameter of 15 mm. Dialysis membrane (Dialysis membrane 110, mwc 12000-14000, Hi Media, India), immersed in water for one hour to remove the preservatives followed by rinsing in phosphate buffered saline (PBS) solution, acted as corneal epithelium, was tied to one end of open cylinder which acted as donor compartment. An ocular insert was placed inside this compartment with 0.7 mL of simulated tear fluid (STF). Then, the glass tube was suspended in the dissolution flask of a USP dissolution apparatus such that entire surface of the membrane was in contact with the receptor compartment containing 250 mL of STF (pH 7.4). The content of the receptor compartment was stirred continuously at 25 rpm. Samples of 1 mL were withdrawn from the receptor compartment at periodic intervals and replaced by equal volume of fresh solution. The samples were analyzed spectrophotometrically at 274 nm against reference standard using STF as blank.

i) Data treatment and analysis

The following parameters were determined from the *in vitro* data obtained for the diffusion of Betaxolol hydrochloride through the membrane.

Diffusion rate [17]

Flux [18]

Permeability coefficient [18]

ii) Kinetics of permeation

For finding out the mechanism of drug release from Ocular system, the diffusion data obtained from the above experiments was treated with the different release kinetic equations[19].

Zero order release equation $Q = K_0 t$ [7.5]

Higuchi's square root of time equation $Q = K_H t^{1/2}$ [7.6]

Where, Q is amount of drug release at time t, K_0 is zero order release rate constant, K_H is Higuchi's square root of time release rate constant.

iii) Mechanism of release

Using Korsmeyer and Peppas equation [20, 21]

$$F = (M_t/M) = K_m t^n \quad [7.7]$$

Where, Mt is drug release at time t, M is total Amount of drug in dosage form, F is fraction of drug release at time t, K_m is constant depend on geometry of dosage form and n is diffusion exponent indicating the mechanism of drug release.

7.2.3.3 Sterility testing [22]

Sterility is one of the most vital requirements for an ophthalmic preparation. The tests for sterility are intended for detecting the presence of viable forms of microorganisms in ophthalmic preparations. The principle governing these tests is that if the microorganisms are placed in a medium which provides nutritive material and water, kept at a favorable temperature, the organisms will grow and their presence can be indicated by turbidity in the originally clear medium. In the present study, two media namely, alternative thioglycolate medium (ATGM) and soyabean-casein digest medium (SBCD) were used to investigate the presence/absence of aerobic, anaerobic bacteria and fungi, in the formulated sterilized ocular inserts.

Sterilized inserts were dissolved in fluid A and directly inoculated in above medium aseptically as described in IP 2007 and labeled as 'Test'. Simultaneously, positive and negative controls were also prepared and all the three tubes were incubated at specified temperatures.

7.2.3.4 *In vivo* studies

Approval for the use of animals in the study was obtained from the Institutional Animal Ethics Committee (1338/c/CPCSEA). New Zealand rabbits of either sex weighing 2.5 to 3.1 kg were used for *in vivo* studies. The rabbits were housed singly in restraining cages during the experiment and allowed food and water *ad libitum*. Free lag and eye movement was allowed. No ocular abnormalities were found on external and slit-lamp examination prior to beginning of the study.

i) Corneal residence time evaluation

Precorneal resident time of ocular drugs has been assessed by certain invasive technique[23] and non-invasive technique [24, 25]. These approaches however, require isolation of ocular tissues or the use of radioisotopes. In the present study, an effort was therefore made to develop a non-invasive method to assess the precorneal residence of the drug from the formulated delivery system based on HPLC technique.

a) Tear sampling and analysis

Tear samples equivalent to 1 µL were collected from the left eye after application of test delivery system at 0, 0.10, 1, 2, 4, 6, 10, 22, 24 hr post dosing. Glass capillary tubes having 320 µm internal diameter and 1 µL Premark were placed near the canthus of the eye without applying pressure. Tear fluid was drained into the tubes due to capillary action. Samples equivalent to 1 µL were mixed with 50 µL of mobile phase and injected into HPLC chamber. Data were collected and interpreted and results were tabulated.

b) HPLC Condition [26]

Mobile phase: methanol:acetonitrile:0.1% diethylamine (pH adjusted to 3 with orthophosphoric acid) (30:60:10, %v/v)

Flow Rate: 1.0 mL/min

Column: Phenomenex C18 Column. (Luna C, 25034.6 mm, 5 mm)

Detector: SPD-M20A Prominence Diode array detector

Retention Time: 4.08 min

Injection Volume: 20 µl by Rheodyne 7725*i* injector

Standard solution: 2 µg/mL of Betaxolol in HPLC grade water.

ii) Ocular safety study [27, 28]

The ocular safety of administered delivery system can be tested based on the Draize Irritancy Test (as discussed in section 5.4.7.3). The observations based on scoring approach were used to establish the safety of the developed ocular inserts in rabbit eye. Three healthy albino rabbits (2.7–2.9 kg) were selected to evaluate the ocular irritancy effects of the optimized formulations. For feeding, conventional laboratory diets were used with an unrestricted supply of drinking water. A single formulation was instilled into the conjunctival sac of left eye of each animal (initially to one animal) and the untreated eye served as a control. Each of the animals was observed visually with a slit lamp for the severity of ocular reactions such as corneal ulceration, iritis, conjunctival redness, and conjunctival edema at various intervals of 1, 24, 48, and 72 hr. The animal experiment was conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations, as per the spirit of ethics committee. This study was approved by IAEC (Approval no. 1338/c/CPCSEA).

iii) *In vivo* anti-glaucoma activity evaluation [9, 29]

a) Animal care and handling

The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2 °C relative humidity 44–56% and light and dark cycles of 12:12 hr, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1338/c/CPCSEA).

b) Steroid induced model [30]

Eyes of the 12 rabbits were treated 3 times daily by 0.5% dexamethasone eye drops for 2 weeks. Dexamethasone sodium phosphate (Taj Pharmaceuticals Ltd., Mumbai) was dissolved in phosphate buffer saline to a final concentration of 0.5 % at pH 7.4. The intraocular pressure was measured at noon twice a week by Schiotz tonometer.

c) Antiglaucoma activity

For evaluation, the rabbits with induced glaucoma were randomly divided in three groups (n=3). First group was treated with marketed preparation of betaxolol HCl eye drops equivalent to 0.5 mg. In second group formulation BNS5 and in third group niosert BNS6 were inserted in to lower *cul de sac* of rabbits. The intraocular pressure (IOP) changes were recorded up to 26 hr at specified time intervals. The ocular hypotensive activity is expressed as the average difference in IOP according to the equation $\Delta IOP = IOP_{'0'\ time} - IOP_{'t'time}$ [31].

7.2.3.5 Ageing study [16]

The optimized nioserts were stored in amber colored glass bottles at 3 different temperatures 4 ^{o}C, Room temperature and 40±0.5 ^{o}C for a period of 6 months. The samples were withdrawn after 30, 60, 120 and 180 days and analyzed for physical appearance, drug content, folding endurance and sterility.

7.3 Results and discussion

In the present study ocular films containing Betaxolol HCl niosomes were prepared using combination of polymers (PVP and PEO) and termed as nioserts. Nine formulations were prepared. Films were evaluated for their use as ocular delivery systems with a view to obtain sustained release.

7.3.1 Water absorption test for polymers

PVP and PEO were found to take up 2.1 folds and 1.5 folds of its own weight of water respectively over a period of 24 hr. This property is very much useful as it enhances the mucoadhesive behavior. The water absorption capacity of 200mg of PVP and 200mg of PEO were found to be 0.42 g and 0.3 g respectively after 24 hr.

7.3.2 Drug-excipients compatibility studies

Interpretation of IR spectrum of Betaxolol (Figure 7.1) shows characteristic peaks at 3237 cm^{-1} (hydroxyl group O-H stretching), 2928 cm^{-1} (methyl C-H stretching), 2858 cm^{-1} (methylene C-H stretching), 1612 cm^{-1} (aromatic ring C=C-C stretching), 1557 cm^{-1} and 1513 cm^{-1} (secondary amine N-H bending), 1474 cm^{-1} (methylene C-H bending), 1376 cm^{-1} (methyl C-H sym. bending), 1246 cm^{-1} (alkyl aryl ether c-o-c stretching), 1179 cm^{-1} (aromatic in plane C-H bending), 1087 cm^{-1} (aliphatic ether c-o-c stretching) and 1050 (amine C-N stretching). FTIR spectra of mixture, in comparison to IR spectra of pure drug, show no substantial shifting of the position of the functional groups, indicating no major interaction between drug and polymers.

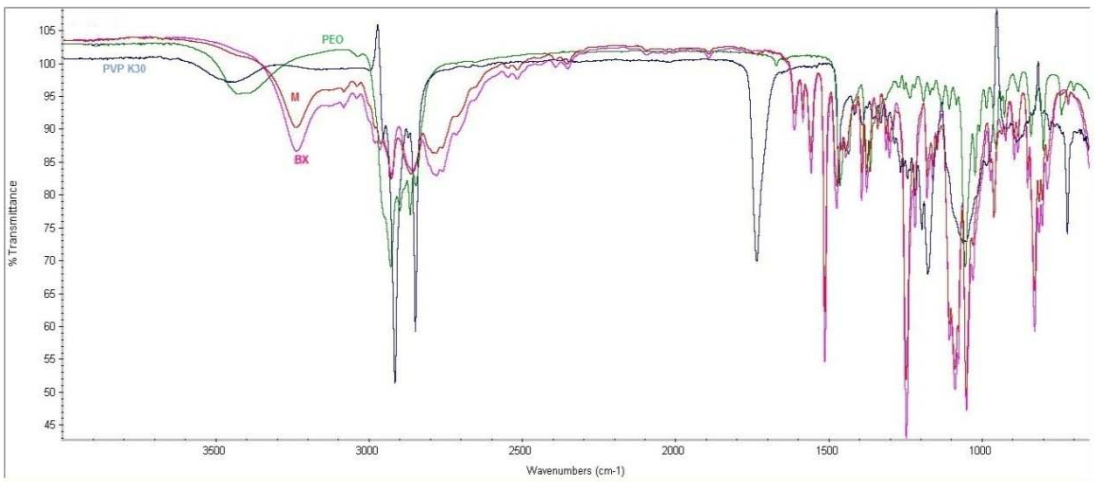

Figure 7.1: IR spectra overlay of pure drug (BX), Mixture (M) of drug/PEO/PVP and polymers (PEO, PVP)

7.3.3 Physicochemical evaluation

In the present investigation solvent evaporation technique is adopted and it was found to be giving thin uniform films. All prepared ocular films have good appearance with slightly rough surface due to presence of niosomes on surface. Films prepared were translucent and pale whitish in color.

Weight and thickness measurement of films was carried out and low standard deviation values in film weight and thickness measurements ensure the uniformity of weight and thickness in each film. A good weight uniformity of all formulation indicates an equal distribution of niosomes and the polymers in the matrix film prepared by solvent evaporation technique. It was also accounted that weight and thickness of films were increasing with increasing polymer concentration. Formulations were not thick enough to produce any irritation while placing and being in *cul-de-sac.*

Table 7.3: Physical properties of prepared nioserts of betaxolol

Nioserts	Weight of films(mg)*	Thickness (mm)[a]	Tensile strength[a] Kg/cm^2	%Elongation at break[a] (%EB)	Folding endurance[a]
BNS1	3.79±0.048	0.122±0.022	0.55 ± 0.02	3.78± 0.11	103±2.5
BNS2	4.81±0.053	0.140±0.015	0.46 ± 0.03	2.63± 0.21	95.6±2.8
BNS3	4.74±0.077	0.138±0.029	0.43 ± 0.01	2.56± 0.14	82.5±3.4
BNS4	5.53±0.061	0.163±0.038	0.64 ± 0.03	5.29± 0.08	112.3±4.16
BNS5	6.59±0.057	0.188±0.009	0.52 ± 0.02	4.02± 0.16	104±3.41
BNS6	6.67±0.063	0.187±0.007	0.51 ± 0.03	4.09± 0.24	94.3±2.18
BNS7	8.62±0.048	0.216±0.013	0.71 ± 0.05	6.49± 0.07	121.2±3.52
BNS8	9.29±0.039	0.241±0.008	0.62 ± 0.02	5.04± 0.31	108.8±2.28
BNS9	9.24±0.072	0.238±0.014	0.64 ± 0.04	4.87± 0.09	98.5±3.53

[a] Average ± SD of three determinations has been reported

*Average ± SD of ten determinations has been reported

The **pH** values of the prepared inserts were found in between 7 to 7.5, indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range[(32)].

Tensile strength and %EB values of all inserts are shown in Table 7.3. It shows that formulation BNS7 showed highest tensile strength and % EB among all batches i.e. 0.71 ± 0.05 kg/cm^2 and 6.49± 0.07% respectively. It was also found that films containing more amount of polymer show higher tensile strength.

Folding endurance values shows that maximum folding endurance was found at high concentration of polymer. Formulation BNS7 shows highest folding endurance. Decrease in folding endurance of nioserts may be due to presence of niosomes causing the reduction in flexibility of films.

Ocular films were also evaluated for % moisture content, % moisture uptake and water vapour transmission rate. Results were tabulated (Table 7.4).

Table 7.4: Evaluation of prepared ocular films of betaxolol

Nioserts	% Moisture content (%MC ± SD)[a]	% Moisture uptake (%MU ± SD)[a]	Water vapor transmission rate[a]× 10^{-3} gm/cm^{2}h	Drug content[a] (% ± SD)
BNS1	4.50 ± 0.03	03.37 ± 0.14	3.95 ± 0.09	100.75±1.32
BNS2	4.78 ± 0.08	03.20 ± 0.09	3.08 ± 0.12	101.14±1.08
BNS3	4.67 ± 0.09	03.18 ± 0.16	3.04 ± 0.04	100.42±1.62
BNS4	6.84 ± 0.11	04.83 ± 0.12	4.83 ± 0.17	101.13±0.48
BNS5	6.77 ± 0.13	04.75 ± 0.08	4.18 ± 0.05	99.85±0.85
BNS6	6.67 ± 0.08	04.72 ± 0.12	4.12 ± 0.19	101.07±1.22
BNS7	7.85 ± 0.12	06.23 ± 0.07	6.02 ± 0.12	100.27±1.09
BNS8	7.81 ± 0.06	06.14 ± 0.09	5.22 ± 0.06	99.73±0.92
BNS9	8.05 ± 0.14	06.10 ± 0.11	5.28 ± 0.04	98.88±1.24

[a] Average ± SD of three determination has been reported

Moisture content values of films were found in range of 4.50 ± 0.03 to 8.05 ± 0.14 percent. It also shows that moisture content of inserts increase with increasing amount of polymers. This is due to Hydrophilic properties of polymer. Same observation was also found in water vapor transmission rate of inserts. Water vapour transmission rate was found in range of 3.04 × 10^{-3} to

6.02 × 10^{-3} gm/cm^2h. The results showed that presence of niosomes in polymer matrix offers resistance to water transmission. The water uptake or absorption behavior of the polymeric film plays an important role at the beginning stage of drug release from dosage form[33]. Thus, the film with higher moisture uptake supposed to give higher drug release rate initially.

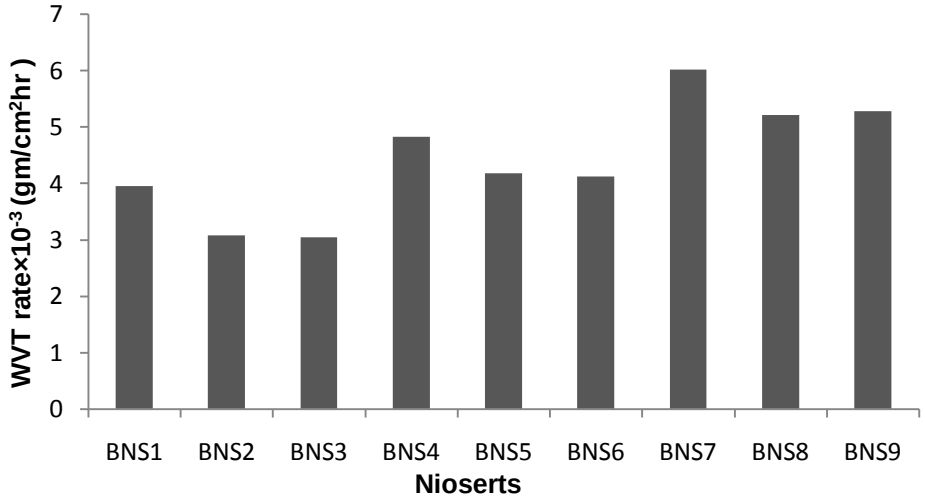

Figure 7.2: Comparative WVT rate of nioserts

Good uniformity in the **drug content** among the batches was observed for all the formulations. % Drug content was found in the range of 98.88±1.24% to 101.14±1.08%. The drug content analysis of the prepared formulations have shown that the process employed to prepare films in this study was capable of giving films with uniform drug content and minimum batch variability.

Scanning electron microscopy

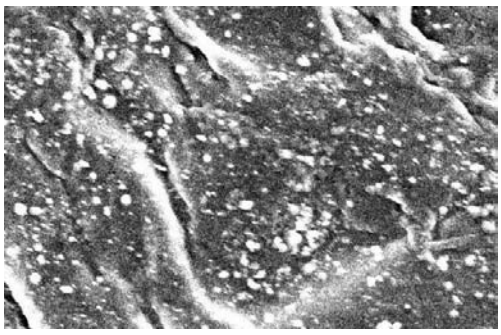

Figure 7.3: SEM image of niosert BNS6

SEM image of Niosert indicated that niosomes were found uniformly distributed throughout the matrix bed of polymer.

7.3.4 *In vitro* drug release studies

In this study, Matrix-type inserts were prepared using polymer blend (PVP and PEO). All formulations prepared were subjected to *in vitro* diffusion study to ensure the effect of variables. The data obtained for *in- vitro* study were tabulated and represented graphically.

Table 7.5 shows percentage cumulative drug release (average of 3 readings) profile for all formulation BNS1 to BNS9. The Maximum drug release was found at lower polymer concentration and as the polymer amount was increased from 1% to 3% w/v, the release was found to be decreased. Niosert BNS8 shows minimum drug release i.e. 69.48% in 24 hr. Initial rapid rate of drug release was observed with nioserts with loading dose as predicted. Initial rate of release affects onset of action.

Table 7.5: Percent cumulative amount of betaxolol permeated through membrane

Time (hr)	BNS1	BNS2	BNS3	BNS4	BNS5	BNS6	BNS7	BNS8	BNS9
	% CP	% CP	%CP	% CP	% CP	% CP	% CP	%CP	% CP
0.5	8.8	1.2	3.25	6.9	0.9	2.68	5.11	0.64	2.16
1	15.42	2.86	7.62	13.26	1.96	7.12	10.43	1.08	6.74
1.5	27.84	3.78	11.42	22.43	3.16	10.86	15.22	1.98	9.32
2	42.32	7.21	18.42	35.92	5.85	17.16	22.43	4.09	15.32
4	72.65	13.18	26.68	60.21	11.08	24.14	45.27	8.46	22.08
6	89.36	19.84	34.98	75.23	16.82	32.43	66.14	13.87	29.32
8	100.78	27.12	43.18	89.21	22.82	40.42	80.18	19.28	36.87
10		33.82	51.26	100.21	29.14	47.82	90.12	25.82	44.24
12		41.26	58.25		36.38	54.12	100.32	32.29	51.12
22		74.32	93.26		68.14	89.32		63.29	83.86
24		82.46	101.14		74.55	96.28		69.48	90.59

Order of cumulative drug permeated was found in following manner:

BNS1>BNS4>BNS7>BNS3>BNS6>BNS9>BNS2>BNS5>BNS8

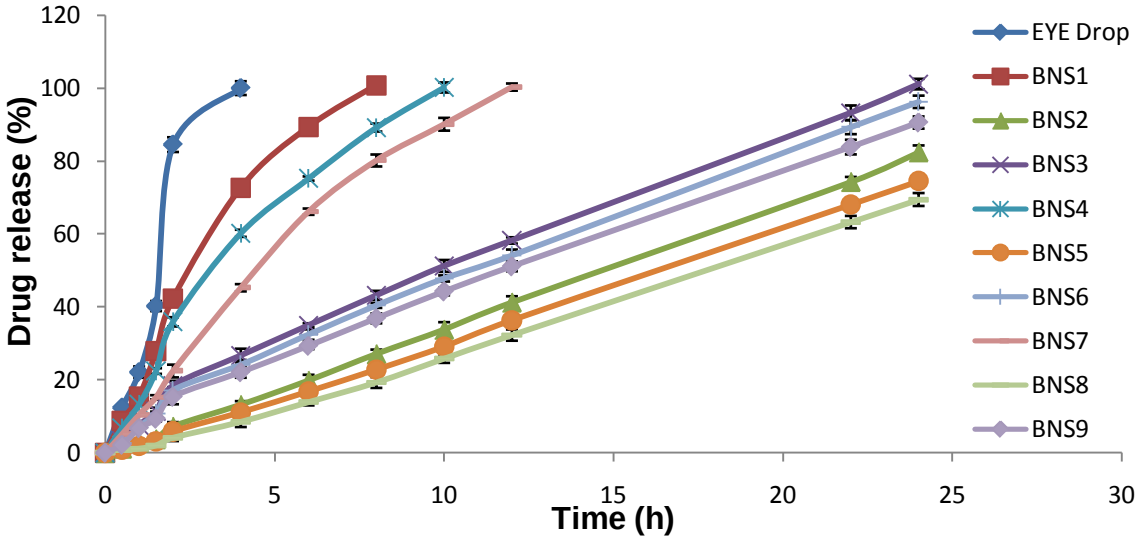

Figure 7.4: *In vitro* release of betaxolol from nioserts

Drug permeation profile of all formulations is shown in Figure 7.4. It shows that formulation BNS1, BNS4 and BNS7 showed almost 100 percent drug release within just 12 hr. Lowest drug release was found in case of BNS8 as it contained more amount of polymer compare to all others.

Diffusion parameters like Diffusion rate, flux and permeability coefficient were also determined.

In vitro permeation profile shows that concentration of polymer in the film plays a very important role on drug permeation. Results show that as concentration of polymer increased there was decrease in drug permeation.

The Maximum average diffusion rate found was 0.0715 mg/hr in case of formulation BNS1 whereas BNS8 shows minimum drug release with diffusion rate of 0.0159 mg/hr. Flux values attained for all formulation are shown in Table 7.6. Result of flux also favors that drug release was inversely proportional to polymer concentration. Diffusion of molecule through inserts was rate limiting step for permeation of molecule. The process of drug release in most controlled release devices is governed by diffusion, and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. It has been reported that diffusion rate of molecule is inversely proportional to diffusion path[34]. Thickness of inserts increases with increasing the amount of polymer. This lead to an increase in diffusion path of molecules and so less

release was observed in case of formulation containing high concentration of polymer.

Table 7.6: Permeation parameters of betaxolol through membrane

Nioserts	Diffusion rate D_r	Flux J_S	Permeability coefficient K_P
	mg/h	mg/cm^{2}h	cm/h
BNS1	0.0715	0.040515289	0.081031
BNS2	0.0175	0.009937712	0.019875
BNS3	0.0254	0.014399773	0.028800
BNS4	0.0567	0.03211778	0.064236
BNS5	0.0168	0.009535674	0.019071
BNS6	0.0237	0.013413647	0.026827
BNS7	0.0379	0.021479049	0.042958
BNS8	0.0159	0.009048698	0.018097
BNS9	0.0221	0.012539638	0.025079

The drug release was found to increase on increasing the concentration of hydrophilic polymer in the polymer matrix. This is due to the fact that dissolution of aqueous soluble fraction of the polymer matrix leads to the release of niosomes which release the drug for prolonged period of times.

Diffusion profiles of all formulation were subjected to data treatment. Diffusion data were treated with zero order, first order, Higuchi and Peppas equation.

The diffusion data treatment of different batches is shown in Table 7.7. It can be concluded from the results that zero order release model fit the best for all the niosert batches as correlation coefficient values for all the inserts were more than 0.99. This is followed by korsemeyer-peppas model and higuchi equation. From the n value it can be seen that all the formulations followed non fickian diffusion of drug release[19]. Nioserts BNS2, BNS5 and BNS8 shows super case-II transport mechanism of release. All other formulations showed anomalous transport of drug release.

From the above results, BNS5 and BNS6 were taken as optimised formulations for further evaluations.

Table 7.7: Kinetic modeling for release profiles of betaxolol nioserts

Insert	Zero order	First order	Higuchi	Korsmeyer -Peppas	n	Release mechanism
BNS1	0.9309	0.7789	0.9819	0.9492	0.8859	Anomalous transport
BNS2	0.9997	0.8066	0.9606	0.9976	1.032	Super Case-II transport
BNS3	0.9920	0.7919	0.985	0.9877	0.7650	Anomalous transport
BNS4	0.9506	0.7806	0.9927	0.9574	0.8429	Anomalous transport
BNS5	0.9994	0.7945	0.9534	0.9948	1.106	Super Case-II transport
BNS6	0.9942	0.8023	0.9812	0.9884	0.7729	Anomalous transport
BNS7	0.9700	0.8297	0.9968	0.9885	0.9184	Anomalous transport
BNS8	0.9983	0.7719	0.9447	0.9912	1.260	Super Case-II transport
BNS9	0.9958	0.8158	0.9792	0.9919	0.7792	Anomalous transport

7.3.5 Sterility testing

The sterility testing of nioserts was performed for aerobic, anaerobic bacteria and fungi by using alternative thioglycollate medium and soyabean casein digest medium as per the IP'07 procedure.

7.3.5.1 Test for aerobic bacteria: Here, *Bacillus subtilis* was used as a test organism. As shown in Table 7.8, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' tube. The results suggest that the nioserts tested for aerobic bacteria were passed the test for sterility.

7.3.5.2 Test for anaerobic bacteria: Here, *Bacterioides vulgatus* was used as test organism. As shown in Table 7.8, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' tube. The results suggest that the nioserts tested for anaerobic bacteria were passed the test for sterility.

Table 7.8: Sterility test observations in ATGM

Sr. no.	Samples	Days				
		1	3	5	7	14
1	Negative control	-	-	-	-	-
2	Positive control	√	√	√	√	√
3	Surface sterilized					
	10 min	-	-	√	√	√
	30 min	-	-	-	-	-

(-) Absence of microbial growth, (√) Presence of microbial growth

7.3.5.3 Test for fungi: Here, *Candida albicans* was used as test organisms. As shown in Table 7.9, there was no evidence of growth found in the 'test' and 'negative control' tubes and there was macroscopic evidence of microbial growth in 'positive control' test tube. The results suggest that the nioserts tested for fungi were passed the test for sterility.

The overall results of the sterility test showed that the surface sterilized (for 30 min) nioserts passed the sterility test and hence they were sterile preparations.

Table 7.9: Sterility test observations in SBCD medium

Sr. no.	Samples	Days				
		1	3	5	7	14
1	Negative Control	-	-	-	-	-
2	Positive contol	√	√	√	√	√
3	Surface Sterilized					
	10 min	-	-	√	√	√
	30 min	-	-	-	-	-

(-) Absence of microbial growth, (√) Presence of microbial growth

7.3.6 *In vivo* studies

7.3.6.1 Corneal residence evaluation

The precorneal residence of betaxolol after application of equivalent doses containing nioserts and eye drops in rabbit eyes is shown in Table 7.10 and Figure 7.5.

Table 7.10: Tear fluid concentration of betaxolol

Time (h)	Tear fluid concentration (µg/mL)		
	Eye drop	BNS5	BNS6
0.1	31.18	0	0.75
1	12.35	0.12	3.16
2	6.85	0.68	4.42
4	2.14	1.24	6.36
6	1.24	2.12	6.88
8	0.18	4.22	6.46
10	N.D.	5.28	5.82
12	N.D.	5.02	5.12
22	N.D.	4.78	4.02
24	N.D.	4.32	3.23

N.D. - Not detectable

There was a significant improvement in precorneal residence of betaxolol after application of the formulated nioserts as compared to eye drops. In case of nioserts, the levels of drug concentration in tear fluid were maintained for

24 hr while for eye drops concentration was very less after 6 hr. It was also observed that in case of BNS5, significant tear concentration of drug was found after 2 hr. This may ultimately affect onset as well as peak effect time of the drug. The increase in corneal residence may be attributed to the controlled release of drug from the nioserts as proved by *in vitro* release studies.

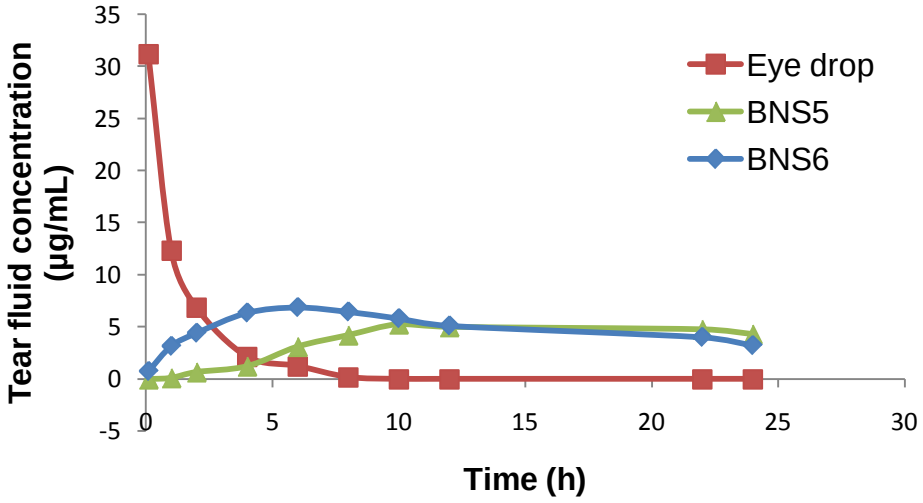

Figure 7.5: Tear fluid concentration of betaxolol

7.3.6.2 Ocular safety studies

The ocular safety score of the formulation BNS6 and BNS5 was found to be 1.5 and 2 at the end of 24 hr respectively and therefore, considered as practically non irritating[27]. Thus, it can be concluded that they were safe for ocular administration.

7.3.6.3 *In vivo* antiglaucoma efficacy evaluation

In vivo antiglaucoma activity was carried out on rabbits using steroid induced glaucoma model. The data obtained was represented graphically as shown in Figure 7.6. Niosert BNS 6 showed faster peak effect (t_{max}) at 6 hr as compared to BNS5 which showed peak effect at 10 hr, this is due to the fact that BNS6 contains free drug as loading dose which started releasing fast due to polymer matrix erosion after insert administration followed by release of niosome encapsulated drug. The results indicate that nioserts showed better control over IOP as compared to eye drops. On the administration of nioserts, IOP was under control for the period of 24 hr while eye drops was effective in controlling IOP for the period of 10 hr.

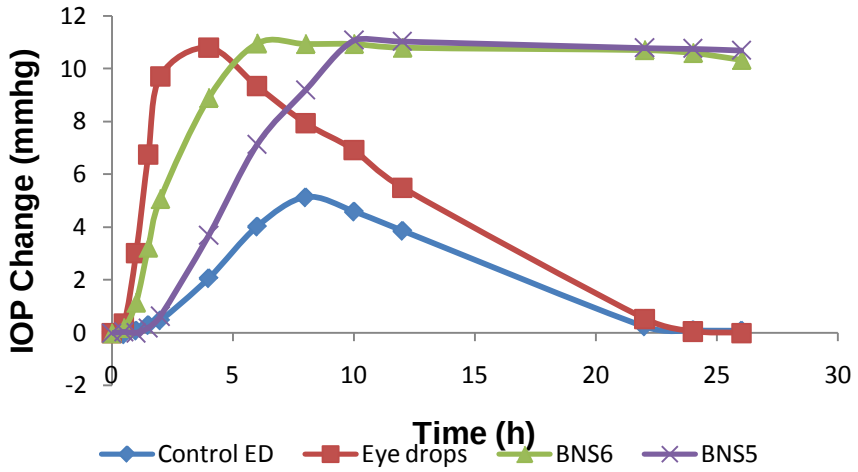

Figure 7.6: IOP lowering activity of betaxolol nioserts and eye drops

Important finding of the study was that control eyes of eye drops treated rabbits also showed reduction in IOP after 4 hr indicating systemic absorption of betaxolol due to nasolachrymal drainage of eye drops supporting the possibilities of side effects on long term use of eye drops. However, no such IOP reduction in control eyes were seen in case of niosert treated rabbits indicating no systemic absorption and hence side effects. Thus nioserts were also found better than eye drops in terms of preventing side effects of betaxolol.

7.3.7 Ageing study

Ageing study of the optimized nioserts was performed at R.T., 4 ^{o}C and 40 ^{o}C for the period of 6 months. The results showed that there was no change in physical appearance of ocuserts. The drug content (Tables 7.11 and 7.12) showed no marked change after six months. Folding endurance values were also comparable to initial values indicating good physical integrity after six months of storage at R.T. and 4 ^{o}C but at 40 ^{o}C, folding endurance was reduced by 40 to 50 % at the end of study possibly due to loss of moisture level of nioserts at high temperature. All the nioserts complied the test for sterility. These results concluded that nioserts were chemically, physically and microbiologically stable at RT for six months. It is better to store nioserts at refrigeration temperature to increase stability. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life.

Table 7.11: Ageing studies of niosert BNS5

Time (days)	4 °C		R.T.		40 °C	
	RDC	FE	RDC	FE	RDC	FE
0	99.85±0.85	103±2	99.85±0.85	103±2	99.85±0.85	103±2
60	99.98±1.05	99.6±3.5	100.12±0.24	94.3±2.5	99.62±0.64	75.3±2.5
90	99.48±0.79	96±1.5	99.81±0.69	91.6±2	98.94±1.08	60.6±2.6
120	99.85±1.13	95.3±3	99.85±0.92	88±2.5	99.18±0.77	54.6±2.5

Table 7.12: Ageing studies of niosert BNS6

Time (days)	4 °C		R.T.		40 °C	
	RDC	FE	RDC	FE	RDC	FE
0	101.07±1.22	94.3±2.18	101.07±1.22	94.3±2.18	101.07±1.22	94.3±2.18
60	100.92±0.85	96±3.5	99.98±1.25	90.33±2.5	101.28±0.63	74.6±2.5
90	100.12±0.28	97.6±1.5	100.28±0.82	85.6±2.8	98.82±0.85	62±2.6
120	99.80±1.68	93.3±3.4	100.52±1.09	80±2.5	99.68±1.77	52.6±2.5

7.4 Conclusion

Attempt has been made to incorporate niosomes in blend of polymers to design sustained release ocular delivery system of betaxolol. The ocular inserts prepared and evaluated in the current study were translucent and uniform in physicochemical properties. Due to use of mucoadhesive polymer, the surface of inserts was hydrophilic enough to be easily wetted by tear film and adhere to corneal surface. Niosomes were found to be uniformly distributed throughout the matrix of polymer as revealed from SEM. Thus the present work showed that incorporation of niosomes into polymeric matrix system can be successfully done in order to model ocular inserts providing promising controlled release delivery system. Niosome loaded ocuserts (Nioserts) showed sustained drug release for 24 hr. The control of IOP, systemic absorption and hence possible side effects using inserts was found to be better than conventional eye drops. Thus, on the basis of *In vivo* antiglaucoma activity, ocular safety test and ageing studies, it can be concluded that this betaxolol nioserts can be a promising once-a-day controlled release formulation.

7.5 Reference

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CHAPTER 8

SUMMARY

8. SUMMARY

Eye being a most delicate organ, ocular drug delivery is a challenge for the formulator. Recent trend in ocular research is to formulate a dosage form which not only prolongs the residence of system in eye but also helps to reduce the elimination of the drug and side effects. In the present study, successful efforts were made to develop such dosage forms.

The ocular inserts were prepared using composite polymeric blend of PMMA and PEO by solvent casting technique. A 3^2 full factorial design was employed to optimize the formulation. Inserts prepared in the current study were semitransparent and uniform in physicochemical properties. Blending of PEO into PMMA matrix was uniform. All the inserts showed drug release for the period of 24 hr *in vitro*. The control of IOP, systemic absorption and hence possible side effects using inserts was found to be better than conventional eye drops.

Betaxolol containing niosomes were prepared using two different surfactants span and tween and evaluated for *in vitro* and *in vivo* tests. A 3^2 full factorial design was employed to study the effects of independent variables on selected responses. Morphological studies revealed that all the formulations were spherical in shape and existed as separate particles. Drug entrapment was higher enough to incorporate required dose of drug in minimum possible concentrated niosomal suspension. The release of drug from niosomes was controlled by diffusion for a prolonged period of time. Both the optimized formulations showed better control of IOP as compared to eye drops.

In nioserts, niosomes were found to be uniformly distributed throughout the matrix of polymer as revealed from SEM. Thus the present work showed that incorporation of niosomes into polymeric matrix system can be successfully done in order to provide promising controlled release delivery system. Nioserts showed sustained drug release for 24 hr. *In vivo* study showed that the control of IOP using nioserts was found to be better than conventional eye drops.

CHAPTER 9

PUBLICATIONS AND PRESENTATIONS

9. PUBLICATIONS AND PRESENTATIONS

9.1 Publications

1. Hitesh B. Gevariya and Jayvadan K. Patel. Design and statistical optimization of betaxolol ocuserts using response surface methodology. ***International Journal of Modern Pharmaceutical Research*.** 2012; 1(2):61-76.

2. Hitesh B. Gevariya and Jayvadan K. Patel. Long acting betaxolol ocular inserts based on polymer composite. ***Current Drug Delivery.*** 2013, 10(2) (In press) (**Impact Factor 1.73)**

3. Hitesh B. Gevariya, Jayvadan K. Patel and Bhanubhai N. Suhagia. Novel vesicular systems for sustained ocular delivery of betaxolol. ***Journal of Microencapsulation.*** (Communicated)

9.2 Presentations

1. Formulation and optimization of betaxolol niosomes using 3^2 full factorial design. **Indo American Pharmaceutical Regulatory Symposium**. Nirma University, Gujarat. Nov 11-13, 2011.

2. Physico-chemical characterization of composite polymeric ocular inserts of betaxolol hydrochloride. **Workshop on 'Practical Approach to Bioavailability and Bioequivalence'**. Uka Tarsadia University, Gujarat. Oct 13-14, 2012.

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Current Drug Delivery, **2013**, *10*, 000-000

Long Acting Betaxolol Ocular Inserts based on Polymer Composite

Hitesh B. Gevariya[1,*] and Jayvadan K. Patel[2]

[1]*Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India;* [2]*Nootan Pharmacy College, Visnagar, Gujarat, India*

Abstract: Poor bioavailability and therapeutic response of conventional therapy due to many pre-corneal constraints necessitate the development of novel controlled and sustained ocular drug delivery to become a standard one in modern pharmaceutical era. This investigation aimed to study the drug release kinetics of betaxolol hydrochloride from a hydrophobic matrix system of PMMA cast with incorporating different proportions of polyethylene oxide (PEO) and evaluate its ability to improve ocular bioavailability and duration of action for the drug. Matrix type ocular inserts were prepared by the film casting technique and characterized *in vitro* by drug release studies using a flow through apparatus that simulated the eye conditions. All the formulations were subjected to physicochemical evaluation. Rabbit model with steroid induced glaucoma was used to establish *in vivo* efficacy of inserts. Polymer composition and concentration significantly affected the drug release based on change in diffusional path length and formation of gelaneous pores by polymer erosion. Formulations released the drug by non-fickian diffusion including anomalous transport ($0.5<n<1$) and super case II transport ($n>1$). It was also observed that increasing the proportion of PEO in to PMMA does not affect the blend miscibility. IVIVC suggested no significant difference ($P< 0.001$) between *in vitro* and *in vivo* release of drug from inserts. *In vivo* IOP lowering activity was better for optimized insert F8 (for 24 h) as compared to eye drops (10 h). This ocular insert could be a promising once-a-day sustained release formulation for treating glaucoma.

Keywords: Betaxolol hydrochloride, ocular delivery, once-a-day ocuserts, polymer composite, release kinetics, sustained release.

INTRODUCTION

Continuous delivery of drugs to the eye offers major advantages over conventional therapies that involve administration of drug solutions or suspensions as eye drops. Eye drop administration often results in poor bioavailability and therapeutic response due to rapid precorneal elimination of the drug and is also associated with patient compliance problems [1, 2]. After instillation of an eyedrop, typically less than 5% of an applied dose reaches the intraocular tissues. This is due to tightness of the cornea1 barrier and rapid loss of the instilled solution from the precorneal area [3-5].

There are two main strategies for improvements of ocular drug delivery: increasing the corneal permeability and prolonging the contact time on the ocular surface. Most formulation efforts aim at maximizing the absorption through prolongation of the drug residence time in the conjunctival sac [6, 7]. Many colloidal drug carriers like nanoparticles, liposomes, niosomes have been investigated as an alternative approach to deliver the drug at right dosage to right target organ, to prevent degradation, metabolism and cellular efflux in the course of drug delivery [8-10]. A basic concept in ophthalmic research and development is that the therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. Ophthalmic inserts offer many advantages over conventional dosages forms, like increased ocular residence, possibility of releasing drug at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf life. Design, construction and technology of ocular insert in a controlled and sustained ocular delivery device are gaining rapid improvement to overcome these constraints [11, 12].

Betaxolol is selective beta-1-adrenegic receptor blocker and used in the treatment of ocular hypertension and chronic open angle glaucoma. There are only a few ocular inserts available on the market, made of EVA as a rate controlling membrane [13, 14]. Likewise, poly (methyl methacrylate) (PMMA) is also an excellent film-forming polymer but the films of PMMA alone are brittle [15]. It offers more resistance to the diffusion of drug molecules, and is less explored as a polymer for ocular delivery of drugs. The current literatures indicate that no inserts are made of hydrophobic monolithic systems using betaxolol. Hence this investigation has been designed to study the drug release kinetics of betaxolol from a hydrophobic matrix system of PMMA cast with incorporating different proportions of polyethylene oxide (PEO). With the addition of hydrophilic polymer to PMMA, the films become resilient and do not break easily and it was ascertained that the diffusion might improve.

MATERIALS AND METHODS

Betaxolol HCL was obtained as a complimentary gift sample from Ciron Drugs and Pharmaceuticals Pvt. Ltd., Mumbai, India. PMMA (molecular weight 120,000) was purchased from Loba Chemie, Mumbai, India. PEO powder

*Address correspondence to this author at the Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India; Tel:/Fax: ??????????????????; E-mail: hitu.phd@gmail.com

1567-2018/13 $58.00+.00

(molecular weight 1000,000) was purchased from Alfa Aesar Inc., USA. Other reagents and chemicals used in the research were of analytical grade.

Preparation of Ocular Inserts

The matrix films were prepared by film casting method from PEO and PMMA using 3^2 full factorial design (Table **1** and **2**). Briefly, weighed quantities of the drug and polymers were solubilized in chloroform with continuous mixing using magnetic stirrer at 25 rpm. The solutions were then sonicated for 30 seconds to remove the air. Polymeric drug solutions were poured on to Teflon coated petridish. The matrix films were dried constantly under the ambient conditions. In all the films DBP (30% w/w) was incorporated as a plasticizer [16-18]. Inserts were sterilized under UV for 1hr [19] and inserts were packed individually in sterilized aluminum foils which were further stored in amber colored glass bottles at room temperature.

Physicochemical Evaluation of Ocular Inserts

Prepared inserts were evaluated for physical appearance, surface pH, thickness, weight variation, folding endurance, tensile strength, moisture content, water transmission rate, water uptake and drug content uniformity.

Surface pH was determined by allowing inserts to swell in a closed petridish at room temperature for 30 minutes in 0.1ml of distilled water [20]. PH paper was kept on surface and after one minute the color developed was compared with the standard colour scale. Thickness was evaluated using a micro meter of sensitivity of 0.001mm (mitutoyo, Japan); the average of ten readings was taken [21]. From each batch ten inserts were weighed individually using digital balance (Shimadzu, Japan) and mean weight was recorded [22]. Folding endurance was determined by repeatedly folding a small strip of ocular film at the same place till it broke. Drug content was estimated by triturating ocular inserts in 20 ml of phosphate buffer pH 7.4 with the help of mortar and pestle. The solution was filtered and one ml solution was withdrawn, diluted and measured by UV-Visible Spectrophotometer at 274 nm [23].

Tensile strength was determined with an instrument assembled in the laboratory. A small strip of ocular film measuring 5 cm×1 cm was cut with a sharp blade. One end of the film was fixed by placing in the film holder. Another end of the film was fixed with the help of forceps having triangular ends to keep the strip straight while stretching and a hook was inserted. A thread was tied to the hook, passed over the pulley and a small pan attached to the other end to hold weights. A small pointer was attached to the thread that travels over the graph paper affixed on the base plate.

To determine elongation and tensile strength, the film was pulled by means of a pulley system. Weights were gradually added (5gms/min) to the pan to increase the pulling force till the film was broken. Elongation was determined simultaneously by noting the distance traveled by the

Table 1. Independent Factors for Formulation of Betaxolol Ocular Inserts.

Independent Variable	Factor A Drug to Polymer Ratio (Drug : Total Polymer)			Factor B Polymer to Polymer Ratio (PMMA : PEO)		
	Low	Medium	High	Low	Medium	High
Coded Levels	-1	0	1	-1	0	1
Actual Levels	1:2	1:4	1:6	9:1	8:2	7:3

Table 2. 3^2 Full Factorial Design of Betaxolol Ocular Inserts.

Formulation	Factor A Drug to Polymer Ratio		Factor B Polymer to Polymer Ratio (PMMA:PEO)	
	Coded	Actual	Coded	Actual
F1	-1	1:2	-1	9:1
F2	-1	1:2	0	8:2
F3	-1	1:2	1	7:3
F4	0	1:4	-1	9:1
F5	0	1:4	0	8:2
F6	0	1:4	1	7:3
F7	1	1:6	-1	9:1
F8	1	1:6	0	8:2
F9	1	1:6	1	7:3

pointer on the graph paper before the film was broken. The weight necessary to break the film was noted as break force. Percentage elongation at break and tensile strength was calculated using the following formulae [24].

% elongation at break = IB $-$ Io $\times$ 100

Where Io is the original length of the film and

IB is the length of the film at break when stress was applied.

$$\text{Tensile strength} = \frac{\text{Break force}}{ab\,(1 + \Delta L / L)}$$

Where a, b and L are width, thickness and length of the strip respectively

ΔL is the elongation at break.

Break force = weight required to break the film (Kg).

Percentage moisture content, moisture uptake [24] and water vapor transmission studies [25] were carried as per the methods prescribed elsewhere.

Surface Morphology

Surface characteristics of polymer blend were studies by Scanning electron microscopy. Films were mounted on an aluminum stub using double-sided adhesive carbon tape and coated with gold palladium using JEOL JFC 1600 auto fine coater for 90 seconds. Samples were examined using scanning electron microscope JSM-6380 LV (Jeol Ltd., Tokyo, Japan) at 20 kv accelerating voltage.

Hydrophilicity

The measurement of contact angle was performed at R.T. by optical tensiometry using contact-angle meter (Theta optical tensiometer, Biolin scientific AB, Sweden) equipped with T200 60 fps digital camera. Drops of STF (pH 7.4) were prepared with a precision syringe (1 ml, Hamilton 1001TPLT) and were dropped onto the surface of the polymer. The static contact angle was measured at contact time t= 10 s.

Drug-Excipients Compatibility Study

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of the pure drug and physical mixture (betaxolol, PEO and PMMA) were taken as KBr pellets in the range of 4000–650 cm^{-1} (FT/IR-4100 type A spectrophotometer, Jasco, Japan). The infrared analysis of optimized insert was carried out in the same range by ATR-IR spectroscopy (Perkin Elmer Model 1600 FT-IR spectrophotometer with ATR mode Perkin Elmer, USA).

Physical State of Drug

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) scans of pure drug and drug loaded ocular insert were performed using DSC 1/700 (Mettler Toledo, Germany). The analysis was performed with a heating range of -20 ^{0}C to 250 °C and at a rate of 10°C per minute in nitrogen atmoshere. The sample weight was approximately 6 mg.

X-Ray Diffraction

X-Ray diffraction (XRD) patterns of pure betaxolol and ocular film were recorded using a powder X-Ray diffractometer (XRD-D8 Advance, Bruker, Germany) over the interval10-90° $2\theta^{-1}$. The experimental condition were: generator tension (voltage) of 38 kV, generator current of 34 mA, scan step time of 30.6 sec^{-1} and scan step size of 0.049° $2\theta^{-1}$.

In Vitro Drug Permeation Study

Since there was no specific official method prescribed for *in vitro* studies of ocular inserts, we fabricated an open flow through assembly, simulating the condition of the ocular cavity, by using the classical standard cylindrical tube which has the diameter of 15 mm. Dialysis membrane (Dialysis membrane 110, mw cut-off 12000-14000, Hi Media, India), immersed in water for one hour to remove the preservatives followed by rinsing in phosphate buffered saline (PBS) solution, acted as corneal epithelium, was tied to one end of open cylinder which acted as donor compartment. An ocular insert was placed inside this compartment with 0.7 ml of simulated tear fluid (STF pH 7.4). Then, the glass tube was suspended in the dissolution flask of a USP dissolution apparatus such that entire surface of the membrane was in contact with the receptor compartment containing 100 ml of STF. The content of the receptor compartment was stirred continuously at 25 rpm. Samples of 1 ml were withdrawn from the receptor compartment at periodic intervals and replaced by equal volume of fresh solution. The samples were analyzed spectrophotometrically using STF as blank [26, 27].

From the drug permeation data, diffusion rate, steady state flux and permeability coefficient were derived [28]. The results of diffusion study were also fitted to zero-order (%release Vs time), higuchi (%release Vs sq. root of time) and Korsmeyer and Peppas (log %release Vs log time) equation [29].

In Vivo Release Study

Approval for the use of animals in the study was obtained from Institutional Animal Ethics Committee (1338/c/CPCSEA). On the day of experiments, the sterilized ocular inserts were inserted into one eye of seven rabbits at the same time and contralateral eye served as control. After 1, 2, 4, 6, 10, 22 and 24 hrs, the inserts were carefully removed and analyzed for remaining drug content by UV spectrophotometer [26, 27].

Ocular Safety Study

The ocular safety of administered delivery system was studied based on the Draize Eye Test. The observations based on scoring approach established the safety of the developed ocular inserts in rabbit eye [30].

In Vivo Antiglaucoma Activity

In vivo intra ocular pressure lowering activity of optimized Ocular insert of Betaxolol was studied in normotensive albino rabbits of either sex. The animals were housed under well controlled conditions of temperature, humidity and 12/12 – h, light-dark cycle, with free access to food and

water. No ocular abnormalities were found on external and slit-lamp examination prior to beginning of the study.

Glaucoma was induced using topical steroid (dexamethasone) by the method prescribed elsewhere [31]. The basal intraocular pressure was measured by schiotz tonometer. The drug formulation was placed in *cul-de-sac* of rabbits. Total 12 rabbits were divided in to three groups each containing 4 rabbits. First group was treated with marketed preparation of betaxolol HCL eye drop equivalent to 0.50 mg. In second group placebo film and in third group medicated film (F8) of betaxolol HCL was inserted in to lower cul de sac of rabbits. The intraocular pressure (IOP) changes were recorded up to 26 h at specified time intervals. The ocular hypotensive activity is expressed as the average difference in IOP according to the equation $\Delta IOP = IOP_{'0,\ time} - IOP't'time$ [32].

Stability Study

Stability studies were carried out according to ICH guidelines [33]. Ocular inserts (F8) were stored in amber colored glass bottles at 3 different temperatures 4°C, Room temperature (R.T.) and 40±0.5 °C for a period of 6 months. The samples were withdrawn after 30, 60, 120 and 180 days and analyzed for physical appearance, drug content and folding endurance.

RESULTS AND DISCUSSION

Physico-Chemical Evaluation

In the present investigation solvent evaporation technique is adopted & it was found to be giving thin uniform films. The films were transparent with smooth surface indicating good miscibility of both the polymers. The physicochemical evaluation data presented in Table **3** indicates that the thickness of the matrix films varies from 0.118±0.06 mm to 0.25±0.054 mm. All the formulations exhibited thickness with low standard deviation values ensuring the uniformity of the films prepared by film casting method. Formulations were not thick enough to produce any irritation while placing and being in *cul-de-sac* as inferred from ocular irritancy test.

The results showed that weights of formulations ranged from 4.09±0.069 mg to 9.09±0.04 mg. The drug content of all the formulations was found to be within the range of 0.495±0.002 mg to 0.51±0.007 mg for matrix films. The minimum intrabatch variations revealed the suitability of the process used to prepare the ocular inserts.

The folding endurance for all formulations was good. The maximum folding endurance of formulation F3 was 99.3±2.51 foldings and formulation F7 showed minimum folding endurance of 61.3±2.08 foldings. This showed that as the concentration of polymer increased in the formulation, folding endurance was decreased. It was also observed that as the proportion of PEO in PMMA increases, flexibility of films increases as indicated by increasing folding endurance values. High tensile strength values indicate good physical strength of the films. As polymer ratio increases, tensile strength decreases. The surface pH of the prepared inserts varied between 6.5 to 7.5, indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range [34].

Surface Morphology (SEM)

SEM study (Fig. **1**) revealed that surface of the ocular films are smooth indicating the complete miscibility of PEO with PMMA. This finding is similar to that of J.baldrian [35] who suggested that when the concentration of PEO is less than or equal to 20wt. %, the polymers are completely miscible and the blend is amorphous. This result is also supported by DSC and XRD studies (Figs. **2** & **3**).

Hydrophilicity

Static STF Contact angles (θs) of films were below 50 (data not shown) indicating increasing hydrophilicity of the surface as the concentration of PEO increased in the matrix. As θ_s is only related to the outermost 10Å of each samples, the surface composition of blend is greatly correlated with θ_s [36]. This result also reveals that PEO was uniformly distributed throughout the bulk of PMMA and concentration of PEO at surface increases with bulk concentration, making

Table 3. Physicochemical Evaluation Data of Different Batches of Ocular Films.

Formulation	Weight of Films (mg)*	Thickness (mm)#	Tensile Strength# Kg/cm²	%Elongation at Break#	Folding Endurance#
F1	4.09± 0.069	0.118± 0.06	0.75 ± 0.03	3.26± 0.21	69±2
F2	4.2± 0.046	0.13± 0.0063	0.68 ± 0.01	8.13± 0.23	90.3±2.08
F3	4.11± 0.052	0.148± 0.0075	0.57 ± 0.01	12.63± 0.4	99.3±2.51
F4	6.43± 0.094	0.19 ± 0.05	0.84 ± 0.02	2.95± 0.32	65.3±3.51
F5	6.49± 0.082	0.20± 0.007	0.72 ± 0.01	7.18± 0.43	81±2.61
F6	6.44± 0.044	0.21± 0.0054	0.65 ± 0.01	12.17± 0.21	94.6±2.08
F7	8.89± 0.057	0.24± 0.003	1.84 ± 0.05	2.28± 0.32	61.3±2.08
F8	9.09± 0.04	0.248± 0.004	0.92 ± 0.02	7.89± 0.46	73.3±2.52
F9	8.91± 0.052	0.25± 0.054	0.80 ± 0.02	11.93± 0.54	89.6±3.78

All readings are in the form of Mean±SD, # Average of 3 runs, *Average of 10 determinations.

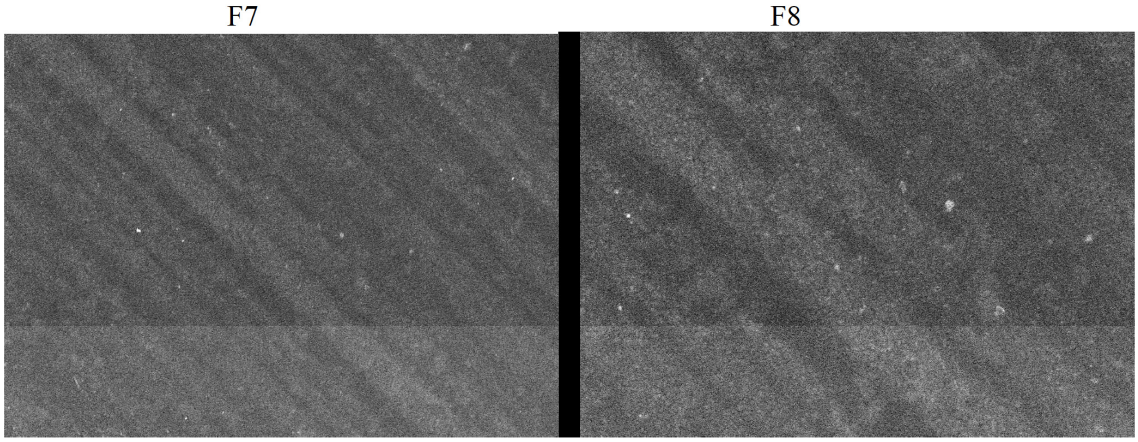

Fig. (1). Scanning electron microscopy (SEM) images of inserts F7 and F8. Experimental condition: magnification= ×1000, Acc. V 20 kV, signal SEI, ___ 10 µm.

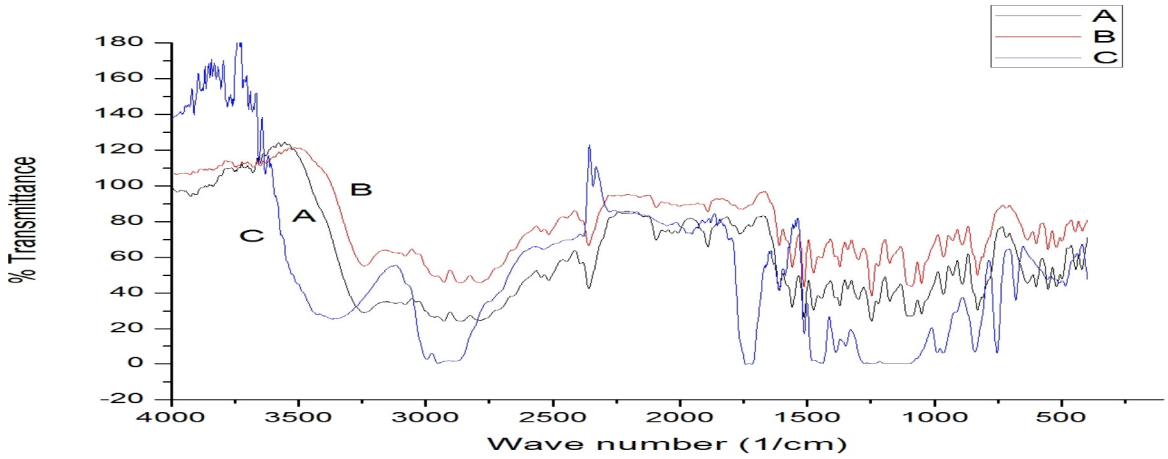

Fig. (2). IR Spectra of (A) Betaxolol HCL (B) Mixture of PMMA/ PEO/BX (C) Ocular Insert F8.

the insert hydrophilic enough to be wetted by tear fluid and adhere to corneal surface for longer duration.

Drug-Excipients Compatibility Study

Interpretation of IR spectrum [37, 38] of Betaxolol (Fig. **2A**) shows characteristic peaks at 3237 cm^{-1} (Hydroxyl group O-H stretching), 2928 cm^{-1} (Methyl C-H stretching), 2858 cm^{-1} (Methylene C-H stretching), 1612 cm^{-1} (Aromatic ring C=C-C stretching), 1557 cm^{-1} and 1513 cm^{-1} (Secondary amine N-H bending), 1474 cm^{-1} (Methylene C-H bending), 1376 cm^{-1} (Methyl C-H sym. bending), 1246 cm^{-1} (alkyl aryl ether c-o-c stretching), 1179 cm^{-1} (aromatic in plane C-H bending), 1087 cm^{-1} (aliphatic ether c-o-c stretching) and 1050 (Amine C-N stretching). FTIR spectra of mixture (Fig. **2B**) and insert F8 (Fig. **2C**), in comparison to IR spectra of pure drug, show no substantial shifting of the position of the functional groups, indicating no major interaction between drug and polymers. However, broadening and reduced intensity of peaks in IR spectra of inserts indicates encapsulation of betaxolol in polymeric matrix. Although shifting and broadening of drug peak at 3237 cm^{-1}in the IR spectra of Insert F8 indicate that hydrogen bonding has occurred between the lone electron pairs of the oxygen atom of polymers and the hydrogen atom of the hydroxyl group of the drug.

Physical State of Drug

DSC

From the overlay of the DSC thermograms, it has been observed that Betaxolol is crystalline in nature (Fig. **3**).

Thermogram exhibited a sharp melting endotherm at an onset temperature of 112.82 ^{0} C, a peak temperature of 119.76 ^{0} C and a heat of fusion of 6.97 J/g. While the thermogram of film shows crystallization of betaxolol from glass at 67.18 ^{0} C followed by fusion at 116.89 ^{0} C. The thermal behavior of film suggested that the drug is present in the film as semicrystalline form as the fusion peak in the film is very weak compared to the pure drug.

XRD

XRD spectrum of Betaxolol (Fig. **4**) revealed that the drug is crystalline in nature. XRD pattern of film showed that characteristic peaks of betaxolol were reduced in number and intensity indicating that the drug crystallinity was decreased in the inserts.

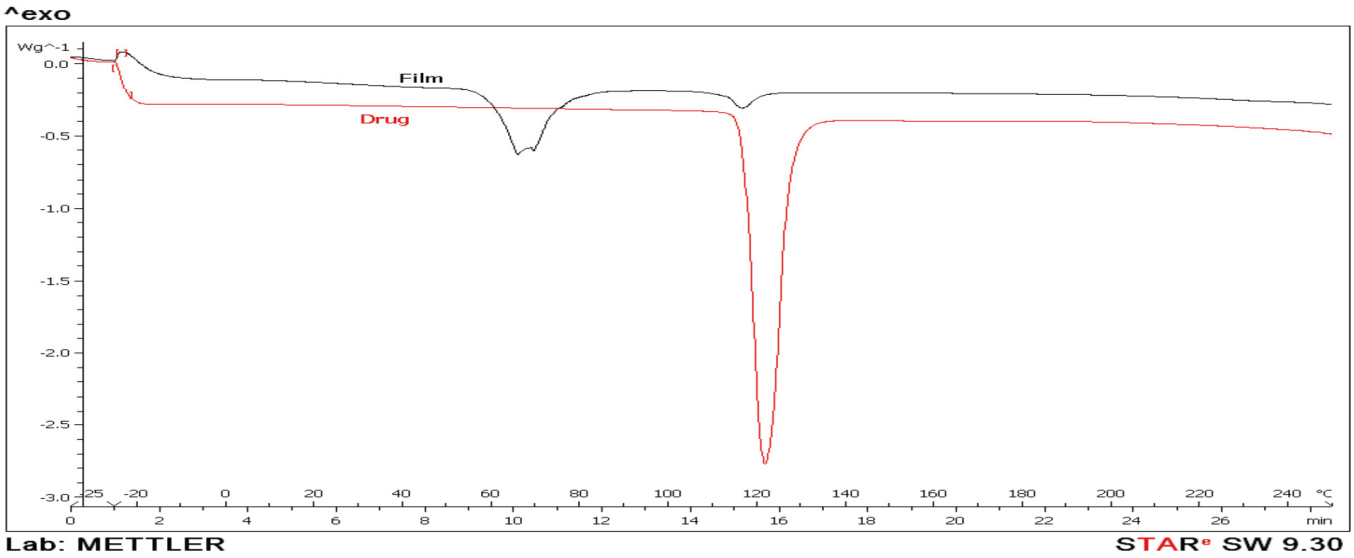

Fig. (3). DSC spectra of pure drug and ocular film.

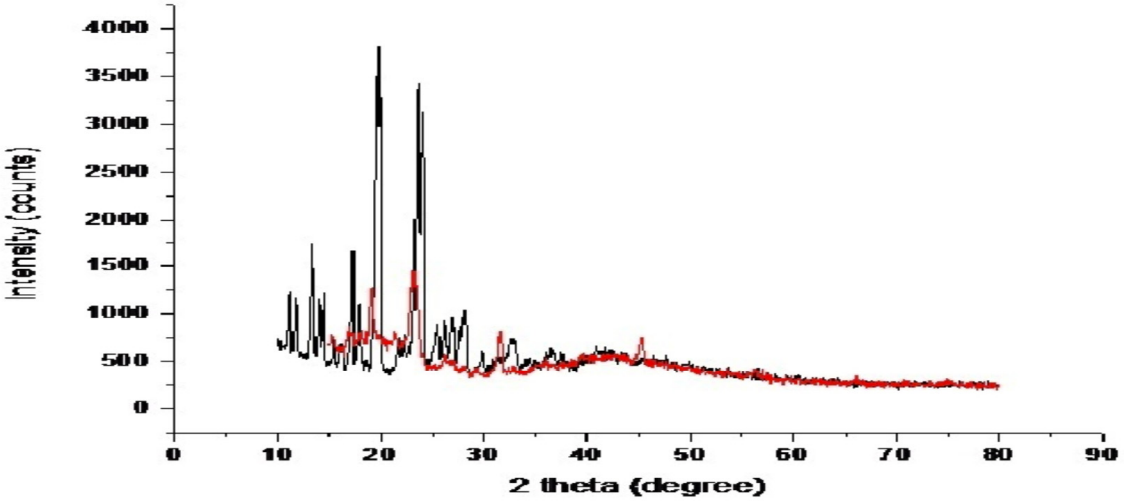

Fig. (4). XRD patterns of Betaxolol (black line) and Insert F8(red line).

In Vitro Diffusion Study

In vitro results revealed that drug release from inserts was influenced by both the factors namely polymer concentration and polymer composition.

Effect of Polymer Concentration

In vitro permeation profile shows that concentration of polymer in the film plays a very important role on drug permeation. ANOVA study shows that factor A (drug to polymer ratio) has significant effect on maximum drug permeated i.e. (Fig. **5**) shows that as concentration of polymer increases there was decrease in drug permeation.

Result of flux (Table **4**) also favors that drug release was inversely proportional to polymer concentration. Among all batches, maximum flux was found in case of F3 i.e. 0.01730 mg/cm^{2}hr.

Diffusion of molecule through polymer matrix was rate limiting step for permeation of molecule. The process of drug release in most controlled release devices is governed by diffusion, and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. It has been reported that diffusion rate of molecule is inversely proportional to diffusion path [39]. Thickness of inserts increases with increasing the amount of polymer. This lead to an increase in diffusion path of molecules and so less release was observed in case of formulation containing high concentration of polymer. Descending order of cumulative drug permeated was found in following order:

Eye drops>F3> F6 > F2 > F5 > F1 > F9 > F4 > F8 > F7

Effect of Polymer Composition

In vitro permeation study shows that change in polymer blend will alter the drug permeation profile. ANOVA study

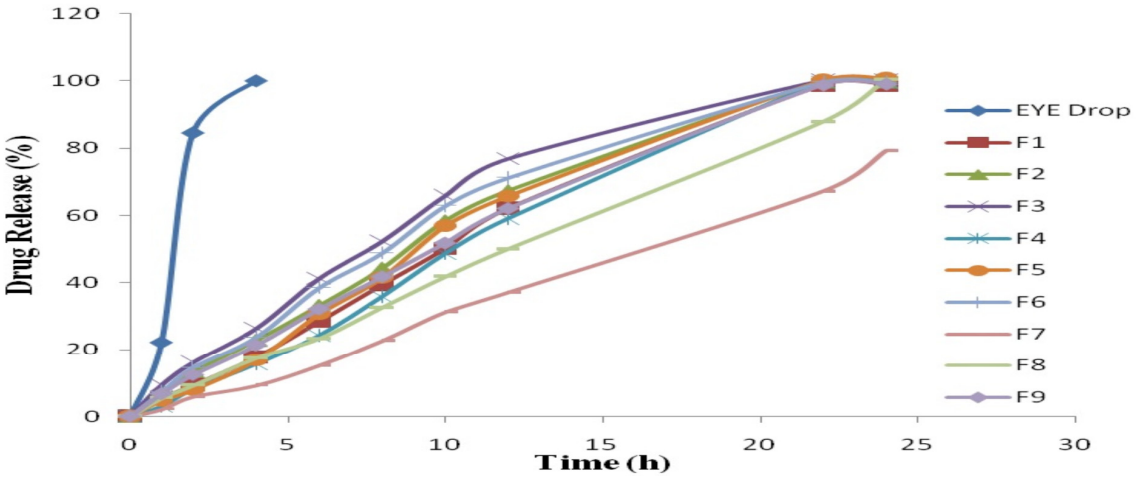

Fig. (5). *In vitro* drug release profile of ocusert.

Table 4. Permeation Parameters of Betaxolol Through Membrane.

Formulation	Diffusion Rate D_r	Flux J_S	Permeability Coefficient K_P
	mg/h	mg/cm^{2}h	cm/h
F1	0.0247	0.013958	2.79×10^{-2}
F2	0.0273	0.015459	3.09×10^{-2}
F3	0.0306	0.017299	3.46×10^{-2}
F4	0.0236	0.013364	2.67×10^{-2}
F5	0.0252	0.014275	2.86×10^{-2}
F6	0.0286	0.016195	3.24×10^{-2}
F7	0.0170	0.009609	1.92×10^{-2}
F8	0.0221	0.012514	2.50×10^{-2}
F9	0.0256	0.014499	2.90×10^{-2}

shows that factor B (HPMC to EC ratio) has significant effect on drug permeation profile.

Average diffusion rate of formulation F1 to F3 was found 0.0247, 0.0273 & 0.0306 mg/h respectively. This shows that diffusion rate was increased with increase in PEO concentration. Similar observations were also found in case of batches F4 to F6 and F7 to F9. Result of flux also indicates that drug release was directly proportional to PEO concentration.

Results showed that increasing concentration of PMMA will retard the drug release and so drug permeation. Release rates were increased when the concentration of PEO increased in the formulations. This is because as the proportion of this polymer in the matrix increased, there was an increase in the amount of water uptake and hydration of the polymeric matrix and thus more drugs was released [40]. The polyether chains of PEO can form strong hydrogen bonds with water, therefore, when inserts are brought into contact with an aqueous medium, the polymer tends to hydrate, forming a superficial gel which eventually erodes as the polymer dissolves [41]. This is due to the fact that dissolution of aqueous soluble fraction of the polymer matrix leads to the formation of gelaneous pores. The formation of such pores leads to decrease in the mean diffusion path length of drug molecules to release into the diffusion medium and hence, to cause higher release rate.

The kinetic treatment of diffusion data is shown in Table **5**. It can be concluded that Korsemeyer and Peppas model fit the best for all the formulations as correlation coefficient value for all the inserts were more than 0.98. This is followed by Higuchi and zero order equation. Inserts F4, F5 and F7 released drug according to super case II transport mechanism while drug release from rest formulations followed anomalous transport. Drug release from such matrices may be controlled by polymer swelling or erosion, or drug diffusion in the hydrated gel, or by these processes altogether [41].

In Vivo Studies

The ocular insert showed 99.87% of drug release *in vivo* at the end of 24 hours which was comparable to *in vitro* drug

Table 5. Kinetic Treatment of Release Study Data of Ocular Inserts.

Formulation	Zero Order	Higuchi	Korsmeyer – Peppas	
	R^2	R^2	R^2	N
F1	0.9869	0.9789	0.995	0.9573
F2	0.9714	0.9869	0.9944	0.8522
F3	0.933	0.9836	0.9867	0.7785
F4	0.9904	0.9721	0.9937	1.095
F5	0.9752	0.9826	0.9871	1.024
F6	0.9517	0.9891	0.9881	0.8423
F7	0.9951	0.9445	0.9935	1.107
F8	0.9986	0.9552	0.9963	0.9215
F9	0.9859	0.9847	0.9979	0.8661

release (Fig. **6**). Thus there was good *in vitro – in vivo* correlation for the ocular insert F8 indicating the effectiveness of the formulation to be used *in vivo*. Difference factor of 0.0004 and similarity factor of 80.29 for the *in vitro-in vivo* release data of formulation F8 indicates no significant difference between *in vitro* release and *in vivo* performance of the inserts.

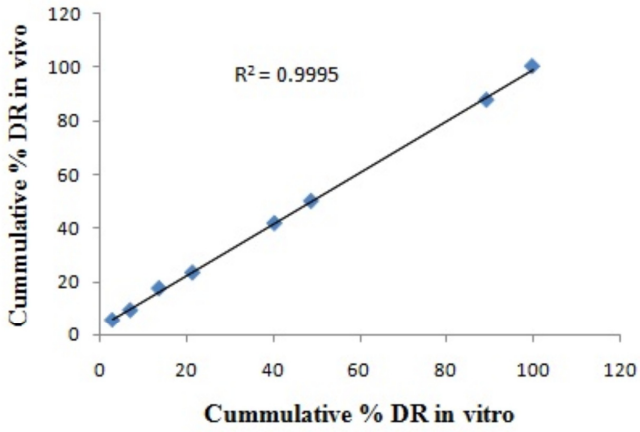

Fig. (6). *In vitro – in vivo* correlation for formulation F8.

The ocular safety score of the formulation F8 was found to be 3 at the end of 24 hours and therefore, considered as minimally irritating [30]. This irritation might be due to the organic solvent used in the preparation of inserts. Thus, it can be concluded that inserts were safe for ocular administration.

In vivo IOP lowering study results (Fig. **7**) revealed that in comparison to eye drops, formulation F8 showed better control of IOP up to 24h ($P< 0.001$).

A single instillation of one drop of 0.5% betaxolol eye drops controlled IOP well to the base level up to the period of 8 h and then after IOP was increased, while same dose of betaxolol from insert F8 controlled IOP up to 24 h. Peak effect was observed at 4 h and 6 h in case of eye drops and F8 respectively. The interesting finding of the study was that IOP was also lowered effectively in control eyes of eye drop treated group, which was not the case for inserts treated group. This can be an indirect measure of systemic absorption of the drug following eye drops treatment, indicating that ocusert provides better control over systemic side effects.

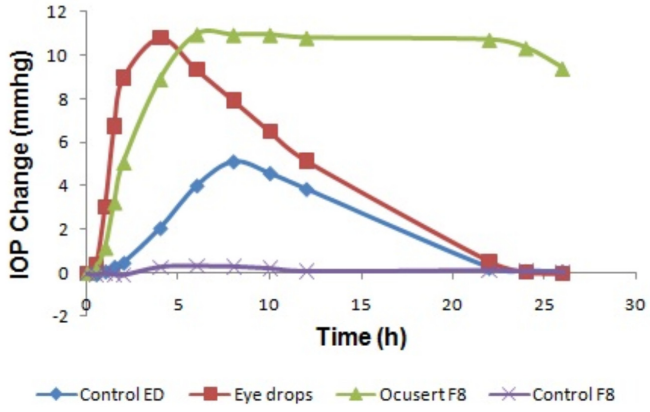

Fig. (7). IOP lowering study.

Stability Study

Stability study of the ocular insert F8 was performed at R.T., 4°C and 40°C for the period of 6 months. The results showed that there was no change in physical appearance of ocular inserts. The drug content showed no marked change after six months and folding endurance were good indicating no change in flexibility of the films. These results concluded that ocular insert F8 was chemically and physically stable at RT for 6 months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life.

CONCLUSION

The ocular inserts prepared and evaluated in the current study are transparent and uniform in physicochemical properties. Due to addition of hydrophilic polymer, the surface of inserts was hydrophilic enough to be easily wetted by tear film. The blend of PEO in PMMA matrix was found to be homogenous and blend was amorphous in nature. No phase separation was observed in polymer composite as revealed from SEM, DSC and XRD studies. Thus the present work showed that incorporation of hydrophilic polymer into hydrophobic matrix system can be successfully done in order to model ocular inserts providing promising controlled release delivery system. Blending of PEO into PMMA matrix was uniform and it was observed that increasing the proportion of PEO in to PMMA increases the rate of release of betaxolol. The control of IOP, systemic absorption and hence possible side effects using inserts was found to be better than conventional eye drops. Thus, on the basis of *In vivo* antiglaucoma activity, ocular safety test and stability studies, it can be concluded that this betaxolol ocular insert can be a promising once-a-day controlled release formulation after due considerations of human *in vivo* studies.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

ACKNOWLEDGEMENTS

The authors thank Dr. D.O. Shah, Director, Shah-Schulman center for surface science and nanotechnology for providing facilities of XRD, DSC and goniometry. We thank Dr. B. N. Suhagia, Rajnikant Mardia and Atinder shukla for their help in IR interpretation. We are grateful to Ciron Drugs and Pharmaceuticals Pvt. Ltd. for gift sample of betaxolol.

PATIENT CONSENT

Declared none.

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Received: ??????????? **Revised:** ??????????? **Accepted:** ???????????

ANNEXURE

Scores for Grading the Severity of the Ocular Irritation (As per Draize Eye Irritancy Test)

Sr. No.	Observations	Rabbit No		
1	**CORNEA**	**1**	**2**	**3**
A	Opacity - Degree of density (area which is more dense taken for reading)			
	Scattered or diffused area-details of iris clearly visible			
	Easily discemible translucent `areas, details of iris slightly obscured			
	Opalescent areas, no details of iris visible, size of pupil barely discemible			
	Opaque, iris invisible			
B	Area of cornea involved			
	One quarter (or less) but not zero			
	Greater than one quarter-less than one-half			
	Greater than one half less than three quarters			
	Greater than three quarters up to whole area			
	Score equals – A×B×5 Total possible maximum = 80 Total Score			
2	**IRIS**			
A	Values			
	Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)			
	No reaction to light hemorrhage; gross destruction (if any or all of these)			
	Score equals – A×5 Total possible maximum = 10 Total Score			
3	**CONJUNCTIVA**			
A	Redness (refers to palpebral conjunctiva only)			
	Vessels definitely injected above normal			
	More diffuse, deeper crimson red, individual vessel not easily discernible			
	Diffuse beefy red			
B	Chemosis			
	Any swelling above normal (include nictitating membrane)			
	Obvious swelling with partial aversion of the lids			
	Swelling with lids about half closed			
	Swelling with lids about half closed to completely closed			
C	Discharge			
	Any amount different from normal (doesn't include small amount observed in inner cul-de-sac)			
	Discharge with moistening of the lids and hairs just adjacent to the lids			
	Discharge with moistening of the lids and considerable area around the eye			
	Score equals – (A+B+C)×2 Total possible maximum = 20 Total Score			

Institutional Animal Ethics Committee approval certificate

Application for permission for animal experiments:

From B per rule 8(a)

APPLICATION FOR PERMISSION FOR ANIMAL EXPERIMENTS

Application to be submitted to sent either to the CPCSEA (address in form A above) or Institutional Animal Ethics Committee (IAEC)

Part A

*1. Name and address of establishment
Faculty of pharmacy, Dharmsinh Desai University, College road, Nadiad- 387 001

*2. Registration number and date of registration **No. 1338/c/CPCSEA.**

3. Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C : Vaccine research centre, Gandhinagar.

4. Place where the animals are presently kept (or proposed to be kept)
Animal house of Faculty of Pharmacy, DDU, Nadiad

5. Place where the experiment is to be performed (Please provide CPCEA Reg. Number)
Department of Pharmacology and Toxicology, Faculty of pharmacy, DDU Nadiad. CPCSEA Reg No. **1338/c/CPCSEA**

6. Date on which the experiment is to commence and duration of experiment
Date of experimental commence: After approval (Proposed-15th March 2012)
Date of completion (Proposed): 14th September 2012
Duration of experiment: 6 month

7. Type of research involved (Basic Research/Educational/Regulatory): Basic research

Signature:

Mr. Hitesh Gevariya
Assistant Professor
Faculty of Pharmacy, DDU, Nadiad
(Name & designation of Investigator)

Date: 05/03/12
Place: Nadiad

*Applicable only for application to be submitted to CPCSEA

CERTIFICATE

This is certifying that the project title "Formulation and Evaluation of Sustained Release Ocular Drug Delivery System for an Anti-glaucoma Drug" has been approved by the IAEC.

Member Secretary IAEC : **Ms. Roshni P. Solanki**

Name of CPCSEA nominee : **Dr. Rajesh U. Posia**

Signature with date

5/3/12

(Ms. Roshni P. Solanki)
Member Secretary IAEC

05/03/12

(Dr. Rajesh U. Posia)
CPCSEA nominee

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)